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Advances in biodegradation of organophosphorus pesticides

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ABSTRACT

Several hundred pesticides of different chemical moietiesare currently used for agricultural purposes all over the world. Because of their widespread use, they are detected in various environmental matrices, such as soil, water and air. Pesticide fate in the environment is affected by microbial activity. Microorganisms readily degrade some pesticides; others have proven to be recalcitrant. Recently the use of microbes for effective detoxification, degradation and removal of toxic compounds from contaminated soils has emerged as an efficient and cheap biotechnological approach to clean up polluted environments.This paper focused on the problem of soil contamination with pesticides, and present a review onthe potential applications of various biological agents for remediation of these types of soils, which have been polluted with continuous and higher doses of pesticides through process of biodegradation with special emphasis on advancements in organophosphorus pesticides biodegradation and clear understanding of its mechanism for the efficient onsite remediation of the contaminated environment.

Keywords: Biodegradation, Bioremediation, Organophosphorus Pesticides, Soil Quality.

INTRODUCTION

Environmental pollution is one of the serious predicaments of the modern world. Environmental contamination is increasing day by day because of increase in population, industrialization and urbanization. Worldwide pesticides usage has increased dramatically during the last two decades, coinciding with changes in farming practices and the increasingly intensive agriculture. This widespread use of pesticides for agricultural and non-agricultural purposes has resulted in the presence of their residues in various environmental matrices, such as soil, water and air **[1]**.

Pesticides are indispensible to modern agriculture. Currently among the various groups of pesticides that are being used world over, organophosphates form a major and most widely used group accounting for more than 36% of the total world market. Organophosphorus pesticides are one of the major groups of pesticides that replaced organochlorines to a greater extent against crop loss by pest attack and improving crop yield. Organophosphorus insecticides are all esters of phosphoric acid and are also called organophosphates, which include aliphatic, phenyl and heterocyclic derivatives and have one of the basic building blocks as a part of their complex chemical structure. They are used to control a variety of sucking, chewing and boring insects, spider mites, aphides and pests attacking crops like cotton, sugarcane, peanuts, tobacco, vegetables, fruits and ornamentals. Some of the main agricultural products are parathion, methyl parathion, chlorpyriphos, malathion, monocrotophos, quinalphos and dimethoate.

Continuous and excessive use of organophosphorus compounds has led to the contamination of several ecosystems in different parts of the world. The widespread use of these pesticides over the years has resulted in problems caused by their interaction with the biological systems in the environment. Considering the toxic effects of pesticides, it is essential to remove these chemo-pollutants from the environment.

Several aspects are involved in the fate and behavior of OPs in the environment: effluent irrigation **[2],** photodecomposition mechanisms **[3]**, volatilization **[4]** and finally biodegradation **[5,6]**. Although organophosphates are biodegradable in nature, their residues are found in environment. Considering their toxicity, research on biodegradation of organophosphates is being carried out all over the world. Bioremediation is the most effective management tool to manage the polluted environment and recover contaminated soil.

This paper mainly aims at addressing onthe problem of soil contamination by pesticides, andthe literature of its degradation or transformation with special emphasis on advances in microbial degradation of organophosphates and clear understanding of its mechanism for the efficient onsite remediation of the contaminated environment is reviewed.

Soil Contamination Of Pesticides.

Extensive application of external agricultural inputs to agricultural production systems leads to deterioration of soil quality.Organic (carbon-based) pollutants that impact soil quality include pesticides. Pesticides, which are very persistent in soil, slowly break down and result in source of contamination**[7].**Soil acts as filter, buffer and degradation potentials with respect to storage of pollutant with the help of soil organic carbon **[8],** but it is recognized that the soil is a potential pathway of pesticide transport to contaminate water, air, plants, food and ultimately to human via, runoff and sub-surface drainage; interflow and leaching; and the transfer of mineral nutrients and pesticides from soil into the plants and animals that constitute the human food chain **[9].**

The capacity of the soil to filter, buffer, degrade, immobilize, and detoxify pesticides is a function or quality of the soil. Soil quality also encompasses the impacts that soil use and management can have on water and air quality, and on human and animal health. The presence and bioavailability of pesticides in soil can adversely impact human and animal health, and beneficial plants and soil organisms. Pesticides can move off-site contaminating surface and groundwater and possibly causing adverse impacts on aquatic ecosystems.

