

## Review

## Microbial degradation of pesticide: A review

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Excessive use of pesticides has been known to be hazardous to the environment, affect soil fertility as well may impart toxicity in living beings. Presently there have been physical, chemical, biological and enzymatic approaches implicated to reduce pesticides. Although aimed to eradicate, physical and chemical methods are inefficient. Curiously, microbial pesticide remediation has been cost effective and thermodynamically more affordable, which may use any physical mater soiled with pesticide. Under favourable conditions microbes have been reported to use pesticides as source of carbon, sulphur and electron donor. Microbes; bacteria, actinomycetes and fungi have been found to help remove or detoxify chlorinated pesticides; polychlorinated diphenyl, polycyclic aromatic hydrocarbons, organophosphorus. Major bacterial genera includes; *Bacillus*, *Pseudomonas*, *Flavobacterium*, *Moraxalla*, *Acinetobacter*, *Arthrobacter*, *Paracoccus*, *Aerobacter*, *Alkaligens*, *Burkholderia* and *Sphingomonas*. Fungi with pesticide degradation potential includes; *Fusarium*, *Aspergillus niger*, *Penicillium*, *Lentinulaedodes*, *Lecanicillium*, *Oxysporum*. Among the Actinomycetes the *Streptomyces* have been found to successfully detoxify pesticides. Persistent organic pollutants in the form of pesticides have also been reported to be taken care by the microbial enzymes *viz-a-viz*; dehydrogenase, ligninase, oxygenase, peroxidises, phosphotriesterase, hydrolases, dehalogenase, laccase and organophosphorus acid anhydrolase. Microbial strategies and tools; enzymes and genes involved in pesticide catabolism are reviewed.

**Key words:** Chlorinated pesticides, organophosphors pesticides, bacteria, fungi, enzyme.

### INTRODUCTION

Pesticides are organic chemical purposely presumably intended for increasing agricultural yield, soil productivity, products quality, minimizing losses of agricultural products caused by crop peet and to control the insect vectors for prevention of the outbreak of human and animal epidemics. Increased use of the pesticide and herbicides in agriculture has also been implemented for

food storage. Recently, over 500 compounds are registered and used worldwide as pesticides or metabolites of pesticides. After World War II the use of pesticide in agriculture field has progressively increased leading to increased world food production. Amongst the South Asian countries of the total pesticide consumption, India is largest of pesticide consumer country which

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account for 3% of the world consumption for crop protection. The most commonly used pesticides in India include organophosphates, organochlorins, neonicotinoids etc. As per definition of ideal pesticide, a pesticide must be lethal to the targeted pests, but not to non-target species, including man but unfortunately, this is not so, hence the controversy of use and abuse of pesticides has come into the light. However, due to their unplanned and indiscriminate use, only 10% of applied pesticides reach the target organism and the remaining high percentage of it is deposited on non target areas such as soil, water, sediments and causes serious environmental pollution. Similarly they also causes impacts onto non target organism such as wild life, besides affecting public health. There are now overwhelming evidence of some of these chemicals that cause unwanted side effects to the environment and do pose potential risk humans and other life forms (Jeyaratnam, 1985; Igbedioh, 1991; Forget, 1993). According to the ICMR bulletin report about 1 million deaths and chronic illnesses are reported per year due pesticide poisoning world-wide. Because of the potential hazards to the nature and humans many of the pollutants which are toxic in nature were subsequently banned. Although their use has been discontinued from long time, these chemicals accumulate in soils and sediments where they can enter the food chain directly or percolate down to the water bodies. One of the features of modern agriculture for higher crop yield is the use of insecticides for controlling plant pests. After application of the insecticides by missing the 'target' they may reach the soil or run-off from stems and leaves (Omar, 1998). Most of the pesticides becomes persistent pollutant because of their relative stable nature, and most of the time the extreme toxic nature results in to the severe cases of pesticide poisoning, which is becoming the issue of concern nowadays. The environmental awareness has increased which resulted into development of regulatory measures that help to remediate past mistakes and protect the environment from future contamination and exploitation (Frazer, 2000). The overview of the present scenario of hazardous compounds needs the promising tools for remediation of these hazardous compounds from the environment. Nowadays the governments and higher authorities of different countries are taking firm steps towards these critical issues. For this reason, it is necessary to generate strategies for the bioremediation of polluted sites and waste treatment. From an economical and environmental point of view, the biological treatment is an important technology. Recently, the use of native or genetically modified organism to degrade or remove pesticides has emerged as a powerful technology for *in situ* remediation. There are many investigation reports of different organisms like bacteria, algae, yeasts, fungus and plants, characterized in relation to their genome and the enzymes that they produce, that can be utilized for waste treatment or bioremediation of soil and water.

## STRATEGIES FOR PESTICIDE REMEDIATION

Due to the problems of pesticide contamination, for effective clean-up of a particular polluted site, the development of technologies that guarantee their elimination in a safe, efficient and economical way is important. Ideally, treatment should result in the destruction of the compound without the generation of intermediates (Frazer, 2000). Nowadays different methods have been developed in order to reduce effects of pesticides on the environment and health, for remediation of contaminated sites and for the treatment of pesticide residues. The existing technologies are those that apply physical treatments, such as adsorption and percolator filters; chemical treatments such as the advanced oxidation which involve the use of powerful transient species, mainly the hydroxyl radical. One of the popular technique used to study pesticide degradation is the heterogeneous photocatalysis with  $\text{TiO}_2$  (Ferrusquía et al., 2008). The high temperature incineration in special furnaces is currently used method, in which pesticides are packaged in the places where they were abandoned, then transported to a country that has special facilities to dispose off hazardous wastes. The estimated cost of these operations by FAO varies is between 3,000 and 4,000 USD/ton (Ortiz-Hernández et al., 2002). The degradation of these compounds also carried out by other strategies that include the photodegradation (Torres-Duarte et al., 2009). However all these methods are not cost effective and have several disadvantages. In the case of organophosphates, the alkaline hydrolysis is used, which must include a rigorous control of the conditions under which the experiments are performed, such as maintenance of alkaline pH, as well as the presence of complexes formed with metal ions, which involves the formation of secondary pollutants. All of these conventional physicochemical approaches have many drawbacks like their high expensiveness and their remediation is often incomplete due to the conversion of the parent compound to metabolites which are more persistent and equally or more toxic to non-target organisms (Singh and Thakur, 2006). To remove pesticide from contaminated environment following measures are used.

### Thermal desorption (at low temperature)

The *ex situ* clean up technology frequently used to decontaminate pesticide polluted sites is thermal desorption at low temperature. But these strategies require highly specialized facility and carry a comparatively high cost. The semi-volatile and volatile organic compounds, including pesticides, are removed from soils and it is also able to remove pesticides from sludge, sediments and filter cakes. The process involve heating of media between 300 and 1000°F, which results into the

volatilization, but not the destruction of organic compounds. The formed organic compounds in the contaminated gas stream are then treated by either passing through an after burner where the contaminants are completely destroyed or condensed. This process would convert the gas into a liquid phase for further treatment or they are captured by carbon adsorption beds which make their immobilization, but do not destroy the contaminants.

### Incineration

Another proven and most frequently used technology to remediate pesticide contaminated sites is incineration. Unlike desorption approach incineration causes complete destruction of contaminant. The soil, sludge or sediments with organic contaminants are best been removed by incinerator. The organic compounds from the contaminated media are subsequently oxidized by applying heat and oxygen. The first stage of incineration involves heating of the contaminated media at temperature between 1,000 and 1,800°F that result in partial oxidation and the volatilization of the organics. The second stage results in the complete destruction of organics which operates at temperatures between 1,600 and 2,200°F. The resulting ash can then be disposed off in a landfill, if it meets safety regulations.

### Phytoremediation

The cost-effective and aesthetically-pleasing method of remediating contaminated sites is an innovative technology is the phytoremediation (U.S. EPA, 1999, 2000). In natural ecosystems, substances generated by nature are metabolized by plants, which act as filters. Phytoremediation is new emerging technology that uses plants to remove contaminants from soil and water (U.S. EPA, 1999, 2000; Raskin et al., 2000). Many studies have been carried out to determine the effectiveness of remediating persistent pollutants with various plant species and more results are frequently being reported. Pesticide biotransformation in plants with reference to similarities and dissimilarities has been documented by Hall et al. (2000). Phytoremediation of ethion, a phosphorus pesticide was studied with water hyacinth; *Eichhornia crassipes* (Huilong et al., 2006). The study reported that ethion disappearance rate constants in culture solutions for non-sterile planted were 0.01059, sterile planted were 0.00930, non-sterile unplanted were 0.00294, and sterile unplanted were 0.00201 h<sup>-1</sup>. These rate constants were significantly divergent and indicated that plant uptake and phytodegradation contributed 69% and while microbial degradation contributed 12% to the removal of the applied ethion.

Capacity of *lemna minor*, *Elodea Canadensis* and

*Cabomba aquatic* to uptake pesticides; copper sulphate, flazasulfuron and dimethomorph were studied by Olette et al. (2008). All of these three plants were able to conduct phytoremediation of pesticides successfully.

