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Bioremediation and Biotechnology, Vol 2

Degradation of Pesticides and Heavy Metals

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Dedicated to our beloved Parents

Foreword

While the world is remarkably progressing each day towards an advanced age of science and technology, at the same time the pace at which the pollutants are being produced and released into the environment is alarming. The dilution in the values of sustainability has produced pollutants that have impacted the air, soil and water environments to such an extent that their restoration in the present scenario seems to be an impossible task. Diverse pollutants having varied and serious effects have created a hostile environment which is not fit for a healthy life to thrive in. The survival of humans on a polluted planet pumped with heavy metals, chemical fertilisers, pesticides, industrial chemicals and toxins is under threat. Continuous efforts have been made from time to time to combat these pollutants with the use of various techniques, however due to various reasons pertaining to ecological sustainability and economic viability; these technologies have failed to touch the mark of success. With the advent of biotechnology, a breakthrough has been achieved and many success stories have been recorded. The book titled *Bioremediation and Biotechnology: Degradation of pesticides and heavy metals in contaminated Environs* is a brilliant effort towards documenting the bio-techniques and technologies that are and can be used to address the problems of pollution in a sustainable manner. These technologies are not only eco-friendly but also cost-effective. The latest research pertaining to biotechnology and bioremediation has been presented in a lucid manner. The book has 15 chapters. Chapter 1 addresses the main causes and consequences of pollution through pesticides and heavy metals which are persistent pollutants capable of bio-magnification and accumulation in the food chain. Chapter 2 highlights the necessity for the development of mechanism or strategies to overcome metal exposure and to identify substances that devote significantly to overcome their toxic effects within living organism's body. In the present scenario of degraded environments particularly due to heavy metals and pesticides, there is a need to develop techniques which can keep a check on these pollutants. In this regard, Chap. 3 highlights the role of modern innovative techniques for monitoring the pollution due to heavy metals and pesticides using sensors and biosensors. A global scenario regarding the remediation techniques like biodegradation, bioventing, biosparging, phytoremediation, bioreactor, biopile and composting used to combat pesticide pollution is elaborated in Chap. 4 while Chap. 5 details the sustainable technique of mycoremediation for abatement of pesticide pollution and ecological reconstruction. Chapter 6 deals with the types of bio-pesticides and the mechanism of action, their advantages over the conventional and chemical pesticides as well as their role in maintaining the sustainability of the environment. With a mushrooming population in the world, there is a huge pressure on the agricultural sector for food production. However, the chemical fertilisers are reducing the soil fertility as well as crop productivity; therefore, there is an urgent need to use alternatives to meet the need for nutrient sources for crops. In this context, Chap. 7 elaborates the values of biofertilisers for sustainable management in agricultural industries. Macrophytes execute optimisation and self-purification tasks and are an important tool for the hyper-accumulation of micro- and macro-trace elements. Chapter 8 discusses in detail the role of macrophytes in phytofiltration thus helping in restoration of lacustrine environments. Certain plants are potent tools for the removal of pollutants from the environment. Chapter 9 discusses the role of *Salix* (Willows) for phytoremediation of heavy metals. Chapter 10 discusses biotechnological tools and applications as easy, time saving, safer and sustainable way to remediate pesticide pollution.

Industries produce chemical effluents that are toxic to the environment. A number of methods are used for the treatment of these chemicals, which however prove ineffective. Chapter 10 details the technique of photocatalysis as the most effective technique for the treatment of these industrial effluents which completely degrade the toxic organic pollutants and dyes. Chapter 11 focuses on another reliable technique, i.e. micellar-enhanced ultrafiltration, which sustainably removes dyes from wastewater. Another innovative modern technology for degradation and removal of heavy metals and recalcitrant pollutants from the polluted aquatic ecosystems is biofilms and elaborated in detail in Chap. 12.

Chapters 13 and 14 focus on the importance of using eco-friendly and economically viable techniques of biotechnology and bioremediation restore the degraded environments in a sustainable manner. The future prospects of these techniques are also delibrated upon in these chapters. The final chapter provides an overview of global environmental regulations framed for management of pesticides for global environmental safety, human welfare and sustainable development.

This book is a valuable resource material for researchers of biotechnology and allied sciences. The authors and editors have put in a lot of effort to make a noteworthy contribution to the area of biotechnology and bioremediation. I wish them all the best for their future endeavours.

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Preface

The world is advancing towards an advanced age of science and technology, but at the same time the ever-increasing anthropogenic interferences are disrespecting the principles of sustainability causing serious pollution problems. These problems not only threaten the survival of humans on the earth but also make this planet a hostile place to live on. The seriousness of the pollution is directly related to the type of pollutant and its impacts. The pollutants of concern include heavy metals, pesticides, fertilisers and chemical dyes. Pollution from heavy metals and pesticides is a major problem as these pollutants can cause irreparable damage to health or end the life of living beings exposed to them. Chemical substances are introduced in the environment through fertilisers, herbicides, pesticides and phytohormones. Chemical pesticides lead to the depletion of the natural fertility of the soil and deterioration of the environment as a whole. The residual effects of these chemicals create an ecological imbalance as well as contribute to the promotion of modern diseases. Due to these and other associated anthropogenic stresses, the environment has changed drastically causing environmental problems including biodiversity loss and public health issues which has attracted the attention of environmentalists for the remediation and restoration of environment at an earliest mode. As compared to conventional and natural methods, the biotechnological tools and applications are an easy, safe and sustainable way to achieve the goal of remediating and restoring the contaminated environs. Environmental biotechnology is the integration of scientific and engineering knowledge that is employed to remediate and restore the degraded environment. This book focuses on the biotechnological approaches used to combat different environmental pollutions with new technologies to rejuvenate the degraded environments alongwith the future prospects and new developments for sustainable future. The book is an elaborate effort towards bringing together the knowledge related to the problems associated with the contamination of the environment and thereof the strategies to combat them.

The book highlights the necessity for the development of mechanism or strategies to overcome heavy metal exposure and to identify substances that devote significantly to overcome their toxic effects within living organism's body. During the present era, it is necessary to develop innovative techniques for assessing and monitoring the pollutants and develop new methodologies to battle their effects. The content of the book conveys how different types of pollution will be addressed and emphasises on the innovative ways of detecting these pollutants. The sustainable approach to reducing pesticide pollution by using mycoremediation has also been aimed at in an elaborate manner. Several techniques including photocatalysis as the most effective technique for the treatment of industrial effluents including toxic organic pollutants and dyes has also been discussed in detail.

The book elaborates bio-pesticides as an alternative to synthetic pesticides as well as a noteworthy part of numerous vermin control programmes. The future prospects for the improvement of new bio-pesticides are likewise discussed. The role and importance of biofertilisers as sustainable tool in the arena of modern agricultural sciences have also been delibrated on with emphasis on their role in agriculture by way of maintaining the soil sustainability and long-term fertility. A good portion of the book is dedicated to phytoremediation which during the present times is considered to be a a safe and eco-friendly mechanism to restore the degraded ecosystems. Some terrestrial plant species which are a potent tool to remove the contaminants from the environment are appropriately discussed. The macrophytes have been highlighted as potential metal and mineral removal tools acting as biofilters in the aquatic environments. Latest research studies have been presented in the book which reflect the importance of these biofilters in the eco-restoration of the degraded aquatic systems.

The use of bioremediation as an eco-friendly and economically viable technique has been discussed appropriately. Biofilm-based technologies have also been presented as tools with tremendous potential in cleaning pollutants with special focus on the potential role of innovative biofilm technologies to remediate aquatic pollution.

The book is a repository of advanced research in biotechnology and bioremediation. It shall act as a useful reference for the latest advances in research in the concerned area. The content of the book is diverse and shall attract the attention of students, researchers and scientists world over.

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About the Book

This book addresses the grave concerns stemming out due to conventional treatment techniques. The main focus of this book revolves round the central kernel of novel technology (bioremediation and biotechnology) which has emerged as an independent warrior to clean up and restore the disturbed environs. Furthermore, this book is a coherent assortment of diverse chapters relevant to the role of biotechnology and bioremediation for restoration of the ecosystems degraded by pesticide and heavy metal pollution. The inaugural chapters deal with the quantification of problem and its magnitude due to pesticides and heavy metals, followed by innovative modern biotechnological and bioremediation treatment technologies and sustainable techniques to remediate the persistent pollutants. It is a detailed comprehensive account for the treatment technologies from unsustainable to sustainable. Academicians, researchers and students shall find it as a complete wrap up regarding biotechnological intervention for sustainable treatment of pollution and shall suffice for the diverse needs of teaching and research.

Contents

About the Editors

Rouf Ahmad Bhat, Ph.D. is working in Cluster University Srinagar (J&K), India, in the capacity of Assistant Professor and has his specialisation in Limnology, Toxicology, Phytochemistry and Phytoremediation. Dr. Bhat has been teaching graduate and post-graduate students of Environmental Sciences for the past 3 years. He is author of more than 50 research papers and 15 book chapters and has published more than 10 books with international publishers. He has presented and participated in numerous state, national and international conferences, seminars, workshops and symposium. Dr. Bhat has worked as an associate Environmental Expert in World Bank-funded Flood Recovery Project and also Environmental Support Staff in Asian Development Bank (ADB)-funded development projects. He has received many awards, appreciations and recognitions for his services to the science of water testing, air and noise analysis. He has served as editorial board member and reviewer of reputed international journals. Dr. Bhat is still writing and experimenting with diverse capacities of plants for use in aquatic pollution.

Khalid Rehman Hakeem, Ph.D. is Professor at King Abdulaziz University, Jeddah, Saudi Arabia. After completing his doctorate (Botany; specialisation in Plant Eco-physiology and Molecular Biology) from Jamia Hamdard, New Delhi, India, in 2011, he worked as a lecturer at the University of Kashmir, Srinagar, for a short period. Later, he joined Universiti Putra Malaysia, Selangor, Malaysia, and worked there as Postdoctoral Fellow in 2012 and Fellow Researcher (Associate Professor) from 2013 to 2016. Dr. Hakeem has more than 10 years of teaching and research experience in plant eco-physiology, biotechnology and molecular biology, medicinal plant research, plant–microbe–soil interactions as well as in environmental studies. He is the recipient of several fellowships at both national and international levels; also, he has served as the visiting scientist at Jinan University, Guangzhou, China. Currently, he is involved with a number of international research projects with different government organisations. So far, Dr. Hakeem has authored and edited more than 35 books with international publishers, including Springer Nature, Academic Press (Elsevier) and CRC Press. He also has to his credit more than 80 research publications in peer-reviewed international journals and 55 book chapters in edited volumes with international publishers. At present, Dr. Hakeem serves as an editorial board member and reviewer of several high-impact international scientific journals from Elsevier, Springer Nature, Taylor and Francis, Cambridge and John Wiley Publishers. He is included in the advisory board of Cambridge Scholars Publishing, UK. He is also a fellow of Plantae group of the American Society of Plant Biologists, member of the World Academy of Sciences, member of the International Society for Development and Sustainability, Japan and member of Asian Federation of Biotechnology, Korea. Dr. Hakeem has been listed in Marquis Who's Who in the World, since 2014–2019. Currently, Dr. Hakeem is engaged in studying the plant processes at eco-physiological as well as molecular levels.

Moonisa Aslam Dervash, Ph.D. is a young scholar who has earned her Ph.D. in Environmental Sciences from SKAUST-K, Shalimar (J & K), whereas, her B.Sc., M.Sc. and B. Ed. from Kashmir University. She has qualified many prestigious national and state level examinations like UGC-NET, ICAR-NET, JK-SET (twice) etc. Presently, she is working in All India Radio-Akashvani Srinagar (Vivedh Bharati) as a broadcaster on part time basis and also extends her services as a guest lecturer in J & K Higher Education Department. Her specialization is in Environmental monitoring, Ecology, Soil biology and Carbon sequestration. She has authored more than three books with national and International publishers. Moreover, she has published number of scientific research articles and book chapters in the journals of national & international repute. She has also participated and presented her papers in national and international scientific conferences.

She is recipient of many prestigious awards and felicitations like Bharat Excellence Award and Best Educationist Award for her dedicated accomplishments. Her focus has remained on many facets of society especially on environmental conservation and women empowerment. She has been felicitated by state government (J & K Department of Ecology, Environment and Remote Sensing) for her outstanding contribution in environmental awareness and conservation through electronic media. Moreover, she is blessed with the poetic tint which keeps her intact with literary world as well. She has participated in number of poetic symposia organized by Sahitya Akademi New Delhi, Akashvani Srinagar, Jammu & Kashmir Academy for Art, Culture & Literature and Adbi Markaz Kamraaz from time to time. She has been also awarded honorary D. Litt. (Vidya Sagar) by Vikramasheela Vidyapeeth (Bhagalpur, India) for her literary works. Recently, she represented J & K (Kashmiri language) in "All India Young Writers Meet (Young Harvest Programme in Annual Festival of Letters-2020)" which was held at Sahitya Akademi, National Academy of Letters Rabindra Bhawan, New Delhi.

Chapter 1
Causes and Effects of Pesticide and Metal Pollution on Different Ecosystems

Flávia Cristina Policarpo Tonelli and Fernanda Maria Policarpo Tonelli

1.1 Introduction

The interference of human beings on the environment is harmful if it does not respect the principles of sustainability (Qadri and Bhat 2019). It can end up inserting synthetic substances in the environment that become persistent pollutants or promoting the disposal of a large amount of naturally occurring elements in a quantity that represents risk to the living organisms. In the first described scenario, the use of pesticides, and in the second scenario the pollution promoted by heavy metals are examples (Mehmood et al. 2019; Rashid et al. 2019; Singh et al. 2019). So, respectively, organic and inorganic pollutants can threat ecosystems as consequence of human activities like the attempt to improve agricultural production, mine exploitation, industrial production of different products, etc. (Jafari et al. 2013; Morillo and Villaverde 2017; Barrios-Estrada et al. 2018; Mateos et al. 2018; Bilal et al. 2019; Mendes et al. 2019; Pesantes et al. 2019; Pu et al. 2019; Rosculete et al. 2019; Aruliah et al. 2019; Vázquez-Luna and Cuevas-Díaz 2019: Zhang et al. 2019).

1.2 Causes and Effects of Heavy Metal Pollution

Heavy metals are naturally present in trace amounts in the environment and they can reach living forms through the occurrence of natural processes like volcanic eruptions and forest fires. However, when it comes to pollution, anthropogenic processes, involving water, soil and/or air, as causes of environments contamination with these

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harmful elements are the most relevant source. Human interference can cause these elements to accumulate, in a way dependent on metal characteristics and climate factors (Zupancic 2017; Herath et al. 2018), reaching quantities that are considered unsafe.

These elements (for example: arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), and zinc (Zn)) with high atomic weight (Duffus 2002; Busairi and Syahir 2018) can, even at low concentration, threat health of living forms (Muszynska and Hanus-Fajerska 2015). The maximum allowable concentration for Hg, for example, is, according to the United States Environmental Protection Agency, 0.002 μg/mL. Zn is more efficiently tolerable possessing a limit of 5 μg/mL (Odobašić et al. 2019).

These inorganic contaminants represent a serious environmental problem due to the fact that their pollution is persistent, lasting for long time periods (long half-life) on contaminated sites. And besides that, they possess the ability to bioaccumulate through the food chain and cause biomagnification (Mehmood et al. 2019; Rashid et al. 2019; Singh et al. 2018).

The presence of organic matter can favor toxic metals pollution as it can improve the capacities of elements like Hg^{2+}, Ni^{2+}, and Pb^{2+} to binding and accumulate (Rikta et al. 2018).

Mining regions are a very relevant source of contamination where not only soil but also watercourses can be polluted. But different kind of industries (for example, dealing with dyes and pigments or petroleum) can also relevantly contribute by discharging their effluents containing heavy metals into water like rivers, for example (Salomons et al. 1995; Goyer 2001; Alkorta et al. 2004; Karthik et al. 2017; Zhu et al. 2017; Jacob et al. 2018; Ali et al. 2019; Wang et al. 2019).

Among the damages that heavy metals can cause to human health is cancer, once these contaminants are teratogenic and carcinogenic agents (Mushtaq et al. 2019). By poisoning different organs and tissues these elements can also affect the spleen, lungs (causing asthma, for example), skin, bones, liver, pancreas, cornea, gut/stomach, heart and vessels, reproductive organs, brain, and kidney (Farombi et al. 2012; Abarikwu et al. 2013; Tepanosyan et al. 2017; Liu et al. 2018; Boateng et al. 2019). However, they can also cause the organism death after poisoning once they can induce high rates of oxidative stress that, on its turn, can induce cells to die. They can accumulate in soft tissues after entering the body through mainly: skin absorption during industrial activities by adults or ingestion by kids (Jaishankar et al. 2014; Manzetti et al. 2014; Jacob et al. 2018; Masindi and Muedi 2018; Xiong et al. 2019; Yang et al. 2019).

Air can be highly polluted, especially in big cities with intense industrial activities and transportation based on the use of fossil fuel. Particulate matters can arise from natural events like volcanic eruptions, dust storms, and soil erosion but mainly from anthropogenic activities like waste incineration (Li et al. 2017; Weibel et al. 2017) causing the environment, especially near the incinerator, to contain, for example, As, Cd, Cu, Hg, Pb, and Zn (Ma et al. 2018). Eye irritation and respiratory problems are the most quickly harmful effects observed as a consequence of air pollution. Heavy metals present on air pollution can be delivered to the soil by acid rain, for example (Zheng et al. 2012; Ventura et al. 2017).

Soil can accumulate nondegradable heavy metals in high amounts being an important source of food contamination. The presence of heavy metals in soil also affect microorganism, impairing the degradation of biodegradable organic pollutants making the pollution scenario worse. Soil quality is also decreased as heavy metals can, for example, alter soil pH and natural chemistry (Musilova et al. 2016). Landfill is a relevant source of soil contamination with heavy metals risking the life of people who directly deal with the wastes and also people far from the pollution site who can be indirectly exposed through water collaterally contaminated or through air (Wittsiepe et al. 2017). Agriculture soils can also contain heavy metals like Mn, Zn, and Pb in high amounts exceeding the safe exposure values for humans (Hu et al. 2018; Shen et al. 2019; Yang et al. 2019).

A contaminated soil can pollute water nearby and/or groundwater through leachates, endangering the entire ecosystem (Jaishankar et al. 2014; Nagajyoti et al. 2010). As previously mentioned, the presence of organic matter can favor heavy metal pollution and it can also originate hidden pollution. Bottom sediments containing these elements adsorbed into matter can be generated (Liang et al. 2015; Ali et al. 2016; Petrosyan et al. 2019).

Therefore, water can also be polluted by heavy metals from diverse sources directly or indirectly and end up endangering all food chains and food webs (exposing human beings, that are commonly at the extreme of this chain, to a high level of pollutant, and causing serious health problems (Lee et al. 2002)). Water from mining areas, for example, experiences a reduction in biodiversity (Netto et al. 2013; Masindi 2016). Acid mine drainage containing heavy metals can induce lowering of water (and soil) pH making the environment unsuitable for the survival of organisms that are not acidophile (Tutu et al. 2008; Simate and Ndlovu 2014; Torres et al. 2014).

1.3 Causes and Effects of Pesticide Pollution

According to Food and Agriculture Organization (FAO 2019), pesticides are any substance or mixture of substances of chemical or biological ingredients used in agriculture to protect plants against weeds, pests, and diseases. They are capable of repelling, destroying, or controlling any pest, or regulating plant growth. In addition, it is also used against ectoparasites for animal protection. They have a variety of chemical structures.

These substances can be classified according to the target group they attack, their chemical structure, or origin: insecticides (chlorinated hydrocarbons, organophosphates, carbamate insecticides, pyrethroids, botanical and biological products, and others not elsewhere classified); mineral oils, fungicides, and bactericides (inorganic, dithiocarbamates, benzimidazoles, triazoles, diazoles, diazines, morpholines, others not elsewhere classified); herbicides (phenoxy hormone products, triazines, amides, carbamate herbicides, dinitroanilines, urea derivatives, sulfonylurea, bipiridils, uracil, others not elsewhere classified); disinfectants, plant growth regulators, rodenticides (anti-coagulants, cyanide generators, hypercalcemics, narcotics, others not elsewhere classified); seed treatment—fungicides (dithiocarbamates, benzimidazoles, triazoles, diazoles, diazines, morpholines, botanical products and biological, others not elsewhere classified), seed treatment—insecticides (organophosphates, carbamate insecticides, pyrethroids, others not elsewhere classified); and other pesticides not elsewhere specified. In addition, they can also be classified into synthetic (carbamate (nitrogen), chlorinated, phosphorus, and chlorophosphorus), botanical (based on nicotine, pyrethrin, sabadine, rotenone), and inorganic, as some elements (including heavy metals) can also possess pesticide's activity (arsenic, thallium, barium, nitrogen, phosphorus, cadmium, iron, selenium, lead, copper, mercury, and zinc) (FAO 2019).

In recent years their effects on the environment and living organisms have attracted great attention because they are highly toxic substances to human beings. According to the Pesticide Actions Network UK (PAN UK 2019), after a single episode of inhalation, skin contact or ingestion, the symptoms may be immediately evident or can arise within 48 h in the acute toxicity (allergic sensitization, nausea, vomiting, diarrhea, eye and skin irritation, respiratory tract irritation, sore throat and/or cough, headache, loss of consciousness, extreme weakness, seizures and/or death). The effects of long-term toxicity include depression and anxiety, Parkinson's disease, asthma, cancer, attention deficit and hyperactivity disorder (ADHD).

When pesticides are released or applied to the environment, their effects are affected by environmental conditions, such as soil interaction and properties, agricultural practice characteristics, and local climatology (Bhat et al. 2018a; Quaglia et al. 2019).

Agricultural chemicals, for example, consist of fertilizers and pesticides. The main cause of pollution by these substances is their improper use: due to their excessive use or lack of knowledge when they are used, problems were already reported in several plantations such as cotton, maize, wheat and rice (Jin et al. 2015). The amount of insecticides, fungicides, herbicides' use is also increasing to try to provide the growing population with food (Gavrilescu 2005).

Pesticide soil pollution affects soil quality in different ways. The microorganisms present in it, for example, may have their growth rate and activities modified by the chemical, which will lead to modification of the nutrient cycle in the soil. The accumulation of macro and micronutrients such as sulfur (S), manganese (Mn), magnesium (Mg), phosphorus (P), and potassium (K) present in the hybrid Bermuda grass leaf tissues can be reduced, for example, due to the use of isoxaben, prodiamine, and indaziflam (Brosnan 2015). In addition, the root mass of this plant was also reduced compared to plants not treated by these pesticides (FAO 2019).

Another effect to be highlighted is the influence of pesticides on nutrient availability in the soil. For example, sulfur is an essential nutrient especially for crop agricultural production. The monocrotophos application significantly changes the oxidation rate of this nutrient in black and red soils in India. Thus, this rate increases after 7–14 days of application of this substance alone or in combination with mancozeb in black soils (Srinivasulu et al. 2015).

In addition, the presence of pesticides in the soil may lead to changes in enzymatic activity. For example, the arylamidase and myrosinase enzymes may be stimulated by the acetamiprid and carbofuran insecticides. However, these same substances have a toxic effect on enzymatic activity in black and red clay soils (Mohiddin et al. 2015).

Thus, it is necessary to apply pesticide remediation techniques in order to try to eliminate these pollutants from the environment. However, it is also necessary to realize that remediation efficiency depends on factors such as chemical and physical properties of pesticides, climatic conditions, extent and concentration of pesticides, and the presence of other contaminants mixed in the soil/water. Remediation can be done by physical treatments such as soil washing or electro-remediation, or by bioremediation, through phytoremediation and microbial remediation in which plants and microorganisms, respectively, sequester, metabolize, or even release enzymes capable of destroying pesticides (Bhat et al. 2018a; Sun et al. 2018).

When it comes to water pollution, it can be divided into two types: diffuse sources and point sources (Vasilescu and Medvedovici 2005). Diffuse source pollution comes from unlicensed sources and dispersed land use activities. Examples of pollutants are: sediments, toxic substances, salts, acid sulfate soils in drained wetlands, raw pollutants, fertilizers, soil erosion by pathogens such as septic tank leaking bacteria. Pesticide spraying is one of the prime examples of diffuse source and, near the water surface, leads to its contamination. Pesticides enter the surface and soil through water runoff especially in agricultural areas, but also along roadsides, forested areas, golf courses, suburban, and urban landscape areas (Srivastava et al. 2019).

According to FAO, pesticide water contamination is related not only to agriculture but also to forestry in the forest industry, especially in Canada. In this industry, these substances are used to control forest pests, mainly insects, and are sprayed over large areas by aircraft. This use is mainly due to the generation of a habitat conducive to the creation of disease-bearing insects by modifying the water regime in irrigated agriculture in tropical and subtropical environments. In addition, to control disease vectors it is necessary in some cases to add insecticides such as DDT (FAO 2019).

Several factors are associated with the effects of pesticide on water quality. Highlights include impurities contaminating the active ingredient, additives mixed with the active ingredient such as buffers, preservatives, solvents, etc., active ingredients in the pesticide formulation, degraded by microbiological, photochemical, chemical degradation of active ingredients. Some factors are employed as criteria for measuring the effects of pesticides on the aquatic environment. They are: toxicity (expressed in Lethal Dose concentration), degraded, persistence (measured in half-life that may be influenced by metabolism and biodegradation in biotic factors, and hydrolysis, photolysis, and oxidation in abiotic factors), fate (this factor is influenced by the chemical's affinity with the environmental compartment: liquid, solid matter, gaseous form, and biota (FAO 2019)).

The effects of pesticides are varied and generally interrelated, and depend on the organism and the type of substance. Chronic, non-lethal effects are not noticeable, although they still have consequences in the food chain. The consequences are cancer, tumor, injury to fish and animals, suppression of the immune system, genetic and cellular damage, inhibition or failure of reproduction, death, disruption of the endocrine system, impoverishment of fish health marked by low red blood cells and white, intergenerational effects (effects are not apparent until subsequent generations of the organism) (Srivastava et al. 2019).

The main cause of air pollution by pesticides is the indiscriminate use of these substances especially in agriculture in an attempt to improve crop quality. Misuse of pesticides includes use of substances prohibited by local governments, lack of protective equipment, excessive spraying, reuse of washed pesticide containers such as food and drinking water containers, and improper storage. Pesticide use can be influenced by gender, age, chemical knowledge, cultural differences, and perceptions. In air, the main source of contamination is due to inhalation by crop sprays. Thus, the humans who are most affected by this pollution are those who live or work on farms (Özkara et al. 2015).

Organophosphates can enter animals through inhaled particles, ingestion, or skin contact. These pesticides are stored in their muscles and fat and can be found in the liver, lungs, and brain. In addition, these substances can be brought to humans through milk and other animal foods (Zhu et al. 2015).

Pesticide use is highly dangerous as poisoning can be lethal (Mushtaq et al. 2018). Organophosphate poisoning can lead to cholinergic syndrome with symptoms such as coma, blurred vision, delayed neuropathy, blockage of the respiratory center, headache, slurred speech. Although it is possible to reverse an acute bout of contamination, there are likely to be long-term nervous system effects. Other consequences of contamination with these substances are immunological endocrine disruption, function damage, female spontaneous abortions and preterm, and children neuro-developmental delays. In this way, it is essential to control the use of these substances and to study alternatives to their use and forms to revert their effect (Schierow and Esworthy 2012; Niti et al. 2013; Cheng et al. 2016; Liu et al. 2018; Reeves et al. 2019).

1.4 Conclusion

Human intervention on environment to economically explore it without considering sustainability induces an increasing concern and threat to life: pollution. As pollution increases in number of sites affected and its effects are increasingly noticed, researches have been dedicating attention to understand its causes and effects. Heavy metals and pesticides are two important persistent pollutants capable of biomagnifying and accumulating in the food chain. So, they are harmful to air, soil, and water, and consequently to the existence of life on the planet.

1.5 Future Perspectives

It is expected that humanity can understand the importance of interacting with the environment in a sustainable way to reduce the amount of pollutants accumulated. It is also necessary to find new ways to detect the presence of heavy metals and pesticides in water, air, and soil, and quantify their concentration to verify their compliance with the legislation parameters. Through multidisciplinary work, a strategy for monitoring these substances that is most economically viable, fast, reproducible, specific, and practical should be achieved. A good strategy is to adapt and refine existing techniques. Perhaps, this is the first step towards a sustainable environmental model, always remembering that each human being is part of it and by avoiding contamination he/she can protect his or her own health and the health from the others.

The great challenge of this process is the considerable diversity of pesticide's chemical structures, the interactions of heavy metals in each organism, and the interaction of contaminated living beings in the food chain. However, it is expected that in the future a viable alternative or, preferably, viable alternatives, will be reached.

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Chapter 2
Ecotoxicology of Heavy Metals: Sources, Effects and Toxicity

Maya Verma

2.1 Introduction

Environmental pollution is considered as a biggest challenge for whole world, mainly in developing countries. Though produced by anthropogenic activities, it has deleterious effects on environment and its resources. Aquatic habitats, mainly freshwater ecosystems are more prone to pollution as compared with others. Within limits, aquatic ecosystem can cope with pollution, but in severity its get reflected in community structure of flora and fauna. HMs play an important role in environmental pollution. Being toxic and persistent in nature, they are a major threat to the world and of course are of serious concern.

HMs' natural sources include weathering of rocks and volcanic eruptions. Mining, agricultural applications (pesticides, fertilizers especially phosphate fertilizers), industrial emissions come under anthropogenic sources. Being persistent in nature, they pose threats to food chains in ecosystem. Due to their toxic nature, they are also responsible for different health problems. HMs above threshold range heavily affect rhizosphere and reduce soil fertility. In an aquatic ecosystem, bioaccumulation of HMs creates adverse effect on aquatic animals.

HMs are those chemical elements which have density greater than 5 g/cm^3. They are also called trace elements. The toxic HMs commonly present in wastewaters are Hg, Cd, Cr, Pb, As, Cu, Ni, etc. The continuous release of HMs into water bodies creates many environmental problems. Their existence and amplification in environment is an outcome of natural and anthropogenic activities such as urbanization, industrial wastes, etc. Adverse effects of HMs on plants include reduction in seed germination rate and lipid content by cadmium, decreased plant growth and enzymatic activity by chromium, inhibition of photosynthesis by copper and mercury, reduction of chlorophyll production by lead (Gardea-Torresdey et al. 2005).

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In animals, major effects include reduced growth and development, cancer, nervous system damage and multiple organ damage. To prevent toxicity in wastewater, there is an urgent need for enough treatment strategies of wastewater effluents before their discharge into aquatic water bodies.

2.2 Sources of HM Pollution

Natural sources include volcanic eruptions, soil erosion, urban wastes and aerosol particulates (Mushtaq et al. 2020; Qadri and Bhat 2020). Anthropogenic sources include electroporating processes, metal finishing, mining and smelting, textile industries and nuclear power activities. It is concluded that volcanic activities create hazardous impacts to environment, climate and health. During eruptions, many gases are released, i.e., CO_2, H_2S, CO and SO_2. Besides this, several organic compounds and HMs are also released. These HMs deteriorate the quality of water bodies. Volcanic activities have been regarded as responsible for release of toxic HMs (Hg, As, Pb, Cu, Ni) (Amaral et al. 2006).

Soil erosion is accomplished by wind and water. During rainfall, sediment bound HMs reach the soil. HMs during their run off are picked up and distributed in the environment. While in some cases, HMs reaches to poor drainage system and later on in nearby rivers (Taiwo et al. 2011). Aerosol particulates (fine colloidal particles) carry several kinds of contaminants (HMs). These particulates accumulate on leaf surfaces and enter interior of leaves through stomata. HMs enter groundwater and surface water through natural and anthropogenic sources. In present time, anthropogenic sources have exceeded the natural means of heavy metal pollution. The basic sources of metal pollution are the burning of fossil fuels, mining, smelting, sewage plant, fertilizers and pesticides (Fig. 2.1). Electroplating and metal finishing activities involve deposition of thin layers of metals using electrochemical method. During which, HMs are released into wastewater (Table 2.1). It can be either through rinsing of the product or dumping of process baths. It is also noted that cleaning of

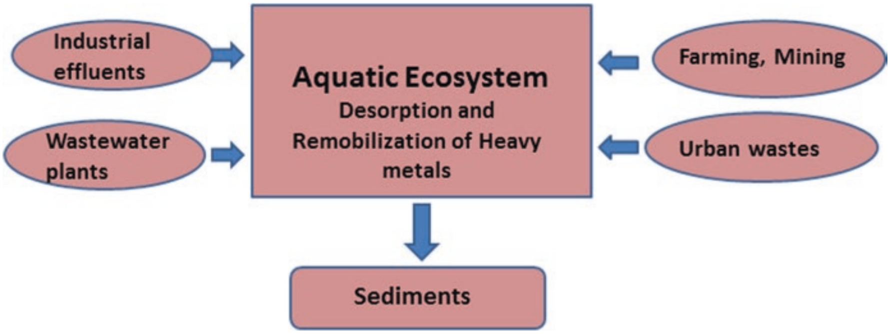

Fig. 2.1 Anthropogenic sources of HMs pollution in aquatic ecosystem

Table 2.1 Major sources of HMs in environment

HMs	Sources (natural and anthropogenic)
Cd	Alkaline batteries, fertilizers, plastic and electrical instruments
Fe	Refineries and textile industries
Cu	Fuel, pesticides, fertilizers, batteries and electrical instruments
Zn	Steel industries, coal burning, mining, pesticides, fertilizers and refineries
Pb	Electrical instruments, batteries, glass industries, fertilizers, refineries and fuel
Cr	Fertilizers and textile industries
Ni	Batteries, fuel and fertilizers
Co	Alloy, aircraft engines and magnets

tanks produces substantial quantity of wet sledge having high concentration of toxic metals (Cushnie 1985).

In the same way, mining activities release toxic metals. Smelting and mining have been considered as major sources of metals in environment. Similarly, textile industries effluents are also a major source of heavy metal pollution as they basically originate from dyeing process. The colouring compounds used in this process include Cu, Cr, Pb, Ni, etc. In nuclear power plant, effluents which are dumped into nearby aquatic bodies affect aquatic biota (Hagberg and Lofgren 2007; Begum et al. 2011).

2.2.1 *Mercury (Hg)*

Mercury exists in metallic form and inorganic salts in nature. It has been considered toxic HMs. Mercury enters air through mining and burning processes and in aquatic ecosystem through municipal wastewater discharge and industrial wastewater discharge (Jan et al. 2009; Chen et al. 2012). Mercury is used colossally in equipment, i.e., thermometer, mercury arc, fluorescent lamp. Its other applications include manufacturing of batteries and some dental applications. Mercury in nature prevails in both mercuric (Hg_2) and mercurous (Hg) forms. Being lipophilic in nature, it can permeate across cell membrane. Mercury in bound form such as methyl mercury (MeHg) enters food chain through fish. Another bound form ethylmercury enters human body via antiseptics and vaccine preparations (Farina et al. 2011; Geier et al. 2015). In soil, mercury (inorganic form) is acted upon by bacteria in soil and water which ultimately results its transformation into methylmercury, it accumulates in fishes and other animal tissue (Hintelmann 2010). Methylmercury induced toxicity includes lipid peroxidation, mitochondrial damages and microtubule destruction while ethylmercury is metabolized into inorganic salts rapidly (Patrick 2002; Carneiro et al. 2014). According to WHO, permissible limit for mercury in drinking water is 0.001 mg/L (WHO 2004). Mercury induced symptoms include lung damage, changes in mucous membrane, diarrhoea, skin rashes, hypertension, renal dysfunction and severe neurological abnormalities (Asano et al. 2000; Bates 2003).

2.2.2 Lead (Pb)

Lead has wide industrial applications such as smelting, refining, mining and battery manufacturing (Malekirad et al. 2010; Karrari et al. 2012; Mehmood et al. 2019; Rashid et al. 2019). It is also used in fertilizers and pesticides and for improving the octane rating of gasoline in vehicles. Due to rapid industrialization and urbanization, increase in effluent discharge from industries, sewage has resulted in contamination of aquatic bodies. Besides this, industrial and agricultural activities are a major cause for contamination of groundwater resources (Ebrahimi et al. 2011). In present times, prescribed limits for lead in drinking water is 0.01 mg/L (Karrari et al. 2012). Being a stable pollutant, its clinical manifestations include from subclinical to life threatening complications (Nelson et al. 2011). Mild exposure of lead results in loss of appetite, headache, abdominal pain, hypertension, renal dysfunction, arthritis, vertigo while severe exposure can cause autism, allergies, dyslexia, hyperactivity, paralysis and brain damage (Papanikolaou et al. 2005). Inhalation through airborne dust and ingestion of contaminated water and food have been considered the most probable routes of lead exposure. In comparison to adults, children are more prone to its toxicity. After absorption through intestine, lead is transferred to soft tissue (liver) and mineralizing tissue (bone) as part of constituent of blood. In evaluation of lead toxicity it was found that lead mobilization from bone to blood increases during lactation, pregnancy, chronic diseases and physiological stress (Gulson et al. 2003). Increased level of lead in pregnant women results in miscarriage, spontaneous abortions and children having low body weight.

2.2.3 Arsenic (As)

Arsenic holds the 20th rank among the most abundant elements in earth's crust (ASTDR 2013). Being inorganic and organic in nature, it is present throughout the world and is major cause for adverse effects on human beings and their environment (Hughes et al. 2011). It exists in more than 200 forms such as arsenate (As V) compounds, as sulphides/sulfosalt, arsenates, arsenides, oxides and silicates (Mandal and Suzuki 2002; Singh et al. 2007). Natural source of arsenic includes volcanic eruptions, rock weathering and geothermal water. Arsenic is primarily used in glass, ceramics, herbicides, pesticides, metals related applications. It is abundantly present in food originated from sea (Meharg 2004; Sohn 2014). Arsenic has been considered as group I carcinogen and in present times its contamination through drinking water has become a serious concern over worldwide (Kulshrestha et al. 2014). Arsenic occurs in two main oxidation states—arsenate (pentavalent) and arsenite (trivalent) anion in nature. After ingestion, it is absorbed by gastrointestinal tract. Their toxicity and level of cellular damage depend upon their valent states. As (III) is regarded as more toxic than As (IV) (Styblo et al. 2000). The permissible limit of this metal in drinking water is 10 μg/L (Smith et al. 2000). Arsenic induced toxicity includes wide range of health problems such as neoplasms, liver, kidney, skin and lymphatic cancer (Shi et al. 2004). Arsenic induced superoxides are also responsible for disrupting cell signalling pathway. Arsenic exposure at low intensity causes reduction in number of RBCs and leucocytes, nausea, vomiting and damage to blood vessels (Yoshida et al. 2004; Hopenhayn 2006).

2.2.4 Cadmium (Cd)

Cadmium is a toxic heavy metal which has its own considerable environmental concerns. Natural sources of cadmium are sedimentary rocks and marine phosphates (Gesamp 1987). It has many industrial applications such as manufacturing of alloys, batteries, mining and smelting (Wilson 1988). Basic route of cadmium exposure is either ingestion of contaminated food or inhalation. Exposure of cadmium in human beings is possible through many ways, e.g., smoking cigarettes, working in cadmium contaminated workplaces (IARC 1993; Paschal et al. 2000). Cadmium inhalation in chronic exposure results in emphysema, decrease in olfactory function. Certain research findings have documented the cadmium exposure in low intensity results in osteoporosis and reducing bone mineral density (Mascagni et al. 2003; Gallagher et al. 2008; Schutte et al. 2008). Cadmium has been regarded as severe pulmonary and gastrointestinal irritant. After acute cadmium ingestion, several health problems arise which include abdominal pain, salivation, muscle cramp, vertigo, shock, loss of consciousness within half an hour (Baselt et al. 1995). While chronic exposure of cadmium has a depressive effect (Singhal et al. 1976). Cadmium has been categorized as human carcinogen by the International Agency for Research on Cancer (IARC 1993). Lung is only the main established site of carcinogenesis in human beings. In animals, other targeted tissues are adrenal testes and haematopoietic system (Waalkes et al. 1995; Waalkes et al. 1996).

2.2.5 Chromium (Cr)

Chromium is naturally present in earth's crust. It occurs in many oxidation states such as Cr (II), Cr (III), Cr (IV), Cr (V) and Cr (VI). Out of these, Cr (VI) form is stable and is regarded as second most stable form (Patlolla et al. 2009). Chromium is produced from natural as well as anthropogenic sources, which includes metal processing industries, chemical industries and tanning industries. Hexavalent form of chromium is classified as human carcinogen by several regulatory authorities (ATSDR 1990; IARC 1990). WHO limit for chromium in drinking water is 50 μg/L (Velma et al. 2009). The main route of chromium exposure in human being is through inhalation and lung is considered as primary target organ (EPA 1992; Velma et al. 2009). More common incidence of dermatitis which has been noticed among workers, working in cement industries, is attributed to their exposure to chromium.

Cr (VI) may cause irritation to the lining of nose, ulcers in stomach and small intestine, anaemia. Incidental ingestion of extremely high doses of Cr (VI) results in cardiovascular, gastrointestinal, haematological, hepatic, renal and neurological effects. Toxicity of chromium related compounds depends upon their oxidation state and their solubility. Cr (VI) compounds are more powerful oxidizing agents. They tend to be corrosive, appear to be much more toxic systemically than Cr (III) compounds (Connett and Wetterhahn 1983; De Flora et al. 1990).

2.3 HMs in Aquatic Ecosystems

In aquatic water bodies, metals can exist either in dissolved, particulates or some other complex forms. Some common physiological processes, i.e., sedimentation, dispersion, absorption and desorption are governing factors for their distribution. Heavy metals exist either in suspended form or sediments in aquatic environment.

2.3.1 HMs Bioavailability to Aquatic Organisms

One of the unique features of HMs is that they are nondegradable. As soon as they enter in aquatic ecosystem, either they are accumulated in sediments or consumed by living organisms (Frémion et al. 2016). Further sediments act as a long term reservoir of contamination in trophic food chain. HMs bioaccumulate in aquatic plants and animals through which they reach up to human beings and cause many health problems (Varol and Şen 2012; Hasan et al. 2016). In sediments, metal accumulates through precipitation, binding and coprecipitation with some metal oxides, i.e., manganese oxide. The process of accumulation depends upon biochemical and physical status among sediment and associated water column (Vasile et al. 2005; Equeenuddin et al. 2013).

2.3.2 Trophic Transfer of HMs

Heavy metals bioaccumulation in trophic food chain depends upon their rate of accumulation rate of removal from body. Therefore all HMs possess different half lives in different species. HMs enter into organism body directly from the water, sediments, soil, etc. (Singh et al. 2018). The confinement of HMs in any organism depends upon speciation of the concerned metal and some physiological processes such as homeostasis, synchronization and detoxification of HMs. Methyl mercury accumulates in biotic environment readily and biomagnifies in food chain due to their lipophilic nature. Metallophytes have developed unique mechanism for copying with high concentration of HMs in soil and they have been categorized into three groups, i.e., (1) excluders, (2) indicators, (3) hyperaccumulators (Ali and Khan 2019). These HMs are taken up from contaminated soil and are transferred to successive trophic levels through food chain (Nica et al. 2012).

2.4 Assessment of HMs in Ecosystems

Specific terms are being used for quantification of HMs in environment, i.e., bioconcentration factor (BCF) and bioaccumulation coefficient (BCF). Bioconcentration factor is defined as the ratio of HMs in an organism tissue to its concentration in abiotic environment (specifically soil and water) (Ali and Khan 2017):

$$\text{Bioconcentration Factor} = \frac{C_{\text{organism tissue}}}{C_{\text{abiotic medium}}}$$

While bioaccumulation coefficient is the ratio of HMs concentration in plants to heavy metal concentration in soil (Ali et al. 2013):

$$\text{Bioaccumulation Coefficient} = \frac{C_{\text{plant}}}{C_{\text{soil}}}$$

2.5 Biomagnification of HMs

Ingestion and biomagnifications of HMs in abiotic environment depend upon variable factors. Ingestion of HMs in plants depends upon bioavailability of metals in soil, which is further dependent upon metal speciation, organic matter and pH of soil. Heavy metals accumulate in plants more simply which are absolutely bioavailable to soil and they would have increased bioaccumulation potential. Plants appear to be more responsive against environmental changes in comparison to soil (Shtangeeva 1995). Many plants and animal species have been regarded as bioindicators of heavy metal pollution, i.e., Lithophaga lithophaga has been reported as a valid bioindicator of heavy marine pollution (Miedico et al. 2016).

2.6 Human Exposure to HMs

HMs affect cell organelles and cellular component, i.e., mitochondria, lysosome, cell membrane, nucleus and endoplasmic reticulum. They are also involved in cellular metabolism and cell detoxification (Fernandes and Nayak 2012). They interact with DNA, nuclear proteins, causing DNA damage which results in cell cycle modulation, apoptosis and severe carcinogenicity (Zhao et al. 2014; Sanyal et al. 2015). Production of ROS and oxidative stress contribute an important role in carcinogenicity of toxic HMs, i.e., Hg, Pb, Cd, etc. (Semu and Singh 1996; Decena et al. 2018). The major HMs (Cd, Cr, Pb, As, Hg) are systemic toxicants which induce multiple organ damage even at lower concentrations (Mehmood et al. 2019). According to International Agency for Research on Cancer (IARC 1993), these metals have been regarded as human carcinogens on the basis of ecotoxicology and epidemiological studies on human beings and animals (Rashid et al. 2019). HMs induced toxicity involves many aspects which are still not clear. Some metals induce synthesis of RNA in nucleus which specifically codes for metallothioneins (MT). These are peptides having low molecular weight, high in cysteine residues (-SH group), found in cytoplasm, lysosome and nucleus. This thiol group induces MTs to bind HMs.

2.7 Conclusion and Suggestions

HMs are nondegradable and persistent in nature. Metals are environmental pollutant for aquatic and terrestrial ecosystem. They are considered toxic due to their unique properties, i.e., persistence in nature, bioaccumulation in successive trophic levels and hazardous in nature. Therefore it is important to access and monitor their concentration in abiotic and biotic environment. Study of environmental chemistry and ecotoxicology of HMs show that following steps should be taken to lessen the impact of toxic metals on human health and environment.

The following suggestions are made:

- There should be regular assessment and monitoring of potentially toxic HMs concentrations in soil, sediment and water.
- Considerable efforts should be made to reduce heavy metal concentration in aquatic and terrestrial ecosystem to protection of biota.
- Industrial effluents and wastewater from industries should be treated more effectively before their discharge.
- Public should be aware about toxic HMs effect on environment and health.
- Research on assessment and monitoring concerned with HMs should be encouraged for improving health of environment.

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Chapter 3

Role of Modern Innovative Techniques for Assessing and Monitoring Heavy Metal and Pesticide Pollution in Different Environments

Fernanda Maria Policarpo Tonelli and Flávia Cristina Policarpo Tonelli

3.1 Heavy Metal and Pesticide Pollution

Heavy metals (Aruliah et al. 2019) and pesticides (Morillo and Villaverde 2017) are relevant environmental pollutants that require attention once they are prone to cause significant damage to various life forms on the planet, especially to humans (Mateos et al. 2018).

3.1.1 Heavy Metal Pollution

Heavy metals as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), zinc (Zn) consist in elements that are expected to be normally found on Earth's crust. However, when their concentrations exceed the naturally occurring safe range limit values they can accumulate (for example in water or soil) and offer high toxicity (even at low concentration), causing serious heath complications (Muszynska and Hanus-Fajerska 2015). Not all heavy metals possess the same toxicity. Actually, it varies from one heavy metal to another. According to United States Environmental Protection Agency, and as previously mentioned in Chap. 1, while the maximum allowable concentration (µg/ml) of Hg is 0.002, the one for Zn is 5 (Odobašić et al. 2019).

These metals possess relatively high atomic weight and specific gravity greater than 5.0 g/cm^3 (Duffus 2002; Busairi and Syahir 2018). Their deposition and the

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pollution distribution on environment depend upon metal characteristics but also environmental specificities like climate factors (Zupancic 2017; Herath et al. 2018). As relevant features, heavy metals possess long half-life persisting in contaminated sites. If not removed by anthropomorphic intervention, they can bioaccumulate and cause biomagnifications (affecting food chain and crop production, for example), present teratogenic and carcinogenic properties, and end up presenting lethal effect (Manzetti et al. 2014; Xiong et al. 2019; Yang et al. 2019).

Besides teratogenic and carcinogenic effects, by poising living organisms heavy metals can affect human beings causing asthma, cardiovascular, neurological, renal, liver diseases, gastrointestinal disorders, skeletal deformities, impairment to reproductive tissues among other severe damages (Farombi et al. 2012; Abarikwu et al. 2013; Tepanosyan et al. 2017; Liu et al. 2018; Mehmood et al. 2019; Rashid et al. 2019; Boateng et al. 2019).

The contamination of a small soil portion (Singh et al. 2018), for example, can end up polluting water. Leachates can drag pollutants to surface water nearby and to groundwater, causing severe undesirable effects not only in human health (Jaishankar et al. 2014), but also ecotoxicological damage (Nagajyoti et al. 2010). Leachates also contain dissolved organic matter which can favor toxic metals (like Ni^{2+}, Pb^{2+}, and Hg^{2+}) binding and accumulation, especially on young samples (Rikta et al. 2018). The kind of pollution that goes through adsorption into matter under water to become bottom sediments is called hidden pollution that is also studied, once it can become real pollution under external factors (Liang et al. 2015; Ali et al. 2016; Petrosyan et al. 2019).

However, what kind of phenomena can cause these elements accumulation in the environment? Human interference through activities involving these metals such as industrial activities related to the use of these elements (e.g., industries dealing with dyes and pigments, textiles, petroleum, fertilizer), mining, smelting, etc. (Goyer 2001; Zhu et al. 2017; Wang et al. 2019).

Waste incineration can also contribute to contamination especially from air, vegetation, and areas surrounding the incinerator (Li et al. 2017; Weibel et al. 2017). Ashes, vegetables, and soil samples collected in areas close to the incinerator can contain Cu, Pb, Zn, Hg, As, and Cd (Ma et al. 2018). Landfill is also a source of environmental contamination with heavy metals. It can contaminate soil, groundwater, water nearby, and plants growing on the area. The latter can accumulate high levels of these pollutants. *Tanacetum vulgare L.*, for example, accumulates Cd, Cr, Ni, and Zn mostly in leaves, and Co, Cu, Fe, Hg, Mn, and Pb mainly in the roots (Adamcová et al. 2017). This environmental pollution represents a wide risk to health, specially the health of the people who work directly with the wastes (Wittsiepe et al. 2017).

Agriculture soils can also contain high amounts of heavy metal (Hu et al. 2018). In China, for example, As, Cd, Cr, Ni, Pb, and Zn accumulate in a level that exceeds the exposure guideline values for children and adults, offering to these people an elevated cancer risk, especially regarding to As, Cd, and Cr (Yang et al. 2019). An interesting study that analyzed in China the surface sediments from an area with increased soil erosion determined, through statistical analysis, that Cr, Co, Ni, and

Cu present in the samples came from natural sources but Mn, Zn, and Pb originated from vehicular traffic, mining, and agricultural and industrial activities (Shen et al. 2019).

Human interference is also important to remove these pollutants from the environment and, consequently, it is necessary to develop innovative techniques for assessing and monitoring heavy metal (and also pesticide) pollution in order to determine pollution's hotspots.

When it comes to remediation of sites polluted by heavy metals physical, chemical, and biological methods (for which researchers continually try to develop improvements aiming cost reduction, more efficiency, and less generation of toxic sludge) can be combined and applied to decrease the amount or to promote total removal of pollutants. Using only one of remediation methods commonly offers worse results when compared to the results obtained by the combination of methodologies generating remediation processes.

In order to perform heavy metals remediation methodologies as generating genetically modified organisms to bioremediate or using species that already possess this ability, performing chemical extraction, bioleaching and/or electrokinetics or other method such as thermal treatment, chlorination, ion-exchange and membrane separation, can offer satisfactory results especially if combined. It is possible, for example, to isolate bacteria from contaminated environments (*Serratia marcescens* from tannery effluent contaminated sludge) and use them for metal removal (zinc) combining bioleaching and electrokinetic remediation in a bioleaching enhanced electrokinetic remediation (BEER) to achieve better results (93.08%) than the ones obtained using single methods as bioleaching (72.86%) or electrokinetic remediation (56.67%) (Selvi and Aruliah 2018; Aruliah et al. 2019).

3.1.2 Pesticide Pollution

Pesticide is the term by which is called a substance or a mixture of substances that can be mainly used to eliminate or control the presence of any pest (living organisms capable of causing damage to environments: commercial, agricultural, and/or domestic) (Schierow and Esworthy 2012; Bhat et al. 2018; Mushtaq et al. 2018).

After the Second World War the use of these substances (e.g., insecticides, fungicides, herbicides, and acaricides) increased in an attempt to improve agricultural production to provide food in a sufficient amount to the growing population (Gavrilescu 2005). However, not only benefit comes from pesticides' use. In fact, the damages that they can cause are various as they are an ecological risk and can threaten human health and life and the survival of other life forms. A large amount of pesticides sprayed may never reach their targets being dispersed instead, contaminating agricultural crop products, soil, water, and air (Niti et al. 2013; Liu et al. 2018; Reeves et al. 2019). For food and drinking water, there are legal limits considered safe (not harmful to human health) by government agencies as the Environmental

Protection Agency (in USA) and the European Environment Agency in European Union (Colmati et al. 2019).

The source of environmental contamination can be punctual (the application points themselves) or diffuse, and even if the soil is commonly the first spot of contamination, the pollutants transferring to waters can occur contaminating not only surface water but also underground water to a greater extent, the more pesticide properties favor their migration into the soil to deeper layers (Vasilescu and Medvedovici 2005).

As pesticides can take long time to be degraded and if metabolized their metabolites can, as same as heavy metals, accumulate in the environment, these substances represent, as previously mentioned, a risk. The less water-soluble the substance, the longer its half-life period and its partition coefficient, and the longer it tends to remain contaminating the environment in which it is present (Arias-Estévez et al. 2008).

Besides being toxic, they can also be biomagnified in the food chain (Ali et al. 2014). Thus, the remediation of sites contaminated by these pollutants is an important goal in order to preserve health and promote sustainable development (Cheng et al. 2016).

This kind of remediation is challenging because it is common that polluted sites contain a mixture of different pesticides and their metabolites. However, they are mainly organic compounds and can be reduced in quantity, eliminated, isolated, or stabilized by applying physical, chemical, and/ or biological processes.

3.2 Innovative Techniques for Assessing and Monitoring Heavy Metals and Pesticide Pollution

In order to detect the necessity of environment heavy metal and pesticide remediation and also to monitor the quality of the remediation process being carried out, and to determine if the present levels no longer represent risks allowing this process to be stopped, it is desirable the development of innovative techniques.

The main motivation to the development of these techniques to assess and monitor the level of environment contamination with pollutants are the disadvantages evidenced in methods conventionally used for this purpose such as the mass spectrometry (Hepel and Stobiecka 2008; Petrosyan et al. 2019; Shen et al. 2019; Xiong et al. 2019; Pesantes et al. 2019) and the chromatographic ones that are still being used by some research groups (Pan et al. 2019). In general, the development of simple methods (not requiring trained operators to apply) that can exhibit the desirable sensitivity within minimum time and also present low cost is the main goal.

When it comes to pesticide pollution, sensors and biosensors are interesting options (Muenchen et al. 2016; Mishra et al. 2018) (Table 3.1). Sensors can identify the presence of a target analyte and convert this information into a signal that can be measured (Hulanicki et al. 1991).

Table 3.1 Some examples of sensors developed in twenty-first century to detect pesticides in different environments

Sensor	Pesticide (s)	Reference
Fiber optic biosensor with acetylcholinesterase-immobilized	Organophosphorus	Choi et al. (2001)
Cantilever based biosensor functionalized with DDT hapten	Dichlorodiphenyltrichloroethane	Alvarez et al. (2003)
Optical microbial biosensor with *Flavobacterium* sp.	Methyl parathion	Kumar et al. (2006)
Carbon electrode modified with mercury film	Atrazine	Maleki et al. (2007)
Liposome nanobiosensors with acetylcholinesterase	Dichlorvos and paraoxon	Vamvakaki and Chaniotakis (2007)
Alkaline phosphatase electrochemical biosensor	2,4-Dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, carbofuran, α-endosulfan, Hg^{2+}, Cd^{2+}, Ag^{2+}, Zn^{2+}, and Cu^{2+}	Shyuan et al. (2008)
Electrochemical biosensor with multi-walled carbon nanotubes	Isoproturon, voltage and dicofol	Manisankar et al. (2008)
Fluorescent nanoimmunosensor	Chlorpyrifos	Zhou et al. (2010)
Biosensor containing photosystem II from spinach	Terbutryn	Ventrella et al. (2011)
Biosensor containing photosystem II from *Synechococcus elongatus f. thermalis*	Atrazine, isoproturon, diuron	Masojídek et al. (2011)
Amperometric biosensor containing tyrosinase	Chlorpyrifos, profenofos, and malathion	Liu et al. (2011)
Electrochemical (carbon electrode modified with copper nanowires)	Trifluralin	Mirabi-Semnakolaii et al. (2011)
Electrochemical biosensor with nanomaterials functionalized with acetylcholinesterase	Demalathion, chlorpyrifos, monocrotophos, and endosulfan	Chauhan and Pundir (2012)
AFM tip nanobiosensor with acetolactate synthase	Metsulfuron-methyl	Silva et al. (2013)
AFM tip nanobiosensor with acetolactate synthase	Imazaquin and metsulfuron-methyl	Deda et al. (2013)
Liquid crystal and paraoxonase enzymes	Paraoxon and other organophosphorus	Chen and Yang (2013)
Biosensor containing tyrosinase attached to a carbon electrode with carbon nanotubes	Organophosphorus pesticides	Vicentini et al. (2013)

(continued)

Table 3.1 (continued)

Sensor	Pesticide (s)	Reference
Biosensor containing alkaline phosphatase attached to a carbon nano-powder paste electrode	Carbofuran	Samphao et al. (2013)
AFM tip nanobiosensor with acetyl-CoA carboxylase	Diclofop	Amarante et al. (2014)
Biosensor containing thylakoids from spinach	Diuron	Rasmussen et al. (2014)
Immunosensor based on a nanocomposite	Chlorpyrifos	Ding et al. (2014)
Immunosensor based on printed carbon electrode modified	Atrazine	Deep et al. (2014)
Amperometric biosensor with carbon nanotubes and acetylcholinesterase	Organophosphorus	Kesik et al. (2014)
Tyrosinase/ZnONPs/SPCE biosensor	Chlortoluron	Haddaoui and Raouafi (2015)
Photoelectrochemical biosensor with nanomaterials and acetylcholinesterase	Paraoxon and dichlorvos	Li et al. (2015)
Acetylcholinesterase-functionalized gold nanoparticles biosensor	Dichlorvos	Wei and Wang (2015)
Quartz crystal microbalance sensor	Atrazine	Gupta et al. (2015)
Amperometric based on mushroom tyrosinase	Atrazine	Tortolini et al. (2016)
Amperometric biosensor containing the enzyme hydroxyphenylpyruvate dioxygenase	Sulcotrione	Rocaboy-Faquet et al. (2016)
Electrochemical biosensor with acetylcholinesterase functionalizing nanocomposites	Malathion and chlorpyrifos	Chauhan et al. (2016)
Electrochemical biosensor containing a nano-stabilizer and acetylcholinesterase	Organophosphorus pesticides	Xu et al. (2018)
Biosensor containing *Chlorella mirabilis*	Simazine and irgarol	Moro et al. (2018)
Modified screen-printed electrodes containing acetylcholinesterase	Chlorpyrifos and methamidophos	Hou et al. (2019)

It is possible to develop biosensors containing enzymes related to the photosynthetic process to detect the presence of herbicides although with a low selectivity. They are able to detect especially phenolic herbicides and they are useful as a first evaluation tool to determine if a sample is contaminated or not by the presence of these substances. Interesting examples are: a) a biosensor to detect Terbutryn through a colorimetric method using ultrathin film multilayers of Photosystem II (from spinach)-enriched photosynthetic membranes (Ventrella et al. 2011); b) a biosensor based in amperometry to detect the pesticides atrazine, isoproturon, diuron, thanks to the Photosystem II complex from *Synechococcus elongatus* and *F. thermalis*. The latter proved to offer more sensitivity to herbicides than the standard algal growth inhibition tests (Masojídek et al. 2011). However, entire organisms and thylakoids can also be successfully used as the bio part of biosensors to detect pesticides. Diuron presence could be detected by thylakoids from spinach-based system (Rasmussen et al. 2014) as same as simazine and irgarol presence by *Chlorella mirabilis* algae biosensor based on fluorescence (Moro et al. 2018).

The pesticide atrazine could be efficiently detected in wastewater using a quartz crystal microbalance sensor made on a gold chip surface (Gupta et al. 2015), and in soil and water samples by a sensor using a carbon electrode that, in the presence of thiocyanate, was modified to contain a mercury film. Atrazine protonates in acidic environment being able to establish an ionic interaction with complexes of mercury–thiocyanate on the surface of the electrode; atrazine adsorption could be determined by adsorptive stripping voltammetry (Maleki et al. 2007). Voltammetry also made possible the detection of the pesticides isoproturon, voltage and dicofol by using a glassy carbon electrode modified with multi-walled carbon nanotubes and with versions of this electrode functionalized with polyaniline or polypyrrole (Manisankar et al. 2008); the detection of trifluralin in soil samples could be performed using a composite of carbon with copper nanowires (that enhanced the conductivity potentializing the current) (Mirabi-Semnakolaii et al. 2011). Nanomaterials are commonly used in electrochemical sensors once they possess large surface-to-volume ratio improving desirable characteristics like being efficient transducing platforms, accepting well the immobilization of biomolecules, and offering the possibility of system miniaturization (Kurbanoglu et al. 2017).

Biosensors, in turn,differently from regular sensors possess a biological recognition element able to recognize the desirable analyte. After recognition, a signal is produced and can be detected and transformed into measurable information by a transducer. Then, this signal can finally be exhibited to the equipment operator as data (Korotkaya 2014). The biological recognition element is immobilized to other sensor components by different strategies as adsorption (noncovalent) or covalent binding (Pereira et al. 2018), and it is necessary to take into consideration during its development the concerns regarding stability, possibility of mass production, reproducibility, and if miniaturization is feasible (Uniyal and Sharma 2018).

