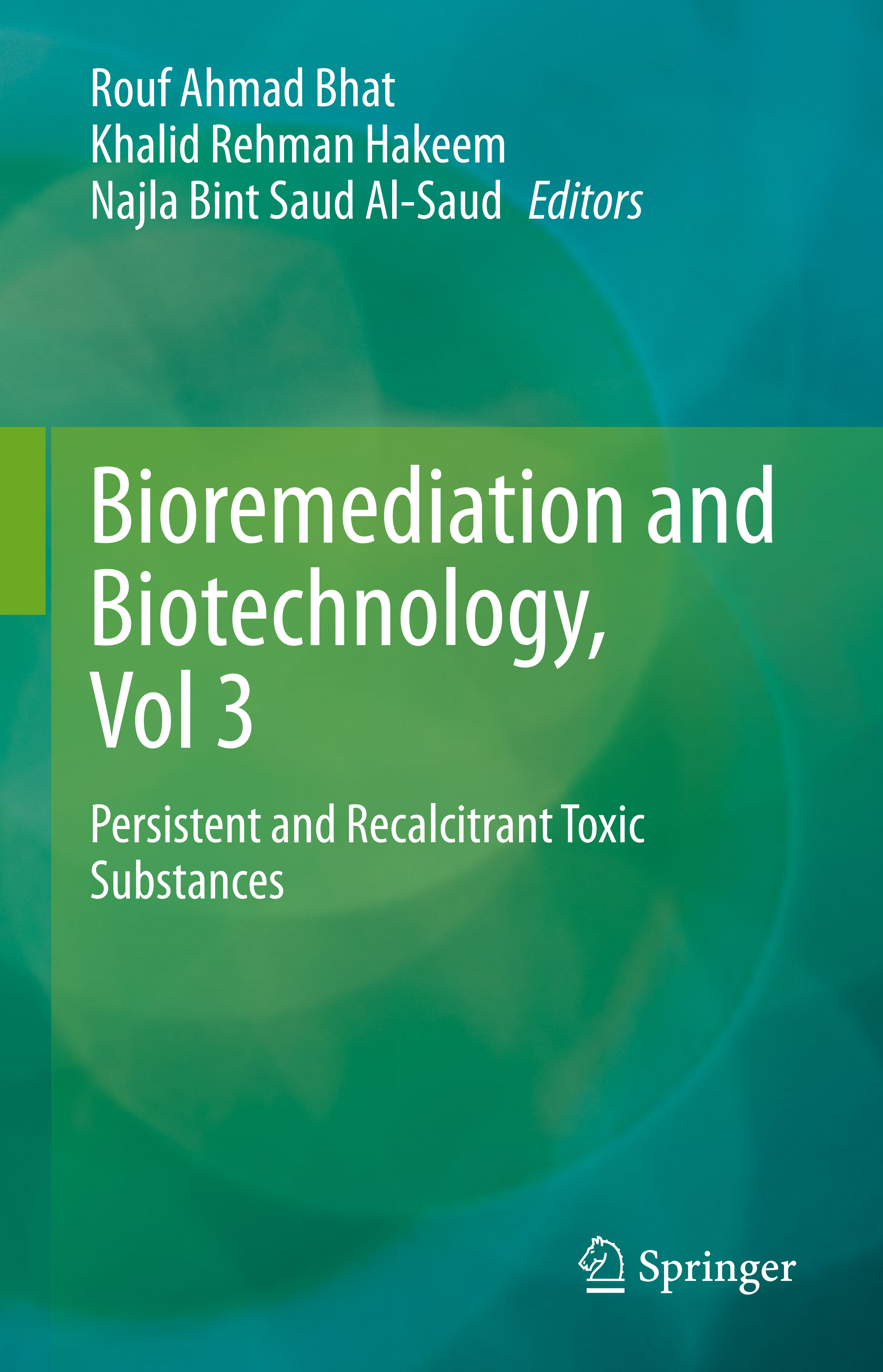

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Najla Bint Saud Al-Saud *Editors*

Bioremediation and Biotechnology, Vol 3

Persistent and Recalcitrant Toxic Substances

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Dedicated to our beloved Parents

Foreword

A healthy environment is essential for any biota, without which survival is not possible. The qualities of the environment are continuously being degraded by persistent and recalcitrant pollutants released into different ecosystems due to anthropogenic activities viz., improper wastewater discharge, unscientific solid waste management, rapid industrialization, urbanization, and application of recalcitrant substances and pesticides. These persistent pollutants not only affect the living organisms in soil and water but also have deleterious impacts on human health. Undoubtedly, synthetic substances like pesticides, herbicides, etc., are helpful for controlling unwanted pests and plants; hence, they are essential for increasing agricultural yield. But, the presence of these substances in the environment has long-term implications as these substances have a long residential period in any ecosystem. Pesticide application on crops directly affects nontarget organisms, soil health, and also reaches nearby freshwater bodies. There are numerous technologies available for monitoring, assessing, and remediation of these recalcitrant pollutants, but they are expensive as well as inefficient for a sustainable future. Seeking sustainable approaches for remediation of persistent substances, biotechnology, and bioremediation has proven to be a purposeful tool for the restoration of disturbed environs. Several scientists, researchers, and workers are exploring different compartments and the use of adopted biota and bioactive compounds for the degradation of toxic substances. Bioremediation and biotechnology are regarded as sustainable approaches to remediate environmental contaminants. These technologies nowadays play a key role in the degradation of contaminants. Furthermore, these sustainable techniques can remove persistent contaminants from the environment in a versatile, cost-effective, and eco-friendly manner.

The book is a lucid assemblage of varied topics in the form of book chapters, pertinent to the credibility of biotechnology and bioremediation for degradation and remediation of persistent pollutants in any ecosystem. The book contains 15 chapters from diverse regions of the world. Chapter 1 entitled "Impact of Heavy Metal Contamination on Quality Environs" is written by a group of authors from North India. It presents information about the toxicity of heavy metals on soil structure, animals, and plants due to anthropogenic stress. Furthermore, the authors urge on the fact that the presence of these substances in any ecosystem not only affects the life process of animals but also critically damages the life processes of plants and microbes and conclude that these substances have deleterious impacts on any biota. The scientists from Brazil provide a valuable picture about the "Concerns and Threats of Xenobiotics on Aquatic Ecosystems" in Chap. 2. This chapter addresses specifically the concerns and threats related to the pollution of aquatic ecosystems by xenobiotics and highlighting the risks posed due to pesticides and pharmaceuticals. Chapter 3 entitled "Approaches and Methods for Evaluation of Persistent Pollutant Load in Different Aquatic Environs" is written by a group of scientists from Brazil. The authors present information pertaining to human health risks due to the contamination of organic and inorganic persistent substances. Persistent chemicals (mainly pesticides and heavy metals), even in small amounts, can represent an enormous risk to the forms of life in contact with the polluted water. Therefore, this chapter focuses on approaches and methods for evaluating the persistent pollutant load in aquatic environs. In Chap. 4, a team of workers from Saudi Arabia and India address on the work of "Chemical Pesticides: Integrated Methods in Assessment and Monitoring." They have added information on the extensive use of pesticides and their negative impacts on human health. Furthermore, the authors have recognized that these contaminants are lethal at low concentrations. Chapter 5 entitled "Chemical Transformations of Synthetic Persistent Substances" is written by a group of researchers from Romania. They have scrutinized the effects of toxic substances at individual as well as at population level on different biota and recommended that the concentration of contaminants which can reach in an organism is an amalgam of several processes: viz., absorption, extraction, accumulation, transformation, and volatilization. For a second time, scientists from Romania present their work on "Ecotechnologies for Persistent Pollutants" in Chap. 6. In this chapter, the authors demonstrate that continental aquatic ecosystems are vulnerable to pollution with persistent organic pollutants (POPs) and advise that a mechanism is needed to obtain coherent data at a regional and global level regarding the concentration of these substances in the sediment, water, and aquatic organisms. A group of workers from Pakistan have expanded the information on the title "Microbiological Aspects of Pesticide Remediation" in Chap. 7. It confers the credibility of microbial biota for the decontamination of pesticides. Moreover, it provides a detailed account of the importance of microbes and the mechanisms involving enzymes associated with pesticide remediation. Techniques to enhance the biodegradation rate and genetics involved in microbial degradation of pesticides have also been briefly explained in this chapter. The authors conclude that the involvement of various methods like nanobioremediation, genetic engineering, and immobilization associated with microbes can significantly enhance the biodegradation of pesticides. Researchers from India have shared information on the "Eco-friendly Approaches for Remediation of Pesticides in Contaminated Environs" in Chap. 8. The information shared discusses the fact that bioremediation by using microorganisms (bacteria, algae, and fungi) can remove pesticides from the environment in a versatile, cost-effective, and eco-friendly manner. Chapter 9 titled "The Reliability of Nanotechnology for Sustainable Industries" is written by scientists from Romania.

They have presented intricate facts pertaining to nanotechnology as a sustainable industrial tool for improving biomass production, production of fireproof building materials, and in the medical system, a possible tool that can extend life expectancy and eradicate diseases. Another group of authors from India and Oman prepared Chap. 10 tilted "Zooplankton: The Significant Ecosystem Service Provider in Aquatic Environment." It deals with the fact that zooplanktons are vital in maintaining the biological configuration of the food web and the overall balance of the aquatic ecosystems. Besides, it presents the information that these valuable assets can be utilized to monitor the physicochemical and biological alterations in aquatic ecosystems due to climate change and anthropogenic influences. Scientists from Mexico have pooled up information on the "Phytoremediation Technology: Sustainable Solution for Cleaning Up of Recalcitrant Pollutants from Disturbed Environs" in Chap. 11. The authors have reviewed more than 90 plants pertaining to their potential in the process of phytoremediation. Among them, 80 plants are capable of removing heavy metals and 13 removing organic pollutants from disturbed environs. In addition, a case study pertaining to the phytoremediation potential of *Typha latifolia* has been discussed in a lucid manner. As per the authors, *T. latifolia* is a hyperaccumulator for Pb and removes other heavy metals at a good rate in disturbed environs. Therefore, the chapter concludes with the suggestion that plants are capable of degrading toxic substances and should be employed for the restoration of contaminated environs. Chapter 12 titled "Applications of Macrophytes as Environmentally Sound Technique for Cleaning of Contaminated Ecosystems" has been prepared by researchers from India. The authors provide a picture about the importance of water resources which support life on earth. Besides, it discusses the impacts posed by diverse categories of pollutants (toxic metals, agrochemicals, synthetic pesticides, and organic substances) that have drastically altered the quality of aquatic ecosystems. The authors accentuate that macrophytes could be applied as an effective, efficient, and better choice to clean the wastewaters contaminated with metals, nutrients, and pesticides. Chapter 13 entitled "Role of Biotechnology in Pesticide Remediation" has been critically overviewed by workers from India. They compare conventional with modern remediation approaches and concluded that biotechnological tools and their applications are easier, time-saving, safer, and sustainable to remediate the contaminated sites. A group of scientists from India have presented a valuable description under the title "Role of Ectomycorrhizal Biotechnology in Pesticide Remediation" in Chap. 14. The authors reported the application of ectomycorrhizal fungal strains or their prominent fungal enzymes via contemporary biotechnological tools for sustainable degradation and remediation of disturbed environs. Chapter 15 entitled "Biosensors: A Biotechnological Tool for Monitoring Environmental Pollution" as the closing chapter has been presented by researchers from India. They have mentioned that biosensors have gained huge significance in the field of defense, biomedicine, food industries, drug discovery, and environmental pollution monitoring. Furthermore, they have highlighted the modern trend in the monitoring of environment is utilization of novel and innovative biotechnological techniques with a focus on genetic or protein engineering. Besides, synthetic biology to program microbes may also be utilized to enhance the specificity, selectivity, and accuracy of biosensors. As per the authors, the application of biosensors has therefore a great potential for ecological scrutiny and recognition of pollutants.

The sequence of chapters incorporated and information demonstrated in this book encompass the most imperative facets of scientific evaluation. The editors must be applauded for their commendable work spent on bringing out this volume.

Centre of Research for Development (CORD) Bashir Ahmad Ganai

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Preface

A healthy environment is essential for any biota, without which survival is not possible. Persistent and recalcitrant pollutants are continuously degrading the qualities of the environment. These substances not only affect the living organisms in soil and water but also have deleterious impacts on human health. Certainly, human-made chemical constituents like pesticides and herbicides play a pivotal role in controlling unwanted pests and plants. However, the existence of these chemical constituents in the environment has extended insinuation due to their long residential period. The application of these substances directly affects nontarget biota, soil health, and also affects freshwater ecosystems. Numerous technologies are available for monitoring, assessing, and remediation of these recalcitrant pollutants, but are expensive as well as inefficient for a sustainable future. Bioremediation and biotechnology nowadays play a vital role in the degradation of persistent pollutants. Furthermore, these sustainable techniques can remove pesticides from the environment in a versatile, cost-effective, and eco-friendly manner.

The book is a smooth-tongued grouping of diverse titles in the shape of chapters, relevant to the integrity of "biotechnology and bioremediation" for decontamination of persistent substances in different environs. It provides a prime position to the chapters about sources, classification and inferences, and threats of recalcitrant substances on different ecosystems. A valuable space has given to the monitoring techniques for recalcitrant substances. Besides, the book contains some chapters encompassing the available conventional technologies for the degradation of recalcitrant pollutants. Furthermore, this book has given a valuable place for innovative technologies, viz. mycoremediation, nanotechnology, phytoremediation, bioremediation, biotechnology, and GMPs for decontamination of recalcitrant pollutants. In general, the introductory chapters of this book deal with the contamination of quandary and its enormity followed by innovative modern treatment technologies and sustainable techniques to remediate the persistent substances in different environs.

The book is a detailed, comprehensive description of the remediation technologies from flawed to sustainable. Academicians, researchers, and students find it a perfect bind about biotechnological intrusion for sustainable treatment of persistent pollutants and is adequate for the different requirements of training, teaching, and research.

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About the Book

This book is a cogent assemblage of assorted topics pertinent to the trustworthiness of biotechnology and bioremediation for degradation and remediation of recalcitrant substances. The book relies on chapters encompassing classification and implications, ecotoxicity, evaluation methods, biosensors, and techniques for assessing and monitoring of recalcitrant contamination. Besides, it contains comprehensive knowledge pertaining to biological tools viz., micro- and macro-biota, biotechnology, and nanotechnology for degradation and restoration of disturbed environs. Therefore, the introductory chapters focus on environmental contamination and its atrociousness followed by pioneering recent treatment approaches and sustainable techniques to remediate the contaminants. Furthermore, it is a meticulous complete portrayal of remediation technologies from quiver to sustainable. Academicians, researchers, and students find it as an absolute bind about biotechnological intrusion for sustainable degradation of recalcitrant substances and adequate for the assorted requirements of training, teaching, and research.

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About the Editors

Rouf Ahmad Bhat, Ph.D is working in Cluster University Srinagar (J&K), India on the capacity of Assistant Professor and has his specialization in Limnology, Toxicology, Phytochemistry, and Phytoremediation. He has been teaching graduate and postgraduate students of Environmental Sciences for the past 3 years. He is an author of more than 50 research papers and 25 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 symposiums. He has worked as an associate Environmental Expert in World Bank funded Flood Recovery Project and also an 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. He is still writing and experimenting with diverse capacities of plants for use in aquatic pollution.

Khalid Rehman Hakeem, PhD is professor at King Abdulaziz University, Jeddah, Saudi Arabia. After completing his doctorate (Botany; specialization in Plant Ecophysiology 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. He has more than 10 years of teaching and research experience in plant ecophysiology, 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 visiting scientist at Jinan University, Guangzhou, China. Currently, he is involved with a number of international research projects with different government organizations. So far, he has authored and edited more than 50 books with international publishers, including Springer Nature, Academic Press (Elsevier), and CRC Press. He also has to his credit more than 100 research publications in peer-reviewed international journals and 60 book chapters in edited volumes with international publishers. At present, he 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. He has been listed in Marquis Who's Who in the World, from 2014 to 2019. Currently, he is engaged in studying the plant processes at ecophysiological as well as molecular levels.

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Chapter 1
Impact of Heavy Metal Contamination on Quality Environs

Rouf Ahmad Bhat, Dig Vijay Singh, Mohammad Ramzan Mir, and Gowhar Rashid

1.1 Introduction

Industrialization and technical progression have put enhancing load of heavy metals (HMs) on the environs by discharging huge magnitude of perilous waste (Table 1.1). HMs and organic pollutants have wreaked severe harm to the environment. The upsurge of HMs in “soils and waters” prolonged severe global health problems. These toxic substances (HMs) cannot be despoiled into non-hazardous forms, but remain in the biotic and abiotic systems. Environmental contamination with HMs has amplified ahead of the safe limits and is unfavorable to both flora and fauna (Tak et al. 2013; Gaur et al. 2014; Dixit et al. 2015). The highest tolerable concentrations of some HMs in water, as declared by the “Comprehensive Environmental Response Compensation and Liability Act (CERCLA), USA” are presented in Table 1.2. The examples of “common HMs include Pb, As, Cr, Ni, Zn, Cd, Cu, and Hg” (Wuana and Okieimen 2011). HM contamination at present is a foremost environmental concern because metal ions stick within the environment owing to their non-degradable character. The “toxicity and bioaccumulation” potential of HMs in the environment is a severe danger to all the biota (Rashid et al. 2019). Unlike organic pollutants, HMs cannot be wrecked by “chemical or biological” processes, but can

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Table 1.1 Different sources of HMs pollution in soil environs (Nriagu and Pacyna 1988; Su et al. 2014)

	As	Cd	Cr	Cu	Hg	Pb	Zn
Source	per year (1000 t/a)						
Crop cultivation and food processing	0.0–0.07	0.01–0.3.3	5.0–85.50	2.5–40.0	0.01–2.0	1.4–30	10–145
Farmyard fertilizer	1.0–4.0	0.21–1.3	15.0–55.0	14–80	0.01–0.23	2.9–19.5	14–315
Logging and timber	0.1–3.2	0.01–2.4	2.5–15.0	3.3–52	0.01–1.9	6.9–7.9	11–70
Domestic waste	0.10–0.60	0.9–8.0	7.0–35.0	13–40	0.02–0.30	20–58	18–89
Macrobiotic wastes	0.01–0.30	0.01–0.03	0.12–0.50	0.04–0.61	0.00	0.03–1.5	0.12–2.7
Metallurgy waste	0.011–0.24	0.02–0.09	0.7–2.6	0.95–7.6	0.01–0.09	3.9–10.99	3.0–16
Coal residue	7.0–12.0	1.7–12.5	150.0–460.0	93–335	0.40–50	50–235	110–479
Chemical fertilizers	0.01–0.22	0.02–0.35	0.04–0.41	0.05–0.58	0.00	0.40–2.5	0.3–1.3
Goods and services	34–42	0.8–1.8	315–622	395–790	0.60–0.79	189–389	299–599
Aerosol deposition	8.5–20.0	2.2–8.4	5.1–38	14–36	0.63–4.3	202–263	49–135

Table 1.2 Heavy metal concentration limits in water and soils

HM (mg/L) limit in water as per the standards of CERCLA						
Ar	Cd	Cr	Pb	Hg	Ag	References
0.01	0.05	0.01	0.015	0.002	0.05	Chaturvedi et al. (2015)
HMs (mg/kg) limit in soils as per Indian standards						
Cd	Cu	Ni	Pb	Zn	Nagajyoti et al. (2010)	
3–6	135–270	75–150	250–500	300–600		

be altered to less toxic. Most of the HMs are lethal at small concentrations and are accomplished of “entering the food chain,” where they hoard and impose damage to “living organisms.” All metals have the potential to reveal detrimental impacts at elevated concentrations and “the toxicity of each metal depends on the amount available to organisms’, viz. the absorbed dose, the route, and the duration of exposure” (Mani and Kumar 2014). Owing to the lethal impacts of these toxic substances, there are increasing environmental and human well-being issues and ensuing requirement for expediting the wakeful and responsiveness in order to treat the contaminated environs. Therefore, it is essential to eliminate or trim down HM pollution in order to avert or diminish polluting the environment. To attain this, “bioremediation should be employed in order to increase metal stability, which in turn decreases the bioavailability of HMs” (Abbas et al. 2014; Akcil et al. 2015; NdeddyAka and Babalola 2016). Bioavailability of HMs establishes the "physiological and toxic effects" of a metal on biota (Olaniran et al. 2013). Bioremediation is a futuristic technique applied for HM elimination and/or resurgence from contaminated environs. The method utilizes intrinsic biological systems to exterminate perilous pollutants by way of employing the microbes and plants to reinstate contaminated environs to their original state (Mani and Kumar 2014; Akcil et al. 2015; Dixit et al. 2015). Microbial remediation by way of applying microbes to execute reduction of HMs in the soils has gained vital position in the field of treating the disturbed environs (Su et al. 2014). Microbes have amazing "metabolic pathways" which consume diverse noxious substances as a source of food for "growth and development, through respiration, fermentation, and cometabolism." Owing to their distinctive destructive enzyme package for a specific pollutant, hence, developed varied mechanisms for sustaining "homeostasis and resistance" to HMs in any ecosystem (Brar et al. 2006; Wei et al. 2014). Most of the HMs destroy "microbial cell membranes," but microbes can build up protection mechanisms that support them in eradicating the lethal effect. Thus, the reaction of microbes to HM contamination is of significance for restoration of degraded environs. The chapter provides insights into the sources and deleterious impacts of HMs on different environs.

1.2 HMs Effluence in Different Environs

Naturally occurring HMs are present in the complexes, which are not quickly accessible for plants. They are on average occurring in insoluble compounds, like in "mineral structures," or in "precipitated or complexes" unavailable for plants' life. HMs present in nature have enormous adsorption ability in soils and hence are not readily obtainable for biota. The adsorption capacity between HMs and soil in nature is massive in relation to man-made sources (Singh et al. 2018). The natural processes by which HMs bring into the environs are: "erosion, volcanic eruptions, and the weathering of minerals." HMs released from man-made cause have elevated "bioavailability" due to their soluble and transferable imprudent forms. The man-made sources comprise "alloy fabrication, automobiles, battery manufacturing, coating, galvanization, explosive manufacturing, discharge of untreated sewage, leather tanning, pesticides, phosphate fertilizer, photographic materials, printing pigments and dyes, and wood preservation" (Fulekar et al. 2009; Dixit et al. 2015; Singh et al. 2018; Bhat et al. 2018) (Table 1.2). The HMs accumulated in soil and then transferred to the food chain depend upon the concentration of HMs in soil, pollution load, and potential of uptake by plants (Bolan et al. 2014). According to D'Amore et al. (2005), "the geochemical cycle of HMs results in the increase of HMs in the environment, which could cause risk to all life forms, when they are greater than permissible limits." Metal extraction from ore is the chief source of HMs contamination in soil environs, and the restoration of these disturbed environs may possibly take long durations. Drawing out of metals and "ore processing" are chief sources of HM contamination in soil environs, and the restoration of disturbed environs from mining activities may possibly take some decades. These activities yield huge quantities of pollutants, which are habitually dumped without treatment and contaminate freshwater environs via “chemical run-off and particulates” that accrue in freshwater sources (Adler et al. 2007).

1.3 Ecotoxicity of HMs

Although some HMs play imperative functions in the “physiological, biochemical, and metabolic processes” of all biota, working as co-factors for various enzymes, “micronutrients, regulators of osmotic pressure, and stabilization of molecules, the majority of them have no known biological function in living organisms and are toxic when generated in excess” (Fashola et al. 2016). The ill effects of HMs are the capacity of a HM to cause detrimental effects on living creatures. This “depends on the HM bioavailability and the absorbed dose” (Rasmussen et al. 2000; Mehmood et al. 2019). The danger produced by HMs to the healthiness of biota is further affected by their endlessly relentless nature in the environs. The toxic effects of HMs worsened in the mediums having acidic pH and low nutrient content, and “when the soil structure is poor, especially in mining environments” (Mukhopadhyay and Maiti 2010). In acidic mediums, HMs lean to produce metal ions, with extra H^+ vacant to inundate metal adsorbing sites, which implies that at higher H^+ concentrations, “the adsorbent surface is further positively charged, thus reducing the attraction between adsorbent and metal cations” (Olaniran et al. 2013). In this way, HM becomes more “bioavailable,” thus mounting the grave effects to flora and fauna. In basic medium, metal ions substitute H^+ to produce new group complexes, ^{-}OH metal complexes. These complexes are soluble in some cases (Cd, Ni, Zn), while those of Cr and Fe are insoluble (Olaniran et al. 2013). The “solubility and bioavailability” of HMs are capable of being influenced by a slight change in the pH values. Slight alteration in the composition of soils, viz. organic matter (OM) content and nutrients, further tighten the effects of HMs toxicity. It has been observed so far that the soils which contain low OM relatively have high concentration of HMs and vice versa. OM content has a forceful impact on “cation exchange capacity (CEC), buffer capacity,” as well as on the availability of HMs. Thus, “metals present in organic soils contaminated with a combination of HMs are less mobile and less bioavailable to microorganisms and plants, than metals present in mineral soils” (Olaniran et al. 2013). Hotness of environs also has a pivotal role in the adsorption of HMs. Temperature has two main impacts on the adsorption route. Firstly, rising temperature will enhance the pace of “adsorbate diffusion across the external boundary layer and in the internal pores of the adsorbate particles,” since fluid gumminess diminishes as temperature rises. Secondly, temperature alters the impacts on the immovability of the metal ions primarily located in solution; immovability of the “microorganism metal complex” relies on the bioactive absorption sites, “microbial cell wall configuration and ionization of chemical moieties on the cell wall” (Arjoon et al. 2013). An “increase in the sorption capacity of lead, from 0.596 to 0.728 mg/g, was obtained when the temperature was raised from 25 to 40 °C” (Arjoon et al.

2013). Introduction to HMs (Pb and Hg) can affect the improvement of autoimmunity, having consequent effects on joint disorders, viz. "rheumatoid arthritis, kidney diseases, circulatory and nervous system disorders," and the detrimental to human fetal brain. Exposure to Pb and Hg in kids diminishes intelligence, impaired improvement, and enhanced risks of cardio disorders. Cadmium is known to be "carcinogenic and mutagenic and can disrupt the endocrine system, harm the bones and respiratory organs, and impinge on the balance of calcium in living organism" (Mani and Kumar 2014). Chromium damages normal hairs and causes baldness, headaches, diarrhea, gastric disorders, and queasiness in humans (Table 1.3). HM infected soils reduce plant growth because of toxic effects, follow-on ecological, "evolutionary, and nutritional troubles" (Abdul-Wahab and Marikar 2012). The HMs contamination in plants fluctuates, depending on the plant variety, involvement of specific metals, and absorption of metal, chemical state of metal, and soil structure and pH (Nagajyoti et al. 2010). Rise in HMs in plant tissues distresses or hampers nutrient uptake, "homoeostasis, growth, and development" (Chibuike and Obiora 2014; Sankarammal et al. 2014; Fashola et al. 2016). They interrupt metabolic functions, such as "physiological and biochemical processes, biochemical lesions, cell organelles destruction, chlorosis, delayed germination, induced genotoxicity, inhibition of photosynthesis and respiration, loss of enzyme activities, oxidative stress" (Salem et al. 2000; Nagajyoti et al. 2010; Dixit et al. 2015), "premature leaf fall, reduced biomass, reduced crop yield" (Fashola et al. 2016) "senescence, stunted growth, wilting, and can even cause the death of plants" (Wang et al. 2012; Ali et al. 2013; Fashola et al. 2016; Ayangbenro and Babalola 2017) (Table 1.3). HM toxicity "affects microbial population size, diversity, and activity, as well as their genetic structure" (Wang et al. 2012; Ali et al. 2013; Fashola et al. 2016; Ayangbenro and Babalola 2017). It affects "the morphology, metabolism, and growth of microorganisms by altering the nucleic acid structure, disrupting the cell membranes, causing functional disturbance, inhibiting enzyme activity and oxidative phosphorylation, and causing lipid peroxidation, osmotic balance alteration, and protein denaturation" (Chibuike and Obiora 2014; Fashola et al. 2016; Xie et al. 2016).

1.4 Effects of HMs on Soil Structure

Soil is the key recipient of HMs via a range of man-made activities. HMs "may persist in soil for a considerably long time" (Adriano et al. 2004). They change the composition and activity of essential soil micro- and meso-fauna groups (Xie et al. 2016). Above permissible limit concentrations of HMs in soil environs caused high ecotoxicity soil toxicity (Su et al. 2014). Chander et al. (1995) have reported that the HM contamination reduced the enzyme activities manifolds. Marques et al. (2009) have determined that the HMs consist of "exchangeable ions" that are "absorbed by inorganic solids on the surface." Metals that are present previously in the soil environs cause no pollution; however, HMs that are added in the soils via anthropo-

Table 1.3 Deleterious effects of HMs on different biota

HMs	Source	Impacts on			References
		Humans	Plants	Microorganisms	
As	"Atmospheric deposition, mining, pesticides, rock sedimentation, smelting"	"Brain damage, cardiovascular and respiratory disorder, conjunctivitis, dermatitis, skin cancer"	"Damages cell membrane, inhibition of growth, inhibits roots extension and proliferation, interferes with critical metabolic processes, loss of fertility, yield, and fruit production, oxidative stress, physiological disorders"	"Enzyme deactivation"	Bissen and Frimmel (2003), Abdul-Wahab and Marikar (2012), Finnegan and Chen (2012), Ayangbenro and Babalola (2017), Mushtaq et al. (2018)
Cd	"Fertilizer, mining, pesticide, plastic, refining, welding"	"Bone disease, coughing, emphysema, headache, hypertension, itai-itai, kidney diseases, lung and prostate cancer, lymphocytosis, microcytic hypochromic anemia, testicular atrophy, vomiting"	"Chlorosis, decrease in plant nutrient content, growth inhibition, reduced seed germination"	"Damages nucleic acid, denatures protein, inhibits cell division and transcription, inhibits carbon and nitrogen mineralization"	Nagajyoti et al. (2010), Sebogodi and Babalola (2011), Chibuike and Obiora (2014), Sankarammal et al. (2014), Fashola et al. (2016), Ayangbenro and Babalola (2017)
Cr	"Dyeing, electroplating, paints production, steel fabrication, tanning, textile"	"Bronchopneumonia, chronic bronchitis, diarrhea, emphysema, headache, irritation of the skin, itching of respiratory tract, liver diseases, lung cancer, nausea, renal failure, reproductive toxicity, vomiting"	"Chlorosis, delayed, senescence, wilting, biochemical lesions, reduced biosynthesis germination, stunted growth, oxidative stress"	"Elongation of lag phase, growth inhibition, inhibition of oxygen uptake"	Cervantes et al. (2001), Barakat (2011), Mohanty et al. (2012), Ayangbenro and Babalola (2017)

(continued)

Table 1.3 (continued)

HMs	Source	Impacts on Humans	Plants	Microorganisms	References
Cu	"Copper polishing, mining, paint, plating, printing operations"	"Abdominal pain, anemia, diarrhea, headache, liver and kidney damage, metabolic disorders, nausea, vomiting"	"Chlorosis, oxidative stress, retard growth"	"Disrupts cellular function, inhibits enzyme activities"	Salem et al. (2000), Nagajyoti et al. (2010), Dixit et al. (2015), Fashola et al. (2016), Ayangbenro and Babalola (2017)
Hg	"Batteries, coal combustion, geothermal activities, mining, paint industries, paper industry, volcanic eruption, weathering of rocks"	"Ataxia, attention deficit, blindness, deafness, decreases rate of fertility, dementia, dizziness, dysphasia, gastrointestinal irritation, gingivitis, kidney problem, loss of memory, pulmonary edema, reduced immunity, sclerosis"	"Affects antioxidative system, affects photosynthesis, enhances lipid peroxidation, induced genotoxic effect, inhibits plant growth, yield, nutrient uptake, and homeostasis, oxidative stress"	"Decreases population size, denatures protein, disrupts cell membrane, inhibits enzyme function"	Wang et al. (2012), Ali et al. (2013), Fashola et al. (2016), Ayangbenro and Babalola (2017)
Pb	"Coal combustion, electroplating, manufacturing of batteries, mining, paint, pigments"	"Anorexia, chronic nephropathy, damage to neurons, high blood pressure, hyperactivity, insomnia, learning deficits, reduced fertility, renal system damage, risk factor for Alzheimer's disease, shortened attention span"	"Affects photosynthesis and growth, chlorosis, inhibits enzyme activities and seed germination, oxidative stress"	"Denatures nucleic acid and protein, inhibits enzyme activities and transcription"	Nagajyoti et al. (2010), Wuana and Okieimen (2011), Mupa (2013), Fashola et al. (2016), Ayangbenro and Babalola (2017)
Zn	"Brass manufacturing, mining, oil refinery, plumbing"	"Ataxia, depression, gastrointestinal irritation, hematuria, icterus, impotence, kidney and liver failure, lethargy, macular degeneration, metal fume fever, prostate cancer, seizures, vomiting"	"Affects photosynthesis, inhibits growth rate, reduced chlorophyll content, germination rate, and plant biomass"	"Death, decrease in biomass, inhibits growth"	Chibuike and Obiora (2014), Gumpu et al. (2015), Ayangbenro and Babalola (2017)

genic activities alter drastically quality characteristics of soils (Ramos et al. 1994). The existence of HMs in soil environs also impacts the pH of the soil (Harter 1983), OM, compactness and category of charge in soil microcell, degree of coordination with type of ligand and soil, soil comparative active surface sites (Norvell 1984), and “soil soluble concentrations” (Marques et al. 2009). HMs tempt noxious effects on soil microorganisms, and the level of effect changes with relation to physicochemical attributes, viz. “pH, temperature, clay minerals, OM, ions, and metal compounds” (Bååth 1989). They also decrease the decomposition of OM in the soil and disturb the nutrient recycling (Su et al. 2014).

1.5 Effects of HMs on Plant Life Cycle

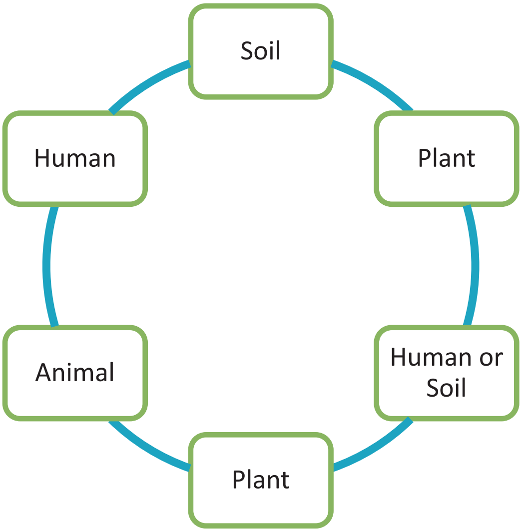
HM accretion above the acceptance point in plants also has undesirable effects on the plants. The plant turns extremely toxic, and it possibly will die. However, a few plants bear HMs toxicity up to the permissible limits. Two characteristics of plant HM interaction are as follows: (1) “metal-induced impairment of plant development” and (2) “resistive adaptation of plants to metal toxicity through modified metabolism” (Cheng 2003). HMs can interact with the bio-physiological processes that are vital to plant development (Chibuike and Obiora 2014). HM infected plants may possibly reveal decreased yield production, which accordingly impacts the food chain. “Cytoplasmic enzyme inhibition and cell structure injuries with oxidative stress occur when the plant is contaminated with a HM” (van Assche and Clijsters 1990; Jadia and Fulekar 2009). An example of “an indirect toxic effect is the replacement of essential nutrients at the cation exchange sites of plants” (Taiz and Zeiger 2002). For example, Zaier et al. (2010) reported that “the water content of *Brassica juncea* plants remarkably decreases under Pb toxicity, although this species is considered tolerant.” Servilia et al. (2005) “demonstrated that plants exposed to HM exhibit stunted growth, deformation, reduced physicochemical activities, and overall alteration of cellular metabolism.” Hg, As, Pb, Cd, and Cr are ranked among the most toxic metals that display considerable effects on plants (Tchounwou et al. 2012).

1.6 Effects of HMs on Human Health

HM pollution in different environs can unswervingly influence humans via suspended particulate matter inhalation or skin absorption (Table 1.3). HM pollution in soil has adverse impacts on humans and ecosystems via direct ingestion or makes contact with HM infected soils (McLaughlin et al. 2000; Ling et al. 2007). In the food chain, toxicity occurs in the order as depicted in Fig. 1.1.

Their contamination in soil also causes health disorders in humans via drinking polluted groundwater and eating of HM infected plants; this infectivity also causes

Fig. 1.1 Cyclic representation of heavy metal toxicity in food chain



disease in agricultural yield (Wuana and Okieimen 2011). Yabe et al. (2010) reported that “about 30% of Chinese children possess blood containing Pb levels in excess of the 100 g/L limit.” Besides, he concluded that HM revelation occurs through “water, fish, soil, food crops, food animals, and toys” (Yabe et al. 2010). This revelation of HMs causes toxicity in children, which in turn can cause multi-organ disorders. Cd “emissions have increased remarkably in recent years, especially with its presence in household waste” (Järup 2003; Ogunkunle et al. 2013; Emenike et al. 2018). Another example of metal toxicity affecting human health is evident in a study conducted at the cement facility in Sagamu, Nigeria (Emenike et al. 2018). Findings revealed significant non-carcinogenic risks to children and adults who are 6–30 years old due to oral exposure to Cd and Cr from the facility (Emenike et al. 2018). Moreover, in Heshan Village, China, farmers were affected by As due to a As processing factory located in the village between 1951 and 1978 (Phoenix Satellite TV 2014). Since this period, “the surrounding environment has been highly polluted with high As levels” (Phoenix Satellite TV 2014). “Soil and water are highly contaminated with As above the prescribed level, and workers suffer from severe As poisoning” (Phoenix Satellite TV 2014). “The surrounding plant and crops die or become too toxic for consumption.” According to the Phoenix Satellite TV (2014), “a total of 400 workers died due to As-induced cancer from 1951 to 2014.”

1.7 Conclusion

Heavy metals release in the quality environment has wide range of negative significant impacts on different life forms from microscopic to macroscopic. These toxic substances are lethal at their low concentrations. Anthropogenic load has further magnified the HMs concentrations in the quality environs. There are multiple health issues caused due to the exposure of HMs on plants, animals, and microorganisms. Therefore primary conclusion is that the priority should be given to the reliable treatment technologies to remediate the HMs from contaminated substances prior to their final discharge and awareness about the sustainable management of different natural sources.

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Chapter 2
Concerns and Threats of Xenobiotics on Aquatic Ecosystems

Flávia Cristina Policarpo Tonelli and Fernanda Maria Policarpo Tonelli

2.1 Introduction

When it comes to pollution of different environs, xenobiotics are synthetic compounds synthesized in large amounts for agricultural, domestic, and industrial use (for example, pesticides, pharmaceuticals, and dyes) but thrown (in large amounts or as micropollutants) in the environment without any concern (Meckenstock et al. 2015; Atashgahi et al. 2018). Generally, authors consider as xenobiotics just organic substances. However, some authors also consider heavy metals (inorganic elements) as belonging to this category of environmental pollutants. These are persistent pollutions that can accumulate in living organism performing trophic levels transfer in food chain, and that are highly toxic. They can induce in human beings, depending on some factors like time of exposure and dose, from signs of poisoning to death (Ali et al. 2019).

Xenobiotics usually are difficult to be degraded (being pH and temperature factors able to influence on their persistence/biodegradation), consisting in a long-term pollution interfering causing imbalances in biosphere, affecting especially human heath in a negative way (Magan et al. 2010; Godheja et al. 2016). It is also possible that xenobiotics partial degradation results in substances that are worst threats. The xenobiotic carbamazepine, for example, an antiepileptic drug, can suffer photolysis promoted by solar irradiation to generate the acridine: a highly toxic substance that can induce mutations and cause cancer (Ebele et al. 2017).

In aquatic ecosystems, it is common to find the hydrophilic compounds in water samples and lipophilic compounds attached to organic matter present on the

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ecosystem or in organisms that live there (Peake et al. 2016). As aquatic forms of life can be exposed to both kinds of substances, researches study these organisms to access the primary risk of xenobiotics that pollute water like hydrocarbons. Aliphatic and polycyclic aromatic hydrocarbons are prone to interfere with PPAR's signaling pathway offering an obesogenic effect in fish from Eleyele lake, in Nigeria (Ibor et al. 2019).

Some of the most important examples of xenobiotics are the pharmaceuticals, pesticides, dyes, and bisphenol A. The worldwide increase of medicines available for human and veterinary use also caused concern converting pharmaceuticals into emerging contaminants that can be detected even in treated wastewater. These bioactive compounds after being discharged in water can affect aquatic species and species that will consume this water causing damage; and the most dangerous feature is that they are already synthetize to be able to act in low concentrations to affect living beings (Bebianno and Gonzalez-Rey 2015; Sangion and Gramatica 2016; Mezzelani et al. 2018). The main pathways that enable water pollution by these xenobiotics are places dedicated to treat water (wastewater still contains these substances), runoff of soils and discharges from industrial areas and from hospitals, aquaculture, and animal farming (Klatte et al. 2017; Yang et al. 2017).

Pesticides are substances with a diverse chemical structure or a mixture of them that possess the effect of protecting plants against weeds, pests, and diseases (repelling, destroying or controlling them) and animals from ectoparasites. They can be insecticides, fungicides, bactericides, herbicides, rodenticides among other categories, and even inorganic elements like heavy metals (for example: cadmium, iron, lead, and zinc) can act as pesticides. In order to attempt to increase agricultural production to attend the necessities of the growing population the use of pesticides considerably increased after the Second World War (Gavrilescu 2005). As pollutants they are considered persistent (lasting for a long time on the polluted site or spreading from there) represent a serious threaten to all living forms (Schierow and Esworthy 2012; Morillo and Villaverde 2017; FAO 2019).

Synthetic dyes are also xenobiotic pollutants. They normally contaminate water through wastewaters from industrial areas as they are widely used to insert color in food, cosmetics, paints, and textiles. The textile dyes are the major pollutants among dyes, and especially azo dyes (containing one or more azo groups (–N=N–) substituted with benzene or naphthalene groups) can resist to the treatments performed in wastewaters from textile mills and dyestuff factories (El-Sikaily et al. 2012). These dyes are already toxic to living beings being able to present carcinogenic and/or mutagenic effect, but they can be partially degraded in the environment or during water treatment generating substances (like aromatic amines) even more toxic (Suzuki et al. 2001; Bhaskar et al. 2003; Pinheiro et al. 2004; Khan and Malik 2018). Already in the early 80s, the benzidine-based azo dyes Direct Blue 6 and Direct Black 38 were related to hepatocellular carcinomas and neoplastic liver nodules development in rats exposed to them for 13 weeks (Robens et al. 1980). By simply coloring the water, they can induce negative effects on the aquatic ecosystem, negatively impacting, for example, the photosynthesis rate due to the reduction of light penetration through water and also reduced dissolved oxygen levels (Hassan and Carr 2018)

Bisphenol A, an emerging contaminant used to produce, for example, epoxy and phenol resins, polycarbonates, and polyesters, is also a xenobiotic pollutant present in aquatic environments due, mainly, to human industrial activities (Peteffi et al. 2018). Wastewater treatment plants can only partially retain this pollutant that is still present in surface water; in a study developed in Leipzig, Germany, Bisphenol A concentration was reduced from a maximum of 21,343 ng/L to 914 ng/L (Musolff et al. 2010). This pollutant threats the reproductive systems (from male and female) not only from humans, but also from wildlife being able to induce, for example, abnormalities in the reproductive tract and infertility (Wu et al. 2015).

In this chapter the concerns and threats regarding xenobiotics as water pollutants will be discussed.

2.2 Concerns and Threats of Xenobiotics on Aquatic Ecosystems

Aquatic ecosystems can be polluted by xenobiotics through industrial and sewage discharge and also agricultural runoff. In this chapter is highlighted a group of pollutants that are a concern since Second World War end, the pesticides, and a group of emergent pollutants with a increasing occurrence, the pharmaceuticals.

2.2.1 Pharmaceuticals

The main pharmaceuticals present as pollutants in aquatic environments are non-steroidal anti-inflammatory drugs, psychiatric drugs (Silva et al. 2012), steroid hormones (Aris et al. 2014), cardiovascular drugs (Godoy et al. 2015), antibiotics (Carvalho and Santos 2016; Välitalo et al. 2017), and hypocholesterolaemic drugs (Neuparth et al. 2012). Non-steroidal anti-inflammatory drugs deserve to be highlighted and from these groups, especially diclofenac and ibuprofen, highly present in samples analyzed worldwide (Mezzelani et al. 2018).

Pharmaceuticals can bioaccumulate in various aquatic organisms such as algae (Vannini et al. 2011), mussels (Maruya et al. 2014), fish (Du et al. 2012). This fact was also demonstrated through analyzes on samples collected from Taihu Lake, China. It was analyzed not only surface water and sediment from the lake to detect the presence of 23 pharmaceuticals (roxithromycin, erythromycin, ofloxacin, norfloxacin, ciprofloxacin, tetracycline, chloramphenicol, sulfamethoxazole, sulfamerazine, sulfadiazine, ibuprofen, diclofenac, naproxen, indomethacin, clofibric acid, gemfibrozil, bezafibrate, 17b-estradiol, 17a-ethynylestradiol, propranolol, carbamazepine, ketoconazole, and sertraline); it also analyzed, to access bioaccumulation, samples from zooplankton, zoobenthos, bivalves, shrimps, and fish species (silver carp, common carp, crucian carp, lake anchovy, whitebait, redfin culter, and yellow catfish (Xie et al. 2017). *Gambusia holbrooki* (the mosquito fish) could uptake in a quick way the pharmaceuticals caffeine, diltiazem, and carbamazepine and as diphenhydramine and ibuprofen was present in the organisms' body after 5 h of exposure. The last two pharmaceuticals were also eliminated in a fast way (half-life of 34 and 32 h, respectively), but the other ones could persist (half-life of 141, 117, and 71 h, respectively) (Wang and Gardinali 2013). Roxithromycin, erythromycin and ketoconazole (antibiotics), ibuprofen and diclofenac (anti-inflammatories), propranolol (β-blocker), carbamazepine (antiepileptic), and 17α-ethinylestradiol (steroid hormone) could be widely detected not only in surface water but also in fish samples in samples collected from Rivers downstream sewage treatment plants in Nanjing, China. Bioaccumulation had as the main target the liver tissue, been followed respectively by brain, gill and muscle (Liu et al. 2015). In a study performed involving the xenobiotic benzodiazepine oxazepam, as expected the main target of bioaccumulation was the brain of *Pimephales promelas* followed by, respectively, plasma, liver, and muscle. At the concentration of 4.7 μg/L the pharmaceutical was able to affect the fish's behavior (Huerta et al. 2016).

Conventional water treatment procedures are designed to offer water with desirable physico-chemical characteristics and fecal coli form counts. So, they cannot efficiently remove these pollutants from water and humans can end up consuming them (Snyder 2008). There are substances that are classified as low persistent in water like Paracetamol and Ibuprofen, some can last for more time being moderately persistent like Iopromide and Ivermectin, and some are considerate highly persistent like Diazepam and Carbamazepine (Löffler et al. 2005). However, the pollutants can migrate from one category to the other in this classification depending on their concentration in water. Oxazepam (an anxiolytic drug), classified in 2005 as moderately persistent was reclassified in 2015 as more persistent (long-term persistent) than in the previous study due to an increase in its use and pollution (Klaminder et al. 2015).

The effects induced in aquatic life by these pollutants also depends on exposure and from the species exposed to them. Oxazepam, for example, does not affect the behavior of *Coenagrion hastulatum* (an invertebrate prey); however, its predator (the fish species *Perca fluviatilis*) became more active. Interestingly researchers noticed that 46% of the pharmaceutical ingested by the prey, accumulated in the predator (Brodin et al. 2014).

2.2.2 Pesticides

When pesticides are sprayed with the intention to improve crops quality and combat pests, it is possible that a large amount of the chemicals not even reach their targets. The sprayed substances can be dispersed contaminating not only surface water nearby but also groundwater, soil, air, and also people and animals especially next to the application spot (Bhat et al. 2018; Liu et al. 2018; Reeves et al. 2019).

Government agencies such as Environmental Protection Agency (in USA) and the European Environment Agency (in European Union) establish legal limits for their presence in food and drinking water (Colmati et al. 2019).

They are highly toxic substances and after a single episode of skin contact or ingestion of contaminated water, for example, a human being can experience acute toxicity symptoms within 48 hours including nausea, allergic reactions and or irritation of eyes and skin, vomiting, diarrhea, cough and respiratory tract irritation, headache, among other; depending on the extension and intensity of the exposure it can cause loss of consciousness, seizures, and even death (Salameh and Abi-Saleh 2004; Mushtaq et al. 2018; PAN UK 2019).

The negative effects observed as consequence of exposure to these pollutants vary depending on chemical properties of the pollutant and also on the exposed organism. Chronic effects can also be unnoticed (as suppression of the immune system, genetic and cellular damage, inhibition or failure of reproduction) or only noticed after a serious diseases development like cancer (Bhat et al. 2018; Srivastava et al. 2019).

The sources of pesticide pollution can be diffuse or point sources (Vasilescu and Medvedovici 2005) and its highly common that the reason of their environmental contamination are related to excessive or inadequate use (Jin et al. 2015; Mushtaq et al. 2018); for example, a person can be contaminated after reusing washed pesticide containers such as drinking water container. Thus, the humans most affected by this kind of water pollution are those who live or work on or near areas that apply pesticides especially in large amounts (Özkara et al. 2015).

When sprayed in large areas, especially near water the pesticides can cause its contamination, but water can also receive pesticides residues by entering soil and reaching groundwater (Srivastava et al. 2019). And although some pesticides have no maximum level of occurrence in environmental samples previously determinate by legislation, it is not desirable their presence in living beings like fish tissues, for example. In this way, new methods to detect these pollutants in water samples are continuously being developed. A rapid analysis of water samples with high performance liquid chromatography containing diode–matrix detector was recently proposed to detect the organochlorine pesticide Imidacloprid (Hrybova et al. 2019).

2.3 Conclusions

Over time the number of areas severely affected by pollution and the number of different types of pollutants has been increasing. So, researches have been dedicated attention trying to understand the concerns and threats related to them and developing ways to detect the pollutants and to remediate them. Therefore, to stop this vicious cycle it is crucial that humans develop environmental awareness in order to establish sustainability. As long as a change in attitude is not adopted by humanity, pollution by xenobiotics will continue to be a growing problem with great potential to endanger the existence of life on Earth.

2.4 Future Perspectives

Xenobiotics are a challenging group of pollutants once they possess a diverse chemical nature and can resist to the water treatments conventionally applied to improve water quality for human use. These substances are highly dangerous and can cause severe sickness like cancer and even death.

In order to understand the concerns related to these pollutants and the threats they represent to the different forms of life, it is expected that studies on these topics continue to be developed worldwide. However, it will not be an easy task, once differences in target organisms, exposure time and quantity, characteristics of the environment can influence on the results observed.

It is also expected the development of new studies on remediation strategies of polluted sites and on more efficient ways to treat water to eliminate xenobiotics.

However, it is urgent that humanity understands the importance of interacting in a sustainable way with the environment to reduce the amount pollution production.

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Chapter 3

Approaches and Methods for Evaluation of Persistent Pollutant Load in Different Aquatic Environs

Fernanda Maria Policarpo Tonelli and Flávia Cristina Policarpo Tonelli

3.1 Introduction

The environment can be polluted in a persistent manner as a consequence of some human actions performed in an unsustainable way (Bhat et al. 2017). For example, the disposal of garbage in landfills, the use of pesticides to improve agricultural production, industrial activities related to the production of explosives, dyes, fuels, organic solvents, vehicular exhausts, and mine exploitation can generate persistent contaminants that can be either organic or inorganic (Bhat et al. 2018; Singh et al. 2018). When it comes to aquatic environments, wastewaters are an important source of pollution, affecting the biological equilibrium and chemical composition of the water bodies where they are thrown (Kazlauskienė et al. 2012; Bhat et al. 2017). And it is important to highlight that not only superficial water can be contaminated; groundwater is also exposed to the damaging effects of the persistent contaminants (Kim et al. 2017; Sposito et al. 2018).

As it is a worldwide concern to treat water in order to provide the population with good quality one (Albering et al. 2016; Shahpar and Esmaeilpoor 2018), remediation strategies are necessary to remove organic persistent pollution in aquatic ecosystems like aromatic substances possessing high cytotoxicity and that are hard to degrade (Parrilli et al. 2010; Mushtaq et al. 2018; Bilal et al. 2019). And it is also possible that they generate even more toxic products after been partially degraded or reacted with other substances present in contaminated water (Rousis et al. 2017; Salvia et al. 2017). These organic persistent pollutants can, for example, be found in wastewater from fields in which pesticides (fungicides, herbicides, or insecticides)

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were applied to kill pests, from industries that apply dyes in the productive process, from mining areas, and sea can also be polluted by not sustainable exploitation of offshore oil (Dhanarani et al. 2016; Bilal et al. 2017; Chatha et al. 2017; Carpenter 2018; Craig 2018; Li 2018; Shabbir et al. 2018).

Heavy metals are worrying representatives of inorganic pollutants commonly present in watercourses from mining regions. These contaminants are very difficult to be removed from the polluted areas, can induce oxidative stress in living organisms causing cell death, present carcinogenic effect, and/or bioaccumulate (Mehmood et al. 2019; Rashid et al. 2019) being a persistent threat: also requiring human intervention to be removed from contaminated spots (Alkorta et al. 2004; Jaishankar et al. 2014; Bansod et al. 2017; Karthik et al. 2017; Jacob et al. 2018; Ali et al. 2019).

Persistent pollutants are commonly resistant to standard water and wastewater treatments (Barbosa et al. 2015). So, it is necessary to develop not only strategies to remove the persistent pollutants from the contaminated aquatic environs, but also ways to detect these contaminants and evaluate how risky they are to that environ.

This chapter will be focused on approaches and methods for evaluation of persistent pollution in aquatic environs.

3.2 Approaches and Methods for Evaluation of Persistent Pollution in Aquatic Environs

When a water sample is analyzed for pollutants presence, physico-chemical analysis, such as determining the sample's conductivity, pH, and turbidity, is an important primary indicator of the presence of these unwanted substances. Although it does not allow contaminant elucidation, it is an important initial screening to determine which samples should receive attention for further robust analysis (Alam et al. 2007; Chagas et al. 2017).

In order to really detect the presence and access the risk related to the level of contamination detected in the environment, it is possible to use conventional methods such as the ones involving chromatography and/or the mass spectrometry (García-Córcoles et al. 2019; Riva et al. 2018; Shahpar and Esmaeilpoor 2018). For example, the presence of the metal atoms of Pb, Cd, Hg, Ni, and Zn could be analyzed in aqueous samples, after chelates generation using 2-(2-quinolinylazo)-4-methyl-1,3-dihydroxidebenzene, by using chromatographic separation and spectrophotometric properties of these atoms (Yang et al. 2005). Triazine herbicides (atrazine, prometon, propazine, and prometryn) were detected in environmental water samples after sample exposure to graphene-based Fe_3O_4 magnetic nanoparticles followed by high performance liquid chromatography-diode array detection (Zhao et al. 2011). The presence of the toxic heavy metal contaminants Pb, Cd, and Ni could be simultaneously detected in the aqueous phase used to extract soil samples by applying the ion chromatographic method (Gao et al. 2018).

Although they have already been used for a long time to evaluate persistent pollutants presence, the techniques involving their use have been constantly innovated and improved; for example, even environmental metabolic footprinting approaches have been developed (Salvia et al. 2017). Liquid chromatography-tandem mass spectrometry can be used to detect the presence and perform quantitation of twelve pesticides (carbendazim, imidacloprid, imazethapyr, hexazinone, imazaquim, tebuthiuron, azoxystrobin, ametryn, atrazine, diuron, propiconazole, and tebuconazole) in surface, ground, and drinking water obtained from Tibagi River in Brazil (Almeida et al. 2019). By using a multi-determination method water samples from Maipo River in central Chile could be screened by gas chromatography coupled to triple quadrupole mass spectrometry to detect the presence of polychlorinated biphenyls, polycyclic aromatic hydrocarbons, polybrominated diphenylethers, and pesticides (Retamal et al. 2013). An online solid phase extraction–high performance liquid chromatography-tandem mass spectrometry multiresidue method (that uses an atmospheric photoionization source) allows the detection of insect repellent residues in water from Sava and Evrotas basins with high sensitivity and precision. Hazard quotients allowed the environmental risk assessment which revealed a risky concentration in some areas (Molins-Delgado et al. 2018). Worried with the use of contaminated wastewater to perform irrigations in farms Polish scientists developed a method using HPLC–Q-TOF–MS to evaluate the presence of contaminants (especially emergent ones) that could be considered markers. Substances like acesulfame-K, carbamazepine, and sucralose proved to be important markers when it comes to communal sewages in that country (Kempińska and Kot-Wasik 2018). Spain and Italy's surface and wastewater could be screened for the presence of 450 pesticides and its subproducts by using liquid chromatography coupled to high-resolution mass spectrometry. Residues of 17 pesticides were found in wastewater and 12 in surface water (Rousis et al. 2017).

UV spectrophotometry is also a methodology that has been successfully applied to study water persistent contaminants. Environmental water samples could be analyzed to detect U^{6+} ion presence after being exposed to an extractant-impregnated resin followed by spectrophotometric analyses (Hosseini-Bandegharaei et al. 2013). Organophosphorous pesticides also had their concentration determined in water samples after degradation (under UV light in the presence of ZnO nanocrystals) by using UV-Vis spectrophotometer analyses (Sharma et al. 2016). A spectrophotometric method to acquire electronic absorbance spectra using a xenon lamp made it possible to analyze the presence of the popular herbicide glyphosate in potable water, groundwater, and treated wastewater (Romero-Natale et al. 2019).

Atomic absorption spectrometry (AAS) makes possible to analyze aqueous solutions (including waste and sea water) for the presence of ions from the metallic elements Au, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Pd, and Zn after exposure to chemically modified chloromethylated polystyrene-PAN ion-exchanger (Kenawy et al. 2010). The AAS also made possible to detect in groundwater the presence of Cu, Cr, Cd, Pb, Zn, and Fe (Boateng et al. 2019). Thermospray flame atomic absorption spectrometry made it possible to determinate Cd and Pb after cloud point extraction from freshwater (Garcia et al. 2015). Cold vapor AAS made possible to detect, in water samples from industrial zone, the presence of the atoms Pb, Cd, Cr, Fe, Cu, Ni, and Hg (Pobi et al. 2019).

After being preconcentrated by silica sequentially modified with polyhexamethylene guanidine and Arsenazo I, the concentration of metal ions from Al, Ca, Cd, Co, Cu, Fe, Mg, Ni, Pb, and Sr ions could be evaluated in river waters of Krasnoyarsk Krai by inductively coupled plasma optical emission spectrometry (ICP-OES) (Losev et al. 2015). By ICP-OES it was also possible to analyze the presence of Al, Bi, Cd, Co, Cu, Fe, Ga, In, Ni, Pb, Tl, and Zn (12 heavy metals) in real water samples (Sereshti et al. 2012). Freshwater sediments were analyzed in order to detect the pesticides triphenyltin hydroxide, azocyclotin, and fenbutatin oxide by using ICP methodology after high performance liquid chromatography and before mass spectrometry (Gui et al. 2016). ICP-OES also made possible the detection of As, Ni, Cr, Pb, Co, Se, Hg, and Cd in groundwater samples from the coastal area of SPSR Nellore District in India (Raju et al. 2014).

Water analyses can also be performed by total reflection X-ray fluorescence spectrometry (TXRF); it made possible to evaluate the levels of Cr, Mn, Fe, Cu, and Pb in water samples from one of Mexico's most important rivers that drain highly polluted regions: the Lerma (Zarazua et al. 2006). Dispersive microsolid phase extraction followed by TXRF consists of a very sensitive methodology that also allows Co, Ni, Cu, As, Cd, and Pb detection in low concentrations even in high salinity samples (samples that challenge other spectroscopy techniques) (Sitko et al. 2015).

Raman spectroscopy is also useful to detect pesticide residues in paddy water. In a rapid way the surface-enhanced Raman spectroscopy together with chemometric methods made possible to detect fonofos, phosmet, and sulfoxaflor in these samples with high accuracy (Weng et al. 2019).

Aqueous environments can also be analyzed from the presence and quantification of Ti, Cr, Mn, Ni, Cu, Zn, Pb through a methodology called micro-X-ray fluorescence core-scanning. It involves the exposure of the sample to cation-exchange resin sachets to concentrate the heavy metals present and then the X-ray analysis is performed. This fast and non-destructive analyses allow the comparison of the results acquired to the ones from analyzed resins containing reference standards (Huang et al. 2019).

However, these methods mentioned so far possess some disadvantages such as being time-consuming, requiring expensive reagents and equipment, requiring well-trained operators, difficulties associated to prepare some samples that limit their use and motivate scientists to develop new methodologies to evaluate the presence of persistent pollutants in water samples (Feldmann et al. 2009, Bansod et al. 2017, Tian et al. 2019).

In order to facilitate the detection of persistent pollutants in aqueous samples, new methodologies have been continuously developed. They generally aim to perform analyses rapidly, facilitate sample preparation and detection procedure, save financial investments, and provide results in a way that is more accurate and trustworthy. For example, to rapidly detect and evaluate Cd presence in drinking water it is possible to use laser-induced breakdown spectroscopy (LIBS) with chelating resin (to enhance signals) (Tian et al. 2019). LIBS also made possible to detect in industrial wastewater Cd, Cr, Cu, Ni, Pb, and Zn and the capacity to analyze Cd, Pb, and Ni can be improved by graphite enrichment combined with plasma spatial confinement (Zhao et al. 2019); the sensitivity can be increased to detect ppb by electrode's aluminum enrichment (Meng et al. 2017).

However, most of the recent strategies use some kind of sensor or biosensor to detect pollutants (Asal et al. 2018; Muenchen et al. 2016; Mishra et al. 2018) (Table 3.1). They can convert the information related to the presence of the pollutant into a signal possible to be measured (Hulanicki et al. 1991); when it is a biosensor, the analyte recognition element is biological.

A quartz crystal microbalance sensor on a gold chip surface could efficiently detect the pesticide Atrazine in wastewater (Gupta et al. 2015). Glassy carbon electrode could be modified with electrochemically reduced graphene oxide to allow detection, with a detection limit of 5 ng/mL, of Zn ions (Kudr et al. 2016). Using a three electrode electrochemical sensor screen printed on a polyethylene terephthalate film it was possible to detect at nanomolar concentration the presence of ions from Pb and Cd (Avuthu et al. 2014).

There are biosensors, for example, that use nanomaterials to improve some characteristics like signal transduction due to their large surface-to-volume ratio being suitable for electrochemical devices constitution (Kurbanoglu et al. 2017; Priyadarshini and Pradhan 2017; Wu et al. 2019). The nano-stabilizer carbon dots functionalized with *N*-carbamoylmaleimide offered ultrahigh sensitivity to detect the organophosphorus pesticides parathion-methyl, paraoxon, dimethoate, and *O*,*O*-dimethyl-*O*-2,2-dichlorovinyl-phosphate; the nanomaterials could avoid thiocholine signal loss (Xu et al. 2018). Iron oxide nanoparticles can be used to modify a glassy carbon electrode and poly(indole-5-carboxylic acid) to attach the acetylcholinesterase enzyme capable to detect with accuracy pesticides able to inhibit that enzyme and present in water samples (Chauhan et al. 2016). In a electrochemical biosensor also developed containing this enzyme and multiwalled carbon nanotubes, the oxidation of acetylthiocholine was impaired in samples containing the pesticides paraoxon, parathion, and chlorfenvinphos, making it possible to detect these pollutants in fortified tap water samples and in a way comparable to the use of the classic chromatographic methods (Kesik et al. 2014). Polymeric electrospun nanofibers of polyamide 6 (PA6)/polypyrrole (PPy) were modified on the surface with two forms of graphene to generate an electrochemical sensor to detect the organophosphorus pesticide malathion in tap and river water (Migliorini et al. 2019).

An amperometric biosensor can be built using the enzyme tyrosinase from mushrooms; in the presence of the pesticide Atrazine the catalysis of the enzyme's substrate catechol oxidation to o-quinone is inhibited allowing the detection of the pollutant in water samples (Tortolini et al. 2016). Screen-printed carbon electrodes modified with ZnO nanoparticles and the same enzyme could detect the herbicide chlortoluron (that inhibited the enzymatic catalysis) in samples of river water, well water, and tap water (Haddaoui and Raouafi 2015). The pesticide sulcotrione could also be detected by an amperometric biosensor containing the enzyme hydroxyphenylpyruvate dioxygenase attached to a screen-printed electrode modified with

Table 3.1 Examples of sensors and biosensors developed from 2014 to 2019 with the intention to detect the persistent pollutants pesticides and heavy metals in aqueous samples

Sensor or biosensor	Pesticide(s) or heavy metal(s)	References
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 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)
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)
Electrochemical sensor based on polymeric electrospun nanofibers of polyamide 6 (PA6)/polypyrrole (PPy) surface-modified with two forms of graphene	Malathion	Migliorini et al. (2019)
Biosensor with double encapsulated algae strains *Chlorella vulgaris* and *Pseudokirchneriella subcapitata* in alginate beads/silica gel	Diuron, atrazine, and isoproturon	Gosset et al. (2019)
Biosensor containing alkaline phosphatase from lactic acid bacteria	Organophosphorus pesticides	Chu et al. (2019)
Biosensor containing bioluminescent bacteria	Cr	Thacharodi et al. (2019)
Biosensor containing recombinant *Escherichia coli*	Cd	Lee et al. (2019)

poly(3,4-ethylenedioxythiophene) polystyrene sulfonate; the presence of the pollutant impaires the oxidation of hydroxyphenylpyruvate in homogentisic acid, resulting in less homogentisic acid being oxidized to generate a detectable current (Rocaboy-Faquet et al. 2016). Carbon paper electrodes containing immobilized thylakoids from spinach could be used to detect the pesticide Diuron (Rasmussen et al. 2014).

Lactic acid bacteria are an interesting source of enzymes to build biosensors to detect pesticides. Biosensors developed to contain alkaline phosphatase, for example, can allow the detection of organophosphorus pesticides in aqueous samples (Chu et al. 2019).

Nanoscale biosensors like AFM tip nanobiosensors could also be developed to detect persistent pollutants; the pesticide Diclofop could be detected by a tip functionalized to contain the enzyme acetyl-CoA carboxylase in experiments in a way similar to the one predicted by theoretical measurements acquired by computer simulation (Amarante et al. 2014).

Immunosensors can be built using antibodies that can attach to pollutants in order to detect them. A highly sensitive detection of atrazine in aqueous samples from 0.01 to 50 ng/mL could be performed using anti-atrazine antibodies attached to a carbon screen-printed electrode (Deep et al. 2014).

Algae strains *Chlorella vulgaris* and *Pseudokirchneriella subcapitata* were double encapsulated in alginate beads/silica gel to generate a biosensor (based on fluorescence of the chlorophyll) that could detect in situ and in an optimized way the pesticides diuron, atrazine, and isoproturon in aqueous samples (Gosset et al. 2019). Seawater samples were analyzed to detect through fluorescence measurement the pesticides simazine and irgarol by a biosensor based on the algae species *Chlorella mirabilis* (Moro et al. 2018). The bacterium species *Escherichia coli* was genetically engineered to produce the enhanced green fluorescent protein in a version modified to contain metal-binding loops; by doing that it was possible to use this transgenic as biosensor that emits fluoresce in the presence of Cd in samples (Lee et al. 2019). Hexavalent Cr present in water samples was detected through an impairment in the luminescence process of the bioluminescent bacteria VIT-JAAK. This species, naturally present in the gut of Sea urchin, could be used in a sensor based on nutrient agar cubesand (Thacharodi et al. 2019).

It is also important to highlight that there are some researches who want to evaluate the effect of the water pollution on aquatic life. In order to do so, not only detection and quantification of the contaminant should be accessed. It is necessary to perform experiments to determine the toxicity of the pollutant and also to detect possible emerging pollutants and complex chemical mixtures. Bioassay panels are an interesting approach for a more holistic scenario comprehension (Altenburger et al. 2019). And in order to surpass obstacles related to some biological material availability throughout the year, cryopreservation arises as an elegant solution (Paredes and Bellas 2019).

3.3 Conclusions

Persistent pollutants, especially heavy metals and pesticides, represent a serious risk to the various forms of life on the planet. So, it is necessary that the human beings have ways to identify their presence in different environs and quantify their presence in order to conclude what kind of danger/threat the concentration present in the sample analyzed represents. When it comes to aquatic environs and aqueous samples, the pollution can spread easily and not only superficial water but also groundwater can be contaminated. Thus, it is a worldwide concern to treat water in order to offer a good quality one to the population that depends upon that to survive.

Nonetheless it is necessary that not only manners of efficiently remediating the environs continue to be developed, but also approaches and methods to evaluate persistent pollution presence in different samples. However, some techniques that can perform these analyses are adequate only to some kind of samples or possess some characteristics that limit their use. That is why it is crucial that new ways to assess and monitor these pollutants are continuously proposed by different research groups, especially to analyze aqueous samples.

3.4 Future Perspectives

Water, present in the Earth surface or groundwater, can be polluted directly or indirectly by contaminants that are difficult to eliminate and can easily persist in environs, threatening different forms of life survival. Therefore, considering how risk this kind of pollution is, it is expected that, first of all, human being develops environmental awareness. Models of sustainable development urge to be adopted once natural resources are being seriously and irreversibly compromised. It is crucial that anthropogenic interference in the environment becomes more harmonic and less aggressive to the environment and to living beings. However, it is also necessary to remediate areas highly polluted, especially the ones containing persistent pollutants like heavy metals and pesticides, starting from the ones in which the pollution load represents more risk. In order to determine if some area is polluted and to evaluate the threaten, it is necessary to detect and quantify the presence of environmental contaminants there. So, it is also expected that new approaches and methods to evaluate this contamination in different kinds of samples, especially aqueous ones, continue to emerge. These approaches and methods should be inexpensive, allow, preferentially, direct and continuous monitoring and should also be more versatile, reliable, practical, reproducible, offering results in a rapid way.

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Chapter 4
Chemical Pesticides: Integrated Methods in Assessment and Monitoring

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4.1 Introduction

Human population escalated with the progress and development of human culture, which led to an increase in the needs and demands of human beings for the available resources. As a result of this change, the organisms with whom human beings shared these resources, became their competitors. These competitors are now treated as threat to human beings or to their interests and labelled as pests (Encyclopedia Britannica, Pest Vermin 2019). A pest may be defined as "any species, strain or biotype of an organism which interferes with human activities or desires" (Norris et al. 2003; Sponsler et al. 2019). Any organism may acquire a pest status when it competes with human beings for food, transmits a disease to human and/or his crops or domesticated animals, damages buildings, deteriorates natural ecosystems, spoils aesthetic value of environment or causes direct displeasure or harm to humans.

Pest population after reaching to economic threshold manifests a large amount of loss to human beings. Therefore, it is necessary to take some measures to keep them checked from reaching to the Economic Threshold Level (ETL). Generally, pest control measures are either preventive or protective. The preventive measures are actions which are taken prior to the attack of pests, while protective measures are taken after the attack of a pest. The method of killing, attracting, repelling or

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regulation of growth of a pest with the help of chemicals is called chemical control method which comes under protective methods. These chemicals are termed as pesticides. This method is widely used as is more effective than other employed methods. However, many of these chemicals might contaminate our environment, foods, water bodies, soil, etc. after application. These may enter the food chain and affect organisms of different tropic levels. Due to the ill effects of many of these chemicals on the environment, this method has become menacing to environment (Metcalf and Luckmann 1994).

Therefore, it became necessary to monitor the effects of these pesticides on environment and ecology of the locality of their use along with the detection of these chemicals as contaminants in different constituents of the environment. This chapter is assembled and discussed for the monitoring and assessment of pesticide contaminants.

4.2 Pesticides

Any material or combination of substances utilized to repel, destroy or control a "pest" is known as "pesticide". The rapid growth in world population demands the growth in production of food with same or higher rate. Pests of agricultural crops are major constraints in enhancing the rate of production. Controlling these pests by chemical means which is easy in its application and cost effective encourages a rapid growth in the application of pesticides.

Wide range of pesticide products exist in market and is difficult to classify all pesticides on the basis of single characteristic. Generally, pesticides are classified on the basis of:

4.2.1 Toxicity Based Classification

Pesticides are used worldwide to augment, enrich and improve lifestyle of mankind. In conjunction with the advantages of use of pesticides, there is also possibility for undesirable and harmful effects to people or the environment. There are mainly two factors which decide the toxicity of a pesticide: dose and time. Dose in toxicity refers to the amount of active ingredient of pesticides exposed to concerned subjects, while the time factor is related to frequency, interval or regularity of exposure of the pesticide. This classification is based on the lethal dose (amount of active ingredient of substance which on administration to an organism causes death of the organism) which is generally expressed as median lethal dose (LD_{50}). The LD_{50} value is the amount of active ingredient of pesticide which on administration through either dermal or oral route kills 50% of the test animals (Rang and Hill 2013). To determine the LD_{50} value of pesticides, these are administered in milligrams per kilogram body weight (mg/kg body weight) to the test animals. A lower LD_{50} value indicates that it is more toxic than the pesticide with a higher LD_{50} value because it takes less of the pesticide to kill half of the test animals (Table 4.1).

Under "The Globally Harmonized System of the Classification and Labelling of Chemicals (GHS)", The United Nations came up with a way to bring different countries under one agreement for chemical regulations and standards. The GHS system classifies hazardousness into five categories from number I to V which are shown in Table 4.2.

4.2.2 *Type of Target Pest Based Classification*

This is the simplest method of grouping pesticide. Under this classification, pesticides were generally but not necessarily named by using Latin word -*cide* as suffix to the group name of the target pest. Latin word -*cide* means killer. Few pesticides are also categorized on the basis of their action. For instance, growth regulators

Table 4.1 Pesticide Classification Scheme recommended by WHO

Class	Intensity of Hazardousness	LD_{50} in rats (mg/kg body weight)		Examples
		Dermal	Oral	
Ia	Extreme	Less than 50	Less than 5	Aldicarb, brodifacoum, chlorethoxyfos, dieldrin, parathion, phorate, etc.
Ib	High	50–200	5–50	Aldrin, cadusafos, dichlorvos, dicrotophos, etc.
II	Moderate	200–2000	50–2000	2,4-D, DDT, chlordane, chlorpyrifos, etc.
III	Slight	More than 2000	More than 2000	Acetochlor, ethephon, malathion, metolachlor, etc.
IV	Unlike	≥5000		Carbetamide, cycloprothrin, etc.

Table 4.2 Hazardousness classification on the basis of GHS

Category	Classification criteria			
	Dermal		Oral	
	LD_{50} (mg/kg body weight)	Hazard status	LD_{50} (mg/kg body weight)	Hazard status
I	Less than 50	Fatal if contacted	Less than 5	Fatal when swallowed
II	50–200	Fatal if contacted	5–50	Fatal when swallowed
III	200–1000	Toxic if contacted	50–300	Toxic when swallowed
IV	1000–2000	Harmful if contacted	300–2000	Harmful when swallowed
V	2000–5000	Might be harmful	2000–5000	Might be harmful

(regulate growth of the target pest), defoliants (make plants to shed leaves), desiccants (help in drying target organisms), repellents (repel target pests), attractants (attract target pests) and sterilants (sterilization of target pests). Table 4.3 represents different types of pesticides based on their target pests.

Table 4.3 Pesticides classification on the basis of pest organism they kill (Akashe et al. 2019)

Type of pesticide	Target organism	Modus operandi	Example
Acaricides	Ticks and mites	Killing or disruption of growth or development	Bifenazate, DDT, dicofol, chlorpyrifos, permethrin
Algaecides	Algae	Killing or inhibition	Copper sulphate, diuron, oxyfluorfen
Antifeedants chemicals	Prevents feeding of an insect or other pest	Prevent from feeding	Chlordimeform, azadirachtin, etc.
Avicides	Birds	Killing	Avitrol, strychnine, fenthion
Bactericides	Bacteria	Killing or inhibition	Copper complexes, Streptomycin, tetracycline
Bird repellents	Birds	Repulsion by odour	Methiocarb
Chemosterillant	Insect	Incorporation of infertility and thus preventing them from reproducing	Diflubenzuron
Desiccants	Dry the tissues of plants	Dry the tissues of plants	Boric acid
Fungicides	Fungi	By preventing, curing or eradicating the fungi	Azoxystrobin, cymoxanil, thiabendazole, Bordeaux mixture
Herbicides or weedicides	Unwanted growth of weeds and plants	Killing or by growth or development inhibition	Atrazine, alachlor, paraquat, 2,4-D
Insect attractant	Insects	Trap is used to attract the pests and hence they are removed from crops, stored products and animals	Gossyplure, gyplure
Insect repellents	Insects	Repel insect from landing on humans, animals or crops	Citronella oil, Permethrin
Insect growth regulators	Insects	Disruption of the growth or development	Diflubenzuron
Insecticides	Insects	Killing or disruption of growth or development	Aldicarb, azadirachtin, DDT, chlorpyrifos, malathion

(continued)

Table 4.3 (continued)

Type of pesticide	Target organism	Modus operandi	Example
Lampricides	Larvae of lampreys (jawless fish like vertebrates)	Killing	Trifluoromethyl nitrophenol, Nitrophenol
Larvicides	Larvae	Inhibition of the growth or by killing	Methoprene
Mammal repellent	Mammals	Mammals are deterred from crops or stored products by using chemicals	Copper naphthenate, trimethacarb
Mating disrupters	Insects	Interfere with the reproduction of male and female insects by preventing them to locate each other	Disparlure, gossyplure
Microbicide	Micro-organisms		
Miticide	Mites		
Molluscicides	Molluscs	Killing	Metaldehyde, thiodicarb
Moth balls	Moth larvae or moulds that damage cloths		Dichlorobenzene
Nematicides	Nematodes	Killing	Aldicarb, Carbofuran, chlorpyrifos, methyl bromide
Ovicides	Eggs of insects and mites	Inhibition of the growth of eggs	Benzoxazin
Parasiticide	Parasites		
Pediculicide	Head lice		
Piscicides	Fishes	Killing	Rotenone
Plant growth regulators	Plants	Alteration of expected growth, flowering or reproduction rate	2,4-D, gibberellic acid
Repellents	Repel target pests by its taste or smell		Methiocarb
Rodenticides	Mice and other rodents	Killing	Strychnine, warfarin, zinc phosphide
Scabicide	Scabies		
Silvicides	Woody vegetation	Killing	Tebuthiuron
Teniacide	Tape worms		
Termiticides	Termites	Kill termites	Fipronil
Vermicide	Parasitic intestinal worms		
Virucides/ viricide	Viruses	Destruction or inactivation	Scytovirin, ribavirin

4.2.3 Chemical Nature Based Classification

Pesticides are classified on the basis of chemical nature, chemical composition and presence of active ingredients in the pesticide. This classification also helps in knowing the efficacy, chemical and physical properties of the pesticide and also helpful in determining the mode of application and precautions for application.

Insecticides, fungicides, herbicides and rodenticides are pesticides which can further be classified in different categories based on their chemical compositions. Based on chemical nature, insecticides are classified as carbamates, organochlorine, organophosphorus, pyrethroids, neonicotinoids, miscellaneous pesticides such as spinosyns, benzoylureas , antibiotics, etc. Aliphatic nitrogen, amide group, aromatic, dicarboximide and dinitrophenols are different classes of fungicides divided on the basis of their chemical nature. Herbicides may also be classified as anilide, phenoxyacetic, quaternary ammonium, chlorotriazine, sulfonylurea herbicides, etc. Rodenticides are classified as inorganic compounds (like zinc phosphide, aluminium phosphide) and organic rodenticides (bromadiolone, coumatetralyl) (Akashe et al. 2019).

On the basis of chemical composition, pesticides are mainly divided into four broad classes, namely: carbamates, organochlorines, organophosphorus and pyrethroids and pyrethrin (Buchel 1983). The classification of pesticides based on chemical nature is much complex. Generally, modern pesticides include chemicals of both natural and synthetic origin. Whereas, few inorganic compounds have also been used as pesticides. Classification of insecticides on the basis of chemical nature is provided in Table 4.4.

