



## Review Article

# A Review on In situ Biodegradation of Methyl Parathion through Soil Microbes

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## ABSTRACT

### Keywords

Organo-phosphorus, Methyl parathion, Bioremediation, Soil Bacteria

Methyl parathion (*O*, *O*-dimethyl *O*-*p*- nitrophenyl phosphorothioate,MP) is an popular organophosphate insecticides, used extensively and globally for the control of a wide range of insect, pests. Its residues are detected in fruit and vegetables and classified by the world health organization (WHO) in the class of extremely hazardous pesticides. It inhibits the acetylcholinesterase activity an important enzyme in the nervous system. Bioremediation is a technique by which the reduction or elimination of poisonous chemicals from soil, sediment water, or other contaminated materials is possible with the help of microorganism. Soil is a good habitat for microorganisms. Soil microflora is potential candidate for detoxification of pesticides. Soil microbes attack on wide range of organophosphorus insecticides. In this review we focused on rapidly degradation of methyl parathion by soil bacteria at optimized environmental conditions.

## Introduction

Orgnophosphorus pesticides (OP) were first developed in Germany by Schrader in 1930 during World War II in the form of tetraethyl pyrophosphate as a byproduct of nerve gas production (Dragun *et al.*, 1984). Various groups of pesticide are used world over, but OP insecticides are most widely used in agriculture field for protecting the crops which are harmed due to attack of insects, bacterial, viral, and fungal pests. OP pesticides also used in homes, garden and in veterinary practices. In the United States, over 40 million kilograms of OP pesticides are applied annually

(Mulchandani *et al.*, 1999). OP pesticides are acutely toxic and act by inhibiting the acetylcholine esterase, an important enzyme in the nervous system (Kanekar *et al.*, 2004). When animals are exposed to OP, this enzyme is unable to function causing the accumulation of acetylcholine, which interferes with the transmission of nerve impulses at the nerve endings (Synapses). OP pesticides are mobile in soil. It is hydrophilic in nature and its little amount is absorbed by soil particles. Continuous use of OP pesticides contaminates different ecosystem of the world (US EPA, 1995;

McConnell *et al.*, 1999; Cisar and Snyder, 2000; Singh and Singh, 2003; Tse *et al.*, 2004). It is highly toxic substance to birds with the LD<sub>50</sub> being 0.9-6.7 mg Kg<sup>-1</sup> (IPCS, 1993). Karalliedde *et al.* (1999) and Sogorb *et al.* (2004) has reported that it is causing around 3 millions poisoning with 20, 0000 deaths annually.

OP insecticides are esters of phosphoric acid, which include aliphatic, phenyl and heterocyclic derivatives and have one of the basic building blocks as a part of their complex chemical structure. Some of the main OP insecticides are parathion, methyl parathion, chlorpyrifos, malathion, monocrotophos and dimethoate. But in this we are focusing about only methyl parathion organophosphorus pesticide.

Methyl parathion is an organophosphate pesticides used extremely for agriculture crop protection (Kumar *et al.*, 1996). It has been banned in many countries because of its higher toxicity for mammals (Keprasertsup *et al.*, 2001; Sharmila *et al.*, 1989). It is highly toxic pesticide with trade name Dimethyl parathion, Mepaton, Mepatox, Methyl E-605 and Methylthophos displaying insecticidal activity against a wide range of insect and arthropod pests (FAO/UNEP, 1996; US EPA, 2003; Barbalace, 2006; Orme and Kegley, 2006). Methyl parathion may also be found in compounds with other insecticides such as acephate, camphechlor, carbaryl, carbophenothion, cypermethrin, dicofol, ethylparathion, ethion, lindane, methoxychlor, monocrotophos, phosal, propargite, petroleum oils, and tetradifon (Kidd and James, 1991; US EPA, 2003). Methyl parathion is formulated as a number of different commercial products. The most commonly available formulations include a wettable powder (WP), emulsifiable concentrate (EC), dustable powder, and

Ultra-low volume liquid and microencapsulated product (FAO, 1997; US EPA, 2003). It is used for control of insects and mites having chewing and sucking type of mouth parts, including thrips, weevils, aphids and leafhopper in a very wide range of crops, including cereals, fruit, nuts, vines, vegetables, ornamentals, cotton, and field crops (Kidd and James, 1991; US EPA, 2003). It kills insects and mites by contact stomach and respiratory action (Balamurugan *et al.*, 2010). Methyl parathion is highly toxic for warm blooded animals the mammals and birds (CICOPLA-FEST, 1997). It is potent inhibitors of acetylcholinesterase activity, an important enzyme in the nervous system. It is applied to agricultural crops by aerial or ground spraying equipment. Methyl parathion has been detected in surface waters and sediments, rainwater, aquatic organisms, cereals and pulses.