As organophosphorus and carbamate are inhibitors of the enzyme acetylcholinesterase, it is common to use this enzyme to generate biosensors to detect these pesticides (biosensors using enzymes are normally based in inhibition). This enzyme catalyzes the cleavage of the neurotransmitter acetylthiocholine (the enzyme's substrate) into acetic acid and thiocholine; the latter can be oxidized by applying voltage allowing current detection by the sensor circuit. However, if a pesticide able to inhibit the enzyme is present, the higher the concentration of the inhibitor present in the sample, the lesser is the detectable current (Pundir and Chauhan 2012). An electrochemical biosensor can be constructed, for example, based on a glassy carbon electrode. It could be modified by iron oxide nanoparticles and poly (indole-5-carboxylic acid) to attach the enzyme, generating a sensing device to detect pesticides in water samples with satisfactory accuracy (Chauhan et al. 2016). An indium tin oxide-coated glass plate can also be used to generate a biosensor by attaching to it iron oxide nanoparticles decorated with carboxylated multi-walled carbon nanotubes and immobilized acetylcholinesterase. The obtained results regarding to the pesticides malathion, chlorpyrifos, monocrotophos, and endosulfan were in good agreement with the ones obtained through gas chromatography–mass spectrometry (Pundir and Chauhan 2012). An amperometric biosensor can also be constructed using a graphite electrode modified with the conducting polymer (poly(4-(2,5-di(thiophen-2-yl)-1H-pyrrol-1-yl) benzenamine)) and multi-walled carbon nanotubes attached to acetylcholinesterase. Electrochemical oxidation of acetylthiocholine was impaired by the presence of the pesticides paraoxon, parathion, and chlorfenvinphos, and it could be measured offering results comparable to the ones obtained using chromatographic methods (Kesik et al. 2014). The enzyme can also be attached to modified screen-printed electrodes. The modification mesoporous carbon-chitosan, for example, proved to be able to enhance the electrochemical reaction improving the biosensor sensitivity to detect chlorpyrifos and methamidophos in different samples (Hou et al. 2019). Acetylcholinesterase could also be attached to an electrochemical sensing platform in the presence of a nanostabilizer (*N*-carbamoylmaleimide-functionalized carbon dots that avoided signal loss of thiocholine) to detect the presence of multiple organophosphorus pesticides (parathion-methyl, paraoxon, dimethoate, and *O*, *O*-dimethyl-*O*-2,2-dichlorovinylphosphate), achieving ultrahigh sensitivity (Xu et al. 2018). Functionalized gold nanoparticles can also be used together with this enzyme for the development of a biosensor. ([BSmim]HSO_4)-AuNPs-porous carbon composite modified boron-doped diamond electrode attached to acetylcholinesterase could efficiently detect dichlorvos presence (Wei and Wang 2015). Biosensors that produce photocurrent could also be built using a glass electrode covered by graphene and CdSe-ZnS quantum dots attached to acetylcholinesterase. It could generate a sensitive photoelectrochemical biosensor capable to detect pollutant pesticides paraoxon and dichlorvos present in fruit samples (Li et al. 2015).

It is also possible to detect pesticides using acetylcholinesterase and optic methods. At a wavelength near 420 nm, *o*-nitrophenyl acetate is colorless. However, after this enzyme action over this substrate *o*-nitrophenyl is generated and this substance is yellow and there is absorbance at 420 nm. Therefore, contamination of organophosphorus in water could be detected by using a fiber-optic biosensor containing an acetylcholinesterase-immobilized Langmuir–Blodgett film and *o*-nitrophenyl acetate. The pesticides caused decrease on the yellow product generation due to the enzyme's inhibition which could be detected by this colorimetric method (Choi et al. 2001). The organophosphorus pesticides dichlorvos and paraoxon in water samples could be detected by liposome-based nanobiosensors. The liposomes contained the enzyme acetylcholinesterase, the substrate acetylcholine, and a pH-sensitive fluorescent indicator called pyranine. In the presence of pesticides able to inhibit the enzyme, it was observed a decrease in the hydrolysis of the acetylcholine and, consequently, a decrease in fluorescent signal (Vamvakaki and Chaniotakis 2007).

Besides acetylcholinesterase there are other enzymes that can be used to assess and monitor the presence of pesticides in samples, once these molecules are characterized as possessing high selectivity to substrates. To detect atrazine, for example, it is possible to use the enzyme tyrosinase from mushrooms immobilized in a carbon based electrode such as multi-walled carbon nanotubes. The pesticide can inhibit the catalysis of catechol's (enzyme's substrate) oxidation to *o*-quinone, allowing the detection of the pesticide pollution in water samples (Tortolini et al. 2016). An amperometric biosensor with a glassy carbon electrode modified with graphene containing tyrosinase immobilized on platinum nanoparticles could also detect organophosphorus pesticides (chlorpyrifos, profenofos, and malathion) based on the enzymatic inhibition of catechol oxidation; the nanomaterials improved the sensor's sensitivity (Liu et al. 2011).This enzyme could also be attached to a glassy carbon electrode modified with a nanocomposite of multi-walled carbon nanotubes-1-butyl-3-methylimidazolium chloride within a dihexadecylphosphate film, to detect the presence of catechol in water samples (Vicentini et al. 2013). The herbicide chlortoluron could also be detected using the enzyme tyrosinase (inhibited by the pesticide) attached to screen-printed carbon electrodes modified by ZnO nanoparticles (Haddaoui and Raouafi 2015). Paraoxonase 1, for example, can hydrolyze organophosphates (for example, the pesticide paraoxon) changing the medium pH as a consequence. This pH change could be detected as a change in color suffered by a biosensor containing also a pH-sensitive liquid crystal (Chen and Yang 2013). Alkaline phosphatase also allows not only pesticide but also heavy metal presence detection in samples and nowadays efforts are still being made to isolate these enzymes from different organisms like lactic acid bacteria (Chu et al. 2019). An electrochemical biosensor containing this enzyme immobilized in a screen-printed carbon electrode modified with hybrid sol-gel/chitosan film could detect alterations caused by the pollutants (2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, carbofuran, α-endosulfan, Hg^{2+}, Cd^{2+}, Ag^{2+}, Zn^{2+}, and Cu^{2+}) in the current generated by the oxidation of the cleavage product (ascorbic acid) from ascorbic acid 2-phosphate (Shyuan et al. 2008). This enzyme could also be attached to a carbon nano-powder paste electrode generating a biosensor capable of detecting carbofuran in spiked water and chilly samples (Samphao et al. 2013). Hydroxyphenylpyruvate dioxygenase is also an option of enzyme to be used to build a biosensor to detect the presence of the pesticide sulcotrione. An amperometric biosensor containing this enzyme could, in the absence of the pollutant, promote the oxidation of hydroxyphenylpyruvate in homogentisic acid, which could be oxidized generating a detectable current. In the presence of the pesticide the activity of the enzyme attached to poly(3,4-ethylenedioxythiophene) polystyrene sulfonate-modified screen-printed electrode and the current diminished, in a measurable way (Rocaboy-Faquet et al. 2016).

Antibodies can also be used as part of biosensors: the immunosensors. Anti-atrazine antibodies were attached to a carbon screen-printed electrode to perform a highly sensitive detection of atrazine in different concentrations (Deep et al. 2014). Anti-trichloropyridinol antibodies are also an interesting option to make an immunosensor once trichloropyridinol is a metabolite of chlorpyrifos: a highly used organophosphorus pesticides in agriculture. The sensor mechanism based on a competitive immunoreaction between the antibody and the pesticide (if present in a sample), or the antibody and 3,5,6-trichloropyridinol attached to quantum dot (for fluorescent readout) from the immunochromatographic test strip assay (Zhou et al. 2010). Antibodies anti-chlorpyrifos could also be used to detect this pesticide in agricultural products through a portable immunosensor based on gold nanoparticles and polyaniline/carboxylated multiwall carbon nanotubes-chitosan nanocomposite (Ding et al. 2014).

Not only molecules like antibodies and enzymes can be used in biosensors. Whole organisms are also viable options as pollution detectors. *Flavobacterium* sp., that contains the organophosphorus hydrolase enzyme, could convert the pesticide methyl parathion into a product (*p*-nitrophenol) detectable by the optic fiber system of the biosensor (Kumar et al. 2006).

It is also necessary to highlight the role of nanobiosensors of cantilevers (Lang et al. 2017). Cantilevers are movable beams, generally containing a tip at their free end, essential to atomic force microscope (AFM) operation. If these structures are functionalized (chemically modified), they can work as nanoscale sensors (with rapid response and high sensitivity) and not anymore as tools for topographic characterization (Peiner and Wasisto 2019). If the AFM tip is functionalized it also generates biosensor, but not cantilever based ones, AFM tip nanobiosensors, and both can be designed to detect the presence of pesticides in various samples (Table 3.1). Different types of functionalization generate sensors with different sensitivity and detection limit (Muenchen et al. 2016). A cantilever based biosensor was generated by immobilizing to the gold-coated side of the cantilever a synthetic hapten of the pesticide dichlorodiphenyltrichloroethane (DDT). The assay was performed in a competitive way comparing the differential surface stress causing nanometer-scale bending of a microcantilever exposed to a sample containing only monoclonal antibody anti-DDT or antibody and the pollutant pesticide DDT (Alvarez et al. 2003). AFM tip nanobiosensor functionalized with the acetolactate synthase enzyme could detect the herbicide metsulfuron-methyl; this detection was based on variation of relative adhesion force observed through the acquisition of force curves in the presence or absence of pesticide. In the presence of the pollutant the adhesion force values were higher due to the specific interaction enzyme-herbicide (Silva et al. 2013). It was also possible to develop this type of biosensor with specificity to detect diclofop by using the enzyme acetyl-CoA carboxylase and the measures obtained experimentally were in agreement with theoretical ones obtained through computer simulation (Amarante et al. 2014).The herbicides imazaquin and metsulfuron-methyl could also cause an increase of the adhesion force being detected using acetolactate synthase enzyme attached to a functionalized AFM tip (Deda et al. 2013).

When it comes to detecting pesticide's presence in water, surface-enhanced Raman spectroscopy (SERS) is a relevant tool. The technique is fast, possess a weak signal interference of water molecules, the sample pretreatment is simple and it can determine with high accuracy (fingerprinting characterization) the pollutant presence. Fonofos, phosmet, and sulfoxaflor (pesticides that if present in paddy water can damage rice production) could be detected efficiently (Weng et al. 2019). However, SERS is not limited to water analysis. Rape plants leaf could be analyzed for the presence of the pesticide thiabendazole, after mixed with acetonitrile and sodium chloride and the supernatant obtained (Lin et al. 2018). SERS also made possible the detection, for example, of residues of: isofenphos-methyl in corns (Liu et al. 2016), chlorpyrifos on spinach (without sample preparation, by just dropping silver colloids onto samples) (Zhai et al. 2016), carbaryl in Fuji apples (Fan et al. 2015), malathion in tomatoes' peels and damson plums (Albuquerque and Poppi 2015), and phorate and fenthion in apple skin (Li et al. 2014). Soil analyses through this technique were able to detect residues of deltamethrin and carbofuran by using gold nanoparticles to increase stability and sensitivity (He et al. 2019).

Another spectroscopy methodology has also proved to be useful to detect pesticide presence in samples. Laser induced breakdown spectroscopy (LIBS) when performed in the presence of metal nanoparticles acquired the capacity to detect not only trace pesticide (chlorpyrifos) but also heavy metal residues (cadmium) in apple and lettuce (Zhao et al. 2019b).

Regarding the heavy metal pollution LIBS could also be used to analyze industrial wastewater performing an online detection of Cd, Cr, Cu, Ni, Pb, and Zn (Zhao et al. 2019a) and the electrode graphite enrichment combined with plasma spatial confinement could improve the technique capacity to analyze Cd, Pb, and Ni faster in this kind of sample. However, if the electrode suffers aluminum enrichment the sensitivity increases and the detected concentration can be as low as ppb (Meng et al. 2017). Actually, LIBS could be used for analyzing different sample types like rock and sea salts detecting Cu, Cd, and Ni (Rehan et al. 2018).

It is possible to use Atomic fluorescence spectrophotometer to determine Hg and As soil samples, vegetables, and fly ashes (Ma et al. 2018). A fast and non-destructive technique called micro-X-ray fluorescence core-scanning was also useful to quantify Ti, Cr, Mn, Ni, Cu, Zn, Pb. Samples were first exposed to cation-exchange resin sachets that concentrated heavy metal cations; then the X-ray was performed and the results compared to the ones from scanned resin reference standards (Huang et al. 2019).

Functionalized cantilevers (modified, for example, with proteins or with chemicals to generate desirable functional groups on the surface) were also a viable option to detect heavy metals like cesium (Ji et al. 2000), mercury (Cherian et al. 2003; Kadam et al. 2006), zinc (Cherian et al. 2003), and cadmium (Velanki et al. 2007).

However, as same as for pesticide detection and measurement, biosensors (Table 3.2) represent a quick, reliable, and simple strategy to monitor heavy metal pollution in different samples (Asal et al. 2018). The antibodies able to detect EDTA–Cd^{2+}, DTPA–Co^{2+}, 2,9-dicarboxyl-1,10-phenanthroline–U^{6+}, and cyclohexyl–DTPA–Pb^{2+} could be used to build immunosensors to detect the presence of these heavy metals (Blake et al. 2001). Groundwater samples could be analyzed for the presence of U^{6+} thanks to an immunosensors with antibody to detect

Table 3.2 Some examples of biosensors developed in twenty-first century to detect heavy metals in different environments

Sensor	Heavy metal (s)	Reference
Immunosensor	Cd, Co, Pb, and U	Blake et al. (2001)
Biosensor containing synthetic phytochelatin fused to a maltose binding domain	Hg, Cd, Pb, Cu, and Zn	Bontidean et al. (2003)
Biosensor containing the enzyme urease	Cd	May and Russell (2003)
Biosensor containing mammalian metallothionein	Cd, Zn, and Ni	Wu and Lin (2004)
Biosensor containing the enzyme glucose oxidase	Hg	Malitesta and Guascito (2005)
Cardiomyocyte-based biosensor	Hg, Pb, Cd, Fe, Cu, and Zn	Liu et al. (2007)
Biosensor containing the enzyme 6His-Tag glutathione *S*-transferase	Zn, Cd, and Hg	Saatci et al. (2007)
Biosensor containing the enzyme glutathione *S*-transferase theta 2-2	Zn	Saatci et al. (2007)
Biosensor containing rodhamine-labeled metallothionein	Cd	Varriale et al. (2007)
Immunosensors: battery-operated or requiring grounded power source	U	Melton et al. (2009)
A colorimetric biosensor containing papain-functionalized gold nanoparticles	Hg, Pb, and Cu	Guo et al. (2011)
Biosensor based on structure-switching	Hg	Long et al. (2013)
Biosensor containing bioluminescent bacteria	Cr	Thacharodi et al. (2019)
Biosensor containing recombinant *Escherichia coli*	Cd	Lee et al. (2019)

U^{6+}-dicarboxyphenanthroline; one immunosensor battery-operated that took 5–10 min to analyze and the other that required a grounded power source and possessed the ability to, in a single experiment, autonomously analyze multiple samples (Melton et al. 2009). Not only immunosensors are able to detect heavy metals. Biosensor containing the enzyme urease (that can be inhibited by Cd) could detect cadmium through surface plasmon resonance determination of changes in the protein's conformation (May and Russell 2003). The mammalian metallothionein protein was also successfully used as part of a biosensor to detect, by surface plasmon resonance, Cd, Zn and Ni (Wu and Lin 2004). Metallothionein (MT) is a cysteine-rich protein capable of binding to Zn, Cu, and Cd, but the affinity for Cd was the highest one. Thus, other kind of biosensor could be developed after the binding equilibrium was reached among rodhamine-labeled MT exposed to Zn^{2+} −saturated chelex resin. In a reversed-displacement format the biosensor was exposed to contaminated water samples and could detect Cd when it was present (Varriale et al. 2007). A synthetic phytochelatin ($(Glu\text{-}Cys)_{20}Gly$) fused to a maltose binding domain was part of a biosensor capable of detecting Hg^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, and Zn^{2+} ions (Bontidean et al. 2003). A colorimetric biosensor containing papain-functionalized gold nanoparticles could detect Hg^{2+}, Pb^{2+}, and Cu^{2+} (Guo et al. 2011); nanomaterials are components with interesting characteristics to build sensors (Priyadarshini and Pradhan 2017). The enzyme glucose oxidase immobilized in poly-o-phenylenediamine could allow a biosensor to detect Hg^{2+} and to also respond to Cu^{2+} presence (Malitesta and Guascito 2005). 6His-Tag glutathione *S*-transferase in a biosensor made possible the detection of Zn^{2+}, Cd^{2+}, and Hg^{2+}; a different isozyme of glutathione *S*-transferase (the glutathione *S*-transferase Theta 2-2) was more selective to Zn^{2+} and also presented a wider dynamic range for it (Saatci et al. 2007). DNA can also be the bio portion of a biosensor to detect pollutants; an optical biosensor for Hg^{2+} detection based on structure-switching DNA was developed to perform rapid analyses. The biosensor surface contained DNA probes to bind to fluorescence-labeled DNA containing T-T mismatch. A decrease in fluoresce could be observed when, in the presence of the heavy metal in water samples, the DNA containing mismatch dehybridized from the sensor to generate T-Hg^{2+}-T complexes (Long et al. 2013). There are also biosensors that are cell-based: cardiomyocytes in a light-addressable potentiometric sensor could rapidly detect Hg^{2+}, Pb^{2+}, Cd^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} (Liu et al. 2007); genetically modified *Escherichia coli* expressing a version of enhanced green fluorescent protein containing metal-binding loops could emit fluoresce indicating, in a biosensor, the presence of Cd in samples (Lee et al. 2019); the bioluminescent bacteria VIT-JAAK, isolated from the gut of sea urchin, could be used in a sensor (based on nutrient agar cubesand) to detect hexavalent Cr that inhibited the luminescence process when present in water samples (Thacharodi et al. 2019).

Sensors without the biological portion are also useful. Hg^{2+} was detected by layered titanate nanosheets containing sodium cations between them. Cation-exchange allowed voltammetric detection of mercury ions in real mushroom samples (Yuan et al. 2013). Silver nanoparticles and hydrogen peroxide in a colorimetric sensor were able to detect Hg^{2+}. Other metallic ions were not able to induce system's color change as mercury ions could (Wang et al. 2012).

Some conventional methods to detect heavy metals, other than the mentioned previously in this text, are not totally banned. Actually, they are still being used in a lot of different studies. Atomic absorption spectrophotometers are also useful to determine heavy metal's concentration in samples. Groundwater could be successfully analyzed for Cu, Cr, Cd, Pb, Zn, and Fe presence (Boateng et al. 2019) and soil samples, vegetables, and fly ashes for the presence of Cr, Pb, Cu, Ni, Zn, and Cd (Ma et al. 2018). This methodology also made possible to analyze tree barks of the species *Poincianella pluviosa*, after being digested in a nitroperchloric mixture, for the presence of Pb, Cd, Ni, Cu, Zn, Fe, and Mn (de Souza et al. 2019), and soil of steel limited dumpsite for the presence of Zn, Cu, Ni, and Cd (Bello et al. 2017). Water samples from industrial zone could also be analyzed for Pb, Cd, Cr, Fe, Cu, Ni presence, and for the presence of Hg through cold vapor atomic absorption (Pobi et al. 2019). Plasma optical emission spectroscopy also made possible the heavy metal analysis (Cd, Cu, Mn, Pb, Zn) on *Parmotrema arnoldii* and *Tillandsia usneoides* deposited by air pollution. Lichens and bromeliads are useful organisms to be used as biomonitors of air pollution (Benítez et al. 2019).

With the values detected through the techniques mentioned in this chapter are commonly performed pollution indices to assess the pollution status on the area and some statistical analysis, for example, multivariate analysis. Contamination factor, enrichment factor, and geo-accumulation index were recently performed to investigate the presence of Fe, Cd, Co, Cr, Cu, Ni, Zn, and Pb in soil surface sediments (Anbuselvan et al. 2018). These three parameters and also the pollution load index were performed to determine Cu, Pb, Zn, Ni, As, Cd, Sb, Sr, and Hg in sediments in Ecuador's artisanal and small scale gold mining area and compared to Canadian guidelines (Pesantes et al. 2019). To propose a new assessment method, the hazard load index, scientists compared the results with the ones obtained using pollution load index. The novel method could perform the assessment of Zn, Cu, Ni, and Cd pollution in top soils of Dana steel limited dumpsite concluding that the samples were in fact polluted. However, the pollution load index overestimated the risk. The authors attributed this fact to an improper control sampling that assessment through pollution load index requires (Bello et al. 2017).

3.3 Conclusions

Given the risk that heavy metals and pesticides pose to the existence of life on the planet, it is necessary to detect their presence in different environments. It is crucial that techniques make possible to determine their levels in order to discover if these pollutants are present and if so, if the concentration threatens health. Thus, new ways to assess and monitor the presence of heavy metals and pesticides in different samples continue to emerge. These new methods generally aim to save financial investment, reduce analysis time, facilitate the detection procedure, and make results more accurate and the analysis truer to reality.

3.4 Future Perspectives

New ways to access and monitor the presence of pollutants such as heavy metals and pesticides as many different samples as possible are expected to continue to be proposed. The work of multidisciplinary teams aims to gather knowledge from different areas so that these modern innovative techniques for assessing and monitoring these pollutants have advantages over the conventional ones: offering more reliable, reproducible, fast, economical, and practical analyzes. There is, for example, a tendency to adapt equipments (that allow this feature) to perform direct and continuous (called online) monitoring of soil, water, and air's quality and safety in polluted spots. It is also desirable that human beings develop their environmental awareness so that they can get as close as possible to a sustainable development model, and can convert the human action over nature into a less harmful and more harmonic one.

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Chapter 4
Global Scenario of Remediation Techniques to Combat Pesticide Pollution

Alina Butu, Ioana Grozea, Ioan Sarac, and Monica Butnariu

4.1 Background

Any agriculture is, in a way, an intervention against nature, which, when practiced unfairly, leads to a decrease in fertility, the modification of biosensors and ecosystems. It would be wrong to argue that chemicals used in agriculture would have no biological effect. Agriculture has, on the one hand, a positive role in the purification of the atmosphere; on the other hand, it sometimes has a negative role through its pollution. The positive effect of agriculture is due to green plants, which, by absorbing CO_2 from the atmosphere for its use in photosynthesis and O_2 removal, purify the air. A series of volatile substances that contaminate the atmosphere following industrial or other processes, $CaSO_2$ and NH_3 can be directly absorbed by green plants, which leads to its purification. Some of the industrial objectives also contribute to the purification of the atmosphere, affecting not only man and the biosphere, but also agriculture. Atmospheric pollution with SO_2, NH_3, NO_2, and cement powders can affect by inhalation or ingestion of animal organisms (Masbou et al. 2018).

Concentration above acceptable tolerance limits has harmful effects on the environment and hence on human life. Too many fertilizers, too many pesticides, as well as an excessively high concentration of livestock in a farm vitiate the quality of the environment. In the soil, excess nitrogen is transported in the form of nitrates in the groundwater canvas, which can then be used as drinking water or at the shelter of the animals. Increasing the water content in phosphorus stimulates the growth of algae and other aquatic vegetal organisms. It has been found that the intensive use of pesticides with nitrogen, phosphorus, and potassium leads to the increase of soil

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content in some elements such as Zn, Pb, Ni, Cr, previously found in soluble forms only as traces (Song et al. 2019).

The term “pesticides” refers to any substance or mixture of substances, including mixtures with ingredients, for use in agriculture, forestry, storage, and other activities for the purpose of preventing, mitigating, removing, or destroying pests, phytopathogenic agents, weeds, and other forms of animal or plant life, including plant and domestic animal or plant viruses, insects, and rodents carrying diseases communicable to humans, and products used to regulate plant growth, defoliation, or desiccation. In fact, pesticides are toxic substances, meaning substances which, after entering the body at a relatively high dose once or more, in a very short period, or in small doses repeatedly for a long time, provoke temporary, transient, or permanent impairment of one or more functions, impairments that can go through to complete annihilation or death (James et al. 2019).

In the fight, pesticides cross different paths in ecosystems. A particular place is occupied by highly persistent, highly biodegradable pesticides, especially those organochlorinated. In the case of herbicides, the useful fraction is 5–40%. In fact, this extremely large, unused in terms of combating, creates all the known ecological problems, hence the need to ensure greater agro-availability of pesticides. Pesticides, once in the soil, besides their action on diseases, pests, and weeds, extend their action to microorganisms so that quantitative and qualitative changes occur both in the edaphic population structure and in physiological activities. The importance of knowledge of these changes is supported both by the requirements of scientific agriculture and by the protection of edaphic ecosystems.

In this context, knowing the influence pesticides have on the biochemical processes that take place in the soil is absolutely necessary. Also, negative influence on denitrifying microorganisms and soil denitrification process was highlighted (Alho 2008).

Referring to the possible damage of micro-organisms by soil active substance residues, some researchers show that this is an ecological feature of micro-organisms to be permanently exposed to natural disasters, losing them together. These occur, for example, in the case of rapid changes in temperature or soil saturation. Therefore, reductions in the population density of microorganisms by 50% and above are very common. When repairing favorable environmental factors, the population is doubled every 10 days, so that at a 12.5% drop in density from the initial level, within 30 days, a 100% restoration can be observed. Consequently, absolute population reduction and delay in regeneration of the initial population density were proposed as criteria, with the importance of delaying rather than the absolute value of population decline. In this case, it is indicated as the regeneration rule: a 30-day period can be neglected; duration of 60 days is tolerable; intervals longer than 60 days are critical, which in fact reflect forms of manifestation of soil pollution phenomena. The application of pesticides—with all the technological measures used in agriculture—represents human intervention in the biosphere and as it intensifies, the stronger and sometimes more aggressive it becomes. The task of agriculture has always been to ensure the need for food, and agricultural production has been energy-consuming since the beginning. In the past, when the population of our country did not exceed one million inhabitants, it averaged 3 ha of arable land per capita and another 6 ha were kept fallow land, food requirements through agricultural production were solved on account of solar energy and to some extent through human labor. Today, with the growth of the population, the need for food and, implicitly, the task of producing more have increased. Agriculture has intensified and its energy resources have changed with regard to the participation ratio: some energy resources are unrestricted, such as solar and wind power, which are considered inexhaustible; others are renewable: organic matter, human, and animal labor; some are limited: fossil energy (Rasheed et al. 2019).

The ratio of participation of different energy resources in the agricultural production process differs accordingly with the agricultural system. In intensive agriculture, this is in favor of limited resources given by fossil energy; the biconversion ratio ranging from 1 to 3–4 in mechanized, irrigated, and chemistry agriculture compared to the 1:129 ratio in extensive pastoral farming, when agricultural output was much lower.

At present, the applied pesticides represent a fossil energy consumption of 6.3 Gj/ha, meaning >50% of the fossil energy used to obtain the agricultural crop production. If we add the energy consumption incorporated in other substances used to fight diseases and pests and in fuels, consumption amounts to 2/3 of the total fossil energy used in the plants hoeing.

Therefore, in the context of the implications of the global energy crisis, in light of the tasks of the "Energy Research and Development Directive" program, it is necessary to rethink and improve the culture technologies, including the application of chemical pesticides, to be done only by certified humans and only as a result of a chemical control of the plant and soil to avoid any waste, which in essence means unnecessary fossil energy consumption.

There is also a need for a more rational choice of pesticide product because there are also differences in energy consumption used for their production. At present, for the synthesis of 1 kg of active substance, 73–91 Mj for nitrogen, 13–20 for Mj for phosphorus, and 8–14 Mj for potassium are consumed; consumption is different in relation to the basic energy source (Atma et al. 2017).

Another aspect related to the energy crisis is the ratio of biconversion in plant products of energy incorporated into pesticides. From the aspect of the biconvex ratio of fossil energy incorporated into pesticides, based on the synthesis of research data from our country for the main crop plants, they differ in relation to the species. The overall biconvex ratio of fossil energy incorporated into pesticides, in the energy contained in plant products, places phosphorus pesticides first, followed by potassium, the latter being nitrogenous pesticides, which are also the largest fossil energy consumers.

The biconversion must be judged also in terms of economic efficiency, which is due to the difference between the value of the yield harvest obtained and the cost of the fertilizer used, as well as the biological value of the vegetable products. Knowing this index is important because the same crop yield can be obtained with different doses and fertilizer ratios, or the fossil energy incorporated in it is not the same. Therefore, it is necessary to choose the right dosages according to the equally efficient curves and their application following a systematic control of the fertility status, the use of new types of fertilizers obtained with less energy consumption, as well as the recirculation of some vegetal residues, in order to increase the transformation coefficient of the energy incorporated in fertilizers into vegetable products, which on average is currently only 1: 3–4.

The tasks facing agriculture in the conditions of the increase of vegetal production imply the increase of the fertilizers used in the conditions of the energy economy, which means not a restriction of their use but an increase of the biconversion coefficient, by the rigorous pursuit of energy efficiency. For this, a new strategy is needed to attract natural organic fertilizers into the circuit of the nutrients in the household, as well as a series of plant and animal organic residues that, composted according to scientific rules, contribute to raising the rate of use of chemical fertilizers (Asano et al. 2014).

Integrated protection is a combination of chemical, physical, and biological methods and means in together with agro-technical (compulsorily compatible) methods applied harmoniously within agricultural ecosystems. Plant protection works are executed according to the emergency disposal technology (EDT) to minimize the number of phytosanitary interventions and to favor the natural control factors that regulate/limit the pest population.

It is necessary to radically reconsider the importance of various harmful and/or pathogenic species. Specifically, the economic importance of each species and the level of losses that could be produced must be known. Ecological and economic data has shown that most of the species recognized as harmful should not be treated. The following categories of species can be found in an agricultural crop: the main harmful species; secondary harmful species; harmful migratory species; indifferent species (Shi et al. 2017).

The evolution of modern agriculture is closely linked to changes in the traditional way of working. The resistance of the varieties to the main diseases and especially to pests is still weak. There are also situations where a variety known as resistance becomes sensitive after many years due to the emergence of new pathogen races. Combat control complexes are used against primary and secondary harmful organisms. At the same time, the measures applied must protect as many useful organisms as possible. Sometimes it is considered that combining methods means the same thing as integrated protection. In reality, combining means and methods is only part of the integrated struggle, but an essential part.

First of all, many prophylactic treatments, after the spraying calendar or healing treatments, have to be replaced with curative treatments. EDT is the most important means of regulating all types of phytosanitary interventions and is a concept of integrated combat that allows treatment to be applied only when the density and/or degree of attack can cause economically justifiable losses, 5% of the harvest, equivalent to the cost of phytosanitary treatment. EDT allows the treatment to be located only where the attack limits are exceeded and varies according to the variety, biological category, plant development stage, pedoclimatic conditions, technological conditions, etc. If all these are taken into account, a “critical situation” that requires phytosanitary intervention can be established in culture.

An assembly of methods and means of struggle structured with interdependent elements, an ensemble which also relies on natural factors to regulate the density of harmful organisms populations, must not exceed EDT (Shi et al. 2018).

From the point of view of the systemic approach, in the framework of the integrated struggle, the following structure can be made: the sub-site of the technological processes itself, which consists of all the plant protection works, together with the apparatuses, equipment, installations, plant protection machines, resistance varieties, methods of application of chemical, biological, physical, etc.; the control subsystem, of great importance for obtaining high quality agricultural products or mitigating the effects of pollution, and which is essentially based on the correct execution of the works; the handling site includes the technical processes of transport and storage of plant protection products, together with the machines specific to these processes, which can be considered as plant protection machines as well. To some extent, in this system, it is also possible to introduce biological products in commercial units; the management or organizational overhead ensures the correct application in time and space of different works, depending on the sequence of the technological links, depending on the EDT and the biological cycles of the plant and the harmful species. From a toxicological point of view, the highly toxic red and green group products (products with acute toxicity below 100 mg/kg) have to be phased out of the range of pesticides. Products with dangerous side effects (carcinogenic, embryotoxic), very persistent or high residue residues in the crop should also be excluded from the ICS (*integrated control systems*).

In their evolution, the models of intensive, superintensive, or agricultural type industrial technologies (using high productivity agricultural machines, new agrotechnical methods, high productivity varieties, and especially chemical means of combating, models which, through their content was approaching industrial production) have begun to be abandoned in favor of models of sustainable agriculture, organic farming, precision agriculture, etc.

The interdisciplinary approach is dictated by the complexity of the agro-system, especially the great variety of pests (phytopathogenic viruses, bacteria, phytopathogenic fungi, nematodes, mites, insects, rodents, weeds, etc.) and the complexity of the methods and the means of fighting. We must use results obtained in biology, physics, chemistry, ecology, systematics, agronomy, physiology, phytopathology, entomology, herbology research.

After a very brief review of the basic rules for the development of integrated protection systems we can see that the basic elements are the economic criteria and the ecological criteria (the ecological criteria being finally economic criteria, because it is now obvious that the environmental risk is an economic criterion, as defined above–danger–damage) (Munns et al. 2017).

4.2 Potential Risk Factors and Prevention of Contamination with Dangerous Chemical Substances and Mixtures

The European Chemicals Agency (ECHA) indicates the following categories of chemical substances and mixtures as high risk to human health and the environment (European Commission 2012). Biocides are products that contain or generate active substances and are used against harmful organisms in the domestic environment (disinfectants, rodenticides, repellents, and insecticides) or to protect natural or artificial products in industry and agricultural applications.

Considering that because of their intrinsic properties the use of biocidal products may pose health risks, the EU has set up a regulatory framework aimed at significantly increasing the safety of biocidal products used and sold in the EU, namely EU Regulation 528/1012. It stipulates the removal from the market of biocides that are carcinogenic, mutagenic, toxic for reproduction, endocrine disrupting, or environmentally hazardous.

Pesticides are products used to combat harmful organisms from agricultural crops such as weeds and insects. However, their use can endanger health and the environment (Bhat et al. 2018; Mushtaq et al. 2018).

Many studies have shown the link between exposure to pesticides and effects on the human body such as cancer, fertility and reproductive problems, respiratory diseases, disruption of the hormonal system (endocrine), damage to the immune system and the nervous system. EU pesticide legislation (EC Directive 128/2009) imposes prohibitions on the use of certain hazardous chemicals in these products. Endocrine disrupting substances are chemicals that interfere with hormones (the endocrine system) causing adverse health effects.

A wide range of substances, both natural and man-made, are thought to cause endocrine disturbances, including pharmaceuticals, pesticides, and organic industrial chemicals. The European Community Endocrine Disruptors Strategy, adopted in 1999, outlined the actions required to counteract the impact of endocrine disrupting substances on human health and the environment. Thus, endocrine disruptors have been introduced into the REACH Regulation lists and in January 2012, the European Commission launched an important study called "Endocrine Disrupting Substance Evaluation Status." The study, based on a thorough scientific documentation, was commissioned to inform the European Commission about the identification of endocrine disrupting substance regulatory criteria in line with EU legislation.

Of these, cadmium, lead, and mercury (from the content of some pesticides) are particularly toxic to the human body and therefore the EU has made considerable progress in addressing the contamination of these metals. Thus, in 2005, the EU Mercury Strategy was launched, providing restrictions on the sale of mercury-containing measuring devices, banning exports of metallic mercury from the EU, and new safety rules for its storage. This strategy was revised in 2010. The implementation of the EU's Industrial Emissions Directive, adopted in 2010, will further reduce mercury emissions from industrial processes.

The EU also firmly supports the international process of developing a global legal instrument on mercury under the aegis of UNEP. Cadmium, lead, and mercury levels are strictly controlled in accordance with the restrictions in the Dangerous Substances Directive (EC 95/2002). Strict limit values are required for these metals at certain specific types of electronic equipment. Also, the Vehicle Disposal Directive (EC 53/2000) further restricts the use of these metals. In addition, the use of cadmium in the manufacture of jewelry and plastic products has been banned under the

REACH Regulation, while in the Water Framework Directive (EC 60/2000) cadmium and mercury are indicated as priority hazardous substances, whereas lead as a priority substance. (Mech et al. 2019).

Persistent organic pollutants (POPs) are chemicals that remain intact in the environment for long periods of time, from where they are transported and penetrate into living organisms, including humans, where they accumulate in their fatty tissue. Due to their toxic characteristics, persistent organic pollutants have harmful effects on human health and the environment. The Stockholm Convention is the world's leading tool to protect against persistent organic pollutants. In doing so, the EU is committed to implementing the Stockholm Convention prescriptions and encouraging the additional listing of chemicals under this Convention. Furthermore, in the EU, the Persistent Organic Pollutants Regulation (EC 850/2004) supports and strengthens the provisions of the Stockholm Convention.

The Persistent Organic Pollutants Regulation contains provisions on the production, placing on the market and use of chemical substances of this type, management of stocks and wastes of persistent organic compounds, and measures to reduce unintended emissions of persistent organic compounds (Hennebert and Filella 2018). Statistics on the effects of pesticides include a physical, chemical, biological database collected through techniques and methods of comparative analysis of their impact on the ecosystem, as can be seen from Table 4.1.

Table 4.1 Effects of pesticides on specific subpopulations

The effect on human health	Pesticides causing the effect	Subpopulations at risk
Cancer	DDT, toxafen, 2,3,7,8-TCDD, mirex, HCH, PCB, HCB	First of all in adults (breast cancer, prostate cancer, and testicular cancer); cancer related to children
Effects on the device reproductively	PCB, dibenzodioxins, dibenzofurans	Fetuses, newborns, women of childbearing age
Growth retardation	PCB, dibenzodioxins, dibenzofurans	Fetuses, newborn babies, children (length, body weight, skull circumference)
Neurological disorders	PCB, dibenzodioxins, dibenzofurans	Fetuses and children (cognitive, memory, and attention problems); adults (Parkinson's and Alzheimer's)
Altering behavioral development	PCB, dibenzodioxine, PentaBDE	Children, continuing into adulthood (disorders with deficiency attention, learning difficulties)
System suppression immune	PCB, dibenzodioxins, dibenzofurans	Newborns, children (severe ear infections, impaired resistance to diseases); adults (suppressing the immune system)
Cardiovascular effects	PCB	Children and adults (variations in blood pressure and rhythm cardiac)
Effects on thyroid	PCB, PFOS, PBDES	Women (hypothyroidism)
Metabolic disorders	PCBs, POPs in general	Adult women and men (diabetes and obesity)
Bone disorders	PCB, dioxin, HCH	Adult women and men (osteomalacia, osteoporosis)

Nanomaterials are chemicals or materials that are manufactured and used in extremely small dimensions (nm, i.e., 10^{-9} m). At present, many products containing nanomaterials are already in use and this market is expected to flourish in the coming years. Nanomaterials have the potential to improve the quality of life and contribute to industrial competitiveness (Singh et al. 2020). However, new materials can also raise health and safety issues. Main technologies for bioremediation of pesticides-contaminated soils can be seen from Table 4.2.

These risks, and how these can be addressed through existing risk assessment measures in the EU, have been the subject of several scientific opinions issued by the European Commission's Scientific Committee on Emerging and Newly Identified Health Risks. Combinations of chemical substances and chemical mixtures, according to some studies, can have harmful effects on human health even when individual chemicals are below the "safety level." In the EU, the regulatory risk assessment for chemicals is based on individual products. However, there are concerns that this approach cannot provide sufficient protection against the risks generated by simultaneous exposure to several chemicals and that another approach is needed. Pharmaceutical residues have become an important source of environ-

Table 4.2 Bioremediation technologies for pesticides-contaminated soils

Method	Technologies	Advantages	Disadvantages
In situ	Biodegradation	Offers the possibility of simultaneous contamination of both soil and groundwater	The equipment requires a delicate operation of specialty
	Bioventing	The equipment is generally easy to place	During the operation it is difficult to appreciate the volume treated, its configuration, and the efficiency of the depollution process
	Biosparging	It can be applied to several types of pollutants	
	Phytoremediation	Can be used in combination with other remediation technologies	Removal efficiency may vary from site to site
	Natural attenuation controlled	It can be used for both long term and freshly contaminated sites	Long treatment time (6 months–3 years)
Ex situ	The bioreactor	Rapid and relatively complete removal of polluted components	High cost of transportation; the need for excavation and soil preparation
	Biopile	The possibility to continue the activity on site	The risk of partial dispersion of pollutants during evacuation work, loading, transporting, and unloading
	Composting	High depollution efficiency, offered by treatment in specialized plants	
	Land farming		Imposing limits of concentration in pollutants, before treatment

mental contamination. Many pharmaceutical chemicals are designed to be non-degradable and thus present a risk when they reach the environment where they persist. The European Commission recognizes that environmental pollution with pesticide residues is both an emerging issue and a public health problem and is committed to addressing this issue (Brinch et al. 2016).

4.3 Management of Plant Protection Products (Pesticides/Biocides)

The management of plant protection products is governed by the law, the rules of approval and placing on the market, storage, application, use of their specific technological means under the conditions of soil and water protection are presented in the Code of Good Practice. The document stipulates the specific rules for the approval and placing on the market, storage, and application of plant protection products.

4.3.1 The Risk of Soil Contamination with Persistent Organic Compounds and Its Prevention

As has been shown before, persistent organic compounds (POPs) are chemicals that remain intact in the environment for long periods of time, from where they are transported and penetrate into living organisms, including humans, where they accumulate in their fatty tissue. In our country, from the point of view of the pollution of soils with persistent organic compounds, significant was the pollution of agricultural soils with organochlorine insecticides (xx). Organochlorine insecticides, namely those based on HCH (hexachlorocyclohexane) and DDT (pp′-dichlorodiphenyl-trichloroethane), were introduced into use in the 1940s and have long been used to protect crops against vector insects. The organochlorinated insecticides HCH and DDT (isomers and metabolites) are very persistent, accumulating in the soil; half-life in soil is 2 years for HCH and decades for DDT. The widespread use of these pesticides and their high persistence have made their residues and metabolites highlighted in all the elements of the environment. Studies have been conducted that have shown that in areas where the soil is polluted with DDT, residues are present and its metabolites and in plants, in animal and human fat. Although it has been forbidden to treat these compounds throughout the country since 1985, HCH and DDT residues are now found in agricultural soils.

To remedy pesticide pollution, the methods of Fig. 4.1 are feasible:

Therefore, their monitoring is necessary; the following compounds are: α, β, γ, δ-hexachlorocyclohexane, p-dichlorodiphenyl trichloroethane (DDT), and the opium dichloride diphenyl trichloroethane (DDT), dichlorodiphenyl-dichloro-ethane, with

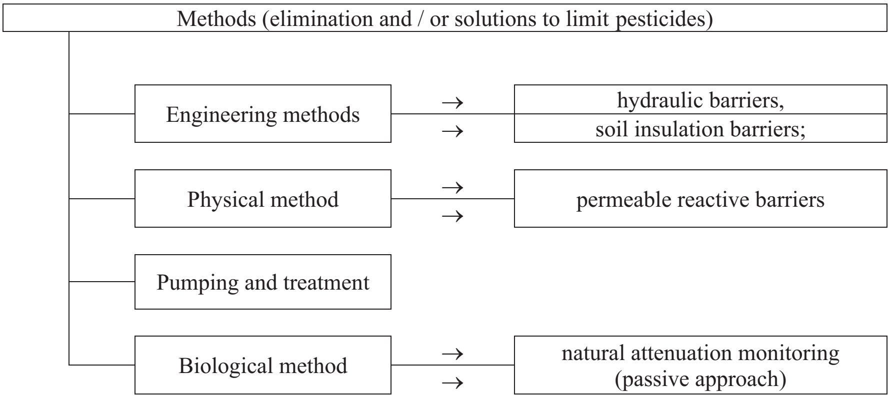

Fig. 4.1 Methods to reduce pollution with pesticide

the two isomers (op′-DDD and pp′-DDD), and dichlorodiphenyldichloro-ethene (DDE). Practically, by summing up these compounds the total concentration of HCH is obtained (De Rop et al. 2019).

4.3.2 *European Policies and Instruments for the Protection of Human Health and the Environment Against Dangerous Chemicals and Mixtures*

The European Union has developed policies and tools to protect human health and the environment against hazardous chemicals and mixtures such as: (European Commission 2012): REACH Regulation (EC 1907/2006) on the Registration, Evaluation, Authorization, and Restriction of Chemicals is a key element of the European Union's set of rules to make the use of chemicals as safe as possible. The REACH regulation addresses long-term exposure to chemicals and the implications for human health and the quality of the environment. The REACH Regulation creates a single regulatory system dealing with industrial chemicals and seeks to eliminate the information deficit related to them by placing accountability on chemical manufacturers who are required to provide safety information on their substances and to manage risks from chemicals.

The philosophy of the REACH regulation is that no chemical, in any form, should be placed on the market without proper documentation. Each manufacturer and importer of chemicals that distributes more than 1 ton per year is required to register them with the European Chemicals Agency (ECHA) and to provide information on their properties and uses and safe methods of handling them. The REACH Regulation also calls for the progressive replacement of the most dangerous chemicals with appropriate alternatives. The first registration deadline ended on November 30, 2010, with approximately 5000 chemicals registered with ECHA. This marks an important step towards safe chemicals management. CLP Regulation (EC 1272/2008) on Classification, Labelling, and Packaging of Chemicals that aligned the existing EU classification system to the United Nations international system, called the Globally Harmonized System of Classification and Labelling of Chemicals (UNGHS). By classifying, labeling, and packaging chemicals in accordance with the CLP Regulation, it is ensured that chemical users and manipulators are aware of the dangers of their use and handling (Jess et al. 2014).

All manufacturers and importers of hazardous chemicals have been compelled to label chemicals in accordance with the CLP Regulation and to notify the European Chemicals Agency (ECHA) as of 1 December 2010. Thus, more than 100,000 chemicals have been notified to ECHA, which allowed ECHA to make an inventory of chemical classification and labeling and to provide chemical users and manipulators with the information they need to limit the risks and to select safer chemicals.

The human biomonitoring HBM system involves the collection and analysis of human tissue or body fluids to identify the presence and levels of chemicals in the human body. Chemicals are taken directly or indirectly from the environment. The HBM system is an important tool for assessing human exposure to environmental substances. The EU Scientific Research Commission has funded a research program to develop a human biomonitoring framework at EU level called DEMOCOPHES.

It was tested the feasibility of a coherent approach to human biomonitoring in Europe by identifying biomarkers of mercury, cadmium, phthalates, and tobacco smoke in the human environment and in human urine. The project will collect biological samples and information from 120 parent–child pairs from each participating country within the EU (Lillebø et al. 2019).

4.4 Methodology for Managing Potentially Contaminated Sites

The historical contaminated area is: “A contiguous site (land and/or aquifer) where anthropogenic activities have determined the presence of pollutants in concentrations which are presenting and/or could be presenting an immediate risk to both the existing site and the surrounding areas or on long term for the health of the population and the environment.” On a contaminated site there may be one or more sources of contamination. The potentially contaminated historical site is: “A contiguous site (land and/or aquifer) on which historical and/or present activities could have a significant impact on the health of the population or the environment for both the existing site and neighboring areas (Storck et al. 2017).

This definition focuses on contamination caused by past industrial practices that led to contamination as a result of inappropriate storage/handling/use of chemicals, soil, and/or aquifer. The management system for contaminated sites includes 4 phases: identification and registration; preliminary assessment; detailed assessment; and remediation. A potentially contaminated site may be classified as a “suspect”

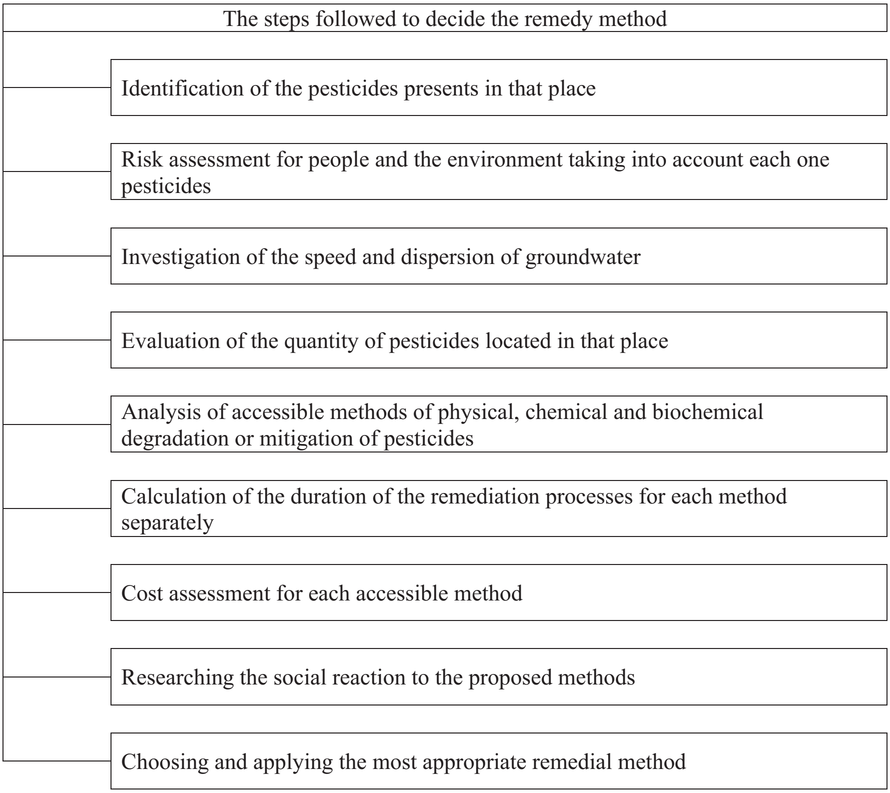

Fig. 4.2 The steps that need to be followed to decide which remediation method can be used

site following an analysis based on the following elements: assessment of the site based on owner's information; regular or irregular site inspections by local authorities and control bodies; analyzing the effects of natural disasters on the site; the owner's notification to the regulatory bodies. On all "potentially contaminated" sites, a preliminary assessment should be carried out. It is recommended that before deciding what remedial method can be used to go through the phases of Fig. 4.2.

The purpose of the preliminary assessment is to determine whether the degree of contamination is real or not and whether a detailed assessment is required on site. For this purpose, laboratory analyses will be performed for samples taken from sites. The corresponding indicator parameters for each industry must be listed. There are three possible outcomes of the preliminary site evaluation, namely: (a) No further investigation is required—the site will be kept in the inventory; (b) Additional investigations are required—detailed site assessment will be carried out; (c) Remedial—cleaning and remediation of the contaminated site will be initiated immediately.

The detailed assessment of the contaminated site requires the following steps: site characterization and contamination: on this occasion, information and location data will be collected; the Conceptual Placement Model (CPA) is being developed; all possible links are identified:

- the source–transfer–receiver path; samples of the surface and of the soil profile will be taken and laboratory contaminants will be determined by laboratory analysis;
- samples will be taken from the groundwater to know if it is contaminated or not; generic risk assessment; the measured contaminant concentration compares with the soil quality standard to define the transfer paths, and the generic scenario will include inhalation of fugitive particles, volatile inhalation, ingestion of groundwater, dermal absorption; if the values in the standard are exceeded, risk assessment will begin for all potential open transfer routes; Also, all the transfer paths will be identified;
- detailed risk assessment; information about site specificity is collected; the specific risk levels are calculated; if the level of risk is higher than the acceptable risk level, the site is listed as contaminated, requiring it to be rectified, the site cleaning level is established, all the alternatives to cleaning technologies will be identified and the technology selected appropriate to meet the remediation targets (Breysse et al. 2018).

During the remediation phase, the designing and execution of remediation works is carried out. The remedial effects will be monitored for both execution and post-execution.

Technologies for soil remediation of POPs that are more frequently applied are bioremediation technologies (in situ technologies presented above) as well as thermal desorption technologies which will be presented below:

- Thermal desorption is a thermal method that works on the principle of polluting soils so that pollutants can evaporate, so they can easily move through the soil to the area of a well system that collects and evacuates them. Thermal desorption can be applied in situ or ex situ.
- Thermal in situ desorption can be done by: steam injection—steam injection is injected into the soil by means of wells dug in the polluted area; the steam heats the area and mobilizes, evaporates and/or destroys the pollutants, the water and the gases produced being collected in special wells from where they are directed to purification plants; hot air injection—a procedure similar to steam injection except that hot air is injected through wells instead of steam, hot air warms the soil causing evaporation of pollutants; hot water injection—a procedure similar to the two above-mentioned processes, introducing hot water this time instead of steam or hot air; heating by electric resistance—a process by which an electric current is applied to the ground through a network of steel electrodes; the heat generated by the electrical resistance of the soil at the passage of the electric current vaporizes the underground water and the water from the soil, creating the conditions of the evaporation of the pollutants; radio waves heating—the process of placing an antenna that emits radio waves in a well; radio waves warm the soil causing the evaporation of pollutants. In some cases, thermal desorption is also

ex situ, which is a more expensive process but where the treatment process is much better controlled.

- Typical ex situ thermal desorption systems work in several successive phases, namely: pretreatment, desorption, and post-treatment of solid material and resulting gases; Pretreatment involves processes such as sorting, dehydration, neutralization, and blending; the desorption is done in thermal installations called desorbers, whose classification is usually made according to the heating system used;—post-treatment of waste gases—depends on equipment-specific factors and may include: combustion at high temperatures (over 1400 °C) followed by treatment and disposal, combustion at moderate temperatures (200–400 °C) using catalysts or gas purification and elimination burned (Butler 2018).

4.5 Monitoring of Soil Quality

The Soil Quality Monitoring System is characterized by four basic elements: spatial distribution; density of the observation network; set of indicators; and periodicity of the determinations. These basic elements of the monitoring system are tracked on three levels of detail:

- level I—consists of carrying out a minimum of investigations at all points of a fixed network, to identify the areas with degraded soils, in various stages and processes, periodically following their evolution through a set of mandatory indicators;
- level II—consists of the detailed investigation in some points of the level I network and in other representative additional points, in order to identify the causes of soil degradation processes (conducting intensive studies);
- level III—consists of sufficiently thorough investigations to verify the hypotheses, from detailed analyses of soil quality alteration processes, elaboration of forecasts of the evolution of soil quality alteration processes, and recommending possible remedial measures (Gorito et al. 2017).

The first level is characterized by the following elements:

- at the macroscale, the spatial distribution of the soil sampling points (sites) at the intersections of a fixed observation network, covering the entire country, with a density of 16 × 16 km (in accordance with the recommendations of the document "Convention on Long Transboundary Air Pollution"), resulting in a total of 942 sites, of which 670 sites in areas with agricultural soils and 272 sites in areas with forest soils;
- at the microscope, the choice and location of an observation point is made inside a square with a 400 m side, which has the center (the point of intersection of the diagonals) in the nodes of the observation network;
- from each observation point a set of samples will be collected to determine the basic indicators that contains: an average sample in the disturbed structure,

composed of 20–25 individual samples from the first 10 cm of soil depth, respectively, 3 individual samples for each soil horizon and 4 samples in the natural structure on the horizon;
- the periodicity of the determinations is of 4–10 years, for the observation points without special problems, and of 1–2 years for the observation points with problems (in which processes of degradation of the quality of the soil take place, as for example: pollution) (Panizzi et al. 2017).

4.6 Control of Residues in Plants and Plant Products, as a Result of Metabolism/Migration of Hazardous Chemical Substances and Mixtures in the Soil

Food security—refers to food availability and easy access to people. The World Health Organization defines the following criteria of food security: availability—sufficient quantity; access—to have sufficient resources (economic and physical) to have the foods corresponding to the individual diet; use—to have sufficient information about nutrition, water, and proper hygiene; stability—ensuring the first 3 conditions. Food safety—ensuring the handling (during the technological process) in hygienic conditions of food ingredients and food from the ground to the consumer so that they are not a source of disease of the body, moreover, to prevent the occurrence of diseases. The food has the capacity to accumulate polluting chemicals from the environment (accidental contamination) to which an additional contribution of chemical compounds from agricultural and/or food technologies (deliberate contamination) is accumulated. The toxicity of chemical compounds depends on a number of factors such as periodicity and amount of chemical contaminant carried; synergism or antagonism between chemical pollutant and food; attenuation or enhancement of the toxicity of the pollutant depending on the metabolic changes of the animal or plant tissue; alteration—modification of sensory and (sometimes) nutritional properties, possibly acquiring harmful properties; impurification—foreign compounds of the nature of the food (biological, chemical, physical contaminants); degradation—loss of nutritional (and/or organoleptic) properties that depend on: frequency of processing, non-compliance with the recipe, heat treatments, exposure to oxygen action, reheating, keeping of hot foods, presence of heavy metals, washing of fragmented foods, keeping in water, and removal of aqueous solution or brine; sanitation—transformation into a product harmful to the body; this does not imply other changes (alteration, degradation, impurification) (Handford et al. 2015).

Contamination of food with chemical substances or mixtures can come from the pollution of environmental factors: soil, water, atmosphere through the trophic chain soil–plant–animal–man, as well as from agricultural and food technologies (Singh et al. 2018).

According to the Codex Alimentarius Commission, the main types of contaminants and pollutants of food are classified into the following classes: pesticides: insecticides, herbicides, defoliants, rodenticides, fungicides, etc.; industrial chemicals: dioxins, polychlorinated biphenyls, chemical compounds from inappropriate packaging materials, etc.; heavy metals (Singh et al. 2018) and other elements; microbiological contaminants (Dar et al. 2020). In addition to these classes of contaminants and pollutants, some antinutritional toxic compounds can be added which can be formed under certain conditions during the processing or storage of foods. Also, parts of the contaminant's category are animal parasites. All these classes of contaminants and pollutants are under the influence of the legislation and the sanitary control. Pesticides are a special class of chemical contaminants, which, in modern agriculture, are widely used (Bhat et al. 2018). The control of pesticide residues and other hazardous chemicals, especially of organic nature from plants and plant products, derived from their migration/metabolization in soil, is based on modern analysis techniques that allow increasingly easy detection of the molecules found in infinitesimal quantities, namely chromatographic methods, such as gas chromatographic (GC) and liquid chromatographic (LC) methods, which will be described below (Adams et al. 2017).

Over 800 active substances are formulated in thousands of pesticides, these substances forming over 100 chemical classes of which the most important would be: benzoylureate, carbamate, organophosphorus compounds (OP), organochlorine (OC), pyrethroids (PYR), sulfonylureas, triazines, dithiocarbamates, azoles, phenoxy acids. Their physical and chemical properties differ greatly, which makes the determination of the residues of these chemicals quite difficult in any matrix and causes problems in the development of a universal method of analytical determination of residues, which would be the most desirable thing. In order to detect pesticide residues from plants and plant products, a very important first step is to prepare the samples for analysis. Most sample preparation methods for gas chromatographic (GC) and liquid chromatographic (LC) determination include the following steps:

- Homogenizing the sample to obtain a uniform matrix.
- Solvent based extraction of pesticide residues.
- Purification—eliminating interference between matrix components.
- Elution and/or fractionation of the extracted analytes.
- Concentration of the eluent and reconstitution in a solvent that is compatible with GC or LC conditions.
- Introduction of the solution containing pesticides in GC or LC. Methods of analysis to determine the content of pesticide residues in plants and plant products can be targeted or non-targeted (Núñez et al. 2012).

An example of targeted analysis is the inspection of MAL (maximum allowed limit) in vegetables, fruits, and foods; the relevant elements are fixed to be determined according to the definitions given by the MAL regulations. The most commonly used methods of analysis for the detection of pesticides and other chemical compounds, especially of organic nature, are gas chromatography and liquid chromatography. In gas chromatography the eluent (a chemically inert gas, e.g., nitrogen, helium, hydrogen) passes through the sample input device (where the sample is volatilized), takes it, and enters it into the chromatographic column, in which the separation process takes place. Due to the interactions between the sample components, with the stationary phase and/or the mobile phase, they migrate through the column at different speeds, due to the differences between the distribution coefficients of the 2 phases. The difference of the component migration speeds is significant so that they are separated at the exit of the column, from where they are taken by the eluent to the detector.

The gas chromatograph can be coupled with: flame ionization detector (FID), electron capture detector (GC–ECD), GC–MS mass spectrometer (quadrupole triple, TOF—“time of flight”—flight time or ion trap), or with high resolution mass spectrometer (HRGC–HRMS). Unlike gas chromatography, in which the mobile phase has the role only of transporting the components through the column, in the case of liquid chromatography the mobile phase, which is a liquid, participates directly in the separation process, through various interactions (adsorption, distribution, exchange, etc.). Also, the liquid mobile phase participates and directly influences the selectivity of the separation process. In the determination of pesticide residues, liquid chromatography (LC) can be coupled with UV–VIS detectors (HPLC UV–VIS) or coupled with a mass spectrometer (with triple quadrupole QQQ, ion trap, or TOF—flight time). In our country the most complete works in the field of pesticide residue analysis are by GC–MS and LC–MS techniques (Ferrer et al. 2007).

Another class of hazardous chemical residues from plants and plant products, derived from their migration/metabolism in soil, is the heavy metals that can contaminate food in the form of combinations of arsenic, cadmium, copper, lead, mercury, zinc, tin, and even of iron (Mehmood et al. 2019). Modern analytical techniques used especially at present that allow the detection of heavy metal residues in plants and plant products are flame atomic absorption spectrometry (AAS) and inductive coupled mass spectrometry (ICP–MS). Atomic absorption spectroscopy (AAS) can be performed in graphite furnace or flame. The principle of atomic spectroscopy is based on the property of the atom to emit or absorb electromagnetic radiation specific to a particular element under certain physical conditions. Thus the elements to be determined from a sample are released from their compounds by an energy absorption making them available as free particles. Since elementary mass spectrometry is based on the fact that electrically charged particles can be separated and detected in a mass spectrometer by energy absorption, the electromagnetic radiation that crosses the sample is provided by a special source called “hollow cathode lamp.” The cathode of this lamp is made from the element to be determined (it follows that for each chemical element a specific lamp is therefore required).

Thus the elements to be analyzed are transformed into a free atomic state in an atomization device by thermal energy absorption. The free atoms have the ability to absorb the radiation specific to the analyzed element. Thus, depending on the concentration of the analyzed element, part of the radiation intensity of the hollow cathode lamp is absorbed by the free atoms formed from the sample. A detector consisting of two photomultipliers measures both the intensity of the unintended radiation and the radiation after leaving the atomizing device, the concentration of the element analyzed in the sample being obtained on the basis of the difference between the two intensities.

The preparation of the samples for analysis consists of calcining the samples at 450 °C with the gradual increase of the temperature, dissolving the ash in hydrochloric acid and evaporating to dryness the obtained solution, redissolving the final residue in nitric acid 1:6, and determining the metal content by atomic absorption spectrometry. Inductive coupled plasma mass spectrometry (ICP–MS) has now become one of the most powerful analytical techniques, highly sensitive, for analyzing a wide range of metals and some non-metals, at very low concentrations, at the level of 1–10 ppt. Inductive coupled plasma (ICP) was developed in 1960 by Reed and was used for the first time by Greenfield Group and collaborators for spectrochemical analysis. The first papers on the inductively coupled plasma mass spectrometer were published in the 1980s, and the first commercially available ICP–MS instrument being introduced in 1983. Since then, many improvements have been made for each generation of ICP–MS instruments. The ICP–MS method is based on the combination of inductively coupled plasma, as the ionization method, with mass spectrometry, as the ion separation and detection method. Combined with various chromatographic separation techniques, the ICP–MS method is a powerful and versatile method for analyzing elemental species, including isotopic species. Besides classical soil, water, plant products, food samples, currently a wide range of biological samples, both solids and liquids can be analyzed by ICP–MS (for example: blood, urine, plasma, serum, interstitial fluids, internal organs, tooth, hair, bones, and even cells) (Gui et al. 2016).

