Chapter 1. Introduction

1. 1. General Introduction

The usage of pesticides greatly benefit the modern agriculture and health of human being, but their hazards to nontarget organisms caused numerous environmental problems. Therefore the persistence and transformation of pesticides in the environment especially in soil and water receive much concern since the long term persistence and side-effect on wildlife of DDT was revealed in 1950s.

Pesticides entered into environment could be transformed by physical, chemical and biological means. All may lead to the detoxification of pesticides, but it is only the biological means that could finally mineralize these synthetic organic chemicals. In general, biodegradation which refers to microbial metabolism of pesticides is the dominant mean leading to the mineralization of pesticides in the environment (Cork & Krueger, 1989). It is also one of the major factors that determines the persistence of pesticides in the environment.

One of the most active field of research in biodegradation of pesticides concerns with soil microorganisms, because most pesticides used in agricultural land finally reach the soil by direct or indirect ways. In the last few years, many studies had established the effects of many pesticides on indigenous populations and the ways in

which the microflora alters a multitude of chemicals to which they are exposed. By now, most studies have been done *in vivo*. Pure cultures which could degrade pesticides have been enriched and isolated from the soil. Their degrading ability , kinetics, pathway and affecting factors have been determined. It had been reported that many genera of herterotrophs use pesticides as substrates, either by cometabolizing the molecules or using them as nutrients. Species of *Agrobacterium, Arthrobacter, Bacillus, Clostridium, Corynebacterium, Flavobacterium, Klebsiella, Pseudomonas,* and *Xanthomonas* among the bacteria; *Alternaria, Aspergillus, Cladosporium, Fusarium, Glomerella, Mucor, Penicillium, Thizoctonia,* and *Trichoderma* among the fungi; and *Micromonospora, Nocardia,* and *Streptomyces* among the actinomycetes were capable to degrade many of the pesticides (Alexander, 1977). Since biodegradation usually lead to the mineralization of pesticides, it may provide a mean to eliminate unwanted residues from the environment. These studies are aimed to establish a fundamental understanding of microbial degradation kinetics under various conditions, its biochemical systems, and its molecular biology that may maximize the potential benefit of the usage of biodegradion.

There has been a steady increase in the use of pesticides in most tropical countries in recent years. It is expected that the loss of organic pesticides, through processes involving volatilization, photo-decomposition, and more importantly microbial degradation, is more rapid than in the temperate regions. For example, lindane known for its long-term persistence under aerobic and temperate conditions, undergoes fairly rapid decomposition in tropical soils upon flooding, the most widely used cultural

practice in rice cultivation (Sethunathan, *et al.* 1982). However, the knowledge of biodegradation of pesticides in this hot and humid condition is quite limited. The criteria of pesticides use in tropical countries are based on the knowledge from temperate regions. So it is essential to study the persistence of pesticides and their impact on the ecosystem under actual tropical conditions.

1.2. Literature Review

1.2.1. Microbial degradation of pesticides

In modern agriculture, it has been a common practice to apply different groups of pesticides either simultaneously or in rotation for effecting broad-spectrum control of a variety of pests, that leading to combined residues in the soil. Significant interactions between pesticides applied in combination, in terms of their persistence in soils and toxicity to crops and insects, have been demonstrated. (Liang and Lichtenstein, 1974; Kaufman, 1977). For instance, parathion is generally short-lived in tropical flooded soils when applied singly, but benomyl, applied at a concentration as low as 5 ppm in combination with parathion, significantly increase the persistence of parathion by inhibiting microbial mediated reactions of both nitro group reduction and hydrolysis (Sudhakar-Barik and Sethunathan, 1979)

Since, herbicides are used regularly each year in a large scale. It is also very important to know whether or not these chemicals have the adverse effects on those

properties of soil. One aspect is the effects of herbicides on soil microorganisms. The soil microbes are responsible for the nutrient cycle, therefore their activity can have the effect on the soil fertility. Under the Toxic Substances Control Act, one of the proposed microbiological assays requires microorganisms involved in nitrogen cycling to be tested for their sensitivity to environmental toxicants (Stern, 1980). Ferrer *et al* (1986) reported the potential inhibitory effect of 2,4-D , 2,4,5-T and 2,3,6-TBA on nitrogen fixation, oxygen consumption and growth by pure cultures of *A. vinelandii.*