Two fungicides; dimethomorph and pyrimethanil removal from water by five macrophytes; *L. minor*, *S. polyrhiza*, *C. aquatica*, *C. palustris* and *E. candensis* were studied by Dosnon-Olette et.al (2009). Experiments conducted in laboratory were able to remove 10 to 18% dimethomorph and 7-2% pyrimethanil during four days incubation window period. The maximum removal rate during the test period was found to be 48 mg/gm fresh weight for dimethomorph and 33 mg/g for pyrimethanil. Out of five, the *L. minor* and *S. polyrhiza* showed the highest removal efficiency towards these fungicides (Dosnon-Olette et al., 2009). Phytoremediation of Chlorpyrifos in greenhouse conditions with water lettuce; *Pistia stratiotes* L and dukeweed *L. minor* L were addressed by Prasertsup et al. (2011). Relative growth rate (RGR) studies at initial chlorpyrifos concentrations up to 0.5 mg/L did not affect plant growth however RGR was found to be affected at or above 1 mg/L chlorpyrifos concentration. RGR inhibition was found to be -0.036 mg/g/day for *P. stratiotes* while it was -0.023 mg/g/day for *L. minor*. The maximum removal of chlorpyrifos by *P. stratiotes* and *L. minor* was noticed when chlorpyrifos was at an initial culture concentration of 0.5 mg/L, was 82 and 87%, respectively. The bioconcentration factor (BCF) of *L.minor* was significantly greater than that for *P. stratiotes* and therefore, at least under this greenhouse-based conditions; *L. minor* was more efficient than *P. stratiotes* for the accelerated removal of chlorpyrifos from water. In their study Riaz et al. (2017) indicated phytoremediation of pyrethroids by macrophytes and algae in freshwater was superior over organochlorine. The *Saccharaum officinarum* (sugarcane) along with *Candida* was found to exhibit augmented removal of lindane in doped soil (Salam et al., 2017). Removal rate for atrazine from eutrophicated lakes in China with *Potamogeton crispus*-planted and *Myriophyllum spicatum*-planted sediments could reach over 90%. The half life of atrazine was significantly reduced from 14.3 to 8.6 days with *P. crispus* and 9.72 days with *M. spicatum* (Qu et al., 2017). In an alternative studies removal of atrazine was effectively achieved with prairie grass (Khrunyk et al., 2017) and shrub willows (Lafleur et al., 2016). Wang et al. (2016) under laboratory conditions assessed the potential of *Acorus calamus* to remove chlorpyrifos at low concentration from water. While phytoremediation of terbuthylazine from polluted solution was found be effective with *Festica arundinacea*. Studies reported that herbicide detoxification was due to Glutathion S-transferase and ascorbate peroxidase (Buono et al., 2016). Removal of terbuthylazine with Italian ryegrass *Lolium multiflorum* L (Mimmo et al., 2015) was studied in liquid solutions. Phytoremediation of imazalil and tebuconazole (Lv et al., 2016) from

contaminated water was studied with four wet land plants; *Typha latifolia*, *Phragmites australis*, *Iris pseudacorus* and *Juncus effuses*. The use of edible crops such as; tomato, sunflower, soyabean and alfalfa for removal of endosulfan was reported by Mitton et al., (2016) and with *Vetiveria zizanioides* L Nash in two cottons soils from Burkina Faso (Abaga et al 2014). Among this sunflower was found to efficiently remove endosulfan from contaminated soil. Human p450 transgenic *A. thaliana* was able to remove herbicide simazine of up to 250  $\mu$ mole while nontransgenic plants were unable to tolerate simazine of 50  $\mu$ mole (Azab et al., 2016). *In situ* cultivation of second generation biofuel crop, *Miscanthus X giganteus* was found to efficiently eliminate risk associated with both environmental and health due to contamination of organochlorines (Nurzhanova et al., 2015). Removal of pentachlorophenol (PCP) was studied (Hechmi et al., 2014). Four plant species, white clover, ryegrass, alfalfa and rapeseed were grown in soil with or without PCP. Mixed cropping could almost remove 89% of PCP from contaminate soil. Transgenic tobacco plants expressing bacterial *bphC* gene was found to remove polychlorinated biphenyls with more efficacy. The specificity of bacterial enzyme and transgenic plant remains unaltered (Viktorovta et al., 2014). An innovative *ex situ* soil washing technology was developed in this study to remediate organochlorine pesticides (OCPs) and heavy metals in a mixed contaminated site. This was effectively achieved with maize oil and carboxymethyl- $\beta$ -cyclodextrin (Ye et al., 2014). Cyanophos pesticide removal with the use of *Plantago major* L in water was reported by Romeh (2014). The performance of *Cytisus striatus* in association with different microbial inoculant treatments on the dissipation of the insecticide hexachlorocyclohexane (HCH) was studied (Becerra-castro et al., 2013). Soil dependent dissipation of HCH was found to be modified with microbial inoculation. Ability to dissipate HCH without microbes was improved by 2.5 times with the use of microbial inoculation. Ability of *Ricinus communis* L, tropical plant species, to promote the degradation of 15 persistent organic pollutants (POPs) in a 66 days period was assessed by Rissato et al. (2015). The pollutants used include; hexachlorocyclohexane, DDT, heptachlor and aldrin are a few to mention. The vegetation could efficiently remove various POPs between 25 and 70% (Rissato et al., 2015).

### Bioremediation

Bioremediation is an innovative technology that is frequently being used for the clean-up of polluted sites. This technology is cost effective and becoming an increasingly attractive clean-up technology. The solid sludge, soil, and sediment as well as groundwater pollution can be treated by bioremediation. The rate of the natural microbial degradation of contaminants is

enhanced by bioremediation. This enhancement is carried out by supplementing these microorganisms with nutrients, carbon sources or electron donors. The process can be carried out by using indigenous microorganisms or by adding an enriched culture of microorganisms. Microbes utilize their inherent specific characteristics to degrade desired contaminant at a quicker rate. The result of bioremediation is the complete mineralization of contaminants to H<sub>2</sub>O and CO<sub>2</sub> without the build-up of intermediates. For effective bioremediation, microorganisms must enzymatically attack the pollutants and convert them to less toxic products. An effective bioremediation can be achieved only where environmental conditions permit microbial growth and activity; its application often involves the manipulation of environmental conditions to allow microbial growth and degradation to proceed at a faster rate (Lacey and Goettel, 1995; Vidali, 2001). Bioremediation processes can be broadly classified into two categories, *ex situ* and *in situ*. The *ex situ* bioremediation technologies involves the use of bioreactors, biofilters, land farming and some composting methods whereas *in situ* includes bio-stimulation, bioventing, biosparging, liquid delivery systems and some composting methods. The low cost and its effectiveness are the most positive parts of this technology.

### APPROACHES FOR BIODEGRADATION OF PESTICIDES

The various biological systems, as microorganisms, have been used to biotransform pesticides. The bacteria and fungi are the major entities involved in the pesticide biodegradation. The fraction of the soil biota, when are continuously applied to the soil, they can quickly develop the ability to degrade certain pesticides. For certain soil microorganisms, these chemicals provide adequate carbon source and electron donors (Galli, 2002), and thus establishing a way for the treatment of pesticide-contaminated sites (Qiu et al., 2007). The absence of the microbial systems that has the pesticide degrading enzymes that leads to the persistence of the pesticide in the soil. In such cases, where innate microbial population of the soil can not be able to manage pesticides, the external addition of pesticide degrading microflora is recommended (Singh, 2008). Thus for bioremediation of other chemical compounds to whom any microbial degradation system is known, the isolated microorganisms capable of degrading pesticides can be used (Singh and Thakur, 2006). However, the transformation of such compounds depends not only on the presence of microorganisms with appropriate degrading enzymes system, but also a wide range of environmental parameters (Aislabie et al., 1995) such as; temperature, pH, water potentials and available nutrients. Some of the pesticides are readily transformed by the microbes

however; some are recalcitrant in nature (Richins et al., 1997; Mulchandani et al., 1999). Additionally, some other aspects such as physiological, ecological, biochemical and molecular play important roles in the microbial transformation of pollutants (Iranzo et al., 2001; Vischetti et al., 2002).

## BACTERIAL DEGRADATION OF PESTICIDES

There are various sources of microorganisms having the ability to degrade pesticides. Generally, microorganisms that have been identified as pesticide degraders have been isolated from a wide variety of pesticide contaminated sites. The soil is the medium that mostly gets these chemicals, when they are applied to agricultural crops; additionally, the pesticide industry's effluent, sewage sludge, activated sludge, wastewater, natural waters, sediments, areas surrounding the manufacture of pesticides are also rich source of pesticide degrader. In different laboratories around the world, presently there are collections of microorganisms identified and characterized for their pesticides degradation ability. The isolation and characterization of pesticides degrading microorganisms that is able to give the possibility to count with new tools to restore polluted environments or to treat wastes before the final disposition. Upon complete biodegradation of the pesticide, the carbon dioxide and water are formed by the oxidation of the parent compound and this process provides the energy to the microbes for their metabolism. The intracellular or extracellular enzymes of the microbes play major role in the degradation of chemical compounds.