Methyl parathion can cause death by oral, dermal or inhalation exposure and may cause deaths. Males may be more susceptible than females to acute effects, and children are more susceptible than adults (PAN AP, 2008). Methyl parathion cause different types toxicity, acute toxicity, sub-chronic toxicity, chronic toxicity, genotoxicity, oncogenicity, reproductive toxicity, developmental toxicity, developmental neurotoxicity, immune toxicity and hematologic effects (CEPA, 2010).

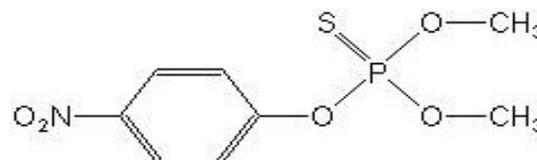
Methyl parathion degradation is very slow and takes several months to degrade when applied in soil as a pesticide. When large concentration of methyl parathion applied in soil as in an accidental spill, the degradation occurs after many years (Howard, 1989). According to many authors (Pritchard *et al.*, 1987; Mathew *et al.*, 1992; Buerger *et al.*, 1994; Reddy *et al.*, 1994; Ortiz *et al.*, 1995) the half life of methyl parathion in natural

water is about one month, which is long enough to be toxic to many aquatic and terrestrial organisms.

Insecticides and their degradation products generally get accumulated in the top soil and influence not only the population of various groups of ecofriendly soil microbes but also their biochemical activities like nitrification, ammonification, decomposition of organic matter and nitrogen fixation (Agnihotri *et al.*, 1981).

### Physical and chemical properties of methyl parathion (IPCS, 1992)

The physical and chemical properties of this compound are given below Structure of MP:-



\*Structure according to California Department of Pesticide Regulation (1999)

Molecular formula	C <sub>8</sub> H <sub>10</sub> NO <sub>5</sub> S
IUPAC name	O, O- dimethyl O-4-nitrophenyl phosphorothioate
CAS No	298-00-0
Common name	Methyl parathion, Parathion methyl, metaphos (World health Organization, 1993)
Melting point	35-38°C
Boiling point	143°C
Vapour pressure	1.3m Pa at 20°C
Water solubility	55-60 mg/litre at 25°C
Log octanol- water Partition coefficient	1.83-3.43
Freezing point	about 29°C (technical product)
Non aqueous solubility	Soluble in ethanol, Chloroform, aliphatic solvents and slightly soluble in light petroleum. (World health Organization, 1993)
Odour	like rotten eggs or garlic (Technical grade) (Midwest Research Institute, 1975; Anon, 1984)

### Fate of Methyl parathion in environment

The environment consists of a series of compartments like soil, water, air and other living macro and microorganisms. Once a pesticide enters the environment, its continuous transformation from one component to another occurs. These processes, in turn, are mediated by several significant biological activities which can be determined by the quantity of residual pesticides that persists in anyone compartment for a certain period of time.

The distribution of MP in air, water, soil, and organism in the environment is

influenced by several physical, chemical and biological factors (WHO, 1993). Methyl parathion is an organophosphate compound, which have been used for the control of a wide range of insect pests all over the world. When dispersed in the air, water and soil it becomes pollutant. Pollution caused by both excessive and continuous use of pesticide. These pesticides enter the environment by diverse modes such as accidental spills,

direct applications residuals due to facility cleaning of containers etc (Ortiz-Hernandez *et al.*, 2001). Many scientists have studied the degradation of MP and the concentration of its metabolites present in soil sediment, air and water. According to Howard (1989), MP takes several months to degrade when applied in soil but when it is applied in higher concentration in soil, as in an accidental spill, its degradation takes several years. According to Pritchard *et al.* (1987), Buerger *et al.* (1994) the half life of MP in natural water is about one month, which is long enough to be toxic to many living organisms in the environments.

