STUDIES ON THE DECAY OF SOME PESTICIDES AND IDENTIFICATION OF THEIR METABOLITES

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By

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Gratis

CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **STUDIES ON THE DECAY OF SOME PESTICIDES AND IDENTIFICATION OF THEIR METABOLITES** in fulfilment of the requirement for the award of the Degree of **Doctor of Philosophy**, submitted in the **Department of Biosciences & Biotechnology**, of the **University of Roorkee** is an authentic record of my own work carried out during the period from November 1992 to December 1996 under the supervision of **Dr. S.N. Tandon** and **Dr. R.P. Mathur**.

The matter embodied in this thesis has not been submitted by me for the award of any other degree of this or any other university.

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ABSTRACT

Pesticides are among the few toxic materials deliberately disbursed into the environment to preserve the agricultural produce. The growth of population is making increasing demands on the natural resources thus resulting into a large scale use of the pesticides. The lack of proper training to the farmers sometimes leads to their indiscreet use and subsequent proliferation of these biological poisons into the environment. A problem inherent to pesticide application technology is the drift or disposal of the pesticides from the point of application. Only 10 to 15% of the applied pesticides actually reach the target with the remaining 85 to 90% dispersed off the target to air, soil and water. Awareness on the deposition of the pesticides outside the target area and the potential health effects due to these chemicals, nevertheless,is growing among the general public and the regulatory agencies. The widespread occurrence of pesticide residues in the different agricultural commodities in developing nations like India has been accepted as an unrefutable fact. In order to keep a proper track of the cycle of these materials a careful study on the decay profiles and formation of metabolites in various segments of the ecosystem is the need of the hour. Although some of the pesticides have been banned due to their toxicity and tendency to bioaccumulate in the living organisms their use still continues owing to their effectiveness and economic reasons. But before restricting the use of certain pesticides, it is imperative to follow their persistence behaviour and toxicity of the metabolites.

The decay of the pesticides takes place through complex mechanisms. The persistence and the type of the metabolites formed, to a large extent, depend

upon nature of the medium and the environmental conditions. The medium can be water, soil or plant and the environmental conditions may include temperature,humidity,wind velocity etc. In a particular type of environmental segment there can be large variation which cannot be controlled. Even in the simplest case like water there are variations in different physico-chemical parameters. Therefore large scale variations may be observed in the data on the decay profiles. For any meaningful interpretation of the results it becomes important to generate a database on decay patterns of the pesticides under controlled conditions. It remains a fact that the laboratory results cannot be necessarily extrapolated to the field. Thus a correct approach to the problem is to conduct the laboratory and field studies simultaneously.

With the awareness in pesticides pollution, voluminous literature has accumulated on residue analysis which essentially involves the identification and quantification of the pesticides on different food products. Studies have been conducted on the kinetics of decay of some of the pesticides under different laboratory conditions. Also substantial information exists on the photodegradation and subsequent identification of the metabolites. However, the field data on the persistence profiles of the pesticides under different meteorological conditions are scarce. Out of the different categories of the pesticides the organochlorines have received maximum attention with regard to the above mentioned studies.

Among the pesticides, organophosphorus compounds enjoy a favoured position due to their relatively fast decomposition and low accumulation in the biological food chain. The economic considerations also dictate their use particularly, in the developing countries. A market survey revealed that out of the organophosphorus pesticides, malathion, methylparathion and dimethoate

are the most commonly used ones. Most of the organochlorines are banned on an international level but some of them are still in use in India. Endosulfan, a pesticide of organochlorine group with cyclodiene moiety, is being used in India liberally. A survey of literature reveals that studies have been conducted on the decay profiles of the above mentioned organophosphorus compounds in soil and water under laboratory conditions but the effect of various parameters affecting the decay and the identification of metabolites have not been systematically investigated. There are some results available on the decomposition and the metabolic pathways of endosulfan. However, no field data are available on the decay profiles of the said pesticides under different environmental conditions.

In view of the above premise it was planned to investigate the decay of malathion and methylparathion under controlled laboratory and environmental conditions and identify the metabolites formed. It was also important to look into the effect of various variables on the decay rate of endosulfan in water and soil.

For the sake of clarity and convenience in presentation the work embodied in the thesis has been divided into the following five chapters:-

I. General Introduction.

II. Experimental Methodology.

III(a). Degradation of Malathion and Methylparathion in Water and Soil Under Laboratory Conditions.

III(b). Degradation of Endosulfan in Water and Soil Under Laboratory Conditions.

IV. Degradation of Malathion and Methylparathion in Plant and Soil Under

Field Conditions.

V. Identification of Metabolites of Malathion and Methylparathion.

Chapter I deals with the role of the pesticides and their classification. The problem of pesticide pollution is highlighted. The parameters affecting the decay of the pesticides and the different metabolites formed are discussed. Finally the aims and objectives of the present study are defined.

The relevant literature on the different aspects has been included in the respective chapters.

Chapter II details out the optimum operating conditions developed for the analysis of malathion, methylparathion and endosulfan. The malathion and methylparathion were analysed by using RP-HPLC and endosulfan by employing GLC with an electron capture detector. This is followed by a description on the extraction procedures adopted in the present investigations. Different procedures were explored to develope a method for each pesticide and the percentage recovery was noted. Methanol-water extraction proved to be most efficient for the plant and soil samples and methylene chloride for water samples. Methanol- water extracts from the plant and the soil were partitioned into methylene chloride, suitably cleaned and finally analysed by the appropriate chromatographic technique. The details of the equipments and the reagents used are also given in this chapter.

Chapter III presents results of the effect of temperature, pH and organic content (humic acid) on the decay of malathion,methylparathion and endosulfan in water.The findings about the decay profiles of the pesticides in three different

types of soil are also included . The samples were spiked with a known amount of the pesticide, extracted at different time intervals and analysed. The degradation was monitored for four weeks and in all the cases the decay is exponential in nature. The degradation rate in water was found to increase with the increase in the temperature and change in the pH from acidic to alkaline region. A similar effect of pH was observed in the soil. The presence of humic acid decreases the half-life of the organophosphates but increases that of endosulfan. The degradation of endosulfan is distinctly slower in soil than in water.

Chapter IV incorporates the degradation study of malathion and methylparathion in radish and carrot and the adjoining soil in three different seasons namely winter, summer and postmonsoon. Pesticide formulations of appropriate concentration were sprayed and the plants were harvested for the analysis at various time intervals. The pesticide from the whole plant was extracted and analysed using the procedure described in chapter II. The decay in summer showed two distinct profiles, initially it was faster and subsequently it slowed down. The decay in winter and postmonsoon followed the usual profile of pseudo first-order kinetics. The rate of decay follows the sequence winter $\simeq$ postmonsoon $<$ summer.

Chapter V describes the possible metabolic pathways of organophosphorus pesticides in radish,water and soil. The metabolites were identified by GC-MS in samples employed for the decay studies. The results indicate that the different metabolites are formed by de-esterification,hydrolysis, oxidation and/or reduction. In water the cleavage occurs at C-S or P-S bond of malathion whereas hydroxylation of phenyl moiety occurs in methylparathion. In soil and radish both the pesticides follow different routes.

It can be concluded that degradation of pesticides follow a first order kinetics. The laboratory data are more or less similer to field results in winter and postmonsoon. However, in summer two different profiles are observed. The study on the identification of metabolites of malathion and methylparathion indicates that they are formed as a results of hydrolysis, oxidation reduction and/or de-estrification. At some stage during the decay cycle the oxons of malathion and methylparathion are formed which are more toxic than their parent compounds.

At the end some important findings of the present work have been summarized.

LIST OF PUBLICATIONS

Papers Presented / Accepted for Presentation

1. Determination and Decay Study of Some Organophosphorous Pesticides by HPLC - Presented in XXI FACSS Meeting, St. Louis, MO, USA, Oct., 1994.

2. HPLC Optimization and Decay Study of Organophosphorous Pesticides., Presented in Conference on New Horizon in Analytical Chemistry, IICT, Hyderabad, India, Feb., 1995.

3. Parameters Affecting the Decay of Organophosphorous Pesticides., Presented in Indian Science Congress Meeting, Patiala, India, Jan., 1996.

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2. Degradation of Some Organophosphorous Pesticides Under Different Environmental Conditions (Accepted in Environmental Technology).

3. Rate of Degradation of Endosulfan in Water and Soil (Accepted in Environmental Technology).

4. Identification of Metabolites of Malathion in Radish, Water and Soil by GC-MS (Accepted in Journal of Biomedical Chromatography).

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CHAPTER I

GENERAL INTRODUCTION

Chapter - I

The green revolution in India has, no doubt, brought self-sufficiency in food grains and prosperity to farmers but in the process the relentless use of pesticides has played irrevocable havoc on health and environment of the nation. The pesticides constitute a group of chemicals which are used to control pests in order to save the agricultural produce. These compounds are mainly organophosphates, organochlorines and carbamates. Although 99.9 % of the insect population is considered as beneficial some parasitic species, however, affect agroproducts profusely and result into their loss. In order to save the crops heavy reliance is put on the use of pesticides. These agricultural chemicals have come to stay as essential inputs in modern agricultural strategy. In India, from a meagre 2,000 tonnes a year, in the fifties, the use of plant protection chemicals in both agriculture and public health has touched a level of 90,000 tonnes during 1989-90 and by the year 2000 A.D., their demand is estimated to cross 200,000 tonnes. These chemicals during 1989-90 covered an area of 80 million hectares as against 6.4 million hectares during 1960-61. Inspite of the increased reliance on these chemicals the losses due to pests including grain loss during storage have doubled from 33,000 million rupees in 1976 to over 66,000 million rupees in 1988. This clearly exposes the inadequacy of the measures taken for the storage and protection of crop in India, no wonder Giddings [1] stated

"The dream of the man that synthetic chemicals could dismantle the universal scourge of insects lies scattered–a monumental ecological absurdity"

Due to explosive population growth the need for more food is increasing steadily and simultaneously the farmers are getting aware of the benefits that can be harvested by the application of these agrochemicals. It is quite well-known that they do so without understanding the implications of the enhanced rate of usage of these chemicals. This may result into development of pest resistance leading to decreased pesticide efficacy, mortality of natural enemies of pest and other nontarget organism, pest resurgence, chemical pollution of the ecosystem and the like. The problem of environmental pollution arising due to their excessive and non- judicious use has already started restricting the boundaries of growth of the pesticide industry. The pesticide use in the modern agriculture has to conform to several specifications of ecological conservation and environmental hygiene and this has necessarily escalated the unit cost of development of commercial pesticides.

Pesticides are inherently toxic and have to be used with caution to avoid environmental pollution and hazards to biota. The toxic effects from accidental use, spillage or misuse of these chemicals damage practically all the segments of the environment. The common side effects [2] on different environmental components are listed in Table-1.1. Moreover, any accident in the process of manufacture and lapse in the use can cause tragedies of the type of Bhopal (India) disaster or epidemic isomalathion poisoning of malaria control workers in Pakistan [3]. In both the cases the main compound viz, carbamate or malathion as such were reasonably safe but the hazardous raw material in the former and a minor contaminant in the latter caused the tragedies.

The use of pesticides in agricultural products is a major source of involuntary exposure of the general public to suspected carcinogenic

Table -1.1 Some Side Effects of Pesticides.

Environment Component	Effect
Abiotic	Residues in soil, water and air.
Food	Presence of residues.
Plants	Presence of residues . Phytotoxicity vegetation changes.
Animals; avians, insects etc.	Residues in domestic animals and wild life. Physiological effects. Mortality in certain wild life species (e.g. mammals, birds, fish). Mortality of beneficial predatory and parasitic insects. Insect population changes (e.g. the development of secondary pest as a result of above). Genetic disorders, biochemical changes.
Man	Residues in tissues and organs. Mortality and deformations.

compounds due to contamination of fresh and processed food by persistent pesticide residues some of which cannot be washed off or degraded by cooking. An average Indian, according to a survey [4], daily ingests half a milligram of DDT and BHC with smaller amounts of malathion and endosulfan. This is forty times more than what an average American ingests and equals the danger levels permitted by the World Health Organization for daily intake. We are not only slowly poisoning ourselves but slowly jeopardizing the future of the coming generations. A recent study conducted in California (USA) by Schomburg *et al.* [5] showed that pesticides concentrate in fog. Even the breast milk of the women in USA was containing pesticides. The environmental scenario may be slightly better in the developed nations but not necessarily free from pesticide problems. Strangely many of the pesticides related accidents in the developing nations remain unreported. Some of the major accidents [6] which occurred in India are reported in Table-1.2.

In view of the problems associated with a large scale application of chemical pesticides and their long term impact on environment and health the need for developing and adopting safer alternatives of pest control mechanism is getting prominence. As a result of this the pesticide manufacturers are going for safer pesticides and the market is getting flooded with a variety of agrochemicals. The pesticides can be classified on the basis of target use like, fungicide, insecticide, herbicide or on their chemical composition. The most acceptable classification includes the following five major categories:-

(1) Organochlorine Pesticides

(2) Organophosphate Pesticides

(3) Carbamate Pesticides

Table-1.2 Some Important Pesticidal Accidents in India.

Pesticide	Place (State)	Year	Cause
Parathion	(Kerala)	1958	Food contaminated due to leakage
	Bombay (Maharashtra)	1962	Inhalation in manufacturing plant
H.C.H.	Bombay (Maharashtra)	1963	Rice contamination
Endrin	Bombay (Maharashtra)	1964	Food contamination
DDT	(Punjab)	1965	"Chutney" contamination with DDT
Diazinon	Pune (Maharashtra)	1968	Food contamination
H.C.H.	Lakhimpur (Uttar Pradesh)	1976	Mixed with wheat
Endrin	(Karnataka)	1977	Contaminated crabs in rice field
Aluminium-phosphide	(Rajasthan)	1983	Contaminated food grain
Methyl-isocyanate	Bhopal (Madhya Pradesh)	1984	Leakage from storage tank

(4) Inorganic Pesticides

(5) Botanical and Biopesticides

The organochlorine pesticides include the chlorinated ethane derivatives of which DDT and methoxychlore are the well known examples. Organochlorine compounds do not easily break down into the original elements or harmless compounds. Once applied they remain in the environment for longer duration and find their way into waterways, air, soil and the food chain. Most of these are banned for utilisation but their illegal use continues particularly in the developing countries.

Organophosphates, derivatives of phosphoric acid, are generally more toxic to vertebrates. These are related to the "nerve gases" so far as their mechanism is concerned. They inhibit enzymes related to nervous system and have been responsible for a number of systemic poisoning and deaths of agricultural workers. They are absorbed by skin as well as by the respiratory and gastro-intestinal tract. Most of them get easily decomposed in environment. The commonly used pesticides in this category are dimethoate, malathion and methylparathion.

Carbamates are the derivatives of carbamic acid($HO-\overset{\overset{O}{\|}}{C}-NH_2$)and are used in different situations, including homes, gardens and farms. Their toxicity to mammals and persistence in the environment may vary from low to medium and they inhibit the vital enzymes like cholinesterase (ChE). Some commonly used carbamates are, carbofuran, baygon, carbaryl and aldicarb.

A large number of elements like arsenic, copper, lead and mercury are the

basic constituents of several inorganic agrochemicals. Copper compounds such as copper sulfate and copper oxychloride are effectively used as fungicides. Sulfur dust and lime sulfur also find application as fungicide. There are several compounds of arsenic and mercury which are very poisonous. Inorganic pesticides have practically lost ground to the organic compounds due to their lethal effects.

The widespread use of the above category of chemical pesticides has resulted into several environmental problems and there is an emphasis to develop safer alternatives. A significant advancement in this direction is the work on botanical and biopesticides. Botanical or phytochemical pesticides are the derivatives of plants traditionally used by farmers to ward-off household insects and pests of agricultural and medical importance. A handful of botanical insecticides are currently approved for use as plant protectants [7] and a number of others are under development and evolution. These are mainly pyrethrum (Chrysanthemum cinerariaefolium), neem (Azadirachta indica), nicotine (Nicotiana sp.), ryania(Derris lonchocarpus). Biopesticides formulation containing bacteria, fungi, virus, protozoa or nematodes are now becoming available in the market. Some of these are dipel (Bacillus thuringiensis, bacteria), bicon (Paecillomyces lilacimis, fungi) and spod-X (Insect virus). Large scale use of these pesticides by a common farmer is still awaited due to the cost constraints and their accessibility.

There are about 1,250 agricultural chemicals registered throughout the world of which about a quarter have been phased out or banned. The list of banned pesticides as now includes 324 compounds and is likely to swell for reasons of human and environmental safety. The Indian Insecticide Act (1968) has a scheduled list of about 340 pesticides to which an addition of 21

chemicals was made in July 1982 and many more subsequently. However, the list of the pesticides registered for use in India under this Act is small and covers only 134 synthetic and biological pesticides. Interestingly, many of the pesticides which have been banned for use in the developed countries find entry in the developing nations. But to some extent they find their way back to the country of the origin on imported food and food products – *a phenomenon aptly dubbed as the circle of poison.*

There is a growing concern throughout the world about the deleterious effects of pesticides to man and environment. No doubt the use of pesticides is an important input for the safety of the agriculture produce but it leaves an everlasting problem for human safety. Once the pesticides are released into the environment they residually impair the quality of water, soil and air, depending on their persistence. A problem inherent in current pesticide application technology is drift or disposal of the pesticide away from the site of application. Only 10 to 15 % of the applied pesticides actually reach the target with the remaining 85 to 90 % dispersed off from the target to air, soil and water.

The degradation of the pesticides and the types of metabolites formed involve complex mechanism and depend upon chemical nature of the pesticide, type of the medium and the environmental conditions. The medium can be air, water, soil or plant. Even in the simplest case like water there are variations in different physico-chemical parameters which affect the persistence of the pesticides. The climatic factors such as temperature, humidity, rainfall, wind velocity etc. also affect the decay rate. The various monitoring programmes need to be designed to fulfil the obligation to know as to where does pesticide go after it is released into the environment, what

happens to it subsequently , where does it accumulate if at all, how fast it breaks down, what compound is formed and which can be disregarded and what are the effects of these residues ? These are some important aspects for which answers should be available. Many Western countries have established intensive monitoring programmes to estimate the levels of pesticide residues in both abiotic and biotic components of the environment. However, in India national level programmes to monitor pesticide residues and their biological effects are still in infancy.

In order to understand the fate of a pesticide in totality there is a need for determining pesticide residue in soil, water and plants in different environmental conditions. Pesticide residues in the environment are usually present in very small quantities varying from ppb to ppm levels. To add to this is the formation of metabolites which may have different degrees of toxicity. Some of these are more toxic than their parent compounds. For example, aldrin and heptachlor are known to be converted into their epoxides, both on the surface of plant and in soil. Parathion and other sulfur containing thio- and dithio-organophosphates are oxidised into oxons. Both epoxides and oxons show more mammalian toxicity than their parent compounds. In the cases where metabolites are more toxic it is imperative to include them for final evaluation of the pesticide toxicity. The pesticides have a tendency to bioaccumulate in the living cells. Bioconcentration has been defined as the amount of a pesticide residue accumulated by an organism by adsorption and absorption via oral or other portal of entry. It depends on the temporary equilibrium of residue concentration reached initially by adsorption on organism in competition with air, water and soil segments of the environment and the redistribution of the adsorbed pesticides in organisms and their tissues by ingestion, absorption,

metabolism partitioning, storage and elimination. In measuring bioconcentration factors such as transformation products of the pesticide, consumption rate,and ecological response of various organism to pesticides at recommended dosages are important. Kenaga [8] has emphasised that low water solubility, high fat solubility, high partitioning coefficient from water to environmental components and high stability under various hydrolytic, light, heat and microbiological conditions are some of the properties of pesticides resulting into higher bioaccumulation.