### Organochlorine pesticide and their degradation

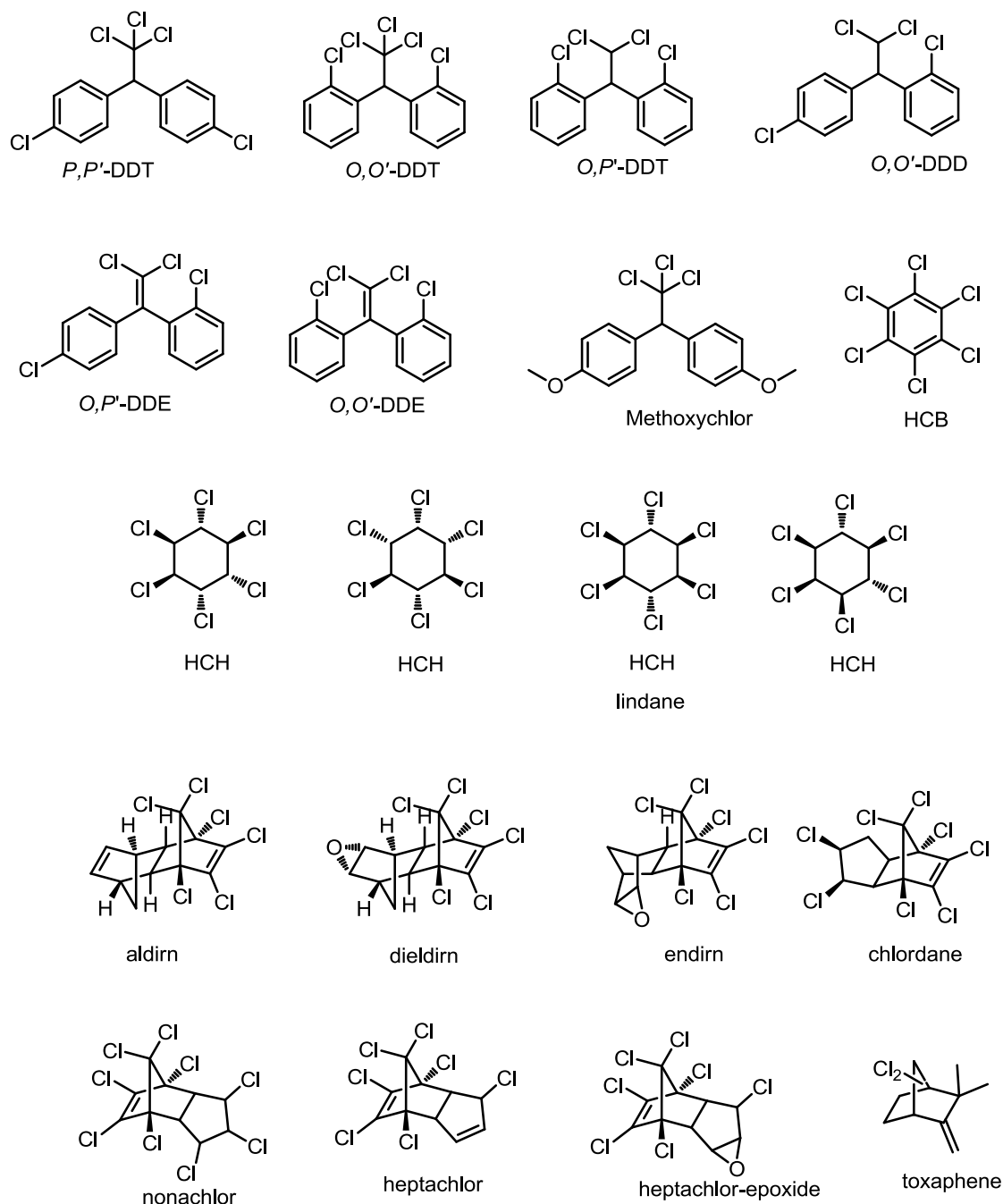
These are organic compound containing at least one covalently bonded chlorine atom and are insecticides containing primarily of carbon, hydrogen and chlorine. These include a chlorocarbon, chlorinated hydrocarbon, organochloride, organochlorine or chlorinated solvent. Organochloride pesticides are synthetic. In the 1970s, these were widely used, mainly in the United States. Because of their wide structural variety and divergent chemical properties it has broad range of applications. Because of the effects of these compounds on the environment use of many derivatives are controversial. Although their use has been banned in many countries, they are still used in developing countries. Many xenobiotic compounds, especially the organochloride pesticides are recalcitrant and resistant to biodegradation (Diaz, 2004; Dua et al., 2002; Chaudhry and Chapalamadugu, 1991). The rate of breakdown of most of them is slow and they can remain in the environment for long time after application and also in organisms long after its

exposure. They act by hyper excitation in the nervous system of the animals, causing acute toxic effects and their death frequently occurs due to respiratory failure after the disruption of nervous system function. Organochloride pesticides are cumulative in the organisms and causes chronic health effects, like cancer and neurological and teratogenic effects (Vaccari et al., 2006).

In general, these highly toxic and carcinogenic compounds persist in the environment for many year. Some of the most widely used organochlorine pesticides include, dieldrin, aldrin, alpha-BHC, beta-BHC, delta-BHC, gamma-BHC (Lindane), heptachlor, endosulfan, methoxychlor, aroclor and dichloro diphenyl trichloro ethane (DDT) (Menone et al., 2001; Patnaik, 2003) (Figures 1 and 2).

In 1939, the use of organochlorine pesticides started when Paul Hermann Müller realized that the DDT was an efficient insecticide (Matolcsy et al., 1988). During the World War II, for prevention of epidemics of typhus transmitted by lice, DDT powder was pulverized on the population's skin. In other countries, this insecticide was also used to control the malaria-bearing mosquitoes (Konradsen et al., 2004). Although most organochlorine were banned from some countries, organochlorine pesticides are still widely studied due to their recalcitrant nature, that is, even after years since the use has been banned, organochlorine contaminated sites are not rare. Both biotic and abiotic factors determine the fate of pesticides in the environment. The rate of different pesticides which are undergoes biodegradation varies widely.

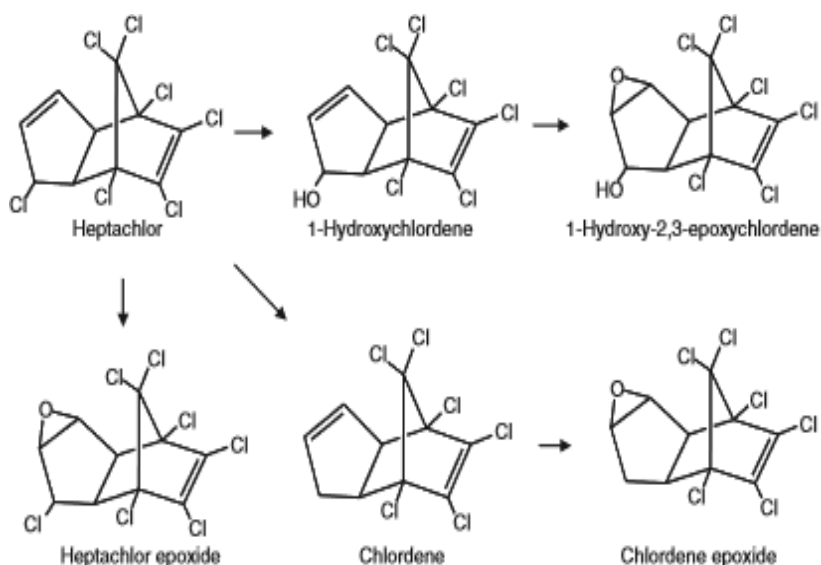
Some pesticides like DDT and dieldrin are recalcitrant and they accumulate in the environment for a long time and enter into food chains and accumulate for decades after their application to the soil (Kannan et al., 1994). The *in situ* degradation of organochlorine pesticides by pure cultures has been proven. Among microorganisms, bacteria comprise the major group involved in organochlorine degradation, especially soil habitants belonging to genera *Bacillus*, *Pseudomonas*, *Arthrobacter* and *Micrococcus* (Langlois et al., 1970). Matsumura et al. (1968) reported that the evidence of the breakdown of dieldrin in the soil by a *Pseudomonas* sp. Studies with fungi have also evidenced the biodegradation of organochlorine pesticides. Ortega et al. (2011) evaluated marine fungi collected off the coast of São Sebastião, North of São Paulo State, Brazil for the biodegradation of organochlorine. The fungi strains were obtained from marine sponges. The fungi like *Penicillium miczynskii*, *Aspergillus sydowii*, *Trichoderma* sp., *Penicillium raistrickii*, and *Bionectria* sp. were previously tested in solid culture medium for their degradation capability. Table 1 presents some of the microorganisms that were able to degrade organochlorine pesticides. An extensive study on fungal degradation of heptachlor and heptachlor epoxide was carried out on the 18 species of white rot



