2. REVIEW OF LITERATURE

In recent years, serious concern over the long-term persistence of organochlorine pesticides and consequent ecological disturbance has led to their partial or total replacement with generally less persistent organophosphorus pesticides in most developed countries in the temperate region. Organophosphorus insecticides form the largest and most diverse group of currently used pesticides. These compounds with phosphorus as the active nucleus are esters of alcohols with phosphoric acid or anhydrides of phosphoric acid with another acid (O'Brien, 1976).

Researches on and development of the organophosphorus insecticides originated in Germany in the late 1930s (Schrader, 1963). The organophosphorus insecticides affect the nervous system of the insects by inhibiting the action of several ester-splitting enzymes particularly acetyl cholinesterase at the synapse (O'Brien, 1967; Corbett, 1974). Therefore, many organophosphorus insecticides, though short-lived, exhibit relatively higher mammalian toxicity than organochlorines.
Over the last four decades, considerable literature has accumulated on the metabolism of a wide range of pesticides in soil and water ecosystems as evident from excellent reviews on this aspect (Kearney et al., 1969; Helling et al., 1971; Adamson and Inch, 1973; Alexander, 1973; Pionke and Chesters, 1973; Bollag, 1974; Haque and Freed, 1974; Hiltbold, 1974; Kaufman, 1974; Parr, 1974; Goring et al., 1975; Kunc, 1975; Tomizawa and Kazano, 1975; Yoshida, 1975; Tu and Miles, 1976; Laveglia and Dahm, 1977; Sethunathan et al., 1977; Williams, 1977; Sethunathan and Siddaramappa, 1978; Mulla et al., 1981; Lal, 1982; Lal and Saxena, 1982; Sethunathan, 1982; Rajagopal et al., 1984). However, information on the metabolism of organophosphorus pesticides was fragmentary until 1970 (Alexander, 1969). Since 1970s, following increasing use of organophosphorus pesticides, considerable literature has accumulated on their fate and metabolism in the soil and aquatic ecosystems (Sethunathan et al., 1977; Miyamoto, 1979; Lal, 1982; Barik, 1984). Available literature indicates that organophosphorus pesticides are readily metabolized in plant and animal tissues (Alexander, 1969) and are therefore less persistent than organochlorines. Kearney et al. (1969) observed that pesticides belonging to organophosphorus group were the least persistent in soils among the pesticides of 12 classes tested.

Parathion has been banned in several countries including India, because of its high mammalian toxicity (LD50
13 mg/kg of body weight of white mice; Pimentel, 1971). Structurally related and relatively less toxic analogues, methyl parathion and fenitrothion are increasingly used as effective replacement for parathion. Nevertheless, parathion is still being used as a model organophosphorus insecticide in studies on the metabolism of pesticides in soil and water. This review is concerned with the progress made hitherto in studies on the persistence of the two insecticides chosen for the study viz., methyl parathion and fenitrothion in soil and aquatic systems with emphasis on chemical versus microbial aspects of their degradation.

2.1. METHYL PARATHION

Methyl parathion, synthesized in 1949 (Hall, 1950), like parathion, is a non-systemic insecticide with broadspectrum action but with lower mammalian toxicity (LD$_{50}$ 33.1 mg/kg of body weight of white mice; Mundy et al., 1978). Methyl parathion is widely used in rice culture as a contact insecticide for controlling stem borer, grasshopper, swarming caterpillar, case worm, rice hispa, thrips, rice bug and other common rice insect pests.

2.1.1. Persistence and degradation in soil, water and sediments

Environmental fate of methyl parathion has received less attention than that of more extensively used parathion.
Methyl parathion is short-lived, and generally less persistent than parathion as it is rapidly degraded in natural soils (Barik, 1984). More than 87 per cent of the methyl parathion added to the soil was degraded in seven days (Lichtenstein and Schulz, 1966). However, in a bioassay study with the larvae of the eye gnat (Hippelates collusor), methyl parathion appeared to persist for < 2 months (Mulla et al., 1961). The persistence of methyl parathion applied as 50 per cent emulsifiable concentrate was certainly lower than that of several organochlorines. Methyl parathion is degraded in soils (nonflooded) principally by hydrolysis (Lichtenstein and Schulz, 1964). After 12 days, only 6 per cent of the applied methyl parathion was recovered from a silt loam soil and p-nitrophenol was detected as the sole metabolite (Lichtenstein and Schulz, 1964).