Bioconcentration and biotransfer potential of various pesticides and their interaction with various components of the environment play a very important role in deciding the fate of the pesticides. The complex interrelationship of these systems has been illustrated in Figure-1. The problem due to contamination by pesticide residues may appear to be removed at the initial point of introduction but can always create serious implications in some other part of the ecosystem. Attri [9] , Kalra and Chawla [10] have reviewed the status of pesticide residues in Indian environment. The presence of residues in the edible portions of eleven species of fresh water fish trapped from river Gomati in Lucknow belt has been reported by Kaphalia *et al.* [11]. The biotransformation, decay and consequent formation of metabolites and the mobility create problems in tracing the pathways of the pesticides. Invariably the pesticide residues occur in very low concentrations. The problem of analysis is further compounded due to the interference from matrix constituents and the metabolites. However, in some situations metabolites may have to be traced. For the analysis of pesticide residues, various chromatographic techniques particularly Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) have gained prominence and the

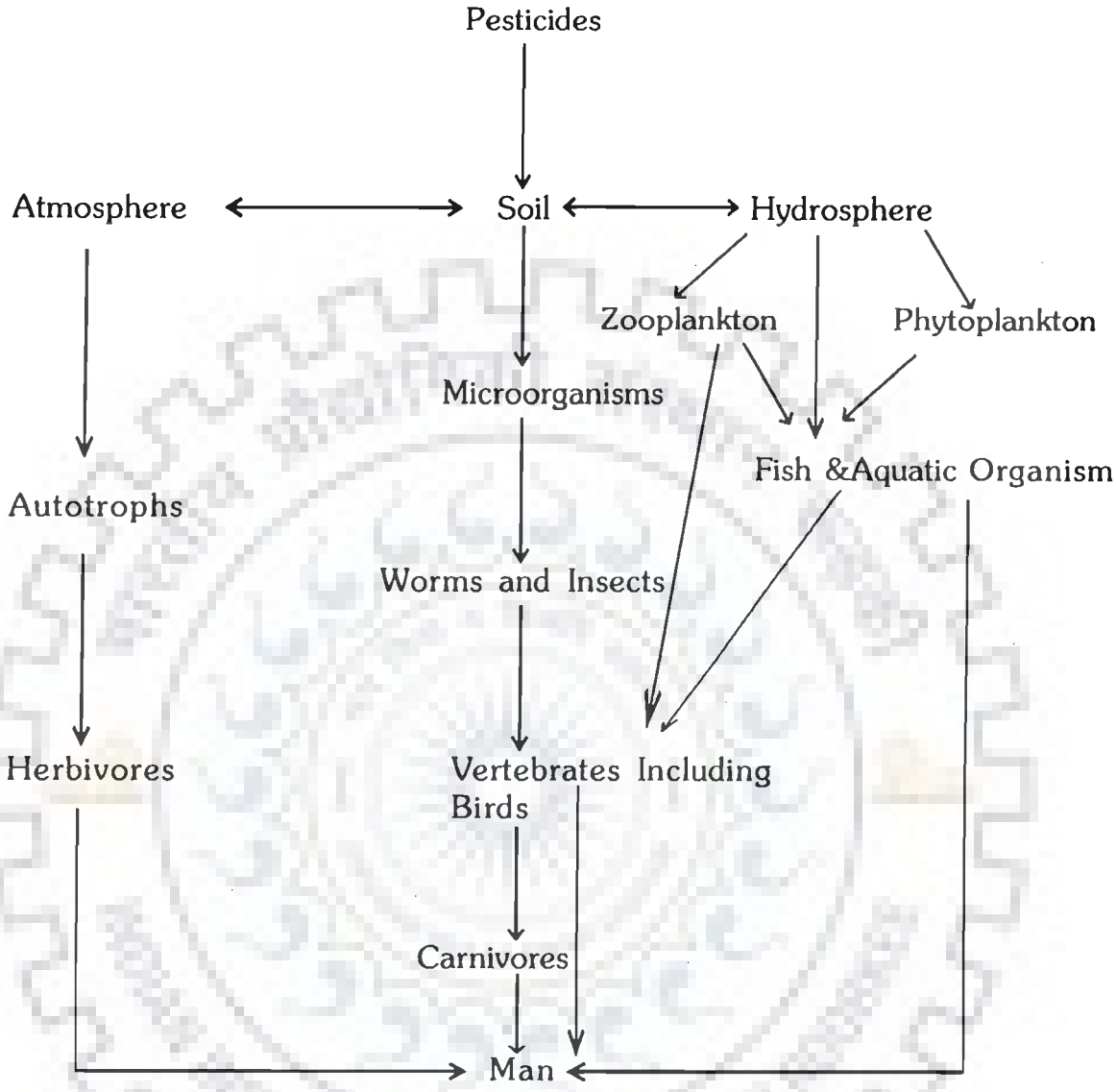


Figure - 1 Biological Transfer of Pesticides to Man

effectiveness of these techniques is improved by hyphenating them with mass spectrometry. The relevant literature on the use of these techniques and the methods employed for extraction is reviewed in chapter II.

On screening the available information from various sources it was found that organophosphates, namely, dimethoate, malathion and methylparathion are extensively used in India. They occupy a favoured position due to their relatively fast decomposition and low accumulation in the biological food chain. The cost constraints also partly dictate their use in developing countries like India. Most of the organochlorines are banned on an international level but some are still being used in India. The most common of these is endosulfan, a chlorinated pesticide with cyclodiene moiety. Its use is probably being overlooked by the concerned authorities because of its supposedly faster decay than the other organochlorines.

The organophosphate pesticides, no doubt are effective, but cannot be rated as safe or less toxic. These compounds inhibit the enzyme acetylcholinesterase resulting into the accumulation of acetylcholine in nerve tissue. The nerve starts firing in an uncontrolled manner and with the result organism (pest/insect) is quickly killed [12]. Snamchaiskul [13] studied the systematological toxicity and the residual effect of methylparathion alongwith other pesticides and found that methylparathion caused longer convulsive effect to worms. The effect of methylparathion on dietary discrimination ability was assessed in two-week old bobwhite chicks (Colinus virginianus) by Bussiere *et al.* [14]. The study demonstrated that feeding behaviour and taste discrimination ability of bobwhite chicks were impaired due to exposure to methylparathion. Meyers and coworkers [15] found that methylparathion had no apparent adverse effect on

reproduction in redwinged blackbird (Agelaius phoeniceus) although it caused ataxia, lacrimation, lethargy and depressed cholinesterase activity.

Resistance of methylparathion, malathion, dimethoate and endosulfan alongwith other insecticides by mustard aphid (Lipaphis erysimi) was studied for more than two decades by Dhingra [16]. He observed that initially upto eighteen years methylparathion, malathion and endosulfan did not show change in toxicity to L.erysimi but in the later years the development of resistance in L.erysimi to these pesticides was not rapid except for malathion. Mittal *et al.* [17] studied the acute toxicity of some organophosphates, organochlorines and synthetic pyrethroid and microbial insecticides to mosquito fish (Gambusia affinis) and found that organochlorines were less toxic than the pyrethroid followed by organophosphates. Dhingra and Lal [18] observed the relative toxicity of different insecticides to the adults of Phyllotreta cruciferae and found malathion to be more toxic than endosulfan. In vitro effects of organophosphates namely malathion and methylparathion on calmodulin activity (CaM) were studied by Pala and coworkers[19]. They suggested the conformation and regulation of Ca^{2+} transport and transduction of enzymes in CaM. Gupta *et al.* [20] found that malathion in vitro conditions even at low concentrations altered the level of enzymes associated with glutathione cycle and antioxidant defence system in human fetal brain and liver.

Malathion, besides inhibiting acetylcholinesterase, also causes other enzymological changes that seemingly are the results of interference with cell membranes and other organelles in metabolism[21]. Neskovic and coworkers [22] investigated the toxic effects of malathion bound to wheat grain and its effect on rats and observed that the main route of (C^{14})-malathion

excretion was through the urine and this signified that grain bound malathion was bioavailable. Toxicological analysis on Drosophilla melanogaster strains demonstrated the resistance of organophosphorus pesticides with the amount of acetylcholinesterase in the central nervous system. They finally concluded that qualitative and quantitative changes in acetylcholinesterase confer resistance to insecticides [23]. Effect of malathion on serum and glandular thyroxine (T-4), tri-idothyronine (T-3), the T-3/T-4 ratio, peroxidase activity and extra-thyroid conversion of T-4 to T-3 have been studied by Sinha *et al.* [24] in fresh water catfish (Clarias batrachus) during the pre-spawning and spawning phases of its annual reproductive cycle. They observed that malathion inhibited T-4 secretion in the kidney but accelerated T-4 synthesis in the pharyngeal thyroid and also inhibited the extra thyroidal conversion of T-4 to T-3 in serum. Pope [25] observed the dose related inhibition of brain and plasma cholinesterase (ChE) in neonatal and adult rats following sublethal organophosphate exposures. He suggested that in vivo inhibitory potency of the methylparathion and chloropyrifos pesticides is towards either brain or plasma. ChE activity was highly correlated with sensitivity to acute toxicity to both neonatal and adult rats.

Malathion and malaoxon alter post-translation modification by collagen with resultant morphological defects in connective tissues [26]. The effect of malathion on cellulase, protease, urease and phosphatase activity has been investigated in a laterite type tropical soil under laboratory conditions and a marked suppression of urease and phosphatase activities in malathion treated soil was observed [27]. Lohar and Wright [28] concluded that malathion changes the lipid content in haemolymph, fat body and oocytes of Tenebrio molitor adult females which might be due to the effect on the neurosecretory adipokinetic

hormone controlling the lipid metabolism. In pregnant rats higher levels of cyctochrome P450 observed in estrogen plus malathion administered rats might have stimulated the increased conversion of malathion to more potent malaoxon. It might have also blocked the glutathione S- transferase and carboxyl esterase related detoxification process resulting in increased malathion toxicity [29]. The toxic effects of malathion and dimethoate alongwith other organophosphates insecticides on Caribbean fruit fly (Anastrepha suspensa) were studied by Nigg *et al.* [30] . They observed that females were significantly more tolerant to malathion than males by oral administration but not by topical application. Villar and coworkers [31] also studied the effect of organophosphate insecticides on Dugesia trigrinia in cholinesterase activity and head regeneration.

The economic conditions prevailing in developing countries dictate the use of cheaper broad spectrum organochlorine pesticides. The indiscriminate use of these pesticides in agriculture, forestry and public health leaves considerable amount of residues and their metabolites in the environment. Endosulfan, a chlorinated pesticide, and its pollutant metabolites are often detected in the natural environment. The two isomers of endosulfan and the biologically active oxidation product detected in waterways are extremely toxic to fish. Higher salt content of water does not change the toxicity of endosulfan towards aquatic organisms. The studies conducted by Knauf and Schulze [32] with golden orfe (Idus idus) have confirmed this fact. On the other hand the same group of workers and Schoettger [33] during their investigations on endosulfan metabolites observed that its sulfur free metabolites possess a significantly lower toxicity against fish. Dalela *et al.* [34] investigated the influence of sublethal long term in vivo exposure of endosulfan on Channa gachua, a fresh water

teleost of India and concluded that endosulfan has influence on various energy dependent processes on the fish tissue. The systematological toxicity and the residual effect of endosulfan on silk worm (Bombyx mori) showed the specific action of high toxicity as well as occurrence of more convulsions [13]. Rao[35] observed the relative toxicity and metabolism of endosulfan to the Indian major Carp (Catla catla) with special reference to some biochemical changes induced by the pesticide. Gill *et al.*[36] studied the effects of endosulfan and phosphamidon poisoning on the peripheral blood of fish (Barbus conchonius).

Reddy *et al.* [37] observed the toxic potential of commercial grade endosulfan on the protein metabolism of fresh water crab in-vivo. Das and Sengupta [38] observed the effect of endosulfan and malathion on the ovarian steroidogenesis and brain acetylcholinesterase activity of cat fish (Clarias batrachus). They found that the exposed fish ovaries showed the loss of stage II and III oocytes and the absence of DELTA-5-3-beta HDS and G-6-PD activity and confirmed the inhibition of the brain AchE activity. Udeaan and Narang [39] studied the ratio of LC_{50} of endosulfan for the green peach aphid (Myzus persicae) and suggested the localised emergence of endosulfan. Ferrando and Moliner [40] studied the effect of temperature, exposure time and other water parameters on the acute toxicity of endosulfan to European eel (Anguilla anguilla) and found that the toxicity increased when the water temperature increased. Ferrando and coworkers [41] also found that the endosulfan was more toxic than diazinon, fenitrothion, chloropyrifos and lindane. Bioaccumulation [42] and chronic toxicity [43] of endosulfan in the tissues of Louisiana cryfish (Procambarus clarkii) was studied by Naqvi and Deborah. The acute toxicity of endosulfan to the fresh water rotifer (Brachinous calyciflorus) was determined by Casalderrey *et al.* [44]. Claudio and Toledo [45] observed the acute toxicity to the yellow

tetra (Hyphessobrycon bifasciatus) and zebra fish (Brachydanio rerio). They found that the behavioural changes included hyperactivity, erratic swimming and convulsions. Histological studies showed predominant acute effects in the gills, with inflammatory infiltration, necrosis and separation of respiratory gill epithelium.

The above reports merely act as a pointer to project the toxicity of organophosphates and endosulfan. Most of these studies have been made on aquatic fauna. The toxic effects with some reservations can be extrapolated to different terrestrial forms . In order to assess the effects of these pesticides quantitatively the decay profiles and the metabolites formed should be precisely known. As indicated earlier the decay of pesticides follow a very complex mechanism and will depend on a number of parameters. The simplest approach to the problem should be to follow the decay under controlled laboratory conditions and identify the metabolites at various stages of decay. Once the effects of different variables on decay and metabolites formation are known the study could be extended to the field. Substantial literature is available on the residue analysis of these pesticides. This essentially reports the identification and quantification of the pesticides in different components of the ecosystem. Scattered references are also documented on the degradation of these pesticides under laboratory conditions. However, the field data on the persistence profiles under different field conditions are scarce. The detailed literature on these aspects is reviewed in chapter III and IV.

There has been a growing concern about the nature and toxicity of the breakdown products and a good deal of information exists on the identification of photodegradation products of the pesticides. No systematic study has been

carried out on the identification of metabolites at different stages of decay. Keeping this in mind it was planned to carry out investigation on the decay profiles and identification of the metabolites of the three organophosphorus pesticides namely dimethoate, malathion, methylparathion and the organochlorine, endosulfan. But during the analysis of dimethoate there were serious interferences due to matrix constituents and the proposed investigation on this pesticide could not be pursued. A sizable amount of literature on the metabolites of endosulfan is already available and the work on this pesticide was confined to its decay kinetics. In the first phase, studies were undertaken on the degradation of malathion, methylparathion and endosulfan under controlled laboratory conditions. Subsequently studies on the persistence of malathion and methylparathion were extended to plants in three different seasons of the year. Finally the metabolites of malathion and methylparathion were identified at various stages of decay in water, soil and plant.

For convenience and clarity of presentation the subject matter of the thesis has been divided into the following five chapters :-

I. General Introduction.

II. Experimental Methodology.

III. (a) Degradation of Malathion and Methylparathion in Water and Soil under Laboratory Conditions.

(b) Degradation of Endosulfan in Water and Soil under Laboratory Conditions.

IV. Degradation of Malathion and Methylparathion in Plant and Soil under Field Conditions.

V. Identification of Metabolites of Malathion and Methylparathion.

Chapter I presents a brief background on the role of the pesticides and their classification. The environmental problems caused by pesticides are highlighted. The parameters affecting the decay of pesticides and the toxicological effects are discussed. Based on the available information the objectives of the work embodied in the thesis have been defined.

The related literature on the different aspects has been included in the respective chapters.

Chapter II details out the optimum conditions for the analysis of malathion, methylparathion and endosulfan. Malathion and methylparathion were analysed by RP-HPLC and endosulfan by GLC. This is followed by a description of the extraction procedures adopted in the present investigations. Extraction procedures were explored with a view to develop a single extraction method for all the three pesticides keeping the percentage recovery in mind. Methanol-water mixture proved to be most efficient for the extraction from plant and soil and methylene chloride for water samples. Methanol-water extracts from the plant and the soil were partitioned into methylene chloride, suitably cleaned and finally analysed by the appropriate technique. This chapter also includes the details about the different materials and the instruments used during the investigations.

Chapter III contains the results of the effect of temperature, pH and organic content (humic acid) on the decay of malathion, methylparathion and endosulfan in water. The results on the decay profiles of the pesticides in three different types of soils are also included. The samples were spiked with a known amount of the pesticide and extracted according to the established procedures. The degradation was monitored for four weeks and it is found to be exponential

in nature. The decay rate increases with the increase in temperature and pH. There is no marked difference in the decay behaviour of malathion and methylparathion in both water and soil. However, the decay rate of endosulfan is slower in soil than in water. The presence of humic acid upto a certain concentration increases the decay rate.

Chapter IV incorporates the degradation study of malathion and methylparathion in radish (Raphanus sativus), carrot (Daucas carota) and the adjoining soil in the different seasons namely winter, summer and postmonsoon. Pesticide formulations in appropriate concentrations were sprayed and the plants were harvested for analysis at different time intervals. The pesticides were extracted from the plant and analysed using the procedure described in chapter II. The degradation in summer showed two distinct decay profiles, initially it is faster and then slows down. The decay rate follows the sequence winter $\simeq$ postmonsoon <summer.

Chapter V details out the possible metabolic pathways of malathion and methylparathion in water, soil and radish. Identification of metabolites was carried out by using GC-MS in samples collected at different stages of decay of the pesticide. The metabolites are formed by de-esterification, hydrolysis, oxidation and/or reduction.

The thesis ends with a note on some important findings.

In the end the author would like to mention that though utmost care has been taken to cite the relevant literature, however, if there are any omissions they are purely inadvertent. Furthermore, in presenting the work, care has been taken to minimize repetition nevertheless, at times, it has not been feasible to avoid it.

CHAPTER II

EXPERIMENTAL METHODOLOGY

Pesticide residue analysis is generally a time consuming and complicated excercise. The major steps in the analysis are :-

(i) Collection and preservation of the sample.

(ii) Extraction of the pesticide.

(iii) Clean-up of the undesirable constituents.

(iv) Determination of the pesticide.

Sampling is the most vital step because the collected sample must represent the exact scenario of the environment from which it is withdrawn. It is very important that the sample intended for residue analysis does not undergo any degradation or chemical change during collection and storage. Also it should not be contaminated with the impurities which may affect the results of the analysis. Invariably the environmental and biological samples cannot be subjected to analysis directly as the concentration of the pesticides encountered is very low and the levels of the interfering constituents may be too high. It is, therefore, necessary to extract the desired compound from the sample ensuring that the extraction is quantitative and there are no changes in the composition. Besides this the components of the matrix should not be coextracted with the pesticide to the extent possible. Subsequently the sample is to be cleaned from the remaining interfering impurities before subjecting it to assay. Adsorption column chromatography finds an extensive use for the clean-up of the sample and now a days standard cartridges are available

for the said purpose. For the measurement of the pesticide concentration various analytical techniques mainly chromatography in its different forms and spectrophotometry are in vogue. Amongst chromatographic techniques HPLC and GC are more popular and whatever improvements are taking place in the pesticide analysis are mainly directed towards these two techniques.

The first documentation on the use of HPLC for pesticide residue analysis was in the year 1971 by Henry *et al.* [46] who determined pesticide in pond water. Although GC is senior and more sensitive than HPLC but for the pesticide analysis it has been partly overtaken by HPLC. A real break through in the technique came with the introduction of versatile ODS column for HPLC by Kirkland and De Stefanos [47]. According to them, two thirds of all the desired separations in the pesticide analysis can be achieved by reversed-phase high performance liquid chromatography . HPLC technique is quite advantageous as it provides the option of collecting the fraction for subsequent testing. The polar fractions of the different matrices which generally constitute more than 70% of the organic carbon on account of high polarity or boiling point can be analysed speedily and effectively by HPLC alone. Barcelo [48] made an indepth review of the use of HPLC in environmental pesticide analysis. Furthermore, HPLC quickly and accurately determines the pesticides which are not easily amenable to analysis by GC at room temperature. Both GC and HPLC are very effective as far the separation is concerned but they are not equally good for identification or confirmation. Therefore they are required to be hyphenated with mass spectrometry particularly in situations where the metabolites are also to be identified alongwith the parent compound.

Inspite of vast literature on the extraction procedures for pesticide residue analysis none of the methods can be rated as standard. The method will depend upon the nature of the pesticide and the medium from which it is to be extracted. On the other hand it is true that the existing information on extraction, clean-up and quantitation can provide a handy guide for developing the analysis procedure. The available literature on the different aspects of analysis of organophosphorus pesticides and endosulfan is briefly reviewed as under.

The biennial reviews by Joseph Sherma which appear in " Analytical chemistry " provide an exhaustive and upto date information on the status of pesticide analysis. Several extraction procedures have been used to extract pesticides from plant, water and soil for the analysis. The methods include simple shaking of solvent with the substrate, centrifugation and soxhlet extractions. The basis for selecting an optimal extractant has not been very clearly defined in the literature. Burke and coworkers [49] evaluated the utility of acetonitrile for extracting organophosphate and organochlorine pesticides from green vegetables and soils. Wheelar *et al.* [50] extracted ten pesticides including organophosphates and organochlorines using methanol, acetone and acetonitrile from the crops and found that the methanol extraction was best in each case. Farrington and coworkers [51] extracted pesticides from grain and soil with methanol and from water with dichloromethane and developed the use of a wrist action shaker for thermolabile compounds. Ambrus *et al.* [52] found that acetone extraction was useful for organophosphates, organochlorines and several other classes of pesticides from soil, plant and water. Dao *et al.* [53] presented a rationale

on the solvent selection for the extraction of residues and concluded that simply on the basis of solubility it is not possible to predict the suitability of a solvent as an extracting medium.

Di Corcia and Marchetti [54] outlined a simple procedure for the extraction of thirty five pesticides from drinking water. They used carbapak β -graphitized carbon black cartridges for their isolation and the detection was done using HPLC with an uv detector. Bowan *et al.* [55] compared a number of solvents for extracting organophosphates from field treated crops. Extraction of the pesticide with 10% methanol in chloroform was found to be the best method and the residues were subsequently analysed by GLC with a flame photometric detector. Liquid chromatographic retention behaviour of seventeen pesticides has been studied on the Vydac-reversed phase using water-methanol and water-ethanol mixtures. Based on the response of an ultraviolet detector the limit of detection for each compound was reported [56]. Kvalvag *et al.* [57] removed parathion from soil/dust samples with acetone-water mixture and analysed it on a HPLC system. Paschal and coworkers [58] have made the use of resin XAD-2 for a good recovery of ethyl and methylparathion. Lawrence and Turton [59] estimated one hundred and sixty six pesticides by HPLC. It was reported that RP- HPLC, using an octadecyl bonded silica column and acetonitrile-water mixture as the mobile phase gives the most efficient separation of the pesticides [60]. LeBel *et al.* [61] isolated sixteen organophosphorus pesticides from drinking water using Amberlite XAD-2 resin and acetone-hexane (15+85) as an eluent and the recovery for each of them was more than 90%. Desmarchelier *et al.* [62] found that methanol or ethanol extracted higher levels of organophosphate

pesticides from crops than hexane. Rajendra Babu *et al.* [63] extracted organophosphates by n-hexane. Kjoelholt [64] estimated organophosphorus residues in soil and sediments by Capillary GC with a N-P detector.