The presence of pesticides in food of plant origin is the result of their contamination from the atmosphere, water, and soil. In the atmosphere pesticides are present due to the fact that they are administered by air, as well as due to the volatilization of pesticides on the soil surface and plants. The binding of pesticides in soil is determined by Van der Waals forces, hydrogen bonds, hydrophobic bonds, chemical absorption, and ion exchange.

Some of the pesticides fall to the ground where they are solubilized by the rainwater and are entrained on the surface or in the depth of the soil where they are biodegraded, entrained in the groundwater, or absorbed by the lattice system of plants. The degree of soil migration in plants depends on the initial amount of pesticides, their properties, and the type of plants. In the body of animals, pesticides can enter through the respiratory tract, the skin, and the oral route.

When using pesticide combinations there are situations when the toxicity of the combination increases 5–10 times compared to the toxicity of the components. Among the most dangerous pesticides from the point of view of food contamination, organochlorine pesticides selectively accumulate in the animal body, the decreasing order being: mesenteric fatty tissue, depositional fat tissue, perirenal fat tissue, and then muscle tissue.

In ruminants, a greater accumulation is observed in pigs. Concentrated feed (cereals, meats, beets) is responsible for a significant accumulation of pesticides. It should be mentioned that the reduction of the amount of organochlorine in the animal body is done only after 3–4 weeks (Tran et al. 2019).

4.7 Regulation on Biocidal Products

Companies must request the approval of an active substance by submitting a file to ECHA. The active substances must be approved before the authorization of a biocidal product containing them. The active substances are first evaluated by the competent authority of the evaluating Member State, and the results of the evaluations are transmitted to the Committee for Biocidal Products of ECHA, which draws up an opinion within 270 days.

The opinion serves as the basis for the European Commission to adopt the decision regarding the approval of the product. The approval of an active substance shall be granted for a defined period, not exceeding 10 years, and may be renewed.

Biocidal Products Regulation introduces formal exclusion and replacement criteria, which is applied to the evaluation of active substances. After approval of an active substance, companies wishing to introduce biocidal products on the market of a Member State must apply for product authorization (Wynendaele et al. 2018).

4.7.1 *Regulation Regarding the Prior Consent Procedure in the Knowledge of the Case*

The Regulation on Prior Informed Consent (PIC Regulation) regulates the import and export of certain hazardous chemicals and imposes obligations on companies that intend to export such products to countries outside the EU. The PIC regulation contains three sets of provisions regarding the export of chemicals: The chemicals mentioned in the list in Annex I of the regulation are subject to the export notification procedure and the explicit consent requirement. This list is updated regularly as a result of regulatory measures under EU law, as well as evolutions under the Rotterdam Convention. The chemicals mentioned in the list in Annex V of the regulation are prohibited for export. All chemicals that are subject to export must comply with the rules on packaging and labeling in accordance with CLP (Classification, Labelling, and Packaging) Regulation (Pouokam et al. 2017).

4.7.2 *The Effects of Pesticides/Dangerous Substances on Humans*

In assessing the probability of survival during an accident occurring in a chemical installation, it is important to consider the following factors: information prior to the accident, information on the progress of the accident, staff reaction time, emergency procedures implemented, evacuation time and distance until at a safe place, the type of danger (toxic gas, thermal radiation, explosion, etc.), protection and mitigation of effects (shielding or reflection), the level of damage as a function of time, total exposure time. From the point of view of toxicity, the most widespread indicator is LC_{50}, respectively, the pollutant concentration which is lethal to 50% of the population after a certain period of time (usually 14 days). LC_{50} is specific for each species (mice, fish) and is determined by standard toxicity assays over a period of time (often under 24 h).

However, because ecosystems include species with different susceptibilities to various chemicals, it is necessary to test more than one species, and the tests to be repeated periodically.

In general, the LC_{50} indicator is specified in the hazardous substance sheet. As a rule, LC_{50} is expressed in mg substance/L air or ppm (1 ppm = 1 cm^3 of gas contained in 1 m^3 of air, or can be determined from the expression in mg/m^3 if gas density is known). In addition to toxicity, persistence and diffusion are considered, both for pure substances and for solutions or mixtures. This has resulted in a broad set of properties and risk indicators of substances, as well as multiple classification systems.

For example, MPC represents the maximum permissible concentration of a substance that appears in the form of gaseous, vapor, or suspended matter, which is not harmful to the health of the humans and does not cause any inconvenience, even in the case of prolonged and repeated exposure. The uncontrolled emission of pollutant represents the release into the environment of a toxic substance for humans and the environment as a result of an accident.

From the place from which it is released, a place called the source of pollution, the substance spreads in the environment to great distances, gradually being taken over by it. In order to estimate the effects of a pollutant, the following parameters must be followed: the concentration level at different source distances, respectively, the pollutant concentration at a specified distance relative to the source (concentration field)—the critical area in which the concentration exceeds an allowed threshold (action distance of the toxic).

In order to estimate the effects of a risk agent it is necessary to have the possibility to quantify the exposure, from the point of view of the intensity, the duration of exposure, and the consequences (Bravo et al. 2011).

This is usually done by estimating the received dose and comparing it with the statistical processing of the experimental data. Vulnerability criteria can be established to determine the exposure dose levels that have specific consequences. Criteria provide the threshold beyond which the protection is needed to prevent impairment of the functions necessary for an individual to be safe.

There are two approaches to determining the effects of the dose received: using the probit functions and determining the harmful dose (applied in case of toxic or thermal risks).

Probit functions take into account the variation of tolerance to harmful effects for an exposed population.

The death rate of humans exposed to harmful agents over a period of time can be calculated using the probit functions, which usually take the form:

$$Y = k_1 + k_2 \ln\left(V\right)$$

in which, Y is probit (with a range of values between 2.67 and 8.09 representing fatality between 1 and 99.9%, a measure of the percentage of vulnerable resources that can suffer damage. The probability of death can thus be determined by evaluating Y on a transformation diagram probit that of Finney (1971). k_1 and k_2 are constants, V—is the product of the intensity or concentration of the toxic compound at an exponent n with the exposure time (expressed in seconds or minutes).

The concentration can be expressed in parts per million (ppm) or milligrams per cubic meter (mg/m^3). Probiotics can be obtained for almost any dangerous agent. Probit analysis is an approximate methodology, but it allows quantifying the consequences of exposure.

It has to be taken into account. However, the estimated probability of death can vary significantly for the same toxic compound, depending on the choice of the probit equation. Also, care must be taken in choosing the units of measure suitable for the equation used (Oribhabor et al. 2016).

4.7.3 Determination of the Harmful Dose

The specified level of toxicity (SLOT) or significant likelihood of death (SLOD) is alternative approaches for estimating the level of fatality upon exposure to a toxic agent.

The SLOT approach involves the use of available toxicity data, which are then extrapolated for human use. The SLOD used in risk analysis estimates the dose needed to produce a significant probability of death. The consequences of exposure (inhalation of toxic substances, exposure to explosion pressure, or exposure to thermal radiation) are usually assessed from existing information, preferably data obtained from previous accidents to which humans have been exposed or from animal experiments.

Uncertainties in translating the data obtained from animals to data relevant to humans are large and, therefore, it is necessary to include "safety factors" in modeling. In general, SLOT is considered to be equivalent to LC1–5 derived from animal experiments. The SLOT and SLOD approaches extrapolate toxicity data to determine the hazardous toxic load (A), which causes a specific level of harm for a given dose. These values are calculated as the product of exposure and time and usually have the form:

$$C^{n \cdot t} = A$$

where t = the exposure time, in minutes, C = the concentration of the substance, in ppm, n = the exponent.

The exponent "n" is derived from the extrapolation of the toxicity data used. SLOT is usually defined as the dose that leads to the death of the vulnerable population and to severe suffering that requires medical attention for the rest of the exposed population and, as such, is equivalent to LD1 or LD1–5. SLOD is defined as the dose that determines the death of the exposed population in proportion of 50%, being therefore equivalent to DL50.

Knowing the SLOT or SLOD values and the exponent, one can calculate the concentrations needed to produce these effects, depending on different exposure time values.

Basically, the dynamic balance in an ecosystem/agrosystem is based on the complementarity of the genes of organisms placed in different ecological niches. Over time there is a re-adaptation of the genetic material of the harmful organisms but also of the plants attacked in regard to the virulence/avirulence gene system—genes of resistance or sensitivity, which is why the process of improvement in this direction is a continuous one.

In general, in plant protection, harmful organisms are not fully combated, an operation that would be exceptionally expensive and difficult to perform. However, for some harmful organisms whose development is rapid, in large areas producing adequate epiphytes or epizootics, the phenomenon of eradication can be practiced. To do this, we call on the methods and means of the operative sector of plant protection.

The close monitoring of the outbreaks in which such organisms appear and the prevention of the widespread of their propagates are elements of phytosanitary quarantine.

They are supported by an adequate legal framework and are within the competence of the government administration. Phytosanitary quarantine belongs to the operating sector of governmental structures but the cultural hygiene elements associated with preventing the spread of quarantine damage agents propagate are into the integrated protection structure (Eason and O'Halloran 2002).

The prediction of the onset of the attack of a certain harmful organism or groups of harmful organisms and the subsequent issuance of warnings for carrying out a plant protection intervention are of great importance for the correct conduct in time and space of the combat activities. It is good to know since harmful organisms exist in an area for agricultural crops in vegetation as well as potential levels of attack to know what spectrum of means of control should be used, what system of machines should be used for their application and optimal times of intervention so that the damages are as small as possible, the interventions as little as possible, the economic effects are maximum and the ecological ones are minimal.

4.8 Conclusions and Recommendations

Ideally, the pesticides used will run out once the purpose is achieved. But besides the undeniable advantages of pesticides, they also have a number of disadvantages. Being toxic to a life form, pesticides pose a risk of harm to humans, pets, hunting, birds, and microflora.

At the same time, the use of natural organic fertilizers and other organic residues contributes not only to raising soil fertility and to protecting the environment.

However, if we really want to reduce the risk posed by the growing diversity of synthetic chemicals released into the environment, we need to rethink some of the basics of industrial development.

This would allow the removal or at least the considerable diminution of one of the major causes that maintains and accentuates the processes of deterioration of the ecological system that produce a series of goods and services necessary for the development of the human society.

The use of pesticides to diminish their impact on the environment implies:

- the use of substances with lower toxicity and remanence, at which the dose of the active substance and the applied quantity per hectare will be optimal, to destroy pests but to affect less the useful species;
- decrease the frequency of pesticide application by using certain products or combinations of products, acting on several categories of pests;
- establishing the mode and surface of application, by editing on large surfaces and applying as much as possible to localized treatments;
- choosing when to use pesticides, which should take into account not only the most sensitive stage of the pest but also the period when the useful species are least exposed;
- increasing the weight of mechanical and physical methods in combating pests;
- the proper application of agrofitotechnical methodologies, emphasizing the components that disadvantage the pests and diminish their effects;
- maintaining or creating portions of land on the edge of crops or roads, on the springs of irrigation channels, in the woods, in parks, and nature reserves, in which the associations of spontaneous vegetation offer to the useful species places of wintering and of refuge during unfavorable time and also additional food;
- cultivation of honey plants, to attract the adults of the parasites, around the crops;
- spraying some nutrients on plants in order to provide additional food for parasites and predators;
- cultivation of nectariferous plants, pollinifers, to attract the adults of the parasites and predators, near the crops;
- recourse to granulated, encapsulated pesticides, with reduced remnant applied strictly localized only if there is no other solution, to minimize their negative effects.

Awareness of the danger posed by pesticides has led to encouraging consumers to develop organic farming. Organic farming does not use pesticide-like substances and the use of mineral fertilizers is much more rational.

This encourages crop diversification and extensive animal husbandry. By increasing the diversity of cultures, the possibility of spreading the attack and the impact of pests compared to the one recorded in monocultures is reduced.

Also, although the production of a particular crop may decrease, the total agricultural production obtained in organic, multifunctional farms is as high as in the intensive ones and the incomes can be even higher.

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Chapter 5
Mycoremediation: A Sustainable Approach for Pesticide Pollution Abatement

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5.1 Background

Environmental problems related to pesticide pollution are complex (chemical, biological, technological, social) due to the high load of harmful substances but especially due to the imminent danger they pose to the environment and health, and their solution is only possible through cooperation of specialists from various fields that propose innovative technologies and services, the classical ones, that were applied until now, proving insufficient.

The sustainable approach contributes to the creation of an environmental product, materialized in the installation of ecosystems as close as possible to the natural ones on anthropic deposits polluted with pesticides and remedied by mycoremediation methods. The common denominator of all life forms in the soil is that every organism needs energy to survive.

While some bacteria, known as chemosynthesizers, get energy from sulfur, nitrogen, or even iron compounds, the rest must consume something that contains carbon to get the energy they need to sustain life. Carbon can come from organic material supplied by plants, residues produced by other organisms, or from the bodies of other organisms (Chanda et al. 2016).

Primarily, all life forms in the soil are getting carbon to fuel metabolism—it is an "eat-and-be-eaten" world, in and on the ground. Fungi are the main decomposition agent in the soil trophic network (Khanday et al. 2016; Bhat et al. 2017). The enzymes they release allow them to penetrate not only lignin and cellulose from plants (dead or alive) but also the harsh chitinous carapace of insects, animal bones,

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and even hard nail proteins from the foot or hand. Bacteria can manage on their own, but require easily digestible foods, often derived from the decomposition made by fungi and only after such elements have been decomposed or broken by fungi or other organisms. Compared to fungi, bacteria are beginners in terms of decomposition abilities.

The fungi in the soil form two extremely important mutual relations with the plants. The first is the association of certain fungi with green algae, which leads to the formation of lichens. In this symbiotic relationship, the fungus receives food from the algae, which uses its power of photosynthesis, while the fungal filaments create the slope or body of the lichen in which the pair lives. The chemical substances secreted by the fungus break down the stone and the wood on which the lichen lives. It creates minerals and nutrients for soil, soil microbes, and plants (Chan-Cupul et al. 2016).

The second type of relationship is mycorrhiza (in Greek, meaning fungus root), symbiotic associations between fungi and roots. In exchange for the exudates from the roots of the plants, mycorrhizal fungi look for water and nutrients and then return them to the plant. The plant becomes dependent on the fungus, and this, in turn, cannot survive without the exudates of the plant. Through a too strong soil compaction, the fungal tubes are crushed and the fungi are destroyed. It is obvious that fungicides and pesticides, inorganic fertilizers, and soil physical modification (soil milling, deep digging) destroy fungal hyphae. Pesticides destroy fungal hyphae by extracting the cytoplasm from the fungal body (Hage-Ahmed et al. 2019).

5.2 Mycorrhizal Fungi Are of Two Types

Ectomycorrhizae grow close to the surface of the roots and can form networks around them. They are associated with conifers and hardwood trees. The second are endomycorrhizal. They actually penetrate and grow inside the roots and also extend outward into the soil. Endomycorrhizal fungi are favored by most vegetables, annuals, perennials, grasses, shrubs, softwood trees. Both types of mycorrhizal fungi can extend their range, as well as the surface of plant roots; the effective surface of the roots of a tree, for example, can be increased 700–1000 times by association (Song et al. 2016; Khanday et al. 2016; Bhat et al. 2017).

Mycorrhizae can be classified in at least 4 groups of mycorrhizae consisting of only three taxonomic classes, and the hosts are, at the moment, various gymnosperms and angiosperms (Table 5.1).

Ectomycorrhizae are usually found on the surface of the rhizoderms of the different forest trees between gymnosperms (*Pinus sylvestris, Picea excelsior, Larix decidua*), and among angiosperms, beech trees (*Fagus sylvatica*), evergreen (*Quercus petraea*), oak (*Quercus petraea*), and hornbeam (*Carpinus betulus*). The installation of an ectomitosis is done through a mycelial filament that approaches the rhizoderm and forms around the root an external hyphae mantle. Over time, the hyphae form a network in the space between cells that ultimately generates short

Table 5.1 Classification of mycorrhizae

The classification method	The type of mycorrhiza	Examples
After selectivity	Non-selective or generalist	The mushrooms *Amanita rubescens*, *Amanita pantherina*, *Boletus edulis*, or *Russula cyanoxantha* make mycorrhiza with several species of woody plants
	Preferentially selective	*Suillus luteus* preferentially pine mycorrhizal communities (trees), but also migrates to the oak tree; *Tricholoma sulphureum* establishes predominant symbiotic relationships with phage, but also passes on *Quercus species*
	Strictly selective	*Leccinum carpinus* makes mycorrhizae only with the hornbeam, *Boletus elegans* only mycorrhiza larch, *Boletus luteus* establishes symbiotic relations only with the pine forest
According to the relationships established between the fungal hyphae and the cells of the plant roots	Ectomycorrhizal or mycorrhizal ectotrophs	The hyphae penetrate intercellularly, so among the cortical cells of the plant root, in the interstitial space
	Endomycorrhizal or endotrophic mycorrhizae	The hyphae enter the cortical cells of the root, localizing intracellularly
	Ectoendomycorrhizal or mycorrhizal ectoendotrophs	Combination of the previous types
	Perimycorrhiza or mycorrhiza peritrofe	The mycelium of the fungus develops around the roots

intracellular hyphae. The entire construction serves to transport water and mineral salts from the root through the stem to the crown of the tree to feed the foliage. The species of fungi in this mycorrhiza group belong to the genera: Russula, Amanita, Lactarius, and other species with the hat formed of gills, consisting of the classes Basidiomycetes, Ascomycetes, and Zygomycetes.

Another group is the mycorrhizae that grow in the root rhizoderma of the order Ericales (fam. Ericaceae) with the following most well-known representatives: blueberry (*Vaccinium myrtillus L.*), cranberry (*Vaccinium macrocarpon A.*), *Erica tetralix*, bear grape (*Arctostaphylos uva ursi)*, *Andromeda polifolia*, *Calluna vulgaris, Rhododendron myrtifolium*, and others. In this group we can distinguish two categories of mycorrhizae: the type of arbutoid (living on the roots of shrubs in warm countries) and the type ericoid (living on semi-shrubs of low height) on the hedgehogs of boreal forests. The first type is composed of basidiomycetes, the second type of Ascomycetes class (Igiehon and Babalola 2017).

Mycorrhizae are specific to grassy plants in the Orchid family. These mycorrhizae are part of the ectomycorrhizal basidiomycetes class and are usually located within the root cortex (endomycorrhizal fungi). Most live in tropical forests. In the litters, gorunets, and oaks we find three species of orchids that possess inside the endomycorrhiza roots, these are: the saprophytic orchid *Neottia nidus avis* (bird's nest), on moist and humus-rich soil; *Epipogium aphyllum*, with green stalk and flowers, but without leaves and characteristic to our beech trees and *Corallorhiza trifida* living on the floor of the beech tree and our spruce, also devoid of leaves.

Mycorrhiza vesicular mycorrhiza that picks on the gymnosperms from us, the tis (*Taxus baccata*) or the exotic *Ginkgo biloba* (today only cultivated in the botanical gardens, brought to Europe from the Chinese Buddhist monasteries for the phytotherapeutic properties), then the *Sequoia sempervirens* and the shrubs, weed all the species of Fragaria from us and the bryophytes (leafy and ground muscles) in the forests. Fungi that produce such mycorrhizae belong to the class of Zygomycetes. Although these mycorrhizae live inside the root cortex, they form their sporangia and spores on the outside, as opposed to the other endomycorrhizae whose fruiting bodies are found within the roots (Mohamed et al. 2019).

Fungal arbuscular mycorrhizas form associations with the roots of a wide variety of vascular plants. The consequences of this association for the host plant vary continuously from positive (most often) to negative. In the classical literature, it has been suggested that the positive effects on plants are due to the increased nutritional supplement in one mycorrhizal plant compared to the other non-mycorrhizal one. Subsequently, it has been shown that plants can also benefit from mycorrhizal capacity in other ways. The presence of fungal associates can increase the resistance to stress, for example, under limited water conditions—hydric stress, in case of pathogenic attack, etc.

Tree mycorrhizal fungi are some of the most important symbionts for most plants. Under phosphate-limited conditions, mycorrhizal tree associations may influence the development of plant communities, nutrient, water intake, and aboveground productivity. They can also act as bioprotectors against pathogens or toxic stress. It has been suggested that, for any plant, there is a curvilinear relationship between the extension of the mycorrhizal fungal colony and the beneficial contribution to the plant exposed to infection. For some plants, a high density of fungal colonies may have a positive effect, while for others even at low levels, fungal colonies may result in decreased plant performance (Mallmann et al. 2018).

The reasons for the alleged negative effects of some mycorrhizal fungi species on some plant species are unclear, but they include loss of mycorrhizal photosynthesis products, immobilization of nutrients, alteration of root exudates to allelopathy but also negative effects of other components of the rhizosphere microflora. Fungal arbuscular mycorrhiza is recognized on the basis of specific characteristics such as biotrophy, asexual reproduction, large spores, and multinucleate with stratified walls, unexpected hyphae, and the formation of shrubs at the root of the plant. Research shows that the species of arbuscular mycorrhiza can grow up to the stage of spore production in vitro, in the absence of plant roots and in the presence of bacterial lines associated with the spores. Mycorrhizal tree fungi have asexual reproduction through spores. There is no evidence of sexual reproduction of arbuscular mycorrhizal fungi (AMF). A study reports the formation of zygospores sexed in Gigaspora but this has not been confirmed. Genetic recombinations, or only a low level, were not detected using molecular marker genes. Therefore, it is generally accepted that the spores of mycorrhizal fungi are asexually formed. The spores are relatively large (40–800 μm) with stratified walls having lipids in the cytoplasm. They are important in identifying mycorrhizal fungi. Traditionally, the taxonomy of arbuscular mycorrhizal fungi has been based on the morphology of the spores, in particular on the structure of their walls and on the methods of formation on the hyphae (Lekberg et al. 2017).

5.2.1 Life Cycle of Mycorrhizal Fungi

The life cycle of arbuscular fungi generally starts from the stage of spores lying in the soil or coming from the adjacent mycorrhizal roots. Hyphae (H) resulting from spores or mycorrhizal roots grow in the same direction as the root of the plant. At the root surface, the terminal portions of the hyphae swell and form a specific structure called apresor (Ap). Infective mycelial filaments start from the oppressors. The hyphae penetrate the cell walls of the root epidermis with the help of penetrating hooks. The point at which the hyphae form any kind of propagule and which enter the root for the first time is called the primary entry point (E). The number of primary points/moments made by the fungus at the root is equivalent to its inoculation potential. At the root, the hyphae grow intercellularly towards the internal cortical layers and in the internal region of the cortex, the hyphae begin to grow intracellularly. After that, the host cell membrane invaginates and envelops the fungus forming a new compartment called apoplastic space. This space allows the efficient transfer of nutrients between the two symbionts but prevents the direct contact between the fungal and plant cytoplasm. Inside the cortical cell, but outside the cytoplasm, the hyphae form shrubs and vesicles. Shrubs are intracellular hyphae structures, with dichotomous branching, which can be the place of exchange for phosphorus, carbon, or other nutrients. The vesicles are intracellular structures full of lipids, assuming they also store carbon, and also serve as reproductive propagules. It has been found that not all mycorrhizal fungi form vesicles, such as Gigasporaceae. The formation of the vesicles depends on both the fungal symbiont and the environmental conditions. With the beginning of the infection process, in the root colonization is accomplished both through the intraradicular mycelium inwards and through the extraradicular mycelium on the outside. Intraradicular mycelium colonizes the root in different ways. Depending on its structure, the mycorrhizae are separated into several types: Arum, Paris and Intermediate.

The Arum model implies a longitudinal growth of the intercellular hyphae as well as the penetration of the root cortex, followed by the formation of the shrubs. The shrubs form short branches on the intercellular hyphae, usually at right angles to the axis of the main root. Arum type is found in cultivated plants. In the Paris type, intercellular hyphae are absent and the existing hyphae are entirely intracellular and irregularly curled, some of them forming shrubs that are not terminal but are located in well-defined layers. Shrubs formed as intercalated spaces are called arboreal twigs. This type is usually found in plants in natural ecosystems. Sometimes both types of structures meet in the same root, as for example in pumpkin or tomatoes and this type has been called Intermediate (Spagnoletti et al. 2016).

Mycorrhizae are associations between fungi and the roots of grassy or woody plants. It is a symbiosis because it offers benefits to both parties. In this case, the plants increase their absorption range thanks to the fungi, while they receive nutrients synthesized by the plants. A single fungus can join several plants, thus forming an extended network. In fact, many forests are connected by mycorrhizae. In this regard, studies have been conducted that show that mycorrhizal trees are capable of living longer than those without mycorrhizal. There are different levels in which plants depend on mycorrhizae. Studies conducted in the area show that the plants may have high concentrations of mycorrhizae, intermediate concentrations, or their absence (Trocha et al. 2016).

Plants with high concentrations of mycorrhizae are dependent on fungi to survive. This is a mandatory relationship because the plant would die without mycorrhizal survival. Plants with intermediate concentrations of mycorrhizae do not depend on this type of association, but take advantage of this to improve their conditions (such as drought resistance and certain diseases). This relationship is optional.

Finally, there are plants whose roots resist the formation of associations with fungi. This happens when the soil conditions are sufficiently good (so the plant does not need the benefits of mycorrhiza) or when the plant is young and stable (so it does not require additional supplements). Mycorrhizae generate hundreds of advantages that can be highlighted in different fields. Thus, mycorrhiza benefits plants, soils, fungi, and human beings.

The mycorrhizae form a network of filaments that increase the degree of absorption of the plant roots. For example, the fungus attached to a plant can extend for hundreds of meters, which puts the plant in contact with more water and nutrients. Fungi are responsible for the breakdown of organic matter, from which they obtain essential minerals, such as nitrogen and phosphorus. These nutrients are sent to the plant through the roots.

By themselves, plants cannot degrade these substances. In addition, micro-germs make plants capable of withstanding certain conditions that they cannot withstand unless they have been connected to fungi. For example, thanks to the fungus, the plants survive drought and create resistance to certain soil pathogens.

Other benefits of micorrhiza for plants are: it helps plants grow faster and stronger, promotes flowering and fruit production in the plant, increases the tolerance of the plant to the salinity of the substrate, they reduce the incidence of diseases, protect the plant from predators, such as worms, increase the transfer of carbon between trees, even among different species, they extend the life of certain species of plants (Liu 2010).

Not only do mycorrhizae benefit from plants, but they also generate benefits for fungi.

Fungi are organisms that do not photosynthesize, so they cannot synthesize products independently. In this sense, they depend on other individuals to be able to feed. By forming mycorrhizae, the fungus receives nutrients produced by plants, such as

Table 5.2 The advantages of mycorrhizae

The major advantage	Intensifies the nutritional exchanges at the root level, can change the reaction of the soils, acting as living buffers. For example, Penicillium species in the rhizosphere change soil pH, making it possible to develop quails on alkaline soils (*Quercus species* do not support basic soils, in the absence of these fungi, the existence of trees on alkaline soils is compromised)
The main advantage is to increase the absorption of mineral nutrients from the soil by the plant	The extension of the external mycelium of the fungus over the rhizosphere is the main consequence of this effect, because it allows the absorption of nutrients outside the area of action of the roots
Other benefits	High resistance of plants to stress and water salinity
	Increased resistance and tolerance to soil pathogens
	Higher absorption of micro and trace elements
	Greater and more uniform production
	Improves plant growth
	More efficient use of fertilizers and water
	Better adaptation of plants to the environment
	Minimizing the influence of the state of fatigue of the land on the complaints

carbohydrates. In general, the presence of mycorrhizae makes the soil more fertile. It avoids erosion of the substrate when establishing connections in the basement. Increase nutrient circulation in the substrate, which creates more fertile soils. The benefits of mycorrhiza use are presented in Table 5.2.

Mycorrhizae are important in economics and medicine. Then, there are mentioned some aspects in which these associations intervene in human life. The fruits of ectomycorrhizae (fungi and truffles) offer nutritional values. For this reason, they are marketed, which makes them economically important. Many of these fruits also have medicinal properties, which is why they are used in the pharmaceutical industry. By increasing the nutrient absorption of plants, the mycorrhizae increase the nutritional value of products, such as fruits and nuts.

The salts absorbed by the mycorrhizal fungi are quickly transported to the upper symbiont, the fungus having a role in supplying the top plant with mineral salts. In turn, the upper symbiont supplies the mycorrhizal fungus with the organic substances needed.

Fungi hyphae are an important means available to the upper plants for the purchase of water and mineral salts. These associations are found in plants that live in poor resorts in mineral salts, in which mycorrhizal fungi—due to their chemotropism—can easily detect even very small amounts of mineral salts, so that only plants with a highly developed root system could enter into competition with them to obtain these salts. In addition, mycorrhizal fungi can absorb organic nitrogen from the soil, so that the upper symbiont is well supplied with nitrogen even in cases where inorganic combinations of this element are in small quantities in the substrate; this is the case of swamp plants, where the mycorrhizae are very widespread;

therefore, plants grow in conditions where the process of nitrification is very low in the soil, and the fertilizers with NH_3 have not proved effective (Koricheva et al. 2009).

5.3 Mode of Action of Pesticides

Pesticides are products that contain or generate active substances and are used against harmful organisms in the home environment (disinfectants, rodenticides, repellents, and insecticides) or to protect natural or artificial products in industry and agricultural applications. The mode of action of mycorrhizae on pesticides is shown schematically in Figs. 5.1 and 5.2. Given that their pesticide use intrinsic properties can pose health risks, the EU has set up a regulatory framework aimed at significantly increasing the safety of pesticides used and sold in the EU, namely EU Regulation 528/1012. It provides for the release of biocides that are carcinogenic, mutagenic, toxic to reproduction, which disrupt the endocrine system or which are dangerous to the environment.

Pesticides, in general, are organic compounds with low molecular weight and different water solubility. The chemical character, molecular shape and configuration, their acidity or basicity, water solubility, polarity of the molecule, size and polarizability of the molecule, all these properties, alone or together, influence the adsorption–desorption processes on soil colloids. Given these properties of pesticides and the character of the relationships involved in the adsorption–desorption process on soil colloids, they can be grouped into two major classes: polar and

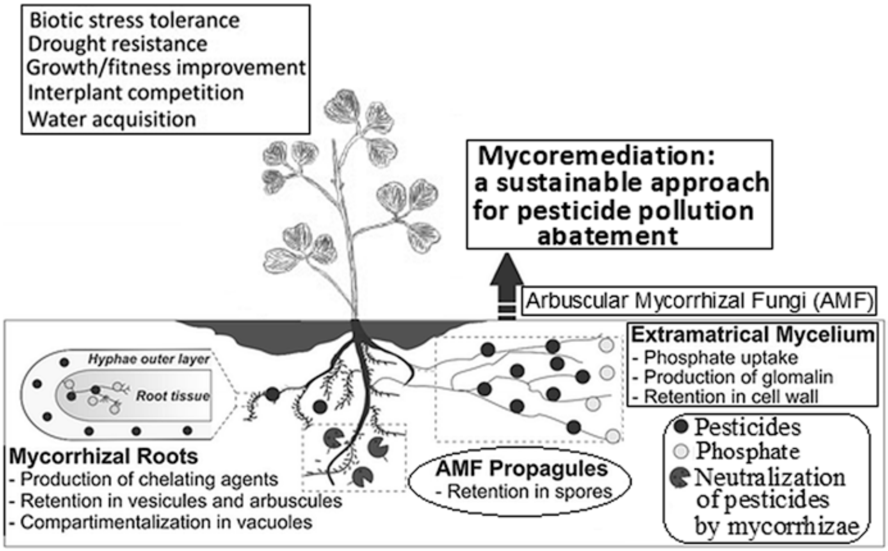

Fig. 5.1 Some processes that take place through mycoremediation: a sustainable approach for pesticide pollution abatement

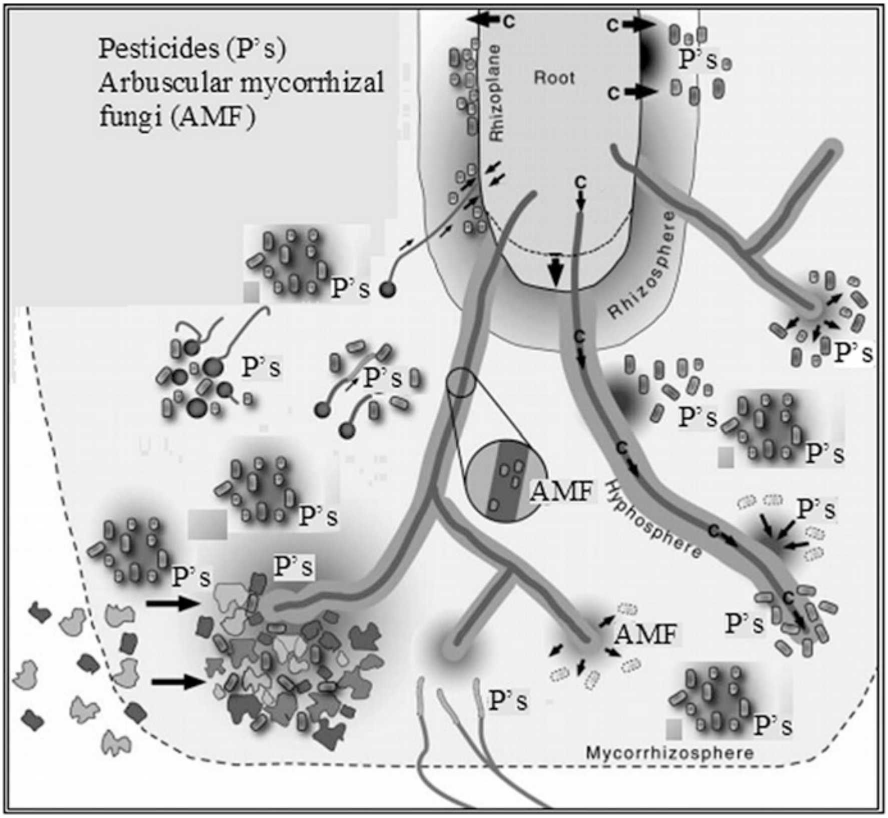

Fig. 5.2 Schematic view of possible interactions among different pesticides of the mycoremediation: a sustainable approach for pesticide pollution abatement

nonpolar. Pesticides containing compounds of an acidic, basic, or dissociative character act as cations, forming the group of ionic compounds. Products that have neither basic nor acidic character make up the group of non-ionic compounds.

Among the structural characteristics that determine the chemical character of the compounds and which influence their adsorption and desorption on soil colloids, the following are mentioned: the nature of the functional groups, the nature of the substitution groups, the position of the substitution groups against the functional groups, and the degree of unsaturation of the molecule. In high pH medium, acidic compounds, by dissociation, become anions. In contrast, in a low pH environment, weakly basic or neutral compounds behave similar to organic cations.

In general, the soil acts as a receptor and reservoir for pesticides, in which, either they degrade or are gradually dispersed into the environment or translocated into plants, some of which can still persist in the soil many years after application (Mushtaq et al. 2018). Pesticides, finely dispersed substances, in soil are subject to numerous influences, abiotic and biotic, which determine their behavior and, finally, their mineralization (Bhat et al. 2018; Garg and Kashyap 2019).

5.3.1 Adsorption

The adsorption of pesticides in soil is a complex phenomenon, influenced by many factors. Adsorption plays an important role in the movement of pesticides in soil and refers to the temporary retention of pesticides in vapor phase, dissolved or suspended on the surface of soil particles. Particularly important in the adsorption of pesticides are the clays and the organic matter in the soil that make up the "colloidal complex" of the soil.

Adsorption mechanisms are based on different types of bonds created between pesticide molecules and soil colloids through soil solution. The main mechanisms of pesticide adsorption by soil include: van der Waals force adsorption which is implicit in the adsorption of non-ionic pesticides of the molecules in an undissociated state on soil adsorbents.

Adsorption by hydrophobic bond is responsible for the retention on the hydrophobic areas of soil organic matter of nonpolar pesticides or compounds whose molecules have nonpolar portions significantly to the polar side. Hydrogen bond adsorption represents a special type of interaction in which the hydrogen atom forms a bridge between two atoms with negative electrons. Adsorption in charge transfer occurs through the transfer of electrons from an electron-rich donor to an electron deficient acceptor. Adsorption by ion exchange that takes place in the case of compounds present in the form of cations or those compounds which, by protonation, gain the positive charge. Adsorption by the formation of coordination bonds by exchange of ligands that takes place when the ions of the transition metals can form an adsorption center on the surface of the clays in the soil and is very important for the behavior of pesticides in the soil (Bruckner et al. 2019).

5.3.2 Circulation of Pesticides in Soil

The movement of pesticides into the soil occurs in solution, by volatilization or with the movement of the colloidal particles on which they are adsorbed. Soil migration, as a solution, is dependent on both the diffusion and mass transport processes. The summation of diffusion and mass transport processes determines the rate of migration of pesticides into the soil. Molecular diffusion motion—which occurs based on the concentration gradient. Movement by mass transport—which occurs with the migration of water or soil particles with which pesticide molecules are associated.

Degradation of pesticides in soil. Pesticides in the soil are subject to various factors that influence both the stage that ensures their effectiveness and the phase in which the product is no longer useful and becomes a residue. Once in the soil, pesticides are subject to degradation, a widespread phenomenon that plays an important role in the dissipation of many such substances. Pesticide degradation is determined by abiotic and biotic factors and processes.

5.3.3 Abiotic Degradation

In soil, pesticides undergo chemical transformations as a result of reactions with soil organo-mineral compounds. The physical and chemical properties of soil are the most important factors that influence the transformation of pesticides into soil.

5.3.4 Biotic Degradation

Numerous papers highlight the role of soil microorganisms in the decomposition of pesticides, as well as the fact that there are few active substances that are not biologically degraded. Some active substances in pesticides can serve as a source of nitrogen and carbon for soil microorganisms. In a synthesis on the biological degradation of herbicides, the role of microorganisms in soil is noted in a whole series of degradation processes, including beta oxidation, de-halogenation, and opening of cycles.

Persistence of pesticide residues in soil. A pesticide residue in the soil is considered to be any substance or mixture of substances that is found on or in the soil as a result of using a pesticide. Concern over the long-term effects of pesticide residues in soil has highlighted the idea that the persistence of a chemical is a quantifiable indicator that represents its resistance to degradation. For our purposes, the term "persistence" means the period of existence in the soil of a given pesticide, which may be expressed in units of time. In terms of persistence, the concept of half-life is used extensively, i.e., the time required for 50% of the pesticide to disappear. The persistence of a pesticide in the soil depends on a number of factors conditions such as content of organic matter, nature and content of clay minerals, pH, microflora and soil microfauna, temperature, ion exchange capacity, cultural practices, exposure to wind and light, precipitation. Above all, the most important role is played by the chemical nature of the pesticide (Zaller et al. 2018).

Due to the potential danger of soil and environmental pollution due to the high remnants of pesticides, we have used easily degradable pesticides such as carbamic insecticides, most organo-phosphoric insecticides, pyrethroid insecticides. However, some of the easily degradable pesticides are characterized by degradation products that can persist in the soil for up to several months and can be very toxic, which must be carefully considered in their use in crop production.

Translocation of soil residues into soil and plant fauna. In soils polluted with pesticide residues, many invertebrates from the soil absorb and accumulate residues in their bodies in concentrations several times higher than in the soil around them, thus having a bioconcentration process. Also, the presence of pesticides in the soil leads to the loading of plants with their residues. The absorption of insecticide residues in plants occurs only when they are in excessive quantities in the soil. Pesticides are absorbed by plants more easily from sandy soils and to a lesser extent from turbid soils that have a high content of organic matter.

Soil deposition of pesticide residues. Under normal conditions, decontamination takes place slowly due to soil cultivation or climatic conditions. For this purpose, different processes are used depending on the chemical composition of the pesticide, the degree of soil loading and its characteristics, the cultivated plants, and the cultivation technology. Thus, to reduce the activity of nitralin or fluometuron, activated charcoal is incorporated into the soil. Diffenamide, dicamba, and ambien products are degraded in soil if a certain microbial culture medium is administered. The fastest depollution processes are those based on the administration in soil of adjuvants, products that retain or degrade the pesticides. Other soil pollution measures are based on the ability of maize, sorghum, sugar cane, as well as some weeds, to pollute the soil of atrazine residues through absorption and metabolic degradation.

The process of atrazine degradation could be faster if the following factors were combined: early hybrid with high growth rate, high doses of organic and chemical fertilizers, suitable density to extract residual atrazine from soil. In this way, the depollution process could be intensified and reduced to a period of 60–70 days. Based on extensive research, mathematical methods have been developed to forecast the dynamics of pesticides in agro-ecosystems, taking into account the environmental factors that allow to know the concentration of a pesticide in the agro-ecosystem at a given time. For the same purpose, maps are developed on the application of pesticides and the conditions of depollution, which take into account a series of criteria, on the basis of which the following groups are established: the most toxic, totaling over 21 points; medium toxic, 21–24 points; relatively weak toxic, below 13 points (Buysens et al. 2015).

5.4 Mycoremediation Methods

Mycorrhizal fungi obtain the necessary carbohydrates from the exudates of the host plant and use them as energy to expand into the soil, pumping moisture and undermining nutrients in places that the plant is inaccessible to. These fungi are not lonely miners. They form complex networks and sometimes transport water and nutrients to the roots of different plants, not just the ones from which they started. The mechanisms by which the alternative agriculture system determines the manipulation of the plant metabolome to obtain the predominant expression of certain genes of agricultural interest.

The biotechnological management system of the agricultural culture was developed based on the new concepts of alternative agriculture by manipulating the plant metabolome for the preponderant expression of certain genes of agricultural interest and stimulating the photosynthesis and radicular translocation of the sap elaborated by using mycoremediation methods with near IR reflection. The use of mycoremediation methods for plant protection reduces the need for pesticide application. Pesticides also have an action to reduce the activity of mycorrhizal fungi (AM). The studies performed have shown to date a good compatibility between the bioproducts based on AM fungi and the antagonistic bacteria *Bacillus subtilis*.

The mechanisms by which the alternative agriculture system determines the manipulation of the plant metabolome for the predominant expression of certain genes of agricultural interest (Hashem et al. 2016).

By cultivating plants using mycoremediation methods as vegetative mulch/decaying plant residues of *V. villosa*, the following genes are predominantly activated: the genes responding to nitrogen—*NiR*, *GS1*, *rbcL*, *rbcS*, and *G6PD*; genes encoding chaperon proteins—*hsp70*, *BiP*; the genes of the plant defense system—chitinase and osmotine; cytokinin-dependent genes of *CKR*; GA 20 oxidase. The predominant activation of these genes in the crop plant leads mainly to the following effects: increasing the efficiency of the use of nitrogen; stimulation of photosynthetic carbon fixation; reducing the incidence of plant diseases; increasing the longevity of the culture. Apart from these effects (explained by the predominant activation of some genes) it is generally acknowledged that the use of mycoremediation methods by using the mulch–carpet of hairy moss has beneficial effects on the soil (reduces erosion, improves water infiltration, stimulates soil tillage) and on protection of plants (reduces the spread of pathogens specific to the main crop, promotes the development of predatory insects and thus reduces the attack of harmful insects, eliminates weeds) (Motaharpoor et al. 2019). All the effects resulted by applying this technological sequence of alternative agriculture are also useful for mycoremediation methods.

Of maximum interest for the final purpose of mycoremediation methods (respectively, increasing the amount of carbon dioxide fixed by sequestering photosynthesis in organic matter; from the soil) is the stimulation of photosynthesis, which, associated with the action of mycoremediation methods with IR reflection close to stimulating the radicular translocation of the sap elaborated, creates the premises to increase the secretion of glomalin in the soil (Wang et al. 2018).

Finding and returning phosphorus is so important for plants that it seems to be a major function of many mycorrhizal fungi; acids produced by mycorrhizal fungi can block, recover, and transport the chemically blocked phosphorus back to the host plant.

Mycorrhizal fungi also release copper, calcium, magnesium, zinc, and iron to be used by the plant. As always, any nutrient not delivered to the root of the plant is trapped in the fungus and is released when it dies and is broken down.

Mycoremediation is a form of bioremediation in which fungi technology is used to decontaminate the environment. Fungi have proven to be a cheap, efficient, and environmentally friendly way to help remove pollutants from damaged environments or wastewater.

Toxins include pesticides and herbicides. The by-products of remediation can themselves be valuable materials, such as enzymes (such as *Laccases* have copper-containing oxidase enzymes found in many fungi, microorganisms, and plants), edible or medicinal fungi, which makes the remediation process easier, even profitable. Fungi, due to nonspecific enzymes, are capable of decomposing many types of substances. Most plants can form symbiosis with fungi, a relationship called mycorrhiza.

Mycorrhizal fungi, in particular AMF, can improve the phytoremediation ability of some plants. This is mainly due to the fact that the stress caused by plants due to pollutants is very low in the presence of arbuscular mycorrhizal fungi, so that they can grow longer and produce more biomass. Fungi provide nutrition, especially phosphorus, and promote general plant health. Also, the expansion of the mycelium can greatly extend the area of influence of the rhizosphere (hyposphere), giving the plant access to more nutrients and contaminants. Increasing the general health of the rhizosphere means an increase in the bacterial population, which can also contribute to the bioremediation process.

This relationship has proven useful with many pollutants, such as *Rhizophagus intraradices* and *Robinia pseudoacacia* in lead-contaminated soil, *Rhizophagus intraradices* with *Glomus versiforme* incorporated in vetiver grass for lead removal, mycorrhizal fungi and *Calendula officinalis* and *Althaea officinalis*, in general, has been effective in increasing the bioremediation capacity of pesticide plants. In wetlands, shrub mycorrhizal fungi greatly benefit from biodegradation of organic pollutants such as benzene, methyl-tertiary butyl ether, and ammonia from groundwater when inoculated into *Phragmites australis* (Huang et al. 2017).

Perennials, shrubs, and trees prefer soils dominated by fungi. Annual plants, herbs, and vegetables prefer soils dominated by bacteria. The explanation for this is the way nitrogen is fixed in the soil. Depending on the soil environment, nitrogen may remain in the form of ammonium (NH_4) or be converted to nitrate (NO_3) by specific bacteria.

Soils dominated by bacteria tend to have an alkaline pH, which is a good living environment for bacteria that have this function of converting nitrogen into nitrates. In the fungi-dominated soils, most of the nitrogen remains in the form of ammonium. Fertilizers are salts that absorb water from bacteria, fungi, nematodes, and soil protozoa, destroying the entire feeding system of microscopic living creatures. Fungi are the main decomposers in the soil. Bacteria often consume matter that has already been processed by the fungus. Like bacteria, fungi should be regarded as containers with fertilizers (Sudová et al. 2018).

Phosphorus is almost always blocked in soils. Even when applied as a fertilizer, phosphorus becomes inaccessible to plants in seconds. The fungi not only seek it, but process it so that it can be absorbed by the roots of the plants. Also, similar processes take place with copper, calcium, magnesium, zinc, and iron. In soils dominated by fungi, nitrogen is in the form of ammonium. The fungi often come into symbiosis with the roots of plants.

In exchange for the exudates from the roots of the plants, the fungi look for water and nourishing substances that they bring to the plants. It was only in the 1990s that the term mycorrhiza (eng. Mychorriza) began to enter in the vocabulary of the agricultural industry, but not at all in the vocabulary of gardeners. Most conifers and hardwood species (birch, oak, beech) form mycorrhizal symbiotic relationships with ectomycorrhizal fungi (which live near the root surface and form networks around it). Most species of shrubs, softwoods, and perennials form mycorrhizal symbiotic relationships with endomycorrhizal fungi (which enter and grow in the root, but also outside it). However, there are exceptions—rhododendrons, azaleas, or blueberries need hedgehog fungi, which are not yet on the market. Some plants prefer the soil dominated by fungi, while others prefer the soil dominated by bacteria. Plants need nitrogen to produce amino acids; it is crucial in the development and survival of plants.

For this reason, soluble nitrogen-based inorganic fertilizers give such good results in plant growth, even if they are harmful to trophic networks. In aqueous solutions, these nitrates (NO_3^-) are immediately accessible to the roots of the plant, which absorb them like a sponge.

Like anions, they remain in aqueous solutions instead of fixing on humus or clay, as do cations loaded with positive charge. In a healthy soil trophic network we have two forms of nitrogen, nitrates and ammonium (NH_4) and, as most often in life when there are more options, some plants prefer nitrates nitrogen in the form of nitrates, others prefer ammonium. When nematodes and protozoa consume fungi and bacteria, they excrete nitrogen in the form of ammonium (Leberecht et al. 2016).

Ammonium oxidizes rapidly or, if found in sufficiently large amounts in the soil, the nitrifying bacteria turn it into nitrates. In the soil dominated by bacteria, things happen almost always after this scenario, because the viscous substance produced by them has a pH above 7, which represents the ideal life environment of the nitrifying bacterium bacteria.

In general, nitrifying bacteria thrive in soils dominated by bacteria. Fungi favor a lower pH because they use organic acids to break down organic matter into nutrients. If sufficient organic acids are available to annihilate the viscous matter produced by the bacteria, the soil pH drops below 7, making the acid environment to be acid and thus increasingly unfavorable for nitrifying bacteria. Therefore, more and more ammonium will remain unchanged ammonium. At first the soil is dominated by bacteria. But as more organic residues accumulate from debris discharged from these organisms and the plant life they support, fungi spores come in handy enough to provide enough nutrients to germinate. Having available a space to occupy and resources to maintain themselves, the fungi have increase emerged. The process is influenced by many other factors, but let us focus on what interests us: as plant life and soil trophic network diversify, the number of fungi populations increases, and plants with a short life span make room for plants with a longer existence, make room for existing plants. Longer, evergreen, shrubs (Castro-Rodríguez et al. 2017).

More organic matter is produced, ensuring the food of fungi populations is which always growing. The shrubs stabilize, then are followed by soft essence trees, evergreen shrubs that expand, hardwood trees that fully develop, and eventually coniferous forests as a whole. Throughout the process, the biomass of fungi grows in proportion to that of bacteria, but they cannot compete, being limited to digesting simple sugars and other carbohydrates—existing in limited quantities, given the increasing mass of complex plants, rich in lignin and cellulose. With the passage from the sandbank, so to speak, to the shrubs bushes, to old coniferous forests, the decisive influence of fungi on the soil is intensified step by step (Bargaz et al. 2018).

5.5 Mycorrhizal Relations/Intraspecific Relations

Organisms grow, reproduce, and die. They are affected by the conditions in which they live and the resources they obtain. But no organism lives in isolation. Each, at certain stages of life, is a member of a population. Individuals of a species have similar requirements for survival, growth, and reproduction, and their cumulative requirements may exceed at any given time the existing stock. As a result, competition for resources intervenes, which undoubtedly causes the deprivation of some of the organisms.

We can say that, the relationships between individuals (individual organisms) originate in the need for survival in the concrete environmental conditions. However, intraspecific relationships should not be viewed unilaterally (competition or relations of perfect harmony).

These relationships can be considered as individual–population relations or system relations (between the individual and the population system or between different subsystems of the population). Intraspecific relationships are appreciated for their role within the population system (Kranabetter et al. 2015).

The most important relationships are the trophic ones (which result from the necessities of getting the food), of protection (of defense against the variations of the ecological factors, of predators), of reproduction, of numerical adjustment of the population, of adjustment of the population structure. Apart from the inclusion of new biotechnological solutions within the agrotechnical sequences with a role in promoting the development of arbuscular mycorrhizal fungi and the accumulation of organic matter in the soil (including through the use of biofertilizers and biopesticides) the proposed technology has a pronounced “bio” and for that they are used, for monitoring the efficiency in the recovery of organic matter from soil, biochemistry methods (extraction and dosing of glomalin from soil; extraction and dosing of humic acids from soil) developed relatively recently based on new knowledge of cellular and molecular biology (Mei et al. 2019).

The proposed technology has the following stages: land management for stabilization and drainage in accordance with the recommendations of codes of good practice in land management; establishment on the degraded and landscaped land of a crop of *Vicia villosa* (the hairy vetch, fodder vetch, or winter vetch), by sowing a norm of 125 kg seeds per ha; the development until the spring of the vegetal carpet of *V. villosa*; mowing/rolling the vegetable carpet of *V. villosa* in strips 2 m wide; formation of a biocomposite mulch by depositing on the mulch of *V. villosa* of a biodegradable film formed by direct spraying of a peliculogen solution based on polyvinyl alcohol, protein isolate, glycerine/glycerol waters, and a red dye; seeding in the biocomposite mulch the seeds of the crop plant; maintaining the resulting crop according to the agricultural framework technology recommended for the crop area; harvesting of useful production and incorporation into soil of plant residues from both crops (*V. villosa* protective culture + basic culture).

This technology presents the following advantages: elimination of biological risks, the composition used containing exclusively the microorganisms useful for the growth of the crop plants and for the restoration of the organic matter from the soil, whose safety is generally recognized; restoration of organic matter from soil by accumulation of glomalin excreted by fungi from endomycorrhizal symbiosis on account of root exudates (whereby plants translocate up to 40% of photosynthetically fixed carbon dioxide); increasing the efficiency of the dark phase of photosynthesis due to the activation of Calvin cycle enzymes as a result of applying the alternative agriculture system of selective activation of agricultural genes by cultivation on *V. villosa* mulch; stimulation of the light phase of photosynthesis and radicular translocation of the sap produced by the use of mulches with red reflection and near IR, reflection produced by the red dye embedded in the biocomposite mulch (Pena et al. 2014); protection of plants against edaphic pathogens due to the presence of *Bacillus subtilis* bacteria, antagonists for phytopathogens; plant nutrition predominantly on the basis of biologically fixed nitrogen by nitrogen fixing bacteria, both naturally occurring in the soil that develops nitrogen fixing symbioses with *V. villosa* and bacterial endophytes of the genus *Azospirillum*, which fix nitrogen in association with the resins of plants, vegetables, or cereals.

Reducing the attack of diseases, pests, and weeds due to the intercalated culture, element of biodiversity that favors the development of populations of antagonists for phytopathogens and predators/parasitoids for harmful arthropods and reduces the development of weeds (Himmelstein et al. 2016).

Basically the technology described above is the contemporary (re)interpretation of some old agricultural practices. Protective crops transformed into vegetable mulch have been used since ancient times, by Romanians and ancient Greeks. Used as a protective crop, *V. villosa* determines nitrogen fixation, recycling of nutrients, reducing soil erosion and compacting, improving the quality of soil organic matter (by rhizodeposition).

When converted to mulch, *V. villosa* plant debris limits weed growth, increases soil organic matter content, reduces water loss, and acts as a controlled release fertilizer. The agricultural crop technology described above, which can also be used for the rehabilitation of degraded land, is part of the European priorities described above, and can be subsidized under the agri-environment measures (Smith et al. 2017).

5.5.1 Increasing Biodiversity

The system proposed obviously determined the increase of biodiversity in the field of vegetable production. In practice, the system involves the promotion and support of environmentally friendly agricultural practices that are directly beneficial to biodiversity (intercalated protective culture and main biocomposite mulch culture) or indirectly (*V. villosa* protective culture is a refuge for other organisms—species of feedable birds).

5.5.2 Reduction of Greenhouse Gas Emissions from Agriculture

The alternative system determines the growth of organic matter in the soil and will reduce the need to fertilize with nitrogen. In the original system a reduction of 50% of the demand of nitrogen fertilizers was demonstrated. Therefore, the proposed system will reduce the need for nitrogen fertilization and/or manure and reduce greenhouse gas emissions from agriculture: N_2O (nitrogen oxide) emissions from the soil, mainly due to nitrogen fertilization; CH_4 and N_2O emissions due to manure handling. In addition, the proposed system offers new solutions for sequestration of carbon in the soil, by re-depositing glomalin due to the symbiosis between the arbuscular mycorrhizal fungi and the root of the basic culture.

5.5.3 Conservation and Sustainable Use of Genetic Resources in Agriculture

The proposed system offers competitive advantages for the native varieties of tomatoes, much more resistant to the competition of the weeds and less productive in superintensive crops than the international varieties. In addition, the system offers a solution for using the genetic resources of microorganisms beneficial to crop plants (Zhang et al. 2016).

5.5.4 Protection of Soil from Erosion and Maintenance of Organic Matter and Soil Structure

The positive impact of the proposed system is evident in this case, resulting from the protective culture and from the rhizodeposition of organic matter by the basic culture in the symbioses formed with the beneficial microorganisms released controlled from the matrix of the biodegradable film. Rhizodeposition/elimination of photosynthetically fixed carbon from the root cells of the epidermis and root cortex of cultured plants leads to a proliferation of microorganisms inside (endorhizosphere), surface (rhizoplane), and outside root (ectorhizosphere).

The release of carbon fixed in the rhizosphere also causes important changes in the chemical, physical, and biological characteristics of the soil. The magnitude of these changes in soil properties is significantly determined by the amount and type of carbon released from the roots as well as by the intrinsic characteristics of the soil. Arboreal mycorrhizal fungi can secrete large amounts of an adhesive glycoprotein and high resistance to degradation (glomalin) in the surrounding soil, which represents for some soils (which increase their natural erosion resistance) a *long-lasting form of sequestration for carbon*. The R/FR reflexive mulch is included in the system not only due to the function of increasing the output by stimulating the light phase of photosynthesis, but also because of its effect on (phytochrome-mediated) stimulation of photosynthetically fixed carbon reallocation to the root system (Husna et al. 2016).

5.5.5 Reduce the Use of Pesticides in Order to Reduce the Adverse Impact on the Environment

This system aims to significantly reduce the use of pesticides. From the elimination from technology of the total herbicide in the initial phase of plant mulch formation to the reduction of the use of fungicides (due to the antagonistic activity of PGPR strains and due to the activation of the systemically acquired resistance mechanism, induced by *V. villosa* residues and mycorrhizal fungi and insecticides (due to the growth of predator/pest populations for insect pests).

5.5.6 Reduce the Pollution of Waters with Nitrates from Agricultural Sources

This is a consequence already presented of reducing the use of mineral nitrogen fertilizers for the basic crop (Lovett et al. 2009).

5.6 Reducing Pesticide Pollution

At EU level, the initiation of the process of integrating agriculture into the environment was determined by the complexity of the relationship between agriculture and the environment, even in terms of reducing pesticide pollution. In this complex relationship between agriculture and the environment are included aspects regarding greenhouse gases, and soil is the main factor of interconnection. In order to achieve this objective of agriculture in line with the environment, a set of good farming practices (GFP), considered necessary at European level, has been established, especially after the reform of the Community Agricultural Policies within the Agenda 2000.

European GFP priorities are practically related to soil protection: maintaining/increasing the organic matter content in the soil; maintaining/improving soil structure; reducing greenhouse gas emissions from agriculture; increasing biodiversity; conservation and sustainable use of genetic resources in agriculture; soil protection against erosion; decreasing the share of pesticide use to minimize their environmental impact; reducing pollution of waters with nitrates from agricultural sources (and above all from the soil).

All these priorities are supported by economic measures, as the main objective of the Community is sustainable development, with a balance between competitive agricultural production and the preservation/improvement of useful resources. The most spectacular economic measures were those included in the Community Agricultural Policy (CAP). According to the Agenda 2000 program document, the CAP has two pillars, the policy of ensuring income and a secure market for farmers ("the first pillar") and the sustainable development of rural areas ("the second pillar"). The 2003 CAP reform brought a new level of integration of agriculture into the environment, with new measures related to promoting the protection of the environment used in agriculture (and first of all soil) through both CAP pillars. These economic measures presented above have created a current stage at European level, with a significant demand for alternative agricultural practices, capable of meeting the requirements of a competitive agriculture in harmony with the environment (Torres et al. 2013; Asemaninejad et al. 2017).

Worldwide, commitments to reduce pesticide pollution began with the signing of the United Nations Framework Convention on Climate Change (Rio 1992), in agreement with Sosa-Hernández and collab. By this convention the signatory countries have assumed a global political commitment to reduce greenhouse gas emissions. In 1997, under the Convention, the Kyoto Protocol was signed, whereby the countries of Annex I (41 countries plus the European Union states) committed to reduce net greenhouse gas emissions (global reduction by 5% of CO_2 emissions) in the period 2008–2012 compared to 1990) in all economic-social activities on economically and socially efficient ways, by engaging the politics at national level, appropriate production technologies, and specific non-polluting practices. Added to these traditional ways were some new specifics, the so-called flexible instruments ("joint implementations," "clean development mechanisms," and "carbon market"). Through these instruments, the political commitment becomes effectively a financial one, materialized in concrete projects. At present, the Kyoto Protocol is only ratified by some of the signatory states, but the instruments created are functional and provide increasing financing in the field of energy efficiency and soil protection (by restoring the carbon recalcitrant stock to the use of land for agriculture and forestry (Sosa-Hernández et al. 2019).

The Kyoto Protocol implicitly refers to the field of carbon fixation in soil fixation in Art. 3.3 about net changes in emissions (sources) or absorption (deposits) of greenhouse gases, resulting from the direct human activity of land use in agriculture and forestry, starting in 1990 and in Art 3.4 about additional activities with net reduction effect on emissions (sources) or absorption (deposits) of greenhouse gases applied to the agricultural lands, changing land use, and forest land. Currently, projects that fall under Art 3.4 are accepted only for national emissions inventory reporting and are not financed through the Carbon Fund.

The reason is due to the fact that there are a number of major scientific difficulties in quantifying the carbon units actually stored through the different agricultural practices/crops (the net absorption from the atmosphere). The difficulty of quantification comes from the lack of scenarios associated with quantitative process models that allow a correct estimation of the addition of carbon units stored in all 720 soil types for over 100 potential crop plants. Under the incidence Art. 3.3 falls the afforestation activity, where the CO_2 storage is simulable by mathematical models based on the afforestation composition, the potential production class of the species in the composition and the allocation of biomass at the individual tree level in relation to the age, during the project implementation (which can be superimposed over the entire production cycle or only part of it) (Loeser and Treede 2008; Metcalfe et al. 2018).

An afforestation project funded for carbon fixing must meet a number of strict parameters defined by the Climate Change Convention: additionally fixed carbon (additional to current of business as usual practice), verifiability and transparency, replicability, durability, risk management and uncertainties and as a tool for regional and local sustainability by addressing multifunctionality.

The application of pesticides should be done only on warning. Treatment warnings are made when a pest has a tendency to develop above the economic damage threshold (EDT). The economic threshold of pest represents the level of the population of pests that produces a damage that exceeds the total costs (ecological and economic) of the treatment with means of protection of the plants (pesticides, biopreparados). Considering the central role of computer systems for forecasting and warning in good agricultural practices regarding pesticide use.

These expert systems were developed due to the development of microprocessors and reliable sensors, as well as due to advances in (radio) telecommunications. Thus, complex, flexible forecasting and warning systems have been developed, whose operation allows a rational use of methods and means of plant protection, with corresponding reduction of the impact produced by harmful organisms. The computer systems for forecasting and warning are actually expert systems, which work on the basis of (mathematical) models of specific biological processes.