Kishk et al. (1976) implicated soil enzymes in the hydrolysis of methyl parathion in soil. The enzyme exhibited optimum activity at pH 7 and two saturation constants (K_m) viz., $1.25 \times 10^4$ and $5.0 \times 10^4$ in a Lineweaver-Burke plot. In a study on the influence of soil-water tension on the mineralization of methyl parathion in a sandy loam (Cecil) and a silty clay loam (Webster) soil, mineralization of methyl parathion was rapid in both soils at 10 and 33 KPa, considerable at 100 KPa and very slow at 1.5 MPa (Ou et al., 1983). Bound residues (non-extractable $^{14}$C) accumulated rapidly during 714 days in the soils maintained especially at
100 KPa. Since no aminomethyl parathion was detected, it was suggested that the insecticide was initially hydrolyzed and p-nitrophenol was subsequently reduced to p-aminophenol. The reduction was considerable in moist soils (10 and 33 KPa) but was negligible in dry soil (1.5 MPa). Thus, degradation of methyl parathion was accelerated at a higher soil-water tension (Ou et al., 1983). Earlier studies have provided evidence for accelerated degradation of parathion under flooded conditions essentially by nitro group reduction (Sethunathan and Yoshida, 1973).

2.1.2. Chemical versus microbial degradation

Methyl parathion, like parathion, can undergo degradation by hydrolysis and/or nitro group reduction. Hydrolysis of methyl parathion can be chemical and/or biological. In situations where hydrolysis is the major pathway of degradation, it has been difficult to distinguish between chemical and microbial participation in the hydrolysis of methyl parathion, because of its extreme susceptibility to chemical hydrolysis.

Methyl parathion is rapidly hydrolyzed at alkaline pH (Faust and Gomaa, 1972). There is evidence for adsorption-catalyzed hydrolysis of methyl parathion on clay surface
(Saltzman et al., 1976). Adamson and Inch (1973) found that methyl parathion was degraded 3 times faster than parathion in soil. There was no evidence for a lag phase or for decomposition of methyl parathion in soil washings. Also, hydrolysis of these insecticides was faster on an ion exchange resin. It was, therefore, concluded that the rates observed result from surface catalysis and that microbial degradation plays very little part in the removal of these insecticides from soil.

There is evidence, mostly circumstantial, suggesting microbial role in the degradation of methyl parathion in soil environment (Barik, 1984). Methyl parathion was rapidly decomposed in nonsterile, but not in sterile soils and 99.9 per cent of the added methyl parathion was degraded at 0.5 per cent to 1 per cent concentration level (Nauman, 1970). Methyl parathion was degraded essentially by hydrolysis in nonflooded soil and microorganisms were implicated in the hydrolysis (Lichtenstein and Schulz, 1964). Soil samples, sterilized by autoclaving, lost their capacity to hydrolyze methyl parathion (Getzin and Rosefield, 1968). Kishk et al. (1976) implicated soil enzymes, probably of microbial origin, in the hydrolysis of methyl parathion.

Bourquin et al. (1977) found that degradation of $^{14}$C-methyl parathion in an artificial salt marsh ecosystem led
to the substantial evolution of $^{14}$CO$_2$. Evolution of $^{14}$CO$_2$ was presumably mediated by microbial community; because, $^{14}$CO$_2$ was not evolved from sterile systems. Likewise, methyl parathion was converted to aminomethyl parathion and CO$_2$ in nonsterile, but not in sterile, sediment-water microcosm (Pritchard et al., 1979). Spain et al. (1980) reported mineralization of both methyl parathion and p-nitrophenol to carbon dioxide by preadapted ecocore microorganisms from river sites. Nauman (1959) reported that the increase in the degradation of methyl parathion in a soil was associated with a distinct increase in the population of Pseudomonas sp.; but degradation in pure culture was not demonstrated.