Stanley *et al.* (1971) collected the ambient air sample from both high and low usage seasons. After 12 or 24 hrs they found no detectable concentration of MP at the site in California near the cities of Fresno and Riverside and detectable concentration as high as 148 ng/m<sup>3</sup> (average 29 ng/m<sup>3</sup>) in the rural areas of three of the eight states. Arthur *et al.* (1976) reported the maximum concentration of MP 2,060 ng/m<sup>3</sup> in ambient air. Kutz *et al.* (1976) monitored ambient air at sites located in 15 different states where the MP was used. No detectable concentration of MP was observed in 85% samples. The higher concentration was detected in Oklahoma. Kutz, (1983) monitored ambient air at 10 locations in eight states (two urban locations in California) for detecting breakdown product of MP and ethyl parathion. Out of 123 samples, 15 samples showed the positive result. Average equivalent concentration was 2.9 ng/m<sup>3</sup> with maximum level as high as 160 ng/m<sup>3</sup>. Sava (1985) monitored the air of three residential sites of agricultural land in Salinas and California. They detected 17 ng/m<sup>3</sup> MP only in one sample in cotton field. According to IPCS (1992) MP was mostly used for protecting cotton field and reported that partition of MP found in air

and soil in environment and less in amount in plant and animal. It also concluded that no movement of MP and its breakdown product was through soil to ground water. It also reported that the concentration of MP after spraying declined rapidly over 3 days and returned to background level after 9 days. WHO (2004) examined in agricultural area of USA and observed that the concentration of MP in natural water was up to 0.46 µg/litre but higher level was recorded in summer.

WHO (2004) have reported the concentrations of MP in food is also present in very small amount, in different regions the world. In the USA residues of MP in food have generally been reported at very low level. Methyl parathion residues were highest in leaf up to 2 mg/kg and root vegetables up to 1 mg/kg reported in the USA. Zhang *et al.* (2002) reported the MP from soil near Shanongda Pesticides Company in Hubei China. MP has been detected in ambient air, surface water, groundwater, rainfall, coastal fog, soil, sediments, fish, human breast milk, umbilical cord blood, and foods (PAN AP, 2008). Methyl parathion is relatively more mobile in soil (US EPA, 2003). It degrades rapidly in flooded non aerobic soil where its half life period is 7 days but in aerobic flooded soil its half life period has been estimated as 64 days (ATSDR, 2001). Castilho *et al.* (2000) studied that the half life of MP in water is 24-28 days. They studied the degradation of MP in 12 rivers of US cotton and rice growing regions, where the concentration of MP was 0.42 ppb to 6 ppb. Tariq *et al.* (2004) detected the MP in ground water in cotton growing districts of Punjab and Pakistan. MP was also detected in Ganges in India (Rehana *et al.*, 1996). Hermanson *et al.* (2005) examined the residues of MP in ice cores collected from the Austfonna ice cap in the Svalbard

archipelago in the arctic. Coupe *et al.* (2000) measured the highest concentration of MP in rain water.

WHO (1993) reported the concentration of MP in environment. They performed experiment in the towns in the centre of agricultural areas of the USA. WHO also examined the seasonal variation of MP concentration and observed mainly in the range of 100-800 ng/m<sup>3</sup> in August/September. They reported the concentration of MP in natural water of agricultural area in the USA and recorded the level ranged up to 0.46 ug/L in summer.

### **Application of Methyl parathion**

Methyl parathion is an OP insecticide and acaricide, used to control boll weevils and many insect pests of agricultural crops, having the biting or sucking type of mouth parts (Adhya *et al.*, 1981). Methyl parathion, after ingested by the insect, is oxidized by the enzyme Oxidases in the insect body, to give paraoxon, replacing the double bonded sulphur with oxygen. This nature of MP offers the fundamental advantages in controlling the pests in better way. It kills insects by contact, stomach and respiratory action (Tomlin, 1994).

WSDA (2006) reported that MP at 0.25% to 0.5% is active ingredient per acre to control the Alfa alfa caterpillar, Aphids, Army worms, Grass hopper. NRA reported that MP is used in agriculture field to control *Helicoverpa* larvae, mites scale aphids, mealy, bugs, lucerne, fleas and thrips.

### **Residues of Methyl parathion in environment**

According to the WHO (1993) reports the low concentration MP residue in the food in the USA with few exceptions of individual

vegetables samples, it was up to 1 mg/kg in leaf and in root up to 1 mg/kg, in survey in the USA between 1966 and 1969.

OP pesticides are degraded quickly in water. There is always the possibility that residues and by products will present in relatively harmful level in the organism (Silva *et al.*, 1999; Ragnarsdottir, 2000). Residues of MP have been recorded in many foods, even occasionally in milk (ATSDR, 2001) and in drinking water sources such as in China (Na *et al.*, 2006).

Sanghi *et al.* (2003) reported residues of organochlorine and organophosphorus pesticides in breast milk samples from Bhopal. They observed that through breast milk infants consumed 8.6 times more endosulfan and 4.1 times more malathion. Organochlorine and organophosphorus insecticide residues in market samples of meat were also reported by Suganathy and Kuttalam (2003).