The final objective of the forecast and warning programs, namely the efficient and sustainable use of agronomic resources, is subsumed by the economic and ecological objectives of sustainable agriculture and precision agriculture. The use of expert forecasting and warning systems leads not only to ecological effects (reducing environmental and food pollution) but also has direct economic consequences. The optimization of the treatments leads to significant savings of pesticides, fuel, labor force, which allow the rapid amortization of the investment in a forecasting and warning system. In EU countries, investments in forecasting and warning systems are considered environmental investments and benefit from fiscal facilities (Solarin et al. 2018).

5.7 Conclusions and Recommendations

In the long term, the evaluation of the expected effects on the environment is highlighted, with the follow-up of details regarding the efficiency of the micro-remedy, the use of biotests as tools for assessing the ecotoxicological risk, the real costs that support the implementation of these processes, and the modeling of the types of interactions between the species of fungi and the categories of pollutants.

No one has ever fertilized a forest.

The advantages of ecological reconstruction by means of mycoremediation of degraded soils are the following: it intervenes in the depollution of the soil by reducing hydrocarbons, herbicides, pesticides, heavy metals, and radionuclides; contributes to the aeration, decomposition, and transport of organic and inorganic substances, without leakage of alluvium; determine the reduction of soil erosion by protecting the useful fauna; favors the restoration of the indigenous mycobiota (mycorrhizae and saprophyte) with a role in the solving process; contributes to the filtration of parasitic protozoan species; are natural barriers for harmful insects that pierce the wood and improve the nutritional quality of the soil, favoring the development of the useful mycobiota; it can develop practical skills for educational purposes in the field of conservation and sustainable use of natural capital.

The ecological reconstruction by means of mycoremediation of soil degraded by pesticides is relevant in the following directions: remediation of soil quality through the use of mycelium in decomposing toxic residues and different categories of pollutants; forestry through the use of by-products resulting from the cultivation of fungi in the nutrition and protection of plant diversity; biological control of pests by the use of complex action substances produced by fungi; controlling the erosion of biodiversity through the microfiltration of biotic and abiotic components.

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Chapter 6
Bio-Pesticides: Application and Possible Mechanism of Action

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6.1 Introduction

As per oxford dictionary, the word "pest" originated from the French word peste or Latin word pestis denoting the bubonic plague in fifteenth century. Pest is any destructive living organism that harms crops, food, livestock etc. Pesticides are synthetic chemicals that are applied usually to crops to destroy pests. The major group

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of pesticides comes under insecticides (e.g., phosphate esters, organochlorines, carbamate esters) and others are as rat poison (e.g., anticoagulants), herbicides (e.g., paraquat, diquat, 2,4-dichlorophenoxyacetic acid [2,4-D]), fungi killing substances (e.g., dithiocarbamates, ethyl mercaptan), and volatile poisonous gases (e.g., ethylene dibromide, methyl bromide) (Ellenhorn et al. 1997; Stamati et al. 2016). Meanwhile in 1960s, synthetic organic pesticides were the most common pesticides used for pest control, developed in the 1940 which begun with DDT usage then other pesticides like phosphate esters and carbamate esters pesticides (Nicholson 2007; Stamati et al. 2016). For successful enactment of Green Revolution technology through the application of pesticides to kill and control the pest population, pesticides get used in excess and their indiscriminate use becomes an important cause to several detrimental effects on plants, animals, and their environment. For instance, in human beings, pesticides induce free radical generation, lipid peroxidation, and inhibit the mechanism that generates total antioxidants for the body (Salazar-Flores et al. 2019; Abdollahi et al. 2004). Identifying the harmful effects of chemical pesticides such as development of pesticide resistant insect species, pest reappearance after application of pesticide, development of pests that were not considered in the category of pests before due to their natural death by application of pesticides, accumulation of pesticide residues in food such as fruits and vegetables consumed by humans, deteriorate their health and also causing ecological disturbances like accumulation in outer layer of lithosphere, atmosphere and hydrosphere (Salazar-Flores et al. 2019; Al-Zaidi et al. 2011). Thus without deteriorating environment and human health and also to generate high yield from diminished natural resources, it becomes need of the hour to develop environmental friendly alternatives for pest management.

Bio-pesticides are described as a broad spectrum of industrially generated products that are active agents of biological origin to control the population of insects, weeds, and disease causing microorganisms (Glare et al. 2012). As per FAO, bio-pesticides differ from biological control agents. Bio-pesticides are inactive agents of biological origin while biological control agents actively search for the pest and kill them such as parasites, nematodes pathogenic to insects. The US Environmental Protection Agency grouped bio-pesticides under microbial pesticides like bacteria, fungi, viruses, protozoa, and nematodes pathogenic to insects, second with genetically modified plants having herbicide, pesticide resistant traits and third are pheromones and other substances of natural origin (Fig. 6.1).

The first biocontrol agent, used as a rat poison was a *salmonella enteric* (Ratin) applied in Sweden and other European countries belonging to European continent in 1904s (Sundh and Goettel 2013) (Table 6.1).

Advantages of bio-pesticides over conventional pesticides/chemical pesticides (EPA 2006; Kumar 2012; Senthil-Nathan 2013; Bhat et al. 2018; Mushtaq et al. 2018):

- They are safe for consumers because of no residue problem specifically for edible fruits and vegetables.

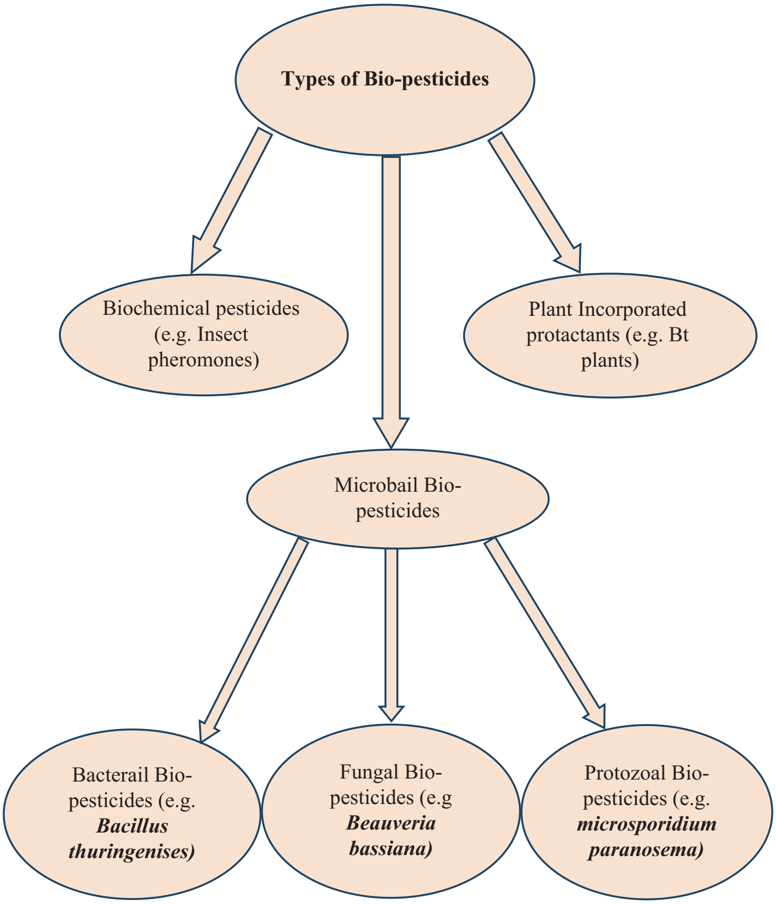

Fig. 6.1 Classification of bio-pesticides (US-EPA 2019)

- The efficacy of bio-pesticides is nearly same to chemical pesticides if we utilize them as one of the component of integrated pest management specifically for the cultivation of fruits, vegetables, and nuts.
- Since bio-pesticides have low pesticide resistance potential and are environment friendly thus do not face the same monitoring problems seen with chemical pesticides.
- Bio-pesticides do not cause environmental quality degradation problems, are gentle to beneficial insects, frequently target specific and also agricultural crops can be reentered soon after the treatment.
- When we want to cultivate organically, microbial bio-pesticides are a good alternative due to low detrimental effects to human health and low potential of resistance developed by pests.

Table 6.1 Types of bio-pesticides and their possible mechanism of action

Organism	Type of bio-pesticide	Possible mechanism of action	References
Bacillus thuringiensis	Bacterial bio-pesticide	Cry delta-endotoxin produced by *Bacillus thuringiensis* bind specifically insect gut epithelial cells, generate pores lead to cell content leakage and ultimately insect death	Jisha et al. (2013) and Shingote et al. (2013)
Bacillus subtilis	Bacterial bio-pesticide	*Bacillus subtilis* derived lipopeptides such as surfactin, Iturin A have antibacterial and antifungal properties by vacuolizing and necrotizing the cells	Ghribi et al. (2011, 2012)
Metarhizium anisopliae	Fungal bio-pesticide	Attach to insect cuticle, produce degradative enzymes which help the fungi to germinate, and grow on insect	Zheng et al. (2012)
Beauveria bassiana	Fungal bio-pesticide	After attachment to insect by appressoria, produces degradative enzymes, insecticidal proteins like 60 kD bassiacridin, helps the fungus to destroy the cuticle, germinate, and grow on the insect	Ortiz-Urquiza and Keyhani (2013) and Borgi and Gargouri (2016)
Baculovirus	Viral bio-pesticide	After ingesting polyhedral enveloped virus by the insect, outer protein matrix is dissolved in alkaline pH, releasing OBV that destroy peritrophic membrane, enter into mid gut cells, multiply and producing new generation of (OB) viral particles	Passarelli (2011)
Heterorhabditis bacteriophora	Nematodal bio-pesticides	After entering through the insect body, nematodes invade the hemocoel and then release their symbiotic bacteria into the intestine that spreads to whole body of insect causing septicemia, killing the host within 24–48 h	Han and Ehlers (2000) and Lu et al. (2016)
Bt plants	Plant-incorporated protectants (PIP)	Exotic DNA sequences that code for insecticidal toxins and others code for siRNA—20 nucleotide sequences that specifically inhibits mRNA translation leading to insect growth retardation or death are incorporated into plant genome	Ramaseshadri et al. (2013) and Fishilevich et al. (2016)
Azadirachta indica	Botanical pesticides	Azadirachtin acts on the prothoracic gland and insects, it is also a strong antifeedant of insects	Weinzierl (2000)

6.2 Microbial Pesticides

During the period of Green Revolution, indiscriminate use of synthetic chemical pesticides to produce maximum yield and this objective was fulfilled, but other problems arise especially the extinction of beneficial microbial and insect species. Hence the need for integrated pest management arose which persuaded us for the application of microbial biocontrol agent based pesticides as an effective and environmental sensitive approach (Chandler et al. 2011). As per US environmental agency (EPA), microbial pesticides are based on prokaryotic organisms like bacteria, eukaryotic organisms like protozoa, fungi, nematodes pathogenic to insects, microbial (entomopathogenic) agents, and acellular obligate parasites like virus.

6.2.1 Bacteria (Mechanism of Action and Applications)

6.2.1.1 *Bacillus thuringiensis*

Bacterial bio-pesticides contribute about 74% of the market (Thakore 2006). The first *Bacillus thuringiensis* subspecies named as *Bacillus thuringiensis* israelensis (Bti) was presented in the year of 1975 and found to be lethal to mosquito (Goldberg and Margalit 1977). *Bacillus thuringiensis* is a gram-positive spore generating bacteria, producing insect specific crystalline delta-endotoxins after lysis at the end of sporulation process. After ingestion by the insect, death occurs within 48 h (Jisha et al. 2013).

Several hours after ingestion of delta-endotoxin by the insect, a cascade of events starts in its mid gut leading to insect death by the increased pH. In mid gut of insect, delta-endotoxin is solubilized, and then fragmented by insect proteolytic enzyme into core toxic fragment (Jisha et al. 2013). The insect protein cleaving enzyme cleaves full protein into N and C terminus fragment which makes individual fragments active. These active fragments interact with the receptors present on the plasma membrane of epithelial cells lining the apical microvilli. This interaction causes conformational change in protein, leading its incorporation into the semipermeable cell membrane and the oligomeric complex forms on the cell membrane that develops an opening into the cell membrane causing leakage of cell contents, disruption of cell transport, endosmosis cause cell bursting and ultimately insect demise (Jisha et al. 2013; Schünemann et al. 2014). Another interesting binary toxin (vegetative insecticidal protein) produced from *Bacillus thuringiensis* strain is Vip1-Vip2 with coleopteran specificity such as store grain pest (*Sitophilus zeamais*) (*Curculionidae* family) causing 60% death (Yu et al. 2011; Shingote et al. 2013).

Lepidoptera is the major insect order containing species susceptible to *Bacillus thuringiensis* followed by Diptera and Coleoptera. Lepidoptera contains pest families that are of major concern in agriculture such as Cossidae, Thaumetopoeidae, Gelechiidae, Lymantriidae, Noctuidae, Pieridae, Pyralidae, Yponomeutidae, Tortricidae, and Pyralidae (Gathmann and priesnitz 2014). Diptera also contains agriculturally major pest families namely Tephritidae, Culicidae, Muscidae, Simuliidae, and Tipulidae whose some species are found susceptible to *Bacillus thuringiensis* toxicity (Azizoglu et al. 2017; Martins et al. 2018). In addition delta-endotoxin released by *Bacillus thuringiensis* which has antifungal activity, it produces acyl homoserine lactone lactonase enzyme that (blocks quorum sensing system in bacteria) and linear aminopolyol antibiotic called zwittermicin A (ZmA) having stronger lethal activity towards oomycota (water molds) and their relatives and also gram negative bacteria. As all fungi are covered by chitin layer, chitinase enzyme released by *Bacillus thuringiensis* has an essential role in biocontrol of phytopathogenic fungi such as 100% growth inhibition in *Sclerotium rolfsii*, 55–82% in *Aspergillus terreus*, *Aspergillus gillus flavus*, etc. (Reyes-Ramirez et al. 2004; Zhou et al. 2008; Lucon et al. 2010). Thus *Bacillus thuringiensis* may be used as a potential biocontrol in plant diseases.

6.2.1.2 *Bacillus subtilis*

Bacillus subtilis is a ubiquitous soil bacterium whose suitable habitat may be plant root system. It easily grows in plant root system by competing with root pathogens for food and shelter which attack the roots. *Bacillus subtilis* has been found as an effective biological agent for controlling population of plant virulent species of nematodes, bacteria, and fungi (Ongena et al. 2010). It has been found that *Bacillus subtilis* SPB1 strain causes death of an insect by bioactive lipopeptide. This bioactive lipopeptide actually enters into insects' tissue by vacuolizing and necrotizing of the epithelial cells by causing obvious histopathological changes like destroying the cells and their boundaries; its lipopeptide biosurfactant enters into insects' tissue. This has been found as an effective biological agent for the Olive moth (*Prays oleae*) population with a LC_{50} value of 142 ng/cm^2 (Ghribi et al. 2011, 2012).

It was found that the cells as well as spores of *Bacillus subtilis* are lethal to nematodes such as *Anguina tritici*, *Heterodera cajani M. incognita*, *Heterodera and cajani*, *Heterodera avenae*. As observed by Kavitha et al. (2012) plant dwelling (endophytic) *Bacillus subtilis* strains that produce a high amount of lipopeptide antibiotics effectively inhabit the egg laying and stop the metamorphosis process of *Meloidogyne incognita* at the second stage juvenile. This nematode is the most destructive pathogen, destroying a broad spectrum of crops under *in vitro* conditions.

Bacillus subtilis derived lipopeptides (e.g., surfactin, Iturin A) have been found to have biocontrol potential against several plant pests especially fungal phytopathogens (Ongena and Jacques 2007). A *Bacillus subtilis* derived lipopeptide (surfactin) has bioactive lethal activity against virulent fungi *Aspergillus flavus*, *Colletotrichum gloeosporioides*, and also exhibits growth inhibition of phytopathogenic fungi like Fusarium species, Aspergillus species, etc. (Mohammadipour et al. 2009; Velho et al. 2011). A novel linear bioactive lipopeptide called Gageotetrins A-C are obtained from a marine *Bacillus subtilis* that showed good time course motility inhibition and also possesses lytic activity towards late blight pathogen *Phytophthora capsici* that is a major pest causing damage to cucumber, chili, beans, and tomato at 0.02 mM concentration (Tareq et al. 2014). Some *Bacillus subtilis* strains synthesize mycolytic enzymes that cause lysis of pathogenic fungi cell wall, thus act as biological control agent of plant rot disease (Yan et al. 2011; Khanday et al. 2016; Bhat et al. 2017). *Bacillus subtilis* bacteria produces enzymes like chitinase, glucanase, and cellulase whose activities help in controlling anthracnose disease of chili caused by *Colletotrichum gloeosporioides* OGC1 (Ashwini and Srividya 2014).

6.2.2 Fungi

6.2.2.1 *Metarhizium anisopliae*

Metarhizium anisopliae is ubiquitous and found everywhere in the soil, and a large spectrum of insect pest species are susceptible to this fungi. Under natural conditions, *Metarhizium anisopliae* is induced by moist conditions of soil to produce infectious spores called conidia. This subspecies was first described in 1879 by Metschikoff as named *Entomophthora anisopliae* described as pathogen of wheat cockchafer and in 1883 name changed to *Metarhizium anisopliae* by Sorokin. A large variety of different strains come under this species (Tulloch 1976; Mnyone et al. 2010).

Entomopathogenic fungi attack the insect by infiltrating cuticle layer anywhere on the insect body, but other organisms like viruses, bacteria, and nematodes attack their host through some particular routes. Infection begins by attachment of entomopathogenic fungi with insect cuticle by the help of infectious conidia. After attachment to insect cuticle, it begins to release many degradative enzymes such as proteases that degrade proteins, chitinases that degrade chitin polysaccharide, lipases ultimately helps the pathogen to grow and germinate on the cuticle of insect (Zheng et al. 2012; Xiao et al. 2012).With the passage of time, novel structures appear from the growing fungi namely infiltration pegs or appressoria or both. These specific structures empower the rising hyphae to infiltrate deep into the insect integument. Brazil is one of the South American countries that uses this fungi on a large scale on their crop fields to protect them from spittle bugs (*Mahanarva fimbriolata*), its larval forms suck water and nutrients from the plant roots increasing stress while adults use leaves as food and insert toxins that cause leaf necrosis reducing production to 40% in Brazil (Dinardo-Miranda et al. 2002; Alves and Teixeira 2014). Above 100,000 ha of sugarcanes are applied with this fungi every year (Faria and Magalhaes 2001).

6.2.2.2 *Beauveria bassiana*

This fungus was discovered as bio-pesticide unintentionally by Agostino Bassi (1835) when he first investigated the disease of silk worms called white muscardine caused by this fungus. *Beauveria bassiana* is a diverse fungus targeting insects that are found in temperate and tropical regions. The suitable habitats of *Beauveria bassiana* range from desert soils to forests and cultivated soils.

The entomopathogenic infection begins with appearance of infection structures called appressoria and other virulent factors such as proteins, and enzymes, which facilitates *Beauveria bassiana* conidia to attach to the host arthropod, breach the cuticle, grow in number within the hemocoel as blastospores (hyphal bodies that escape from the surveillance of host immune system (Lewis et al. 2009) and finally the host is destroyed. Then *Beauveria* bassiana hyphae reappears, covering the whole body of insect corpse, and generates novel conidia, thus finishing the parasitic life cycle (Toledo et al. 2010; Ortiz-Urquiza et al. 2010; Ortiz-Urquiza and Keyhani 2013). Hydrophobins that are expressed on the surface of fungi make it hydrophobic

(Hyd1 and Hyd2).This hydrophobic surface easily adheres to the epicuticular layer of pest and thus contributing in its virulence (Cho et al. 2007; Zhang et al. 2011). After attachment, it produces degradative enzymes such as cytochrome $P_{450,}$ catalases, esterases, long-chain alcohols, and aldehyde dehydrogenases, which facilitates degradation of lipid, hydrocarbon, protein, and other compounds found in insect cuticle. *Beauveria bassiana* also generates insecticidal, 60 kD protein called bassiacridin that possess many enzymatic activities such as beta-glucosidase, beta-galactosidase, and N-acetylglucosaminidase activities (Quesada-Moraga and Vey 2004; Borgi and Gargouri 2016). In addition to this degradative and detoxifying enzyme cocktail, *Beauveria bassiana* also generates some of the organic acids mainly oxalic acid that has a role in *Beauveria* bassiana pathogenicity since oxalic acid facilitates to loosen the structural rigidity of insect cuticular surface (Bidochka and Khachatourians 1991).

In addition, smaller sized secondary metabolites synthesized in vitro and in vivo participate in parasitization process. They play important roles as (a) toxins that triggers insect's death, (b) immunomodulators that enable the fungus to evade from the surveillance of the host immune system, (c) generating some biochemicals that have an activity against rival microorganisms, and (d) defensive molecules against mycophagous organisms (Charnley 2003; Feng et al. 2015a, b; Lozano-Tovar et al. 2017). The known secondary metabolites produced by this entomopathogenic fungus include cyclic peptides, such as beauvericin, bassianolide, and beauverolides, and polyketide-derived pigments, such as oosporein, tenellin, and bassianin, but only those genes involved in the biosynthesis of beauvericin, bassianolide, tenellin, and oosporein have been functionally verified (Halo et al. 2008; Molnar et al. 2010; Feng et al. 2015a, b). Beauvericin is probably the most studied cyclic peptide compound produced by *Beauveria bassiana*. Beauvericin is an effective anti-pesticidal molecule (Hamill et al. 1969) but the exact mode of action is not fully understood (Wang and Xu 2012). In addition, this hexadepsipeptide has cytotoxic and proapoptotic activities in several human cell lines including leukemia cells (Wang and Xu 2012). Beauvericin seems to act as an ionophore, forming cation-selective channels and increasing intracellular Ca^{+2} (Kouti et al. 2003), for example, in case of erythrocytes in which increased intracellular calcium cause stimulation of KCL channels leading to water loss, cell shrinkage (Qadri et al. 2011) and also have been suggested to trigger calcium sensitive cell apoptotic pathways in some other cells (Wang and Xu 2012).

6.2.3 Protozoa

6.2.3.1 *Nosema*

There are almost 1000 entomopathogenic protozoans. They are lethal and act as biocontrol agent for many invertebrates. Grasshoppers and heliothine moth are common insect species susceptible to *Nosema,* for example, Henry et al. (1981) recently developed *microsporidium Paranosema* (*Nosema*) for grasshoppers control (Lange and Sokolova 2017). Nosema species and *Vairimorpha necatrix* are two well recognized entomopathogenic protozoan species. These two species come under Microsporidia (unicellular spore generating organisms) generally host specific and produce spores that are taken by the pest and germinate in its mid gut. The infection process is slow and chronic. Massive demolition of organs and tissues starts when the sporoplasm ruptures to release germinating spores. Sporulation process recycles from the infected tissues and upon ejection and assimilation by a susceptible host induces an epizootic (wide spread outbreak) within insect population (Brooks 1988; Milner et al. 2003). They are obligate parasites that thrive only in living hosts and some species need another host for the asexual development that is intermediate host. Microsporidia species have benefits over other entomopathogenic protozoa such as perseverance and repeating of their reproductive cycle in susceptible host populations and their debilitating effect on reproduction and overall fitness of susceptible host insects (solter and Becnel 2000).

Nosema locustae (entomopathogenic protozoan) is the only species that showed effective activity towards grasshoppers and later on was registered and recognized commercially (Henry et al. 1981). The first reported microsporidium is *Nosema bombycis* which is the causative agent of devastating silkworm disease called silkworm pébrine which continued in some parts of Asia, Europe, and North America, during the period of 1850 AD (Becnel and Andreadis 1999). Unfortunately, pébrine is still causing a wide spread outbreak among the silk-producing countries like China leading to large financial sufferings (Cai et al. 2012).

6.2.4 Virus

6.2.4.1 *Baculovirus*

Baculovirus is the most important class of arthropod virus (Herniou and Jehle 2007). The use of Baculovirus started in nineteenth century as a biocontrol agent for forestry and agriculture pests. They are the most deeply studied virus pathogenic to insect (Inceoglu et al. 2001). Baculovirus have two different observable phenotypes, one is the budded virus phenotype that is generated after initial infection by the budding through the plasma membrane of cells infected by the virus. Budded virus particles contribute to spread the viral particles to another region from mid gut of insect (Jehle et al. 2006). The other is occluded phenotype (OV) that is sealed in a crystalline protein matrix covering called occlusion body (OB) generated in the final phase of infection. Occlusion bodies enable the virus to remain alive even outside the host (Cory et al. 2000). Two groups of baculovirus are observed, one is called the nuclear polyhedrosis virus (NPVs) and the other one is granulovirus (GVs). In nuclear polyhedrosis virus, occlusion body contains many viral particles, but in granulovirus, occlusion body usually contains just single viral particle. Nuclear polyhedrosis virus constitutes three genera, Alphabaculovirus that is effective against lepidopteran, Gammabaculovirus which is effective towards hymenopteran, and last one is Deltabaculovirus effective for dipteran insects; granuloviruses constitute one genus Betabaculovirus that specifically controls the population of lepidopteran pests only (Jehle et al. 2006). Occlusion bodies in insect excrements spread to other insects when present on plants ingested by insect larva (Szewczyk et al. 2006).

In 1982 AgMNPV was developed which is a commercially well recognized viral bio-pesticide of Alphabaculovirus to be effective against *Anticarsia gemmatalis* that is a common and copious soybean pest in Brazil. It was used to treat around 2000 ha of soybean (Moscardi 1999) and its use starts growing. The application of AgMNPV to manage the insect population of *Anticarsia gemmatalis* in Brazil was a very effective program and is considered as the most important one through the world (Yang et al. 2012). Many other South American countries also followed the same program (Sosa-Gómez et al. 2008). Globally, *Cydia pomonella* is considered as a frequent pest of apples, pears, and walnuts (Arthurs et al. 2007). Since 1964 *Cydia pomonella* granulovirus (CpGV) was developed that is an effective viral bio-pesticide for insect control, safeguarding fruits from financial losses in integrated pome fruit production especially in European countries and some other countries (Moscardi 1999; Lacey et al. 2008; Kutinkova et al. 2012). CpGV viral bio-pesticide is possibly the well-established viral bio-pesticide on a commercial scale that is applied worldwide in terms of treated area (Vincent et al. 2007). Globally, the genera such as Heliothis and Helicoverpa signify important insect pests. They are accounted for million dollars economic losses every year (Moscardi et al. 2011). *Helicoverpa armigera* (cotton bollworm) is a highly polyphagous insect and one of the most serious pests recognized globally. This pest attacks numerous crops namely some kinds of beans, cotton, maize, tomatoes, tobacco, etc. belonging to wide range of plant families (Sun et al. 2002). HaSNPV viral bio-pesticide is manufactured, produced, and treated to the crops by many countries such as Australia, India, and in China, it is considered as one of the most important baculovirus bio-pesticides applying different NPVs on over 100,000 ha of crops per year (Moscardi et al. 2011; Yang et al. 2012).

Apart from wide application of baculovirus, the slow mode of killing mechanism becomes a hurdle in their wide spread practical use. So, many tactics are established to progress their killing action by altering the baculovirus genome using genetic engineering, to insert specific kinds of genes taken from insects such as genes coding for hormones or enzymes or toxins (Kamita et al. 2005; Li and Bonning 2007; Gramkow et al. 2010).

6.2.5 Entomopathogenic Nematodes

6.2.5.1 *Steinernema and Heterorhabditis (Rhabditida)*

There are 23 nematode families from which many nematodes are known to parasitize insects. Such nematodes are called entomopathogenic nematodes (EPN) (Koppenhofer 2007). Steinernema and Heterorhabditis were the first two active entomopathogenic nematode genera revealed and documented as a biocontrol agent towards insects during the beginning of 1990s (Copping and Menn 2000). The Heterorhabditidae and Steinernematidae entomopathogenic nematode families have received the greatest consideration because they keep many of the characteristics of an ideal biocontrol agent (Kaya and Gaugler 1993; Grewal et al. 2005; Koppenhofer 2007) and are applied as conventional, protective, and proliferative biocontrol agents. Steinernematidae have two genera represented by Steinernema and Neosteinernema while Heterorhabditidae has one genera indicated by the genus Heterorhabditis and used as biocontrol entities in agriculture for pest management (Kaya and Gaugler 1993; Kaya and Stock 1997; Lacey and Georgis 2012). The alimentary canal of all entomopathogenic nematodes is usually occupied with symbiotic bacteria like Xenorhabdus bacteria present in Steinernematidae, while Photorhabdus bacteria are found in Heterorhabditidae (Boemare 2002). Such nematode and bacterium complex works mutually as a biocontrol unit to destroy an insect pest. The above two nematode families are not closely related and belong to the order Rhabditida (Blaxter et al. 1998) but because they possess many biological similarities, they will be considered together. Entomopathogenic nematodes attack insects at soil-lodging stages and destroy them within 48 h by ejection of pathogenic bacteria (bacteria beneficial to nematode) that are present in alimentary canal of nematode. The infectious stages of the nematode become adult and an army of infective juveniles is produced from the insect cadaver. There have been various efforts to use nematode IJ to control insect pest population via spraying but little is known about the capability of native species of nematodes about their impact on the pest population (Peters 1996). However their capability can be enhanced by using in combination with other bio-pesticides such as entomopathogenic fungi, e.g., Wakil et al. showed that application of *Heterorhabditis bacteriophora* in combination with *Beauveria bassiana* exhibited greater effectiveness against the larval stages of *Rhynchophorus ferrugineus* (Olivier) commonly called red palm weevil (Wakil et al. 2017).

Once the non-feeding IJ have entered through the insects natural body pores like the egestion opening, ingestion opening, and respiratory openings (spiracles), nematodes invade the hemocoel and then release their symbiotic bacteria into the intestine that spreads to whole body of insect causing septicemia, killing the host within 24–48 h. The uptake of infective juveniles of nematode is quickly manipulated by the bacteria and decays the host tissues. Recently it has been also found that activated infective juveniles produce some lethal proteins called venom proteins that also contribute to its pathogenicity (Lu et al. 2017). Almost two to three progeny of the nematodes are produced within the host cadaver (Pomar Jr and Leutenegger 1968; Bird and Akhurst 1983; Grewal et al. 1997). Earlier it is found that the important insect pest families observed in stockpiled goods such as Pyralidae and Curculionidae can be efficiently controlled by entomopathogenic nematodes (Duncan and McCoy 1996; Shannag and Capinera 2000; Shapiro and McCoy 2000). Higher concentration of nematodes has been found to be susceptible to insect pests present in stockpiled goods like *Ephestia kuehniella Zeller* and *Tenebrio molitor L*. by Morris (1985). Georgis (1990) had also detected a field concentration of >2.5 billion nematodes per ha effective against some of the important insect pests of crops grown in rows (row crops).

6.3 Plant-Incorporated Protectants/Genetically Modified Plants (GMP)

Plant-incorporated protectants (PIPs) are bioactive ingredients having macromolecular nature, produced in transgenic plants whose genome is modified by introducing some exotic gene sequences. First-generation plant-incorporated protectants having insect killing activity are Cry genes (code for bt toxins) taken from bacterium *Bacillus thuringiensis* genome, introduced in specific plants like maize, cotton, etc. Now these plants are called genetically modified crops or transgenic plants. These *Bacillus thuringiensis* crops contain one or more than one gene sequences that code for insect killing Cry proteins chosen from the bacterium *Bacillus thuringiensis* (Bt) (Clark et al. 2005). Their size typically ranges from 63 to 68 kDa (Mendelsohn et al. 2003). A novel group of genetically modified crops are established that possess bioactivity by expression of double stranded RNA (dsRNA) PIPs that silences expression of specific mRNA which are essential for their survival. RNA interference mechanism protects the crops in which dsRNA is introduced which is a preserved eukaryotic gene expression regulation process revealed in 1998. The United States Environmental Protection Agency permitted the first RNA interference crop in June 2017 to biocontrol corn rootworm which is a major insect pest (Ramaseshadri et al. 2013; Fishilevich et al. 2016). This crop also produces a *Bacillus thuringiensis* delta-endotoxin protein and contains some specific genes that make it resistant to glyphosate herbicide (US, EPA 2015). Fifteen years after the dawn of genetic engineering, the first GM crops expressing PIPs were presented in 1987 (Barton et al. 1987; Fischhoff et al. 1987; Vaeck et al. 1987). Several Bt crops have been registered mainly cotton and maize from the year 1995 (GM crop database, EPA-PIPS registrations). Many Bt crops now show a “stacked” phenotype, expressing traits like two or more Cry proteins (Tabashnik et al. 2013, EPA-PIPS registrations) or Cry protein(s) expressed with other traits like immunity to the herbicide glyphosate (Que et al. 2010). In 2016, it is estimated that 98.5 million hectares of transgenic plants including 23.1 million ha of crops expressing cry protein and 75.4 million ha of crops expressing both anti-pesticidal and immunity to herbicide were planted globally, which is nearly equivalent to partial of the global area planted with cotton or maize (GM Crops 2016). Consent of genetically modified crops producing PIPs, developed on a commercial scale in Australia, Asia, and America, and, to a smaller level in parts of Africa. Nevertheless, the application of genetically modified crops producing plant-incorporated protectants is limited in Europe (GM Crops 2016).

Bt toxin protein and siRNA PIPs produce their desirable effects like blocking insect development or leading to insect death by their different mechanisms of action when an insect ingests the tissue of transgenic crop. Bt toxin protein interacts with definite receptors on the plasma membrane of epithelial cells lining the insect mid gut, incorporates into the plasma membrane, and finally generates transmembrane channels through which cell content is leaked leading to ultimately insect pest death (Bravo et al. 2007). There is structural diversity in cry proteins, governing their toxicities towards the insect pests that are specific to certain insect orders (Xu et al. 2014; Crickmore et al. 2016) like Cry1 proteins is lethal to Lepidoptera, e.g., the corn borer and Cry3 protein is lethal to Coleoptera, e.g., the corn rootworm (Clark et al. 2005).

After ingestion of tissue of transgenic plant, double stranded RNA is taken by the specific cells lining the insect mid gut. Within the cell, dsRNA is trimmed by RNase enzymes into siRNA that approximately 20 nucleotides in length which guide the cleavage of matching mRNA (complementary) into small pieces by its association with insect's endogenous RNAi machinery. The degradation of the targeted mRNA by siRNA-RISC complex blocks its translation pathway that is vital to the pest insect, causing sub-lethal consequences (e.g., reduced growth) or pest death. The first double stranded RNA plant-incorporated protectants that have received regulatory consent biocontrol the corn rootworm (*Diabrotica virgifera virgifera*) by preventing the translation of the Snf7 protein, an essential vacuolar sorting protein (Ramaseshadri et al. 2013)

6.4 Biochemical Pesticides

According to US Environmental Protection Agency biochemical pesticides are substances that control pest population by the non-lethal mode of action and also they should be naturally occurring substances. By contrast synthetic pesticides directly destroy or deactivate the pest like pheromones, plant extracts, natural insect growth regulators, etc.

6.4.1 Plant Extract/Botanical Pesticides

Botanical pesticides are usually secondary phytochemicals presented as a substitute to synthetic chemical formulations but they sometimes show little toxicity towards humans. The use of botanical pesticides are cited in Hieroglyph, Chinese, Greek, and Roman ancient times and also in India where the application of extracts obtained from the neem tree (*Azadirachta indica Juss* Meliaceae) was described in the Veda, a body of documents written in ancient Sanskrit dated at least 4000 years ago (Philogène et al. 2005; Regnault-Roger and Philogène 2008)

6.4.1.1 *Azadirachta Indica* (Neem)

Schmutterer (1990) first discovered neem tree as bio-pesticide when he found that crowded desert locusts defoliated almost all native flora but neem tree was resistant to its defoliating action in Sudan. It is found that neem seed oil is active against soft-bodied insects, mites, and management of plant pathogens due to the presence of disulfides contributing to its bioactivity (Dimetry et al. 2010; Dimetry 2012). The neem seed extract after separating oil from it contains a variety of bioactive secondary metabolites. The essential ingredient is a limonoid called azadirachtin, the remaining minor analogs which contribute little to overall efficacy of the extract. Seed extracts include substantial amounts of other triterpenoids such as salannin, nimbin, and their modified forms whose role is debatable but most observations suggest that azadirachtin as the most important active secondary metabolite (El-Sayed 1983a, b; Isman et al. 1996). Neem product activity is measured against 450–500 insect pest species in many countries worldwide, and from that, 413 insect pest species are found to be susceptible at various concentrations (Schmutterer and Singh 1995).

Azadirachtin acts on the prothoracic gland of insect, prevents the formation and exit of molting hormones from the gland cells (ecdysteroids) which lead to incomplete ecdysis in immature insects. A comparable mode of action cause infertility in adult female insects, in addition azadirachtin is an effective agent of antifeedant towards numerous pest insect species. Indeed, azadirachtin was first isolated based on its extraordinary antifeedant activity towards the desert locust, and this plant secondary metabolite becomes the most effective locust antifeedant discovered till now. Neem quickly became the modern standard for the progress of botanical pesticides in the USA (Weinzierl 2000).

6.4.1.2 *Annona muricata* (Custard Apple) and *Annona squamosa* (Graviola)

Botanical pesticides are conventionally developed from the seeds of tropical Annona species, namely (*Annona* squamosa) and soursop (*Annona* muricata) that are the species of the custard apple family (Annonaceae). They are major sources from which fruit juices are prepared in Southeast Asian countries. A detailed study in 1980 revealed that they contain a number of long-chain fatty acid derivatives termed acetogenins, later isolated, found accountable for anti-pesticidal activity. The important acetogenin isolated from *Annona* squamosa seeds is annonin I or squamocin. Later, an analogous compound, asimicin, was obtained which is observed in the bark of the American pawpaw tree (*Asimina triloba*) (McLaughlin et al. 1997; Johnson et al. 2000).

Earlier it was found that these secondary phytochemical compounds are sluggish stomach poisons and masticating pest insects namely Lepidopterans as well as Colorado potato beetle (*Leptinotarsa decemlineata*) are particularly susceptible. Additional study uncovered that the mechanism of action of acetogenins is undistinguishable from rotenone, i.e., it blocks ATP generation in mitochondria in mammals as well as in insects (Londershausen et al. 1991). Certain acetogenins in their pure form are lethal to mammalian species (LD_{50} is <20 mg kg^{-1}), an obstacle to regulatory consent; however, homogenous extracts obtained from the Annona seeds and Asimina plant tissues like seeds, bark show minimum toxicity. Annona seed extracts seem to prove more beneficial in countries where the seeds are left behind after fruits are consumed or used to produce the juice. For example, Leatemia and Isman (2004a, b) recently demonstrated that the diamondback moth is susceptible to crude ethanolic extracts or even aqueous extracts produced from seeds of Annona squamosa collected at several sites in eastern Indonesia (*Plutella xylostella*) (Isman 2006).

6.4.2 Pheromones

Insects release biochemicals in their environment called pheromones to evoke a specific behavioral response such as mating, aggregation, and alarm from other individuals. On the basis of their effects, they are categorized as sex hormones, aggregation hormones, alarm hormones, etc. Most of the pheromones stimulate the trail following behavior, egg laying behavior, and aggregation, whereas few other sex attractant pheromones attract partners of opposite sex for mating. Pheromones released by insects evoke the behavioral response only in the same species, for instance, maiden female insects are releasing sex pheromones in the surrounding environment that is recognized by the male of the same species. Aggregation pheromones are released by insects such as wood attacking beetles releases aggregation biochemicals when they found a rich food source to inform and aggregate the other members of the species (Copping and Menn 2000).

A large number of pheromones and sex attractants whose number exceeds 1600 have been found and many of them have a potential to become important tools to control and monitor the pest population that causes heavy losses to agricultural crops (Witzgall et al. 2004). Environmental protection agency (USA) registered 30 pheromone based products that have potential to disrupt mating in insect pest species belonging to order Lepidoptera that is responsible for heavy losses in agricultural crops (Copping and Menn 2000). Environmental conservation, food security, and pest resistance managing are some of the main issues guiding current pest management guidelines in commercial agriculture (Witzgall et al. 2010). While the needs of the rapidly growing world population and at the same time retaining sustainability development and ecological stewardship is an important issue faced by current agricultural system (Kogan and Jepson 2007). The usage of pheromones and/or plant defensive chemicals released against herbivores (allelochemicals) as pest behavioral disruption tools can substitute or counterpart existing pest management policies (Witzgall et al. 2008) resulting in a lesser dependence on pesticides that have wide range of targets including beneficial insects present in agricultural fields. At the present time, pheromones and other semiochemicals are applied to crops that monitor and control pests in millions of hectares. There are many benefits of utilizing pheromones as bio-pesticides over others, like less expensive, specific to target insect, easy to utilize, and highly sensitive compared to conventional pest management system (Wall 1990; Laurent and Frérot 2007; Witzgall et al. 2010). Plants and its derived attractants have been found to interact with the insects to a valuable food source and when combining with insect-obtained attractants can be developed an effective attraction to some insect pests (Copping and Menn 2000).

6.5 Conclusion

The use of natural products with business esteem is legitimately showed by the various compounds present in the market and that have stayed there much of the time after numerous years. These estimations of natural products are considered as a wellspring of new systems/ mechanisms and their ensuing fuse into high-yield screens is difficult to assess. As of late, a few works have been practiced and stretched out to be embraced to upgrade the timeframe of realistic usability, quick demise, the natural plan, proficient in the field and constancy, and the impact of average cost for basic items frameworks and there have been some striking achievements in circumstances where some disturbance to the yield is satisfactory.

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Chapter 7
Values of Biofertilizers for Sustainable Management in Agricultural Industries

Sajad Ahmad Raina, Rouf Ahmad Bhat, Humaira Qadri, and Ashut Dutta

7.1 Introduction

Growing human population led to the dependence on pesticides and elemental fertilizers due to conventional agriculture that fulfills the food needs of population growth (Santos et al. 2012; Bhat et al. 2018; Mushtaq et al. 2018). Chemical fertilizers are elements composed of known amounts of nitrogen, phosphorus, and potassium, and their utilization causes adulteration in air and groundwater through the process known as cultural eutrophication (Youssef and Eissa 2014). In this sense, latest efforts must be more channelized to produce "high quality nutrient rich foods" in ecologically compatible manner to safeguard biosecurity. The inventive vision of agricultural production fascinates the increasing demand for biological fertilizers limited to agrochemicals (Raja 2013). In order to improve nutrient intake and preserve field management that helps to promote the alternative methods of soil fertilization is based on organic inputs (Araujo et al. 2008). Organic farming is one of the important strategies that increase soil biodiversity leading to the assurance of food safety (Megali et al. 2013). The additional benefits of biofertilizers include extended shelf life devoid of any postharvest adverse effects on the ecosystem (Sahoo et al. 2014).

The distinguished effects of the increased use of element enrichers in agriculture result in alterations in soil reaction, cause nutritional disproportions in plants, greater vulnerability to pests and diseases, decline of the root nodulation of legumes and mycorrhizal linkages existing among various crops which ultimately result in soil humus reduction and water pollution. The harmful effects of chemical fertilizers continuously used in large quantities without the use of organic components

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have aroused an interest to substitutes in which organic fertilizers play an important role to supply nutrients to plants in an integrated manner resulting in the Integrated Nutrient System for Plants (IPNS). Environmental problems, such as soil erosion, energy saving and freshwater pollution, might the farmers to acquaint with the new cultivation methods that have less effect on the environment. In this regard, the use of chemical fertilizers has reduced by the greater applicability of organic fertilizers that is considered a mandatory way to remove the pressure on the environment resulting from conventional agricultural practices. Increase in human population has led to a growing dependence on chemical fertilizers and pesticides in order to meet the food needs due to conventional agriculture practices (Santos et al. 2012). The exploitation of chemical fertilizers composed of known quantities of nutrients such as nitrogen, phosphorus, and potassium manufactured in industries causes air and groundwater pollution through the process known as cultural eutrophication (Youssef and Eissa 2014). In this regard, the new efforts have been channeled more in the direction of producing of "high quality nutrient rich foods" in sustainable behavior to safeguard biological security. The main environmental problems associated with the use of fertilizers lead to the pollution of water with nitrates and phosphates. These nitrates find their way into groundwater reservoirs through percolation and the consumption of such drinking water with high levels of nitrates is reflected as a pronounced disease (methemoglobinemia) that is lethal to human beings.

Phosphorus binds and moves with the soil particles and cannot be easily removed from the ground. Therefore, the soil that is eroded contains phosphorus can be washed in surface water together with the soil particles. Phosphorus stimulates the growth of algae in water bodies and is not considered dangerous. The algae causes the death of fishes due to removal of oxygen content in water bodies by death and decomposition, this process is called eutrophication.

There are different sources of these pollutants such as industrial waste, detergents, sewage disposal, and fertilizers. The decomposition of crop residues and organic residues produces nitrates and the main problems of high levels of nitrates in groundwater have been recorded since 1860, long before the use of fertilizers became common.

Synthetic fertilizers are an interesting alternative, but they have undesirable negative effects. Some are discussed as below.

7.2 Motility of Microorganisms and Plants

Some fertilizers possess highly acidic attributes which transform soil chemistry and thereby result in killing of earthworms and other microorganisms that are beneficial to plants. Earthworms perforate into the soil and result in increasing the passages in soil which help to ventilate the soil for boosting up the fertility. They also deposit their castings into the soil. The most important feature of earthworms and microorganisms is that organic matter decomposes. These microorganisms are difficult to find in soils treated with synthetic fertilizers. This soil is not rich, since it does not have the benefit of the nutrients released by these organisms (Shafi et al. 2018).

7.2.1 Increases Soil Toxicity

The chemicals contained in synthetic fertilizers are toxic to useful soil microorganisms. The distribution of earthworms is quite rare in a synthetically fertilized soil. And chemical toxicity of synthetic fertilizers makes the soil less beneficial for plants.

7.2.2 Increases Plant Diseases

Since plants grown in synthetically enriched soils do not have the nutrients that introduce useful microorganisms, they are more prone to plant diseases. They also have a weaker immune system.

7.2.3 Makes Waterways Impassable and Damage Marine Life

Nutrients from this soil penetrate into sewers and waterways. While doing so, they encourage algae growth. The proliferation of algae is sometimes large enough to prevent the flow of water through drainage and sewage systems.

7.2.4 These Fertilizers Have a Short Life

Most of these petroleum based fertilizers are water soluble. They release their nutrients quickly, but these nutrients disappear so quickly. A common complaint among gardeners using such fertilizers is that they often have to continue adding them, increasing their operating costs.

7.3 Synthetic Fertilizers Damage Humans and Pets

Synthetic fertilizers are not beneficial to humans and pets. Exposure can cause respiratory problems in humans. Studies also show that exposure to such fertilizers causes cancers in pets. Synthetic fertilizers produce vegetables with nutritional deficiencies that do not necessarily provide optimum vitamin concentration that our body requires for carrying day-to-day life.

7.4 Costs

"A professional lawn care contractor will charge on average between $ 50 and 80 to apply fertilizer on an 8000 square meter construction site." This number varies depending on whether one chooses an organic or synthetic fertilizer. Most contractors apply a fixed price based on the services provided and the size of the area. If the contractor charges hourly rates, then it is expected to pay an average of $ 65 per hour. An average of 8000 ft^2 of lawn will cost between $ 50 and 95 for the application of organic fertilizer. Futhermore, synthetic fertilizers are costilier and accounts around $ 25 and 80.

7.5 Environmental Concerns

Organic fertilizers are biodegradable, renewable and are ecologically sustainable. Organic fertilizers are made from unprocessed and natural ingredients as manure, cornmeal, compost and are considered one of the best solutions to produce a healthy environment for plant growth. These fertilizers are often acquired locally and mass scale production can be achieved through installing the greenhouses and farms, which further reduce the environmental impact.

Synthetic fertilizers are processed from a variety of different products, including mineral salts and petroleum products in order to ensure the stability of the nutrient content. The treatment and regular use of these fertilizers use more water and are potentially harmful to the environment due to additional chemical inputs that can lead to the accumulation of poisonous chemicals, such as arsenic and uranium, making adverse effects on people, pets, and plants.

7.6 Effects of Chemical Fertilizers on Soil

Chemical fertilizers are artificial fertilizers or industrially made fertilizers used to increase the nutrient level in the soil and are essential for plant growth as these nutrients are produced synthetically from inorganic materials and applied to the soil as similar to natural nutrients present in the soil such as nitrogen, phosphorus, and potassium. These fertilizers increase the yields in a short duration of time, but have some harmful effects on beneficial soil microbes and soil structure by the use of chemical fertilizers instead of organic fertilizers made from natural sources.

7.7 Groundwater Pollution

The continuous use of chemical fertilizers causes groundwater pollution or leaching. Chemical fertilizers with high solubility are absorbed by the soil more quickly than absorbed by the projected plants. Plants absorb a given level of nutrition and leaving the extra fertilizer to leach at a time. Leaching of chemical fertilizers is dangerous for groundwater, besides that it also affects the subsoil because these chemicals bind with the clay particles to form hard layers in soil called as hardpan.

7.8 Soil Friability Effect

The presence of various acids such as hydrochloric and sulfuric acids in the soil produces a harmful effect on the soil called soil friability. The various acids in the soil dissolve the crumbs in the soil that helps to keep the rock particles together. The crumbs of the soil are due to the combination of humus, or decomposed natural material, like dead leaves, with clay. These soil-rich mineral crumbs are essential for soil drainage and greatly improve air circulation in the soil. Since the chemicals contained in chemical fertilizers destroy the crumbs in the soil, the result is highly compacted soil with less drainage and air circulation.

7.9 Causes of Destruction of Microorganisms

The continuous use of synthetic chemicals as chemical fertilizers altering the pH of the soil thus has adverse effects on the health of beneficial soil microorganisms present naturally in the soil. Thus the altered level of acidity in the soil removes the beneficial microorganisms that are helpful to increase the defense mechanisms of plants against pests and diseases. These microorganisms are found in healthy soils to produce antibiotics and include bacteria, mycorrhizae, and other fungi. The continuous use of chemical fertilizers damages the bacterial community which helps in nitrogen fixation and balance the soil health. These bacteria are accountable for converting atmospheric N_2 into nitrates that are easily available to plants.

7.10 Effect of Fertilizers on Human Health

The continuous use of fertilizers produces toxins into the soil that enter the humans through food chain by the consumption of contaminated vegetables and cereals as these fertilizers are made of a mixture of toxic chemicals absorbed in plants and also the runoff from agricultural fields finds their way into aquatic environ that eventually affects the human health (Table 7.1).

These fertilizers also cause dangerous diseases such as kidney diseases, lung and liver problems, and cancer because of the metal toxicity of fertilizers and it depends

Table 7.1 Toxic substances released by using synthetic fertilizers and their impacts on human health

S. no.	Pollutants associated with synthetic fertilizer	Impacts on human health
1	Contaminated water (runoff)	It may contain high levels of nitrates and nitrites, causing hemoglobin disorders
2	Heavy metals	Heavy metals such as mercury, lead, cadmium, and uranium have been found in fertilizers, which can cause kidney, lung, and liver problems and cause cancer. More than 29 popular fertilizers tested positive for 22 toxic heavy metals, including silver, nickel, selenium, thallium, and vanadium, all directly related to human health risks
3	Exposure to ammonium nitrate causes	Exposure to ammonium nitrate causes other health problems, such as eye and skin irritation, which produce a burning sensation. Exposure by inhalation may cause irritation to the nose, throat, and lungs. You can also experience nausea, vomiting, redness of the face and neck, headache, nervousness, uncontrolled muscle movements, fainting and collapse
4	Potassium chloride	It interferes with nerve impulses and virtually interrupts all bodily functions and mainly affects the functioning of the heart. It can cause all types of stomach and stomach pain, dizziness, bloody diarrhea, convulsions, headaches, mental disorders, redness or itching of the skin or eyes
5	Cadmium	It eventually enters human tissues and produces diseases such as tracheobronchitis, pneumonia, pulmonary edema, kidney failure, osteoporosis, and many others. Lettuce (*Latuca sativa* L.), which is found in large quantities in countries such as Brazil and others in South America, very easily absorbs Cd, with an average quantity of 870 mg Cd/kg. Through the gastrointestinal system, they absorb only about 5% of the Cd through food. However, those who do not have iron can absorb up to 6% more. Therefore, this can be harmful to them because cadmium poisoning, which results from their excessive consumption, can cause damage to the kidneys, bones, and lungs. The “Itai-Itai” (it hurts, hurts, in Japanese), discovered in Japan in 1912, is caused by cadmium poisoning, which produces acute pain in the joints and the spine
6	Major health risks (Cancer)	It increase the risk of cancer death by more than six times, including brain cancer, lymphoma (non-Hodgkin’s lymphoma, NHL), prostate cancer, leukemia, and large bowel cancer
7	Methemoglobinemia	Methemoglobinemia is a blood disease in which an abnormal amount of methemoglobin, a form of hemoglobin, is produced. It is produced in place of essential hemoglobin, which is responsible for supplying oxygen to red blood cells. This causes difficulty in delivering sufficient oxygen to all body tissues and is related to birth and developmental disorders such as “blue baby syndrome”

upon the amount of consumption of fertilizers. Fertilizers remove essential nutrients from the soil, thus damages the soil and environment. The soil fertility decreases with the increasing use of fertilizers.

7.11 Why Biofertilizers?

Biological fertilizers more commonly known as biofertilizers that contain live soil microorganisms result in increasing the absorption and availability of mineral nutrients for plants (Vessey 2003). Their activities produce additional materials for plant growth and also support the soil ecosystem. They are available in the soil due to the interaction of microorganisms or its associations with plants and include organic fertilizers such as manure, etc. The seeds or soil inoculum multiply and participate in the nutrient cycles by the application of biofertilizers and increase the crop productivity. The process of nitrogen fixation, solubilization, or phosphate and potassium mineralization keeps the soil environment rich in all kinds of micro- and macronutrients by the application of biofertilizers, substances that are released by the plants regulate the growth, antibiotic production, and biodegradation of organic materials in soil which increases the tolerance to stress due to famine and humidity and also provides greater nutrient absorption.

The production of rice increases by the use of biofertilizers in comparison with chemical fertilizer. Thus, biofertilizers help to solve the problem of feeding growing world population when agriculture is facing several pressures and environmental changes by the use of chemical fertilizers.

7.12 Microbes as Biofertilizer

The microorganisms that have found application as a biofertilizer include *Rhizobium*, *Azospirillum*, *Cyanobacteria*, *Azolla*, liquid biofertilizers, *Azotobacter*, *Acetobacter*.

7.12.1 Rhizobium

Rhizobium, a symbiotic organism can easily inhabit the existing spaces in vegetable roots and its infection aids in fixation of atmospheric nitrogen. Their morphology and physiology will change from an open living condition to a bottom bacterium, they are the most productive biofertilizers based on the quantity of nitrogen deposited in question. They have seven genres and are very specific to shape the lump in vegetables, referred to as a cross-immunization group (Raja 2013).

7.12.2 *Azospirillum*

The essential pollutants of the soil are *Azospirillum lipoferum* and *A. brasilense*, the rhizosphere, and the intercellular spaces of the bark of the roots of herbaceous plants. They perform a harmonious cooperative connection with the grass. The microbes of the *Azospirillum* genus are creatures that alter nitrogen and are detached from the roots or other parts of the soil of a variability of plants and are gram negative, *Vibrio* or *Spirillum* which have an ample amount of poly-beta-hydroxybutyrate in the cytoplasm. To date, only five kinds of *Azospirillum* have been defined: *A. brasilense*, *A. lipoferum*, *A. amazonense*, *A. halopraeferens,* and *A. irakense*. The way of living is multiplied both in anaerobic conditions and in terms of oxygen consumption; however, it is above all the small scale aerofilia in the proximity or the non-occurrence of the nitrogen bond in the middle.

7.12.3 Cyanobacteria

Both cyanobacteria and free-living cooperatives have been affected by the development of rice in India. A composite of blue green algae with heterogeneous *Nostoc*, *Anabaena* and *Aulosira*, etc., is administered as an important inoculum in the plate and pots coated with polyethylene and then doubled in mass in the field used as soil chips for development of rice at the rate of 10 kg/ha.

7.12.4 *Azolla*

It is an aquatic fern that moves freely and floats in the surface of water and fixes the atmospheric nitrogen in relation to the blue green algae *Anabaena azollae*. The *Azolla* fronds are composed of sporophytes with a cabotage rhizome and bilobate leaves and roots that cover little. Rice development ranges in Southeast Asia and other underdeveloped nations have recently shown greater interest for the use of the valuable nitrogen *Azolla* sedimentation water plants as an another source of nitrogen or as a complement to commercial nitrogen fertilizers. *Azolla* is used as a biofertilizer for rice in wet areas and it is known to use 40 kg of N/ha at 60 kg N/ha for rice cultivation.

7.12.5 *Azotobacter*

It is imperative and certain that the high-impact nitrogen-free sedimentation bacteria are understood. In non-leguminous plants, it is used as a biofertilizer particularly in rice, cotton, vegetables, etc. *Azotobacter* cells are not present in the flat rhizosphere, but are abundant in the leguminous roots.

7.12.6 Acetobacter

This is a sacharophillic microbe and is associated with sugarcane, sweet potatoes, and sweet sorghum plants and can fix 30 kg/N/ha per year. Basically, the bacterium is marketed for the cultivation of sugarcane. It is known that it is used from 10 to 20 t/section of soil and the sugar content is about 10–15%.

7.13 Equipment Used in the Manufacture of Biofertilizer

In the field of biofertilizer creation, gear types (equipment and other manufacturing gadgets) represent the significant basis, which comprises 70% of capital assumption. Any negotiations on the usage of these kinds of equipment that are accompanied can finally reduce the nature of the biofertilizer. After observing the standards behind the use of all instruments, some part of the instruments can be changed by a company room equipped with a UV lamp. Hot air ovens, incubators, autoclaves, and fixing machines are manufactured locally with suitable specialized details. The correct use of the kinds of equipment will give a continuous demonstration with the quality inoculations.

7.14 Mechanism of Action of Numerous Biofertilizers

Mycorrhizal fungi are the association with the roots of the larger floors (Khanday et al. 2016; Bhat et al. 2017). While still a paradox, it assists as a model system for understanding the device behind stimulating radical cell growth as a result of the presence of mycorrhizae. Genome sequencing of two EMs (ectomycorrhizaes), bicolor L. *T. melanosporum* (Megali et al. 2013) and fungi (black truffle) (Molina-Favero et al. 2007), helps to identify factors that control the mycorrhizal development and its role in the plant cell (Bonfante and Genre 2010). Fifteen genes regulated during symbiosis were identified as putative hexose transporters in bicolor L. *T. melanosporum*. However, melanosporum has an invertase genetic factor and, unlike bicolor L., and can host sucrose directly (Bonfante and Genre 2010). Upregulation of carrier genes during symbiosis called transport action of beneficial complexes such as amino acids, oligopeptides, and polyamines through the symbiotic interface of one organism to another organism. The mycelium takes nitrates of the soil of life and ammonium for free. Subsequently, these compounds reach the soil and then transferred to the plants. A cysteine-rich protein (MISSP7) fungus plays an important role as effectors and mediators in the formation of symbiotic interfaces (Plett et al. 2011). Many genes that associated with the biosynthesis of auxins and root morphogenesis exhibited upregulation during mycorrhizal colonization (Ansari et al. 2013; Splivallo et al. 2009; Abdel-Raouf et al. 2012). Furthermore, *G. versi-*

forme transports of inorganic phosphate (Pi) in their hyphae that helps through absorption of soil phosphate and glutamine synthetase gene in *G. intraradices* found, which reinforces the opportunity of nitrogen metabolism in the fungal hifon that can be later available to the plant (Salvioli et al. 2012). It is suggested that bioactive compounds call for factors similar to *Rhizobium* Nod. Myc factors are secreted by mycorrhizae and *Rhizobium* and are perceived by the roots of host-activating signal transduction or by common symbiosis (SYM) (Kosuta 2003). The routes that prepare the plant for AM and rhizobium infection have some common characteristics. MGS commonly prepared by the host plant to produce changes at the molecular and anatomical level in the first contact of the fungal hyphae. Until now, it is assumed that calcium is the secondary messenger, increasing Ca^{2+} in the nuclear region of the absorbent hairs (Sieberer et al. 2009). *Leguminosarum biovarviciae* can induce *Rhizobium* various genes in plants (peas, alfalfa, and sugar beet) as shown by microarray studies (Ramachandran et al. 2011). The PGPR produces IAA, in turn, induces the production of nitric acid (NO), which serves as a second messenger to activate a complex signaling network that improves the processes of growth and development of the root (Molina-Favero et al. 2007). The expression of ENOD11 and many defense-related genes, as well as the root remodeling genes, is adjusted upward upon entry. Subsequently, this allows the formation of a pre-penetration device or PPA (Bucher et al. 2009). Once deposited, causes a decrease in arbuscular growth (Bapaume and Reinhardt 2012). It is known that many other genes, including the subtilisin 65 proteases, the transporter 66, or two phosphates of the transporter 67, are involved in the formation of arbusculi (Zhang et al. 2010). Today's scientists commonly use nitrogen fixer genes to create technical structures that can repair atmospheric nitrogen. The induction of genes in the case of nitrogen-fixing bacteria occurs at a low concentration of nitrogen and oxygen in the rhizosphere (Santos et al. 2012). Interestingly, sugarcane seedlings inoculated with a wild strain of *G. diazotrophicus* demonstrated radioactive N_2 fixation compared with *G. diazotrophicus* mutant having the mutant nif D gene demonstrated the importance of nif genes. The efficiency of nitrogen fixation depends on the use of carbon (Sevilla et al. 2001). A bacterium like *Bacillus subtilis* (UFLA285) can differentially induce 247 genes in cotton plants compared to the control in which PGPR was not supplied to the cotton plant (Baharlouei et al. 2011). Many disease resistance genes that work with jasmonate/ethylene signals, as well as osmotic regulation from proline synthesis genes, have been expressed differentially with the induction of UFLA285 (Baharlouei et al. 2011). Several differently uttered genes have been identified, including the type 1 metallothionein protein, an integral membrane protein similar to NOD26, ZmNIP2–1, a protein of the thionine family, a precursor of the gamma chain orycin, an associated protein to emphasize 1 (OsISAP1). They induce the PBZ1 and auxin protein and the probenazole genes that respond to ethylene (Brusamarello-Santos et al. 2012). It is also a well-established fact that the expression of the proteins related to the PBZ1 and thionine defense is suppressed in the seropedica association of rice-H, signifying the modulation of the defense responses of the plant in colonization (Brusamarello-Santos et al. 2012). Among PGPR species, it has been proposed that *Azospirillum* helps in regulation of gibberellins, auxins, and ethylene (Perrig et al. 2007). Some bacteria associated with plants can also induce the synthesis of phytohormones, as *Pinus contorta* when *Paenibacillus polymyxa* inoculated with IAA had elevated levels in roots (Bent et al. 2001). *Rhizobium* and *Bacillus* IAA can be synthesized under different growth conditions such as pH, temperature, and the presence of agricultural waste as a substrate (Sudha et al. 2012). Ethylene, unlike additional phytohormones, is accountable for inhibiting the growth of dicotyledonous plants (Ansari et al. 2013*)*. It was initiated by Glick et al. (1998), PGPR could improve the growth of plants by suppressing ethylene expression. Interestingly, a model in which, the synthesis of ethylene from 1-aminociclopropane-1-carboxylate (ACC), an immediate precursor of ethylene, from the bacterial enzyme ACC deaminase hydrolyzes the need for nitrogen and carbon which ultimately acts as the source. It is also one of the mechanisms of induction of good conditions for growth. ACC deaminase activity has also been found in bacteria such as *Alcaligenes* sp., *Bacillus pumilus, Pseudomonas sp.,* and the *variovorax paradox* (Ansari et al. 2013). The participation of ACC deaminase in the indirect influence on plant growth has been demonstrated in canola, with mutations in the CAC deaminase gene that has resulted in the loss of the growth in *Pseudomonas putida*. Interestingly, the potential for PGPR has been further increased by introducing genes involved in the direct oxidation (DO) and phosphate solubilization (MPS) pathways in some useful PGPR strains. The gene encoding glucose dehydrogenase (gcd) involved in the route of the DO has been cloned and characterized by *Acinetobacter calcoaceticus* and *E. coli* and *Enterobacter asburiae* (Tripura et al. 2007). A soluble form of gcd was also cloned by *Acinetobacter calcoaceticus* and *G. oxydans* (Sashidhar and Podile 2010). In addition, it has been reported that glucose directed to dehydrogenase (GDH) and gluconate dehydrogenase (GADH) mutagenesis increases the activity of this enzyme. The simple S771 M substitution conferred thermal stability to *E. coli*, whereas the 742 mutation of the lysine glutamate improved EDTA tolerance of *E. coli* PQQGDH. The application of this technology has been carried out through the transfer of genes involved in the OD pathway, namely GDH, GADH, and pyrroloquinoline quinone (PQQ) for rhizobacteria and phosphoenolpyruvate carboxylase (PPC) of *P. fluorescens*, offering MPS functionality (Sashidhar and Podile 2010) (Fig. 7.1).