Table 4.4 Chemical nature based classification of pesticides (Jayaraj et al. 2016)

No	Chemical group	Chemical names
1	Organochlorines	Dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD), aldrin, eldrin, dicofol, dieldrin, lindane, chlorobenzilate, methoxychlor, chlordane, BHC, endosulfan, heptachlor, isodrin
2	Carbamates	Carbofuran, methyl: carbaryl, propoxur, aminocarb, dimethan, isolan, dimetilan, carbanolate, pyrolan, aldicarb thio: vernolate, diallate, pebulate
3	Organophosphates	Fenthion, bidrin, dimefox, methyl parathion, mipafox, ronnel, phorate, coumaphos, abate, phosphomidon, dichlorvos, diptrex, fenitrothion, demetox
4	Phenyl amides	Carbetamide, carbanilates: barban, prophan, chlororprofan, monuron, fenuron, bromuron, diuron, chloroxuron, flumeturon, neburon, phenyl urea
5	Pyrethroids	Dimethrin, allethrin, tetramethrin, bonthrin, cyclethrin, pyrethrin, fenvalerate, furethrin, decamethrin, alphamethrin, cypermethrin

Organochlorine Organochlorines pesticides are chlorinated hydrocarbons having five or more chlorine atoms. Organochlorines have prolonged residual effect on the environment.

Organophosphates Organophosphates (OP) class of pesticides are synthesized by the esterification of phosphoric acid and alcohol (Adeyinka and Pierre 2018).

Carbamates Carbamates are chemically similar to organophosphates. These are derived from esterification of alcohols and carbamic acid (Adeyinka and Pierre 2018).

Synthetic Pyrethroids The structure of natural pyrethrins is duplicated to prepare synthetic pyrethroid which makes them much stable and have longer residual effects as compared to their natural parent molecules.

4.2.4 Mode of Entry Based Classification

Pesticides act on target organisms by coming in contact with them or by entering into the target pests. On the basis of mode of entry, pesticides may be classified as systemic pesticides, contact pesticides, stomach poisons, repellents and fumigants.

4.2.4.1 Systemic Pesticides

Pesticides which on application (to soil, seeds, plants or animals) are absorbed by the organisms and translocated despite of the manner of application and entry route to those parts of the organisms which are untreated, i.e., these pesticides act on even those parts which are untreated (Table 4.5). These chemicals are capable of penetrating tissues and able to move through vascular or circulatory system of the treated organisms. Systemic pesticides control pests of the organisms on which it is applied.

Table 4.5 Classification of pesticides on the basis of mode of entry (Akashe et al. 2019)

S. No.	Category	Description	Example
1.	Systemic pesticides	Translocated to untreated parts via absorption by animals or plants	2,4-D, glyphosate
2.	Contact pesticides	Act on target pests by coming in physical contact	Paraquat, diquat
3.	Stomach poisons	Enter the body of target pests during feeding	Malathion
4.	Fumigants	Can produce toxic vapour and enter body of target pest through respiratory system	Phosphine
5.	Repellents	They are distasteful to the pests and keep them away from treated parts. They do not kill the pests, but interfere with the ability of pests to locate the crop	Methiocarb

4.2.4.2 Contact Pesticides

These pesticides are also known as non-systemic pesticides. These pesticides act on target pests by coming in physical contact, which is a must for the pest to be effective. These pesticides can enter the body of target pests through epidermis but it is not necessary for their function. Examples of these pesticides are diquat dibromide and paraquat.

4.2.4.3 Stomach Poisons

These pesticides enter the target pests during feeding through mouth in the digestive system. They get absorbed inside body of the target pest and cause death by empoisoning. Example: Malathion.

4.2.4.4 Fumigants

Fumigants are the chemical pesticides producing toxic vapours, which enter the body of target pest through their respiratory tracts and kill the target pests by poisoning. Fumigants are useful in sterilization of stored agricultural produces, soils, rooms, etc.

4.2.4.5 Repellents

Repellents do not kill but produce unpleasant and repulsive environment for target pests.

4.2.5 Mechanism of Action Based Classification

Herbicides, fungicides and insecticides can also be classified on the basis of their mechanism of action. According to the Fungicide Resistance Action Committee (Fishel and Dewdney 2012), fungicides may be divided on the basis of mechanism of action as shown in Table 4.6.

4.2.6 Sources of Origin Based Classification

Pesticides may be produced from chemical reactions or obtained from biological or other natural resources. On the basis of their source of origin, pesticides are classified as:

Table 4.6 FRAC code list: fungicides classification on the basis of mechanism of action

Code	Target site of action	Group name	Common name	Chemical group
1	β-tubulin: mitosis	Methyl benzimidazole carbamates (MBC)-fungicides	Carbendazim, benomyl, thiabendazole, fuberidazole	Benzimidazoles
			Thiophanate-methyl thiophanate	Thiophanates
2	NADH cytochrome C reductase	Dicarboximides	Vinclozolin, procymidone, chlozolinate, iprodione	
3	Sterol biosynthesis: C14-demethylation	Demethylation inhibitors (DMI)-fungicides	Triflumizole, imazalil, prochloraz, oxpoconazole, pefurazoate	Imidazoles
			Pyrifenox	Pyridines
			Triforine	Piperazines
			Diniconazole, azaconazole, bromuconazole, tetraconazole, bitertanol, difenoconazole, triadimefon, cyproconazole, triadimenol, epoxiconazole, triticonazole	Triazoles
			Nuarimol, fenarimol	Pyrimidines
4	RNA polymerase I	Phenyl amides (PA)-fungicides	Metalaxyl, benalaxyl, metalaxyl-M (mefenoxam), furalaxyl	Acylalanines
			Ofurace	Butyrolactones
			Oxadixyl	Oxazolidinones
5	Sterol biosynthesis: Δ^{14}-reductase and $\Delta^8 \rightarrow \Delta^7$ isomerase	Amines (morpholines)	Tridemorph, aldimorph, fenpropimorph, dodemorph	Morpholines
			Spiroxamine	Spiroketalamines
			Piperalin, fenpropidin	Piperidines
6	Phospholipid biosynthesis: methyltransferase	Dithiolanes	Isoprothiolane	
		Phosphorothiolates	Edifenphos, pyrazophos, iprobenfos (IBP)	

(continued)

Table 4.6 (continued)

Code	Target site of action	Group name	Common name	Chemical group
7	Fungal respiration: formation of complex II: succinate dehydrogenase	Carboxamides	Oxycarboxin benodanil, carboxin, boscalid, flutolanil, fenfuram, mepronil, furametpyr	
8	Adenosine deaminase	Hydroxy-(2-amino) pyrimidines	Ethirimol, dimethirimol, bupirimate	
9	Methionine biosynthesis	Anilinopyrimidines (AP)–fungicides	Mepanipyrim, cyprodinil, pyrimethanil	
10	β-tubulin: mitosis	N-phenyl carbamates	Diethofencarb	
11	Fungal respiration (formation of complex III): ubiquinol oxidase, Qo site	QoI-fungicides (quinone outside inhibitors)	Azoxystrobin, picoxystrobin, enestrobin	Methoxyacrylates
			Pyraclostrobin	Methoxy-carbamates
			Kresoxim-methyl, trifloxystrobin	Oximino acetates
			Famoxadone	Oxazolidinediones
			Dimoxystrobin, orysastrobin, metominostrobin	Oximino-acetamides
			Fenamidone	Imidazolinones
			Fluoxastrobin	Dihydro-dioxazines
12	MAP protein kinase in osmotic signal transduction	PP-fungicides (phenylpyrroles)	Fludioxonil, fenpiclonil	
13	G-proteins in early cell signaling	Quinolones	Quinoxyfen	
14	Lipid peroxidation	Aromatic hydrocarbons (AH)-fungicides (nitroanilines, chlorophenyls)	Biphenyl, dicloran, chloroneb, tecnazene (TCNB), tolclofos-methyl, quintozene (PCNB)	
		Heteroaromatics	Etridiazole	1,2,4-thiadiazoles
15.1	Melanin biosynthesis: reductase	Melanin biosynthesis inhibitors (MBI)-R (reductase)	Fthalide	Isobenzofuranone
			Tricyclazole	Triazolobenzothiazole
			Pyroquilon	Pyrroloquinoline
15.2	Melanin biosynthesis: dehydratase	Melanin biosynthesis inhibitors (MBI)-D (dehydratase)	Carpropamid	Cyclopropane carboxamide
			Fenoxanil	Propionamide
			Diclocymet	Carboxamide

(continued)

Table 4.6 (continued)

Code	Target site of action	Group name	Common name	Chemical group
16	Sterol biosynthesis: 3-ketoreductase during C4 demethylation	Hydroxyanilides (SBI: class III)	Fenhexamide	
17	Sterol biosynthesis: squalene epoxidase	(SBI: class IV)	Terbinafine, naftifine	Allylamines
			Pyributicarb	Thiocarbamates
18	Chitin synthase	Polyoxins	Polyoxin	Peptidyl pyrimidine nucleoside
19	Cell division	Phenylureas	Pencycuron	
20	Fungal respiration: (formation of complex III): ubiquinone reductase, Qi site	QiI-fungicides (quinone inside inhibitors)	Cyazofamid	Cyanoimidazole
21	β-tubulin: mitosis	Benzamides	Zoxamide	
22	Protein synthesis	Hexopyranosyl antibiotic	Kasugamycin	
23	Protein synthesis	Enopyranuronic acid antibiotic	Blasticidin-S	
24	Protein synthesis	Glucopyranosyl antibiotic	Streptomycin	
25	Unknown	Cyanoacetamide-oximes	Cymoxanil	
26	Trehalase and/or inositol-biosynthesis	Glucopyranosyl antibiotic	Validamycin	
27	Cell membrane permeability: fatty acids	Carbamates	Iodocarb, prothiocarb, propamocarb	
28	Oxidative phosphorylation: uncoupler		Dinocap, binapacryl	Dinitrophenyl crotonates
			Fluazinam	2,6-dinitro-anilines
			Ferimzone	Pyrimidinone hydrazones
29	Oxidative phosphorylation inhibitors: ATP synthases	Organotin compounds	Fentin acetate, fentin hydroxide, fentin chloride	Triphenyltin compounds
30	DNA topoisomerase type II (gyrase)	Carboxylic acids	Oxolinic acid	
31	DNA/RNA synthesis	Heteroaromatics	Octhilinone	Isothiazolones
			Hymexazole	Isoxazoles

(continued)

Table 4.6 (continued)

Code	Target site of action	Group name	Common name	Chemical group
32	Unknown	Phosphonates	Phosphorous acid and salts	Ethyl phosphonates
			Fosetyl-Al	
33	Unknown	Benzotriazines	Triazoxide	
34	Unknown	Phthalamic acids	Tecloftalam (bactericide)	
35	Unknown	Pyridazinones	Diclomezine	
36	Unknown	Benzene sulfonamides	Flusulfamide	
37	ATP production		Silthiofam	Thiophene carboxamides
38	Complex I of respiration		Diflumetorim	Pyrimidinamines
39	Phospholipid biosynthesis and cell wall deposition	CAA-fungicides (carboxylic acid amides)	Flumorph, dimethomorph	Cinnamic acid amides
			Mandipropamid	Mandelic acid amides
			Iprovalicarb, benthiavalicarb	Valinamide carbamates
40	Protein synthesis attachment of aminoacyl-tRNA to ribosomal acceptor (A) site	Tetracycline antibiotic	Oxytetracycline	
41	Unknown	Thiocarbamate	Methasulfocarb	
P	Host plant defence induction	P1 salicylic acid pathway	Acibenzolar-S-methyl	Benzothiadiazole BTH
		P2	Probenazole (antibacterial and antifungal activity also)	Benzisothiazole
		P3	Tiadinil	Thiadiazole-carboxamide
U	Unknown	U5	Ethaboxam	thiazole-carboxamides
	Unknown	U6	Cyflufenamid	Benzamidoxime
	Unknown	U7	Proquinazid	Quinazolinone
	Unknown	U8	Metrafenone	Benzophenone
	Unknown	U9	Fluopicolide	Acylpicolide

(continued)

Table 4.6 (continued)

Code	Target site of action	Group name	Common name	Chemical group
M	Multi-site contact activity	M1	Copper (different salts)	Inorganic
		M2	Sulphur	Inorganic
		M3	Ferbam, maneb, mancozeb, thiram, metiram, ziram propineb, zineb	dithiocarbamates and relatives
		M4	Captan, folpet captafol	Phthalimides
		M5	Chlorothalonil	Chloronitriles (phthalonitriles)
		M6	Tolylfluanid, dichlofluanid	Sulphamides
		M7	Dodine, iminoctadine, guazatine	Guanidines
		M8	Anilazine	Triazines
		M9	Dithianon	Quinones (anthraquinones)
NC	Not classified	NC	Mineral oils, potassium bicarbonate, organic oils	Diverse

4.2.6.1 Biopesticides

Biopesticides contain live micro-organisms or compounds isolated from living organism. These pesticides may be of three types:

Microbial Pesticides

When micro-organisms or their products are utilized for pest control, they are called as microbial pesticides. For example, toxins obtained from *Bacillus thuringiensis* and *Bacillus sphaericus*.

Pesticides Originated from Plant Incorporated Protectants

Pesticides produced naturally by plant which is modified by introducing a suitable genetic material, is termed as plant incorporated protectants.

Biochemical Pesticides

Pesticides which include natural materials that have non-toxic mechanism to controls pests. Example: Insect sex pheromones

4.2.6.2 Chemical Pesticides

These pesticides are produced or modified by different chemical reactions and are quite toxic but not always biodegradable. Different classes of chemical pesticides include: organophosphates, organochlorines, pyrethroids and carbamates.

4.3 Monitoring of Pesticides

Pesticides remain present on food items including vegetables, cereals, fruits which result from the excessive usage of pesticides. Human beings consume these pesticides directly from these sources or these pesticides residues are fed to the animals from where it can reach human body through meat, milk and eggs (Usman et al. 2017). Pesticides can also contaminate water bodies which will ultimately reach untreated areas through irrigation or by drinking by animals or humans. The leftover traces of pesticides on any substance are known as residues and the highest level of residues that may be legally permitted for consumption (if used bestowing good agricultural practices for crop production or from animals receiving veterinary medicine) is known as Maximum Residue Levels (MRL). MRLs are not safety limits, these are set far below the considered safe levels for human consumption.

Investigations revealed that large number of factors (Hoerger and Kenaga 1972; Nigg et al. 1978; Timme and Frehse 1980; Willis and McDowell 1987; Spynu 1989; Bentson 1990; Bromilow and Chamberlain 1991; Fletcher et al. 1994; Holland et al. 1996) are responsible for the presence of different levels of pesticide residues, which are as follows:

1. Application rate
2. Crop characteristics
3. Number of applications
4. Pre-harvest intervals (PHI)
5. Climate
6. Growth conditions
7. Physicochemical characteristics of active ingredients and formulations of a pesticide including:
    a. volatilization
    b. foliar-uptake
    c. rainfastness
    d. roots-uptake

e. persistence and degradation on the plant surfaces
f. photostability
g. water solubility and lipophilicity

Generally, pesticides are highly stable and mobile compounds which may take years and decades to break down and are capable of bioaccumulation which is the accumulation in living systems (Dwivedi et al. 2017).

Although, use of organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT) and their metabolites, aldrin, etc. are prohibited years agone in several countries owing to their toxicity on human beings and environment, they still are often found in environmental samples such as water sources, because of their higher persistivity. Annually, around five hundred completely distinct pesticides have been used or detected on the fields worldwide. Regardless of the propagation of various sorts of pesticides, organochlorines including HCH and dichlorodiphenyltrichloroethane still hold for a massive two-third of total utilization in India due to their cheap price and flexibility in effects against numerous pests. Earlier, it was indicated and proved that the Indian foods and feeds get contaminated by such pesticides (Battu et al. 1989).

General people get exposed to the residues of organophosphorus compounds due to intake of intoxicated food items including fruits, vegetables or cereals which get direct treatment from Organophosphorous pesticides (OPPs) and are produced in contaminated crop fields. The OPPs show comparatively lower environmental-persistence in comparison to the organochlorines; however, a greater acute toxicity is associated with them. Therefore, pesticide residues inside food have strictly been watched by government of different countries so as to work out whether or not the pesticides concentrations exceed their MRL values (European Commission Directive 1993).

Pesticides have a vital role to play in agricultural field as they display different unwanted and harmful adverse effects on non-target organisms. The in-depth or inapt use of these pesticides result in transfer of these pesticides and the metabolites produced thereafter to the surroundings, which lead to contamination of the environment. A number of toxic effects of these pesticides are observed in aquatic organisms, mammals and beneficial insects such as bees, beetles, etc. These insecticide residues are found in vegetables, agricultural merchandise, soil and waters. Hence, increased observance efforts are needed to establish the results of those compounds and to judge their effects on human health.

4.4 Assessment Methods of Pesticides

The assessment of pesticides includes procedures such as sample preparation and the identification of contaminants (Ikeda et al. 2003; Belmonte et al. 2005; Da Silva et al. 2007).

4.4.1 Sample Preparation

Before carrying out the instrumental analysis, sample preparation methods are usually mandatory. It involves compound extraction, pre-concentration and clean-up steps.

4.4.1.1 Liquid–Liquid Extraction (LLE)

This extraction involves the separation of mixture of substances from any analyte using two solvents. These solvents are chosen in such a way that they do not mix with each other, i.e., they are immiscible. LLE method is also known as solvent extraction and partitioning. For instance, chloroform and water are two immiscible solvents. These are chosen according to differential solubility of analytes as the solute should have better solubility in one solvent than the other. The distribution among these solvents for any drug is known as partitioning. It involves the distribution of drug among these solvents depending upon the chemical characteristics such as pH and polarity of these analytes (Berk 2013).

4.4.1.2 Solid-Phase Extraction (SPE)

Sometimes, due to the similarities in hydrogen ion concentration (pH) or polarity, it is not possible to differentiate the two compounds by LLE. To overcome these limitations, another technique known as SPE is used. SPE depends on the principle of adsorption, where analyte in any phase (Solid, liquid or gas) is adhered to the solid material's surface. If only one constituent of a mixture is adsorbed on the surface, it may be separated by exploiting this technique. Sometimes, two or more constituent of any analyte mixture having different affinities for the adsorbent, can be separated using this technique. Using SPE, analytes may be isolated from a range of matrices including water, animal tissue, urine, blood, soil, beverages, etc. This technique utilizes the affinity of analytes for the process of adsorption. The liquid in which solutes are dissolved or suspended is known as the mobile phase and the solid on the surface of which analytes are adsorbed is known as the stationary phase. To separate undesired components from any mixture, mobile phase is passed on to the stationary phase which results in the adsorption of either desired analytes or undesired impurities on to the stationary phase. If the desired analyte is adsorbed on to the stationary phase, it can be eluted by rinsing with an appropriate eluent (known as solid-liquid extraction, leaching or elution, Fig. 4.1). During pesticides extraction method development by SPE, several factors are needed to be considered for better recovery. SPE method of pesticides may be divided into four steps:

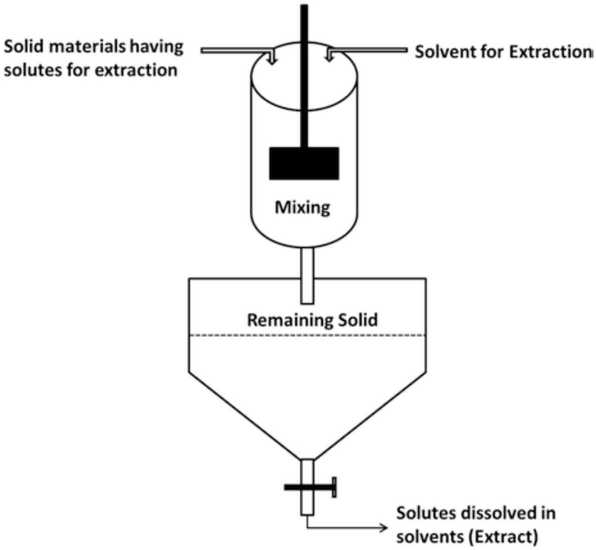

Fig. 4.1 A graphical representation of steps and procedure involved in the solid-liquid extraction process (Berk 2013)

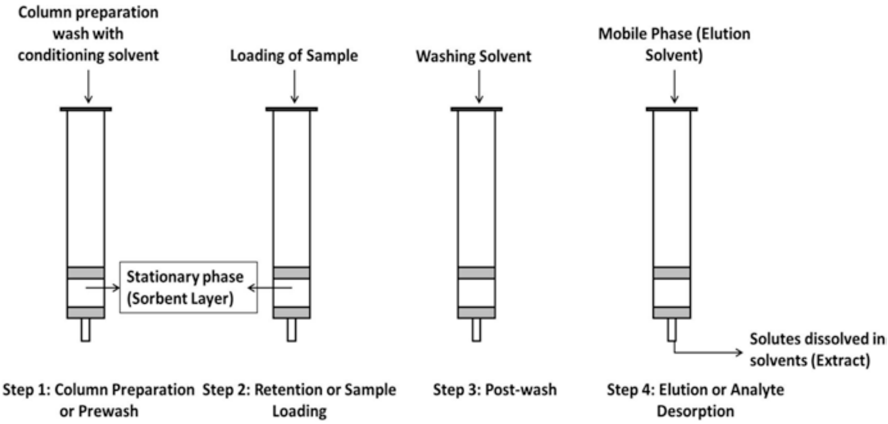

Fig. 4.2 Graphical representation of steps and procedure involved in the SPE method (Żwir-Ferenc and Biziuk 2006)

column preparation or prewash, retention or sample loading, post-wash and elution or analyte desorption (Bhuiyan and Brotherton 2002). Figure 4.2 explains the different steps of SPE (Żwir-Ferenc and Biziuk 2006)

4.4.1.3 Supercritical Fluid Extraction (SFE)

A substance beyond the critical temperature and pressure is known as supercritical fluid (SCF). Any pressure cannot convert a gas into liquid above critical temperature, but higher pressure can make SCF. SCF has nearly same density but lower viscosity than that of their liquid form (Berk 2013). These two characters provide high solubilization power with more permeability to SCFs as an extractant (King 2000). Carbon dioxide is the most commonly used supercritical fluid as extraction medium (Sahena et al. 2009).

4.4.1.4 Solid-Phase Micro-Extraction (SPME)

The conventional solid-phase extraction technique requires relatively large amount of analyte for an effective separation to be achieved. To combat the problem, modification in the technique was achieved in recent years, which is called as the solid-phase micro-extraction. The advantage of this technique is that a very small amount of sample is required. In this technique, an adsorbent (charcoal) is coated on a small wire and is attached to a holder which can withdraw or extend the wire. This wire is introduced to the liquid or vapour on which adsorption would take place and subsequently placed in a gas chromatograph instrument where the adsorbed substance can be eluted and analysed (Houck and Siegel 2015).

4.4.1.5 Liquid Phase Micro-Extraction (LPME)

LPME is a modification of LLE method in which the use of water-immiscible solvent for sample pre-treatment is minimized. There are three classes of LPME: (1) Single-Drop Micro-extraction (SDME), (2) Dispersive Liquid–Liquid Micro-extraction (DLLME) and (3) Hollow Fibre Micro-extraction (HF-LPME) (Sarafraz-Yazdi and Amiri 2010).

4.4.1.6 Single-Drop Micro-Extraction (SDME)

The SDME involves the partitioning of analytes among the extractant and sample solution and is a kind of miniaturized sample preparation method. High Elution Factors (EFs) are achieved using SDME owing to the reason that highly reduced extractant to sample ratio is needed (Sarafraz-Yazdi and Amiri 2010).

4.4.1.7 Dispersive Liquid–Liquid Micro-Extraction (DLLME)

Recently, a new method known as the DLLME method of LLE technique was reported (Rezaee et al. 2006; Birjandi et al. 2008; Rezaei et al. 2008). In this method, a definite quantity (1 L) of extraction solvent is used with a few millilitres of dispersive solvents (Sarafraz-Yazdi and Amiri 2010). It results into the formation of a cloudy mixture which is injected into the aqueous sample having analytes to be detected. The lipophilic solutes are mixed in the extraction solvent by dispersing into the bulk aqueous phase. The mixture is then centrifuged and the analytes can be determined in the bottom phase by conventional techniques. In this method, dispersive solvent has a major role to play as it helps the extraction solvent to form fine droplets in the aqueous phase, which constitute the majority of total volume of the mixture. This increase in the surface area due to fine droplets increases the mass transfer of analytes to organic phase from aqueous phase. Along with overcoming the problem of the time consumption in other methods this method also enhances extraction efficiency. Non-selectiveness is the major disadvantage associated with this method.

4.4.1.8 Hollow Fibre Micro-extraction (HF-LPME)

This technique was first discovered by Pedersen Bjergaard and Rasmussen in the year (1999).The concept of supported liquid membrane (SLM) was used for the first time to carry out LLME as a simple, disposable and cost-effective unit. The membrane used for the technique was made of polypropylene hydrofluorides (Sarafraz-Yazdi and Amiri 2010).

4.4.1.9 Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) Method

Presently, “quick, easy, cheap, effective, rugged and safe” (QuEChERS) method of preparation of samples has been identified as one of the most commonly used technique for the analysis of multiple residues of pesticides in food items in particular in fruits and vegetables (Lehotay 2006). The Association of Official Agricultural Chemists (AOAC) has widely accepted this technique and has approved it for the purpose. The major advantage of this technique is that a large number of samples can be extracted in a shorter period of time. It was first tried on extracting 10–20 samples (Lehotay and Mastovska 2004) and after successful trial, the follow-up studies were performed on larger samples (200 pesticides) which were also validated successfully (Lehotay et al. 2005).This technique gives better results for problem causing analytes and has also been tested in fatty matrices (Table 4.7).

Table 4.7 Possible pesticide analytes recovered by using the QuEChERS Method (Lehotay 2006)

Yield recoveries (%)	GC-amenable	Analysed by LC-MS/MS	Analysed by either technique
>70	Chlorothalonil	Cycloxydim, florasulam, methiocarb sulfone, pymetrozine, sethoxydim	Acephate, dichlofluanid, fenhexamid, methamidophos, tolylfluanid
>90	Acrinathrin, aldrin, bifenthrin, bromopropylate, bupirimate, cadusafos, (α-, γ-)chlordane, (α-,β-) chlorfenvinphos, chlorpropham, chlorpyrifos, chlorpyrifos-methyl, chlorthal-dimethyl, chlozolinate, coumaphos, (λ-)cyhalothrin, cypermethrin, (2,4′-, 4,4′-) Dichlorodiphenyldichloroethylene (DDE), (2,4′-, 4,4′-)dichlorodiphenyltrichloroethane (DDT), deltamethrin, diazinon, dichlorobenzophenone, dicloran, dieldrin, diflufenican, diphenyl, diphenylamine, (α-, β-)endosulfan, endosulfan sulphate, EPN, esfenvalerate, ethion, etridiazole, famoxadone, fenazaquin, fenithrothion, fenpiclonil, fenpropathrin, fenpropidine, fenvalerate, flucythrinate I and II, fludioxonil, fluconazole, flutolanil, fluvalinate, fonophos, furalaxyl, heptachlor, heptachlor epoxide, heptenophos, hexachlorobenzene, iprodione, lindane, mecarbam, mepronil, metalaxyl, methidathion, mepanipyrim, mevinphos, ofurace, parathion, parathion-methyl, (cis-, trans-)permethrin, o-phenylphenol, pyriproxyfen, quinalphos, quinoxyfen, quintozene, tetradifon, tetrahydrophthalimide, terbufos, terbufos sulfone, tolclofos-methyl, triazophos, trifluralin, vinclozolin	Acetamiprid, aldicarb, aldicarb sulfone, aldicarb sulfoxide, azaconazole, azamethiphos, bitertanol, butocarboxim, butocarboxim sulfone, butocarboxim sulfoxide, carbendazim, 3-hydroxycarbofuran, chlorbromuron, clofentezine, cymoxanil, demeton, demeton-O-sulfoxide, demeton-S-methyl, demeton-S-methyl sulfone, desmedipham, dichlorvos, diclobutrazole, dicrotophos, dimethomorph, diuron, dodemorph, epoxiconazole, etaconazole, ethiofencarb sulfone, ethiofencarb sulfoxide, ethirimol, fenbuconazole, flufenacet, fosthiazate, furathiocarb, hexythiazox, imazalil, imidacloprid, iprovalicarb, isoprothiolane, isoxathion, linuron, metconazole, methiocarb sulfoxide, methomyl, methomyl-oxime, metobromuron, metoxuron, monolinuron, nuarimol, oxamyl, oxamyl-oxime, oxydemeton-methyl, paclobutrazol, phenmedipham, phosphamidon, prochloraz, spinosad, tebufenozide, thiabendazole, thiacloprid, thiamethoxam, thiodicarb, tridemorph, vamidothion, vamidothion sulfone, vamidothion sulfoxide	Azinphos-methyl, azoxystrobin, bromuconazole, buprofezin, carbaryl, carbofuran, cyproconazole, cyprodinil, dimethoate, diniconazole, disulfoton, disulfoton sulfone, DMSA, DMST, Ethoprophos, etofenprox, fenamiphos, fenamiphos sulfone, Fenarimol, fenoxycarb, fenpropimorph, fenpyroximate, Fenthion, fenthion sulfoxide, flusilazole, furmecyclox, hexaconazole, kresoxim-methyl, malathion, malathion oxon, mephosfolan, methiocarb, monocrotophos, myclobutanil, omethoate, oxadixyl, penconazole, pencycuron, phorate, phorate sulfone, picoxystrobin, pirimicarb, pirimicarb-desmethyl, profenofos, propiconazole, propoxur, pyridaben, pyridaphenthion, pyrifenox, pyrimethanil, spiroxamine, tebuconazole, Tebufenpyrad, tetraconazole, triadimefon, trifloxystrobin, triflumizole, triphenyl phosphate

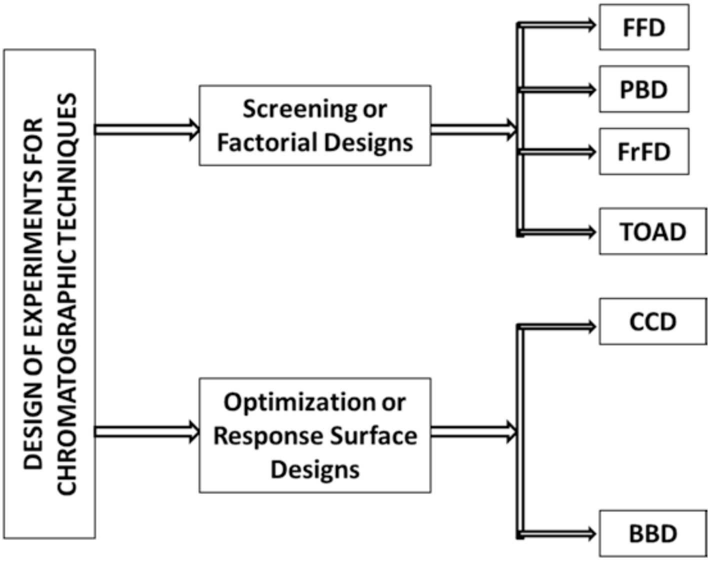

Fig. 4.3 Classes of design of experiments for chromatographic techniques. *FFD* full factorial design, *PBD* Plackett–Burman design, *FrFD* fractional factorial design, *CCD* central composite design, *BBd* Box–Behnken design (Narenderan et al. 2019)

The extraction process is a very crucial step for the analysis of pesticide residues available in trace amount. Due to complexity of matrix, extraction process becomes tedious and time consuming. Therefore, various factors such as pH, temperature, time, rainfall, soil condition, extraction solvent, amount of sorbent desorption time, ultrasound time, centrifugation time, molar ratio, ionic strength, etc. need to be optimized during the extraction of pesticides due to the complexity of the matrix which is time consuming and tedious. Optimization of the extraction process is generally done by a two-step process, screening and optimization. Narenderan et al. (2019) classified these two processes (full factorial, fractional factorial, Plackett–Burman Design for screening and central composite design, Box–Behnken design, Taguchi design, Doehlert design, D-optimal design for optimization) in detail which is summarized as chart in Fig. 4.3.

4.5 Analytical Methods for the Monitoring of Pesticide Residues

Table 4.8 summarizes pesticide residues found in various sources and their extraction and detection methods.

Table 4.8 Extraction and detection methods of pesticide residues

Sources	Extraction method	Method of detection/ analysis	Pesticides	References
Water samples	SPE (solid-phase extraction)	GC-ECD (gas chromatography-electron captured detector)	Diazinon, hexachlorocyclohexane isomers (α, β, γ, δ), aldrin, heptachlor, chlorpyrifos, malathion, endosulfan, heptachlor epoxide, dieldrin, dichlorodiphenyl trichloroethane (DDT) family (4,4′-DDT 4,4′-DDE, 4-4′-DDD), endosulfan and endocrine	Abdullah et al. (2017)
Fruit juices and vegetables paste	Easy, quick, effective, rugged, cheap, and safe extraction method and then clean up using d-SPE	GC/MS triple quad, with electron ionization detector (EID)	Organochlorine pesticides (Aldrin, 4,4-DDT, 2,4-DDT, chlordane-cis, chlordane-trans, heptachlor, endosulfan-I, endosulfan-II, iprodione, fluchloralin, epoxide B, dieldrin and dicofol), pretilachlor, organophosphorus pesticides (chlorpyrifos, dichlorvos, parathion and chlorfenvinphos cis + trans and parathion-methyl), chlorpyrifos-methyl, synthetic pyrethroids pesticides (cypermethrin, β-cyfluthrin, deltamethrin, λ-cyhalothrin fenvalerate, fenpropathrin and permethrin); others including oxyfluorfen, trifluralin, etofenprox, and pyrazosulfuron-ethyl	Dwivedi et al. (2017)
Sour cherries	Simple QuEChERS solvent-based extraction	Multi-class LC-MS/MS (liquid chromatography with tandem mass spectrometry) method, used in the positive mode with Electrospray Ionization (+ESI)	Acetamiprid-d3, acetamiprid, azoxystrobin, carbendazim, carbofuran, carbofuran-d3, clothianidin, cyproconazole-1, cyprodinil, cyromazine, dimethoate, difenconazol, dimethomorph, fenhexamide, fenoxycarb, fenpropimorph, fluroxypyr-methyl, flusilazole, flutriafol, imazalil, imidacloprid, indoxacarb, metalaxyl-M, metconazole, methomyl, ethoxyfenozide, metsulfuron-methyl, myclobutanil, oxadixyl, pencycuron, phoxim, pirimiphos -methyl, propoxur, propyconazol, pymetrozin, pyraclostrobin, pyrimethanil, pyriproxyfen, spiroxamine, tebuconazole, tebufenpyrad, thiamethoxam	
Vine leaves	Sample preparation based on ethyl acetate	GC-MS/MS	Chlorpyrifos, chlorpyrifos-methyl, diazinon, and dimethoate	Maestroni et al. (2018)

Blood samples Bottled water Soft drinks	United States Environment Protection Agency (USEPA) protocols for organochlorine pesticides	Gas chromatograph with the ^{63}Ni selective electron-capture detector (for organochlorine) and gas chromatograph with nitrogen phosphorus detector (for organophosphorus)	Organochlorines and organophosphorus pesticides	Mathur et al. (2003, 2005) Johnson et al. (2006)
Winter fruits	Soxhlet extractors	Gas chromatograph with electron-capture detector (ECD)	g and b BHC, a BHC, dimethanoate, malathion, methyl parathion, endosulfan, ethion, dieldrin, DDT, DDE	Kannaujia et al. (2012)
TEA	Extraction by using organic solvent	GC-ECD	A-Endosulfan and bifenthrin	Styarini, et al. (2014)
Leafy vegetables	Extraction by using organic solvent	Thin layer chromatography (TLC)	Acephate	Elgailani, and Alghamdi (2018)
APPLE JUICE	SPE (solid-phase extraction)	RP-HPLC (reversed phase high performance liquid chromatography) with UV diode array detection.	Atrazine, malathion, fenitrothion, parathion	Velkoska-Markovska et al. (2018)

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Chapter 5
Chemical Transformations of Synthetic Persistent Substances

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5.1 Background

Biocides, which are synthetic persistent substances (SPSs) that contain or generate active substances, are used against harmful organisms in the home environment (disinfectants, rodenticides, repellents and insecticides) or to protect natural or artificial products in industrial and agricultural applications. Given that due to their intrinsic properties, the use of biocidal products can pose health risks, the EU has established a regulatory framework aimed at significantly increasing the safety of biocidal products 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 are products used to combat harmful organisms in agricultural crops, such as weeds and insects. Their use can endanger the health and the environment. Many studies have revealed the link between pesticide exposure and effects on the human body such as cancer, fertility and reproductive problems, respiratory diseases, disruption of the hormonal (endocrine) system, impaired immune system and nervous system. EU pesticide legislation (EC Directive 128/2009) imposes prohibitions on the use of certain SPSs in these products.

Endocrine disrupting SPSs are chemicals that interfere with hormones (the endocrine system) causing adverse health effects. A wide range of SPSs, both natural and man-made, are considered to cause endocrine disruption, including pharmaceuticals, pesticides and industrial organic chemicals. Pesticides are SPSs with a particularly high biological action, used to control diseases, pests and weeds, which have a

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varied chemical structure. In addition to the undeniable benefits offered by the use of pesticides, such as high yields from intensive agriculture, increased food production, evolution of agronomic techniques, improved food quality, however, many adverse effects have been observed since 1960 on the aquatic environment, soil and human health (Handford et al. 2015).

Exactly the same properties that make them effective against pests transform them into polluting agents of the environment. The s-triazine family comprises some of the most widespread pesticides in the world. They are used as selective herbicides to control spontaneously grown plants in many types of agricultural crops. These herbicides and their metabolites are very persistent in the environment and their prolonged use involves some risks. Urea herbicides (PUHs) such as diuron, isoproturon and chlorotoluron are used in large quantities for different purposes, such as herbicides in cereal crops (isoproturon and chlorotoluron), total herbicide in agriculture (diuron) and as an algicide in paints and different coatings (diuron), renovation of sports, golf and green areas in cities. Another important group of SPSs are biocides, organic synthetic substances, commonly encountered in aquatic systems. They are used in the manufacture of anti-deposition paints for both boats and static structures, to prevent the accumulation and development of aquatic organisms, especially mollusks and algae. However, some studies have evaluated the toxicity of biocides and concluded that they are growth inhibitors for some freshwater and marine waters, influencing key species such as seagrass and corals. Linear alkylbenzene sulfonates (LAS) are one of the most common and widely used classes of anionic surfactants, in the composition of household detergents, laundry, dishes, shampoos and other personal care products, with an estimated global consumption of 2.8 million tons in 1998 (Rotter et al. 2018).

The most direct impact of pollution on human health comes directly from contaminated drinking water, but SPSs enter also indirectly into the body being absorbed through the skin or ingested through the food chain. Previous studies have shown a link between water pollution in China and the promotion of cancer in the stomach and liver, due to the consumption of water contaminated with a high concentration of SPSs. The European Union has established various directives, such as the Water Framework Directive (WFD) 2000/60/EC, on water quality. One of the objectives of this directive is to achieve good ecological status of river waters by 2020 in European countries and to develop water management according to the hydrographic districts. Several studies have shown that treated wastewater, if properly managed, can be an important water resource to meet the needs of a growing economy. The biggest challenge in implementing this strategy is the adoption of technologies that are less expensive in water purification, which maximize the efficiency of using limited water resources and ensure that all safety and health standards related to reuse of wastewater treated effluent are respected (Robin and Marchand 2019).

5.2 Management of Plant Protection Products (Pesticides/Biocides)

The management of the plant protection products is regulated by law, the norms of approval and placing on the market, storage and application, use of the technological means specific to them in the conditions of soil and water protection being presented in the Code of good practices. The document specifies the specific rules for approval and placing on the market, storage and application of plant protection products. From the point of view of toxicity, the most widespread indicator is LC50, respectively, the concentration of pollutant that is lethal to 50% of the population after a certain period of time (usually 14 days). LC50 is specific to each species (mouse, 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 different chemicals, it is necessary to test more than one species, and the tests to be repeated periodically. In general, the LC50 indicator is specified in the SPSs file. Typically, LC50 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 the gas density is known). In addition to toxicity, persistence and spread are considered, both for pure SPSs and for solutions or mixtures. This has resulted in a broad set of properties and risk indicators of SPSs, as well as multiple classification systems. MAC represents the maximum allowed concentration of an SPS that is in the form of gaseous, vapour or suspended matter, which is not harmful to the health of the personnel and does not cause any discomfort, even in the case of prolonged and repeated exposure. The uncontrolled emission of pollutant represents the release into the environment of an SPS 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 up to great distances, gradually being taken over by it. To estimate the effects of a pollutant, the following parameters must be observed: the concentration level at different source distances, respectively, the pollutant concentration at a specified relative distance to the source (concentration field), the critical area in which the concentration exceeds an allowed threshold (the action distance of the toxic). To estimate the effects of a risk agent, it is necessary to be able to quantify the exposure, in terms of intensity, duration of exposure and consequences. This is done by estimating the received dose and comparing it with the statistically processed experimental data. Vulnerability criteria can be established to determine the exposure dose levels that have specific consequences. The criteria provide the threshold beyond which protection is needed to prevent impairment of the functions required of an individual to be safe. There are two approaches to determining the effects of the dose received: using the probity functions and determining the harmful dose (applied in the case of toxic or thermal risks) (Chen 2007).

5.2.1 Determination of the Harmful Dose

The specified level of toxicity (SLOT) or significant likelihood of death (SLOD) are alternative approaches for estimating the level of fatality upon exposure to a toxic agent. The SLOT approach is described in 1993 by Turner and Fairhurst and involves the use of available toxicity data, which are then extrapolated for human use. The estimated dose is referred to as SLOT. The SLOD, used in the risk analysis, estimates the dose needed to produce a significant probability of death. The consequences of exposure 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 high and, therefore, it is necessary to include "safety factors" in the modelling. SLOT is considered to be equivalent to LC1-5 derived from animal experiments. Knowing the SLOT or SLOD values and the exponent *n*, the concentrations necessary to produce these effects can be calculated, depending on different exposure time values. The general characteristics of SPSs, in terms of their effect on the environment, can be summarized as follows: they are very widespread; present a great diversity, can migrate, sometimes over very long distances from the source, the determining factors being the nature of SPSs, the configuration of the land and the currents of air or water, can accumulate in the environment and/or in terrestrial and aquatic organisms (some SPSs accumulate in tissues and are very difficult to remove); they have a wide range of effects on health (toxicological effects) and on the environment (ecotoxicological effects); may have other undesirable effects on the environment (fire, explosions, corrosivity); it has synergistic effects, which represent the interaction and accumulation of SPSs effects in organisms. The elements used to quantify the effects of an accidentally discharged SPSs on the environment are: the effects of SPSs on the environment and the associated dangers; the intrinsic properties of SPSs (toxicity, persistence, bioaccumulation capacity, water solubility, etc.); behaviour of SPSs in the environment (evaporation, dilution, degradation, sedimentation, chemical reactivity, etc.); the physical conditions of the environment (land configuration, wind speed and direction, the flow of rivers, the size of the lakes, the physical–chemical properties of the water, the background pollution, etc.); population and sensitivity of existing habitats in the area (Kura et al. 2015). The strategy of environmental sensitivity indices in designing and modifying a process has four main goals:

- Minimization: the use of small amounts of SPSs (use of continuous reactors instead of discontinuous ones; reduction of the number of raw materials and intermediates; use of high efficiency heat exchangers);
- Substitution: replacement of a material with a less dangerous one (use of water-based coatings and paints; use of alternative chemical compounds; use of less flammable and toxic solvents);
- Moderation: use of less hazardous process operating conditions, less hazardous material or facilities that minimize the impact of hazardous emissions (use of dilution; cooling of volatile SPSs; use of granules instead of powders);

– Simplification: the design of facilities that eliminate the operation with a high degree of complexity, that minimize the errors or correct them (simple technologies, with less chemical reactions and operations; elimination of the excessive equipment, consideration of the human factor in operation, even from the phase of design) (Maggi et al. 2015).

5.2.2 SPSs Behaviour in the Environment

Following the production process, the transport and the use, for various purposes, of the SPSs, their emissions in the environment take place. These substances, once released, will be distributed among the major compartments of the environment: water, air, soil and biota.

The distribution of SPSs is governed by the transport processes within each compartment and between compartments, transport processes that take place in parallel with biotic and abiotic transformation processes of the compounds. The fraction of the chemical compound to be found in each compartment will be determined, on the one hand, by the physio-chemical properties of the compound, and on the other hand, by the characteristics of the respective compartments. Knowing the chemical structure of the compound and the intrinsic physio-chemical properties derived from it, as well as the main structural characteristics of the environmental compartments in which the compound is subjected to transport and transformation processes, will allow an assessment of its behaviour in the environment.

The assessment of the behaviour in the environment of SPSs or potentially dangerous ones is an important and absolutely necessary step in assessing the risk that these SPSs can generate on the environment, in general, and on human health in particular. The main reactions involved in SPSs entering the atmosphere are oxidation reactions.

Organic compounds from both natural and anthropic sources can be directly activated by solar radiation with their photodissociation, or they can interact with active species that have been generated in other photochemical processes, such as hydroxyl radicals (·OH), oxygen atoms (O) or ozone molecules (O_3). The mechanism by which the oxidation process proceeds will also depend on the altitude at which it takes place, because the wavelength of the radiation as well as the presence/absence of chemical species differs with the altitude. Hydrocarbons can interact in the troposphere or stratosphere with hydroxyl or ozone radicals. In their reaction with hydroxyl radicals, aliphatic hydrocarbons generate unstable radical species, alkyl radicals. These, following the reaction with an oxygen molecule, lead to the formation of other reactive radical species, alkylperoxy radicals, which can act as oxidants losing an oxygen atom (in favour of NO with NO_2 formation).

When the attack of the hydroxyl radicals takes place on the aromatic nucleus, are produced compounds from the class of phenols and a reactive radical species, the hydroperoxyl radical. If aromatic compounds have a side chain, the attack of the hydroxyl radical will first occur on the side chain. The oxidation process represents a mechanism for the removal of organic compounds from the atmosphere (Boethling et al. 2009).

Even though organic SPSs do not undergo complete oxidation in the atmosphere (oxidation to inorganic compounds-mineralization), they are converted to organic compounds in a higher oxidation state (phenols, aldehydes, carboxylic acids) which have a higher solubility in the water. They are more easily removed from the atmosphere by wet deposition and may undergo biodegradation processes in soil or water. Photo-oxidation of hydrocarbons, when they are in a high concentration in the atmosphere, together with nitrogen oxides (in urban areas), can also be a stage in the process of photochemical smog formation.

The formation of photochemical smog is a very complex process, difficult to fully explain. In an atmosphere polluted with hydrocarbons and nitrogen oxides, exposed to strong solar radiation and characterized by stagnation of air masses, there is the tendency to form oxidizing species. The main oxidant is ozone, along with other oxidants such as hydrogen peroxide (H_2O_2), organic peroxides (ROOR′), organic hydroperoxides (ROOH) and peroxiacil nitrates (such as peroxiacetyl nitrate $CH_3C(O)OONO_2$–PAN).

Behaviour in the atmosphere of halogenated organic compounds is important. Following photochemical reactions, they can release halogen atoms (X·), radical species (containing a non-coupled electron) that can participate in the decomposition of stratospheric ozone. The decomposition of organic matter can take place in a shorter or longer time depending on the conditions in the soil (Brambilla et al. 2016).

Molecular exchange capacity is based on molecular adsorption, a physical process of retaining on the surface of colloidal particles of the soil some SPSs in molecular dispersion state. The richer a soil in clays is, the higher its molecular adsorption capacity is.

Soil is retained by molecular adsorption: water molecules (soil particles are coated with a film of water = film water, hygroscopic water), ammonia molecules (NH_3) resulting from the decomposition of organic matter (its retention protects it from loss through volatilization), humic acid molecules, iron and aluminium oxides (which form films around the soil particle). It is appreciated that a chemical species does not need to be initially electrically charged in order to be retained in the soil. Although it is a very complex process, the decomposition can be framed in a general scheme (Table 5.1) in which the main stages are distinguished: hydrolysis, oxidation–reduction, total mineralization and humification.

The molecules in the soil solution can be converted to charged species and then adsorbed as anions/cations or they may remain non-ionic and be adsorbed as a consequence of polarization that produces electrical charge located inside the molecule (Kohay et al. 2015).

Molecular retention is achieved by protonation, deprotonation, hydrogen bonds, Van der Waals forces, hydrophobic sites. Protonation allows certain non-ionic chemical species to receive protons in acidic solutions, becoming cations that can be adsorbed on negative colloidal soil compounds. Thus, the herbicides S-triazine, S-triazole and NH_3 are retained. Hydrogen bonding is a special type of dipole–

Table 5.1 Transformations of the main components of organic matter through hydrolysis and oxidation–reduction processes

Components of organic matter	Transformation processes	
	Hydrolysis	Oxidation–reduction processes
Protein substances	Peptides, aliphatic and aromatic amino acids, purine and pyrimidine bases	Organic acids, fatty acids, NH_3 alcohols, CO_2, H_2O, CH_4, H_2S, etc.
Sugars	Pentoses, hexoses, amino acids, uronic acids, cellobiose	Oxyacids, organic aldehyde acids, alcohols, CO_2, H_2O, CH_4
Firewood, tannins	Phenol type hydrolytic products	Phenols, quinones, CO_2, H_2O, etc.
Lipids, resins	Glycerine, fatty acids, resin acids	Unsaturated acids, oxyacids, volatile organic acids, hydrocarbons, CO_2, H_2O

dipole bond in which the hydrogen atom serves as a bridge between two electronegative atoms. Hydrogen is retained by electrostatic bonding to one electronegative atom and strong covalent bonding by another negative atom. A more complex arrangement involving hydrogen bonding is the water bridge between hydrated, exchangeable cations and non-ionized water molecules. Bridge water is the most common type of hydrogen bond in wet soils. Strongly hydrated Me^{x+} cations increase the polarization of water and intensify the association of hydrogen in water with oxygen in R–CO–R.

Hydrogen bonding, individually, is a relatively weak bond, but the addition of several such bonds leads to strong molecular retention. Van der Waals forces, although they exert a weak and short-acting attraction in space, are additive and are considered the main adsorption forces only for nonpolar molecules, generally overshadowed by the stronger electrostatic forces involved in the retention of electrically charged species. The hydrophobic bond is specific to the organic matter of the soil, especially to the fats, resins, aliphatic chains of fulvic and humic acids which have some hydrophobic surfaces/sites.

Highly polar water molecules compete poorly with nonpolar molecules for occupying these sites. Thus, the nonpolar molecules accumulate on these sites taking place hydrophobic binding. Molecular adsorption depends on the chemical character of the molecules, their shape and configuration, the acidity or basicity of the soil solution, the solubility in water, the distribution of the charge, the polarity, the size of the molecules, their polarizability.

The soil is a complex, inhomogeneous matrix, in which very varied processes take place. The Different soil type is depending on the conditions of formation, so: relief, rock type material, climate (precipitation and temperature), vegetation type, with specific characteristics regarding the type and content of clay, humus content, salts, oxides and hydroxides, determines how SPSs behave. The soil, by its morphological, physical and chemical characteristics, controls the interaction with different SPSs, the retention of compounds at the level of colloids (adsorption), their release (ion exchange), volatilization, vertical and horizontal transport flows of the different compounds (percolation in groundwater, surface waters). Among the abiotic processes, hydrolysis and photochemical degradation have particular importance. Regarding process chemistry, it can be said that the photo-degradation of SPSs in the atmosphere, water and soil occurs through similar mechanisms. Photochemical degradation in surface waters will occur only in the first centimetres of the water column, since penetration of solar radiation is limited in depth. Photo-degradation of SPSs from the soil is possible only at the soil–atmosphere interface. However, even under these conditions, photo-degradation may be an important mechanism for removing SPSs from surface or soil waters. It is appreciated that the removal of diclofenac (2-[(2,6-dichlorophenyl) amino] benzenacetic acid) from the water of a lake occurs predominantly by the photochemical pathway. Photo-oxidation can ensure the conversion of hard biodegradable compounds into higher biodegradable compounds, facilitating the elimination of refractory compounds (Kohay et al. 2015).

5.3 Biodegradation Processes

Biodegradation plays an important role in removing SPSs from aquatic and terrestrial ecosystems. Biodegradation of organic compounds is mediated by microorganisms, especially bacteria and fungi. Fungi are aerobic microorganisms, while bacteria can be active in aerobic or anaerobic environments. In the process of microbial degradation, a number of intermediate compounds can result, but successive transformations, in toxic environment, tend to convert SPSs into carbon dioxide, water and salts (nitrates, sulphates), a process called mineralization. In anoxic (anaerobic) environment, along with carbon dioxide and water, methane and compounds of the organogenic elements are reduced (ammonia and hydrogen sulphide).

Microbial degradation of SPSs often occurs through the same processes that are involved in the degradation of naturally occurring organic substances in the ecosystem.

Microorganisms mediate transformations that are energetically favourable.

The distribution of SPSs in the environment can be appreciated based on the general tendency of physical systems to evolve towards a more stable state that is to lower energy states (the third principle of thermodynamics). SPSs tend to migrate from higher chemical potential states to lower chemical potential states. In a homogeneous environment, the chemical potential is directly proportional to the concentration of the compound. The concept of fugacity is a criterion of the thermodynamic equilibrium suitable to be used for complex systems, heterogeneous than that of the chemical potential. The fugacity model, in order to describe the distribution of SPSs in the environment, allows obtaining sets of equations that can account for the complex behaviour of the SPSs in a multi-phase environment that is not in equilibrium. The fugacity can be defined as the "escape tendency" of the SPS from a certain phase/environment compartment. High leakage of one SPS in one phase expresses the marked tendency of the substance to migrate from the phase/compartment in which it is located in another. Each phase is characterized by a fleeting ability to an SPS, which can be regarded as the affinity of the phase to that SPS (Petsas and Vagi 2019).

For the same concentration of SPS, the escape tendency, which has as a driving force the gradient of fugacity, is lower in the case of a phase with high fugacity capacity, than in the case of one with low fugacity capacity. For an equivalent thermodynamic level, a phase with a higher fugacity constant may accept a higher concentration of a given compound than another with a lower fugacity capacity. The relation between the concentration of an SPS in a certain component of the environment and its fugacity is expressed by the equation: $C = Z \cdot f$, where C the concentration of SPS ($\mathrm{mol \cdot m^{-3}}$); Z the fugacity of the compound in the respective phase ($\mathrm{mol \cdot m^{-3} \cdot Pa^{-1}}$); f fugacity of the compound (Pa). There are four levels of fugacity models applied in evaluating the behaviour of SPSs in the environment. These models, whose complexity increases from level 1 to level 4, are briefly presented in Table 5.2. The simplest and most frequently applied models are level 1. The values of fugacity capabilities are calculated using the distribution coefficients of SPSs between two constituent phases of a binary system.

The partition coefficient represents the ratio of the concentrations of the compound in thermodynamic equilibrium in a binary system. Because the distribution between groups of two phases/environmental compartments is of interest in terms of practical applications (soil/water distribution; sediment/water; air/water) for determining such distribution coefficients there are standard protocols (Yu et al. 2017).

Table 5.2 Levels of the fugacity models of SPSs

Level	Description of the system	Model description
1	Closed system in equilibrium and in steady state	The phases/compartments of the system are considered to be at thermodynamic equilibrium (according to the distribution/ distribution coefficients). The processes of transformation and transport by advection are not introduced in the model
2	Open system in equilibrium and steady state	In addition to level 1, the continuous emission of SPS and its transformation (biotic or abiotic) are taken into account
3	Open system in steady state, but not in equilibrium	In addition to level 2, the active transport and emission specific to each compartment are taken into account
4	Open system that is not in a steady state nor in a stationary state	In addition to level 3, the dynamics of emissions are taken into account and therefore the variation in concentration over time resulting from it where

K_{AW}–air–water distribution constant

5.4 Physio-Chemical Properties of SPSs in the Assessment of Distribution in the Environment

A number of properties of SPSs such as water solubility, vapour pressure, dissociation constant, specific density, distribution constants between binary systems (octanol–water; organic carbon–water; air–water) are considered to be determining characteristics regarding the environmental behaviour of SPSs. Water is found, in various weights, in all environmental compartments, being an essential component of ecosystems, the water solubility of an SPS is an important property. This can be defined as the concentration of SPSs in water in contact with another phase at equilibrium (saturation concentration). Most often, water solubility is expressed relative to the pure substance (the phase with which the water is in contact).

However, there are cases where solubility is not expressed in relation to the pure substance, such as atmospheric oxygen. The solubility of SPSs in water is dependent on the nature and strength of the chemical bonds involved and the geometry of the molecule (in the case of molecular compounds). It can be said that the presence of ionic bonds in SPSs can confer a high solubility in polar solvents, implicitly in water. However, not all ionic SPSs have high water solubility. In fact, depending on the strength of the bond/energy of the crystalline lattice, the solubility of ionic SPSs can vary greatly, from high-solubility SPSs, of the order of hundreds of grams per litre, to quasi-insoluble SPSs. The solubility in water of organic SPSs is the highest when the intermolecular interactions that can be established between their molecules and those of water are stronger. Nonpolar SPSs (such as hydrocarbons; only C–C and C–H bonds) whose molecules can only interact with water via London dispersion forces are weakly soluble or insoluble in water.

SPSs that contain functional groups that can participate in the formation of hydrogen bonds (bridges) with water tend to dissolve in it. Thus, the presence in the molecule of functional groups such as carboxyl (–COOH), hydroxyl (–OH), amino ($-NH_2$) and even carbonyl (=C=O) or ether (–O–) may confer a hydrophilic character to the molecule, favouring its solubility in water. The greater the number of such functional groups in SPSs molecule is, the higher the solubility. In contrast, an increase in the hydrocarbon skeleton, which represents the hydrophobic part of the molecule, leads to a decrease in solubility.

The ionization constant can be used to describe the dissolution in acidic or basic SPSs (weak acids and weak bases) in water. The Henderson–Hasselbalch relation is obtained based on which it is possible to assess the weight in which the acid is in ionized form, as a function of pH. Thus at pH values higher than pKa the acids are predominantly in dissociated form. Similarly, it can be deduced that the bases are predominantly in ionized form for pH values lower than (14-pKb). The solubility of SPS in ionized form is higher compared to that of molecular form, because in this case, strong ion–dipole interactions occur between ionic species resulting from dissociation and water molecules. The solubility of SPSs in water may vary depending on the conditions encountered in the environment. Temperature is an important factor that affects the solubility of SPSs differently. Gas solubility decreases with increasing temperature while solubility of solids, in most cases, increases with increasing temperature. Solubility of liquid SPSs varies slightly with temperature. Other factors that can influence the solubility are the presence of other dissolved substances, and in the case of gases, the partial pressure at which they are found in the atmosphere. SPSs with high water solubility are characterized by increased mobility and bioavailability (Admire et al. 2015).

The vapour pressure of SPSs varies inversely in proportion to the boiling point of the compound. A high vapour pressure indicates increased volatility of the compound. The vapour pressure of SPSs is even greater as the cohesion forces between molecules (intermolecular forces) are weaker. The lower the polarity of the molecule and the lower the molecular mass, the higher the volatility of SPSs will be. A volatile compound found in surface water or in soil will tend to leave these phases to reach in the atmosphere. From the point of view of the environmental factors, the vapour pressure is influenced by the temperature, the increase of the temperature leading to an increase of it. The specific density (ρs) represents the density of SPSs, in solid or liquid aggregate state, relative to the density of water for a given temperature. Knowing the specific density value for a liquid hydrophobic substance allows one to assess whether it can form a pellicle on the surface of the water ($\rho S < 1$) or a pellicle on the water–sediment interface ($\rho S > 1$). Similar to the notion of specific density applied to liquid and solid SPSs, it is that of vapour density, used for SPSs in gas phase.

Gases with a molar mass greater than 29 g mol^{-1}, due to their higher vapour density than air, may tend to accumulate near the earth's surface if air mass movements do not favour mixing/homogenization. This aspect is important to know when evaluating the behaviour of some gas emissions. The distribution constants between the different phases are characteristics of SPSs derived from the fundamental physico-chemical properties, important for the assessment of their distribution in the environment. Henry's constant (H) is a constant of air/water distribution of substance (K_{AW}). It can be determined directly, experimentally, or can be estimated as the ratio between vapour pressure and water solubility.

By correlating the two properties through Henry's constant one can appreciate the water–atmosphere transport of the compounds. Compounds with a high Henry's constant are easily transported from water into the atmosphere. The octanol–water partition coefficient (K_{OW}) represents the ratio of concentrations at equilibrium between two phases: 1-octanol and water. K_{OW} varies inversely proportional to the water solubility of SPSs. This coefficient is frequently used to assess the passage of the compound from the aqueous phase into lipids and thus characterize the bioaccumulation capacity. Often the value of the octanol–water partition coefficient is given for the logarithmic form ($\log K_{ow}$). The repartition coefficient organic carbon–water (K_{oc}) is given by the ratio of the equilibrium concentrations of the compound in the organic carbon fraction of soil or sediment and in water. It is the repartition coefficient or distribution soil/sediment-water normalized to the organic carbon content of the solid phase. This coefficient was introduced because it has been experimentally found that low polarity organic SPSs (hydrophobic SPSs) are predominantly retained by the soil/sediment organic fraction. K_{OC} can be determined experimentally for each particular situation, but given the high diversity of the solid material (soil/sediment) on which adsorption takes place, empirical relations of K_{OC} calculation based on K_{OW} have been developed for a number of SPSs of interest (Table 5.3).

A series of relationships correlating K_{OC} and K_{OW} for hydrophobic SPSs, in particular organochlorine and triazine pesticides, are summarized, and polycyclic aromatic hydrocarbons $K_d = f_{OC} \cdot K_{OC}$, where K_d solid/water distribution coefficient; f_{OC} soil/sediment organic carbon fraction; K_{OC} the repartition coefficient (distribution) of organic carbon–water. K_{oc} values are useful in assessing the mobility of organic SPSs in soil and aquatic systems. High K_{oc} values are correlated with reduced mobility of organic substances and vice versa. The soil/water partition/distribution coefficient (K_p or K_d) is given by the ratio of the concentration of the substance in the soil and that of the water. It varies similar to K_{OC} (Admire and Yalkowsky 2013).

5.4.1 SPSs Group and the Approach by Extrapolation

The vast majority of SPSs that are studied from the behavioural point of view in the environment are organic SPSs (it should also be taken into account that the number of organic SPSs is incomparably higher than that of the inorganic compounds). But there is a whole series of organic SPSs which are of interest from this perspective, such as inorganic SPSs containing heavy metals, arsenic, phosphorus and nitrogen compounds and others. Inorganic SPSs (ionic or molecular) are treated individually, while organic SPSs can be more easily classified into a well-defined class of compounds with specific properties. For organic SPSs, it is easier to estimate the behaviour in the environment, based on other compounds with similar structures or being part of a homologous series (in a homologous series the properties vary in a systematic manner).

Table 5.3 Empirical relations of K_{oc} calculation based on K_{ow} values

Equation	Classes of chemical compounds
$\log K_{oc} = 0.544 \cdot \log K_{ow} + 1.377$	Large variety of compounds, especially pesticides
$\log K_{oc} = 0.937 \cdot \log K_{ow} - 0.006$	Aromatic compounds, polycyclic aromatic hydrocarbons, triazines and dinitroaniline herbicides
$\log K_{oc} = 1.00 \cdot \log K_{ow} - 0.21$	Especially aromatic compounds and aromatic polynuclear compounds, chlorinated organic
$\log K_{oc} = 0.94 \cdot \log K_{ow} + 0.02$	S-triazine and dinitroaniline herbicides
$\log K_{oc} = 1.029 \cdot \log K_{ow} - 0.18$	Various insecticides, herbicides and fungicides
$\log K_{oc} = 0.524 \cdot \log K_{ow} + 0.855$	Phenylurea derivatives and alkyl-*N*-phenylcarbamates

The European Chemicals Agency (ECHA) recommends that in the process of evaluating SPSs on their environmental behaviour and the potential dangers that they may present, a grouping of SPSs should be done and an approach by extrapolation may be undertaken. This involves extrapolating a property from one substance to another within a category of SPSs with a similar structure. Compounds from the class:

- aliphatic halogenated derivatives, more specifically chlorofluorocarbons (CFCs) (freons), halons (fully halogenated compounds also containing bromine in the molecule) and hydrochlorofluorocarbons (HCFCs) or hydrofluorocarbons (HFCs) and
- polychlorinated biphenyls (PCBs). The completely halogenated compounds of the freon and halon class are volatile compounds of exclusively anthropogenic origin that have remarkable chemical stability and consequently persistence in the environment.

Given these characteristics, once released into the environment, these compounds will be found predominantly in the atmosphere where they can be transported over long distances and evenly distributed up to the stratosphere level. The low reactivity of these compounds is due to the strong carbon–halogen (C–X) bonds that cannot be cleaved in the troposphere by interaction with hydroxyl radicals or by photodissociation under the action of the electromagnetic radiation available in this area of the atmosphere. However, the electromagnetic radiation present in the stratosphere has the energy required to cleave the C–X bonds. Halogen atoms resulting from photodissociation can interact with stratospheric ozone by decomposing it. If the freons and halons do not undergo reactions in the troposphere, alkyl halides which also contain hydrogen in the molecule, hydrochlorofluorocarbons (HCFCs) and hydrofluoro-carbides (HFCs), are likely to react with HO· radicals in the troposphere (Mathieu 2017).

The potential for depletion of the ozone layer of halogenated compounds depends on the constituent elements of the compound. The nature of the halogen and the degree of halogenation will influence the extent to which these compounds are degraded in the troposphere before reaching the stratosphere and the altitude in the stratosphere where photodissociation of the C–X bond occurs. Thus, the presence of one or more hydrogen atoms in the halogenated compound molecule will make it possible to eliminate it in the troposphere by reaction with HO· radicals. The altitude at which the photolytic dissociation of the alkyl halides takes place depends on the nature of the halogen. The strength of the carbon–halogen bond varies in the order: C–F> C–Cl> C–Br. The required energy for photodissociation differs depending on the halogen atoms in the molecule of the halogenated derivative. The C–F bond needs the highest energy and therefore radiation with a lower wavelength. In order to have lower wavelength radiation required for photodissociation, the compound must reach high altitudes, sometimes up to 50 km. The dissociation of C–Cl and especially C–Br bonds requires lower energies and will therefore occur at lower altitudes. It can be appreciated that the replacement of chlorine atoms with fluorine atoms in the chlorofluorocarbon molecule leads to an absorption displacement to smaller wavelengths (dissociation at higher altitudes).

Thus, $CFCl_3$ photolysis occurs at approximately 25 km, CF_2Cl_2 at 32 km, while a predominantly fluorine-containing compound such as $CClF_2CF_3$ photo dissociates at 40 km.

So, as a chlorofluorocarbon contains more Cl than F in the molecule, it will dissociate at lower altitudes, having a higher efficiency in stratospheric ozone decomposition. Halogenated derivatives that also contain bromine in the molecule, halons have an even more severe impact on the ozone layer because their photolysis takes place at even lower altitudes, thus having the possibility to interact with ozone molecules from the whole layer. The trends manifested in the behaviour of this series of compounds are illustrated by the variation of the ozone-depletion potential (PDO) of the compounds. The PDO of a halogenated compound is defined as the diminution of the amount of ozone resulting per mass unit from the relatively evaluated compound to that obtained per mass unit from $CFCl_3$ (CFC–11) vertically integrated in the stratosphere area, in stationarity regime. Polychlorinated biphenyls (PCB) are exclusively anthropogenic compounds, situated today in the priority SPSs class. They formally derive from the biphenyl molecule by substituting 1–10 H atoms with Cl atoms, thus resulting in 209 different compounds (Li et al. 2015).

The common characteristics of the PCB series members are: high stability, low vapour pressure (10^{-7}–10 Pa at 25 °C), low water solubility (0.0027–0.042 ng/L) and a high water–octanol partition constant ($\log K_{ow} \geq 4.54$). Being characterized by low or very low vapour pressure and pronounced hydrophobic character, PCB tend to be accumulated in soil and at the level of suspended matter and sediments in aquatic systems, reaching in a smaller share in the atmosphere. However, the high stability of the PCB allows, under certain conditions, their migration over long distances. And the lipophilic character favours their bioaccumulation.

The physico-chemical properties and the tendencies that these compounds show in their behaviour in the environment present systematic variations depending on the number of chlorine atoms and their position in the molecule. It can be appreciated that, in this series, the increase of the number of chlorine atoms leads to the decrease of the vapour pressure and the solubility in water and to the increase of the lipophilic character. An increase in the number of Cl atoms in the molecule leads to decreased aerobic biodegradability of PCB.

The first stage of the oxidative degradation process, hydroxylation proceeds as fast as there is a smaller number of chlorine atoms in the nucleus. The presence of chlorine atoms on only one of the two nuclei leads to a faster metabolization than in the case of biphenyls with the same number of chlorine atoms, but which are distributed on the two nuclei (3,4-dichlorobiphenyl will be more rapidly transformed than 3,4′-dichlorobiphenyl), the cleavage of the aromatic ring occurs faster if it is unsubstituted.

Highly chlorinated PCBs having a successive arrangement of chlorine atoms (in a 2,3,4-trichlorophenyl sequence) as opposed to an alternating one (in a 2,4,6-trichlorophenyl sequence) are more biodegradable (Mühlebach et al. 2006).

5.4.2 Aerobic Biodegradation

Aerobic biodegradation occurs at higher speeds for substituted biphenyls at ortho-positions (such as 2,2′,6,6′-tetrachlorobiphenyl) than for other biphenyls with the same number of chlorine atoms in the molecule (substituted at meta-/para-positions).

Biphenyls with a high degree of chlorination have resistance to the aerobic degradation process, but they can undergo anaerobic chlorination. Studies on river sediments have shown that in the anaerobic environment the concentrations of tri-, tetra- and penta-chlorobiphenyls decrease, while for mono- and di-chlorobiphenyls the concentrations remain higher. If the speed of the aerobic degradation process varies inversely in proportion to the degree of chlorination of the PCB, that of the anaerobic process of dechlorination increases with the degree of chlorination.

But, as with the aerobic degradation process, not only the number of chlorine atoms in the molecule, but also their position influences the process. The dechlorination is mainly performed for Cl from meta- and para-positions, leading to the accumulation of substituted terms in ortho (Dolinová et al. 2017).

5.5 Retrieving SPSs from the Environment

The retrieving of SPSs by an organism can be carried out in several ways, being direct (from water, air) or indirect (take over by food). Regardless of the path of retrieval, the first process consists in the passage of SPSs through the biological membrane.

According to studies, it can be done in three ways: lipidic, aqueous and endocytosis.

The lipid pathway involves the passage of the lipophilic compound through the lipid bilayer of the cell membrane, which requires its diffusion through the membrane. Transport by *aqueous pathway* is made through two types of membrane transport proteins: “channel” proteins (which form channels) or “carrier” proteins (or transporters), which transfer hydrophilic contaminants into the cell. In the first case, a channel is formed from β-folded bounded proteins, which combine to form a structure with the nonpolar components of the amino acids facing outward from the membrane and with the polar components to the inside of the channel. The functioning of these channels, such as sodium, may be influenced by the presence of other chemicals, including toxic ones.

“Carrier” proteins such as Na^+, K^+-ATP-ase facilitate the transport of a specific class of compounds through the membrane. Another protein that can function as a carrier is calmodulin, a messenger protein that provides calcium transport. It is considered as a possible takeover mechanism: adsorption, passive diffusion, active transport, facilitated transport or diffusion and endocytosis. Adsorption is the first act of retrieval through the cell membrane and consists of its accumulation on the contact surface of the two phases between which the exchange takes place. The process can be described by a Freundlich or Langmuir isotherm. The graphical representations have the same allure, pointing out that a level of the adsorbed SPSs concentration is reached even though its concentration in solution, after the adsorption is complete, has high values. Passive diffusion does not require energy input and is performed against an electrochemical or chemical gradient (Rainey et al. 2017).

5.5.1 Identification of SPSs

The production and use of SPSs can have a significant negative impact on the environment, so the risk assessment of these substances has become a priority in recent years. In order to identify SPSs that could represent a danger to the environment (including human health), a number of factors have been proposed, with general character, which may serve as criteria for the selection of substances:

- The amount of substance produced (including by-products resulting from a process of production of another substance)/transported/used. This quantitative aspect must take into account the quantity of substance that is produced, but also the extent in which it can reach the environment depending on the usage mode. Some uses involve the release of small quantities into the environment, while others may lead to the total release of SPSs into the environment, such as the application of pesticides on agricultural land.
- Persistence. SPSs with high persistence may tend to accumulate in the environment. If the inflow of SPSs (emissions) is greater than that of the output (biotic or abiotic degradation) the substance will accumulate, which will result in an increase of its concentration in one or more of the environmental compartments. A high concentration can have implications for exposure and adverse effects (toxic or of other nature) on ecosystems. SPSs (lifetime in the order of years) have the opportunity to migrate and act at great distances from the source. It should be kept in mind, however, that the persistence of SPSs depends not only on its properties but also on the characteristics of the environmental compartment in which it is located.
- Possibility to generate various adverse effects. Particular attention is paid to SPSs that have the ability to influence atmospheric chemistry (organohalogenated compounds that contribute to the decomposition of atmospheric ozone); produces pH changes (sulphur and nitrogen oxides that can produce acid precipitation with effect on aquatic systems, soil, vegetation); heavy metal chelating, increasing their mobility and bioavailability; to generate toxic secondary SPSs.
- Bioaccumulative potential. A substance with high bioaccumulation potential is likely to have a significant risk to the organism if the substance or its metabolites are toxic.

– The potential for long-distance transport. Risk management for SPSs that get to be transported over long distances is much more difficult.

Society is based on a series of activities that involve the use of a significant number of SPSs, the challenge that arises is the most appropriate use of these SPSs, so that the adverse effects on the environment are minimal. Thus, in order to achieve this goal, an important step is the evaluation of the behaviour of these SPSs in the environment, an assessment that must be based on the knowledge and understanding of the transport/distribution and transformation processes of the SPSs in the environment. SPSs are important when is performed the assessment of the risk (García-Santiago et al. 2016).

SPSs are usually lipophilic and tear resistant, which gives them the ability to reach high concentrations and toxic potential in prey and humans. A very important principle in toxicology was formulated by Paracelsus (1493–1541): “All substances are poisonous; there is none that is not poisonous. The correct dose distinguishes a poison from a remedy”. This principle was formulated almost 500 years ago, but it is still valid today. This concept is the basis for the safe use and restriction of all chemicals, as most chemicals are considered harmless if the dose is below a certain level. The lowest dose of a substance that causes an adverse effect is called LOAEL (lowest-observed-adverse-effect level)—the lowest level with observed adverse effect, and the highest dose that does not cause an adverse effect is called NOAEL (no-observed-adverse-effect level)—level without any observed adverse effect. Adverse effects include many types of end results, which can be graded or “all or nothing”. Examples of graded effects include lowering blood pressure, reducing sperm motility and inhibiting an enzyme. Among the effects of the all or nothing type are any effects from death to the production of tumours and infertility. NOAEL and LOAEL result in particular from animal studies and eventually lead to estimates of safe or acceptable levels of exposure for humans, such as DNEL (level with no derivative effect) and risk estimates such as RCR (ratio of risk characterization), which are essential for regulatory toxicology. An important aspect of the chemicals and their potential toxicity is that the effect may differ from human to human. NOAEL and LOAEL result from a dose–response curve, which specifically has a sigmoid shape. This curve is relevant to all compounds, including nutrients, micronutrients and oligominerals (Zarn et al. 2011).

An important feature of this curve is that it has a toxicity threshold. This means that doses below this threshold are non-toxic, and doses above this threshold are toxic. For some SPSs, especially those that can cause mutations, there may not be a threshold, and the dose–response relationship may be linear or may follow more complex models. SPSs can enter the body in different ways, determined by the mode of use and the nature of the substance. Gases such as arsine or cyano enter the body through inhalation, while fluids may be a problem associated with skin contact. SPSs and food additives generally enter the body by buccal route. There are three routes of exposure through which SPSs can enter the body: inhalation, skin contact and ingestion. For a substance to have an effect, it must also be absorbed, which happens either locally or systemically. Local toxicity is relevant for SPSs that can cause skin irritation, while absorption is the process by which a substance is taken up by the bloodstream. After absorption, the substance can be distributed. From the bloodstream the substance can penetrate and cause effects in all cells of the body (Nelms et al. 2019).

Substances can be metabolized by various enzymes inside the body and eventually they can be eliminated from the body by excretion. Together absorption, distribution, metabolism and excretion describe the arrangement of compounds in the organism.

5.5.2 Absorption and Distribution of SPS

The four processes of absorption, distribution, metabolism and excretion (ADME) are vitally important factors for the toxic effect of SPSs on humans or animals. ADME describes the evolution of SPSs in animal or human organisms. Another concept, toxicokinetics (TK), describes the speeds of these processes, for example, how quickly a himic SPS enters the body and what happens to it inside the body. Systemic absorption is the transfer of SPSs from the exposure site into the systemic circuit, from where it can be distributed in organs with diverse biological compositions. There are three main routes of absorption: ingestion (through food), inhalation (through breathing) and skin absorption (through the skin). In order for the toxic substances to be absorbed, they must pass through the cell membranes. The distribution can be measured at different times after absorption into the circulation, when the compounds begin to distribute from plasma to tissues, by taking samples from blood plasma, organs, bile, urine and faeces. In order for the toxic substances to be absorbed, they must pass through the cell membranes.

The distribution can be measured at different times after absorption into the circulation, when the compounds begin to distribute from plasma to tissues, by taking samples from blood plasma, organs, bile, urine and faeces. First the samples are homogenized or extracted in a certain way, then they are analysed by various chemical methods. Small hydrophilic molecules (<600 Da) penetrate into membranes through the pores of the junctions between cells, a process called paracellular diffusion. However, most toxic substances are larger hydrophobic SPSs, which pass through membranes through transcellular diffusion (passive transfer) as non-ionized compounds. Diffusion is a physical process that complies with Fick's law, in that SPSs are transported from high-concentration regions to low-concentration regions. Some SPSs run through special transportation systems, active transport or facilitated diffusion. Membrane transport is divided into passive transport, diffusion, filtration (kidney), special transport (in humans: 5% of genes), active transport: ABC transporters, facilitated diffusion: SLC transport proteins, additional transport processes.

One requirement for membrane diffusion (passive transport) to occur is that the molecule is soluble in the membrane. The transfer speed is proportional to the solubility of the lipid molecule. Hydrophobic (lipophilic) SPSs without charge dissolve well, while compounds with pregnancy and/or hydrophilic ones dissolve less well. The state of charge [cations (positive charge (+)), anions (negative charge (−)) or without charge (0)] of the molecules depends on their availability to donate (acid) or absorb (base) protons (H^+). This condition is called pKa value for acids and pKb for bases, which becomes pH when the chemical is 50% ionized. The pH in the environment is also of maximum importance. An acid may exist in a protonated form without charge (R–COOH) at a low pH (in the stomach, pH is 1.5–4.0), but it may also be unprotected with charge (R–COO–) under neutral conditions/basic (in the duodenum, pH is 7.0–8.5) after donating its proton. On the other hand, the bases will have an increasing load as the pH decreases (the base $R–NH_2$ forms $R–NH_3^+$), thus becoming less suitable for dissolving and penetrating the membranes. The solubility of SPSs is described by the value of Log P2 or Log D3 (logarithmic scales), which can be found experimentally by analysing the distribution of the compound between the octanol and water phases in equilibrium. To find the Log P values, the pH of the aqueous phase is adjusted so that the predominant form of SPSs is non-ionized. Log D values are determined at a certain pH, for example, at pH 2.0 or 7.4. Log D ranges from −5 to +10.

Values can be positive or negative: Log D < 0 means the preference for the aqueous environment, around 0—no preference and Log D > 0 means the preference for the lipophilic environment. For both hydrophilic and hydrophobic SPSs the size of the molecules matters; smaller molecules are more easily absorbed, whereas lipophilic compounds with large molecules present in foods may not be well absorbed.