### **Toxicity of Methyl parathion**

OP insecticide exhibits the high oral and moderate dermal toxicity with a half life of 14-21days. The toxicologically relevant mode of action is the inhibition of choline esterase activities (Skripsky and Loosli, 1994) and also various clinical effects occur due to OP poisoning in human (Serdar *et al.*, 1985; Serdar, 1996). MP is lipid soluble and it can penetrate in skin also. It also enters the body through the respiratory and gastrointestinal tracts and when absorbed through alimentary canal, it is stored in adipose tissue. Its primary mechanism for toxicity is its slow release into the blood stream and subsequently to the nervous system. Some also enters the liver, where it is changed into the more harmful methyl paraoxon. Methyl parathion and its metabolite may be transferred via the

placenta to the developing foetus (Edwards and Tchounwou, 2005).

### **Acute toxicity**

Normal functioning of the nervous system and brain is interfered by MP (ATSDR, 2001). Methyl parathion acetylcholinesterase inhibiting activity causes the accumulation of the neurotransmitter acetylcholine in the nerve synapse. According to CEPA (2010), when the rats are treated with MP in the laboratory, they appeared to be most sensitive species. In the rats, the median oral lethal doses (LD<sub>50</sub>) ranged between 6-50 mg/kg (Category I oral toxicant), the dermal rat LD<sub>50</sub> was 67 mg/kg (Category I dermal toxicant) indicating that the toxicity of MP via oral route or via skin is comparable. An acute (Single dose) oral exposure of rats to MP caused decrease in cholinesterase activities in the brain.

### **Sub-chronic toxicity**

When the rats were exposed by oral and dermal routes with the sub-chronic MP, the Inhibition of the ChE activities in brain was recorded and due to this the plasma and erythrocytes became most sensitive for toxicological endpoint.

The cholinergic signs including constricted pupils tremors, gait abnormalities decreased activity, abnormal breathing and impairment of the cognitive and motor functions. The deaths were observed in 5 to 95-day treatment (CEPA, 2010).

### **Chronic toxicity**

Decreased hematocrit and erythrocyte level caused the retinal regeneration and sciatic nerve degeneration also by the MP due to the chronic toxicity of methyl parathion (US EPA, 2003).

Sub chronic exposure to low doses of MP, caused abnormalities in the functions of enzymes in the liver and plasma, which is the indication of the cellular toxicity (Kaur and Dhanju, 2004). Chronic dietary exposure to MP of rats decreased the cholinesterase activities, neurological signs hematological effects and nerve demyelination. The reduction of the ChE activity in the mice brain was the most sensitive toxicological endpoint (CEPA, 2010).

### **Genotoxicity**

MP was observed as genotoxic in *in vitro* and *in vivo* causing gene mutations in bacteria, chromosomal aberrations in mammalian cells, sister chromatid exchange (SCE); and was positive on the sex-linked recessive lethal assay in *Drosophila*. *In vitro*, MP showed binding directly to the cellular DNA (CEPA, 2010).

### **Reproductive toxicity and developmental toxicity**

The effects of MP on reproduction included: alteration in the levels of the luteinizing hormone in serum and early menopause in humans, decreased pup survival in rats, possible ovarian toxicity in rats and sperm abnormalities in mice. The lower fetal body weight, increased resorption, reduced pup survival, abnormalities and variations of ossification, and cleft palate are the main reproductive abnormalities caused by MP in rats, mice and rabbits (CEPA, 2010).

Detrimental effects on the reproductive organs of both male and female along with the degeneration of placental cells have been reported by Edward and Tchounwou (2005). Methyl parathion caused fibrosis and hemorrhage of the endometrium during pregnancy and significant changes in the duration of oestrus cycle (Edward and

Tchounwou, 2005). It also causes cytotoxic damage and tubular atrophy in the testis of rats (Narayana *et al.*, 2006). Newborn animals are more sensitive for acute lethality than adults (Liu *et al.*, 1999; US EPA, 2003).

### **Neurotoxicity**

Studies of developmental neurotoxicity revealed an increased sensitivity of immature rats to the inhibition of the ChE activity compared to adult rats after single or repeated exposures to MP (CEPA, 2010). The exposure of MP caused the neuropathology and acetylcholinesterase inhibition in the central nervous system, red blood cells and plasma (US EPA, 2003).

Edward and Tchounwou (2005) reported that MP also causes tremor, irritation and purposeless chewing, lacrymation, reduced spontaneous locomotor activity, neuromuscular coordination, and impaired memory due to exposure of MP.