Usually, the distribution or microorganisms is not uniform throughout the soil profile. The microorganisms are most abundant and active in the upper most layer of soil and declined with depth. Torstensson (1987) indicated that once a herbicides is mobile and pass through the top soil layer into the subsoil, the chance of its micobial decomposition taking place is considerably less. The half-life of trichloroacetic acid (TCA) in the subsoil was extended several-fold compared with the topsoil. Therefore the possibility of this herbicides entering into the ground water was increased. It also may be true for other pesticides other than herbicides. Several cases of herbicides found in ground water have been reviewed by Hance (1987). So one of the aspect of pesticides behaviour in soil concerns their degradation rate in the subsurface layer where microbial population and their activity may be less than the surface layer. Harper *et al* (1989) reported that the degradation rate of metribuzin which is a potential groundwater contaminant was decreased below the surface soil. This decrease was related to the soil organic matter and microbial population.

Many researches have been done with pesticide degrading soil microrganisms. Some were done with pure culture or mixed culture isolated from the soil, some were done with soil sample directly.

Marty *et al* (1986) reported a bacterial strain isolated from soil and identified as *Pseudomonas alcaligenes*, was able to hydrolyze four phenylcarbamate herbicides (CIPC, BIPC, IPC and swep) to corresponding anilines and alchols by co-metabolism.

Roberts *et al* (1993) reported that a stable mixed bacterial culture which was isolated from the soil, was able to utilise linuron as a source of both nitrogen and carbon and was also able to degrade the related herbicides monolinuron and chlorbromuron and the possible intermediate degradation products of linuron. The culture contained gram-negative aerobic rods, and gram-possitive aerobic non-spore-forming rods and cocco-bacilli. None of the single isolates from the mixed culture was able to degrade linuron.

Barua *et al* (1990) reported that several soil fungi, *Aspergillus flavus, A. terreus, Fusarium solani, F. oxysporum, Penicillium citrinum* and *P. simplicissimum* could effectively degrade herbicide pendimethalin supplied as sole carbon source in mineral solution.

A particularly interesting phenomenon of microbial degradation of pesticides is enrichment. Enrichment is an increase in the number and /or activity of the microorgnisms capable of metabolizing a particular pesticide following the addition of the pesticide to the soil. This phenomenon could lead to the failure of pest control. However, it offers a tool to eliminate the contaminants in the environment. The first microbial enrichment was reported for 2,4-dichlorphenoxyacetic acid (2,4 - D) about 40 years ago. Recent studies were concentrated on methyl carbamate insecticides, especially carbofuran (Sethunathan, *et al.* 1982).

Williams *et al* (1976) studied the degradation of carbofuran by soil microorganisms.They reported that in a soil containing high level of actinomycetes, rapid degradation of carbofuran may be expected.

Turco and Konopka (1990) compared the degradation rate of carbofuran between the soils previously treated and untreated with carbofuran. The experiment showed a rapid breakdown of carbofuran in previously-exposed soils, however degradation in the unexposed soils was minimal.

There are many factors effecting the microbial degradation rate of pesticides. One of the factors is the presence of other organic molecules. Visoottiviseth *et al* (1992) studied the kinetics of degradation of triphenyltin pesticides by *Pseudomonas putida*

No. C, and reported that glucose and sucrose can enhance the degradation, however starch and xylose had much less effect on the degradation.

1.2.2. Carbaryl

Carbaryl (1-naphthyl methylcarbamate, C.A.) is a broad-spectrum contact carbamate insecticide, with slight systemic properties. It is used for control of over 150 major pests on more than 120 crops,
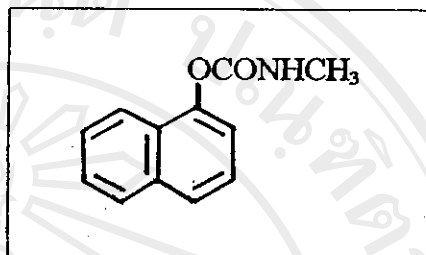
including field crops, forage, vegetables, fruits, nuts, shade trees, ornamentals, forests, lawns, turf, and range land, as well as control of pests of domestic animals.