A gas chromatographic method has been used for the analysis of seventeen pesticides in the food samples from Food and Drug Administration (FDA) (USA) [65]. Kelley *et al.* [66] have presented a compendium on the procedures for the enrichment, clean-up and determination of organophosphorus pesticides in water and solid samples by GC and HPLC. Martindale [67] determined the residues of a range of fungicides, antisprouting agents and insecticides (organophosphorus and organochlorines) in potatoes by HPLC and GC. Neicheva *et al.*[68] extracted organophosphorus pesticides with acetonitrile and analysed them by GC on a glass column. The organophosphorus pesticides were quantitatively recovered from potatoes with acetone and determined by GC [69]. Sharp *et al.* [70] have reviewed the literature on the extraction, clean-up and determination of organophosphorus pesticides, pyrethroid and carbamate by GC and HPLC in grain and grain products. Organophosphorus pesticides have been determined in industrial and municipal waste water by dichloromethane extraction, florisil column clean-up and final estimation by GC [71]. Twenty nine organophosphorus pesticides were simultaneously determined in food by acetone extraction followed by methylene chloride and hexane (2:8) partition and estimating them by capillary gas chromatography [72]. Branca and Quaglino [73] determined organophosphorus pesticides and triazine herbicide in food and water by acetone extraction followed by analysis with GC. Hernandez *et al.*[74] presented a comparative study of different multi-residue methods for the determination of pesticides in fruit samples

by GC. Bayers and coworkers [75] have developed an analytical method using C_8 solid phase extraction (SPE) column to extract malathion and carbaryl from well and pond water. Seven pesticides were analysed by SPE using glass columns and 47 mm disks of octyl and octadecyl bonded silica. Better extraction efficiency was obtained with C_8 disks [76]. Pinto *et al.* [77] used dual electrochemical detection for the liquid chromatographic analysis of organophosphorus pesticides after cloud point preconcentration with a non-ionic surfactant, Triton X-114.

Like the organophosphorus compounds there are numerous methods for extracting endosulfan from different media. The more popular ones involving the use of various liquid-liquid partition systems have been reviewed by Beroza *et al.* [78]. Beard and Ware [79] have extracted the isomers and metabolites of endosulfan in a mixture of hexane-ethanol and petroleum ether. Ethanol was removed by shaking it with water. Archer [80] recovered endosulfan from plants by soxhlet extraction using a mixture of benzene and propanol. Ranga Rao and Murty [81] extracted endosulfan from the soil samples with 10% acetone in distilled water. Zweig and Archer [82] described a gas liquid chromatographic procedure for the determination of endosulfan in a technical grade material using a column containing chromosorb coated with 30% silicon grease at 250°C. It was thus possible to estimate the two isomers of endosulfan. Ballschmiter and Tolg [83] reported the gas chromatographic behaviour of endosulfan and its few metabolites on three columns of different polarity. Schuphan *et al.* [84] discussed in detail the stationary phases suited for the separation of α and β endosulfan from their metabolites.

Burke and coworkers [85] have extracted endosulfan (α and β), its metabolite and endosulfan sulfate by acetonitrile. Zoun *et al.* [86] determined endosulfan in water and fish samples by GC. Riebal and coworkers[87] recovered β-endosulfan from the solid sample using GC on a glass column of chromosorb WHP containing 1% each of QF-1, XE-60 and OV-17. Wylie and Oguchi[88] estimated α and β-endosulfan in apple by using P-selective detector. Gopal and Mukherjee [89] separated isomers of endosulfan (α and β) alongwith endosulfan sulphate metabolite on a glass column packed with 3% OV-25 on chromosorb W. Szeto and Price [90] extracted endosulfan from moist soil with ethylacetate and mixed it with sodium sulfate. Endosulfan from aqueous media was extracted with a mixture of hexane, acetone, methanol and medium (15:5:2:2) in Mixxor reservoirs [91].

A comparison of the different commonly used clean-up methods based on adsorption column chromatography has been presented by Versino and coworkers [92]. Sparacino and Hines [93] have recommended the use of sep-pak cartridges for the enrichment and partial clean-up. Ambrus *et al.* [52] found that mixed adsorbants were suitable for samples with high chlorophyll content and alumina for purifying lipid contents of the extract. Neicheva *et al.*[68] cleaned up the apple extract and used a mixed adsorbent column containing sodium sulfate, florisil, celite and charcoal (10:10:8:1).

The above literature survey clearly indicates that both GC and HPLC can be used effectively for the estimation of organophosphate residues. But because of certain inherent advantages of HPLC it has been preferred

for the analysis of malathion and methylparathion in the present investigation. For chlorinated pesticides it is well known that GC with an electron capture detector is more sensitive than HPLC. The fact is amply reflected in the literature by the scanty use of HPLC for endosulfan. Moreover, it was observed that HPLC did not give reproducible result with endosulfan. Therefore in the present studies GC has been used for endosulfan. The commonly used organic solvents for extraction of the pesticides are methanol, acetone, dichloromethane and chloroform. A trial of all these solvents showed that methanol-water is best suited for the extraction of malathion, methylparathion and endosulfan from soil and plants. Dichloromethane gave the desired results for extraction from water. A mixed adsorbant column containing silica, charcoal and magnesium sulfate (2:1:1 by weight) was effective for the clean-up of the samples.

The present chapter describes in detail the methods developed for the extraction, clean-up and quantification of malathion, methylparathion and endosulfan from water, soil and plant.

REAGENTS

Acetonitrile, methanol, dichloromethane, chloroform, acetone and water of HPLC grade from E-Merck (India) were used. Sodium sulfate, magnesium sulfate , charcoal and silica gel (mesh size 80-120) used for column chromatography were of desired analytical purity from E - Merck (India). Double distilled water was prepared after pretreatment with $KMnO_4$. The solvents and the water used in chromatography were degassed and prefiltered through 0.45μm millipore filter. Potassium dihydrogen

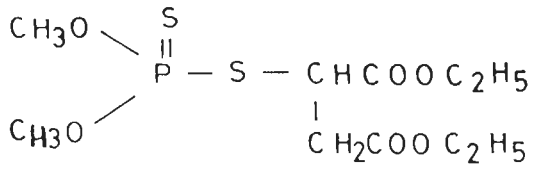
phosphate and di-sodium hydrogen phosphate-2-hydrate of analytical grade (E.Merck) were used for preparing buffer solutions. Sodium salt of humic acid (Aldrich, USA) was used as a representative of organic matter. Authentic samples of pure pesticides were procured from different sources. Pure (99%) samples of malathion (m.p. 2.85^{0}C) and endosulfan (m.p. 80-90^{0}C) were received as a gift from Indian Agricultural Research Institute, Pusa, New Delhi India. Methylparathion (m.p. 34-35^{0}C) of 99% purity was obtained from Bayers India Ltd, Bombay, India. The purity of all the three pesticides was checked by chromatographic techniques. The chemical structures of malathion, methylparathion and endosulfan (α and β, 70:30) are shown in Figure-2.1. The Formulation of malathion (35% E.C.) used for the study was of Anu, India and formulations of methylparthion (50% E.C.) and endosulfan (50% E.C.) were from Bayers, India Ltd.

EXPERIMENTAL

Apparatus

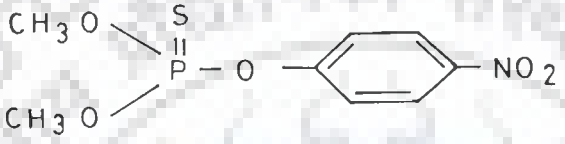
The Waters Associates (MA, USA) high performance liquid chromatographic system consisting of two high pressure pumps (M 501) with a pressure and flow capability of 6000 p.s.i. and 9.9 ml min^{-1}, respectively and Model 680 Automated Gradient Controller was used to programme the elution system and produce the gradient profile. Eluents were monitored using Lamda-Max model 481, variable wavelength spectrometer with a 5 µl cell and recorded using a Waters 740 Data Module. The column was octadecyl bonded phase µ Bondapak C_{18}, dimensions 30 cm x 3.9 mm. U6-K universal injector (syringe loop) and 25µl Hamilton syringe (Model No. 802) were used.

Malathion



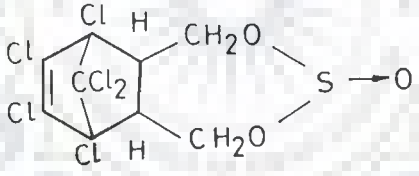
Diethyl Mercaptosuccinate S-Ester
with O,O-Dimethyl Phosphorodithioate

Methylparathion



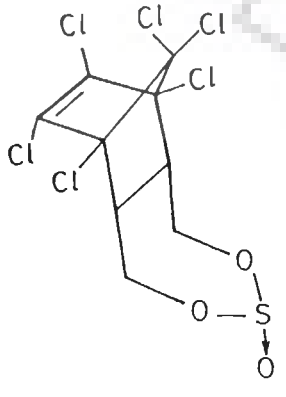
O,O-Dimethyl O-(p-Nitrophenyl) Phosphorothioate

Endosulfan



6,7,8,9,10,10-Hexa Chloro –
1,5,5a,6,9,9a-Hexahydro
6,9-Methano 2,4,3-Benzodioxathiepin
3-Oxide

∝-Endosulfan



β-Endosulfan

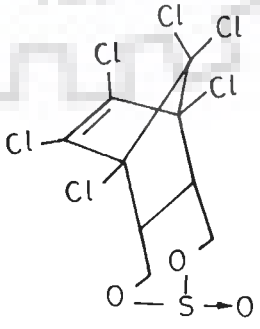


Figure 2·1 Chemical Structures of the Pesticides

Gas chromatograph of Hewlett Packard Model 5890 A fitted with an electron capture detector was used.

GC - MS system with Hewlett Packard(HP) G1800 A mass spectrometer coupled with GCD series gas chromatograph and an electron ionization detector was used for the identification of metabolites of malathion and methylparathion at the following operating conditions :-

Column : HP 5 (30m X 0.25 mm, i.d.)
Temperature : 100°C to 250°C,@ 10°C/minute
Run time : 22 minutes.

Optimization of Parameters for the Analysis by HPLC

The conditions were optimized by varying different parameters mainly mobile phase composition, uv detector wavelength, flow rate of the mobile phase and injection volume. Methanol-water and acetonitrile water mixtures in different ratios were tried as mobile phase with a view to get the best recovery and resolution. Acetonitrile-water was found to be a better mobile phase. In order to get the desired response of the uv detector wave length range from 210-254 nm was scanned. The flow rate was varied from 0.50 to 1.25 ml/min for speedy elution of the peak of interest without any compromise on the resolution. Under these conditions the injection volume was varied from 1 µl to 10 µl. The optimum conditions for the malathion and methylparathion are given below :-

	Malathion	Methylparathion
Mobile phase	70%Ac - 30%W	75%Ac - 25%W
Injection volume	3µl	3µl

Detector	uv	uv
Wave length	215nm	221nm
Flow rate	1.25ml min^{-1}	1.25ml min^{-1}

Ac → Acetonitrile

W → Water

Column was operated at ambient temperature.

Optimization of Experimental Conditions for the Analysis with GC

OV-17 (5m x 0.53 mm, i.d.) silicon column and glass column (2m x 2mm, i.d.) packed with 3% OV-225 on chromosorb W were tried to get a better response and resolution. The column temperature was varied from 160 - 230^{0}C keeping the injector and detector temperature at 300^{0}C. The two isomers of endosulfan can be separated on OV-17 silicon column at 180^{0}C but the resolution was poor. However, glass column packed with 3% OV-225 on chromosorb W operated at 230^{0}C gave a clean resolution of the two isomers. The operating conditions are given as under :-

Column	-	Glass column packed with 3% OV-225 on chromosorb W.
Detector	-	Electron capture detector (ECD)
Column temperature	-	230^{0}C
Detector temperature	-	300^{0}C
Injector temperature	-	300^{0}C
Injector volume	-	2 µl

N_2 with a flow rate of 36 ml min^{-1} was used as a carrier gas.

The chromatograms of the pesticides are shown in Figures-2.2 and 2.3

It is important to note that these conditions are equally effective for the analysis of the pesticides in the formulations as well as the environmental samples.

Extraction and Clean-up

Several extraction procedures [51,52,94,95] were assessed to evolve a single analytical method for different substrates namely water, soil and plants (radish and carrot) . Since the purpose of the determination was eventually to measure trace amount of the pesticide in the field samples it was logical to use a procedure which may give maximum recovery of the pesticide and require a minimum clean-up. Extraction procedures involving the use of acetone, methanol - water and chloroform were tried for the recovery of the pesticide from plant and soil samples. Acetone and methanol - water gave better recovery than chloroform but in acetone many constituents of the matrix were coextracted. The most efficient way to recover the pesticides from water samples was found to be direct partitioning with dichloromethane. The methods adopted for the extraction and clean-up from water, soil and plant (radish and carrot) are briefly summarized below:-

Water

10 ml of water sample containing the pesticide was extracted with 10 ml of dichloromethane. The organic layer was passed through an anhydrous sodium sulfate column and rinsed with 20 ml of dichloromethane. Dichloromethane was evaporated at the ambient temperature and the residue was dissolved in an appropriate solvent.

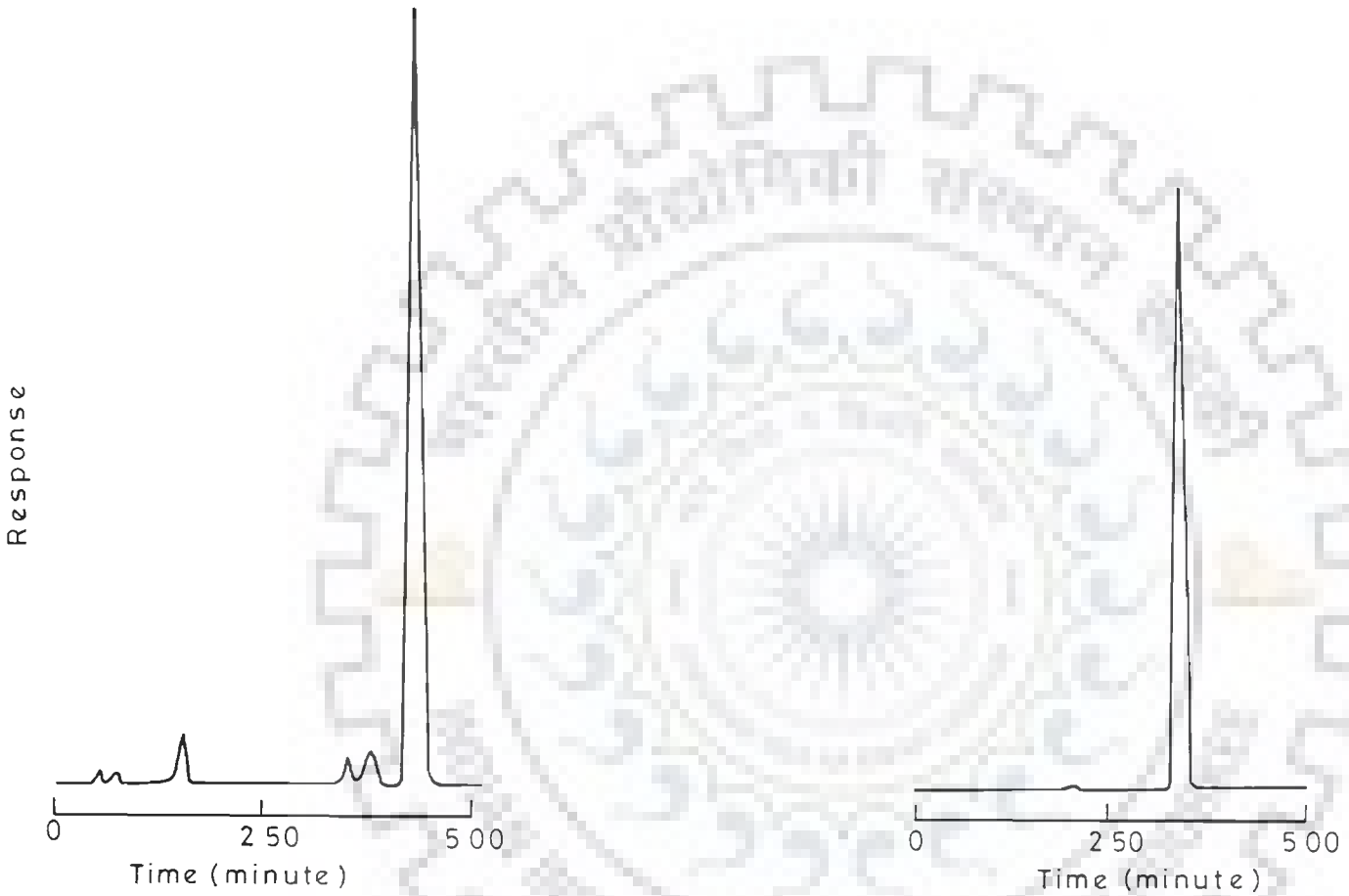
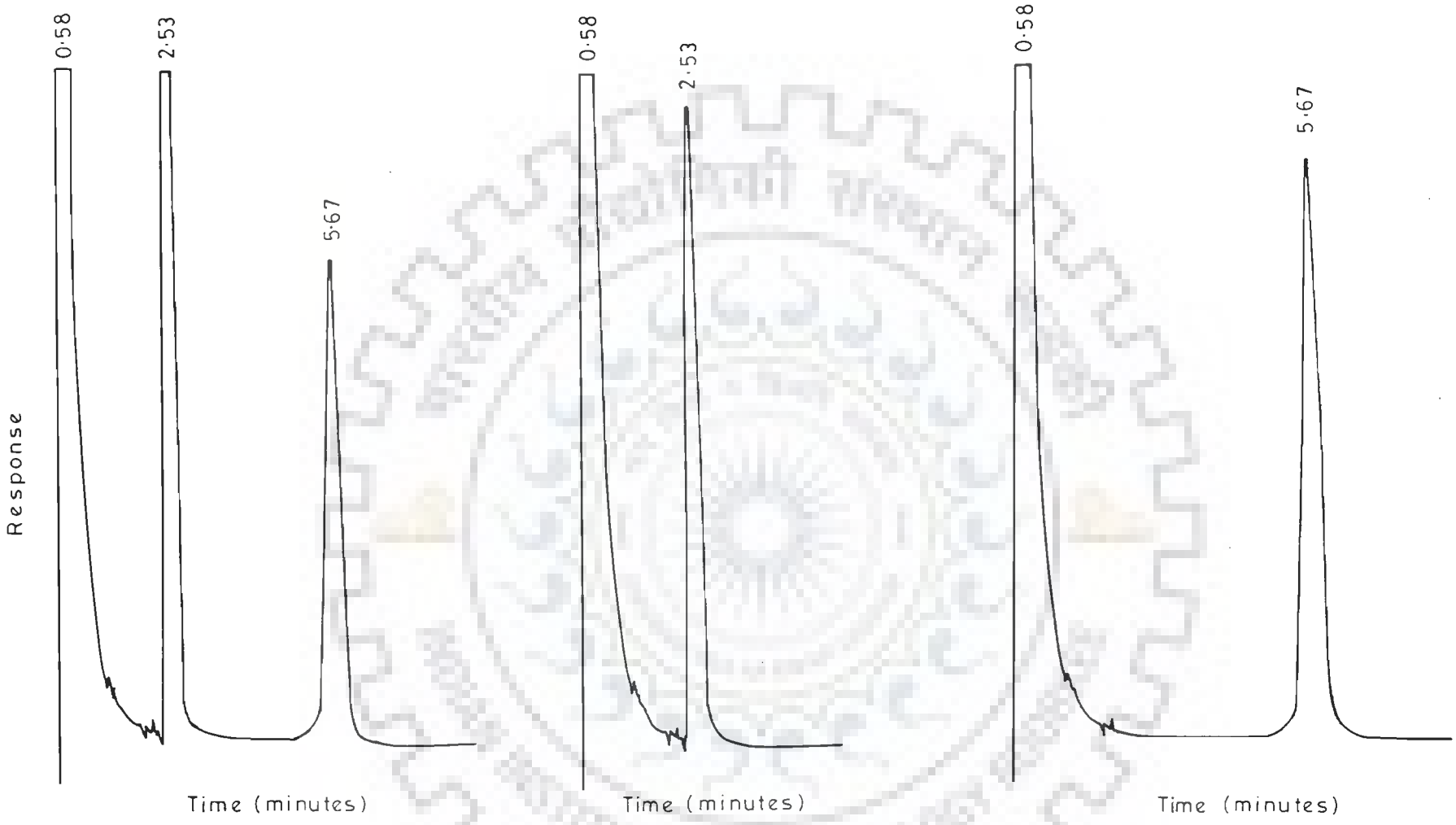


Figure 2·2 Chromatograms of Malathion and Methylparathion



(a) Endosulfan (technical) (b) α-Endosulfan (c) β-Endosulfan

Figure 2·3 Chromatograms of Endosulfan (technical) and Its Isomers

Soil

A known amount (ca 2g) of soil sample was shaken with 20 ml of methanol-water in 5:1 v/v ratio and centrifuged. The supernatant was partitioned with an equal volume of dichloromethane and the extract was passed through anhydrous sodium sulfate column. For the removal of interferences the extract was further passed through a column containing silica gel, charcoal and magnesium sulfate (2:1:1 by weight).