### **Immunotoxicity**

Repetto and Baliga (1996) reported the effect of MP on the immune system. The weight of the thymus gland in rat and rabbit was decreased due to its effects. They also recorded the decrease in the secondary antibody response in rat. Exposure of a mixture of MP and chlorpyrifos, at 1/30<sup>th</sup> the LD<sub>50</sub>, affected immune function in rats (Liu *et al.*, 2006). CEPA (2010) reported that in rats MP the lymphoid depletion and necrosis of spleen and thymus increased due to the MP exposure. It also decreased IgG production

### **Endocrine disruption**

US EPA, (2003) reported that MP is an endocrine disruptor in mammals. ATSDR

(2001) reported its weak oestrogenic activity. Liu *et al.* (2006) reported the exposure of a mixture of MP and chlorpyrifos at 1/30<sup>th</sup> the LD<sub>50</sub> increased oestradiol level in male and female rats.

WHO (1993) reported the short term toxicity of MP, using various routes of administration on the rat, dog and rabbit and observed the inhibitions of plasma, red blood cell and brain ChE, and related cholinergic signs. Long term toxicity/carcinogenicity studies were also carried out on mice and rats.

When MP was inhaled, the first adverse effects were a bloody or runny nose, coughing chest discomfort and difficulty in breathing. Skin contact may cause localized sweating and involuntary muscle contractions. The further continuous effects may include nausea, vomiting, diarrhoea, abdominal cramps, headache, dizziness eye pain blurred vision, constriction or dilation of the pupils, tears, salivation, sweating and confusion. In severe cases its poisoning affected the central nervous system, caused slurred speech, loss of reflexes, weakness, fatigue and eventual paralysis of the body extremities and respiratory muscles. Death may be caused by respiratory failure cardiac arrest (EPMP US, 1994).

### **Effects of methyl parathion and other Organophosphorus insecticides on the activities of soil microorganisms**

The recycling of essential plant nutrients, humus formation and soil structure stability are main functions of soil microorganisms. Soil fertility depends on type of microorganisms involved in ammonification, nitrification, denitrification etc. The wide use of organophosphorus pesticide has created the pollution of the environment and also may disturb the

equilibrium and thus fertility of the soil is ultimately affected.

Sultan *et al.* (2010) conducted the experiment in laboratory to study the effect of various OP pesticides on soil bacterial population. Various concentrations of Chlorpyrifos (1000 ppm), Polytrin (2000 ppm), Endosulfan (100 ppm) and phorate (10,000 ppm) were used to check the effect on total number of bacterial population. Chlorpyrifos showed significant reduction in the bacterial colonies than other OPs pesticides.

Farrukh and Ali (2011) reported the effect of Dichlorvos on growth, reproduction and avoidance behavior of earthworm *Eisenia foetida*. They observed that the weight of earthworm was decreased and reproduction and behavior was significantly affected by dichlorvos exposure. Gilani *et al.* (2010) studied the effects of 100 ppm and 1000 ppm chlorpyrifos on microorganisms of agriculture soil from a depth of 0-25 cm. Samples were kept under darkness; microbial activity was monitored after 6 months and one year. The result showed that the growth of *Bacillus* sp. was sensitive but a number of colonies of *Klebsiella* sp. were enhanced during the same treatment in the experiment. Adiroubane *et al.* (2003) conducted study to observe the impact of monocrotophos 36 SL and phosphamidon 85 EC on the total heterotrophic bacterial and nitrogen fixing bacterial population in the rhizosphere soil and phyllosphere of irrigated rice ecosystem. They observed a sudden reduction in bacterial population immediately after spraying, but subsequently the recovery was also recorded the influence of insecticides on soil microorganisms. The influence of pesticide on biochemical activities such as nitrification, ammonification, respiration,

nitrogen fixation *etc* was studied by Agnihotri *et al.* (1981).

Aggarwal and Gupta, (1996) worked on the effect of organophosphorus pesticides amidithion, DDVP and phosphamidon on nitrification and ammonification by soil microbes. The nitrification and ammonification processes was not affected but slightly stimulated at lower concentrations (25 and 125 ppm), but it was decreased at higher concentration of 1250 ppm. Garg and Tandon (2000) conducted research on the effect of OP pesticides on bacteria and actinomycetes of salt affected alkaline soil of Banasthali region. The effect variation in *Bacillus*, *Arthrobacter*, *Xanthomonas* and *Actinomyces* population was an indication of either stimulatory or inhibitory effects on different microbial groups and had some role to play in the degradation of OPs pesticides.