Unequivocal evidence for the microbial role in the degradation of pesticide is generally provided by demonstrating its degradation in isolated cultures of microorganisms. There are some reports of degradation of methyl parathion in mixed cultures of microorganisms mostly from systems enriched with a related pesticide, parathion and not methyl parathion. Thus hydrolysis of methyl parathion has been demonstrated in cell-free extracts from parathion adapted mixed culture of microorganisms. Munnecke and Hsieh (1974) isolated an inducible enzyme, parathion hydrolase from a mixed culture adapted for growth on parathion. This enzyme hydrolyzed methyl parathion at a relative rate of 0.20 (parathion = 1.0) at 1000 mg (active pesticide) substrate concentration (Munnecke, 1976). Methyl parathion was hydrolyzed faster than parathion at 5000
mg/l substrate concentration (Munnecke, 1976). When parathion and methyl parathion were combined together and tested for enzymatic hydrolysis by the same enzyme preparation, parathion was first hydrolyzed and hydrolysis of methyl parathion was delayed until parathion was hydrolyzed completely (Munnecke, 1980).

A mixed bacterial culture obtained from oligotrophic or eutrophic waters cometabolically degraded methyl parathion (Chou and Bohonos, 1979).

Microorganisms in batch cultures were adapted to methyl parathion and diethyl phthalate (Lewis and Holm, 1981). Loss of methyl parathion from the culture vessel was due to microbial action, as no degradation occurred with autoclaved aufwuchs biomass.

Reports on the degradation of methyl parathion in pure cultures of isolated microorganisms are scanty. A \textit{Bacillus} sp., isolated from a sewage sludge, cometabolized methyl parathion (Maleszewska, 1974). A \textit{Flavobacterium} sp. ATCC 27551 that had been isolated from a diazinon-treated rice field (Sethunathan and Yoshida, 1973 b), readily hydrolyzed not only diazinon, but also methyl parathion, parathion and fenitrothion as a sole source of carbon (Adhya \textit{et al.}, 1981). In contrast, a \textit{Pseudomonas} sp. ATCC 29353, isolated from a parathion-enriched flooded soil (Siddaramappa \textit{et al.}, 1973),
hydrolyzed parathion and diazinon, but not methyl parathion and fenitrothion (Adhya et al., 1981). In another study, a phosphotriesterase from the Flavobacterium sp. ATCC 27551, isolated from diazinon-treated rice field, hydrolyzed methyl parathion and parathion as a sole source of carbon in addition to diazinon (Brown, 1980).

Until recently, there had been no report relating to the isolation of microorganisms capable of degrading methyl parathion from soils exposed to methyl parathion before. Recently, however, Chaudhry et al. (1988) found that two mixed bacterial cultures, isolated from soils that were previously treated with methyl parathion, utilized methyl parathion and parathion as a sole source of carbon and nitrogen. A Pseudomonas sp., isolated from this mixed culture, hydrolyzed methyl parathion and parathion to p-nitrophenol, but required glucose or another carbon source for growth. The crude cell extracts prepared from this Pseudomonas sp. also hydrolyzed both methyl parathion and parathion. The hydrolysis of methyl parathion in cell extracts proceeded at a faster rate than that of parathion (Chaudhry et al., 1988).

2.2. FENITROTHION

Fenitrothion, synthesized in 1957 (Drabek and Truchlik, 1957) and introduced in 1961 (Nishizawa et al.,
1961), is a broadspectrum insecticide with a very low mammalian toxicity \( \text{LD}_{50} 988 \text{ mg/kg of body weight of white mice; Mundy et al., 1978} \). Fenitrothion has been used widely since 1967 as a replacement for more persistent DDT and other organochlorines in controlling forest defoliators and insect pests of several economically important agricultural crops including rice.

2.2.1. Persistence and degradation in soils, water and sediments

Fenitrothion, like other related organophosphorus insecticides, parathion and methyl parathion, is generally short-lived in the environment.