Plant

ca 100 g of plant sample (radish or carrot) was homogenized and ground before subjecting it to the extraction procedure. 100 ml of mixture of methanol and water in 5:1 ratio were added and shaken for 30 minutes on a mechanical shaker. Methanol-water extract was partitioned with 100 ml of dichloromethane and the extract was passed through anhydrous sodium sulfate column. Due to high pigmentation the extract was cleaned on a column containing silica gel, charcoal and magnesium sulfate in 2:1:1 (by weight) ratio.

The details of the above procedures are schematically shown in Figures- 2.4 - 2.6.

The above procedures were developed by spiking water, soil and plant samples with 100 ppm of malathion and methylparathion and 10 ppm of endosulfan, respectively. The percentage recovery for malathion, methylparathion and endosulfan is 92%, 97% and 95% (σ ±1, n=5), respectively. Blank determinations were run but the contribution was found to be negligible.

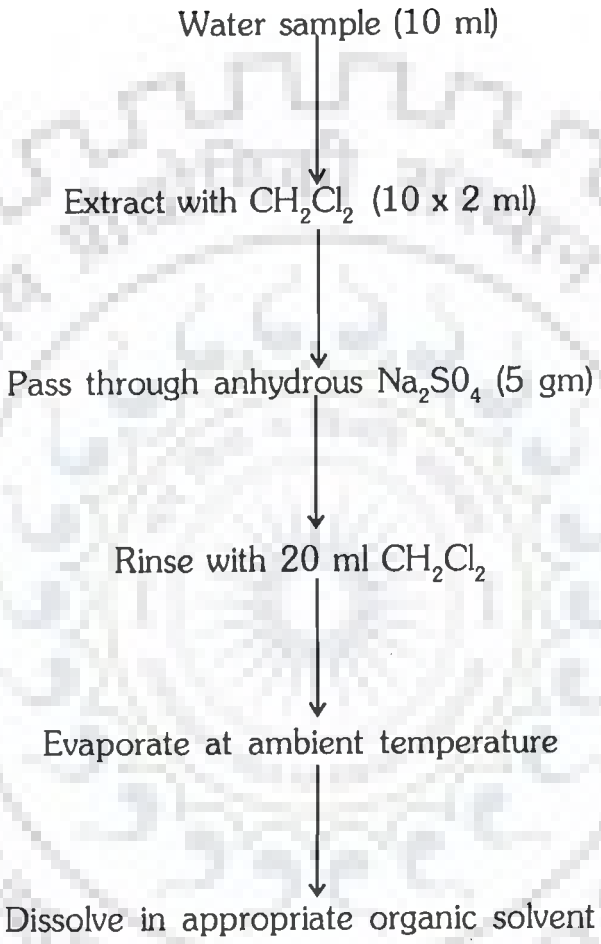


Figure-2.4 Extraction Procedure for Water Sample

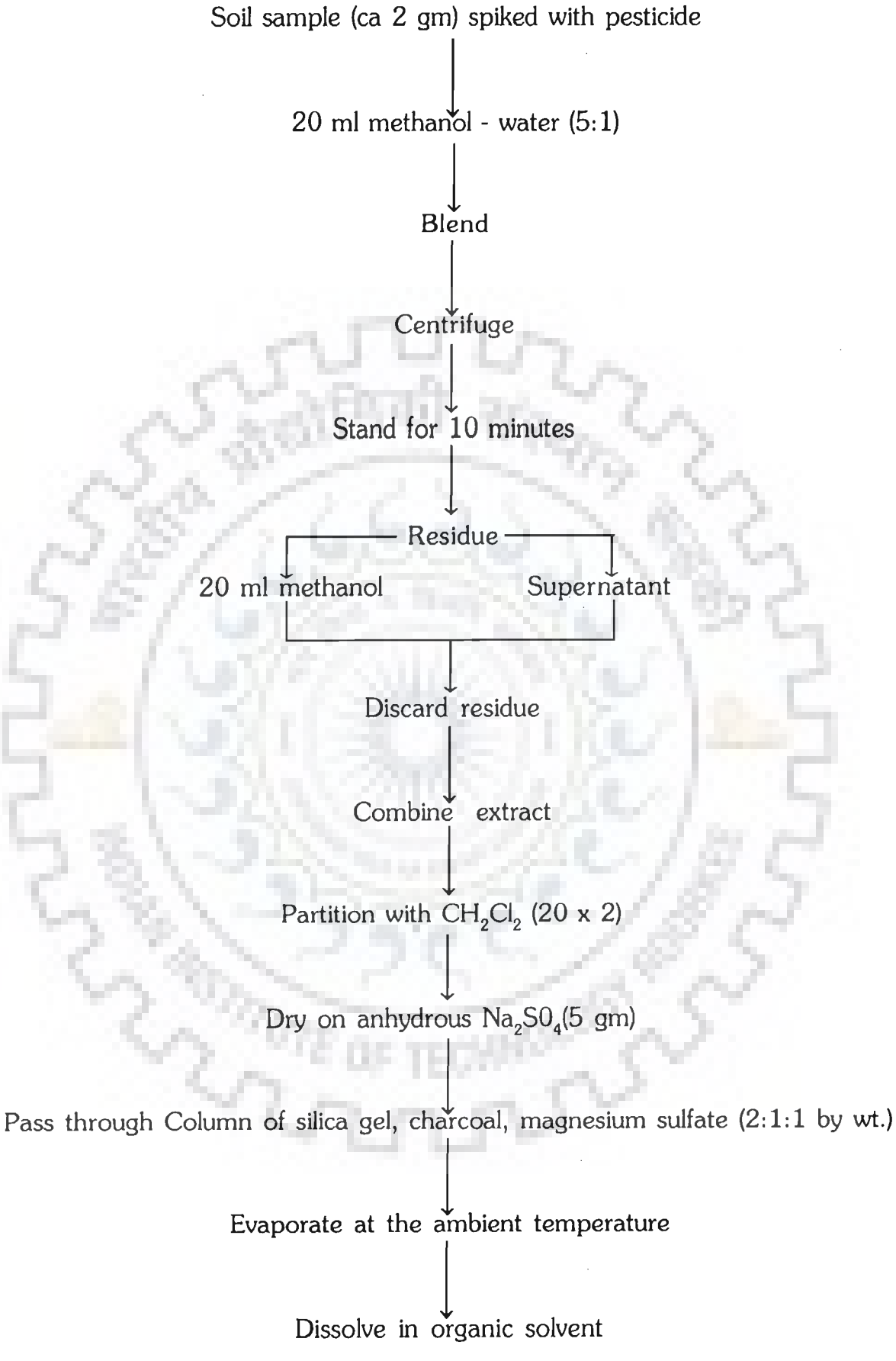


Figure-2.5 Extraction Procedure for Soil Sample

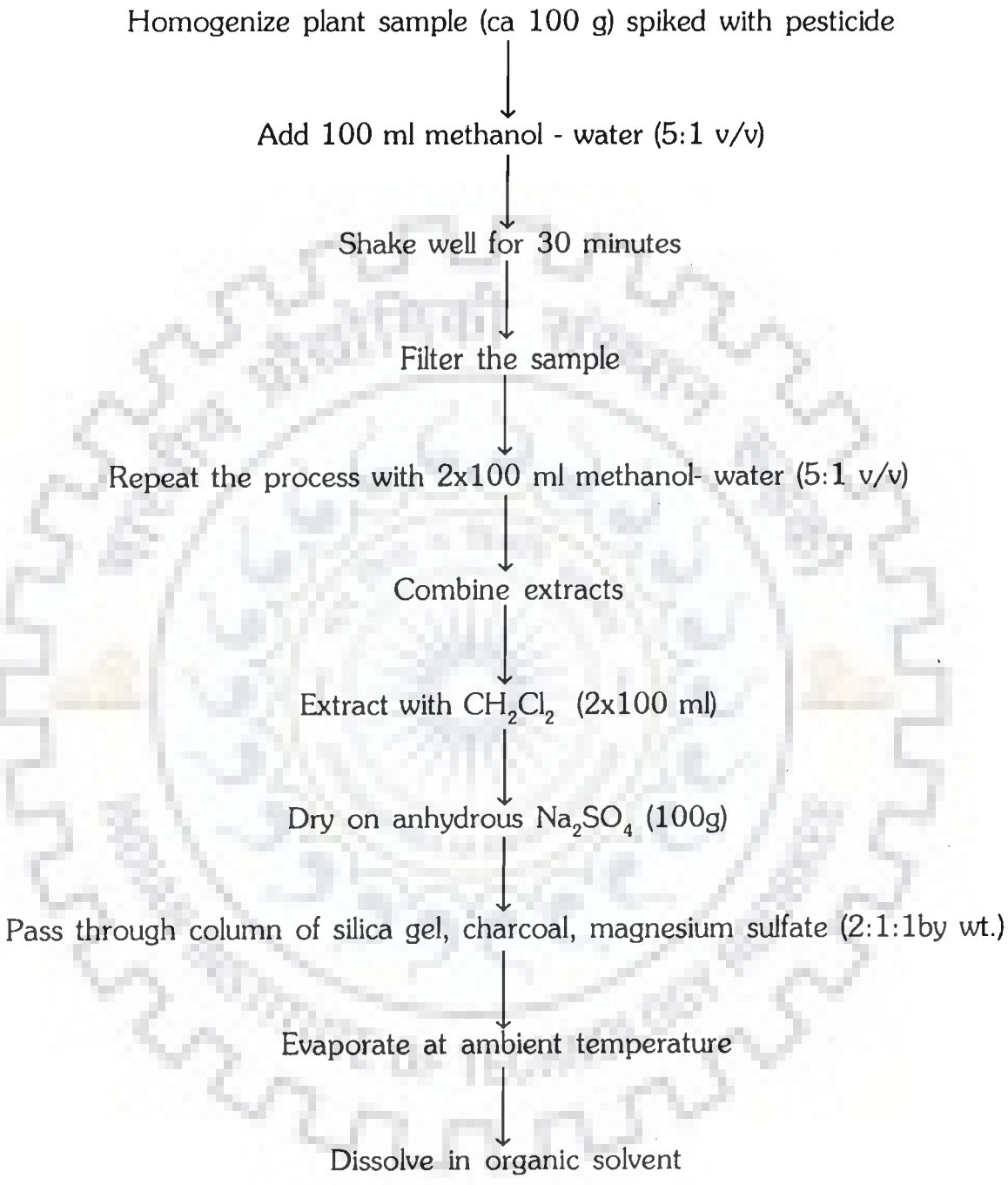


Figure-2.6 Extraction Procedure for Plant Sample

CHAPTER III

(a). DEGRADATION OF MALATHION AND METHYLPARATHION IN WATER AND SOIL UNDER LABORATORY CONDITIONS.

(b). DEGRADATION OF ENDOSULFAN IN WATER AND SOIL UNDER LABORATORY CONDITIONS.

Pesticides enter in soil or water bodies accidentally or through their intended use for the control of pests. They may cause unprecedented damage to the ecology of the area. Invariably after their application a large amount of the pesticides persist in agricultural and forest soils. They reach the soil mainly as fallout during aerial spraying. The remains of plants and animals which get burried in the soil also contribute towards their entry into the open environment. Waste water from pesticide manufacturing industries further adds to the load on the environment. The presence and the persistence of the pesticides in soil and water affect the innocent biota. The deleterious effects, to a large extent, will depend upon the decay rate and the toxicity of the pesticide and its metabolites. The persistence, no doubt, is primarily determined by the chemical nature but is significantly affected by the nature of the medium and the environmental factors like temperature, humidity and pH. The interaction of the pesticide with a medium like soil is more complicated than with water. The persistence in soil may vary from a few hours to many years depending on its type. The fate or the behaviour of the pesticide in soil depends upon many factors like adsorption on clay and organic matter, leaching with the downward percolation of water, volatilization, uptake by the soil micro-organisms or plants, movements with runoff water and chemical and photochemical degradation (Figure -3.1).

In a particular study it may not be feasible to consider all the above

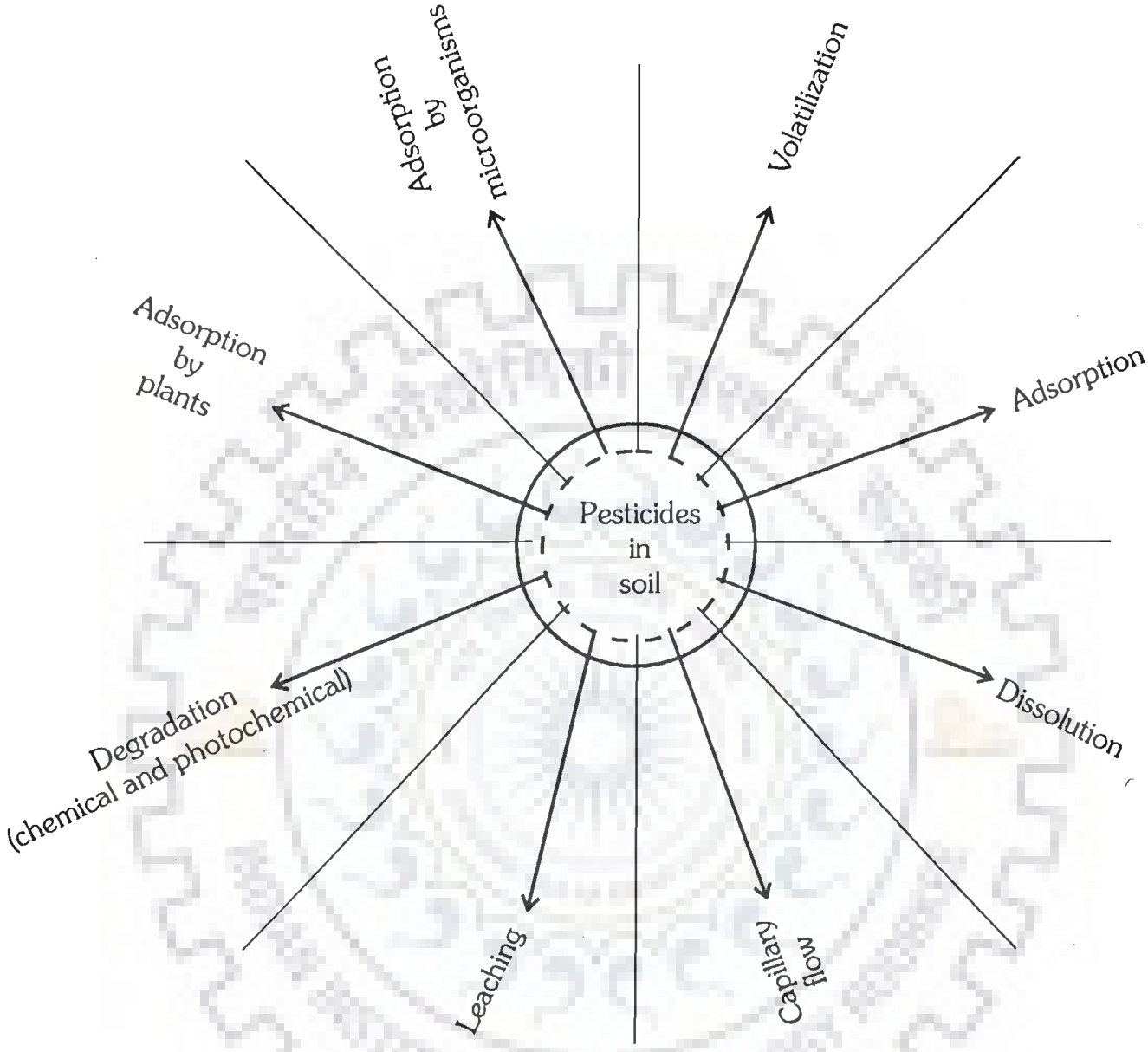


Figure 3.1 : Fate of Pesticide in Soil Environment

factors resulting into the change in the concentration of the pesticide. Generally, the emphasis is laid on chemical interactions. The degradation has been mainly reported as a first order or pseudo-first order reaction. In exceptional cases where the microbial activity in the matrix is significant a second order rate kinetics has been observed [96]. The persistence of a pesticide is expressed in terms of half- life ($t_{1/2}$) or rate constant (k) of the reaction.

Some isolated studies have been carried out by earlier workers on the parameters affecting the decay of organophosphates and endosulfan in soil and water. Wolfe and coworkers [97] found that the degradation of malathion in laboratory water is strongly influenced by temperature and pH and the decay rate is slower in water than in soil. Holm *et al.* [98] evaluated the laboratory ecosystem for assessing the chemical fate of methylparathion. Gile and Gillett [99] also studied the transport and fate of organophosphates in a laboratory model ecosystem. Freed and coworkers [100] evaluated the degradation of some organophosphate pesticides in water and soil and indicated that half-lives are pH and temperature dependant. Because of the importance of soil in biologically reducing the quantity and retarding the rate of pollutant movement into ground water a laboratory study was undertaken by Davidson *et al.* [101] to evaluate the adsorption, mobility and degradation of a large number of pesticides including methylparathion. Kelkar and coworkers [102] monitored the decay of methylparathion and concluded that in soil, under tropical conditions, it completely decays within two to three weeks. Ishikura *et al.* [103] monitored the evaporation and thermal decomposition of organophosphorus pesticides during cooking of rice. They determined pesticides after thermal decomposition in water and divided them into three

categories on the basis of their stability. To determine the effect of pH and temperature Noble [104] studied breakdown rate of dimethoate in fruit and vegetable dips and concluded that the half-life decreases with the increase in temperature. Rajukkannu *et al.* [105] studied the decay profile of malathion in different types of soils and confirmed that the reaction follows a pseudo first order kinetics. Wolfe *et al.* [106] followed the kinetics of disappearance of methylparathion in anaerobic sediment samples as a function of concentration, pH and E_h and described its decay by a first order rate equation. Tratnyek and Macalady [107] observed first order kinetics in the abiotic reduction of nitro -aromatic pesticides including methylparathion in a homogeneous solution of hydroquinone. Miles and Takashima [108] opined that the half-life of malathion in soil ranges from four hours to several days and the rate increases with the increase in moisture and alkalinity of the soil. Kolbe and coworkers [109] studied the persistence of dimethoate in different soils under laboratory conditions at different temperatures. Chapman and Cole [110] observed the influence of pH on the persistence of phosphates and several other insecticides in water and soil.

Endosulfan (thiodan) is often detected in natural environment. Chopra and Mahfouz [111] studied the confinement of endosulfan (α and β) and endosulfan sulfate on tobacco leaves. Ranga Rao and Murty [81] monitored its decay in dry and wet soil and found that the persistence depends on the initial dose. They observed that in different soils the rate of degradation of α endosulfan is more than its β isomer. Miles and Moy [112] reported that at 22^0C, in a phosphate buffer and under aerobic conditions, the half-lives of α and-β endosulfan are 88 and 40 days, respectively. Cotham and Bidleman [113] followed the degradation of endosulfan in incubated sea-water

and sediments and reported the half-lives. The half-life of α and β -endosulfan in nonsterile seawater (pH 8.0) was observed to be 4.9 and 2.2 days, respectively. Guerin and Kennedy [114] studied the dissipation of α and β - endosulfan in sterile aqueous system and found higher half-life values than those observed by Cotham and Bidleman[113]. Singh *et al.* [115] carried out a laboratory study on the decay of α and β– endosulfan by simulating conditions of tropical agro-ecosystem. The study included the measurement of rates of volatilization, hydrolysis and photolysis. Results describing the fate of endosulfan in a simple well-defined, nonbiological aqueous system are also available [116, 117].

The literature reports clearly point out that studies have been conducted on the decay of malathion, methylparathion and endosulfan in soil and water under different conditions; a majority of them being on soils. Invariably, the results reported for both soil and water by different groups of workers are at variance. One thing which clearly emerges out of these reports is that the decay is very susceptible to the experimental conditions and in several of these investigations the conditions are not properly defined. In the absence of such data no regular trend could be predicted. In order to arrive at meaningful conclusions about the decay rate the studies need to be conducted under controlled laboratory conditions and the effect of different variables like temperature, pH and organic content has to be monitored. The present work is an attempt in this direction and includes the effect of temperature, pH and organic content on the decay profiles of malathion, methylparathion and endosulfan.

EXPERIMENTAL

Persistence Studies

Laboratory experiments on decay were conducted in double distilled sterilized water (pH-5.5) and phosphate buffers of pH-5.5 and 8.0. It was ascertained that the constituents of buffer do not have any affect on the decay rate. Known amounts of water, buffer solution of the required pH or the buffer solution (pH-8.0) containing a known amount of humic acid were taken in glass stoppered test tubes. The humic acid solution had to be buffered because its addition to double distilled water shifts the pH to an alkaline range. The soil samples from different regions of India namely, West Bengal (W.B.), Andhra Pradesh (A.P.) and Uttar Pradesh (Roorkee) (RK) were air dried, homogenized and sterilized (dry) to make them bacteria free. The characteristics of the soils as determined by I S I methods [118] are given in Table-3.1. To different sets of sample tubes, 100 ppm of malathion, methylparathion or 10 ppm of endosulfan were added. The samples were kept at a constant temperature (± 0.2^0C) in a thermostatic water bath. The decay profile of the pesticides under different conditions were followed by determining the residual pesticides in the samples. Initially the samples were analysed at shorter intervals but later a longer gap was allowed. Extraction and measurement were done as per the procedures listed in Chapter II. Care was taken that there was no loss of water from the solution. Blanks for water and soil samples were run to exclude the possibility of any contamination. The coefficient of variation(CV) based on five replicate measurments of the amount of pesticides left in water and soil immediately after first half- life is 2% and 5%,

Table -3.1 Physicochemical Characteristics of Soils.