The effects of pesticides such as MP, DDT and lindane on soil microflora, under laboratory conditions, were investigated (Singh and Alka, 1998). Slight changes in the fungal and bacterial population were recorded after 5 days but the higher inhibitory effect was observed after 20 days. MP showed maximum inhibitory effect than the lindane and DDT. Babu *et al.* (1998) studied the effect of pesticide on protease activity of soil. The effect of two pesticides, singly and in combination of Quinalphos and Cypermethrin, was studied at concentration ranging 5 to 25µg/g soil. Protease activity of soils in combination with the pesticides was greater than or equal to sum of protease activity obtained from soils treated with one pesticide at lower concentration. They concluded that it is due to synergistic or additive interactions respectively. While the same combinations at higher concentrations, reacted

antagonistically by inhibiting 10-20 % higher protease activity in soil.

Singh *et al.* (2002) studied the effect of organophosphorus pesticide chlorpyrifos, fenamiphos and chlorothalonil alone and in combination on soil microbial activity. They observed that the measured soil microbial parameters, especially the enzyme activities and total microbial biomass, was stable in the pesticide free control soils throughout 90 days of inoculation period, but they were all adversely affected in the presence of added pesticides. Menon *et al.* (2004) reported that the arginine ammonification activity of rhizosphere microorganisms was inhibited by chlorpyrifos and its metabolites 3,5,6-trichloro-2-pyridinol and 3,5,6-trichloro-2-methoxypyridine (TMP) in loamy sand and sandy loam soils after seed treatment with chlorpyrifos (5 gm/kg seed). They also observed the stimulation of rhizospheric N mineralization by parent compound but inhibition was recorded by its metabolites.

Martinez-Toledo *et al.* (1992) studied the effect of two methyl pyrimifos and chlorpyrifos, OP pesticide on soil microflora in an agricultural loam. The methylpyrimifos of 100 to 300 $\mu\text{g g}^{-1}$  or chlorpyrifos 10 to 300  $\mu\text{g g}^{-1}$  concentrations, showed the significant decreased aerobic dinitrogen fixing bacteria and dinitrogen fixation activities. The fungal and denitrifying bacterial populations were not affected by the addition of the OP pesticide to the agricultural soil.

Ahmed and Ahmad (2006) studied the effects of Chlorpyrifos 40EC, Imidacloprid 200SL, Cypermethrin 10EC, Endosulfan 35EC, Carbofuran 20EC, Bifenthrin 10EC and Cypermethrin 10EC (OP pesticides). The effects of four concentrations of i.e. 125, 250, 500 and 1000 ppm were studied on the number of soil bacterial populations.

It caused the significant reduction in number of soil bacteria. However in the field experiment, effect disappeared after 21 days of application. Roger *et al.* (1994) reported the effect of pesticide on soil and water microflora and mesofauna in wetland rice field. They reported damaging effects on microbial population or their activities when pesticide was applied on soil and water.

### **Bioremediation**

Biodegradation or bioremediation is techniques to use the living organisms to solve an environmental problem such as contamination and pollution of soil and groundwater. It's based on that different or single microbe functioning together to detoxify or remove the hazardous or pollutants, such as pesticides from the environment (Gibson and Sayler, 1992). Bacteria and fungi are significant detoxifiers of pesticides, herbicides and other toxic compounds. Microbes use these compounds for their metabolism and growth. They convert toxic organic compounds into non-toxic intermediate compounds, CO<sub>2</sub> and Methane gas (Singh, 2008). These organisms may be naturally occurring or cultured in laboratory. Bioremediation processes apply for numerous applications, including clean up of ground water, soil lagoons, sludge and process waste streams (Boopathy, 2000). Uses of such pesticide degrading microbial systems for bioremediation thus have received a great attention because of its low cost effectiveness and ecofriendly nature. This technology offers the potential to treat contaminated soil and groundwater on site without the need for excavation (Balba *et al.*, 1998; Kearney, 1998). It requires little energy input for the preservation of the soil structure (Hohener *et al.*, 1998). Perhaps the most attractive feature of bioremediation is the reduced impact on the natural

ecosystems, which should be more acceptable to the public (Zhang and Chiao, 2002). Bioremediation has also several limitations because it depends on the nature of organisms, enzyme which involved, compounds concentration and availability and finally survival of micro-organisms. .

There are two basic approaches to accomplish the process of microbial degradation of organic wastes. The aerobic and the anaerobic bacteria are the major tools for the bioremediation. Production of methane gas may serve as fuel and sludge obtained as a byproduct thus produced may be used as fertilizer. With the advancement of biodegradation technology, different types of techniques have been designed and used for the operation, which eventually maximized the optimum conditions for bacterial growth. The success of any bioremediation process depends upon the physical and chemical characteristics of the substrate such as nutrient status, moisture content, pH and temperature, which influence as the environmental factors (Comeau *et al.*, 1993; Boopathy, 2000; Zhu *et al.*, 2004; Hazen, 2010). The biotic factors such as inoculum density play a significant role in such process (Ramadan *et al.*, 1990).