For an effective absorption of SPSs, “Lipinski’s Rule of 5” states that an orally active SPSs cannot violate more than one of the following criteria: maximum 5 hydrogen bond donors (total number of N–H and O–H bonds), maximum 10 hydrogen bond acceptors (all N and O atoms), a molecular mass of less than 500 Da, an octanol–water partition coefficient (log P) of no more than 5 (very fatty compounds may not be extensively absorbed in the gastrointestinal tract). All numbers are multiples of five, from which the rule’s name comes. There are exceptions to Lipinski’s Rule (Wang et al. 2018).

Special transport of SPSs can be mediated by membrane proteins that normally only allow the transport of selected chemicals, such as nutrients, peptides and small ions. All transporters with ATP binding cassettes (ABC transporters) have the characteristics of active transport contrary to concentration gradients, a process that requires energy, the energy coming from ATP binding and hydrolysis. SLC transporter proteins facilitate diffusion through the cell membrane, but not against concentration gradients, so energy is not required.

Special transport can take place both inside and outside the cells. The carriers can only be located in one part of the cell, to mediate “directional” transport. ABC and SLC carriers constitute both a large family of multiple genes/proteins. As an example of an additional transport process, the particles can be embedded and transported by macrophages. The lymphatic system can carry chemicals (Yang et al. 2012).

The content of the gastrointestinal (GI) tract (i.e., food, bacteria) is outside the body. Throughout the GI tract conditions can vary greatly, in terms of pH (gastric juice has pH = 2, intestine pH = 7), bacterial composition (microflora, largely anaerobic), oxygen pressure (high in stomach, very small in the intestines) and the type and quantity of specialized transporters from enterocytes (a type of endothelial cells) in contact with the interior (lumen) from different areas. Most SPSs are absorbed by simple diffusion; pH influences the ionization of molecules and their absorption. Absorption may also be influenced by other factors, such as the presence of food and the duration of resistance. There are differences between species in terms of absorption; the biology and anatomy of the GI tract are specific to each species. To reach the circulatory system (where they will be absorbed), the compounds of the GI tract must first cross the intestinal membranes and pass through the liver. Small particles can also be absorbed. For oral absorption, it is considered that SPSs present in the intestinal wall (not in the intestinal lumen), in the portal circulation and in the bile were absorbed (along with the chemicals present anywhere inside the body). Various gases, vapours, aerosols and particles are inhaled from the air. Toxic gases come from liquids, and the concentration of a gas in the air depends on the vapour pressure of the liquid; the higher the vapour pressure, the easier it evaporates and the concentration in the air will be higher. The partition of gas between air and blood in the alveolar sacs of the lung is determined by the ratio of gas solubility (also called the blood–gas partition coefficient). This represents a constant value, which describes the balance between the concentration of gas in the blood and the concentration in the gas phase. For gases with very low solubility ratio, the rate of transfer depends in particular on the blood flow in the lungs (limited by infusion), whereas for gases with high solubility ratio the rate of transfer depends on the frequency and depth of respiration (limited by ventilation).

The nasal mucosa is covered by a liquid film, which is why the gas molecules (water soluble or highly reactive) can dissolve in this film and the nose can retain them (the nose acts as a “gas purifier”) so as not to get into the lungs. Particles larger than 5 μm are deposited in the nasopharyngeal region, and particles 2–5 μm in size reach the tracheobronchial region. The particles in the alveoli can be phagocytosed or attacked by macrophages, which can be harmful to surrounding cells. By moving in the opposite direction, the chemicals can be expired through the lungs (Yin et al. 2018).

The skin is the largest organ of the body, constituting a good protective barrier, because it is not easily permeable. Small lipophilic SPSs (below 400 Da) can penetrate through passive diffusion, a relatively slow process because the skin (epidermis) contains several layers of cells, including a barrier represented by the outer corneum layer (stratum corneum), consisting of densely arranged keratinized cells (biologically inactive, which have lost their nuclei), which are replaced in 3–4 weeks. SPSs can be absorbed through the appendages (sweat and sebaceous glands and hair follicles). In the biology of the skin there can be large differences between regional organisms and between species (thickness, vascularization).

Solvents may increase percutaneous absorption. SPSs that have passed through the stratum corneum into the epidermis are considered absorbed. Depending on the characteristics of SPSs, they can reach different parts of the body, such as the adipose layer, liver, kidney, bones and blood plasma. In the plasma, it can occur the binding on the transporter protein—albumin, but also on various globulins (globulins are higher than albumin, but exist in lower concentrations), on transfer in and on lipoproteins. The fraction bound to a protein is in equilibrium with the free fraction from the plasma (Bhat et al. 2018).

Thus, a strong bond with the protein results in a smaller free fraction. It creates a balance between blood plasma and organs. When unbound SPSs are removed from the plasma by filtration by the kidneys, the chemical balance in the plasma is rapidly restored by other toxic molecules that become free in the plasma. The concentration of toxic substance from albumin or organs is somewhat diminished. Albumin carries various nutrients, including fatty acids. Perfluorooctanoic acid (PFOA) resembles fatty acids and binds to proteins, having in humans a long half-life by elimination ($t_{1/2} \approx 3$ years). PFOA can disrupt fatty acid sensitive receptors. Persistent lipophilic SPSs are located in the adipose layer of the body (SPSs deposit), where they are released slowly, and starvation (which causes their rapid release) can therefore cause intoxication. The liver and kidney are permanently infused with large amounts of blood and contain active transporters that can largely absorb toxic substances. The blood–brain barrier (BBB) is the most effective barrier against SPSs. But this is not absolute and is not fully developed in newborns. BBB consists of capillary endothelial cells that have tight junctions between them and contain absorption and efflux transporters. The placenta protects the foetus, but it is not as accurate as the BBB, and the placenta can differ greatly from one species to another. However, fat-soluble toxic substances can also overcome these barriers. Foetuses have very little fat, so they do not accumulate very lipophilic chemicals. Newborns can receive lipophilic toxic substances through breast milk (Cheng and Ng 2017).

5.5.3 Toxicokinetics

It describes what happens to SPSs inside the body using mathematical models, such as absorption rate or plasma elimination half-life ($t_{1/2}$) for a given substance to a particular species. The concepts refer to the study of SPSs, but they are also relevant to SPSs.

Following contact with SPSs, further chemical analyses of the substance (and its metabolites, if relevant) in plasma and/or in the tissues at various times are required to track how quickly it is absorbed, distributed (where, how quickly and how much?), metabolized and/or excreted. The data can be used to calculate how high the dose of SPSs should be given to have a certain concentration in plasma (or in the tissue) at a given time and how often it should be administered to maintain a certain minimum concentration. When the rate of absorption exceeds the rate of elimination, the toxic compounds accumulate. Following oral, skin or inhalation administration, plasma concentration depends on absorption, distribution and elimination rate.

Bioavailability is the fraction F of an administered dose from SPSs that reaches unchanged in the systemic circulation (may vary from 0 to 1, absorption being complete when $F = 1$). Bioavailability and absorption are not the same thing. The difference between bioavailability (i.e., presence in systemic blood flow and tissues) and oral absorption (i.e., presence in the intestinal wall and portal circulation) may arise from chemical degradation caused by the metabolism of the intestinal wall, or from efflux back into the intestinal lumen, or the presystemic metabolism in the liver. Elimination includes biotransformation (metabolization), expiration and excretion. The half-life by elimination $t_{1/2}$ is the duration (*h*) required for the plasma concentration to reach half its initial value. The rate of elimination constant, kel (*h* − 1), is the rate at which the compound is eliminated from the body. Any SPSs in a deposit (organ) are in equilibrium with the plasma free fraction of toxic substance and, as the unbound plasma fraction is eliminated, more substance is released from the deposit.

The extent to which SPSs are distributed in tissues and not in plasma is called apparent volume of distribution (VD). VD is a theoretical volume representing a constant value of SPSs (chemical substance). The measure unit for VD is the litre (or occasionally litre per kg body). VD is the apparent space in which a quantity of chemical substance is distributed in the body to result in a certain plasmatic concentration. VD can be from ~5 L up to 15,000 L. Non-ionized lipophilic SPSs and some basic lipophilic compounds (R_3N^+ can form ion–pair interaction with acid phospholipids from membranes) typically have higher VD values than acid lipophilic compounds. If VD is known, the dose required to have a certain plasma concentration can be determined (Robert et al. 2017).

5.5.4 Biotransformation and Elimination of SPSs

SPSs are often substrates for enzymatic reactions of biotransformation (metabolization), in order to be excreted. The most important organ is the liver, but also the small intestine, lungs and kidneys have a significant capacity for biotransformation (present in a small extent in all cells). Biotransformation involves the enzymatic transformation of lipophilic SPSs into hydrophilic SPSs more easily to be excreted. Usually, SPSs are first subjected to a phase I modification (introduces functional groups), then to a conjugation associated with phase II, in which a hydrophilic substrate is often attached.

However, there are many exceptions, a compound can go through two phase I modifications and two phase II conjugations and only then it can be excreted. Many substrates (phenols and alcohols) support only phase II conjugation reactions. In some cases, biotransformation reactions generate even more toxic compounds such as, for example, reactive metabolites resulting from phase I reactions, but occasionally and from phase II reactions. In order to prevent the release of toxic intermediates, often reactions in phases I and II occur in close proximity, within cells, for example, inside the endoplasmic reticulum.

Biotransformation enzymes are primarily located in the endoplasmic reticulum or in the cytoplasm and, in smaller quantities, in the mitochondria, nuclei and lysosomes. Most often, enzymes are constitutively expressed, but some can be induced by SPSs. Endogenous compounds can also undergo these transformation reactions. Due to hereditary differences (nucleotide deletions/mutations), the structure and function of the enzymes may differ from one species to another, but also from one individual to another. In humans there are also genetic differences, polymorphisms: to an individual it may be completely missing a certain set of genes or may have two or more sets compared to another. A well-known example is that of the weak and ultra-fast metabolizers that have different CYP2A6 activity and differently activate the nitrosamines related to cancer induction (Abu-Bakar et al. 2013).

Phase I In the first phase, SPSs are subjected to oxidation, reduction or hydrolysis. These processes expose or introduce a functional group (–OH, $–NH_2$, –SH or –COOH), which often leads to a slight increase in hydrophilicity. *Cytochrome P450 monooxygenases (CYPs).* Oxidation by cytochrome P450 (abbreviated P450 or CYP) containing monooxygenase systems is a major metabolic pathway for many SPSs, but also for many endogenous substrates, including steroids and cholesterol. The highest CYP concentrations are found in the hepatic endoplasmic reticulum, but CYP enzymes are actually present in all cells. CYP enzymes function as terminal oxygenases in multicomponent electron transport chains, in which the second enzyme transports electrons into CYP. Using O_2 as a co-substrate, an O atom is activated and introduced into an aliphatic (RH) position of the substrates; the other O atom is reduced to H_2O. CYP enzymes are identified in all life forms—animals, plants, fungi, protists, bacteria, archaebacteria and even viruses.

In humans, 57 CYPs were identified belonging to 18 gene families, numbered from CYP1 to CYP51. Mice have genes for 101 CYP enzymes. A letter indicates the subfamily, for example, CYP1A, B, C, and the last digit indicates the gene, for example, CYP1A1, 2, etc.

In the mitochondria, electrons are transferred from NADPH to CYP via ferredoxin and ferredoxin reductase. However, in some cases related to endogenous substrates, CYP can act as an isomerase and does not need a second enzyme to function. CYP catalyses various types of oxidation reactions, including hydroxylation of aliphatic or aromatic carbon groups, double bond epoxidation and oxygenation of a heteroatom (S, N, I) and N hydroxylation. The following are some important aspects of CYP enzymes. *Induction of CYP enzymes:* Some aromatic SPSs, such as polyaromatic hydrocarbons (PAH), TCDD (a type of dioxin) are detected by the cytoplasmic aryl (Ah) hydrocarbon receptor, which is activated and transported to the cell nucleus, where it acts as a transcription factor, increasing the mRNA transcription that encodes for certain CYP enzymes, which can biotransform PAH and TCDD. In this way, the substance itself induces its own biotransformation. Another example: phenobarbital activates the constitutive androstane receptor (CAR), leading to the induction of certain CYP enzymes. *CYP enzyme inhibition*: CYP enzyme inhibitors present in food may affect the absorption and half-life of SPSs. Certain flavonoids in grapefruit juice inhibit CYP3A4 and may lead to longer and more potent effects of certain SPSs. In snakes, tributyltin (TBT) inhibits CYP19, causing the imposex effect. Piperonyl butoxide inhibits CYP enzymes by binding iron (a suicide substrate) added to pyrethrum preparations to prolong the insecticidal effect. *Individual*

CYP differences: As for CYP2A6, 1–3% of Europeans have the inactive enzyme and in some Asians the gene suffered a deletion. This could induce the risk of lung cancer because nitrosamines are not activated in the carcinogen substance when the enzyme is inactive or the gene is not expressed. *CYP differences between species*: In insects, malathion is metabolized to maloxone by CYP enzymes. Maloxone is a cholinesterase inhibitor and a potent neurotoxin, which can cause spasms and eventually the death of the insects. In mammals, however, malathion is metabolized to diacid malathion by hydrolysis of esters, conjugated and excreted. In addition to FMO and CYP enzymes, there are many other Phase I enzymes that can oxidize SPSs (Diaz et al. 2010).

Peroxidase SPSs can be oxidized into free reactive radicals by donating an electron to different peroxidases during the reduction of hydroperoxides (H_2O_2 and ROOH). An important peroxidase is prostaglandin H synthetase (PHS; also known as prostaglandin endoperoxide synthetase, PTGS) which contains cyclooxygenase (COX) and the catalytic activity of peroxidase, which are involved in the endogenous conversion of arachidonic acid into prostaglandin H_2, a lipid precursor signalling molecule (prostaglandins can cause inflammation and pain). In tissues containing weak CYP activity, PHS can activate SPSs by transporting peroxides (H_2O_2) to the substrate, forming reactive products. *Xanthine oxidoreductase*: xanthine dehydrogenase (XD) and xanthine oxidase (XO) are two forms of the same enzyme, which is a molybdenum hydroxylase. SPSs that are good substrates for XD and XO tend to be weak substrates for CYP enzymes and vice versa.

The XO reaction involves the reduction of O_2 which can generate reactive oxygen species (ROS). *Aldehyde oxidase (AO)*: Cytosolic molybdenum hydroxylase has similar XO action to catalyse the oxidation of aldehydes and to mediate the catabolism of biogenic amines and catecholamines. Reactions involve the reduction of O_2 which can generate reactive oxygen species (ROS). Sulphite oxidase: Molybdenum hydroxylase. It oxidizes sulphite, an air-irritating pollutant, into sulphate. *Monoamine oxidase*: Oxidative examination of primary, secondary and tertiary amines, including a large number of SPSs.

Alcohol Dehydrogenase (ADH) Alcohol dehydrogenases are dimeric cytosolic enzymes that oxidize primary and secondary alcohols to aldehydes and ketones. There are five major classes (I–V). *Aldehyde dehydrogenase (ALDH)*: Some SPSs and endogenous aldehydes can be highly reactive and can form additives with toxic potential following reactions with proteins and nucleic acids. The ALDH enzymes present in the mitochondria and in the cytosol oxidize the aldehydes in carboxylic acids in redox reactions.

Dihydrodiol Dehydrogenase Cytoplasmic oxidoreductases, which require NADP, belong to the aldo-keto reductase (AKR) superfamily, which oxidizes various aromatic hydrocarbons into potentially toxic metabolites (Matyja et al. 2016).

Reduction of SPSs Certain metals and SPSs that contain an aldehyde, ketone, disulphide, sulfoxide, quinone, N-oxide, alkenes, azo or nitro group are often reduced in the organs or tissues of the body. Reactions can be both enzymatic and non-enzymatic (electron donor reducing agents are present intracellularly, e.g., glutathione, FAD, FMN and NADPH). Some enzymes, such as alcohol dehydrogenase (ADH), aldehyde oxidase and cytochrome P450, can catalyse both reduction and oxidation reactions, depending on the substrate or conditions. *Reduction of carbonyl group:* NADPH-dependent aldo-keto reductases (AKR) and short chain dehydrogenases/reductases (SDR) reduce certain aldehydes in primary alcohols and ketones in secondary alcohols. *Reduction of azo and nitro groups*: In the anaerobic environment of the lower gastrointestinal tract, azo and nitro reductions are catalysed by the intestinal microflora. Cytochrome P450 from the liver and quinone oxidoreductase–NADPH can also mediate azo and nitro reductions under reduced oxygen pressure conditions. *Reduction of quinones*: NADPH–microsomal cytochrome P450 reductase can reduce certain quinones to semi-quinone free radicals that can be extremely toxic, because they can reduce oxygen (O_2) to superoxide anions ($O_2{\cdot}^-$; react with themselves and form O_2 and H_2O_2, a strong oxidant), then they can be reduced again to semi-quinone free radicals for the next round of reaction with O_2 (redox cycle of the chinone), producing large amounts of H_2O_2 that can cause high oxidative damage to biomolecules. *Dehalogenation:* Halogen atoms in aromatic hydrocarbons are very difficult to remove (Cl from polychlorinated biphenyl compounds and Br in the decaBDE flame retardant). They oxidize hard, but can undergo reducing halogenation reactions. CYP dehalogenation can occur by oxidative or reducing pathways. The presence or absence of O_2 determines whether oxidation or reduction reactions occur. Also, it matters and availability reductant (i.e., NADPH, donor electron). Bacteria possess enzymatic systems similar to animals and over 99% of the bacteria in the intestines are anaerobic. *Reductions of sulfoxide and N-oxide:* They may be mediated by thioredoxin-dependent enzymes in the liver and kidneys (Yanagita et al. 1997).

Hydrolysis According to an old definition, enzymes that hydrolyse organophosphate (OP) compounds are A-esterases, those that are inhibited by OP compounds are B-esterases, and those that do not interact with OP compounds are C-esterases. *Carboxylesterase*: Carboxylesterases are glycoproteins present in most tissues. They hydrolyse numerous endogenous lipid compounds and activate ester- and amide-based prodrugs in pharmacologically active SPSs. Carboxylesterases (and cholinesterases, below) are serine esterases that hydrolyse carboxylic acid esters, amides and thioesters. *Cholinesterases*: Serine esterase. It limits the toxicity of organophosphates (war gases, insecticides, pesticides) that inhibit acetylcholinesterase, acting as cholinergic neurotoxins. *Paraoxonase:* A plasma enzyme that hydrolyses lactones and phosphoric acid esters. Epoxide hydrolase: Present in five forms in all tissues, epoxide hydrolase eliminates electrophilic epoxides (which can react with proteins and nucleic acids) by the addition of enzymatic water. Microsomal epoxide hydrolase (mEH) and soluble hydrolase epoxide (sHE) detoxify xenobiotic epoxies, especially those formed during enzymatic oxidation of cytochrome P450 substrates. There are three forms that mainly eliminate endogenous epoxides (Maxwell and Brecht 2001).

Phase II Following the enzymatic modification of SPSs in phase I, the reactions in phase II involve the enzymatic conjugation with a substrate which, with the exception of methylation and acetylation, results in a large increase in the hydrophilicity of SPSs, which facilitates excretion. Glucuronidation, sulfonation, acetylation and methylation conjugations depend on energy-rich co-substrates, whereas amino acids or glutathione conjugations involve reactions with activated SPSs. The substrate and co-substrate must be available simultaneously for conjugation: thus, reduced availability of co-substrate may limit the rate of detoxification. *Glucuronidation of UDP–glucuronosyltransferases (UGTs)* catalyses the reaction between uridine diphosphate–glucuronic acid (UDP–GA) and electron-rich heteroatoms (N, O and S) from the substrate, with the addition of a sugar fraction. Typical substrates are hydroxyl groups, carboxyl groups, amino groups, sulfhydryl groups, phenols, aromatic amines and aliphatic alcohols. Endogenous substrates include bilirubin, steroid and thyroid hormones (Jeong et al. 2017). Glucuronidation contributes 35% to the metabolism of SPSs by enzymes in phase II. There are four main types belonging to two gene families (UGT1, UGT2). UGT enzymes have high capacity but low specificity and are induced (de novo synthesis) by some SPSs, including those that induce CYP 1, 2, 3 and 4. Glucuronidated substrates are called glucuronides (negatively charged at pH = 7.4), are recognized by the liver and kidney anion transport systems and are excreted in the bile or urine; especially those with low molecular weight conjugated in the urine. The intestinal microflora can contain α glucuronidase, which can deconjugate the SPSs glucuronides released by the liver into the bile, the result being that SPSs can be reabsorbed into the intestine (Wang 2005).

Sulfation (Sulfonation) Sulfotransferases (SULT) transfer sulfonate (SO_3^-) from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) cofactor to substrates that are similar to those for glucuronidation: hydroxyl groups, amino groups, phenol, aromatic amines and aliphatic alcohols. Compared with the cellular concentrations of UDPGA (~350 μM), the PAPS concentration is lower (~75 μM). A lower concentration of SPSs seems to promote sulfonation and a higher concentration glucuronidation. Membrane-bound sulfotransferases (located in the Golgi apparatus) and soluble cytoplasmic sulfotransferases differ in that membrane-bound sulfotransferases sulfonate large molecules, whereas cytoplasmic sulfotransferases sulfonate small molecules, including most SPSs. The sulphonated conjugates of SPSs are excreted primarily through the urine.

Acetylation of N-acetyltransferases (NAT; in human NAT1 and NAT2) mediates the transfer of an acetyl group to the substrate from the acetyl-coenzyme A (acetyl-CoA) co-substrate. Acetylation occurs at SPSs with aromatic amine ($R–NH_2$) or a hydrazine group ($R–NH–NH_2$) that are converted to slightly soluble amides in water and hydrazides, but many of them are also eliminated through urine. Depending on the structure of the aromatic amines, by acetylation could result not only detoxification, but also reactive intermediates.

Methylation (i.e., O–, N– and S–methylation) is a minor biotransformation pathway that reduces the solubility in water of the substrate. Methyltransferases add a methyl group from S-adenosylmethionine (SAM) to the substrate (such as SPSs, neurotransmitters), usually to its functional groups O–, N– or S–, which mask them by conjugation by other enzymes. Some neurotransmitters in the catecholamine group and some catechol SPSs (such as L–DOPA, which contains a functional phenol group) are subjected to O-methylation. S-methylation is an important pathway for SPSs containing the sulfhydryl group (R–SH). Methylation may increase toxicity. Glutathione conjugation (GSH, a tripeptide) is an important detoxification reaction, because a large number of electrophilic SPSs formed in Phase I reactions can bind to nucleophiles such as proteins and nucleic acids. Glutathione conjugates are thioethers, which are formed by the nucleophilic attack of glutathione thiolate anion (GS–) on an electrophilic C, O, N or S atom from the xenobiotic compound. GSH exists in high concentrations (~5 to 10 mM) inside cells and the reactive substrates can react spontaneously with GSH as a necrophage. Glutathione enzyme S-transferase (GST) increases the rate of conjugation by deprotonating GSH to GS–. GST enzyme is located in the cytoplasm (>95%) and in the endoplasmic reticulum. *Conjugation with amino acids:* Substrates for conjugation with amino acids (glycine, glutamine or taurine) are restricted to certain aliphatic, aromatic, heteroaromatic, cinnamic and arylacetic acids. Reaction mediated by many different enzymes. Thus, benzoyl-CoA scans the fraction of coenzyme A with glycine (forming hippuric acid) with the aid of the enzyme acyl-CoA: amino acid N-acyltransferase (Demirel et al. 2015).

Excretion In short, toxic compounds are eliminated in various ways, such as via the liver in the bile and through the kidneys in the urine. Also, the chemicals exit the body and through the saliva, through cell losses (skin, intestine), semen, menstruation, milk, hair and nails. *Elimination of SPSs by kidney in urine:* The kidneys work by filtering out small compounds and proteins in the urine, while larger cells and proteins remain in the blood. The higher pressure in the cardiovascular system is the one that activates the glomerular filtration process. Then, the compounds in question are reabsorbed from the inner tubular lumen of the nephrons (the functional unit of the kidneys) into the peritubular capillaries (small blood vessels) using special transporters. From the peritubular capillaries that surround the long tubules of the nephrons the compound can be eliminated again in the lumen of the tubes by passive diffusion and with the help of special transporters. *Elimination of the SPSs in the bile at the level of the liver*: Liver cells have a high capacity for biotransformation of toxic compounds (see above) and for their elimination in bile acid. In this way, the toxic compounds absorbed from the food are prevented from reaching the general circulation (metabolism of the first passage) (Reiter et al. 2014).

At the plant level, absorbed SPSs enter either in the phloem or xylem. Those taken up by the root system are transported to the aerial parts by xylem, while the compounds taken up by the leaves are transported by phloem. The storage of toxic substances takes place in those areas where they are not subjected to biotransformation ("metabolization") and do not interact with the centres of action. Such lipophilic areas are the fat deposits, lipoprotein mycelia and cell membranes that do not contain the centres of action of the toxic substances or do not contain the enzymatic systems that can catalyse their transformation (Kot-Wasik et al. 2016).

The amount of fat in an organism can vary widely over time. If an organism is well nourished, the lipid deposits can be considerable and can store a significant amount of SPSs. During periods when food is insufficient, the deposits decrease and SPSs are released, being transported to the centres of action and biotransformation. In the short term, storage in lipid-rich areas results in reduced toxicity of SPSs and protection of the body. However, in the long term, the release from the storage centres is accompanied by the manifestation of toxic effects. Long-term storage of lipophilic SPSs was evidenced by analyses of organisms of predatory species at the top of the trophic pyramid. Biotransformation may result in intensification of the elimination, detoxification, redistribution or reactivation of SPSs. By detoxification is meant to reduce the toxicity of SPSs by transforming it into a less toxic compound. Activation (or bioactivation) is the consequence of converting an initial SPSs into a compound with less pronounced toxic effect. The oxidative transformation of the parathion insecticide, with the formation of paraoxon, has an increased toxic effect.

In phase I of transformation SPSs undergo oxidation, reduction, hydrolysis reactions. The resulting compounds are more hydrophilic, as a result of the introduction of functional groups –OH, $-NH_2$, –COOH. Oxidation reactions are catalysed by a group of enzymes belonging to the enzymatic system "mixed function oxidation" (MFO). These enzymes are present in the endoplasmic reticulum of cells from many tissues. The reduction reactions are specific to halogenated compounds, oxygenated compounds (carbonylic), azo compounds and nitroderivatives. The enzymes that catalyse this type of reaction are reductases.

Hydrolysis is a common process, the substrates being esters, amides, epoxy compounds. The enzymes involved in these reactions are specific to the compounds that make up their substrate. Thus, the hydrolysis of esters is catalysed by a group of enzymes called esterases. Esterases of type A catalyse the hydrolysis of organophosphorus esters, and those of type B, also called carboxylesterases, ensure the hydrolysis of carboxylic esters. Being more polar than the initial compounds, biotransformation phase I products can be excreted more easily. They tend to move from the endoplasmic reticulum (headquarters of phase I transformations) to the cytosol, where they undergo further transformations, in phase II of biotransformation.

The reactions of phase II consist of the coupling of the products of phase I with chemical compounds containing functional groups such as –COOH, –OH, $-NH_2$ and are catalysed by specific enzymes, in the category of transferases. By coupling, there is an increase in the molecular mass of the phase I metabolites and an increase, most often, of the polarity. The most common couplings are with glucuronic acid, acetyl, sulphate and glutathione (Tomberg et al. 2015).

A synthetic comparison of the two phases of biotransformation of the toxic substances is presented in Table 5.4.

The term biotransformation is very appropriate for describing the transformations of organic SPSs and seems more inappropriate for describing the behaviour of metals in the body. However, they are subject to biological transformations at the level of organisms, which result in their storage or their disposal. Specific transformations of metals consist of complexing with different ligands of biological nature (Seidel 2015).

5.6 The Action Mode of the Toxic Compounds

Molecular interaction between a toxic compound and the centre of action may be highly specific for certain organisms/species or non-specific. This interaction results in the manifestation of toxic effects. Depending on the toxic effect generated, SPSs are classified into the following main categories: 3/4 genotoxic compounds; immunotoxic compounds; 3/4 carcinogenic compounds; 3/4 neurotoxic compounds. SPSs were classified according to the action centre in: 3/4 genotoxic compounds; 3/4 neurotoxic compounds; 3/4 mitochondrial toxins; 3/4 Vitamin K contaminants (anticoagulants); 3/4 thyroxine antagonists; 3/4 ATPase inhibitors; 3/4 herbicides regulators of plant growth. Genotoxic compounds can have a carcinogenic effect and act on the genetic material by degrading the DNA. The effects of DNA degradation on the body are complex. Examples of SPSs with genotoxic effect are: polycyclic aromatic hydrocarbons (PAH), aflatoxin, vinyl chloride. In all these cases, the compound does not initially react with DNA but, following biotransformation in phase I (oxidation), a reactive compound that binds to DNA is formed. This type of molecular interaction is widespread and many SPSs are known to have mutagenic properties (Fowles et al. 2017).

Table 5.4 The main characteristics of the biotransformation phases of the toxic compounds

Characteristic		Phase I	Phase II
Reaction type		Oxidation–reduction hydrolysis	Glucuronic acid coupling, sulphate coupling, glutathione coupling, acetyl coupling
Involved enzymes (general class)		Mixed function oxidase system	Transferases
Physico-chemical consequences	Structure	Functional groups appear: –COOH, –OH, $–NH_2$	Increases the molecular mass by attaching the molecule to which it is coupled
	Hydrophobicity	Hydrophobicity decreases	Hydrophobicity decreases
Toxicological consequences		Most often the toxicity decreases, but can occur also bioactivation	Toxicity decreases

Neurotoxic compounds can be natural or of anthropogenic origin. Among those in the latter category are organochlorinated, organophosphoric, carbamic insecticides, all acting as toxic to the nervous system by affecting the normal transmission of nerve impulse. However, the mechanism of action is different in some of these classes of compounds. The cyclodiene organochlorinated insecticides have as their centre of action the GABA receptors, which function as a channel for the Cl^- ion in the nerve cell membrane. Organophosphorus insecticides act on acetylcholinesterase, inhibiting its hydrolysis. Mitochondrial toxins act on the mitochondrial membrane, affecting the proton gradient and thus stopping ATP production. In this category are the natural insecticide rotenone and the cyanide ion. Tyrosine antagonists disrupt thyroid hormone transport by coupling with transthyretin. Such an effect is presented by biphenyl polychlorinated compounds with non-planar structure. The inhibition of ATP–Aases is accompanied by the disruption of the metabolism of ions with regulating role of the osmotic pressure (Na^+, K^+) and Ca^{2+}. Such an action is presented by the DDE, the metabolite DDT. Herbicides with regulatory role in plant growth are chlorinated derivatives of phenoxy-alkyl-carboxylic acids, whose most known representatives are 2.4 D; 2.4 DB. The mechanism and centre of action are not fully elucidated. The toxicity of these herbicides is low for vertebrates and insects (Lewis and Botham 2013).

5.7 Elimination of SPSs from Organisms

Elimination of toxic compounds implies a decrease in their concentrations as a result of biotransformation and/or excretion. The same effect can be registered as a result of the growth of the organism, phenomenon known as “growth dilution”. This is not an actual elimination as the total amount of SPSs has not changed as a result of the body’s growth.

The mechanisms of the excretion process are well studied in vertebrates and less well known in invertebrates, with the exception of some insect species. At the plant level, the elimination of SPSs occurs through biotransformation, evapotranspiration, leaf fall, in the form of root exudate. Mechanisms of elimination of SPSs in animal species consist of transport by the gills, biliary secretion, expiration, secretion of hepatopancreas, secretion of intestinal mucosa, removal of granules, faecal matter, excretion through urine, egg laying, loss of hair/feathers/fur or desquamation. In the upper animals, elimination can occur through lactation, sweating, saliva and genital secretions. Large molecules can be incorporated into the bile and removed with the faecal matter. As with the retrieval process, elimination depends on the lipophilicity of the compound. Low lipophilic SPSs with log Ko/a values between 1 and 3 can be rapidly eliminated through gills and teguments. The products of phase II of biotransformation have a high polarity and are easily eliminated by faeces. If, on the other hand, the coupling products of phase II are decomposed in the intestine, they are reabsorbed into the circulatory system by passive diffusion, and they return to the liver and the coupling cycle is repeated. This process is called enterohepatic circulation and can be performed several times until the final elimination by faeces. The extent to which excretion occurs through bile or urine depends on the molecular weight of the toxic compound and the nature of the chemical species. Most excreted coupling compounds are in the form of organic anions. When the molecular weight is below 300 u.a.m., excretion by urine predominates, and when it is over 600 u.a.m, by bile. Many aquatic vertebrates excrete lipophilic SPSs in water through passive diffusion. As with passive takeover, elimination can also be described by mathematical relationships. Considering that at moment 0, which marks the initial moment of elimination, SPSs have the Corg concentration in the body and that the elimination process follows a first-order kinetic, the speed of the process can be expressed in differential form. The mathematical treatment presented is based on the assumption that the elimination of SPSs takes place at the level of a single elimination centre. In fact, this process is carried out simultaneously in several ways. In this case, the elimination rate of c SPSs is the sum of several processes, each having a specific elimination constant (Van der Molen et al. 2000).

5.8 Bioconcentration, Bioaccumulation and Bioamplification

The net result of the processes of taking over, biotransformation and elimination is the concentration of SPSs in the body. The term "bioconcentration" is used to describe the process for organisms in aquatic ecosystems, to which it predominates, most of the time, direct take-over and elimination through passive diffusion. Bioamplification involves the concentration of SPSs in the body as a result of taking over by feeding. But, SPSs are taken over in several ways, a process that is designated by the term bioaccumulation.

The Bioconcentration Process To estimate the toxic effects of SPSs, it is particularly useful to model the processes mentioned above. The simplest toxicokinetic model that describes the variation of their concentrations in the body is the two-compartment model between which the transfer of compounds is performed. This model assumes that the absorption and elimination of SPSs respects a first kinetic order, specific to the bioconcentration process, in which the exchanges take place between water and the body. The concentration of the compound in the body in the stationarity phase represents the maximum level that the SPSs can reach under the given exposure conditions. When this level is reached, the ratio of the concentrations of c SPSs in the body and in water becomes equal to the ratio of the take-off and elimination constants, this being the bioconcentration factor.

If the concentration of SPSs in the body is determined before the stationary state is reached, the bioconcentration factor is underestimated. The bioconcentration process is influenced by a variety of factors related to the nature of SPSs, environmental conditions, as well as biological factors. The most important properties of SPSs on which their bioconcentration depends are: 3/4 the size and geometry of the molecule, 3/4 its ionization degree, 3/4 the chemical species in which it is presented.

Those molecular structures that have larger dimensions than the pores of cell membranes, as well as chains longer than the thickness of the lipid layer, are poorly taken over by aquatic organisms. An important aspect that influences the acquisition of metals from the aquatic environment is their possibility to form complex combinations with different stabilities depending on the nature of the present ligands. The pH value of the aquatic environment determines the formation of metal compounds with different solubilities, which influences their bioavailability.

The biological factors relevant to the bioconcentration process are: 3/4 the specific area of takeover, 3/4 the development stage of individuals, 3/4 the intensity of the biotransformation processes. In the literature it is frequently mentioned that the take-up and elimination rates of SPSs are higher in the small-sized organisms, which have a large specific area. Organisms in the lower stages of development have a more active metabolism, which leads to increased rates of uptake and elimination. The intensity of the biotransformation processes greatly influences the toxicity of SPSs and their elimination capacity from the body. A number of external factors are determinants for the bioconcentration process: pH, temperature, nutrient availability, oxygenation regime, etc. (Mathieu-Denoncourt et al. 2016).

5.8.1 The Bioamplification Process

Bioamplification is the increase of the concentration of SPSs from one trophic level to the next, as a result of its accumulation from food. The process can be modelled similar to that of bioconcentration, with the difference that SPSs are taken up through the feeding process. Feeding rate constant depends on the biological species through the feeding rate and the efficiency of taking up the feed compound. The latter depends, in turn, on the mechanisms by which SPSs cross the lipid barrier of the digestive tract (its desorption from the matrix in which it is located, the digestion of food) and the chemical structure of the compound. Some researchers have shown that for most fish species the retrieval efficiency from food of polychlorobiphenyls varies around 50%. Similar to the way in which the bioconcentration factor was defined, the bioamplification factor can be determined, as the ratio between the concentration of SPSs at a certain trophic level and that of a lower trophic level, under stationary conditions. It has been found that, in reality, in many cases there has not been a steady increase from one trophic level to another. Bioamplification involves increasing the level of contamination towards the trophic pyramid peak (Volschenk et al. 2019).

5.8.2 Bioaccumulation Processes

The uptake constants of SPSs from the sources mentioned above depend on the amount of ventilated water, filtered sediment and ingested food and on the uptake efficiencies from them. Small organisms ventilate a large volume of water per body weight compared to larger ones. With similar take-up efficiencies, it turns out that small organisms have a higher take-up rate from water. Taking SPSs from the food is more intense for larger organisms. By reporting the take-up rates of SPSs from different compartments, the main way of reception can be established. Such a calculation made for Guppy fish highlights that reception from food becomes important for chemical compounds having a bioconcentration factor greater than 10^5. Similar calculations have shown that even in the case of terrestrial invertebrates from the soil it becomes important the path of feeding through highly hydrophobic SPSs with logKo/a > 5. This result is explained by the fact that only highly hydrophilic SPSs can cross the intestinal membrane and thus may be taken from the food. The bioaccumulation process is influenced by the factors mentioned in the case of bioconcentration and bioamplification processes. It is emphasized the importance of the size of the body in establishing the major way of taking SPSs from the environment. The concentration that of SPSs reaches at an organism level following the processes of bioconcentration, bioamplification and bioaccumulation is essential in assessing its ecotoxicological effect (Liu et al. 2015).

5.8.3 Bioconcentration, Bioaccumulation and Bioamplification Processes of SPSs in Biocenosis

Plant and animal organisms take up the toxic substances from the environment, which can reach at the level of biocenosis higher concentrations than in the compartment from which they were taken. In order to be able to estimate the effects of toxic SPSs at the level of an individual and then at the level of a population, it is firstly necessary to evaluate to what extent it can accumulate. The concentration that a contaminant can reach in an organism is the result of several processes: retrieving over from the environment, storage, biotransformation and eliminating it, all these processes being influenced by a multitude of factors.

5.9 Ecotoxicological Tests to Evaluate the Risk of SPSs on the Environment

Ecotoxicology aims to study the effect of SPSs on ecosystems and the entire biosphere. This includes the knowledge of the processes of contamination of the ecosystems by the different classes of SPSs, the behaviour in the environment of the

SPSs, the knowledge of the mechanisms of uptake by the plant and animal organisms and the study of their effects on the biocenoses and the fundamental ecological processes. Specific to ecotoxicology is the study of the effects of SPSs at all levels of the living matter organization, from the cellular to the super individual level. Ecotoxicological studies are needed to determine the potential hazard and risk of an SPS or a mixture of substances on the environment.

The potential danger refers to the potential of the substance to cause an undesirable effect, while the risk represents the likelihood that effect will occur. The action of an SPS or a mixture of SPSs is largely dependent on exposure (duration) and concentration, and determining its toxic potential involves analysing the toxicity data to highlight the relationship between the concentration or dose of SPSs and the effect that it produces. By conducting toxicity tests, the response of different species or groups of species to the action of SPSs is tracked by following specific parameters. In order to determine the risk that an SPS can present, respectively, a mixture, several elements must be known, such as the quantity released into the environment; its extension, respectively, its spread; the maximum reached concentration. These are compared with acute and chronic toxicity, accumulation and concentration factors, fundamental toxicological "limit values" or those established by law. Acute toxicity is the effect caused by intense, short-term exposure of organisms to SPS action. The usual duration of acute exposure is generally 96 h or shorter.

The REACH Regulation defines, for example, acute oral toxicity by the adverse effects that occur after oral administration of one or more doses of the substance within 24 h (REACH, Commission Regulation No. 440/2008). There is a scale for quantifying the effects, depending on the percentage of organisms where the toxic effect is observed (for example, DL50/CL50—the dose/concentration that induces the death of 50% from the tested individuals). The scale for quantifying the effects also applies to the irritating or sensitizing effect, such as weak, moderate, strong or severe, depending on the values. Depending on the pathways into the body, acute oral, dermal or inhalation toxicity is differentiated. Chronic toxicity refers to the effect caused by prolonged exposure. It is a hidden toxicity with much more subtle effects, which can be combined with other effects, such as mutagen (genotoxicity), carcinogenic (carcinogenicity), reproductive (repro or embryo-toxicity), teratogenic (teratogenicity) or neurotoxic (neurotoxicity) (Brandt et al. 2015).

According to a convention established in ecotoxicology, a chronic test must be performed for at least 10% of the life cycle of the tested species. Sometimes chronic toxicity tests are shorter in duration. An important aspect is the testing of mortality according to the stages of development of the tested species. Thus, a study developed throughout the life cycle must determine mortality, growth, reproduction, development or other variables at the level of all stages of development of the species. More recent ecotoxicology tests are focused on determining non-lethal parameters, such as birth, behavioural parameters, development, reduction of photosynthesis. The toxic response of a tested group is estimated by certain limit values. Limit values refer to a maximum, minimum or average value, usually established by experimental means or a toxicological limit value established by law, such as the maximum allowable limit for residues of products in food (vegetables, fruits, meat, eggs, fodder, etc.) or water (drinking water, groundwater, surface water). In the ecotoxicological tests reference is made to the following values: 3/4 NOEC (No Observed Effect Concentration)—the concentration of a substance/mixture with no effect on a particular tested organism; 3/4 LOEC (Lowest Observed Effect Concentration)—the lowest concentration of a substance that produces an effect/ mortality to the tested organism; 3/4 PEC (Predicted Environmental Concentration)—the concentration of a substance/mixture that is supposed to reach the environment after application; 3/4 PiEC—the initial concentration of a substance/mixture that is supposed to reach the environment after application.

Frequently, several SPSs are released simultaneously in the form of mixtures, which manifest their toxic effects in complex ways. As a result, estimating the adverse effects is much more difficult compared to the situation of exposure to the action of a single SPS. In this case, it is important to know the potential new effects that arise as a result of the action of the mixture. Four such situations are distinguished: potentiation of toxic effects, additivity, synergism and antagonism of effects (Amorim et al. 2019).

The potentiation of effects may occur if a non-toxic chemical increases the toxicity of another substance in the mixture. Potentiation is used to improve the effectiveness of some insecticides. One such case is the addition of piperonyl butoxide to the formulas of some insecticides, in order to enhance their action by inhibiting the decomposition of the pesticide following the action of the cytochrome p450 monooxygenase system. Additivity consists in accumulating the toxic effects of the components of the SPSs mixture. Such a response is obtained when the SPSs of the mixture act independently one from other, through different mechanisms of action that do not influence each other. Synergism occurs if the observed effect of the mixture is greater than the sum of the expected effects of the constituent SPSs.

Some authors use the term “enhancement” in the context of synergism. The antagonism is manifested by decreasing the observed effect of the mixture, compared to the sum of the individual effects. In the specific used terms for the notion of “antidote”, the chemical whose effect is reduced is called “agonist”, and the one that reduces its effect is “antagonist” or “antidote”. Antagonism can be chemical, of receiver or of distribution at the level of the action centres. Regarding the ecotoxicological effects, the exposure to mixtures of SPSs with different toxic action having low, but potentially relevant concentrations, is a cause for concern, even if their individual values are below the concentration with no expected effect. It is necessary to improve the knowledge base and the methodologies used, as well as the holistic approach to ecological risk assessment. The use of the REACH Regulation database provides conditions for achieving this goal (Heye et al. 2019).

5.10 Conclusions and Recommendations

The general methods of estimating the effects at the community level are based on the use of the indicator species (tolerant and sensitive), the estimation of the CFE value at the community level and the biomonitoring. The toxic effects of SPSs depend, of course, on the emitted quantity and the duration of action and will occur first in the populations of the most sensitive species, then in the communities and the entire ecosystem. In addition to the information obtained from following the changes among sensitive, indicator species, the potential role of the factors that mirror the interactions between species, which is a very difficult problem, must be estimated. The interactions between species are of the following types: 3/4 competition-based interactions; 3/4 mutualism; 3/4 prey–predator interactions. The difficulty lies in the fact that one species establishes different relationships with each of the other species of the biocenosis, whose knowledge is required for the correct interpretation of the results. If, following the action of SPSs, a sensitive species of biocenosis is affected, which reduces its population, the species of its predator will also be affected due to the reduction of the number of prey. Instead, the species with which it is in a relationship of competition will be favoured by reducing the competitor. In recent years, the approach based on the response of the species most sensitive to the action of SPSs has led to the development of protective measures at the level of biocenosis. However, there is concern that this approach does not guarantee the protection of a community if sensitive species are affected, with a key role at the ecosystem level. By tradition, but not always right, ecotoxicology has focused its attention on organizational levels down to the ecosystem. There are studies addressing the higher levels of integration (complexes of regional, macro-regional ecosystems, up to global level). The special difficulty in ecotoxicology is the extrapolation of the results of the toxicity tests from the laboratory in the field. This is because, on the one hand, it is impossible to test the multitude of newly synthesized SPSs and their mixtures, and on the other hand, the species response to the action of the same SPSs is sometimes very different, depending on factors that cannot be simulated in the laboratory, and the standardized tests are performed on a very small number of species.

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Chapter 6
Ecotechnologies for Persistent Pollutants

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6.1 Introduction

Persistent organic pollutants (POPs) are chemicals that remain intact in the environment for long periods of time, from where they are transported and enter the living organisms, including humans, where they accumulate in their fatty tissue. Due to their toxic characteristics, POPs have harmful effects on human health and the environment. The Stockholm Convention is the world's leading instrument for protection against POPs. In this regard, the EU is committed to implementing the Stockholm Convention's provisions and encouraged the further listing of some chemicals under the cause of this convention. Furthermore, in the EU, the Persistent Organic Pollutants Regulation (EC 850/2004) supports and strengthens the provisions of the Stockholm Convention. Table 6.1 shows the POPs, based on the recent Stockholm Convention list and their remediation technologies.

The persistent organic pollutants regulation contains provisions on the production, placing on the market, use of this type of chemicals, managing of the stocks and waste of persistent organic compounds, and measures to reduce unintended POPs emissions.

POPs are chemicals that have extremely dangerous toxic qualities; they have a high degree of resistance to degradation and accumulation in living organisms and the environment; they can be easily transported into the atmosphere over long distances and are deposited far from the place of emission; they can harm human health and the environment, either near or far from their sources (Fernandes et al. 2019).

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Table 6.1 POPs and their remediation methods/technologies

POPs	Method/technology	Advantages	Disadvantages
Aldrin	Organic remediation	Environmental friendly	Needs time
	Degradation by oxidation with persulfate	Degradation 99.9%/180 min	Possible gas emissions
Dieldrin	Organic conversion with fungi	39%/30 days	Needs time
Chlordane	Ultrasounds/Fe	96%	Energy consumption (costs)
Endrin	Modified chemical techniques	85–90% remediation	Loss of soil microorganisms
Chlordecone	Microbial degradation	50 μg/mL	Needs time
Heptachlor	Organic remediation	Genetic accuracy mechanism	Still in study
Hexabromobiphenyl	Liquid extraction	Reduced time	Reduced recovery
Hexabromocyclododecane	Organic remediation microbial communities	57–60%/40 days	Needs time
Hexabromodiphenyl ether	Organic remediation	Genetic accuracy mechanism	Still in study
Heptabromodiphenyl ether	Oxidative decontamination	Acts directly	Reduced capacity
Hexachlorobenzene (HCB)	Chemical and microbiological methods	Conversion 52–59%	Energy consumption (costs)
α-Hexachlorocyclohexane (α-HCH)	Catalytic dechlorination	Conversion 99%	Gas emissions
β-Hexachlorocyclohexane (β-HCH)	Catalytic dechlorination	Conversion 99%	Gas emissions
γ-Hexachlorocyclohexane (Lindane)	Catalytic dechlorination	Conversion 99%	Gas emissions
Mirex	Ultrasounds	Conversion 97%	Energy consumption
Pentachlorobenzene (PeCB)	Apolar water remediation zeolites	High degree of treatment	Limited
Polychlorinated biphenyl (PCB)	Organic remediation with catalytic enzymes of thermophilic bacteria	POPs reduction	Energy consumption (costs)
Technical endosulfan (2 isomers)	Organic remediation	Conversion 69–100%	Needs time
Tetrabromodiphenyl ether	Liquid extraction	Reduced times	Reduced recovery
Pentabromodiphenyl ether	Organic remediation	Cost efficiency	At the laboratory level

(continued)

Table 6.1 (continued)

POPs	Method/technology	Advantages	Disadvantages
Toxaphene	Phytoremediation	Environmental friendly	Needs time
Dichlorodiphenyltrichloroethane (DDT)	The technology of commercial zero valent Iron (ZVI)	Positive effect in remediation	Reduced applicability in the future
Perflorooctane sulfonate (PFOS)	Gravitational nanomembrane	Conversion 36–90%	Still in study
Polychlorinated dibenzodioxin	Organic remediation with catalytic enzymes of thermophilic bacteria	POPs reduction	Energy consumption (costs)
Polychlorinated dibenzofuran	Organic remediation with catalytic enzymes of thermophilic bacteria	POPs reduction	Energy consumption (costs)

There are 12 POPs with toxic properties, which negatively influences the living organisms. They are grouped as follows: pesticides: aldrin, chlordane, dichlorodiphenyltetrachloroethane (DDT), dieldrin, endrin; heptachlor, mirex, taxopen; industrial chemicals: hexachlorbenzol (HCB)—also used as a pesticide, polychlorinated biphenyl (PCB); secondary combustion products: dioxins, furans (Schön et al. 2017).

Pharmaceutical residues have become an important source of environmental contamination (Puckowski et al. 2016).

Many pharmaceutical chemical products are designed to be non-degradable and thus represent a risk when they reach the environment where they persist.

The European Commission recognizes that environmental pollution with pharmaceutical residues is both a developing and a public health issue and is committed to addressing this issue. Most of the chemicals in the POPs category are non-polar organic compounds, as a result they are hydrophobic, with extremely low water solubility, they are lipophilic, which causes the accumulation in the fatty tissues of organisms.

Due to the phenomenon of bioaccumulation and bioamplification, the concentration of POPs may be much higher in the tissues of organisms at the end of the trophic chains (species of fish, mammals, birds, and humans) than the concentration in the environment.

POPs are chemicals that remain intact in the environment for long periods of time, from where they are transported and enter the living organisms, including humans, where they accumulate in their fatty tissue. From the point of view of the pollution of soils with persistent organic compounds, the pollution of agricultural soils with organochlorinated insecticides was significant.

Organochlorinated insecticides, namely those based on HCH (hexachlorocyclohexane) and DDT (pp′-dichlorodiphenyltrichloroethane), were introduced in use in the 1940s and have long been used to protect agricultural crops against vector insects of certain diseases. Organochlorinated insecticides HCH and DDT (isomers and metabolites) are very persistent, accumulating in the soil; the half-life in soils being 2 years for HCH and 10 years for DDT. The widespread uses of these pesticides, as well as their high persistence have made their residues and metabolites evident in all elements of the environment. Studies that have been conducted show that in areas where soil is polluted with DDT, its residues and its metabolites are present in plants, in animal, and human adipose tissue (López-Fernández et al. 2016). According to Table 6.2 POPs can result from:

Although treatment with these compounds has been prohibited, residues of HCH and DDT are still found in agricultural soils. Therefore, and monitoring the following compounds is necessary: α, β, γ, δ-hexachlorocyclohexane, pp′-dichlorodiphenyltrichloroethane (pp′-DDT) and its isomer op′-dichlorodiphenyltrichloroethane (op′-DDT), dichlorodiphenyldichloroethane with the two isomers (op′-DDD and pp′-DDD), and dichlorodiphenyldichloroethylene (DDE) (Denk and Milutinović 2018).

Basically, by summing the concentrations of these compounds the total HCH concentration is obtained. The degradation of chlorinated compounds and pesticides can be done chemically with a rate of 95% with the recovery of by-products such as sulfates, phosphates, bromates, etc. At present, the production of hexachlorocyclohexane is stopped, but there are deposits of inactive isomers that present a risk to health and the environment.

Table 6.2 Classification of POPs by origin

Agriculture	→	Application of pesticides on soils
		Use of products with POPs and storage of expired POPs or waste of POPs
		Off-road vehicles (tractors, trucks, and other machinery)
Industry	→	Industrial combustion processes
		Metalworking operations
		The production of chlorinated chemicals
		Use and application of products containing POPs
		Liquid and solid waste resulting from the production of POPs
		Waste water from cellulose and paper production
		Chemical production processes
		Waters from the use of preservatives and dyes used in the textile, leather, wood industry
		Domestic wastewater
Energy industry	→	Combustion operations for commercial, institutional, and residential energy users
		Energy production in industry
		Use of electrical equipment and transformers
Transport	→	Maintenance, fuel quality, and reduced efficiency of the combustion in the means of transport
Other sources	→	Incinerators for medical waste
		PCB deposits and other POPs

Used in agriculture, energy and industry, as well as a result of the burning of materials in which they are contained, these chemical compounds are extremely dangerous, because remains in the environment for a very long time until partial or complete decomposition are transported over long distances from the sources; they are deposited in the tissues of living organisms, where they arrive with food, water, inspired air; exert toxic actions in a wide range. POPs action on the human body leads to increased cancer morbidity, abnormal development, low fertility, weakened immunity, reduced intellectual capacity. The consequences of the action of these substances on the embryo, fetus, and young children are major (Miniero et al. 2005).

6.2 Displacement Ways of POPs

Migration of POPS into the environment has a complex character. Air is the most favorable way of rapid spread and on large areas of any substance, including toxic ones. The spread in the aquatic environment is slower, but also in the case of hydrographic systems, the mobility of POPS is considerable and, in a few days, or even hours they can be transported at distances of tens or hundreds of kilometers. In the soil, organic pesticides, PCBs, and other POPs move less. The presence of pesticides in foods 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 the following types of interactions and processes: Van der Waals forces, hydrogen bonds, hydrophobic bonds, chemical absorption, and ion exchange. A part of the pesticides falls to the ground where they are solubilized by rainwater and are entrained on the surface or in the depth of the soil where they are biodegraded, are entrained in groundwater or absorbed by the reticular system of plants (Qin et al. 2017).

The degree of migration from soil to plants depends on the initial amount of pesticides, by their properties and the type of plants. In the body of animals, pesticides can enter by the respiratory tract, by skin, and oral route. It should be mentioned that as the effectiveness of pesticides against pests increases, risk factors for warm-blooded animals increase as well.

When using pesticide combinations there are situations when the toxicity of the combination increases 5 up to 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 organism, the decreasing order being: mesenteric fatty tissue, depository fatty tissue, perirhinal fat tissue, and then the muscle tissue. In ruminants, a more pronounced accumulation is observed than in pigs.

Concentrated feed (cereals, mackerel, 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 (Tsai 2010).

6.2.1 Impact on Air

The presence of POP_S in the air is explained by the releases from the chemical companies and the factories of waste burning, the chemical treatment of the agricultural plants and of the forests against the diseases and pests, their evaporation from the surface of the soil, etc. When spraying agricultural crops with pesticides (against diseases and pests) there is a strong odor that irritates the respiratory organs and even causes headaches. The biggest impact on the air is given by the dioxins and furans that come from the burning of household and industrial waste. In the emissions of waste combustion plants are contained only 3% of POPs out of their total quantity obtained by burning. However, this amount constitutes about 50–60% of the total volume of dioxins and furans that reach the atmosphere annually (Majewski et al. 2014).

6.2.2 Impact on Waters

Pesticide concentrations and other POPs are detected in most aquatic basins, in rivers, seas, and oceans. These compounds are also found in the rainwater, as well as in the one from the melting of the mountain glaciers. In small concentrations POPs do not directly influence the quality of the waters and their appearance. In the case of larger quantities, the water acquires a specific odor, characteristic of the given type of pesticides. As a result of the migration processes, the pesticides along with the rainwater infiltrate the groundwater and even the artesian waters. Deposits on the bottom of lakes contain usually higher amounts of pesticides than basin water (Barizon et al. 2019).

6.2.3 Impact Over the Soil

There is virtually no place where any traces of POPs would be found, they are even found in the natural reservations. It is considered that they do not significantly influence the soil, but they have the property to increase their concentrations in the trophic line: plants, insects, prey animals, humans. The existence in soil of various bacteria that consume organic substances as food gradually reduces pesticide concentrations. Thus, occurs their natural destruction (decomposition). The most common way is to disperse solutions on the soil surface, suspensions, and powders containing these compounds.

Even if pesticides are destined to reach the leaves of plants, much of them reach the soil surface. Such method was also practiced during the use of DDT. Another way of polluting the soil with POPs is to incorporate pesticides directly into the soil with seeders or special aggregates. In this case, all of the POPs remain in the soil, influencing microorganisms and plants. Large quantities of pesticides are introduced into the soil with the seeds treated against diseases and pests. Soil pollution also occurs at loading-unloading of pesticides or during the station time of aggregates when they introduce the herbicides. After decades of such accidental pollution, on the fields or on the edge of them are seen pieces of land without vegetation (Barron et al. 2017).

6.2.4 The Impact on the Plant Kingdom

For the protection of plants against diseases and pests, insecticides, fungicides, and other chlororganic compounds are used. When treating plants with these pesticides, the leaves, stems, roots of the plants come into direct contact with POPs, which rarely affect their biological cycle. A property of plants is their ability to accumulate toxic chemical compounds in tissues (Casida 2017).

6.2.5 Impact on the Animal World

Insecticides are highly toxic substances, often with a lethal effect on organisms from soil, water, and air. One of the first preparations of chlororganic origin, widely used in the fight against various pests and carriers of infection was DDT, synthesized in 1874 by the Swiss chemist Paul Muller. Used as a disinfectant, it has been shown to be very effective in fighting infectious diseases (typhus, malaria, etc.), diseases of which the carriers are the insects. Later it has been found that its massive use leads to extremely serious consequences for the animal world.

The danger of using DDT and other pesticides lies in the fact that, along with the harmful insects, the useful ones are destroyed in great numbers. This POPs have a harmful influence on all living organisms. It accumulates in the tissues of animals, exerting carcinogenic, mutagenic, embryonic, neurotoxic, immunotoxic actions and it changes the hormonal system, causes anemia, hepatitis, etc.

DDT greatly changes the structure of the eggshell of birds, by thinning it, it significantly reduces the reproductive capacity of birds, fish, and snakes. Another POPs, toxaphene, causes damage to the vertebrae of the fish that become very fragile and at sudden movements they break easily, which leads to paralysis of the anterior part of the body and the death of the fish. The concentration of this substance is high in the ichthyofauna of the entire planet (Dinis-Oliveira et al. 2008).

6.2.6 Sources of Pollution and Environmental Risk Outbreaks

Potential source of pollution with POPs can be considered any enterprise producing POPs or territory where are stored chlorogenic substances that can spread to the environment naturally or through human activities.

Although DDT and HCH have been out of use for several decades, considerable quantities of these POPs remain in chemical storage facilities. Another risk factor is represented by dioxins and furans coming from the burning of waste containing plastics, used tires, and other chlorogenic waste. As a result of burning some of them, the dust and ash, which contains most of the dioxins and furans, are driven by wind at considerable distances, landing in the soil, in the surface waters and acting toxic on the pedobionts and the hydrobionts (Hall et al. 2015).

6.2.7 Sources of POPs in Environmental Pollution

Chemical industry enterprises; remained and destroyed pesticide deposits; thrown or buried stocks of chemicals under the open sky; power plants and capacitors containing contaminated soils; the places of authorized storage of waste and spontaneous waste; waste combustion plants, if they do not comply with the technological requirements; factories for the preparation of asphalt, without facilities for the collection of harmful substances; unauthorized companies that produce construction materials; transport (Cui et al. 2020).

6.2.8 The Impact on Human Health

From the soil, pesticides reach the fruits and vegetables consumed by humans, they reach the grass that the animals graze and then in their body. Epidemiological studies show an increased frequency of neurotoxic (priority from the peripheral system), hepatotoxic, carcinogenic disorders, as a result of people's long exposure to DDT (this pesticide was used extensively in the 70–80s agriculture).

Research in the USA has confirmed the direct link between human exposure to DDT and the frequency of breast cancer. The mammary gland can be considered as the target organ, because DDE, a metabolite of DDT, was determined in the blood serum in sick women and those who contacted DDT. Chlorinated cyclodienes (aldrin, dieldrin, heptachlor, chlordane, endosulfan, etc.) have been shown to have more toxic properties than DDT. In contrast, the toxic effect produced by chlorinated cyclodienes is manifested by convulsions, dizziness, chronic spasms, headaches, etc. Most of these POPs have a carcinogenic effect, frequently causing liver tumors. The toxic effect of pesticides is also manifested on the woman's body.

Epidemiological investigations have shown that, with the intensification of the application of pesticides, the frequency of pathological conditions and functional disorders of the reproductive sphere in women also increases.

A similar impact is also occurring on the male reproductive sphere, with an increase in male infertility cases in recent decades.

At the same time, there is an increase in mortality, especially in rural areas. In 1985 the overall rural areas mortality was 13.2, and in the cities areas—8.0 per 1000 inhabitants. To this has contributed the intense application, during 20 years, of pesticides, primarily the chlororganic ones.

Currently mortality remains high and constitutes 11–12 deaths per 1000 inhabitants, the highest continuing to be in the rural area. The mortality rate caused by chronic hepatitis and liver cirrhosis exceeds that of the neighboring countries. This index varies depending on the level of application of POPs in previous years, evidence of the harmful influence of these substances on the health of the population (Mubushar et al. 2019).

6.2.9 Measures to Prevent the Pollution of the Nature and POPs Intoxication of the Population

The Stockholm Convention (May 2001) provides for each country to develop and implement strategies and plans that would contribute to reducing or excluding the use of these toxic substances. But the respective measures must be taken at the district or village level. They can be realized operationally, without great efforts and financial allocations.

The main danger is the existing stocks of pesticides and other toxic compounds that have accumulated over the last 20–30 years.

This is why it is very important for every community to know all the existing sources of pollution and to inform the population about them. In the case of the lack of a specialized service, the inventory initiative must belong to the local council which, with the support of the specialists, will investigate the situation, will draw up a register of the objects, the territories where toxic substances are contained and will make the obtained information public.

Enterprises of any kind are required to manage the deposits that belong to them in an appropriate, efficient, and rational way.

In the near future it will be preceded with the accumulation of used pesticides from all over the country in one or a few specially arranged warehouses. It is the responsibility of the local public authorities to contribute to their packing and transport to the place of destination and to carry out the measures of ecological rehabilitation of the territories occupied by them.

The population must be aware of the real danger posed by the sources of pollution, including the packaging and containers after the use of pesticides that are kept in the households of citizens. As far as possible, they can be collected from the population and stored centrally for detoxification or subsequent destruction (Ramón and Lull 2019).

6.3 Remediation Technologies for Soils Contaminated with POPs

The most frequently applied technologies are organic remediation technologies (in situ technologies presented above) as well as thermal desorption technologies. Thermal desorption is a thermal method that works on the principle of heating the polluted soil so that POPs evaporate, thus being able to move easily through the soil to an area with a system of wells that collects and evacuates them. Thermal desorption can be applied in situ or ex situ. In situ thermal desorption can be done by:

- steam injection—steam is injected into the soil using wells dug in the polluted area; steam heats the area and mobilizes, evaporates and/or destroys POPs, the water and gases produced being collected in special wells from where they are directed to wastewater treatment plants;
- hot air injection—a process similar to steam injection except that hot air is injected through wells instead of steam, hot air heats the soil causing evaporation of POPs;
- hot water injection—a process similar to the two processes presented above, introducing hot water this time instead of steam or hot air;
- heating by electrical 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 groundwater and the water from the soil creating the conditions of evaporation of POPs;
- radio-wave heating—a process that involves placing an antenna that emits radio waves in a well; radio waves heat the soil producing evaporation of POPs.

In some cases, thermal desorption is done also ex situ, which is a more expensive process but in which the treatment process is much better controlled (Abhilash et al. 2013).

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

Environmental processes that govern the behavior and fate of POPs in soil, surface, and groundwater can be classified into three categories:

- transport processes, associated with POPs migration,
- transfer processes, which control the movements of POPs in environmental compartments (water, biota, suspensions, sediments, atmosphere); the transfer refers to the manner of distribution of a chemical compound between the solid and the liquid phase (for example, between soil and water), or between the solid and gaseous phase (between soil and the air contained therein) or between a liquid and a gaseous phase (water–air).
- transformation processes refer to the biological and chemical processes that change the structure of a substance or degrade it completely.

In general, the short-term behavior and long-term fate of POPs in the soil-water system are determined by the physical, chemical, and biological properties of POPs (determined by its chemical structure) and by the environmental conditions. Organic compounds undergo a multitude of transformations in the environment. Most POPs degrade over time as a result of several chemical and microbiological reactions in soil. Some decay due to the action of sunlight. At the end of the degradation process of the compound results mineral compounds, CO_2, H_2O, HCl, SO_2, etc. During degradation, some compounds form degradation intermediates whose biological effects can be significant for the environment. The most important degradation processes are biological processes (biodegradation) and physico-chemical processes—hydrolysis, photolysis, etc. (Zhang et al. 2014).

6.3.1 Photochemical Degradation

An important physical parameter is light, especially ultraviolet solar radiation. The intensity of this energy is high enough to degrade some chemicals. Factors that influence photodegradation of pesticides are: light intensity; exposure time; site properties; method of application; properties of the pesticide.

6.3.2 Hydrolysis

In the aquatic environment, in moist soil and in groundwater, hydrolysis can be an important degradation mechanism for some compounds.

6.3.3 Biological Processes

The ability of microorganisms to decompose organic molecules depends in part on their ability to adapt to a new potential food source. Physical factors such as temperature, light, and nutrient availability in the environment play an important role in microbial degradation. Chemicals must be accessible to microorganisms or to be within the range of enzymes to be degraded by microbial processes.

6.3.4 Microbial Degradation

Biologically mediated structural alteration of an organic compound resulting in metabolite formation is the main process that affects the persistence of pesticides in the environment. This process is dependent on many factors including: chemical structure of POPs, its concentration, microbial population density, temperature, concentration of the present nutrients, oxygen content, pH, climatic conditions, as well as the presence of other POPs that can increase or decrease the reaction rate (Abhilash et al. 2012).

Modelling of environmental systems takes into account their complexity and the interactions they have with various other systems and which are sometimes difficult to define.

Patterns of dispersal of POPs in rivers. Accidental pollution of rivers is a problem of major importance for the society and economy, with short- and long-term impact on the environment. Industrial and municipal waters contain a wide range of POPs and contaminants: organic compounds, inorganic compounds, microorganisms, solid suspended substances. The sources of pollution of surface waters, and in particular of rivers, are diverse:

- POPs of domestic origin (from wastewater from the city sewerage network);
- POPs of industrial origin (from industrial units discharges);
- POPs of agricultural origin (rainwater that washed agricultural lands treated with chemical fertilizers, herbicides, pesticides, waters resulting from technological processes related to animal complexes or waters infested with the liquid fraction of animal waste).

In the context of the documentary and experimental studies elaborated within this stage it can be stated that the analyses regarding the problems related to air, water, and soil pollution generated by crossing over certain limits of the concentration of different POPs are in a relationship with the ecological impacts and health risks. Based on them, mathematical models of different complexities regarding contamination will be developed and validated, most of them focusing on air, soil, water, air-water, air-soil, soil-water pollution. The main types of models are based on deterministic and stochastic differential equations (ordinary differential equations, partial derivative equations), static algebraic equations, Petri networks, mathematical programming, stochastic programming, optimal control theory, Markov chains, Markov processes, Monte Carlo simulation, models based on mathematical equations (Nøst et al. 2018). The properties of POPs and the factors that influence the persistence, mobility, bioavailability, and bioaccumulation, systematized based on the documentary studies performed are presented in Table 6.3.

Such a model, regarding the phenomenon of air pollution, is the one based on the theory of stochastic processes, where it is considered that, for longer periods of time, the degree of pollution can be described by means of a multidimensional stochastic process: $X = (Xt)\mathrm{t} \geq 0$. It can also be used to construct the empirical distribution function of the studied stochastic process. This construction is made on the basis of historical data regarding the concentration in the atmosphere of polluting factors. With the help of the empirical distribution function, it is possible to estimate the possible risk that the level of concentrations in the atmosphere of polluting factors will exceed various limits.

Regarding the risks associated with the impacts of chemicals on the environment and on human health-existing actions and new initiatives, the studies performed at this stage show that the task of evaluating the effects of POPs and designing regulations to ensure a safe framework for use has become one of great importance, due to the large number of substances that must be covered.

It has proved that is impracticable to examine each potentially negative property of the substances. It is therefore important to minimize the amount of substances released into the environment as a precautionary measure, even when their harmful properties have not yet been proven. The discharge of hazardous substances can be reduced by prohibiting and/or imposing restrictions on the manufacture and use as well as reducing emissions from industrial plants. There is also a need for a scientific certainty to justify risk reduction measures. For this reason, there are debates on the reorientation of policy in Europe, in the field of chemicals towards a greater weighting of the precautionary principle with environmental groups belonging to EU Member States (Oterhals et al. 2010).

The impact on the environment in case of accidental pollution of the rivers can be manifested on short or long term and is a problem of maximum importance for both the quality of life and the economy. For this reason, a large number of models of dispersal of POPs in rivers have been developed, at a certain distance downstream from the discharge point. In the dispersion zone, the dilution of POPs is caused by several processes taking place simultaneously: convection due to river flow, turbulent diffusion due to concentration gradient, degradation of POPs due to reactions, accumulation of POPs in river sediments.

The resulting mathematical model taking all these aspects into consideration is extremely complex, complicated, difficult to perform, and inoperable for real-time simulations. The fluid dynamics in rivers is strongly influenced by the geometric shape and roughness characteristics of the riverbed, and the approximation of the diffusion characteristics requires the introduction of a turbulence model. If is added the biochemical processes of degradation of POPs and the eventual processes of thermal transfer between the stream and river, it results a complex mathematical model which describes all these aspects in detail (Trivedi and Majumdar 2013).

Table 6.3 Properties of POPs and factors of influence

Propriety	Category	Definitions	Factors of influence
Adsorption in the soil	Weakly adsorbed $K_{oc} < 300$ Moderately adsorbed $K_{oc} =$ 300–1000 Highly adsorbed $K_{oc} > 1000$	The accumulation/adhesion of POPs on the soil surface as a result of the electrostatic attraction between the opposite sign loads of the POPs, respectively, the soil and is expressed by: The adsorption coefficient (partition). The adsorption coefficient (partition) (degree/rate of adsorption/sorption) K_{oc}, which represents the ratio between the amount of POPs in the soil solution and the amount adsorbed by the soil	Physico-chemical properties of POPs: water solubility, polarity, stability (related to chemical structure). Soil properties: content of organic matter and clay, texture, humidity, pH, superficial loading, permeability, distribution of soil particles, temperature
Bioaccumulation	Hydrophilic log $K_{OW} = -3$ to 3 Moderately hydrophobic log $K_{OW} = 3$ Very hydrophobic log $K_{OW} > 6$	The gradual increase in the amount of POPs in an organism or part of it due to the fact that the rate of penetration of the substance into the body far exceeds its capacity to eliminate the substance and is expressed by the partition coefficient. The octanol/water Kow partition coefficient, which indicates how POPs can be distributed between two immiscible solvents: water (polar solvent) and octanol (relatively non-polar solvent, representing fats)	Physical properties of POPs: insolubility in water, solubility in fatty tissues
Bioavailability		The speed and degree of adsorption of POPs or metabolites in an organism and the extent to which it is available for metabolism or for interaction with significant biological receptors. This involves both release from an environment (if applicable) and absorption by an organism	Physical and chemical properties of POPs: octanol/water solubility, concentration, pKa sorption/desorption on soil particles. Environmental compartment characteristics: dissolved organic matter (concentration and molecular structure), total organic carbon content (concentration, composition), soil particle size distribution, pH, temperature, presence of co-POPs. Characteristics related to the body: lipid content, eating habits and life history, elimination processes

(continued)

Table 6.3 (continued)

Propriety	Category	Definitions	Factors of influence
Persistence	Non-persistent <30 days Moderate persistent 30–100 days Persistent >100 days	Lifespan of a chemical species (primary chemical product or by-product) in a specific compartment (air, soil, water and living organisms). The resistance of a POPs to any modification of its chemical structure	Composition and characteristics of environmental compartments: clay/sludge/sand ratio of soil, water content, organic matter, pH, microbial biomass, temperature. Environmental conditions: intensity of sunlight, temperature, wind, humidity. Chemical properties of POPs: susceptibility to oxidation, reduction, hydrolysis, photolysis, substitution. Physical properties of POPs: vapor pressure, water solubility, dissociation constant, partition coefficient, soil adsorption, water and soil volatilization
Solubility in water	Insoluble $S_w < 1$ ppm Moderate soluble $S_w =1–30$ ppm Very soluble $S_w > 30$ ppm	The tendency of POPs to dissolve in water. The maximum concentration of POPs that dissolve in one liter of water and is expressed in ppm. Parts per million ppm, representing the ratio of quantities expressed as POP parts per sample (1 mg/L)	Soil characteristics: moisture. Environmental conditions: soil/water temperature, air pressure. Physical and chemical properties of POPs: chemical structure, dissociation constant, affinity for water or soil
Toxicity	Extremely toxic LD50 < 50 mg/kg—Very toxic LD50 = 50–200 mg/kg—Moderate toxic LD50 = 200–1000 mg/kg—Slightly toxic LD50 > 1000 mg/kg	The ability to cause harm to living organisms by the amount of substance administered or absorbed, how the substance is administered and distributed over time, the type and severity of the damage, the time required to produce the damage, the nature of the affected organism and other relevant conditions and is expressed by the Lethal Dose. Lethal dose 50, LD50, defined by the minimum amount of substance, usually given in mg/kg, capable of killing 50% of the population of tested animals	Properties related to the structure of POPs: term of guarantee, dose, duration of exposure, route of entry or absorption by the living organism

(continued)

Table 6.3 (continued)

Propriety	Category	Definitions	Factors of influence
Volatility	Non-volatile $H < 3 \times 10^{-7}$ A little volatile: $H = 3 \times 10^{-7}$ to 1×10^{-5} Moderate volatile $H = 1 \times 10^{-5}$ to 1×10^{-3} Very volatile $H > 3 \times 10^{-3}$	The conversion of POPs from the solid/liquid phase to the gas/vapor phase by heating, pressure reduction, by a chemical reaction, or a combination of these processes is expressed by Henry's Constant. Henry's constant, which is the ratio between vapor pressure and solubility of POPs	Soil composition and characteristics: texture, organic matter content, humidity. Environmental conditions: temperature and air movement, relative humidity, temperature. Method of application (in the case of pesticides) on the soil or on the crop. Physical properties of POPs: vapor pressure, solubility, heat of vaporization, diffusion rate in air

The disadvantage of such a model is the difficulty of making and solving it because of the many parameters that must be known. The flow conditions can also be included in reduced mathematical models, by adopting simplifying hypotheses, with a greater or lesser degree of approximation of the real phenomenon. In practice, the used dispersion models have the degree of complexity correlated with the adopted simplifying hypotheses and with the inclusion, or omission, of some terms in the transport equations.

The bidimensional dispersion model consists of the continuity equation and the equations of motion on two directions (x, y) in which the property gradients are considered significant, supplemented by the mass balance of the POPs. The third equation in the system describes the conservation of the mass of POPs discharged into the river and contains the chemical, biological reaction rates (ri) of the POPs in the river. The obtained solution is of parabolic form, taking into account the simplifying hypothesis of the constant depth of the river in the direction of flow ($dh/dy = 0$) (Song et al. 2019).

6.4 Treatment of the Water Used in Wastewater Treatment in Watershed

For optimum operation, the wastewater treatment in watershed (WWT) is designed to take over a certain wastewater flow, with a certain maximum load of POPs. A classic wastewater treatment station consists of a series of sections linked in sequence: mechanical-chemical treatment (primary), biological treatment (secondary), and sometimes advanced (tertiary) treatment. The first mechanical step consists of retaining suspended solid materials, with dimensions larger than 0.2 mm, with the help of grills and desanders, followed by decanting of solid suspensions (with particle sizes up to 2–5 μm) and separation of non-immiscible liquids with water (with lower density, such as fats or petroleum products).

The chemical stage is combined with the mechanical one and involves the elimination, in an advanced degree, of the small solids (colloids) by precipitating them with the help of coagulants, as well as reducing the pH of the acid waters. The biological step consists in the passage of waste water over an active sludge (formed by colonies of microorganisms) and the biological degradation of soluble POPs (such as nitrates, nitrites, phosphorus compounds, organic compounds). A biological treatment unit also includes an active sludge separator, provided with recirculation and purge of surplus sludge. Advanced wastewater treatment involves complex methods, with high-costs, in an additional stage (tertiary). The methods used include: the use of ion exchangers, desalination membranes by reverse osmosis, electrodialysis, chemical processes (oxidation/reduction) for the transformation of recalcitrant POPs into simple, easy to remove compounds (Thevenon et al. 2013).

The optimization and advanced control of each stage of treatment leads to the improvement of the WWT station's performance and the reduction of the risk of uncontrolled discharges, with the possibility of taking an overload of treatment (respectively higher flows and/or higher loads of POPs). The biological stage is the most sensitive regarding the quality of the influencers (concentrations, flows) and the operating conditions (temperature, pH, the presence of additives/inhibitors, the efficiency of aeration, the liquid and biomass stationary times, the mixing mode in the basins, the flexibility of the sludge separator, and efficient recirculation of active sludge). The removal of POPs is done in this step by the action of microorganisms, in aeration basins, in the presence of nutrients and other additives that contribute to the maintenance of the biological process. The evolving and adaptable community of microorganisms is highly sensitive to changes in flow characteristics that are processed in WWT (concentrations of POPs, the presence of certain chemical or biological compounds), due to the many variables that influence the process being difficult to reproduce and presenting a wide range of time constants, nonlinearities, and even instabilities. To reduce the risk of overloading the station, of malfunctioning, and uncontrolled discharges into rivers, there were proposed a large number of constructive, operating, or control variants of the biological step:

- the use of several biological steps, operated differently, arranged in series; thus the conditions of bioaccumulation are created in each sub-unit of specialized microorganisms for the removal of certain types of POPs;
- the use of a complex liquid and sludge circulation scheme between the biological treatment sub-units with active sludge;
- integration of chemical treatment with the biological treatment, in various variants: chemical pre-treatment and induction of increased biodegradability;
- the use of modern, high-performance biological treatment units (membrane reactors, with biofilms, etc.);
- optimal operating policies having as criteria the reduction of the WWT station's risk to the influencer overload.

The organic constituents of the soil are nonhumic compounds (fresh organic debris, intermediate products of decomposition of plant mass and fauna found after death in soil and microorganisms participating in different biological processes) and humic compounds (macromolecular organic substances with very complex structure, resulting from polycondensation and polymerization of decomposition products). Soil microorganisms play an essential role in the catalysis of redox processes. The roots of the plants release in the soil organic acids, along with the exudates and play a very important role in the circuit of the elements in the soil. The soil area strongly influenced by the activity of the plant roots is called the rhizosphere. Pollution with biodegradable organic compounds occurs when large quantities of organic compounds that are substrate for microorganisms are discharged into natural receptors. In the processes of decomposition, the present oxygen in the natural receptor is consumed and it results in the decrease of the concentration in oxygen which causes negative effects on the water biota. Organic effluents contain large amounts of solid suspensions that prevent or absorb the light required for photosynthesis, which leads to alteration of the characteristics of the natural receptor (Le et al. 2017).

Organic compounds consist of proteins (65%), carbohydrates (25%), lipids (10%), nucleic acids, and a multitude of combinations thereof. The most typical pollution with biodegradable organic compounds is that with domestic water (fecaloid-domestic). In nineteenth century England, the first sewers were built to solve the problem of waterborne epidemics; the domestic wastewater was discharged into the Thames which they transformed into a dead river that released a pestilential odor and because of that in the windows of parliament were hung cloths soaked with calcium chloride. As a result, the first water treatment plants were built (Urase et al. 2008).

