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 physio-chemical 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

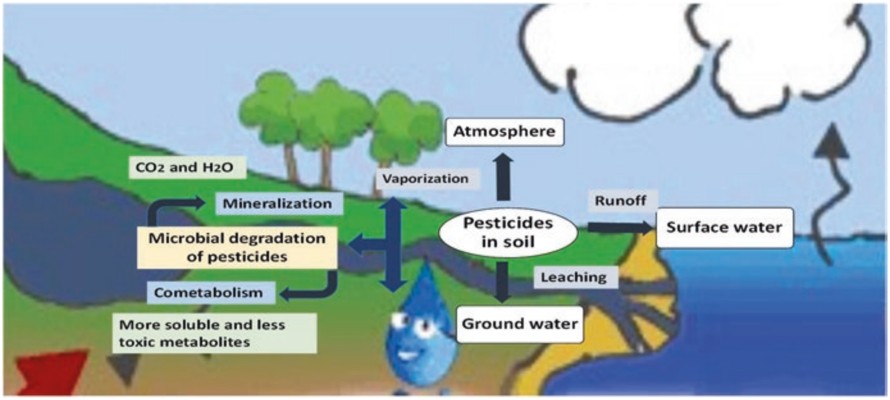

Fig. 7.1 Pesticides in the environment and their microbial degradation

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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