### **Factors affecting the bioremediations**

Several factors affect the bioremediation process and are of physical and chemical characteristics of the substrate, concentration of contaminants, amount of catalyst, temperature, pH, Moisture, additional energy source soil salinity inoculums density etc.

### **Structure of pesticide**

Structure of pesticide determines its physical and chemical properties and inherent biodegradability. Introduction of polar groups such as  $\text{OH}^-$ ,  $\text{COOH}^-$  and  $\text{NH}_2^{3-}$  may

provide the microbial system, a site of attack. If the molecule is present as substituent of halogen or alkyl, it makes more resistant to biodegradation (Cork and Krueger, 1991). Minor difference in the arrangement or nature of substituent in pesticides of the same class can influence the rate of degradation (Topp *et al.*, 1997).

### **Concentration of pesticides**

The rate of pesticide degradation depends on the concentration of pesticides and amount of catalyst present in the microbial density, which involved in the degradation process. The rate of degradation decreases generally quantitatively with the residual pesticide concentration (Topp *et al.*, 1997).

### **Effect of temperature**

The report of biodegradation reveals that the influence of temperature is outstanding. It has been emphatically stated that the entire process of biodegradation is carried out at mesophilic and thermophilic temperature ranges. Whereas, the process becomes viable at the mesophilic 30°C-37°C temperature range, optimal temperature being at 35°C, the thermophilic digestion occurs between 50° to 60°C temperature range, with the optimum being at 55°C (Alexander, 1977). The optimal temperature required for both the ranges, is not invariably critical for the biodegradation.

### **Effect of pH**

Soil pH is an important factor, which affects the adsorption of pesticides for the abiotic and biotic degradation processes (Burns *et al.*, 1975). According to Hicks *et al.* (1990) it influences the adsorption behavior of pesticide molecules on clay and organic surfaces. This also affects the chemical speciation, mobility and bioavailability.

Racke *et al.* (1997) reported that degradation of a given pesticides depends mostly on the soil alkaline or acidic pH. In fact the biodegradation depends upon the susceptibility of the microorganism in the optimum pH of the medium.

#### **Effect of moisture**

Moisture is an environmental factor and affects the rate of biodegradation. Water acts as medium for the movement and diffusion of pesticides it is necessary for microbial functioning. Moisture (water) influences the rate of contaminant metabolism because it influences the type and amount of soluble materials that are available in the environment. It maintains the osmotic pressure and pH of terrestrial and aquatic systems. The amount of water in the pore spaces of soil affects the exchange of reparatory gases. Under saturated conditions, oxygen can be consumed faster than it is replenished in the soil space and the soil becomes anaerobic. This retards the rate of biodegradation and causes major changes in microbial metabolic activity to occur. Generally the soil moisture content should be between 25-85% of the water holding capacity, and a range of 50-80% is optimal for biodegradation.

#### **Effect of salinity**

Not more information is available concerning the effects of salinity on the degradation of pesticides, although, salinity is a severe problem in many arid, semiarid and coastal regions. According to Reddy and Sethunathan, (1985) the parathion degradation is faster in non saline soils. However reports on the stability of pesticides in estuarine and sea water of varying degrees of salinity are available. A high salt content in seawater may be unfavorable (Walker, 1976) or inhibitory pesticidal degradation (Weber, 1976; Kodama and Kuwatsuka, 1980).

#### **Bioremediation of Methyl parathion in soil**

The environmental conditions and microbial degradation decide the fate of OPs in the soil. Microbial degradation is the most significant and successful key of disappearance of organophosphorus pesticides from the soil. Microorganisms possess the unique ability of completely mineralization of many aliphatic, aromatic and heterocyclic compounds.

Several microorganisms have been isolated from soil which degraded the MP in the environment. Increase in cell biomass stimulated increase in the biotransformation rate (Lewis *et al.*, 1984). It is hydrolyzed in soil (Kishk *et al.*, 1976) and also in flooded soil (Sharmila *et al.*, 1989).

