

## Review Article

# A Review on Microbial Degradation of Organophosphorous Pesticide: Methyl Parathion

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Organophosphorous pesticides are widely used in agriculture, though they are biodegradable in nature, some are acutely toxic and their residues are found in the environment. Various traditional physico-chemical techniques are used to detoxify these chemicals such as incineration, land filling excavation etc. But because of their tedious procedure and high expenditure cost, they are not used frequently. Microbial degradation is considered to be a major factor determining the fate of organophosphorous insecticides in the environment in a cost effective way. Thus the present paper reviews the efficacy and usefulness of bioremediation technique for the detoxification of methyl parathion pesticide in soil.

**Keywords:** Microbial degradation; Bioremediation; Pesticides; Organophosphorous pesticide; Methyl parathion; Xenobiotics

## Introduction

India is an agro based country, with agriculture being the main resource of living, for most of its population, thus in order to meet the requirement for food and to feed the growing population, tremendous pressure is exerted on the agricultural sector. Thus use of synthetic chemicals, good quality seeds and effective protection strategy are some of the techniques by which the farming community can increase the annual crop yield [1-3]. One of the major hindrances in the adequate production of these commodities is the loss caused by a range of biological entities known as pests, which include plant pathogens, weeds, insects, rodents and various other agents which causes significant losses in field, during transit and storage. Thus in order to protect the damages caused by these pests, a lot of novel synthetic chemicals are used [4]. As a result the air, water and soil in which our crops are grown and the environment in which the population of animals and plants grow is contaminated with a variety of synthetic chemicals. One such synthetic chemical is a pesticide. It is a substance/mixture of substances used to kill pest [5]. It may be a chemical substance, biological agent (such as virus or bacteria), antimicrobial disinfectant or device used against any pest. Pests include insects, plant pathogens, weeds, molluscs, birds, mammals, fish, nematodes (roundworms) and microbes that compete with humans for food, destroy property, spread or are a vector for diseases or cause a nuisance. The common formulations types of pesticides are liquids, wet table powder, emulsifiable concentrates and dusts. There are used for different purposes like to sanitize drinking and recreational water, to disinfect indoor areas (e.g. kitchens, operating rooms, nursing homes) as well as dental and surgical instruments. There are 4 major routes through which pesticides get in touch with the water, they may drift outside of the intended area when sprayed, they may percolate or leach through the soil, may be carried to the water as runoff or they may be spilled for e.g. accidentally or through neglect. They may be also carried to water by eroding soil. Factors that affect a pesticide's ability to contaminate water include its water solubility, the distance from an application site to a body of water,

weather, soil type, presence of a growing crop and the method used to apply the chemical [6]. Apart from air and water, migrating birds and oceanic currents may also contribute in pesticide migration from thousands of miles away from the site of application.

A rough estimate shows that about one third of the world's agricultural production is lost every year due to pests. In India, pests' cause crop loss of more than Rs 6000 crores annually of which 33% is due to weeds, 26% by diseases, 20% by insects, 10% by birds and rodents and the remaining 11% is due to other factors. The magnitude of the problem would speed up further, as different types of pests and diseases are likely to attack crops and therefore the need to use pesticides in different forms will be necessary in the years to come [7]. The manufacture of pesticides began in India in 1952 with the construction of a plant for producing BHC (Benzene Hexa Chloride) followed by DDT (Dichloro-Diphenyl-Trichloro-Ethane) [8]. Among the South Asian and African Countries, India is the largest manufacturer of basic pesticides next to Japan [9]. Presently, India is the 13th largest exporter of pesticides and disinfectant in the world. United Phosphorous Ltd, Gharada Chemicals and Excel are some of the Indian Companies which have not only developed products indigenously but have also emerged as prominent exporters.