Carbaryl was first introduced into use in 1960s (Sly,1977). The use of carbamate pesticides was widely spread in tropical countries in 1970s because of the tremendous buildup of brown planthoppers (*Nilaparvata lugens*) in rice fields. The N-methylcarbamates carbofuran and carbaryl are the most effective insecticides for controlling this pest (Sethunathan, *et al.* 1982).

Carbaryl has the empirical formula $C_{12}H_{11}NO_2$ and a molecular weight of 202.20. Pure carbaryl forms colorless to light tan crystals. It has a melting point of 142°C and a vapor pressure of less than 4×10^{-5} mm Hg at 26 ° C. The solubility of carbaryl in water is 40 ppm at 30 ° C. It is moderately soluble in most polar organic solvents, such

as dimethylformamide, dimethyl sulfoxide and acetone; it is slightly soluble in hexane,

benzene and methanol and about 5 % soluble in petroleum oils. Carbaryl is stable when

temperature is lower than 70 °C and to the light, hydrolyzed rapidly at pH 10 or above.

(Worthing & Hance, 1991)

Table 1.1 shows some mammalian toxicity data of carbaryl. The Environmental

Protection Agency (EPA) of the United States has established four categories of

toxicity for pesticides in its Toxicology Guidelines (Ashton, 1974). Carbaryl is in the

third category, and it is moderately toxic to mammals.

Table 1. 1. Acute oral toxicity of carbaryl (Worthing and Hance, 1991)

Species	Sex	LD_{50} (mg/kg)
Rat	Both	233-850
Mouse	Both	108-650
Guinea pig	Female	280
Gerbil	Female	491
Rabbit	Female	710
Dog	Female	250-795
Cat	Female	125-250
Monkey	Female	>1,000
Swine	Female	1,500-2,000

Carbaryl is toxic to fish; the LC_{50} (8-d) for bluegill is 10 mg /l; for rainbow

trout is 1.3 mg/l; for sheepshead minnow 2.2 mg/l. It is also toxic to honey bees.

The 1973 FAO / WHO joint meeting on pesticides residues estimated a human acceptable daily intake (ADI) for carbaryl to be 0.01 mg/kg body weight. (Anonymous 1978)

Carbaryl is a widely used insecticide in Thailand, it ranks the 7th of the top ten pesticides imported into Thailand (Sangphagdee, 1994). Though carbaryl is a moderate toxic insecticide, research during the last few years has shown that carbaryl has possible chronic toxic effects. The United States Department of Agriculture has recently changed the health hazard status of carbaryl to a higher risk category. So many studies were carried out to detect the residues of carbaryl in water, soil, biological samples, raw fruit, vegetable and fruit juice. (Bushway, 1988) The method of carbaryl analysis by high performance liquid chromatography is well developed.

The knowledge of persistence and biodegradation of carbaryl in tropical soils are quite limited. It was found, in neutral soils and in acid soils, that degradation of carbaryl was both chemical and biological (Aly and El-Dib, 1972). Venkateswarlu et al (1980) found that heat treatment of the flooded soils prior to their incubation with carbaryl increased the persistence of carbaryl. Rodriguez and Dorough (1977) noted that carbaryl could condition the soil for enhancement of its own biodegradation. A strain of Achromobacter sp. was isolated from soil, which could use carbaryl as a sole carbon source with concomitant formation of 1-naphyl, hydroquinone, catechol, and pyruvate (Sud et al., 1972). Many other researches were related to the cross adaptation of carbaryl and carbofuran, both are the N-methylcarbamate insecticides. For instance, a

Pseudomonas sp. isolated from a flooded soil amended with carbofuran could metabolize both carbofuran and carbaryl in a yeast-extract-supplemented mineral medium (Venkateswarlu *et al.*, 1980).