The host roots perceived bioactive ligands called Myco factors and NOD factors secreted by the mycorrhiza as well as *Rhizobium* trigger via signal transduction (Kosuta 2003), which initiates an additional indication of transduction pathway via unknown receptor (SYMRK and NORK) (Bonfante and Genre 2010), that causes the release of Ca^{2+} in the cytosol (Sieberer et al. 2009). All pathways involve receptor kinases like DMI and phosphorylating their SYM71 protein substrates (Maillet et al. 2011). The nuclear pore complex (NPC) and some of its proteins (NUP) play a role in the addition of calcium. The DM1 proteins play a role in maintaining the periodic oscillation of calcium ions inside and outside the nucleus. Many protein channels (Ca^{2+} protein channels) also facilitate this process with different carriers (Sieberer et al. 2009). CCaMK is calcium-dependent calmodulin, which phosphorylates the CYCLOPS protein product, thus initiating the activation of several genes that involve the formation of structures such as nodule and pre-penetration apparatus (PPA) protein kinase (Maillet et al. 2011).

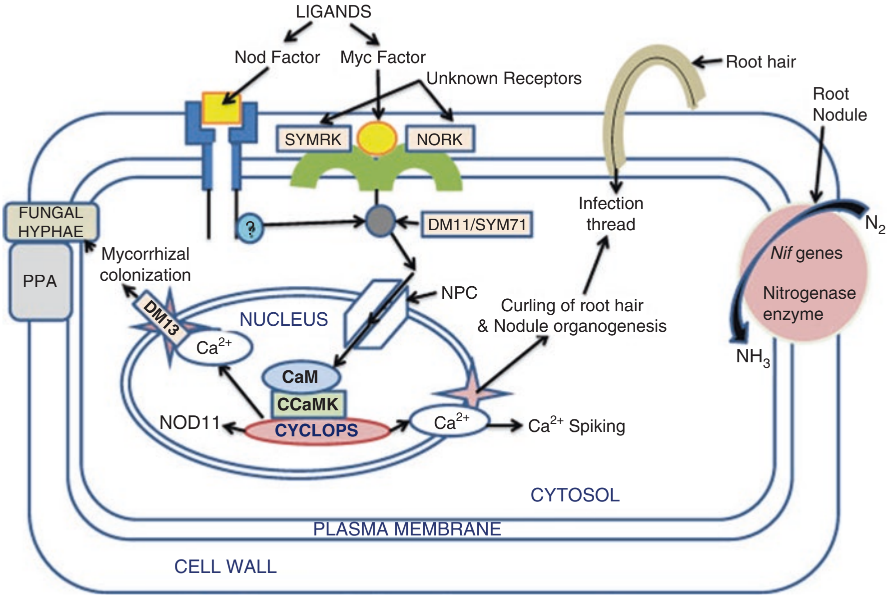

Fig. 7.1 Mechanism action of biofertilizers in the "root cell"

7.14.1 Mode of Action of Biofertilizer

1. They fix atmospheric nitrogen in the soil through the root nodules of the legume plants and make it accessible to the plant.
2. Solubilize the phosphate as tricalcium, iron, and aluminum phosphate that are available in insoluble form.
3. Produces hormones and antimetabolites that promote root growth.
4. Decompose organic matter.
5. By the application of biofertilizers on the soil and seeds, there is increase in the yield up to 10–20% and also an increase in the nutrients pertaining to plant growth without producing the adverse effects on the environment. Therefore, the height of the plant, the number of branches, the number of roots, the length of the root, the length of the shoot, the accumulation of dry matter in the plant organs significantly increase the growth parameters of the plant and the index of vigor, etc.

7.14.2 Application of Bio-Fertilizers

There are different four types of methods for the application of biofertilizers which are seed treatment, set treatment, seedling treatment, and soil treatment (Table 7.2).

Table 7.2 Methods and application of biofertilizers

S. no.	Method	Application
1	(a) The seeds needed to sow an acre in a pile are kept in a clean floor or cemetery (b) The culture suspension is prepared by mixing 1 pack (200 g) of biofertilizer in approx. 400 mL of water (1: 2) (c) The culture suspension is sprayed onto the seed stack and mixed by hand so that the thin coating is uniformly applied to the seeds (d) The seeds are spread in the shade for a while to dry and then sow (e) Instead of water, rice glue can also be used for better results	*Seed treatment* For the inculturation of cereals such as rice, wheat, sorghum, corn, etc. And oily seeds like peanuts, sunflower seeds, mustard, safflower, legumes such as cowpea, green gram, black gram, and soy. One pack (200 g) is sufficient to treat 10–12 kg of seed. On this basis, the dose of biofertilizer per acre can be calculated based on the seed rate
2	(a) The culture suspension is prepared by mixing 1 kg (five packs) of biofertilizer in 40–50 L of water (b) The cut pieces of sowing material that are required to sow one acre remain immersed in the suspension for 30 min (c) The cut pieces are removed and dried in the shade for some time before planting (d) After sowing, the field is watered in 24 h (e) For the setting treatment, the ratio of biofertilizer to water is about 1:50	*Set treatment* This method is generally recommended to treat sugarcane sets, chopped potato pieces, and the base of banana shoots
3	(a) The suspension is prepared by mixing 1 kg (5 packs) of biofertilizer culture in 10–15 L of water (b) The necessary seedlings are obtained for one hectare and small packages of seedlings are made (c) The root portion of these seedlings is immersed in the suspension for 15–30 min and immediately transplanted (d) In general, the proportion of inocula and water is 1:10 (approximately), i.e., 1 kg of biological fertilizer in 10 L of water (e) For vegetables like tomato, cabbage, cauliflower, one pack of biofertilizer is sufficient for 0.1 ha (10,000 m^2) of soil	Seedling treatment This method is recommended for crops such as rice, tobacco, tomato, cold, onion, cabbage, cauliflower, etc.
4	*Soil application* This method varies from one crop to another depending on its duration. In general, for a short-term harvest (<6 months), 10–15 packets (each of 200 g) are mixed with 40–60 kg of well-decomposed cattle manure or 40–60 kg of soil for an acre of earth. The mixture of biofertilizer and manure of livestock/soil sprayed with water is then transmitted to the soil at the time of sowing or at the time of irrigation in permanent crops. For long-term crops (perennial crops), 20–30 packs of biofertilizer (each with 200 g) are mixed with 80–120 kg of manure or soil per acre	For its application in permanent crops, perennials are pruned once a year. After pruning, the soil of the bed is dug up with a fork with due care to avoid damaging the roots. Then a mixture of biofertilizer and FYM/soil is applied, incorporating it into the soil, followed by irrigation

7.15 Advantages of Synthetic Fertilizers

7.15.1 Improve Soil Quality and Add Micronutrient Benefits to the Soil

Organic fertilizers improve the soil structure and soil quality. The creation of compost with a balanced combination of cooking residues, garden pruning, rodent dung, and straw encourages microorganisms to grow and enrich the soil. The vegetables produced are healthier and more beneficial for the body.

7.15.2 Organic Fertilizers Are Slow-Release

A lot of compost can take longer. It also releases its nutrients slowly. However, it does this constantly. Its nutrients are also more useful and stimulate the growth of healthier plants.

7.15.3 They Are Not Toxic

Chemicals in synthetic fibers are toxic, acidic and can burn the roots of seedlings. Organic fertilizers, without toxins, have a neutral pH level and do not produce this effect.

7.15.4 They Are Cheap

These fertilizers are also less expensive. Creating compost, with natural and organic ingredients from your kitchen, does not cost much. In contrast, some synthetic fertilizers are quite expensive.

With this persuasive drive to use organic fertilizers, it is prime time to start composting.

7.16 Advantages of Using Biofertilizers

Some of the advantages associated with the use of biofertilizers include:

- They are ecological and profitable
- Improves the soil enrichment and soil quality over time

- Although these fertilizers do not show immediate results, the results shown after some time are spectacular
- These fertilizers directly use atmospheric nitrogen and makes it available to plants
- Increasing the dissolution of phosphorus content of the soil and releasing the unavailable phosphorus
- Biofertilizers help to release the growth-promoting hormones and improve root proliferation
- The microorganism converts compound nutrients into simple nutrients available for plant growth
- Microorganisms that are present in biofertilizers promote the adequate supply of nutrients to host plants and to ensure its proper development of growth and regulation in its physiology
- Help increase the crop yields by 10–25%
- Biofertilizers can protect plants from ailments transmitted through the soil to some extent

7.17 Conclusion

Biofertilizers contain microorganisms that increase soil microbial activity. Often, organic matter is included to enhance the rate of production. And since "soil fertility is decreasing in most soils throughout the world due to soil erosion, loss of nutrition, accumulation of toxic elements, unbalanced accumulation of water and nutrients." Compost and organic fertilizers are alternatives to meet the need for nutrient sources for crops. Microbes which have the capacity in the production of biofertilizers include *Azotobacter*, *Azospirillum*, *Phosphobacter,* and *Rhizobacter*. Biofertilizers have great importance in agricultural production. They play a fundamental role in maintaining soil sustainability and long-term fertility by fixing atmospheric nitrogen, thus mobilizing macro- and micronutrients fixed in the soil. In this context, the excessive use and dependence on chemical fertilizers are not feasible on the long-term basis because of the costs and other negative implications on environment. In this context, the biofertilizers would be the feasible option for farmers to increase in productivity per unit area without harming the quality of the environment.

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Chapter 8
Role of Macrophytes in Spontaneous Lacustrine Phytofiltration

Umar Nazir Bhat and Anisa B. Khan

8.1 Introduction

Aquatic plants mainly comprise hydrophytes like phytoplankton, periphyton and multicellular macrophytes occupying varied ecological niches in the water environment. Majorly fresh water vascular plants and macroalgae collectively form macrophytes (Wetzel 2001). Macrophytes consist principally of aquatic vascular flowering plants but also include the aquatic mosses, liverworts, ferns and the larger macroalgae (Chambers et al. 2008). Macrophytes being predominant primary production sources play an important role in energy input, nutrient budgeting and recycling (Algesten et al. 2003), biofiltration (Dhote 2007) and sedimentation processes in the water bodies. Macrophytes are ecologically dominant community of fresh water bodies especially lakes performing basic functions of energy and oxygen production, biogeochemical cycling, regulation of water quality, sediment stabilization and furnishing habitat to micro and macro water-organisms (Gurnell et al. 2012; Bhat et al. 2018). They influence hydrochemical processes and serve as substrate for periphyton and phytophilous invertebrates thus creating specific ecological conditions necessary for functioning of other aquatic biota (Raspopov et al. 2002; Vymazal 2002). Invertebrates and macrofauna including fish and waterfowl find them as suitable breeding, nesting and sheltering niches (Cvetkovic et al. 2010; Grabas et al. 2012; Jurca et al. 2012; Sierszen et al. 2012). The provision to act as fodder and green manure is an option too. Eutrophication is associated with transition from macrophyte dominated clear water state to turbid phytoplankton dominated state (Scheffer 1989; Bhat et al. 2017) and loss of submerged macrophytes (Körner 2002; Xiangcan 2003). Aquatic weeds scavenge inorganic and some organic compounds from water, incorporating the dissolved materials into their own structure and as such act as 'biological sinks' (Scholz 2003) for large

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quantities of minerals which could otherwise cause ‘algal blooms’ (Mushtaq et al. 2020). The presence and dominant assemblages of macrophytes reflect differences in trophic status, therefore, are very good indicators of water quality. Macrophytes have been evaluated not only for stripping nutrients (Gottschall et al. 2007) but also in altering physicochemical environment of waters (Bhat et al. 2018). For instance, nutrient assimilation during photosynthetic productivity reflects alkaline pH (Hart et al. 2002). The relationship between water column nutrient concentrations and macrophyte growth is extensively documented (Moore and Wetzel, 2000). Harpole et al. (2011) studied colimitation of N and P for productivity, while Carrow et al. (2001) observed growth patterns at nutrient gradients exhibiting growth limited at low, improved at higher and toxic at the highest levels allied to osmotic stress and pH primarily. The quality and quantity of vegetation facilitate insight of environmental state (Lenzi et al. 2012). Vegetation reflects lower C:P and C:N in eutrophied waters. Either due to low physiological requirement or tolerance for nutrients, macrophytic group shifts occur in water bodies as the opportunistic species prevail (Lenzi et al. 2012).

‘Green Clean’—a popular term for phytoremediation is an acceptable and eco-friendly in situ alternative to conventional remediation approaches (Rai 2008). It can be an ecologically responsible practice to detoxify, remove, sequester and stabilize persistent pollutants in aesthetic manner (Meagher, 2000) effectively in water bodies (Peng et al. 2009). This plant based removal, stabilization, transfer or decomposition technology utilizes natural or GM flora in varied ecosystems in eco-restoration programmes (Amaya-Chavez et al. 2006; Padmavathiamma and Li 2007; Tian et al. 2007). The widespread application of plants as toxic site (land, water or air) cleaners of all pollutant sorts of organic and inorganic nature are spearheading from lab to field and market place (Ibañez et al. 2016). Phytofiltration uses plants to remove contaminants from water (Arthur et al. 2005). Despite the nuisance character of aquatic plants, their capability to improve water quality has ecological significance for pollution abatement (Qadri and Bhat 2020). A growth (metabolic) property of aquatic macrophytes removes pollutants (Hasan et al. 2007; Mishra et al. 2008) as the knowledge range of cleanup duly applies on aquatic and semi-aquatic vascular species (Bhatia and Goyal 2014) like *Azolla*, *Carex, Ceratophyllum*, *Cyperus*, *Eichhornia*, *Elodea*, *Glyceria*, *Hydrocotyle*, *Juncus*, *Lemna*, *Phragmites*, *Pistia*, *Potamogeton*, *Sagittaria*, *Scirpus*, *Spartina*, *Typha* and the like. Hydrophytes entrap nutrients in the water column both by physical obstruction (biomass adsorption) and by uptake from soil solution (Lukács et al. 2009). Yang et al. (2007) list pollution tolerance, faster growth and high harvestable biomass as positive indices for a good phytoremediator. The frequent abundance of macrophytes in aquatic ecosystems prescribes them as preferred and potent bioagents for pollution abatement and biomonitoring. The phytoremediation technology addresses the global problem of pollution in air, water and soil. The macrophytic role in purification of aquatic ecosystems involves removal, detoxification or stabilization of contaminants in their growth matrix, viz. water and sediment (Becker 2000). Phytoextraction possesses several appealing attributes compared to the traditional established methods of remediation. It is natural, spontaneous, efficient both in situ and ex situ, eco-friendly, aesthetically pleasing, cost effective, solar driven, sustainable, least site invasive, applicable on large scale, demands minimal maintenance and amenable to amelioration of a broad range of contaminants (Lasat 2000; Morikawa and Erkin 2003; Tian et al. 2007). Further, phytoremediation offers maintenance of natural biochemical activity and geophysical structure along with sites' self-restoration. The bioaccumulation property of wetland plants has resulted in their widespread use as bioindicators and as bioremediative agents (Qian et al. 1999; Samecka-Cymerman and Kempers 2002). So the overall complex structural and functional integrity imparted to an aquatic ecosystem, indicative of its environmental quality conditions is regulated by macrophytes. Henceforth, an endeavour is made in the current study to evaluate the comparative spontaneous efficacy of unlike life-form macrophytes in the phytofiltration of nutrients and traces for their in situ role of self-remediative lacustrine function.

8.2 Methods

The city of Srinagar (33°59′14″–34°12′37" N latitudes and 74°41′06″–74°57′27″ E longitudes) sited on the banks of the Jhelum (*Vyeth*) spreads across the plains of Kashmir vale. It has a moderate physiography ≥1580 m a.s.l. representing a hill topography. The general climatic conditions resemble the sub-Mediterranean characterized by yearlong precipitation episodes except a few summer and autumn dry-periods, besides, both seasonal and diurnal extremes of temperature. The focuses of present limnological research remain two fluvial urban valley lakes, namely Anchar and Dal (average depth of 1.5 m) situated in the summer capital (Srinagar) of Jammu and Kashmir. A preliminary comparison indicates the Anchar as unmanaged, while the Dal as fairly managed lake with substantial differences in their physical appearance and open-water surface area (Fig. 8.1).

However, both the lakes are eutrophied and majorly engulfed in urban and agricultural transformations as evident from satellite imagery (Fig. 8.2). The inter-annual survey, sample collection and hydrobiochemical study of the two lakes are based on their five select stations. The five locations designated as A1, A2, D1, D2 and D3 were each further fragmented into ten sampling stations (indicative as red spots in Fig. 8.3) in order to obtain composite representative samples in triplicate for enhanced precision. The site selection criterion implemented here represents the distinct spatial features within the lakes. A1 adjoins the urban locale highly infested with vegetation and diffuse sewage inputs; A2 is situated closer to the lakes' exit and recipient of run-off from paddy fields; D1 vicinity is contributed with key inlet and STP discharges; D2 typifies the floating garden area; and D3 nearby the outlet has hotel and house-boat zone.

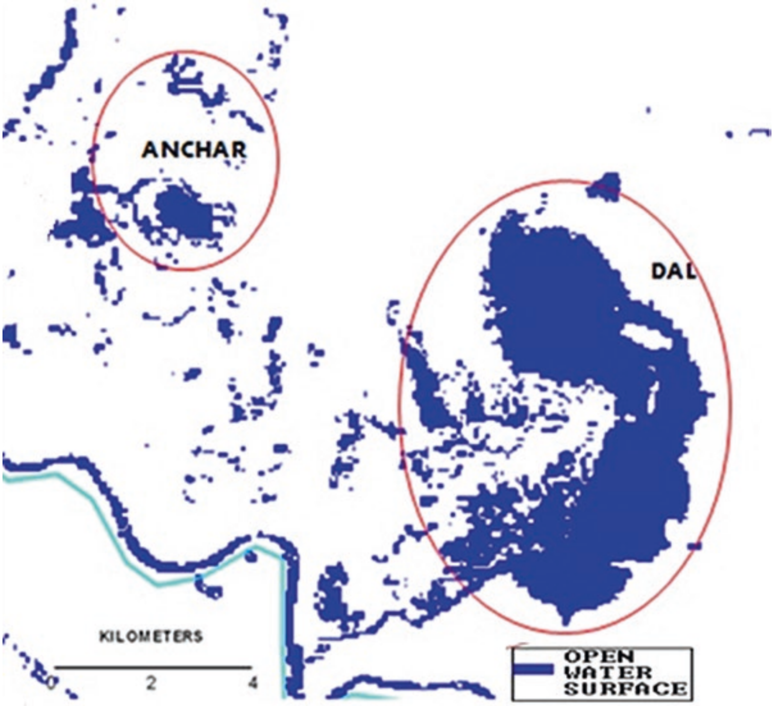

Fig. 8.1 Open water expanse of the study sites (Source: NRSC/ISRO—Bhuvan)

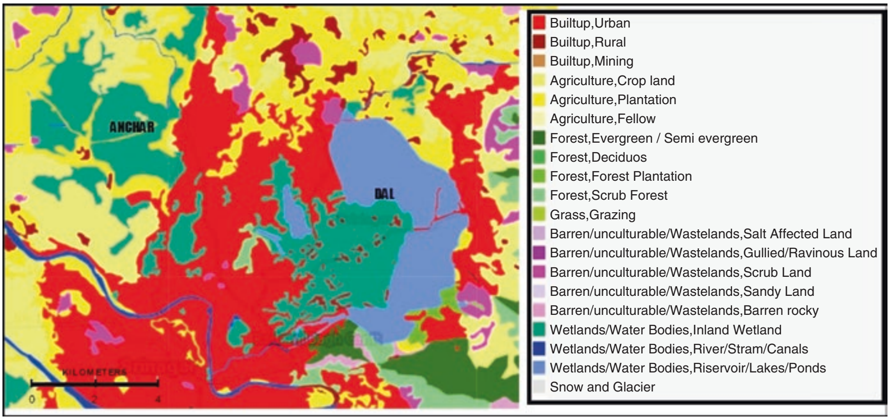

Fig. 8.2 Visualization of land-cover/use for study site catchment (Source: NRSC/ISRO—Bhuvan)

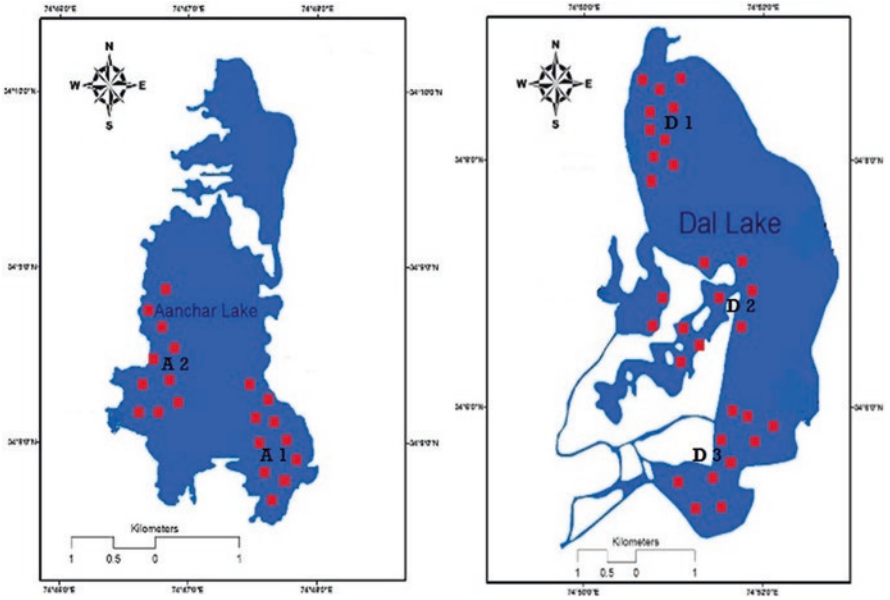

Fig. 8.3 Sample collection stations

8.3 Macrophytes

The biological component of macrophytes employed in the study includes select species chosen from emergent, free-floating, rooted-floating and submerged categories, namely *Myriophyllum aquaticum*, *Salvinia natans*, *Nelumbo nucifera* and *Ceratophyllum demersum,* respectively. Their selection represents omnipresent abundance at each sampling location besides being high biomass yielding, eutrophication tolerant and hyperaccumulators. The preferred producer species are recognized while referring to standard taxonomic scheme of Cook (1996), Ghosh (2005) and Arshid et al. (2011). A brief account is provided herewith species wise indicating taxonomic and morphological aspects.

8.3.1 Myriophyllum aquaticum *(Parrot-Feather Watermilfoil)*

- Family = Haloragaceae
- Genus = Myriophyllum
- Species = aquaticum

Commonly referred to as ‘Parrot-feather Watermilfoil’, *Myriophyllum aquaticum* is an amphibious, invasive, herbaceous and perennial rooted shallow water plant with more emergent than submerged shoots (Arshid et al. 2011). Its feather-like pinnate leaves appear whorled on an elongated stem (40–90 cm) growing into a population of dense stands. It is a tolerant heterophyllous colonizer of diverse habitat ranging from fresh to saline waters exhibiting phenoplasticity in response to extreme environmental gradients (Trémolières 2004; Wersal et al. 2011). Its growth is chiefly regulated with temperature and light conditions. It shows vegetative reproduction via fragmentation of shoots.

8.3.2 Salvinia natans *(Floating-Moss or -Fern)*

- Family = Salviniaceae
- Genus = Salvinia
- Species = natans

Floating-moss is a noxious annual perennial weed. It is a deciduous, free-floating, rootless and clonal aquatic fern emerging on water surface. It has horizontal stem with opposite hair covered water resistant leaves (1–3 cm). Amongst its leaf whorl of three, two elliptic leaves perform the floating function, while the profoundly dissected third one submerges to perform as a root alike. Its strong ability to duplicate mats of foliage makes it capable to engulf all the water. Its sexual reproduction occurs after a winter diapause by fertilization of male microspore and female macrospore on the water surface at temperatures >12.4 ± 0.2 °C (Gałka and Szmeja 2013). The spores release gradually from the reproductive organs called sporocarps lying at the bottom as their walls disintegrate. The resultant zygote develops into a structurally clonal sporophyte. Again the vegetative means entail the mature clone fragmentation.

8.3.3 Nelumbo nucifera *(Asian Lotus)*

- Family = Nelumbonaceae
- Genus = Nelumbo
- Species = nucifera

Lotus is an attractive ornamental and sacred aquatic plant associated with Indian cultural heritage besides being accorded the status of National Flower. Its phenotypic diversity and thermoplasticity grants it wide naturalization globally (Yang et al. 2013). It is a rooted-floating type perennial macrophyte possessing ≥3 cm long petioles. The leaves are orbicular, palmate but slightly concave, peltate and tough leathery structured with waxy cuticle (glaucous) on their upper side. The circular leaf blades range from 10 to 100 cm in diameter. Its flowering period is June to September displaying large (10–25 cm across) solitary bisexual pinkish-red blossoms. Its fruit called torus is an enlarged receptacle enclosing embedded seeds.

It multiplies by seed and vegetative rhizome propagation. It has commercial, medicinal and pollution management utility in addition to palatable flowers, leaves, petioles, rhizomes and seeds (Sinha et al. 2000). It can tolerate acidic conditions but grows luxuriantly in alkaline nutrient rich waters.

8.3.4 Ceratophyllum demersum *(Hornwort or Coontail)*

- Family = Ceratophyllaceae
- Genus = Ceratophyllum
- Species = demersum

Ceratophyllum demersum is commonly referred to either as 'Coontail' due to its resemblance to a racoon's tail or 'Hornwort' attributed to its horny plant appearance. It has a brittle branched body bearing long, thin and flexible stem (1–3 m) with nodes and internodes. Its leaves (8–40 mm) are flat, forked, vertical, denticulate at the apex, whorled (6–12 leaves) and more crowded near tip of stem. It is a monoecious plant owning both male and female flowers (2 mm long). The spiny fruit (a small nut) about 5 mm long contains a single seed. It is a rootless submerged free-floating herb but can develop rhizoids (modified leaves) to anchor. Calm, deep and permanent waters serve as its habitat. It is a fast grower nutrient sponge (Khang et al. 2012) in semi-hard or hard waters. It is almost undemanding for light levels and CO_2 supplements. It is known to combat phytoplankton and blue-green algae (cyanobacteria) by producing allelochemicals. This invasive plant shows proliferated growth and dense mat formation (Keskinkan et al. 2004). It develops turions—the buds forming new plants in spring after residing at the lakebed during winters.

8.4 Bioassays

The survey, sample collection and limnological analysis of macrophytes, sediment and water from selected sites of the two lakes were carried out from March, 2011 to February, 2013. For the purpose of present research, macrophytes, sediment and water are collected simultaneously at the same sampling locations. Aquatic plant samples are obtained manually on growth-phase basis during the stretch of the annual macrophytic progress from emergence to decay distributed into three durations: (1) March to May as sprouting (sp.), (2) June to October as peak growth (pg.) and (3) November to February as senescence (sn.) phases. Quadrat method (Havens et al. 2004; Gunn et al. 2010) is followed for macrophytic sample collection. Hooks and Ekman dredge are used in case of the submerged plants falling within a floatable quadrat. The specific bioassays of macrophytes include:

8.4.1 Photosynthetic Pigments

Photosynthetic pigments, viz. total chlorophyll, are estimated in 1 g fresh weight of macrophytic sample applying Sadasivam and Manikam (2005) procedure. The isolated pigment extracts' colour intensity is measured in visible region on Elico SL-27 spectrophotometer and the chlorophyll content measured according to the equation:

$$\text{Total Chlorophyll}\left(\text{mg g}^{-1}\right)=\left[6.45\times A_{665}+17.72\times A_{649}\right]/1000\times V/W$$

where V = volume of the total extract (50 mL); W = fresh weight of plant material (1 g) and A_{649}/A_{665} are optical densities of the extract at 649 nm and 665 nm.

8.4.2 Biomass, Productivity, NPP, Turnover and Growth Rate

Biomass of the selected species is estimated by harvest method (Johnson and Newman 2011) as dry weight per unit area (1 m^2 quadrat) after drying adherent free collection in an oven at 80 °C for 24 h to attain constant weight. At this temperature besides averting thermal decomposition, complete moisture removal ensures insistent reference value (Mishra et al. 2008; Peng et al. 2009). The values expressed as g m^{-2} denote mean biomass for five random quadrats for each species. The rate of production is measured as a net positive increment in dry biomass at seasonal intervals by deducting the initial biomass value (B_1) at time, t_1 from final biomass value (B_2) at time, t_2.

Therefore, productivity (g m^{-2} day^{-1}) is change in biomass (ΔB) between the time interval (Δt), i.e., productivity (g m^{-2} day^{-1}) = $\Delta B/\Delta t = B_2 - B_1/t_2 - t_1$. Net primary production (NPP) for each species is calculated by employing Caçador et al. (2007) formula: NPP (g m^{-2}) = [Maximum Biomass − Minimum Biomass]. Turnover period of every species is the ratio of its biomass parameters of NPP and maximum dry weight biomass (Duarte et al. 2010), viz. Turnover Rate = NPP (g m^{-2})/ Maximum Biomass (g m^{-2}). And growth rate describes weight gain in a definite phase interim relative to mean weight figures (APHA 2005), i.e., growth rate (day^{-1}) = Weight Gain, g/Time Interval, d × 1/Mean Weight, g where mean weight = [initial weight, g ÷ final weight, g ÷ 2].

8.4.3 BCF, Removal Potential and Element Turnover

Bioconcentration factor (BCF) designates apportionment fraction of an element concentration in biomass at harvest, E_B and initial element concentration in its medium, E_M (Wang et al. 2008; Nguyen et al. 2011) given by:

BCF = E_B/E_M. BCF exceeding the reference value of 1 implies hyperaccumulation. Nutrient storage [macro- and micro-elements in mg while traces' in µg] is defined as the product of harvest biomass 'B' and plant nutrient quantum 'C_N' per unit area 'A' (Wu et al. 2011), i.e., nutrient storage = $B \times C_N / A$. So, a plant's element composition, C (mg g^{-1} or μg g^{-1}) multiplied by the yield, Y (g m^{-2}) gives removal potential (mg m^{-2} or μg m^{-2}) of a specific species. Therefore, removal potential = $Y \times C$. Element turnover is given as the quotient of two element accumulation parameters (Duarte et al. 2010), i.e., g of net primary accumulation (NPA) and the g of element pool (EP). EP can provide effective instantaneous amount of metal retention as the same stands as sample biomass = $^{[\text{BIOMASS}]}{}_t$ times the metal content per gram = $^{[\text{METAL}]}{}_t$. So, element turnover = NPA/EP where NPA = maximum metal pool minus minimum metal pool and EP = $^{[\text{BIOMASS}]}{}_t \times {}^{[\text{METAL}]}{}_t$.

Besides, the water samples were analysed for temperature, pH, conductivity, bicarbonates, chloride, calcium, magnesium, sodium, potassium, nitrate nitrogen and total phosphorus on monthly basis, while trace elements of Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn were measured for spring, summer and winter seasons. The collection, preservation, preparation, storage and estimation of water samples follow standard methods of APHA (2005), Radojevic and Bashkin (2006) and Estefan et al. (2013). The sediment analysis includes H-ion concentration, electrical conductivity, bicarbonates, organic-C, organic matter, total-N, C/N ratio, elemental composition of Ca, Cl, K, Mg, Na, P, S, Si and the given trace elements for each season. Again, the macrophytic biomass were analysed for mineral composition (Ca, Cl, K, Mg, N, Na, P, S, Si) and the trace elements. For plant and sediment samples, the common parametric evaluations are performed following Gupta (2004), Ryan et al. (2007), Radojevic and Bashkin (2006) and Estefan et al. (2013). The multi-elemental quantification of Ca, Cl, K, Mg, Na, P, S and Si in sediments and plant biomass is performed using wavelength dispersive X-ray fluorescence spectrometer (WD-XRF) at central instrumentation facility (CIF), Pondicherry University. However, the trace elements in all samples were determined at sophisticated analytical instrument facility (SAIF) IITM, Chennai using ICP-OES (Perkin Elmer Optima 5300 DV) instrument. Further, the descriptive and illustrative statistical analysis is performed using MS-Excel 2010, PAST 3 and SPSS 19.

8.5 Findings

The biochemical assays of select macrophytes for suitable parameters carried out in tandem with water and sediment associates divulge their cohesive and codependent networking. A marked difference in mean total chlorophyll content (mg g^{-1}) of fresh weight across the hydrophyte species and their growth phases of sprouting (sp.), peak growth (pg.) and senescence (sn.) is shown in Fig. 8.4. The maximum total chlorophyll concentration is noted in *Ceratophyllum demersum* (0.92 mg g^{-1}) during its peak growth at site D3, while a minimum 0.02 mg g^{-1} is recorded both in case of *Myriophyllum aquaticum* at site A2 and *Salvinia natans* at site D3 throughout senescence. The individual ranges of chlorophyll content (mg g^{-1}) for *Myriophyllum*

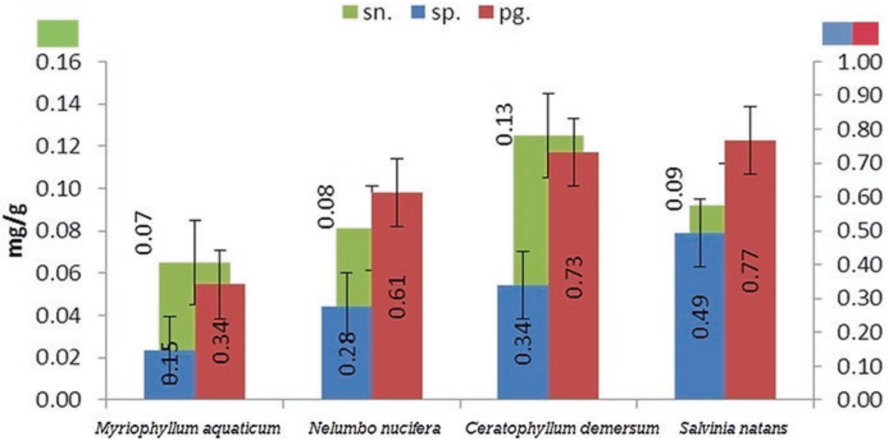

Fig. 8.4 Average total chlorophyll concentration of the macrophytes. *sn.* senescence phase, *sp.* sprouting phase, *pg.* peak growth phase

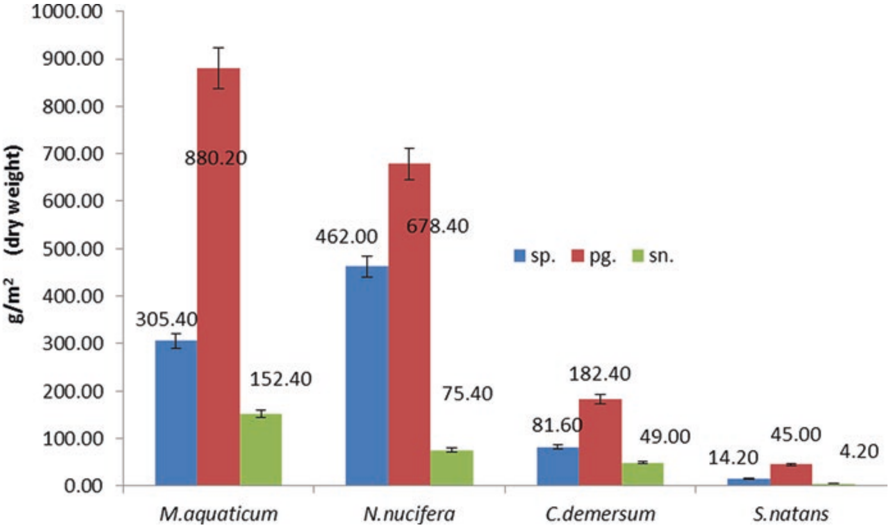

Fig. 8.5 Average biomass values (g m^{-2} dry weight). *sp.* sprouting phase, *pg.* peak growth phase, *sn.* senescence phase

aquaticum, *Nelumbo nucifera*, *Ceratophyllum demersum* and *Salvinia natans* include 0.02–0.37, 0.05–0.72, 0.10–0.92 and 0.02–0.87, respectively.

The productive capacity of the investigated macrophytes calculated on the basis of dry weight mean biomass (g m^{-2}) for each experimental species in the three major growth phases is presented in Fig. 8.5. The biomass calculations reflect subsequent ranges for each species as 107–960 g m^{-2} for *Myriophyllum aquaticum*, 66–750 g m^{-2} for *Nelumbo nucifera*, 39–231 g m^{-2} for *Ceratophyllum* *demersum* and 1–55 g m^{-2} for *Salvinia natans*. *Myriophyllum aquaticum* (880.2 g m^{-2}) dominated *Nelumbo nucifera* (678.4 g m^{-2}), *Ceratophyllum demersum* (182.4 g m^{-2}) and *Salvinia natans* (45 g m^{-2}) in terms of mean peak dry weight biomass values. A similar fashion low occurred in dry weight (g m^{-2}) during their senescence period ranking by way of 152.4 > 75.4 > 49 > 4.2, respectively.

The percentage contribution of various life-form classes among the studied macrophytes towards production (dry weight biomass, g m^{-2}) as well as specific percent relative difference during sprouting, peak growth and senescence phases is described in pie-chart diagrams accessible in Fig. 8.6.

Other biomass parameters like productivity, net primary productivity and specific growth rate establish uniform variations in the experimental species of the order: emergent *M. aquaticum* > rooted-floating *N. nucifera* > submerged *C. demersum* > free-floating *S. natans* but species turnover is highest in case of *S. natans* and lowest for *C. demersum* as illustrated in (Table 8.1).

The average tissue concentration of analysed elements fluctuated not only between the select species (Figs. 8.7 and 8.8) but also depicted growth-phase variations within the same species. However, no significant site contrasts were noticed.

The ANOVA test conducted between the species ('df' = 11) for the mineral composition of macrophytes conveys significance for Cu ($p < 0.05$), K ($p < 0.001$), Na ($p < 0.05$), Se ($p < 0.01$) and Si ($p < 0.01$) only, whereas ANOVA for their growth phases ('df' = 11) reflect significance in case of Al ($p < 0.05$), As ($p < 0.001$), Ca ($p < 0.05$), Co ($p < 0.05$), Cr ($p < 0.05$), Fe ($p < 0.05$), Mg ($p < 0.05$), Mn ($p < 0.001$), Ni ($p < 0.01$), Pb ($p < 0.01$) and Zn ($p < 0.001$).

The bioconcentration factor (BCF) criterion adopted indicates hyperaccumulation phenomenon for most of the micro- and trace-elements in *C. demersum* and *S. natans* except for Pb, Sn and Zn, and *M. aquaticum* hyperaccumulates Cd and Mn while *N. nucifera* Mn only. The removal potential of the macrophytes clearly stipulates species-specific variation (Figs. 8.9 and 8.10) ensuring a generalized arrangement of emergent *M. aquaticum* > rooted-floating *N. nucifera* > submerged

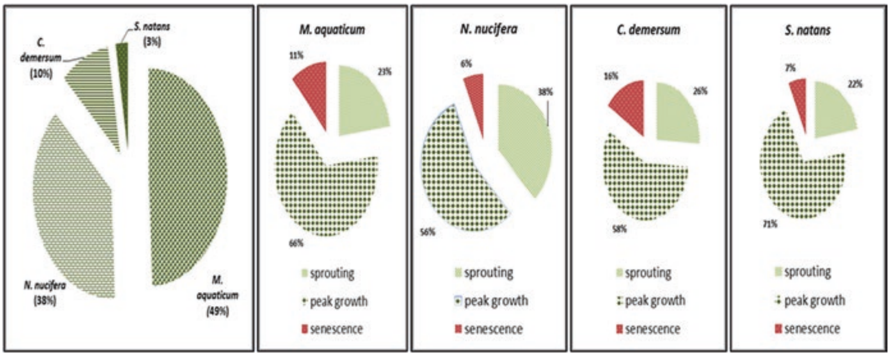

Fig. 8.6 Comparative of percent dry weight biomass (g m^{-2}) across growth phases and species

Table 8.1 Trends in productivity, NPP, specific growth rate and species turnover

Biomass parameter	*M. aquaticum*	*N. nucifera*	*C. demersum*	*S. natans*
Productivity (g m^{-2} day^{-1})	4.78 ± 0.32	1.81 ± 0.73	0.84 ± 0.24	0.26 ± 0.07
NPP (g m^{-2})	853	684	192	54
Specific growth rate (day^{-1})	29.68 ± 8.13	7.39 ± 2.18	3.96 ± 1.64	1.83 ± 0.87
Species turnover	0.89	0.91	0.83	0.98

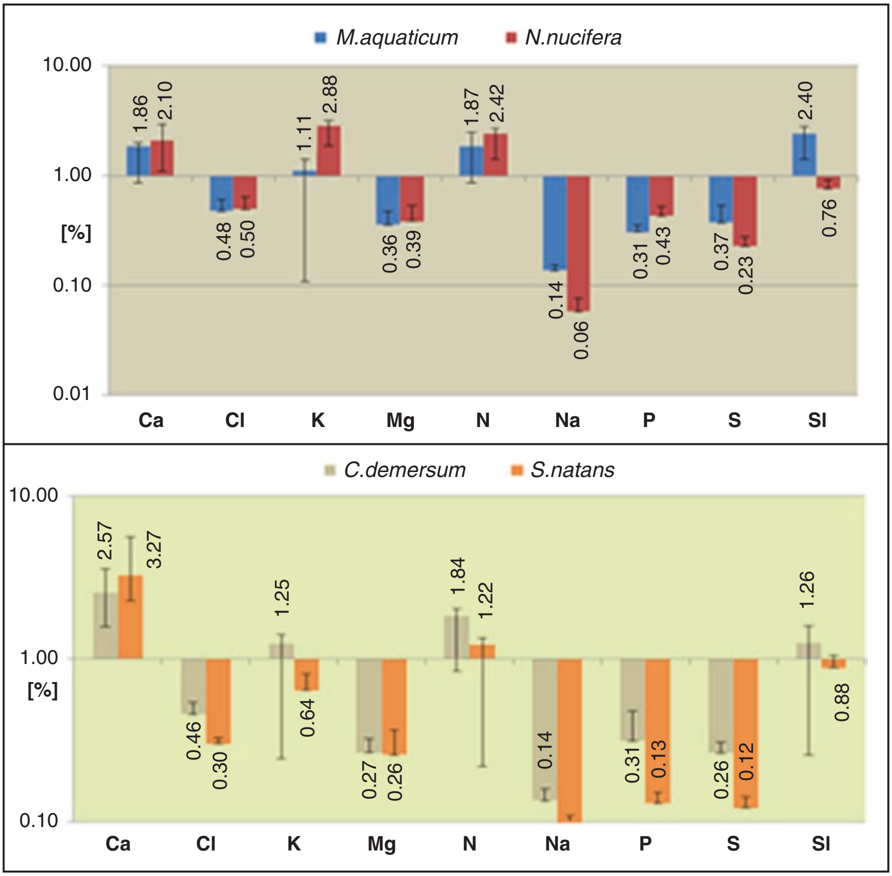

Fig. 8.7 Average elemental bioconcentration (%) in *M. aquaticum*, *N. nucifera*, *C. demersum* and *S. natans*

C. demersum > free-floating *S. natans*. Nevertheless, element-wise removal potential of each studied macrophyte for accumulation of (a) nutrients and (b) traces, respectively, follow the following pattern:

1. *M. aquaticum*: (a) Si > N > Ca > K > S > Cl > Mg > P > Na and (b) Fe > Al > Mn > Zn > Cu > Cr > Ni > As > Co > Sn > Pb > Se > Cd.

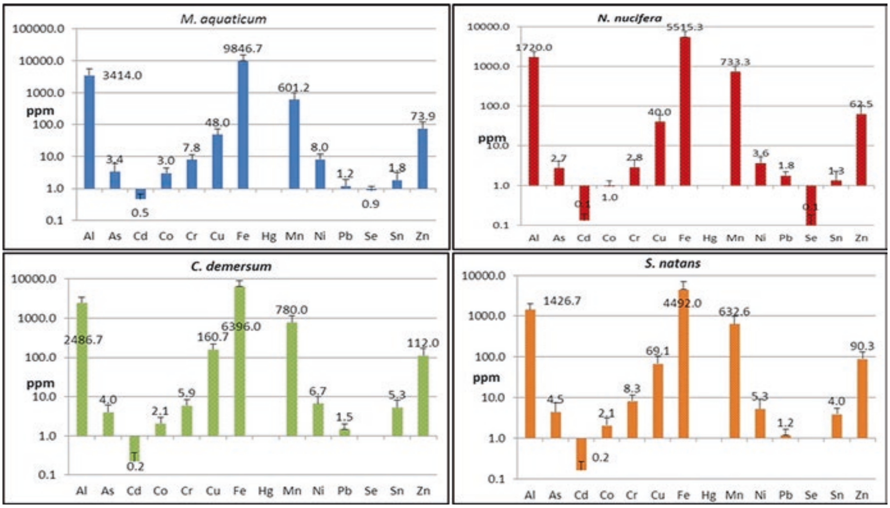

Fig. 8.8 Mean trace element bioaccumulation (ppm)

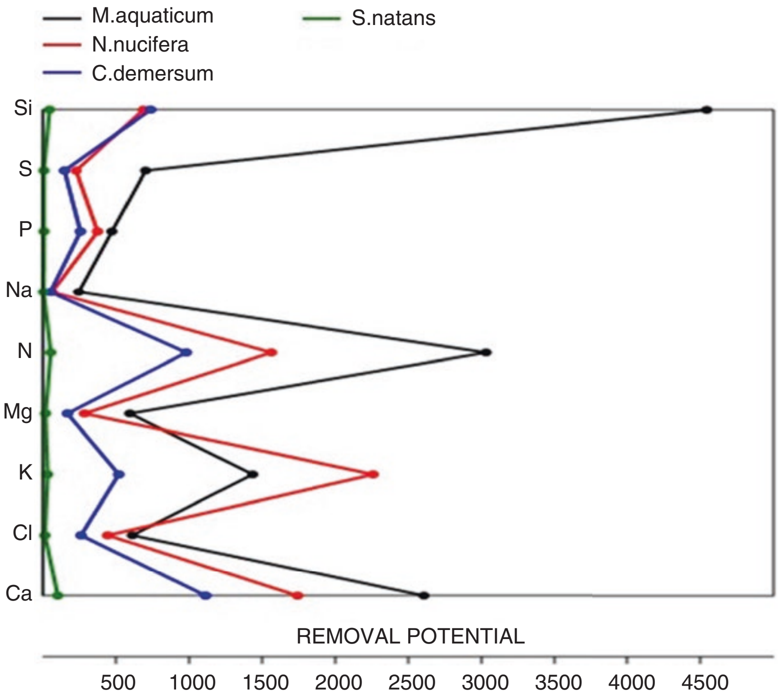

Fig. 8.9 Removal potential for major or minor elements (mg m^{-2})

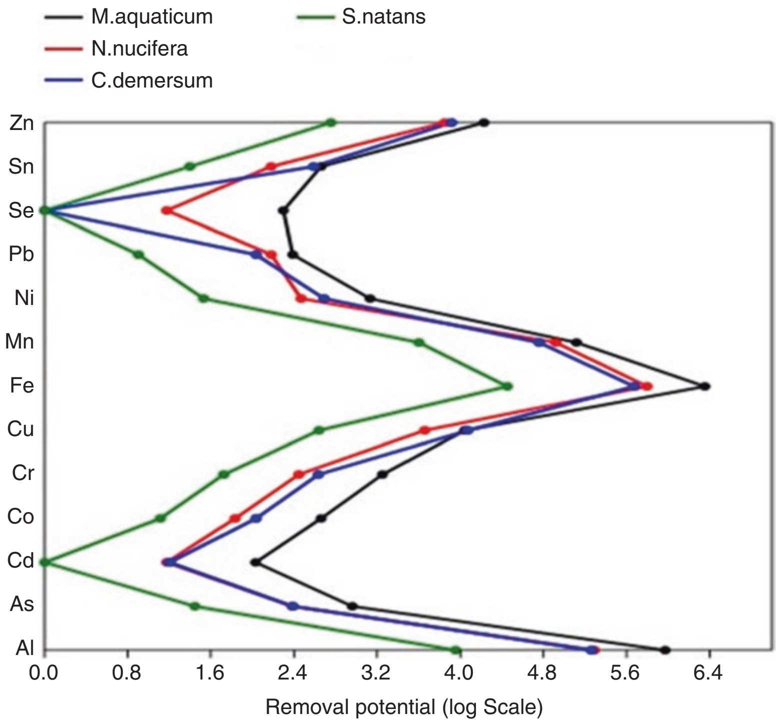

Fig. 8.10 Removal potential for traces (μg m^{-2})

2. *N. nucifera*: (a) K > Ca > N > Si > Cl > P > Mg > S > Na and (b) Fe > Al > Mn > Zn > Cu > Ni > Cr > As > Pb > Sn > Co > Cd > Se.
3. *C. demersum*: (a) Ca > N > Si > K > Cl > P > Mg > S > Na and (b) Fe > Al > Mn > Cu > Zn > Ni > Cr > Sn > As > Co > Pb > Cd > Se.
4. *S. natans*: (a) Ca > N > Si > K > Mg > Cl > P > S > Na and (b) Fe > Al > Mn > Zn > Cu > Cr > Ni > As > Sn > Co > Pb > Cd > Se.

Lastly, significant element turnover rate is observed for almost all minerals except Mg and Na in case of *M. aquaticum*. However, *N. nucifera* has significant turnover for Ca, Cl, Mg, As, Cd, Co, Cr, Cu, Ni, Pb, Se, Sn and Zn. Similarly, less significance is reflected for K, Mg, Na, Al and Se turnover by *C. demersum*. And *S. natans* has lesser turnover rate for Mg, N and Se only.

8.6 Discussion

The phyto-assessment of aquatic environments involving vascular plants determine phytostimulation as well as phytotoxicity due to nutrient inputs from sewage, sediments and catchment surface run-off and toxins from commercial chemicals, industrial effluents, agrochemical and municipal wastes, hazardous wastes, etc., respectively. The in situ biomonitoring technique utilizing vascular aquatic vegetation gives information about environmental quality and plant responses to toxins and nutrients. The various parameters of study during phyto-assessment include measurement of biomass and pigment content, determination of bioaccumulation pattern, productivity, physiological effects and community composition. All of these diagnostic attributes reflect the trophic status and effect of all sources and types of contaminants present in the study area. The selected biomonitors among the macrophyte community manifest both contribution (command) and subservience to its abiotic allies. Macrophytes dominate mineral regulation between sediment and water (Kissoon et al. 2013). They are capable to remove nutrients even at low loading rates (Gottschall et al. 2007). Their adaptive advantage to lock up minerals from nutrient pool is helpful to check degradation primarily and restore mineral loading later on (Ismail et al. 2014). N and P are intimately related to biological productivity of aquatic ecosystems (Kalff 2002). The productivity also lies in consonance with developmental stage, ambient nutrient medium and physiognomy of a species (El-Otify 2015). Higher temperature favours bio-production and elevates carbonate driven pH significantly (Hasler et al. 2016). Among the evaluated species *M. aquaticum* accumulated 49% of the dry weight biomass annually, whereas *S. natans* contributed only 3% but it outdid in species turnover comparison. Biomass parameters like dry weight, productivity, NPP and specific growth rate establish similar variations in the experimental species but species turnover is highest in case of *S. natans* and lowest for *C. demersum.* Substantial OM productions by macrophytes contribute towards nutrient immobilization and provide OC requirement for denitrification while decaying (Lin et al. 2002; McElarney et al. 2010). The upgraded biomass configuration property establishes an additional nutrient and trace element interception feature of hyperaccumulation from the growth medium (Shaltout et al. 2014).

Macrophytes are tested ecological indicators when compared against watershed (Søndergaard et al. 2010, 2013), catchment development (Sass et al. 2010) and even climate (Beck et al. 2014) in order to validate ecosystem changes. Pertinently submergeds are least pollution and stress resistant species (Pulido et al. 2011; Sierszen et al. 2012). Environmental variations are more influential compared to interspecific competition in governing the community structure (Grabas et al. 2012). A combination of limnochemical and physicomorphic factors of water bodies describes the macrophyte community characterization (Schneider et al. 2015) but nitrate commonly influences their growth, height and biomass profile as against ammonium supplements (Wersal and Madsen 2011). The gaining momentum for comprehending phyto-nutrient dynamics and their implications for water quality management or eco-restoration is a subject of site specific (habitat) application of a suitable phytoremediator (Udeigwe et al. 2015). A number of determinants like light, temperature, redox, pH, solubility, water flow, chelation of metal compound, metal concentration, metal species, exposure duration and cation exchange capacity altogether affect extent and rate of bioaccumulation for decontamination. The mere presence of nutrients is not the condition for rising or reducing uptake of toxic metals (Göthberg et al.

2004). Though the solubility of target element is not a problem in aqueous media but its bioavailability matters which consequently controls plant accumulation and translocation (Zaier et al. 2010). The peak nutrient uptake and bioconcentration coincided with peak biomass in summer and autumn (Garbey et al. 2004). The diminishing conductivity at improved temperature conditions suggest higher bioaccumulation rate of nutrients (Liu et al. 2014). The structural (physiognomy) difference between the species and the micro-habitat occupied within aquatic ecosystem are major factors related to bioconcentration of various elements (Lukács et al. 2015). The removal potential pattern of the emergent accrued more Si, the rooted floating retained greater K, while the rootless submersed and free floating ones amassed Ca. Although the quantum of removal potential for different elements in the analysed species is divergent but the pattern is related which suggests unselective absorption (Sarwar et al. 2010). The elemental turnover rates in select macrophytes closer to the reference value of 1 are significant. The vascular aquatic plants possess higher productivity (C:N and C:P ratios) with organic polymer body capacitating their slowed decomposition that improves nutrient and C capture potential and as a result net autotrophy (Cotner et al. 2009). The concurrence of peak bioaccumulation and biomass production empowers the concept of high yield harvesting (Quilliam et al. 2015). The harvesting of macrophyte biomass suitably during peak growth phase is perfect to avoid nutrient and metal remobilization from belowground vegetative parts or during senescence leaching on ultimate OM mineralization (Wang et al. 2015). Similarly, a mono annual harvesting practice improves height, shoot density and biomass of macrophytes, although leading to nominal increment for mass TN and TP removal rates (Zheng et al. 2015). The ANOVA comparing the species shows uptake preference mode for Cu, K, Na, Se and Si indicating specific role in the unlike macrophytes (Sistla et al. 2015). Contrarily, the growth-phase variance for Al, As, Ca, Co, Cr, Fe, Mg, Mn, Ni, Pb and Zn possibly implies their short-term raised presence or bioavailability during the annual developmental cycle (Wiik et al. 2015). BCF criterion indicates hyperaccumulation for most of the metals in case of *C. demersum* and *S. natans*. The BCF apportionment clearly summarize *M. aquaticum* and *N. nucifera* to dissipate the PTE in their rhizosphere zone but the rootless *C. demersum* and *S. natans* concentrate them in their foliage parts (Harguinteguy et al. 2014).

Anthropogenic disturbances arising from land-use change, altered global biogeochemistry and biodiversity interferences are clearly reflected by community structure and function with macrophytic communities being no exception. The ecological disturbances experienced by freshwater biota vary in magnitude, frequency, predictability, duration and on spatial-scale as well (Lake et al. 2000). Macrophytes react to the ambient physicochemical environment changes not only by altering species composition but also by displaying plant biomass variations. Their presence, density and morphology are determined by factors like sediment type, water turbidity, water current, nutrient concentration, water depth, shoreline disturbance, herbivore grazing and human activities (Bornette and Puijalon 2011; Wood et al. 2012). They exhibit a stress response of depletion in diversity (genetic erosion) associated with predominantly excessive proliferation of a selected few (Michelan et al. 2010; Chappuis et al. 2011). Although the tissue concentration of nutrients and trace elements differs species wise but well correlates with ambient water and sediment media (Amari et al. 2014). The biological sinks scavenge the deleterious proportions of nutrient supply from lakes preventing them from being overtaken by blooms. Even if slight phyto-extraction of inorganics would have occurred physically compared to large-scale quanta of the in situ media, still the biogenic calcification evidenced in the summers is capable of lowering them mediated via temperature, pH, HCO_3^- and Ca changes (Boyd et al. 2016). Hence, a selective and scaled deweeding practice can thereby work wonders in lake management and restoration programmes (Novak and Chambers 2014).

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Chapter 9
Phytoremediation of Heavy Metals Using Salix (Willows)

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9.1 Introduction

Due to demotechnic growth, naturally occurring potential toxic elements in soils recorded an upward trend, subsequent to which these areas have been rendered contaminated (Singh et al. 2018). To quote, increased pyrite ore roasting in Torviscova area leads to contamination which creates various effects on living entities and their health aspects (Moosavi and Seghatoleslami 2013). In fact such areas remain devoid of vegetation which cause soil degradation and pollution off-site (Ali et al. 2013). High bioavailability is the other problem of industrial pollutants, contrary to metalloids are naturally present in soils which may not be available for the use of plant (Park et al. 2011).

Phytoremediation is the implementation of plants for the degradation and decontamination of soils (Bhat et al. 2018) and render them harmless. In fact phytoremediation is a low cost alternative remediation technique (Vishnoi and Srivastava, 2008) which is environmentally acceptable. Since this technique is not toxicant specific, hence phytoremediation could be used for many pollutants (Moosavi and Seghatoleslami 2013).

The present chapter aims at the extent of phytoremediation of *Salix* spp. which grows on the polluted soils under natural factors.

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9.2 Ecology of *Salix alba*

Salix spp. has about 450 species distributed all over the globe but mostly dominated in the Northern Hemisphere (Argus 1997). They are mostly found in temperate and arctic zones. However, willows have also been recorded from tropical and subtropical areas which comprises of trees, shrubs and groundcovers. Geographically they are found in every continent of the world with the exception of Antarctica and Australia. Of the total flora available, trees offer a costless, eco-friendly and sustainable sound solution for phytoremediation of trace metal contaminated land. Moreover, the use of plants such as willow and poplar species with trace metal contamination have also been found to be promising in phytoremediation as they accumulate Cd and Zn in stem. So, poplar and willows could be used for harvesting not only the nutrients but also the heavy metals as they:

1. Are easy to propagate with an extensive root system (Aasamaa et al. 2010) with high evapotranspiration (Kowalik and Randerson 1994) that can stabilize pollutants
2. Can thrive on soils with polluted conditions (Justin et al. 2010)
3. Have high biomass production (Kowalik and Randerson 1994)
4. Can resist the reasonable extent of PTE in the roots (Borišev et al. 2009).

Realizing their importance in heavy metal harvesting, Belarus planted willow trees for the phytostabilization and phytoremediation of land with polluted soils, in mining landscapes and on sanitary landfills. But in India, studies on the potential of phytoremediation have remained restricted under the field conditions. Vervaeke et al. (2004) premeditated that the degraded sediments with raised hydraulical aspects are viable with *Salix* species for phytoextraction or stabilization of sediment disposal sites. Needless to mention poplar and willow offer better mechanisms for the effective dexotification of overall metal compositions to a less effective level. A paramount concern with planting trees for metal defiled soils will be the potential impacts with respect to biological community to remove metals.

Lebrun et al. (2017) observed that *Salix alba* and *Salix viminalis* that have been raised on arsenic and lead concentrated mine technosol concentrate higher concentrations of arsenic and lead in the underground system compared to aboveground parts. Since such soils have extreme pH, poor nutrient content and high PTE concentrations, it has been established that application of organic amendment like Biochar improves the nutrient value of soil through an increase in pH, dissolved organic carbon (Beesley et al. 2010), nutrient availability (Agegnehu et al. 2016), water retention capacity (Omondi et al. 2016), electrical conductivity (Kloss et al. 2014), plant productivity (Hossain et al. 2010) and decrease in greenhouse gas emission (Janus et al. 2015) while decreasing bulk density.

Moreover, branches of Salix and related species revealed comparatively elevated contents of elements at early stages of development, as they uptake higher nutrients, while the effect of dilution resulted in very less levels of metals till the flowering stage. Needless to mention the natural process of senescence often resulted in an augment in the absorption of metals in the vegetation due to loss of fluid.