## Classification of pesticides

On the basis of mode of action, pesticides may be classified as contact or non-systemic and systemic pesticides [10]. The contact or surface pesticides used in olden days were effective only at the surface level. In contrast, many of the current pesticides are systemic in character i. e. these can effectively penetrate the plant cuticle and move through the plant vascular system. Examples are provided by the phenoxyacetic selective herbicides, organophosphorous insecticides and the more recently discovered systemic fungicides like, benomyl. Unlike the contact pesticides these can not only protect the plant from fungal attack, but also cure or inhibit an established infection. These are little affected by weathering and also confer immunity on all new plant growth. Owing to their systemic, they are likely to be more

**Table 1:** Classification of pesticides on the basis of target.

Type	Target
Bactericide(sanitizers or disinfectants)	Bacteria
Defoliant	Crop foliage
Dessiccant	Crop foliage
Fungicide	Fungi
Herbicide	Weeds
Insecticides	Insects
Miticide(acaricide)	Mites and ticks
Molluscicide	Slugs and snails
Nematicide	Nematodes
Plant growth regulator	Crop growth processes
Rodenticide	Rodents
Wood preservative	Wood-destroying organisms

selective in their toxicity than contact pesticides. Synthetic pesticides have played a very crucial role in the success of green revolution and 1960s and 1970s are considered as the golden period for the synthetic pesticides. The (Tables 1-3) shows the classification of pesticides on the basis of target, mode of action and chemical nature [11].

The extensive use of pesticides exerts profound and lethal effects on wildlife populations and humans [12]. Nearly 500,000 illness and 20,000 deaths can be attributed annually to chemical pesticides. According to an estimate made by the WHO, each year 3,000,000 cases of pesticide poisoning including 2,20,000 deaths are reported across the globe [13]. Humans come in contact with these chemicals, whether in the field, during pesticide application, weeding, pruning, harvesting, re-entry to collect fire wood or vegetables, or in their homes, to kill mosquitoes, cockroaches, fleas and flies. Storing pesticides can cause acute and /or chronic exposures, with adverse health consequences. Although the inhalation, dermal and oral routes of exposure are most common but pesticide residues in food and water may add to indirect exposures commonly in the general population [14]. Farm workers who are engaged in thinning, cropping, loading, transplanting and transporting plants, mixing different types of pesticides and involve in spraying these pesticides show elevated level of organophosphates in their urine [15]. Previous studies revealed that the children’s of farm workers were highly exposed to different types of pesticides and as a result suffer from leukemia, cancer of brain, and kidney [16]. It was observed that the loss of fatal and miscarriage is quite common in spouses of pesticide applicators than the spouses of non- agricultural workers and is more common during the spray season [17].

Animals may be poisoned by pesticide residues that remain on food after spraying for e.g. When wild animals enter in the sprayed fields or nearby areas shortly after spraying. Poisoning from these chemicals can travel up to the food chain e.g. birds can be harmed when they eat insects and worms that have consumed pesticides. Some pesticides can also bio-accumulate or build up to toxic levels in the bodies of organisms that consume them over the time [18]. Residues of DDT, aldrin, dieldrin, deltamethrin are observed in fish tissues, lakes adjacent to the agricultural farms [19,20]. A number of food items have shown alarming level of pesticides, like tomatoes and its products have shown the presence of Lindane, Dieldrin, DDT,

**Table 2:** Classification of pesticides on the basis of mode of action.

Type	Action
Contact	Kills by contact with pest
Eradicant	Effective after infection by pathogen
Fumigants	Enters pest as gas
Nonselective	Toxic to both crop and weed
Post – emergence	Effective when applied after crop or weed emergence
Pre – emergence	Effective when applied after planting and before crop or weed emergence
Preplant	Effective when applied prior to planting
Protectants	Effective when applied before pathogen infects plant
Selective	Toxic only to weed
Soil sterilant	Toxic to all vegetation
Stomach poison	Kills animal pests after ingestion
Systemic	Transported through crop or pest following absorption

**Table 3:** Classification of pesticides on the basis of chemical structure.

By Chemical Nature
1. Pesticides can be either organic or inorganic chemicals. Most of today's pesticides are organic
2. Commonly used inorganic pesticides include copper-based fungicides, lime-sulfur used to control fungi and mites, boric acid for cockroach control, and ammonium sulfamate herbicides
3. Organic insecticides can either be natural (usually extracted from plants or bacteria) or synthetic. Most pesticides used today are synthetic organic chemicals. They can be grouped into chemical families based on their structure.