S.No.	Characteristics	W.B. Soil	A.P. Soil	RK Soil
1.*	Clay (%)	1	3	1
2.*	Sand (%)	71	53	80
3.*	Silt (%)	28	44	19
4.	Organic Content (%)	0.48	0.46	0.27
5.	pH	6.3	7.5	7.3
6.	Moisture Content (%)	23	24	27

*Characteristics 1, 2 and 3 are as per dry weight.

respectively. For the purpose of convenience and clarity, the results of this chapter have been presented in two sub divisions, one on malathion and methylparathion and the other on endosulfan.

RESULTS AND DISCUSSION

Malathion and Methylparathion

The effect of temperature (10^0, 20^0 and 30^0C) on the decay of malathion and methylparathion in water is shown in Figures -3.2 (a, b). The degradation of both malathion and methylparathion almost follows similar trends. As expected the decay rate increases with the increase in temperature. This is in agreement with the observations made on the effect of temperature on the decay of malathion in water [100] and dimethoate in different soils [109]. There is an exponential decrease in the concentration of the two pesticides. The data plotted on the natural log scale by least square method gives a straight line indicating that the process of disappearance of the pesticides obeyed the first order rate equation.

The effect of pH (5.5 and 8.0) on the degradation of malathion and methylparathion in water at 20^0C is shown in Figures -3.3 (a, b). The results of the same study conducted on soil (pH-6.3, 7.5 and 7.3) are shown in Figures -3.4 (a, b). The decay profiles indicated that the persistence of the pesticides in both water and soil increases with the decrease in pH. The decay in the soil is known to be affected by its different characteristics. Since the moisture and organic content of the A.P. and W.B. soils are not much different the faster decay rate in former soil can

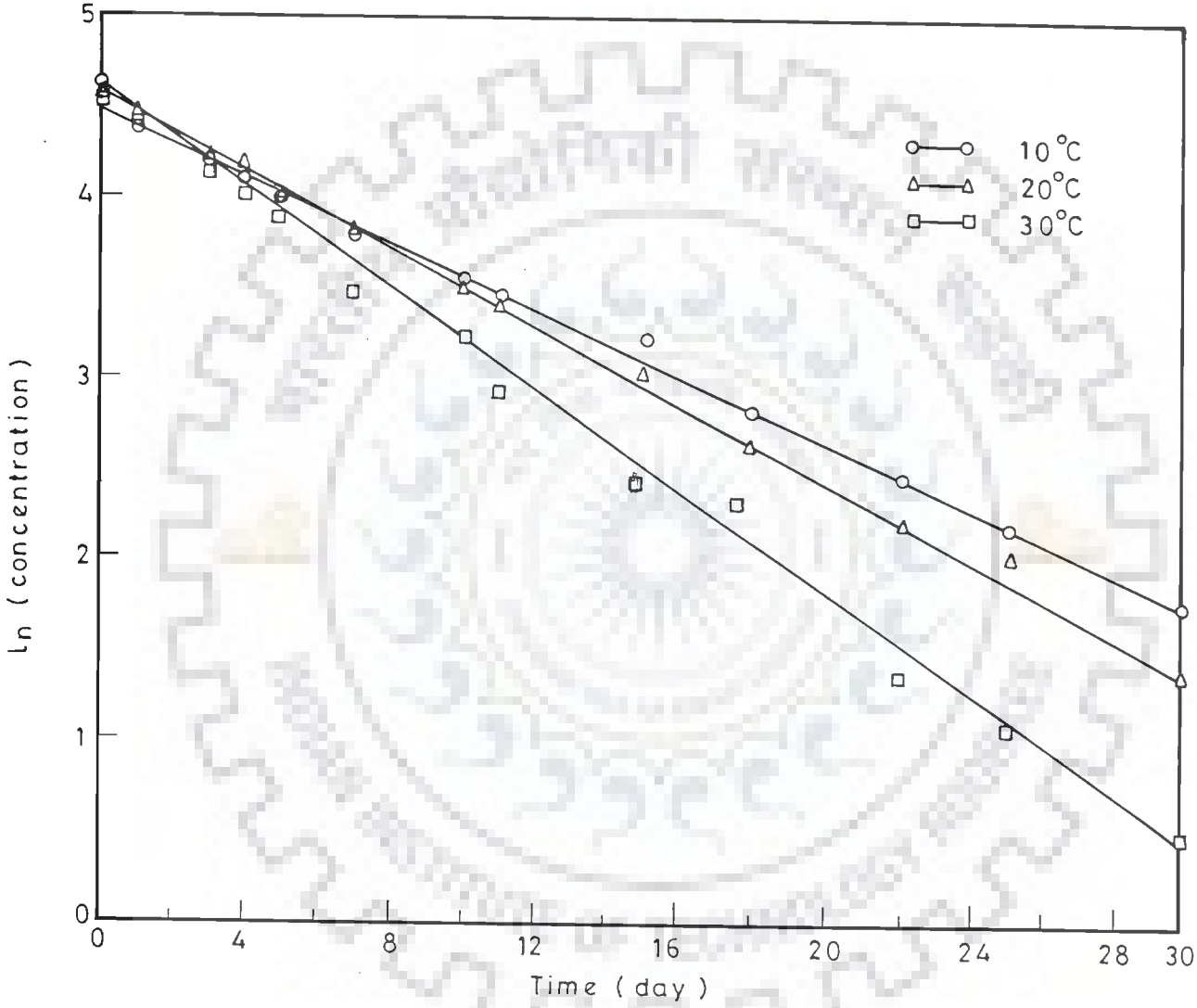


Figure 3·2 (b) Decay of Methylparathion in Water at Different Temperatures

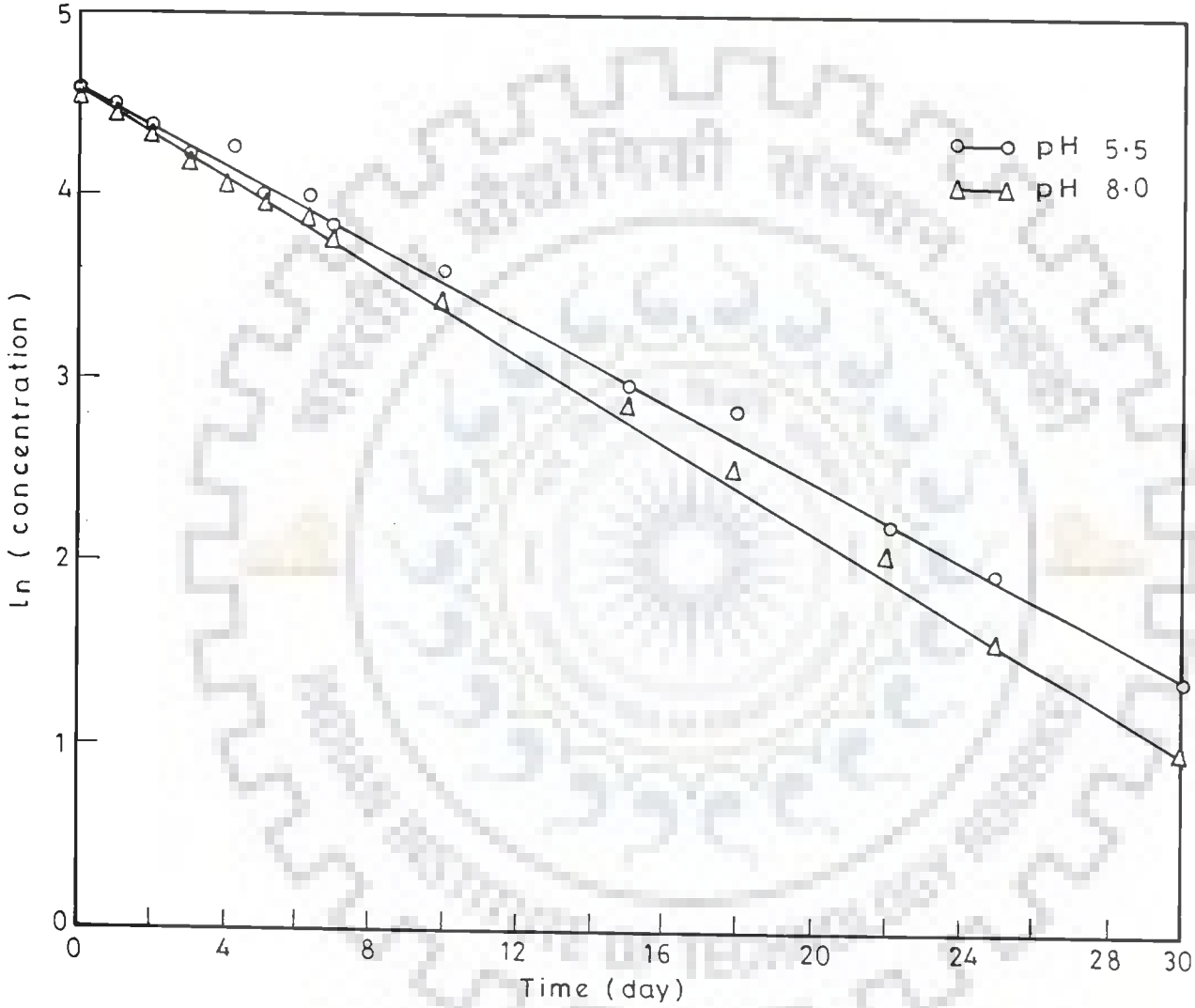


Figure 3·3 (b) Decay of Methylparathion in Water at Different pH (temperature 20°C)

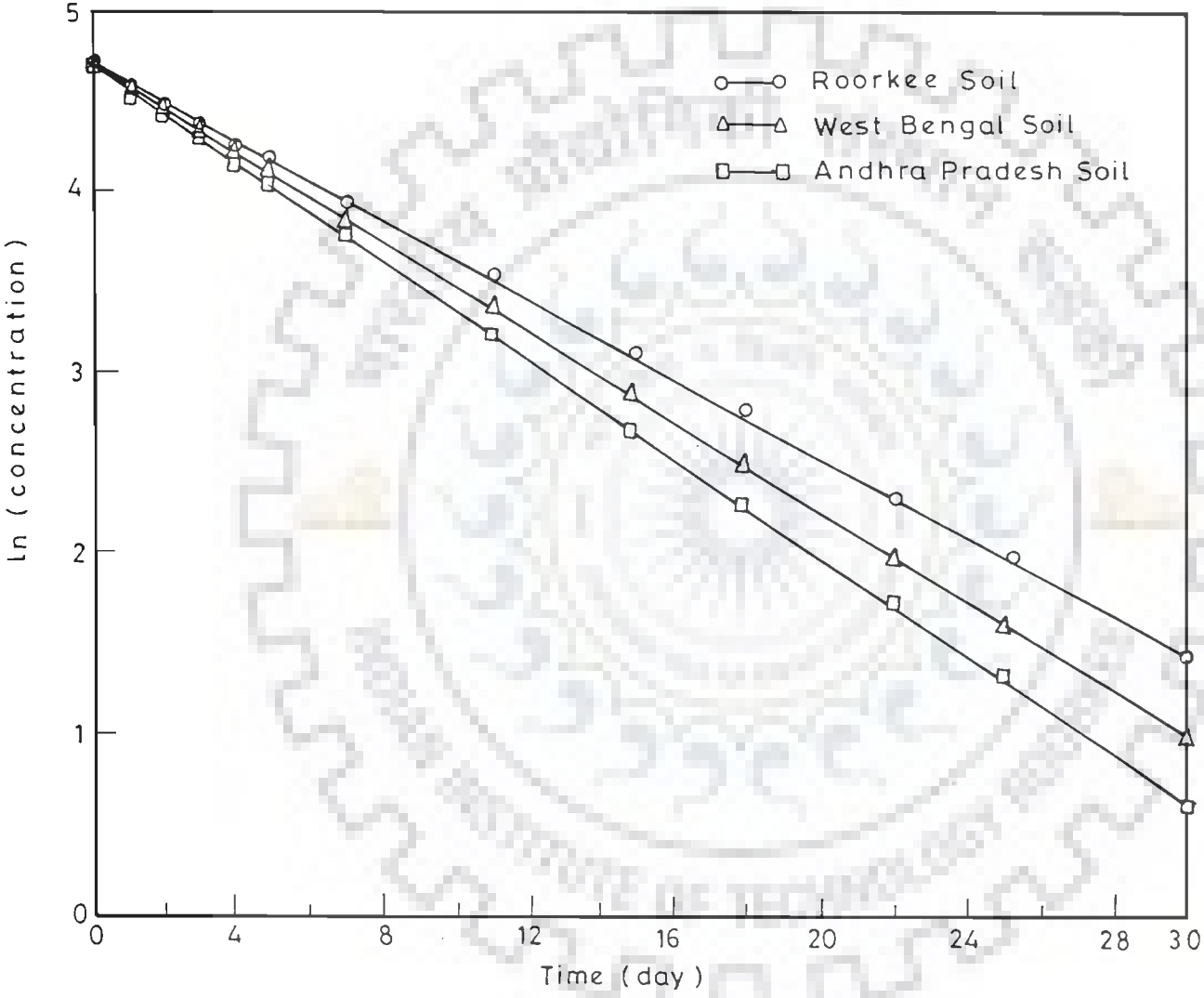
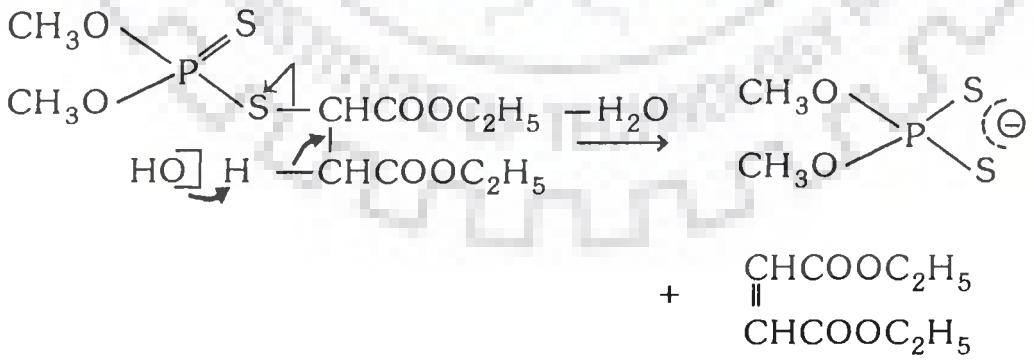


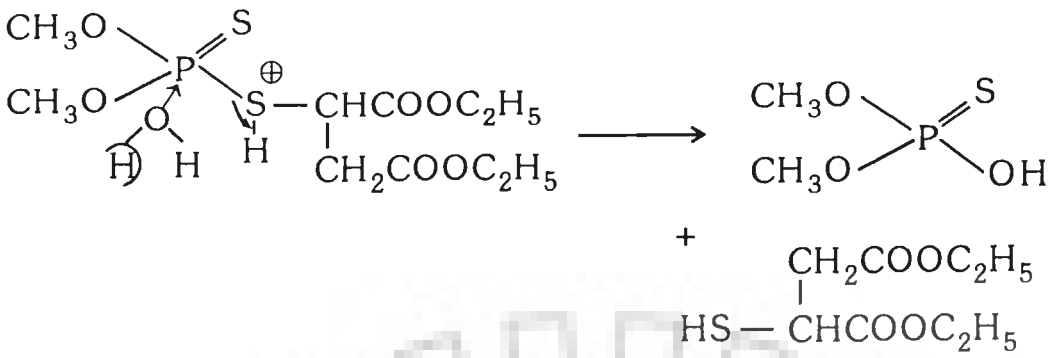
Figure 3·4 (a) Decay of Malathion in Soil

be assigned to its higher pH. The A.P. and RK soils have nearly the same pH and moisture but distinctly different organic content therefore the slower decay in RK soil is probably due to its lower organic content. This clearly points out that the increase in pH and organic content makes the decay faster in soil. These results on the effect of pH on decay in soil and water fall in line with the observations made by earlier workers on malathion [100,108,110], parathion [100,110] and dimethoate [14].

Faust and Gomaa [119] have pointed out that the hydrolysis of organophosphates under alkaline conditions proceeds at higher rates than in acidic medium. The reaction is more effectively catalyzed by the hydroxide ion than the hydronium ion. In the case of malathion [120] the faster decay kinetics in alkaline medium can be argued on the fact that in alkaline medium the cleavage takes place at C-S bond and the resulting compound formed due to rupture of this bond is stabilized by resonance. On the other hand in the acidic medium it is the P-S bond where the cleavage takes place. The probable mechanisms are depicted below:-



In Alkaline Medium



In Acidic Medium

The organic content of the matrix is known to affect the persistence of pesticides. The effect of humic acid concentration on the decay profile of malathion and methylparathion in the presence of different molar ratios of humic acid at pH-8.0 is shown plotted in Figures -3.5 (a, b). Apparently the presence of humic acid up to equal proportions increases the rate of decay and subsequent increase even to hundred fold excess does not bring any significant change in the degradation pattern. Rajukkannu *et al.* [105] have also observed that higher organic content lowers the persistence of malathion.

The half-lives and the rate constants of malathion and methylparathion at different temperatures and pH and in the presence of varying organic content are given in Tables - 3.2-3.4. The results indicate that the decay of these pesticides becomes faster with the increase in temperature, pH and organic content up to a certain concentration. It is important to point out that there is no significant difference in half-lives and behaviour of malathion and methlyparathion. The degradation trends ($r>0.80$) in all these cases followed the first order kinetics.

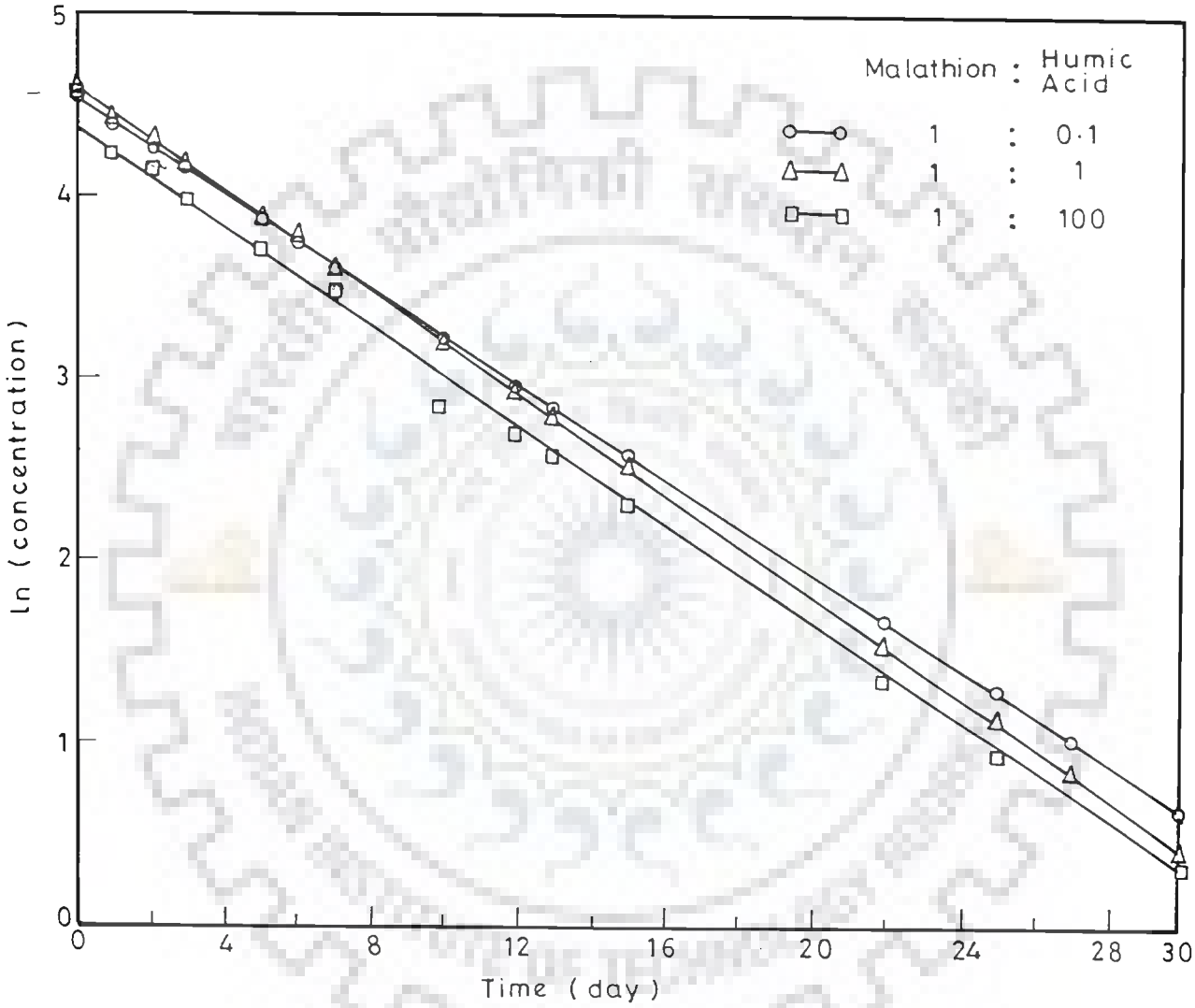


Figure 3·5 (a) Decay of Malathion in the Presence of Humic Acid

Table -3.2 Half-lives of Malathion and Methylparathion in Water at Different Temperatures.