The pesticidal application reduces the biodiversity of the soil. The microorganisms of soil are more spoiled by soil disturbance by application of chemicals than any other parameters. The communities of beneficial microorganisms in soil have declined due to overuse of pesticides, which has a negative impact on the available nitrogen, phosphorus and potassium (NPK) from soil **[10]**, thereby degrading the soil quality. Several studies have been made to detect the contaminated soil in which different kinds of pesticides have been found **[11,12].**

Some fertilizers and pesticides are known to contain various levels of heavy metals, including Cd and Cu **[13].** Therefore, continuous and heavy application of these agrochemicals and other soil amendments can potentially exacerbate the accumulation of heavy metals in agricultural soils over time **[14,15].**

Biodegradation Of Pesticides

Availability of different pesticides in field provides exposure of several different kinds of microorganisms to pesticides. Most of the organisms die under toxic effect of pesticides but few of them evolve in different ways and use pesticide compounds in metabolism.

Microbial degradation (biodegradation) is the result of microbial metabolism of pesticides, and is often the main source of pesticide degradation in soils**[16,17,18].**It occurs when fungi, bacteria, and other microorganisms in the soil use pesticides as a source of carbon and energy, or consume the pesticides along with other sources of food or energy.

A pesticide in soil solution has to move to these microbial colonies and cross the microbial cell membrane into the cell to metabolize. Some microbes produce enzymes, which are exported from the cell to predigest pesticides that are poorly trans- ported. Once inside an organism, a pesticide can metabolize via internal enzyme systems. The ability of microorganisms to degrade or modify compounds depends on the ability to produce requisite enzymes and ideal environmental conditions for the reactions to occur. In addition, sufficient biomass and communication between the pollutant and the enzymes (intracellular or extracellular) is necessary.

The biodegradation rate is dependent on an important group of factors such as:

- ***Soil conditions*** - Soil organic matter content, moisture, temperature, aeration, and pH all affect microbial degradation. Microbial activity is high in warm, moist soils with neutral pH.

- ***Frequency of pesticide application*** (alternating between different classes, groups or formulations of pesticide can minimize the potential for microbial degradation problems as well as pest resistance).

Recent studies have revealed that microorganism consortia often are involved in the degradation phenomenon**[19,20,21].**Several reports are available indicating degradation of different pesticides when they are available in nature in excess **[22, 23, 24].**

Different approaches for biodegradation
(a) Bacterial degradation. (b) Fungal degradation. (c) Phytodegradation.

- ***Role of bacteria in biodegradation:***

The use of bacteria for the degradation and detoxification of numerous toxic chemicals such as pesticides is an effective tool to decontaminate the polluted sites. Isolation of indigenous bacteria capable of metabolizing pesticides provides environmentally friendly means of *in situ* detoxification **[25].** Successful removal of pesticides by the addition of bacteria (bio augmentation) had been reported earlier for many compounds, including chlorpyrifos, endosulfan, malathion, parathion, quinalphos, ethoprop, and atrazine **[26].**Most bacterial species, which degrade pesticides, belong to the genera *Flavobacterium, Arthrobacter, Azotobacter, Burkholderia and Pseudomonas*. Degradation by microbes depends not only on the presence of degradative enzymes, but also on a wide range of environmental parameters. Temperature, pH, water potential, nutrients and the amount of pesticide or metabolite in soil may also act as limiting factor for pesticide degrading microorganisms, which requires further exploration in relation to total microbial population and their biochemical activities **[27].**

The nature of degradation varies among species and the target compound. *Pseudomonas sp. and Klebsiella pneumoniae* have been shown to possess hydrolytic enzymes that are capable of breaking down s-triazine herbicides, such as atrazine. Similarly, a number of enzymes such as oxygenases, hydroxylases, hydrolases and isomerases present in *Pseudomonas* and *Alcaligenes* sp. have been shown to degrade herbicide 2, 4-D **[28]**. Introducing microbial population, which can degrade the pesticides completely and by optimizing the environmental condition, can enhance the degradation of pesticides and its metabolites in the soil. Microbial degradation of most of the recalcitrant organic compounds is limited by the presence of anionic species in the compound. The anions like chloride, sulphate etc. are strongly bonded to the hydrocarbon ring which prevents the microbes from attacking the ring structure. This may be due to increased toxicity of anionic group **[29].**