Dimethoate, Profenfos [21]. Organocholine pesticides like DDT was determined in food items such as milk, bread, coffee [22]. Bollen, et al. [23] emphasized the side effects of pesticides on soil microorganisms, since then several reports and reviews have appeared on the impact of pesticides on soil microorganisms and their activities [24-27]. Persistent organic pollutants, mainly emitted to the environment from anthropogenic sources, are in most cases lipophilic and can be characterized as toxic, stable and having a tendency to bioaccumulate and biomagnify [28].

A large number of pesticides manifest their toxicity through functional and biochemical action in central and peripheral nervous system, but there are also exceptions to this. In addition to acute effects, there is evidence that links to long term exposure to some pesticides with chronic diseases, including cancer, e.g. chloracne following exposure to chlorophenoxy acid derivatives, porphyria due to hexachlobenzene, and delayed neuropathy from exposure to some organophosphates. Cancers of the soft tissues (sarcoma), brain, lung, gonads, liver, the digestive system and the urinary tract have also been associated with exposure to some pesticides [29]. Illustrations of delayed toxicity are neuropathy caused by organophosphates esters, lung damage by paraquat and sensitization by pyrethroids [30]. Due to the environmental concerns associated with the accumulation of pesticides in food chain, there is a great need to develop safe, convenient and economically feasible methods for pesticide detoxification. Presently, incineration is the most proficient method for disposal of chemical compounds. But, environmental groups have opposed incineration as a remediation method as it releases several byproducts, which may pose significant environmental deterioration. Transport of these toxic compounds from storage to

disposal sites is both times consuming and risky, considering the danger of potential accidents. Further as expected, the population has openly opposed the installation of more incinerators within their communities near storage sites [31]. There are several other methods of disposal available, but most of these have undesirable limitations. Landfills are an adequate method, but leaching into the soil and groundwater may cause further contamination [32]. Other disposal methods involve removal, alteration or contaminant isolation. Usually, for treating contaminated soil, these techniques consist of excavation followed by incineration or containment. Moreover, these remediation technologies are expensive, and most of the time they do not destroy the contaminating compound but rather transfer it from one environment to another [33].

### Organophosphorous pesticide-methyl parathion

Organophosphorous pesticides are all esters of phosphoric acid and also called organophosphates, which include aliphatic, phenyl and heterocyclic derivatives and have one of the basic building blocks as a part of their much more complex chemical structure. Organophosphates 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. They were first developed by German chemists during the Second World War, since then some of them have proved to be chemical warfare agents [31]. Organophosphorous especially Methyl Parathion (MP) is acutely toxic. Methyl Parathion (O-dimethyl O-4-nitro-phenylphosphorothioate), has a molecular formula of  $C_8H_{10}NO_5PS$  with a molecular mass of 263.23. The trade names of MP are Azofos, Ekatox, Mepatox, Paratox. Pure MP is a white crystalline solid or powder, technical grade (80%) is a light to dark tan liquid. Solubility of pure Methyl Parathion in water is 55-60mg/L at 25°C, in addition it is readily soluble in most organic solvents viz, ethanol, chloroform and aliphatic solvents and slightly soluble in light petroleum. The odor of MP is like rotten eggs or garlic. Technical MP is available as a solution containing 80% active ingredient, 16.7% xylene and 3.3% inert ingredients [34]. It is one of the highly active thiophosphorous ester insecticides developed in the 1940s by the German pesticide company Bayer, and subsequently being produced worldwide by many companies. The production of MP in the year 1966 was 31,700 tones, from which 14,800 tones were produced in the United States [34]. MP was originally registered in the United States in 1954. However, its use has been restricted in this country since 1978 [24]. It is registered in at least 38 countries and widely used throughout the world [35]. It is used to control chewing and sucking insects such as aphids, boll weevils, and mites in a wide variety of crops, including cereals, fruits, vines, vegetables, ornamentals, cotton and field crops. It is a broad spectrum non-systemic pesticide that kills pests by stomach poisoning, it is an acaricide, and has some fumigant action. Since the cost of the pesticide is comparatively low and its effect is high, it is the most preferred chemical by many [36]. It acts by inhibiting acetylcholine esterase, an important enzyme in the nervous system. On exposure to MP, the enzyme is unable to function, causing accumulation of acetylcholine, which interferes with the transmission of the nerve impulse at the nerve endings. In human beings MP poisoning causes the following symptoms—general weakness, headache, excessive sweating, salivation, nausea, vomiting, diarrhea, abdominal cramps and tremors. In very severe