Fenitrothion persisted in forest soils for a period of 30 to 64 days (Yule and Duffy, 1972, Spillner et al., 1979), with a maximum half-life of 64 days (Krehm, 1973). Residues of fenitrothion in forest soil samples reached 0.01 ppm after 64 days of its application (Yule and Duffy, 1972). Most of the residues of fenitrothion was confined to the top 30 mm of the soil when applied as granules to a pasture at the rate of 2.24 kg a.i./ha. Only traces of fenitrothion were recovered from the soil 7 weeks after treatment (Martin, 1974). When fenitrothion was sprayed from a helicopter, concentration of fenitrothion in soil increased after rain as a result of washing from tree crown canopy. However, leaching of fenitrothion from
soils was negligible due to its strong sorption to the soil (Mochida et al., 1981).

Fenitrothion can disappear rapidly from agricultural soils under both aerobic and anaerobic conditions (Takimoto et al., 1976). An emulsion of $^{14}$C-fenitrothion labelled at the m-methyl position was incorporated at the rate of 10 ppm into two silty loam soils, one sandy loam soil and one sandy soil under nonflooded (60 per cent maximum water holding capacity) and flooded conditions. Under nonflooded conditions, 3-methyl-4-nitrophenol was the major metabolite, accounting for 10-20 per cent of the applied radioactivity, but decreased on longer duration of incubation due to its further metabolism. Major end product, carbon dioxide amounted to 12-40 per cent of the $^{14}$C in fenitrothion after 60 days. In all soils except sandy soil, under flooded conditions, the decrease in the concentration of fenitrothion was more rapid than in nonflooded soil, the half-lives ranging from 4 to 20 days with a concomitant increase in soil-bound radioactivity. Aminofenitrothion was the major product under flooded conditions accounting for 50 per cent of the applied radioactivity at the maximum; 3-methyl-4-nitrophenol and carbon dioxide were detected as minor products. Soil-bound radioactivity as well as water soluble metabolites increased at later stages of incubation under both moisture regimes. Correlation analyses did not reveal any direct relationship
between decomposition rate of fenitrothion and physico-chemical properties of soil such as clay content, organic matter content, cation exchange capacity and pH (Takimoto et al., 1976).

Periodic sampling and analysis of two forest soils treated with ring-\(^{14}\)C-fenitrothion applied at the rate of 7.4 ppm in wet soil revealed that 50 per cent degradation of fenitrothion occurred in 3 days (Spillner et al., 1979). Distribution of radioactivity after 15 days of incubation of the soils amounted to 3-6 per cent of the originally applied \(^{14}\)C as fenitrothion, 5-7 per cent as 3-methyl-4-nitrophenol and 48-50 per cent as 3-methyl-4-nitro-anisole. The bound residues were associated mainly with humic acid and fulvic acid fractions (Spillner et al., 1979).

Fenitrothion is also short-lived in pond and stream water samples with a half-life of 0.3 to 3.5 days (Sundaram, 1973, 1974). Fenitrothion was incubated in the dark in buffered distilled water, natural lake water and buffered lake water (Greenhalgh et al., 1980). Above pH 8, fenitrothion was readily hydrolyzed to form 3-methyl-4-nitrophenol. Below pH 7, dealkylation also occurred to form dimethyl fenitrothion. Aminofenitrothion was also detected as a reaction product, but only in natural lake water systems (Greenhalgh et al., 1980).

After application of fenitrothion to an aquatic
ecosystem (stagnant pool), fenitrothion levels were higher in
the surface than in the subsurface water (Moody et al., 1978).
Fenitrothion levels fluctuated in fast flowing water, probably
due to rainfall during sampling period. Aminofenitrothion was
detected in substantial amount and desmethyl aminofenitrothion
and S-Me fenitrothion in small amounts in water after
fenitrothion application. Aminofenitrothion and desmethyl
fenitrothion were the major products of fenitrothion metabo-
lism in river water incubated under laboratory conditions(Zitco
and Cunningham, 1974). When sprayed to pond water,3-methyl-4-
nitrophenol accumulated only in water whereas aminofenitrothion
accumulated in sediment fractions and not in water (Maguire and
Hale, 1980).

2.2.2. Chemical degradation

Fenitrothion is relatively nonvolatile (NRCC, 1975),
stable under acid conditions (Zitco and Cunningham, 1974), but
susceptible to alkaline hydrolysis (Truchlik et al., 1972).
The half-life of fenitrothion in water at 25°C was 60 h at pH
5.42 and .22 h at pH 8.22 (Sundaram, 1973). Fenitrothion at
23°C and pH 7.5 in natural lake water showed a half-life of
49.5 days in the dark and 1.5 to 2 days in the field
(Greenhalgh et al., 1980).