Rani and Lalithakumari (1994) studied the degradation of MP by *Pseudomonas putida* and reported that the MP is utilized by this bacterium as sole carbon and phosphorus source and hydrolyzed the MP to *p*-nitrophenol and it is further into degraded into hydroquinone and 1, 2, 4-benzenetriol. Three isolates *pseudomonas diminuta*, *pseudomonas putida* and *pseudomonas aeruginosa* were isolated from soil of Madhya Pradesh (M.P.) which were capable of degradation of 1200mg/L MP at pH 7.0 and 30°C temperature after 48 hrs of incubation. However when optimized different parameters such as pH, temperature, salinity, and growth sources, these three isolates showed rapid growth and degradation proximate at 36 hrs of incubation (Sharma *et al.* 2014). Many researchers studied on MP degradation. Chaudhry *et al.* (1988) isolated two bacterial species of *Pseudomonas* and *Flavobacterium* from agricultural soil by enrichment method. *Pseudomonas* sp was capable of utilizing MP and parathion as a sole source of carbon and *Flavobacterium*

sp. was able to degrade the p-nitrophenol to nitrite. For the degradation of MP by *Pseudomonas* species, glucose and another carbon source as supplements were added in MSM. Degradation of MP in an aqueous medium by soil bacteria has been studied by Keprasertsup *et al.* (2001). He isolated mixed and pure bacterial cultures from agricultural soil treated with MP. They obtained the *Burkholderia cepacia*, which showed the best degrading ability of MP in the BMM in the absence of glucose. Balamurugan *et al.* (2010) isolated two bacterial species *Pseudomonas aeruginosa* and *Trichoderma viridae*, from monochrotophos and MP pre-treated soil. These isolates potentially degraded monochrotophos and MP. *Pseudomonas aeruginosa* was comparatively more efficient in degrading the monochrotophos and MP.

Qiu *et al.* (2006) studied the MP degradation by soil bacteria. They isolated a bacterial strain from soil capable of mineralizing MP. This bacterial strain was identified as *Ochrobactrum anthropi*. This strain totally degraded MP and its four metabolites products, were also analyzed as p-nitrophenol, 4-nitrocatechol, 1, 2, 4-benzenetriol and hydroquinone by HPLC and gas chromatography-mass spectrometry (GC-MS) analyses. Pakala *et al.* (2007) isolated bacterial strain from soil by enrichment method in minimal medium containing MP. This strain was identified as *Serratia* sp. strain DS001 and was capable of utilizing MP, p-nitrophenol, 4-nitrocatechol, 1, 2, 4-benzenetriol as a source of carbon and energy sources but could not grow using hydroquinone as a source of carbon.

Ghosh *et al.* (2010) studied the biodegradation of MP by the *Pseudomonas and Fransicella* and concluded that these

microbes utilised MP as a source of carbon and energy. These two isolates were obtained from pre-treated MP agricultural field. Both bacterial strains degraded 2 ppm MP in basal mineral medium. Biodegradation of MP by microalgae and cyanobacteria was also reported by (Megharaj *et al.*, 1994). He reported that microalgae or cyanobacteria utilized 1 ml of 1000 ppm commercial MP as a carbon and nitrogen source.

Shunpeng *et al.* (2001) isolated the *Plesiomonas* sp strain M6 from MP contaminated soil and they also determined the *mpd* gene, which was responsible for such expressed in *Esherichia coli*. Sharmila *et al.* (1989) reported the effect of addition of yeast extract on the degradation of MP by bacterial sp. They isolated *Bacillus* species from laterite soil which degraded MP in the presence of yeast extract.

Fenitrothion was also degraded by this species but diazinon was not degraded and showed the highest degradation at 35°C. Biodegradation of MP was reported by Ali *et al.* (2011). They isolated the *Bacillus pumilus* Ti, having ability to degrade the MP concentration of 500 ppm, in the presence of glucose. This strain was also degraded 70% p-nitrophenol, a metabolite product of MP, with in 24 hrs.

Degradation of MP in soil by strain DLL-1 was studied by Zhang *et al.* (2004). The best period of degradation by microbes, was recorded 3 days after the application of the pesticide. *Bacillus* and *Pseudomonas* species isolated from pre-treated OP pesticides ground nut fields (Madhuri and Rangaswamy, 2009).

These species degraded several insecticides such as chlorpyrifos, phorate, Dichlorvos, MP and methomyl. Karunya and Reetha

(2012) worked on the degradation of malathion and parathion by soil bacteria, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Klebsiella*. These three species degraded 50 ppm of both the pesticides. The maximum growth was recorded at 35°C and 6 pH. They also recorded the carbon and nitrogen source as a growth promoter in minimal salt broth.

Methyl parathion is an organophosphorus pesticide used tremendously for agriculture crop protection, industrial and home use and represents a significant potential health risk. It is highly toxic pesticide. This review is useful in the detoxification of organophosphorus contaminated soil and may lead to development of a possible bioremediation in the near future for renovation of contaminated soil. Environmental factors such as physical and chemical characteristics of the substrate, nutrient status, pH, temperature, biotic factor and inoculums density interfere the accomplishment of any bioremediation process. Optimization of environmental factors play important role for enhancement of the biodegradation activity of soil isolates.

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