cases respiratory failures may lead to death. Most of them have a short residual activity, which is desirable in case of food crops, but a problem when longer protection is required then repeated application becomes necessary [37].

### Bioremediation—a potential detoxification technology

Bioremediation overcomes the limitations of traditional methods of hazardous chemical disposal by bringing about the actual destruction of many organic contaminants at reduced cost. Many extraordinary characteristics of microorganisms such as their small size, ubiquitous distribution, high specificity, surface area, potentially, rapid growth rate and unrivaled enzymatic and nutritional versatility make them, as one of the most important recycling agents of pesticides in nature. Biodegradation is a metabolic process that involves the complete breakdown of an organic compound and when this compound is broken down into its inorganic forms, the process is referred to as mineralization. In the context of environmental sciences, it is defined as the use of biological agents to eliminate hazardous substances from the environment [38]. Xenobiotic compounds are those having a molecular structure, to which microorganisms have not been exposed, therefore, they, may be recalcitrant or resistant to bioremediation or not completely degraded. Biodegradability represents the susceptibility of substances to be altered by microbial processes. The alteration may occur by intra or extra cellular enzymatic attack that is essential for growth of the microorganisms. The attacked substances are used, as a source of carbon, energy, nitrogen or other nutrients or as final electron acceptor. Co metabolic reactions occur when enzymatic attack is not necessarily beneficial to the microorganism's i.e. a physiologically useful primary substrate induces production of enzymes that fortuitously alter the molecular structure of another compound.

Bioremediation is the collection of number of processes like bioaccumulation, biodegradation, biostimulation and bioaugmentation; all involves living organisms but using different strategies for using them. The technique that involves the stimulation of microorganisms by adding the nutrients and other chemicals required for the metabolism of microorganisms that encourage the degradation of contaminants of native microorganisms at the contaminated site, is biostimulation. This is one of the most preferred and cheapest techniques to decontaminate the environment. If the contaminated site have no native microorganisms, then another way is to introduce some microorganisms that have ability to degrade these contaminants that is known as bioaugmentation, in this technique genetically modified microorganisms can also be used. Another way, is to isolate and purify the enzymes from the microorganisms and introduce into the contaminated area either land or water, to decontaminate it from the contaminants. Decontamination by the organisms depends on three factors, the type of microorganisms, the type of contaminant and the geological and chemical conditions of the contaminated site [39,40].

There are two main approaches proposed for the bioremediation with microorganisms. In the first case microorganisms can be applied directly to degrade pollutants and wastes in a reactor or in situ. In second case, cell extracts or purified enzymes preparations of microbial origin could be used for decontamination purposes. [41].

Direct application of microbes in contaminated sites for the

remediation of toxic chemicals is a cheapest way of remediating the toxic compounds, when conditions are favorable for the growth of microorganisms, but if the conditions are unfavorable, their growth would be very slow and sometimes they die. In these conditions the addition of nutrients plays a vital role in the bioremediation process, that enhances the growth of microbes and ultimately the rate of biodegradation [42].