Another source of pollution with organic compounds is the industry, especially the cellulose and paper industry, the food industry. Organic compounds that reach lakes and rivers decompose in the presence of oxygen with the help of microorganisms. If there is sufficient dissolved oxygen, degradation is aerobic with oxygen consumption, with the elimination of carbon dioxide (respiration) and water. If oxygen is insufficient, conditions are created for anaerobic degradation processes in which carbon is converted to methane and carbon dioxide. In this case nitrogen compounds participate in denitrification reactions; the sulfate is reduced to hydrogen sulfide. These processes occur frequently in lakes and marshes, but very rarely in rivers. A particular case of pollution with organic substances is the pollution with petroleum products that even in low concentrations give color, odor, and taste to the waters in which they are eliminated. The main processes that petroleum products undergo are transport, transfer, and transformation. Petroleum products are lighter than water and therefore form a film on the surface of the water that prevents oxygenation which can lead to ecological imbalances (Xu et al. 2019).

Suspensions in rivers and lakes carry nutrients, organic and inorganic pollutants. These suspensions result from natural erosion, from the endogenous production in waters from algae (maximum 20 mg/L in eutrophic waters), following the precipitation of calcium carbonate in hard and high alkalinity waters, but also from the anthropic activities. Drinking water should not contain suspensions. Fine suspensions are difficult to remove and cause clogging of transport pipes, of filters, confer unpleasant taste and odor, disrupt the disinfection process, transport toxic substances, heavy metals, and cause CBO_5 growth.

A problem associated with the presence and persistence of metals in the environment, especially in waters, is bioaccumulation (a biochemical process that causes an increase in the concentration of a chemical in an organism in a larger proportion than in the surrounding environment including food) and biomagnification (the process by which the concentration of the chemical becomes higher than that in the food when the main way of entering the body is the ingested food) which results in a higher amount of exposure to the contaminant of some organisms in relation to its value from the environment (species of coastal fish, seabirds are monitored for the presence of heavy metals). POPs can produce lethal or non-lethal effects through direct or indirect exposure, both for short and long term (Zhong et al. 2017).

Pesticides cause the death of some species; they cause behavioral and physiological changes of wild species. As a result, the reproductive function of individuals of these species is altered. The result can be seen due to their concentration in the food chain. The toxic effects of POPs in general and of pesticides in particular are related to their chemical structure, by the mechanism of action and transformations that occurs in the animal body. Animals respond differently to exposure to a particular POPs and the response is dependent on species and individuals. Thus, some animal species metabolize POPs into non-toxic metabolites, while others cannot provide a specific response. The biological (biodegradability) and chemical degradability of the various synthetic POPs is extremely different. In various environments, some persist for weeks (organophosphorus insecticides), others for months (triazines), and others for a long time (over 10 years for DDT). Some POPs are retained or decomposed by the usual water purification procedures, while others are almost unchanged (lindane, pentachlorophenol) and accumulate in aquatic organisms (Chen et al. 2014).

6.5 Models of Dispersal of POPs in Air

The problem of the prediction of atmospheric pollution aims to determine, based on dispersion models, the conditions for the discharge of POPs into the atmosphere, from an intermittent or continuous source.

These models determine, depending on the characteristics of the source (continuous, instantaneous, low, high), the quantity discharged, the meteorological conditions (wind, temperature), and the topology of the area (surface obstacles), the dynamic evolution of the concentration field of POPs over a distance of up to 10 km, as well as the size of the affected area. Since most of the discharges are in jet form, the dispersion model is usually solved in two stages:

- the evolution of the effluent plume in the jet and transition area above the waste basket;
- the evolution of the effluent in the dispersion zone, in which the effluent loses its own energy and evolves only by the action of the dynamics of the external environment.
- From the point of view of the evacuation mode, respectively, of the duration of POPs emission, there are three types of models: instantaneous emission (in the form of cloud, “puff”), continuous emission with constant flow (in the form of plume, “plume”), continuous emission with variable flow.

From the point of view of relative density in relation to the air of the emitted POPs, there are three possible cases: gas/light vapor (average density lower than air density), gas/normal vapors (average density comparable to air density), gas/dense vapors (average density higher than air).

Weather conditions from the time of POPs evacuation play an essential role on the mode and speed of its dispersion in the air. To be used in dispersion models, the atmospheric conditions are classified into six Pasquill stability classes, noted from the letters A (the most unstable) to letter F (the most stable). Atmospheric stability is related to wind speed and intensity of sunlight, defined in terms of vertical thermal gradient.

In an expression quantified by Richardson’s number (ALR = decrease rate of the atmospheric temperature under adiabatic conditions = 0.98 °C/100 m), the thermal gradients lower than the ALR define the stable conditions, and the higher than the ALR define the unstable ones (Tao and Vidrio 2019).

6.6 Ecotechnologies for the Degradation of POPs

Bioremediation, including stimulation of native microflora, bio-augmentation, phytoremediation, and rhizoremediation, are methods of purification for the soil contaminated with POPs that have already become used. Technologies for using microorganisms for soil and water decomposition of POPs (pesticides, petroleum products, detergents, drug residues) include creating conditions for activating their destructive capacity, use of bioreactors as appropriate for detoxifying highly polluted soils.

The stimulation of the destructive capacity of the native microflora can be done by inducing additional sources of carbon, nitrogen, hydrogen peroxide, by oxygen aeration, maintaining high humidity, etc.

In the cases of organochlorine pesticides, the most effective decomposition pathway is reductive bioremediation. The depletion of the oxygen supply from the soil creates a very low redox potential, which promotes the dehalogenation of chlorinated organic compounds.

Oxygen limitation and supplementation with specific nutrients for anaerobic/optionally anaerobic soil-destroying microorganisms (peptone, yeast extract, ethanol, etc.) result in efficient disposal of existing POPs residues.

Another widely used principle of anaerobic bioremediation is the conventional composting of soil contaminated with manure or bovine blood, to stimulate the growth and development of native anaerobic microflora, as well as different mineral salts based on P, N, etc. (Sagarkar et al. 2014).

6.6.1 Phytoremediation of POPs

Organic pollutants are released into the environment through leakage (fuel, solvents), military activities (explosives, chemical weapons), agriculture (pesticides, herbicides), industry (chemical, petrochemical), wood treatment, etc. Unlike inorganic pollutants, organic pollutants can be degraded to stable intermediates or even mineralized to inorganic compounds (e.g., CO_2, H_2O, N_2, Cl_2) by some plants and their associated enzymes.

This process is called phytodegradation. Degradation of POPs can be accomplished by phytoremediation due to a combination of mechanisms including microbial degradation promoted by plants, absorption and accumulation of plants, phytovolatilization, and phytodegradation. POPs are either degraded in the rhizosphere (rhizodegradation) by root exudates, meaning enzymes that catalyze the degradation of contaminants to simple organic molecules, or by the action of microbes in the rhizosphere.

The microbial activity in the rhizosphere is amplified by the root exudates, so that combining the growing plant with the microflora creates an environment in the rhizosphere suitable for the degradation of the contaminants.

Plants can also take up POPs and then they will be degraded to simpler molecules by enzymatic transformation into the plant tissues (phytodegradation). The phytoremediation term was first used in the 1980s, but its rapid expansion in remediation of POPs began at the end of the last century. Low molecular weight POPs, such as some pesticides, can be transported through plant membranes and removed from the soil. These can be released through the leaves by evapotranspiration (phytovolatilization) processes (Haith et al. 2002).

Non-volatile components can be degraded (phytodegradation) or become nontoxic through enzymatic modifications and sequestration in the plant (phytoextraction) or are degraded by the microorganisms present in the rhizosphere (rhizodegradation).

The compounds isolated in the plants can be removed with the biomass for incineration. The activity of the plants depends on the environment that we want to recover, the type of plant used, and the properties of the contaminant. Some plants have been shown to be able to absorb organic pollutants from soil contaminated by the root mass and leaf cuticle, POPs that can be translocated to other parts of the plant for enzymatic degradation or storage.

The absorption of POPs by plants depends on the physico-chemical properties of the compounds, the application mode, soil type, climatic factors, and plant species. POPs absorbed through plant roots can be translocated elsewhere by xylem. Permeation from plant roots to xylem is, however, optimal for those compounds that are only slightly hydrophobic.

Hydrophilic compounds, on the other hand, have limited absorption through waxy cuticles of the leaves. Microbial activity in the rhizosphere also plays a crucial role in transforming POPs, which can help root absorption and subsequent degradation in plants. Therefore, microbial transformations in the rhizosphere should be considered as an integral component of phytoremediation. A number of studies have attempted to determine the absorption and translocation of persistent pesticides in plants.

For example, rapeseed seedlings grown on lindane-treated seeds (γ-HCH) or maize plants grown on lindane-treated soil were reported to absorb and translocate the pesticide. For semi-volatile POPs, the application form also plays an essential role in the uptake of the plants. The application of 14C-lindane together with the nutrients for the coffee plants resulted in the absorption and accumulation of pesticides in the roots of the plants.

Compared to this, almost 90% of the pesticide was lost due to volatilization when applied to the leaves of coffee plants in pots. There are significant differences in plant tolerance to pesticides. One study showed that the absorption of DDE (an organochlorine insecticide) was specific for certain subspecies (Qiu et al. 2019).

The Cucurbitaceae family achieved significantly higher absorption; for example, in this case there were found specific mechanisms of absorption for the pesticide dieldrin. It has also been observed that sunflower has the highest phytoextraction capacity of endosulfan compared to tomatoes, soy, or lucerne. However, in the case of soils polluted with DDT (organochlorine pesticide), tomato plants seem to be the most suitable candidates for phytoremediation. Phytoremediation of POPs is affected by a number of factors. Their low bioavailability in the soil may limit the success of this technology.

To increase the ability of POPs to be transferred from soil to plants, some of the naturally occurring low molecular weight organic acids excreted through the roots have been used to improve phytoremediation; a study showed that more organic acids increased the desorption of DDE (Dichlorodiphenyldichloroethylene).

The effects of citric and oxalic acids exuded in the root of plants on the desorption of DDT (dichlorodiphenyltrichloroethane) in soils have also been studied. These natural organic acids alter the organo-mineral bonds that disrupt the soil structure and lead to the release of metals and fractions of organic carbon in the aqueous phase. Pesticides are usually combined with this fraction of organic carbon and therefore, desorption has been increased. Also, surfactants can increase the desorption of pesticides from the soil to be degraded.

Studies have shown a substantial translocation of heptachlor epoxide, a smaller translocation of T-chlordane, but no translocation of dieldrin in the types of feed from pastures from soils contaminated with residual pesticide. Residues of dieldrin, lindane, DDT, and DDE, absorbed from soil contaminated with pesticides, mainly accumulated in roots of *Lolium perenne* grass, while only traces were detected in plant shoots (Khairy et al. 2016).

Similarly, DDT and aldrin residues absorbed from soil were concentrated in the shell of the contaminated sweet potato tubers. A study showed that in 12 cultures of tested food plants, chlordane residues were accumulated from the soil in the root tissues, which were also translocated into the aerial tissues. Chlordane residues were detectable in edible root tissues of carrots, beets, and potatoes, as well as in edible aerial tissues of spinach, salad, dandelion, and pumpkin, small quantities in edible portions of eggplants, but not in the edible parts of tomatoes, peppers, and corn. As a recent progress in phytoremediation, transgenic plants that have specific pesticide degradation enzymes have been developed over the last decade.

The so-called new genes involved in metabolism, absorption, or transport of specific POPs in transgenic plants allow overcoming some of the disadvantages of phytoremediation, such as high concentrations of pesticides or elimination of plants that accumulate POPs. Once pesticides are degraded by transgenic plants specific to some non-toxic or completely mineralized metabolites, the plants can be safely disposed of (Cao et al. 2015).

Although field applications have not yet been regulated due to their potential impacts on the environment and biodiversity, in the near future, this strategy could receive an increased attention. Most products of POPs transformation from plants are not intended for excretion, but remain in plant tissues either in soluble conjugated forms or in insoluble bound forms.

Although mineralization (complete decomposition) of pesticides is the desired end point in phytoremediation, usually a few transformation reactions are sufficient for the organic substance to drastically change its biological activity. Three main types of reactions lead to the transformation of pesticides in the plants:

- degradative such as oxidation, hydrolysis,
- rearrangement (epoxide formation),
- syntheses, e.g., conjugations.

The first step in the degradation of chlorinated aliphatic hydrocarbons is chlorination. This is supposed to be a reductive dechlorination with the necessary electrons, obtained from the reduction of the organic compound. Reductive dehalogenation was reported to be responsible for the degradation of the following compounds: chloroform, carbon tetrachloride, 1,2-dichloroethane, 1,1,1-trichlorethane, 1,1,2,2-tetrachlorethane, trichloroethylene, and tetrachloroethylene under methanogenic conditions with the addition of acetate as the main carbon source. The degradation test was performed on chloroform and carbon tetrachloride. C-14 carbon measurements indicated that these compounds were almost completely oxidized to carbon dioxide by the methanogenic population.

The described experiments had good results in the presence of a carbon source much higher in concentration than in the studied compound. In the presence of molecular oxygen only a few chlorinated aliphatic hydrocarbons are biodegradable. Degradation of dichloromethane by a mixture of microbial population was observed. In more detailed studies the degradation pathway was better clarified with isolated bacteria growing on dichloromethane as the single source of carbon and energy (Jesus et al. 2016).

Both *Hyphomicrobium DM2* and *Pseudomonas* sp. strain DM1 were observed to convert Dichloromethane (DCM or methylene chloride) by nucleophilic substitution reaction with a halidohydrolase to Dichloromethane (DCM or methylene chloride). This intermediate could then be spontaneously broken down to formaldehyde which is assimilated by the serine pathway. In one study it was found that dichloromethane could be preferentially degraded on acetate by *Pseudomonas species LP*.

Halogen substitution in aromatic hydrocarbons leads to compounds that are generally less susceptible to microbial degradation.

However, microbial degradation of halogenated aromatic hydrocarbons was observed and microorganisms capable of being developed on a number of these compounds were isolated. It is suggested that catabolic reactions can occur through two types of mechanisms: the cometabolic type of the process and by the common mineralization process.

Under anaerobic conditions, together with the processes called dechlorination, the degradation of chlorinated aromatic compounds also requires a stage of breaking the aromatic cycle. Under aerobic conditions this rupture is preceded by a hydroxylation in which molecular oxygen is incorporated through an oxygenase.

Anaerobic bacteria cannot carry out oxygen reactions. That is why they are forced to use different enzyme systems to break the cycle. In anaerobic conditions, non-halogenated cycles are the first to be dearomatized. The breakage of the cycle subsequently occurs through a hydrolytic step. The basic requirement for breaking the cycle under anaerobic conditions, however, is the presence of oxygen in a substituent on the cycle. Most of the chlorinated monoaromatic hydrocarbons that have been examined so far are aerobically degraded by microorganisms that leave the C–Cl bond intact until the formation of chlorcatehins with or without an additional carboxyl or phenol group as a substituent. They can be transformed into chlorcatehine through different stages by different enzymes. Chlorocatechols may undergo ortho and meta breakdown, which is accomplished by dioxygenase catalyzed reactions, reactions in which both molecular oxygen atoms are incorporated. The isolation of bacteria capable of using chlorobenzene and 1,2- and 1,4-dichlorobenzene, 1,2,4-trichlorobenzene as the single source of carbon and energy, indicates that there must be a degradation path leading to the total mineralization of these compounds (Elango et al. 2011).

The resistance of halogenated aromatic compounds to microbial degradation is dependent on the number and position of the halogen atoms in the cycle. An increase in the number of halogen atoms in the ring would result in a higher resistance to microbial attack. However, it has been found that the widely used pesticides based on pentachlorophenol represent the single source of carbon and energy for a few bacteria.

Most of the chlorinated monoaromatic hydrocarbons that have been examined so far are degraded by microorganisms that leave the C–Cl bond intact until the formation of chlorcatehins with or without an additional carboxyl or phenol group as a substituent, can be converted to chlorochlorine at different stages by different enzymes.

6.6.2 Removal of Identified POPs, Ultrasound and Biodegradation

After irradiation of ββ dichlorodiisopropyl ether from aqueous solutions it was observed that the decomposition of the ether occurs faster in the solution in which air was bubbled than in the other. Thus, after 40 min of ultra-acoustic irradiation, the ββ′ ether was completely decomposed, for the bubbled solution, while for the second case; the efficiency was about 90%.

The TOC and CCO–Cr analyses indicated that ββ′ ether was not completely mineralized to CO_2 and H_2O_2, being transformed into other organic compounds. After 40 min of acoustic irradiation, the amount of carbon initially present in the solution was almost 85% removed for the first case and about 60% for the second.

From the experiments of ultra-acoustic irradiation of waters containing propenoxide, 1,2,-dichlorpropane and ββ′ ether, after 90 min., for CCO–Cr are observed removal yields of 32%, respectively, 44.8%, for propenoxide oxide removal yields of 88.7%, respectively, 89.3%, for 1,2-dichloropropane, removal yields of 91.7% and 92%, and for ββ ether, removal yields of 86.6% and 92.5%. The analysis of the kinetic parameters that describe the dynamics of the biodegradation process of dichlorodiisopropyl ether, 1,2-dichlorethane, and 1,2-dichlorpropane led to the conclusion that all substances can be considered as biodegradable, the basic condition being the adaptation of the biological sludge to the substrate and the conductance of the biodegradation process with permanent pH control in the case of 1,2-dichloropropane and 1,2-dichlorethane (Hauck et al. 2001).

Regarding the degree of biodegradability of the tested compounds, they can be classified according to the biodegradability potential as follows: ββ dichlorodiisopropyl ether, 1,2-dichlorethane, 1,2-dichlorpropane.

From the biodegradation experiments a trichloroethylene removal efficiency of 65% is observed, taking into account the results of the TCE stripping experiments ($\eta = 35\%$ by stripping), for 70% perchloroethylene, taking into account the results of the PCE stripping experiments ($\eta = 30\%$ by stripping), for 1,2,4-trichlorobenzene by sludge adsorption and biodegradation, of 90%, taking into account the results of the stripping experiments, ($\eta = 10\%$), for hexachlorobutadiene of 30%, taking into account the results of the active sludge stripping and adsorption experiments for hexachlorobutadiene ($\eta = 70\%$).

From the data obtained for all variants of experiments of biological treatment with active sludge in the continuous flow micropilot system, it is observed that the best removal yields of the organochlorine substances were obtained for the contact time 10 h, with removal yields for all three substances tested over 96.9%, while the best purification yields for organic loading expressed in CCO–Cr and CBO_5 were obtained for the contact time 12 h, with purification yields of over 85% for CCO–Cr and over 80% for CBO_5 (Cernava et al. 2019).

The very low biodegradability and high solubility in lipids of pesticides, especially organochlorines, cause problems regarding bioaccumulation in animal and plant tissues.

Because organochlorine pesticides are not metabolized and excreted by fish, they are biomagnified in the food chain. Organophosphorus and carbamide pesticides degrade rather quickly in the environment, therefore they will have a smaller ecological impact but will have a more toxic effect on human organisms, being classified by the World Health Organization, in Class I pesticides (extremely dangerous). Over the decades, numerous epidemiological studies have established correlations between different diseases and the presence in the animal organisms of some pesticides or industrial POPs.

The acute toxicity of different pesticides differs significantly. Very toxic and toxic are considered raticides and insecticides, moderately toxic are considered fungicides and a small number of insecticides and non-toxic are a large part of herbicides.

For organochlorine pesticides included in the Stockholm Convention, acute toxicity ranges from low (Mirex), low to moderate (DDT), moderate (Chlordane, Toxafen, Heptachlor), and high (Aldrin, Dieldrin, and Endrin). Under the action of Toxafen, the vertebrae of the fish become very fragile, at sudden movements they break easily, which leads to paralysis of the front part of the body and death. In terms of bioaccumulation/bioconcentration-DDT and toxaphene are highly hydrophobic contaminants and tend to attach to suspended particles, sedimenting and accumulating at the bottom of lakes and seas, as well as in aquatic biota. Also, the concentration of chlordane is much higher in fish than in the waters in which they live due to its tendency to bioconcentrate.

Many dyes are visible in water at low concentrations (1 mg L^{-1}). The waste water from the textile industry, with a dye content of between 10 and 200 mg L^{-1}, is therefore intensely colored and their discharge into the effluent leads to a series of aesthetic problems.

Because the dyes are chemically and photolithically stable, they are persistent in natural environments. Their presence in these environments presents an ecotoxic and bioaccumulation danger which, in turn, can have a negative impact on the food chain.

The acute toxicity of dyes is generally reduced. The most acute action on algae was presented by cationic dyes (basic). And in the case of fish, the most acute toxic action was presented by the basic dyes, but they also showed a relative sensitivity to a quite large number of acid dyes. Rats mortality showed that only 1% of the 4461 commercial colorants tested had LD50 values below 250 mg kg^{-1} body (Commendatore et al. 2015).

Therefore, the danger of human mortality due to acute dye toxicity is quite low. However, some of them cause allergic reactions such as eczema or contact dermatitis. The value of the bioconcentration factor (BCF) for different groups of dyes determined by the partition coefficient n-octanol-water (K_{OW}) shows that, in general, water-soluble dyes that have low K_{OW} values (acid, basic, or reactive dyes) do not bioaccumulate (BCF log <0.5).

In these cases, there is a linear dependence between log K_{OW} and log BCF and, therefore, water-soluble dyes whose K_{OW} coefficient is high will bioaccumulate.

Organic pigments (water-insoluble compounds) that have extremely high partition coefficients do not bioaccumulate, probably due to their extremely low solubility in water and fats. Also, the BCF values of the dispersed dyes, moderately lipophilic, are quite low.

In both cases, log BCF <2, indicating that none of these pigments or dyes does not show a substantial bioaccumulation (Aranda et al. 2017.).

Table 6.4 presents a series of processes for eliminating pharmaceuticals, hygiene, and endocrine disrupting compounds as well as the efficiencies of these processes.

It is observed that from the point of view of efficiency the best results are obtained by the reverse osmosis process and for certain compounds by degradation under the action of radiation or biodegradation. Pharmaceutical and hygiene products (PFI) have a persistence and bioaccumulative potential similar to those of organohalogenated POPs.

Part of the PFI, such as, for example, nitro- and amino-nitro-mosses has a very high acute toxicity in the aquatic environment. Others, such as selective serotonin inhibitors, can lead to subtle but significant effects among many species, which are not necessarily detectable quickly but lead to ecological changes that can be mistakenly attributed to natural causes. Aquatic life can be extremely sensitive to the action of PFIs, the effects between species, sexes, and types of medicines varying to a large extent (Chen et al. 2017).

Alkylbenzene sulfonate molecules (LASs) contain a sulfonated aromatic core in the para position, attached to a straight alkyl chain in any position, except for the terminal carbons. LASs are the most widely used anionic surfactants in numerous industrial, commercial, and household applications. The overall LASs consumption was estimated at about 22,109 kg in 2000 and 25,109 kg in 2002.

The consumption at European level was about 43,108 kg in 2005. In addition to analyzing the elimination of linear alkylbenzene sulfonate isomers (LASs) from wastewater treatment plants (WWTPs), the efficiency of eliminating POPs from different interest classes has also been studied: antioxidants (BHA and BHT), triazines (Prometon, Atrazine, Propazine, Ametrin), urea herbicides (Diuron, Isoproturon), flavors (Tonalides), antimicrobials (Triclosan), antiepileptic drugs (Carbamazepine).

The mass spectra EI of the homologous series C10–, C11–, C12–, and C13–LAS in the form of methyl ester derivatives depend very much on the position of the chain branch. The structure of the compounds can be obtained by comparing the relative intensity of the ion produced by the rearrangement and fission processes. The ion corresponding to the fission of the adjacent bond to the chain branch ($i = m$ and $i = k$) can be recognized from the ions ratio $(A + 1)/A$. The ratio is greater than 30%, due to the ions produced by the rearrangement processes. The molecular distribution of LAS isomers in water samples can be described by the chromatogram profile of the characteristic ions (m/z 199 as a common ion for all compounds and

Table 6.4 Processes for elimination of pharmaceuticals, hygiene, and endocrine disrupting compounds and the efficiencies of the individual processes

Group	Classification	AC	O3/AOPs	UV	Cl_2/ClO_2	Coagulation/flocculation	NF	RO	Degradation (B/P/AS)
CED	Industrial products	E	F–G	E	P	P–L	E	E	G–E (B)
	Steroids	E	E	E	E	P	G	E	L–E (B)
Ph	Antibiotics	F–G	L–E	F–G	P–G	P–L	E	E	E (B)
	Antidepressants	G–E	L–E	F–G	–	P–L	G–E	E	G–E (P)
	Anti-inflammatory	E	E	E	P–F	P	G–E	E	G–E
	Lipid regulators	E	E	F–G	P–F	P	G–E	E	E (B)
	X-ray contrast media	G–E	L–E	F–G	P–F	P–L	G–E	E	P (B)
CIP	Psychiatric control	G–E	L–E	F–G	P–F	P–L	G–E	E	E (B and P)
	Synthetic musks	G–E	L–E	F	P–F	P–L	G–E	E	G–E
	Solar screens	G–E	L–E	F–G	P–F	P–L	G–E	E	E (B)
	Disinfectants	G–E	L–E	F–G	P–F	P–L	G–E	E	F (P)
	Surfactants /detergents	E	F–G	F–G	P	P–L	E	E	L–E (B)

CED compounds endocrine disruptors, *Ph* pharmaceutical compounds, *CIP* hygiene compounds, *B* biodegradation, *P* photodegradation (solar), *AC* active coal, *AS* active sludge, *NF* nanofiltration, *RO* reverse osmosis, *E* excellent (>90%), *G* good (70–90%), *F* sufficient (40–70%), *L* less (20–40%), *P* poor (<20%)

molecular ions 312, 326, 340, and 354 corresponding to each homologous series). The compounds LASs detected in WWTP contain chains with the number of carbon atoms between 10 and 13, having the concentration of: 47% (C10–LAS), 40% (C11–LAS), 11% (C12–LAS), and 2% (C13–LAS).

The elimination of the 14 compounds from the treatment plants depends on the amount of zeolite. The average removal rate was 26.9% (for 9 g/m^3) and 49.3% (for 15 g/m^3). A positive highlight was observed in the processes of elimination of C12–LAS compared to the homologous series C10–LAS and C11–LAS. In relation to the studied organic compounds, an average removal efficiency of 19% is observed, (with some exceptions) in the case of flux with 9 g/m^3 zeolite and 31% for that with 15 g/m^3 zeolite.

The amount of POPs eliminated was calculated based on the initial flow, where no adsorbent material was added. In all cases carbamazepine elimination is zero. The best removal efficiency is observed in the case of the higher amount of zeolite (15 g/m^3) added at the beginning of the process in the bioreactor. In the studied wastewater, all pesticide compounds (triazines and urea herbicides) were below the detection limit. This can be considered normal, considering that all the water that reaches the respective treatment plant is domestic (and not agricultural) (Dyer et al. 2008).

Monuron (3-(4-chlorophenyl)-1,1-dimethylurea), a herbicide from the urea derivatives group, is commonly used in agriculture due to its property of inhibiting photosynthesis. Persistence in the environment is quite high (about 10 months). Relatively long service life can be a risk in water contamination. Concern over the soluble forms of metals in the aquatic environment has increased in recent years.

Excessive release of these from industrial waters (mining, galvanizing, metal processing, textile industry, bacterial production, pigment production, paints production, oil refining) in the environment is a problem of interest worldwide. Most metal ions become dangerous when their concentration exceeds a certain level and is closely related to the sensitivity of the organisms that consume them. The strict legislation in the field of environmental protection has led to the search for alternative techniques for the removal of metals from industrial wastewater (Donaldson et al. 1973).

The feasibility of using “lowcost” materials obtained from coffee residues in two forms was investigated: unprocessed coffee grounds and pyrolyzed coffee grounds at 500 °C. These natural adsorbents have been used to remove a pesticide from the urea herbicide class (monuron) and traces of metals from aqueous solutions. To compare the efficiency, the active carbon (a very good adsorbent material) was used as a reference material.

In one study, the feasibility of using residues obtained from coffee in two forms was investigated: unprocessed and pyrolyzed at 500 °C, as natural adsorbents for removing monuron and traces of metals from aqueous solutions.

Unprocessed and pyrolyzed at 500 °C coffee grounds were used as low-cost adsorbent materials to remove monuron and metals from aqueous solutions. Monuron had a low adsorption on these materials compared to the activated carbon.

The elimination rate of 18.9%, respectively, 23.9% for the two used materials is relatively small compared to 90.4%, a value recorded in the case of activated coal. The residues obtained from coffee showed an adsorption affinity for some metals, with a real potential for decontamination of wastewater. The direct use of the material, without preliminary treatments, offers some advantages: ease of obtaining and low costs.

This approach also helps to recycle a waste (coffee grounds) and use it for other purposes. The results show that the coffee grounds can be used as a low-cost material, easy to obtain, without additional treatments, for the recovery of elements from aqueous solutions.

The experiments regarding isotherms were performed for 41 micro-POPs in active sludge for which the adsorption kinetics were also examined, because for these isothiazolones the adsorption equilibrium could not be reached due to their rapid biodegradation.

Furthermore, seven compounds from the triazine group and seven from the urea herbicide group were included in the isothermal studies. Based on the results obtained from the adsorption kinetics, the isothermal experiments were performed without the addition of sodium azide, taking into account the equilibrium time set at 1.5 h (Boudesocque et al. 2008).

Freundlich isotherms were determined by linear regression of the logarithmic form of the compounds concentrations in solid and liquid phase, for all analytes for which the concentrations were higher than the quantification limits, comprising six injection levels and covering three orders of magnitude.

Sodium azide (NaN_3) has been shown to be inadequate for deactivating fresh sludge because: it insufficiently inhibits microbial degradation and thus does not influence the water/sludge distribution. This is the first study that confirms that by increasing the concentration of NaN_3, the amount of compound adsorbed on active sludge can be decreased. In addition, the results showed that deactivation of the sludge is not a necessary condition if biodegradation does not prevent the adsorption equilibrium being reached (Hwang et al. 2003).

However, quantification of the analytes in both phases, both solid and liquid, is crucial, if biodegradation cannot be excluded, because only analyzing the concentration in the liquid phase would increase the distribution coefficients. It has been shown that the Freundlich model is suitable for describing the behavior of different micro-POPs in contact with the active sludge. Because, for most compounds, Freundlich exponents were close to 1 and thus $K_f \approx K_d$, the linear model can be used most often for rapid determination of water/sludge distribution coefficients. For a linear approach, the concentrations should be as close as possible to the actual ones in the treatment plants (WWTPs). The retention by plants and animals of the different POPs from soils, sediments, and water is a complex, dynamic process, involving all levels of the food chain.

Numerous equations have been developed for quantitative evaluation of the impact of persistent and/or dangerous POPs on the environment but also on human health.

Considering the large number of POPs that are constantly released in the environment and their diversity, it is necessary to develop complex analytical methods to correctly determine and quantify the behavior of these contaminants.

Among the studied problems of interest are: adsorption of POPs from the class of biocides, triazines, and herbicides based on urea into the active sludge from the biological stage of a treatment plant, the influence of sodium azide on adsorption, water/sludge distribution coefficients, quantification of analytes in both phases, both solid and liquid, determination of linear alkylbenzene sulfonate type compounds from water samples, their mass spectra, their removal from the adsorption treatment plants by zeolites, application of adsorption on zeolites, and other organic pollutants in the group of antioxidants, triazines, urea herbicides, flavors, antimicrobials, and antiepileptic drugs, development and application of materials, obtained from waste, with low costs, in the treatment of waste waters, characterization of these materials, adsorption of the monuron and some traces of metals (Sáez et al. 2001).

6.7 Conclusions and Recommendations

The justification for the selection and classification of priority hazardous substances lies in their behavior in the environment. They do not degrade, but persist in the environment and accumulate in animal and plant tissues, thus presenting long-term risks to human health and ecosystems. As these substances advance in the food chain, their concentration increases the risks to humans and the ecosystem following an identical trajectory. Therefore, it is necessary to control the emissions of these substances, even when their concentration is very low.

POPs are in great decline due to the implementation and support of the Stockholm Convention at EU level through existing directives and laws. Better management control of POPs at the level of Europe is needed because their monitoring is at a poor level, existing countries that do not monitor these substances because analytical determinations are expensive, and many states consider them to be clean from the point of view of POPs.

To remedy the sites polluted with POPs there are innovative technologies and methods, some only in the laboratory stage, with the possibility of being perfected to reach the targets of sustainability and efficiency. From the realized POPs flow, we can say that the weak point is the unintended emission of POPs in the environment and the appearance in the environment of POPs as a by-product, and the solution would be to reduce the production of POPs as a by-product and to retain POPs at the source.

At the level of the classical water treatment stations, the remediation is not total, and finally the POPs reach the environment affecting the ecosystems, therefore the implementation of innovative technologies and methods is necessary. The new methods of treatment and remediation of the environment must also include technologies for the recovery and recycling of POPs in order to reduce the consumption of natural resources and to be able to implement a sustainable economy for the future. Many of the new technologies look promising for a sustainable future in which the recovery or recycling rate tends to be 100% and the resulting by-products are re-evaluated in order to reach "zero waste."

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Chapter 7
Microbiological Aspects of Pesticide Remediation

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7.1 Introduction

Pesticides refer to a group of synthetic chemical compounds or biological agents that are used to combat with the populations of harmful pests. Pests include the organisms like fungi, rodents, insects, and many others that threaten the survival of beneficial plants, animals, and humans. Such pests need to be mitigated to avoid their destructive impacts and use of chemical pesticides is considered to be the most effective method for pest management. Worldwide enormous increase in the use of pesticides has been observed since the past few decades. Pesticides are used in agricultural field to reduce crop damage from pests as well as in homes to control fleas, mites, ticks, rats, etc. or in food storage facilities to avoid contamination by such insects. Composition of pesticides involve active and inert ingredients i.e. active component is meant for killing the pests while inert material enhances the efficiency of active ingredient (Chandran et al. 2019).

Ancient Romans initiated the use of chemicals like sulfur for treating weeds. Later on different chemical mixtures were used for pest control. The time around World War II is marked as a period of massive bloom in the field of discovery and synthesis of many effective pesticides. The use of pesticides reached at its peak in the years 1950–1960. Although pesticides are produced for multiple purposes but mainly these are designated to increase crop yield by reducing destruction and wastage caused by pests. Pest damage to feedstuff is required to overcome for feeding the growing world population. Green revolution led to extensive pesticide use and the resulting crop yield was increased at exponential rates due to reduction in crop loss by pests. This excessive use steered the harmful consequences of pesticides that derived attention to dangers associated with them. Public understanding and

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research related to pesticide direct and indirect impacts resulted in reduced utilization of such chemicals (Mahmood et al. 2016).

7.2 Classification of Pesticides

Pesticide is a general term used for a huge group of compounds including a variety of substances that differ in their physical and chemical properties. Such compounds can be classified into various categories on different basis. The major groups are classified based on the mode of action, target pests, and chemical composition. According to the mode of action, pesticides can be grouped into systematic and nonsystematic pesticides.

Systematic Pesticides The pesticides that produce the desired effect by penetrating in plant tissues and moving through vascular system. For example, glyphosate and 2,4-D etc.

Nonsystematic Pesticides The pesticides that are unable to penetrate the plant tissues but produce the effect by contacting with the target pest hence known as contact pesticides. Examples include diquat, paraquat, etc.

On the basis of target pest species, pesticides can be grouped as different classes like insecticides for insects, herbicides for weeds, fungicides for fungi, and rodenticides for rodents (Tano 2011).

Based on the pesticide properties, they are classified into various groups. Some major classes include:

Organochlorines These include pesticides that are composed of chlorine, carbon, and hydrogen having diversified structures and functions. These are chemical compounds with at least one covalently bonded chlorine, have non-polar nature, and soluble in lipids due to which they can accumulate in the fatty tissues of animals. These are persistent organic compounds that stay in the environment for longer time periods. For example, aroclor, dichlorodiphenyltrichloroethane, and lindane.

Organophosphates These are phosphorus containing compounds with stable cyclic, aromatic, and aliphatic structures. These are soluble in water as well as organic solvents and are less stable than organochlorines. But they have toxic effect on organism's normal body functioning due to their ability to interact with acetylcholinesterase and cholinesterase resulting in disruption of nervous system. Examples include acephate, disulfoton, diazinon, etc.

Carbamates This group includes derivatives of plant alkaloids similar to organophosphates in mode of action that are used to kill vertebrates. These are less persistent to degradation, e.g., fenoxycarb, carbofuran, dioxacarb, carbaryl, etc.

Pyrethroids These are chemical compounds synthesized to mimic the natural insecticidal behavior of plants. These are considered safest to use in houses, non-persistent, and less toxic than other groups. Examples include permethrin, phenothrin, resmethrin, etc.

Neonicotinoids These are synthetic compounds analogous to nicotine that is naturally occurring insecticide. They are substitutes of organophosphates and carbamates used to treat insects such as aphids, etc. They have an efficient activity rate and less toxicity to mammals. For example, clothianidin, nitenpyram, thiamethoxam, etc. (Sharma et al. 2016).

7.3 Applications of Pesticides

Pesticides have various applications which are not limited to agricultural fields but play a role in mitigating pests in different areas. The major sector demanding pesticides is agriculture constituting about 85% of total world's pesticide usage. Pesticides are required to enhance crop productivity and its quality by controlling the pests damaging to plants. A wide variety of agricultural pesticides are available with great specificity for the target pests. Public health is an important concern and many pests act as vectors to different diseases like yellow fever, malaria, plague, dengue, etc. Controlling the populations of such disease carriers is necessary to ensure disease control and health safety. This is possible by the pesticides developed for killing the vectors responsible for disease spread to avoid disease epidemics. These are also applied for water purification to prevent the growth of bacteria, fungi, and other unnecessary organisms. Another important application of pesticides is in different buildings, storage facilities, vehicles, and other areas to get rid of harmful insects, microbes, weeds, etc. to secure public and environment from harmful impacts of pests. Protection of natural reserves and ornamental areas also requires pesticide use. Different products like shampoos, cosmetics, and soaps contain pest repellents and these are used as disinfectants in homes (Garcia et al. 2012).

7.4 Hazards Associated with Pesticides

Despite the benefits provided by pesticides in different sectors, there are various disadvantages of these synthetic chemicals. Excessive use of pesticides harms the natural ecosystem and its functions. The hazardous impacts of pesticides are mostly associated with their persistence in the environment. These are recalcitrant compounds that are resistant to natural degradation process or converted inefficiently resulting in contamination of environment. Pesticides are classified by WHO on the basis of their associated health risks with an increasing toxicity order from lowest to highest represented by numbers I–IV, referring to extremely toxic, highly toxic, moderately toxic, slightly toxic, respectively (World Health Organization 2010). The toxic effects of pesticides can be described in terms of human health and environment.

7.4.1 Environmental Risks

Pesticides pose harmful impacts to the environment resulting in soil, water, and air pollution. These chemicals not only contaminate the environment but also affect the non-target species resulting in loss of beneficial species diversity. This reduction of useful species due to pesticides' inappropriate use threatens the stability of ecosystem. Pesticides have different modes of action to destroy the pests such as organochlorines that act as hormone disruptors and organophosphates are neurotoxic compounds affecting the signal transmission capability of treated insects. These toxic mechanisms are not only limited to target pests but also affect other species in similar way. These compounds have the ability to enter the food chain by accumulating in organism bodies hence targeting a huge population including the birds, insects, and other biota (Carvalho 2017). One of the common examples of pesticide bioaccumulation is destruction of bird populations due to continuous exposure to DDT. This insecticide is an endocrine disruptor and accumulates in the body fats of organisms due to its lipophilic nature. DDT present in soil is taken by the earthworms which are then consumed by birds resulting in loss of the prey's lives (Jayaraj et al. 2016).

Pesticides used at one place are not only present in that area but they are transported to other sites via water percolation, runoff, or through air. The drift of pesticides from the treated site results in contamination of untreated areas leading to unwanted consequences. The persistence of pesticides to biodegradation helps them stay and travel in the environment for longer periods of time and the resultant harmful effects are increased (Yadav et al. 2015). Water contamination is one of such issues which make the water unsuitable for drinking purposes. The pollution of surface water negatively impacts the aquatic organisms (shrimps, planktons, etc.), posing a threat to their health and survival. Most affected among aquatic organisms are fishes being sensitive to water pollution. Pesticides tend to impair physiological properties and health of fishes leading to various ill effects like decreased growth rate, reproductive and nervous system disorders, etc. (Sabra and Mehana 2015).

Air pollution due to volatile pesticides enhances the extent of exposure to non-target species and destruction of natural environment. This chemical use is also detrimental for crops and plants of interest which actually was not intended. The environmental quality is highly affected by such damaging chemicals such as soil health which in turn impacts both crop yield as well as living organisms exposed to such environments. Physical and chemical properties like solubility, stability, vapor pressure, etc. of pesticides are responsible for their adverse impacts. Pesticide interaction with environment depends on variable factors i.e. soil properties, climatic conditions, application rate, and types of plant species (Damalas and Eleftherohorinos 2011; Odukkathil and Vasudevan 2013).

7.4.2 Risks to Human Health

Humans are exposed to pesticides in two ways: either directly during household or agricultural use or indirectly through food. The direct exposure occurs to general population when pesticides are applied in their vicinity like in parks, houses, on roads, etc. They can enter in the human body by oral ingestion, respiration, skin absorption, or through eyes when present in air, water, or soil present around the humans. Indirectly, contaminated food chain is the source of pesticide delivery to human bodies. Intake of food (plants and animals) containing the residues of accumulated pesticides led to their entry in humans. Toxic impacts of pesticides in humans depend on various factors including chemical properties of pesticides, type and duration of exposure, and concentration of pesticide. Furthermore, some populations like infants, pregnant women, and old people are more susceptible to harmful impacts of pesticides (Kim et al. 2017). The effects of pesticides range from acute to chronic in living organisms. Acute toxic effects are short term and occur dramatically after exposure resulting in eye irritation, nausea, fatigue, headache, dizziness, etc. Chronic impacts occur after a long-term exposure to pesticides even when the intake is in very small quantity, such exposures lead to diseases like cancer, leukemia, diabetes, etc. (Sarwar 2015). Studies have revealed damaging effects of pesticides on immune, respiratory, nervous, reproductive, and endocrine systems. Cancers of different types like lung, prostate, breast, and lymphatic are found to be associated with pesticide presence in body. Pesticides are found to weaken the immune system and increase the risks of allergy, fever, and chemical sensitivity in affected people. Neurodevelopmental disorders are developed due to the action mechanisms of specific pesticides that are actually developed to disrupt the nervous system of pests but in humans they led to the development of dementias, amyotrophic lateral sclerosis, Alzheimer's and Parkinson disease. Endocrine disruptors are responsible for development of reproductive and metabolic diseases. Examples include birth defects, developmental disabilities, miscarriages, fetal death, reduced fertility rate, etc. (Gilden et al. 2010; Blair et al. 2015). Pesticides may lead to epigenetic alterations in humans resulting in diseases. Such modifications include DNA methylation, histone modifications, and changes in miRNAs expression profile which could be responsible for noxious impacts of pesticides in humans (Collotta et al. 2013). Due to these problems, pesticide remediation is an important concern for maintaining ecosystem functionality and protecting biodiversity.

7.5 Methods of Pesticide Remediation

Pesticides show specific behaviors in the environment depending upon their physiochemical properties and environmental conditions. The structure and composition of the molecules determine their interaction with the environment. They may be present in vapor phase or attached to the air particles, adsorbed on the soil particles or water sediments, etc. These are then transported to water bodies either by surface runoff, leaching, or deposition (wet or dry) from atmosphere resulting in pesticide pollution of all environmental compartments (El-Shahawi et al. 2010).

Naturally pesticides are transformed in the environment by various mechanisms and the transformation products are either innocuous or more toxic than parent molecule in some cases. Photolysis involves the breakdown of pesticides by sunlight and direct or indirect photo transformation utilizing the light energy. Hydrolysis of organic pesticides occurs in the presence of water converting them into simpler molecules. Biological transformation by natural species is the most common method of pesticide degradation that involves oxidative, reductive, biotic, and abiotic transformations rendering the pesticides less harmful for the ecosystem. These processes occur at very low rates and are not sufficient for degradation of large quantities of persistent organic pesticides so variety of artificial technologies have been devised to remediate pesticide pollution (Fenner et al. 2013).

7.5.1 Physical and Chemical Methods

Physical methods like adsorption and immobilization are being used to reduce mobility of organic pesticides limiting their dangerous impacts to non-target species and environment. Biochar and compost are commonly used for this purpose. Similarly, various separation and extraction techniques are also utilized for pesticide removal from contaminated sites with the help of different solvents like alcohol and surfactants i.e. Tween-80 and triton, etc. Another effective process is coagulation and flocculation for removing organic pollutants in high quantities. Coagulant tends to destabilize the colloidal particles and flocculation process leads to floc formulation from unstable particles. It enables the remediation of colloidal and suspended particles from a solution. Chemical treatment involves a variety of reactions responsible for pesticide degradation like ionization, oxidation, etc. Different ionic species like iron are used to facilitate the redox reactions resulting in oxidation or reduction of particular pesticide. Advanced oxidation technology refers to the generation of reactive species capable of oxidizing harmful pesticides either transforming them in less hazardous compounds or completely mineralizing the desired chemicals. Common oxidation techniques comprise fenton oxidation, ozonation, plasma oxidation, and photocatalysis (Cheng et al. 2016; Pariatamby and Kee 2016; Morillo and Villaverde 2017).

7.5.2 Biological Methods

Despite the advantages of traditional physical and chemical remediation methods, these are not considered efficient due to various reasons e.g. high cost, use of toxic chemicals, incomplete pesticide transformation forming more toxic compounds, waste management, and production of harmful byproducts. An attractive alternative to such techniques is the utilization of natural potential of biological organisms i.e. plants and animals for abatement of pesticide pollution, known as bioremediation. Living organisms are capable of degrading harmful pollutants by various mechanisms that take place at very slow rates; an increase in the rate of such process provides an efficient way for pollution control. Bioremediation is preferable to other methods because it involves low costs, natural processes, and organisms; mineralizes pesticides completely; no use of harmful chemicals; and absence of management problems. Various organisms including bacteria, fungi, plants, etc. are capable of pesticide degradation which can be employed technically to combat the challenge of pesticide pollution (Ahmad and Ahmad, 2014).

7.6 Bioremediation Technologies

Bioremediation methods can be broadly classified into two categories based on the location at which contaminant treatment is carried out i.e. in situ and ex situ methods.

In situ In this method treatment of pollutants is done at the site of contamination with the help of various procedures including biostimulation, bioaugmentation, bioventing, biosparging, phytoremediation, etc.

Ex situ It involves the treatment of contaminants away from the affected site. Contaminated water or soil has to be moved away from the original location to treat them under controlled conditions (Ajlan 2016).

Some important techniques of bioremediation are discussed below:

7.6.1 In situ Remediation

Natural Attenuation

It is an in situ remediation process that utilizes natural biodegradation method for reducing the pollutant concentration at affected site. It is also known as passive or intrinsic remediation that monitors and verifies the natural degradation process and reduces contaminant load at a specific site by its spreading that makes biological decay of such pollutants easier (Juwarkar et al. 2014).

7.6.1.1 Phytoremediation

The utilization of plants for environmental cleanup operation is termed as phytoremediation. It is a biotechnological approach for pollutant detoxification by application of various plant species (Rani and Dhania 2014; Talukder et al. 2015). Plants in association with microorganisms can degrade the pollutants in different ways including accumulation, absorption, mineralization, or volatilization of organic chemicals (Ijaz et al. 2016). Phytoremediation takes place by any of the possible mechanisms described below:

- Phytodegradation: The degradation of complex pesticides is done by converting them into simpler molecules by plants and microbes associated with them. The breakdown products are then utilized as sources of nutrition by plants and microorganisms.
- Phytoextraction: This method relies on the natural ability of certain plants to accumulate the organic compounds in their body parts like stem or roots, etc. therefore, also known as phytoaccumulation. There are hyperaccumulator plant species capable of storing high contaminant concentrations.
- Phytovolatilization: It involves the uptake of soluble pesticides by the plants and their volatilization in the atmosphere. Plants absorb these compounds along with the nutrients which travel through vascular bundles and reach the leaves from where they are evaporated to the air.
- Phytostabilization: Stimulation of microorganisms is done by plant roots in contaminated region resulting in reduction of pollutants mobility minimizing their transport to other areas.
- Rhizofiltration: Efficient root system of plants tends to degrade contaminants by absorption, accumulation, and precipitation.
- Rhizodegradation: Growth of native microbes is facilitated by plants which then degrade xenobiotic compounds present along with root exudates thus also called as phytostimulation.

Phytoremediation is advantageous for removal of pesticides but in some cases, it is not effective due to certain limitations like requirement of long time periods and area, dependency on climatic conditions, and inability of some plants to bioaccumulate a particular pesticide (Thijs et al. 2017).

7.6.1.2 Bioventing

An in situ treatment method of pesticide involving the provision of suitable conditions to microbes to facilitate their proliferation and enhancing the degradation process. It is based on aerobic degradation method which is enhanced by supplementing the microbes with nutrients and sufficient oxygen to carry out the decontamination (Parween et al. 2018).

7.6.1.3 Bioaugmentation

Introduction of specific pollutant degrading microbes at the site of contamination is referred as bioaugmentation. The microbes naturally capable of detoxification or genetically modified species can be used for this purpose to decontaminate a polluted area. It can be done both in situ and ex situ depending upon the suitable conditions (Baćmaga et al. 2017; Cycoń et al. 2017). Bioaugmentation has been applied successfully for remediation of a variety of pesticides e.g. iprodione and carbamates removal with *Arthrobacter* sp. and *Trametes versicolor* species, respectively (Campos et al. 2017; Rodríguez-Rodríguez et al. 2017).

7.6.1.4 Biostimulation

The amplification of bioremediation processes by overcoming the problems hindering the rates of degradation. Such problems include limiting nutrients for microbial growth, lack of electron donor or acceptors and catalysts, etc. Providing the suitable conditions for enhancing population and degradation rate is called biostimulation because it is used to stimulate the naturally occurring microbes able to degrade the pesticides. This procedure involves addition of nutrients, suitable catalysts, and other conditions required for microbial survival and activity. Levi et al. (2014) used biostimulation for degrading different pesticides by addition of limiting factor i.e. oxygen for aerobic detoxification.

7.6.2 Ex situ Remediation

7.6.2.1 Composting

A process of nutrient recycling through decomposition of biodegradable wastes by microbes. It nourishes the soil with organic nutrients that are consumed by microbial communities involved in pollutant degradation (Chen et al. 2015). Many studies revealed the implication of waste materials for soil amendment caused by its decomposition which in turn enhances microbial density improving biodegradation of pesticides. DDT degradation in the presence of manure was studied by Purnomo et al. (2010) and found maximum degradation of chemical in 28 h. Decomposition of carbofuran and chlorpyrifos by mixing cotton fiber and garden compost with soil was enhanced and complete mineralization can be obtained in this way (Chin-Pampillo et al. 2016). Huete-Soto et al. (2017) analyzed impact of biomixture on removal of a mixture of herbicide, insecticide, and fungicide and found positive results with enhanced removal of pesticides as compared to untreated soil.

7.6.2.2 Bioreactors

The decomposition of pollutants under controlled conditions in different types of containers known as bioreactors where contaminated material is mixed with suitable microorganisms. The process may be aerobic or anaerobic depending upon the nature of pesticide and microbes. Microbes are supplemented with required nutrients and optimum environmental conditions are provided for maximum microbial growth and resultant biodegradation. Bioreactors are being used for treatment of pesticides including different organophosphorus insecticides (chlorpyrifos, forate, diazinon, and malate) and herbicide (linuron). The high degradation is achieved by use of bioreactors with optimized conditions supporting microbial communities for pesticide remediation (Ghoshdastidar et al. 2012; Marrón-Montiel et al. 2014).

7.7 Factors Affecting Pesticide Bioremediation

Bioremediation of pesticides is dependent on multiple factors including various environmental and nutritional conditions. These parameters actually influence the microbial populations that are involved in pesticide transformation. The rate of bioconversion is determined by the suitability of these factors for microbial growth, activity, and bioavailability of pesticides. Some of these factors are discussed below:

7.7.1 Microbial Population

As bioremediation is mainly carried out by native microbes present in the contaminated area so the microbial diversity and their specific ability to degrade complex pesticides is the main factor determining the rate of degradation. The presence of metabolically active microbes engaged in transformation of xenobiotic compounds is an indicator of high decontamination rates. Microbial density, their distribution, and interactions with other microbes greatly affect biodegradation potential. Adaption of microbes to a particular environment and their evolution with time as a result of exposure to different conditions enhances their ability to mineralize the pesticides efficiently. Microbes exhibiting better functional ability and effective mechanisms including production of enzymes provide effective pesticide removal from a contaminated site.

7.7.2 *Pesticide Composition*

The physical and chemical properties of each pesticide differ from the other and affect their biodegradation patterns. Structure, molecular weight, and substituted chemical groups determine the degree of pesticide remediation. The complexity of structure makes the pesticide difficult to degrade by microbes. Simpler compounds with biodegradable chemical substitutes are easy to degrade. Pesticides are xenobiotic compounds that are not native or originally present in an environment but are introduced by anthropogenic activities. Such compounds lack the ability to trigger the microbes for their degradation and microbes possess ineffective mechanisms and enzymes for decontamination of such compounds (Ye et al. 2018). The concentration of pesticides is also an important factor to consider as very high concentrations may be toxic for microbes and very low concentrations may not be recognized by microbes resulting in loss of pesticide remediation.

7.7.3 *Environmental Factors*

Pesticide remediation in a particular environment is influenced by different parameters including pH, temperature, humidity, soil properties, and nutritional sources. Microbes function effectively under specific conditions suitable for them and each organism needs different optimum circumstances. The optimum set of conditions is necessary for survival, growth, and metabolic activity of the particular microorganism. Temperature and moisture content are important factors determining the structure and function of microbial community. It indicates the prevailing microbes that best perform in specific temperature and moisture conditions. Temperature can alter the metabolic rate, enzymatic activity and hinder the growth of some microorganisms. It also affects the physical state and toxicological behavior of pesticides (Reedich et al. 2017). The acidic or alkaline conditions are important for the microbial proliferation and function. Each microbe works best in a particular pH range and the rate of pesticide degradation differs in different pH conditions e.g. *Bacillus thuringiensis* performs maximum activity at 6.5–7.5 pH and has highest quinalphos degradation rate at this pH (Gangireddygari et al. 2017). Soil conditions are important for pesticide remediation in soils both in terms of pesticide adsorption or provision of nutrition to the soil microbes involved in degradation. Different soil types exhibit variable properties like porosity, aeration, and water holding capacity influencing the bioavailability of pesticides. Soils with high adsorptive rates make the pesticides unavailable for microbes that are required for their degradation thus slowing down the bioremediation process. Soils rich in nutrients help better microbial growth and the relevant activity as nutritional deficiency may be a limiting factor for microbial degradation of pesticides (Odukkathil and Vasudevan 2013; Fuentes et al. 2017).

7.8 Microbes Involved in Pesticide Remediation

Microbes play a key role in degradation of pesticides in the environment. Different microbes have developed ability to modify and detoxify the complex recalcitrant compounds into simple innocuous products. Pesticides decontamination by microorganism involves various mechanisms and pathways which lead to the mineralization of these chemicals into carbon dioxide and water. Some species lead to complete mineralization of these compounds while others can only convert them into simpler forms that can be more toxic than the parent molecule; such products need to be completely degraded by other microbial species. Such detoxification reactions involve cometabolism by different species to degrade a particular pollutant. The decontamination procedure is actually a function of enzymes produced by microbes when they are exposed to such pollutants; some of these organisms require an acclimatization period for enzyme synthesis. These intracellular or extracellular enzymes convert the pesticides by catalyzing different reactions. Microorganisms may be present naturally on the contaminated site or inoculated artificially with suitable microbes to support the remediation procedure (Uqab et al. 2016). Microbial degradation is preferable because of naturally occurring microbes with efficient pesticide remediation ability; these can easily be cultured, have high reproduction rates, and microbes evolve degradation potential when exposed to pesticides. Such modifications occur due to mutations and make the microbes adaptable to contaminated environment. These organisms degrade the pesticides enzymatically and utilize the breakdown products as nutrition source (Singh 2014). Principal agents involved in bioremediation of pesticides are bacteria (*Escherichia coli*, *Clostridium*, *Bacillus* spp., etc.), fungi (*Rhizopus*, *Cladosporium*, *Aspergillus fumigatus*, *Penicillium*, *Fusarium*, etc.), algae (*Chlamydomonas*, green algae, diatoms, etc.), and actinomycetes (*Nocardia*, *Streptomyces*, *Micromonospora*, etc.) (Huang et al. 2018).

7.8.1 Pesticide Remediation by Bacteria

Bacteria are considered as the principal agents for pesticide removal both in natural environments and through biotechnological processes. The degradative ability of each differs and can be modified by various techniques like genetic engineering. A broad range of bacterial species are involved in pesticide remediation exhibiting variable mechanisms and behaviors. Bacterial remediation of pesticides is a suitable method because of its cost-effectiveness, high bacterial reproductive/growth rates, and easy manipulations. Pesticide remediation can be in a variety of conditions depending on the specific bacteria adapted to that environment. Degradation takes place both in the presence of oxygen by aerobic bacteria and in its absence by anaerobic species. Various enzymes are produced by bacteria that detoxify the pesticides involving different metabolic processes. Common groups of enzymes responsible for pesticide metabolism include oxygenases, isomerases, hydrolases, hydroxylases, etc. Various environmental conditions like availability of nutrients and the nature of pesticide are the limiting factors in bacterial remediation. Some bacteria can either completely degrade a pesticide or partially transform it which is then attacked by other species leading to entire detoxification of compound. Modification of the conditions suitable for bacteria or introduction of bacterial species capable of degrading pesticides isolated from any other site can enhance the rate of biodegradation (Table 7.1). The properties of pesticides affect the bacterial degrading ability either by being toxic to them or resist bacterial attack due to complex structure and composition (Rani and Dhania 2014).

Diuron is a persistent pesticide that contaminates water, soil, and sediments. This compound is toxic to mammals, birds, and aquatic invertebrates. Degradation ability of three bacterial strains *Arthrobacter sulfonivorans*, *Variovorax soli*, and *Advenella* sp. was evaluated resulting in mineralization from 22 to 69% (Villaverde et al. 2017). Mandal et al. (2014) used *Bacillus firmus* for degradation of a potentially hazardous pesticide fipronil which is applied for killing a variety of insects. Usually pesticides are not completely degraded by a single bacterial strain; bacterial consortia or mixture is found to be more effective in such situations (Geed et al. 2017). Briceño et al. (2018) conducted a comparative study with *Streptomyces* sp. individually or as mixture and findings suggest high diazinon removal rates by mixed culture. Similarly, a microbial consortium resulted in metabolization of phorate resulting in degradation of 97.65 and 98.31% at different pesticide concentration.

A mixture of *Brevibacterium frigoritolerans*, *Bacillus aerophilus*, and *Pseudomonas fulva* isolated from contaminated soil was used in this study (Jariyal et al. 2018). Many contaminated sites contain more than one pesticide which is difficult to degrade. Mixed bacterial culture work in association to metabolize such pesticide blends such as mixed *Streptomyces* culture demonstrated ability to treat chlorpyrifos (CP) and diazinon collectively (Briceño et al. 2018).

7.8.2 Pesticide Remediation by Fungi

Mostly bacterial ability to decompose harmful pesticides is being explored but several fungal species e.g. *Fusarium*, *Aspergillus*, and *Trichoderma*, etc. also exhibit a great potential for pollutant degradation (Table 7.2). Research in this area was provoked in 1980s due to fungal unique properties to transmit hazardous chemicals. Use of fungi for bioremediation is an efficient strategy because of mycelial networks that extend over large areas and excretion of enzymes with less specificity. Fungi have been involved in remediation of different pesticides by various mechanisms like dehalogenation, oxidation, esterification, etc. depending on the pesticide nature. This process is mediated by fungal enzymes like hydrolases, esterases, laccases, peroxidases, etc. (Mahmood et al. 2016).

Fungal specie *Ganoderma* isolated from an agricultural soil was utilized for treatment of a toxic organophosphate insecticide, chlorpyrifos, and its metabolite

Table 7.1 Pesticide degradation by different bacterial species

Pesticides	Bacterial species	References
DDT	*Ochrobactrum* sp. *Stenotrophomonas* *Aerobacter* sp.	Pan et al. (2017) Xie et al. (2018) Neerja Grewal et al. (2016)
Chlorpyrifos	*Pseudomonas aeruginosa* *Klebsiella* sp.	Kharabsheh et al. (2017) John et al. (2018) Farhan et al. (2013)
Lindane	*Streptomyces* consortium *Staphylococcus* sp.	Saez et al. (2014) Kumar et al. (2016)
Endosulfan	*Alcaligenes faecalis* *Pseudomonas* and *Bacillus* *Bordetellapetrii*	Kong et al. (2013) Harikumar et al. (2013) Supreeth and Raju (2017) Zhang et al. (2016)
Atrazine	*Arthrobacter* and *Nocardioides* *Rhodococcus* sp., *Bacillus* sp. *Pseudomonas* sp., *Achromobacter* sp.	Sagarkar et al. (2013) Kolekar et al. (2019) Fernandes et al. (2018)
Iprodione	*Arthrobacter* sp.	Campos et al. (2017)
Hexachlorocyclohexane	*Bacillus circulans* and *Bacillus brevis*	Giri et al. (2014)
Phorate	*Brevibacterium frigoritolerans*, *Bacillus aerophilus* and *Pseudomonas fulva*	Jariyal et al. (2018)
Malathion	*Bacillus* sp. *Pseudomonas putida*	Khan et al. (2016) Kadhim et al. (2015)
Methyl Parathion	*Bacillus* sp. and *Kosakonia* sp.	Alvarenga et al. (2018)

while the strain was found to be effective for the degradation of this compound (Silambarasan and Abraham 2014). Fungal remediation involves various metabolic pathways resulting in the formation of intermediate metabolites that are of particular concern. Studies have been conducted to understand such mechanisms and intermediates produced during the process. Detoxification of pentachlorophenol was obtained with the help of strain *Rhizopus oryzae* CDBB-H-1877 by dechlorination and methylation of the compound (León-Santiesteban et al. 2016). Supplementation of the contaminated site with the limiting nutrients required by fungi for their metabolic processes like carbon, nitrogen, etc. can enhance the rate of degradation. Peter et al. 2015 investigated the malathion remediation ability of *Fusarium oxysporum* and found an increase in degradation potential of fungus in the presence of suitable nutrients. Fungi and bacteria can be used together for pesticide removal, and the interactions between the two organisms can enhance the overall efficiency of the degradation process. An herbicide diuron was mineralized by using consortia of bacteria and fungus and the rate of removal was increased (Ellegaard-Jensen et al. 2014). For the degradation of endosulfan and chlorpyrifos, twenty fungal strains were screened; *Phanerochaete chrysosporium*, *Trichoderma harzianum*, and *Trichoderma virens* were found to be effective for chlorpyrifos removal while *T. hirsuta* and *Trametes versicolor* gave better degradation of endosulfan (Bisht et al. 2019).

Table 7.2 Pesticide degradation by different fungal species

Pesticides	Fungal species	References
Dieldrin	*Penicillium miczynskii* *Pleurotus ostreatus*	Birolli et al. (2015) Purnomo et al. (2017)
Clothianidin	*Phanerochaete sordida*	Mori et al. (2017)
Atrazine	*P. ostreatus* INCQS 40310	Pereira et al. (2013)
Triclosan	*Aspergillus versicolor*	Taştan and Dönmez (2015)
Pentachlorophenol	*T. harzianum* CBMAI 1677	Vacondio et al. (2015)
Phenanthrene	*Cryptococcus*, *Cladosporium*, and *Tremellales*	Schwarz et al. (2018)
Dichlorophenoxyacetic acid	White-rot fungi	Serbent et al. (2019)
Fipronil	*Trametes versicolor*	Wolfand et al. (2016)
Tetraconazole, mefenoxam, metalaxyl	*Rhizopus stolonifer*, *Gongronella* sp.	Martins et al. (2013)
Endrin	*Phlebia acanthocystis* and *P. brevispora*	Xiao and Kondo (2019)
Methyl parathion	*Aspergillus sydowii*, *Penicillium decaturense*	Alvarenga et al. (2018)

Fungal enzymes can be used directly for pollution remediation which exhibit strong catalyzing potential and technical feasibility. Laccases have been used extensively for pesticide remediation and proved to be an efficient tool for degradation of different complex pesticides like isoproturon, atrazine, etc. (Margot et al. 2015; Zeng et al. 2017; Chan-Cupul et al. 2016). Other enzymes like phytase obtained from *A. niger* and cellulase from *Trichoderma longibrachiatum* were found to be active against organophosphorus pesticides and dicofol (Wang et al. 2015; Shah et al. 2017).

7.8.3 Pesticide Remediation by Algae

Algae play an important role in removal and degradation of many pollutants rendering them nontoxic for the ecosystems (Table 7.3). These phototrophic microorganisms can grow faster in a variety of environment, requiring fewer amounts of water and land. Bioremediation with the help of algae is termed as phycoremediation and involves a variety of algal species specially phytoplanktons and microalgae. Algal species have developed various mechanisms for pollutant removal from the environment like bioaccumulation, biotransformation, biosorption, biomineralization, etc. Enzymes produced by algae also have a great potential for pesticide remediation (Baghour 2017).

Various studies have been conducted to monitor the potential of algae for degradation of toxic pesticides. Pesticide biotransformation ability of three phytoplankton species including *Microcystis aeruginosa*, *Synechococcus* sp., and

Table 7.3 Pesticide degradation by algal species

Pesticides	Algal species	References
DDT	*Ulva lactuca*, *Ulva* sp. and *Cystophora* sp.	Qiu et al. (2017) Sudharshan et al. (2013)
Diazinon	*Chlorella vulgaris*	Kurade et al. (2016)
Fenhexamid, metalaxyl, triclopyr, iprodione	*S. quadricauda* and *C. vulgaris*	Baglieri et al. (2016)
Atrazine	*Aspergillus niger* AN 400 *Chlamydomonas mexicana*	Marinho et al. (2017) Kabra et al. (2014)
Tricyclazole	Blue green algae	Kumar et al. (2017)
Phorate, parathion	Small green algae	Tang et al. (2017)
Ciprofloxacin	*Chlamydomonas mexicana*	Xiong et al. (2017)
Sulfamethazine	*Chlorella pyrenoidosa*	Sun et al. (2017)
Lindane	*Nannochloris oculata* *Laminaria digitata*	Pérez-Legaspi et al. (2016) Cunha et al. (2017)
Diflubenzuron	*Laminaria digitata*	Cunha et al. (2017)

Chlamydomonas reinhardtii was evaluated. In this research 15 fungicides were used at different concentrations which were completely mineralized by the phycoremediation, and the reactions were likely to be mediated by enzymes like glutamate, etc. (Stravs et al. 2017). Hussein et al. (2016) examine the ability of *Chlorella vulgaris* for simultaneous degradation of multiple herbicides, pesticides, and insecticides including Pendimethalin, Atrazine, Simazine, Isoproturon, Propanil, Dimethoate, Molinate, Pyriproxyfen, Carbofuran, and Metolachlor. Removal rates up to 99% were achieved by the used algal biomass.

7.9 Mechanisms Involved in Microbial Degradation of Pesticides

Microbes have ubiquitous nature with great diversity, huge populations, and biomass present in the natural environments. They exhibit potential capabilities to synthesize and degrade different compounds including various catalytic pathways involved in these processes. Unique ability of microbes to survive in extreme conditions even tolerating oxygen deficiency and toxic environments make them probable candidates for being utilized as pollution remediation agents. Natural ecosystems contain a diversity of microbial population that works by interacting with each other either synergistically or antagonistically. This well-organized system leads to changes necessary for ecosystem maintenance. Microbes interact with the pesticides both physically and chemically and detoxify by converting them into simple nontoxic substances (Fig. 7.1). This transformation is a function of either needs of organism to gain energy and nutrition from the compounds or detoxify them to

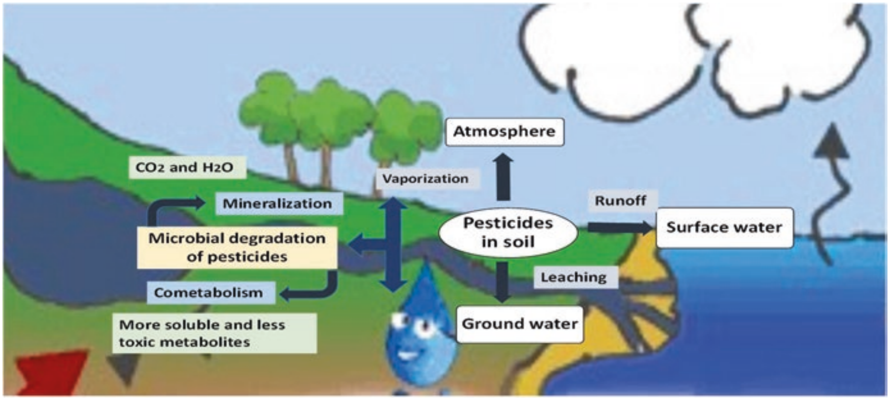

Fig. 7.1 Pesticides in the environment and their microbial degradation

clean up the environment. Bacteria and fungi are the principal degraders of pesticides due to production of large amounts of extra cellular enzymes. Fungi mainly transform the pesticides by rendering minor changes in the structure of compound leading to its detoxification. The major modes of microbial action for pesticide removal are bioconcentration or cumulative effect of different microbes resulting in complete mineralization or cometabolism. Mineralization takes place when microbes need to utilize the pesticide as nutritional and energy source. Microbial degradation of organic compounds results in the formation of inorganic substances, carbon dioxide, and water. This process involves complete degradation of the compound and is the most efficient because the resultant molecules are nontoxic. Cometabolism involves the utilization of an alternative source for their nutrition when pesticides cannot be consumed (Mustapha et al. 2018). Relying on a different energy source, microbes proliferate and carry out the metabolic reactions leading to partial degradation of pesticides. Pesticide metabolism is a three-phase process:

- Step 1: It is the metabolic phase in which the properties of parent compound are usually altered by a series of chemical reactions. The transformation takes place involving the hydrolysis, oxidation, or reduction processes. The main purpose of these alterations is to make the recalcitrant and water insoluble compound into water soluble and less toxic compound that can further be biodegraded.
- Step 2: This phase involves further modifications in the products released in first step changing their physical state and chemical properties. Enhancement of water solubility and reduction in toxicity occur by conjugation of pesticides or their metabolites with sugar or amino acids which is due to the action of microbes.
- Step 3: More secondary conjugates are formed in this process leading to partial or complete mineralization of the pesticide compounds. The role of extra- and intracellular enzymes including oxidases, hydrogenases, and hydrolases is significant during this phase (Ortiz-Hernández et al. 2013).

7.10 Reactions Involved in Pesticide Transformation

Various biochemical reactions are involved in the breakdown of pesticides assisted by microbes which are explained below:

7.10.1 Hydrolysis

It involves hydrolytic enzymes that release –H or –OH groups from the compound of water which are then added to the target pesticide for cleavage of substrate bonds. A variety of such enzymes are produced by microbes that aid the metabolic degradation of different substrates including functional groups of esters, amides, and carbamates. These enzymes may work inside the organism or released outside for the desired action. Such water dependent enzymes are active under both aerobic and anoxic conditions. Esterases are the commonly used enzymes for ester hydrolysis while lipases and proteases are also used to lesser extent. Microbes produce different types of esterases that vary in structure, specificity, and location of production or activity. Malathion, phorate, diazinon, etc., are degraded by hydrolysis (Kumar et al. 2018).

7.10.2 Oxidation

Oxidation is one of the important processes for conversion of xenobiotic pesticides due to their recalcitrant nature that makes them difficult to degrade. Such reactions are mediated by a variety of oxidative enzymes such as peroxidases, oxidoreductases, phenoloxidases, etc. These enzymes are involved in the polymerization of phenols, anilines, and other aromatic compounds with ringed structure. Cytochrome P450s are the most efficient oxidative enzymes involved in hydroxylation of pesticides by catalyzing monooxygenase reactions. Examples of such transformations include detoxification of 2,4-D, carbendazim, etc. (Chen et al. 2013; Doolotkeldieva et al. 2018).

7.10.3 Nitro Reduction

Nitroreductase enzymes produced by microbes are responsible for metabolism of nitroaromatic xenobiotic compounds. Such enzymes reduce the nitro group by converting N_2O to NH_2 making the pesticide nontoxic on complete degradation. However, in some cases, partial degradation may occur resulting in formation of

NH_3. This reaction is useful for the remediation of pesticides containing nitro group like ether herbicides, parathion, etc. (Arora and Bae 2014; Huang et al. 2018).

7.10.4 Dehalogenation

Xenobiotic compounds are recalcitrant to degradation due to presence of strong bonds in their structure. One of such interactions resulting in pesticide stability is the bond between carbon and halogens. Microbes can mediate dehalogenation reactions to treat these bonds by substituting halogen with hydrogen or carboxyl functional group making it less stable. Most important halogen containing group of pesticides is organophosphonates in which the bonding between carbon and phosphorus is resistant to hydrolytic, thermal, photochemical, and chemical degradation. Microbial enzymes have the ability to attack such bonds, and for cleavage of carbon phosphorus bond, C-P lyase enzyme is responsible (Ye et al. 2018).

7.10.5 Conjugation Reactions

Reactions that are involved in the detoxification of such compounds that cannot be directly modified or transformed by microbes are termed as conjugation reactions. It is a metabolic process in which a natural compound is attached with the pesticide to facilitate its bioremediation. The conjugate formed is attacked enzymatically by the microbes rendering the pesticide nontoxic. Conjugation is also mediated by microbial enzymes like pesticide conjugation with glucose occurs due to Uridine diphosphate-glucosyl (UDPG) transferase and the glucose ester is then cleaved by microbial esterases. Alkylation, xylosylation, acylation, methylation, and nitrosation are involved in conjugation reactions. For example, conjugation of phenols with methyl groups to facilitate their degradation by microbes (Tewari and Saini 2017).

7.10.6 Enzymes Involved in Microbial Degradation of Pesticides

The microbial activity leading to the remediation of pesticides is a function of enzymes. Both intracellular and extracellular enzymes are involved in degradation processes. Extracellular enzymes are released in the environment and biotransformation of pesticides is done outside the microbial cells. Intracellular enzymes are produced by the microbes that function within the microbial cells; such mechanism involves three main steps:

- Pesticide adsorption on surface of microbial cell. Physical contact takes place between the microbes and pesticides at the contaminated site.
- Pesticide penetration within the cell through cell membrane. This is a rate limiting step for pesticide degradation because cell membrane possesses selective permeability and penetration of chemical depends on its molecular nature. Cell membrane only allows some pesticides to pass through it and hinders the entrance of others resulting in internal biomineralization of selective pesticides.
- Pesticide degradation takes place inside the organism's body by the action of catabolic enzymes. A series of chemical reactions occur resulting in mineralization of pesticides releasing the harmless residues (Tewari and Saini 2017).

Some of the important enzymes necessary for pesticide removal from a contaminated site are discussed here:

7.10.6.1 Hydrolases

This extensive group of enzymes plays a significant role in pesticide degradation by microbes. These enzymes are involved in the hydrolysis of various classes of pesticide by catalyzing the breakdown of peptide bonds, esters, carbon-halide bonds, thioesters, etc. The action of these enzymes is generally independent of redox cofactors; this property makes them perfect candidates for pesticide bioremediation. Many types of hydrolases are involved in degradation reactions, two of them are phosphotriesterases and esterases.

7.10.6.2 Phosphotriesterases

Phosphotriesterases are produced by a variety of microorganisms involved in pesticide degradation. These are responsible for detoxifying organophosphate pesticides by hydrolyzing phosphoester bonds. Different genes responsible for production of such enzymes have been identified in *Pseudomonas diminuta*, *Flavobacterium* sp., *Pseudomonas moteilli*, etc. known as phosphotriesterases encoding gene e.g. opd. It is a homo-dimeric protein which hydrolyses pesticides in different steps: a proton is removed from water resulting in its activation by the active site of enzyme and then this activated molecule directly targets the phosphorus atom present in the center of pesticide compound leading to its configurational changes.

7.10.6.3 Esterases

Esterases is a diverse group of enzymes with high variability due to which they are involved in detoxification of multiple pesticides depending on their chemical nature. Several biochemical reactions are catalyzed by such enzymes including hydrolysis of amides by amidases, phosphate esters by phosphatases, and carboxylic esters by carboxylesterases. Alcohol and acid components of ester substrates are actually targeted by such enzymes. Different esterases are produced by the organisms, two important types include esterases A and B. Esterases A contains a cysteine residue while a serine residue is present in the active center of esterases B. Organophosphates are interacted with -SH functional group in esterases A and a bond is formed between P=S, that is hydrolyzed by water molecule. In esterase B, pesticides interact with SER-OH group leading to formation of P=O bond which cannot be hydrolyzed. Such interactions inhibit the pesticide degradation. Esterases exhibit great substrate specificity and have the ability to bind with particular groups of amides, peptides, esters, etc.

7.10.6.4 Oxidoreductases

The electron transfer from one molecule to another is catalyzed by this category of enzymes. The transfer occurs from the electron donor to the electron acceptor or oxidant molecule. These enzymes require additional cofactors like electron donating molecule, etc. for their activity. Such enzymes are of particular importance in degradation of endosulfan and its metabolites by a series of oxidation reduction reactions. Oxidoreductases can be divided in various categories and have been classified in 22 subclasses, many of them being involved in pesticide degradation. One important class is monooxygenases that involves reduction of molecular oxygen to water or hydrogen peroxide. Such enzymes are of particular importance in detoxifying xenobiotics resulting in either an increased reactivity or water solubility by adding an atom of oxygen. Flavin dependent monooxygenases contain a flavin cofactor, which is susceptible to reduction by NAD(P)H substrate, and the reaction is facilitated by enzymes themselves. Mixed function oxidases are also involved in pesticide remediation requiring NADPH for their activity. NADPH-cytochrome P450 reductase and cytochrome P450 constitute this enzyme system. This is a well characterized and huge family of monooxygenase enzymes that has a potential ability of oxidizing or hydroxylating substrates with the help of molecular oxygen in an enantiospecific manner. Many of these enzymes constitute a broad substrate array and catalyze biochemical reactions leading to pesticide degradation. These characteristics make them ideal for degradation of persistent pesticides and their residues. Carbamates, organophosphates, pyrethroids, and DDT can be metabolized by these enzymes.

7.10.6.5 Other Enzymes

Several other small groups of enzymes are involved in pesticide remediation. Glutathione S-Transferase is the enzyme catalyzing the association of hydrophobic residues with tripeptide glutathione. This process involves the reaction between thiol groups of glutathione with an electrophile of pesticide forming a conjugate whose metabolism or excretion is easy. These are involved in many detoxification reactions for the treatment of xenobiotic compounds like pesticides. Haloalkane dehalogenases are involved in the degradation of halogen containing pesticide groups. Lyases catalyze cleavage of bonds in absence of redox cofactors or water, including carbon–carbon bonds and bonds of carbon with oxygen, phosphorus, nitrogen, sulfur, and halides. Elimination of halogen atom is the main mechanism that results in structural and compositional changes in the pesticide molecules rendering it less toxic (Ortiz-Hernández et al. 2013a; Sharma et al. 2016).

7.11 Genetic Basis of Pesticide Degradation

Various genes in microbes responsible for pesticide degradation have been identified. These genes are involved in catalytic degradation of pesticides and are mostly located on the chromosomes or on transposons or plasmids in some cases. Advances in the field of genomics led to the discovery of novel genes and the associated regulatory elements that encode for pesticide degrading enzymes. Such genes are characterized from microbes isolated from different geographic locations that influence the specific pesticide metabolizing behavior of organism. Genes including *atz, trz, psb, tri, tfd, puh, ndo*. encode for different enzymes like dehalogenase, urease, dehydrogenase, hydrolase, haloperoxidase, dehydrochlorinase, cytochrome P-450, deaminase, isomerases, dioxygenase, reductases, glutathione-S transferases, etc. These genes present in several microbes are responsible for pesticide degradation in different metabolites (Ahmad et al. 2014). Few catabolic genes identified in microbes are listed in Table 7.4.