- ***Role of fungi in biodegradation**:*

Fungi, from natural sources can be screened out as an effective tool for biodegradation of toxic organic chemicals. Fungi degrade pesticides by introducing minor structural changes to the pesticides rendering it non-toxic and are released to soil, where it is susceptible to further biodegradation by bacteria **[30].** Several fungi such as *Agrocybe semiorbicularis, Auricularia auricula, Coriolus versicolor, Dichomitus squalens, Flammulina velupites, Hypholoma fasciculare, Pleurotus ostreatus, Stereum hirsutum, and Avatha discolor* have shown their ability to degrade various pesticide groups like phenylamide, triazine, phenylurea, dicarboximide, chlorinated and organophosphorus compounds **[31].**Several classes of pesticides such as lindane, atrazine, diuron, terbuthylazine, metalaxyl, DDT, gamma- hexachlorocyclohexane (g-HCH), dieldrin, aldrin, heptachlor, chlordane, lindane, mirex, etc. have been degraded to different extent by white-rot fungi**[32]**. Fungi do not generally metabolize contaminants; degradation occurs extracellular by enzymes excreted by the fungi. Much research remains to be done to identify the fungal strains most capable of degrading specific contaminants.

- ***Phytoremediation:***

A promising biological technology is phytoremediation, which is an in-situ and clear techniquebased in the use of some species of plants with ability to degrade organic pollutants **[33]** being its cost 20-50% inferior to the ones presented by the chemical, physical and thermal in-situ processes.Plants can either accumulate and metabolize organic pollutants (phytodegradation) or stimulate appropriate rhizospheric microorganisms (phytostimulation). Both approaches have been explored to remediate soils contaminated with pesticides.

More than 2400 plant species are reported to possess pest control properties, while some plants kill through poisonous exudates, others control through non killing activities like repellency, feeding deterrence and growth inhibition without adverse effects on environment and human health **[34].** Plant products can also be used as major bio pesticides.

Microbial Degradation Of Organophosphorus Pesticides

Pesticides in soil and water can be degraded bybiotic and abiotic pathways, however biodegradationby microorganisms is the primary mechanismof pesticide breakdown and detoxification in manysoils. Thus microbes may have a major effect onthe persistence of most pesticides in soil **[35].**

When organophosphates are released in the environment, their fate is decided by various environmental conditions and microbial degradation. Moreover, since organophosphorus pesticides and some of their metabolites are toxic, their degradation in contaminated soil is necessary. Microbial degradation is the major reason of disappearance of these pesticides.Bacterial enzymatic detoxification of organophosphorus pesticide has become the focus of many studies, because it is economical, environment friendly and effective. However, the use of microorganisms for bioremediation requires an understanding of all physiological, microbiological, ecological, biochemical and molecular aspects involved in pollutant transformation **[36].**

In 1973, the first bacterium to degrade OP compounds was isolated from a soil sample from the Philippines and was identified as *Flavobacterium* sp. ATCC 27551**[37].**This is the first report of the isolation of OP-degrading bacteria from the environment. A number of other microorganisms with similar degrading capabilities have since been isolated. Since then, several bacteria, a few fungi and cyanobacteria, have been isolated that can use OP compounds as a source of carbon, nitrogen or phosphorus.

Isolation of indigenous bacteria capable of metabolizingOP compounds has received considerableattention because these bacteria provide an environmentallyfriendly method of in situ detoxification**[38, 39].** Insome contaminated environments, autochthonousmicrobial populations have evolved over time toadapt to these contaminants **[40].** These sites are therefore the most appropriateecological niches to find and isolate strains capableof degrading OP compounds **[41,42,43,44].**

The worked on microbial degradation of organophosphates has been reviewed wherein the degradation is reported in plants, soils, water and animals **[45],** in flooded soil and in anaerobic cultures **[46],** and by soil microorganisms **[47].** Bacteria from flooded soil were found to hydrolyze selected organophosphorus insecticides **[48].**

Research studies have revealed that the microbial degradation process to detoxify hazardous wastes has been effectively used to overcome the pollution problem since longtime.For bioremediation of pesticide wastes, several soil bacteria with the ability to degrade organophosphotus pesticides have been isolated. A few consortia with the capability to metabolize OP compounds have also been reported **[5,49]**. Most microorganisms can degrade one OP or a narrow range of OP compounds **[5].**