9.3 *Salix* and Ecological Niches

The willows and poplar trees are referred to colonize new environments, for example, marshes, sand rises and rock bars, roadside trench, deserted farming fields, railways, old mine tailings and rock pits just as glaciated or overwhelmed territories (Argus 1986; Skvortsov 1999) because of their capacity to create harmonious relationship with mycorrhizal parasites that use phosphorus and natural nitrogen (Schramm 1966; Lodge 1989; van der Heijden and Kuyper 2003), yearly generation of various seeds and a successful arrangement of seed dispersal for long separation travel (light weight and hair connection of willow seed), expanding the odds of finding an opening for germination and development; foundation into vegetation holes supporting those plants that can endure full daylight (willows are not adjusted to shade) and high relative development rate with moderately short future (Raven 1992). Needless to say that Salix needs a situation with consistent dampness supply for quick seed germination (Dorn 1976) as seeds of Salix are feasible for just half a month (McLeod and McPherson 1973; Maroder et al. 2000). So moisture or water accessibility is a constraining element in districts with dry conditions in pre-summer. The invasion of disturbed sites by Salix trees serve to recuperate the harmed environments by expanding surface shade, action of roots and humus formation for the improvement of soil structure and soil productivity (Stott 1992).

One of the most significant ecological niche for Salix are the wetlands that are currently disappearing at a very fast rate due to urbanization, encroachment, soil erosion, ill planned development, industrial growth, disposal of wastes, etc. Hence there is a need for the conservation and mitigation of altered wetlands due to anthropogenic intervention and subsequently *Salix* sps., which have a high significance for wildlife, providing rich habitat and food for diverse organisms (Kennedy and Southwood 1984; Hightshoe 1998; Sommerville 1992). The invertebrate herbivores from aphids to caterpillars nourish on willows, and maintain a large food web of complex trophic level organisms. Animals, viz. livestock, caribou, muskrats deer, elk, moose and, porcupines, etc., depend on willows for food and shelter like beavers and birds (Bates 1951; Smith et al. 1978). Salix species thrive well near aquatic ecosystems especially at wetland sites (Fig. 9.1a, b).

9.4 Land Reclamation

Since land surfaces have been degraded by human interferences hence there is an urgent need to address the same. Beyond all doubts, the stabilization of ecosystems degraded by chemicals could be achieved by using Salix species, which have a tendency to survive in environmentally disrupted areas owing to their drought resistance (Logan 1992), less nutrient requirements and capability to bud, root, and sprout in extreme conditions like degraded soils with scarce top soil, extremely eroded soils, waste areas and roadsides (Hartwright 1960; FAO 1979; White 1992; Bungart and Huttl 2001), heavy metal tolerance (Eltrop et al. 1991; Sennerby-

Fig. 9.1 (**a**) *Salix alba* in a wetland near Degree College Bijbehara. (**b**) *Salix alba* growing in wetland near Railway station Srinagar

Forsse et al. 1993; Punshon and Dickinson 1997), etc. Since willows inhabit open areas (Schramm 1966; Clewell 1999) in a spatially random fashion that cannot be predicted, the strategic plantation of willows into degraded landscapes has been found to restore such lands more quickly. For the introduction of plant cover, willows in the forms of mats, stakes, rooted cuttings and stumps with root masses could be used for reclamation and management of wastelands.

9.5 Phytoremediation

Owing to immense potential of Salix in nutrient harvesting and phytoremediation, extensive researches across globe are underway for establishing its degraded lands through synthetic chemical and metabolic processes like phytoextraction (the expulsion of overwhelming metals from land because of the plants' take-up and translocation of metals into aboveground organs), phytodegradation (the aggregation and biochemical change of natural contaminations by plants and related microorganisms), rhizostimulation and rhizofiltration (the expulsion of poisons from fluid arrangements through direct take-up by plants roots) and phytostabilization (substrate drying out and counteractive action of toxin transport). As willows are quickly developing with broad root framework other than having capacity to amass target poisons, *Salix* is the most favoured plant for phytoremediation.

9.6 Phytoextraction

Voluminous literature has emanated on the confrontation of willows to some metals (Cd, Cu, Fe Ni, Pb, Zn) and its ability to decontaminate the polluted soils (Dickinson et al. 1994; Landberg and Greger 1994; Punshon and Dickinson 1997; Ali et al. 1999; Greger and Landberg 1999; Robinson et al. 2000; Klang-Westin and Perttu 2002; Watson et al. 2003; Ali et al. 2003; Keller et al. 2003; Klang-Westin and Eriksson 2003; Lunackova et al. 2003; Kuzovkina et al. 2004). Although heavy metal resistance in *Salix* has been elaborated by many workers but the biochemical mechanisms for tolerance of metals is yet to be examined. Ali et al. (2003) revealed that tolerance of metal in Salix may be due to phytochelatin synthesis. Riddell-Black (1994) and Pulford et al. (2002) observed that different clones and species of willow have variable metal translocation patterns and confrontation to heavy metals. Landberg and Greger (2002) projected that metal tolerance of some clones may be due to variation in lipid peroxidation and enzyme concentration against the levels of metal levels. But Eriksson and Ledin (1999) suggested that there is a need of more exploration to be carried out to optimize the uptake of metals through replicas and management alternatives. Although new willow clones are introduced by plant breeders but the potential impact of metal contamination on vegetation needs to be monitored as it can have devastating effects on ecosystem in general and food web in particular (Mertens et al. 2001; Granel et al. 2002; Watson et al. 2003). Nevertheless, role of *Salix* in phytoremediation has been well-documented technique that can be used as a remedy for highly polluted sites and exhausted farmlands (Perttu and Kowalik 1997; Pulford et al. 2002).

9.7 Phytodegradation

While the capacity of poplars to expel a huge range of natural pollutants by assimilation into the roots of plant is very much recorded, new research demonstrates an ideal effect of *Salix* spp. on the fate of natural contaminations. Prairie Cascade willow (Salix x' Prairie Cascade') has demonstrated overwhelming development on darkened soil created by an oil slick and capacity of soil decontamination through activation of oil-degrading microorganisms related with their root system (Thompson 1998). *Salix* spp. are required to be cultivated in oil-mining territories in the Siberian taiga. *Salix* spp. in degraded areas without soil amendments immediately shaped green cover increased the removal of synthetic chemicals contrasted with exposed land soils. Quicker sequestration of metals than in past endeavours, the plants, when planted on cost effective imported soil layer, had been noticed. It is evident that critical abatement of 57% in mineral oil focus in the land having willow species instead of 15% on neglected plots, occurred on disposal sites for dredged sediment (Vervaeke et al. 2004). The capability of willows' to transport oxygen by root zone through aerenchyma is known to provide better conditions to bacterial development. An investigation of willow plantation on the soil near landfill (Maurice et al. 1999) has demonstrated that those destinations are having more oxidation rates for methane contrasted with soils having no vegetation. Trees with broad root systems will give a superior domain to methane oxidizing microscopic organisms accordingly lowering its outflow into the air. Promising outcomes in remediation of ethanol-mixed pollutants utilizing willow and its resistance to expanded degrees of ethanol, has been accounted for (Corseuil and Moreno 2001). In a research centre examination, cuttings of sobbing willow (*Salix babylonica*) had the option to diminish ethanol and benzene focus in watery arrangement by over 99% in fewer than 7 days through root take-up and plant biomass sorption. It was proposed that the take off by plants was essentially identified with the plant's transpiration. Willows show conceivable utility for the remediation of sites polluted with cyanide mixes. Transport and digestion of cyanide and ferrocyanide by willow has added to the debasement of these contaminants in the wastewater from gold mining (Ebbs et al. 2003). Cyanides (glycosides) are produced by most of the plants for chemical defence and plant cells by and large have a high ability to wipe out free cyanide, as it is a well-established fact that willow roots and leaves have the quickest rates of cyanide removal capacity (Larsen et al. 2004). High biomass generation and proficient control on water in soils, because of high rates of evapotranspiration makes willow a reasonable potential "bioreactor" for cyanide expulsion.

9.8 Rhizofiltration and Rhizostimulation

Recently, in America and Eurasia the utilization of constructed wetlands for the removal of pollutants is gaining much importance. A need is felt to incorporate vegetation filters to enhance nitrogen removal due to hydraulic mass flux by root zone technology. *Salix* sps have been utilized in these types of ecosystems that have reduced the pollution load to greater extent and hence these plants are considered a good source for improving the quality of effluent before being drained into any aquatic system (Martin and Reddy 1997). *Salix* sps is a potential tool for improving the quality of domestic, industrial, municipal wastewater and agricultural runoff (Elowson and Christersson 1994; Kirt 1994; Perttu and Kowalik 1997; Rosenqvist et al. 1997). The ability of Willow spp. having high rates of evapotranspiration, effective nutrient uptake, tolerance of flooded conditions and better biomass productivity are some of the physiological characteristics of *Salix* sps. that make it potential candidate as vegetation filters.

Salix alba has a potential to transport oxygen deep down the root zone via aerenchyma formation that will contribute to suitable conditions for bacterial growth. They have a composite biomass that provides lignin for composting operations and hence used for wetland construction.

9.9 Phytostabilization

The drying of swampy soils by Salix is quite old and well known among farmers. Most of the farmers in malaria affected areas prefer to plant Salix trees as it helps them to dry the accumulated water very quickly that reduces the chances of malaria in these areas. The introduction of willow vegetation buffer zones for coping waste dumping sites has been successfully applied in phytostabilization projects. A general argument is that strategic planting and positioning of willow around the contaminated area will help to decrease the water entering landfill. The type of Salix vegetation may also solve the problems of landfill as most of these species are tolerant to landfill sites.

The planting of trees will be a best remedy to keep away the linked pollutants from soil, because of diminishment of surface water percolating into the groundwater. Planting of *Salix* on municipal sewage deposits enhances the nitrification processes and contaminants in the sludge apparently do not affect the growth of willows (Scheirlink et al. 1996; Wielgosz 2000). The process of nitrification will be enhanced by planting more *Salix* trees on municipal sewage deposits that will decrease contaminants to great level. Extensive root systems and copious litter fall stabilize soil structure, augment cycling of nutrient and endorse biotic communities and creation of forest microclimate.

9.10 *Salix alba* and High Metal Biomagnifications

Pulford et al. (2002) argued that due to luxuriant biomass productivity *Salix* sps have performed goof in the acidic medium of soil in terms of heavy metal accumulation in their shoots, although no benefit was observed by addition of elemental sulphur. However, Cd and Zn levels were highly accumulated in leaves than in stems.

Hinchman et al. (1995) demonstrated that approximately 9.5% of the lead and 0.1% of the arsenic were removed by willows, respectively, from heavy metal contaminated sites. However, newly planted willow trees removed only 1% and 0.1% of the available lead and arsenic, respectively, from the same soil. Similarly, when the experiments were conducted in sand culture amended with 40% of the lead and Arsenic, willows were able to remove 30–40% of the lead and Arsenic from it.

Pulford et al. (2002) argued that one group of *Salix* sps has very less amount of Ni and Cu in the bark and very high Cd and Zn in the wood, with better survival rates and high productivity rates. On the other hand, other group of willow had relatively good content of Ni and Cu in the bark and very little Cd and Zn in the wood and these *Salix* sps were very poor in terms of endurance and biomass production. This is an indication that different factors play an important role in accumulating heavy metals.

Vandecasteele et al. (2005) advocated that willow foliar Cd concentrations in *Salix* sps were highly associated with soil and soil water Cd levels. Both clones exhibited very high accumulation levels of Cd and Zn in shoot system. Roots accumulated Cu, Cr, Pb, Fe, Mn and Ni. Bioaccumulation factors of Cd and Zn in the leaves were the maximum for the treatments with the least soil Cd and Zn levels.

Vervaeke et al. (2004) showed that Cu and Pb, but not Cd, were more available in the root zone by amending it with water and ammonium acetate extraction when compared with the bulk sediment. Sediment of the root zone was better structured and aggregated and thus more porous for downward flow of water that caused leaching of a fraction of the metals and significantly lower total contents of Cd, Cu and Pb.

Vyslouzilova (2003) found that As and Cd uptake enhanced in Suchdol-Zn soil compared to Suchdol-Pb soil very slowly and the element removal from soil was significantly high in Suchdol-Pb soil due to a significant decrease in biomass yield in Suchdol-Zn soil. The yield reduction had decreased the uptake of plant-available elements by biomass which indicates that higher plant-available portions of As and Cd were present in Suchdol-Zn soil.

Mleczek et al. (2010) revealed significant differences among the clones of Salix. The clones most effective in concentrating metals were *S. viminalis* ‘1154’ and ‘1054’. The *Salix* population under study was considerably varied as regards to accumulation efficiency. The removal efficiency in terms of different heavy metals was Cd 84%, Cu 90%, Hg 167%, Pb 190% and Zn 36%.

Marius et al. (2017) presented the impact of simultaneous variations of lead and magnesium levels on growth of radicular and leaf system of *Salix* alba that were cultured in hydroponic cultures. Hydroponic solutions were amended with lead and magnesium in equal concentrations. Willow offshoots were placed in hydroponic solutions having different types of water with varied levels of pollutants for a period of 35 days. The evolution of leaves and roots number in time showed that phytotoxicity limit for simultaneous adding of lead and magnesium ranges from 0.1 % to 0.5 % (w / w).

Popoviciu and Ticuţa (2018) reported highest average concentrations of heavy metals in *Salix alba* L. species: 8.89 mg/kg Cu, 43.94 mg/kg Mn and 94.10 mg/kg Zn, values that are not consistent with hyperaccumulation. *Salix alba* L. was the only species with BAC > 1 for Cu (constant in all samples) and Zn (only as average value).

Bajraktari et al. 2019 determined that the content different heavy metals in *Salix alba*. The different pollutants in water and soil samples are above maximum permissible level and it is recommended that *Salix alba* plantations will reduce the pollutant load (Table 9.1).

Table 9.1 Phytoremediation characteristics of *Salix* sp.

Salix sp.	Metal(s)	Results	Author(s)
Salix alba	Cu, Zn and Mn	Highest accumulation of heavy metals	Popoviciu and Ticuţa (2018)
Salix alba	Pb and Mn	Reduced concentration in hydroponic system	Marius et al. (2017)
S. viminalis x miyabeana, *S eriocephala*	As	Total As accumulation was greater	Puckett et al. (2012)
S. geyeriana, *S. drummondiana*, *S. planifolia*	Cd, Cu, Pb and Zn	Metal concentration accumulated in roots	Meiman et al. (2012)
S. Purpurea, *S. alba*, *S. fragilis*, *S. japonica*	Cd, Co, Cr, Cu, Ni, Pb and Zn	Salix sps accumulated more and other species were effective	Mleczek et al. (2010)
S. Purpurea, *S. alba*, *S. fragilis*, *S. japonica*, *S. nigra*	Cd, Co, Cr, Cu, Ni, Pb and Zn	In general, highest concentration of these metals	Mleczek et al. (2009)
S. Purpurea, *S. eriocephala*, *S. Sachaliensis*	Zn, Mn, Fe, Cu and Al	More Zn, Fe and Al were accumulated than populous	Zalesny and Bauer (2007)
S. Purpurea, *S. viminalis x miyabeana*, *S. Sachaliensis*	AS	By amendment in soil with phosphate As accumulation increased	Purdy and Smart (2008)
S. geyeriana, *S. monticola*	Cd, Mn and Pb	*S. monticola* is better for Cd accumulation as compared to *S. geyeriana*	Boyter et al. (2009)
S. geyeriana, *S. monticola*	Mn and Zn	Good for Mn resistance and better for phytostabilization	Shanahan et al. (2007)
S. nigra	Mn, Ni and Fe	Metals were found to be present in almost all parts	Punshon et al. (2005)
Salix sps	Cd and Zn	These metals were found more in leaves than in stems	Pulford et al. (2002)

9.11 Conclusions

Heavy metals are a cause of concern both for the terrestrial and aquatic ecosystems. The removal of heavy metals from these ecosystems is a priority of experts as heavy metals may render these ecosystems unsuitable for the functioning for which they are meant. Different methods have been tried to recover heavy metals from these ecosystems and phytoremediation is considered to be a safe and an eco-friendly approach. Willows are excellent phytoremediation agents as they accumulate and tolerate heavy metals and follow dominant vegetation even at higher elevations. They have been found to be quite useful in abandoned mine sites across the year world. This is considered a super phytoextraction plant that requires high translocation rate from roots to shoots. They are useful due to their fast growth, high biomass and high metal translocation ability. They are also highly propagated due to highly developed root system that quite often reaches deep down the soil and accumulate heavy metals over there.

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Chapter 10
Photo Catalysis: An Effective Tool for Treatment of Dyes Contaminated Wastewater

Muhammad Saeed, Shahid Adeel, Majid Muneer, and Atta ul Haq

10.1 Introduction

Today the world is facing a number of problems; however, the environmental pollution is the most significant problem which increases continuously and gives rise to irreversible and serious damage to the life of living organism. Environmental pollution can be classified into three major types:

1. Water pollution
2. Soil pollution
3. Air pollution

Among these pollutions, research on water pollution got much attention. There are number of factors including domestic wastes, pharmaceutical residue, fertilizers in agriculture sector, excess use of pesticides and industrial effluent that are responsible for water pollution (Legrini et al. 1993; Lianos 2011; McLaren and Williams 2015). Wastewater contains a variety of pollutants like dyes, chlorophenols, polychlorinated biphenyls (PCBs), pesticides, dibenzofurans, dioxins, and solvents. Among these pollutants, the dyes are considered as major contributors to water pollution because a number of industries use the dyes in various processes. Hence the discharge of these industries contains a large concentration of these toxic and carcinogenic dyes. Most of these dyes have complex aromatic structures due to which they are highly stable and resistant to biological decomposition (Jo and Tayade 2014; Shinde et al. 2014; Nguyen and Juang 2015; Reddy and Kim 2015).

The natural dyes, dyes obtained from natural resources like plants and animals, have been used for dying of fabrics since 5000 years back. However, the synthetic dyes discovered in the nineteenth century have replaced the use of natural dyes. These synthetic dyes are industrially produced in huge amount which are used in

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different industries like electroplating, pharmaceuticals, agricultural research, cosmetics, food, paper, leather, and fabrics. Synthetic dyes can be classified into different groups like direct dyes, disperse dyes, basic dyes, acid dyes, pigments, mordant, metallic, sculpture, vat dyes, solvent dyes, and reactive dyes. These dyes reflect their behavior and functionalities. Presently, vast varieties consisting of more than 100,000 of these synthetic dyes are commercially available worldwide. It is estimated that about one million tons of these synthetic dyes are produced on yearly basis. It has been reported that 10–15% of dyes employed in different industries are lost in industrial effluent to environment during manufacturing and application processes (Saeed et al. 2015a, b; Natarajan et al. 2013a, b; Ajmal et al. 2014).

A huge amount of synthetic dyes released in industrial effluent generate a threat to living organisms and environment. As most of these dyes are brightly colored, therefore a small amount of these dyes can be easily recognized in wastewater. The wastewater contaminated with these dyes is badly affected in terms of transparency and aesthetic merit. These dyes present in water bodies adsorb and reflect the solar radiation resulting in inhibition of penetration of solar rays to interior of the water body. This kind of inhibition of solar radiations into the water body has effects on aquatic life, growth of various aquatic species, and photosynthesis. Furthermore, these dyes have chronic and acute effects on aquatic organisms. The severity of these effects depends on concentration of dyes in wastewater and exposure time of living organisms to contaminated water. Hence, the removal of these dyes is a major concern toward environmental pollution abatement.

In order to minimize the environmental impact of dyes present in wastewater, a number of methods and techniques have been developed for treatment of dyes contaminated wastewater. The developed methods are divided into four groups as given below.

1. Physical methods
2. Chemical methods
3. Biological methods
4. Acoustical methods

Physical methods include filtration (reverse osmosis, nanofiltration, ultrafiltration, and microfiltration), adsorption, and sedimentation. Chemical methods include oxidation, coagulation, electrochemical oxidations, and advanced oxidation processes. Aerobic and anaerobic treatments are included in biological methods. Acoustical methods include radiation and electric processes (Delee et al. 1998; Rai et al. 2005; Barragan et al. 2007; Maezawa et al. 2007; Hong et al. 1999; Aguedach et al. 2005; Bandala et al. 2008; Zhao et al. 2008; Shi et al. 2007; Al-Bastaki 2004; Marmagne and Coste 1996; Linsebigler et al. 1995; Avlonitis et al. 2008; Gupta and Suhas 2009; Hage and Lienke 2006; Namboodri et al. 1994; Dogan and Turkdemir 2005; Wang 2008).

Physical methods (like electro-dialysis, reverse osmosis, nanofiltration, and adsorption) and chemical methods (like electro flotation, flocculation, and precipitation) are suitable techniques for elimination of dyes in aqueous medium; however, these methods are effective in decreasing the concentration of dyes only without being degrading the dyes. These methods transform the pollutants from one form to another form which result in secondary pollution. Similarly, the biological methods which use the enzymes for removal of dyes from wastewater are also not suitable due to longer time. Furthermore, some dyes are very resistant to biological degradation. Additionally, the biological degradation of dyes produces aromatic compounds which are highly carcinogenic (Freeman 1989).

10.2 Photo Catalysis

Recently, the advanced oxidation processes, AOPs, which are highly effective in complete degradation of dyes and economically favorable, have got extra attention for dyes contaminated wastewater treatment. These advanced oxidation processes are performed under irradiation of ultraviolet and visible light. These processes are also assisted with hydrogen peroxide and ultrasonication (Aleboyeh et al. 2012; Srivastava et al. 2013; Rao et al. 2009). In photo catalysis the dyes and other organic pollutants are degraded over semiconductor metal oxides catalysts like cadmium sulfide (CdS), zinc oxide (ZnO), zinc sulfide (ZnS), titanium dioxide (TiO_2), tin oxide (SnO_2), strontium titanate ($SrTiO_3$), bismuth oxide (Bi_2O_3), vanadium oxide (V_2O_5), iron oxide (Fe_2O_3), etc. (Natarajan et al. 2013a, b; Lee and Mills 2004; Mills and Lee 2002; Tayade et al. 2007; Huang et al. 2013; Sobana and Swaminathan 2007).

Photo catalysis is the increase in rate of reaction or initiation of reaction in the presence of a semiconductor metal oxide under irradiation of light which cause chemical transformation that come into contact with it and regenerates its chemical composition after each cycle of such interactions by absorption of light quanta. The semiconductor metal oxide is called as photo catalyst (Fox and Dulay 1993).

Two approaches are used to promote the efficiency of metal oxide semiconductors photo catalysts (Tayade et al. 2008, 2011; Saeed et al. 2018a, b; Mogal et al. 2012).

1. The metal oxide semiconductor photo catalysts are doped with metal atoms like Ag, Zn, etc. These metal islands deposited on semiconductors photo catalyst prevent the recombination of positive hole and photo excited electron. These positive holes and photo excited electrons are generated by irradiation of catalysts under visible or ultraviolet light. These positive holes and photo excited electrons take part in degradation of pollutants.
2. The semiconductor metal oxide photo catalysts are anchored on the surface of a non-catalytic material having high surface area, the support, such as clay, zeolite, silica, activated carbon, etc. These high surface area supports provide maximum dispersion to the active catalytic material. Additionally, these non-catalytic supports have better sorption characteristics due to charge density and high surface area. These semiconductor metal oxide photo catalysts are easily separated from reaction mixture.

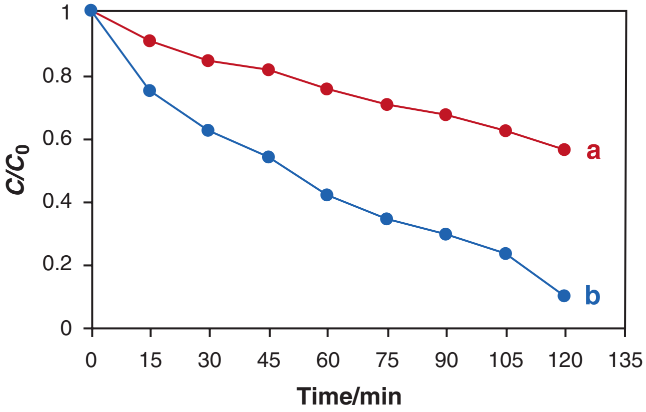

Fig. 10.1 Comparison of performance of (a) Co_3O_4 and (b) Ag-Co_3O_4 on photo degradation of rhodamine B dye. This study was conducted by suspending a 0.05 g of catalyst in 200 ppm solution of rhodamine B. The suspension was agitated at 40 °C under irradiation of visible light

The improvement in catalytic activity of photo catalyst by deposition of metal is the commonly used method. In the first method, dispersion of active catalyst on solid support, some of the dye molecules may be adsorbed on the solid support which will remain un-degraded. In the second method, the dye molecules are completely degraded. Therefore, the latter technique is the commonly used method for enhancement of photo catalytic activity. Saeed et al. (2018a) have enhanced the performance of Co_3O_4 by deposition of Ag on Co_3O_4 for photo degradation of rhodamine B dye. They reported that activity of Co_3O_4 increased from 24% to 57% by deposition of 3%Ag on Co_3O_4. Figure 10.1 shows the increase in catalytic activity of Co_3O_4 by deposition of Ag.

Similarly, in another report the performance of manganese oxide has been enhanced by deposition of silver on manganese oxide (Saeed et al. 2018b). They found that catalytic activity of manganese oxide increased from 45 to 91% for degradation of rhodamine B dye by deposition of manganese oxide with silver. Figure 10.2 shows the enhancement of photo catalytic activity of manganese oxide with deposition of silver.

10.3 Mechanism of Photo Catalytic Degradation Reactions

A photocatalytic process can be described as follows.

1. Generation of pair of hole and electron by irradiation of photo catalyst with ultraviolet or visible radiations.
2. Separation of generated pair of hole and electron by traps.

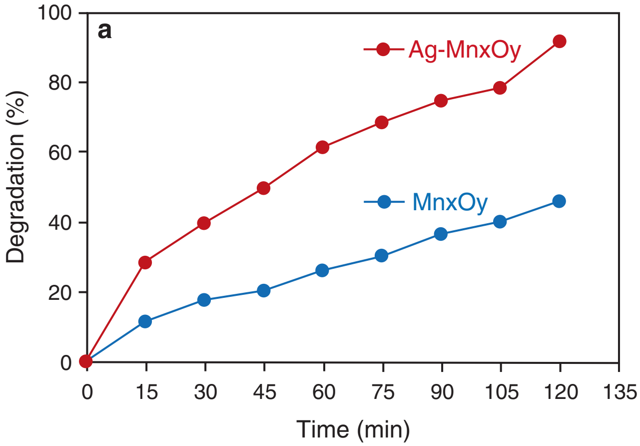

Fig. 10.2 Comparison of performance of Mn_xO_y and Ag-Mn_xO_y towards rhodamine B dye degradation. This study was carried out by suspending a 0.05 g of catalyst in 40 mL of 200 mg/L of rhodamine B dye solution. The reaction mixture was stirred under irradiation of visible light at 40 °C

3. Series of secondary reactions of hole and electron with water and oxygen to produce OH radicals.
4. Degradation of pollutants by reaction with OH radical and regeneration of the catalyst.

These steps are explained in Fig. 10.3 (Natarajan et al. 2018).

The reaction mechanism of photo catalytic reaction can be described as redox mechanism and the role of irradiation of semiconductor photo catalyst is its excitation. The band gap of semiconductor metal oxide photo catalysts has a prominent effect on degradation of pollutants and dyes. The catalytic efficiency of semiconductor metal oxide photo catalyst towards degradation of pollutants varies with catalyst band gap position. The band gap also called as energy gap is the energy difference between valence band and conduction band of the semiconductor metal oxide photo catalyst. Band gap of semiconductor metal oxide is the energy range where electron cannot exist. The position of band gap determines the power of oxidation or reduction of photo catalysts. Most of the semiconductor metal oxides have band gaps from 0.7 to 5 eV. The semiconductor metal oxides are used as photo catalysts on the basis of band gap of these materials. Those semiconductor metal oxides which have band gap from 2.0 to 3.3 eV are generally selected as photo catalysts for treatment of dyes contaminated wastewater.

The mechanism of photo catalytic degradation reactions can be classified as direct photo catalytic mechanism and indirect photo catalytic mechanism.

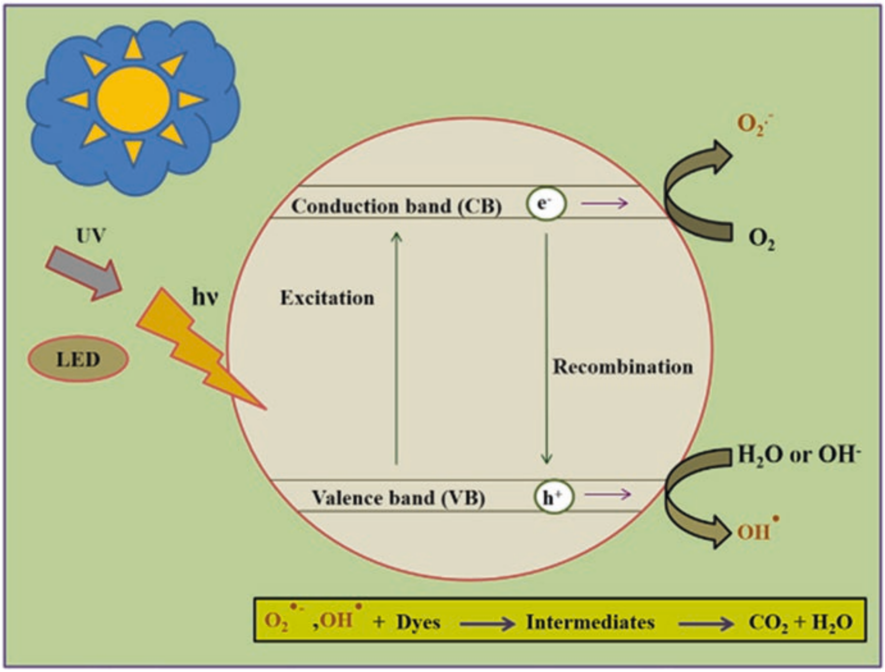

Fig. 10.3 Mechanism of ultraviolet or visible light assisted photo catalytic degradation of dyes (Natarajan et al. 2018)

10.3.1 Direct Photo Catalytic Mechanism

The direct photo catalytic mechanism is also called as Langmuir–Hinshelwood mechanism. According to direct pathway, the Langmuir–Hinshelwood mechanism, the dye molecules gets adsorbed at the surface of catalyst in the first step. In the second step the degradation of dye molecules takes place. According to this mechanism, when catalyst is irradiated with ultraviolet of visible light then a pair of positive hole and photo excited electron takes place. The positive hole is generated in valence band of catalyst. The photo excited electron is generated in conduction band of catalyst. The positive hole generated in valence band of catalyst is trapped by surface defect of the catalyst. This trapping of positive hole by surface defect gives rise to surface-active center (S). Then, the surface-active center reacts with molecules of dye and produces an adduct species $(\text{S-Dye})^+$. This adduct species decomposes to produce degradation products or it can re-combine with photo excited electron. This process can be outlined as:

$$\text{Catalyst} + \text{Dye} \rightarrow \text{Dye}_{\text{ads}} \tag{10.1}$$

$$\text{Catalyst} + hv \rightarrow h^+ + e^- \tag{10.2}$$

$$S + h^{+} \rightarrow S^{+} \tag{10.3}$$

$$S^{+} + e^{-} \rightarrow S \tag{10.4}$$

$$S^{+} + \mathrm{Dye}_{\mathrm{ads}} \rightarrow \left(S - \mathrm{Dye}\right)^{+} \tag{10.5}$$

$$\left(S - \mathrm{Dye}\right)^{+} + e^{-} \rightarrow S + \mathrm{Products} \tag{10.6}$$

The rate expression for Langmuir–Hinshelwood mechanism can be written as (Saeed et al. 2015a, b, 2016, 2018c):

$$r = k\frac{KC}{1 + KC} \tag{10.7}$$

$$\frac{1}{r} = \frac{1}{kKC} + \frac{1}{k} \tag{10.8}$$

where r, k, K, and C is the rate of reaction, rate constant, adsorption equilibrium constant, and concentration of dye, respectively.

Saeed et al. (2018c) have reported Langmuir–Hinshelwood mechanism for degradation of dyes catalyzed by $CoFe_2O_4$. Langmuir–Hinshelwood model (Eq. 10.7) was applied with Curve Expert software to degradation data of dyes. The concentrations of dyes were calculated using Curve Expert software. Both the experimental and calculated concentrations of dyes were close to each other as given in Fig. 10.4. The values of k, the rate constant and K, the equilibrium constant are listed in Table 10.1.

10.3.2 Indirect Photo Catalytic Mechanism

The indirect mechanism is also called as Eley–Rideal mechanism which states that the dye reacts in fluid phase and forms the degradation products. This mechanism also consists of generation of positive hole and photo excited electron under irradiation of catalyst. The positive hole which is produced in valence band of catalyst is trapped by water molecule and produces OH radical and H^{+}. Similarly, the photo excited electron in conduction band is trapped by molecular oxygen to form superoxide radical which can enter into a chain reaction to produce HO radicals. Finally, the OH radicals, which are highly reactive species, attack the dye molecules and produce intermediates and end products (Yue et al. 2002).

This process can be outlined as:

$$\mathrm{Catalyst} + h\nu \rightarrow h^{+} + e^{-} \tag{10.9}$$

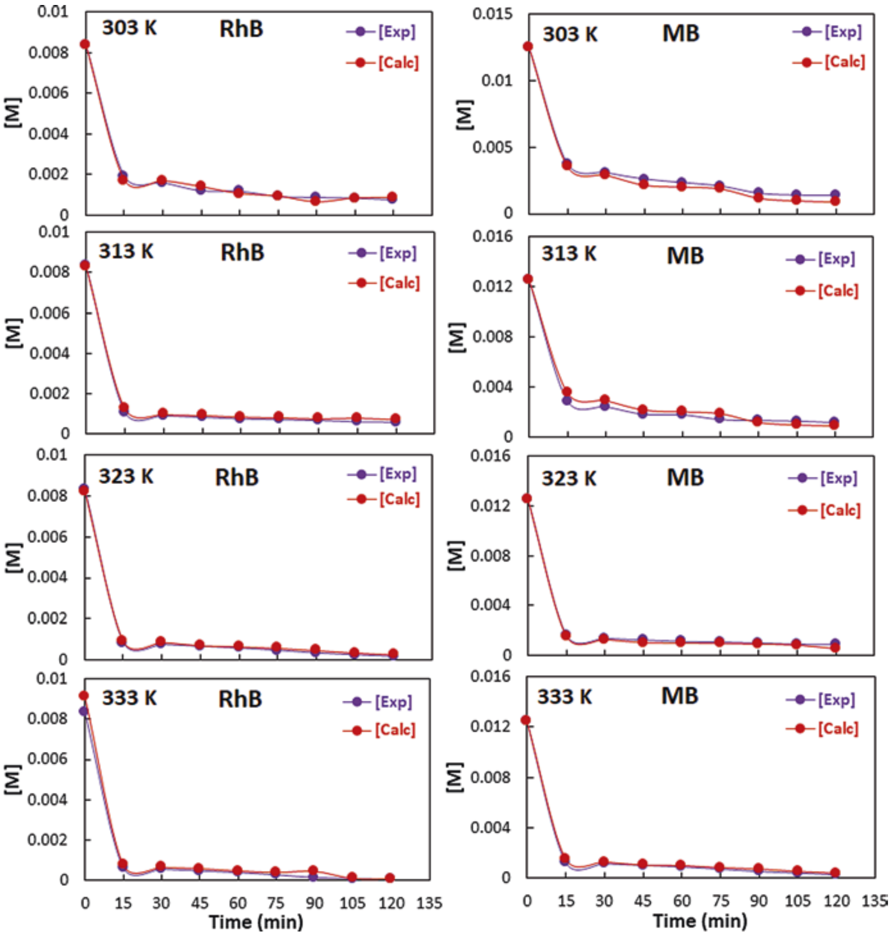

Fig. 10.4 Langmuir–Hinshelwood mechanism of degradation of rhodamine B and methylene blue dyes catalyzed by $CoFe_2O_4$

Table 10.1 Langmuir–Hinshelwood kinetics parameters of $CoFe_2O_4$ catalyzed degradation of dyes

	RhB		MB	
T (K)	*k* (/min)	*K* (L/mol)	*k* (/min)	*K* (L/mol)
303	0.028	1.61	0.031	1.74
313	0.066	1.60	0.069	1.71
323	0.098	1.58	0.098	1.69
333	0.128	1.55	0.131	1.67

$$H_2O + h^+ \rightarrow OH + H^+ \tag{10.10}$$

$$O_2 + e^- \rightarrow O_2^- \tag{10.11}$$

$$H^+ + O_2^- \rightarrow HO_2 \tag{10.12}$$

$$HO_2 \rightarrow O_2 + H_2O_2 \tag{10.13}$$

$$H_2O_2 \rightarrow OH \tag{10.14}$$

$$\text{Dye} + OH \rightarrow \text{Degradation products} \tag{10.15}$$

The reaction of methylene blue dye in fluid phase can be expressed by kinetic expression:

$$r = -\frac{dC}{dt} = kC \tag{10.16}$$

$$\ln\frac{C_o}{C_t} = kt \tag{10.17}$$

Saeed et al. (2018a) have explained the Ag-Co_3O_4 catalyzed photo degradation of rhodamine dye in terms of indirect photo catalytic mechanism. The cited mechanism is given in Fig. 10.5. The rate constants determined according to given mechanism were 0.0198, 0.0140, and 0.0121 at 323, 313, and 303 K, respectively.

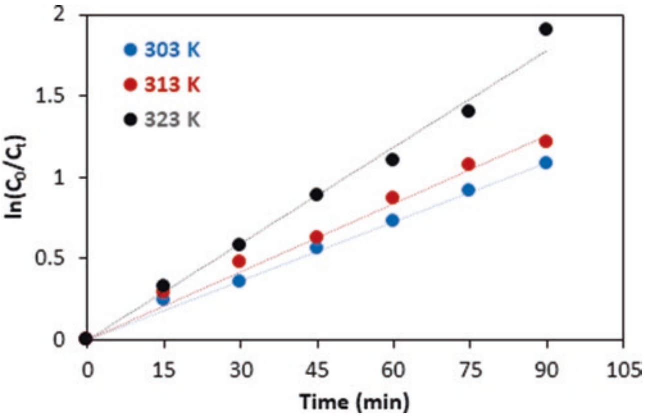

Fig. 10.5 Eley–Rideal mechanism for degradation of rhodamine B dye catalyzed by Ag-Co_3O_4

10.4 Simultaneous Adsorption and Photo Catalysis

Originally the dyes and other pollutants in aqueous medium were removed by adsorption process. In last few decades the semiconductor catalyzed photo degradation of dyes and other pollutants have gained much attention. Up till now, a number of researchers have reported a vast range of catalysts for degradation of dyes. On the basis of vast data on photo catalytic degradation of dyes, it can be concluded that penetration of radiation through catalyst, electron and hole recombination rate and phase composition of the catalyst, crystallinity of the catalyst, band gap and surface area of the catalyst, and ability of pollutants to adsorb on the surface of catalyst are the parameters which play significant role in the photo catalytic degradation performances (Corma and Garcia 2004). Therefore, the researchers have tried to produce photo catalysts with significant surface area and able to adsorb dyes to a significant extent (Anandan and Yoon 2003). To get catalysts with higher surface area, the catalysts like CdS, ZnO, TiO_2, etc., care synthesized needle like, rod like and tubular with nanocrystalline structures. It is reported that nano tube TiO_2 has much higher surface area compared to bulk TiO_2 (Tayade and Key 2010). Different researchers have reported the synergistic effect of carbon and TiO_2 (Matos et al. 2007; Areerachakul et al. 2007). Similarly, Sun et al. (2016) studied the carbon-TiO_2 as photo catalyst to investigate the synergistic effect. The incorporation of adsorbent with photo catalyst provides a new approach to photo catalytic system. Similarly, Li et al. (2014) have explained the synergistic effect by using g-C_3N_4-ZnO composite. They used this composite for removal of rhodamine B dye in aqueous system. Figure 10.6 explains the whole process.

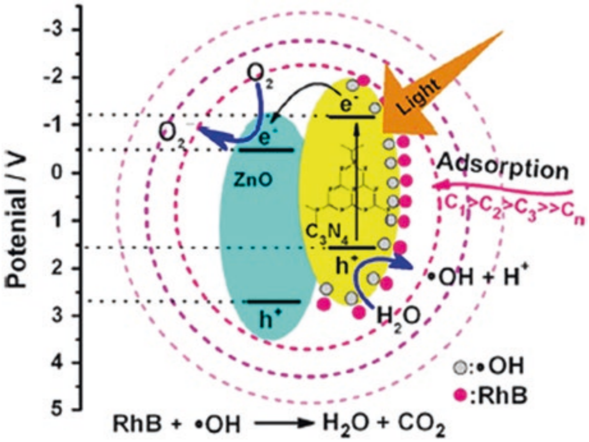

Fig. 10.6 Reversible separation of electron and positive hole and adsorption at composite of g-C_3N_4/ZnO (Li et al. 2014)

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Chapter 11
Removal of Dyes from Waste Water by Micellar Enhanced Ultrafiltration

Amnah Yusaf, Shahid Adeel, Muhammad Usman, Nadia Akram, and Tanveer Hussain Bokhari

11.1 Introduction

11.1.1 Dyes

The dyes are major component of textile industries effluent. The low biodegradability of dyes and other synthetic compounds do not let the biological treatment to get rid of it in effective way and, thus, their presence opposes aerobic biological treatment (Hassani et al. 2008). Lot of work has been done recently on dyes like the microwave radiation effect on the dyeing e.g. with reactive blue 21 dye (Ghaffar et al. 2019), Disperse Yellow 211 dye (Adeel et al. 2018a, b, c) and Reactive Violet H3R (Kiran et al. 2019), Microwave assisted modulation of vat dyeing of cellulosic fiber (Adeel et al. 2018a, b, c), Ultrasonic assisted improved dyeing of cellulosic fabric using Vat Blue 4 (Saeed et al. 2018), and the eco-friendly disperse dyeing of Ultraviolet treated polyester fabric using Disperse Yellow 211 dye (Adeel et al. 2018a, b, c) have been studied. A large quantity of other organics like aromatic hydrocarbons, alkylphenols, aliphatic alcohols, chloroaromatic compounds, and aromatic amines are also present in waste water, in addition to dyes (Dehghani et al. 2011; Pramauro and Prevot 2007). Production of dyes on the earth has been estimated to be more than 10,000 tons each year being consumed in leather industries, additives, cosmetics, paints and ink industry (Abedi and Nekouei 2011). The dyes, being colored compounds, are responsible for far-reaching environmental damage and pose serious threat to aquatic life because suspended dye particles block sunlight and cause decrease in dissolved oxygen in water (DO) and increase in the biological oxygen demand (BOD) of the waste water (Rawal et al. 2018).

Approximately 15% of quantity of used dye is lost during the mechanical procedures for example dyeing, dye assembling, textile finishing, pulp and paper

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production (Chen et al. 2011). The strict environment protection rules have invited research to find new ways for treatment of waste water (Das et al. 2008).

11.2 Removal Methods

Several techniques have been utilized till now to remove dyes contaminants from waste water, for example, utilizing adsorption by activated carbon, ozonations, changing pH of solution, nanofiltration, (Purkait et al. 2004c) cloud point separations, polyglycol systems (Korzystka et al. 2003), physical and chemical techniques such as oxidation, coagulation, chlorination, and adsorption onto different supports, reverse osmosis, solvent extraction, and ultrafiltration. Direct eradication of organic compounds by ultrafiltration of aqueous solutions is not achievable. Such a separation can be accomplished utilizing surfactant solutions. Micellar enhanced ultrafiltration (MEUF) is one of the conceivable membrane techniques to remove traces of organic contaminations from aqueous streams (Bielska and Prochaska 2007).

11.3 Ultrafiltration Process

The uses of ultrafiltration membranes are picking up a ton of interest nowadays because of their higher selectivity, thermal stability, and permeation rate. In recent years, different UF membranes were prepared with generally excellent structural integrity, high selectivity, and fouling resistance. The composite UF membranes establish various sorts of thin polymeric layers, for example, polysulfone, cellulose acetate, polyvinyl acetate, polyvinyl pyrrolidone, polydimethylsiloxane, etc. (Jana et al. 2011). The sulfonated and aminated derivatives of polyethersulfone can likewise be applied for the separation of dyes through MEUF process (Pozniak et al. 2009).

11.3.1 Micellar Enhanced Ultrafiltration

This technique has been recommended as one of the useful ways for removal of harmful contaminants especially dyes (Ahmad et al. 2015) using micelles, the active colloidal aggregates of surfactant molecules. Surfactant molecules are amphiphilic in nature, i.e., there are both hydrophobic and hydrophilic moieties in their molecules. Surfactant molecules may be ionic (cationic, anionic, zwitterion) or nonionic. As surfactant concentration increases in solution, its properties deviate from ideality and at the concentration where accumulation of monomers into micelles happens, a pronounced change is observed. This concentration is known as the critical micellization concentration (CMC) (Taşcıoglu 2018). Above the critical micellar concentration, monomers of surfactant will assemble and aggregate in order to form large isotropic clusters called micelles having hydrodynamic diameter greater than the pore size of membrane (Puasa et al. 2011). Organic pollutants are expelled toward interior of micelles (Luo et al. 2010) and get entrapped there. Micelles having incorporated organic dyes are retained by the membrane and a permeate stream going through the membrane has minimum amount of dyes (Ahmad et al. 2015).

Unbound surfactant monomers and dye molecules/ions go through the ultra-filter membrane to the permeate side. In the MEUF procedure, rejection efficiency and permeate flux depend upon the nature of solute and membranes as well as on working conditions (Bade and Lee 2011). MEUF has been also used for recovery of hazardous organic compound or heavy metal ions from waste water (Schwarze et al. 2009). The benefits of MEUF are low operating pressure and low energy requirement. The required pressure expected to drive MEUF ranges from 97 to 587 kPa. MEUF using mixed surfactant systems include the combination of ionic (cationic or anionic) and nonionic surfactants to improve efficiency of MEUF (Puasa et al. 2011). It is to be noticed that MEUF gives surprisingly higher filtration as compared to UF treatment (Deriszadeh et al. 2010). It has been discovered that the effectiveness of dye retention depends upon molecular weight and concentration dyes in feed solutions in addition to hydrophilicity of the membrane (Korzystka and Prochaska 2006).

11.3.2 Selection of Surfactant for Removal of Dyes

Although large number of surfactants are available but we need those having low critical micellar concentration, little or no adsorption to the surface of membrane, large micelles, low cost, greater solubilization limit, high aggregation number, and being biodegradable. Unfortunately no individual surfactant can satisfy all these conditions simultaneously. For the maximum removal of dyes from fluid, the main point has to be very good dye–surfactant interaction. The post-treatment steps, like recovery of surfactant after removal of dye should be ensured (Schwarze 2017).

11.4 Experimental Procedure

In the ultrafiltration cell a membrane of suitable pore size is placed in a compartment. A pump is used to push the aqueous solution towards that membrane. In this setup, two rotameters and a manometer were positioned for monitoring and controlling the rate of permeates flux (Huang et al. 2010).

A cylinder of nitrogen gas is used to apply pressure on membrane and permeate from the bottom of the cell is collected and analyzed using UV/Visible spectrophotometer or atomic absorption spectrophotometer (Purkait et al. 2004a). Washing of membrane is done, with distilled water, after each run. The experiment is usually performed at room temperature (Ahmad and Puasa 2007). Zeta-potential of the micelle of surfactant is controlled by dynamic light scattering and estimated by instrument known as Zetasizer (Huang et al. 2010).

11.5 Types of Membrane

MEUF requires membrane of suitable material, pore size, and hydrophilicity/hydrophobicity depending on nature of dye and other experimental conditions i.e. pH and temperature of the solution (Schwarze 2017). Usually regenerated cellulose (RC), polysulfone, cellulose acetate, polyacrylonitrile, polyethersulfone, polyvinyl difluoride (molecular weight 10,000 and permeability 6.28×10^{-11} m/Pas) PVDF, polyamide (PA), and polytetrafluoroethylene (PFTE) are used (Ahmad and Puasa 2007). Sometimes ceramic membrane is also used because it has the advantage to be used under highly basic or acidic conditions. The main problem with MEUF is membrane contamination. In laboratory scale testing, the membrane is gradually cleaned between two experiments to restore its performance and to remove any impurity. The purpose of the purification process is to eliminate adsorbents i.e. dyes or surfactant molecules. A survey revealed that membranes of hydrophobic nature are preferred as they cause less fouling. In most of cases membranes of low MWCO are used i.e. 5 or 10 kDa but in some cases membranes of 20 or 30 kDa may also be used depending on nature of dye. The washing of membrane is most often accomplished by placing it in distilled water to remove adsorbed surfactant. Sometimes dilute solution of hydrochloric acid and sodium hydroxide may also be used. In addition, combinations of water and minor amounts of alcohols such as 2-propanol are also useful.

The permeability of the membrane is checked after cleaning and the membrane is recycled as long as the deviation of the groundwater flow is less than 5–10% (Schwarze 2017). The polyethersulfone membrane showed slightly better separation than the regenerated cellulose membrane. The high molecular weight dye e.g. Direct Black was rejected almost 100% independent of the nature of membranes (Majewska-nowak 2009).

11.6 Filtration Mode

The filtration cell should not be filled till maximum volume limit. The pressure is usually applied using a cylinder of inert gas like argon or nitrogen gas. A cross-flow filtration process has been found to be much better; consequently, many researchers have used such setup. A driving force is provided by the rotational speed of the rotator in case of the centrifugal micellar enhanced ultrafiltration (Schwarze 2017).

11.7 Ultrafiltration Study

Since dye molecules are smaller than membrane pores, the retention causes the dye adsorption at surface or in the pores membrane. After micelle formation, incorporation of dye takes place leading to its solubilization and, then, held by membrane for

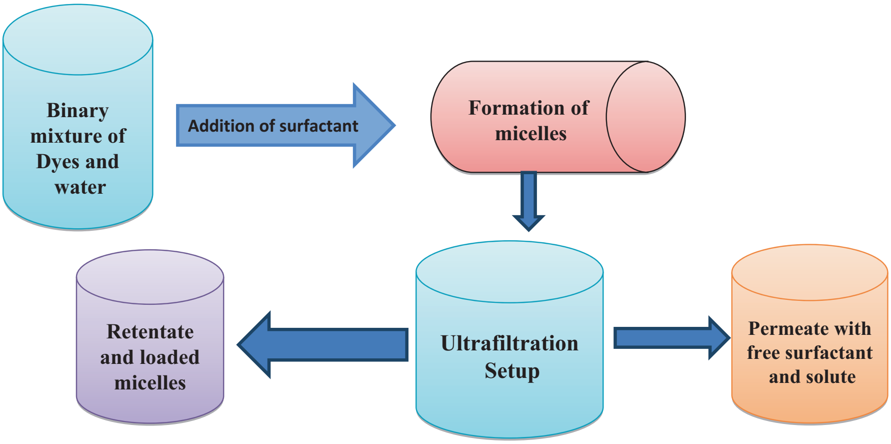

Fig. 11.1 Simple ultrafiltration setup by MEUF

ultrafiltration. The micelles, after some time, get attached to the surface of membrane, bringing about high concentration of surfactant close to the membrane (Zaghbani et al. 2007). Most of researchers have kept trans-membrane pressure (TMP) constant at 1.4 bars. The membrane is completely washed with distilled water. It has been seen that in the presence of dye, without surfactant, the flux decreases with time whereas, in the presence of surfactant reduction in the flux is observed in the beginning and then constant flux is noticed (Zaghbani et al. 2008).

A simple schematic diagram showing micellar enhanced ultrafiltration setup has been represented in Fig. 11.1.

11.8 Removal of Dyes by MEUF

Different dyes have been removed by MEUF using suitable surfactant and membranes are as follows.

11.8.1 Removal of Mordant Black 11

The removal phenomenon was performed by using three different membranes such as cellulose acetate (CQ), polysulfone (PES), and polyvinylidene fluoride (PVDF) and combination of surfactant has been made, so for this purpose cetyl trimethylammonium bromide (CTAB) and oxyethylated coconut fatty acid methyl esters (OMC-1) were used. A trans-membrane pressure of 0.2 MPa was applied. By using cellulose acetate (CQ) membrane along the CTAB, the retention of mordant black

11 was found to be 99.3% whereas for CTAB+ OMC-10, 98.6% was obtained. For polysulfone (PES) with CTAB resulting in a 99.2% for CTAB+ OMC-10 it was observed about 98.5%. For polyvinylidene fluoride (PVDF) with CTAB and CTAB+ OMC-10, the retention was found to be 82.6 and 77.1%, respectively (Bielska and Prochaska 2007).

11.8.2 Reactive Black 5

For the removal of dye by micellar enhanced ultrafiltration, a thin film composite membrane having 10,000 MWCO was used with a cetyl pyridinium chloride CPC as a surfactant. A pressure of 300–400 KPa was applied. The pH ranges 2–11 and at a temperature of 50 °C. The retention of reactive black 5 was found to be 99.7% (Ahmad and Puasa 2007; Majewska-nowak 2009). At a pressure of 1.5 bars and at concentration greater than their CMC value at pH in the range of 2–10, using 30,000 MWCO regenerated cellulose membrane, retention of 83% was observed with CPC while 98% was obtained with CTAB at room temperature of 25 °C (Khosa and Shah 2011).

11.8.3 Crystal Violet Dye

A rejection of 92% was observed using CTAB as a surfactant and a polysulfone ultrafiltration membrane was used. The variation in pressure was made in the range of 50–250 KPa (Rawal et al. 2018).

11.8.4 Removal of Mordant Black 17

Cellulose acetate (CQ), polysulfone (PES), and polyvinylidene fluoride (PVDF) membrane was used with a combination of surfactant such as cetyl trimethylammonium bromide (CTAB) and oxyethylated coconut fatty acid methyl esters (OMC-1). A trans-membrane pressure of 0.2 MPa was applied. By using cellulose acetate (CQ) membrane along the CTAB, the retention of mordant black 17 was found to be 98.9% whereas for CTAB+ OMC-10, 97.6% was obtained. For polysulfone (PES) with CTAB resulting in a 98.9% for CTAB+ OMC-10 it was observed about 97.1%. For polyvinylidene fluoride (PVDF) with CTAB and CTAB+ OMC-10, the retention was found to be 74.6 and 69.6%, respectively (Bielska and Prochaska 2007).

11.8.5 Reactive Orange 16

For the removal of dye by micellar enhanced ultrafiltration, a thin film composite membrane was used with a cetyl pyridinium chloride CPC as a surfactant. A pressure of 300–500 KPa was applied. The pH ranges 2–11 and at a temperature of 50 °C. The retention of reactive black 5 was found to be 99.6% (Ahmad and Puasa 2007); a rejection of 99.98% was also observed with same setup above (Ahmad et al. 2006).

11.8.6 Removal of Methylene Blue

By using single and a combination of surfactants i.e. sodium dodecyl sulfate (SDS) and oxyethylated coconut fatty acid methyl esters (OMC-1), the retention percentage of dye was measured by using cellulose acetate (CQ), polysulfone (PES), and polyvinylidene fluoride (PVDF) membranes. A trans-membrane pressure of 0.2 MPa was applied. By using cellulose acetate (CQ) membrane along the sodium dodecyl sulfate (SDS), the retention of methylene blue was found to be 94.3% whereas for SDS+ OMC-10, 72.3% was obtained. For polysulfone (PES) with SDS resulting in a 91.4% for SDS+ OMC-10 it was observed about 95.5%. For polyvinylidene fluoride (PVDF) with SDS and SDS+ OMC-10, the retention was found to be 88.8 and 73.6%, respectively (Bielska and Prochaska 2007). The methylene blue was also removed by using SDS and polysulfone hollow fiber membrane. The effective membrane area was noted as 0.8 m^2. The pressure was set at 0.03 MPa. The pH ranged 2–13 and a temperature of 5–45 °C. A rejection of 93.3% was observed (Huang et al. 2010). A 95–99% rejection of methylene blue was also observed with a cellulose membrane of MWCO (molecular weight cut off) 10 kDa by using SDS. The pressure was maintained at 1.4 bars. The pH was ranging in between 2 and 11 at a temperature of 30 °C (Zaghbani et al. 2007). A mixed surfactant system has been made using SDS and oxyethylated coconut fatty acid (OMC-10). A rejection of 93–94% was obtained by a hydrophilic membrane made of cellulose along a pressure of 0.2 MPa in the pH range of 2–11 (Bielska and Szymanowski 2006). Using SDS with ultrafiltration regenerates cellulose membrane 30,000 MWCO at room temperature with a pressure of 1.5 bar, a retention of 92.53% was observed at pH ranging 2–10 (Khosa et al. 2011a).

11.8.7 Methyl Orange

Twenty to Sixty percent rejection of methyl orange was found using CTAB with a polysulfone and cellulose acetate membrane at a pressure of 0.2 MPa (Korzystka and Prochaska 2006).

11.8.8 Removal of Eosin (Acid) Dye

The removal of dye by micellar enhanced ultrafiltration was done using 10,000 MWCO ultrafiltration membrane and cetyl pyridinium chloride CPC was used as a surfactant. The molecular weight of eosin dye was 691.86. A pressure of 276–414 KPa was applied and a maximum operating pressure was 518 kpa. The temperature was set at 32 °C. The retention of eosin was found to be 68–74% (Purkait et al. 2004a, b). Rejection of 74% was also observed at a pH of 6–7 and a pressure of 500 KPa (Purkait et al. 2006).

11.8.9 Removal of Safranin T

The molecular weight of Safranin T is 350.85. A rejection percentage of 99% was found by using SDS and a cellulose membrane of MWCO 10KDa at room temperature within a pH range of 2–11 and at a pressure of 1.4 bar (Zaghbani et al. 2008).

11.8.10 Direct Black

Almost a 100% rejection of direct black was found using CTAB with a polyethersulfone membrane at a pressure of 0-1-0.20 MPa (Purkait et al. 2004c).

11.8.11 Eriochrome Blue Black R (EBBR)

Eriochrome blue black R is an anionic dye and is removed by using CTAB with a cellulose membrane with 10,000 MWCO at 30 °C. The pH was maintained in the range of 2–13. Retention of 99% was observed at a pressure of 1.4 bars (Zaghbani et al. 2009).

11.8.12 Alizarin Red S

Rejection of 98.8% was obtained at pressure of 2 bars and in the pH range of 4–9 using CTAB and a regenerated cellulose ultrafiltration membrane at room temperature (Khosa et al. 2011b).

11.9 Factor Effecting MEUF

Number of factors are responsible for the removal efficiency of dyes through micellar enhanced ultrafiltration process which are discussed below:

11.9.1 Effect of pH

The effect of pH varies and is dependent on the type of surfactant and solute. For anionic surfactants, protons are attracted by micelles, at lower pH values, and there is competition between dye cations and protons for adsorption on micelle. Nature and structure of the solute get altered with the pH, and the rejection percentage depends on original structure of solute. An example is the cationic nature of crystal violet dye, having variety of structures and is pH dependent. Under the influence of acidic medium, dye has the two positive charges. Usually the increase of the flow is reported with the pH (Schwarze 2017). At the pH of 3, removal of reactive Black 5 and reactive Orange 16 dyes decreases from 90.64% and 77.50%, while 79.59% and 61.72% at pH 9, respectively (Ahmad et al. 2006). Different experiments have been performed at pH 4 and using 5 ml of Britton–Robinson buffer (Abedi and Nekouei 2011). The retention of methylene blue (MB) was found to be same, nearly 99%, using 50 mM of SDS solution, in the pH range of 2–11. Similar results were obtained in the presence of the cationic surfactant CTAB. The influence of pH on MB retention cannot be elucidated by the ion exchange model. Hydrophobic interactions appear to contribute to binding between MB and SDS micelles. The influence of the pH on the permeation flux has also been investigated. The flow increased as the pH was increased to 2–11. This may be due to deprotonation of the carboxyl groups in the dynamic membrane layer (Zaghbani et al. 2007, 2008).

11.9.2 Effect of Ionic Strength

The discharges from the textile and dyeing industries possess high salt contents that also affect the dye removal because of increase in concentration of free counter ions. In this manner decreasing repulsion force between the charged heads of micelle causes reduction in critical micellar concentration and increase in the aggregation number of micelles. Subsequently, adding little amount of the electrolyte to the ionic nature of surfactants increases the solubilization of the hydrocarbons and decreases that of polar compounds. For methylene blue, the solubilization and afterward the retention rise slightly after the addition of sodium chloride and permeate flux accordingly decreases (Zaghbani et al. 2007). The salt addition, e.g. NaCl,

Na_2SO_4, and $NaHPO_4$, is responsible for changing ionic strength in solutions. Salt addition also causes salting out of many impurities (Zaghbani et al. 2007). The effect of the addition of NaCl on the retention of Safranin T (ST) retention has been investigated, where a decrease in the ST retention has been observed after increasing concentrations of NaCl because of decreases in available space for dye in the outer region of micelle (Zaghbani et al. 2008).

11.9.3 Effect of Electrolyte

The rejection of methylene blue remains constant by increasing the concentration of NaCl i.e. at 99.3%. A reduction in the forces of repulsions between charged surfactant head groups has been observed by the addition of electrolyte in the aqueous medium, causing lower critical micellar concentration and, consequently, an increase in solubilization of hydrocarbons, to be solubilized in the inner core of micelle but a decrease in that of polar solutes that get incorporated in the outer portion of the palisade layer. The rejection capacity of surfactant (SDS) increases, with the concentration of NaCl, from 63.48% in the absence of NaCl to 96.0% at 200 mM or 300 mM of NaCl (Huang et al. 2010).

11.9.4 Effect of Trans-Membrane (TMP) or Operating Pressure

Trans-membrane pressure can be defined as the pressure needed to press the water through membrane or gradient of pressure that exists between the feed sides and the permeate side (Rawal et al. 2018). For micellar enhanced ultrafiltration, the pressure is considered as a driving force and much lower pressure (0–1.0 MPa) is required as compared to nano-filtration. High pressure may affect membrane performance due to membrane compression. In some cases, the membrane is pretreated at high pressure before the main filtration experiment (Puasa et al. 2011; Korzystka et al. 2003; Hankins et al. 2005; Schwarze 2017). It has been observed that by increasing operating pressures, the rejection of dye decreases. Dye rejections for Reactive Black 5 (RB5) and Reactive Orange 16 (RO16) decreased from 97.0% and 98.8% at 300 kPa to 91.1% and 97.4% at 400 kPa. Decreasing dye rejection may be due to fact that at higher operating pressures, micelles are compressed and, therefore, their solubilization capability is decreased. By increasing pressures, the effective driving force for the transportation of solvent is greater that will overcome the resistance of membrane. Thus, more water will pass by the membrane by increasing pressure, resulting in a high permeate flux (Ahmad and Puasa 2007). Although for nonionic surfactant, the rejection of the dyes decreases with the trans-membrane pressure but in case of anionic, the rejection increases at the start and then decreases. The reason is the increase in the thickness of the polarization and the gel layer appears by increasing trans-membrane pressure. Hence, more adsorption of pollutants will be possible on the surface of membrane and hence increase in rejection is observed (Salmani 2016). It has been noticed that MB and SDS rejection decreased a little with the increase of operating pressure, ranging from 99.62 to 62.5% at 0.01 MPa to 98.96 and 57.84% at 0.09 MPa, the permeate flux increased linearly with the operating pressure. By increasing the pressure, more solutes will move towards the surface of membrane, creating a rise in the adsorption on membrane adsorption and a concentration polarization (Huang et al. 2010). So, an increase in operating pressure leads to increase in the gel layer, and therefore rejection of CPC increases at the permeate stream (Ahmad et al. 2006).