**Figure 1.** Structures of organochlorine pesticide.

They proposed the metabolic pathways in five species for degradation of these pesticides. The *P. tremellosa*, *P. brevispora* and *P. acanthocystis* removed about 71, 74 and 90% of heptachlor, respectively, after 14 days of incubation. The metabolic products of heptachlor from most fungal cultures were heptachlor epoxide, 1-hydroxychlordene and 1-hydroxy-2,3-epoxy-chlordene. *P. acanthocystis*, *P. brevispora*, *P. lindtneri* and *P. aurea* removed about 16, 16, 22 and 25% of heptachlor

epoxide, respectively, after 14 days of incubation. In these fungal cultures, heptachlor diol and 1-hydroxy-2,3-epoxychlordene were produced as metabolites, suggesting that the hydrolysis and hydroxylation reaction occur in the epoxide ring and in position 1 of heptachlor epoxide, respectively (Xiao et al., 2010). Successful degradation of the hexachlorocyclohexane in *Spingobium indicum*, *Spingobium japonicum* and *Spingobium francense* has been due to uptake and metabolism of the



**Figure 2.** Organochlorine: Heptachlor degradation.

pesticide by the *lin* genes present in these three species (Pal et al., 2005). Latter on Liu et al. (2007) demonstrated complete removal of hexachlorocyclohexane chlorinated pesticide from the soil with *S. japonicum*, with an efficacy of 2 mg/L/h. Studies by Jayashree and Vasudevan (2007) conducted studies on edosulfan remediation from the soil. In their experimental design they used synthetic surfactant Tween 80 and efficacy of *P. aeruginosa* to degrade endosulfan at neutral pH and at 8.5 pH was tested. In their studies they could demonstrate that surfactant could positively influence bacterial degradation of endosulfan. Bacterium could remove as high as 94% endosulfan at pH 8.5 producing endodiol and endosulfan sulphate, those were less toxic to soil (Jayashree and Vasudevan, 2007). When mixed cultures of *Stenotrophomonas maltophilia* and *Rhodococcus erythropolis* were used for degradation of endosulfan (Kumar et al., 2008), studies revealed that 73 and 81% of  $\alpha$  and  $\beta$  endosulfan were removed, respectively. When tested individually the *S. maltophilia* had better efficacy over *R. erythropolis*. The metabolites produced by mixed culture degradation were found to be close to non-toxic to that of endosulfan when toxicity was tested against human polymorph nuclear cell detected by micronucleus assay (Kumar et al., 2008). Bacterium *Achromobacter xylosoxidans* CS5 was found to be able to utilize edosulfan as the sole carbon, sulphur and energy source from the activated sludge of Jiangsu, China (Li et al., 2009). Culture analysis with reference to pH, cell growth and residual endosulfan demonstrated CS5 strain could degrade endosulfan producing endosulfan diol and endosulfan ether as the major metabolites. Studies Tas et al. (2011) investigated role for *Dehalococcoides* sp. in degradation of hexachlorobenzene dechlorination in river sediment. Curiously they

could identify organism was able to perform activity anaerobically at various range of temperature throughout the year and the *cbrA* gene product; trichlorobenzene reductive dehalogenase was found to be more important enzyme. Chaussonnerie et al. (2015) isolated and characterized two closely related species of *Citrobacter amalonaticus* those were able to transform recalcitrant chlordene from pesticide contaminated soil. Endosulfan degradation by *P. aeruginosa* G1 (88.5%), *Stenotrophomonas maltophilia* G2 (85.5%), *B. atrophaeus* G3 (64.4%), *Citrobacter amalonaticus* G4 (56.7%) and *Acinetobacter lowffii* G5 (80.2%) was reported (Ozidal et al., 2016). Effect of biochar on the water soluble arsenic concentration and the extend of organochlorine degradation in a co-contaminated historic sheep-dip soil during 180d glasshouse incubation experiment was studied (Gregory et al., 2015). In this study microbial degradation activity was found to be enhanced after 60 day incubation. The biochar amended soil contained *Chryseobacterium*, *Flavobacterium*, *Dadobacterium*, and *Pseudomonadaceae* members. Increased microbiological dehydrogenase activity due to biochar amendment was responsible for enhanced degradation of organochlorine that was otherwise attenuated due to arsenic contamination. Abundance and distribution of potential microbes and functional genes associated with pentachlorophenol anaerobic mineralization in a continuous flow reactor was studied by Li et al. (2016). Microbes with potential reductive dechlorinators; *Dehalobacter*, *Sulfospirillum*, *Desulfitobacterium* and *Desulfovibrio* spp. and phenol degrader *Cryptanerobacter* and *Syntrophus* spp. and putative functional genes; chlorophenol reductive dehalogenase *cprA*, benzoyl-CoA reductase *bam A* and seven types of putative nitrogenase reductase genes were determined (Table 1) (Li et al., 2016).

**Table 1.** Studies on organochlorine pesticide degrading microorganisms.

Pesticide	Microorganism	Reference
DDT	<i>Alcaligenes eutrophus</i> , <i>Aerobacter aerogenes</i>	Nadeau et al., 1994.
	<i>Sphingobacterium</i> sp.	Wedemeyer, 1967.
	<i>Penicillium miczynskii</i> , <i>Aspergillus sydowii</i> , <i>Trichoderma</i> sp., <i>Penicillium raistrickii</i> , <i>Aspergillus sydowii</i> and <i>Bionectria</i> sp.	Fang et al., 2010.
	<i>Aerobacter aerogenes</i>	Ortega et al., 2010.
	<i>Trichoderma viridae</i> , <i>Pseudomonas</i> sp., <i>Micrococcus</i> sp., <i>Arthrobacter</i> sp., <i>Bacillus</i> sp. <i>Pseudomonas</i> sp. <i>Sphingobacterium</i> sp.	Wedemeyer, 1967. Patil et al., 1970. Patil et al., 1970; Kamanavalli and Ninnekar, 2005; Fang et al., 2010; Pesce and Wunderlin, 2004
Dieldrin	<i>Pseudomonas</i> sp.	Matsumura et al., 1968.
Endosulfan	<i>Pseudomonas aeruginosa</i> , <i>Burkholderia cepaeia</i> , <i>Arthrobacter</i> sp. KW, <i>Aspergillus niger</i>	Bhalerao and Puranik, 2007.
PCP	<i>Arthrobacter</i> sp., <i>Flavobacterium</i> sp.	Stanlake and Finn, 1982; Crawford and Mohn, 1985
1,4- Dichlorobenzene	<i>Pseudomonas</i> sp. <i>Sphingomonas paucimobilis</i>	Spain and Nishino, 1987. Pesce and Wunderlin, 2004.
Lindane	<i>Pleurotus ostreatus</i> , <i>Streptomyces</i> sp. <i>Ganoderma australe</i>	Benimeli et al., 2008 Rigas et al., 2007.
DDE	<i>Phanerochaete chrysosporium</i>	Bumpus et al., 1993.
DDD	<i>Trichoderma</i> sp.	Ortega et al., 2011.
Heptachlor epoxide	<i>Phlebia</i> sp.	Xiao et al., 2010
	<i>Phanerochaete chrysosporium</i> ,	Arisoy and Kolankaya, 1998; Xiao et al., 2010
Heptachlor O	<i>Phlebia</i> sp.	Lacayo et al., 2006
	<i>Bjerkandera</i> sp.	Patil et al., 1970.
	<i>Trichoderma viridae</i> , <i>Pseudomonas</i> sp., <i>Micrococcus</i> sp., <i>Bacillus</i> sp.	Patil et al., 1970.
Toxaphene O	<i>Trichoderma viridae</i> , <i>Pseudomonas</i> sp., <i>Micrococcus</i> sp.	Patil et al., 1970.
	<i>Arthrobacter</i> sp., <i>Bacillus</i> sp.	Patil et al., 1970.
Aldrin O	<i>Pseudomonas</i> sp. 105	Patil et al., 1970.
	<i>Pseudomonas</i> sp.	Patil et al., 1970.
Endrin O		Patil et al., 1970.
Dieldrin O		Matsumura et al., 1968.

The organochlorine compounds undergo reductive dechlorination that takes place under anaerobic conditions, and dehydrochlorination, occurring aerobically. Several bacterial genera such as *Klebsiella* (Kwon et al., 2005), *Alcaligenes*



(Don and Pemberton, 1981) *Staphylococcus* (Sonkong et al., 2008), and *Pseudomonas* (Barragan-Huerta et al., 2007) carry out the reaction. The ability to degrade organochlorine has been documented in different genera of fungi. Among these, basidiomycetes show more resistance toward these compounds (Gomes Machado et al., 2005; Rigas et al., 2005). Recently it is reported that a strain of *Trichoderma harzianum* has capability to degrade organochlorines through an oxidative system (Katayama and Matsumura, 2009).

Another best example of organochlorine degradation is endosulfan degradation. Microorganisms play a key role in removal of such xenobiotic from the contaminated sites. The dynamic, complex and complicated enzymatic systems of the microbe degrade these chemicals by eliminating their functional groups of the parent compound. The endosulfan can undergo either oxidation or hydrolysis reactions. Several intensive studies on the degradation of endosulfan have been carried out which show, formation of endosulfan sulphate and endosulfan diol as primary metabolites. The *Mycobacterium tuberculosis* ESD enzyme degrades beta-endosulfan to the monoaldehyde and hydroxyether, but transforms alpha-endosulfan to the more toxic endosulfan sulphate. However, oxidation of endosulfan or endosulfan sulphate by the monooxygenase encoded by *ese* in *Arthrobacter* sp. KW yields endosulfan monoalcohol (Weir et al., 2006) alternatively, in some bacteria like *Pseudomonas aeruginosa* and *Burkholderia cepacia* hydrolysis of endosulfan occur which yields the less toxic metabolite endosulfan diol (Kumar et al., 2007).