A novel gene pytH, in *Sphingobium* sp. strain JZ-1, encodes for a pyrethroid-hydrolyzing enzyme i.e. carboxylesterase. *Escherichia coli BL21* was used as PytH

Table 7.4 Microbial catabolic genes involved in the pesticide remediation

Pesticides	Genes involved in degradation	References
Isoproturon	*ddhA* *pdmAB*	Yan et al. (2016) Gu et al. (2013)
3-Phenoxybenzoic acid	*pbaAB*	Wang et al. (2015)
Fenoxaprop-P-ethyl	*feH*	Liu et al. (2019)
2,4-D	*tfdA, tfdB, tfdC, tfdD, tfdE, tfdF, tfdR, cad RABKC operon*	Chakraborty and Das (2016)
Methyl parathion, paraoxon, dimethoate	*mph*	Wang et al. (2018)
Atrazine	*atzA* *Trz*	Vail et al. (2015) Douglass et al. (2017)
Glyphosate	*aroA*	Firdous et al. (2018)
Propham	*MmH*	Sun et al. (2019)
Chlorpyrifos	*tcpA* and *fre*	Fang et al. (2019)
Propanil	*mah*	Zhang et al. (2019)

expression strain and resultant enzyme was purified which was able to transform pyrethroid pesticides. Another esterase gene, sulE, isolated from *Hansschlegelia zhihuaiae* S113 was identified for de-esterification of sulfonylurea herbicide. Transferring of sulE in microbial strain *Saccharomyces cerevisiae* BY4741 resulted in enhanced resistance and de-esterification ability of sulfonylurea herbicides (Jiang and Li 2018).

7.12 Advances in Pesticide Bioremediation Techniques

Research in the field of biotechnology has resulted in the development of new methods for pesticide remediation aimed at improving the remediation of hazardous pesticides. Some of these techniques include:

7.13 Bioinformatics and Electrobioremediation

Bioinformatics is an efficient tool developed in recent years with a great potential for identification and investigation of cellular components important for the biodegradation of pesticides. Such components include genes, proteins, their functions, regulatory elements, interactions, and metabolic pathways involved in the process of pesticide biotransformation. Electrobioremediation is another useful technique in which electric current is applied directly to soil resulting in an increased bioavailability of nutrients and their transformation into simpler components. It helps microbial community to decontaminate the toxic compounds easily (Martínez-Prado et al. 2014).

7.14 Use of Biosurfactants

Biosurfactants are used to increase the bioavailability of pesticides by enhancing their solubility hence improving the biodegradation of contaminants by microbes. This is a broad category of compounds produced naturally by microbes including bacteria, yeast, and fungi. These are either found to be bounded by cell membrane or secreted extracellularly. These are composed of a hydrophilic and hydrophobic region that helps in reduction of surface tension between two insoluble liquids hence increasing the solubility of hydrophobic pesticides. Microbes involved in production of biosurfactants are *Pseudomonas aeruginosa*, *Stenotrophomonas* sp., *Acinetobacter junii*, *Aneurinibacillus aneurinilyticus*, *Candida* sp., and *Bacillus subtilis* (Mnif et al. 2016; Dong et al. 2016; Gargouri et al. 2017; Balan et al. 2017).

Pesticides degrading strains with the ability to produce biosurfactants are responsible for effective bioremediation. Identification of two strains (*Bordetella petrii* I and *Bordetella petrii* II) was reported by Odukkathil and Vasudevan (2013) that possess an ability to detoxify α and β endosulfan and secrete biosurfactant helping in surface tension reduction by 19.6 and 21.4%, respectively. The production of biosurfactants by microbes can be enhanced by addition of different precursors like nutrients. Similar study was done by using residues from oil industry; this soil amendment resulted in the production of glycolipid biosurfactant that enhanced the solubility of ethyl parathion, trifluralin, and methyl parathion. This technique results in reduction of costs required for pesticide remediation and is environment friendly (Bagheri Lotfabad et al. 2017).

7.15 Genetic Engineering

The molecular techniques have opened up the ways to manipulate the microbial characteristics by genetically modifying them to obtain better pesticide degradation potential. Genetic engineering is a valuable tool for enhancing the biocatalytic activity of microbes for degradation of recalcitrant compounds. This technique is used to modify enzymes to exhibit greater specificity and designing of new metabolic pathways or altering the existing ones, enhance the biodegradation ability of microbes by recombinant genes and synthesis of biosensors for detection of hazardous pollutants and their level in a particular environment. In *Pseudomonas putida* KT2440, mpd and gfp genes were incorporated in the chromosome making the strain capable to use chlorpyrifos and carbofuran as carbon source. The resultant hydrolysis rate of pesticides was greater when recombinant strain was introduced in the soil contaminated with pesticides mixture. Green fluorescent protein was also introduced as a biomarker for monitoring of recombinant stain (Gong et al. 2016).

The use of multiple pesticides and contamination of environment with mixtures of such pesticides make their degradation difficult by the microbes. Recombinant strains with better degradation ability are utilized to treat such areas. Zuo et al. (2015) conducted a research by integration of two pesticide degrading genes (mpd and the pytH) in *Pseudomonas putida* KT2440; the coexpression of these genes made the strain capable of completely mineralizing a pesticide mixture including methyl parathion, fenitrothion, chlorpyrifos, permethrin, fenpropathrin, and cypermethrin in 15 days.

7.16 Nanobioremediation

Various factors influence the choice of best suitable method for pesticide remediation e.g. process efficiency, cost-effectiveness, pesticides complexity and hazards, availability of resources, required time, etc. One technology for remediation in such cases may not be sufficient, so combination of various technologies is an attractive option to overcome this issue. Nanobioremediation is the combination of nanotechnology and bioremediation to enhance the degradation of recalcitrant compounds. Nanoparticles used in this process favor the biodegradation of pesticides in different ways. These nanomaterials either break down the chemicals to such a level which can be easily degraded by microbes or act as adsorption medium to enhance the bioavailability of pesticides. Nanomaterials used in this process are synthesized biologically from microbes or plants making the method ecofriendly. Nanoparticles can degrade the pesticides by photocatalysis or catalyzing redox reactions. Bimetallic nano-metals have a great potential for pesticide remediation like DDT, carbamates, etc. Iron and Iron-Pd NPs are capable of reducing pesticides like lindane, PCB, etc. utilizing the metabolic activity of bacteria (Cecchin et al. 2017; Pandey 2018).

7.17 Immobilization Techniques

In recent times, immobilization methods are being employed in bioremediation processes. Immobilization involves limited mobility of microbial cells or enzymes resulting in preservation of catalytic functions and viability. Microorganisms have a natural ability to form biofilms on various surfaces; this property is of particular concern for their immobilization. Immobilization tends to reduce process costs and improves overall efficiency. Advantages of this method include high degradation efficiency, reuse of biocatalysts, stable microenvironment conditions for cells/enzymes, reduction of genetic mutations, resistance to shear stress, adverse environmental conditions and toxins, increased biocatalytic activity, and tolerance to high concentrations of pollutants. A variety of materials can be used for immobilization including natural substances like sawdust, plant fibers, crop residues, etc. that can favor biodegradation without any harmful impact (Dzionek et al. 2016). In order to enhance the biodegradation of diuron, strain *Arthrobacter globiformis* D47 was entrapped in a biocompatible carrier of silkworm excrement composites. Bacterial cells were immobilized on the carriers with high survival rate and resulted in stable catalytic activity degrading target pollutants effectively (Liu et al. 2019). Immobilization of *Micrococcus* sp. strain CPN 1 was done on polyurethane foam, sodium alginate, agar, etc. The rate of cypermethrin degradation was greater by immobilized cells as compared to freely suspended cells with retention of their degradative ability (Tallur et al. 2015).

Immobilization of microbial enzymes is also explored to facilitate the biodegradation of pesticides. Wang et al. (2017) reported the immobilization of fungal laccases and the embedded enzymes were employed to pesticide contaminated soil. Carbofuran was degraded up to 86% with this technique. Nanomaterials have a great potential to act as the matrices for immobilization of microbes enhancing bioremediation rates (Devi et al. 2018). The esterase stabilization by immobilization on magnetic nanoparticles provides better degradation rates than the normal enzyme. The immobilized system was able to effectively remediate organophosphorus i.e. quinalphos, chlorpyrifos, and monocrotophos pesticides (Punitha and Rose 2018).

7.18 Conclusion

Pesticides are persistent compounds that can stay in the environment for longer periods depending upon their physical and chemical nature. These chemicals greatly influence the environmental quality and damage the health of living organisms. Pesticides tend to destroy the normal ecosystem functioning and biotic species by bioaccumulation and biomagnification leading to the food chain spoilage. The persistence of these compounds makes them capable of travelling in the environment and reaching higher trophic levels, leading to an increased toxicity. Risks associated with these pesticides are of particular concern, demanding an effective method for their remediation from the environment. Various physical and chemical methods have been employed for pesticide degradation but these have some associated limitations that make them less effective. An alternative way to overcome these problems is the use of living organisms for removal of pesticides from the contaminated sites. This technique, known as bioremediation, is cost-effective and ecofriendly. Microbes exhibit natural ability to degrade pesticide by various pathways involving enzymatic activity. Bacteria, fungi, and algae are the principal agents for biotransformation of pesticides. This unique capability of microbes is a function of specific genes regulating different degradation mechanisms and enzyme production. Different factors including pesticide nature, pH, temperature, etc. affect the rate of microbial pesticide degradation. To boost up the bioremediation process different techniques have been developed resulting in pesticide treatment either on site or away from the contaminated area. Supplementing the microbial populations with optimum conditions or introduction of efficient microbes can enhance the bioremediation rate. New methods have been exploited to enhance the bioremediation process involving immobilization techniques, genetic modification of microbes, and use of nanoparticles or biosurfactants.

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Chapter 8
Ecofriendly Approaches for Remediation of Pesticides in Contaminated Environs

Dig Vijay Singh, Rohaya Ali, Anita, Mahiya Kulsum, and Rouf Ahmad Bhat

8.1 Introduction

Environmental safety, increasing population, food production and security are the major concern of whole world (Popp et al. 2013). Pesticides are recalcitrant and non-biodegradable that have great property of bioaccumulation and biomagnifications leading to more serious impacts on flora and fauna (Chakravarty et al. 2017). These toxic substances are persistent and harmful to non-target organisms but their usage is continuously rising year by year. They are also carcinogenic in nature and are banned in many countries due to the risk posed by their presence in the environment (Mahmood et al. 2016). Some pesticides banned several decades ago but their residues are still found in the environment. Currently, huge quantity of the crop yield (>45%) is lost due to increasing pest population and diseases (Oerke 2006) and is more in tropical countries due to presence of suitable environmental conditions (high temperature and humidity) for rapid growth of pest population (Hill 2008). Pest and diseases damage the crop and also increase the production cost as pesticides are used to control the damage caused by pests (Rosenzweig et al. 2001). Shrinking agriculture land and increasing losses due to pest infestation are putting extra pressure to use different pesticides to intensify crop yield (Aggarwal et al. 2006). Despite being costly, pesticides are used to kill or inhibit the growth of pest in the agricultural field (Pimentel et al. 1992).

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Pesticides are actually manmade chemicals responsible for controlling the pest population in agricultural sector. Actually, pesticides are the chemical that are used to kill or arrest the growth of pests, insects, rodents and weeds (Cook et al. 2007). The low biodegradability has made these chemicals persistent in nature (Tayade et al. 2013). These synthetic chemicals are being considered as toxic, persistent and stable (Rani et al. 2017) and are often not used in proper manner, hence can easily contaminate air, water and soil ecosystem at alarming rate (Arias-Estevez et al. 2008; Aktar et al. 2009). Among the different pesticides (organochlorine, organophosphorus and carbamates), organochlorine accumulates in the food chain causing serious problems like sterility, kidney disease, endocrine disruption and neurological disorders in the living organisms (Khan and Law 2005). Farmers are also at the risk of exposure to pesticides due to inappropriate use of personal protection and spraying equipment (Issa et al. 2010). Misuse of pesticide can also effect human health as can easily get transferred through food chain (Damalas and Eleftherohorinos 2011) and also leads to degradation of air, soil and water environment (Yadav et al. 2015). Furthermore, pesticides applied to soil can also reach to human body by inhalation (during spraying), ingestion (contaminated food material) and by body contact. Agricultural residues of pesticides can have detrimental effects as these can be easily found in food grains (Aktar et al. 2009). All the food material gets contaminated with different harmful pesticide residues, which can further degrade the health of living organisms (Agrawal et al. 2010). Indiscriminate use of pesticides affects human health, beneficial microorganisms, plants and animals residing in different ecosystems (Corvalan et al. 2005; Abhilash and Singh 2009; Aktar et al. 2009; Gill and Garg 2014). Excessive use of pesticides increases crop production in quick time but the impacts on the surrounding environment are countless (McMichael 2009).

India known as agricultural country has more the half (60%) of the population totally dependent on agriculture activities. Agricultural sector in India has undergone significant changes by evolving the concept of using high yielding varieties and agrochemicals. But with time, damage to the crops by the pests and diseases is also increasing thereby affecting the crop productivity (Rosenzweig et al. 2001). Pesticides are vital part of agriculture and remain in soil for long time and leach to ground and surface water (Arias-Estevez et al. 2008). They are produced mostly from agricultural fields and are transported to different components of environment by posing serious risk to living organisms (Fig. 8.1). Excessive application of pesticides can lead to development of resistance in pests (Gill and Garg 2014) that can also change dosage of pesticide application (Shetty 2004) (Table 8.1). Thus, pesticides were used to arrest the growth of pest population without having any idea of post application impacts on the environment (Desneux et al. 2007). Traditional techniques are not capable to remove pesticides from different ecosystem but pesticide released into the environment can be removed efficiently by using environment friendly technique like bioremediation that is a cheap, efficient and sustainable (Gavrilescu et al. 2015). Bioremediation is the use of living organisms like bacteria, algae, fungi and plants for removing or degrading toxic pollutants from contaminated environment in ecofriendly manner (Murali and Mehar 2014).

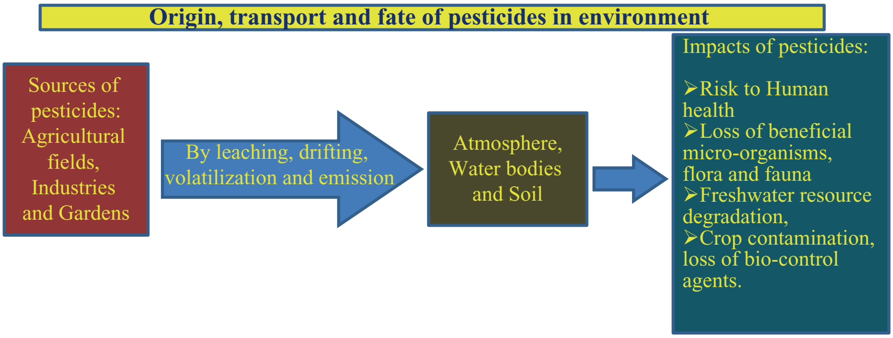

Fig. 8.1 Fate of pesticides in environment

Table 8.1 Doses of pesticides applied for controlling the pests in different agricultural crops (Vaccari et al. 2006; Abhilash and Singh 2009)

Crop	Percentage of pesticide use (%)
Cotton	45
Rice	25
Chillies/vegetables/fruits	13–24
Plantations	7–8
Cereals/millets/oil seeds	6–7
Sugarcane	2–3
And other	1–2

8.2 Pesticide Use in India

After independence (1948–1949), India too have been started the application pesticides (DDT and BHC) for controlling malaria and locust, respectively (Abhilash and Singh 2009). The uses of pesticides on large scale have started after green revolution in order to promote the productivity of the high yielding varieties (Conway and Barbier 2013). Various pesticide industries were set in different parts of the country for increasing the availability of pesticide to the farmers. Pesticides are used in India in huge quantity in order to increase production to feed the rapidly growing population (Trostle 2010). Organochlorine pesticides are mostly used in developing countries because of cheapness but due to persistence and various harmful effects, organochlorine has been banned in the developed countries (Darko et al. 2008). DDT having several harmful impacts is banned but India has got special exemption for using DDT (10,000 tons) in health sector (Dash et al. 2007). India is among the few countries still involved in the production, export and use of chemicals banned by other countries. In whole world, two million tons of pesticides are used annually in which Europe (45%) is at top followed by the USA (24%) and other countries (25%). China (Fig. 8.2) is leading in the use of pesticide among Asiatic countries followed by Korea, Japan and India (Abhilash and Singh 2009). Despite huge pesticide use, almost 33% of production of food crop is lost due to the increasing pest

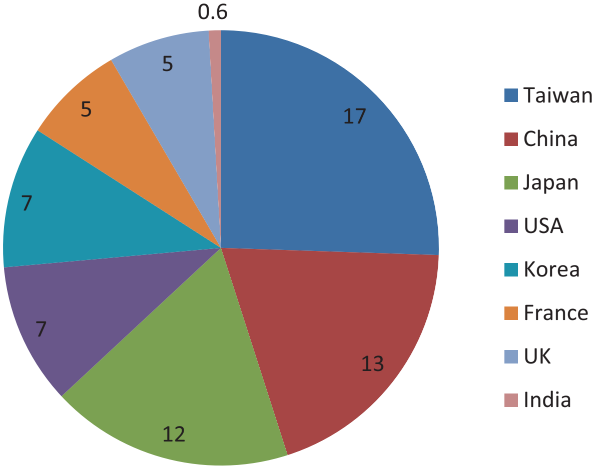

Fig. 8.2 Pesticide consumption in different countries (kg/ha)

incidence. In India, 1/3rd of crop loss is by weeds followed by diseases (26%), insects (20%), birds/rodents (10%) and by other factors (11%) resulting in almost annual loss of Rs. 6000 crores (Jayaraj et al. 2016). Pesticides like organophosphorus (broad spectrum insecticide) directly attack nervous system by inhibiting the activity of enzyme acetylcholinesterase, thus termed as anticholinesterase insecticides (Eyer 2003). In India, among different types of pesticides (Fig. 8.3), organochlorine is mostly used for controlling of pest (Yadav et al. 2015). Pesticide use results in poisoning of farmers, neurological disorders, fatal deformities, miscarriages and lowering the sperm count of applicators (Sabarwal et al. 2018; Kalliora et al. 2018; Kumar and Kumar 2019). Excessive use of pesticide poses numerous risks, thus regular monitoring of environment and minimum use of pesticides should be done on priority (Thokchom and Thacker 2019).

8.3 Impact of pesticides on bio-control agents of pest

Bio-control agents live by attacking pests and helps in controlling the pest population (Flint and Dreistadt 1998). These agents play a crucial ecofriendly role in the management of pests (Dutta 2015). Pesticides interfere in the growth and development of these bio-control agents thus affecting the natural management of pests (Pal and Gardener 2006). They are at the risk of exposure either by direct contact during spraying or by intake of residues from contaminated sites (Stanley 2016). Bio-control agents are natural enemies of the pests that need proper nurturing and care so that dependence on pesticides can be reduced (Abhilash and Singh 2009). *Trichoderma*, *Gliocladium viren*, *Phytophthora*, *Rhizoctonia spp.*

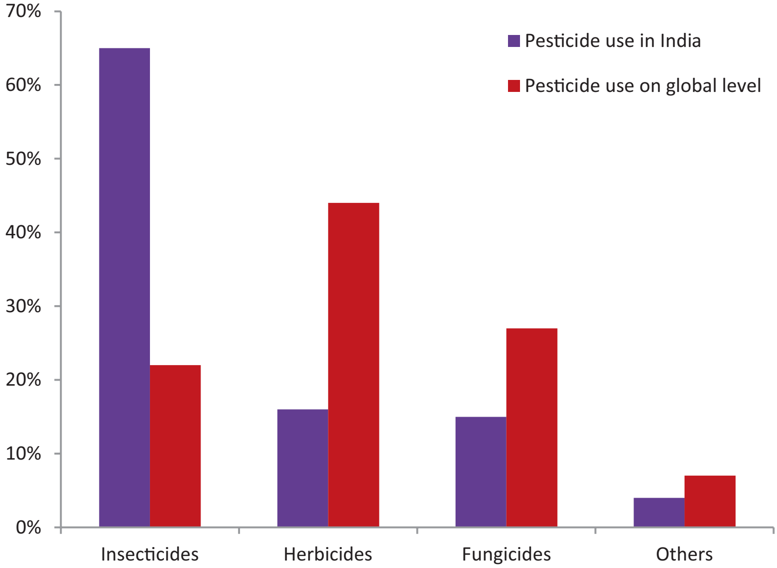

Fig. 8.3 Comparison of pesticide application in India with respect to global level (Devi et al. 2017)

Streptomyces, *Beauveria bassiana* and *Bacillus thuringiensis* are most commonly used bio-control agents for controlling different pests (Warrior et al. 2002; Kao 2007; Maheshwari et al. 2014; Mishra et al. 2018). Presence of bio-control agents is main reason that some insects are not able to damage crop and cannot become economic pest but use of pesticide also eliminate these bio-control agents (Sarwar 2015). Elimination of one pest by pesticides sometimes results in the outbreak of other due to the absence of bio-control agent. Uncontrolled use of pesticides also destroys different bio-control agents responsible for controlling of various types of pests in the nature (Patel and Jasrai 2012). Pesticides reduce population by decreasing egg laying capacity, feeding ability, mobility, sex ratio (less females) and reproductive system of bio-control agents (Kim et al. 2017). Thus, bio-control agents need extra care so that management of pest can be done in sustainable manner (Abhilash and Singh 2009).

8.4 Phytoremediation

Contamination of environment by pesticides can pose serious threat by degrading the health of ecosystem (Perez-Lucas et al. 2018). Pesticides attack non-target flora and fauna thereby creating more problems in maintaining the stability of ecosystem (Gill and Garg 2014). Phytoremediation is low cost, solar power driven technique that involves the use of efficient plant species for the elimination or degradation of toxic pollutants from the contaminated environment (Mir et al. 2017). Plants and crops are directly exposed to pesticide pollution and can enter into plant system mainly through roots from the soil or water environment (Main et al. 2017). Plants eliminate pollutant by various mechanisms like phytoextraction (stored in root, stem and leaves), phytodegradation (using enzymes for pesticide degradation), phytovolatilization (transpiration of contaminants) and rhizodegradation (microbes in rhizosphere help in degradation of pollutants) (Truua et al. 2015). Pesticides uptake by plants lead to conversion of toxic pesticide into less toxic one thus help in the remediation of toxic pollutant from contaminated sites (Singh and Singh 2017). Utilization of plants for remediation of pesticide (Table 8.2) also results in landscape improvement, controlling of soil erosion, prevent leaching of contaminant, acts as habitat for different microorganism and animals (Mishra et al. 2015). Moreover, phytoremediation is safe, economical and green technique that can significantly help in remediation of pesticide from contaminated environment in ecologically sound manner (Kuppusamy et al. 2016).

Table 8.2 Remediation of pesticides from different environment by plants

Plant species	Pesticide	Reference
Typha latifolia	Methyl parathion	Subashini et al. (2007)
Zea mays and *Brassica campestris*	Endosulfan	Mukherjee and Kumar (2012)
Brassica campestris	DDT	Suresh et al. (2005)
Ricinus communis	Chlorpyrifos, methoxychlor, heptachlor epoxide, endrin	Rissato et al. (2015)
Kochia sp.	atrazine, metolachlor and trifluralin	Tripathy et al. (2014)
Wheat (*Triticum* spp) *or* pigeon pea (*Cajanus cajan*)	Lindane	Singh et al. (1991)
Plantago major L.	Cyanophos	Romeh (2014)
Scirpus schoenoplectus, *Cyperus rotundus*	Monocrotophos	Tandon and Deore (2017)
Eichhornia crassipes	Malathion organochlorine and pyrethroid	Xia and Ma (2006) Riaz et al. (2017)
Lemna minor	Dimethomorph	Dosnon-Olette et al. (2010a, b)
Ceratophyllum demersum	Endrin, cypermethrin, methamidophos	Koranteng et al. (2018)
Nymphaea lotus	γ-Lindane, methamidophos, chlorpyrifos, diazinon	Koranteng et al. (2018)
Ceratophyllum demersum	Fenitrothion, methamidophos, chlorpyrifos, diazinon	Koranteng et al. (2018)

8.5 Microalgae and Pesticide Remediation

Environment free of pesticide is the main objective of all the countries of the world. Pesticide residue in environment can pose grave threat and their removal is vital for maintaining balance of ecosystems. Pesticides are one of the common toxic pollutants in aquatic ecosystems (Islam and Tanaka 2004). Surface water bodies are the easy target of the most of the pesticides used in agriculture sector (Agrawal et al. 2010). Rising population, shrinking agriculture land is responsible for increasing use of pesticides over entire world (Carvalho 2006). Continuous use of pesticides is increasing the contamination of the freshwater ecosystem as these pesticides through surface run off reach finally to water bodies (Konstantinou et al. 2006). Pesticides after reaching to freshwater bodies can lead to numerous problems like decreasing the quality of water, death of flora and fauna residing in water bodies (Agrawal et al. 2010). Algae can be potent organisms for removal of different pesticides from the contaminated sites. They are photosynthetic organisms with diverse number of species ranging from single cell microalgae to multiple cell macroalgae. Microalgae like plants convert radiant energy from sun to chemical energy thus are known as photoautotrophic organisms (John et al. 2011). They are simple in structure but are able to sequester carbon dioxide faster (10–50 times) than plants (Subashchandrabose et al. 2013) and are known as potent oxygen producer on the earth. Simple cell structure is responsible for easy transport of nutrient and water (Chacoon-Lee and González-Mariño 2010). Several microalgal species are known to have potential to survive efficiently in contaminated sites by developing internal defence mechanism (Torres et al. 2017). Using microalgae can fulfil various goals like nutrient recovery from wastewater, improvement in physico-chemical characteristics, pollutant removal and biomass production, which can be used for production of industrially important by-products (Rawat et al. 2011). Microalgae utilization also results in oxygen production thus helps in maintaining the balance of the environment. Oxygen produced by microalgae can assist bacteria in enhanced degradation of pesticides from contaminated environment (Munoz and Guieysse 2006). Several authors have reported that microalgae use organic pollutants as energy source and enhance the degradation of pesticides from contaminated ecosystem (Matamoros et al. 2015). Microalgae are potent organism that removes toxic pollutant from contaminated environment mainly by bioaccumulation and biosorption (Chojnacka 2010). Bioaccumulation is active process involving use of living system that by using energy accumulate or metabolize pollutants from the environment (Velasquez and Dussan 2009), while biosorption involves both living and dead organisms as this mechanism is not dependent upon energy for the removal of pollutants in disturbed environs (Aksu 2005). Microalgae are known as efficient biosorbent of heavy metals (Monteiro et al. 2012) as well as pesticide (Priyadarshani et al. 2011) thus can act as the cheap pollutant removal approach (Table 8.3). Microalgae are capable of not only bioaccumulating pesticides but can also transform pesticide into non-toxic compounds (Rath 2012). Degradation of pesticides is dependent upon types of microorganisms, optimum environmental condition and

Table 8.3 Removal of pesticides from contaminated environment by different microalgae/cyanobacteria

Pesticide	Microalgae/cyanobacteria	References
Prometryne, fluroxypyr, isoproturon	*Chlamydomonas reinhardtii*	Chekroun et al. (2014)
Bisphenol A	*Monoraphidium braunii*	Gattullo et al. (2012)
α-endosulfan, mirex	*Chlorococcum* sp.	Sethunathan et al. (2004), Kobayashi and Rittmann (1982)
α-endosulfan	*Scenedesmus* sp.	Sethunathan et al. (2004)
Dimethomorph and pyrimethanil	*Scenedesmus quadricauda*	Dosnon-Olette et al. (2010a, b)
Atrazine	*Chlamydomonas Mexicana*	Kabra et al. (2014)
Malathion	*Chlorella vulgaris*, *Scenedesmus quadricuda* and *Spirulina platensis*	Abdel-Razek et al. (2019)
DDT	*Chlorococcum* sp., *Anabaena* sp. *and Nostoc* sp.	Megharaj et al. (2000)
DDT, parathion	*Scenedesmus obliquus*	Kobayashi and Rittmann (1982)
Atrazine	*Isochrysis galbana*, *Dunaliella tertiolecta*, *Phaeodactylum tricornutum*, *Pseudokirchneriella subcapitata and Synechococcus sp.*	Weiner et al. (2004)
Diclofop-methyl	*Chlorella vulgaris*, *Chlorella pyrenoidosa and Scenedesmus obliquus*	Cai et al. (2007)
Fenamiphos	*Pseudokirchneriella subcapitata*, *Chlorococcum* sp.	Caceres et al. (2008)
Mirex	*Dunaliella* sp.	Rath (2012)

composition of pesticide. Enhanced degradation of pesticides is also possible due to the presence of appropriate microorganisms in contaminated ecosystem (Ortiz-Hernandez et al. 2013). Degradation of pesticides is dependent upon their solubility in water, molecular weight, functional group attached and also concentration of pesticide in the contaminated environment (Subashchandrabose et al. 2011). Microalgae can adapt to any environmental condition easily and are known to be capable to grow efficiently in the various stress conditions (Mata et al. 2010). Microalgae under light and nutrient stress are known to grow mixotropically by using light and organic carbon as energy source thereby providing extra advantage compared to other microorganism (bacteria and fungi) in pollutants degradation (Subashchandrabose et al. 2013). Microalgae used for remediation of pesticide contaminated sites are an integrated cultivation approach for sustainable future.

8.6 Bacterial Degradation of Pesticide

Pesticides are persistent in nature for many years and can also reach to human body through different food chains (Jones and De Voogt 1999). The structure of a pesticide molecule determines its physico-chemical properties and inherent biodegradation (Chowdhury et al. 2008). Some pesticides are insoluble in water and adsorb tightly to soil particles. Thus, these pesticides are relatively unavailable for biodegradation and thus their residues remain in the soil for a long time, thereby adversely affecting the ecosystem (Chowdhury et al. 2008; Purnomo et al. 2011, Wang et al. 2013). Xenobiotic compounds may become accessible to microorganisms in different environmental compartments where they are further transformed and degraded (Fetzner 2002). Organophosphate pesticides are the most commercially favoured group of pesticides, with large application areas all over the world (Kumar et al. 2018). Several methods for pesticide remediation have been adopted in order to minimize the impact on environment (Azubuike et al. 2016). Physical degradation, chemical degradation, and physico-chemical degradation are the traditional methods and result in secondary pollution thereby adding more problems in the conservation of environment (Huang et al. 2008). Traditional techniques being costly cannot be used for pesticide removal from contaminated sites (Wilson and Tisdell 2001). Bioremediation of pesticide by using beneficial living organisms is the cost effective, ecofriendly and is one of the best alternative available for the pesticide removal (Gavrilescu 2005). Microorganisms are diverse and efficient to live in extreme condition (oxygen deficient condition) and can show synergistic or antagonistic relation between different organisms. Surging need of the green technology for the degradation of pesticides leads to isolation of bacteria from different ecosystems which are capable of degrading pesticides at good rate (Singh et al. 2011). Bacteria belonging to genus *Flavobacterium*, *Arthrobacter*, *Azotobacter*, *Burkholderia* and *Pseudomonas* are efficient in removing of pesticides from different contaminated sites. Microbial remediation is a suitable technique for pesticide degradation without causing any further risk to the environment as microbes degrade pesticides for nutrients and results in the release of CO_2 and H_2O (Huang et al. 2008). Several microorganisms like bacteria have been used to degrade the toxic pesticides from contaminated sites (Singh and Walker 2006). Bacteria having potential to degrade pesticide (Table 8.4) have been isolated from different sites. Pesticides applied regularly to the soil are degraded by the available soil biota as these organisms use pesticides as carbon source as well as electron donor (Gavrilescu 2005). Degradation of pesticides is dependent upon the environmental conditions, contact time and bacterial species (Arias-Estevez et al. 2008). Activity of bacteria is also affected by several factors like temperature, pH, moisture content, humidity, nutrient content and concentration of the substrate in the environment (Huang et al. 2008). Degradation of pollutants by bacteria is effected by the presence of anions (chloride, sulphate) as anions are strongly bonded and prevents microbes from attacking the compound structure (Rani and Dhania 2014). Bacteria degrade pesticides

Table 8.4 Removal of pesticides by different bacterial species from environment

Bacterial species	Pesticides	References
Sphingobium japonicum	Hexachlorocyclohexane, lindane	Liu et al. (2007)
Burkholderia cepaciastrain CH-9	Imidacloprid, metribuzin	Madhuban et al. (2011)
Acinetobacter calcoaceticus	Bifenthrin	Tingting et al. (2012)
Streptomycetes	Chlorpyrifos	Javaid et al. (2016)
Enterobacter	Chlorpyrifos, parathion, diazinon, coumaphos and isazofos	Singh et al. (2004)
Pseudomonas putida and *Pseudomonas mendocina*	Permethrin and cypermethrin	Mendoza et al. (2011)
Sphingomonas and *Pseudomonas*	HCH	Phillips et al. (2005)
Pseudomonas, *Neisseria*, *Moraxella* and *Acinetobacter*	DDT	Carrillo-Perez et al. (2004)
Pseudomonas bacteria	Atrazine	Wyss et al. (2006)
Escherichia coli	Organochlorine, BHC, DDT, endosulfan, HCH isomers, and 2,4-D	Singh et al. (2003), Gupta (2005), Chaudhary et al. (2006)

and convert them into inorganic constituents which are used by bacteria for different purposes (Laura et al. 2013). Bacterial species capable of growing in contaminated sites are responsible for hydrolysis of bonds between different elements (P–O, P–F, P–S and P–C) thus enhance the degradation of organophosphorus pesticides (Singh and Walker 2006). Interaction of microorganism with pesticides may result in structural changes or degradation of pesticides from contaminated environment (Huang et al. 2008). Bioremediation of pesticides from affected environment occurs mainly by natural attenuation (native species degrade pesticides), biostimulation (nutrient and oxygen are supplied to enhance rate of degradation) and bioaugmentation (potent bacterial species from other site is introduced) (Adams et al. 2015). Pesticides accumulation can occur due to the absence of potent bacterial species in the soil but bacterial species can be added from external source to reduce their accumulation. Bioaugmentation is the addition of specific bacteria to the contaminated sites that result in degradation of pesticides from the effected sites (Dejonghe et al. 2001). Bioaugmentation relies on the enhancement of the catabolic potential of soil microbial communities for the degradation of pollutants (El Fantroussi and Agathos 2005). Endosulfan degrading bacteria (*Klebsiella*, *Acinetobacter*, *Alcaligenes*, *Flavobacterium* and *Bacillus*) isolated from different productive ecosystem are known to degrade endosulfan in efficient manner (Huang et al. 2008). A recent study also reported that the *Stenotrophomonas* sp. isolated from sludge collected at a drain outlet of a chlorpyrifos manufacturing plant in China might be an excellent candidate for application in remediating the pollution of diazinon and other organophosphate pesticides, with a removal efficiency of nearly 100% (Deng et al. 2015). Species like *Burkholderia Pseudomonas*, *Sphingomonas*, *Cupriavidus*, *Corynebacterium* and *Arthrobacter* have been isolated from different matrices around Asia and found to be very efficient in degradation of fenitrothion (Tago et al. 2006; Zhang et al. 2006). Bacteria release certain enzymes that lead to the degradation of pesticides into less toxic or non-toxic form (McGuinness and Dowling 2009). The nature of degradation varies with the bacterial species as different enzymes like oxygenases, hydroxylases, hydrolases and isomerases are released that degrade the pesticides in contaminated environment (Karigar and Rao 2011). Hydrolytic enzymes released by *Pseudomonas* sp. and *Klebsiella pneumonia* are efficient in degrading of pesticide like atrazine from environment (Odukkathil and Vasudevan 2013). *Pseudomonas* sp release three enzymes, namely AtzA, AtzB, AtzC that attack atrazine, respectively, and result in degradation of the compound into simple one (CO_2 and NH_3) (Wackett et al. 2002). Enzymes are more resistant to environmental condition and are also efficient in removal of pesticide from effected sites at higher rate (Huang et al. 2008). Furthermore, single bacterial species is able to degrade pesticides at good rate but multiple species are more efficient and thrive well in harsh environment (Díaz 2004). Thus, the need is to identify the bacteria species that can thrive in extreme condition and degrade pesticide completely from contaminated sites.

8.7 Mycoremediation

Fungi (yeast, mould and mushroom) are eukaryotic, heterotrophic, saprophytic and parasitic spore producing organisms growing mostly under cool and humid conditions (Jobard et al. 2010). They can be found in diverse habitats with majority growing on terrestrial habitats (80% associated with terrestrial trees) and some are found also in aquatic environment (Zhang et al. 2015). Furthermore, they can also be found under snow, high temperature condition, in salty as well as sweet environment. On the basis of nutrition fungi can be saprophytic, parasitic and symbiotic. Fungi exhibited both asexual (budding, fragmentation or spore production) and sexual (meiosis and fusion) type of reproduction (Lee et al. 2010). These organisms are very close to animals as their cell wall contains chitin, stores energy in the form of glycogen and feeds on other organism for nutrition similar to animals. Moreover, these organisms feed on the complex organic matter, thus helps in the cycling of nutrient back into the environment (Jacoby et al. 2017). Pertinently, fungi can have symbiotic/mutualistic (consortia development) or antagonistic (pest control) relation with other organisms (Deveau et al. 2018). They are ubiquitous and tolerant to numerous toxic chemicals released into the environment by various human interferences. The increasing use of pesticides is a growing concern for the whole world due to various harmful effects on different ecosystems (Aktar et al. 2009). Several potential microorganisms have been isolated with time from different ecosystems for degradation of toxic pesticides from the environment (Ortiz-Hernandez et al. 2013). The natural chemist has great biogeochemical potential and plays significant

Table 8.5 Pesticide removal potential of fungal species from environment

Pesticide	Fungal species	Removal rate (%)	Reference
Chlorpyrifos	*Cladosporium cladosporioides*	100	Chen et al. (2012)
Chlorpyrifos	*Ganoderma sp.* JAS4	100	Silambarasan and Abraham (2014)
Endosulfan	*Aspergillus niger*	100	Bhalerao and Puranik (2007)
Methamidophos	*Penicillium oxalicum*	99.9	Zhao et al. (2010)
Methyl parathion	*Aspergillus sydowii* CBMAI 935	100	Alvarenga et al. (2014)
Chlorpyrifos	*Cellulomonas fimi*+ *P. chrysosporium*	100	Barathidasan et al. (2014)
Endosulfan	*B. laricina* JAS6+ A. *tamarii* JAS9+ *Lasiodiplodia sp.* JAS12	100	Abraham and Silambarasan (2014)
Malathion	*Fusarium oxysporum* JASA1	100	Peter et al. (2015)
2,4-D	*Aspergillus* species *Mortierella* species	0–52 11–46	Vroumsia et al. (2005)
Atrazine	*Coriolus versicolor*	86.2	Bending et al. (2002)
Diuron	*Hypholoma fasciculare*, *Stereum hirsutum and Agrocybe semiorbicularis*	70–80	Bending et al. (2002)

role in maintain health and enhances quality of environment (Madigan et al. 2012; Gargano et al. 2017).

Fungi, algae and bacteria can remove pesticides from contaminated environment in economical and environment friendly manner thus conserve the environment (Kumar et al. 2011) (Table 8.5). Utilization of fungi for pollutant removal from the contaminated environment is called mycoremediation (Kulshreshtha et al. 2014). Mycoremediation of pollutants can be done by accumulating toxic pollutants inside the body (bioaccumulation) in which fungi uses pollutants as carbon source and pollutant is enzymatically degraded into simple compounds (Adenipekun and Lawal 2012). Fungi are capable of transforming as well as detoxifying pollutants from contaminated environment and are efficient in removing pesticides from soil and water ecosystem (Tortella et al. 2005). They are preferred for remediation of pesticides because of extended hypae network and unconventionality from utilizing organic compounds as substrate for growth thus making them potent candidate for remediation of pesticides from contaminated environment (Harms et al. 2011; Chen et al. 2012). Among microorganisms, fungi are preferred because of their structure, i.e., hypae which makes pesticide molecules easily accessible through micropores. Extended mycelia network not only increases the rate of degradation of pesticides, but also increases the nutrient and water availability to the plant (Huang et al. 2008). These organisms secrete enzymes to degrade complex matter as food source and this capacity of fungi also helps by converting toxic pollutants into non-toxic (Tortella et al. 2005). Ligninolytic fungi are one of the potent groups of organisms that secrete various extracellular enzymes thus helps in the transformation of recalcitrant compounds (Anastasi et al. 2013; Harms et al. 2017). Enzymes are excreted dominantly by saprotrophic fungi followed by white rot fungi, soft rot fungi and brown-rot fungi (Wu et al. 2015). White-rot fungus is considered as an efficient biodegrader of contaminants includes pesticides due to non-specific extracellular enzymatic system which is composed of lignin peroxidase, manganese peroxidase and laccase (Gago-Ferrero et al. 2012). Having an excellent ligninolytic properties, fast growth and easy handling in culture favours the use of white rot fungi in biodegradation of toxic substances (Diez 2010). Different white-rot fungal strains such as *chrysosporium*, *Trametes hirsutus*, *Phanerochaete sordida*, *P. ostreatus*, *Pleurotus* and *Cyathus bulleri* have been reported for their ability to degrade lindane, diuron and other recalcitrant pesticides (Jauregui et al. 2003; Sagar and Singh 2011). Fungi also produce some extracellular enzymes like lignin peroxidase, manganese peroxidase, laccase and oxidases that act upon broad range of chemicals thus results in their degradation (Janusz et al. 2017). Degradation of toxic pesticide by enzymes can also be effective in long run. Fungi may introduce minor changes in the structure of chemical pesticide resulting in the production of non-toxic molecules which can be easily degraded in the soil by microflora (Hai et al. 2012). Different processes like hydroxylation, demethylation, dechlorination, dioxygenation, esterification, dehydrochlorination are adopted by fungi for pesticide degradation as pesticide with varied functional groups are used in order to minimize the crop damage (Maqbool et al. 2016). Fungi cause structural changes by attacking on functional groups thus enhance the degradation of pesticides. Some fungi (Ascomycetes, Basidiomycetes and Zygomycetes) are known to degrade pesticide using the processes like polar hydroxylation, demethylation (Badawi et al. 2009), esterification, dehydrogenation and dioxygenation (Pinto et al. 2012; Deng et al. 2015). Pesticide degradation by fungi is dependent upon several factors like pH, moisture content, nutrient availability and temperature (Maqbool et al. 2016)**.** Presence of optimum moisture content in contaminated environment can enhance the degradation of pesticides as both binding and distribution of pesticide are influenced by water availability (Philippoussis et al. 2001). Monoculture of fungi is not much efficient in degrading different pesticide but consortia of different fungi not only degrade different pesticide but the system produced can thrive under different hostile environmental conditions. Mixture of various potent fungal species is also known to degrade pesticides (DDT and chlorpyrifos) at rapid rate (Maqbool et al. 2016). Thus, focus should be on culturing of versatile fungi species that can accelerate the degradation of pesticides from the effected environment.

Keeping in view the long-term impacts of using fungicides, biopesticides can be helpful in controlling various pathogens. Biopesticides are beneficial natural organism used to inhibit or control the population of harmful pests (Sandhu et al. 2012). Fungi, namely *Trichoderma* sp. is mostly used as bio-control agents for controlling various diseases and pathogens in the field. *Trichoderma* releases protease that partially inhibits the enzyme activity related to pathogenicity (Steyaert et al. 2003) and can also induce plant defence system (Sarrocco et al. 2017) and by mycoparasitism (Druzhinina et al. 2011) control pathogens. *Trichoderma* as efficient fungicides thus can limit the use of these chemicals and increase the crop production in efficient and sustainable manner (Haggag and Mohamed 2007). The need is to adopt and cultivate these biopesticides at wide level so as to feed the growing population without contaminating environment. Use of biopesticides can have positive implication on soil, crop and as well as on the environment (Ranga Rao et al. 2007). Biopesticides are cheap, easy to use and can significantly reduce the production cost of crops (Debashri and Tamal 2012). Use of biopesticides in different parts of world is growing due to increasing demand of organic food. Biopesticide boosts agricultural yields and ensuring food safety and security.

8.8 Conclusion

Indiscriminate use of pesticides will further strengthening the problems associated with its mismanagement in the environment. Removal of pesticides from the contaminated environment is expensive and no such techniques are available that can remove pesticides in environment in ecofriendly manner. Bioremediation is a cheap, acceptable and ecofriendly technique that can degrade toxic pesticides in efficient manner. Pesticides are persistent and their residues remain in the environment for numerous years. Thus it is crucial to screen microorganism (algae, bacteria and fungi) that can use toxic pesticides as energy source and degrades them in cost effective manner. The need of an hour is to minimize the use of pesticides and utilize green techniques like bioremediation for removal of pesticides from the contaminated environment. To reduce the intensive use of pesticides, it is an urgent need to promote the organic farming practices and search for the effective biopesticides or biological agents to control agricultural pests in order to reduce the degradation of quality environs.

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Chapter 9
The Reliability of Nanotechnology for Sustainable Industries

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9.1 Introduction

Nanotechnology is a multidisciplinary science that shows how a matter can be manipulated, both in the molecular and atomic levels; to do this, is working on a nanometer scale—a scale so small that we can't see even to a microscope.

A nanometer is a billionth part of a meter. Atoms are even smaller. It is difficult to quantify the size of the atom—they do not tend to have a certain shape. But in general, a typical atom is about one-tenth of a nanometer in diameter. There is an unprecedented multidisciplinary convergence of scientists dedicated to the study of such small particles.

Nanotechnology is so new that no one is sure what will happen in the future with this discipline. Even so, the forecasts range from the ability to reproduce things like diamonds and food to a world that will be devoured by self-replicating nanorobots.

Nanotechnology is fast becoming an interdisciplinary field. Biologists, chemists, physicists, and engineers are all involved in the study of substances at the nanoscale level.

Thus, it is hoped that different disciplines will develop a common language and communicate with each other. Only then can nanoscience be taught effectively, because the world of nanotechnology cannot be understood without solid knowledge related to multiple fields of science.

Nanotechnology, the general term for technological developments on a nanometric scale, is the manufacture of a product with a controlled geometric size in which at least one functional component has a particle size of less than 100 nm.

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In a broad sense, nanotechnology is any technology whose nanometric finite results are fine particles, chemical synthesis, advanced microlithography, etc. In a narrow sense, nanotechnology is any technology that is based on the ability to build complex structures at atomic level specifications using mechanical synthesis (Mohajerani et al. 2019).

The nanometric structures are not only very small, stretching even to the atomic scale, but they possess unique and unexpected properties, compared to the same substance taken at the macroscopic level. Due to the large surface/mass ratio, nanosized materials have special energy properties, properties that can be used for a multitude of effects that cannot be achieved with conventional products.

Nanotechnologies represent engineering on a very small scale. These can be applied in many areas, such as health and medicine, information and communications technology, energy and the environment.

Nanotechnologies work at the nanoscale—the scale of individual molecules. Some foresee the emergence of unimaginably small computer processors or tiny devices to detect and repair damaged arteries in the human body. Nanotechnologies could generate alternative energy, could extend our life, and improve many existing technologies. And they are already making an appearance.

There are already over 1000 products on the market that use nanomaterials. A nanometer is one-billionth part (10^{-9}) of a meter, that is, 000000000.1 m, or one-millionth part of a millimeter. A nanometer is smaller than an apple just as an apple is smaller than the planet Earth (Ramachandraiah et al. 2015).

Nanotechnologies handle materials with dimensions on the nanometer scale, approximately between 1 and 100 nm. This scale is called the nanoscale. Known materials at the nanoscale may have new properties. For example, an aluminum foil is a convenient way to keep the sandwiches fresh until lunch. When the aluminum foil is broken into a very small pieces (nanometric in size), something special happens—the pieces become extremely reactive/even explosive. This makes aluminum nanoparticles excellent for introduction in the rocket fuel, but is probably something you do not want your lunch to come in contact with!

Nanotechnology, the "Twenty-first Century Manufacturing Technology," encompasses high precision engineering, the main direction in biomedical applications in areas such as gene therapy, drugs-controlled transport, new types of drugs, nanoscale synthesis techniques, or more concise *art of manipulating individual atoms and of molecules to build structures with managed properties*. Nanotechnology represents the ability to construct objects by assembling atoms in well-defined time sequences. To build structures starting from atoms and molecules, it is necessary to invent assembly devices at nanometric scale.

Their role is to assemble atoms and molecules into billions of configurations specific to a nanometric structure. The ability of the assemblies to self-replicate is one of the basic requirements of nanometric instruments.

Each nano-instrument will have to operate properly and be programmable. Synthesized materials whose characteristic dimensions are between 1 nm and 100 nm have dimension-dependent properties. As far as the size of a material is smaller, the quantum phenomenon is more pronounced and the defects are less important than in the sintering processes as example (Shafique and Luo 2019).

The industrial interests are enormous: overcoming the limitations of lithography for semiconductor technology, the demand for control at the molecular level, the simplification of processes, the hardening of surfaces, photonic applications, the control of permeability, biocompatibility, to name a few of them.

Nanotechnology is determined by the results of interdisciplinary sciences and the new nanometric investigation tools of matter.

It has generated a wide intermingling of the sciences considered fundamental and has propelled new fields of research unimaginable a few decades ago. In a restrictive sense is the science of materials whose properties depend on size and include:

- Nanosciences: the fundamental phenomena and rules of physics, chemistry, and biology applied at the nanometric scale.
- Nanoengineering: manipulating molecules to build new types of materials; the tools and methods by which devices are designed and manufactured at the nanometric scale, their assembly into consumer products and for new advanced research (Iavicoli et al. 2014).

Nanotechnology is a multidisciplinary field through which the great scientific achievements of physics, chemistry, biology, mathematics, and materials science; and can be applied to construct with atoms and molecules material at the nanometric scale with artificial intelligence, biocompatible structures, unconventional energy sources, nanorobots for medicine, and chips with high density of components and self-replicating biomaterials, etc. (according to Table 9.1).

Table 9.1 Common applications of chemo and biosensors

Field	Applications
Agriculture	pH detection, herbicides, pesticides
Food, drinks	Determination of chemical composition, odors, degradation level, odorants, fermentation control, etc.
Defense	Military applications, countermeasures in biological and chemical weapons
Cars	Fuel supply control system, monitoring of gas and pollution emissions
Customs control	Detection of illegal, dangerous substances, drugs, explosives, radioactive substances
The aeronautical industry	Air quality monitoring systems in the aircraft cabin
Chemical industry	Toxic gas emission monitoring systems, material testing
Medicine	In vivo/in vitro clinical diagnostics, determination of anesthetic gas concentration, telemedicine, intensive therapy
Environment, environmental protection	Detection of air, water and soil pollutants, BOD, detergents control
Civil security	Gas detection

The bottom-up construction method is known as “building from bottom-up.” It is the domain where biological structures (DNA, proteins, oligomers, and bio-oligomers) are architectured with materials synthesized at the nanometric scale using combined techniques of physics and chemistry (atomic force microscopy, nanolithography, molecular beams, nano-electrochemistry, soil–gel) with those in genetics; the domain where molecules and polymers become electronic devices (molecular electronics); the domain where the properties of the materials are extremely exploited for the spatial technique; the field where the entire information technology is developed.

The multidisciplinary aspects are complex for understanding the engineering of these systems covering large areas: nanostructures, nanoelectronics, microfluidics, high resolution biochemical sensors, detection of chemicals and harmful biological agents, microsensors for radioactivity, medical research, power applied noninvasive sensors, biomimetic, rapid DNA sequencing, targeted drug transport, electronic polymers, nanooptics, nanometric analytical techniques, nanoassembly, nanointegration, nanoscale information technology, multifunctional nanosystems, and bionanointerfaces (Beattie et al. 2011).

As the implication of nanomaterials in biosensors and medicine treats various aspects, it is necessary to define them:

Nanobiotechnology: applies nano/microfabrication tools and processes to build devices for studying biosystems, microanalysis of biomolecules by interfacing a single cell, raising the profile of its metabolism and biochemistry, analyzing the response to different analyses; nanoparticles inspired by biology; biosensors, microbial combustion cells, new types of markers, etc.

Nanomedicine: describes a set of capabilities of molecular machine systems that can be used in the diagnosis, treatment, and repair of tissues or organs: nanorobotic medical devices; the ability to recognize, sort, and transport molecules; self-generation; communication with the physician; ability to migrate throughout the body to manipulate microscopic objects, to disassemble cells and viruses, and to highlight the genes responsible for cancer eventually repairing them.

Econanotechnology: nanotechnology in environmental protection and pollution, environmental purification and new renewable energy solutions, biodiversity recovery and maintenance, toxicity of nanoparticles (Nanotoxicology) (Aziz et al. 2019).

9.2 Classification of Nanomaterials

Even the word itself, “nanotechnology,” has an exotic air, which suggests to the engineers the unbelievable, difficult to understand. In synthesis, nanotechnology is the science of making objects by working on the scale of atoms.

The raw material is made up of atoms themselves, which, by certain methods, are “obliged” to form groups that give special qualities to the materials. Then, by making mechanical structures from the created molecules, nanorobots can be obtained, capable of performing certain tasks, according to a program.

Nanomaterials have dimensions between 0.1 and 100 nm. It is a size difficult to imagine; it is difficult to feel/see how small a nanometer is; it is 1 m divided by one billion. For the time being, there is no single method of working with atoms, each scientist inventing it's nanotechnology method; currently scientists estimate that at least one new nanotechnology is invented every day.

For example, some methods involve changing the quality of materials using nanotubes. Nanotubes are constructed from spheres created from 60 carbon atoms, called buckminsterfullerene or, more simply, bucky balls.

Materials that contain the "bucky" spheres are six times lighter and one hundred times more resistant than steel. To isolate cancer cells, researchers at the University of Michigan have developed a method that uses "mean of transport" as pieces of DNA molecules (portions called dendrimers) (Miernicki et al. 2019).

9.2.1 Products from Nanotechnology

The new functions, associated with the nanomaterials, are dependent on the type of materials and their dimensions. Examples of nanomaterials that can be manufactured in one-, two-dimensional, or three-dimensional structures are thin films, nanotubes, and nanoparticles, respectively.

Classification of nanomaterials is not easy, due to their complex structures and diverse properties. Also, those structures that are deliberately produced on a nanometric scale and have new properties are considered nanomaterials, as opposed to structures that may be naturally present (e.g., sugar molecules, fats) or have resulted from conventional methods (for example, ricotta cheese nanoparticles).

The application of nanomaterials in the food system is manifested primarily in the production of food ingredients with nanostructure and in the distribution systems of supplements and nutrients.

Areas of investigation of meat products include reformulation by minimizing and modifying fat content, decreasing the amount of sodium, phosphate, or nitrate and including probiotics, prebiotics, and other materials, such as seaweed and walnut. Also, improving bioavailability, forming compounds that can promote health and reducing unhealthy compounds are possible areas of study for the processing and storage of meat products. Many products contain zinc oxide or titanium nanoparticles.

Older sunscreen formulas use larger particles, thus providing a whitish color to most products. The smaller particles are less visible, which means that when you get on the skin, it does not leave a whitish tint on the skin.

Scientists use nanoparticles to make clothes more interesting. By coating the fabrics with a thin layer of zinc oxide nanoparticles, manufacturers can create clothing that offers greater protection against UV radiation (Lutz-Bueno et al. 2018).

Some garments have nanoparticles in the form of hairs that help repel water and other materials, making clothing resistant to stains.

Scientist Robert Burrel has created a process for making antibacterial bandages using silver nanoparticles. Silver ions block cellular respiration of microbes. In other words, silver suffocates the harmful cells, killing them. New products that contain nanotechnology appear every day.

Non-wrinkle materials, cosmetics that penetrate deep into the skin, LCDs, and other facilities that use nanotechnology are now on the market. Not long after, we will see dozens of other products that benefit from the benefits of nanotechnology, from Intel microprocessors to bio-nanobatches and capacitors with a thickness of only a few nanometers.

While all this seems interesting to us, they are only the tip of the iceberg in terms of how nanotechnology can affect us in the future (Avilov et al. 2017).

9.2.2 Natural Nanomaterials

Many living beings use a phenomenon that depends on structures with nanometric dimensions. Therefore, a gecko lizard that goes on the ceiling, leaves that reject water, vivid colors of butterflies, and resistant and flexible materials such as spider webs and all use natural nanotechnologies. In addition to nanoparticles produced by living things, some are created by natural phenomena, such as erosion and volcanic eruptions. They are also produced as a result of some types of chemical reactions, especially combustion.

The human body and in general all living beings have developed, during evolution, specific sensors that maintain the physiological and metabolic balance concerning the interaction and exchanges with the environment.

Unfortunately, the environmental changes, the increasingly technologized society, the abusive and irrational involvement of man on the regulatory biosystems of nature have led to imbalances that living beings can no longer regulate through their sensory systems. We increasingly need artificial sensors to be the interface for monitoring and change.

People have already been exposed to some forms of nanoparticles existing in some consumer goods. For example, mineral titanium dioxide—used as a bleach in toothpaste and food coloring—contains a fraction of nanoparticles.

These are not introduced later, but are part of the natural composition of the mineral, in the form of sand granules of different sizes.

Although scientists have been researching nanoscale matter for many years, in physics and chemistry, atoms and molecules could be directly visualized and manipulated with the invention of a new generation of microscopes in the 80 years, at the IBM laboratory in Switzerland. This paved the way for the systematic investigation of nanomaterials and the realization that their exceptional properties could be used to create innovative materials and devices. Frequently, nanomaterials observed in nature are used as inspiration for designing innovative ones.

Many researchers believe that science is not a revolution, but rather an evolution of traditional scientific disciplines, but nanotechnologies can have some revolutionary implications for our society in terms of applications or tools whose realization can facilitate it.

Thus, the sensors offer portals between the “real” or the analogous world in which we live and the digital world of computers and modern communication systems through which we connect to the environment.

Sensors make it possible to get real-time information about things we can see, touch, smell, and hear and about other things we cannot detect—things that may be harmful or useful to us. The electronic signal collected from the sensor is passed to a circuit where it is digitized by an analog-digital converter (ADC).

The digital information can then be stored in memory, reproduced visually on a monitor, or made accessible to the real world through a digital communication port (Lowry et al. 2019).

A chemical or biological sensor works on the principle described by emitting a signal (voltage or electric current, photonic) in response to a chemical reaction such as the bond between two molecules.

This event involves a chemical or biological receptor, R (macrocyclic ligand, antibody-enzyme, etc.) that binds to a specific target molecule in a sample to be analyzed, the analyte.

The signal transmission is accomplished by coupling it with a transducer, which interfaces the processes from the sensor with the processing-transformation unit into a measurable signal. The biosensor is a device sensitive to a physical or chemical stimulus (such as heat or acidity, metabolism) that transmits information about vital processes.

Biosensors deal with the detection of physiological signals and their transformation into standardized “technical” signals, most often electrical, to be quantified from analog to digital. The analysis of signals in medicine and biology aims to process the signals recorded by measurements to extract the maximum useful information in diagnosis and monitoring.

Nanotechnology represents the ability to construct objects by assembling atoms at a given time in well-defined sequences.

Currently, with the tools provided by nanotechnology, genetic engineering, proteomics, we are building “bottom-up” objects, that is, we assemble atoms and molecules in structures. In this context, the notion of biosensor is evolving mainly as a field of application. In the last decade the term biosensor has been applied in various ways to a number of devices either for monitoring live systems or for those that have incorporated biotic elements.

Recently, the IUPAC committee has tried to clarify the definition of the term biosensor by systematizing the specialized literature that over time has been used to describe thermometers, mass spectrometers, and devices for determining Daphne in the water ecosystem, electrophysiological equipment, and identification of chemical elements by ion-selective electrodes.

Currently, biosensors are used in many fields such as medicine, chemical, food, pharmaceutical, military, and therefore it is necessary to know the principles of construction and their operation (according to Table 9.2).

Table 9.2 Classification of biosensors by biological activity

Bio-affinity biosensors $A + R \rightleftarrows AR$ Changing electrons density		Metabolic biosensors $A + R \rightleftarrows AR \rightarrow P + R$ Substrate consumption and product formation	
Receiver R	Chemical signal A	Receiver R	Chemical signal A
Antibody	Antigen	Enzymes	Substrate
Apoenzyme	Glycoproteins	Microorganisms	Cofactor
Dye	Prosthetic group	Microbes	Inhibitive
Lectin	Hormone	Striated tissue	Activator
Transport system	Protein		Enzyme activity
	Analog substrate		
	Substrate inhibitor		
	Saccharides		

By consensus, it has been established that the term should be reserved for use in the modern context as a sensor that incorporates biological elements such as enzymes, antibodies, nucleic acids, microorganisms, or cells.

IUPAC suggests that this term be fully used in this context. With the developments in nanotechnology that have brought extremely wide interdisciplinarity and the materials are currently being manipulated at the nanoscale, the notion of biosensor is getting a wider definition: *The biosensor will be defined as a compact analytical device incorporating a biological sensory element or biological derivative integrated intimately with a physical-chemical translator. The purpose of the biosensor is to produce an analog or digital electronic signal that is proportional to the concentration of a single analyte or a group of analytes* (Scott et al. 2018).

The biological component of which the molecular recognition element is made up is represented by different active species which may be: enzymes or enzymatic systems, antibodies (Ab) or antigens (Ag), receptors, populations of bacteria or eukaryotic cells, tissue fragments, sometimes even embryonic cells.

Analyses or substances that can be analyzed (A) are: glucose or other sugars, amino acids, alcohols, lipids, nucleotides.

They can be identified by their specific interaction or their concentration can be measured by various methods. Both R and A represent distinct molecular species, with high macromolecular specialization (antibodies, antigens, enzymes, receptors, etc.), or are complex systems (cells, tissues, protozoan cysts, eggs of intestinal parasites).

Thus, biosensors can generally be classified into two groups according to the biological component:

- *Catalytic sensors* use enzymes, microorganisms, or cells to catalyze a reaction with a target substance.
- *Affinity sensors* use antibodies, receptors, and nucleic acids to bind to a target substance. The reactions are quantified by electrochemical, optical, evanescent, etc. translators.

According to the active biological component they can be subclassified as follows:

The Enzymatic Biosensor Enzymes are high energy proteins, characterized by their catalytic function. Modified substrate molecules lead to oxidation, reduction, hydrolysis reactions, which can be measured using the enzyme biosensor. The enzymatic biosensor produces a linear response depending on the substrate concentration.

The Immunosensor Antibodies are glycoproteins produced by the immune system at the intervention of an outside substance, the antigen. Theoretically, it is possible to produce antibodies without identifying an antigen. The immunosensor is a high sensitivity sensor. The operating principle is based on the Ag–Ac interaction, molecular recognition.

The Biosensor with Receptors The normality of biological processes is ensured by high sensitivity molecular processes based on the specialization of structural proteins, called receptors capable of recognizing several physiological signals.

This is also the case of neurotransmitters, whose action is mediated by the presence of receptors in the plasma membrane, in sites or cell targets. In this case, the activation of the biologically active site is via ion channels. The acetylcholine receptor is the first receptor known in neurotransmission phenomena.

The Biosensor Based on Cells or Tissues The measurement of the molecular species in this case is not limited to the interaction with the compounds to be analyzed; the transformations that take place can be measured as the resulting products. It is desirable to operate with populations of cells whose main metabolic pathways are known (Zappi et al. 2019).

A relevant example is provided by the L-arginine biosensor, which associates bacterial cell populations of *Streptococcus faecium* in combination with an ammonia electrode.

Arginine is metabolized by microorganisms; according to the reactions in Scheme 9.1 three enzymes catalyze this pathway EC 3,5,3,6=arginine deiminase (ADI), EC 2,1,3,3=ornithine transcarbamylase (OTC), and EC 2,7,2,2=carbamatekinase (CK):

Such complexes of reactions outside cellular structures are difficult to obtain. Similar to the use of cell populations as sensitive elements, fragments or parts of tissues, plants, or animals can be used. In this case, the advantage is greater, because no further efforts are made to maintain viable cells, in a natural arrangement.

$$\begin{gathered}
L\text{–}arginine+H_2O \rightarrow L\text{–}citruline+NH_3\ (ADI)\\
L\text{–}citruline+Pi \rightleftarrows L\text{–}ornithine+carbamyl\text{–}P\ (OTC)\\
carbamyl\text{–}P+ADP \rightleftarrows ATP+CO_2+NH_3\ (CK)
\end{gathered}$$

Scheme 9.1 Mechanisms of transformation of L-arginine through *Streptococcus faecium*

For the adenosine biosensor, a tissue biosensitive element, obtained from the mucosa of the small intestine from a mouse, has been proposed. For the dopamine biosensor, the specialists focused on banana fruit pulp, considering that it has remarkable biocatalytic properties (Herdendorf and Geisbrecht 2018).

Biosensors with Redox Proteins Redox proteins are involved in biochemical processes such as cellular respiration and reactions characteristic of the photosynthesis system. The main types of redox proteins involved and known are:

- *cytochromes,* contain iron ions in the prosthetic group, and cytochrome "c" is involved in the transfer of electrons into the mitochondria;
- *ferredoxins,* contain iron and sulfur ions, in dimeric combinations of chloroplasts (2Fe–2S) ferredoxin and tetrameric combinations of bacterial ferredoxin 2(2Fe–2S), involved in photosynthesis and nitrogen transfer-fixed ion transfer processes;
- *blue proteins,* contain copper bound to the smallest cysteine residue, involved in a tetrahedral structure, such as plastocyanin and azurine, which mediate electron transfer in photosynthesis and possibly reduce the nitrites;
- *flavoproteins,* which contain a prosthetic group and an organic conjugate, are involved in the transfer of proteins such as flavotoxins. These proteins play an important role in nature, due to the location on their surface of redox centers. The subtle architecture of the molecules provides selectivity and specificity of these molecules in their interaction with other proteins or enzymes, such as the structure of the cytochrome "c." Porphyrin iron (heme) is located in the center of the molecule and well coated or hidden; it is exposed to solvents in a small proportion of 0.06%, of the total molecular surface. The protein supports a positive potential of +9 mV due to the excess of basic lysine residues. A dipole moment of 324 Debye is manifested, which produces an imbalance in the balance of the spatial distribution of the acid chains. Several of lysine residues are distributed around the solvent to which the center of the heme is exposed that interacts with the redox proteins.

The field in which these devices have been widely used is the medical one; it is necessary to know the reaction mechanisms and the affinity of enzymes and microorganisms for different substrates of interest (Kostyuk et al. 2018).

9.3 Nanotechnology: The Technology of the Near Future

Nanotechnologies could develop with the advent of techniques such as tunneling scanning microscope and atomic force microscope. These instruments, combined with lithography, allow the observation, manipulation, and creation of nanostructures. Physicist Richard Phillips Feynman is the first to talk about molecular technologies.

It was 1959 and few understood how the British Encyclopedia could be written on a needle tip. In 1985, two scientists in England made spheres of 60 carbon atoms, which resembled the dome designed by architect Buckminster Fuller.

They called them "buckminsterfullerene," but everyone tells them bucky balls. They are six times lighter and one hundred times stronger than the best steel. Bucky balls can be conglomerated in the form of cylinders, called "nanotubes."

Fibers are made from nanotubes, which can be included in various materials, which become incredibly resistant. Ottilia Saxl, executive director of the US Institute of Nanotechnology, said: "The drugs we make today are very ineffective because of precautionary measures."

When we make an antibiotic, which is meant to kill the bacteria, we must take care that it does not kill the cells of our body. A nanorobot, capable of administering the poison only to the bacteria we target, would greatly simplify things.

"Nanotechnology is the technology of the future, of the near future." It allows the introduction of new functionalities in a product, for the future already working on some interesting projects. Nanotechnology-related: Carbon fiber and nano-sized carbon black wall paint act as a screen against 99% of high frequency electromagnetic waves.

They can also act as a shield against electromagnetic field interference in laboratories where measurements are made and in computer centers (Kamali et al. 2019).

The paint industry will be of major importance; about 30% of the total paints to be sold in the future will be based on nanotechnologies. In the paint industry this will lead to products with essentially improved properties:

- Use of painted surfaces with products based on nanotechnologies for the generation of solar energy.
- Self-regenerating paints will repair their small scratches on their own without any external intervention.
- Piezoelectric paints will show the fatigue of materials in real time, for example, on bridges.
- Chameleon paints that change their color reversibly. Thus, on the fog, the vehicles will be able to become much more visible by simply pressing a button.

In recent decades, it has changed radically due to the use of electronic devices. Think about how cell phones have evolved over the past 20 years. Nanotechnologies have played an important role in producing smaller, more efficient, and multifunctional devices. In the future, our life could change through many technological innovations, such as:

- "Tattoos" on the skin to monitor the levels of salts and other metabolites and to warn athletes or diabetics.
- Intelligent food packaging with sensors for detecting the way used to transport the product, for detecting contamination, which is provided with a tracking/communication system that warns the manufacturer and the trader.
- Nanometric devices for transporting drugs and targeting cancer cells.

- The introduction into the circulatory system of drugs that can be activated and controlled from outside the human body; they could collect data and send them to the doctor to modify the (theranostic) treatment.
- Footwear or clothing with sensors that collect data during training.
- Flexible and transparent solar panels, integrated in windows, ceramic tiles, etc., with high efficiency of solar energy conversion.
- Integrated energy harvesting systems (in textiles, footwear, etc.) that collect solar and mechanical energy to charge electronic devices.
- Surfaces and textile materials that remove nitrogen oxides and other smog gases from the urban atmosphere (Kah et al. 2019).

Atoms and molecules stick together because they have complementary shapes that block each other or have charges that attract. Like magnets, a positively charged atom will adhere to a negatively charged atom. As millions of atoms are assembled by nanomachines, a specific product will begin to take shape.

The purpose of molecular manufacturing is to manipulate atoms individually and place them in a model to make a particular object.

According to the general definition, an ideal sensor is a device that will detect an "analyte," the target undergoing the analysis and which is present in a given sample. Most samples also contain other "analytes" that may interfere with the biosensor response (Fig. 9.1). It must possess a *specific selectivity* to identify the target "analyte."

Therefore, it is necessary to design biosensors with selectivity for an "analyte" with the ability to discriminate the interferences produced by the other components of the analyzed sample.

The ability to identify and specific selectivity is the key component of *molecular recognition*. Molecular recognition is accomplished through the sensor component consisting of a *host molecule* (e.g., host-*chemoreceptor*) that selectively binds to the target "guest" analyte ("guest" molecule/complex) that needs to be identified.

For each "host-guest" system there is always a specific chemical reaction from the multitude of possible reaction channels. When the specific host-guest-reaction assembly has been identified then the host molecule is immobilized or incorporated into the sensor, usually on a membrane that interfaces with the transducer or a contact electrode.

Finally, you need to find a way to signal that the binding/recognition event has taken place (transduction to the translator).

Five key areas of science and technology development are identified that have contributed to facilitating the large-scale implementation of biosensors: sensitivity, selectivity, stability, integrated systems, patterning (modelling and multiplication as sensor areas with multiple functions and applications).

Biosensors use biological systems with different levels of recognition of the substances to be determined. The first step in this interaction is the formation of the specific complex of the biologically active substance, immobilized, R (the receptor, the sub-layer with the sensitive biological component) with the analyte A (often defined as the chemical signal) (Beyene et al. 2016).

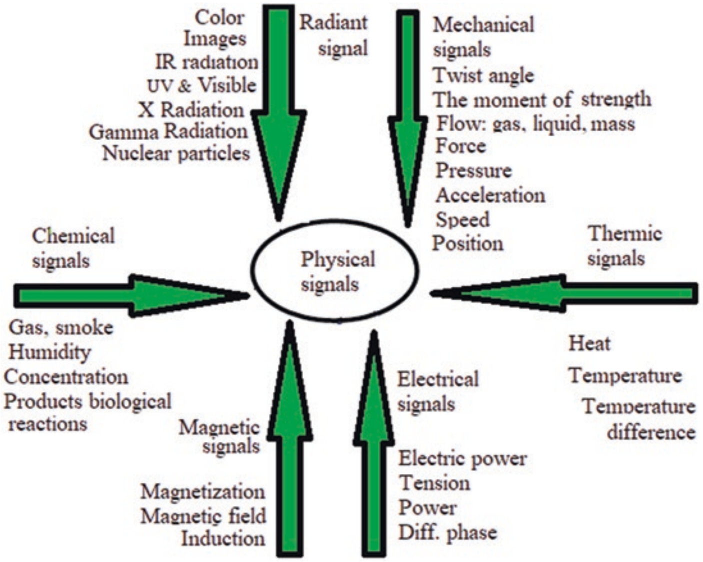

Fig. 9.1 Sizes transformed into measurable physical signals

9.3.1 The Principles of Molecular Recognition

One of the key requirements for molecular recognition is the existence of groups or centers with specific reactivity in the host molecule that can "close" or bind ions, atoms, molecules, biomolecules.

Examples of this kind of host-guest systems with recognition capabilities can be seen in everyday life processes. All living organisms use enzymes, which are proteins containing "pockets," active centers, designed to recognize a specific analyte. This means that only a specific analyte can enter the pocket of the enzyme.

Enzymes can be used in biosensors as host receptors with molecular recognition capability, but are generally unstable.

It is thus necessary to synthesize new classes of macromolecules/biomolecules that are capable of playing the role of *host-receptor* molecules. To design host molecules that can be used in a biosensor, the following criteria are considered:

- ideally, it should release the analyte after detection so that the host is free to be reusable;
- must be able to selectively link the analyte in the sample;
- must be capable of being immobilized in a film/membrane that is in contact with the sample;
- the host molecule must be stable under the conditions under which it will be used;
- must report that a host-guest binding event has taken place.

Biological receptors include antibodies, membrane receptors, enzymes, ribosomes, lectins, etc. They link the analytes using molecular recognition mechanisms of the “lock-and-key” type (key-lock, identification-immobilization).

Biological receptors are generally not practical solutions for many applications because their specificity, sensitivity, and stability cannot be optimized.

Artificial receptors are immobilization media that can be optimized by molecular design for any type of application. The design and synthesis of synthetic receptors are based on instruments developed by proteomics and genetic engineering producing recognition components that can respond to the occurrence and identification of unknown diseases, in bioterrorism, the development of anticancer, antiviral drugs, rapid test kits, clinical and veterinary diagnostics (Mu et al. 2014).

Currently developing platforms and areas of artificial receivers based on combinatorial mathematics techniques, interface biology, surface chemistry. These have led to the development of various mediums of artificial receptors with rapid and diversified selection capabilities for any target analyte.

The current technique for producing synthetic receptors is called CARA—combinatorial array of receptor analysis.

We will present some examples of molecular recognition on relatively simple structures of synthetic receptors, immobilizers with immobilization capacity and identification for metal ions. These examples will create a deeper understanding of the mechanisms involved in biosensors with enzymes, antibodies, cells, etc., where they play an important role in receptor systems (Beyene et al. 2016).

Supramolecular chemistry has developed a diverse range of macrocycles with the role of synthetic receptors. The most common feature for macrocycle classes is that they contain cavities that act as host pockets for guest molecules.

Host selectivity can be achieved in “read” mode by varying the size of the preformed cavities. For example, 12-crown-4 has a small cavity ideal for binding small ions such as Li^+, while 18-crown-6 has a large cavity that better fits larger ions such as K^+.

It is obvious that the size of the cavities is important for the selectivity of the host, but the question remains what exactly attracts an ion or molecule in a preformed cavity and what factors stabilize the host-guest complex.

In enzymes, weak noncovalent interactions (hydrogen bonding, electrostatic, dipole–dipole, van der Waals) are used to bind the guest in the enzyme pocket.

These interactions stabilize the host-guest interaction. Macrocycles contain polar functionalities that are capable of interacting with guests through hydrogen bonding, electrostatic interactions, and dipole–dipole interactions.

It is also desirable that the cavity binding is not too strong; because it is important that the analyte, the guest, be released from the host after it has been detected and measured.

Crown ethers and calixarenes are ideal for the binding of metal cations, based on the size of their cavity but also on the high electron density present on the oxygen atoms in the cavity (Wang et al. 2014).

Tetraethylestercalix arena was prepared from the basic calix macrocycle arena. Although the base compound selectively binds Li^+ to other metal cations, the modified version of the base macrocycle has a very good selectivity for Na^+.

Thus, through synthetic modification it is possible to increase the capacity of the host cavity, and new functionalities can be introduced that will favor the binding of specific molecules and ions. Another example of modified calixarenes, which further demonstrates this principle, is the group of calix tetraphosphorus arena.

By changing the binding groups on the same calix template arena from esters into phosphorous hydrogen oxides, the selectivity from Na^+ to Ca^{2+} is changed. By increasing the number of repeatable units in esters and phosphorous hydrogen oxides to six, the cavity capacity increases and the selectivity changes in favor of larger cations such as Cs^+ and Pb^{2+}, respectively.

This type of sensors has been used to detect metallic cations of biomedical interest and for the environment. Some host compounds have also been developed for selective detection of small neutral molecules without charge. Calixarene structures have been found to have wide application in this area. An example involves the use of amide of tetra-(S-propanol) calyx arena containing four chiral lateral halves for selective differentiation between phenylalaninol enantiomers (Chi et al. 2017).

Other techniques of supramolecular chemistry can be employed in the synthesis of synthetic receptors that simulate the properties of enzymes.

We mention basic structures that can be modified: porphyrins, semiconductor polymers of the tetrathiafulvalene (TTF) and poly(p-phenylene vinylene) or polyphenylene vinylene (PPV), Other templates can be considered modified polysaccharides. A linear archetype is polyanilines containing the two types of redox states (Mukherjee and Menon 2001).

9.3.2 The Molecular Basis of the Ag–Ac Interaction

Of the many classifications of biosensors, those of bio-affinity have a wide range of applications and antigen–antibody (Ag–Ac) interactions play an important role and are increasingly considered as an important tool in the development of molecular recognition principles. We continue to present the notions strictly related to biosensors.

Ag–Ac interactions in vivo are always reversible. The factors that condition the Ag–Ac interaction are:

- *Structural complementarity* between the antigenic determinant and the combination site of the antibody. This is the exclusive factor of the specificity of the reaction. The structural complementarity implies the conformational adaptation of the two reactant groups and was thought in structural terms, on the key-lock principle similar to the mechanism described above.
- *The chemical complementarity* of the reactant groups is the consequence of the structural complementarity and signifies the entry into the action of intermolecu-

> lar forces that stabilize and strengthen the interaction of the two groups. The formation of intermolecular bonds requires the existence of atomic groups close enough to the two molecules. The distance between them is inversely proportional to the degree of complementarity.

Although structural complementarity is not strictly required, a higher spatial fit is more favorable to the interaction. It is expressed by the congruence of the contact surfaces that provide intermolecular attraction forces that stabilize the complex.

The following types of noncovalent bonds are involved in the Ag–Ac interaction: H bonds, electrostatic forces, van der Waals bonds, and hydrophobic bonds. They are all non-specific forces of little value and their nature makes the reaction reversible. H bonds are formed when two atoms share an atomic nucleus of H (a proton).

The common proton is found between two N or O atoms or between one N and one O. The H nucleus is covalently linked to one of the two atoms (N or O). The H bond has a binding energy of 3–7 kcal/mol.

The *intermolecular forces* involved in the formation of the Ag–Ac complex. The action of these forces requires close contact between the two reactive groups.

The H bonds result by forming an H bridge between two close atoms.

The electrostatic forces are due to the attraction of ionic groups with opposite charges located at the periphery of the two protein chains.

Van der Waals forces resulting from the interaction between different electronic clouds, represented in the form of oscillating dipoles. The van der Waals bonds, the weakest interaction forces, are active over very small distances between the reactant groups. The binding energy is 1–2 kcal/mol.

Van der Waals connections are not based on a permanent separation of electrical charges, but on their fluctuations, induced by the proximity of molecules.

At an intermolecular boundary distance instantaneous electric fields are formed, with polarizing effect on the neighboring molecules. Between the atoms close enough, there is a force of mutual attraction induced by the fluctuating dipole charge, which a dipole induces in the neighboring dipole. These forces are also called *dispersion forces*.