Photolysis is one of the most important degradative
mechanisms of fenitrothion in the environment. Fenitrothion was exposed to radiation available in the solar spectrum i.e. $\sim 300$ nm in vapour and liquid phases. Both in the vapour phase and in ethanol solution, two primary products were produced, the major one being 3-methyl-4-nitrophenol (Brewer et al., 1974). Fenitrothion exhibited a lower half-life in fine weather (1.02 days) than in cloudy or rainy weather (2.00 days) suggesting that the difference in half-life is attributed to more rapid degradation under sunlight (Mochida et al., 1981). Ohkawa et al. (1974) found that photodecomposition of fenitrothion in various solvents and as films by both UV and sunlight led to the formation of five products involving isomerization, oxidation, hydrolysis and solvolysis.

During ultraviolet irradiation of fenitrothion in hexane, both the P=S and aryl methyl group were oxidized to give fenitrooxon and formyl fenitrothion. Small amounts of dinitrofenitrothion were also formed. Irradiation in methanol gave carbomethoxyfenitrothion formed from oxidation by solvolysis (Greenhalgh and Marshall, 1976).

$^{14}$CH$_3$-fenitrothion applied on to thin-layer soil plates was hydrolyzed rapidly when irradiated by natural sunlight with a half-life of 1.1 days; fenitrooxon and 3-methyl-4-nitrophenol were the principal products. With longer irradiation, the concentration of 3-methyl-4-nitrophenol
increased, with eventual formation of humic acid and soil-bound radioactivity (Miyamoto, 1977).

2.2.3. Microbial degradation

Almost all added fenitrothion was recovered (<90 per cent) after 30 days of incubation with sterilized forest soils (Spillner et al., 1979). Likewise, no degradation of fenitrothion occurred in sterile soil suspension (Takimoto et al., 1976). More rapid degradation of fenitrothion in nonsterile samples than from sterile samples provides evidence, but indirect, for microbial role in its degradation. Although fenitrothion rapidly disappeared from forest soils, there was no evidence for its use as a carbon source by the microflora (Salonuis, 1972). In certain nonsterile soils, fenitrothion was degraded to aminofenitrothion (and subsequently to nitroso compound) or 3-methyl-4-nitrophenol (Miyamoto, 1977). There is evidence, but limited, for degradation of fenitrothion in mixed and pure cultures of microorganisms. A mixed microfloral culture from 2 silty loam soils (Utsunomiya and Moriyama) aerobically degraded fenitrothion to aminofenitrothion, formyl aminofenitrothion, acetyl aminofenitrothion and 3-methyl-4-nitrophenol as major products. One *Fusarium* sp. and two *Bacillus* spp., isolated from this mixed culture, decomposed fenitrothion in a rich medium containing glucose under aerobic conditions (Takimoto et al., 1976).
Several species of soil and water bacteria, including *Bacillus subtilis*, *Escherichia coli*, *E. freundii*, *Pseudomonas reptilovora* and *P. aeruginosa* metabolized or inactivated fenitrothion (NRCC, 1975).

Hirakoso (1968) found that chlorpyrifos was stable in media containing 32 isolates of the bacteria tested; but when individually tested, insecticidal activity of fenitrothion, dichlorvos and fenthion was retarded by four, seven and one bacterial species, respectively.

Inactivation of fenitrothion in polluted water samples in Japan was attributed to its rapid degradation by microorganisms. *Bacillus subtilis* was the most effective in inactivating fenitrothion through its reduction to aminofenitrothion (Yasuno *et al.*, 1965). When the liquid medium (polypeptone, yeast extract, sucrose, NaCl) containing fenitrothion added at a concentration of 0.5 ppm and 60 ppm was inoculated with *Bacillus natto*, almost all the added fenitrothion disappeared within 48-72 h at 37°C. Degradation was less pronounced in cooked soybeans than in the liquid medium. In both media, aminofenitrothion and 3-methyl-4-nitrophenol accumulated (Ikeda and Suzuki, 1981).