Temperature (^{0}C)	Pesticide	$t_{1/2}$(days)	k(sec^{-1})*	r
10	Malathion	7.8	9.62×10^{-5}	0.993
	Methylparathion	7.5	8.68×10^{-5}	0.999
20	Malathion	6.7	7.75×10^{-5}	0.999
	Methylparathion	6.4	7.40×10^{-5}	0.993
30	Malathion	5.2	6.01×10^{-5}	0.991
	Methylparathion	4.5	5.20×10^{-5}	0.960

*$k = 0.693/t_{1/2}$ (Expressed in proper units).

Table -3.3 Half-lives of Malathion and Methylparathion in Water and Soil at Different pH (temperature 20^0C).

pH	Medium	Pesticide	$t_{1/2}$(days)	k(sec^{-1})	r
pH -5.5	Aqueous	Malathion	6.8	$7.87x10^{-5}$	0.999
		Methylparathion	6.4	$7.40x10^{-5}$	0.993
pH -8.0	Aqueous	Malathion	5.9	$6.82x10^{-5}$	0.991
		Methylparathion	5.7	$6.59x10^{-5}$	0.977
pH-6.3	Soil (W.B.)	Malathion	5.4	$6.25x10^{-5}$	0.990
		Methylparathion	6.2	$7.17x10^{-5}$	0.989
pH-7.5	Soil (A.P.)	Malathion	5.0	$5.78x10^{-5}$	0.996
		Methylparathion	5.5	$6.36x10^{-5}$	0.933
pH -7.3	Soil* (RK)	Malathion	6.4	$7.40x10^{-5}$	0.993
		Methylparathion	6.6	$7.63x10^{-5}$	0.994

* Lower Organic Content

Table -3.4 Half-lives of Malathion and Methylparathion at Various Humic Acid Concentrations in Water (temperature 20^0C, pH-8.0).

Pesticides : Humic acid (Molar ratio)	Pesticide	$t_{1/2}$ (days)	k(sec^{-1})	r
1:0.1	Malathion	5.3	$6.13x10^{-5}$	0.999
	Methylparathion	5.4	$6.25x10^{-5}$	0.996
1:1	Malathion	5.0	$5.78x10^{-5}$	0.991
	Methylparathion	4.9	$5.67x10^{-5}$	0.987
1:100	Malathion	4.9	$5.67x10^{-5}$	0.999
	Methylparathion	4.7	$5.43x10^{-5}$	0.997
Absence of Humic Acid	Malathion	5.9	$6.82x10^{-5}$	0.991
	Methylparathion	5.7	$6.59x10^{-5}$	0.977

Endosulfan

The study on the rate of degradation of endosulfan in water at 10^0C indicated that there is no significant decay upto thirty days. The decay profiles of α and β isomers at 20^0C and 30^0C are shown in Figure-3.6. The plots represent that both α and β form decay at the same rate and undergo faster degradation with the increase in temperature. The decay rates at different temperatures followed first order kinetics with statistically significant r values greater than 0.80. The rate constant and half-lives obtained by least square analysis of first order plots for different temperatures are presented in Table-3.5. The half-lives of both the isomers of endosulfan have been reported [112-115, 121, 122] covering a broad range of time period depending upon the experimental conditions. In some studies the two isomers are reported to have nearly the same value of $t_{1/2}$. The results are in agreement with those of Gopal and Mukherjee [89] who on mustard leaves observed the same half-life (~4 days) for both the isomers.

The decay of both the isomers in water at pH-5.5 and pH-8.0 at 20^0C suggests that the rate significantly increases (Figure-3.7) with the change in pH to a higher side. Singh *et al.* [115] have also observed a similar effect of pH on the decay rate in aqueous medium. Endosulfan persists longer in soil than in water (Table-3.6) and like water the decay is accelerated with the change of pH of the soil from acidic to alkaline region (Figure-3.8). Goebel *et al.* [123] have reported that endosulfan isomers are more susceptible to alkaline hydrolysis.

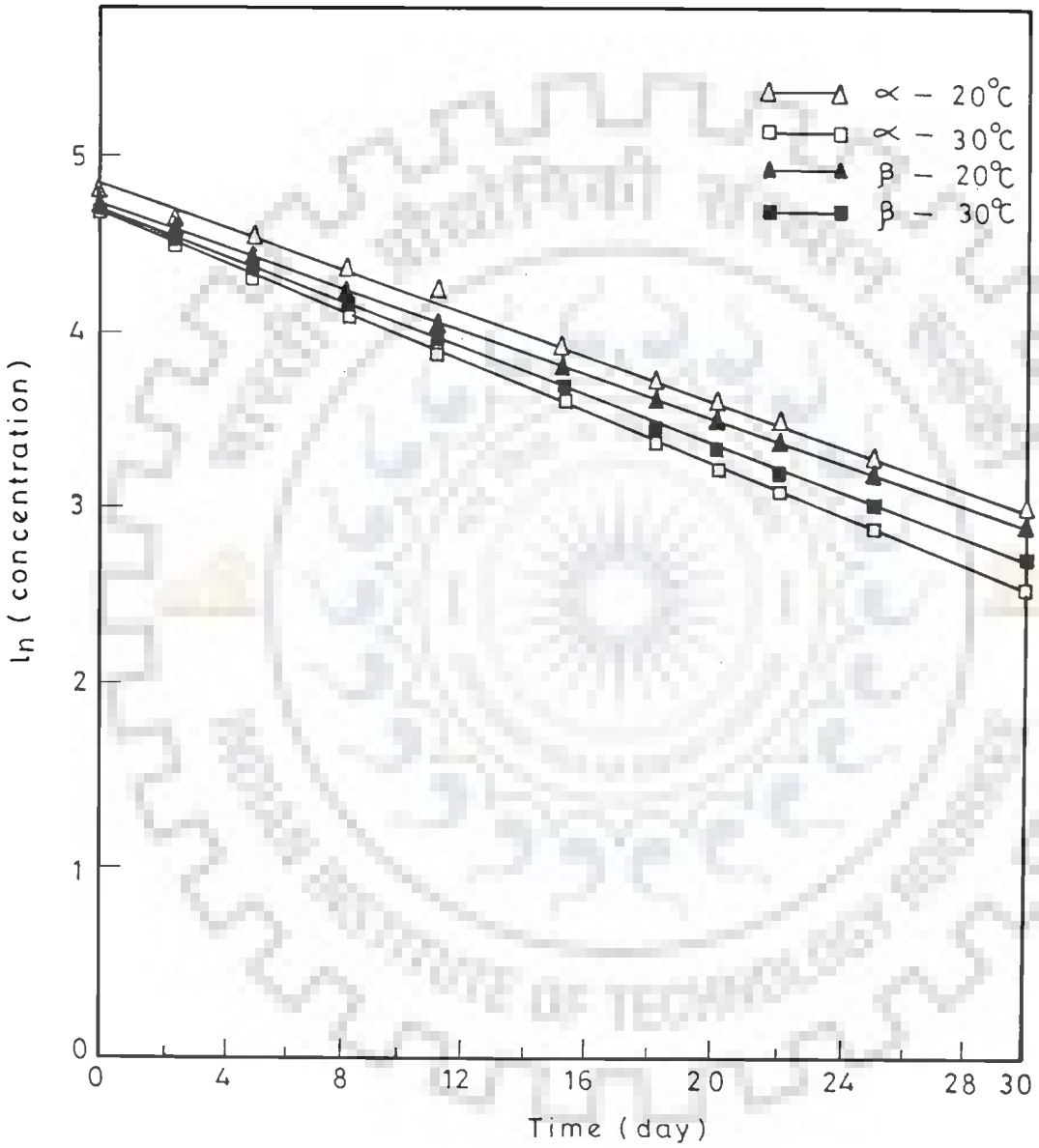


Figure 3·6 Decay of Endosulfan Isomers (∝-and β-) in Water at Different Temperatures

Table -3.5 Half-lives of Isomers of Endosulfan in Water at Different Temperatures.

Temperature (^{0}C)	Isomers	$t_{1/2}$(days)	k(sec^{-1})	r
10	Alpha	No Detectable Decay Upto 30 Days		
	Beta			
20	Alpha	11.3	$1.31x10^{-4}$	0.976
	Beta	11.8	$1.36x10^{-4}$	0.993
30	Alpha	9.8	$1.13x10^{-4}$	0.971
	Beta	10.6	$1.22x10^{-4}$	0.993

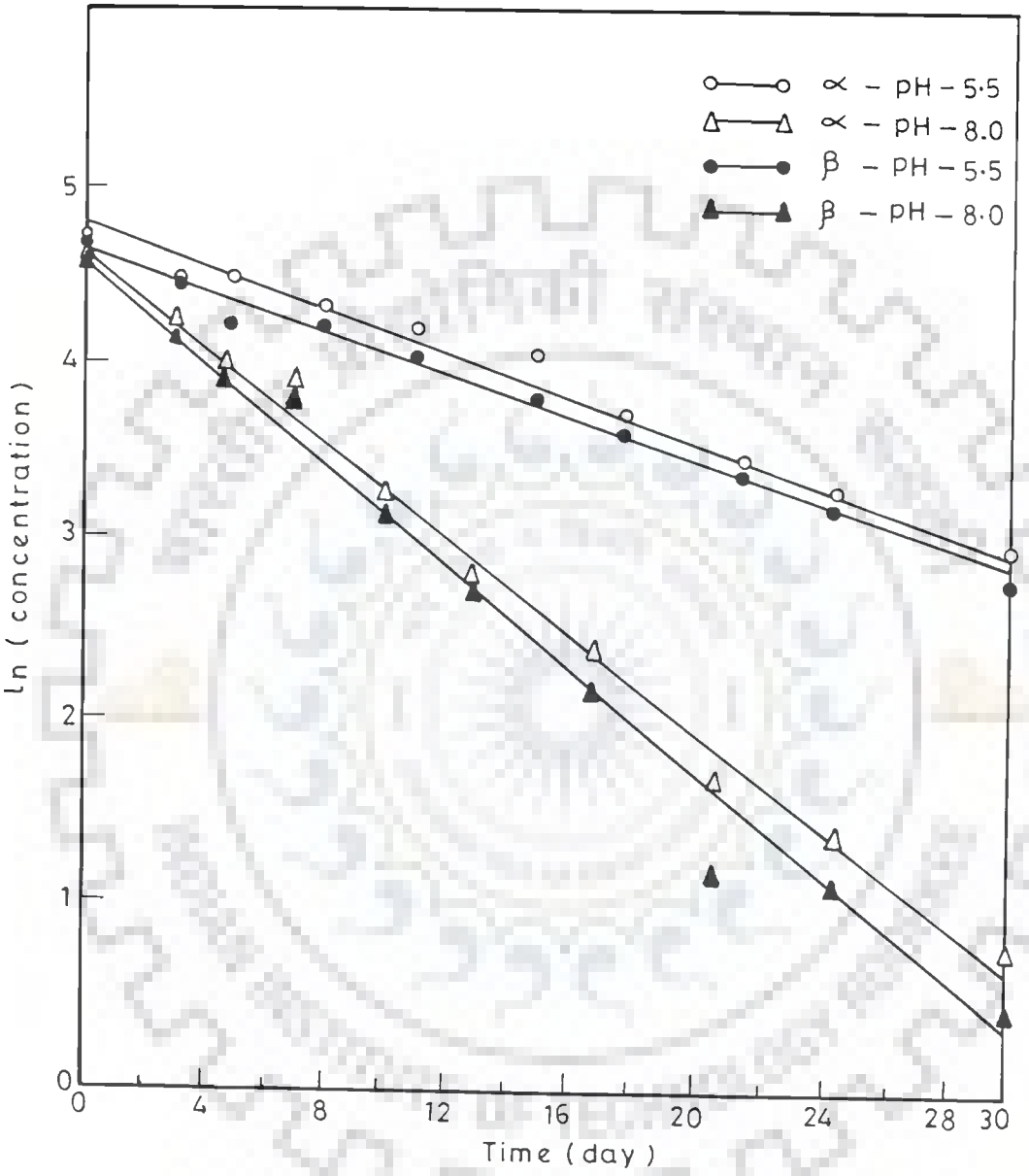


Figure 3·7 Decay of Endosulfan Isomers (∝-and β-) in Water at Different pH (temperature 20 °C)

Table - 3.6 Half-lives of Isomers of Endosulfan in Water and Soil at Different pH (temperature 20°C).

pH	Medium	Isomers	$t_{1/2}$ (days)	k(sec^{-1})	r
pH-5.5	Aqueous	Alpha	11.8	1.31×10^{-4}	0.976
		Beta	11.8	1.36×10^{-4}	0.993
pH-8.0	Aqueous	Alpha	5.3	6.13×10^{-5}	0.995
		Beta	5.0	6.13×10^{-5}	0.986
pH-6.3	Soil (W.B)	Alpha	27.4	3.17×10^{-4}	0.810
		Beta	27.5	3.18×10^{-4}	0.843
pH-7.5	Soil (A.P.)	Alpha	14.1	1.63×10^{-4}	0.973
		Beta	15.1	1.75×10^{-4}	0.974

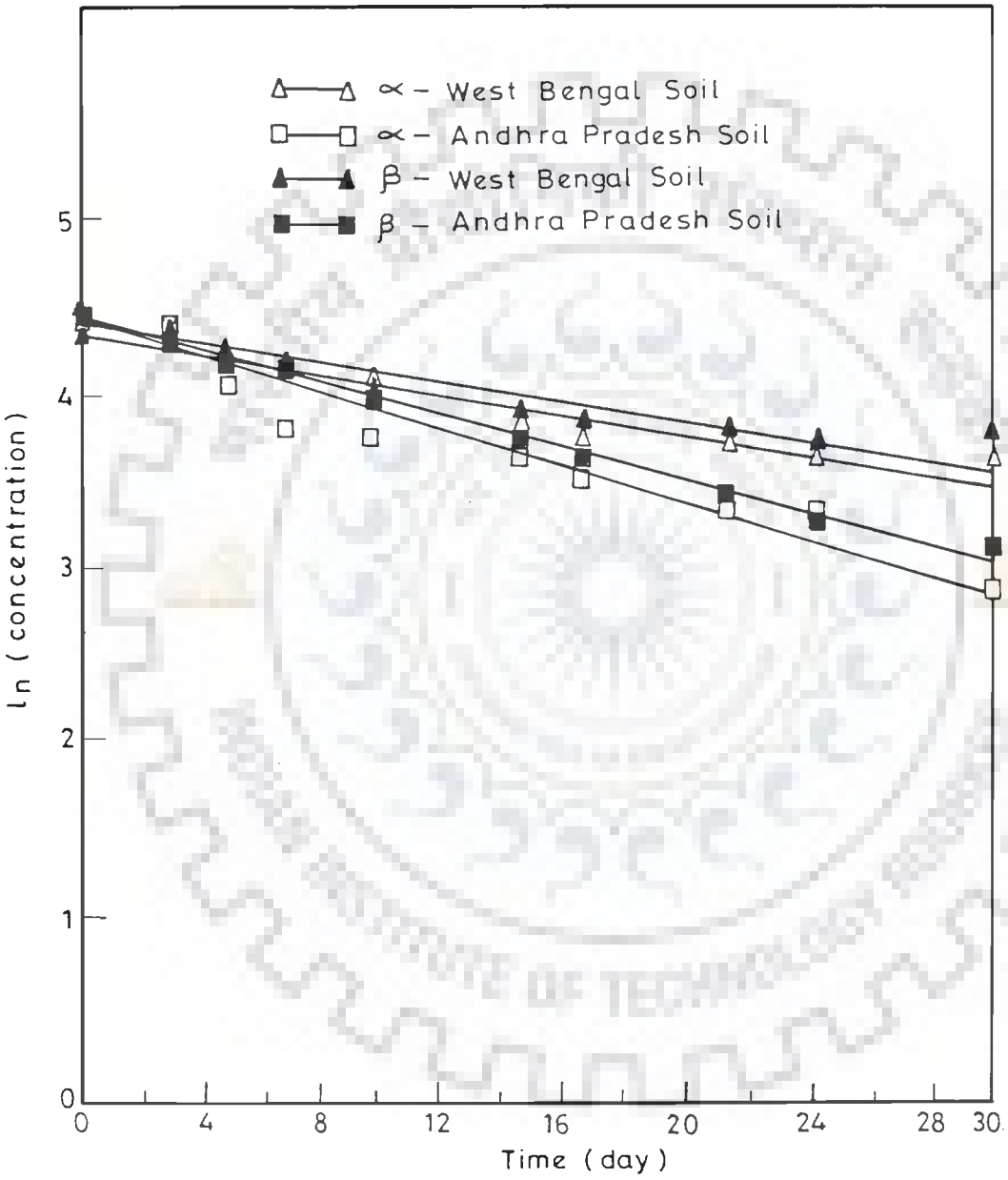


Figure 3·8 Decay of Endosulfan Isomers (∝ and β) in Soil

The decay of endosulfan in the presence of humic acid is shown in Figure-3.9 and the half-lives with rate constants are tabulated in Table-3.7. Unlike the organophosphates the decay of endosulfan decreases with the increase in the humic acid concentration, behaviour at 1:1 ratio being an exception. No proper explanation can be offered for this exception in behaviour.

The different degradation profiles follow the first order kinetics ($r>0.80$) and both the forms of endosulfan decay faster with the increase in temperature and pH.

The results show that endosulfan decays at a slower rate than malathion and methylparathion. A comparison of these two classes of pesticides suggests that endosulfan persists longer in soil than malathion and methylparathion but the half-lives in water are comparable. Both the organophosphates and endosulfan decay at a faster rate with the increase in temperature and change of pH from acidic to alkaline region. An unusually slow decay of endosulfan at 10^0C poses a greater threat to its use in cold countries than in tropical conditions. The effect of pH in water and soil is more pronounced in endosulfan than organophosphates. The presence of humic acid affects the decay of these two classes of pesticides but apparently in different directions.

It is a little difficult task to compare the decay profiles of the pesticides investigated in this chapter with other organophosphates and organochlorines. The available results on other compounds are not exactly under the same experimental conditions. However, one can dare

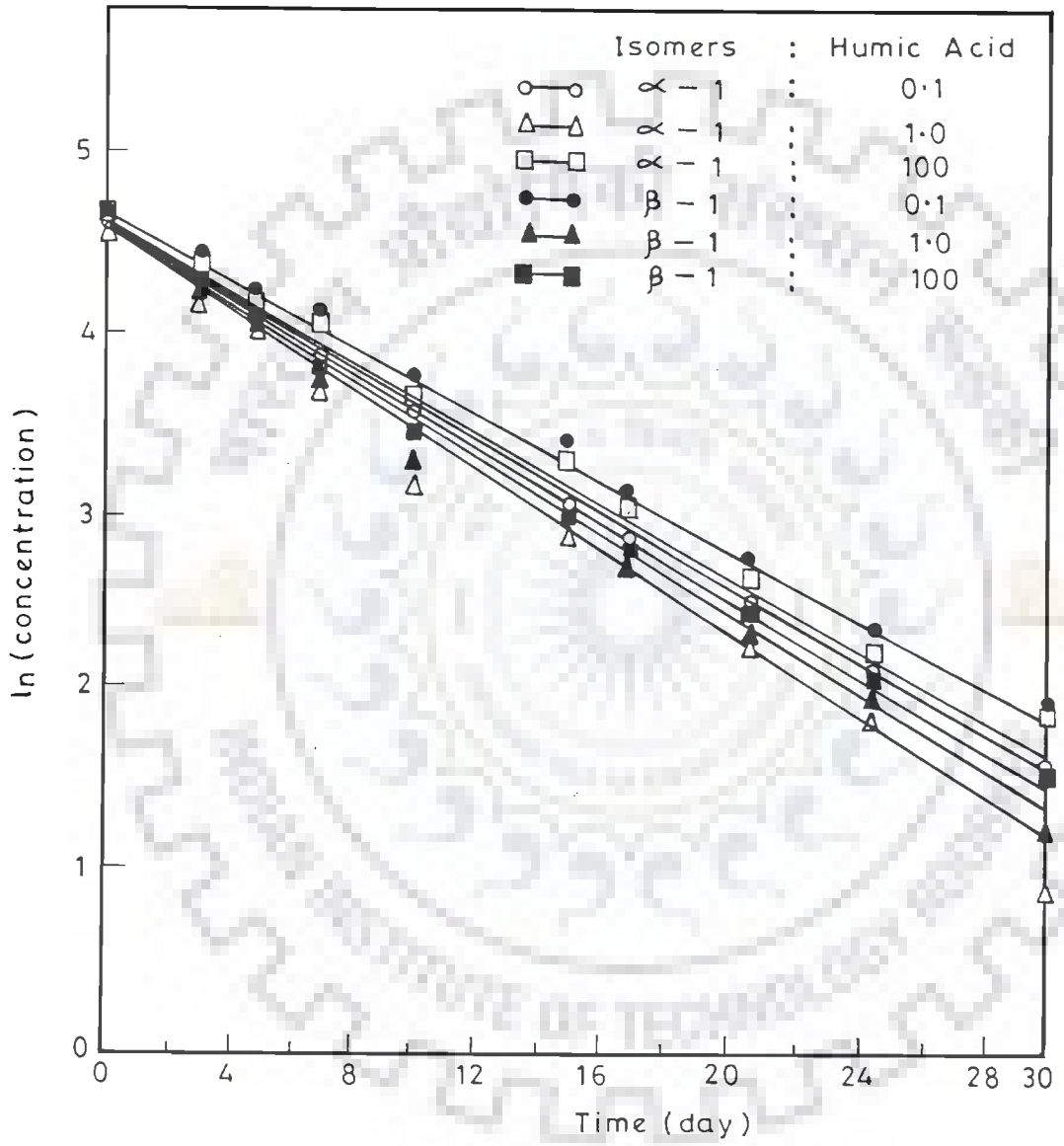


Figure 3·9 Decay of Endosulfan Isomers (∝ and β) in the Presence of Humic Acid

Table-3.7 Half-lives of Isomers of Endosulfan at Various Humic Acid Concentrations in Water (temperature 20^0C, pH-8.0).