The permeate flux decreases with feed CPC concentration at a fixed operating pressure. At constant CPC concentration, the flux increases with pressure. For example, at CPC-to-eosin ratio of 2500, (i.e., feed CPC concentration 25 kg/m^3), the flux increases from 3.5×10^{-6} to 4.25×10^{-6} m^3/m^2 s while the pressure increases from 276 to 414 kPa. With increase in the surfactant concentration, the micelle concentration in the solution increases. This leads to a stronger solubilization of eosin in CPC micelles, which increases the retention of eosin. At a fixed ratio of CPC to eosin, eosin retention increases with decreasing operating pressure. As the permeate concentration increases, the eosin retention observed at high pressure decreases. At constant pressure, the increase in eosin retention is faster at lower CPC-to-eosin concentration ratios and slows as this ratio increases. Therefore, a lower operating pressure and a ratio of CPC-to-eosin feed concentration of 2000 may be considered optimal for the maximum removal of eosin (Purkait et al. 2004b).

11.9.5 Effect of Temperature

Normally MEUF is performed at room temperature (from 20 to 40 °C). Solubility of surfactant, CMC, size, shape, and aggregation number of micelle depend on temperature. The rise in temperature causes an increase in the CMC. Viscosity is another temperature dependent factor responsible to alter the performance of ultrafiltration and it decreases with temperature and flux increases (Zaghbani et al. 2007). The increase in solubility of micellar solution with temperature results in a high extraction of dye. The results described that the effects of operating temperature on the efficiency of dye extraction increases with temperature at various concentration of the dye (20, 50, and 100 mg L^{-1}) till 75 °C then becomes constant (Abedi and Nekouei 2011).

11.9.6 Effect of the Nature of Surfactant

Rejection of methylene blue has been studied in the presence of cationic (CTAB), anionic (SDS), and nonionic (TX-100) surfactants, and the retention of 2.10, 2.82, and 99.45% was obtained, respectively. So from these results it is clear that a higher retention was observed due to anionic surfactant SDS owing to opposite charge of dye and surfactant. So, it is concluded that for retention of dyes, the electrostatic interaction between dye and surfactant plays important role (Zaghbani et al. 2007).

11.9.7 Effect of Concentration of Surfactant

It has been observed that, as the concentration of surfactant (CPC) increases, the dye rejection also increases. At the 1000 ppm concentration of CPC, the highest rejections of dyes (RB5 and RO16) were found to be 99.7% and 99.6%, respectively. At operating pressure of 300 kPa, a decrease in the permeate flux was observed by increasing the concentration of surfactant (CPC). Comparing to reactive black 5, the flux of reactive orange 16 was found to be very high at a same concentration of surfactant i.e. 0.500 g/L due to higher molecular weight of RB5 dye (MW 991.82) in comparison to RO16 (MW 617.54). Low molecular weight dyes can pass through the membrane quiet easily, whereas dyes having high molecular weight will remain present on the surface of membrane. Some of particles of dye were deposited on the surface of membrane, offering resistance for solvent flux and leading to a decrease in the flux of RB5 dye as compared to RO16 (Ahmad and Puasa 2007). In a suitable pressure range and in the absence of surfactants, the concentration of permeate of dye started from 5 to 6 ppm and the concentration further increases (up to 9 ppm) because of the forced convection of dyes through pores of membrane and the concentration reached 7–9 ppm. So, in both cases, with and without surfactants at low operating pressure, minimum concentration is present leading to maximum retention. When the micelles of CPC is present, the extent of the unsolubilized dyes governs the concentration of permeate (Purkait et al. 2004a). As compared to nonionic surfactant, a more permeate flux has been observed for anionic (Salmani 2016). When the concentration of surfactant (SDS) is lower than the critical micellar concentration, the obtained results were credited to three reasons: (1) the reactive precipitates of dye (MB) and surfactant (SDS), aggregated and gathered forming a cake on the surface of membrane as well as in the pores of membrane and (2) the fouling of the membrane by a MB adsorption. Whereas, above the CMC at high concentrations of SDS, the deposited layer was due to aggregates of micelle and dye molecules causing more resistance to the flux of solvent through membrane (Huang et al. 2010). Solute having low solubility in water showed a greater solubilization constant compared to solute with high water solubility in a micellar solution. Reactive orange O16 have a high tendency to be solubilized in micelles as compared to Reactive Black 5 and, thus, RO16 have a greater rejection percentage. The surfactant present in the filtrate can be precipitated and recovered and, thus, reused and recycled (Ahmad et al. 2006). It has been observed at a fixed temperature and feed dye concentration, the extraction efficiency of dye increases with surfactant concentration (Abedi and Nekouei 2011). Spectral studies revealed that hydrophobic and electrostatic forces are the reason behind the interaction between ionic dyes and surfactant and, thus, surfactant causes change in spectra of dyes. For the oppositely charged surfactants, the interaction of dyes is mainly of a Coulombic nature. Hydrophobic interaction has been able to overcome the electrostatic repulsion, if the interaction between dyes and surfactants of same charge occurs. If only the nonionic surfactant is present, just the hydrophobic interaction arises. As the hydrophobic interaction is weak as compared to electrostatic repulsion, the dye will not interact with surfactant. If no interaction is present between dye and surfactant, then it was due to the weakness of hydrophobic interaction (Zaghbani et al. 2007).

11.9.8 Effect of Concentration of Dyes

It has been noticed that by increasing dye concentration, permeate flux decreases due to adsorption of dye on membrane and, thus, formation of deposited layer. In most of cases almost 99% rejection of dye has been observed, showing that the concentration of dye in the feed has little effect on the rejection efficiency. The reason behind greater dye rejection is large scale solubilization of dye molecules in micelles. Sometimes presence of dye decreases CMC and increases aggregation number of micelles and, thus, solubilization capacity is enhanced (Huang et al. 2010). The change in dye concentrations, at constant surfactant concentration, results in almost constant retention efficiency (Purkait et al. 2004b).

11.9.9 MEUF Using Mixed Micellization

Anionic surfactants provide the most suitable and cost-effective micellar media to remove cations of metals and dyes from waste water by MEUF. Although nonionic surfactants have lower CMC than ionics but they are not able to attract ions. However, addition of small quantity of nonionic surfactant into solution of ionic surfactants can facilitate solubilization because they reduce repulsions between ionic heads and thus decrease CMC and, therefore, increase aggregation number and size of micelle. The mixed micellar solution, being formed by the combination of two or more surfactants provides better media for removal of dyes. Although presence of nonionic surfactants decrease charge density and thus cation attracting ability of micelle but they increase solubilization capacity to much greater extent (Schwarze 2017).

11.9.10 Effect of Coagulant Concentration

By increasing the dose of coagulant, the percentage removal efficiency of dye increases. For dyes, i.e. Reactive Black 5 (RB5) and Reactive Orange 16 (RO), in the dosage range of 1.0 and 4.0 g/L, respectively, a major enhancement in the removal efficiency was found. At the pH of 3 and with a concentration of coagulant between 4.5 and 9.0 g/L for RB5 and RO16, the removal percentage has been observed to be 95.61 and 79.39%, respectively. For maximum removal, the required concentration of coagulant is low for RB5 as compared to RO16. At a very high dose, Nalco coagulant represents a reversal effect due to the coagulation charge reversal phenomenon where the colloidal system becomes destabilized because coagulant charge becomes greater than total charge on colloidal system (Ahmad et al. 2006).

11.10 Summary (in Tabulated Form)

Removal of a variety of dyes by MEUF has been studied, and the effects of various factors like the type of surfactant, the effect of pH, temperature, pressure, type of membrane, etc. have been reviewed. The removal efficiency is summarized in Table 11.1.

Table 11.1 Percentage rejection of dyes by MEUF

Dyes	Membrane	Surfactant	Pressure	Rejection percentage	References
Mordant black 11	Cellulose acetate (CQ)	CTAB	0.2 MPa	99.3	Bielska and Prochaska (2007)
	Cellulose acetate (CQ)	CTAB+ OMC-10		98.6	
	Polysulfone (PES)	CTAB		99.2	
	Polysulfone (PES)	CTAB+ OMC-10		98.5	
	Polyvinylidene fluoride (PVDF)	CTAB		82.0.6	
	Polyvinylidene fluoride (PVDF)	CTAB+ OMC-10		77.1	
Reactive black 5	Thin film composite membrane having 1000 MWCO	CPC	300–400 KPa	99.75	Ahmad and Puasa (2007) and Ahmad et al. (2006)
Crystal violet dye	Polysulfone ultrafiltration membrane	CTAB	50–250 KPa	92	Rawal et al. (2018)
Mordant black 17	Cellulose acetate (CQ)	CTAB	0.2 MPa	98.9	Bielska and Prochaska (2007)
	Cellulose acetate (CQ)	CTAB+ OMC-10		97.6	
	Polysulfone (PES)	CTAB		98.9	
	Polysulfone (PES)	CTAB+ OMC-10		97.1	
	Polyvinylidene fluoride (PVDF)	CTAB		74.6	
	Polyvinylidene fluoride (PVDF)	CTAB+ OMC-10		69.6	
Reactive orange 16	Thin film composite membrane	CPC	300–500 KPa	99.6	Ahmad and Puasa (2007)

(continued)

Table 11.1 (continued)

Dyes	Membrane	Surfactant	Pressure	Rejection percentage	References
Methylene blue	Cellulose acetate (CQ)	SDS	0.2 MPa	94.3	Zaghbani et al. (2007)
	Cellulose acetate (CQ)	SDS+ OMC-10		72.3	
	Polysulfone (PES)	SDS		91.4	
	Polysulfone (PES)	SDS+ OMC-10		75.5	
	Polyvinylidene fluoride (PVDF)	SDS		88.8	
	Polyvinylidene fluoride (PVDF)	SDS+ OMC-10		73.6	
	Ultrafiltration regenerate cellulose membrane 30,000 MWCO	SDS	1.5 bar	92.53	Khosa et al. (2011a)
	Hydrophilic membrane made of cellulose	Oxyethylated coconut fatty acid (OMC-10) and SDS	0.2 MPa	93–94	Bielska and Szymanowski (2006)
	Cellulose membrane of MWCO 10 kDa	SDS	1.4 bars	95–99	Zaghbani et al. (2007)
	Polysulfone hollow fiber membrane	SDS	0.03 MPa	93.3	Huang et al. (2010)
Methyl orange	Polysulfone and cellulose acetate membrane	CTAB	0.2 MPa	20–60	Korzystka and Prochaska (2006)
Eosin (acid) dye	1000 MWCO ultrafiltration membrane	CPC	276–414 KPa	74	Purkait et al. (2004a, b, 2006)
Safranin T	Cellulose membrane of MWCO 10KDa	SDS	1.4 bar	99	Zaghbani et al. (2008)
Direct black	Polyethersulfone membrane	CTAB	0–1-0.20 MPa	100	Purkait et al. (2004c)
Eriochrome blue black R (EBBR)	Cellulose ultrafiltration membrane/10,000 MWCO	CTAB	1.4 bars	99	Zaghbani et al. (2009)
Alizarin red S	Cellulose ultrafiltration membrane	CTAB	2 bars	98.8	Khosa et al. (2011b)

11.11 Conclusion

Industrial waste having dye content are source of water pollution and cause irreparable damage to ecosystem. Conventionally, the waste water is being treated by several physical and chemical processes, extraction, adsorption, chlorination, oxidation, nanofiltration and ultrafiltration, etc. Among the said techniques, micellar enhanced ultrafiltration (MEUF) is a wonderful technique for dye removal from the aqueous stream. The key to success of MEUF is the selection of most suitable micellar media, preferably mixed micellar system, and optimum operating conditions. Generally, the surfactant with charge opposite to that of pollutant is the best choice so as to develop a strong dye–surfactant interaction. Micellar solution has proved itself to be an effective medium to capture dye molecules. In this way true solution of dye is converted to colloidal solution and, resultantly, can be filtered using ultrafiltration membrane of suitable pores size. The effects of various factors like trans-membrane pressure, pH, temperature, ionic strength, nature of surfactant, concentration of dye, concentration of surfactant, etc. have been studied. It has been concluded that low trans-membrane pressure, low pH, room temperature, high ionic strength, combination of ionic and nonionic surfactants, and surfactant concentration higher than CMC are optimum factors to accomplish maximum dye removal.

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Chapter 12
Biofilm: An Innovative Modern Technology for Aquatic Pollution Remediation

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12.1 Introduction

Water is the basic necessity of life and its use is increasing globally by 1% per annum. According to Burek et al. (2016), increase in water use at this rate will lead to the total increase of 20–30% by 2050. Water bodies are seriously polluted due to increase in the rate of urbanization, industrialization, and population explosion (Qian et al. 2007). Pollution of surface water bodies mainly occurs by domestic waste effluent, surface runoff due to precipitation, and storm drainages. Besides this, industrial activities are responsible for creating tons of waste globally and its ultimate disposal into the aquatic environment (Edwards and Kjellerup 2013). World Health Organization has attributed 30% of all global diseases and 40% of global deaths to the water pollution (Kantawanichkul et al. 2009). There are different types of contaminants present in water such as detergents, pesticides, fats, and oils; microorganisms such as bacteria, viruses, protozoa, and helminthes; nutrients such as nitrogen, phosphorus, and ammonia, etc.; metals and inorganic materials such as mercury, lead, cadmium, nickel, and hydrogen sulfide, etc. (Sehar and Naz 2016). All such contaminants are responsible for the deterioration of water quality and make it unfit for use which in turn adds to the global water stress. Therefore the need of the time is to restore and remediate polluted water bodies that will ultimately help in fulfilling the demands of growing population. Different techniques have been adopted by scientists throughout the world for the remediation of pollutants from water bodies. One such technique is bioremediation of pollutants from the water. The key tools

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used for decontamination of water involve chemical precipitation, electrochemical deposition, evaporation, membrane process, ion exchange, and activated carbon adsorption. But the applicability of these methods is usually in question due to certain limitations such as high operational cost, inefficiency, and limited use. Therefore, the development of innovative, effective, sustainable, and low-cost technique for the remediation of the environmental pollutants has become necessary (Das et al. 2012). One such technique is the bioremediation of pollutants. "Bioremediation is the application of biological processes for the removal of hazardous pollutants present in the environment (Gianfreda and Rao 2004)." In the year 1972, bioremediation was used for the first time in Pennsylvania for the removal of gasoline leaked from the pipeline (Wang et al. 2012). Bioremediation may be natural or artificial and may find its applicability as in-situ or ex-situ. Among bioremediation techniques biofilm remediation of pollutants has recently gained much more importance particularly in remediating xenobiotic compounds from water (Das et al. 2012). The term biofilm was coined by Costerton et al. (1978). Biofilm can be defined as "An as an aggregation of bacteria, algae, fungi and protozoa enclosed in a matrix consisting of a mixture of polymeric compounds, primarily polysaccharides generally referred as extracellular polymeric substances (EPS) (Das et al. 2012)." Biofilm based bioremediation techniques of water are much better due to the sessile nature of biofilms and their ability to tolerate the extremely stressful conditions and the very nature of accumulation and stabilization of contaminants. In this chapter, emphasis has been laid on the use of aquatic biofilm community as an innovative strategy for remediation of pollutants from water.

12.2 Benefits of Using Biofilms for Remediation of Pollutants

The diverse group of microorganisms in a biofilm is surrounded by extracellular polymeric substances. They easily communicate and there is also exchange of food, materials, and energy which ultimately helps the community to tolerate variety of external pressures. This range of features demonstrates a multifaceted and strong mechanism to diverse kinds of cells working as an organ (Fux et al. 2005). Biofilms are sessile in nature and therefore continuously exposed to the contaminants in water bodies due to which these communities have developed the potential to degrade contaminants (Dar and Bhat 2020). The potential to tolerate and detoxify hazardous contaminants in water is of prime attention. While applying biofilms for bioremediation and biotransformation of contaminants, the chemicals to which biofilms are exposed may be in such concentrations that may cause severe damage to microorganisms living in isolation. Thus the accumulation of microbes in sessile or moving biofilms has the main benefit of augmented tolerance towards changing environmental conditions (Hall-Stoodley et al. 2004; Edwards and Kjellerup 2013). Other major characteristics that make biofilms suitable for bioremediation are:

1. their stress tolerance ability against toxic chemicals, changing nutrient conditions, etc. (Boles et al. 2004; Accinelli et al. 2012)
2. diverse metabolism and symbiotic relations for utilization of waste from surrounding environments (Boles et al. 2004; Chen et al. 2013)
3. their unique physical structure provided with channels of water for the transport of different nutrients, metals, and waste products (Latch et al. 2003; Wang et al. 2011; Xiao et al. 2012; Edwards and Kjellerup 2013)
4. help in treatment of xenobiotic substances such as surfactants and nutrient cycling (Rodriguez and Bishop 2008; Seo et al. 2009; Galiana et al. 2008; Verhagen et al. 2011).

12.3 Application of Biofilms for the Bioremediation of Aquatic Pollutants

12.3.1 Biofilm Based Remediation of Heavy Metals

Heavy metal remediation from aquatic environment can be achieved by immobilization of metals, biotransformation, bio-stabilization, complexation, etc. Researchers have reported the successful removal of heavy metals by using biofilms. Removal of Cr(III) by bacterial biofilm in a continuous flow biofilm reactor has been reported by Sundar et al. (2011). *Bacillus subtilis* and *Bacillus cereus* biofilms developed on coarse sand have the capacity to remediate approximately 98% of Cr(III) (Sundar et al. 2011; Das et al. 2012). A study on Cr(VI), Cd(II), Fe(III), and Ni(II) removal from the polluted water by using biofilm forming *Escherichia coli* grown on NaY zeolite proved to be an effective tool for the remediation of metal pollutants (Cristina et al. 2009a, b). The common representative groups of biofilm forming microbes on different substrates with their potential of remediation of specific types of heavy metals are presented in Table 12.1.

Table 12.1 Remediation of heavy metals by biofilm forming microbes on different substrates

Heavy metal remediated	Biofilm forming microbes	References
Cr(III)	Biofilms of *"Bacillus subtilis* and *Bacillus cereus"* on coarse sand substrate	Sundar et al. (2011)
Cr(VI), Cd(II), Fe(III), Ni(II)	"*Escherichia coli*" biofilm grown on zeolite NaY substrate	Cristina et al. (2009a, b)
Cr(VI)	Biofilm of "*Escherichia coli*" used on granulated activated carbon substrate	Rabei et al. (2009)
Zn(II)	"*Pseudomonas putida*" biofilm sorption of a Zinc metal by forming Zn-phosphoryl complex	Brandy et al. (2005)
Cu(II), Zn(II), Cd(II)	Activated sludge from a sewage	Costley and Wallis (2001)
Cd(II), Cu(II), Zn(II), Ni(II)	"*Pseudomonas* sp. NCIMB (11592)" biofilm bound on granular activated carbon (GAC) substrate by extracellular polymeric substance for metal and organic pollution removal	Scot et al. (1995)

12.3.2 Mechanism Behind Metal-Microbe Interaction

Microbes interact with metals by different ways and the classification of interaction between the two is based on type of metabolism involved for the uptake of metals. Thus on the basis of metal-microbe interaction, the mechanism of heavy metal remediation is divided into active and passive interactions (Alluri et al. 2007; Gupta and Diwan 2017). The benefit of metal-microbe interaction is that metal is changed into less toxic, immobile, and unusable form and is no longer available to cause injury. The capacity of a microbe to resist or tolerate the metal contamination in a water body may be specific such as bio-methylation of elemental mercury into more toxic methyl mercury or non-specific such as formation of cysteine rich protein by metallothioneins by *Cyanobacterium synechococcus*, *E. coli, Pseudomonas putida,* etc. (Rajendran et al. 2003; Gupta and Diwan 2017). Similarly in a constructive non-specific mechanism, a slime layer or exopolysaccharide substance is produced which is the main characteristic of biofilms. The metal-microbe mechanism and various ways of fixing of metal pollutants are shown in Fig. 12.1 (Gupta and Diwan 2017). Biosorption of metal by biofilms is beneficial over other mechanisms due to its ability to work passively without the help of metabolic activity and may also work with live and dead biomass. The advantage of dead cells over living cells is that dead cells are not influenced in terms of viability. Besides, dead cells are free from any hazard created by live cells.

12.3.3 Mechanism of Extracellular Polymeric Substance (EPS) to Remediate Metal Pollutants

Extracellular polymeric substances have the potential to show the phenomenon of biosorption by attracting positively charged metal ions towards its negatively charged surface. There are number of functional groups and non-carbohydrate groups such as acetamido group of chitin, structural polysaccharides of fungi, amine groups, sulfhydryl groups, carboxylic groups, proteins, phosphate, hydroxyl groups that ultimately give the negative charge to EPS (Wang et al. 2010; Gupta and Diwan 2017). In comparison to homopolysaccharides, extracellular heteropolysaccharides are frequently polyanionic due to presence of certain functional groups with polysaccharide mainstay (Pal and Paul 2010). The mechanism of sorption happens by ion exchange, complexation, precipitation, etc. (DePhilippis et al. 2011; Wang et al. 2010). As already discussed, biofilm forming microbes are potential players of remediating metal pollution from polluted ecosystems. *Herminiimonas arsenicoxydans* has the ability to induce biofilm formation and remediate arsenic through EPS up to a tolerance limit of 5 mM metal ion concentration (Marchal et al. 2010; Muller et al. 2006; Muller et al. 2007, p. 104). *Thiomonas sp*. CB2 strains remediate arsenic by using its exopolysaccharide and induce biofilm formation

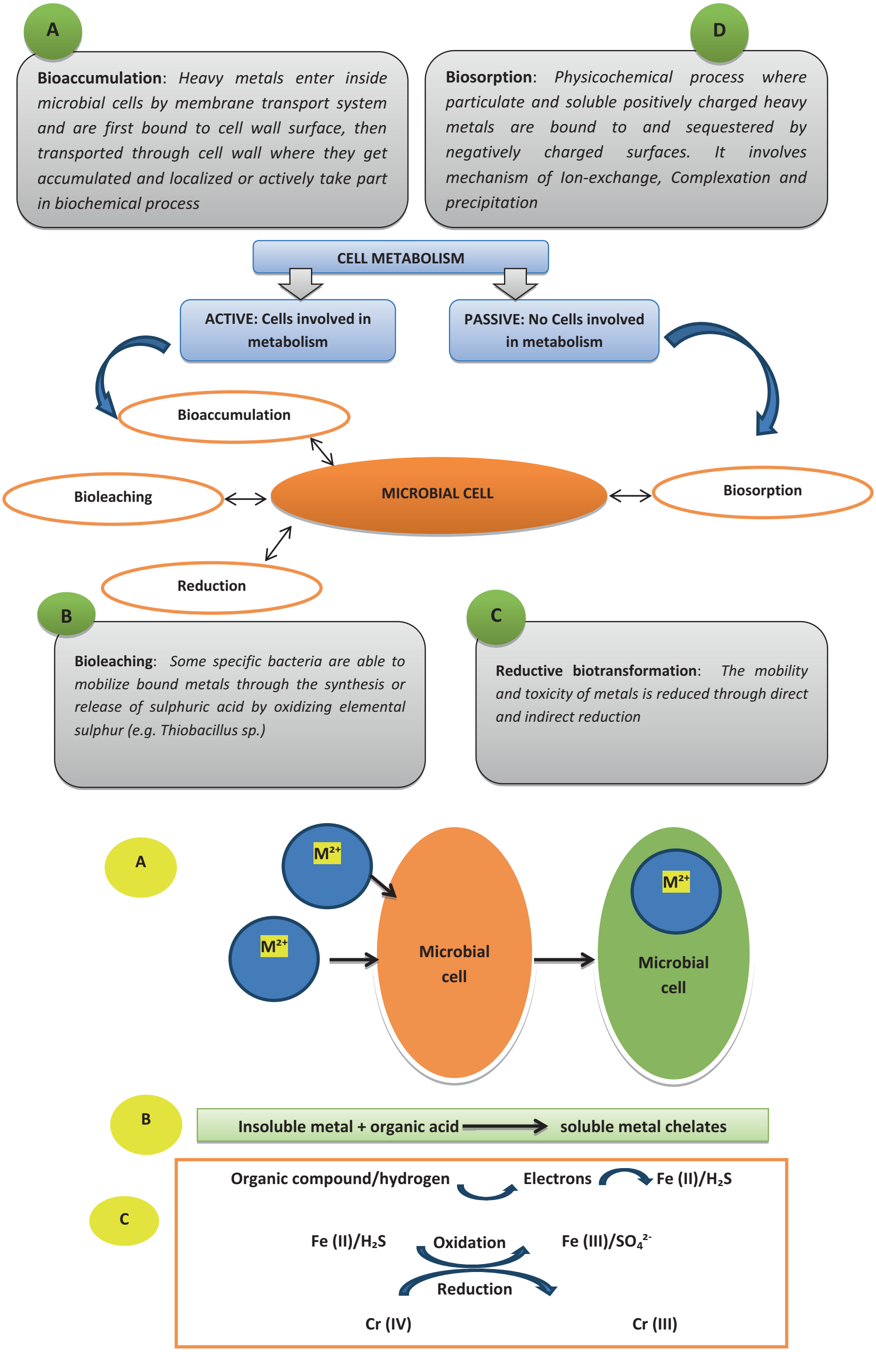

Fig. 12.1 Microbial-metal interaction mechanism (Active and Passive) showing bioaccumulation, biosorption, bioleaching, and redox arbitrated reactions for metal remediation (Gupta and Diwan 2017)

under stress conditions created by arsenic pollution (Marchal et al. 2011). Similarly, EPS and cell biomass of *Pseudomonas* isolates activated sludge scavenge metal ions from water (Lau et al. 2005). Some *Cyanobacteria* and marine microalgae (*Halomonas* sp.) have been found to have chelating ability and thus help in bioremediation of metals.

12.4 Innovative Way of Surface Water Bioremediation by Biofilms Supported by Filamentous Bamboo

Cao et al. (2012) modified batch and continuous flow bioreactor by introducing in it biofilm laden filamentous bamboo (Fig. 12.2). They reported that biofilm can remove COD, Cr, up to 66.1% in a batch hybrid reactor which can further be improved by adding glucose in a reactor. In case of continuous flow reactor, the rate of removal of COD, Mn, NH_4-N, turbidity, and bacteria was found to be 11.2–74.3%, 2.2–56.1%, 20–100%, and more than 88.6%, respectively. They further concluded that water surfaces with refractory organic pollution, low transparency, and high nitrogen pollution can easily be remediated by using biofilm on filamentous bamboo substrate.

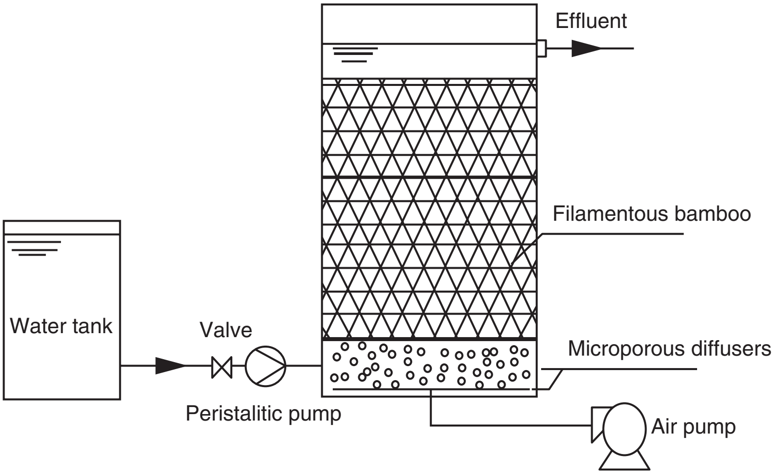

Fig. 12.2 Experimental setup for biofilm based surface water treatment plant with filamentous bamboo substrate used by Cao et al. (2012)

12.5 Biofilm Based Remediation of Recalcitrant and Toxic Chemicals

The urge for development has resulted in production of certain types of recalcitrant and toxic pollutants in water bodies that not only deteriorate the quality of water but are also dangerous for the ecosystem health. Much of the toxic chemicals like polychlorinated biphenyls, polynucleated aromatic hydrocarbons, and DDT, etc. have been banned by environmental agencies. But years after their ban, these recalcitrant compounds still exist in the environment in some form. The best way to remediate such pollutants is to use sustainable and low-cost remediation techniques. Recently yeast biofilm (*Candida tropicalis*) grown on gravel substrates has been utilized to degrade 97% of oil polluted water just in a short time span of 10 days. This biofilm can be potentially used for treating liquid waste in trickling filters on gravel bed before releasing the waste into water bodies (Chandran and Das 2011). The recent innovative technique of removal of left over organic pollutants of toxic nature after treating water has been developed by Rafida et al. (2011). The technique involved the application of PVC made vertical flow biofilter (VFB) of anaerobic nature. The upper part of this reactor was filled with small bottles of polyethylene terephthalate (PET) and lower part was filled with gravel. The biofilm of diverse microorganisms was grown on it and the efficiency of this technique proved to be 90% in terms of removal of hydrocarbon pollutants. Chlorinated ethene is usually added to drinking water to kill pathogenic microbes in it. A specific group of bacteria *Dehalococcoides* have the potential of reductive de-chlorination of chlorinated ethenes (Löffler et al. 2013). Different types of biofilm based microbes that help in the remediation of toxic chemicals from water are shown in Table 12.2.

12.6 Biofilm Based Remediation of Plastic and Micro-Plastic Wastes

The main constraint with plastic waste is its non-degradable nature in the environment. Biofilm based microbial consortia has been successfully used to degrade plastic wastes which causes several physical and chemical and biological obstructions in the aquatic ecosystem. In recent times, remediation of polyethylene wastes by the fungal biofilm formed of *Aspergillus niger* (ITCC No.6052) isolated from dump sites of plastic waste has been in use (Mathur et al. 2011). The fungi show proliferous growth when grown on medium with optimum environmental conditions. The biofilm of this fungus was able to reduce polythene by 3.44% and 61% of mass and tensile strength, respectively, in a period of 1 month. This property of *Aspergillus niger* (ITCC no. 6052) to remediate plastic waste was related to its high cell surface hydrophobicity and thermal oxidation potential. A wide variety of algal species including *Chaetophora, Cymbella, Navicula, Cocconis, Oocystis, Oedogonium,* etc. have been evaluated to show the potential of degrading plastic waste. “*Rhodococcus*

Table 12.2 Biofilm based remediation of toxic chemicals (Das et al. 2012)

Toxic compound	Biofilm forming organisms	References
Hydrocarbon compounds	*"Candida tropicalis"* biofilm grown on gravel	Rafida et al. (2011)
	"Filamentous cyanobacteria, picoplankton, and diatom based biofilm grown on gravel and glass plates" "*Acinetobacter calcoaceticus* and *nocardioforms* were partly found attached to filaments of cyanobacteria"	Al-Awadhi et al. (2003)
	Gravel supported biofilm of *"Cyanobacteria Dermocarpella* sp. *and Acinetobacter calcoaceticus, Acinetobacter calcoaceticus, Micrococcus* and *nocardioforms*"	Radwan and Al-Hassan (2001)
2,4-Dichlorophenol	Rotating perforated tubes biofilm reactor (RTBR) laden with *"Pseudomonas putida"* to remove 2,4-Dichlorophenol pollutant from water	Kargi and Eker (2005)
2,4,6 trichlorophenol "2,3,4,6-Tetrachlorophenol and pentachlorophenol"	*"Pseudomonas sp., Rhodococcus sp"* based fluidized bed biofilm for chlorophenol mineralization	Puhakka et al. (1995)
2 chlorophenol	Hydrogenotrophic biofilm cultivated in three silicone-tube membrane bioreactors under denitrification, sulfate-reduction, and dechlorination conditions for remediation of anaerobic sludge from a swine wastewater	Chang et al. (2004)
4 chlorophenol	Bacterial consortium from rhizosphere of *Phragmites australis*	Caldera (1999) and Das et al. (2012)
Polychlorinated biphenyl (PCB)	Anaerobic bacteria isolated from secondary sludge wastewater	Josephine et al. (2006)
4,6-Dinitro-ortho-cresol	"Biofilm based bacterial culture and fixed bed culture" biodegradation	Gisi et al. (1997)
Dinitrotolune (DNT)	Fludized biofilm reactor containing sludge from wastewater treatment plant; various aerobic and anaerobic bacteria	Lendermann and Spain (1998)

rubber strain (C208) has the capacity to degrading polythene at the rate of 0.86% per week due to its very nature of using polythene as a sole source of carbon and also it's potential to use polythene as a substrate during biofilm development (Suseela and Kiran 2007)". "Micro-plastics are defined as particles less than 5 mm in size with various chemicals and toxins attached to their surfaces (Bakir et al. 2016)". Though microplastic pollution and its impact on water bodies has been extensively studied, its remediation by using biofilms remains unexplored. The biofilm grown on the surfaces of plastics could be utilized to degrade microplastics and biotransform them into usable form. This goal can be achieved by pilot and micro-level studies by using microbial biofilms of diverse nature.

12.7 Bioremediation of Pharmaceuticals and Personal Care Products by Aquatic Biofilms

Pharmaceuticals and other personal care products are disposed into the environment as a part of effluent. These products are usually toxic in nature and prove fatal to the aquatic ecosystem. Sometimes these products may get transformed into more toxic pollutants. One such pollutant is triclosan, an antimicrobial drug which gets phototransformed into dioxin congeners in water (Latch et al. 2003; Buth et al. 2009). Removal of pharmaceuticals such as diclofenac, trimethoprim, metoprolol, etc. is efficiently removed by membrane biofilm reactors (MBRs) as compared to simple activated sludge processes (Sui et al. 2011). Onesios and Bouwer (2012) reported the in-situ bioremediation of pharmaceuticals and personal care products by using activated sewage sludge as inoculum for the formation of biofilm on sand bed column filters. In this experiment out of 14 pharmaceuticals and personal care products, 10 were completely degraded. Thus the use of biofilms for degradation of pharmaceuticals is an easy and more reliable way to get rid of these types of aquatic pollutants.

12.8 Biofilm Based Bioremediation of Synthetic Dyes from Water

Most of the synthetic dyes are carcinogenic and pose serious threat to the environment. Aquatic pollution due to synthetic dyes may also retard esthetic value of the water besides other problems by changing its color. Jiang and Bishop (1994) reported the aerobic degradation of azo dyes. Out of Acid Orange 8 (AO8), Acid Orange 10 (AO10), and Acid Red 14 (AR14), AO8 was the only dye that was decomposed aerobically. But under anaerobic conditions, all the dyes were decomposed easily. Pilot scale rotating drum biofilm reactor (ex-situ) also showed bioremediation of azo dye Everzol Turquoise Blue G by *Coriolus versicolor* activated sludge-based biofilm (Zhang et al. 1995).

12.9 Application of Periphytic Biofilm in Quantification of Denitrification Rate in Water Bodies

Periphytic biofilms are widely found in shallow water and sediment systems. It plays an important role in nitrogen cycling and helps in sustaining aquatic ecosystems and its role in denitrification by constructing the model. They divided water and sediment into three separate parts, viz. water column, sediment, and periphytic biofilm. They further used species, periphytic biofilm, and bacterial nitrifiers and denitrifiers as

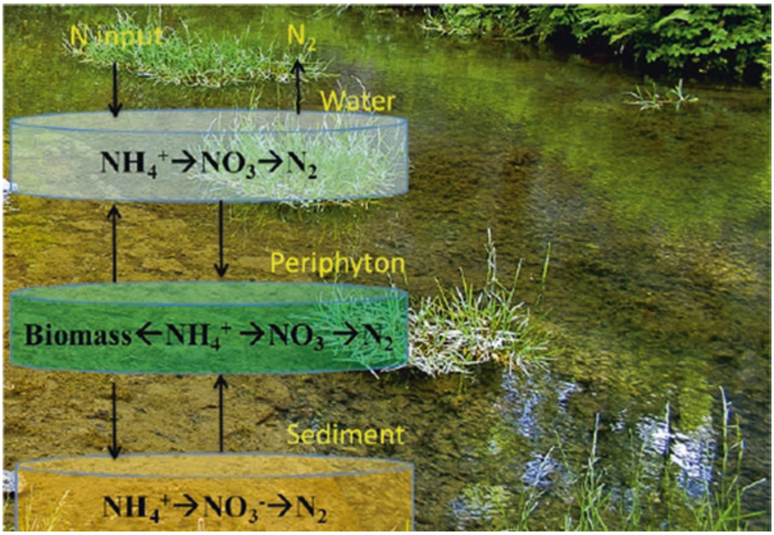

Fig. 12.3 Denitrification process by periphytic biofilm (https://www.thescientificnews.com/periphyticbiofilm)

potential factors. For this research structural modeling was used. "The study pointed that periphytic biofilm increased net denitrification directly as well as indirectly through the pH, the dissolved oxygen, dissolved organic carbon, and Nconcentrations in the water column and sediment" Fig. 12.3. Further research can be helpful in utilizing the potential of periphytic biofilm in nitrogen removal from wastewater.

12.10 Conclusion

Biofilm is an important and useful tool for the remediation of potentially hazardous pollutants from water bodies. Biofilm based innovative techniques are helpful in the degradation and removal of heavy metals and recalcitrant pollutants from the polluted aquatic ecosystems. The microbial biofilm application is a sustainable way of remediating toxic chemicals, synthetic dyes, pharmaceutical contaminants, plastic waste, and hydrocarbon pollutants from the wastewater. This type of substrate on which biofilm is grown plays an important role in removal of pollutants.

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Chapter 13
Heavy Metal Soil Contamination and Bioremediation

Malik Muzafar Manzoor, Pooja Goyal, Ajai P. Gupta, and Suphla Gupta

13.1 Introduction

Heavy metals are naturally occurring inorganic chemical hazards of high atomic weight, which are usually toxic at lower concentrations. Metals like zinc (Zn), copper (Cu), mercury (Hg), arsenic (As), lead (Pb), chromium (Cr), cadmium (Cd), and nickel (Ni) appear in the WHO list of chemicals of public concern (Kirpichtchikova et al. 2006; Adriano 2001). The significant portion of heavy metals released by anthropogenic activities into the environment finds their way into the soil. Unlike organic contaminants which are oxidized to carbon (IV) by microbes, most of the metal contaminants are not degraded by microbial and chemical processes after their introduction into the soil (Adriano 2001), as a result of their total concentration persists for an extended time (Maslin and Maier 2000). However, they may change in their chemical forms (speciation) and bioavailability in due course of time. The point of concern is that the biodegradation of organic contaminants can get affected by heavy metal contaminated soils (McLaughlin et al. 2000b). Heavy metal contamination of soil has adverse influences on human health and environment through drinking of contaminated groundwater, reduction in land usability for agriculture, and also land tenure. This demands regular characterization and remediation of soil and sufficient reclamation of soil ecosystem systems contaminated by heavy metals. Contemporary enactment respecting environmental protection and general well-being over the world depends on the data that characterizes environmental phenomena based on their chemical properties, particularly that resided in our food chain (Zhao and Kaluarachchi 2002). Soil categorization would give an insight of heavy

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metal bioavailability and their speciation, remediation of soil contaminated with heavy metals would necessitate information of the type of contaminant and source of contamination, respectively. This will help in calculating the associated health risks. The scientific assessment of associated risk would facilitate decision-makers to manage contaminated sites cost-effectively while preserving the ecosystem and public health. Phytoremediation, immobilization, and soil washing strategies used for remediation of heavy metal-contaminated sites are among the best demonstrated available technologies (BDATs) for remediation purposes (Kirpichtchikova et al. 2006). In spite of being ecologically suitable and cost-effective application of these technologies has been reported only in developed nations. Due to lack of awareness for their ecological benefits and principles of operation for their field application, these technologies are not commercially available in underdeveloping nations. Due noteworthy mindfulness by the legislature and the general public of the hazardous effects of the soil contaminated with heavy metals on animal and human health, researchers have shown their keenness in remediating contaminated sites using novel technologies (Pierzynski et al. 2000). Developing nations have limited funds for environmental restoration programs thus they need cost-effective and ecologically sustainable alternatives for the restoration of contaminated soil to lessen the associated risks, enhance food security, availability of the land resource for agricultural production. This could enable scaling down the land tenure problems. In this chapter, we have compiled the literature from various sources related to the contamination and health hazardous of heavy metals and their bioremediation.

13.2 Heavy Metals: Their Origin

Heavy metals exhibit properties like conductivity, ductility, cation stability, malleability, and ligand specificity which are also characteristic properties of metals. Several heavy metals like traces elements (Co, Mn, Cu, Mo, Fe, Ni, V, and Zn) are beneficial for organisms and are required in minute quantities. These metals are found naturally in the soil environment at micro levels and are hardly toxic (Pierzynski et al. 2000; Kabata-Pendias and Pendias 2001). However, excess of these elements can be harmful to organisms. The contaminated sites are found to be predominantly rich in As, Cu, Pb, Zn, Cr, Cd, and Hg (Jiang et al. 2001) having relatively high density and atomic weight (<20) (Smejkalova et al. 2003). Other heavy metals (Hg, Cd, Pb, and As) are not beneficial to organisms and pose major threats to the ecosystem. Over-exploitation of natural resources and other anthropogenic activities causes a disturbance in the delicate balance of the ecosystem, resulting in the accumulation of different heavy metals (Fig. 13.1) above defined values jeopardizing ecosystems, plants, animals, and human health (D'amore et al. 2005). The natural balance between heavy metal formation and degradation gets disturbed essentially because of rapid generation/usage of commercially important minerals through human made techniques altering their natural concentrations. This may also influence their abundance and bioavailability in the soil environment (D'amore

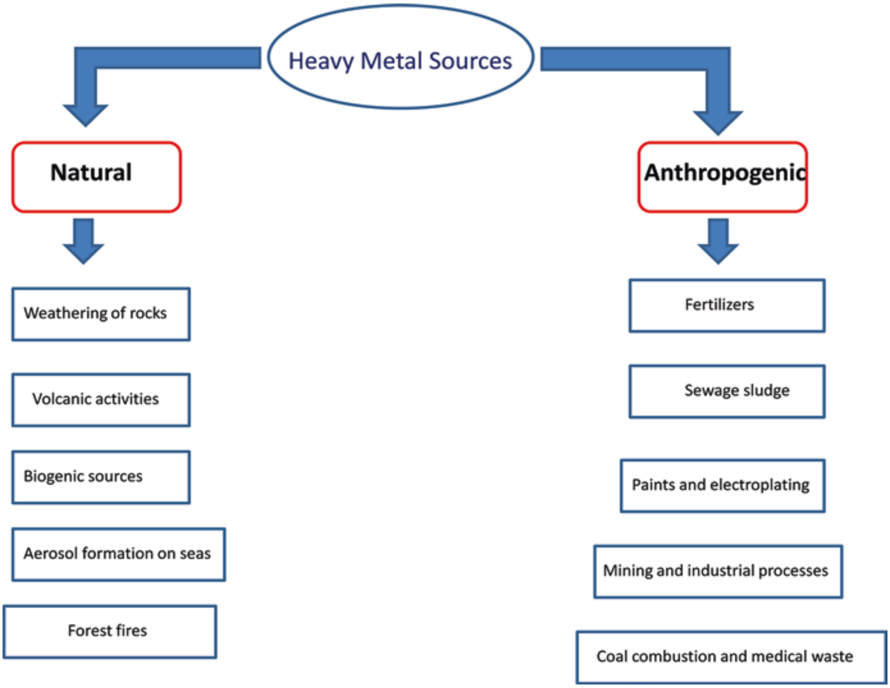

Fig. 13.1 Sources of heavy metals

et al. 2005). According to Lombi and Gerzabek (1998) the mass balance of heavy metal (M) is the sum total of parent material (p), atmospheric deposition (a), fertilizer sources (f), agrochemical sources (ag), organic waste sources (ow), and other inorganic pollutants (iP) minus activities due to crop removal (cr) and losses by leaching, volatilization (l).

$$\mathrm{M\ total} = \mathrm{Mp} + \mathrm{Ma} + \mathrm{Mf} + \mathrm{Mag} + \mathrm{Mow} + \mathrm{Mip} - \left(\mathrm{Mcr} + \mathrm{Ml}\right)$$

Emission through natural fluxes of several metals into the environment is three folds lower as compared to emission caused due to anthropogenic activities (Sposito and Page 1984). Bioavailability and mobility of heavy metals present in contaminated soils, which are added through anthropogenic activities vary from pedogenic or lithogenic ones (Kuo et al. 1983; Kaasalainen and Yli-Halla 2003). Several anthropogenic sources like metal mine tailings, leaded gasoline and paints, petrochemicals, sewage sludge, fertilizer, compost, pesticides, residues from coal combustion, disposal of high metal wastes in inappropriately protected landfills are the originator of metal-bearing solids at contaminated sites (Khan et al. 2008; Basta et al. 2005; Zhang et al. 2010). To estimate environmental, health effects of heavy metals and to understand their speciation, possible remedial options, and bioavailability, it is prerequisite to have the knowledge of their basic chemistry. It is the chemical form and speciation of heavy metals which determines their fate and transport in soil.

Adsorption of heavy metals on the soil is a complex process which took years. After adsorption on soil heavy metals get reallocated into various chemical forms with changeable toxicity, bioavailability, and mobility (Shiowatana et al. 2001; Buekers 2007). There are several factors that control re-distribution which include adsorption and desorption between the metals, ion exchange, precipitation of mineral and dissolution, aqueous complexation, and plant uptake, biological immobilization and mobilization (Levy et al. 1992).

13.2.1 Effects of Fertilizer Application

Historically the first and foremost human influence on soil was in the form of agriculture. Not only macronutrients (S, Ca, Mg, N, K, and P) but also important micronutrients are acquired by plants for their complete lifestyle and growth. Heavy metals such as (Cu, Co, Fe, Ni, Mn, Mo, and Zn) which are crucial for plant growth and development are deficient in some soils (Lasat 1999), and they are supplied to crops as an addition to the existing soil nutrients. Similarly, Cu and Mn are supplied to cereal crops which are growing in deficient soils in addition to the soil composition. In intensive farming system, a large quantity of fertilizers is added to soil to provide sufficient N, P, and K for the growth of a crop. Heavy metals like cadmium and lead, in trace amounts, are generally present in these fertilizers. Their content in soil is significantly increased because of the continuous and large scale use of fertilizers (Jones et al. 1981). Though metals like Cd and Pb do not show any noted physiological activity; however, certain phosphatic fertilizers used in farming unknowingly add Cd and other toxic elements to soil together with Pb, F, and Hg. Application of fertilizer though is beneficial for short-term plant productivity but in the long term it leads to more harm if used non-judiciously.

13.2.2 Pesticides

In the past pesticides were commonly used in horticulture and agriculture containing significant concentrations of metals. About 10% recently approved chemicals for use as fungicides and insecticides in the UK contained metals like Mn, Cu, Hg, Zn, or Pb. They include copper oxychloride and Bordeaux mixture which is a copper-containing fungicidal sprays (Jones et al. 1981). In fruit orchards lead arsenate was used to control parasitic insects for several years. Similarly to control banana pests and cattle ticks arsenic-containing compounds were extensively used in New Zealand and Australia. For the preservation of wooden logs formulations of

Cu, Cr, and As (CCA) were used in various parts of the world, and there are currently several polluted sites where the concentration of these elements in the soil is greatly surpassing the background concentrations. The use of such formulations is generally localized to a particular site or job and for a specific application. However, problems arise when such contaminated locations are used for different agricultural or non-agricultural purposes (McLaughlin et al. 2000a).

13.2.3 Natural Fertilizers: Manures and Biosolids

Accumulation of heavy metals such as Cd, Pb, Hg, Mo, As, Cu, Ni, Sb, Se, Cr, Tl, Zn in the soil is primarily due to the application of various biosolids like composts, sewage sludge, and livestock manures that unintentionally leads to their buildup (Lasat 1999). Poultry, pig manures, and cattle waste are considered as valuable fertilizers and are thus applied to crops as slurries or solids (Sumner 2000). Copper (Cu) and zinc (Zn) metals are generally added to pig and poultry diet as growth promoters (Sumner 2000; Chaney and Oliver 1996). In the long run, repeated application of manure to restricted areas of land from animals on such diets containing high concentrations of Zn and Cu metal can cause a substantial buildup of these heavy metals in the soil. Potential of biosolids with heavy metals to contaminate the soil has produced abundant concern regarding their application for agricultural purposes (Canet et al. 1998). Cd, Cr, Pb, Ni, and Cu are the most commonly found heavy metals in biosolids, and it is their nature, intensity of the industrial process, and the process employed for their treatment which governs their concentration (Canet et al. 1998). Groundwater usually gets contaminated because of leaching down of metals through the soil profile (McLaren et al. 2005). In New Zealand, the recent analysis had shown that soils treated with biosolids increased Zn, Cd, and Ni levels in drainage leachates (McLaren et al. 2004; Keller et al., 2002).

13.2.4 Wastewater and Industrial Effluents

Since ages, municipal and industrial wastewater were used for watering land in several countries (Reed et al. 1995). It is estimated worldwide that wastewater is utilized to irrigate about 20 million hectares of arable land. Studies suggest that in numerous African and Asian cities, about 50% of agriculture land is based on wastewater irrigation (Bjuhr 2007). Irrigation of land for a long duration of time with industrial effluents and wastewater containing heavy metal in less concentrations ultimately results in their accumulation in the soil. Now several developed countries have invested hugely in wastewater treatment plants for its use in irrigation and other activities with minimum or no harmful effects.

13.2.5 Industrial Waste and Mining Activities

Modernization and infrastructure development have left the legacy of a wide range of heavy metal contaminants in soil, air, and water equally. Direct discharge of byproducts from the mining process (tailings) into natural or human-made reservoirs results in elevated concentrations (DeVolder et al. 2003; Mushtaq et al. 2020; Qadri and Bhat 2020). Large scale smelting and mining of Zn and Pb ores have resulted in contamination of soil that hugely affects the health of the living organism. In spite of being lengthy and expensive, many strategies used for the reclamation of these sites may not restore soil productivity with high efficiency. Heavy metals accessibility in the soil in large amounts causes environmental risk directly and indirectly to humans, directly and indirectly, both. Assimilation or entry pathways of metals include the oral bioavailability that is contaminated soil ingested directly or contaminated soil ingested by plant materials (Basta and Gradwohl 1998). Different materials are released in the environment by several industries like tanning, accidental oil spills, textile, pesticides, petrochemicals, and pharmaceutical manufacturing units and have high variability in their composition. Among these, some are beneficial for forestry or agriculture when disposed on land. But many are potentially hazardous and pose risks to ecosystem and human health because of high contents of toxic organic components and heavy metals like Pb, Cr, and Zn (Sumner 2000). Several toxic influences of heavy metal published are compiled in Table 13.1.

13.3 Bioremediation

The unlimited release of industrial waste to the environment because of the growing industrial sector, especially in the urban sector, has lead to the accumulation of heavy metals. Gradually these heavy metals are depleted through various processes like erosion, deflation, leaching, and plant uptake. Heavy metals which are released into the water and soil are least amenable to changes thus cannot be transformed into less toxic forms and consequently have long-term influences on the ecosystem. Several studies have advocated bioremediation approach to be one of the most effective solutions for the protection of the environment as well as human health (Martin and Ruby 2004; Shafi et al. 2018).

Generally, remediation processes are subjected to an assortment of regulatory necessities. However, for processes where no known standards occur or where standards are recommended, their assessment is based on ecological risks and their effects on human health. The regulatory authorities usually allow that remediation strategies that are centered on decreasing metal bioaccessibility only if it demonstrates the decreased risk and manifests long-lasting effect (Martin and Ruby 2004). The choice of suitable remediation treatment depends on the type of metal-contaminated soils and their physical and chemical form. Several techniques are

Table 13.1 Toxicity of heavy metals

Heavy metal	EPA regulatory limit (ppm)	Toxic effects	Reference
As	0.01	ATP synthesis and oxidative phosphorylation	Tripathi et al. (2007)
Ni	0.2 (WHO permissible limit)	Skin rashes, lung cancer, nose and throat infections, neurotoxic, genotoxic, affects fertility	Khan et al. (2007), Das et al. (2008), Duda-Chodak and Blaszczyk (2008)
Hg	2.0	Tremors, fatigue, temper outbursts, depression, restlessness, hair loss, vision disturbance, brain damage, failure of kidney and lung	Gulati et al. (2010), Ainza et al. (2010)
Cd	5.0	Mutagenic, carcinogenic, influences calcium regulation in biological systems, lung damage	Salem et al. (2000), Degraeve (1981)
Cr	0.1	Causes hair loss	Salem et al. (2000)
Pb	15	Cardiovascular disease, impaired development in children, short-duration memory loss, reduced intelligence, difficulties in coordination and learning debilities	Padmavathiamma and Li (2007), Salem et al. (2000)
Se	50	Influences endocrine function, hepatotoxicity, and gastrointestinal disorders, impairment of NK cells activity	Vinceti et al. (2001)
Zn	0.5	Fatigue, dizziness	Hess and Schmid (2002)
Ba	2.0	Elevated blood pressure, cause cardiac arrhythmias, muscle twitching, gastrointestinal dysfunction	Jacobs et al. (2002)
Ag	0.10	Stomach pain, exposure causes skin and tissues to turn gray or blue-gray, irritates in lung and throat, breathing problems	Abadin et al. (2007)

available which are employed to remove these heavy metals, which include oxidation or reduction, membrane technology, electrochemical treatment chemical precipitation, ion exchange, and reverse osmosis.

However, most of these techniques are expensive or ineffectual to lower concentrations of the metal (>100 mg/L) (Ahluwalia and Goyal 2007). Alternately, the attractive and the best alternate to physicochemical methods for removal of heavy metals are biological approaches such as bioaccumulation and biosorption (Kapoor and Viraraghavan 1995). To resolve and restore the natural condition of the soil usage of plants and microorganisms are the best possible options for remediation purposes. Heavy metals despite being important for microbial growth and development at relatively low concentrations, they exert considerable stress on microbial communities or desired plant community utilized for the bioremediation (Doleman 1994). Microbial makeup is mainly modified when the essential metal ions of biological molecules are displaced or modified; it is the blockage of essential functional groups that can also change the microbial makeup (Wood and Wang 1983; Li and

Tan 1994). Heavy metal contaminations trigger a complex response in microbial communities depending on their concentration, availability and kind of heavy metal, the nature of the medium, and microbial species (Coblenz and Wolf 1994).

Despite being a nondisruptive strategy for soil remediation, Bioremediation is time-consuming and is influenced by geological and climatic conditions of the remediation site (Schmöger et al. 2000). The process of bioremediation of heavy metals adopts a different approach. Here the heavy metals are not degraded but are transmuted from one organic complex or oxidation state to others. Because of the change in the oxidation state of the heavy metals, change in volatility, toxicity, bioavailability, and solubility can occur (Garbisu and Alkorta 1997, 2003). If they become more or less water soluble, they may be removed through leaching or precipitation from the environment. Since metal contamination varies from site to site and type of effluent, no single process may be used for its bioremediation. Plants, microorganisms, or the combination of both organisms can be employed to achieve the target.

13.3.1 Microbial Remediation

The microbial remediation involves a cocktail of several microorganisms like bacteria (*B. subtilis*), *Enterobacter* (*E. cloacae*), and *Pseudomonas* (*P. putida*) have been effectively used for the reduction of Cr(VI) to the less toxic Cr(III) (Garbisu et al. 1997; Garbisu et al. 1998; Ishibashi et al. 1990; Wang et al. 1989; Shafi et al. 2018). It is also reported in the literature that *B. subtilis* can reduce non-metallic elements. For example, Garbisu et al. (1998) studied that selenite has been decreased to the less toxic elemental selenium using *B. Subtilis*. Further, Ajaz et al. (2010) revealed that *B. thuringiensis* and *B. cereus* could increase the cadmium and zinc extraction from the soil rich in Cd and soil contaminated with sewage from the metal industry. Studies have revealed that heavy metals stimulated the siderophore production (Fe complexing molecules) using bacteria, thereby modulating their bioavailability (Van der Lelie et al. 1999). In a study, it was observed that *Azotobacter vinelandii* showed increased siderophore production in the presence of Zn(II) (Huyer and Page 1988). The change in bioavailability could have led to the natural extraction of these metals from the soil. Besides the change in bioavailability, precipitation by sulfate-reducing bacteria (*Desulfovibrio desulfuricans*) could also lead to bioremediation. This process is indirect and mostly ex-situ where the sulfate-reducing bacteria convert sulfate forms of heavy metal into their hydrogen sulfate, as in heavy metals Zn and Cd, to form insoluble forms of these metal sulfides (White et al. 1998). Mercury is a very toxic pollutant, and its bio-magnification has created havoc to land and aquatic life in the past. In in-situ setup soluble mercuric ions Hg(II) were converted to volatile metallic mercury Hg(0) by mercury-resistant bacteria (Hobman 1997). This microbial assisted remediation process is very crucial in controlling mercury-based contamination. The reduced form of mercury Hg(0) can be easily volatilized, subsequently decreasing atmospheric level (Lovley and Lloyd 2000).

Genetic engineering is the recent and designer technology that can be adapted to modify microbes to make microbe assisted remediation more effective. Genetically modified *Ralstonia eutropha* was used to sequester Cd-contaminated soils (Valls et al. 2000). Using this approach, the *R. eutropha* was genetically engineered and designed to produce a cysteine-rich metal binding protein (metallothionein) on its cell surface. These receptors could quench Cd available in the soil. Presence of sequestered metals in soil is less harmful because of their less bioavailability. Two major loop holes for this remediation approach are the controversies for the use of genetically modified organisms (Urgun-Demirtas et al. 2006) and the remains of sequestered metals in soil.

One of the strategies used during bioremediation of contaminated soils to make it favorable for soil microbes is biostimulation which involves the use of organic amendments and addition of nutrients to the soil. Organic matter in the form of manure serves as a carbon source for soil microbes and the growth and activities of microorganisms involved in the process of remediation increase due to added nutrients, the only limitation here is to identify the right strain for biostimulation. Typically biostimulation is used to biodegrade organic pollutants (Abioye 2011) and can also be employed for the bioremediation of soils contaminated with heavy metals. Since heavy metals are less amenable to biodegradation, biostimulation indirectly enhances their bioremediation by altering soil PH. It is the introduction of organic matter that actually reduces the soil pH (McCauley et al. 2009). Solubility and bioavailability of heavy metals get increased by low pH of the soil, which subsequently increase its extractability from the soil (Karaca 2004). Biochar is an organic material which is known for its potential in the management of soils contaminated with heavy metals. Currently, it is being used for remediation purposes in various parts of the world. Experiments conducted by Namgay et al. (2010) stated that the presence of heavy metal in the contaminated soil amended with biochar was decreased. Unlike most other organic amendments biochar because of its ability to increase soil pH (Novak et al. 2009) reduces the bioavailability of metals for plant uptake due to increased sorption. Biochar properties differ broadly depending on the feedstock used in its production, and it also depends on the method employed during its production. Thus it is essential to note the effect of different amendments of biochar will have on its effectiveness when using for soil remediation. Further, in-depth research is required to comprehend how soil microorganism are influenced by biochar and how remediation process of contaminated soils is amended because such reports are rare in literature.

13.3.2 Phytoremediation

Phytoremediation ("Phyto" means plant) is a broad term used for a group of technologies that uses plants and associated microorganisms for in-situ partial or complete removal of selected contaminants from soil, sediments, sludge, groundwater, and wastewater. Phytoremediation can be employed to remove heavy metal contaminants, organic pollutants, as well as radionuclides (Ali et al. 2013; Bhat et al. 2018). Plants because of their unique absorption, metabolic capabilities, and remarkably efficient transportation systems selectively absorb nutrients or contaminants from the growth matrix and environment. Over the years, the emphasis was on phytoremediation since plants because of this unique property can be used to remediate soils contaminated with heavy metals. Phytoremediation is a technique that includes growing plants in a contaminated matrix, to eliminate contaminants or simplify immobilization (containment/binding) or degradation (detoxification) of the pollutants. The plants can be consequently processed, harvested, and disposed of. Driven by solar energy phytoremediation is an efficient, cost-effective, and environmentally friendly in situ remediation technology. There are different processes involved in phytoremediation which contain phytoextraction, phytofiltration, phytostabilization, photo-degradation, and phytovolatilization (Alkorta et al. 2004). The preliminary step of phytoremediation is the uptake of contaminants (phytoextraction) by plant roots from soil or water and their translocation and accumulation in plant tissues (Sekara et al. 2005). Translocation of metals to the aerial tissues of a plant is an important biochemical process. During photo-filtration, the plant absorbs water along with soluble components through roots (rhizofiltration), seedlings (blast filtration), or excised plant shoots (call filtration) (Mesjasz-Przybyłowicz et al. 2004). In phytoremediation, movement of metals in underground water is restricted because of their adsorption and absorption by the plants. In addition to this, other processes like phytoimmobilization or phytostabilization also reduce metal bioavailability and movement, which ultimately prevent their migration into the food chain and groundwater (Erakhrumen 2007). There are several plant-based approaches which immobilize metals present in contaminated soils which include precipitation, sorption through roots, reduction in the rhizosphere, and complex formation or metal valence (Poschenrieder and i Coll 2003). To metabolize organic pollutants, plants employ enzymes like oxygenase and dehalogenase, which do not need rhizospheric microorganisms for their action (Vishnoi and Srivastava 2007).

However, several volatile heavy metals like Hg and Se present in the contaminated soils are made ineffective by the process of phytovolatilization. In this process, plants convert them into volatile forms and are consequently released into the atmosphere (Karami and Shamsuddin 2010). However, phytovolatilization has limitations that it does not remove metals completely from the contaminated site. Instead, they are transferred from the site of contamination to the atmosphere from which they have chances to reenter the previous medium. The list of plants used in phytoremediation is given in Table 13.2. Further research in this area to identify and experimentally validate plant species resistant to heavy metal contaminated soil would be highly valuable in saving the environment.