Their intensity depends on the distance between the groups involved and is inversely proportional to the power of the 7th distance. Their value is optimal at 1–2 Å.

Hydrophobic bonds, which can contribute half of the Ag–Ac binding force, are produced by the association of nonpolar and hydrophobic groups, from which water molecules are excluded. The optimal distance between the reactive groups varies with the type of bond.

Electrostatic forces (Colombian or ionic) are the results of the attraction between atoms or groups of atoms of opposite electrical charge, located on the two reactant groups: for example, between a cation (Na^+) and an anion (Cl^-) or between COO– and NH_3^+.

The binding energy of these forces is significant at very small distances (below 100 Å) between the reactant groups. The exact juxtaposition of ions favors the action of these forces. The binding energy is 5 kcal/mol and varies inversely in proportion to the square of the distance between the two reactant groups ($1/d^2$).

The hydrophobic (or apolar) *bonds* occur between nonpolar (non-ionized) groups in aqueous solutions and are the consequence of the tendency to exclude the ordered network of water molecules, between the antigen molecule and the antibody molecule.

These links are favored by amino acids with apolar groups, which tend to associate, decreasing the number of water molecules in their vicinity. By eliminating the water molecules between the reactant groups, the distance between the active sites decreases greatly and the value of the stabilizing forces increases.

Spatial complementarity or intermolecular forces are not, in part, sufficient to form stable bonds. Both conditions are required for the stability of the Ag–Ac interaction.

The higher the binding energy of the reactants, the more stable the Ag–Ac complexes (Wang et al. 2019).

The interaction of reactive groups of antigen and antibody is defined by two parameters: *affinity and avidity* of antibodies.

Measurement of antibody affinity can be achieved by equilibrium dialysis Ag–Ac interaction is reversible. Inside the dialysis bag, the hapten is partially free and partly bound to antibodies, depending on the affinity of the antibodies.

Only the free hapten can diffuse through the dialysis bag membrane and its external concentration will equal the concentration of the free hapten inside the bag. Measuring the concentration of hapten in the dialysis bag allows calculation of the amount of hapten bound to antibodies. The constant renewal of the buffer results in the total dissociation and loss of hapten in the dialysis bag, which denotes the reversible nature of the Ag–Ac bond.

The affinity of antibodies measures the *binding force* between an antigenic determinant and the complementary binding site to a specific antibody. Affinity is the result of the forces of attraction and rejection that mediate the interaction of the two reactants. The strength of these interactions is measured in the reaction between a monovalent antigen (hapten) with specific antibodies (Sagawa et al. 2005).

A high-affinity interaction implies perfect complementary structures while the imperfect complementarity of the reactive groups determines a low affinity, because the attraction forces are active only over very short distances and are diminished by the rejection forces. Ag–Ac complexes formed by antibodies with low affinity persist in circulation and are deposited on the basement membrane of renal glomeruli.

Complexes formed by antibodies with high affinity are rapidly eliminated from the circulation, without adverse effects on renal function.

The Ag–Ac interaction is permanently characterized by the formation and cancellation of different types of intermolecular bonds. In vivo, probably all Ag–Ac reactions are reversible, but the secondary reactions, in vitro (agglutination, precipitation), under the equilibrium conditions of the reactants, are irreversible (Yoshida et al. 2019).

9.3.3 Host Molecule Immobilization

Once a receptor compound has been developed to identify a specific analyte, it is necessary that it must be immobilized in the sensory device. The most widely used method is the incorporation of the host molecule into flexible membranes of polymers attached to the sensor surface. Most membranes are formed either of solution of polymers or polymers laminated and preformed into thin films.

The polymers used are generally soluble in the usual organic solvents. The host receptor-molecule must be soluble in a variety of organic solvents. This is achieved by introducing lipophilic groups such as t-butyl into calixarene (Cagil et al. 2018).

Problems with these immobilization methods arise when the membranes come in contact with the sample (which is usually aqueous). It has been found that the host molecules filter over time from the membranes thus reducing the sensor's lifetime.

One way to avoid this problem is to covalently bind the host molecule in the membrane polymer. This approach can be quite cumbersome from a synthetic point of view because the reactive units must be incorporated into the host molecule, which can affect selectivity. In case the reactive groups have been introduced into the molecule they can be used to covalently bind the host in polymers and to the surfaces of the substrate (for example, silica) with simultaneous coupling to the electrode (platinum).

Other immobilization techniques currently addressed are inkjet printing, plasma polymerizations, electrophoretic transport, electro-polymerizations, each with specific advantages (Murray et al. 2017).

9.4 Applications of Nanobiotechnology

9.4.1 Biosensors, Biodiagnostics, Nanobiotechnology, Nanomedicine

Diagnostic platforms, molecular imaging and molecular diagnostics, characterization of biological processes and structures, are based on the foundation and development of biosensors. Thus several areas have been developed such are detection and identification systems, molecular recognition, functionalized nanoparticles with biological systems, sensor areas with high sensitivity and rapid response, imaging with scanning microscopy by atomic forces—SPM (scanning microscopy) of biological structures, intracellular detection of biomolecules, and engineering of nanobio-interfaces.

As we can see we are approaching the point where single-molecule detection, the in vivo determination of the biological properties, and the specific interactions will be possible.

Achieving this goal will allow researchers, physicians, technicians: obtaining of the reliable detection of biomolecules in minimal sample volumes, evaluation and rapid diagnosis of unprocessed, directly sampled materials—for example, rapid diagnosis of the disease or early detection of biological contamination; studying the real-life cycle of organisms at the molecular level, for example, for determining the intracellular interaction of viral proteins with their target cells, thus leading to a better understanding of the associated diseases; and investigating the proteomic dynamics and thus differentiating the pathways and the cellular response to changes in the environment.

The implementation of the conquests in biology supramolecular chemistry and biochemistry on the support of nanotechnology and nanometric scale instruments developed in physics has generated the new domain, that of nanobiotechnology, where biosensors and biochips occupy a preferential place.

Their incorporation into medicine has generated a new domain: nanomedicine, the field where noninvasive and self-repairing techniques are the essential elements of the future of medicine (McDonagh et al. 2009).

Soon we will be attending systems and nanorobots that will take the place of surgery and invasive treatments: targeted transport of drugs to the target, injectable nanobots with precise information that will repair organs, internal generation of biofuel cells to maintain the activity of the heart or lung, neuronal systems, regulation of blood glucose by miniaturized biofuel cells, etc.

Biosensors have propelled border areas and created new fields and sciences: supramolecular chemistry; bioelectronics, biofuel cells, nanoanalytic systems; conception of new synthetic receptors using combinatorial methods; molecular imprinting; immunosensors, sensors on enzymatic structures; natural and synthetic receptors; engineering of biomolecules capable of additional functions such as signal transduction (molecular wires) or mechanical transmissions (molecular motors, molecular arms; engineering of proteins, nucleic acids and saccharides) as receptors and catalytic systems; nanosystems; proteomics and individual cell analysis; nucleic acid sensors, DNA chips; self-assembly techniques.

The success of the biosensor for glucose determination was the point of biosensor development. It has been developed due to the extraordinary demands of diabetes patients and the ability of biosensors to provide a convenient, hygienic, and compact method of personal monitoring (Tric et al. 2017).

Biosensors offer great potential for detecting a wide variety of analyzes in current medicine specific to clinical laboratories or outpatient treatments; in the food industry, in monitoring environmental polluting factors.

The most common enzymes used in enzyme electrode design are those that contain redox groups that change their redox state during the biochemical reaction.

Redox enzymes are oxidase and dehydrogenase, pyrroloquinoline-quinone (PQQ). They act by oxidizing the substrate accepting electrons during the process and then transforming it into a reduced state.

These enzymes return to the oxidized active state by transferring electrons to the oxygen molecule resulting in oxygenated water (H_2O_2).

The oxygen and the peroxide being electrochemically active, continue by reducing the cosubstrate (oxygen) or oxidizing the peroxide (reaction product).

The method based on oxygen reduction at the O_2 electrode is one of the simplest methods but suffers from several disadvantages, namely slow response, miniaturization difficulties, accuracy, and low reproducibility.

Measurements on the oxidation of peroxide overcome the above difficulties and are currently the most popular methods (Han et al. 2017).

9.4.2 Microbial Biosensors

There are two classes of microbial biosensors that use the same principle: measuring the activity of metabolism in the presence of the analyte. The first class uses immobilized microorganisms from which the products resulting from metabolism are measured. This class has become commonly defined as microbial biosensors.

The second class measures the electrical activity of the metabolism of microorganisms when consuming a "biofuel," for example, glucose. This class is known as generic bioelectrochemical cells or biofuel cells.

Being developed as a separate domain of electrochemical cells generating electricity, based on the consumption of biofuels, the aspect of their ability to be true biosensors has been neglected.

Microbial biosensors contain immobilized microorganisms and a much more diverse transduction chain. They are generally used for a single biochemical process (Ravikumar et al. 2017).

Microbial sensors have the following advantages: easy preparation because the cultivation of microorganisms is simple; have a longer lifetime than enzyme electrodes; high variability because it adapts easily to specific environmental conditions; independence from cofactors; lower sensitivity to inhibition or impurification of the analyte; physiological response to toxic products; they are cheaper than enzyme electrodes; they have a higher tolerance in terms of pH and temperature.

The disadvantages of these devices are the following: they have a longer response time than enzyme electrodes; reusing them in a new measurement requires a longer time.

In principle, the layout of a microbial sensor is identical to that of an enzyme biosensor.

The scheme consists of immobilizing intact cells in close contact with a specific translator that converts the products or effects of metabolism into a biochemical signal.

Microbial sensors have found many industrial applications in biochemical and microbiological processes in fields such as drug production, food industry, wastewater treatment, and energy production, where fermentation and electron transfer reactions play an important role. In the field of clinical laboratories—microbiology, parasitology—where intensive culture media are used microbial sensors have an important potential for implementation.

Most materials in the culture media cannot be determined by spectrophotometric methods being optically inactive or non-transparent. A medium combination of culture-immobilization substrates with proper coupling to a translator is feasible with advantages over reducing investigation times and responding to a specific diagnostic.

Continuous, rapid, sensitive monitoring and control of the variable factors already mentioned allowing the evaluation of substrates and metabolism products, the number of viable cells present in the cultures, the types of microorganisms (Hassan et al. 2016).

Electrochemical Microbial Biosensors Microbial biosensors contain microorganisms immobilized in a membrane and an electrochemical device.

They are classified according to the type of respiratory activity measured or the type of electrochemical, metabolic, optical, etc.

In case of a change in the respiratory activity of microorganisms, immobilized in a substrate, it is detected by an oxygen electrode.

By measuring respiratory activity or oxygen consumption, the concentration of the substrate is estimated.

Other electrochemical methods are used in the measurement of metabolism products. The electrochemical microbial sensor shows important differences from the enzyme sensor. It is composed of living components that produce a physiological response, while the enzyme sensor includes alteration of the analyte.

The microorganisms used in this case are aerobic.

The sensor is introduced into an oxygen saturated buffer solution. After addition to the substrate, the respiratory activity of the microorganisms increases, causing the concentration of oxygen to decrease near the membrane. Using the oxygen electrode, the substrate concentration can be measured by detecting the decrease in oxygen concentration.

Another type of microbial biosensor detects metabolic, electrochemical activity, such as hydrogen, carbon dioxide, ammonia, and organic acids, which are secreted by microorganisms. Both aerobic and anaerobic microorganisms can be used.

Most microbial biosensors are based on the measurement of respiratory activity and use the activity of aerobic microorganisms (Plekhanova et al. 2019).

Photomicrobial Biosensors It is based on the optical phenomena that microorganisms can have in metabolic processes: photoluminescence, electroluminescence, chemoluminescence, specific optical activity (chirality dependent polarization, refractive index, absorbance, etc.).

For example, the luminescence intensity of photobacteria (luminobacteria) is dependent on metabolic activity. It is thus possible to build highly sensitive microbial biosensors by combining these photobacteria with a photodetector.

In vivo luminescence of photobacteria is produced by the following enzymatic reactions in the presence of *oxidoreductase* and NAD(P)–FMN:

$$\mathrm{NAD(P)H} + \mathrm{H}^{+} + \mathrm{FMN} \rightarrow \mathrm{NAD(P)} + \mathrm{FMNH}_2$$

$$\mathrm{E}(\text{luciferase}) + \mathrm{FMNH_2} \rightarrow \mathrm{E - FMNH_2}$$

$$\mathrm{E - FMNH_2 + RCHO + O_2 \rightarrow FMN + RCOOH + H_2O + hv}$$

where RCHO–aliphatic aldehyde and RCOOH–fatty acid, FMN–flavin mononucleotide.

Luminescence is strongly affected by changes in external conditions for bacteria that can subsequently lead to the accidental accumulation of intracellular concentrations of NAD(P)H, FMN, H_2, ATP, and aldehydes (enzyme synthesis of aldehydes requires cofactor ATP). Using this principle, the food of these microorganisms (e.g., glucose and amino acids) and their inhibitors (e.g., toxins and heavy metals) can be detected.

Luminescence intensity is a more sensitive parameter for metabolic activity than for heat generation activity.

Moreover, the metabolic response of cells can cause luminescence in photobacteria. The behavior of microorganisms is given by their physiological state, and this is given by the extreme limitation of nutrients (Deriabin and Aleshina 2008).

Metabolism is a condition that ensures cell survival.

The first critical condition for forming a signal at the microbial sensor is the use of the substrate. The solute can enter the cells through the specific translocation system, first by active transport or by forced diffusion.

Passive transport carried out only by diffusion due to the concentration gradients of oxygen and nutrients is of minor importance. Active transport ensures the accumulation of nutrients in the substrate in the sense of concentration gradient.

This causes the substrate to accumulate proteins with high specificity that is consumed in energy metabolism. Also, the coupling of cells with the energy phenomena transduction system, especially in the respiratory chain, is an important aspect of active transport, being crucial for signal formation in the microbial sensor, for example, the use of glucose, sucrose, and other oligopeptides.

After using the substrate, it is specifically degraded by the enzyme sequences present and immobilizing the cells. Under aerobic conditions, oxygen is consumed.

Thus, organic acids such as lactic acid and pyruvate, carbon dioxide, ammonium ions, and hydrogen sulfide are secreted and accumulate in products of cellular metabolism. It is worth mentioning the importance of iron in the biology of bacteria, in general and especially in gram-positive ones, in particular.

The efficient multiplication of pathogenic bacteria in the tissues of the host organism is conditioned by the obligatory presence of ferric iron, in a form accessible to their metabolism. Iron is required for certain enzyme reactions essential for growth but is also a key component of the electron transport chain in the cell membrane—cellular respiration. Therefore, the microorganisms cultivated for the preparation of a microbial biosensor must come from cultures on metabolically balanced environments concerning ferric iron, which will ensure good cellular respiration to the microorganisms.

Other types of electrodes can be used in microbial biosensors, in combination with electrodes for H_2, CO_2, NH_3. At a constant temperature and pressure, as a standard, the proportion of oxygen in the air, in volumes, is 20.9%.

In aqueous solution and equilibrium with atmospheric air, the proportion of oxygen is also 20.9%, of the total dissolved gases. In both cases in air and solution, the nitrogen balance is greater than the carbon dioxide balance.

The concentration of dissolved gases in the solution varies with temperature, and may be affected by the solute or other solvents present in the solution. For example, a warm solution retains less oxygen than a cold solution.

The presence of ethanol significantly increases the ability of an aqueous solution to retain oxygen. In the specialized studies it is shown that small temperature differences can affect the balance of the environment with the air in the room, as well as some reagents that we add to the working cell (e.g., ethanol).

The selection of the transducer device is given by the physiology of the cells that we use in the sensor structure (respiration, photoluminescence, and gravimetry) (Karube and Nakanishi 1994).

The direct combination of microorganisms with a field-effect transistor was developed for estimating glucose of alcohol with *Acetobacter* and cell-based xylose of *Gluconobacter oxidans*.

It has been found that a number of soluble mediators with redox potential, such as phenazine ethosulfate, fericianide or fericianide in combination with benzoquinone, have the possibility of direct measurement of electrons tracking the metabolic activity of cells.

Insoluble mediators such as ferrocene, tetrahydra-fulvalene, and tetracyanoquinodimethane have recently been used, which have been incorporated into carbon pastes in contact with *Paracoccus denitrificans*.

Another interesting technique of microbial biosensor was created by the luminescence of photobacteria connected with an optical detector. Thus, a microbial sensor was created and described for the determination of metal ions and aromatic ions.

Through genetic engineering the genes responsible for light emission from the *Vibrio photobacteria* were transferred into the genetic structure of the *Escherichia coli* or *Serratia marcescens* bacteria (Kim and Kwon 1999).

However, the future of translators in microbial biosensors is held by piezoelectric crystals, quartz microbalances with one of the functionalized electrodes.

By combining microorganisms with enzymes, it is possible to increase the selectivity of the microbial sensor. These combinations are capable of determining biopolymers, such as starch, proteins, and lipids, which cannot be appreciated by microorganisms.

For these microorganisms were combined with hydrolases (Maillard et al. 2011).

This resulted in a hybrid, amperometric sensor for the determination of NAD^+, based on *E. coli* and NAD-ase cells. Urea and creatinine were determined using a combination of nitrifying bacteria and urease or creatinase.

From the experiments with the BOD sensor it was concluded that several species of microorganisms can be combined and several substrates can be used in the same experiment. Another typical example of biosensor containing mixed populations of different species of microorganisms having specific metabolic capacities is the biosensor with nitrifying bacteria.

This sensor, which was developed especially for wastewater investigation, contains mixed cultures of *Nitrosomonas sp*. and *Nitrobacter sp*. In this case, an amperometric measuring system was used to determine ammonia.

Oxygen control by the oxygen sensor is required to measure the ammonia concentration in the sample to be measured.

Because the biosensor reacts with other nitrites or urea, the amounts of nitrifying substances are usually added up. The main impediment is the surplus of substrate (ammonia or urea).

This process, of combining different species of microorganisms, is also successfully used in the field of toxicology. An inhibitor can usually be used when the substrate concentration is too high. One possibility is to use dialysis membranes, when the substrate is in excess (Lei et al. 2005).

9.4.3 The Effects of Nanotechnology on Health

Any emerging technology may be associated with unknown health risks when it first reaches consumers. Thus, mobile technology: the health risk involved in the use of mobile phones has been discussed after years of using them even now this risk is not fully understood. Despite this, we are currently using mobile phones.

The safety of nanotechnologies has been in the spotlight for many years, being discussed by several civil society organizations, which raised health problems and called for early action to ensure the responsible development of nanotechnologies.

The common desire is for this technology to progress, while ensuring that workers and consumers are not exposed to risk.

Nanotechnologies use extremely small nanoscale materials that are extremely small, so there is a concern that they could harm people by penetrating protective barriers, such as the skin, causing damage to the human body.

For example, sunscreen lotions containing nanomaterials have been analyzed. However, to date, scientific evidence shows that nanoparticles do not penetrate the skin. So far, there has been no evidence that consumer goods containing nanomaterials would be harmful to consumers.

However, research is ongoing to verify and detect any toxicity of nanoscale materials, especially for products that come into direct contact with the human body.

Sensitive and selective determination of a large number of compounds was and is still very useful for scientific research, as well as for some industrial branches (chemical and food). In the field of health, it became indispensable to find methods for diagnosing diseases and the sensors with a fine selectivity and easy to handle became a key problem in the improvement of the laboratory analyses.

During this period a series of sensors on solid structures were used to determine physical parameters such as temperature, pressure, sound energy but qualitative and quantitative analyses made on the chemical composition remained difficult.

The best-known at this stage was the pH meters that do not apply to the measurements of important physiological substances such as urea, cholesterol. They became useful in the first investigations in the case of macromolecules such as enzymes, antibodies, or microorganisms (Li et al. 2009).

However, living beings can adapt to changes in their metabolism and the environment through the help of so-called receptors, which are composed of complex protein structures and are largely linked to cell membranes. They have a high affinity for specific factors, such as hormones, enzymes, or antibodies.

The link between these factors causes the activation of "cascades" of enzymes, which cause changes in the receptor protein and amplification of the signal. Clark extends the use of this "oxygen measuring electrode" to determine the level of glucose in the blood.

He "coated" the oxygen sensor with a thin layer of gel containing a biocatalyst, the enzyme glucose oxidase, followed by a semi-permeable dialysis membrane that allows glucose to diffuse into the sensor, but stops the enzyme from diffusing. The more glucose enters, the more oxygen is consumed by the enzyme (Leso et al. 2019).

Therefore, a small amount of existing oxygen translates into a high level of glucose. The term enzyme electrode is introduced, and the details necessary to construct an enzyme electrode for glucose are described experimentally.

The first description of a biosensor was made in 1962, where a platinum electrode with the enzyme oxidoreductase was presented, in sandwich construction.

The platinum anode polarized at +0.6 V responds to the peroxide produced by the enzyme in reaction with the substrate. This type of biosensor opens the way for blood glucose measurement.

While Clark-type sensors measure the flow of current through the electrode (amperometric sensor), the potentiometric sensor measures the voltage required to keep the current at zero. The electrode does not consume any reactants so it is less susceptible to errors caused by changes in the external environment.

The potentiometric system has a logarithmic response curve so it can track a concentration more than 100 times higher than other types. Later, more than 100 enzymes were used in the construction of biosensors.

Researchers in the field have realized that not only single enzymes can be used, but also tissues that react to amino acids and other biomolecules.

For example, the use of banana pulp for dopamine measurement, corn kernel for pyruvate, cucumber leaf for cysteine, sugar beet for tyrosine, rabbit liver for guanine, and rabbit muscle powder for adenosine monophosphate. went even further in using parts of biological systems; one of its sensors contains an antenna, a small sense organ, from a blue crab, subjected to dissection to use the nerve fibers connected to an electrode.

This device can measure the concentration of drugs, as well as toxins of the environment (Nikoleli et al. 2018).

In 1975, biosensors take a new revolutionary path when it is suggested that bacteria can be used as a biological element in a microbial electrode to measure alcohol concentration. The article marks the beginning of research efforts in Japan and subsequently in all countries of biosensor applications in environmental protection and biotechnology. In 1975, to describe the optic fiber sensor which has immobilized an indicator for the measurement of carbon dioxide and oxygen, the researchers extend the concept by developing an optical biosensor for alcohol by immobilizing the alkoxide oxidase at the end of the optic fiber sensor for oxygen. In 1976, an electrochemical glucose biosensor was incorporated into an artificial pancreas.

The biostat was not widely marketed and was replaced by a continuous catheter glucose analyzer. In the same year La Roche (Switzerland) introduced Lactate Analyzer LA 640 in which the soluble mediator hexacyanoferrate is used for electron-mediated transfer from lactate dehydrogenase to the electrode.

Although it did not gain commercial use, the generation of biosensors with mediators in the transfer of electrons with applications in dairy analyzers for sports and chemical applications was opened.

A breakthrough for in vivo applications of the glucose sensor was reported by researchers describing a needle-like enzyme electrode for subcutaneous implantation, 1982, a scientific success but without commercial use (Damborska et al. 2017).

The idea of constructing immunosensors with direct antibody binding on a piezoelectric or potentiometric transducer has been explored since the 1970s.

This principle is the basis of the enzyme electrodes released by MediSense (Cambridge, USA) in 1987 as a pencil measuring instrument for the monitoring of blood glucose in outpatients. Its entire electronics were designed on the credit card model in the format of a "mouse–computer."

The yeast-based biosensor performs a reading of the results in 30 min as opposed to 5 days to obtain the results by conventional methods. Biosensors are also used to determine the quality of the food and its freshness.

They are very successfully used in industrial processes to determine the chemical composition of materials. These measurements are especially important in biotechnology where cultures of microorganisms that ferment fermentation processes that produce active proteins and other products such as interferon or insulin cannot currently be monitored (Dosekova et al. 2017).

For measuring glucose in beverages was use of pyrroloquinone as a mediator in a glucosoxidase enzyme electrode.

Alternatively, the electrocatalytic detection of the reaction products resulting from the enzymatic reactions can be improved by chemically modified electrodes such as rodinized electrodes or hexacyanoferate modified carbon electrodes.

For example, the use of Prussian blue to modify the electrode surface at the amperometric detection of oxygenated water at both oxidation and reduction potentials for the enzyme electrode in the detection of lactate and glucose (Krushinitskaya et al. 2011).

A more elegant solution is to search for the redox centers of the enzyme via a molecular wire to perform the transfer of electrons to the electrode. In this regard, much has been published about enzymes linked by molecular yarns, but in general concerns have focused on immobilizers mediated on different polymer chains.

Molecular strands are regarded as intermediates in electron transfer over long distances being made up of groups of two pyrimidines linked to thiophene of different lengths.

Wires of this type can be used in conjunction with self-assembly techniques to produce an isolated electrode that transfers electrons by predetermined molecular pathways. This would lead to enzymatic electrodes free from electrochemical interference.

Modeling and computer-aided design allow us to model electron transfer reactions, receptor binding, and their interactions with high accuracy.

This enhances our understanding of the receiver/translator interface thus allowing us to develop new types of receivers with high selectivity.

To obtain improved ligands for use in optosensors such as glycohemoglobin (HbA1c), a new synthetic peptide, it was constructed from a chemometric combinatorial library consisting of 1 million L-aminoacid hexapeptides starting from 10 amino acids.

The hexapeptide library was selected, and analyzed for glycohemoglobins HbA1a, HbA1b, HbA1c and HbA0 (major hemoglobins) and corresponding sequenced ligands were found.

Individual ligands or areas of ligands in conjunction with the shape recognition technique are areas that will contribute to the increase of selectivity and stability (Zaitouna et al. 2015).

9.4.4 The Effects of Nanotechnology on the Environment

All products become waste at the end of their life.

One question that citizens and researchers ask is: do nanomaterials from nanotechnology products produce harmful effects when they reach the trash?

Could these residues interfere with animals and plants and cause harmful effects?

These are complex questions, and finding answers takes time.

A lot of research is taking place all over the world. Scientists are also looking at whether there are safety issues when washing garments containing nanomaterials. Some civil society organizations argue that consumer goods containing nanomaterials should not be marketed until there are clear answers to these questions.

Others claim that we have already coexisted with many natural nanoparticles in the environment, nature, and pollution.

Nanoparticles are not just a novelty and, although we should do more research on their safety, we should try not to raise too many obstacles in the development of this field of research.

Nanotechnology can have a positive effect on the environment. For example, scientists could program nanobots into the air to rebuild the thin ozone layer. They could remove contamination from water sources and could clean up oil leaks.

Manufacturing materials using the reverse method of nanotechnology pollute even less than conventional manufacturing processes.

Our dependence on non-renewable resources would decrease with the help of nanotechnology. Cutting down trees, mining, or drilling oil would no longer be necessary—nanomachines could produce these resources (Wu et al. 2017).

9.5 Compliance, Risks, and Ethics

Potential benefits of using nanomaterials in food are improved bioavailability, antimicrobial effects, increased acceptance of sensors, and targeted delivery of bioactive compounds.

However, there are challenges in the applications of nanomaterials due to knowledge gaps in the production of ingredients, such as nanopowders, the stability of meat delivery systems, and the health risks caused by the same properties that they offer, also benefits.

For the success of nanotechnology in meat products, the challenges in public acceptance, economics, and regulation of processed foods with nanomaterials, which may have the potential to persist, accumulate, and lead to toxicity, must be addressed.

So far, the most promising area for nanotechnology application seems to be meat packaging, but it is necessary to further study the long-term effects on human health and the environment, due to the migration of nanomaterials from the packaging.

The future of nanotechnology in meat products depends on the roles of governments, regulatory agencies, and producers in addressing the challenges of applying nanomaterials in food.

Currently, there are no specific EC regulations on nanomaterials.

The official position, after numerous revisions and estimates, is that nanomaterials are well controlled by current regulations.

Nanomaterials are treated like any other chemical, a substance that has to comply with a set of regulations to be used in consumer goods and industrial processes.

However, some civil society organizations require specific regulation for nanomaterials, considering that nanomaterials have special properties, which require special attention.

At present, there is no law requiring the inclusion on the label of a product of the specification for the content of nanomaterials, except cosmetics and foodstuffs, which must state this in the list of ingredients. The most immediate challenge in nanotechnology is that we need to learn more about materials and their properties at the nanoscale.

Universities and corporations around the world are rigorously studying how atoms fit together to form larger structures.

Because the elements behave differently on a nanometric scale than their large-scale shape, there is concern about certain nanoparticles that may be toxic.

Some doctors fear that nanoparticles are so small that they could easily cross the blood–brain barrier, a membrane that protects the brain from harmful chemicals in the blood. If we are going to use nanoparticles to cover everything from clothes to highways, we must be sure that they will not poison us.

There are also some social concerns related to nanotechnology (Resnik 2019).

Nanotechnology could also create more powerful weapons, both lethal and non-lethal. Some organizations are concerned that we will only end up examining the ethical implications of nanotechnology in terms of weapons, after the devices are already built.

They urge scientists and politicians to carefully examine all the possibilities of nanotechnology before designing ever more powerful weapons.

Not all questions involve the modification of the human body; some also refer to the world of finance and economy.

If molecular manufacturing becomes a reality, how will this affect the global economy?

Assuming we can build whatever we need with a single click, what happens to all jobs in factories?

If we can create anything using a replicator, what will happen to the currency?

Did we permanently move to an electronic economy?

Do we need any more money?

Eric Drexler, the man who introduced the nanotechnology to the world, presented an apocalyptic vision-nanorobots that replicate billions of times, rapidly consuming the entire world while drawing carbon from the environment to build more of their offspring.

This is called the "ash matter" scenario, where a nano-size synthetic device replaces all organic materials (Sohal et al. 2018).

Another scenario involves nanodevices made from organic material that destroys the Earth–this is called the "green matter" scenario.

9.6 Conclusions and Recommendations

Twenty-fold increase in energy production could also be possible with the help of nanotechnology. It would reduce the costs required for carbon and organic nanoparticles waste, improving the potential of biomass to produce energy.

Nanotechnology could also be an ecological solution for electricity generation.

Nanostructured materials, as well as carbon nanotubes and ceramic nanoparticles could help develop more efficient materials. The acquisition of new physicochemical properties of the materials opens the perspective of some areas of fundamental research and applications grouped under the name of nanosciences.

Contaminants from wastewater could be removed using nanotechnologies.

The presence of contaminants such as natural organic substances and small amounts of organic substances that accumulate in the body create great problems when treating water.

Coagulation/flocculation and chlorination technology is the most widely used technology for removing contaminants.

However, with this technology, not all contaminants can be completely removed. Also, exposure to aluminum is suspected to play a role in the onset of Alzheimer's disease.

Natural organic substances react with most disinfectants used in conventional treatment plants (chlorine, ozone, chloramine, etc.), giving rise to by-products such as trihalomethanes (THM), haloacetic acids (HAA), bromoform ($CHBr_3$), dibromic acid acetic acid (DBAA), and 2,4-dibromo phenol (2,4-DBP), which are carcinogens.

The use of micro/ultra-filtration membranes in the water treatment process is a modern method of producing quality drinking water.

However, the classic membranes tend to lose material, and through the pores created they pass the natural organic substances, the organic substances that accumulate in the body and the microorganisms.

The auto industry could produce tires with higher grip qualities, it could improve the rigidity of the car body, auto reflective windows to prevent blindness, prevent the effect of condensation on windows, etc.

The building materials industry would produce materials with high fire retardant and thermal insulation properties, high hardness, and more.

Extending life expectancy by implementing nanotechnologies used in the medical system, eradicating diseases that cannot be cured at this time, repairing clogged arteries, or stimulating the heart are some of the advantages of nanotechnology.

Currently, the market for nanotechnology products is more than 100 billion euros and is expected to grow to more than 1000 billion in the coming years.

According to European Union forecasts, in the coming year, the global market for nanotechnologies could represent between 750 and 2000 billion euros.

Thus, soon, the useful creative potential could reach 10 million uses related to nanotechnology (about 10% of the total creations used in manufacturing industries worldwide).

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Chapter 10
Zooplankton: The Significant Ecosystem Service Provider in Aquatic Environment

Yahya Bakhtiyar, Mohammad Yasir Arafat, Saima Andrabi, and Hamid Iqbal Tak

10.1 Introduction

Zooplankton comprise very small organisms but the valuable assets of the aquatic biota with size ranging from few microns to few millimetres or more, which are actually representatives of almost every taxon of the animal world (Goswami 2004). Among zooplankton, some spend their whole life as plankton (holoplankton) or some part of their life as plankton (mesoplankton) (Lindeque et al. 2013). In general, zooplankton represents the organisms living in the water column and are collected with plankton net (the so-called as net zooplankton). Zooplankton community may be non-motile or weak swimmers which drift in water bodies via water currents and are correlated with respect to changes in the phytoplankton communities (Zannatul and Muktadir 2009; Perbiche-Neves et al. 2016). Since they feed on either phytoplanktons or other members of zooplankton species, they play an important role in food web (Ward et al. 2012) and also act as a means to transfer energy between phytoplankton and fish (Telesh 2004). Zooplankton community is affected by both biotic and abiotic components of an aquatic ecosystem. Among the biotic components, food quality and predation (Danielsdottir et al. 2007) and among the abiotic components temperature, pH, turbidity, conductivity, biological oxygen demand (BOD), chemical oxygen demand (COD), etc. alter or affect the distribution, abundance and seasonal variation of zooplankton community (Sebastian and Yamakanamardi 2014; Raut and Shembekar 2015; Das and Kar 2016). Some of the other important ecological factors viz., dispersal through atmospheric wind and water flow regulate zooplankton distribution (Havel and Shurin 2004). The main

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five types of freshwater zooplankton groups include protozoa, rotifera, copepoda, cladocera and ostracoda, most of which depend on various bacterioplankton and phytoplankton for their food (primary consumers), and some feed on smaller zooplankton, therefore playing a role of secondary consumers in the food chain (Dhanasekaran et al. 2017). Zooplankton community play an important role in the overall balance of an aquatic ecosystem (Midya et al. 2018) as they hold very good importance in various biological phenomena viz., regulating the allocation of matter, energy and pollutants among trophic levels (from lower to higher) of food webs (Bettinetti and Manca 2013), act as major consumers of primary production (Calbet and Landry 2004) and also regulate sedimentation and cycling of nutrients especially nitrogen, phosphorus and carbon (Eyre and McKee 2002).

Rotifers called as wheel animalcules are the soft bodied metazoan protostomes with cosmopolitan distribution (Shah et al. 2015). The habitats of these diverse assemblages of rotifers include the slightly acidic oligo to mesotrophic waters (Segers 2008). They possess filter feeding mechanism of the mode of nutrition by consuming different kinds of food of varying sizes (Wallace et al. 2006; Wallace and Snell 2010). The community composition and density of rotifers vary greatly with respect to eutrophication level (Zankai 1984; Park and Marshall 2000); therefore, they often act as bioindicators of water pollution (Bonecker and Lansac-Tôha 1996; Shayestehfar et al. 2008). The family brachionidae of rotifer indicates eutrophication of water (Pal et al. 2015) wherein abundance of *Brachionus calyciflorus* indicates the organic pollution (Pandey et al. 2013). They also play an important role in energy relocation and nutrient cycling, therefore act as a food source for commercially important crustaceans and fish (Telesh 2004).

Copepods are the dominant members of the zooplankton which are abundant in freshwaters in planktonic, benthic and groundwater forms (Reid and Williamson 2010). Copepods occupy the position of consumers (primary and secondary) in the aquatic food chain, therefore play a crucial role in food webs and form a main portion of biomass of consumers in the aquatic ecosystem (Sladecek 1983). Copepods also act as chief source of diet for invertebrates as well as vertebrates together with fish and prawn (Turner 2004) which are commercially very much important. Copepods are found in both fresh as well as marine water habitat (Fathibi et al. 2017) and among freshwaters, they are divided into five orders, which include Clanoida, harpacticoida, Cyclopoida, Gelyelloida and Siphonostomatoida (Boxshall and Defaye 2007).

The cladocerans, commonly called as water fleas, inhabit pelagic, littoral and benthic zones, but are rare in oceans (Fathibi et al. 2017). In the lake plankton, cladocerans especially the genus *Daphnia* are the significant herbivore (Sommer and Sommer 2006) which can be easily cultured and possess short generation time and hence represent an important model organism as far the ecological and evolutionary studies are concerned (Forro et al. 2008).

Ostracods are the most diverse group of crustacean living in fresh, marine as well as brackish waters and also act as model group for several ecological and evolutionary studies (Perez et al. 2010). They possess calcified carapace which reflects the variation in the ecological factors of its habitat, such as temperature, pH, salinity, etc. (Ruiz et al. 2013); therefore, they can be used as bioindicators of climate change.

10.2 Zooplankton and Trophic Interactions in Food Webs

In order to channelize the flow of matter (nutrients) and energy, the biotic constituents of an ecosystem are well organized by the trophic structure (Casini et al. 2008; Llope et al. 2012). The proportion of prey production that is converted into predator production (i.e. trophic transfer efficiency) largely determines the strength of trophic interactions and is very much fundamental for understanding many natural processes (Pauly et al. 1998). The "trophic transfer efficiency" influences many of the ecological parameters like food chain length and trophic structure (Post 2002), estimation of amount of carbon transfer and fishery production (Richardson and Jackson 2007). Studies on the aquatic food webs over the past eras have revealed that in structuring aquatic ecosystems and trophic interactions, the specific species play a principal role through trophic cascades or through the influence of "keystone species" (Paine 1992; Power et al. 1996). Some other factors which influence the structure of aquatic food webs are species invasion and fishery exploitation (Jennings and Polunin 1996; Pinnegar et al. 2000). In the so-called planktonic food webs in aquatic ecosystems, the zooplankton are functionally very much important as they establish a key link between primary producers and the species in the higher trophic level; therefore, these zooplankton herbivorous species can greatly influence the production of phytoplanktonic biomass and their species composition (Sommer et al. 1986) and also constitute foremost nutrient source for many invertebrate predators and the fish (Boudreau and Yan 2003). The increase in body size of zooplankton is dependent on the quantity as well as the quality of food (Vrede et al. 2002). Since zooplankton play a central role in trophic cascade mechanisms, the concept first proposed by Paine (1980) was primarily applied to pelagic systems by Carpenter et al. (1985) so as to designate the downhill transmission of top-down effects from fish to phytoplanktons of a lake (i.e. predatory fish more in number result in less number of planktivorous fish, which further results in abundance of zooplankton and fewer number of phytoplankton). Therefore, zooplankton community can play a central role in lake restoration via the so-called biomanipulation phenomena. In order to reduce the phytoplankton blooms in the water body, so as to enhance the light attenuation in water column successfully, zooplanktivorous fish was completely removed which in turn enhance the herbivorous zooplankton population, so as to reduce phytoplankton blooms (Wright 1984). These are the examples of Trophic cascade phenomena, which exist more often in freshwater planktons as compared to marine planktons because of the zooplankton–phytoplankton associations are different with respect to the lakes and sea (Sommer and Sommer 2006).

Trophic communications include not only the energy transference but also biochemicals, so it is of considerable importance to identify the composition of plants and animals which are in focus and their metabolism (Brandt 1899). Sometimes, many of the required biochemical constituents cannot be produced by heterotrophs themselves; therefore, if the disproportions of vital biochemicals amongst heterotrophic consumers and their target prey are large, then those biochemical constituents become limiting (Muller-Navarra 2008). Aquaculture research has shown that the subset of C_{20} and C_{22} “highly unsaturated fatty acids” (HUFA) and “polyunsaturated fatty acids” (PUFA) is of great importance in the diet of fish larvae for their proper development (Sargent et al. 1999; Hamre et al. 2005; Morais et al. 2007), which are provided essentially by zooplankton. For several decades the research revealed that marine creatures discharge free amino acids into water (Parry 1960; Prosser and Brown 1961). Some studies pointed out that marine zooplankton like copepod and others release substantial amount of glycine, taurine and alanine (Johannes and Webb 1965) and Daphnids, through feeding also discharge some amino acids (Gardner and Miller 1981). The laboratory experiments point out that these dissolved free amino acids (DFFA) might serve as the source of nitrogen and energy in addition to growth components for bacteria as well as other marine saprotrophs (Ostroff and Henry 1939; Provasoli 1963). There are two arguments regarding why zooplankton release dissolved organic compounds. One argument is regarding the concept of “community stability”, which states that particulate organic derivatives from dissolved organic matter offer a constant fuel or sustenance for zooplankton that phytoplankton alone ensures, which lead to more community stability (Riley 1963). Alternative argument provided by Wangersky (1965) is the matter of “cost accounting”, which states that by keeping the small organic molecules quantitatively in an aqueous ecosystem might entail more energy than if vanished by “diffusion” when made in excess of abrupt needs.

10.3 Zooplankton as a Source of Chemical Compounds

In natural ecosystems like bogs, the mineralization of organic matter is the source of nitrogen and phosphorous (Fellman and D’Amore 2007). The microbial mineralization is not only the source of release of minerals, but some ciliates and rotifers excrete significant amount of nutrients in bog ecosystems (Bledzki and Ellison 2002; Mieczan 2012). The primary production in freshwater ecosystems is maintained by the nutrient regeneration (Vanni and McIntyre 2016) and the rotifers are reported from various lakes for their ability of nutrient regeneration (Hudson et al. 1999; Ejsmont-Karabin 2004). About 40% of the primary production in marine environments is being removed by zooplankton (Calbet 2001), some part of the assimilated organic matter is used for growth and reproduction, while the non-assimilated is discharged out as dissolved mixtures by means of excretion (Moller 2007; Saba et al. 2011). Rotifers excrete significant amounts of nitrogen (NH_4-N and NO_3-N) and phosphorous (PO_4-P) by well-studied biochemical pathways (Gulati et al. 1995; Bledzki and Ellison 2002). Therefore they constitute chief assemblage of aquatic invertebrates responsible for nutrient renewal by elimination of organic nutrients after consumption of organic matter (Ejsmont-Karabin 1984;

Den Oude and Gulati 1988). So this group of zooplankton performs a great job of nutrient cycling in bogs and freshwater at local, regional and global scales. Carbon cycle is an important concept of ecosystem ecology which poses a profound effect on climate change and health of an aquatic ecosystem because even in waters supersaturated with carbon dioxide (CO_2), it may co-limit primary production (Matveev and Robson 2014). In the herbivore food chain of aquatic ecosystem, the carbon present in phytoplankton flows towards zooplankton grazers and finally to fish. Many a time of top-down effects in aquatic food web, there is suppression of zooplankton grazers by which carbon accumulation by phytoplankton increases (Carpenter et al. 1987), which implies increased capture of CO_2 and increased sedimentation (Flanagan et al. 2006).

Copepods stay significant suppliers of bioreactive mixtures for marine bacteria through faecal pellet production and the elimination of dissolved compounds, nevertheless the quality and composition of zooplankton excreta is reliant on the quality as well as the quantity of food they consume (Miller and Roman 2008; Alcaraz et al. 2010). The dissolved matter so discharged supplements to vertical transport of bioelements due to “diel vertical migration” as well as acts as a source of dissolved compounds for microbial community and phytoplankton (Steinberg et al. 2002; Perez-Aragon et al. 2011). Dissolved organic matter (DOM) is regarded as the main reservoir of nutrients in the marine environment which consequently become available to phytoplankton, and the addition of DOM can reshape the bacterial community composition (Sarmento et al. 2013). Copepod feeding can increase the uptake of phosphorous and bacterial activity in a phosphorous limited system (Titelman et al. 2008). The excretory products of copepods viz., dissolved organic nitrogen and dissolved organic phosphorus generate a variety of changes in bacterial community and recycling of nitrogen and phosphorus, consequently redesign the composition of dynamic marine bacterioplankton community (Valdes et al. 2017).

10.4 Zooplankton and Bioaccumulation Phenomena

Bioaccumulation process is the concentration of contaminants into biota which reach the higher trophic levels by the process of biomagnification, which increases the chemical concentration of a contaminant at each successive trophic level (also known as trophic biomagnification) (Gobas et al. 1988; Suedel et al. 1994). Organochlorine compounds accumulate at a faster rate in zooplankton than their predators (i.e. zooplanktivorous fish) and is likely to react much faster towards the changing concentrations of contaminants present in water column (Bettinetti et al. 2010). It is also reported that in the water column, zooplankton also react much rapidly than fish, to changes in organochlorine caused by thermal stratification events (Bettinetti et al. 2010). These attributes provide a clear idea that zooplankton (an important component of pelagic food web) are potential organisms having ability to be used as a biological tool for transfer of pollutants and an early warning tool for contamination of water resources. The hepatotoxins e.g. microcystins get released into water body due to the death or senescence of cyanobacterial blooms (Dawson 1998). These toxic microcystins get accumulated into higher trophic levels (Sotton et al. 2014; Preece et al. 2017). The cyanobacterial toxins (microcystins) get accumulated in the tissues of planktivorous animals through ingestion of toxic cyanobacterial cells via food web transfer (Ferrao-Filho et al. 2014). Cyanobacterial toxins affect the health of aquatic organisms especially commercially important fish fauna, disrupt the food webs and overall interrupt health and balance in aquatic ecosystem (Corbel et al. 2014; Valerio et al. 2016; Preece et al. 2017). Some of the zooplankton constitute a major part of diet of many fish during early ontogenic stages as well as in later life (DeVries and Stein 1992). Several studies carried out by different workers highlighted the presence of microcystins not only in fish species (Martins and Vasconcelos 2009; Sotton et al. 2011) but also in zooplankton species, which clearly indicates or reveals the presence of microcystins at multiple trophic levels (Ibelings et al. 2005; Lehman et al. 2010). Studies carried out in the Lake Hallwil to provide insights into the pathways of transfer of microcystins into zooplanktivorous white fish clearly revealed that some assimilated portion of microcystins were found in muscle and liver of white fish originated from the consumption of microcystin toxin containing zooplankton especially Chaoborus larvae, and that source of microcystin (i.e. *Planktothrix rubescens*) was clearly found in the gut of various zooplankton taxa via targeting cyanobacterial nucleic acids (DNA) (Sotton et al. 2014).

10.5 Zooplankton as Bioindicators of Aquatic Ecosystem

Bioindicators comprise of organisms, for instance, plants, animals, microbes and planktons, used to assess the condition and well-being of natural ecosystem and biogeographical or environmental changes owed to the presence of contaminants or pollutants which can disturb the biodiversity of the environment along with the species present in it (Gerhardt 2002; Holt and Miller 2010). Bioindicator is a cumulative term covering entire sources of abiotic and biotic responses against the ecological fluctuations (Parmar et al. 2016). Bioindicators possess very much potential to predict the natural state or the degree of contamination of a certain ecosystem (Khatri and Tyagi 2015). There are great advantages of bioindicators in monitoring process as: biological influences can be monitored/determined; advantageous and non-advantageous influences of various pollutants on taxa can be predicted (Fig. 10.1); monitoring in early stage of harmful impacts of toxins on human beings; early warning signal against the dangerous effects of pollutants, etc. (Parmar et al. 2016). Presently various bioindicators (like plants, animals, microbes, plankton, etc.) are utilized by various organizations, e.g. “World Conservation Union” (WCU), “International Union for Conservation of Nature” (IUCN), etc. as biological tools to evaluate human impacts and to effectively perform the process of biomonitoring (Parmar et al. 2016). The organisms fundamentally utilized to describe the characteristic features of biosphere are actually called as “bioindicators” or

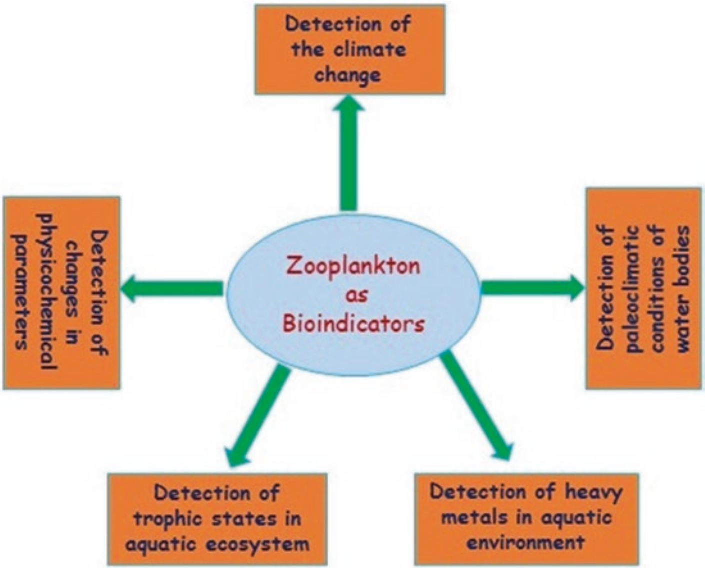

Fig. 10.1 Various ecological indications shown by zooplankton community

"biomonitors", both of which may vary considerably (Lilian 2009; Offem et al. 2011). Simply "bioindicators" determine the quality of deviations taking place in the environment, while "biomonitors" are used to get quantitative data on the quality of environment and the impact of several variables (Gaston 2000; Chakrabortty and Paratkar 2006). Therefore the monitoring can be performed for numerous biological processes in order to observe changes in health status of ecosystem (due to changes in species diversity), impact of environmental and anthropogenic stressors, assessment of anthropogenic measures (reclamation, remediation and re-introduction), water quality assessment (pollution), reflect the impact over other creatures of their ecosystem, etc. and that too with minimum preparation and training (Fadila et al. 2009; Hosmani 2013).

Though all the natural species to some point can be treated as biomonitors, but all the above key points apply very nicely to planktons and co-species category as far the pollution is concerned (Singh et al. 2013). Meanwhile planktons effectively respond to natural changes and act as best indicators of water quality and are the source of food for many fish and many other marine species (Thakur et al. 2013). Besides their role as a food source for aquatic organisms, they have a better potential likely to be used as indicators of water quality, eutrophication and production of freshwater body. Their bioindicator potential relies on the ground that their growth, development and conveyance are dependent on various abiotic parameters (e.g. pollutants, stratification, salinity, temperature, etc.) and some biotic parameters (competition, food limitation, predation, etc.) (Ramachandra et al. 2006). The status of a freshwater body can be analysed by measuring the seasonal variation and presence of zooplankton (Zannatul and Muktadir 2009). Different species of zooplankton hold their role as bioindicator species viz., *Trichotria tetractis* which indicates pollution due to phosphorus and heavy metal ion accumulations (Aslam et al. 2012); *Thermocyclops* indicates eutrophic conditions (Markert et al. 2003); cladoceran group indicates low concentration of contaminants (Hosmani 2014); some copepods (*Cyclops* and *Phyllodiaptomus*) and cladocerans (*Daphnia*, *Bosmina* and *Moina*) indicate well-being of the marine environment (Aslam et al. 2012); *Keratella tropica and Hexarthra mira* indicate high turbidity caused by the suspended sediments (Thakur et al. 2013) and *Brachionus calyciflorus* indicate organic pollution in lakes as well as their eutrophic conditions (Jain et al. 2010). Some significant properties of zooplankton viz., highly sensitive, short-lived, poikilothermic nature, commercially not harboured and free floating nature make them key indicators of climate change (Richardson 2008). In order to recognize the anthropogenic effects on numerous aquatic ecosystems, zooplankton could serve as model organisms and also play a domineering role in determining the magnitude and speediness of climate change (Richardson 2008).

10.6 Evidences of Zooplankton as Bioindicators

The Lake Mirik in Darjeeling Himalayas was very much polluted due to toxins from outer sources which had resulted in increased acidity level due to decrease in its pH (Jha and Barat 2003). These researchers investigated the planktonic community and other physicochemical parameters wherein cladocerans (*Daphnia*, *Bosmina* and *Moina*) and copepods (*Cyclops* and *Phyllodiaptomus*) were found, which confirmed that these organisms functioned as bioindicators to focus on health of this water body. *Trichotria tetractis* can be employed as contamination indicators keeping in view their presence in phosphorus rich and heavy metal rich water bodies and also in sewage contaminated tanks (Siddiqui and Chandrasekhar 1996; Zannatul and Muktadir 2009). Since for the zooplankton development, phosphorus and metal particles, high hardness, high conductivity (130 ms m^{-1}) and high alkalinity of the lake water are the limiting constituents for the zooplankton development (Ramachandra et al. 2006). There are some abiotic factors (viz., salinity, temperature, pollutants, stratification) as well as biotic factors (viz., competition, food limitation and predation) that influence the growth and distribution of zooplankton (Marzolf 1990; Ferdous and Muktadir 2009). For copepods, *Thermocyclops decipiens* has been employed to indicate eutrophic waters (Landa et al. 2007; Nogueira et al. 2008) as this species maintains high population density even in the presence of algal blooms (Rocha et al. 2002). In some species, the reproduction and development of zooplankton is influenced with respect to changes in water quality (Matsumura-Tundisi and Tundisi 2005; Landa et al. 2007; da Silva 2011). The water qualities of two Brazilian reservoirs, one eutrophic (Irai reservoir) and one oligo/mesotrophic (Verde river reservoir) were indicated by using rotifers and cyclopoid copepods via studying their ecological parameters viz., species richness, frequency and abundance, wherein it was found that among rotifers, *Brachionus* were higher in richness, frequency and abundance in "eutrophic" reservoir, but in the oligo/mesotrophic reservoir only *Plationus patulus* was found. *Tropocyclops prasinus* dominated in the eutrophic reservoir among copepods, but dominant taxa in oligo/mesotrophic reservoir were *Microcyclops anceps*, *Thermocyclops prasinus*, *T. inversus* and *T. minutus*. These species were also related with total phytoplankton, total phosphorus and chlorophyll-A and were thus indicators of the trophic state. Among rotifers, the dominance of *Brachionus* and among copepods, the dominance of *Acanthocyclops robustus* and *Tropocyclops prasinus* stood indicators of "eutrophic" situations (Perbiche-Neves et al. 2013).

Zooplankton assemblage plays a significant role in monitoring heavy metal load or pollution in aquatic ecosystems due to their capacity to accumulate heavy metals and encroachment of some polluted compounds in food chains (Prosi 1981; Stemberger and Chen 1998). Farkas et al. (2003) worked on such aspects of zooplankton community, wherein they worked out on biological indicators via crustaceans for detection of contamination in lake Balaton (Hungary) due to heavy metal pollution, wherein some trace metals such (Zn, Pb, Cd, Cu) in mixed zooplankton samples were measured by atomic absorption spectrophotometry to evaluate seasonal and spatial variations in heavy metal loads during the year 1996–2000. During the investigation the results revealed the overall significant increase in heavy metal (Cd, Cu and Pb) load of crustacean zooplankton mass. The Pearson's correlation analysis revealed the strong relationship between heavy metal concentration of crustacean plankton biomass and that of water (for Cd, Cu and Zn), whereas for Pb, correlation proved to be non-significant. During the course of their study, the results clearly demonstrate that zooplankton proved to be well-suited indicators of Cd, Cu and Zn load in ambient water. Nowadays eutrophication, a gradually worsening quality of water is a prevalent and widespread problem, affecting water bodies all over the world. The untoward hydrodynamic conditions pose an influence on the aquatic environment (as far as their physical and chemical parameters are concerned) and on the aquatic organisms (Kruk 2012; Paturej et al. 2012). At present, the methods being adopted to evaluate the trophic conditions of water bodies are based on some physical and chemical factors such as "Carlson's trophic indices" (Carlson 1977) and "Vollenweider method" adopted by OECD (Vollenweider and Kerekes 1982). But the recent legal regulations (Directive 2000) involve the biological aspects that could be utilized for monitoring of water quality with other parameters supporting such an evaluation. As the life cycle of zooplankton is short especially that of rotifers, they respond rapidly to fluctuations in ecological conditions and their biological parameters not only represent/specify the contamination level of aquatic environment but with the passage of time, they also aid to display general inclinations of deviations in environmental conditions (Duggan et al. 2001; Ejsmont-Karabin 2012). Many researchers analysed increased biomass and number of rotifers with advanced eutrophication (Duggan et al. 2001, May and O'Hare 2005; Tasevska et al. 2010). But as far the analysis of brackish water bodies is concerned, some other ecological conditions such as salinity and temperature must also be taken into kind attention that could considerably have an impact on the structure

Table 10.1 Various zooplankton as bioindicators of ecological conditions

Zooplankton	Environmental indication	Response	References
Cladocerans (*Simocephalus sp*; *Ceriodaphnia reticulata*)	Eutrophication	Species richness, density and fecundity adversely affected	Loria (2017)
Rotifera (*Brachionus sp*), Copepoda (*Tropocyclops prasinus*; *Thermocyclops decipiens*)	Eutrophication	Rotifer species abundance, frequency and richness high Copepod population dominates	Rocha et al. (2002), Perbiche-Neves et al. (2013)
Rotifera (*Plationus patulus*)	Oligo/mesotrophic condition	Dominance increases	Perbiche-Neves et al. (2013)
Copepoda (*Metacyclops mendocinus*; *Thermocyclops decipiens*)	Hypereutrophic condition	Dominance increases	Landa et al. (2007), Zaganini et al. (2011)
Cladocera (Ceriodaphnia *sphaericus*; *Leydigia acanthocercoides*; *Alona rectangula*)	Eutrophic condition	Abundance increases	Amsinck et al. (2005)
Cladocera (*Disparalona rostrata*)	Mesotrophic condition	Abundance increases	Amsinck et al. (2005)
Cladocera (*Daphnia magna*)	Heavy metals (Cd, Hg and Pb)	Mobility negatively affected and oxidative stress	Kim et al. (2017)
Ciliate protozoans	Eutrophy, low O_2 concentration and high temperature	Successful growth	Brönmark and Hansson (2010)
Cyclopoid copepods	Eutrophic condition	Abundance increases	Conde-Porcuna et al. (2004), Jeppesen et al. (2007)
Calanoid copepods	Oligotrophic condition	Abundance increases	Pinto-Coelho et al. (2005), Hsieh et al. (2011)
Ostracoda	Low pH	Absent at pH <5 because of difficulty in carapace calcification	Boomer et al. (2006)
Ostracoda (*Heterocypris sorbyana*; *Candona neglecta*)	Hypoxia	Tolerant to hypoxic conditions	Altinsacli and Griffiths (2001)
Ostracoda (*Cyprideis torosa*)	Salinity	Different ecophenotypic ornamentation of valves	Zhai et al. (2010)
Lacustrine Ostracods	Paleaoclimatic reconstruction	Altered phenotypic response by carapace	Mischke (2010)

of these aquatic biota (Joyce et al. 2005; Marques et al. 2006), together with rotifers as well (Bosque et al. 2001; Kaya et al. 2010) (Table 10.1).

10.7 Conclusion

Zooplanktons form an important component of aquatic ecosystems due to their profound role in trophic interactions viz. trophic food chain and food webs, energy transfer and the overall stability. Besides these important ecological properties they possess some dynamic role in pollutant transfer, bioaccumulation and biomonitoring phenomena due to which polluted zones of the aquatic part of the world are being identified and analysed properly and that too at their early stages for which remedial measures are properly operated against their restoration. As zooplankton assemblages and their ecology are dependent on both biotic and abiotic factors, their overall role as bioindicators can be fully formulated by taking together into consideration all their influencing factors.

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Chapter 11
Phytoremediation Technology: Sustainable Solution for Cleaning Up of Recalcitrant Pollutants from Disturbed Environs

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11.1 Introduction

Water is a fundamental substance for the sustenance and reproduction of life since it is indispensable for the development of biological processes (Mongue 2004). However, we currently find that various water sources such as wells and rivers are contaminated with large amounts of recalcitrant's pollutants (Wong Argüelles 2009). The term recalcitrant applies to compounds whose presence in the environment is prolonged due to their difficult degradation. These compounds can be from simple halogenated hydrocarbons to complex polymers. The recalcitrants can persist even in effluents that have received a previous treatment, which causes the presence of these compounds in the receiving environments causing cause health and

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environmental problems (Gupta and Thakur 2017). The low concentrations of recalcitrant in the environment make its treatment and elimination difficult using conventional treatments such as activated carbon, chemical precipitation, exchange resins, and filtration (Sandhya et al. 2005).

This chapter summarizes the information obtained, by scientific sources, about plants used in the phytoremediation of organic and inorganic recalcitrant contaminants. In addition, a study we conducted with *Typha latifolia* in the removal of heavy metals is presented.

11.2 Main Recalcitrants Pollutants

The recalcitrant pollutants are a variety of compounds such as heavy metals, chlorinated phenolic compounds (CPCs), complex dyes, explosive compounds, polyaromatic hydrocarbons (PAH), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), pharmaceutical active compounds (PhACs) (Ahmadi et al. 2017) (Fig. 11.1).

Heavy Metals Heavy metals are natural components of the Earth's crust, and some are indispensable for cell growth and biological mechanisms, however, when released into the environment, and exceed permitted limits are considered toxic. Most heavy metals come from industrial sources and are considered hazardous soil contaminants because they have chronic and acute toxicity (Abdel-Shafy and

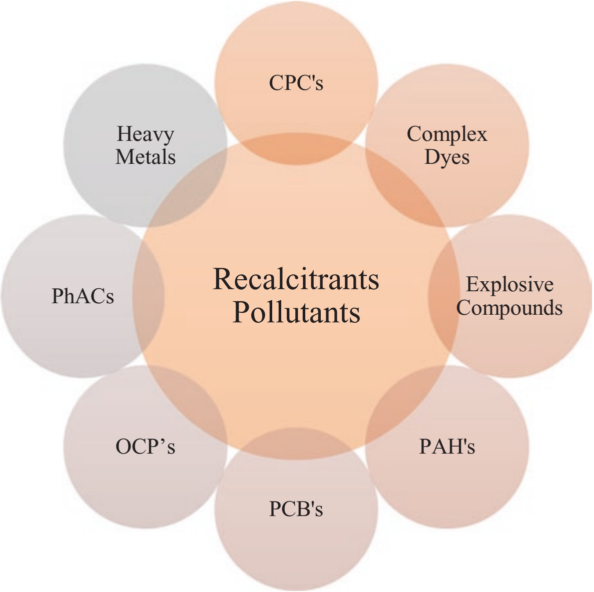


Fig. 11.1 Mains groups of recalcitrants pollutants

Mansour 2018). Heavy metals have a prolonged persistence because they do not degrade, which causes them to accumulate in living organisms causing toxic and potential adverse effects on humans and ecosystems (Khatoon et al. 2017). The major toxic heavy metals are Hg (I), Cd (II), Pb (II), Cr (IV), Fe (III), and Cu (II) (Sud et al. 2008).

Complex Dyes Most synthetic dyes are toxic and highly resistant to degradation due to their complex chemical structures. The textile industry is one of the largest generators of liquid effluent contaminants due to the high amounts of water used in dyeing processes. In recent years, interest in environmental control of dyes has increased due to their toxicity and carcinogenicity, as it contains carcinogenic compounds such as benzidine and other aromatic compounds (Ali 2010).

Explosive Compounds Explosives are highly stable compounds and have the tendency to bind with soil organic matter and contaminate it. Explosive materials are energy substances that, when released, present toxic hazards to the environment and biota. Around the world, soils are contaminated by such pollutants, whether due to manufacturing operations, military activities, conflicts of different levels, open burning/open detonation, ammunition discharge, etc. (Chatterjee et al. 2017).

Poly-Aromatic Hydrocarbons (PAHs) They are considered as one of the most dangerous chemical pollutants in the environment. These chemicals have carcinogenic, toxic, and mutagenic effects on the living body. In addition to their carcinogenic activity, these PAHs are recalcitrant, ubiquitous, and have bioaccumulative potential. Diesel and all hydrocarbon fuels are composed of an excessive amount of PAHs. Therefore, PAHs cause abundant pollution to ecosystems (Abdel-Shafy and Mansour 2018).

Polychlorinated Biphenyls (PCBs) They are among one of these groups of notorious contaminants. Polychlorinated biphenyls (PCBs) are members of chlorinated organic chemicals that theoretically can contain 209 different congeners. Highly chlorinated congeners are more stable and tend to have lower solubility in aqueous solution and also have high octanol water partition coefficients (K_{OW}) than low molecular weight PCBs. The high K_{OW} is partly responsible for their persistence and allows them to absorb strongly in the soil. Due to their environmental and ecological impacts, PBCs have been included in the Stockholm Convention as priority persistent organic pollutants (POPs), which must be removed from the environment by 2025.

Pharmaceutically Active Compounds (PhACs) In aquatic environments, PhACs have become an important issue that attracts the attention of the public due to their pseudo-persistence, possible toxic effects on microorganisms, and the widespread occurrence in surface waters (Yan et al. 2016). The aromatic side-chain compounds used in the pharmaceutical industry are difficult to biodegrade due to their high persistence in the environment and their resistance to degradation. These compounds reach water systems from different sources, such as human excretion (wastewater), improper disposal, landfill leaching, water drainage, or industries. It has been found that PhACs have the potential to cause aquatic toxicity, development of resistance in pathogenic microbes, genotoxicity, and endocrine alteration (Couto et al. 2019).

Chlorinated Phenolic Compounds (CPCs) The contamination of the environment by chlorinated aromatic compounds is a serious problem due to its large-scale applications such as biocides, lubricants, solvents, etc. and its high stability to biodegradation (Colomban et al. 2014). CPCs are the precursors or intermediates in many process industries, such as the dye, resin, and plastic, pharmaceutical, and pulp and paper industries. CPCs are irritating at low levels and have a very negative impact on the central respiratory and nervous systems at higher doses. They also have adverse effects of acute toxicity and carcinogenic properties (Miran et al. 2017).

Organochlorine Pesticides (OCPs) These compounds are of environmental concern due to their recalcitrance in soils and sediments, global distribution, and toxicity. The OCPs had been applied to the soil to protect the crops until they were banned. Due to their properties, they could bind strongly to organic matter and become trapped within the soil matrix, which would lead to persistence several years after their last use. The presence of pesticides released into the soil represents a risk to the environment since they can migrate through the profile; be absorbed by biota and transported to aquatic systems by erosion (Mitton et al. 2012).

11.3 Phytoremediation Technology

Phytoremediation is a term that applies to a group of technologies that use plants to reduce, eliminate, degrade, or immobilize toxic compounds in the environment through biological processes that develop the same plants or through synergistic plant-microorganism processes that develop in the rhizosphere, with the objective of restoring the areas to a condition in which they are usable (Manahan 2007; Peer et al. 2006). This technology is used to remove heavy metals, radionuclides, nutrients (nitrate, phosphate, etc.), solvents, explosives, crude oil and organic pollutants such as persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), and pesticides from wastewater and soils (Anand et al. 2019).

Phytoremediation of contaminated sites has gained popularity as a non-invasive, relatively inexpensive, and esthetically pleasing technology to the public compared to alternative remediation strategies that involve excavation, removal or stabilization, and chemical conversion in situ (Peer et al. 2006). The phytoremediation is a sustainable technology for cleaning up of recalcitrant pollutants from disturbed environs. The phytoremediation strategies have a number of promising advantages in comparison with other remediation techniques, in particular (Fig. 11.2).

There are factors that influence plant phytoremediation, which include the ability of the plant to absorb pollutants through its roots and transfer it to the shoots; the level at which plants tolerate contaminants and the available concentration of contaminants so that the plant can absorb them; and the rate of transforming toxic substances into less toxic by plants. Other factors that also influence phytoremediation are the root length of the plants, the types and concentration of the target metals, the environmental condition of the soil, and the interaction of the plants and associated soil microorganisms (Naila et al. 2019).

11.4 Plants Used in Phytoremediation Technology to Cleaning Up of Recalcitrant Pollutants

This chapter provides information of plants considered hyperaccumulants of recalcitrant contaminants mainly heavy metals in Table 11.1 and other recalcitrant pollutants are presented in Table 11.2. The information was searched by consulting the following electronic sources: ScienceDirect, SCOPUS, Web of Science, SpringerLink, SCIELO, PubMed, and Google Scholar finding 77 studies with different heavy metal accumulator plants such as Cd, Pb, Hg, Mn, Ni, B, Zn, Tl, Co, and Cu. Table 11.1 shows the plant species and the recalcitrant pollutant that removes the part where it accumulates.

Table 11.2 presents the 13 studies with plants removing other recalcitrant pollutants such as polycyclic aromatic hydrocarbons, oil hydrocarbons, phenol, nitrogen compounds.

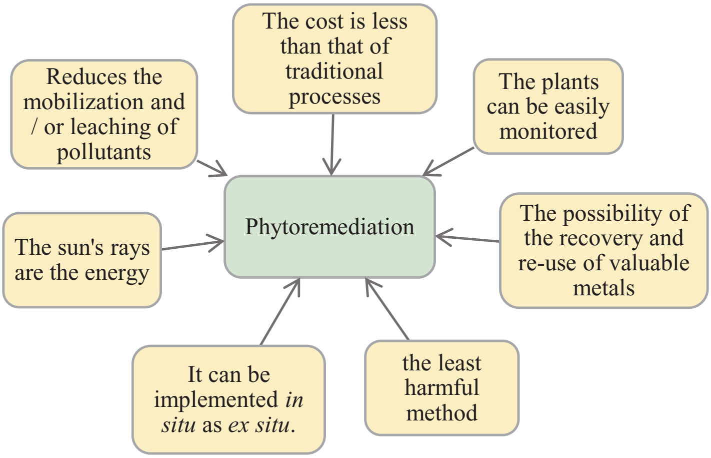


Fig. 11.2 Main advantages of phytoremediation (Dubchak and Bondar 2018; Abdel-Shafy and Mansour 2018)

11.5 *Typha latifolia* Plant Used in Phytoremediation Technology

Typha latifolia (cattail) is an emergent macrophyte; it is a perennial plant belonging to the Thyphaceae family, which grows in swamps, lakes, and lagoons. In addition, it has the advantage of growing in diverse climatic conditions and in environments contaminated with heavy metals (Alonso-Castro et al. 2009). The aquatic macrophyte presents rapid growth, easy spreading, high-pollutant uptake capacity, and easy harvesting, properties required for aquatic plants used in treatment system (Boyd 1970).

The accumulation of toxic elements by *T. latifolia* is a gradual process and depends on the concentration of elements and time of exposure. Additionally, this plant presents characteristics desirable for the remediation of aquatic sites such as a well-developed root–rhizome system, easy harvesting, and high biomass production. Further analyses are necessary to characterize the cellular mechanisms involved in the internalization of these elements in this plant. Previous research conducted by our research group indicated that *T. latifolia* presents the ability to remove and accumulate As, Cd, Pb, Ni, Cr, Mn, and Fe from *in situ* (an artificial lagoon) and controlled conditions (Carranza Álvarez 2005; Alonso-Castro et al. 2009; Carranza Álvarez et al. 2008; Leura Vicencio et al. 2013). In addition, promoter bacteria were isolated from the root of *T. latifolia*, finding that these exert a protective role in the phytoextraction of heavy metals. Nevertheless, the response to the toxic elements stress depends on the plant species.

11.5.1 **Typha latifolia** *as Biosorbent*

The heavy metals present in waters come mainly from industrial processes such as mineral extraction, wastewater treatment, metal casting and coating, nuclear and nuclear generation industries, among others. Heavy metals are introduced into food chains resulting in accumulation in the human body. Even at low concentrations they have important health effects such as stunted growth, spread of cancer, damage to organs, deterioration of the nervous system, and, in more severe cases, death (Aghababai and Beni 2020).

Lead is a non-essential element in the human body and its accumulation can cause some damage to the brain, leading to decreased learning and memory. It also affects bone metabolism, causing genetic mutations (Chu et al. 2019). There are several processes that can be applied in water treatment to reduce the concentration of heavy metals and other pollutants such as ion exchange, membrane separation, electrodialysis, reverse osmosis, solvent extraction, evaporation, etc. These methods are expensive and ineffective. Adsorption has become an economical and effective method for treating heavy metal wastewater due to its easy availability of raw materials and simple operation (Elhafez et al. 2017). Biosorption, considered as a type of

Table 11.1 Plants considered hyperaccumulants of recalcitrant contaminants

No.	Plant species	Recalcitrant pollutants that remove the part where it accumulates	References
1	*Abelmoschus manihot*	Cd$^{R, L, St}$	Wu et al. (2018)
2	*Achyranthes* sp.	Fe$^{R, L}$, Zn$^{R, L}$, and Mn$^{R, L}$	Chandra and Kumar (2017)
3	*Actephila alanbakeri*	Ni^{L}	Van Der Ent et al. (2016)
4	*Ageratina sp*[a]	Zn^{Sh}	Bech et al. (2016)
5	*Alcea aucheri*	Pb$^{Sh, R}$ and Cd ^{L}	Ravanbakhsh et al. (2016)
6	*Amaranthus spinosus* L.	Fe^{R}, Zn $^{R, Sh}$, and Mn$^{R, Sh}$	Chandra and Kumar (2017)
7	*Amaranthus viridis*	Pb	Eid and Shaltout (2016)
8	*Anisopappus chinensis*	Cu^{L} and Co^{L}	Lange et al. (2016)
9	*Antidesma tomentosum Blume*	Mn^{L}	Nkrumah et al. (2018)
10	*Antidesma coriaceum Tul.*	Mn^{L}	Nkrumah et al. (2018)
12	*Antidesma leucopodum* airy shaw	Mn^{L}	Nkrumah et al. (2018)
13	*Antidesma montanum* blume	Mn^{L}	Nkrumah et al. (2018)
14	*Antidesma Montis-silam*	Mn^{L} and Ni$^{L, B, St}$	Nkrumah et al. (2018)
15	*Antidesma neurocarpum Miq.*	Mn^{L} and Ni^{L}	Nkrumah et al. (2018)
16	*Antidesma neurocarpum* var. *Linearifolium* (Pax and K. Hoffm.) Petra Hoffm	Mn^{L}	Nkrumah et al. (2018)
17	*Antidesma puncticulatum Miq.*	Mn^{L} and Ni^{L}	Nkrumah et al. (2018)
18	*Antidesma stipulare Blume*	Mn^{L}	Nkrumah et al. (2018)
19	*Basella alba*	Fe$^{R, Sh, L}$, Zn$^{R, L}$, and Mn$^{R, L}$	Chandra and Kumar (2017)
20	*Bassia indica*	Fe and Pb	Eid and Shaltout (2016)
21	*Blumea lacera*	Fe$^{R, Sh, L}$, Zn$^{R, L}$, and Mn$^{R, L}$	Chandra and Kumar (2017)
22	*Brassica oleracea* var. *capitata*	Tl	Jia et al. (2013)
23	*Brassica rapa* var. *rapa*	Cd$^{St, L}$	Li et al. (2016)
24	*Calendula officinalis*	Pb^{R} and Cu^{R}	Shao et al. (2019)
25	*Camellia oleifera*	Mn$^{L, St}$	Yu et al. (2019)
26	*Cannabis sativa*	Fe$^{R, Sh, L}$, Zn$^{R, L}$, and Mn$^{R, L}$	Chandra and Kumar (2017)
27	*Carduus nutans*	Cd$^{R, Sh}$	Palutoglu et al. (2018)
28	*Celosia argentea* Linn.	Mn$^{L, St, R}$	Liu et al. (2014)
29	*Centella asiática*	Fe$^{R, L, St}$	Bhat et al. (2016)
30	*Chenopodium album*	Fe$^{R, Sh, L}$, Zn$^{R, L}$, and Mn$^{R, L}$	Chandra and Kumar (2017)
31	*Chlorophytum comosum*	Pb^{R} and Cu^{R}	Shao et al. (2019)
32	*Clethra barbinervis*	Co^{L}	Yamaguchi et al. (2017)
33	*Coronopus didymus* (L.)	Cd$^{R, Sh}$	Sidhu et al. (2017)
34	*Cortaderia hapalotricha*[a]	Pb^{Sh}	Bech et al. (2016)

(continued)

Table 11.1 (continued)

No.	Plant species	Recalcitrant pollutants that remove the part where it accumulates	References
35	*Croton bonplandianum*	Fe$^{R, L}$, Zn$^{R, L}$, and Mn$^{R, L}$	Chandra and Kumar (2017)
36	*Datura stramonium*	Fe$^{R, Sh, L}$, Zn$^{R, L}$, and Mn$^{R, L}$	Chandra and Kumar (2017)
37	*Eichhornia crassipes*	Cr (VI)$^{R, L, St}$, Cu, Pb, Hg, and Zn	Saha et al. (2017), Romero-Hernández et al. (2017)
38	*Epilobium denticulatum*[a]	Zn^{Sh}	Bech et al. (2016)
39	*Erato polymnioides*	Pb, Zn, Cu, and Cd	Chamba et al. (2016)
40	*Gevuina avellana*	Al^{L}	Delgado et al. (2019)
41	*Glochidion* cf. *sericeum*	Ni^{L}, Co^{L}	Van Der Ent et al. (2018)
42	*Helianthus annuus*	B	Barbafieri et al. (2018)
43	*Isatis cappadocica* subsp. *Cappadocica*	Ni^{AG}	Çelik et al. (2018)
44	*Kalanchoe pinnata*	Fe$^{Sh, L}$	Chandra and Kumar (2017)
45	*Lomatia dentata*	Al^{L}	Delgado et al. (2019)
46	*Macleaya cordata*[a]	Cd$^{R, St, L}$	Nie et al. (2016)
47	*Microthlaspi perfoliatum*	Ni^{AG}	Çelik et al. (2018)
48	*Myriophyllum aquaticum*	Cu, Pb, Hg, and Zn	Romero-Hernández et al. (2017)
49	*Noccaea caerulescens*	Zn^{Ec}	Kozhevnikova et al. (2017)
50	*Noccaea ochroleuca (Boiss and Heldr.) FKMey*	Ni	Salihaj et al. (2018)
51	*Odontarrhena oxycarpa*	Ni^{AG}	Çelik et al. (2018)
52	*Odontarrhena muralis*	Ni^{AG}	Çelik et al. (2018)
53	*Orites myrtoidea*	Al^{L}	Delgado et al. (2019)
54	*Panax notoginseng*	Pb^{R} and Cu^{R}	Shao et al. (2019)
55	*Parthenium hysterophorus*	Fe$^{R, Sh, L}$, Zn$^{R, L}$, and Mn$^{R, L}$	Chandra and Kumar (2017)
56	*Phlomis* sp.	Cd$^{R, Sh}$	Palutoglu et al. (2018)
57	*Phyllanthus balgooyi*	Ni$^{L, St, P}$	Mesjasz-Przybylowicz et al. (2016)
58	*Phyllanthus rufuschaneyi*	Ni	Bouman et al. (2018)
59	*Phyllanthus securinegioides*	Ni$^{pR, pSt, L}$	Van Der Ent et al. (2017)
60	*Pluchea dioscoridis*	Fe	Eid and Shaltout (2016)
61	*Polygonum lapathifolium* L.	Mn$^{L, St, R}$	Liu et al. (2016)
62	*Portulaca oleracea*	Mn	Eid and Shaltout (2016)
63	*Potamogeton pectinatus* L.	Cd	Lu et al. (2018)
64	*Pseudosempervivum sempervivum*	Ni^{AG}	Çelik et al. (2018)

(continued)

Table 11.1 (continued)

No.	Plant species	Recalcitrant pollutants that remove the part where it accumulates	References
65	*Pterocypsela laciniata* (Houtt.) C. Shih	Cd$^{Sh, R}$	Zhong et al. (2019)
66	*Rinorea niccolifera*	Ni	Fernando et al. (2014)
67	*Saccharum munja*	Fe$^{R, Sh, L}$, Zn$^{R, L}$, and Mn$^{R, L}$	Chandra and Kumar (2017)
68	*Salvinia cucullata*	Cu$^{Sh, R}$	Das and Goswami (2017)
69	*Sedum plumbizincicola*	Cd, Zn	Fan et al. (2019)
70	*Senecio conrathii* NEBr.	Ni^{L}	Siebert et al. (2018)
71	*Setaria viridis*	Fe$^{R, L}$, Zn$^{R, L}$, and Mn$^{R, L}$	Chandra and Kumar (2017)
72	*Silene latifolia*	Tl^{Sh}	Escarré et al. (2010)
73	*Solanum nigrum*	Zn$^{R, L}$, Pb$^{L, St, R}$, Co^{R}, Ni Cd, and Fe	Saad-Allah and Elhaak (2017), Eid and Shaltout (2016)
74	*Tagetes erecta* L.	Cd^{Tb}	Liu et al. (2018)
75	*Tagetes patula* L.	Cd^{Tb}	Liu et al. (2018)
76	*Taraxacum ohwianum* Kitam.	Cd$^{R, L}$	Cheng et al. (2019)
77	*Thlaspi rosulare*	Ni^{AG}	Çelik et al. (2018)
78	*Thlaspi triangulare*	Ni^{AG}	Çelik et al. (2018)
79	*Trichosanthes dioica*	Fe$^{R, Sh, H}$, Zn$^{R, H}$, and Mn$^{R, H}$	Chandra and Kumar (2017)
80	*Typha domingensis*	Ni^{R,Sh} and Cd$^{R, Sh}$	Mojiri et al. (2016)

Part where the recalcitrant accumulates: *R* hyperaccumulation in root, *Sh* shoot, *L* leaves, *St* stem, *P* petioles, *Tb* total biomass, *pR* phloem root, *pSt* phloem stem, *B* bark, *Ec* epidermal cells, *AG* above ground parts

[a] Almost considered hyperaccumulator

adsorption using biomass, is a promising method for the treatment of Pb (II) contaminated water due to its low cost and minimization of toxic sludge (Liu et al. 2014). The removal of heavy metals using biomass is attributed to a stable and insoluble matrix with active groups on the surface mainly carboxyl, amino, and hydroxyl groups.