Pesticides : Humic acid (Molar ratio)	Isomers	$t_{1/2}$ (days)	k(sec^{-1})	r
1:0.1	Alpha	6.8	$7.94x10^{-5}$	0.997
	Beta	6.9	$8.02x10^{-5}$	0.993
1:1	Alpha	6.1	$7.07x10^{-5}$	0.987
	Beta	6.6	$7.60x10^{-5}$	0.995
1:100	Alpha	7.5	$8.72x10^{-5}$	0.997
	Beta	7.7	$8.91x10^{-5}$	0.979
Absence of Humic Acid	Alpha	5.3	$6.13x10^{-5}$	0.995
	Beta	5.0	$6.13x10^{-5}$	0.986

to logically extrapolate the results of other pesticides for comparison and arrive at some reasonable conclusions. The half-life of dimethoate [109], in heavy clay has been observed to be 8.5 days at 20°C. Although the characteristics of the clayey soil are not mentioned. The half-life of dimethoate seems to be on the higher side than that of malathion and methylparathion. Chapman and Cole [110] in sterile water-ethanol (99:1), at pH-8.0 and 25±3°C, have reported the half-life of other organophosphates, namely ethion (58.8 days), fensulfothion (406 days), fonofos (48.5 days) and parathion (105 days). If one ignores the small alcohol content of the water and a slightly higher temperature used in the studies all these pesticides have longer half-lives than those of malathion (5.9 days) and methylparathion(5.7 days). The report mentions the half-life of malathion as 3.7 days which is almost the same as observed in the present investigations.

A comparison of the decay pattern of endosulfan with some other organochlorines indicates that endosulfan degrades faster than many other members of its class. Rajukkannu *et al.* [105] have observed that the half-life of DDT in flooded and non-flooded black soil is 44 and 50 days, respectively. Dieldrin, another chlorinated pesticide with cyclodiene moiety, at 30°C (pH-9.5) has a half-life of 88.4 days [115].

The decay profile data on the entire spectrum of commercially available organophosphates and organochlorine pesticides are not easily available. But by stretching the available information it seems that malathion, methylparathion and endosulfan decay faster than some other pesticides of their respective categories.

CHAPTER IV

DEGRADATION OF MALATHION AND METHYLPARATHION IN PLANT AND SOIL UNDER FIELD CONDITIONS.

The decay of pesticide residues on plants is an intriguing phenomenon. When a pesticide is applied to the crop it partitions itself into absorbed (interior) and adsorbed fractions (surface). The absorbed fraction of the pesticide primarily decays through chemical pathways and the adsorbed fraction diminishes primarily due to physical loss, microbial activity and photochemical degradation. The loss due to vaporization from the surface reaches the atmosphere resulting into its deposition in the rain and fog. Organophosphates have been reported in the fog in the states of California and Maryland (USA). The persistence of the pesticide in the environment, whether it is a plant, soil, water or atmosphere, largely depends upon the nature of the matrix and the seasonal variations. From the environmental point of view, the decay in all these segments of biosphere is of great concern but the decay in the plant assumes a greater significance. Pesticide residues are transported to various parts of the plant like root, stem, foliage and fruit in varying forms. It is ultimately important to know as to how much remains in the plant with the passage of time. Scanty information is available on the rate of decay of pesticides under field conditions in different seasons. Most of the available data refer to the residual concentrations rather than the rate with which the concentration of the pesticide changes.

Kavadia and Gupta [124] studied the decay of malathion on onions in field and concluded its rapid dissipation, though some low level of persistence occurred in the bulbs [125]. Shankar [126] followed the

persistence of malathion on tomatoes and observed its complete degradation within seven days. Krishnamurthi [127] compiled a lot of data on the persistence of pesticide residues on various crops in different soils and indicated a rapid decomposition of the organophosphates with only traces being left at the harvest time. Lavanon [128] studied the role of fungi and bacteria on the decay of malathion and other pesticides. The results indicated that the biodegradation of pesticides in soil is facilitated by both fungi and bacteria. Readman and coworkers [129] followed the persistence of organophosphorus pesticides in marine environment and observed methylparathion and malathion below the limits of detection. Kelkar and coworkers [102] carried out investigation on the decay of methylparathion in soil under tropical conditions and concluded that it completely decays in soil within two to three weeks. Sharma [130], without exactly defining the environmental conditions, followed the persistence of some organophosphorus and carbamate pesticides on potato (Solanum tuberosum), cabbage (Brassica oleracea), and brinjal (egg plant) (Solanum brinjales). She concluded that both malathion and methylparathion completely decay within a week.

The decay of a pesticide even in a particular portion of a plant will change with the prevailing environmental conditions in the field. No doubt, it is very difficult to control the meteorological conditions for the field study but an investigation carried out in different seasons by citing average maximum and minimum temperatures, humidity and wind velocity will yield meaningful results. Such an exercise will provide complementary information if it is carried out together with decay studies under controlled laboratory conditions.

In view of the above the present study was planned to follow the degradation of malathion and methylparathion on plants, namely radish (Raphanus sativus) and carrot (Daucas carota) under different climatic conditions. The two root vegetables were chosen because these are generally consumed raw as salad items. Simultaneously the decay rate was followed in the soil adjoining to the plants.

EXPERIMENTAL

The leaves of two week old plants and adjoining soil at some distance were sprayed with a known amount of the pesticide of formulation grade. The distance between the plant and soil sampled for the analysis was such that there was no possibility of any transmigration from one to the other. In order to avoid any error the decay was followed by determining the residual pesticide in the whole plant and a 10cm core of the soil. It was ascertained that there was no movement of pesticide beyond 10cm of the soil. The pH, moisture and organic content values of the adjoining soil (RK) collected in winter at the start of the series of experiments are given in Table -3.1. It was difficult to monitor the subsequent variations in the soil characteristics as the decay was followed for thirty days in each of the three different seasons, namely, winter (December - January), summer (May) and postmonsoon (October - November). The minimum and maximum temperatures, average humidity and wind velocity in winter, summer and postmonsoon were 11-24 ^{0}C, 74.1%, 1.7-4.6 Kmph; 25-35 ^{0}C, 53.1%, 1.5-5.3 Kmph and 15-28 ^{0}C, 70.9%, 0.8-1.8 Kmph, respectively. Control and blanks were run wherever necessary. The precision (CV, n=5) of the amount of malathion and methylparathion left in plants immediately after

first half-life is 5% and 6%, respectively. However, in soil CV is 9% and 10% for malathion and methylparathion. It was checked that in the chromatogram of the formulation there was no interfering peak. The values reported are the average of minimum of duplicate runs. There was no rain or cyclonic activity during the course of investigation to cause an unusual loss of the pesticide.

RESULTS AND DISCUSSION

The decay behaviour of malathion and methylparathion in the three different seasons on carrot and radish and the adjoining soil is shown in Figures -4.1-4.8. The degradation profiles of the two pesticides on the two plants and in the soil are similar. The results clearly indicate that in winter and postmonsoon both the pesticides show smooth linear decay. The behaviour is more or less similar to those shown in chapter III and for the sake of comparison the decay in distilled water at 20 ^{0}C is also shown in Figure -4.7. The half-lives of the malathion and methylparathion in the soil as well as on the plants (Tables 4.1-4.2) in the two seasons do not show any marked difference because the temperature range and average humidity are not much different. But one can make out a slight increase in the half-lives in winter compared to those observed in postmonsoon because a lower temperature persists for longer time in winter. However, in summer the decay of both these pesticides on plants and in soil follows two distinct profiles. The decay is initially faster but it subsequently slows down. The degradation profile of the first seventy five percent of the pesticides is different from that of the remaining twenty five percent. If the first part of curve, that is the decay of the first seventy five percent, is used as the yardstick, the half-life

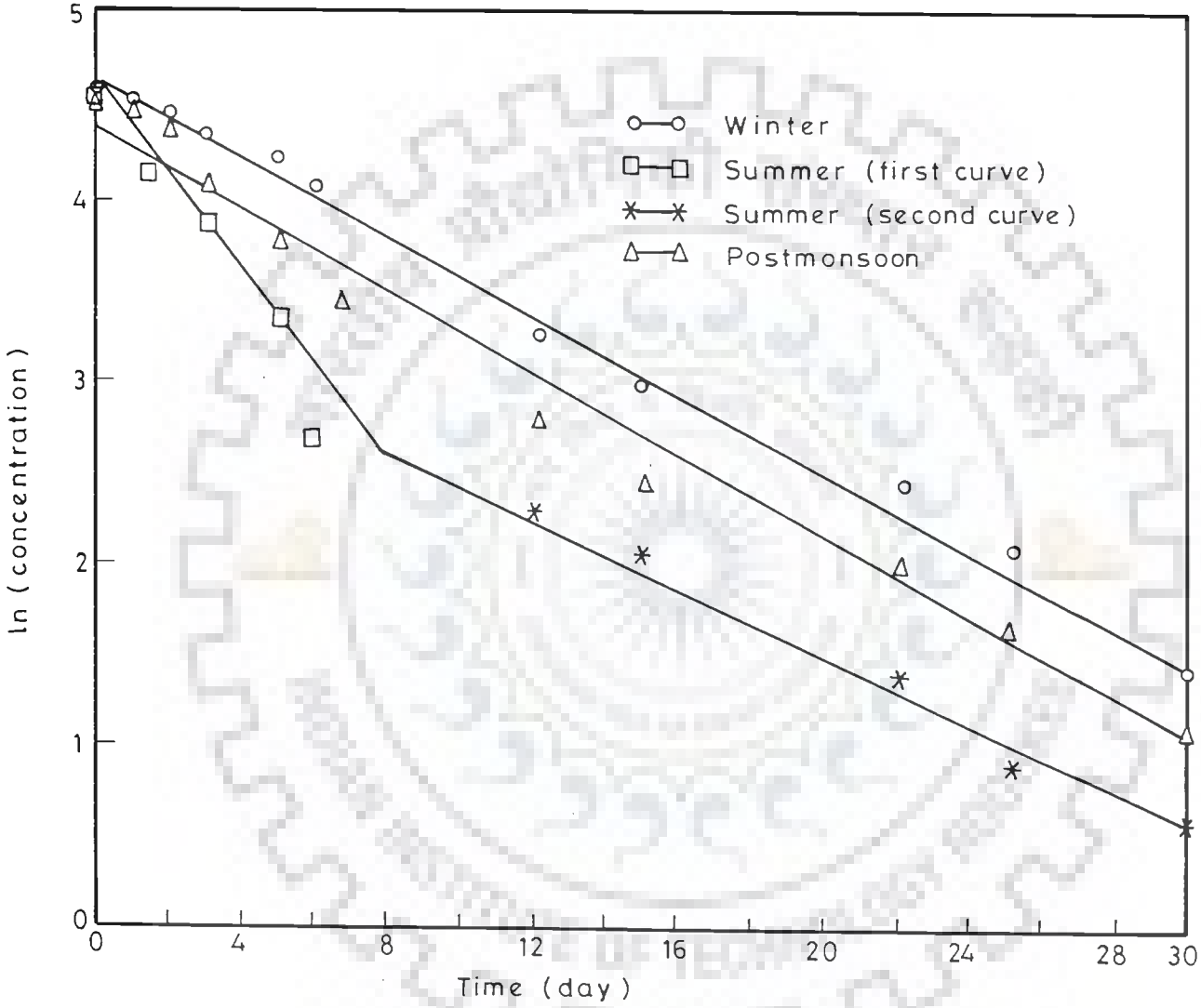


Figure 4.1 Decay of Malathion in Radish

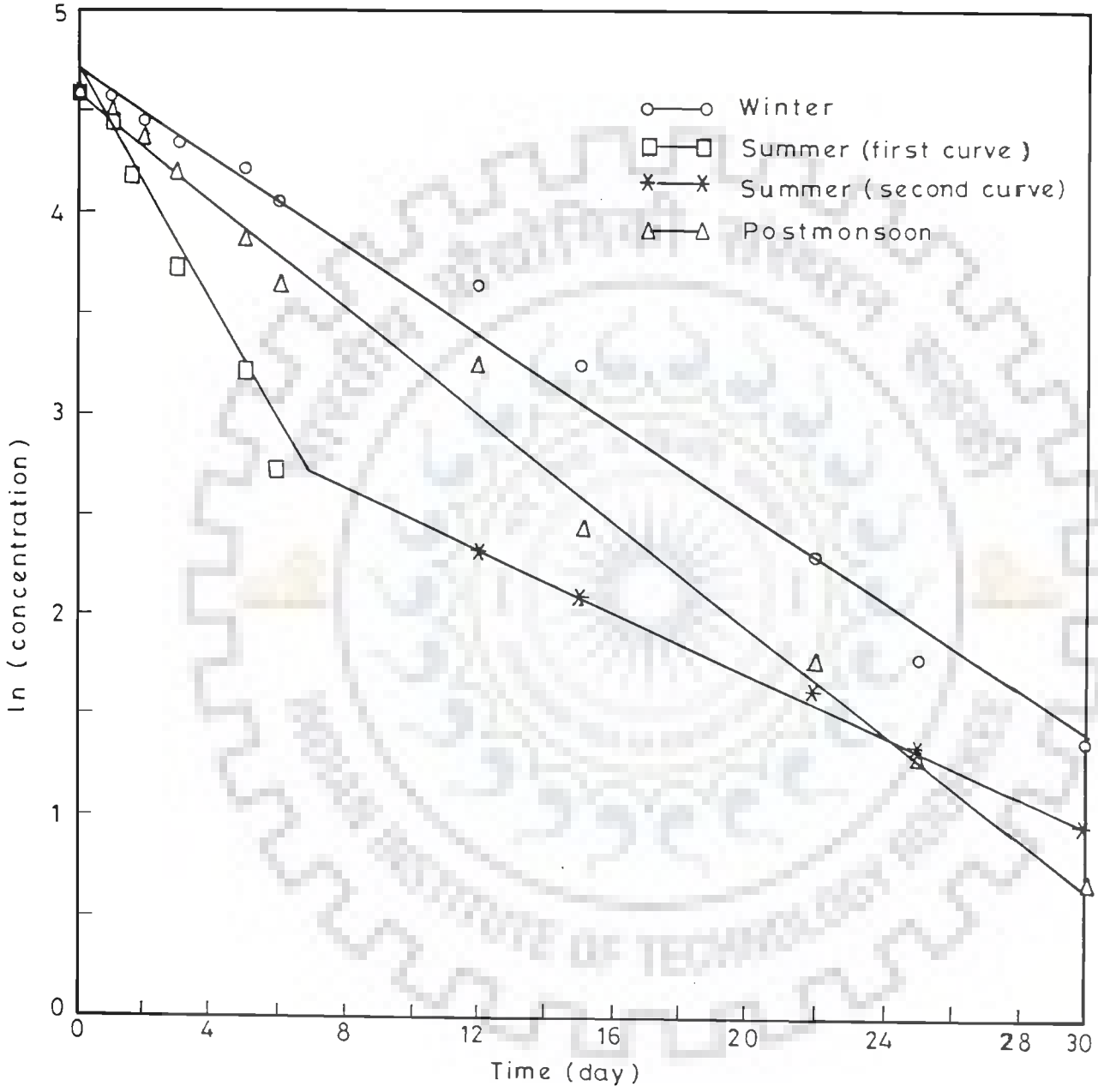


Figure 4·2 Decay of Malathion in Soil (adjoining to the radish)

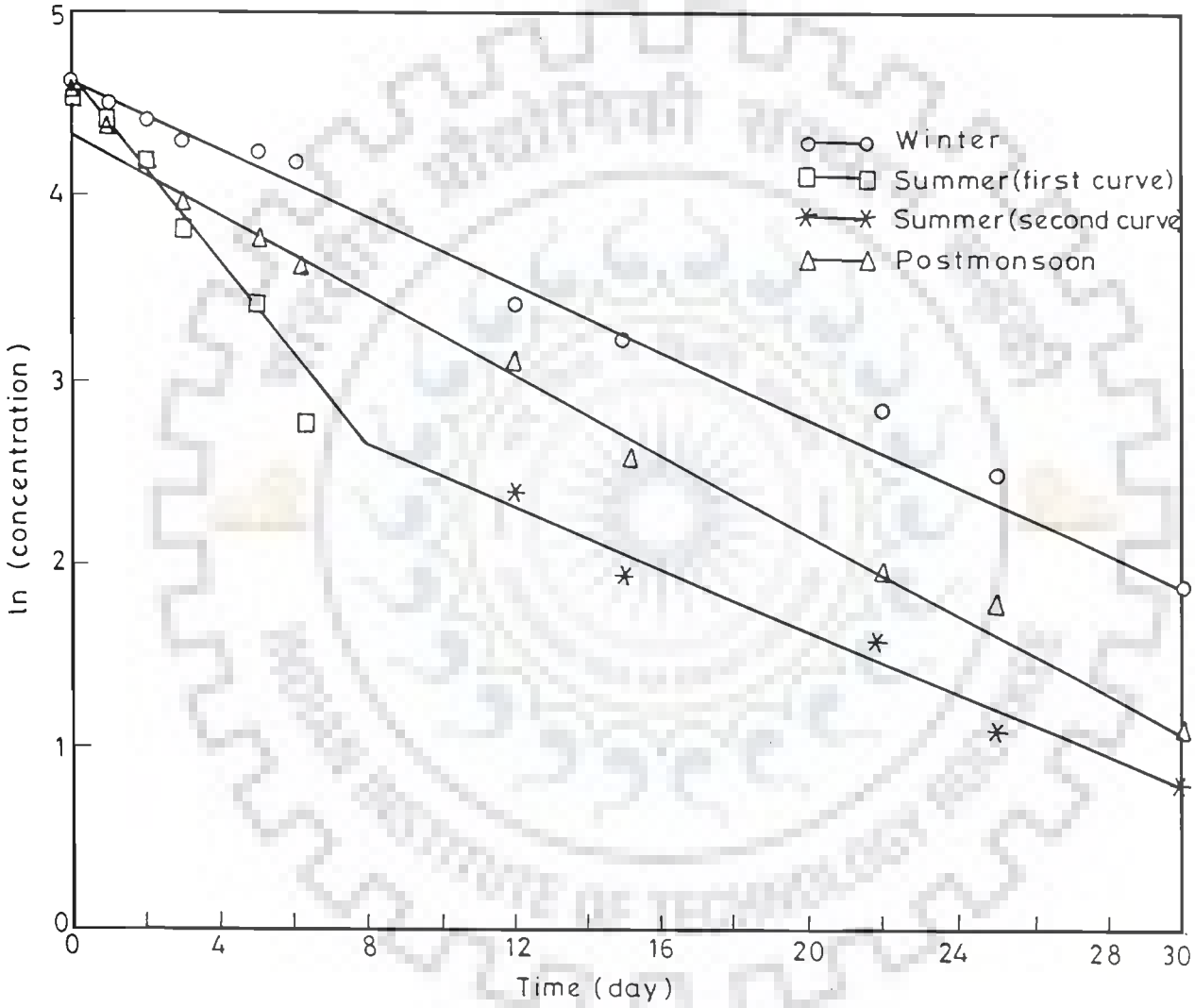


Figure 4·3 Decay of Malathion in Carrot

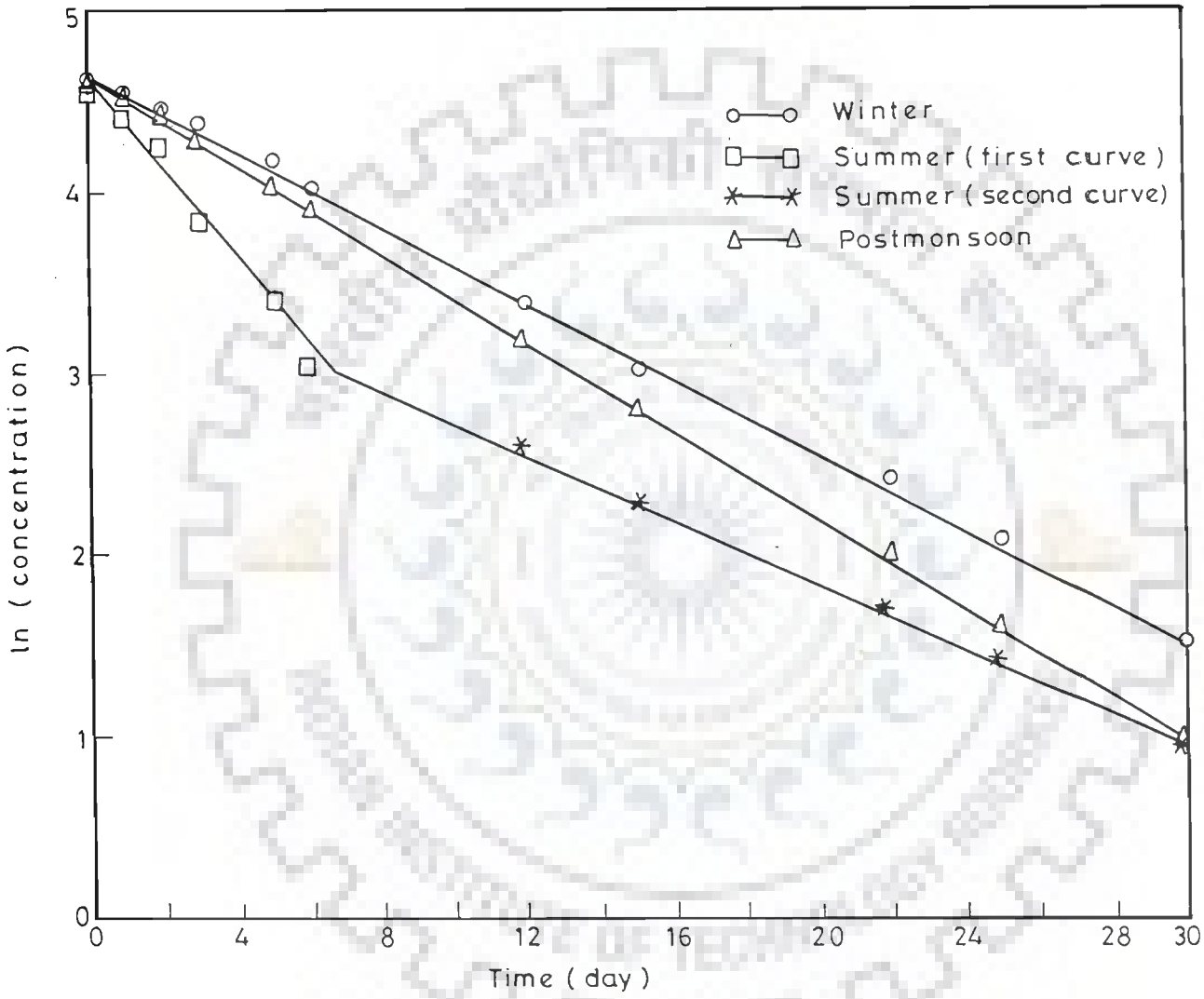


Figure 4·4 Decay of Malathion in Soil (adjoining to the carrot)