A *Bacillus subtilis*, isolated from polluted water, converted fenitrothion to aminofenitrothion (Miyamoto *et al.*, 1981).
1966). Aminofenitrothion was further metabolized, but at a lower rate than the parent compound to desmethyl aminofenitrothion, desmethyl fenitrothion and dimethyl phosphorothioic acid.

Hydrolysis is the major pathway of degradation of fenitrothion in most soils under nonflooded conditions, and in water; but evidence for microbial involvement in its hydrolysis is only circumstantial.

Until 1986, no microorganism capable of hydrolyzing fenitrothion as a sole source of carbon had been isolated from a soil retreated with fenitrothion. However, a Flavobacterium sp. ATCC 27551, isolated from a flooded soil retreated with a related insecticide, diazinon, readily hydrolyzed not only diazinon, but also fenitrothion and other related organophosphorus insecticides, methyl parathion and parathion as a sole source of carbon (Adhya et al., 1981 b). In contrast, a Pseudomonas sp., isolated from a flooded soil retreated with parathion, hydrolyzed parathion and diazinon, but not their methoxy analogues, methyl parathion and fenitrothion (Adhya et al., 1981). Methyl parathion and parathion have a common ring moiety, with differences in side chain while diazinon and parathion differ in their ring moiety with a common side chain. The ability of Pseudomonas sp. to hydrolyze parathion and diazinon and not methyl parathion
would suggest that side chain determines the susceptibility of these organophosphorus insecticides to hydrolysis by this bacterium. But, recently, Baaschers and Heitland (1986) found that a fungus, *Trichoderma viride* enzymatically hydrolyzed fenitrothion and fenitrooxon with great ease in the presence of alternate nutrients. Its hydrolysis product, 3-methyl-4-nitrophenol appeared to undergo further degradation. Cometabolism was implicated in the fungal degradation of the parent compound and 3-methyl-4-nitrophenol.

2.3. Parathion

Parathion, synthesized in 1945 (Fletcher *et al.*, 1948) and introduced in 1947, has been the most widely used organophosphorus insecticide for broadspectrum control of insect pests of economically important agricultural crops and in public health.

2.3.1. Chemical degradation

Several chemical factors in the environment such as pH, salinity, adsorption by various colloidal surfaces, oxidants such as ozone, chlorine, permanganate etc., temperature and sunlight can affect the stability of parathion (Paris and Lewis, 1973; Yaron and Saltzman, 1978). Parathion appears to be more resistant to chemical hydrolysis at acidic
and neutral conditions than other related organophosphorus compounds; but, under alkaline conditions, parathion is readily hydrolyzed at P-O-C linkage to p-nitrophenol and diethylthiophosphoric acid (Faust and Gomaa, 1972). Parathion showed increasing persistence with decreasing pH of aqueous solution, with half-life of 33.2 h at pH 10.4 and 4182 h at pH 3.1. Temperature also affects the chemical stability of parathion. Thus, parathion disappeared 5 to 7 times faster at 37.5°C than at 20°C in aqueous solution at pH 7.4. The surface-catalysis of parathion sorbed on clay surfaces can also be common (Saltzman et al., 1974; Mingelgrin and Saltzman, 1979). Parathion was oxidized to paraoxon on soil dust and clay minerals under laboratory conditions (Spencer et al., 1975, 1980 a,b). Photochemical reactions play an important role in the breakdown of parathion.

2.3.2. **Microbial degradation**

Although parathion can undergo degradation by chemically-catalyzed reaction in the environment, microbially mediated degradation is the major or only means of detoxification of parathion in the soil and water environments (Sethunathan et al., 1977).