There are a large number of reported studies on metal removal using various biosorbents, but few have focused on the use of cattails as the basis for adsorbent materials. Cattails are perennial, herbage plants with high adaptability and large biomass that are usually used in the construction of wetlands for removing nitrogen, phosphorous, and organics from wastewater (Tang et al. 2017). These aquatic plants have taken an interest in the remediation of aquatic ecosystems contaminated by heavy metals. *Typha latifolia, Typha angustifolia* and *Salix matsudana* are low-cost materials that have been used directly or as precursors of activated carbons for the removal of heavy metals such as Pb(II), Cd(II), Cr(VI), Cu(II), Mn(II), Zn(II),

Table 11.2 Plants used in bioremediation of recalcitrant contaminants

No.	Plant species	Recalcitrant pollutants that remove the part where it accumulates	References
1	*alfalfa Crioula*	Polycyclic aromatic hydrocarbons	Alves et al. (2018)
2	*Aspilia africana*	Oil hydrocarbons	Anyasi and Atagana (2018)
3	*Carex praegracilis*	Benzotriazole	Pritchard et al. (2018)
4	*Chromolaena odorata*	Oil hydrocarbons	Anyasi and Atagana (2018)
5	*Cucurbita pepo* L	Polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzo-furans (PCDF)	Urbaniak et al. (2016)
6	*Cyperus alternifolius*	Carbamazepine, sulfamethoxazole, ofloxacin, roxithromycin	Yan et al. (2016)
7	*Lolium perenne*	Anthracene	Yarahmadi et al. (2017)
8	*Nasturtium officinale*	Azo dye C.I. acid blue 92	Torbati et al. (2015)
9	*Pistia stratiotes*	Naphth-1-yl acetic acid, nitrate nitrogen	Pavithra and Hina (2018)
10	*Rhizophora mangle* L.	Oil hydrocarbons	Moreira et al. (2011)
11	*Salix* sp.	*P,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD	Mitton et al. (2012)
12	*Uvaria chamae*	Oil hydrocarbons	Anyasi and Atagana (2018)
13	*Vetiveria zizanioides* L. Nash	Phenol, oil hydrocarbons	Singh et al. (2008), Dudai et al. (2018)

among others. However, few studies evaluate the physicochemical properties of biosorbents and their relationship with the removal of the metal of interest (Bukhari et al. 2013; Kumari and Tripathi 2015; Liu et al. 2014; Rajaei et al. 2013; Song et al. 2013; Tang et al. 2017).

Therefore, the main objective of the present work was to use the root of *Typha latifolia* as an adsorbent material for the removal of Pb(II) from water as a low-cost alternative, as well as to relate its physicochemical properties and adsorption capacity.

11.5.2 Preparation of **Typha latifolia** *Root as Biosorbent*

T. latifolia samples were collected from a temporary lagoon located in the municipality of Ciudad Valles, San Luis Potosí, Mexico, by means of a meticulous collection of plants with similar characteristics such as height between 30 and 50 cm and degree of robustness of their root and rhizome between 10 and 20 cm long and 5 cm wide. Once collected, they were treated using a process of acclimatization at room temperature in a plant nursery for 30 days. During this acclimatization process, the plants were provided with nutrients (K, P, N) and enough water to promote root growth. Next, the plants with the largest amount of root were selected and washed with abundant water to remove impurities, the roots were cut and washed again using distilled water, then introduced into the stove for drying at 70 °C for 72 h. The roots were then crushed and pulverized using an analytical mill. Once the root was pulverized, it was thoroughly washed with deionized water and sieved to an average particle size of 0.16 mm. Finally, the root was dried at 80 °C for 24 h. The biosorbent obtained was packaged and stored in a plastic container for later use.

11.5.3 Characterization of T. latifolia *Root*

The morphology of the surface of the natural root of *T. latifolia* was examined by means of a scanning electron microscope (SEM), Philips, model XL-30-SFEG, equipped with a Link/ISIS-OXFORD microanalysis system of dispersed energy (EDS) which allows to identify the elements present on the surface of the material used as biosorbent. The functional groups present in the natural root of T. latifolia were identified by infrared spectroscopy (IR). The IR spectra of the root were obtained using an infrared spectrophotometer, Thermo-Scientific, model Nicolet iS10 that has the technique of attenuated total reflectance (ATR). The active sites were determined by the acid–base titration method proposed by Boehm (1994). The total acid and basic sites were neutralized with 0.01N NaOH and HNO_3 solutions, respectively. Surface charge and zero point charge (pH_{PZC}) were determined by the procedure proposed by Kuzin and Loskutov (1996).

11.5.4 Determination of the Concentration of Pb(II) in Aqueous Solution

The concentration of Pb(II) in aqueous solution was determined using an atomic absorption spectrophotometer, Varian model SpectrAA-20 and the concentration in aqueous solution was estimated using a calibration curve with standard Pb(II) solutions.

11.5.5 Adsorption Isotherms of Pb(II) on T. latifolia *Root*

Experimental data on the adsorption equilibrium of Pb(II) on the root of *T. latifolia* were obtained in batch adsorbers as follows. In a volumetric flask of 50 mL a solution of a known concentration of Pb (II) was prepared from a 1000 mg L^{-1} standard solution and a buffer solution prepared by mixing NaOH and HNO_3 solutions was gauged. Next, an initial sample of 10 mL was taken and subsequently analyzed to corroborate the initial concentration. To the batch adsorber, a certain root mass and 40 mL of the initial concentration solution were added. The adsorber was placed in a constant temperature bath, and the root and solution were left in contact until equilibrium was reached. In previous experiments, it was found that 5 days was enough time to achieve equilibrium. Once a day, the adsorbers were mixed in an orbital agitator at 200 rpm for 30 min.

The pH of the solution was measured periodically with a potentiometer and kept constant by adding drops of solutions 0.01, 0.1, and 1N of HNO_3 and NaOH, as necessary. The volumes of these solutions were recorded to calculate the final total volume of the solution. Once equilibrium was reached, a 10 mL sample was taken and analyzed to determine the final concentration of the solution. The mass of Pb (II) adsorbed on the root was calculated by means of a mass balance which is mathematically represented as follows:

$$q = \frac{V}{m}\left(C_0 - C_e\right) \tag{11.1}$$

where q (mg g^{-1}) is the mass of Pb(II) adsorbed per unit root mass, V (L) is the volume of the Pb(II) solution, m (g) is the mass of the root, C_0 (mg L^{-1}) is the initial concentration of Pb(II), and C_e (mg L^{-1}) is the concentration of Pb(II) in the equilibrium.

11.5.6 Results and Discussion About the Study of Typha latifolia *as a Biosorbent*

11.5.6.1 SEM, EDX, and IR Analyses of the *T. latifolia*

The surface, morphology, and particle distribution of *T. latifolia* root were observed by scanning electron microscope (SEM) and the elemental chemical composition of the surface was determined by microanalysis (EDX). Figure 11.3a, b shows the photomicrographs of fragments of *T. latifolia* root. Figure 11.3a shows a micrograph of the root increased to 36×, in which the irregular and rough surface is observed. Figure 11.3b is at 3500× and it is shown in great detail that the surface of the root does not have a uniform topography, also can be seen in it some irregular particles which are attributed to Pb, these in size range between ±5 μm.

Figure 11.4 presents the X-ray fluorescence (EDX) microanalysis of the *T. latifolia* exposed to Pb. It can be seen that C and O are the main elements, since it is a lignocellulosic material. The presence of Al, Si, and S was also detected in smaller quantities, as well as the characteristic peak of Pb.

The results of the IR spectra (not shown) revealed that the root of *T. latifolia* exposed to Pb(II) presents a slight change in the band of the functional groups O–H, C–H, C–O, and C–N. The results of the IR spectra revealed that the root of *T. latifolia* exposed to Pb(II) presents a slight change in the band of the functional groups O–H, C–H, C–O, and C–N. However, the increase in the intensity for the groups C=O and C=C was more noticeable and is a clear indication of the interaction between the organic compounds of *T. latifolia* and Pb(II) in solution.

It is very important to mention that the influence of the surface chemistry of adsorbent materials confers a certain degree of selectivity, for example, the presence of carboxylic, lactonic, and hydroxylic phenolic groups is responsible for the acidic surface properties of activated carbon (Böehm 2002). These specific functional groups are essential in the adsorption of heavy metals due to their chelating quality forming complexes (Monser and Adhoum 2002; Kikuchi et al. 2006).

11.5.6.2 Adsorption Isotherms

The experimental Pb(II) adsorption equilibrium data were adjusted to the Freundlich and Langmuir isothermal models. These models are mathematically represented with the following equations:

$$q = kC_e^{1/n} \tag{11.2}$$

$$q = \frac{q_m K C_e}{1 + K C_e} \tag{11.3}$$

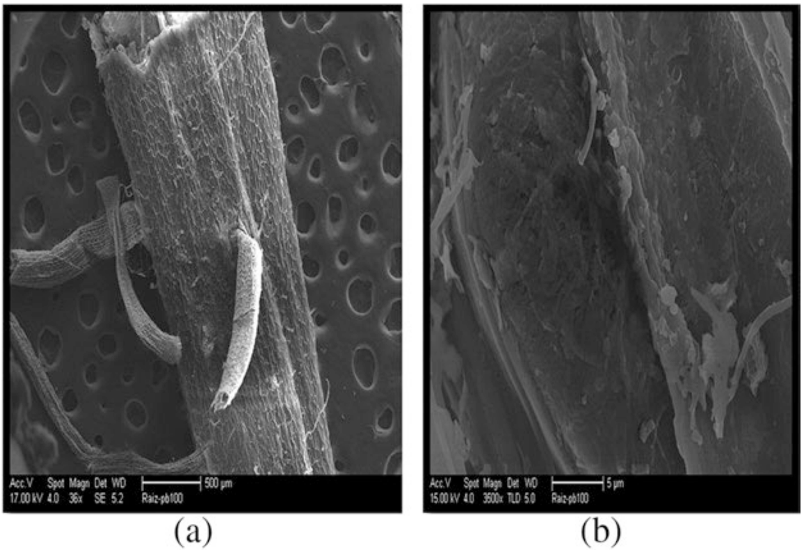
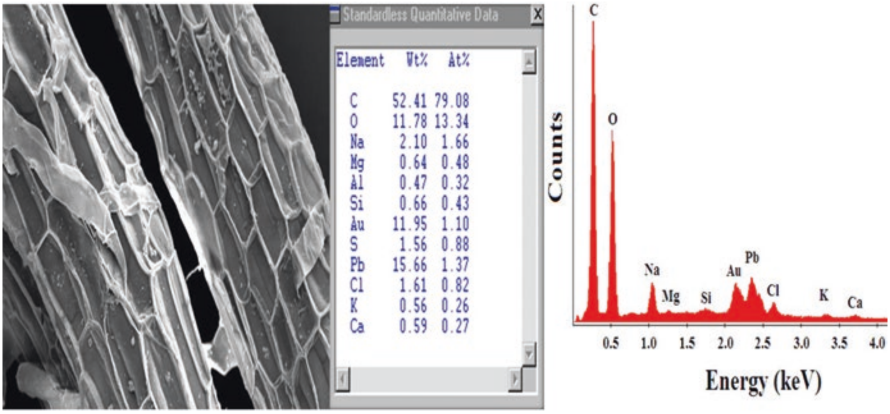


Fig. 11.3 SEM micrographs of *T. latifolia* saturated with Pb(II) (**a**) 36× and (**b**) 3500×



Standardless Quantitative Data

Element	Wt%	At%
C	52.41	79.08
O	11.78	13.34
Na	2.10	1.66
Mg	0.64	0.48
Al	0.47	0.32
Si	0.66	0.43
Au	11.95	1.10
S	1.56	0.88
Pb	15.66	1.37
Cl	1.61	0.82
K	0.56	0.26
Ca	0.59	0.27

Fig. 11.4 SEM micrograph and EDX spectra of *T. latifolia* saturated with Pb(II)

The parameters K (L mg^{-1}) and q_m (mg g^{-1}) are the Langmuir constants for Pb(II) related to adsorption energy and maximum adsorption capacity, respectively. The parameters k (L$^{1/n}$ mg$^{1\text{-}1/n}$ g^{-1}) and n are the Freundlich constants for Pb(II) related to adsorption capacity and intensity, respectively. The parameters of these isotherms were estimated by a method of least squares based on the Rosenbrock–Newton optimization algorithm which uses the following objective function:

$$R = \sum \left(q_{\text{exp}} - q_{\text{cal}} \right)^2 = \text{minimum} \tag{11.4}$$

On the other hand, the average absolute percentage deviation %Dev was estimated using the following equation and the results are reported in Table 11.3.

$$\%\text{Dev} = \left(\frac{1}{N} \sum_{i=1}^{N} \left| \frac{q_{\text{ex}} - q_{\text{cal}}}{q_{\text{ex}}} \right| \right) \times 100\% \tag{11.5}$$

Table 11.3 shows that the isotherm that best adjusted the adsorption equilibrium data was the Freundlich isotherm since the %Dev was lower than the Langmuir isotherm for all cases. The %Dev was lower than 17.2 and 11.5% for the Langmuir and Freundlich isotherms, respectively. Therefore, it was considered that Freundlich isotherm better interpreted the experimental data. It should be noted that the model of the Freundlich isotherm suggests that the surface of the adsorbent is heterogeneous. However, it is important to clarify that the fit of the isotherm model to the adsorption equilibrium data is a statistical mathematical method and not a corroboration that the adsorption mechanism occurs according to the fundamentals that support the model that best adjusted the data.

11.5.6.3 Effect of Solution pH on the Adsorption Capacity

The solution pH is probably the most important factor in the adsorption of ions in aqueous solution onto porous solids. The pH has a considerable influence on the adsorption equilibrium since the surface charge of the adsorbent and the species or ionic complexes formed by the adsorbate are a function of the pH. The effect of solution pH on the adsorption capacity of Pb(II) in aqueous solution on the root of *T. latifolia* was investigated by determining adsorption isotherms at pH 2, 3, and 5 and $T = 25$ °C. The effect of pH on the adsorption capacity of Pb(II) in aqueous solution on the root of *T. latifolia* was investigated by determining adsorption isotherms at pH 2, 3, and 5 and $T = 25$ °C. Figure 11.5 shows the effect of pH on the adsorption capacity of *T. latifolia* root and it can be seen that pH significantly affects adsorption capacity.

The results revealed that maximum adsorption capacity occurs at pH 5 and decreases as it decreases from 5 to 3 and from 3 to 2. On the other hand, the averages of Typha root adsorption capacities were 0.96, 1.72, and 7.74 mg g^{-1} at pH 2, 3, and 5, respectively. This is a clear indication that the adsorption capacity shown by the *T. latifolia* root increases considerably with increasing pH. The effect of the pH of the solution on the adsorption capacity can be explained considering that the pH_{PZC} of the biosorbent determined in this study was 5.6 which indicates that its character is acid and that the surface charge can be positive, neutral, or negative when the $pH < pH_{PZC}$, pH = pHPZC, or pH > pHPZC, respectively.

It is important to mention that during the adsorption of Pb(II), the pH of the solution always remained variable during the adsorption progress and to keep it constant it was necessary to add solutions 0.1 and 0.01N of NaOH or HNO_3. In the experiments carried out at pH 5.0, the solution varied considerably, increasing with the days until reaching equilibrium. On the other hand, in the experiments performed at pH 2 and 3, the pH of the solution hardly changed. This could indicate that the H^+ ions from the root surface were transferred to the solution and the Pb(II) cations in solution were diffused to the *T. latifolia* root surface. In other words, Pb(II) was adsorbed by ion exchange.

Table 11.3 Parameters for the Langmuir and Freundlich adsorption isotherms

		Langmuir			Freundlich		
T (°C)	pH	q_m (mg g^{-1})	K (L mg^{-1})	%Dev	k ($L^{1/n}$ $mg^{1-1/n}$ g^{-1})	n	%Dev
15	5.0	17.3	0.156	17.2	3.39	2.45	8.22
25	2.0	28.8	0.003	10.4	0.10	1.13	7.17
	3.0	7.79	0.062	9.03	0.53	1.34	8.64
	5.0	19.5	0.205	16.3	4.68	2.63	7.23
35	5.0	28.8	0.058	16.6	3.62	2.14	11.5

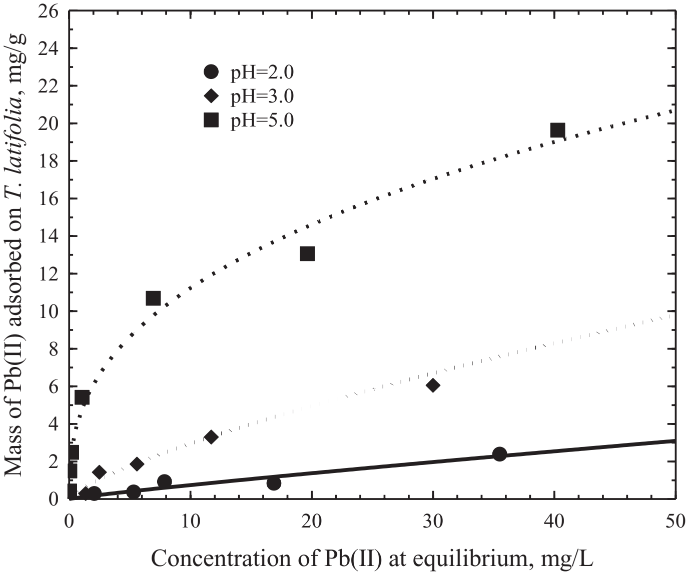


Fig. 11.5 Effect of the solution pH on the adsorption isotherm of *T. latifolia* at T = 25 °C. The lines represent the Freundlich isotherm

11.5.6.4 Effect of Solution Temperature on the Adsorption Capacity

The effect of the temperature of the solution on the adsorption of Pb(II) in the root of *T. latifolia* was analyzed by isotherms of Pb(II) adsorption at temperatures of 15, 25, and 35 °C, at pH of 5. In Fig. 11.6 it is observed that the capacity of the root to adsorb Pb(II) increased as the temperature increased. These results indicate that the adsorption of Pb(II) was endothermic. The effect of temperature can be checked by calculating the heat of adsorption (ΔHads), which when occurring at the same mass of adsorbed Pb(II), but at different temperatures is known as isosteric heat of adsorption. This calculation is made by means of the following equation (Medellin-Castillo et al. 2017):

$$\left(\Delta H_{\mathrm{ads}}\right)_q = \frac{R\,\mathrm{In}\dfrac{C_2}{C_1}}{\dfrac{1}{T_2}-\dfrac{1}{T_1}} \tag{11.6}$$

where $(\Delta H_{\mathrm{ads}})_q$ (J mol^{-1}) is the isosteric heat of adsorption, R (8314 J mol^{-1} K^{-1}) is the universal constant of the ideal gases, C_1 (mg L^{-1}) is the concentration of Pb(II) to T_1 (K) at the same value of q in equilibrium, and C_2 (mg L^{-1}) is the concentration of Pb(II) to T_2 (K) at the same value of q in equilibrium. Thus, at a mass of Pb(II) adsorbed on the root of *T. latifolia* of q = 14.0 mg g^{-1}, the concentrations of Pb(II) in equilibrium were $C_1 = 32.0$ mg L^{-1} and $C_2 = 14.8$ mg L^{-1}, at temperatures of $T_1 = 288.15$ K and $T_2 = 308.15$ K, respectively. The estimated isosteric heat was 28.5 KJ mol^{-1} indicating that the biosorption process of Pb(II) on the root of *T. latifolia* is endothermic ($\Delta H > 0$).

Experimental data from Pb(II) adsorption isotherms on *T. latifolia* root were correlated by Freundlich and Langmuir isotherms. The isotherm of Freundlich better adjusted the data under the criterion of the lowest percentage deviation. The study of the effect of pH and temperature in the Pb(II) adsorption isotherm on the root of *T. latifolia* revealed that the adsorption capacity of this biosorbent to remove lead is considerably dependent on pH and the temperature of the solution, increasing with increasing pH and temperature. The biosorption of Pb(II) on the root of *T. latifolia* is an endothermic process, with an isosteric heat of adsorption of 28.5 KJ mol^{-1}. Finally, it was concluded that Pb(II) can be efficiently removed from aqueous solutions by means of Typha latifolia root, which is widely distributed in the world and can be easily found in the Huasteca region in San Luis Potosi, Mexico and therefore can be considered a viable and low-cost option for the treatment of water intended for human consumption.

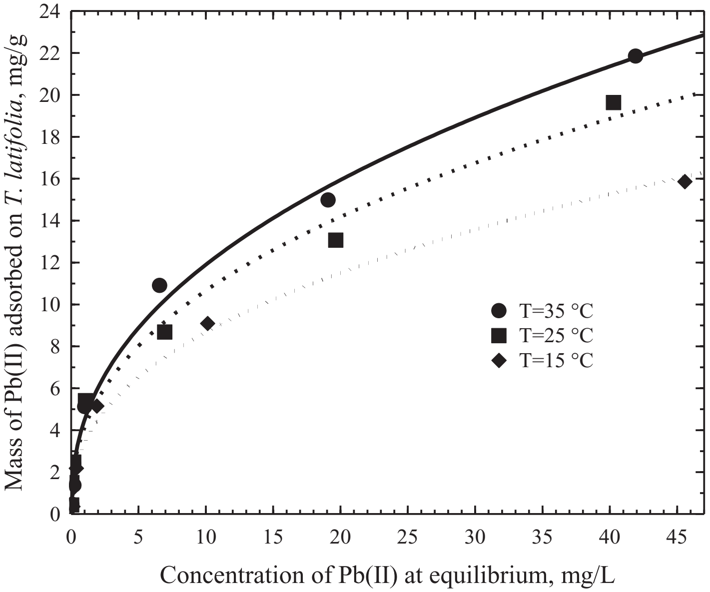


Fig. 11.6 Effect of the solution temperature on the adsorption isotherm of *T. latifolia* at pH = 5.0. The lines represent the Freundlich isotherm

11.6 Conclusions About the Study of *Typha latifolia* as a Biosorbent

Experimental data from Pb(II) adsorption isotherms on *T. latifolia* root were correlated by Freundlich and Langmuir isotherms. The isotherm of Freundlich better adjusted the data under the criterion of the lowest percentage deviation. The study of the effect of pH and temperature in the Pb(II) adsorption isotherm on the root of *T. latifolia* revealed that the adsorption capacity of this biosorbent to remove lead is considerably dependent on pH and the temperature of the solution, increasing with increasing pH and temperature. The biosorption of Pb(II) on the root of *T. latifolia* is an endothermic process, with an isosteric heat of adsorption of 28.5 KJ mol^{-1}. Finally, it was concluded that Pb(II) can be efficiently removed from aqueous solutions by means of Typha latifolia root, which is widely distributed in the world and can be easily found in the Huasteca region in San Luis Potosi, Mexico and therefore can be considered a viable and low-cost option for the treatment of water intended for human consumption.

11.7 Conclusion

Fitorremediation is a sustainable, low-cost technology because it uses the natural capacity of plants to remove contaminants. Plants are able to remove and accumulate in their plant tissues a wide variety of recalcitrant contaminants (organic and inorganic). To facilitate phytoremediation, native plants with a large amount of biomass and rapid growth such as *Typha latifolia* should be used. *T. latifolia* is a suitable plant for cleaning contaminated environments. *T. latifolia* is solution for cleaning up of recalcitrant pollutants from disturbed and can be used in situ or as a biosorbent material.

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Chapter 12
Applications of Macrophytes as Environmentally Sound Technique for Cleaning of Contaminated Ecosystems

Ishrat Bashir, Rouf Ahmad Bhat, and Shafat Ahmad Mir

12.1 Introduction

The quality of water is deteriorating due to prologue of chemicals and nutrients beyond the threshold limits in aquatic environs (Srivastava et al. 2008). Aquatic environs are directly or indirectly as the ultimate beneficiary of potent toxic substances present in the domestic sewage, agricultural, and industrial effluents (Demirezen et al. 2007; Peng et al. 2008), which consequently pose serious threat to the concerned ecosystems (Sudhira and Kumar 2000). The nuisance nutrients (N and P) in aquatic environs have been detected above the permissible levels in most of the surveys reported by researchers and scientists (Kamarudzaman et al. 2011). Presence of these nutrients in aquatic ecosystems has a detrimental impact on the quality of water and aquatic life. Consumption of such contaminated water with high concentration of nitrate-nitrogen could be the base for “methaemoglobinaemia” or “blue-baby syndrome” (Sampat 2000) and also induce toxicity to animals (Annelies et al. 2011). Furthermore, phosphate polluted water leads to excessive aquatic plant growth because it is considered as the “limiting nutrient” and has a critical role in “eutrophication” (Zhou et al. 2001; Varjo et al. 2003; Kamiyango et al. 2011). Population explosion, industrial expansion and urbanization adversely affects the quality of aquatic ecosystems and results in enrichment of nutrients (N and P). Therefore, responsible for mass motility of vital aquatic biota (Lu and Huang 2010). Accumulation and loading of nutrients (N and P) in an aquatic ecosystem is the major root of “eutrophication,” destruction the environmental quality and performance of aquatic ecosystems (Kronvang et al. 2005; Kantawanichkul et al. 2009). Cultural “eutrophication” in surface water bodies is main factor for “algal and cyanobacterial blooms,” mass motility of native flora and declining “biodiversity index” (Pretty et al. 2003; Conley et al. 2009). Besides, there are numerous

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dominant impacts of nutrient enrichment in aquatic environs viz., high growth rate of algae, enhanced organic load, fish motility, increase in sedimentation, and declining of water clearness (Klapper 1991; Correll 1998).

12.2 Consequences of Contamination in Aquatic Environs

Water contamination with toxic substances viz., organic contaminants "(pesticides, detergents, antimicrobials, solvents and cleaning fluids, hormones, and sterols) and inorganic contaminants (heavy metals, nutrients, radionuclides)" have caused imbalance in the natural performance of aquatic environs (Goswami et al. 2017). Among these, heavy metals put tremendous stress on routine functioning of aquatic biota and sometimes cause denudation of body structures in aquatic animals (Sood et al. 2012). The increased anthropogenic and agricultural activity to meet the demands of mammoth population has further strengthening the environmental degradation rate. Anthropogenic activities like metal extraction processes, paint manufacture, application of synthetic fertilizers and pesticides, tanning of leather, and manufacture of electronic gadgets further spoil the quality of aquatic environs by releasing huge quantities of wastewater containing tremendous amount of toxic substances into the aquatic ecosystems (Goswami et al. 2017; Yu et al. 2017). Once freed into the aquatic environs, these substances generally tend to combine with suspended matter present in the water systems due to sedimentation phenomenon and settle down on bottom sediments under gravity. Hence, sediments are considered as the most prominent storehouse of toxic substances (Harguinteguy et al. 2014).

Actually, heavy metals are considered as one of the most lethal substances due to their rigorous effects on plants, animals, and human beings (Ahmad et al. 2018; Kushwaha et al. 2018). The toxic metals which pose grave threats to the humans include Pb, Cd, Hg, As, and Cr (Abbas et al. 2013). Distinguishably, heavy metal contamination poses serious threat to the all forms of aquatic life in comparison to organic contamination due to their high rate of persistence (Miretzky et al. 2004; Khan et al. 2010), bioaccumulation (Doke et al. 2012), and biomagnification properties (Hamidian et al. 2014; Arul Manikandan et al. 2016; Goswami et al. 2017; Bind et al. 2018; Singh et al. 2019), posing deadly effects on biota (Martin et al. 2017) and human health (Etesami 2018). Furthermore, contamination of heavy metals damages the human "respiratory, reproductive, cardiovascular, endocrine and immunological systems leading to insomnia, irritability, anemia, and dizziness and muscles weakness" (Kushwaha et al. 2015, 2017; Goswami et al. 2019).

Discharge of untreated contaminated wastewaters into the freshwater bodies has harmful impacts on humans (Khurana and Pritpal 2012) and aquatic environs (Scholz 2010). In the modern era, artificial wetlands have been constructed to alleviate the problems associated with eutrophication in aquatic ecosystems (Brix and Arias 2005; Mitsch et al. 2005). In artificial wetlands, all types of macrophytes (free floating, submerged, and even emergent) in combination with sediments are employed for the treatment of wastewaters (Vymazal 2007). Macrophytes are considered as "soul component of aquatic ecosystems" not only as a source of food for aquatic organisms but also as capable accumulator of nutrients (N and P) (Bolpagni et al. 2007; Jordan et al. 2011; Boerema et al. 2014; Castaldelli et al. 2015; Filho et al. 2015) and heavy metals (Samecka-Cymerman and Kempers 2002; Samecka-Cymerman et al. 2005; Filho et al. 2015). They act as true filter by accumulating N and P, and heavy metals from the aquatic environs (Vymazal 2011; Alaa and Elsayed 2015). These aquatic macrophytes exhibit species-specific capability to store heavy metals and nutrients and thus, the species mixture might perform well than a single species in the treatment ability (Lin et al. 2002).

12.3 Role of Macrophytes Used to Improve Water Quality in Natural Wetlands

Wetland ecosystems are buffer zones in between terrestrial and aquatic environs, characterized by low depth waters and presence of different categories of macrophyte vegetations. The potential of these water bodies for cleansing of contaminates in natural conditions have been determined by the scientists regularly in recent times worldwide (Nahlik and Mitsch 2006). The rate of removal or degradation of contaminates in wetlands relies on the "sedimentation, adsorption on the root surface" and accumulation by the aquatic plants (Serra et al. 2004). Macrophytes in relation with the "aquatic micro-organisms" and "periphytons" increase the pump out of nutrients (N and P) from bottom sediments and water column (Vymazal 2002). Periphytons have the potential to clean metal cations (Scinto and Reddy 2003) and $PO4^{3-}$ and NO_3^- by direct assimilation from the water column (Khatiwada and Polprasert 1999). The aquatic plants and algae bring approximately 85–89% of O_2 available in the root system. This encourages both aerobic putrefaction of organic matter and supports the nitrogen fixation process (Scholz 2006; Lee and Scholz 2007).

12.4 Role of Macrophytes Used to Treat Wastewater in Constructed Wetlands

A constructed wetland (CW) system is an artificial compound that is the diminutive of natural aquatic environs used for treatment of contaminated waters (Hammer and Bastian 1989). CWs can eliminate momentous quantity of floating solids, organic matter, N, P, heavy metals in association with the micro-organisms (Kadlec and Knight 1996) and pesticides (Cheng et al. 2002). Wastewater cleaning by employing CWs has gained attractiveness due to its efficiency over the conventional treatment systems. The components of CWs include filtering media, microbes, and plants. Contaminated water is poured on filtering media, and contaminants are cleared chiefly by physical processes such as "filtration or sedimentation" and via biochemical degradation (Table 12.1), such as "microbial degradation" (Barko et al. 1991; Sorrell and Boon 1992; Vymazal 2011). Aquatic plants are an imperative constituent of CW systems (Kalff 2002; Hadad et al. 2006). The efficiency of aquatic plants in CWs depends on several factors: kind of CW (vertical, horizontal, surface, or subsurface flow, with or without recirculation), quality and quantity of the wastewater loads (Sklarz et al. 2009), and type of macrophyte group and their associations (Brisson and Chazarenc 2009). Combination of two or more plants species is more effective than single-species vegetation for the degradation of contaminates in CWs (Karathanasis et al. 2003; Fraser et al. 2004). Maehata (2008) carried out a study for the treatment of wastewater by employing combination of submerged and floating macrophytes in CW for the conservation of Biwa Lake and concluded that CW could be vital tool for the conservation of natural water bodies.

The chief vital mechanism by which aquatic plants participate to the CWs for the removal of contamination from wastewaters is not in absorption but moderately in the physical effects of root organization pooled with aeration (Brisson and Chazarenc 2009). Root development is well known to have an effect on some soil-hydraulic behavior (Stottmeister et al. 2003). Physical properties of roots include "filtering, flow velocity reduction, improved sedimentation, decreased re-suspension and even the distribution of water and prevention of clogging" (Stottmeister et al. 2003; Vymazal 2011). The rhizosphere provides better condition for the microbes in CWs.

Table 12.1 Role of macrophytes in constructed wetlands

Physical effects of root structure	References
• Filtering effect	Vymazal (2011)
• Velocity reduction, promotion of sedimentation, decreased re-suspension	Vymazal (2011)
• Prevention of medium clogging	Brix (1994)
• Improved hydraulic conductivity	Petticrew and Kalff (1992), Brix (1997)
Roots as a base for micro-organisms	
• Provision of surface for microbial attachment	Brix (1997), Vymazal (2011)
• Oxygen leakage-increased aerobic degradation	Barko (1991), Sorrell and Boon (1992)
• Oxygen leakage-supports precipitation of heavy metals	Vymazal (2011)
• Oxygen leakage-increased nitrification	Brix (1997), Yang et al. (2001), Fraser (2004), Ruiz-Rueda (2009)
• Release of antibiotics, phytometallophores and phytochelatins	Seidel (1964), Seidel (1976), Vymazal (2011)
• Root exudates promote metal chelation to prevent metal toxicity	Vymazal (2011)
Plant uptake	
• Storage and uptake of nutrients	Gersberg et al. (1986), Lee and Scholz (2007), Vymazal (2011)
• Metal remediation	Salt et al. (1995), Weis and Weis (2004), Lee and Scholz (2007)
• Salt remediation	Shelef et al. (2012)

Since, micro-organisms are vital component for the degradation of contamination present in the wastewaters (Brix 1997; Vymazal 2011). Macrophytes utilize the minerals released from complex substances formed via microbial interactions in CWs and thereby reducing the levels of nuisance pollutants from wastewaters (Dierberg et al. 2002; Hadad et al. 2006). Thus, phytoremediation play a pivotal role for the remediation of contaminants in CWs (Vymazal 2011; Lee and Scholz 2007; Konnerup et al. 2009; Shelef et al. 2012). The choice of using macrophytes in CWs is firstly adapted in stressed environs therefore, halophytes are preferred ones (Brown et al. 1999; Stottmeister et al. 2003).

In general, a dare pertaining to CW efficiency is the limited elimination of alkaline cations like Na, Ca, and Mg (Samecka-Cymerman et al. 2004; Gu et al. 2006). This is due to the reason that these cations are present in huge quantities in wastewaters which exceed the regular plant needs, and consequently their subsequent concentrations are impervious when the wastewater passes through the CW systems (Richardson 1989). The few advantageous roles of aquatic plants in CWs are depicted in Table 12.1.

12.5 Classification of Constructed Wetlands

In general, the classification of CWs relies on three foremost factors: water column, plant category, and flow of water (Hoffmann et al. 2011; Vymazal 2014). Surface and subsurface flow are regarded as the most important kinds of CWs (Vymazal 2013; Wu et al. 2014). The differentiation among these two kinds is that the former one contains extensive aquatic plants and enough water column surface, whereas the later one does not contain clean water surface. Furthermore, on the basis of water flow direction, CWs are categorized into “vertical and horizontal flow” types (Fig. 12.1) and can be pooled into single system (hybrid) to attain towering contamination deduction efficiency (Vymazal 2013, 2014; Wu et al. 2014). “Horizontal-flow” CWs contain plenty of water compared “vertical-flow” CWs (Stefanakis et al. 2014).

12.6 Free Water Surface Flow Constructed Wetlands

This kind of CW is made up of an uncovered system laded with a mixture of different kinds of aquatic plants (Fig. 12.2) and it resembles with natural wetlands in every respect (Vymazal et al. 2006; Wu et al. 2014). This system contains well sealed “shallow pool” to thwart spilling of wastewater, hence protects the ground from contamination (Stefanakis et al. 2014). In these CWs, the contaminated water is encumbered from the apex; it then horizontally runs through the media (Vymazal et al. 2006). In addition, “treatment processes such as sedimentation, filtration,

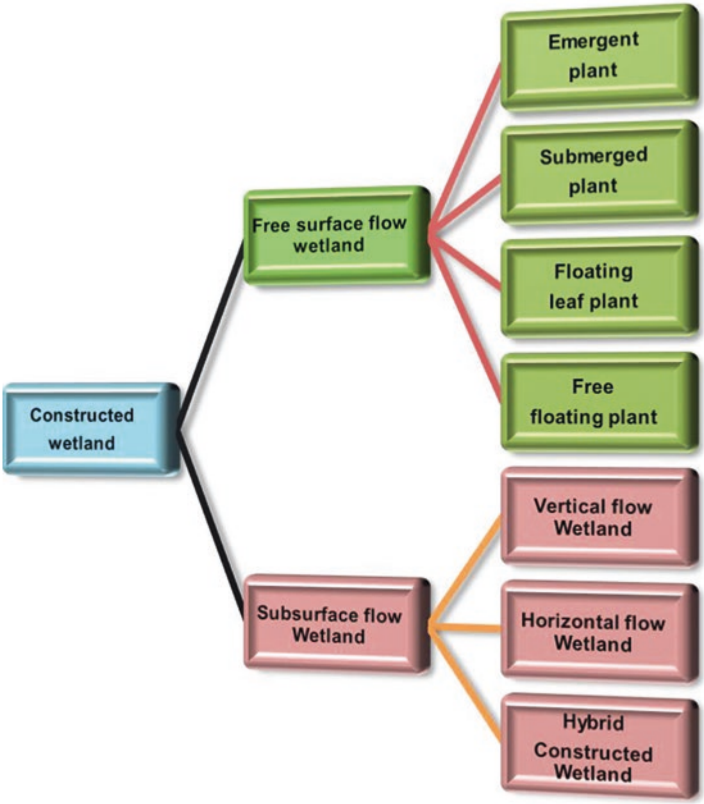

Fig. 12.1 Classification of constructed wetland (Almuktar et al. 2018)

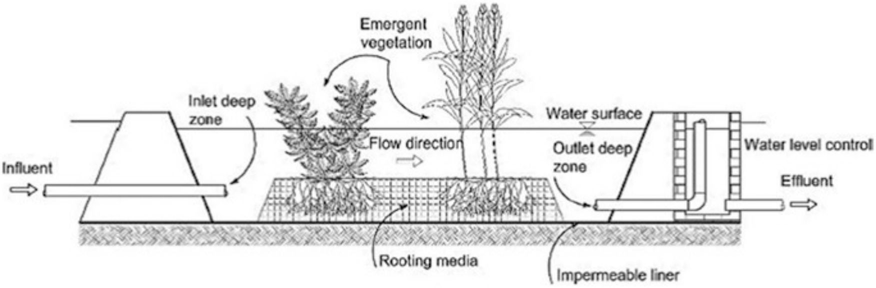

Fig. 12.2 Free water flow constructed wetland

oxidation, adsorption, and precipitation" will occur as wastewater passes through this CW (Kadlec and Wallace 2008). Furthermore, this kind of treatment system removes significantly the suspended solids, N, heavy metals, and organic pollutants in wastewaters (Kadlec and Wallace 2008; Tsihrintzis and Gikas 2010).

12.7 Subsurface Flow Constructed Wetland Systems

This kind of CW systems comprises of planted macrophytes on substrates of sand or gravel, permeating "flooding of the system with wastewater, which will pass through the media by gravity, improving treatment processes" (Knowles et al. 2011). The substrate array in this CW will make available a useful passageway that improves the function of microbes in the system to remove diverse kinds of contaminants (Hoffmann et al. 2011). Fan et al. (2012, 2013) and Nivala et al. (2013) reported that "subsurface flow constructed treatment wetland systems" show towering efficiencies in terms of C and N compound remediation, due to the eminent O_2 accessibility in their media. Moreover, this form of CW shows high-quality effectiveness in limited areas compared to those occupied by "surface flow constructed wetlands" as reported by Hoffmann et al. (2011) and Stefanakis et al. (2014).

12.7.1 Horizontal-Flow Subsurface Constructed Wetland System

The wastewater flows "horizontally through the system substrate," roots and rhizomes of plants are in the direction of the outlet system (Vymazal 2014). Aquatic plants are planted in the system substrate (Fig. 12.3) containing sand and coarse gravel beneath, in the course of which the applied wastewater passes from the system inlet towards the outlet (Vymazal et al. 2006). Moreover, the underneath of the system is packed with a waterproof membrane stopping the leakage of the contaminated water to the groundwater (Kadlec and Wallace 2008). However, the "roots of macrophytes and porous media in this system are responsible for biomass development and subsequently enhance the organic matter and suspended solid removal from the contaminated water" (Gikas and Tsihrintzis 2010; Vymazal 2014). This

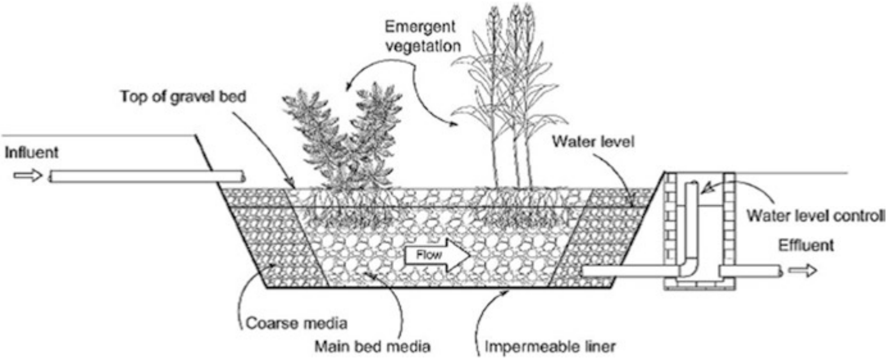

Fig. 12.3 Horizontal-flow constructed wetland system

type of CWs is weak in terms of treating the ammoniacal nitrogen, however, they can remediate N efficiently in absence O_2 (Zhang et al. 2014).

12.7.2 Vertical-Flow Subsurface Constructed Wetland System

These kinds of CWs are gaining the wide range of popularity due to its efficiency for removing the nitrogenous substances (Stefanakis et al. 2014) and accomplish an elevated pace of O_2 shifting (Fan et al. 2013; Li et al. 2015). Hence, enhance the aeration and biological treatment wastewater (Vymazal et al. 2006). In this system, the wastewater is applied erratically (Fig. 12.4) in cycles of filling and draining the substrate media leading to a high rate of oxygen transfer in the system (Wallace 2013; Li et al. 2015).

These types of CWs are extremely capable in terms of removing wide range of contaminants in wastewater (Langergraber et al. 2007; Scholz 2010; Paing et al. 2015; Song et al. 2015; Zhi et al. 2015).

12.8 Hybrid Constructed Wetland System

Currently, the use of "combined wetland systems" (Fig. 12.5) is gaining popularity around the world due to its potential for remediating N compounds from different contaminated wastewaters (Vymazal and Kröpfelová 2011; Ayaz et al. 2012). Furthermore, several studies have determined that a "hybrid wetland system" may possibly be used to degrade different kinds of (Serrano et al. 2011), pharmaceuticals (Reyes-Contreras et al. 2011), water produced from oil fields (Alley et al. 2013), gray water, and industrial wastewaters (Comino et al. 2013; Vymazal 2014).

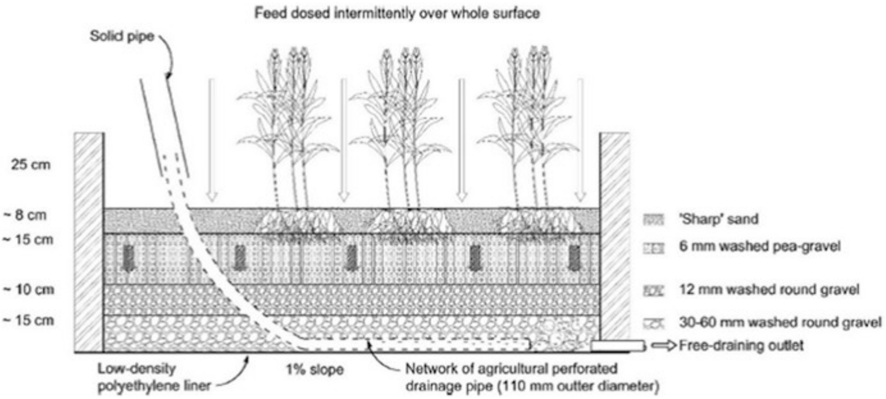

Fig. 12.4 Vertical-flow constructed wetland system

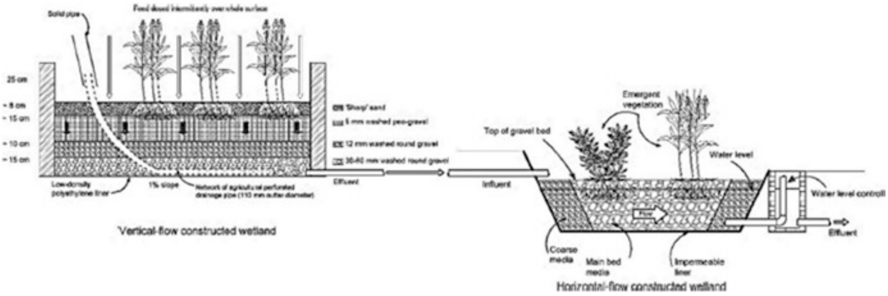

Fig. 12.5 Hybrid constructed wetland system

12.9 Macrophytes in Wetlands

Macrophytes are aquatic plants and are common in wetlands (Stefanakis et al. 2014). They are considered as purifiers due to their property of extracting and accumulation contaminants from polluted waters (Scholz 2010; Villa et al. 2014). Furthermore, they are categorized under four main classes, namely, "free floating, floating leave, submerged or emergent" (Padial et al. 2008).

12.10 Nutrient and Heavy Metal Acquisition and Sequestration by Wetland Macrophytes

Macrophytes play a pivotal role in wetland ecosystems, which can straightforwardly influence the wastewater quality by removing and extracting nutrients (N and P) (Ong et al. 2010; Ko et al. 2011), "antibiotics" (Liu et al. 2013), and "heavy metals" (Scholz 2010; Ha et al. 2011) (Table 12.2). Aquatic plants extract the "pollutants" from wastewater and accumulate them in their tissues and also providing enough space for microbial interaction with complex pollutants (Vymazal 2002). Moreover, "wetland macrophytes" are accountable for transferring O_2 from their roots to the "rhizosphere," providing aerobic conditions to enhance the contaminant degradation in aquatic environs (Moshiri 1993). Furthermore, growth of macrophytes in wetland substrate stabilizes media, which leads to the improvement of the hydraulic conductivity in the system, reduces clogging probability, and provides suitable conditions for microbial growth and release oxygen as reported by Stefanakis et al. (2014).

The quantity of "nutrients and heavy metals" extracting by macrophytes determines the remediating capabilities of these aquatic biota (Figs. 12.6 and 12.7). However, the quantity of accumulated nutrients depends on the "physiological capacity" which varies from plant to plant in aquatic environs (Pieczynska 1990).

Table 12.2 Macrophyte species studied for nutrient and heavy metal acquisition and sequestration (Golda et al. 2014)

Aquatic plants	Category	Efficient in remediating and stabilizing	References
“*Alternanthera sessilis*” (alligator weed)	Emergent	Cu, Cr, Fe, Mn, Cd, Pb	Rai et al. (1995)
“*Azolla caroliniana*” (mosquito fern)	Free floating	Cd, Pb	Stepniewska et al. (2005)
“*Azolla pinnata*” (duckweed fern)	Free floating	pH, tolerance to dye wastewater, Fe, Cu, Hg, Cd	Sharma et al. (2005), Jain et al. (1989), Rai (2008)
“*Ceraophyllum demersum*” (coontail)	Submerged	Turbidity, NH_3, PO_4^-, COD, BOD, Cu, Cr, Fe, Mn, Cd, Pb, Zn	Rai et al. (1995), Keskinkan et al. (2004), Kulasekaran et al. (2014)
“*Chara corallina*” (green algae)	Submerged	Cu, Cr, Fe, Mn, Cd, Pb	Rai et al. (1995)
“*Chara najas* (chara)	Submerged	P, NO_3, Ca, K	Srivastava et al. (2009)
“*Colocasia escuenta*” (elephant-ear)	Emergent	pH, BOD, COD, NO_3^-, PO_4^-, EC, TS, Pb, Cd	Bindu et al. (2008), Bindu et al. (2010), Selvamurugan et al. (2010)
“*Cyperus alopecuroides*” (foxtail flatsedge)	Emergent	Na, K, Ca, Mg, pH, tolerance to dye wastewater, Fe, Mn, Zn, Cu, Ni, Cr, Pb	Sharma et al. (2005), Prusty et al. (2007)
“*Cyperus pangorei*” (korai grass)	Emergent	BOD, COD, TSS and chlorinated phenol	Prabu and Udayasoorian (2007)
“*Echinochloa colonum*” (billon dollar grass)	Emergent	Cd, Co, Cu, Ni, Pb, Zn	Kumar et al. (2008)
“*Eichhornia crassipes*” (water hyacinth)	Free floating	pH, Turbidity, EC, TDS, TSS, DO BOD, COD, TN, N, P, K, Na, TS, tolerance to dye wastewater, SS, NO_3^-, PO_4^-, NH_4^-, Hardness and Coliform bacteria, Cd, Co, Cu, Ni, Pb, Zn, Hg	Sharma et al. (2005), Collins et al. (2005), Caldelas et al. (2009), Dhote and Dixit (2009a, b), Dipu et al. (2010), Tiwari et al. (2007), Kumar et al. (2008), Caldelas et al. (2009), Rana et al. (2011), Jumbo and Campoverde (2012), Kumar (2015)
“*Elodea Canadensis*” (American water weed)	Submerged	Cu, Zn, Cd, Cr, Pb	Nyquist and Greger (2007), Basile et al. (2012), Dogan et al. (2009)

(continued)

Table 12.2 (continued)

Aquatic plants	Category	Efficient in remediating and stabilizing	References
"*Hydrilla verticillata*" (water thyme)	Submerged	pH, turbidity, EC, TDS, TSS, BOD, COD, TN, N, P, K, Na, $NO_{3,}$ Ca, Mg, tolerance to dye wastewater, Cd, Co, Cu, Ni, Pb, Zn, Fe, Mn, Cr, arsenic, aluminum	McFarland et al. (2004), Sharma et al. (2005), Prusty et al. (2007), Srivastava et al. (2009), Dhote and Dixit (2009a, b), Dhote and Dixit (2007), Kumar et al. (2008)
"*Hygrorrhiza arisata*" (Asian water grass)	Emergent	Cu, Cr, Fe, Mn, Cd, Pb	Rai et al. (1995)
"*Ipomoea aquatic*" (water spinach)	Emergent	P, NO_3, Ca, Mg, K, Na, Cd, Co, Cu, Ni, Pb, Zn, Fe, Mn, Cr	Prusty et al. (2007), Srivastava et al. (2009), Kumar et al. (2008)
"*Lemna aequinoctialis*" (lesser duckweed)	Free floating	pH, tolerance to dye wastewater	Sharma et al. (2005)
"*Lemna gibba*" (fat duckweed)	Free floating	As and U	Mkandawire et al. (2004), Mkandawire and Dudel (2005)
"*Lemna minor*" (duckweed)	Free floating	Temperature, pH, turbidity, salinity, EC, TDS, alkalinity, free CO_2, Total CO_2, Cl^-, DO, percentage O_2 saturation, COD, BOD, total hardness, Ca, Mg, NO_3^-, PO_4^-, NH_4^+, Fe, Cu, Hg, Cu, Zn, Cd, Pb, As, Cr	Jain et al. (1989), Jumbo and Campoverde (2012), Kumar (2015), Sekomo et al. (2012), Basile et al. (2012)
"*Ludwigia repens*" (creeping primrose willow)	Emergent	P, NO_3, Ca, K	Srivastava et al. (2009)
"*Marcellia* sp. (goat weed)	Emergent	P, NO_3, Ca, K	Srivastava et al. (2009)
"*Myriophyllum alterniflorum*" (alternate water-milfoil)	Submerged	Cd, Cu	Ngayila et al. (2007)
"*Myriophyllum Spicatum*" (spiked water-milfoil)	Submerged	Cu, Zn, Pb, Co, Ni, Cu	Keskinkan et al. (2003), Lesage et al. (2007)
"*Nelumbo nucifera*" (lotus)	Floating	Cd, Co, Cu, Ni, Pb, Zn	Kumar et al. (2008)
"*Neptunia oleracea*" (water mimosa)	Emergent	Na, K, Ca, Mg, Fe, Mn, Zn, Cu, Ni, Cr, Pb	Prusty et al. (2007)
"*Paspalidium punctatum*" (bristle grass)	Emergent	Na, K, Ca, Mg, Fe, Mn, Zn, Cu, Ni, Cr, Pb	Prusty et al. (2007)

(continued)

Table 12.2 (continued)

Aquatic plants	Category	Efficient in remediating and stabilizing	References
“*Paspalum distichum*” (knot grass)	Emergent	Na, K, Ca, Mg, Fe, Mn, Zn, Cu, Ni, Cr, Pb	Prusty et al. (2007)
“*Phragmites australis*” (common reed)	Emergent	TSS, TDS, TN, TP, COD, BOD, chlorinated phenol	Prabu and Udayasoorian (2007), Baskar et al. (2009)
“*Phagmites karka*”	Emergent	TDS, TSS, BOD, DO, COD, NH_4^+, NO_3^-, Org-N, TKN, pH, tolerance to dye wastewater, Coliform bacteria, Turbidity, TS, PO_4^-	Sengupta et al. (2004), Sharma et al. (2005), Vipat et al. (2007)
“*Pistia stratiotes*” (water lettuce)	Free floating	pH, turbidity, EC, TDS, TSS, BOD, COD, NO_3, Na, TS, TN, P, Ca, K, Cu, Pb, Ni, Zn	Irfan and Shardendu (2009), Srivastava et al. (2009), Dipu et al. (2010), Odjegba and Fasidi (2004), Cruz et al. (2009), Coutinho et al. (2017)
“*Polygonum barbatum*” (smart weed)	Emergent	pH, tolerance to dye wastewater	Sharma et al. (2005)
“*Potamogeton lucens*” (shinning pondweed)	Submerged	Cu, Zn and Pb	André et al. (1999)
“*Potamogeton natans*”(broad leaved pondweed)	Submerged	Hg, Cu, Zn, Cd and Pb	Lacher and Smith (2002), Fritioff and Greger (2006)
“*Potamogeton pectinatus*” (sago pondweed)	Submerged	Cr, Ni, Cu, Zn, Cd and Pb	Demırezen and Aksoy (2004)
“*Pseudoraphis spinescens*” (mud grass)	Emergent	Na, K, Ca, Mg, Fe, Mn, Zn, Cu, Ni, Cr and Pb	Prusty et al. (2007)
“*Salvinia auriculata*” (eared watermoss)	Free floating	Cd, Zn and Pb	Wolff et al. (2009), Wolff et al. (2012)
“*Salvinia cucullata*” (water moss)	Free floating	Cd and Pb	OECD (2002)
“*Salvinia molesta*” (giant salvinia)	Free floating	Cd, Hg, Pb, Ni and Zn	Kumari et al. (2016)
“*Salvinia natans*” (floating fern)	Free floating	Cd, Hg, Pb, Ni and Zn	Kumari et al. (2016)
“*Salvinia rotundifolia*” (butterfly fern)	Free floating	Pb	Banerjee and Sarkar (1997)

(continued)

Table 12.2 (continued)

Aquatic plants	Category	Efficient in remediating and stabilizing	References
"*Spirodela Polyrrhiza*" (gaint duckweed)	Free floating	pH, BOD, COD, NO_3, PO_4^-, sulfate, TDS, TSS and turbidity, Cu, Cr, Fe, Mn, Cd, Pb, Zn, Hg, Co and Ni	Brix and Schierup (1989), Loveson et al. (2013), Rai et al. (1995)
"*Trapa natans*" (water chestnut)	Floating	P, NO_3, K, Ca, reduces current velocity	Hummel and Kiviat (2004), Srivastava et al. (2009)
"*Typha angustata*" (cattail narrow leaved)	Emergent	pH, tolerance to dye wastewater, Cd, Co, Cu, Ni, Pb and Zn	Sharma et al. (2005), Kumar et al. (2008)
"*Typha latifolia*" (broad leaf cattail)	Emergent	pH, turbidity, EC, TDS, TSS, BOD, COD, NO_3, PO_4^-, Na and chlorinated phenol	Juwarkar (1995), Prabu and Udayasoorian (2007), Dipu et al. (2010), Selvamurugan et al. (2010)
"*Chrysopogon zizanioides*" (Vertver grass)	Emergent	BOD, COD, SS, NH_4^+, PO_4^- and pH	Meheesan et al. (2011)
"*Vallisneria spiralis*" (Eel grass)	Submerged	Cd, Co, Cu, Ni, Pb, Zn, Cr, Fe and Mn	Rai et al. (1995), Kumar et al. (2008)

TSS total suspended solids, *EC* electrical conductivity, *TDS* total dissolved solids, *BOD* biochemical oxygen demand, *COD* chemical oxygen demand, *SS* suspended solids, *DO* dissolved oxygen, *TP* total phosphates

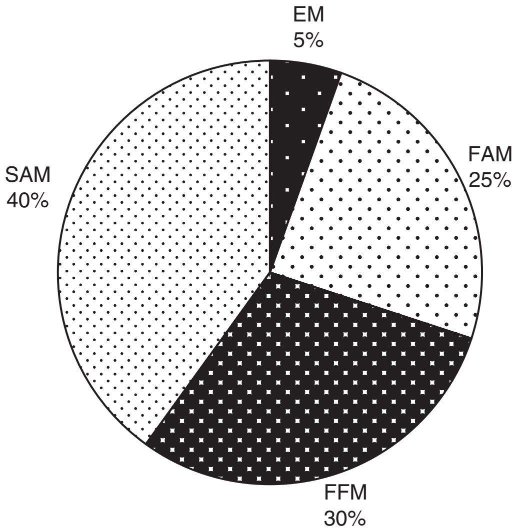

Fig. 12.6 The nutrient ion removal efficiency of aquatic macrophytes (*EM* emergent aquatic macrophytes, *FAM* floating aquatic macrophytes, *FFM* free floating aquatic macrophytes, *SAM* submerged aquatic macrophytes (Srivastava et al. 2008)

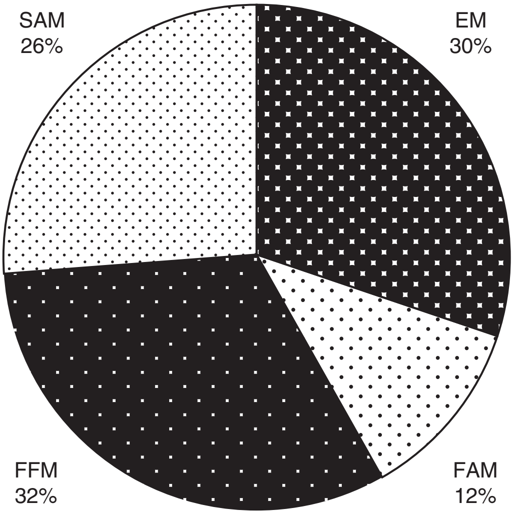

Fig. 12.7 Metal ion removal efficiency of aquatic macrophytes (*EM* emergent aquatic macrophytes, *FAM* floating aquatic macrophytes, *FFM* free floating aquatic macrophytes, *SAM* submerged aquatic macrophytes (Srivastava et al. 2008)

12.11 Conclusion

Macrophytes are important component of constructed wetlands. They help in improving water quality. Constructed wetlands have been proved by numerous scientists an effective, efficient and sustainable alternative to the conventional methods of wastewater treatment. Aquatic plants adapt number of routes and mechanisms to extract nutrients and heavy metals from disturbed environs. Most of the macrophytes have been detected to tolerate stressed environmental conditions. Therefore, focus should be given to identify such kind of aquatic plants in order to employing them for remediation of pollutants from wastewaters and disturbed environs.

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Chapter 13
Role of Biotechnology in Pesticide Remediation

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13.1 Introduction

Rapid industrial and agricultural activity across the globe during the twentieth century has led to the release of considerable amounts of pollutants of hazardous nature in to air, water, and soil systems (Masood and Irshad 2014). These environmental pollutants which are non-degradable type are highly toxic, mutagenic, teratogenic, and carcinogenic which are exerting the negative effect on the microbial diversity, fauna, flora, and human beings; hence removal of these harmful and recalcitrant pollutants from nature has now become a global concern (Fulekar and Jaya 2008). From the last three decades the public health is adversely affected with the increased burden of pollutants in accessories of daily life such as milk, vegetables, oils, fish, other food items (Ozkara et al. 2016; Rehman et al. 2016), and direct contact of humans with these pollutants at industries (pesticide factory workers); agricultural fields (farm workers) and other commercial crops (Ozkara et al. 2016; Kudagammana and Mohotti 2018).

For instance, if we see the list of chemicals or xenobiotic compounds which are entering into the environment, it includes agricultural chemical inputs such as fertilizers, fungicides, weedicides, herbicides, miticides, rodenticide, nematicide, molluscicide, raticides, soil fumigants, plant growth regulators (Sheahan

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and Barrett 2017), and industrial outputs as pollutants such as carbon monoxide, carbon dioxide, chlorofluorocarbons, hydrogen sulfide, heavy metals, nitrogen oxides, ozone, particulates, sulfur dioxide, unburned hydrocarbons, volatile organic compounds (VOCs), etc. (Chen et al. 2007). All the agricultural chemical inputs are resulting from industrial processes; hence, we can say that the industrial revolution was misutilized by the humans and released the pollutants in to environment and has created environmental problems leading to loss of biodiversity and public health issues.

In recent years, due to rapidly evolving technologies in processing and packaging of agricultural products and food items, unknowingly people have been exposed to several chemical pollutants which cause health disorders (Ozkara et al. 2016). Among them the most influential pollutant on humans is pesticides (Pastor et al. 2003; Erdogmus et al. 2015). Through food chain and processed foods, when people consume large quantities of pesticide, it results in acute poisoning and long-term health effects, including cancer, respiratory problems, and adverse effects on reproduction. In the last decade there are several epidemiological reports about the toxic effects of pesticides on public health and correlated the pesticide usage with the occurrence of various types of cancers including leukemia and solid tumors (Merhi et al. 2007; Akyil et al. 2015).

Pesticides are natural or artificial compounds with inorganic or organic type which are toxic and can kill the pests of agriculture such as herbs, insects, nematodes, fungi, rodents, and soil fumigants (Verma et al. 2014). They are used in agriculture systems to prevent the attack of pests or inter growth of weeds in a commercial crop and to enhance the yield and quality of crop for good economic returns (Spina et al. 2018). For beautification, nowadays pesticides are also used in large amounts in private gardens, amusement parks, and other public areas (Grube et al. 2011). The use of pesticides has increased drastically in last three decades and out of applied pesticide spray or solution; only some portion is utilized by the crop systems and remaining enters into the surrounding environments of soil, water, and air (Lozowicka et al. 2014). This has caused bioaccumulation through a series of trophic levels of food chain and ultimately reached the humans and posed the public health issues (Kolpin et al. 1998; Lefrancq et al. 2013).

Indiscriminate usage of highly organic and aromatic pesticides for the eradication of pests and for the increase of agricultural productivity has resulted in the persistence of recalcitrant xenobiotic pesticides in the nature (Arzu et al. 2016). It has ultimately caused the environmental problems leading to risk of mankind and other living organisms which has attracted the scientific community and placed an issue all over the world regarding its remediation. Persistence of pesticides in the nature is either due to their physiochemical properties, their immobile nature in a particular environment, due to their non-degradable nature, where the natural flora of microbes could not able to degrade those persisted chemicals (Velazquez et al. 2012) and by virtue of which environmentalists and other researchers have posed their attention to degrade the deposited pesticides.

Pesticide degradation is a process by which pesticides either get transformed from a toxic to nontoxic form which is mostly carried by eukaryotes (fungi, plants, and animals) or may get totally metabolized by microbial communities (eubacteria and bacteria) in single or in consortia (Copley 2009). One way of natural removal of pesticides from nature is exposure of deposited pesticides to heat, light, and high humidity where a volatilizing chemical be easily removed (Sibanda et al. 2011; Fenner et al. 2013). This natural way of pesticide degradation is time consumable, less effective and more ever most of the pesticides which are used in agriculture are nonvolatile and non-degradable type.

Biotechnology has been regarded as a potential tool for the safe, inexpensive, in situ (on site) operation with effectiveness for the remediation of sites contaminated with hazardous pesticides and other pollutants (Jeffrey 1992). Bioremediation is considered as an effective process where living organisms (bacteria, fungi, and plants) were used to degrade or remove the harmful and toxic pollutants from the environment in an effective and inexpensive manner (Glazer and Nikaido 1995). In last two decades researchers have studied the microbial ecology and physiology in pesticide contaminated sites and also to some extent identified the molecular and biochemical basis of pesticide degradation (Mishra et al. 2001; Watanabe 2001).

With advancements in biotechnology with regard to identification of specific genes and enzymes responsible for the degradation of a specific pollutant, now it is possible to utilize them in ecofriendly and cost-effective degradation of pesticide. This chapter focuses on the role of biotechnology in pesticide remediation from contaminated water and soil through a review study with special emphasis on the role of bacteria, fungi, plant, and plant–microbe interactions.

13.2 Bioremediation

As pesticides are posing serious threats to quality of water, content of air, fertility of soil, and ultimately to human health (Hussain et al. 2007; Yao et al. 2015); hence there is an urgent need to remediate the pesticide pollutants either naturally (recycling, pyrolysis, incineration) or by artificial means of chemical, physical, and biotechnological based applications. Bioremediation is considered as most ecofriendly and cost-effective than the chemical and physical methods of remediation (Song and Bartha 1990). The term bioremediation is defined as a sustainable process where living organisms (bacteria, fungi, plants) or their products (enzymes) were employed to clean up (degrade, destroy, transform, detoxify, disperse, dilute, sorption, volatilize, mineralize) the pollutants from the contaminated sites of environment.

13.2.1 In Situ Bioremediation

In situ bioremediation is the biological treatment of contaminated water and soil at their natural locations. In situ bioremediation is most suitable for remediation of polluted ground water and agricultural lands as treatment occurs directly at the surface or subsurface of contaminated sites (Campbell 2009). In situ bioremediation is further classified into intrinsic and extrinsic types. In intrinsic type, remediation is carried by the innate abilities of natural flora by without adding any supplement and without modifying any condition, whereas in extrinsic type, remediation is carried by the natural microflora or by an engineered strain and needs the addition of some supplements and modifications of condition like provision of ventilation, temperature, and pH.

13.2.1.1 Natural Attenuation or Intrinsic In Situ Bioremediation

Natural attenuation is an intrinsic type of bioremediation (without any inputs) where natural microbes take part in bioremediation of pollutants mostly found in contaminated groundwater and soil wastes comprising of landfills and hazardous wastes (Pedro et al. 2005). Along with sewage water and contaminated soils, recently natural attenuation is proved as a cost-effective remediation process for low risk oil contaminated sites (Hinchee 1998). Even though through natural attenuation the pollutants found in subsurface groundwater and soil can be removed, the major disadvantage of intrinsic bioremediation is: it is a time consuming process as all the required conditions and factors may not be available naturally at the contaminated sites.

In in situ based intrinsic bioremediation, even though there are some microbial strains found naturally in water and soil which are capable of carrying out the biodegradation of pesticides, but due to lack of some primary requirements such as aerobic conditions, suitable pH, appropriate temperature, free carbon source, electron donor or acceptor, and other stimulants; the bioremediation is not adequately done to the extent as expected (Azubuike et al. 2016).

13.2.1.2 Extrinsic In Situ Bioremediation

Among the different types of bioremediation in situ based extrinsic bioremediation is considered to be ideal due to their onsite treatment with minimal damage. By providing the additional requirements and engineered strains of microbes; the in situ based extrinsic biodegradation process can be carried to the adequate level which can eliminate or can convert the pesticides from toxic to nontoxic form. Compared to intrinsic bioremediation by natural microflora, bioremediation by engineered strains can cause total destruction of pesticide pollutants by without generating any intermediated or secondary products. Extrinsic type of in situ bioremediation is further classified into biostimulation and bioaugmentation.

13.2.1.3 Biostimulation

Biostimulation is a process where stimulation of innate ability of degradation of pollutants by indigenous microbes is done by supplementing the additional requirements such as providing aerobic conditions, suitable pH, appropriate temperature, free carbon source, electron donor or acceptor, and other stimulants (Morgan and Watkinson 1989; Margesin and Schinner 1999). Biostimulation is of different types; such as bioventing, bioslurping, biosparging, landfarming, and phytoremediation. In bioventing type, for providing aerobic conditions oxygen is supplied to subsurface areas through small pipes and additional supplements of nutrients are provided to carry the bioremediation at a faster rate (Shah et al. 2001; Philp and Atlas 2005). In 2011, Sui and Li have carried the biodegradation of toluene by injecting the air to subsurface areas of contaminated sites. Bioslurping is an enhanced bioventing technique, where vacuum operated dewatering is carried in combination with supply of oxygen and nutrients (Gidarakos and Aivalioti 2007; Kim et al. 2014), it is mostly suitable for remediation of hydrocarbon contaminated sites.

Biosparging is also a modification of bioventing technique, where oxygen is supplied in to the subsurface air through a specialized pipe with small holes by which the in-blown air is allowed to make upward movement. Biosparging technique allows the volatile pollutants to move from microbe fewer zones to microbe densified zone and there by provides the provision of their removal or remediation. Kao et al. (2007) have reported about the enhanced biodegradation of benzene, toluene, ethyl benzene, and xylene by biosparging technique. In landfarming, the land contaminated with pollutants is excavated and can be treated by adding the nutrients and required microbes (Cerqueira et al. 2014). Landfarming is carried to speed up the bioremediation process by evenly distributing the pollutants and microbes. Landfarming type of bioremediation can be carried both as in situ and ex situ types; the treatment site determines the type of bioremediation.

In phytoremediation, plants are being used to clean up the contaminated sites by their biophysical and biochemical interactions through different mechanisms such as volatilization, extraction, stabilization, filtration, degradation, augmentation, transformation, and stimulation (Kuiper et al. 2004; Peuke and Rennenberg 2005). By using several plant species, the heavy metal contamination was successfully remediated from the contaminated sites (Cobbett 2000; Ali et al. 2013; Yavari et al. 2015).

13.2.1.4 Bioaugmentation

Bioaugmentation is an extrinsic type of in situ bioremediation; in which potential microbes were initially mass cultured under laboratory conditions and then cultured microbes were applied at the contaminated sites for fast and efficient degradation of pesticides than the naturally existing microbes (Alvarez and Illman 2006). As most of the pesticides which were being used in modern world are highly aromatic and recalcitrant type; the bioaugmentation process is gaining more importance than the biostimulation for the faster and effective way of degrading them. Bioaugmentation can be carried either by using pure cultures of a single strain or by mixed cultures of several indigenous bacteria (Zheng et al. 2001; Ulrich and Edwards 2003).

In bioaugmentation, the introduced strain of microbe may face the rapid declinity in their population size; due to several limiting factors such as immovable or non-mobility of introduced strain (Harvey et al. 1987), nutrient availability (Wilson and Lindow 1995), unsuitable physiochemical conditions (Thiem et al. 1994), grazing pressure (Barcina et al. 1997), competition from native microbial species (Goldstein et al. 1985), and availability of micro niches (Postma et al. 1990). Later, to overcome these limitations for carrying out the successful degradation of environmental pollutants several strategies were developed (Brown and Jaffe 2001; Kane et al. 2001; Streger et al. 1988).

Degradation of highly toxic and pesticide compounds (dichlorodiphenyltrichloroethane (DDT), carbendazim, chlorpyrifos, parathion, endosulfan, atrazine, lindane, carbamate, and pentachlorophenol) found in water bodies and soil sediments are the major concerns of remediation with regard to polluted water and soil systems. Some reports are there regarding the degradation of organophosphates (Ramanathan and Lalithakumari 1996), atrazine (Feakin et al. 1995), carbamate (Mulbry and Kearney 1991), organochlorine (Ghadiri et al. 1995), DDT (Aislabie et al. 1997), chlorpyrifos (Singh et al. 2003), lindane (Bintein and Devillers 1996), parathion (Hansch et al. 1995), carbendazim (Tomlin 2003), endosulfan (Tomlin 1997), and pentachlorophenol compounds (McAllister et al. 1996). In removal of deposited harmful pesticide pollutants along with conventional method of using native or cultured microbial strains; there is a need to introduce the constructed or recombinant plasmids possessing pesticide degrading genes (Carlos et al. 2017) for better and efficient removal of pesticides from contaminated water bodies and soils.

13.2.2 Ex Situ Bioremediation

With microbes found in much lesser concentration in greater depths of some contaminated sites, the in situ bioremediation could not be much successful, hence the researchers have adopted the ex situ bioremediation. In ex situ bioremediation polluted water and soil samples were evacuated from contaminated sites and were treated in bioreactors and other equipment by providing all microbial favorable conditions in a laboratory or away from their natural site of contamination (Kuppusamy et al. 2016). Ex situ bioremediation is also carried in those sites where pollutants were present in greater concentrations, as ex situ bioremediation can speed up the process of biodegradation as compared to in situ bioremediation.

Ex situ bioremediation is classified in to aerobic and anaerobic type; slurry phase, liquid phase, and solid phase types based on the requirement of oxygen and state of contaminated material to be treated. Further solid phase of ex situ bioremediation is classified into biopile and windrow types. Biopile is a solid phase of aerobic ex situ bioremediation technique, where contaminated material in solid form is excavated from the natural site and piled up one above the other away from their natural site and monitored regularly by the human intervention for the inoculation of pollutant specific microbes, for the supply of nutrients, oxygen, water, and other factors (Whelan et al. 2015; Dias et al. 2015).

Windrow is a modified type of biopiling technique where periodic turning of piled contaminated solid materials were carried for faster and efficient degradation. The periodic turning of piles with the supply of nutrients, water, and oxygen can speed up the bioremediation process (Barr 2002). Windrow type of treatment has proven better in removal of hydrocarbon pollutants from contaminated soils (Coulon et al. 2010). In slurry phase and liquid phase of ex situ bioremediation the excavated soil with pollutants is placed in a bioreactor and diluted with different quantities of water to form either slurry or liquid. Bioreactor was inoculated with suitable microbial strain for carrying out the removal of pollutants in a faster way under controlled conditions (pH, temperature, aeration, and mixing). Bioreactor based bioremediation was carried for the successful removal of carbofuran (Plangklang and Alissara 2010), petroleum (Chikere et al. 2016), 2,4-dicholorophenoxy acetic acid (Mustafa et al. 2014) from contaminated soils.

Even though ex situ bioremediation is much costlier than the in situ bioremediation process due to the involvement of labor, transporting of contaminated material, and usage of bioreactors, it is much preferred over in situ; as the ex situ type can remove the pollutants in quick and effective way and thereby can reduce the environmental human health risks in an effective manner (Tomei and Andrew 2013).

13.3 Bioremediation of Pesticides by Bacterial Strains

Degradation of pesticides resulted in the discharge of the initial compounds from coal oxygen (CO_2) and air (H_2O). Through pesticide degradation, microorganisms use energy. However, degradation effectiveness relies on different parameters such as heat, pH of land, humidity levels, etc.; the biodegradation of pesticides via microbes has an important effect on the health of agrarian crops. In addition, microbes also have other important benefits, such as variety, wide dispersal and adjustment of different cellular processes. Various methods for early degradation of pesticides have also been used, such as genetic manipulation and the generation of genetically modified organisms (Cui et al. 2012). Following genera *Azotobacter*, *Arthrobacter*, *Burkholderia*, *Flavobacterium,* and *Pseudomonas* possess bacterial species with pesticide degradation ability but the degradation process varies with species and the target compounds (Table 13.1). As s-triazine herbicides, e.g., atrazine used to degrade by *Klebsiella pneumonia* and *Pseudomonas* sp., while as *Alcaligenes* sp. and *Pseudomonas* also possess the ability to degrade herbicide 2,4-D (Mulbry and Kearney 1991); organochlorine pesticide like endosulfan (Jayashree and Vasudevan 2007a, b), lindane (Gupta et al. 2001), organophosphorus insecticide chlorpyrifos (Yang et al. 2005), etc. Generally microbial consortia are

Table 13.1 Bacterial species reported with pesticide degrading capacity around the globe

Species	Pesticide	Reference
Acinetobacter calcoaceticus	Bifenthrin	Tingting et al. (2012)
Acinetobacter johnsonii (MA-19) strain	Organophosphate pesticides	Xie et al. (2009)
Acinetobacter	Esbiothrin	Ha et al. (2009)
Acidomonas sp.	Allethrin	Paingankar et al. (2005)
Azospirillum and *Pseudomonas*	Ethion	Zhang et al. (2007)
A. xylosoxidans JCp4 and *Ochrobactrum* sp. FCp1	Chlorpyrifos	Akbar and Sultan (2016)
Bacillus thuringiensis	Malathion	Zeinat et al. (2008)
Burkholderia cepacia strain CH-9	Imidacloprid and metribuzin	Madhuban et al. (2011)
Bacillus sp. and *Chryseobacterium joostei*	Lindane, methyl parathion, and carbofuran	Foster et al. (2004)
Enterobacter aerogenes	Bifenthrin, cypermethrin	Lio and Xie (2009)
Escherichia coli	BHC, DDT, endosulfan, HCH isomers, and 2,4-D	Xue-Dong et al. (2003) Chaudhary et al. (2006)
Ochrobactrum	Triazophos	Shunpeng and Mingxing (2006)
Photosynthetic bacterium (GJ-22)	Cypermethrin (CMP)	Yin et al. (2012)
Paracoccus sp. strain	Pyridine	Qiao and Wang (2010)
Pseudomonas	Endosulfan, atrazine	Wyss et al. (2006)
Pseudomonas putida and *Pseudomonas mendocina*	Permethrin and cypermethrin pesticides	Mendoza et al. (2011)
Pseudomonas and *Alcaligenes* sp.	Herbicide 2,4-D, endosulfan, lindane, chlorpyrifos	Yang et al. (2005)
Rhodococcus bacteria	Para-nitrophenol	Zhang et al. (2009)
Rhodobacter sphaeroides	Chlorinated pesticides, herbicides, and fungicides	Harada et al. (2006)
Sphingobium japonicum	Hexachlorocyclohexane	Liu et al. (2006)
Stenotrophomonas maltophilia	DDT and endosulfan	Barragán-Huerta et al. (2007)
Sphingomonas	DDT	Shunpeng and Mingxing (2006)
Sphingobacterium sp.	DDT	Fang et al. (2010)
Sphingobium sp. JQL4-5	Fenpropathrin	Yuanfan et al. (2010)
Sphingomonas yanoikuyae	Carbamate and pyrethrin	Ouyang et al. (2008)
Vibrio and *Shewanella*	Methyl parathion	Liu et al. (2006)

more efficient compared to single strains for pesticide degradation. Microbial consortia are generally more effective than single types for degradation of pesticides.

Sphingobium japonicum is another identified microbial line of the chlorined (hexachlorocyclohexane) pesticide degradation strain (Liu et al. 2006). The degradation of imidacloprid and metribuzin is also engaged in the degradation of *Burkholderia cepacia* type CH-9 (Madhuban et al. 2011). *Acinetobacter calcoaceticus* degraded bifenthrin (Tingting et al. 2012), i.e., a synthesized pesticide. The capabilities for cypermethrin (CMP) degrading are noted as photosynthetic bacterium (GJ-22) (Yin et al. 2012).