### Bacterial degradation of methyl parathion

Early studies provided the evidence that microorganisms played a vital role in enhancing the degradation of MP. Siddaramappa et al. [43] reported two bacterial species, *Bacillus* sp. *Pseudomonas* sp. which was able to hydrolyze MP, using the hydrolysis product Para-Nitro Phenol (PNP) as a sole source of carbon. Munnecke et al. [44] isolated a mixed microbial culture which was able to grow on parathion. The strain was able to degrade 50mg of parathion per liter per hour. Rosenberg et al. [9] isolated two *Pseudomonas* sp. that were able to hydrolyze a number of organophosphorous compounds including parathion, and use the ionic cleavage products as a sole source of carbon. Choudhary et al. [20] isolated two mixed bacterial cultures by soil enrichment that was capable of utilizing MP as a sole source of carbon. Singh et al. [32] reported that in case of most of the transformations, co- metabolism is involved where initial growth is mediated by obligatory presence of a growth substrate or another transformable compound. Misra et al. [45] has isolated a *Flavobacterium* sp. the strain was able to use MP as a sole source of carbon. Prakash et al. [46] reported a *Pseudomonas* sp. This was able to utilize Para Nitro Phenol (PNP) as a source of carbon and nitrogen. Ramanathan et al. [47] reported complete mineralization of MP by *Pseudomonas* sp. A3. The strain readily utilized MP as a sole carbon and/or phosphorous source. In 40 hrs the cells completely removed MP (100%) from the medium as a sole carbon source, whereas 93% of MP was degraded while it was provided as a phosphorous source. Over this period, only 65% of MP could be degraded and complete disappearance of this compound was noted in 56 hrs. When the cells derived both carbon and phosphorous sources from this insecticide alone. Mallick et al. [48] reported another organophosphorous pesticide chloropyrifos which was utilized as carbon source by *Flavobacterium* and *Arthrobacter* sp. Zhang et al. [49] reported the maximum MP degradation after 30 days of incubation by a bacterial culture. Yu and Yu [50] have reported that *Pseudomonas aeruginosa* mineralized p-nitro phenol more rapidly when all biodegradable carbon sources completely utilized in the culture medium. Laura et al. [51] recovered a MP degrading consortium of bacteria from agricultural soils in central Mexico, using MP as the only carbon source. Zhongli et al. [37] isolated a MP degrading strain M6 and reported a absorption peak at 310nm which is due to appeared, end product, PNP. Ogel et al. [52] reported that time course required to reach maximum levels of activity may be affected by several factors, like the presence of different ratios of amorphous to crystalline substances. However, further increase in the incubation time, reduced the rate of MP degradation. It might be due to the depletion of macro and micro- nutrients in the aqueous medium with the lapse in time, which stressed the microbial physiology resulting in the inactivation of secretary machinery of the enzyme [53,54]. Keprasertsup et al. [52] reported degradation of MP in aqueous medium by the soil bacteria *Burkholderia cepacia*. This bacterium degraded MP at 50, 100, and