Table 13.2 List of selected plants reported for phytoremediation of heavy metals

Heavy metal	Plant species	References
Cd	Castor (*Ricinus communis*)	Huang et al. (2011)
Cd, Pb, Zn	Corn (*Zea mays*)	Meers et al. (2010)
Hg	*Populus deltoides*	Che et al. (2003)
Se	*Brassica juncea, Astragalus bisulcatus*	Dixit et al. (2015)
Zn	*Populus canescens*	Bittsánszky et al. (2005)
Hg	*Eichhornia crassipes*	
Cr	*Spartina argentinensis*	Nalla et al. (2012)
Cd, Cu, Ni, Pb	Jatropha (*Jatropha curcas* L.)	Abhilash et al. (2009)
As	*Pteris vittata*	Datta et al. (2017)
Cd, Cu, Pb, Zn	*Populus* spp. (*Populus deltoides, Populus nigra, Populus trichocarpa*)	Ruttens et al. (2011)
Ni	*Alyssum bertolonii, Alyssum murale*	Mengoni et al. (2012)
Hg	*Pteris vittata*	Su et al. (2008)
Cd, Cu, Pb, Zn	*Salix* spp. (*Salix viminalis, Salix fragilis*)	Pulford and Watson (2003)
Pb	*Medicago sativa*	Su et al. (2008)

13.3.3 Natural Phytoextraction

Certain plants have the inherent property of metal accumulation. Phytoextraction utilizes the natural hyperaccumulator plants which comprise more than 1% of metal in their dry biomass. Hyper-accumulator plants accumulate 100-fold higher metals compared to non-accumulator ones. Studies have shown that a hyperaccumulator plant can collect more than 10 mg kg^{-1} Hg, 100 mg kg^{-1} Cd, 1000 mg kg^{-1} Pb, Cu, Cr, and metals and up to 10,000 mg kg^{-1} of Zn and Ni (Lasat 2002; Baker and Brooks 1989). This property of high metal tolerance of hyperaccumulator plant species has been employed on the metalliferous sites for bioremediation. Literature reports around 400 plant species belonging to 45 plant families can hyperaccumulate metals (Ghosh and Singh 2005; Henry 2000). The important families include *Euphorbiaceae, Brassicaceae, Lamiaceae, Fabaceae, Scrophulariaceae, and Asteraceae* (Dushenkov 2003; Salt et al. 1998). A report on the evaluation of hyperaccumulation potential of crops has shown that alpine pennycress (*Thlaspi caerulescens*), *Astragalus racemosus,* Ipomoea alpine, *Sebertia acuminate, Haumaniastrum robertii* contain great bioaccumulation potential for Co, Se, Cu, Ni, and Zn, respectively (Lasat 1999).

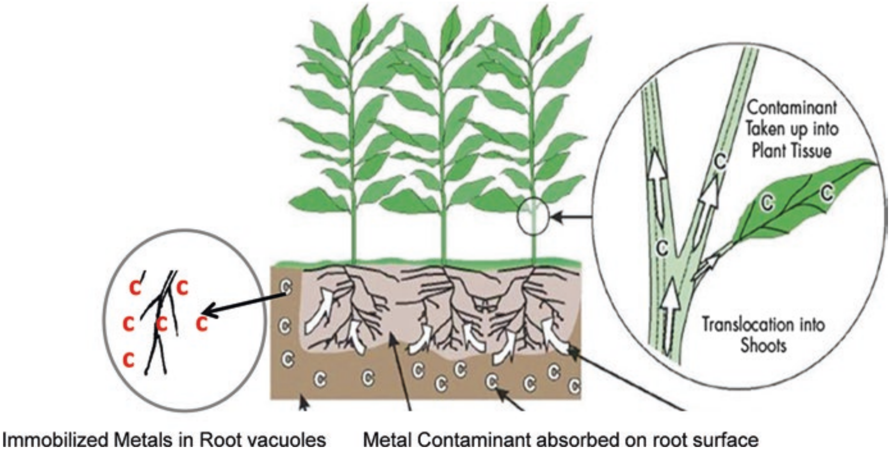

Fig. 13.2 Phytoextraction of metals from soil

Similarly, a report by Schmidt (2003) has shown that following plants have high uptake and tolerance to heavy metals: *Brassica juncea* L., *Helianthus annuus* L., *Zea mays* L., and *Salix viminalis* L. Some of the plants with hyper metal accumulation properties are listed in Table 13.2. Lasat (1999) had proposed steps involving metal absorption from the soil by the plant system. According to him, phytoextraction of metals from soil involves multiple processes (Fig. 13.2). Initially, the metal present in the proximity of the root surface gets sorbed on its surface and enters the root cells through the cellular membrane. Subsequently, some parts of the metal cross the metal cellular membranes into root vascular tissue (xylem), while the remaining part gets immobilized in the vacuole. From the vascular tissues, the metal gets translocated to stem and leaves. Most of the metals once inside the plant become insoluble and cannot move in the vascular system due to optimal cellular pH.

The insoluble metals are generally stored in extracellular (apoplastic) and intracellular (symplastic) compartments as precipitates of sulfate, carbonate, or phosphate (Raskin and Ensley 2000). Metal hyperaccumulator plants though are efficient in absorbing metals in large quantities from the soil, but they produce little biomass and are slow growing. To clean up contaminated sites using these plants, it may take years to decades. To overcome these shortfalls chemically improved phytoextraction has been employed. In this approach, chelating organic acids are used to enhance high biomass-producing crops to take up large amounts of metals (Nowack et al. 2006).

13.4 Combination Approach for the Remediation

To speed up the remediation process of contaminated soil, the combined use of microorganisms and plants has resulted in a faster and more proficient clean-up process (Weyens et al. 2009). Mycorrhizal fungi have been utilized in several heavy metal remediation processes. Studies on mycorrhizal metal uptake have shown that mycorrhiza use different approaches for the remediation of soils contaminated with heavy metals. Few studies have indicated greater accumulation of heavy metals in plants thus improved phytoextraction (Jamal et al. 2002; Weyens et al. 2009; Marques et al. 2006; Joner and Leyval 2001), others reports showed reduced metal concentration in plants and enhanced phytostabilization through metal immobilization (Janoušková et al. 2006; Heggo et al. 1990). The mycorrhizal associations with the plant are not limited to metal quenching only but it extends to water acquisition, plant growth, plant resistance to diseases, and increased nutrient content of the plant (Cardoso and Kuyper 2006; Harrier and Watson 2004; Wright et al. 2007). Many studies revealed that mycorrhizal association enhances the soil fertility, helps in the survival of plants growing in contaminated soils, and assists in the regeneration of remediated soil (Chibuike 2013). But there are also some reports that mycorrhizal association does not always help in soil remediation that is contaminated with heavy metals (Diaz et al. 1996; Joner and Leyval 1997) this is because not all the species of mycorrhizal fungi are effective in all the metals at any concentration (Chibuike 2013; Marques et al. 2009). Also, there are reports in the literature that heavy metals may inhibit the activities of mycorrhizal fungi (Del Val et al. 1999; Chao and Wang 1990). As compared to plants, some mycorrhizal fungi species (*Arbuscular mycorrhizal)* are found more sensitive to pollutants. Microorganism in association with plants is also used for bioremediation process apart from mycorrhiza. Among these are plant growth-promoting rhizobacteria (PGPR) which are present in the rhizosphere. These microbes assist plant growth by several mechanisms such as by supplying of nutrients and phytohormone production (Glick et al. 1995), siderophores production, and other chelating agents (Kamnev and Van der Lelie 2000). These PGPR also assist through specific enzyme activity, nitrogen fixation (Khan 2005), and reduction in ethylene production, which encourages root growth (Glick et al. 1998). In general, plant growth-promoting rhizobacteria (PGPR) have been used to reduce heavy metal associated plant stress in phytoremediation studies (Reed and Glick 2005). It has been observed that inoculation of *B. juncea* and *B. napus* plants with Bacillus species showed enhanced nickel and cadmium accumulation (Sheng and Xia 2006; Reed and Glick 2005; Zaidi et al. 2006). On the other hand, it was shown that inoculation of tomato plant with *Burkholderia species* and *Methylobacterium oryzae* showed improved plant growth due to reduction in Cd and Ni accumulation in the root and shoot tissues of the plant (Madhaiyan et al. 2007). This indicates that the mechanism employed in the process of phytoremediation by PGPR is a complex intertwined network and may dependent on the species of PGPR, the mechanisms employed by PGPR and plant involved in the process. Though reports on phytoremediation including both mycorrhizal fungi and PGPR are unusual, (Vivas et al. 2006) observed increased mycorrhizal efficiency due to PGPR (*Brevibacillus* sp.) which leads to decrease in metal accumulation and enhanced white clover growth on a heavy metal (Zn) contaminated soil.

13.5 Conclusion

Knowledge and possible threats of toxic heavy metals present in contaminated soils are vital for the selection of suitable remedial method. It is necessary to remediate the soil contaminated by heavy metals to make the land resource available for agricultural purposes, reduce the associated risks, scale down the land tenure problem, and enhance food security. Immobilization, soil washing, and phytoremediation are the best available technologies frequently used for cleaning up heavy metal contaminated soils. Bioremediation is the most appropriate disruptive method when used for cleanup of land used for agriculture purposes. Compared to microorganism assisted bioremediation use of plants (phytoremediation) is more common strategy to remediate the heavy metal contaminated soil. There are several methods employed by plants to remediate contaminated soil, which includes the most common method (phytoextraction). Combined use of microorganism and plants to remediate the contaminated soil will increase the efficacy of bioremediation process.

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Chapter 14
Environmental Biotechnology: For Sustainable Future

Malik Muzafar Manzoor

14.1 Introduction

Environmental sustainability is one of the biggest issues faced by humankind. Rapid and rampant industrialization and urbanization have put global land resources under severe threat. To make our planet a sustainable ecosystem and suitable habitable for future generations, we not only need to make corrections in existing methods but also need to remediate the polluted natural resources using natural ways. Recycling of biomass, development of production processes that makes optimal use of natural resources, recovering energy, and minimizing waste generation can have favourable effects on the environment. Despite escalating efforts to prevent waste accumulation and to promote recycling, the amount of environmental damage caused by the high standard of living, over-consumption, the quantities of waste generated, and the degree of unsustainable land use appear likely to continue growing. Environmental biotechnology is one of the options to prevent, arrest, and reverse environmental degradation through the appropriate use of biotechnology in combination with other technologies while supporting safety procedures as a primary component of the program. Biotechnological processes and their products can be applied with a view of long-term ecological security to protect environmental integrity (Dervash et al. 2020). While bioremediation is acknowledged as a promising technology for restoring polluted and degraded lands, its field potential is limited for various reasons. However, recent biotechnological advancements, including producing efficient microbial consortia, applying enzymes with higher degrees of specificity, and designing plants with specific microbial partners, are opening new prospects in remediation technology.

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14.2 Recent Biotechnological Advancements for On-Site Cleaning

To speed up the remediation process of the contaminated environment, the combined use of microorganisms and plants has resulted in a faster and more proficient cleanup process (Weyens et al. 2009). With the advent of the transgenic technology, plants and microorganisms can be engineered to degrade pollutants. Most of the sites are contaminated with different pollutants; thus, onsite bioremediation needs different strains of bacteria with the diverse catabolic potential to degrade pollutants. Advanced biotechnological approaches offer opportunities to isolate and culture these useful bacterial strains for their in-depth study. Next generation sequencing technology offers clues to engineer strains so that their remediation potential can be enhanced (Su et al. 2015).

High-throughput RNA sequencing can identify viable but nonculturable (VBNC) states under unfavourable conditions of biphenyl/polychlorinated biphenyl (PCB)-degrading bacteria *Rhodococcus biphenylivorans* (Su et al. 2015, Khan et al. 2017). VBNC state identification of microorganisms which are used for remediation of the polluted environment is vital for knowing their functional state during field application, and it also assists in providing favourable conditions for enhancing their remediation potential. Microbe capturing using immunomagnetic beads and colorimetric detection of their outer membrane peroxidase by oxidizing 3,3′,5,5′-tetramethylbenzene in the presence of H_2O_2 is another cost-effective method for the rapid enumeration and sensitive detection of uranium-reducing *Shewanella oneidensis* from sediment samples (Wen et al. 2014).

Genetic engineering is the recent and designer technology that can be adapted to modify microbes to make microbe assisted remediation more effective. Genetically modified *Ralstonia eutropha* was used to sequester Cd-contaminated soils using this approach, the *R. eutropha* was genetically engineered and designed to produce a cysteine-rich metal-binding protein (metallothionein) on its cell surface. These receptors could quench Cd available in the soil. The presence of sequestered metals in soil is less harmful because of their less bioavailability. Two major loopholes for this remediation approach are the controversies for the use of genetically modified organisms and the remains of sequestered metals in soil.

14.3 Bioremediation of Heavy Metal Contaminated Soils

Expanding industries, land application of fertilizers, spillage of petrochemicals, coal combustion, animal manures, wastewater irrigation, and sewage slime are the potential sources of heavy metals which contaminate soil. Different techniques of physical, chemical to biological methods for remediating metal contaminated soils exist. Most physical and chemical methods (vapour extraction, solidification, electrokinetics, soil flushing, soil washing, and stabilization) left the soil unsuitable for plant growth. On the other hand, biological approach (Bioremediation) which is achieved through natural processes encourages the use of remediated soil for the growth of plants. Phytoremediation ("Phyto" means plant) is a broad term used for a group of technologies that uses plants and associated microorganisms for in situ partial or complete removal of selected contaminants from soil, sediments, sludge, groundwater, and wastewater. Phytoremediation can be employed to remove heavy metal contaminants, organic pollutants as well as radionuclides (Ali et al. 2013). Plants because of their unique absorption, metabolic capabilities, and remarkably efficient transportation systems selectively absorb nutrients or contaminants from the growth matrix and environment.

To speed up the remediation process of contaminated soil, the combined use of microorganisms and plants has resulted in a faster and more proficient cleanup process (Weyens et al. 2009). The microbial remediation involves a cocktail of several microorganisms like bacteria (*B. subtilis*), Enterobacter (E cloacae), and Pseudomonas (P putida) that have been effectively used for the reduction of Cr (VI) to the less toxic Cr (III) (Garbisu and Alkorta 1997, Garbisu et al. 1998, Ishibashi et al. 1990). It is also reported in the literature that B. subtilis can reduce non-metallic elements. For example, Garbisu et al. (1998) studied that selenite has been decreased to the less toxic elemental selenium using *B. Subtilis*. In a study, it was observed that *Azotobacter vinelandii* showed increased siderophore production in the presence of Zn(II) (Huyer and Page 1988). The change in bioavailability could have led to the natural extraction of these metals from the soil. Besides the change in bioavailability, precipitation by sulphate-reducing bacteria (*Desulfovibrio desulfuricans*) could also lead to bioremediation. This process is indirect and mostly ex situ where the sulphate-reducing bacteria convert sulphate forms of heavy metal into their hydrogen sulphate, as in heavy metals Zn and Cd, to form insoluble forms of these metal sulphides (White et al. 1998).

One of the strategies used during bioremediation of contaminated soils to make it favourable for soil microbes is biostimulation which involves the use of organic amendments and accumulation of nutrients to the soil. Organic matter in the form of manure serves as a carbon source for soil microbes and the growth and activities of microorganisms involved in the process of remediation increase due to added nutrients, the only limitation here is to identify the right strain for biostimulation, which can be identified by using next generation sequencing and other biotechnological approaches. Typically biostimulation is used to biodegrade organic pollutants (Abioye 2011) and can also be employed for the bioremediation of soils contaminated with heavy metals. Since heavy metals are less amenable to biodegradation, biostimulation indirectly enhances their bioremediation by altering soil pH. It is the introduction of organic matter that actually reduces the soil pH (McCauley et al. 2009). Solubility and bioavailability of heavy metals get increased by the low pH of the soil, which subsequently increase its extractability from the soil (Karaca 2004). Biochar is an organic material which is known for its potential in the management of soils contaminated with heavy metals. Currently, it is being used for remediation purposes in various parts of the world. Experiments conducted by Namgay et al. (2010) stated that the presence of heavy metal in the contaminated soil amended with biochar was decreased. Unlike most other organic amendments biochar because of its ability to increase soil PH (Novak et al. 2009) reduces the bioavailability of metals for plant uptake due to increased sorption. Biochar properties differ broadly depending on the feedstock used in its production, and it also depends on the method employed during its production. Thus it is essential to note the effect of different amendments of biochar will have on its effectiveness when using for soil remediation. Further, in-depth research is required to comprehend how soil microorganisms are influenced by biochar and how remediation process of contaminated soils is amended because such reports are rear in literature. The insoluble metals are generally stored in extracellular (apoplastic) and intracellular (symplastic) compartments as precipitates of sulphate, carbonate, or phosphate (Raskin and Ensley 2000). Metal hyperaccumulator plants though are efficient in absorbing metals in large quantities from the soil, but they produce little biomass and are slow growing. To clean up contaminated sites using these plants, it may take years to decades. To overcome these shortfalls chemically improved phytoextraction has been employed. In this approach, chelating organic acids are used to enhance high biomass-producing crops to take up large amounts of metals (Nowack et al. 2006).

14.4 Wastewater Treatment

Conventional wastewater treatment systems are energy inefficient processes and inadequate to achieve discharge standards in effluent prescribed by various agencies (Caputo and Pelagagge 2001). It involves primary and secondary processes followed by disinfection for disposal of wastewater. With the advancement of technologies nutrients and harmful chemicals are removed, which ultimately decrease the oxygen demand of the wastewater and improve the quality of the treated water so that it can be reused. To stop eutrophication and elevated oxygen demand of wastewater an additional operation unit (Tertiary treatment) is added to the conventional treatment process which is generally employed to remove nutrient load (Phosphate and nitrates) which are the major drivers of eutrophication and high oxygen demand (Schaar et al. 2010). Biological wastewater treatments gained popularity in recent years. Conventional aerobic treatments used to reduce nutrients and organic load from wastewater are not efficient enough to remove most of the contaminants and it cannot be achieved without the combined use of both that is aerobic and anaerobic treatments (Okoh et al. 2010). Algae are known to breakdown the organic material to obtain energy and nourishment for their growth and development present in wastewater. They are wonderful biological mediators that own potential to convert solar energy and carbon dioxide into valuable products such as biodiesel, bioethanol, and biobutanol. Due to non-pathogenic nature and capability of algae biomass to grown in different effluents like municipal, industrial, and agricultural effluents, they are used for bioprocessing of wastewater (Chinnasamy et al. 2010). Two stage Sludge digestion system in which organic mass is digested by bacteria in absence of oxygen. The first stage of this system involves the break down of large molecules like proteins and lipids into smaller water soluble molecule by acid forming bacteria which finally ferment these molecules into fatty acids. The second stage involves the movement of soluble molecules into the second tank where bacteria convert soluble molecules into biogas (Carbon dioxide and Methane). Methane is used to generate heat for the operation of the treatment plant and is also used for the generation of electricity (Andreoli et al. 2007). Ballasted floc reactor, integrated fixed-film activated sludge (IFAS), and membrane bioreactor process are some examples of improved treatment methods of wastewater.

14.4.1 Molecular Techniques in Wastewater Treatment

Application of molecular techniques to treat wastewater is quite new and are economical than the already established techniques. Majority of these are used to make wastewater xenobiotic free. Specific nucleic acid probes are used to detect harmful microorganism from wastewater (Khan et al. 2004; Sanz and Köchling 2007). Other techniques which proved to be most effective and interesting for detection process are fluorescent in situ hybridization (FISH), genomics library, and denaturant gradient cell electrophoresis (DGGE) (Sanz and Köchling 2007). Use of recombinant technology has also its own disadvantages and limitations (Timmis et al. 1994) such as instability of recombinant strain, complex xenobiotic degradation pathways, and accidental or deliberate release of genetically modified strains into the environment.

14.5 Microbial Enzymes in Bioremediation

14.5.1 Microbial Oxidoreductase

Various bacteria, fungi, and higher plants detoxify toxic organic compounds through oxidative coupling which is mediated by oxidoreductases (Gianfreda et al. 1999: Bollag et al. 1998). These enzymes are also involved in microbial energy extraction via energy-yielding biochemical reactions. Oxidoreductases also assist in the transfer of electrons from the reduced substrate to acceptor compound, which results in oxidization of contaminant to the harmless compound. Oxidoreductases participate in the humification of the phenolic compound resulting from the decomposition of lignin, and they also can detoxify xenobiotics by binding them to humic substances or through polymerization and copolymerization with other substrates (Park et al. 2006). Enzymes of microbial origin have been exploited in the degradation and decolourization of dyes (Vidali 2001; Williams 1977; Husain 2006). Some bacterial strains have tendency to reduce the radioactive metals from their oxidized soluble form to reduced insoluble form. Some bacteria during their energy production process takes up electrons from organic compounds and make radioactive metals as the final electron acceptor. To reduce radioactive metals some strains of bacteria use intermediate electron donor. It is the redox reactions system of metal-reducing bacteria, which finally precipitate the contaminant (Leung 2004). Effluents generated from paper and pulp industry contain chlorinated phenolic compounds which are produced from the partial degradation of lignin during the process of pulp bleaching. Many fungal species due to their extracellular oxidoreductase enzymes like manganese peroxidise, laccase, and peroxidise are considered suitable for the treatment of environment contaminated with phenolic compounds. Fungi release these enzymes from their mycelium into the environment (Rubilar et al. 2008). Phytoremediation of phenolic compounds using plants from Fabaceae, Gramineae, and Solanaceae families. They degrade contaminants using their oxidoreductases is a promising natural approach to decontaminate the environment. Organic contaminants like explosives, petroleum hydrocarbons, and chlorinated solvents are generally focused (Duran and Esposito 2000, Newman et al. 1998).

14.5.2 Microbial Dioxygenases

Dioxygenases are multicomponent enzyme systems that introduce molecular oxygen into their substrate Rieske nonheme iron oxygenases is one of the large family of dioxygenases which include aromatic hydrogen dioxygenase. These dioxygenases have wide application in environmental remediation because of their involvement in the oxygenation of wide range substrates (Fig. 14.1). All members of this family have one or two electron transport proteins preceding their oxygenase components. The presence of Rieske (2Fe–2S) cluster and mononuclear iron in each alpha subunit of the enzyme has been confirmed from the crystal structure of

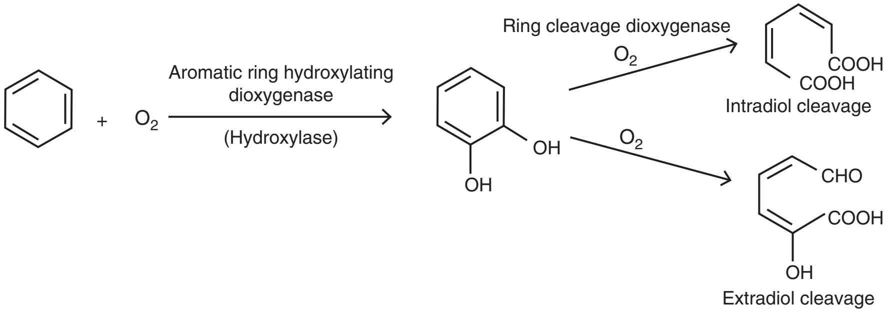

Fig. 14.1 Degradation of aromatic compound by dioxygenase

naphthalene dioxygenase (Dua et al. 2002). Catechol dioxygenases found in soil bacteria are involved in the transformation of aromatic precursors into aliphatic products.

14.5.3 Microbial Hydrolytic Enzymes

The industrialization has led to the pollution of soil and water through the discharge of effluents by the industrial units. Industrial discharge onto land contains many harmful chemicals, petroleum hydrocarbons, mineral acids, bases, and many more chemicals of unknown nature. Bioremediation provides an economical and safe alternative to commonly used chemical treatments. Organic pollutants are mostly hydrolyzed by bacterial activity. It is the extracellular enzyme activity that has the key role in degradation since bacterial cell pores can only allow molecules lower than 600 daltons to enter the cell (Vasileva-Tonkova and Galabova 2003). Disruption of major chemical bonds of toxic compounds by bacterial hydrolytic enzymes results in the reduction of their toxicity. Hydrolytic enzymes of microbial origin are effective in the biodegradation of insecticides (DDT) and oil spill (Vasileva-Tonkova and Galabova 2003; Lal 1982; Williams 1977; Shafi et al. 2018). Hydrolases are considered to be effective players in bioremediation because of their ready availability, tolerance to the addition of solvents which are water miscible and lack cofactor stereo selectivity.

14.6 Solid Waste Biotreatment

Stringent standards for the discharge of waste into the environment, as well as the cost of routine treatment options, have motivated the scientific community to look for the different treatment options (Nicell 2003; Hamer et al. 2007; Mazzanti and Zoboli 2008). Keeping the following objectives in view, these treatment processes are developed by (1) reducing costs and resource conservation, (2) minimal effluent disposal and efficient waste recycling within the facility, (3) reduction in effluent quantity, and (4) transformation of waste into valuable products. There are two ways in which waste and pollutants can be treated one is biological, and the second one is chemical. We produce biowaste in our day to day life, which includes agricultural waste, horticulture waste, wastewater treatment plants, etc. Biowaste can be categorized as: process waste, manures, and plant material. In European countries, biowaste consists of 40–60% of municipal solid waste, and it is being collected separately and used for many purposes like composting or degradation (aerobic) which improves soil texture by supplying nutrients to the soil. Biowaste is also used for the generation of biogas in many countries of the world. Biological treatment of waste involves the careful selection of organisms (Biocatalysts) to degrade the waste. It is considered as most

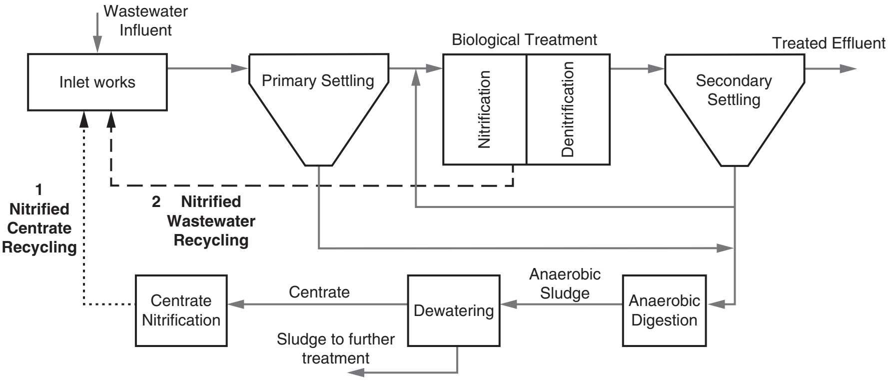

Fig. 14.2 Schematic diagram of biological wastewater treatment

favourable, stable, and bulk, reducing option which reduces the potential adverse effects on health and environment. Generating useful products and reclaiming valuable minerals for reuse are the added advantages of biotreatment. Biological treatment of solid waste can be done in situ or ex situ (Doble et al. 2004). Treatment of biological waste can be done under aerobic conditions or anaerobic conditions (Fig. 14.2). Digestion of organic waste is accelerated in anaerobic conditions under optimum conditions of temperature, pH, and moisture (Mata-Alvarez et al. 2000; Salminen and Rintala 2002), anaerobic processes are energy efficient, maintain enclosure of order, produce low sludge compared to aerobic ones.

14.7 Pollution Detection and Monitoring: Role of Environmental Biotechnology

Measuring a set of selected essential parameters on a regular basis which determine the environmental quality is environmental monitoring. There are two methods available (Biological and physiochemical) to judge the environmental health (Kaiser 2001; Lam and Gray 2003; Hagger et al. 2006). Environmental monitoring a couple of decades before was solely dependent on the measurement of physiochemical parameters and the monitoring of biological parameters were occasionally done. Physiochemical methods have limitations because of their heavy dependence on sophisticated equipment and complex analytical methodology (Gu et al. 2004).

For the protection of environment pollution detection at low concentrations is very important so that protective measures can be placed at the beginning (Durrieu et al. 2006). Monitoring of contaminants typically involves the frequent and regular measurements of various chemicals in soil, water, air, and sediments over a fixed time period. Integration of informational technology with biotechnology has made data collection, and monitoring work quit easy and accurate. It has also revolutionized the real-time analysis and data interpretation.

14.7.1 Bioindicators/Biomarkers

Environmental monitoring, apart from physiochemical measurements of contaminants, also needs to assess various biological/ecological parameters and responses. The temporal and spatial changes of selected biological responses which are used by ecologist and environmental biologists for the assessment of environmental quality (Durrieu et al. 2006; Conti 2008; Lam 2009). Organisms or communities react to changing environmental conditions by showing a measurable change in their chemical compositions/biological processes. These changes/responses are called as biomarkers/bioindicators (Kaiser 2001; Conti 2008). Thus biomarkers are used to give biological information (effect of environmental pollution). Bioindicators can be categorized into three types: those obtained on exposure, on susceptibility, and on effect.

Bioindicators that have potential to be used for biological monitoring are

- Molecular markers (Change in expression, DNA damage)
- Biochemical markers (Enzymatic, specific proteins)
- Histo-cytopathological (Cytological, histopathological)
- Physiological and behavioural

14.7.2 Biosensors

Biosensors are analytical devices consists of biomarker in the form of enzyme, antibody which is in close contact with transducer, which together relate the concentration of an analyte to a measurable electrical signal (Reiss and Hartmeier 2001; Rodriguez-Mozaz et al. 2004). Biosensing research has gained considerable attention in recent years because of the growing environmental pollution. Biosensing devices produce signals exploiting specificity of biological molecules to measure pollution levels. In broader terms biosensors are referred to any system that produces quantifiable signal on the detection of substrate using biological component. The main part of the biosensor is its biological component which can be whole cell, enzyme, antibody, genetically engineered organism. Biosensors represent the wonderful synergistic combination of microelectronics and biotechnology (Verma and Singh 2005). Biosensors can be used to detect both chemical pollutants as well as biological contaminants. Microchip biosensor with very high selective sensitivity was developed by combining biological activity with nanoelectronics (Cui et al. 2001).

Microalgal whole cell biosensors based on (Chlorophyll fluorescence or phosphatase and esterase inhibition) are used for the detection of environmental pollutions. Biosensor based on genetically modified yeast as used to detect endocrine disruptors such as oestrogen or 17-oestradiol. It was initially designed for therapeutic uses in humans. But later it was found to have potential to be used for detection of environmental pollutants (Tucker and Fields 2001). Use of biosensors has addressed many vital environmental issues which arose with other conventional methods.

14.8 Biotechnology for Cleaner Production

Use of biotechnological approach to combat environmental pollution is considered as the motor for the sustainable future. Contrary to other pollution control strategies which struggle for the tail end of the process and manage pollution once it has been generated, biotechnology works to stop pollution at its source by using different practices like (1) use of efficient raw material, (2) substituting less harmful substances for hazardous materials, and (3) eliminating hazardous toxic substances in effluents. Although the primary focus of environmental biotechnology is to develop technologies to treat waste but the basic information has been gained by people that how biotechnology can handle these wastes and the main focus is how to implement these processes as Available Technology Not Entailing Excessive Costs (BATNEEC) (Grommen and Verstraete 2002). Biotechnological processes after their successful set up are considered cost effective than conventional methods. Use of biotechnology for production processes not only contribute for the protection of environment, but also make companies economically sound and continuously improve their market image (Olguín 2000; Willke et al. 2005).

14.8.1 Pulp and Paper Industry

Biotechnological techniques like enzyme engineering, proteomics, metabolomics genetic engineering have helped in developing methods for paper production which are economically feasible, cost effective, and environmental friendly. Paper and pulp industry plays a vital role in world economy. Cellulosic pulp which is the target product is produced from pulping of wood (lignocellulosic material) by chemical or mechanical treatment for the release of fibres from the material. Most of the cellulosic pulp (90%) is produced from woody plants. Sugar cane, straw, and bamboo are the major raw material source for the pulp production (Sixta 2006).

The overall paper and pulp production process can be divided into six steps.

1. Wood preparation
2. Pulping

3. Pulp washing
4. Pulp screening
5. Bleaching
6. Paper manufacturing

From the last few decades, pulp and paper industry mostly in developed countries has been using biotechnological approaches for technological needs, which has resulted in a cleaner environment.

14.8.2 Biopulping

Use of ligninolytic enzymes or fungi cultures for the pre-treatment of raw material or wood is one of the interesting biological approaches used in the pulp and paper industry. Although there are other microorganisms which can be used for the bioprocessing of wood but fungi being the primary colonizers of wood utilizes its sugars without affecting its strength. Soft rot fungi and white fungi have high capability to degrade the wood because of their different extracellular enzymes. It is the white rot fungi in particular which are efficient ligninolytic organism producing all the necessary extracellular oxidative enzymes for the complete degradation of wood (Tekere et al. 2001). There are some fungi which are non-selective (*Trametes versicolor*) that is they simultaneously degrade hemicelluloses lignin and cellulose. While some others like *Phlebia tremellosa*, *Ceriporiopsis subvermispora,* and *Phellinus pini* degrade lignin preferentially (Otjen et al. 1987). The aim of biopulping is to utilize the special ability of selectively delignifying wood with cellulose decomposition of these specific fungi.

Fungal pre-treatment of wood chips before kraft pulping showed a significant increase in pulping yield (Mendonça et al. 2002) and decreased energy consumption (Young and Akhtar 1997). Fungal pre-treatment of woodchips before mechanical pulping process makes the wood soft for grinding. Thus to facilitate pulping this methodology does not require chemical agents, sophisticated equipment, and additional energy. Biotreatments enhance pulp quality, in addition to this biotreatment reduces energy consumption and environmental impact of the refining process, which make it more environmentally friendly than conventional methods used (Tian et al. 2012).

14.8.3 Bio-bleaching

Bleaching process of pulp needs chlorine, which is disastrous for the environment (Bajpai et al. 2006). Presence of lignin content is responsible for the brown colour of pulp. To improve the paper quality and printability, the removal of lignin content is required. Bio-bleaching is an alternative to conventional bleaching treatments by microorganisms such as fungi or their enzymes. Enzyme bio-bleaching is considered as an excellent environmentally friendly alternative due to its cost effectiveness, specificity, and decreased dependence on chemicals. Following are the main advantages for the use of bio-bleaching:

- Mill trials very simple and inexpensive with minimal risk
- Reducing the pollution from bleaching
- Enhance pulp physical properties
- Process can be easily combined with traditional bleaching
- It enhances fibrillation to give stronger paper
- Simplify the process and reduce the severity of treatment of wastewater.

Xylanase enzyme treatment in bio-bleaching reduces the operation cost and consumption of chlorine. Microbial thermostable xylanases are stable under alkaline conditions of pulping and are generally preferred enzymes for bio-bleaching (Raghukumar et al. 2004). It has been reported that xylanase pre-treatment of kraft pulps has reduced AOX discharge by 5–20%. Deposits of pitch are controlled by the use of lipases. Dewatering rate of pulp is improved by using cellulase enzymes (Foster 1986) that are also used for deinking and fibre modification. Catalase also has an important role it converts hydrogen peroxide to water and oxygen. Thus saves water and makes effluent ecologically harmless. Other enzymes like manganese peroxidise and laccase have also been used. Laccase delignify pulp directly thus replaces chemical stages of bleaching such as oxygen or ozone stage. For the control of pitch buildup and for deinking purposes, particularly when inks contain formulations of vegetable oil lipase has been used (Sharyo et al. 1993). It has been reported that in chip pre-treatment ascomycetes albino fungi reduce pitch and saves bleach cost up to 36% (Trivedy and Pathak 2015).

14.9 Bioplastics

The main threat to the environment and ecology because of plastic is due to its non-biodegradable nature and due to its production from synthetic polymers, which consumes the vast wealth of non-renewable resources (Stevens 2002; Reddy et al. 2003). Bioplastic production is based on natural substances (Proteins, fibres, sugar, and oils) thus avoids the use of non-renewable resources which in turn reduces the release of harmful gases into the environment. Microbes have an important role in converting vegetable and plant material into the building blocks of bioplastic (Luengo et al. 2003; Moldes et al. 2004). Bioplastic production from organic waste and enzyme assisted plastic reduction has addressed many environmental issues like the reduction of plastic waste and a decrease in consumption of non-renewable resources. OECD report (2001) assessed the wide spreading of industrial biotechnology, which was based on 21 companies from different sectors like chemical, paper, pharma, textile, etc. In this study, they found that industrial biotechnology has environmentally sound profound character because of its cleaner production.

14.10 Biotechnology and Agriculture

There is no doubt that the use of chemical herbicides, fungicides, and fertilizers has increased the yield and productivity of crops, but they have caused environmental hazards due to their low biodegradability. The use of biopesticides and genetically modified plants may considerably diminish the use of these harmful chemicals. Biopesticides are classified (Table 14.1) as

- Microbial pesticides (Containing microorganism as active ingredient)
- Plant-incorporated protectants (Genetic material incorporated into the plant produces active pesticide)

Table 14.1 Biopesticides and their control targets (MCD 2008)

S. no.	Organism	Target	Example
1	Bacteria	Insects	*Bacillus thuringiensis*
			Bacillus sphaericus
			Serratia entomophila
			Paenibacillus popilliae
2	Fungi	Insects	*Beauveria* spp.
			Metarhizium
			Lecanicillium lecanii
			Nomuraea
			Paecilomyces fumosoroseus
			Zoophthora
			Entomophaga
3	Protozoa	Insects	*Nosema*
			Vairimorpha
			Thelohania
4	Fungi	Weed control	*Colletotrichum gloeosporioides*
			Chondrostereum purpureum
			Cylindrobasidium leave
			Xanthomonas campestris
5	Fungi	Plant disease control	*Ampelomyces quisqualis*
			Candida spp.
			Clonostachys rosea
6	Competitive inoculants	Plant disease control	*Coniothyrium minitans*
			Pseudozyma flocculosa
			Trichoderma spp.
7	Composts, soil inoculants	Plant disease control	*Bacillus pumilus*
			Bacillus subtilis
			Pseudomonas spp.
			Streptomyces griseoviridis
			Burkholderia cepacia
8	Mollusc parasitic nematode	Nematicides	*Phasmarhabditis hermaphrodita*

- Biochemical pesticides (Include substances which control pests by non-toxic mechanisms)

Biopesticides decompose quickly and are often effective in small quantities (Boyetchko et al. 1999). According to Fraser et al. biopesticides have the following characteristics (Fraser 2005):

- Low risk to human health.
- Low potential to contaminate environmental components and resources.

Biopesticides and genetically engineered insect resistant plants have considerably diminished the use of harmful pesticides and chemicals. Thus, ultimately lead to less exposure to chemicals.

14.11 Chemicals and Biofuels

Excellent research and development is already in force across the globe for production of biofuels by the enzymatic conversion of different substrates (Agricultural waste, municipal waste, and vegetable oils) into bioethanol and biogas, etc. (Dale 2006; Willke et al. 2005). Different applications have conceived the idea of anaerobic digestion of wastes from agriculture and food industries for methane production (Fig. 14.3). Complex organic molecules present in the waste are broken-down by anaerobic bacteria for the production of methane rich combustible biogas having energy value between 21 and 28 MJ/m^3 (Doble et al. 2004).

The idea of chemical synthesis from natural renewable resources is still in its infancy, but the conversion of biomass feedstock into biofuels can have a major economic as well as environmental benefit (Gavrilescu and Chisti 2005; Willke et al. 2005; Chisti 2007). Several companies have started to produce their products from renewable resources (e.g., DuPont) (Willke et al. 2005). Production of fine chemicals using traditional methods has created severe environmental problems. Analysis performed by environmental organizations in order to evaluates two production processes, viz., chemical and biotechnological. It was found that it is the biotechnological process which is more economical and coefficient (e.g., Vitamin B_2 production process). It was found that riboflavin can be produced at cost reduced by 50% using biotechnological approaches (BIO–PRO 2008). Hydroxylation of steroids using microbes leads to the development of steroid drug used for arthritis (Dutta and Samanta 1997).

The key intermediate for the production of penicillins used for chemotherapy is 6-aminopenicillanic acid (6-APA). Synthesis of aminopenicillanic acid (6-APA) using biological approach was found to be 20% cheaper than its chemical synthesis.

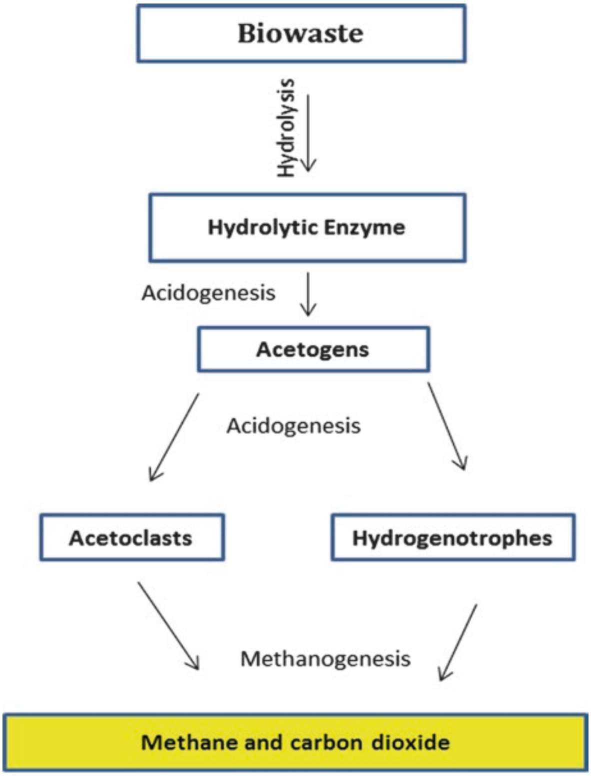

Fig. 14.3 Biowaste methanisation

14.12 Conclusion

Globally there are thousands of environmental challenges, and new challenges are evolving at a faster rate. New technologies are currently under development, and some already established ones are gaining more and more ground in practice. There is a need of a technology which can harness the potential of plants and microbes as robust and eco-efficient agents in a variety of practical situations. Along with a vast group of technologies to achieve the objectives of sustainability, biotechnology is fighting from the front and will continue to play a vital role in the field of environmental protection, food, renewable materials, and bioremediation. Environmental biotechnology is a sustainable approach to develop efficient protocols, clean processes, and products with reduced environmental impact. Environmental and economic benefits that biotechnology can offer in manufacturing, monitoring, and waste management are in balance with technical and economic problems which still need to be solved. All this is being achieved with reduced environmental impact and enhanced sustainability. An evaluation of the consequences, opportunities, and challenges of modern biotechnology is important for both policymakers and the industry.

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Chapter 15
Global Environmental Regulations for Management of Pesticides

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15.1 Introduction

Excessive and improper pesticide usage results in contamination and deterioration of soil and aquatic ecosystems and disturbs ecological equilibrium (Hui et al. 2003; Morillo and Villaverde 2017; Spina et al. 2018; Sun et al. 2018; Bhat et al. 2019; Kumar et al. 2019). Pesticides are extremely deleterious due to their persistence, toxicity, bioaccumulation potential, and long range environmental transport potential (Teran et al. 2012; Pariatamby and Kee 2016; Bharat 2018). During the past decade, pesticides have drawn mass public, political, and scientific concern due to their carcinogenic, immunotoxic, mutagenic, and neurotoxic prospective (Dixit et al. 2019). Although new harmless pesticides with relatively enhanced safety profile and novel modes of action have been developed as a substitute to extremely toxic ones (Gill and Garg 2014; Dixit et al. 2019); however, due to the dearth of a comprehensible pesticides management approach, large amount of obsolete pesticide residues have already stockpiled over the time (Dasgupta et al. 2010).

Pesticide exposure occurs primarily through improper pesticide storage, pesticide mishandling and leakage, inappropriate and indiscriminate use, inadequate disposal, and by consuming pesticide contaminated food and water (Morillo and Villaverde 2017; Dixit et al. 2019). A mixture of toxic banned pesticide residues have been detected in large quantities (above WHO's maximum daily intake) in a wide variety of food items that we eat like eggs, fish, flour, fruits, grains, milk, poultry, pulses, rice, vegetables, and wheat, which cause several chronic human diseases (Pimentel et al. 1992; ICMR 2001; Rani and Dhania 2014; Pandey et al. 2018). World Health Organization (WHO) estimated that globally there occur 3,000,000 cases of pesticide poisoning which result in approximately 220,000 deaths annually (Lah 2011; Dixit et al. 2019).

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Nowadays, besides some traditional means of monitoring pesticides residues, several novel and innovative technologies are being applied (Xing-lu et al. 2019). Several developed regions have established pesticide risk assessment models which are of great significance for environmental sustainability (Xing-lu et al. 2019). These models play a crucial role in creating mass awareness and thus, facilitate involvement of all the stakeholders in successful recuperation and restoration of pesticide contaminated environs.

In addition to the application of various scientific methods for the treatment of pesticide pollutants, there is an immediate need to develop global guidelines, management policies, and standard regulations for overseeing pesticide usage and restoration of pesticide contaminated environments (Gill and Garg 2014; Varjania et al. 2019). Pesticide pollution causes various human health and environmental safety issues, which instigated varied global governmental and non-governmental organizations to frame environmental regulations for management and mitigation of pesticide pollution for global environmental safety, sustainable development, and human welfare. Pesticide pollution is one of the major drivers of ecosystem degradation. The United Nations General Assembly declared 2021–2030 as the “International Decade on Ecosystem Restoration” with one of the main focus on restoration of degraded ecosystems globally for sustainable development (Tripathi et al. 2019).

15.2 Environmental Risk Assessment of Pesticides

Risk assessment is a promising multi-disciplinary procedure inured to assess environmental and health risks propounded by chemical pollutants (Singh et al. 2009). Assessing the environmental providence of pesticide residues and their imminent exposure risks to environment and public health is essential for formulating risk-based management strategies for risk reduction (Rice et al. 2007; Singh et al. 2009); however, the development of environmental risk assessment of pesticides is extremely complex and challenging because of the disparity in nature and quantity of pesticide sprayed, ecotoxicity of pesticides, exposure pathways (direct and indirect), period and intensity of exposure, and environmental characteristics of the pesticide application site (Pandey et al. 2018; Spina et al. 2018; Xing-lu et al. 2019). Moreover, the regulatory and risk assessment approaches are concerned with the impact of the pollutant, rather than the pollutant concentration (Beesley et al. 2011).

Several developed regions like North America, European Union, and others have framed and established their own territory-specific pesticide risk assessment models (Xing-lu et al. 2019). Realistic and valid environmental risk assessment of pesticides furnishes fundamental information for management of risk and formulation of remediation decisions (Sun et al. 2017). SCI-GROW risk assessment model of pesticides was established to envisage the risk of five widely used pesticides in China (Cheng et al. 2007; Xing-lu et al. 2019). Levitan et al. (1995) subsequent to analyzing various categories of environmental risk assessment like anecdotal assessments, composite environmental impact rating systems, directory-format and tabular databases, economic assessments, holistic assessments, single and multiple-parameter assessments, and site-specific assessment tools reported that a comprehensive pesticide impact assessment system is still lacking.

Environmental impact assessment of pesticides influences characterization of previously permitted pesticides, approval of new pesticides, and establishment of remediation goals along with maintenance of environmental quality (Song et al. 2017; Dixit et al. 2019). New pesticides registrations based solely on strict environmental risk assessment procedures can help in combating the menace of hazardous pesticides (Schwarzenbach et al. 2010; Xing-lu et al. 2019). Moreover, biotoxicity tests reflect the biological impacts of toxicants, thus serve as important tools in environmental risk assessment (Prokop et al. 2016).

15.3 Pesticide Management Strategies

Pesticides incur adverse impacts on biodiversity, human health, and environment (Marican and Durán-Lara 2018; Dixit et al. 2019). So, management of pesticide pollution assumes great significance and demands immediate action. Novel pesticide management tools and techniques with greater reliability are being developed for improved safety and mitigation of adverse pesticide impacts.

Pesticide management strategies encompass assorted pest as well as pesticide management strategies (Fig. 15.1). Various pest management strategies are categorized as: biological control strategies (such as adoption of integrated pest management-IPM systems by introduction of biocontrol agents, use of biopesticides like biofungicides, bioherbicides, and bioinsecticides), cultural control strategies (such as

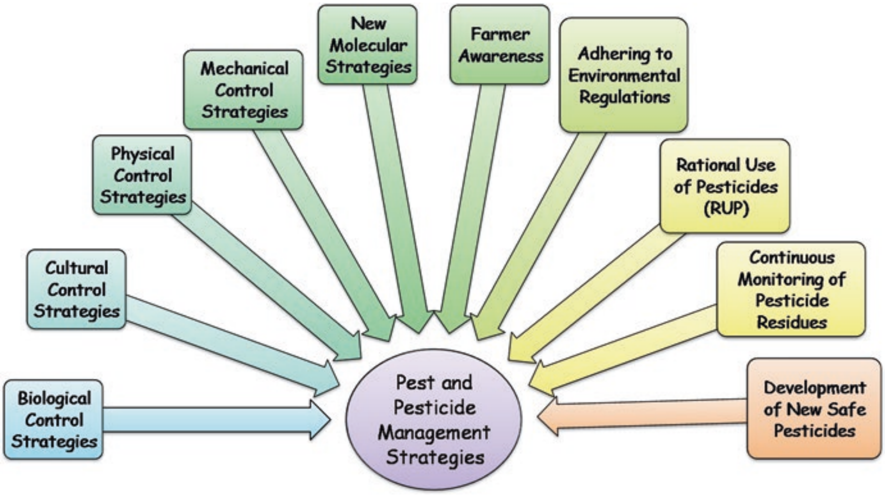

Fig. 15.1 Pest and pesticide management strategies

adoption of integrated crop management-ICM, crop rotation, intercropping, allelopathy, certified seeds, use of pest resistant varieties, farmyard manure, and organic farming), physical control strategies (like sun exposure, light traps, steaming, and moisture management), mechanical control strategies (include use of manual devices like handpicking, clipping, trapping devices, pest exclusion by barriers, and crushing/pruning of pest infested part), new molecular strategies (for instance, development of pest resistant genotypes). Pest management by proficient alternative approaches rationalizes the pesticide consumption (Dhaliwal et al. 2006; Abhilash and Singh 2009; Gill and Garg 2014; Dixit et al. 2019).

Several effective and reliable pesticide management strategies are farmer education and awareness about basic safety guidelines in selection and application of pesticides, adhering to the environmental regulations (precautionary measures as well as safety guidelines) during all the phases of pesticide handling, rational use of pesticides-RUP (apposite pesticide selection and meticulousness in their application over space and time), continuous monitoring of pesticide residues in agricultural commodities, alteration of formerly existing pesticide formulations for secure formulations and development of new pesticides with enhanced safety profiles (Abhilash and Singh 2009; Gill and Garg 2014; Dixit et al. 2019).

15.4 Pesticide Ban

The unsustainable production and use of pesticides over past few decades resulted in colossal environmental contamination. As stated by Food and Agriculture Organization (FAO) inventory (2001), approximately 500,000 tons of obsolete pesticide residues have accumulated in several countries and are threatening the public and environmental health (FAO/UNEP/OECD/SIB 2001). Many cases of pesticide poisoning that either caused adverse impacts on human health or even death have been reported across the world. For instance, (a) The first case of pesticide poisoning in India was reported from Kerala in 1958, wherein around 100 people died due to the consumption of wheat flour that was found to be contaminated with pesticide parathion (Boudh and Singh 2019), (b) In Brazil, 123 persons suffered from severe pesticide poisoning between 1992 and 2002 (Weiss et al. 2016), (c) In 2013, 23 school children died in eastern India, after eating school lunch that was later found to be contaminated with residuals of monocrotophos (banned pesticide) (Weiss et al. 2016). During the former decade, there was an enormous increase in public concern regarding deleterious impacts of pesticide residues.

Pesticide ban is an important regulatory stride taken for the management of deleterious pesticides. Globally, several highly persistent and toxic pesticides have been banned hitherto. In 1995, a convention held in Stockholm proposed to ban 12 (Dirty dozen) toxic persistent organic pollutants (POPs), most of which are pesticides (Morillo and Villaverde 2017). However, pesticides that are currently banned in developed countries are still used or stockpiled in developing countries due to lack of awareness regarding hazardous pesticide impacts, dearth of reliable risk assessment protocols, lack of infrastructure, environmental controls and regulations in such countries (Carneiro et al. 2012). Moreover, pesticides persist even for centuries in ecosystems due to their stability and degradation resistance. The pesticides that have been banned since several decades, their residues are still today found in water and sediments (Ballesteros et al. 2014).

15.4.1 Case Study of Pesticide Dichlorodiphenyltrichloroethane (DDT)

Insecticide "Dichlorodiphenyltrichloroethane" (DDT) was discovered by a Swiss entomologist Paul Muller in 1939. Following World War II, DDT was used in large quantities. DDT was the first organic chemical to be used as a pesticide, and it protected several economical crops and eradicated malaria from numerous parts of the world (Kumar et al. 2018). Alternatively, DDT has resulted in universal environmental contamination due to its slow degradation rate that has been estimated to range from 4 to 30 years (Abhilash and Singh 2009; Pandey et al. 2018; Boudh and Singh 2019). DDT causes eggshell thinning in birds by inhibiting synthesis of prostaglandin in eggshell gland mucosa (Lundholm 1997). DDT is known to have huge bioaccumulation and biomagnification potential at higher trophic levels (Gill and Garg 2014). Thus organisms at the higher trophic levels of food chain are at more risk. DDT exposure causes cancer, endocrine hormone disruption, liver damage, neurological disorders, and reproductive abnormalities (Persson et al. 2012; Pandey et al. 2018; Dixit et al. 2019).

In 1972, production and use of DDT was entirely banned in the USA and other developed countries due to vast array of side effects like non-target toxicity, bioaccumulation, and environmental persistence (Pandey et al. 2018). However, many developing countries (including India) were exempted from DDT ban and were allowed to use DDT till March 2013 for the control of vector-borne diseases (Bharat 2018). Since the ban, large quantity of DDT have stockpiled under improper conditions in developing countries, where it pose a serious risk to human and environmental health (Weiss et al. 2016).

15.5 Global Environmental Regulations for Pesticide Management

Several national and international regulatory authorities conceded various laws and regulations regarding secure manufacture, import, export, sale, transport, proper use, and application of pesticides (FAO 1955, 1988; IPSMC 1999; Lallas 2001; Rotterdam Convention Secretariat 2004; WHO 2004; MEA 2005; FAO 2006a, b, 2007; UNEP 2006; Abhilash and Singh 2009; ECHA 2011; Teran et al. 2012; Pesticide Action Network International 2014; GlobalGAP 2016; Bharat 2018). Numerous initiatives and conventions were instigated for pesticide management during the past two decades. The initial regulations for pesticide management were introduced in the beginning of 1970 with the ban on production and use of pesticide DDT and restricted use of other harmful pesticides in the USA (Teran et al. 2012). Subsequently, several international regulations dealing with pesticide management came into force since 1980. Some of the imperative global environmental regulations for management of pesticides are:

(a) *The International Code of Conduct and Use of Pesticides (Code of Conduct)*: In 1985, the 23rd session of the main governing body of Food and Agriculture Organization (FAO) formulated and adopted the International Code of Conduct and Use of Pesticides (Code of Conduct) for providing universal principles of conduct of pesticide management for pesticide industry and national governments. This code of conduct was once amended in 1989 and then again in 2002 (FAO 2006a, b).
(b) *The Montreal Protocol*: The Montreal Protocol was adopted in 1987 with the aim to limit the production and use of substances (such as pesticide methyl bromide) that cause ozone layer depletion (Abhilash and Singh 2009). This protocol came into force in 1989.
(c) *The Rotterdam Convention*: The Rotterdam Convention was adopted in 1988 for the regulation of international trade of hazardous chemicals and pesticides (Kumer 1999; Rotterdam Convention Secretariat 2004). It later came into force in the year 2004. Its main objective is to control and ban the international trade and stockpiling of perilous pesticides. Furthermore, it promotes labelling of deleterious chemicals with safety directives and pictograms (United Nations Environment Programme, Food and Agriculture Organization 2013).
(d) *The Basel Convention*: The Basal Convention dealing with the trans-boundary passage of perilous wastes and their disposal was adopted in 1989 and came into force in 1992 (Basel Convention 2006).
(e) *The Stockholm Convention*: In 1995, a convention held in Stockholm proposed to ban 12 toxic persistent organic pollutants (POPs) (Dirty dozen), to which more chemicals were added afterwards. Later in 2001, an international legal treaty named as “Stockholm Convention” was opened for ratification and was finally signed by 179 countries, which then came into power in 2004, around after a decade after UNEP’s (United Nations Environment Programme) call for global action on POPs in 1995 (UNEP 2009). This convention proposed to ban or restrict the production, release, and use of selected harmful chemicals including polychlorinated biphenyls (PCBs) and a range of organochlorine pesticides (OCPs) in all participant countries (UNEP 2009; Morillo and Villaverde 2017; Bharat 2018). Furthermore, SC amendments in 2011 engorged the list up to 22 POPs (United Nations Environment Programme 2013a).
(f) *The International POPs Elimination Network (IPEN)*: The International POPs Elimination Network (IPEN) is a non-profit organization that was established in 1998 in Sweden. IPEN deals with the sound chemical management policies and practices, as a strategy for global economic development and environmental protection (Bharat 2018).

(g) *The International Labor Organization Convention No. 184 (ILO Convention 184)*: ILO Convention 184 deals with all aspects of safety and health while chemical application in agriculture sector (ILO 2000). It was adopted in 2001 and later came to force in 2003.

(h) *The Global Harmonized System of Classification and Labelling of Chemicals (GHS)*: The GHS has been devised for the safety of human beings and the environment during the handling, use, and transport of hazardous chemicals (FAO 2006a, b).

(i) *Strategic Approach to International Chemicals Management (SAICM)*: Strategic Approach to International Chemicals Management (SAICM) is a global policy framework for proper chemical management. It was adopted by International Conference on Chemicals Management (ICCM) in 2006 (Bharat 2018). It recognizes the role of chemicals in the current scenario as well as the potential threat to sustainable development by chemical mismanagement. SAICM goals together with the support of United Nations Economic Commission for Europe (UNECE) and UNEP deal with the chemical management, environment, and sustainable development in alliance and deem in achievement of these goals by the year 2020 (Teran et al. 2012; Bharat 2018).

(j) *Registration, Evaluation and Authorisation of Chemicals (REACH)*: In 2007, a legislation called as Registration, Evaluation and Authorisation of Chemicals (REACH) was adopted and enforced by the European Union (EU) with the rationale of information gathering on chemicals entering European markets without prior knowledge vis-a-vis their detrimental impacts on environment and human health (EU 2006; EEA 2007; ECHA 2008). This is achieved through the recognition of appropriate pesticide risk assessment models for the screening, proper approval, and registration of these new pesticides being marketed in Europe, with the purpose of reducing future exposure risks (ECHA 2011). REACH encompass registration, evaluation, authorization, and restriction elements.

(k) *The Clean Sweep Program*: The Convention on Persistent Organic Pollutants along with the Food and Agriculture Organization (FAO) are setting up global efforts for decreasing the occurrence as well as abundance of detrimental pesticides. In this direction, for apposite disposal of pesticides “Clean Sweep Program” was instated in several regions within the USA (Dixit et al. 2019).

(l) *The Codex Alimentarius Commission*: The Codex Alimentarius Commission formulated several standards on the presence of pesticide residues in food (Abhilash and Singh 2009).

(m) *Global Good Agricultural Practices (GlobalGAP)*: The Global Good Agricultural Practices (GlobalGAP) is a European non-profit organization that delineates good agricultural practices like using small quantity of pesticides for sustainable production (GlobalGAP 2016).

(n) *Pesticide Action Network organization (PAN)*: The Pesticide Action Network organization (PAN) is a network of over 600 NGO’s and institutions, operational in around 90 countries. Its main aim is to substitute deleterious chemical pesticides by ecologically sound approaches (Pesticide Action Network International 2014).

Some other international conventions that are of great importance are: The Mediterranean Action Plan for the Barcelona Convention (MAP) (UNEP 1978); The Arctic Monitoring and Assessment Programme (AMAP) (Arctic Council 1991); The Chemical Weapons Convention 1993; The Convention for the Protection of the marine Environment of the North–East Atlantic (OSPAR); Northern Contaminants Program (NCP) (NCP 2003); The Environmental Monitoring and Governance in the East Asian Hydrosphere-Monitoring of POPs (EMGEAH), and European Monitoring and Evaluation Programme (EMEP http://www.emep.int). Furthermore, globally, several acts were passed for management of pesticides such as The Prevention of Food Adulteration Act, 1954; The Insecticide Act, 1968; The Insecticide Rules, 1971; The Environment Protection Act, 1986; and The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (Frazar 2000; Abhilash and Singh 2009).

Environmental regulations play an indispensable role in effectual pesticide management along with maintenance of environment. However, these regulatory frameworks are not appropriately and effectively implemented even in developed countries (Morillo and Villaverde 2017). In developing countries, the environmental regulations governing pesticide application, storage, and disposal are scarcely implemented (United Nations Environment Programme 2013b, 2014). So, relying only on legislation for pesticide management is not a practicable solution. Globally, governments should adopt environmentally sustainable approaches for pest and pesticide management like public awareness campaigns, training programs regarding proper pesticide application, mass awareness about use of minimum-risk products, and adoption of integrated pest management (IPM) system. Nonetheless, a firm legislative and regulatory framework strengthens all of these approaches.

15.5.1 Legal Penalty for Violating Environmental Regulations

For strengthening the environmental regulations, several global organizations framed legal penalty for violating environmental regulations. For instance, depending on the location and nature of landfill site, the landfill operators are bound to make financial assurances and are charged different amounts of licence fees by Environment Protection Agency (EPA). Any kind of unauthorized waste disposal draws legal penalty up to US$60,000 per individual (Kuppusamy et al. 2016).

15.6 Conclusion

Extensive use of pesticides has become a serious ecological and human health hazard globally. Due to the dearth of a comprehensible pesticides management approach, large amount of obsolete pesticide residues have already stockpiled over the time. So, there is an immediate need to develop technique for the degradation and removal of pesticides from contaminated sites. Assessing the environmental providence of pesticide residues and their imminent exposure risks to environment and public health is essential for formulating risk-based management strategies for risk reduction. Environmental impact assessment of pesticides influences characterization of previously permitted pesticides, approval of new pesticides, and establishment of remediation goals along with maintenance of environmental quality. Several developed regions have established pesticide risk assessment models which are of great significance for environmental sustainability. Biological control strategies, cultural control strategies, physical control strategies, mechanical control strategies, new molecular strategies, Farmer education and awareness, adhering to environmental regulations, rational use of pesticides (RUP), continuous monitoring of pesticide residues, and development of new pesticides with enhanced safety profiles are some effective and reliable pesticide management strategies. Pesticide ban is an important regulatory stride taken for the management of deleterious pesticides. Globally, several highly persistent and toxic pesticides have been banned hitherto with DDT ban an exemplary one. This chapter provides an overview of global environmental regulations framed for management of pesticides. The International Code of Conduct and Use of Pesticides, The Montreal Protocol, The Rotterdam Convention, The Basel Convention, The Stockholm Convention, The International POPs Elimination Network (IPEN), The International Labor Organization Convention No. 184 (ILO Convention 184), The Global Harmonized System of Classification and Labelling of Chemicals (GHS), Strategic Approach to International Chemicals Management (SAICM), Registration, Evaluation and Authorisation of Chemicals (REACH), The Clean Sweep Program, The Codex Alimentarius Commission, Global Good Agricultural Practices (GlobalGAP) and Pesticide Action Network organizations (PAN) are some imperative global environmental regulations framed for management of pesticides.

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Index

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