### Bioremediation of organophosphorous

The first OP compounds degrading bacterium was isolated from a soil sample from the Philippines in 1973, and was identified as *Flavobacterium* sp. ATCC 27551 (Sethunathan and Yoshida, 1973). The biodegradation of organophosphorous compounds with their enzyme systems involved in the biodegradation has been extensively studied (Singh, 2008). Many of the pesticide degrading genes harbored on the plasmid DNA are reported (Chung and Ka, 1998; Laemmli et al., 2000). The plasmid carrying genetic system for the degradation of a complex compound is referred to as the catabolite plasmid. In the literature till date, the catabolite plasmids bearing several bacterial species viz, *Klebsiella*, *Moraxella*, *Rhodococcus*, *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Acinetobacter* and *Arthrobacter* are reported (Sayler et al., 1990). Recently, the co-metabolic degradation of organophosphorous compound dimethoate is reported by bacterium *Raoultella* sp. X1 (Liang et al., 2009). Similarly, by using *Paracoccus* sp. Lgjj-3, the biodegradation of dimethoate with detailed biochemical pathway is also reported (Li et al., 2010). Till date number of bacterial species has been reported for dichlorovos degradation. These include *Proteus vulgaris*, *Vibrio* sp., *Serratia* sp.

and *Acinetobacter* sp. (Agarry et al., 2013).

The gas chromatographic analysis of chlorpyrifos degradation with *P. putida* strain MAS-1 revealed degradation was rapid. When growing in minimal salt medium containing 2 mg/ml Chlorpyrifos as the solitary carbon source it was demonstrated that as high as 90% chlorpyrifos was utilized by MAS-1 just in 24 h (Munazza et al., 2012). In a parallel study, Sasikala et al. (2012) isolated nine different bacteria from chlorpyrifos contaminated soil of which four were further used in preparation of consortium for the degradation of pesticide. In another study, it has been reported that 4 strains of *Pseudomonas* isolated from a water waste irrigated agri-soil in India, were able to utilize chlorpyrifos as an exclusive carbon source (Bhagobaty and Malik 2008). Rani et al. (2008) isolated chlorpyrifos degrading *Providencia stuartii* from agricultural soil experience with chlorpyrifos for 10 years in a row. While degradation of Chlorpyrifos by *Spingomonas* sp. Dsp-1 stain from contaminated water was addressed by Li et al. (2007). In an independent study, *B. cereus* mediated chlorpyrifos degradation from Chinese soil was demonstrated by Liu et al. (2012). Actinomycetes *Streptomycetes* strains have been known for their profound industrial application. Strains degrading chlorpyrifos were isolated (Briceno et al., 2012), ability to degrade pesticide was established in agar medium, interestingly degradation was found to be modulated by the changes in the pH of the cultivation medium (Briceno et al., 2012). Yet another pesticide Profenofos degrading bacteria were isolated by enrichment technique. These bacteria predominantly belonged to *Pseudomonas* genus and were able to remediate proficiently 90% pesticide within 90 h (Malghani et al., 2009). Under laboratory conditions Rayu et al. (2017) could demonstrate that *Xanthomonas* sp. 4R3-M3 and *Pseudomonas* sp. 4H1-M3 were able to use both chlorpyrifos and 3,5,6-trichloro-2-pyridinol as a sole carbon and nitrogen source. Degradation of cyhalothrin and other pyrethroids by *B. thuringiensis* strain was reported (Chen et al., 2015).

Studies demonstrated production of 3-phenoxyphenyl acetonitrile and *N*-(2-isopropoxy-phenyl)-4-phenoxy benzamide as metabolites in the degradation pathway of pyrethroids. Cypermethrin degrading *Bacillus* sp. strain G2 was isolated from pesticides contaminate soil. The isolate was found to utilize a novel pathway producing intermediates as 4-propylbenzoate, 4-propylbenzaldehyde, phenol M-tert-butyl and 1-dodecanol (Pankaj et al., 2016). Parte et al. (2013) tested ability of *Pseudomonas demolyticum* NCIM2112 strain to degrade sulfonated aromatic amine, used as precursor in much OP synthesis. Under controlled laboratory condition NCIM2112 strain of *P. demolyticum* was found to successfully remediate sulfonated aromatic amine. In an independent study strain SMK of *Pseudomonas stutzeri* was obtained from the soil that received dichlorovos treatment for consecutive three years. Strain was tested

**Table 2.** Studies on organophosphorous degrading microorganism.

Microorganism	Pesticide	Source	References
<i>Ochrobactrum</i> sp., <i>Castellaniella</i> sp., <i>Variovorax</i> sp., <i>Pseudomonas</i> sp., <i>Psychrobacter</i> sp.	Igepal CO-210 Igepal CO-520	Sewage sludge	DiGioia et al., 2008.
<i>Sphingomonas</i> sp.	Chlorpyrifos	Wastewater	Li et al., 2007.
<i>Enterobacter</i> sp.	Chlorpyrifos	Soil	Singh et al., 2004
<i>Sphingomonas</i> sp.	Isoproturon	Soil	Bending and Rodríguez, 2007
<i>Burkholderia</i> sp.	Fenitrothion	Soil	Hong et al., 2007
<i>Acinetobacter radioresistens</i>	Methyl parathion		Liu et al., 2007
<i>Ochrobactrum</i> sp.	Methyl parathion	Soil	Qiu et al., 2007
<i>Pseudomonas frederiksbergensis</i>	Dimetoate, Malathion	Soil	Al-Qurainy and Abdel Megeed, 2009
<i>Bacillus pumilus</i>	Chlorpyrifos	Soil	Anwar et al., 2009.
<i>Bacillus</i> sp.		Soil	Batissou et al., 2009.
<i>Serratia liquefaciens</i> , <i>Serratia marcescens</i> , <i>Pseudomonas</i> sp.	Diazinon	Soil	Cycon et al., 2009
<i>Pseudomonas putida</i> , <i>Burkholderia gladioli</i> .	Prophenofos	Soil	Malghani et al., 2009.
<i>Pseudomonas putida</i>	Propiconazole	Tea rhizosphere	Sarkar et al., 2009.
<i>Providenciastuartii</i>	Chlorpyrifos	Soil	Rani et al., 2009.
<i>Micrococcus</i> sp.	Diuron	Diuron storage	Sharma et al., 2010.
<i>Sphingobium</i> sp	Methyl parathion	Fenpropathrin sewage sludge	Yuanfan et al., 2010.

for its ability to remediate dichlorovos under controlled conditions (Parte et al., 2017).

Following are the list of microorganism with their isolation source and pesticide that undergoes biodegradation (Table 2).

### Biodegradation of neonicotinoids pesticide

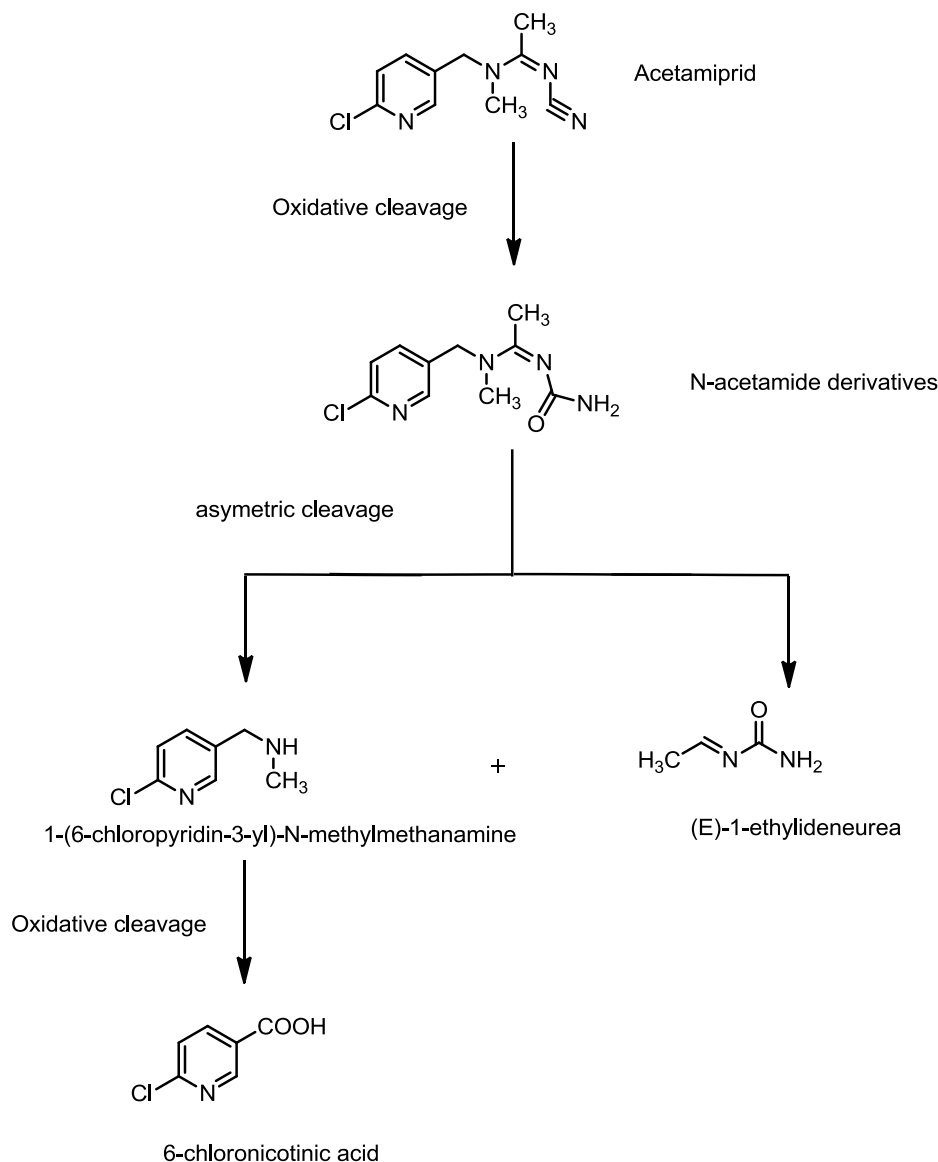
Like organophosphorus compounds, the neonicotinoids are also degraded by bacterial species such as *Stenotrophomonas maltophilia* CGMCC 1.1788, which degrades acetamiprid (Dai et al., 2007; Chen et al., 2008). The *Pseudomonas* sp. 1G is reported for biotransformation of imidacloprid (Pandey et al., 2009), *Leifsonia* sp. (Anhalt et al., 2008). The *Rhodotorula mucilaginosa* strain IM-2 is reported for the degradation of acetamiprid (Dai et al., 2010). Agricultural soil that experienced clothianidine for three years in a row was used for isolation of clothianidine degrading bacterium. Under laboratory condition *Pseudomonas* sp. was able to degrade clothianidine within 14 days (Figure 3) (Parte et al., 2015)