For biotechnological applications, it is important that bacterial degraders have activity against all or most OP compounds. A few suitable species have been isolated; for example, *Flavobacterium* sp. ATCC 27551, which was isolated by virtue of its ability to degrade diazinon metabolism, can degrade almost all known OP bonds, with a range of efficacies, by enzymatic hydrolysis**[37].** Similarly, *Pseudomonas diminuta*, which was isolated from an enrichment culture in the United States, can cleave all known OP bonds**[50].**The microbial cleavage of variousorganophosphorus insecticides was studied and two strains of *Pseudomonas* sp. isolated from diazinon andmalathion enrichments were found most versatile**[51].** Degradation of malathion bybacterial strain *Pseudomonas sp. N-3* isolated from industrial effluents was found to degrade malathion up to 150 ppm only in the presence of ethanol (1% v/v) as a co-substrate **[52].**

These two microorganisms have received the most scientific attention during the past three decades. Another *Flavobacterium* sp. isolated from an Indian soil sample, as well as *Agrobacterium radiobacter* and an *Enterobacter* sp. isolated from Australian soils can also degrade several OP compounds[**53,5444].**

Methyl parathion (O,O-dimethyl-O-(p-nitro-phenylphosphorothioate) is one of the most used organophosphorus pesticides. This product is widely used throughout the world and its residues are regularly detected in a range of fruits and vegetables. Investigation of microbial degradation is useful for developing insecticide degradation strategies using microorganisms. Bacteria with the ability to degrade methyl parathion have been isolated worldwide **[55,56].**

The isolation of pesticide degrading bacteria was carried out by**[57]**and the isolated bacterial isolates were identified as *Pseudomonas fluorescens*, *Bacillus subtilis*and *Klebsiella sp*. The growths of these three pesticide degrading isolates were assessed in Minimal salt broth containing 2 different pesticides *viz*., Malathion and Parathion which were used in the study and found that among the three bacterial isolates, the bacteria *Klebsiella sp.* utilized the pesticides effectively and showed maximum growth.By the end of 7 days, about 40 per cent of monocrotophos supplemented to mineral salts medium and nearly 56 per cent and 76 per cent of quinalphos was degraded by *A.lipoferum*and *Bacillus* sp.isolated from black soil following enrichment culture technique**[58].**

One of the most importantenzymes is phosphotriesterase (PTE), first found in*Pseudomonas diminuta* MG and *Flavobacterium sp*.ATCC 27551, which is able to hydrolyze a considerablenumber of synthesized OP **[59,60,61].** Phosphotriesterases (PTEs) are a group of enzymes that can degrade OP compounds, and are found in microorganisms, animals and plants. There are three different types of well-characterized bacterial PTEs. This enzyme iscalled organophosphorus hydrolase (OPH) **[62]**and exhibits high catalytic activity, hydrolyzinga broad range of organophosphates throughcleavage of P-O and P-S bonds in these OP **[63].**

The most significant step in OP compound detoxificationis hydrolysis, since it makes compoundsmore vulnerable to further biodegradation **[64].** The mechanism of hydrolysis andits kinetic characteristics are well known **[65,66,67,68,69,70].** PTE has potentialfor use for cleaning up OP-contaminated environments.However, in previous laboratory trials using cultures in aqueous medium, it was observedthat this enzyme does not have any effect on someOP, which suggests a specificity in its activity thatdepends on the type of phosphoric acid substitutesused to form the OP **[43,70].**

Concluding statement

Considering demand on organophosphorus pesticides in agriculture, their use and toxicity, it is recommended that research on biodegradation particularly on bioremediation of sites contaminated with these pesticides will be needed in future.Bioremediation has been proven a suitable technique for reducing pesticide poisoning. In this context, isolation of various microbial strains can immensely help degrading various pesticide residues and can help in bioremediation of the contaminated sites. Repeated application of OP to the same soil results in rapid biodegradation of OP by soil bacteria **[5].**

Biodegradation of pesticides with specific microorganisms is economic and environmental and socially acceptable. By understanding the mechanisms for degradation, it is possible to develop technologies to increase the efficiency of degradation, such as the immobilization of cells in different support systems and the construction and use of bio beds for waste degradation *in situ*.

When few or no indigenous degradative microorganisms exist in a contaminated area and practically does not allow time for the natural enrichment of suitable population, inoculation may be a convenient option.In future we can use genetic engineering to improve the efficacy of microorganisms to reduce the environmental burden of toxic substances.There are reports of different organisms viz. bacteria, algae, yeasts, fungus and plants, characterized in relation to their genome and the enzymes that they produce, that can be used for waste treatment or bioremediation of soil and water contaminated with pesticides.

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