Another research shows very elevated biodegradable permethrin capacities and cypermethrin pesticides in *Pseudomonas putida* and *Pseudomonas mendocina* (Mendoza et al. 2011). *Paracoccus* sp. strain is studied for its ability to degrade pyridine (Qiao and Wang 2010). *Enterobacter aerogenes* strain is recorded for degradations of various pesticides, e.g., bifenthrin, cypermethrin, etc. The degradation of organophosphate pesticide is being investigated in the species of *Acinetobacter Johnsonii* (MA-19) (Xie et al. 2009). The effectiveness of para-nitrophenol degradation Rhodococcus bacteria has been investigated by Zhang et al. (2009). Different types of organophosphate pesticide degradation was known to carry by *Bacillus* and L-*Proteobacteria* (Sabdano and Radjasa 2008). In malathion degradation, *Bacillus thuringiensis* works (Zeinat et al. 2008). *Acinetobacter* breaks down the esbiothrin (Ha et al. 2009). The capacity to degrade DDT and endosulfan is demonstrated by Stenotrophomonas maltophilia (Barragán-Huerta et al. 2007). *Pseudomonas* joins endosulfan together with atrazine (Prabakaran and Peterson 2006; Wyss et al. 2006). *Sphingomonas* (Shunpeng and Mingxing 2006) is another gram-negative bacteria species that is extremely efficient in DDT degradation. Diverse pesticides such as chlorinated pesticide, herbicides, fungicides, etc. have been effectively degraded by the fermentation method by Rhodobacter sphaeroides (Harada et al. 2006). Effective methyl parathion degraders are *Vibrio* and *Shewanella bacterium* (Liu et al. 2006). Photosynthetic fungi (chlorpyrifos, phoxims, and triazophos) are revealed to be capable of degrading separate pesticides (Zhang et al. 2007). It has been reported that Ochrobactrum is oxidizing triazophos and degrading the compounds to 95% (Shunpeng and Mingxing 2006). *Acidomonas* sp. is reported to degrade allethrin, a pyrethroid insecticide (Paingankar et al. 2005). Microorganisms also recorded anaerobic aldrin degradation (organochlorine insecticide) (Guohui 2004). The mesophilic bacteria, *Azospirillum* and *Pseudomonas* anaerobically degrade Ethion (Zhang et al. 2007). For biodegradation of lindane, carbofuran, and methyl parathion; researchers applied bacterial consortium, e.g., *Bacillus* sp. and *Chryseobacterium joostei* (Foster et al. 2004). Psychrotrophic bacterium degrades me-parathion (Krishna and Philip 2009). Six bacterial genera have been discovered to be efficient in degrading organochloride pesticides, i.e., endosulfan, including *Micrococcus* and *Pseudomonas* (Li et al. 2004). *Escherichia coli* is effective in the degradation of organochlorine and various pesticides such as BHC, DDT, endosulfan, HCH isomers, and 2,4-D (Gupta et al. 2001). DLL-1 bacterial strain is another effective pesticide degrader (Yu-Suo et al. 2003). The capacity to degrade diazinon and profenofos is also separated from different bacterial types (Abo-Amer 2011).

Ortiz-Hernández and Sánchez-Salinas (2010) undertook research which stated that tetrachlorvinphos and organophosphate pesticide degradation capability was separated from agricultural soil by a six-pure type bacterial consortium. Lactic acid bacteria also possess the ability to degrade organophosphorous insecticides (Kye et al. 2009). Matsumura et al. (1968) researched *Pseudomonas* sp. degradation of dieldrin. It was also found that bacteria with the capacity to degrade dieldrin also performed aldrin, endrin, and DDT biodegradation (Patil et al. 1970). Microbes of the genera *Arthrobacter*, *Bacillus*, *Micrococcus,* and *Pseudomonas* are particularly involved in the degradation of organochlorine (Langlois et al. 1970). *Sphingobacterium* sp. has the biodegradation capability of DDT (Fang et al. 2010). Worldwide, different bacteria with the ability to degrade methyl parathion have been isolated (Liu et al. 2006). *Sphingobium* sp. JQL4-5 is capable of degrading fenpropathrin (Yuanfan et al. 2010). The organophosphate fenamiphos has been degraded by *Pseudomonas putida* and *Acinetobacter rhizosphaerae.* Different microbes have been separated with carbamate pesticides' capacity to biodegrade. Different *Arthrobacter*, *Achromobacterium Pseudomonas*, *Flavobacterium*, *Sphingomonas*, and species are capable of carbofuran degradation. Chlorpyrifos is a popular pesticide used in vegetable and cotton areas for the treatment of pests, although it is a toxic compound that causes contamination of soil and water. A bacterial consortium of six bacterial strains, namely *Stenotrophomonas maltophilia*, *Proteus vulgaris*, *Vibrio metschnikovii*, *Serratia ficaria*, *Serratia* spp., and *Yersinia enterocolitica*, has the capacity to degrade tetrachlorvinphos (Ortiz-Hernández and Sánchez-Salinas 2010). Two bacteria distinct from each other, *A. xylosoxidans* JCp4 and *Ochrobactrum* sp FCp1 were recognized for chlorpyrifos degradation. Different microbes have been separated with carbamate pesticides' capacity to biodegrade. Different *Pseudomonas*, *Flavobacterium*, *Achromobacterium*, *Sphingomonas*, and *Arthrobacter* species are capable of carbofuran degradation. Chlorpyrifos is a popular pesticide used in vegetable and cotton areas for the treatment of pests, although it is a toxic compound that causes contamination of soil and water. A bacterial consortium of six bacterial strains, namely *Stenotrophomonas maltophilia*, *Proteus vulgaris*, *Vibrio metschnikovii*, *Serratia ficaria*, *Serratia* spp., and *Yersinia enterocolitica*, has the capacity to degrade tetrachlorvinphos (Ortiz-Hernández and Sánchez-Salinas 2010; Akbar and Sultan 2016). With prospective implementation in the degradation of chlorpyrifos (CP) pesticide, various types of Streptomycetes were recognized (Briceño et al. 2012). Also separated were various actinomycetes species capable of degrading carbamate pesticides (De Schrijver and De Mot 1999). *Actinomycete* species HP-S-01 has been separated with the capacity to knock down deltamethrin along with bifenthrin, fenvalerate, and fenpropathrin degradation (Chen et al. 2011). *Sphingomonas yanoikuyae* degraded carbamate and pyrethrin (OPs) (Ouyang et al. 2008).

13.4 Bioremediation of Pesticides by Fungal Strains

Different fungal species from artificial sources were also evaluated for their biodegradation effectiveness of various toxic organic chemicals. Researchers researched various fungal species for bioremediation operation. Fungal species, *Fusarium oxysporum*, *Lentinula edodes*, *Penicillium brevicompactum,* and *Lecanicillium saksenae,* have been extremely effective in biodegrading various pesticides (terbuthylazine, difenoconazole, and pendimethalin) (Table 13.2). As lindane was known to be degraded by *Fusarium verticilloides* and later uses the degraded produts as carbon source (Pinto et al. 2012). The biodegradation behavior against aldicarb, atrazine, and alachlor has been shown in another unacclimatized blended community (Nyakundi et al. 2011) of bacteria and white-red funguses. Methomyl and diazinon (pesticides) were degraded by red fungi extracted from contaminated plants (Sagar and Singh 2011). The *Asperger strain* (Javaid et al. 2016) may cause endosulfan degradation (pesticide). Various fungal strains also possess DDD pesticide degradation ability (Ortega et al. 2011). *Mortierella* sp. strains W8 and Cm1-45 are endosulfan-degrading, aerobic fungal strains which were efficient for the bioremediation of soil contaminated with organochlorine pesticides and improved the soil fertility (Kataoka et al. 2010).

Slaoui et al. (2007) reported that genus *Gliocladium* were found very effective for biodegradation of carbofuran as *C. elegans* successfully degrade an insecticide DEET (Seo et al. 2005) and also *Trichoderma viride* and *T. harzianum* possess very

Table 13.2 Fungal species reported with pesticide degrading capacity around the globe

Species	Pesticide	Reference
Aspergillus	Endosulfan	Javaid et al. (2016)
C. elegans	DEET, an insecticide	Seo et al. (2005)
Fusarium oxysporum *Lentinula edodes* *Penicillium brevicompactum* and *Lecanicillium saksenae*	Terbuthylazine, difenoconazole, and pendimethalin	Hai et al. (2012)
Fusarium verticillioides	Lindane	Pinto et al. (2012)
Mortierella sp. strains W8 and Cm1-45	Endosulfan	Kataoka et al. (2010)
Trichoderma viride and *T. harzianum*	Pirimicarb	Romeh (2001)
Rot fungi	Methomyl and diazinon	Sagar and Singh (2011)
White-rot fungi	Aldrin, aldicarb, alachlor, atrazine, chlordane, diuron, DDT, dieldrin, gamma-hexachlorocyclohexane (γ-HCH), heptachlor, lindane, mirex, metalaxyl, terbuthylazine	Nyakundi et al. (2011)

high potential for degradation of pirimicarb (Romeh 2001). Odukkathil and Vasudevan (2013) stated that degradation of various types of pesticides can be achieved by *Phanerochaete chrysosporium.*

Different fungal species, *Auricularia auricula*, *Agrocybe semiorbicularis*, *Avatha discolor*, *Coriolus versicolor*, *Dichomitus squalens*, *Flammulina velutipes*, *Hypholoma fasciculare*, *Pleurotus ostreatus*, and *Stereum hirsutum*, demonstrated biodegradation capacity for various pesticides, namely dicarboximide, chlorinated compounds, phenylamide, phenylurea, triazine, and organophosphorus compounds (Bending et al. 2002). Likewise, Quintero et al. (2007) have indicated that white-red fungi can degrade a number of distinct pesticides such as aldrine, atrazine, chlordane, DDT, dieldrin, diurone, heptachlorine, lindane, and mirex to varying concentrations of gamma-hexachlorocyclohexane and terbuthylazine, etc. (Das and Chandran 2011).

13.5 Biotechnological Approaches for Bioremediation of Pesticides

Along with the conventional methods of using in situ and ex situ type of bioremediation, there is need to utilize the applications of advanced subjects like biotechnology and bioinformatics for reducing the environmental pollutants and public health risks. Some of the biotechnological approaches/products which can speed up the process of biodegradation of pesticides and can eliminate the pesticides in a safer and effective way are listed and described below.

13.5.1 Genetically Engineered Microorganisms

Microorganisms are the pivotal organisms in degrading the pesticide and other environmental pollutants; as they have the ability to produce specific enzyme which can metabolize the environmental contaminants and can use them as their food material. Pesticide pollutants of simpler chemical nature or non-recalcitrant type (nitro compounds, monochlorinated compounds, simple phenols, biphenyls, polychlorinated compounds) can easily be degraded by natural microflora (Parales and Haddock 2004). However, recalcitrant pesticides of highly aromatic, highly nitrated, highly chlorinated type and which are chemically insert cannot be degraded easily by the natural microorganisms (Parrilli et al. 2010). Hence Paul et al. (2005) have stressed about the importance and need of introducing the genetically engineered microorganisms for the faster and easier bioremediation. Brokamp and Schmidt (1991) and Fulthorpe and Wyndham (1991) have proposed for the need of introducing specific traits regarding the degradation of environmental pollutants through genetic manipulation of microbes. Genetically engineered microorganisms with foreign genes of pesticide resistance can confer the better ability for degrading the persistent pollutants which were found in nature.

There are several reports about the efficient degradation of pesticide compounds by using genetically modified microorganisms (Wilson and Lindow 1993; Fujita et al. 1994, 1995; Erb et al. 1997; Chen and Mulchandani 1998; Yee et al. 1998; Sayler et al. 1999; Dejonghe et al. 2000; Sayler and Ripp 2000; Watanabe et al. 2002). The scientific community has succeeded in degrading chemical pollutants like phenol (Erb et al. 1997; Watanabe et al. 2002), 2,4-Dicholrophenoxy acetic acid) (Dejonghe et al. 2000), trichloroethylene (Fujita et al. 1994; Yee et al. 1998) polycyclic aromatic hydrocarbon (Sayler et al. 1999), mercury, toluene, chlorobenzene, indole (Lange et al. 1998) by using genetically modified microbes.

13.5.2 Transgenic Plants

As phytoremediation is considered as the most ideal and non-destructive type of removing environmental pollutants; public has mostly accepted it in cleaning up the environmental pollutants (Ziarati and Alaedini 2014). Researchers have concentrated on identifying different plant species which can accumulate, degrade, volatilize, and transform the harmful and toxic pesticides to inactive or nontoxic form. Along with the native plant species, certain transgenic plants were raised by the researchers by expressing specific enzymes which can metabolize the pesticides. Over the last ten years, a number of transgenic plants such as rice, maize, arabidopsis, mustard, tobacco, soybean, and tomato plants are being produced which expresses herbicide or weedicide degrading enzymes (cytochrome P-450, glutathione-s-transferase, glyphosate oxidase) in order to provide tolerance and degrading capacity against pesticides (Kawahigashi 2009). Sonoki et al. (2005) developed a transgenic tobacco which expresses fungal (*Coriolus versicolor*) based laccase enzyme showing resistance against pentachlorophenol and bisphenol pesticides. Kawahigashi et al. (2007) have raised a transgenic line of rice expressing CYP1A1 transgene gene showing resistance and degradation abilities against atrazine, chlorotoluron and norflurazon pesticides. Behrens et al. (2007) have expressed a bacterial (*Pseudomonas maltophilia* strain DI-6) gene encoding dicamba monooxygenase (DMO) in transgenic plants of tobacco, arabidopsis, and tomato plants. Dicamba monooxygenase is a rieske non-heme monoxygenase type of enzyme which coverts dicamba into an inactive and nontoxic form of dicamba, i.e., 3,6-dicamba dichlorosalicylic acid. Colin et al. (2008) have expressed TfdA gene encoding aryloxyalkanoate dioxygenase enzyme in corn plant showing resistance and causing degradation of herbicides such as pyridyloxy acetate and 2,4-dichlorophenoxy acetic acid.

Similarly, after the identification of pesticide degrading genes and enzymes from different source of organisms and most predominantly form microbes; commercial seed companies have raised the transgenic crop plants which can resist and degrade the pesticide or herbicide. Jerry (2009) in his research reported that several commercial companies like Monsanto, Bayer, Calgene, DeKalb, and Pioneer have developed the commercial transgenic crops (soybean, sugar beet, canola, cotton, alfalfa, and corn) expressing herbicide resistant genes (*cp4 espsps*, *bxn nitrilase*, *goxv-247*, *pat*, *gat*, *gm-hra,* and *zm-hra*) against different herbicides.

13.5.3 Enzymes Based Bioremediation of Pesticides

Since the introduction of xenobiotic and highly aromatic natured pesticide compounds into the nature, their degradation by a specific organism, mode of action, mechanism, and chemical reactions involved in their degradation has gained lot of significance due to the advancements of biotechnological research. Enzymes present in pesticide resistant organisms will either totally metabolize it or they can transform it in to an inactive and nontoxic form. Hence, enzyme based research has gained importance over the last three decades to identify pesticide specific degrading enzymes across different living organisms (fungi, bacteria, plants, and animals).

In enzyme based pesticide degradation, among all the organisms bacterial based enzymes have prominent role as the natural microflora interacts directly with the pesticide pollutants present in contaminated water and soil. Among the bacteria, *Pseudomonas* has been reported to possess several types of pesticide degrading abilities. De Souza et al. (1996) have reported about the AtzA gene of *Pseudomonas* sp. encoding atrazine chlorohydrolase which can degrade chloro-s-triazine. Similarly different species of pseudomonas were reported for their ability to degrade pesticides like glyphosate, coroxon, dicamba, trifluralin (Zylstra et al. 1988; Horne et al. 2002a, b; Herman et al. 2005; Pollegioni et al. 2011). *Klebsiella pneumonia* based enzyme Bxn nitrilase monooxygenase was reported to possess the ability of degrading bromoxynil pesticide (Stalker et al. 1996).

Other bacteria such as *Arthrobacter*, *Sphingobium*, *Plesiomonas*, *Agrobacterium*, *Flavobacterium*, *Burkholderia,* and *Ralstonia* were reported for their degrading abilities through specific enzymes against specific pesticides (Leveau et al. 1999; Shim et al. 2000; Cui et al. 2001; Horne et al. 2002a, b; Iris et al. 2002; Weir et al. 2006; Latifi et al. 2012; Nandavaram et al. 2016). As genes of pesticidal resistance or pesticidal degradation were identified from the above-mentioned different bacterial species; now researchers are successfully creating genetically modified organisms (bacteria and plants) the better cleanup of the environment pollutants.

Apart from bacteria; animal and plant based genes were also identified which can efficiently degrade the pesticidal pollutants. Animals such as *Anguilla japonica*, *Oryctolagus cuniculus,* and *Lucilia cuprina* were possessing pesticidal degrading genes against chlorotoluron, diuron, linuron, simazine, trichloroethane, vinyl chloride, carbon tetrachloride, and pyrethroids (Doty et al. 2000; Heidari et al. 2005). Similarly plant species such as *Helianthus tuberosus*, *Zea mays*; *Coriolus versicolor*, *Glycine max,* and *Arabidopsis thaliana* were reported to possess genes against degradation of pesticides like linuron, chlortoluron, isoproturon, alachlor, pentachlorophenol, phenyl urea, and PPO inhibitors (Siminszky et al. 1999; Iimura et al. 2002; Luc et al. 2002; Karavangeli et al. 2005; Li and Nicholl 2005). Humans based Cytochrome P450 genes of class 1, 2, and 3 were also reported to possess pesticide degradation property (Doty et al. 2000; Inui and Ohkawa 2005; Kawahigashi et al. 2005, 2007).

13.6 Conclusion and Future Perspectives

With an ever growing human population across the world, the application of pesticides in agricultural and horticultural crops has increased rapidly during the last twenty years. Similarly in food industry the addition of chemical additives or chemical preservatives has rapidly increased for providing attractive flavor and for long-term preservation schedules. The applied pesticides and preservatives have found their way to nature including human beings and posed threats to environmental risks and public health. In this context, private biotechnology companies have recognized bioremediation of pesticides as a promising area for providing better human health and for environmental cleanup. Over the last few years, people also realized the recalcitrant nature and harmful effects of pesticides on environment and public health.

Biotechnological based applications and technologies have gained importance for the bioremediation of pesticides. With the initial success of remediation of pesticide contaminated sites with genetically engineered microbes, transgenic plants, enzyme based and bioreactor based biotechnologies, there is a scope for introducing new technologies in environmental cleanup in upcoming years.

The future perspective of bioremediation should be as follows:

1. Need to carry out pesticide to free carbon production by biotechnology based methods, i.e., carrying out total metabolism of pesticides
2. Utilizing bio-control agents for eradication of pests
3. Biotechnology based weed management
4. Has to revolutionize the farming practices with zero input of pesticides and herbicides
5. Need to work out on synergistic effect of plant–microbe interactions in pesticide degradation.
6. Biodegradability and persistence nature of pesticides and other xenobiotic has to be questioned before its approval for commercial utilization.
7. Has to carry out ecological impact and economics of transgenic plants/genetically engineered microbes involved in bioremediation process.
8. More importantly awareness should be created among the people for the safe disposal of xenobiotic compounds.

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Chapter 14
Role of Ectomycorrhizal Biotechnology in Pesticide Remediation

Rezwana Assad, Zafar Ahmad Reshi, Irfan Rashid, Yogesh Shouche, and Dhiraj Dhotre

14.1 Introduction

In global environmental research, unprecedented global environmental change instigated by detrimental anthropogenic activities, is one of the critical issues nowadays. Unsystematic overuse of chemical pesticides is one such anthropogenic practice that degrade soil quality, influence biotic community structure, and pose staid environmental health risk (Chatterjee et al. 2019), which has been the focus of attention for ecologists, environmentalists, and conservationists worldwide. It is an apparent known fact that pesticides pose severe ecological threats because of their toxicity, long-range transport capability, bioaccumulation potential, and persistent nature (Futai et al. 2008; Chatterjee et al. 2019).

During past few decades, the application of microorganisms for pesticide remediation has acquired substantial attention of ecologists, which eventually led to the discovery of vast array of bacteria and fungi that are directly or indirectly involved in pesticide degradation, under natural as well as laboratory conditions (Huang et al. 2007; Chatterjee et al. 2019; Singh et al. 2019).

The term mycorrhiza (Greek; “mykós”: “Fungus”, “riza”: “Root”) that literally signify “Fungus Root”, is a type of symbiotic association involving fungi and plant roots (Sharma et al. 2007; Itoo and Reshi 2013; Kumar and Atri 2017). This term was originally introduced in 1885 by a renowned forest pathologist namely Albert Bernard Frank, in his pioneer study on soil-microbial–plant interactions (Frank 1885). Since 1885, lot of work has been carried out in this arena. Mycorrhizal fungi are known to play a pivotal role in *self-sustenance* of dynamic ecosystems (Quoreshi

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2008; Kumar and Atri 2017). Based on the kind of fungi–plant symbiosis, colonization type and their morphology, mycorrhizae have been categorized into seven types, viz., (a) Ectomycorrhiza, (b) Endomycorrhiza, (c) Ectendomycorrhiza, (d) Ericaceous, (f) Arbutoid, (g) Monotropoid, and (h) Orchidaceous (Brundrett 2004; Finlay 2008). Amongst these types, ectomycorrhizae are most widespread, diverse, and abundant (Smith and Read 1997; Allen et al. 2003; Siddiqui and Pichtel 2008).

Ectomycorrhiza (Greek; "ektos": "Outside, "mykós": "Fungus", "riza": "Root") is a type of obligatory symbiotic association between fungal symbionts and roots of specific plant taxa. Ectomycorrhizal (ECM) fungi represent a functional group of immense importance, that are typified and recognized by their intercellular nature (fungal hyphae do not penetrate their host cells), presence of Hartig net (extremely branched intercellular hyphal network between root epidermal as well as cortical cells) and mantle (dense sheath of fungal hyphae covering the tertiary roots) (Fig. 14.1), and in some cases by the formation of macroscopic sporocarps/aboveground fruiting bodies (Luoma et al. 1991; Agerer 1999; Itoo and Reshi 2013).

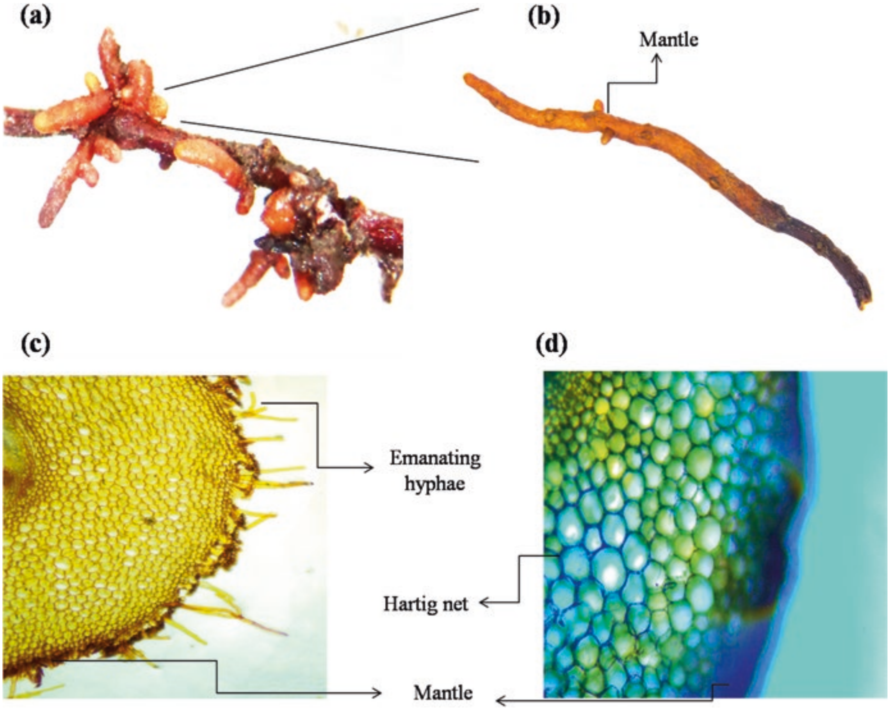

Fig. 14.1 (**a**) Association of ectomycorrhizal fungi with conifer roots (**b**) Morphological characteristics of ectomycorrhizal fungi (**c**, **d**) Hand-cut transverse sections of ectomycorrhizal root showing Emanating hyphae, Mantle, and Hartig net (stained with Trypan blue followed by destaining with lactic acid)

Predominantly, members of division Basidiomycota, some of Ascomycota, and a few members of Zygomycota are known to form ectomycorrhizal associations (Futai et al. 2008; Kumar and Atri 2017).

Worldwide, an estimated 25,000 species of fungi form ectomycorrhizal associations with most dominant woody plant families like Pinaceae, Fagaceae, Betulaceae, Salicaceae, Cistaceae, and Myrtaceae (Suz et al. 2008; Brundrett 2009; Kumar and Atri 2017). Plants belonging to these families, which are the major constituents of temperate, boreal, subtropical and tropical forests, have been reported to form association with diverse ECM symbionts (Futai et al. 2008; Kumar and Atri 2017). Benefits that accrue to the host plants upon such mutualistic association include enhanced growth and survival under normal as well as stressful conditions, enhanced nutrient mobilization (mainly nitrogen and phosphorus) and uptake, increased competitive ability, protection against pathogens and environmental extremes, tolerance to drought and heavy metals, and enhanced seedling regeneration (Kumar and Atri 2017; Velmala et al. 2018). In return, the fungal symbiont benefit by receiving 30–60% of the net photosynthates produced by the host plant (Simard et al. 1997). This symbiotic association is assumed to have originated approximately 125 million years ago, independently from saprophytic fungi (Hibbett et al. 2000; Kumar and Atri 2017).

ECM fungi interconnect plants through below-ground colossal hyphal networks that facilitate inter-plant metabolite movements (Leake et al. 2004; Simard et al. 2012; Chatterjee et al. 2019; Domínguez-Núñez et al. 2019). This below-ground diverse mycorrhizal network that interconnects rootlets of trillions of plants has been named as "Common Mycorrhizal Network (CMN)", and is informally termed as "Wood-Wide-Web" (Martin et al. 2016).

ECM fungi are considered as potential indicators of changes in environmental quality, owing to their specialized-sensitive lifestyle, ubiquity, key stance at soil–root interface, and vital ecological role (Heijden and Sanders 2003; Jiang and Yanbin 2018; Domínguez-Núñez et al. 2019), thus are of great significance in global change biology studies (Heijden and Sanders 2003). ECM fungi, as ecosystem engineers, contribute significantly to ecosystem responses to global environmental changes and help in restoration of degraded ecosystems (Chatterjee et al. 2019).

This chapter provides an overview of ectomycorrhizal ecology, mycorrhizoremediation of pesticides, fungal enzymes entailed in remediation, role of ectomycorrhizal biotechnology in pesticide remediation, and plant-pollutant-mycorrhiza interactions with reference to climate change.

14.2 Role of Ectomycorrhizal Biotechnology in Pesticide Remediation

14.2.1 Mycorrhizoremediation of Pesticides

Use of root-associated mycorrhizal fungi in remediation ventures is designated as mycorrhizoremediation (Kumar 2017). Formerly, the role of root-associated ECM fungi in remediation of pollutants was scarcely premeditated due to their imperceptible below-ground nature (Futai et al. 2008). However, from past few years, endeavours pertaining to application of this intriguing symbiotic association for site remediation are acquiring more attention from global scientific community (Siddiqui and Pichtel 2008; Domínguez-Núñez et al. 2019). Furthermore, several workers have reported that other root-associated microbes function in synergy with the ectomycorrhizal fungi during degradation of range of recalcitrant contaminants (Sarand et al. 1998, 1999; Heinonsalo et al. 2000; Meharg and Cairney 2000).

Currently, mycorrhizoremediation technology is being widely employed for reclamation of polluted ecosystems. Various ECM fungi have been found serviceable for the biodegradation of perilous pesticides (Table 14.1). For instance, pesticide remediation potential of range of ECM fungi belonging to genera *Agaricus*, *Agrocybe*, *Amanita*, *Boletus*, *Cordyceps*, *Gomphidius*, *Hygrophorus*, *Hypholoma*, *Laccaria*, *Leccinum*, *Lepiota*, *Lepis*, *Paxillus*, *Pleurotus*, *Psalliota*, *Radiigera*, *Rhizopogon*, *Russula*, *Suillus*, *Trametes*, and *Trichoderma* has been confirmed (Donnelly and Fletcher 1995; Meharg et al. 1997a, b; Braun-Lullemann et al. 1999; Meharg and Cairney 2000; Huang et al. 2007; Kulshreshtha et al. 2014; Rhodes 2015; Deshmukh et al. 2016).

Meharg and Cairney (2000) reported 33 ectomycorrhizal species possessing pesticide degradation potential. Fourteen ECM fungi capable of degrading polychlorinated biphenyl (PCB) congeners were reported by Donnelly and Fletcher (1995). These ECM fungi bring about pesticide degradation via their ectomycorrhizospheric effects (Meharg and Cairney 2000). In ectomycorrhizosphere, pesticides are incorporated into fungal biomass via mycosorption; wherein these perilous chemicals are either subjected to mycodegradation or relocated to above-ground plant parts (Donnelly et al. 1993; Meharg et al. 1997a; Schnabel and White 2001; Dittmann et al. 2002; Huang et al. 2007; Bücking 2011).

Comprehensive study of mycorrhizosphere processes using modern molecular approaches further widens the applicability of this symbiotic association. Until now, mycorrhizoremediation potential of only a diminutive fraction of ectomycorrhizal fungi has been documented and further studies certainly warrant discovery of scores of novel proficient mycorrhizoremediators.

Table 14.1 Overview of role of ectomycorrhizal fungi in pesticide remediation

S. No.	Type of pesticide	Ectomycorrhizal fungi	References
1.	Atrazine	*Agrocybe semiorbicularis* *Auricularia auricula* *Coriolus versicolor* *Dichomitus squalens* *Flammulina velutipes* *Hypholoma fasciculare* *Phanerochaete velutina* *Pleurotus ostreatus* *Stereum hirsutum*	Meharg and Cairney (2000) Bending et al. (2002)
2.	Benzo[a]anthracene Benzo[a]pyrene Benzo[ghi]perylene Benzo[k]fluoranthene	*Pleurotus ostreatus* *Trametes versicolor* *Trichoderma* sp.	Baldrian (2008)
3.	Benzo[α]pyrene	*Amanita excelsa* *Leccinum versipelle* *Suillus grevillei* *Suillus luteus* *Suillus variegatus*	Braun-Lullemann et al. (1999)
4.	3-chlorobenzoic acid (3-CBA)	*Suillus bovinus*	Dittmann et al. (2002)
5.	Chrysene	*Pleurotus ostreatus* *Trametes versicolor* *Trichoderma* sp.	Baldrian (2008)
6.	Dichlorodiphenyltrichloroethane (DDT)	*Boletus edulis* *Gomphidius viscidus* *Laccaria bicolor* *Leccinum scabrum*	Huang et al. (2007)
7.	2,4-dichlorophenol (2-4-D)	*Paxillus involutus* *Suillus variegatus*	Meharg et al. (1997a) Meharg and Cairney (2000)
8.	Dieldrin and endrin	*Cordyceps brongniartii* *Cordyceps militaris*	Xiao and Kondo (2013)
9.	Diuron	*Agrocybe semiorbicularis* *Auricularia auricula* *Coriolus versicolor* *Dichomitus squalens* *Flammulina velutipes* *Hypholoma fasciculare* *Phanerochaete velutina* *Pleurotus ostreatus* *Stereum hirsutum*	Bending et al. (2002)

(continued)

Table 14.1 (continued)

S. No.	Type of pesticide	Ectomycorrhizal fungi	References
10.	Metalaxyl	*Agrocybe semiorbicularis* *Auricularia auricula* *Coriolus versicolor* *Dichomitus squalens* *Flammulina velutipes* *Hypholoma fasciculare* *Phanerochaete velutina* *Pleurotus ostreatus* *Stereum hirsutum*	Bending et al. (2002)
11.	Polychlorinated biphenyl (PCB)	*Radiigera atrogleba*	Donnelly and Fletcher (1995)
12.	Terbuthylazine	*Agrocybe semiorbicularis* *Auricularia auricula* *Coriolus versicolor* *Dichomitus squalens* *Flammulina velutipes* *Hypholoma fasciculare* *Phanerochaete velutina* *Pleurotus ostreatus* *Stereum hirsutum*	Bending et al. (2002)
13.	Toluene	*Suillus bovinus*	Sarand et al. (1999)
14.	2,4,5-trichlorophenol	*Clitocybe maxima*	Zhou et al. (2015)
15.	2,4,6-trinitrotoluene	*Suillus variegatus*	Meharg et al. (1997b)

14.2.2 Fungal Enzymes Involved in Pesticide Remediation

Mycorrhizoremediation operates through enzymatic conversion of noxious pesticides into innocuous residues (Singh et al. 2019). Soon after the mycosorption and bioaccumulation of pesticides in ECM fungal mycelium, a range of intracellular and extracellular fungal enzymes act and bring about mycodegradation of these detrimental chemicals (Spina et al. 2018). ECM fungi produce variety of non-specific intracellular and extracellular enzymes, viz., catalases, cellulases, chitinases, cytochrome P450 monooxygenases, hemicellulase, laccases, ligninases, lignocellulases, pectinases, peroxidases, oxidases, transferases, tyrosinases, and xylanases, which act synergistically as pesticide decontaminating agents either by chemical conversion or by affecting chemical bio-accessibility (Read and Perez-Moreno 2003; Huang et al. 2007; Ulčnik et al. 2013; Kulshreshtha et al. 2014; Deshmukh et al. 2016; Kumar 2017; Prakash 2017; Spina et al. 2018; Domínguez-Núñez et al. 2019; Singh et al. 2019, 2020; Yadav 2019). These enzymes possess low substrate specificity, thus can act upon array of recalcitrant pollutants with structural similarities, which makes these fungi excellent tools for degradation of variety of pollutants.

Several workers have postulated the detailed pathways of pesticide mycorrhizoremediation (See Donnelly and Fletcher 1995; Meharg et al. 1997a, b; Meharg and Cairney 2000; Huang et al. 2007; Deshmukh et al. 2016). Furthermore, different aspects of mycorrhizoremediation like fungal enzymes involved and detailed degradative pathways can be accessed from various databases such as MetaCyc database (MetaCyc.org) (Caspi et al. 2014).

14.2.3 Role of Ectomycorrhizal Biotechnology in Pesticide Remediation

Ectomycorrhizal biotechnology engrosses application of subsist ECM fungal strains or their prominent fungal enzymes, via contemporary biotechnological approaches, for sustainable remediation and recuperation of gravely disturbed environs (Quoreshi 2008; Yadav 2019). With rapid development in the arenas of ectomycorrhizal ecology and biotechnology, over the past few decades, the trend of using ectomycorrhizal fungi in environmental cleanup by employing various biotechnological methods is currently escalating fast. Various approaches in application of ectomycorrhizal biotechnology for remediation of pesticide contaminated environments are discussed in the subsequent sections and are outlined in Fig. 14.2.

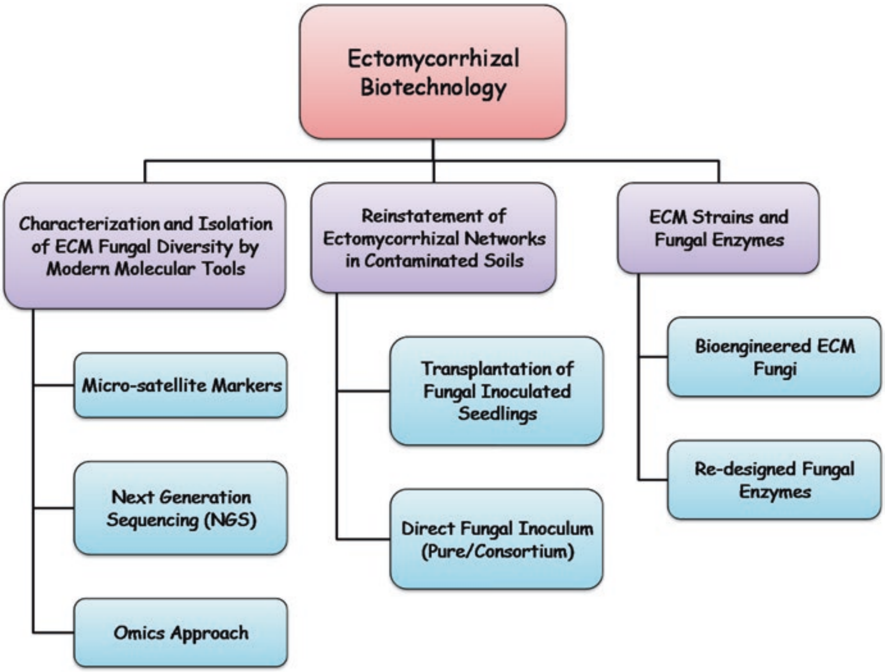

Fig. 14.2 Approaches in ectomycorrhizal biotechnology

(a) Characterization and isolation of ECM fungal diversity by modern molecular tools

The ecological functions and degree of functional specificity of ECM fungi are poorly studied, primarily because of their enormous diversity, cryptic nature, and methodological limitations to the accurate identification of these mutualists. Formerly, the characterization of ECM fungal diversity was largely carried out through conventional sporocarp analysis, morphological observations, and culture-based techniques (Heijden and Sanders 2003), which have several intrinsic limitations. However, with the recent development of molecular techniques, substantial progress has been made in the field of fungal ecology and diversity. These modern molecular methods serve as powerful tools in direct characterization of fungal symbionts in environmental samples (samples with concoction of different taxa), with an elevated degree of resolution (Bridge and Spooner 2001).

DNA sequencing of environmental samples revolutionized the field of ectomycorrhizal ecology and provided novel insights related to below-ground fungal diversity (Kumar and Atri 2017; Jiang and Yanbin 2018). The recent advancements in next generation sequencing (NGS), particularly the development of omics approach (metagenomics, metatranscriptomics, metaproteomics, and metabolomics), have made exceptional contribution to our understanding of ECM fungal diversity, ecology, and biogeography (Nilsson et al. 2011; Smith and Peay 2014). With the advent of these novel robust technologies, further exploration of ectomycorrhizal networks and their sophisticated interactions with other organisms becomes attainable (Deveau et al. 2016).

(b) Reinstatement of ectomycorrhizal networks in contaminated soils

Another eminent remediation approach in ectomycorrhizal biotechnology is reinstatement of ectomycorrhizal networks in contaminated soils. This is accomplished through production and subsequent transplantation of fungal inoculated seedlings (inoculated with appropriate individual/consortium of ECM fungi) to the site of contamination (Quoreshi 2008; Yadav 2019). Or else, direct fungal inoculum can be also used for reinstallation of soil ectomycorrhizal networks (Quoreshi 2008). ECM inoculation enhances seedling growth and survival (Itoo and Reshi 2014; Yadav 2019). This approach proves beneficial for reclamation of disturbed ecosystems (Danielson and Visser 1989; Marx 1991; Quoreshi 2008).

The modern technological tools such as micro-satellite markers and NGS techniques function as potential markers for monitoring the introduction, functioning, and persistence of individual ECM fungal strains at the remediation site (Quoreshi 2008).

(c) Bioengineered ECM fungi and re-designed fungal products

In ectomycorrhizal biotechnology, use of genetically modified ECM fungal strains and re-designed fungal products is one of the important methods, employed for the remediation of recalcitrant pesticide residues (Singh et al. 2019). In several orders of ectomycorrhizal fungi, genes encoding for different enzymes involved in remediation have already been recognized (Ramesh et al. 2008). With recent advances in recombinant DNA technology and biomolecular engineering, the remediation of persistent pollutants employing bioengineered ECM fungi and redesigned fungal enzymes (enzymes with enhanced expression level, substrate specificity, and activity) is a budding area of environmental research (Singh et al. 2019; Yadav 2019).

14.3 Plant-Pollutant-Mycorrhiza Interactions

14.3.1 Influence of Ectomycorrhizal Fungi on Plant Phytoremediation Potential

Ectomycorrhizal fungi possess the capability to survive and thrive under extreme conditions like in contaminated, disturbed, and degraded ecosystems (Colpaert et al. 2011). Furthermore, these myco-symbionts also improve host plant growth, mediate host plant performance, enhance stress tolerance of host plants, and augment host plant phytoremediation ability under such circumstances (Quoreshi 2008; Gil-Martínez et al. 2018; Domínguez-Núñez et al. 2019). Ectomycorrhizae, in integration with other soil microbes, assist host plant mediated degradation and removal of range of persistent pollutants (Meharg and Cairney 2000; Sharma et al. 2007; McGuinness and Dowling 2009; Weyens et al. 2009; Glick 2010; Domínguez-Núñez et al. 2019). However, the community composition of ectomycorrhizal fungi has been reported to significantly influence host plant phytoremediation potential (Gil-Martínez et al. 2018).

Even though, mutual remediation role of merely few fungus-plant combos has been studied hitherto, yet, ECM mediated role of mycorrhizal plants in swift reclamation of degraded ecosystems is intriguing and promising.

14.3.2 Effect of Climate Change on Plant-Pollutant-Mycorrhiza Interactions

Climate change (change in provisos of climatic variables like temperature, precipitation, solar radiations, wind speed, atmospheric pressure, etc., that persevere for an extended time), ensuing predominantly from the detrimental anthropogenic activities (for instance, anthropogenic emanation of greenhouse gases, land use alterations), in concert with some environmental catastrophes (e.g., volcanic eruptions), are resulting in major alterations in global species distribution and ecosystem functions (Solomon et al. 2007; Rummukainen et al. 2010; Intergovernmental Panel on Climate Change (IPCC) 2014). The plant-pollutant-mycorrhiza interactions are multifaceted (Siddiqui and Pichtel 2008), and are significantly affected by rapidly changing climate, either directly or via indirect means (Abhilash et al. 2013). This amendment in these interactions will consecutively influence collective Phyto-Myco-remediation of pollutants.

Climate variability affects environmental behaviour, leaching power, volatilization, environmental emission, mobility, transport, distribution, degradation, and fate of chemical pollutants predominantly of pesticides, to an enormous extent (Lamon et al. 2009a, b; Teran et al. 2012; Tripathia et al. 2015). Various regional and global scale models have been developed and applied for the study of impact of climatic variability on the fate, transport, and spatial/temporal distribution of chemical pesticides. For instance, (a) Eulerian multimedia fate and transport models (Gusev et al. 2005; Semeena et al. 2006; Lammel et al. 2009; Lamon et al. 2012), (b) Global Circulation Models (GCMs) (Stemmler and Lammel 2011). These models amalgamate entire available information of chemical pesticides like emission details, chemical characteristics, and environmental interaction patterns and provide a key module for understanding their environmental dynamics (Teran et al. 2012).

Temperature is one of the key climatic drivers that determine global distribution of pesticides due to its direct impact on pesticide emission, partitioning, deposition, volatilization, half-life, and degradation (Gouin and Wania 2007; Lamon et al. 2009a, b, 2012; TF-HTAP 2010). With increase in global mean temperature, the volatilization/re-volatilization potential and long-distance transport ability of pesticides can augment considerably, which will subsequently lead to effluence of remote pristine ecosystems (Hung et al. 2005; Armitage et al. 2011; UNEP/AMAP 2011; Teran et al. 2012; Tripathi et al. 2015, 2019). However, warming climate diminishes the remediation potential of plant-mycorrhiza remediation systems by restricting the profuse pollutant availability and uptake (Tripathia et al. 2015).

Climate fluctuations in terms of elevated CO_2 concentration will not only augment plant productivity but will also enhance production of root exudates and rhizospheric microbial activity (Phillips et al. 2009; Abhilash and Dubey 2014; Abhilash et al. 2015), which may possibly intensify phytodegradation/mycodeterioration of concoction of pollutants (Kim and Kang 2010; Tripathia et al. 2015). Furthermore, warming climate can affect soil structure, alter composition of soil microbial community, and influence interactions between plant and root-associated myco-symbionts (Castro et al. 2009), which will subsequently either foster or interrupt the mutual remediation approach. The comprehensive scheme through which climate change affects plant-pollutant-mycorrhiza interactions and ensuing remediation processes is still ambiguous. So, further elaborate studies are prerequisite in order to understand the plant-pollutant-mycorrhiza interactions under changing climate scenario.

14.4 Conclusions

Application of ectomycorrhizal fungi for pesticide remediation is an efficient and sustainable environmental decontamination strategy. Pesticide mycorrhizoremediation potential of several ECM fungi belonging to genera *Amanita*, *Boletus*, *Cordyceps*, *Gomphidius*, *Hygrophorus*, *Hypholoma*, *Laccaria*, *Leccinum*, *Lepiota*, *Lepis*, *Paxillus*, *Psalliota*, *Radiigera*, *Rhizopogon*, *Russula*, and *Suillus* has been confirmed. These fungi produce variety of enzymes, viz., catalases, cellulases, chitinases, cytochrome P450 monooxygenases, hemicellulase, laccases, ligninases, lignocellulases, pectinases, peroxidases, oxidases, transferases, tyrosinases, and xylanases, which act synergistically as pesticide decontaminating agents. Although, mycorrhizoremediation potential of only a diminutive fraction of ectomycorrhizal fungi has been documented hitherto. Further studies certainly warrant discovery of scores of novel proficient mycorrhizoremediators.

With rapid development in the arenas of ectomycorrhizal ecology and biotechnology, the trend of using ectomycorrhizal fungi in environmental cleanup by employing various biotechnological methods is currently escalating fast. In ectomycorrhizal biotechnology, reinstatement of ectomycorrhizal networks in contaminated soils is an eminent remediation approach and the modern technological tools such as NGS techniques function as potential markers for monitoring the introduction, functioning, and persistence of individual ECM fungal strains at the remediation site. Moreover, the remediation of persistent pollutants employing bioengineered ECM fungi and re-designed fungal enzymes is a budding area of environmental research.

Ectomycorrhizae, in integration with other soil microbes, assist host plant mediated degradation and removal of range of persistent pollutants. Changing climate significantly affect these multifaceted plant-pollutant-mycorrhiza interactions. Climate variability affects environmental behaviour, transport, and fate of pesticides and can lead to effluence of remote pristine ecosystems. The comprehensive scheme through which climate change affects plant-pollutant-mycorrhiza interactions and ensuing remediation processes is still ambiguous. So, further elaborate studies are must to understand the plant-pollutant-mycorrhiza interactions under changing climate scenario.

Further in-depth research of ectomycorrhizal biochemistry, ecology, molecular genetics, and physiology is crucial to ascertain the molecular mechanism of pesticide mycorrhizoremediation.

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Chapter 15
Biosensors: A Biotechnological Tool for Monitoring Environmental Pollution

Rohaya Ali and Dig Vijay Singh

15.1 Introduction

A biosensor is an analytical and self-sufficient integral device that generates specific, quantitative, and systematic knowledge by means of a biological material (biochemical receptor) which is directly connected to a transduction device. The biological material is fixed by means of conventional methods like covalent or non-covalent binding and physical or membrane entrapment (Olson and Bae 2019). A contact is made between the bioreceptor and the transducer. The analyte binds to the bioreceptor to form a bound analyte which in turn generates an electronic signal that can be calculated. At times the analyte is changed to a product which can be coupled with the release of heat, gas, or any charged species. The transducer then transforms the product related changes into electrical signals that can be amplified and then calculated by means of an electronic method (Dhewa 2015).

Earlier many biosensors have been developed using a range of combination of bioreceptors and transducers (Xu et al. 2019). Biosensors can be categorized according to bioreceptors (enzymes, microbial cells, or DNA) or transducer (thermal, visual, mass based) used. Initially biosensor was invented by Clark and Lyons (1962). It was used to determine glucose level in biological items. For this they used electrochemical recognition method of oxygen and hydrogen peroxide via immobilized glucose oxidase electrode (Fig. 15.1). After that, there has been an implausible development in biosensors with pioneering approaches related nanotechnology, bioelectronics, and electrochemistry.

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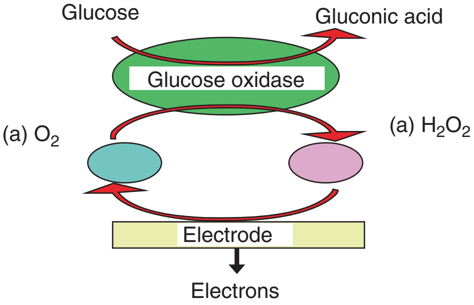

Fig. 15.1 Electrochemical detection of oxygen or hydrogen peroxide using immobilized glucose

Biosensors serve an extensive field with a colossal effect on health system, agriculture, and environmental quality management. In current times, one of the main troubles we are facing is increase in the environmental pollution that is threatening to human lives. Environmental pollution is mainly of five basic types which include water, soil, air, noise, and light pollution. Among these water and soil badly affected due to growing industrialization and urbanization (Antikainen et al. 2008). Soil and water are significantly polluted by unprocessed industrial and agricultural dissipates, including dioxins, heavy metals, pesticides, or herbicides (Kawamura et al. 2005). In addition, anthropological activities have resulted in pollution of water bodies with biological/organic micropollutants (Barbulescu et al. 2018). These pollutants like viruses, bacteria, protozoa, and mycoplasma are the cause of numerous illnesses and serve as a chief cause of death globally. These compounds can badly influence the ecosystem. Majority of these pollutants can mount up and endure for a long time in the environment. This is on account of their non-degradable character. Besides, they can also pierce into the food chain. Consequently, recognition and monitoring of such environmental pollutants are vital for the overall maintenance environment and hence biological system. Hence, ecological safety is one of the chief concerns for the security of living beings. Various initiatives like governmental, scientific, and social actions have been taken up to manage and control the intimidation of environmental contamination, but it still remains a global challenge (Sang et al. 2015)

Conventional techniques like chromatography and spectroscopy consume a lot of time and necessitate a lot of know-how besides being costly. For this reason, there is a growing need of uncomplicated, fast, precise, perceptive, and convenient method for determining environmental security threats. Thus, to surmount limitations of the conventional method, a complementary method is being established wherein living systems are utilized for assessment of various environmental samples. A variety of living systems such as bacteria, fungi, enzymes, etc. are utilized for such analyses. This is attributed to their fast growth rate, huge population size, and least prerequisite for their growth and development (Biran et al. 2000). Besides, the bacterial systems can be genetically engineered to scrutinize environmental contamination. Moreover, proteins present within the cells could also be used for the recognition of a particular analyte. Biological systems producing enzymes or isolated biocatalysts/enzymes in the form of whole cells or as immobilized state could be utilized as source for recognition, measurement, and dilapidation of pollutants. Besides, they can be used for transformation of pollutants to non-polluting compounds to restore the normal environmental equilibrium. Biosensors are therefore ideal tool for the recognition and quantification of environmental pollution in a consistent, explicit, and reliable way (Scott et al. 1997).

15.2 Basic Concept of Biosensor

Biosensor is a versatile and portable analytical tool which comprises of a sensing element (biochemical receptor) of biological origin and a physico-chemical transducer (Senveli and Tigli 2013). Biosensor is principally composed of three main parts, viz. a biological recognition element, a transducer, and a signal processing system (Fig. 15.2). As per to the International Union of Pure and Applied Chemistry (IUPAC), a biosensor is a self-controlled integrated tool that is able of generating a precise quantitative and systematic information by means of a biological recognition element. Biosensors are gaining a lot of popularity on account of their sensitivity and selectivity (Abe et al. 2014). As such, field of biosensors is expanding very fast than current traditional sensors. Two important parts of biosensors are a biological recognition system and a transducer which are discussed below:

15.2.1 *Biological Recognition System/Biomaterials*

Biomaterials are utilized in sensing the presence of a substance along with its concentration. The most widespread biological materials used are enzymes, receptors, antibodies, DNA, and complete/whole cells (Fig. 15.3). Enzymes produce sensitivity to a specific reaction through their catalytic effect when bound to a particular substrate. They can be utilized in unmodified form or may be obtainable in microbes

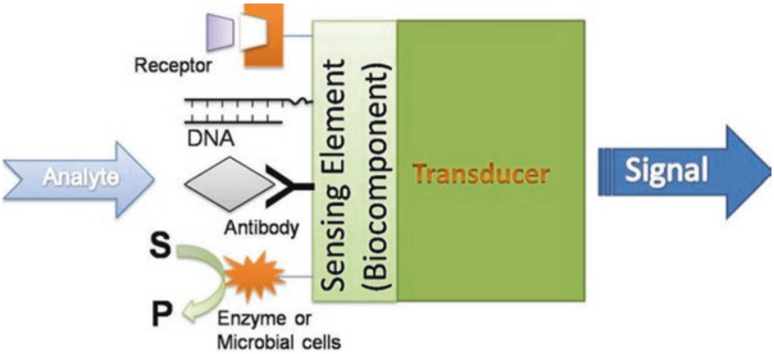

Fig. 15.2 Schematic representation of biosensor

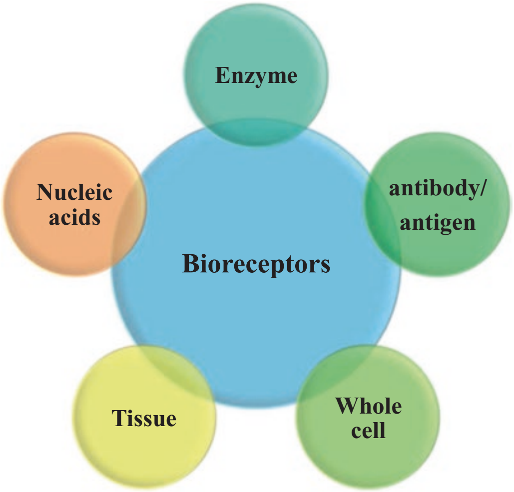

Fig. 15.3 Bioreceptors/biological recognition agents for development of biosensor transducers

or even in the form of slices of unharmed tissues. On the other hand, antibodies are skillfully combined with a substrate to offer a signal for a transducer to sense. In contrast to enzymes, antibodies lack catalytic effect and therefore could be bound to an analogous antigen to eliminate it from the sphere of activity. Sensitivity of biosensors is enhanced greatly when antibodies are utilized as biological agents. Likewise nucleic acids can also be used in biosensors. They can react to specific substrate owing to their unique property of base pairing. They can be used in diagnosis of various genetic disorders. Similarly proteins like receptors which have a unique molecular recognition pattern can also be used in bio-sensing (Dias et al. 2014). Although they are housed in plasma membrane and are difficult to isolate, but their affinity and specificity are similar to those of antibodies when used as biomaterials. Complete or whole cells are outstanding sensors for assessment of noxious waste. Numerous biosensors incorporating entire microbial organism as the sensing element have been made for the speedy and remarkable detection of heavy metals like lead, cadmium, etc. Besides they can also be used to detect the inorganic pollutants (Dickinson and Pulford 2005).

15.2.2 Transducer

Transducer is regarded as the chief stage of a biosensor development. It converts a physical change caused by a reaction into a suitable signal for dispensation in a succeeding phase. Biomaterials are either be linked to or incorporated within a transducer. They can be either bound physically or chemically to a transducer. This phase is dependent on the immobilization technique required to link the biological substance on transducer. Immobilization techniques comprise of cross linking, adsorption, covalent attachment, microencapsulation, inclusion, entrapment, etc. Among the numerous types of accessible transducers are: calorimetric, optical/visual, potentiometric, aural, conductometric, and piezoelectric transducers (Turner 2013).

15.3 Classification of Biosensors

Biosensors are categorized on the basis of type of bioreceptors (nucleic acids, enzymes, antibodies, proteins, etc.) involved in the detection or on the basis of physico-chemical property of transducers (electrochemical, visual, thermal, aural, piezo-electrical, and calorimetric) used for the detection of toxicants (Verma and Bhardwaj 2015). In making of these sensors, the major difficulty that we face is at the time of integration of biological element with the transducers. An appropriate choice of bioreceptor molecule, an appropriate immobilization method, and proper selection of a specific transducer, and lastly packaging in a portable form are important steps in the formation of biosensors. The various kinds of biosensors and their working are discussed below:

15.3.1 Optical Biosensors

Environmental monitoring requires the development of fast, trouble-free, and extremely responsive biosensors. This is possible by means of optical biosensors with immobilizers like silica, quartz, glass or gold, and carbon-based materials. The assimilation of quantum dots or gold nanoparticles with the employment of microfabrication offers novel machinery for the development of extremely responsive optical biosensors for specific purposes. Moreover, fiber optic chemical sensors have huge significance in a variety of fields like drug discovery, bio-sensing, and biomedicine. Furthermore, DNA based sensors like hydrogels are budding resources for immobilization in fiber optic chemistry (Xia et al. 2015). Hydrogels occur in three-dimensional (3D) forms that offer better loading capability of sensor molecules. Hydrophilic polyacrylamide gels are cross linked polymers that can be converted to various shapes for immobilization which range from emaciated sheets to nanoparticles. They are regarded as trouble-free substrates for DNA immobilization that offer wider advantages like proper entrapment, DNA defense, analyte augmentation, etc. These features are distinctive only to hydrogels in comparison to other biomaterials. In addition, fine optical transparency of hydrogels provides suitable approach for visual detection. Comprehensive techniques for immobilizing DNA based biosensors in gel microparticles and mono-lithic polyacrylamide are often regarded as technological improvement in the field of biosensors. Besides, single molecule detection method has also been formulated by means of electrochemical oxidation of hydrazine for DNA recognition (Kwon and Bard 2012).

15.3.2 Electrochemical Biosensors

The first line of discovery of electrochemical biosensors started with the discovery of glucose oxidase based biosensor. Glucose based biosensors are extensively famous in medical science as they are crucial for diabetic patients when the periodic assessment of blood glucose levels is done. Nevertheless, these biosensors also have certain shortcomings due to their instable enzyme action which requires additional calibration. These shortcomings have paved way for discovery of wide variety of biomolecules having differential electrochemistry that could be used for generating suitable and viable biosensors. Of late, these biosensors are characteristically produced by manipulating the surface of metal and carbon electrode using biological elements such as nucleic acids, enzyme, antibody, etc. (Orozco et al. 2016). Fascinatingly, a variety of biomolecules have differential electrode fidelity and specificity, which eventually adds up in the growth of novel types of electrochemical biosensors with number of uses. For example ferroceneboronic acid (FcBA) and its derivatives have been shaped into electrochemical biosensors. This is attributed to the presence of electrochemically active site (Fc moiety) and a binding site (boronic acid moiety) in them. FcBA bears an exclusive characteristic of binding to diol residues of saccharides leading to the generation of cyclic boronate esters. The redox ability of FcBA-sugar adduct varies from that of free boronic acids which forms the basis for electrochemical detection. Similarly, hydrocarbon chains present in the polypeptide chain of HbA1c can be measured using FcBA based electrochemical detection. Electrochemical biosensors used to evaluate the levels of reactive oxygen species (ROS) and their scavengers, i.e., antioxidants in various in vivo and in vitro systems are one more modern invention (Etzov et al. 2015). These techniques can be widely used in estimation of pollutants present in the environment and hence help in assessing environmental security (Bako et al. 2004). One more upgradation in biosensor technology is targeting at genetic levels. It is an established fact that expression of cellular miRNA is a model bio-marker for the analysis or diagnosis of commencement of diseases. Besides, various genetic disorders can be treated via gene therapy by means of miRNA. Generally, polymerase chain reaction, northern blotting, microarray, etc. are used for detection of miRNA. In current times, modern and innovative technologies provide perfect electrochemical biosensors for miRNA which involve label-free recognition of guanine oxidation subsequent to the hybrid formation of miRNA. All such discoveries are the result of contemporary approaches of bio-fabrication for efficient use of electrochemical biosensors in biomedicine, environmental pollution analyses, defense, etc. (Salvador and Marco 2016).

15.3.3 Nanomaterial Based Biosensors

Development of nanotechnology has proved of great use to in the establishment of nanoparticle based material like gold, silicon, copper, and carbon-based materials like graphite, graphene that are utilized for designing biosensor immobilizers (Rotariu et al. 2012). Besides they also offer immense specificity for designing electrochemical and other different kinds of biosensors. Among the nanoparticles, gold based nanoparticles offer best utility owing to their stability against oxidation and have almost zero toxicity. On the other hand, some nanoparticles like silver oxidate have noxious effect, if utilized internally in medication like in drug delivery (Zhang et al. 2016). Use of nanomaterials for biosensors has potential challenges, which needs to be addressed if used for biomedicine. In addition, nanoparticle based signal augmentation comprises of various advantages and disadvantages. Nonetheless, nanomaterials are regarded as crucial elements in bioanalysis as they offer better sensitivity and detection limits for single molecule recognition (Lu et al. 2015). Nanoparticles based on iron oxide and semiconductor quantum dots which possess magnetic as well as optical properties can be efficiently coupled with tumor targeting ligands like peptides, monoclonal antibodies, and other small molecules to target tumor antigens with huge affinity and specificity. Thus, nanoparticle based biosensors offer extensive application in various fields (Maduraiveeran and Jin 2017).

15.3.4 Silica, Quartz, and Glass Biosensors

Silica, quartz, and glass materials are extensively used in the development of modern biosensors due to their distinctive properties. Among these silica based nanoparticles are used widely owing to its bio-compatibility, profusion, electronic, visual, and mechanical properties. Besides, silicon nanomaterials are non-toxic which is vital precondition for biomedical and biological usage. Silicon based nanomaterials provide broader choice in bio-imaging, bio-sensing, and cancer treatment. In addition, fluorescent silicon nanomaterials have long-term usage in bio-imaging (Mishra et al. 2017). Fascinatingly, silicon nanowires along with gold nanoparticles generate hybrids that are utilized nano-reagents for successful treatment of cancer. For efficient hybridization and UV spectroscopy, DNA oligomers are attached covalently to DNA films via their thiol modified residues. Quartz crystal microbalance biosensors offer an additional platform for assessment of the interactions in between biomolecules with huge sensitivity (Yilmaz et al. 2015). Pulsations of quartz oscillators can be measured without any wire connections by means of antennas through electromagnetic waves. Such non-contacting technique is a key feature for generation of ultrahigh sensitive detection method for proteins in liquid utilizing quartz crystal biosensor based technology. Bearing in mind these exceptional features of quartz, glass, and silica, numerous novel biosensors have been designed with high-end technology for efficient use in biomedicine, food safety, environmental pollution analysis, etc. bearing in mind cost effectiveness and bio-safety (Justino et al. 2015).

15.3.5 Microbial Biosensors

New trends in bioremediation and environmental monitoring are to exploit pioneering techniques based on protein or genetic engineering methods in microbes with high sensitivity and specific signal outputs. Live cells possessing enzyme activity to degrade xenobiotic compounds offer huge use in bioremediation. Likewise, microbial fuel based biosensors have been designed which help in monitoring biochemical oxygen demand (BOD) and eliminating poisonous pollutants in the environment (Chen et al. 2017). Steps have been taken in order improve the performance and lessen the expenses with novel systemic methods to design self-powered engineered microbial biosensors. Such pioneering strategy will offer novel biosensors with elevated sensitivity and selectivity. Another field of microbial biosensors is their application in heavy metal and pesticidal recognition with high specificity and sensitivity. Eukaryotic microbes offer huge specificity to diverse toxins and have significance to advanced animals (Yoo et al. 2017). Interestingly, microbial biosensors have huge applications which range from energy production to environmental monitoring (Mittelmann et al. 2002).

15.3.6 Genetically Encoded Biosensors

Growth of tagged biosensors with genetically encoded or artificial fluorescence set forth a ground to know about the biological processes and other molecular pathways operating within the cell (Bidmanova et al. 2016). Of late, fluorescent biosensors have been designed to analyze motor proteins by means of single molecule detection using specific analyte concentration. Despite this advantage, the technique of probe detection is challenging task. Discovery of fluorescent proteins like green fluorescent protein offered many advantages in terms of efficiency and optical probe design. Till last decade, genetically programmed biosensors, targeting molecules associated with energy production, reactive oxygen species (ROS), and cAMP offered good understanding of mitochondrial physiology. The cGMP is a vital signaling molecule and also a drug target for cardiovascular system. Forster resonance energy transfer based biosensors have been designed to visualize cAMP and cGMP within the cells. Electrochemical, optical, and electromechanical biosensors have also been made for detecting miRNA much more proficiently than other molecular techniques. Bearing in mind the dawn of in vivo imaging with small molecule biosensors, an enhanced knowledge of cellular pathways and many other biomolecules like DNA, RNA, and miRNA have been recognized (Kohler et al. 2000). In times ahead, this field requires transformation in terms of whole genome approach utilizing enhanced optical based genetic biosensors. Optical or visual based biosensors have gained momentum in terms of usage and specificity when coupled to fluorescence and small molecules. The growth of biotechnological techniques has largely helped in producing such biosensors (Daunert et al. 2001).

15.4 Application of Biosensors in Monitoring Environmental Pollution

Biosensor technology offers an extensive area with focus on agriculture, food industry healthcare, and environment quality management. Research on biosensors is growing swiftly compared to their commercialization. This is because of their low cost and durability. Biosensor market is dominated by biomedical field and their worth for environmental monitoring has only been investigated in the last decade. At present, one of the important aspects of biosensors is their use in monitoring blood sugar in diabetic patients. It works in the electrochemical transduction procedure. Biosensors are easy, selective, and economical devices which can be used to monitor pathogens in food, water, etc., e.g., *E. coli* (McPartlin et al. 2017). Some thermophilic organisms can also be monitored using biosensors. Numerous other benefits have been studied and commercialized for forensics, environmental monitoring, water quality assessment, and warfare detection. On the whole, the main advantage of biosensors is their ability to determine enormously low amounts of pollutants besides monitoring molecular interactions at a cellular level.

Biosensors can utilized as ecological or environmental quality monitoring devices in evaluation of chemical, physical, or biological pollutants. Biosensors like apta-sensors, immunosensors, geno-sensors, and enzymatic biosensors have been used for the recognition of range of environmental pollutants. These utilize antibodies, aptamers, nucleic acids, and enzymes as detection materials, respectively (Sun et al. 2015). Several applications of biosensors are mentioned beneath for recognition and monitoring of a variety of pollutants like organic and inorganic pollutants, toxins, heavy metals, antibiotics, and contaminating microorganisms like bacteria (Fig. 15.4).

15.4.1 Heavy Metals

Human activity (Anthropogenic pollution) produces an enormous amount of compounds that are released into the surroundings without aforementioned knowledge of their toxicity on biological world. Heavy metals and metalloids such as cadmium, arsenic (As), lead (Pb), aluminum (Al), and mercury (Hg) are chief environmental pollutants, mainly in industrialized countries (Biran et al. 2000). Heavy metals are produced as a result of human activities like cement or mining factories, refineries,

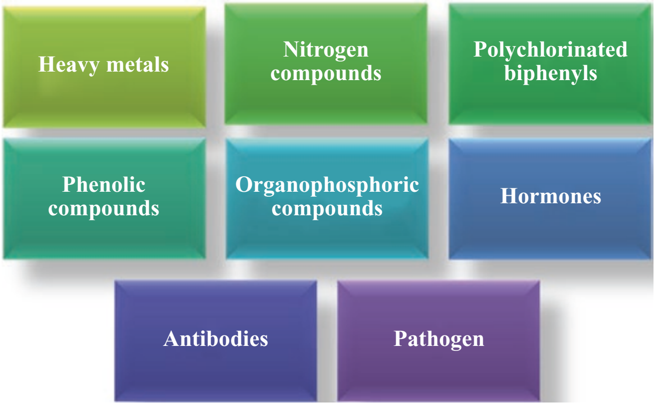

Fig. 15.4 Pollutants (organic or inorganic) that can be monitored by using biosensors

smelting plants, and heating systems. These metals are the basis of grave environmental contamination. They are well known for their soaring toxicity and biomagnification. Various bacterial biosensors have been shaped for the purpose of heavy metal determination in different variety of environmental samples (Guo et al. 2018). These utilize enzymes and DNA as bioreceptor and visual and electrochemical transduction systems. Optical biosensors have been produced to detect lead and cadmium by inhibiting alkaline phosphatase found on the outer membrane of *Chlorella vulgaris*, microalgae used as a bioreceptor (Long et al. 2013). One more instance for determination of cadmium pollution is through lacZ gene expression which is under the control of the cadmium responsive promoter zntA. This whole-cell biosensor can detect nanomolar amounts of cadmium in soil and water samples in lesser time. Apart from this biosensor can detect the presence of a variety of heavy metal like Hg, Zn, and Cu (Li et al. 2017).

The occurrence of heavy metals in the surroundings exerts a very strong selection pressure on the living beings existing in them. High rate of horizontal gene transfer and augmentation in their concentration can select heavy metal resistant microbes. Consequently, detoxification genes and resistance genes have been utilized as biomarkers for studying polluted and unhealthy environments using molecular techniques like polymerase chain reaction (quantitative PCR and real-time quantitative reverse transcription PCR). Among these genes are those involved in the resistance to arsenic (As), ACR3 (arsenite efflux pump), arsC (arsenate reductase), aioA (arsenite oxidase), and arsM (arsenic methyltransferase). In addition those that grant resistance to copper, cusA (copper export system) and copA (copper-exporting P-type ATPase) have also been utilized as biosensors. For cadmium, zinc, and cobalt resistance, czcA (Cd/Zn/Co efflux pump) is used. Similarly for mercury, hgcA (mercury methylating protein) and merA (mercuric reductase) biomarkers are used. Genes like mr and sodA that encode to metallothionein a cysteine-rich and sodA which codes for a superoxide dismutase find an immense use against toxicity of heavy metals (Wu et al. 2016). One more technique to detect the occurrence and abundance of genes involved in resistance to heavy metals by means of the microarrays such as the GeoChip. With the help of this chip it is possible to show a relationship between copper oxidase, alkylmercury lyase, cystathionine beta-lyase, and tellurite methyltransferase genes involved in detection of cadmium, copper, mercury, and sulfur in polluted water (Ko et al. 2013)

15.4.2 Biochemical Oxygen Demand

Biochemical oxygen demand (BOD) is an important factor in the estimation of the amount of biodegradable pollutant found in a water sample. Estimation of BOD of any sample is a time taking process. It takes around 5 days and is hence not suitable for online monitoring process. Quick estimation or monitoring is possible only by means of BOD biosensors. Recombinant cells of *Escherichia coli* with *Vibrio fischeri* gene lux AE based biosensor has been formulated for evaluating BOD. A BOD biosensor has been formulated by means of yeast cell bearing an oxygen probe that is helpful in detection of organic contamination/pollution in just 15 min (Bahadir and Sezginturk 2017). For efficient monitoring of water quality, an integrated smart meter has been formed. This system bears sensors linked to a cloud computing system for smart administration of a water network. Pasternak and his coauthors have reported a self-powered, autonomous BOD biosensor for online water quality assessment which works on signal frequency. The working and the schematic diagram of this sensor are presented in (Fig.15.5). This biosensor works in freshwater with open circuit situation. In presence of urine, the open circuit voltage of the sensor gets augmented. Under this condition energy management system (EMS) turns ON and visual alarm and audio signal are initiated. Thus, pollution of water with urine can be estimated using this sensor. Electro-active microbes are utilized to generate this biosensor. This is self-powered and could function autonomously for about 5 months (Kumar and Rani 2013).

15.4.3 Nitrogen Compounds

Nitrogen compounds like nitrates are extensively utilized in food factories to enhance shelf life and hence act as preservatives. Besides they are also utilized in fertilizer industries. The surplus uses of these compounds result in detrimental effects on wellbeing of humans and also contaminate the surface and groundwater (Petit et al. 2017). This in turn is toxic to aquatic environment. Nitrogen compounds decrease oxygen carrying capacity of hemoglobin by binding irreversibly with hemoglobin (Bari et al. 2015). In order to evaluate the amount of nitrogen based compounds in water, numerous biosensors have been formed. Amperometric based biosensor has been produced by using an enzyme (cytochrome c nitrate reductase) isolated from *Desulfovibrio desulfuricans* for estimation of nitrates. A new quick,

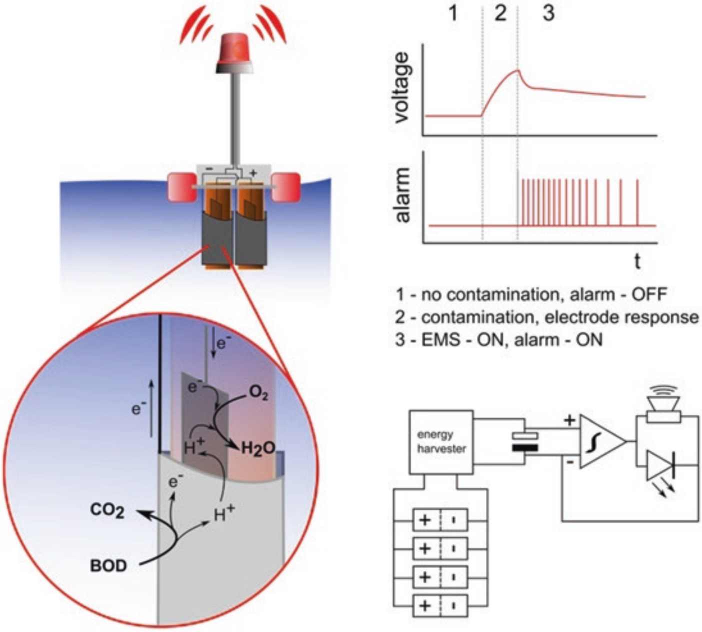

Fig. 15.5 Schematic representation of the BOD biosensor for estimation of water quality (adapted from Pasternak et al. 2017)

extremely responsive and stable enzymatic based biosensor has been developed for the assessment of nitrate in water. Thus, these biosensors aid in the estimation of water quality and hence can be utilized for estimation of environmental pollution (Bahadir and Sezginturk 2017).

15.4.4 Polychlorinated Biphenyls

PCBs are cosmopolitan poisonous organic compounds which have been barred in many countries due to their production long time ago. Many PCBs accumulate in food chain due to their lypophillic nature. Nucleic acid based biosensor with chronopotentiometric detection, immunosensors utilizing antibodies/antigens with fluorescence, and electrochemical sensors are used for estimation of PCBs in the environment. Thus, PCB based biosensors have huge potential in eradication of toxic organic compounds (Gruhl et al. 2013).

15.4.5 Phenolic Compounds

Phenolic compounds like chlorophenol are dispersed widely in the environment. They constitute a huge group of pollutants derived from various industries. These involve industries involved in manufacture of plastic, dyes, drugs, pesticides, etc. Phenolic compounds include various analytes which have huge importance in health care and environmental monitoring (Mauriz et al. 2007). For the determination of phenolic effluents, usually amperometric biosensor with enzyme (tyrosinase) as bioreceptor is used as biosensor. In addition, a flow injection chemiluminescence fiberoptic biosensor for finding of chlorophenols has also been developed. Catechol, a phenolic compound, which is quickly taken up and absorbed by the gastrointestinal tract, is highly toxic compound. It causes liver degeneration, malignancies, vasoconstriction, renal tube degeneration, and neurodegenerative disorders (Mayorga et al. 2014). Tyrosinase, a polyphenolic oxidase is involved in catalyzing the oxidation of phenols through hydroxylation with oxygen to catechol and later dehydrogenation to o-quinones. Therefore, tyrosinase has been employed for the designing of a biosensor for low potential detection of phenols and catechol in pharmaceutical, foods, and environmental samples (Mishra et al. 2017).

15.4.6 Organophosphorus Compounds

Organophosphorus compounds (OPCs) are the assembly of organic compounds which are utilized as insecticides, pesticides, or herbicides in contemporary farming to control vectors, pests, and weeds. Pesticides prevent, destroy, and repel the pests. These are extensively dispersed in water and soil. Persistence or perseverance of pesticides in the environment and their lethality is an alarming situation which needs to be tackled (Meng et al. 2013). Enzyme based sensors are widely utilized for recognition of these compounds. Besides, antibody based amperometric biosensors have also been formed for assessment of pesticides present in water. For estimation of herbicides like triazines and phenylureas, amperometric and visual transducers are employed. Polychlorinated compounds like dioxin are released as by-products in numerous chemical procedures like those related with chlorine. Dioxin is highly poisonous and carcinogenic agent. Besides, it has detrimental effect on the environment due to an inadvertent ingression in the food chain. For detection of these dioxins, surface plasmon resonance (SPR) biosensor has been designed (Guo et al. 2017).

15.4.7 Antibiotics

Antibiotics are widely used in the environment but they pose serious threat as they prop up antibiotic resistance. Prevalence of antimicrobial compounds also raises the issue of food safety as they can ingress in the food web. In past years, numerous types of biosensors have been designed to assess the quantity of antibiotics in various biological samples especially in food. A visual/optical immunobiosensor has been developed to determine sulfamethazine in animal urine. This in turn helps in assessment of sulfamethazine in their bodies and hence in the environment (Zhang et al. 2017).

15.4.8 Hormones

Rising human population and rigorous farming incessantly add up hormone residues both synthetic and natural in the environment (Dai and Liu 2017). Among these estrone, estradiol, and ethinylestradiol cause increased occurrence of cancers especially genital malignancies (Fan et al. 2018). In addition to this they also cause drop in human sperm counts. Hence, advanced automated optical immunosensors have been produced to detect presence of these hormones and other organic pollutants in environment. Thereby, maintaining environmental security (Singh et al. 2017).

15.4.9 Pathogens

Various diseases are transmitted via wastewater by means of a variety of disease causing agents, i.e., pathogens such as bacteria, viruses, fungi, and protozoa (Mittelmann et al. 2002). These pathogens are found in wastewaters polluted or untreated water. These pathogens impose serious threat to human health globally (Hui et al. 2007). Consequently, efficient monitoring of water supply is necessary to lessen the effect of the waterborne pathogens on human health. Novel techniques like biosensors have been engaged for swift detection and real-time monitoring of contaminating microbes. Several types of bio-devices including nucleic acids (DNA/RNA) and immune affinity (antibody/antigen) based sensors are utilized for rapid assessment of contaminating organisms and their toxins (biotoxins). For real-time monitoring of *Listeria monocytogenes, Legionella pneumophila,* and *Salmonella enteritidis* a surface plasmon based sensor has been developed (Neufeld et al. 2003). The occurrence of these pathogens in environment especially and in water could pose serious threat to human health, besides being detrimental to our environment. Of latest, recognition of bacterial RNA by the RNA detector probe immobilized on the biochip gold shell is gaining an impetus (Orozco et al. 2016).

15.5 Conclusion

Over the past years, biosensor technologies have been a budding research area. There has been a rapid growth in the improvement of biosensors for a range of applications especially in the areas of biomedicine, food, beverages, bio-defense, and environmental investigation (Oldach and Zhang 2014). Biosensors are perfect tools for environmental assessment or monitoring as they are highly sensitive, fast as well as reasonably priced. Though, in contrast to biopharmaceutical utility, biosensor techniques for environmental utility are still in their infancy and are highly challenging owing to their intrinsic characteristics of environmental analysis. Specificity for a particular analyte and low detection limit are key factors that govern the growth and use of biosensors in environmental analysis (Bahadir and Sezginturk 2017). Additional efforts are required in bridging innovations that will play a crucial role in the establishment of continuous, programmed, and real-time biosensors with lofty throughput scrutiny of environmental samples. Efforts are made for adopting critical detection techniques for environmental pollution analysis. It has become need of hour. Environmental monitoring requires portable, sensitive, precise, and highly accurate methods. In this pretext, biosensors are ideal tools to scrutinize the environmental pollution. Biosensors can sense or detect variety of pollutants like phenolics, antibiotics, pathogens pesticides, hormones, and toxins (Akki et al. 2015). At times, some biosensors do not generate precise information due to certain limitations like time consumption and interference by other compounds. Consequently, supplementary investigation and research are needed to perk up the ability of the biosensors in original samples at both field and farm levels. So, in this perspective, biosensors for impending environmental assessment need to be designed with focus on the technologies like genetic modification of enzymes and whole cell microbes. Besides genetic engineering techniques will also help in establishment of highly efficient biosensors. Moreover, to enhance the analytical ability, use of novel and highly selective bioreceptors and transducers is the need of hour. Such techniques will help in improvement of detection element immobilization and sensor interference

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