Biodegradation of acetamiprid using *Rhodococcus* sp. strain BCH2 could allow elucidation of degradation pathway with the GC-MS analysis (Phugare and Jadhav, 2013). The acetamiprid was found to be degraded via formation of N-amidoamide derivative, 1-(6-chloropyridin-3yl)-N-methylmethanamine (m/z ¼ 156) and 6-chloronicotinic acid (m/z ¼ 157). Investigations with an

aerobic bacterium *Burkholderia cepacia* strain CH-9 were performed by Madhuban et al. (2011) for degradation of imidacloprid and metribuzin. Bacterial strain over 20 days could remove as high as 69% imidacloprid and 86% metribuzin with initial dose of 50 mg/L in mineral salt medium.

### Biodegradation of carbamate

Carbamates were introduced as pesticides in the early 1950s and are still used extensively in pest control due to their effectiveness and broad spectrum of biological activity (insecticides, fungicides and herbicides). High polarity and solubility in water and thermal instability are typical characteristics of carbamate pesticides, as well as high acute toxicity carbamates are used to control insects and nematodes in soils. Chemically, the carbamate pesticides are esters of carbamates and organic compounds derived from carbamic acid. The carbamates are inhibitors of AChE and are responsible for the greatest number of poisonings in the rural environment. The carbamates are transformed into various products in consequence of several processes such as hydrolysis, biodegradation, oxidation, photolysis, biotransformation and metabolic reactions in living organisms (Soriano et al., 2001). A number of bacterial genera have been identified as carbamate degraders (Parekh et al., 1995). Degradation of the pesticide occurs mainly through the hydrolysis of the methylcarbamate linkage by an enzyme



**Figure 3.** Proposed pathway for Acetamiprid degradation.

called carbofuran hydrolase, codified by the *mcd* gene, which was located on a plasmid is first described in *Achromobacter* sp. (Tomasek and Karns, 1989). Further studies showed that a wide variety of bacteria could degrade carbamates using carbofuran hydrolase. Among other genera *Pseudomonas*, *Mesorhizobium*, *Ralstonia*, *Rhodococcus*, *Ochrobactrum*, and *Bacillus* are the most notorious (Desaint et al., 2000). Fungal degradation of carbamates has also been reported. Of special interest is the report a novel hydrolase from *Aspergillus niger* capable of hydrolysing several N-methylcarbamate insecticides (Qing et al., 2006). Several Actinomycetes that metabolize carbamate pesticides were isolated. In most cases, this is initiated by hydrolysis of the carbamate at the ester linkage.

The bacteria like *Sphingomonas* sp. (Kim et al., 2004) and *Arthrobacter* sp. (De Schrijver and De Mot, 1999) degrade Carbofuran first into carbofuran phenol which was further degraded to 2-hydroxy-3-(3-methylpropan-2-ol) phenol. It has been reported that the fungicide carbendazim was degraded by a microbial consortium obtained from several soil samples in Japanese paddy fields with continuous culture enrichment. Afterwards, the carbamate carbendazim was converted to 2-aminobenzimidazole by *Pseudomonas* isolates (De Schrijver and De Mot, 1999). Following table how the example of carbamate degrader. Shin et al (2012) isolated 37 carbofuran degrading bacteria representing 15 different 16S RNA types. These included *Rhodococcus*, *Spingomonas*, *Spingobium* as known while

**Table 3.** Studies on carbamate degrading microorganism.

Pesticide	Microorganism	References
Carbofuran	<i>Achromobacter</i>	Karns et al., 1986
	<i>Pseudomonas</i> ,	Chaudhry and Ali, 1988
	<i>Flavobacterium</i>	Chaudhry and Ali, 1988
	<i>Flavobacterium</i>	Head et al., 1992
EPTC	<i>Arthrobacter</i>	Tam et al., 1987
	<i>Rhodococcus</i>	Behki et al., 1993
	<i>Rhodococcus</i>	Behki and Khan, 1994

*Bosea* and *Microbacterium* were new bacteria with carbofuran degradation potential. Four different strains of *Pseudomonas* were isolated from soil exhibiting enhanced oxamyl biodegradation. Bacteria could transform oxamyl to oxamin oxime by utilizing methylamine as a source of C and N. All of the four strains carried carbamate hydrolase homologue of the *cehA* gene (Rousidou et al., 2016). Carbamyl degrading bacteria; *Bacillus*, *Morganella*, *Pseudomonas*, *Aeromonas* and *Corrynebacterium* were isolated from local soil ecosystem of the Gaza strip. Hamada et al. (2015) demonstrated that *Bacillus* and *Morganella* was able to efficiently utilize carbamyl as solitary source of C and N, remediating as high as 94.6 and 87.3% respectively. The *Corrynebacterium* indicated moderate carbamyl degradation activity accounting to 48.8%. The soil isolate *Pseudomona* sp. strain C5pp was reported to mineralize carbaryl via 1-naphthol, salicylate and gentisate (Trivedi et al., 2016). The draft genome analysis of C5pp strain revealed that carbaryl catabolic genes are organized into three operons; upper, middle and lower. Studies also suggested role of horizontal gene transfer events in the acquisition and evolution of carbaryl degradation pathway in strain C5pp (Table 3) (Trivedi et al., 2016).

Genetic manipulation techniques can contribute to elucidate biochemical pathways involved in the microbial degradation of organochlorines, which represent promising alternatives towards developing highly efficient strains as well as the isolation and application of enzymes potentially involved in biodegradation.

Genes involved in the degradation of carbamates have been isolated and cloned from *Achromobacter* sp. WMII by Tomasek and Karns (1989) and *Rhodococcus* sp. strain NI86/21 by Nagy et al. (1995). The gene encoding for carbofuran hydrolase in *Achromobacter* sp. WMII was cloned and designated the *mcd* gene (methyl carbamate degradation); the enzyme has a broad spectrum of activity towards carbamates. Plasmid-borne genes have been implicated in the complete mineralization of carbofuran by an unidentified soil bacterium by Head et al. (1992). Similarly, plasmid involvement has also been implicated in the degradation of the carbamate herbicide EPTC by an *Arthrobacter* strain (Tam et al., 1987).

Molecular characterization of this isolate demonstrated that its ability to degrade EPTC was associated with the presence of a gene cluster that codes for an aldehyde dehydrogenase and cytochrome P-450 specific for thiocarbamates degradation. They are the major thiocarbamate-induced enzymes in *Rhodococcus* sp. Strain NI86/21.