Initial evidence, mostly indirect, for microbial role in the degradation of parathion was based on its related
stability in sterile and nonsterile soil samples. Thus, parathion disappeared more rapidly from nonsterile soils (Lichtenstein and Schulz, 1964; Lichtenstein et al., 1968; Miles et al., 1979), estuarines (Walker, 1976) and natural water (Sharom et al., 1980) than from corresponding samples sterilized by autoclaving. Similarly, parathion disappeared rapidly from nonsterile, but not sterile, soils under flooded conditions (Sethunathan and Yoshida, 1973a). Likewise, parathion decomposed faster in nonsterile soils than in soils sterilized by irradiation and autoclaving (Getzin and Rosefield, 1968) or other sterilizing agents such as sodium azide (Lichtenstein et al., 1968), methyl bromide (Sacher et al., 1972) and detergents, alkyl benzene sulfonate and linear alkyl benzene sulfonate (Lichtenstein, 1966). Increased persistence of parathion effected by these sterilizing agents was attributed to reduced microbial activity (Lichtenstein, 1968; Getzin and Rosefield, 1968; Sacher et al., 1972; Sethunathan and Yoshida, 1973a; Walker, 1976; Miles et al., 1979; Sharom et al., 1980).

The metabolism of parathion in isolated cultures of microorganisms is well documented (Sethunathan et al., 1976). Early reports showed that nitro group reduction appeared to be the major pathway of parathion degradation in pure cultures. In these studies, nutrient-rich media were generally used with parathion as additional carbon source. Thus, a Bacillus
subtilis, isolated from polluted water, reduced parathion to aminoparathion (Yasuno et al., 1965). Pseudomonas melophora, a bacterial symbiont of the apple maggot, degraded a number of insecticides including parathion (Boush and Matsumura, 1967). Trichoderma viride readily degraded parathion through its oxidative system. Hirakoso (1968) found that at least 12 bacterial species formed aminoparathion from parathion. Parathion was degraded by Rhizobium japonicum and Rhizobium meliloti primarily by nitro group reduction and to a minor extent (10 per cent) by hydrolysis to diethyl thiophosphoric acid (Mick and Dahm, 1970). Penicillium waksmani, isolated from a flooded acid sulphate soil, converted parathion to aminoparathion as a major metabolite and certain polar metabolites (Rao and Sethunathan, 1974).

Besides bacteria and fungi, algae seem to play a major role in the transformation of parathion in rice paddies and pure cultures. Algae degraded parathion in rice fields (Sato and Kubo, 1964). A pure culture of the alga, Chlorella pyrenoidosa converted parathion to aminoparathion and an unidentified metabolite (Mackiewicz et al., 1969). About 65 per cent of added parathion disappeared in cultures of Chlorella pyrenoidosa (Ahmed and Casida, 1958). In another study, Chlorella pyrenoidosa converted parathion to four metabolites including aminoparathion (Zuckerman et al., 1970). Algae were implicated in the rapid transformation of parathion in sandy loam soil following flooding (Iwata et al., 1973).
According to earlier literature, degradation of parathion in soil and aquatic environments and in pure cultures of isolated microorganisms proceeded essentially by nitro group reduction (Sethunathan et al., 1977). But, Sethunathan (1973 b) reported that an enrichment culture from a flooded alluvial soil rapidly hydrolyzed parathion, but lost its activity upon autoclaving. A Pseudomonas sp., isolated from this enrichment culture (Siddaramappa et al., 1973), readily hydrolyzed parathion and completely mineralized it with nitrite (Siddaramappa et al., 1973) and CO₂ (Barik et al., 1976) as the end products; but methyl parathion or fenitrothion was not hydrolyzed by this bacterium (Adhya et al., 1981 b). A Flavobacterium sp. ATCC 27551, isolated from diazinon-treated rice fields hydrolyzed diazinon and mineralized the ring moiety with great ease (Sethunathan, 1973 a; Sethunathan and Yoshida, 1973 b). This bacterium also exhibited a broad substrate specificity and hydrolyzed closely related organophosphorus insecticides such as parathion, methyl parathion, fenitrothion (Adhya et al., 1981), coumaphos (Kearney et al., 1986; Karns et al., 1986) and diisopropyl fluorophosphate, a structural analogue of nerve gas agents, soman and savin (Attaway et al., 1987). Likewise, another Flavobacterium sp., isolated from a diazinon-treated soil, hydrolyzed diazinon, parathion and paraoxon (Forest et al., 1981). De Andrea et al. (1982) found that parathion was hydrolyzed to p-nitrophenol by a Nocardia sp. isolated from a parathion-treated soil. Nelson
(1982) reported the hydrolysis of parathion by several bacterial isolates mainly belonging to *Bacillus* and *Arthrobacter* from parathion-treated Israeli (Gilat) soil.