150ug/ml in standard Basal Mineral Salts Medium (BMM) rapidly. Furthermore, Para-Nitro Phenol (PNP) - a hydrolysis product of MP was completely degraded by *Burkholderia cepacia*. Singh et al. [55] identified a robust bacterial population that utilized chloropyrifos as a source of carbon in an Australian soil. Singh et al. [56] have reported that *Enterobacter* strains-14 utilized chloropyrifos as a sole source of carbon and phosphorous. Liu et al. [57] reported the utilization of MP as a sole source of carbon, energy and nitrogen by *Pseudomonas* sp. WBC-3. Labana et al. [58] carried out a microcosm study on the bioremediation of PNP contaminated soil using *Arthrobacter protophormiae* RKJ100 and effective biodegradation over the range 1. 4-210 ppm, at 20-40°C, 7. 5 pH when applied @ 2x10<sup>8</sup> CFU/gm soil. Similar results has also been recorded by Ahmed et al. [59] who reported the time course of 15 days for MP degradation from *Pseudomonas* sp. Pakala et al. [60] reported biodegradation of MP and PNP by a gram negative *Serratiasp.* strain DS001. The bacteria were capable of utilizing MP as a carbon source. They reported for the first time the existence of PNP hydroxylase component "A" typically found in gram positive bacteria, in a gram negative strain of the genus *Serratiasp.* Gomes et al. [61] also reported the maximum time for MP degradation of 30 days by *Chrobactrum* B2 and *A. Xylooxidans*. Suresh et al. [62] isolated an isolate of *Serratia* sp. from soil bacterium capable of utilizing MP as sole source of carbon and energy. Duration required for maxima MP degradation depends upon the composition of aqueous medium and bacteria used as studied by Ojumu et al. [63] and Suzuki & Watanabe [64]. Fang-yao et al. [14] reported biodegradation of MP by *Acinotobacter radioresistens* USTB-04. An initial concentration of 130mg/ L was completely biodegraded in 2h in the presence of cell free extract with a protein concentration of 148.0mg/L which was increased with the increase of pH from 5-8. Krishna et al. [65] reported degradation of mixed pesticides (lindane, carbofuran and MP) by mixed pesticides enriched cultures. After seven weeks of incubation enriched cultures were able to degrade 72% lindane, 95% carbofuran and 100% of methyl parathion in facultative co- metabolic conditions. In aerobic systems, degradation % of lindane, MP, and carbofuran were increased by the addition of 2grams/L of dextrose. Ghosh et al. [66] have also reported similar observation with *Pseudomonas* sp. & *Fransicella* sp. which were able to degrade MP with the formation of PNP. Shen et al. [67] reported degradation of MP by a *Stenotrophomonasp.* SMSP-1. This strain could hydrolyze MP to PNP. Pirie et al. [68] undertook a microcosm study on PNP biodegradation in soil slurry by *Alcaligenes faecalis* and stated that temperature, inoculums size, yeast extract concentration, pH and soil-water ratio were the most effective factors responsible for PNP biodegradation. Naqvi et al. [69] reported similar observation in case of biodegradation of carbaryl in soil. Pino et al. [70] isolated a selected microbial consortium capable of degrading MP and PNP from the contaminated soil site. In culture, the consortium was able to degrade 150mg/L of MP and PNP in 120 hrs, but after adding glucose or peptone to the culture media, the time of degradation decreased to 24 hrs. In soil, the consortium was able to degrade 150mg/L of MP in 120 hours at different depths and also managed to decrease the toxicity. Wang et al. [71] reported biodegradation of MP and PNP by a *Agrobacterium* sp. strain Yw12. The strain completely degraded MP and PNP and utilized MP as a sole source of carbon and energy for its growth. Pradeep et al. [72] reported a soil bacteria capable of degrading an organophosphorous pesticide

chlorpyrifos and endosulfan. Greeshma et al. [73] reported the toxicity and bioremediation of pesticides in agricultural soil Gang et al. [74] reported the influence of kaolinite and goethite on microbial degradation of MP and observed that biodegradation was improved by kaolinite and depressed by goethite. Verma et al. [75] reported the pesticide relevance and their microbial degradation in soil. Hassan et al. [76] reported different factors that are responsible for the degradation of pesticides in soil.

## Conclusion

The use of chemical pesticides has bestowed many benefits on the farming community such as the enhancement in agricultural production, soil productivity and products quality which is reflected in economic benefits. But this is only the fruitful side of these synthetic chemical, their extensive utilization causes deleterious effect on the ecosystem. For this reason, it is necessary to generate strategies for waste treatment and /or for the remediation of polluted sites. Uses of microbes for the detoxification of these toxic chemicals have proved to be an important technology from an economical and environmental point of view. Presently, the use of indigenous or genetically modified organism to degrade or remove pesticides has emerged as a powerful technology for in situ bioremediation. This is the review compiling the worked done by the researchers towards the microbial degradation of methyl parathion pesticide and it will be helpful to the researchers who plan in future to work in this direction.

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