### Fungal biodegradation of pesticides

When the bacterial species fails to degrade pesticides then fungus can be used. Recently, bioremediation technologies using fungus has taken considerable attention. For biodegradation of toxic organic chemicals, fungi from the natural source can be screened out as an effective tool. The wood attacking fungi such as *Phanerochaete* and other related fungi possess a powerful extracellular enzyme, the peroxidase that acts on the broad range of chemical compounds. The variety of chemicals including pesticides, polychlorinated biphenyl (PCB) and polycyclic aromatic hydrocarbons (PAH) are degraded by well-known fungus, *Phanerochaete chrysosporium*. Which is well known for xenobiotic metabolism and it was reported to have the ability to degrade isoproturon herbicide via solid state fermentation (Castillo et al., 2001). Endosulfan-degrading, aerobic fungal strains are effective for remediation of soil contaminated with organochlorine pesticides. Water body and soil that are affected by endosulfan can be easily bioremediated by *Aspergillus niger*. The fungus, *A.niger* was tested for their endosulfan degrading ability and it was noted that the culture could tolerate 400 mg/ml of technical grade endosulfan and the complete disappearance of endosulfan was seen after 12 days of incubation (Bhalerao and Puranik, 2007), which was indicated by the evolution of CO<sub>2</sub>. Change in pH of culture broth to acidic range supported the biological transformation. Thin layer chromatography (TLC) analyses revealed the formation of various intermediates of endosulfan metabolism including endosulfan diol, endosulfan sulfate and an unidentified metabolite. In an independent study, Kamaei et al. (2011) indicated successful degradation of endosulfan and endosulfan sulphate by a white rot fungus *Trametes hirsuta*. The fungus was shown to degrade endosulfan and endosulfan sulphate by using multiple pathways. It is reported that, strains such as *Mortierella* sp. strain W8 and Cm1-45 resulted in 50 to 70% degradation of endosulfan in 28 days at 25°C via diol formation of endosulfan firstly and then endosulfan lactone and resulting into enhancing fertility of agriculture land (Shimizu, 2002). A fungal strain *Fusarium verticillioides*, isolated from *Agave tequilana* leaves by enrichment techniques is able to use lindane as a carbon and energy source under aerobic conditions. In nitrogen and phosphorus limiting atmosphere, the higher degradation efficiency is achieved. The concentration of lindane and

yeast extract and environmental factors improved the efficiency of the biodegradation process (Urlacher et al., 2004; Alzahrani, 2009). Fungus uses chlorpyrifos as a sole energy and carbon source and causes its rapid degradation. Another fungus, basidiomycetes, degrades chlorpyrifos very effectively. The fungal strains such as *Fusarium oxysporum*, *Lentinula edodes*, *Penicillium brevicompactum* and *Lecanicillium saxenae* has great potential for the biodegradation of the pesticides including difenoconazole, terbuthylazine and pendimethalin in batch liquid cultures and are investigated to be valuable as active microorganisms for pesticides degradation (Shi et al., 2012). The rot fungi isolates from contaminated soil degrades methomyl and diazinon pesticides. The temperature optima for maximum efficiency are 28°C. The higher rate of degradation is achieved by using mixture of fungal strains (Zhongli et al., 2001). By using mixed fungal strains, there is possibility of degrading mixed insecticides (DDT and chlorpyrifos). At low concentration of mixed insecticides there was the high efficiency of degradation is observed. It is reported in DDT and chlorpyrifos, that the efficiency of degradation was 26.94 and 24.94% respectively (Pieper et al., 2000). The phytopathogenic fungus easily grows up on organophosphonate herbicides and easily degrades herbicides (Shen et al., 2009). The pesticide like pirimicarb is degraded by *Trichoderma viride* and *T. harzianum* with high potential. Upon addition of activated charcoal the degradation capacity increases (Eapen et al., 2007). By enrichment culture method the strain, *Sphingomonas yanoikuyae* can degrade carbamate and pyrethrin (OPs) with high efficiency under harsh conditions, and analyzed by gas chromatography (Cases et al., 2005). The carbofuran is degraded by salt resistant actinomycete (Chougale and Deshmukh, 2007). Up to 95% degradation of carbofuran is carried out by the *S. alanosinicus*. It utilizes carbofuran as a sole source of carbon and is applicable to saline soils for its efficiency (Wu et al., 2004). More than 30 microorganisms have capability of degrading the pesticides, of which *Gliocladium* genus has maximum activity for selective degradation of carbofuran (Fu et al., 2004).

Besides these, various fungal species including *Trametes* sp. and *Polyporus* sp. were able to degrade variety of chemicals including pesticides. Pesticide degradation ability was also reported using *A. fumigates*, *A. sydowii*, *A. terreus*, *A. flavus*, *Fusarium oxysporum* and *Penicillium chrysogenum* (Hasan, 1999).

## MICROBIAL ENZYME SYSTEMS IN PESTICIDE BIODEGRADATION

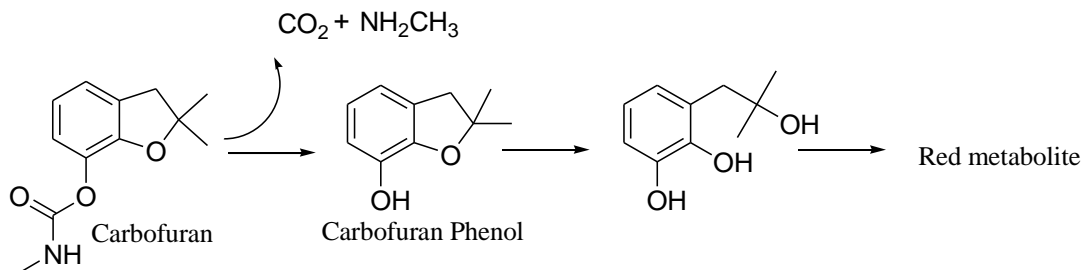
Among various strategies used by the microorganism for degradation of xenobiotic compounds the enzymes play a key role in the biodegradation. The biochemical reactions for degradation are achieved through a number of

different enzymes such as dehydrogenases (Bourquin, 1977; Singh and Singh, 2005), cytochrome p450 (Castro et al., 1985; Jauregui et al., 2003), dioxigenases (Nadeau et al., 1994; Van Eerd et al., 2003), ligninases (Pizzul et al., 2009). Several reports have documented the capability of different genera of fungi to degrade organochlorines. Bacterial enzymes have been found to achieve such detoxifying reactions (Singh et al., 1999; Yañez- Ocampo et al., 2009). The commonly used as a detoxification mechanism, especially in plants and insects, is conjugation with glutathione and this mechanism has also been reported in bacteria (Vuilleumier, 2001; Wei et al., 2001; Chaudhry et al., 2002). In these processes, bacteria and fungi are involved. They produce intracellular or extra cellular enzymes such as hydrolytic enzymes, oxygenases, peroxidases (Van Eerd et al., 2003). The basidiomycetes are more resistant to these compounds (Gomes Machado et al., 2005; Rigas et al., 2005). Recently a strain of *Trichoderma harzianum* is reported to degrade organochlorines through an oxidative system (Katayama and Matsumura, 2009).

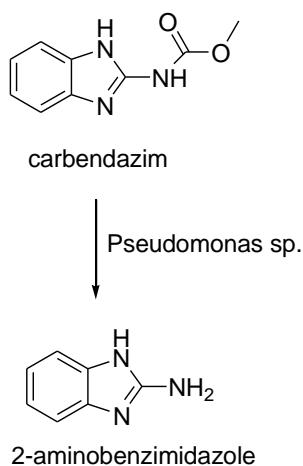
It has been reported that a large group of bacterial genera are able to degrade organophosphates compounds. Hydrolysis is fundamental for the complete degradation of the molecule. The reported and studied enzymes are related to the phosphotriesterase, having capability to hydrolyzing organophosphate pesticides at the central atom of pesticides, phosphorus. Phosphotriesterase activity is the first and most important step in detoxification. A large number of bacterial genera have been reported for the carbamate degradation (Parekh et al., 1995). An enzyme called carbofuran hydrolase carry out the hydrolysis of the methylcarbamate linkage within carbamate (Figure 4).

Kim et al. (2004) studied the carbofuran degradation by bacteria like *Sphingomonas* sp. and *Arthrobacter* sp. by De Schrijver and De Mot (1999) and proposed the metabolic pathway. The Carbofuran first degraded into carbofuran phenol which was further degraded to 2-hydroxy-3-(3-methylpropan-2-ol) phenol. Slaoui et al. (2007) reported more than 30 microorganisms were capable of degrading the pesticides, out of which *Gliocladium* genus had maximum activity for selectively degrading carbofuran. Chougale and Deshmukh (2007) reported salt resistant actinomycete is capable of degrading carbofuran. One of seven actinomycetes, *S. alanosinicus*, is most effective and gives up to 95% degradation. It uses carbofuran as a carbon source and was found to be applicable to saline soils for its efficiency (Figure 5).

De Schrijver and De Mot (1999) reported that the fungicide carbendazim was degraded by a microbial consortium obtained from several soil samples in Japanese paddy fields with continuous culture enrichment. Afterwards, the carbamate carbendazim was converted to 2-aminobenzimidazole by *Pseudomonas*



**Figure 4.** Biodegradation of Carbofuran by *Spingomonas* sp.



**Figure 5.** Biodegradation of carbendazim *Pseudomonas* sp.

isolates.

Tian and Chen (2012) studied carbendazim degradation and reported microbial strain screening and isolation are very effective for degradation of carbendazim in mineral culture medium. Carbendazim is carbon source for the growth of this strain. The pH range, 5.1 to 8.1, and temperature range, 25 to 40°C, are optimum for maximum degradation efficiency, that is, up to 90% in nitrogen atmosphere. Zhu-cai et al. (2008) reported that under harsh conditions, *Spingomonas yanoikuyae* strain can degrade carbamate with high efficiency in enrichment culture method, analyzed by gas chromatography. Yu et al. (2009) expressed methyl parathion hydrolase enzyme within the *Pichia pastoris* to achieve an efficient remediation of parathion.