Hsieh and Munnecke (1972), using a continuous culture method to isolate a parathion-hydrolyzing bacterium for use in the decontamination of environments heavily polluted with parathion, succeeded in preparing an active enrichment culture which hydrolyzed parathion to **p**-nitrophenol. The crude cell-free extract (Munnecke, 1976) of this mixed bacterial culture hydrolyzed a broad spectrum of organophosphorus insecticides diazinon, EPN, chlorpyrifos, triazophos, parathion, aminoparathion, paraoxon, methyl parathion, dursban, fenitrothion and cyanophos.

A bacterium isolated from this adapted mixed culture utilized **p**-nitrophenol, but not parathion as a sole source of carbon (Munnecke and Hsieh, 1974). Daughton and Hsieh (1977) finally isolated two species of *Pseudomonas*, *P. stutzeri* and *P. aeruginosa* from this consortium that could degrade parathion and/or **p**-nitrophenol. *P. stutzeri* cometabolized parathion to **p**-nitrophenol and then mineralized **p**-nitrophenol whereas *P. aeruginosa* mineralized only **p**-nitrophenol to nitrite and CO₂.

There is considerable concern over the large scale contamination of the environment by accidental spills, disposed
pesticide containers and effluents from pesticide plants. Efforts have been made to study the feasibility of using a highly acclimated mixed culture of parathion-hydrolyzing bacteria for detoxification of parathion in heavily contaminated areas (Wolfe and Durham, 1966; Wolfe et al., 1973; Munnecke and Hsieh, 1974; Barles et al., 1979). The acclimated culture of bacteria effected complete degradation of parathion in a silt loam soil upto 1250 kg parathion/hectare within 35 days (Barles et al., 1979). Microorganisms acclimatized to technical parathion were particularly effective in degrading parathion rapidly in nonsterilized soil for at least 8 to 14 days under laboratory conditions; but, their effectiveness was greatly reduced after 3 weeks (Daughton and Hsieh, 1977). Fischer et al. (1980) explored the feasibility of using immobilized enzyme systems for detoxification of high concentrates of parathion in the environment.

Rapid hydrolysis of parathion has been demonstrated in cell-free extracts of adapted pure and mixed cultures of microorganisms (Munnecke, 1978, 1979 a; Munnecke et al., 1982). A cell-free extract of the Flavobacterium sp. ATCC 27551, isolated from a diazinon-treated rice field, exhibited an exceptional capacity to hydrolyze parathion and diazinon (Sethunathan and Yoshida, 1973 b). The reaction in cell-free systems ceased at p-nitrophenol in case of parathion. Brown (1980) characterized the constitutive phosphotriesterase from
this *Flavobacterium* sp. ATCC 27551 which was composed of two protein units, one with a molecular weight greater than 100,000 daltons and the other with a molecular weight of 50,000 daltons. This hydrolase was particularly effective on organophosphorus compounds having an electron withdrawing aromatic or heterocyclic leaving group as in parathion, methyl parathion and diazinon. Compounds with weakly electrophilic group like 4-aminophenol were not hydrolyzed. This hydrolase was active over a broad pH range (8 to 10) and not affected by the presence of EDTA, NAF, or NaN₃ or metal ions such as Zn²⁺, Cu²⁺, Co²⁺, Cd²⁺, Mg²⁺ or Mn²⁺. Likewise, a cell-free preparation of *Pseudomonas* sp. readily hydrolyzed parathion; but p-nitrophenol formed resisted further degradation (Barik and Sethunathan, 1978 b), although resting and growing cells of this bacterium metabolized parathion past p-nitrophenol to CO₂ and nitrite (Siddaramappa *et al*., 1973; Barik *et al*., 1976).

There is evidence that parathion hydrolyzing gene (opd) of *Flavobacterium* sp. ATCC 27551, isolated from diazinon-treated rice fields (Sethunathan and Yoshida, 1973 b), is encoded in plasmids (Mulbry *et al*., 1986) and this opd gene has been cloned to *Escherichia coli* and *Streptomyces lividans* (Steiert *et al*., 1989); there is a great scope for genetic manipulation of this and other parathion hydrolyzing bacteria for extending their degradation range.