1.2.3. Paraquat

Paraquat (1 ,1'- dimethyl, 4 ,4'- bipyridinium, C.A.) is a widely used herbicides in Thailand. It is a non-selective contact herbicide which is first introduced into use in 1960s. It is the only pesticide which is locally produced in Thailand and its production in 1988 was about 750 tons. The consumption rate of this compound increases steadily.
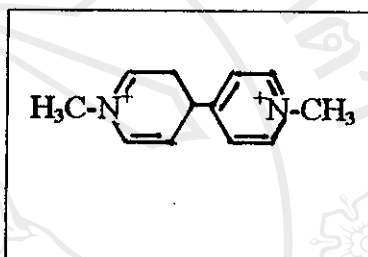
Paraquat destroys green plant tissue by contact action with some translocation. Uses include stubble cleaning (140 - 840 g AI / ha); pasture renovation (140-2,210 g/ha); weed control in plantation crops (280-560 g sprayed/ha). Paraquat is used in either highland or lowland agriculture as pre-planting, pre-harvest desiccant and non-crop land weed control substance. (Viriyahirunpiboon, 1994)

The mechanism of the herbicidal effect and toxicity of paraquat involves the formation of free radical by reduction of the ion and subsequent auto-oxidation to yield the original ion. The free radical itself does not appear to be the primary toxicant but rather hydroxyl radical and /or hydrogen peroxide which is formed during the auto-

oxidation of the free radical to the ion. The photosynthetic apparatus, light and oxygen are the required co-factors for herbicidal action. Symptoms of their action include a rapid discoloration and scorch of target leaves, followed by desiccation; chlorosis of unsprayed leaves follows somewhat later.

Paraquat dichloride has the empirical formula $C_{12}H_{14}Cl_2N_2$ and a molecular weight of 257.2. Pure paraquat dichloride forms colorless crystals decomposing at 300 °C. Paraquat is very soluble in water with its solubility around 700 g/l, but it is slightly soluble in short-chain alcohols and practically insoluble in hydrocarbons. It is nonexplosive and non-flammable in aqueous formulation but corrosive to metals. Its vapor pressure is less than 0.01 m Pa at 20 °C. It is stable in acid or neutral solutions but is readily hydrolyzed by alkaline. Paraquat also readily undergoes a single-electron reduction to the action radical with the standard redox potential, $E°$, -0.54 v (Worthing & Hance, 1991). Table 1.2 shows the acute oral toxicity of paraquat to mammals and birds.

Table 1.2. Acute oral toxicity of paraquat (Worthing and Hance, 1991).

Species	LD_{50} (mg/kg)
Rats	150
Mice	104
Dogs	25-50
Sheep	70
Rhode Island hens	262
Bobwhite quail	981
Japanese quail	970
Mallard duck	4,048

The fish toxicity of paraquat depends on the formulation and wetter used, LC_{50} (96 hr) for rainbow trout is 32 mg/l; for brown trout is 2.5 to 13 mg/l.

According to the WHO recommended classification of pesticides by hazard, paraquat belongs to class II, a moderate hazardous substance with an ADI of 0.004 mg/kg. The oral and dermal toxicity are 150 and 236-500 mg/kg, respectively. The maximum residue limit (MRL) for fruits and vegetables for the European Economic Community is 0.05 mg/kg.

It was accepted that paraquat in soil binds rapidly and tightly to clay minerals and residual phytotoxicity from free-available paraquat is unlikely. Duah - Yentumi and Johnson (1986) studied the biomass change in soil in response to repeated application of some pesticides, and they found that repeated paraquat application significantly lowered soil microbial biomass (chiefly fungal biomass). So it is interesting to study whether or not paraquat has the potential negative effect on the microbial ability to degrade other pesticides, therefore leading to longer persistence of those pesticides in the soil.

1. 3. Research Scope and Objectives

The whole research include:

1. soil sampling : both the upper 10 cm and lower 10 cm layers were sampled.

2. soil analysis: some of the physical, chemical and biological parameters were measured and compared between both layers of soil.

3. enrichment: carbaryl degrading microorganisms were enriched from both layers of soil samples.

4. isolation and purification: carbaryl degrading microorganisms were isolated from the soil and purified.

5. degradation kinetics: the degradation of carbaryl by isolated pure culture was studied, both with carbaryl and in the presence of other carbon source.

6. paraquat interaction: the effect of paraquat at different concentrations on carbaryl degradation was determined.

This research aims to obtain some basic knowledge about carbaryl degrading microbes in soil, its degradation kinetics and the potential effect of paraquat on carbaryl degradation.