Table 4 give show the list of microorganisms and the enzymes degrading pesticides:

## ROLE OF CATABOLIC GENES AND GENETIC ENGINEERING FOR PESTICIDE DEGRADATION

Bacteria considered as elastic material once by the

geneticist now has proven to have significant potential in bioremediation. In order to investigate genetic basis of pesticides biodegradation, several worker studied role of catabolic genes and the application of recombinant DNA technology. Pesticide-degrading genes from different microorganisms have been characterized. Most of the catabolic genes responsible for degradation are extrachromosomal, genomic or found in transposons. The new advancement in the field of metagenomics and whole genome sequencing have opened up new avenues for searching the novel pollutant degradative genes and their regulatory elements from both culturable and non-culturable microorganisms from the environment. The enzymes responsible for the degradation of several pesticides are encoded by these plasmids and transposons which act a mobile genetic element. For successful implementation of the technology for *in situ* remediation, it is important to understand of the genetic basis of the mechanisms of how microorganisms biodegrade pollutants and how they interact with the environment (Hussain et al., 2009). Different microbial enzymes with the capability to hydrolyze pesticides have been reported (Yan et al., 2007), such as organophosphorus hydrolase (OPH); encoded by the *opd* gene found in bacterial strains and have been isolated in different geographic regions. Although these plasmids show considerable genetic diversity, the region containing the *opd* gene is highly conserved. Another enzyme, methyl-parathion hydrolase (MPH) from the *Pseudaminobacter*, *Achrobacter*, *Brucella* and *Ochrobactrum* are encoded by the *mpd* gene and were identified by comparison with the gene *mpd* from *Pleisomonas* sp. M6 strain (Zhongli et al., 2001). The gene for the organophosphorus hydrolase has 996 nucleotides, a typical promoter sequence of the promoter TTGCAA N17 TATACT from *E. coli* (Zhang et al., 2005). Table 5 shows the most studied isolates of microorganisms capable of degrading pesticide with their several genes.

Microorganisms have inherent property against different stresses and respond differently to various kinds of stresses and acquire fitness in the polluted environment. This property can be accelerated by applying genetic engineering techniques. The unique

**Table 4.** Studies on pesticide degrading microorganism with their enzyme.

Pesticides class	Microorganism	Enzymes	Reference.
Organochlorine	Bacteria <i>Klebsiella</i> sp., <i>Alcaligenes</i> sp. <i>Staphylococcus</i> sp. <i>Pseudomonas</i> sp.	Dehalogenases	Franken et al., 1991; Sharma et al., 2006; Kwon et al., 2005; Don and Pemberton, 1981. Sonkong et al., 2008. Barragan-Huerta et al., 2007
	<i>Flavobacterium</i> and <i>Pseudomonas</i> sp. <i>Agrobacterium radiobacter</i> <i>Alteromonas</i> sp. <i>Plesiomonas</i> sp. <i>Achromobacter</i> , <i>Pseudaminobacter</i> , <i>Ochrobactrum</i> and <i>Brucella</i> <i>Alteromonas undina</i> , <i>Alteromonas haloplanktis</i> <i>B. diminuta</i> and <i>Flavobacterium</i> sp	Organophosphorus hydrolase(OPH), organophosphorus anhydrolase (OPAA), Laccase Aspergillus enzyme (A-OPH) Penicillium enzyme (P-OPH)	acid Singh and Walker, 2006; Serdar et al., 1982. Somara and Siddavattam, 1995; Horne et al., 2002; Cheng et al., 1996; Cheng et al., 1997; Zhongli et al., 2001; Zhang et al., 2005; Chen and Mulchandani (1998); Chen et al., 1999; Amitai et al., 1998; Liu et al., 2001; Liu et al., 2004; Liu et al., 2001
Organophosphate	Fungi: <i>Pleurotus ostreatus</i> . <i>Aspergillus</i> sp. <i>Penicillium</i> sp.		
	<i>Achromobacter</i> sp. <i>Pseudomonas</i> , <i>Mesorhizobium</i> , <i>Ralstonia</i> , <i>Rhodococcus</i> , <i>Ochrobactrum</i> , and <i>Bacillus</i> .	Carbofuran hydrolase	Tomasek and Karns, 1989; Desaint et al., 2000.
Carbamate	<i>Serratia</i> , <i>Pseudomonas</i> .	Carboxyl phosphotriesterase.	Grant et al., 2002.
	Fungi: <i>Aspergillus niger</i>	Pyrethroid hydrolase	Liang et al., 2005

insights into the molecular events that lead to the development of enhanced pesticide degradation phenomenon is achieved through advancement in the technique of isolation of pesticide degrading microorganisms and the characterization of genes encoding pesticide degradation enzymes. For optimization of the enzymes, metabolic pathways and organisms relevant for biodegradation in various genetic approaches have been developed and used (Pieper et al., 2000). New information on the metabolic pathways and bottlenecks of degradation is still gathering, requiring the need to

reinforce the available molecular toolbox. Nevertheless, the incorporated genes or enzymes, even in a single modified organism, need to be introduced within the regulatory and metabolic network for their proper expression (Shimizu, 2002; Pieper et al., 2000; Cases et al., 2005). Firstly it was demonstrated that the detoxification of organophosphate pesticides by genetically engineered microorganisms and the genes encoding these hydrolases have been cloned and expressed in *Streptomyces lividans*, *Yarrowia lipolytica*, *P. pseudoalcaligenes*, *E. coli*, and *Pichia*

*pastoris* (Wu et al., 2004; Fu et al., 2004; Wang et al., 2012; Shen et al., 2009). The site-directed mutagenesis has been used to improve the substrate specificity and stereo selectivity of OPH (Casey et al., 2011; Van Dyk and Brett, 2011). To generate OPH variants with up to 25-fold improvements in hydrolysis of methyl parathion, directed evolution have recently been used (Cho et al., 2002). Up to 700-fold improvement was obtained, and the best variant hydrolyzes chlorpyrifos at a rate similar to that of the hydrolysis of paraoxon by wild-type OPH (Cho

**Table 5.** Studies on pesticide degrading microorganism with their catabolic gene.

Microorganism	Gene
<b>Bacteria:</b>	
<i>Pseudomonas diminuta</i>	<i>opd</i>
<i>Alteromonas</i> sp.	<i>opaA</i>
<i>A. radiobacter</i>	<i>opdA</i>
<i>Nocardia</i> sp.	<i>adpB</i>
<i>Escherichia coli</i>	<i>pepA</i>
<i>Pseudomonas monteilli</i>	<i>hocA</i>
<i>Burkholderia caryophilli</i>	<i>pehA</i>
<i>Bacillus cereus</i>	<i>phn</i>
<i>Burkholderia</i> sp. JBA3	<i>ophB</i>
<i>Stenotrophomonas</i> sp.	<i>ophC2</i>
SMSP-1	<i>opdB</i>
<b>Lactobacillus brevis.</b>	
<i>Arthrobacter</i> sp. scl-2	<i>imh</i>
<i>Ochrobactrum</i> sp. Yw28, <i>Rhizobium radiobacter</i>	<i>mpd</i>
<i>Arthrobacter</i> sp	<i>oph</i>
<i>Arthrobacter</i> sp. L1.	<i>mph</i>
<i>Burkholderia cepacia</i>	<i>mpdB</i>
<i>Enterobacter</i> sp.	<i>opdE</i>
<i>Spingobium francense</i>	<i>lin</i>
<i>Spingobium indicum</i>	<i>lin</i>
<i>Spingobium japonicum</i>	<i>lin</i>

et al., 2004). Linuron degrading bacterium *Variovorax* sp strain SRS16 contain the *libA* gene that encodes a hydrolase enzyme, which initiates degradation. An alternative hydrolase; HylA was found to exist in *Variovorax* sp. Strain WLD1. Both the *libA* and *hylA* could contribute linuron degradation in the same environment (Horemans et al., 2016). Aryloxyphenoxy Propanoate (AOPP) degrading strain *Rhodococcus ruber* JPL-2 was isolated by Hongming et al. (2015). A novel esterase gene *feh*, encoding phenoxaprop-p-ethyl hydrolyzing carboxyesterase (FeH) was cloned from *R. ruber* JPL2. The ability of *Streptomyces* strain alone or as mixed culture to remove pentachlorophenol and chlorpyrifos was studied (Fuentes et al., 2013). It was shown that without exhibiting any antagonism growth of mixed culture was superior to that of pure culture. Pure culture of *Streptomyces* sp. A5 and quadruple culture could remove pentachlorophenol efficiently whereas *Streptomyces* sp. M7 presented the best removal of chlorpyrifos. Immobilized cells were curiously able to remediate both pentachlorophenol and chlorpyrifos than that of free cells (Fuentes et al., 2013).

## CONCLUSION

Biodegradation processes can be employed for the

pesticide degradation using microbes. Microbial degradation processes applied for bioremediation involves use of variety of different microbes including bacteria, fungi and actinomycetes.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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