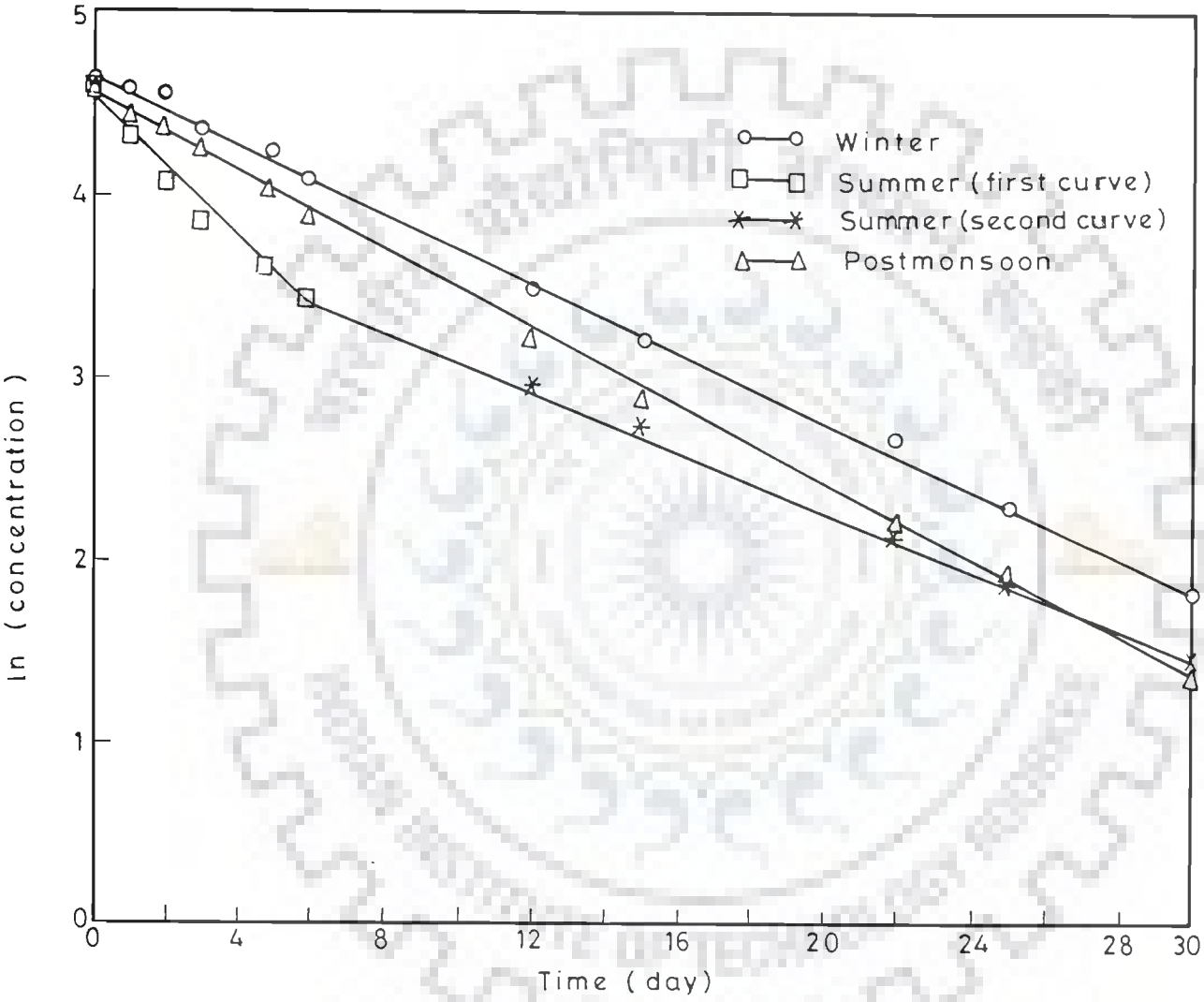


Figure 4·5 Decay of Methylparathion in Radish

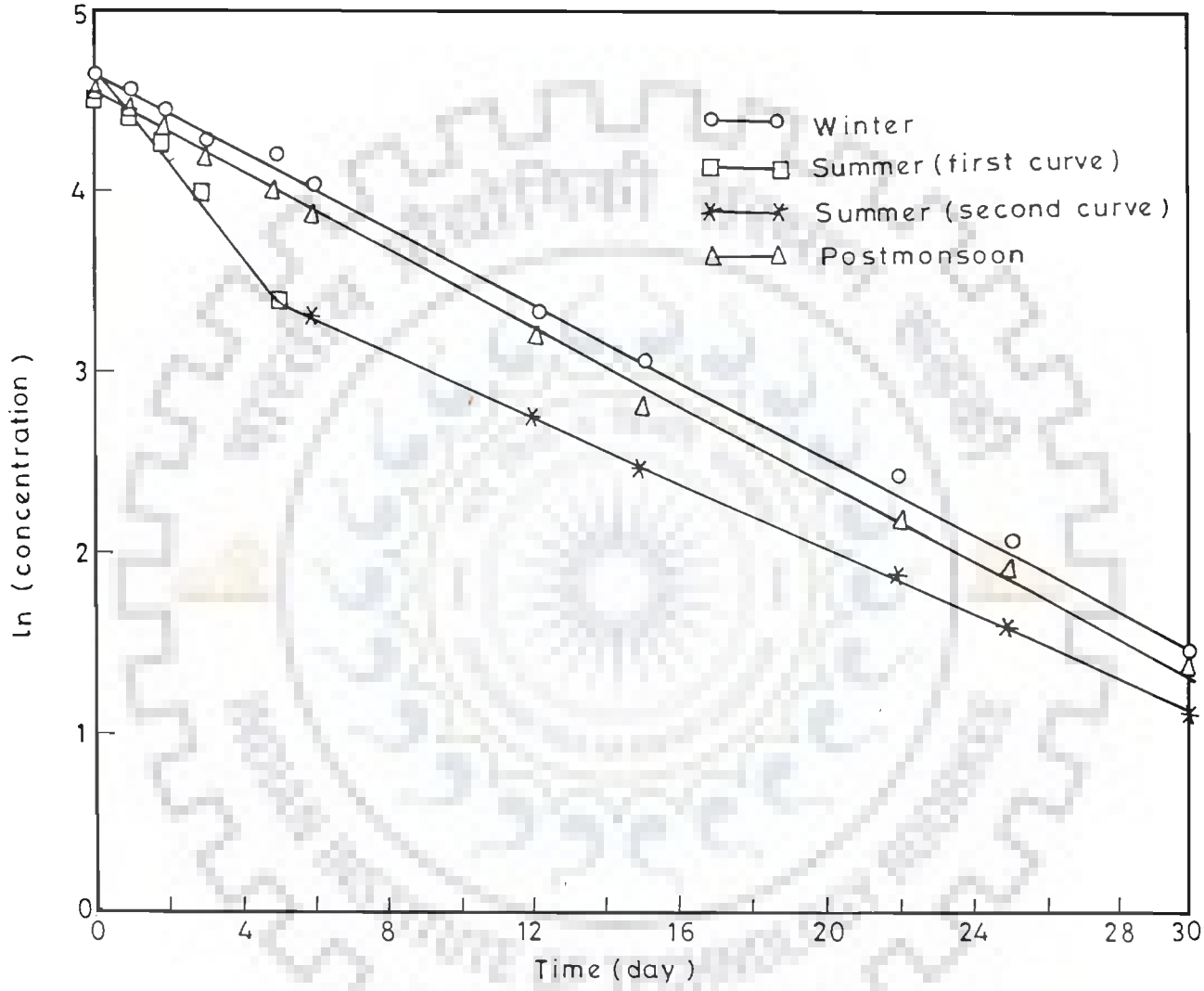


Figure 4·6 Decay of Methylparathion in Soil (adjoining to the radish)

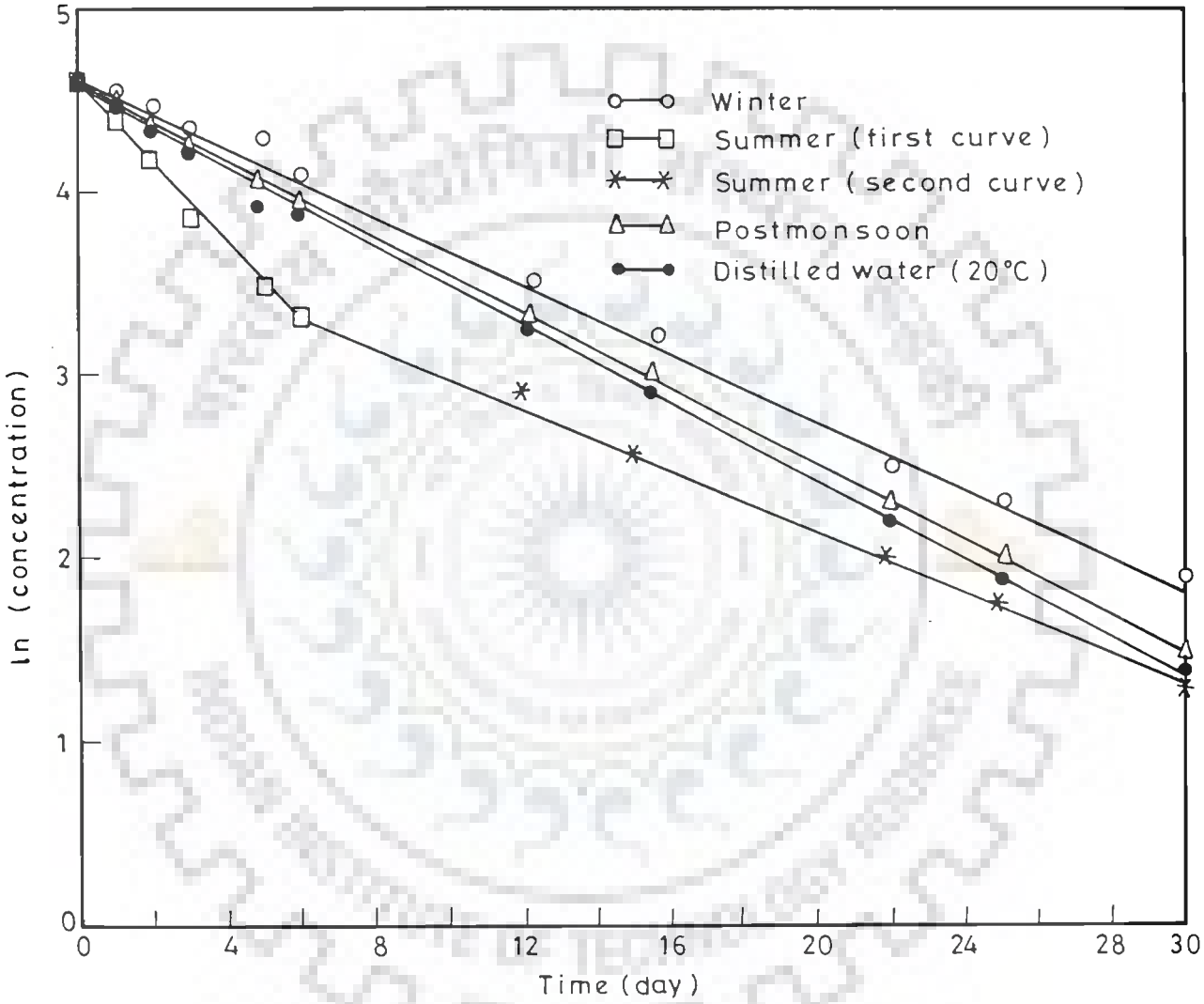


Figure 4·7 Decay of Methylparathion in Carrot

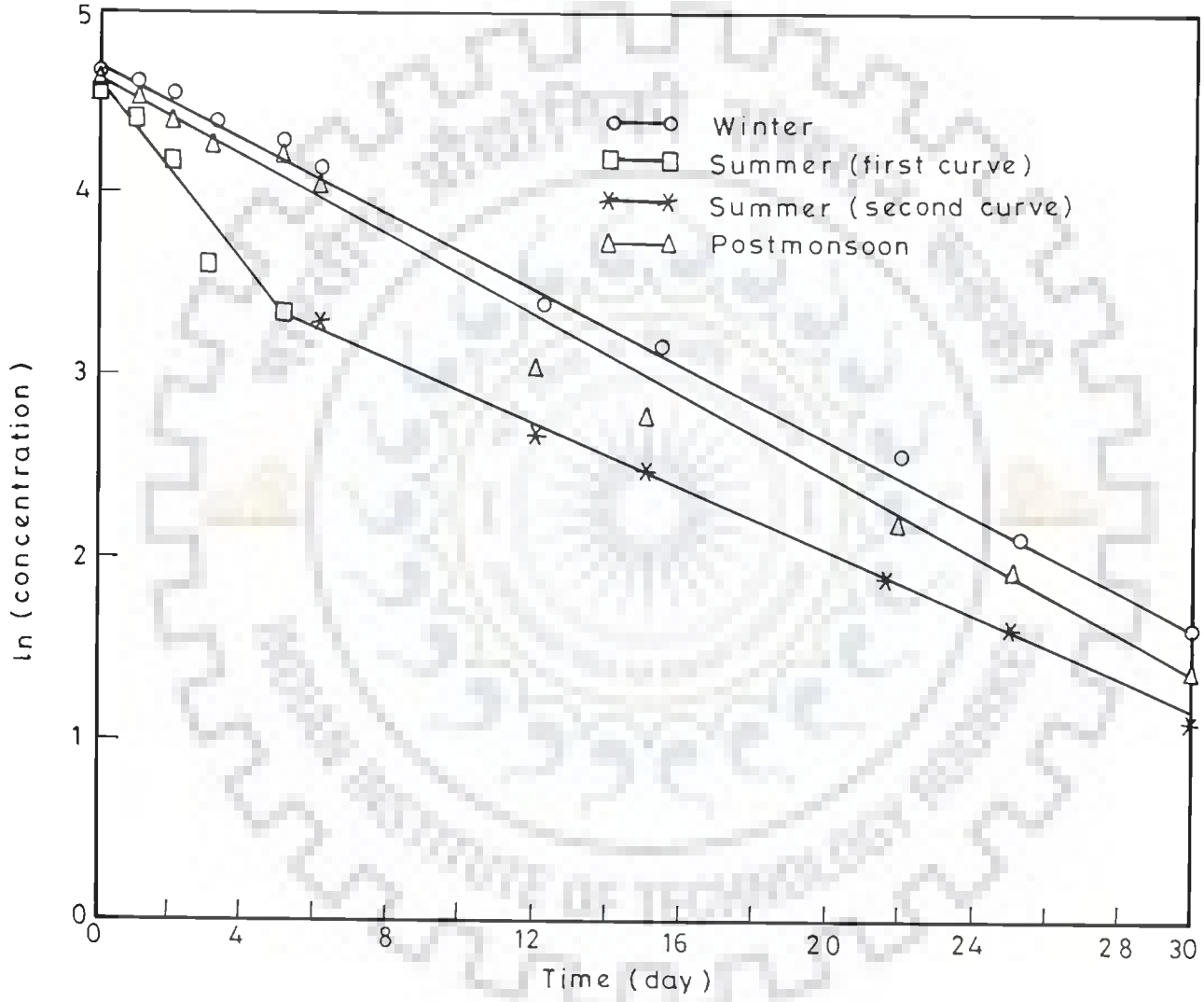


Figure 4·8 Decay of Methylparathion in Soil (adjoining to the carrot)

Table -4.1 Half-life of Malathion in Plant and Soil Under Field Conditions.

Plant/Soil	Season	$t_{1/2}$(days)	k (sec^{-1})	r
Radish	Winter	6.4	7.40×10^{-5}	0.988
	Summer*	2.1	2.43×10^{-5}	0.975
	Postmonsoon	6.1	7.06×10^{-5}	0.981
Adjoining soil	Winter	6.4	7.40×10^{-5}	0.993
	Summer*	2.1	2.43×10^{-5}	0.974
	Postmonsoon	5.3	6.13×10^{-5}	0.992
Carrot	Winter	7.6	8.79×10^{-5}	0.989
	Summer*	2.7	7.29×10^{-5}	0.980
	Postmonsoon	6.3	7.29×10^{-5}	0.974
Adjoining Soil	Winter	6.6	7.63×10^{-5}	0.996
	Summer*	2.6	3.0×10^{-5}	0.992
	Postmonsoon	5.6	6.48×10^{-5}	0.992

* Based on the first part of the decay curve

Table -4.2 Half-life of Methylparathion in Plant and Soil Under Field Conditions.

Plant/Soil	Season	$t_{1/2}$(days)	k (sec^{-1})	r
Radish	Winter	7.4	8.56×10^{-5}	0.993
	Summer*	3.4	3.93×10^{-5}	0.926
	Postmonsoon	6.5	7.52×10^{-5}	0.991
Adjoining Soil	Winter	6.5	7.52×10^{-5}	0.995
	Summer*	2.6	3.0×10^{-5}	0.966
	Postmonsoon	6.4	7.40×10^{-5}	0.989
Carrot	Winter	7.5	8.68×10^{-5}	0.985
	Summer*	3.0	3.47×10^{-5}	0.982
	Postmonsoon	6.8	7.87×10^{-5}	0.982
Adjoining Soil	Winter	6.6	7.63×10^{-5}	0.994
	Summer*	2.5	2.89×10^{-5}	0.952
	Postmonsoon	6.5	7.52×10^{-5}	0.988

* Based on the first part of the decay curve.

(Table -4.1 -4.2) of the pesticide in summer is about one third of that observed in winter and postmonsoon. Initial shorter decay time in summer may be due to faster decay kinetics at higher temperatures and low relative humidity coupled with surface loss. The contribution of the surface loss assumes prominence initially because of the availability of the material at the surface but as the material is absorbed inside the matrix the surface loss becomes insignificant. The above result suggests that a comparison of the half-lives of the pesticides in summer with those of winter and postmonsoon may not give correct projections because of the two different profiles followed in summer. A better way to compare the decay in the different seasons could be the time required for the 90% of the pesticides to decay ($t_{0.9}$). These values for malathion and methylparathion are cited in Table -4.3.

In both, plant and soil the decay follows the sequence winter $\simeq$ postmonsoon < summer. No significant difference could be observed in the decay profile of malathion and methylparathion. Invariably, the half-lives of these compounds on carrot are slightly more than on radish but the difference is not very pronounced. Since the soil adjoining to the two plants is almost similar the half-lives are about the same. Probably due to the contribution of some soil constituents the rate in soil is slightly faster than in plants.

The present investigation clearly indicate that the decay trends under controlled and field conditions are similar with the exception of summer where two clear-cut profiles are observed. No doubt, the decay increases with temperature but in the initial faster decay in summer the contribution of the surface loss assumes prominence. One should not also ignore the longer duration of daylight and bright summer sunshine which may contribute more

Table - 4.3 $t_{0.9}$ (days) of Malathion and Methylparathion.

Plant/ Soil	Pesticide	Winter	Summer	Postmonsoon
Radish	Malathion	21.0	12.0	20.4
	Methylparathion	24.4	18.0	21.7
Adjoining Soil	Malathion	21.0	12.0	17.7
	Methylparathion	21.7	15.0	21.0
Carrot	Malathion	25.2	14.0	20.9
	Methylparathion	24.9	22.0	22.6
Adjoining Soil	Malathion	21.8	13.0	18.7
	Methylparathion	22.1	15.8	21.4

towards the photodegradation at the surface. The decay pattern in plant is similar to that in soil. The half-lives in radish and carrot are nearly of the same order but this similarity cannot be necessarily extended to different crops. In all these cases the decay follows the pseudo-first order kinetics. The half–lives observed in winter and postmonsoon are not much different from those observed under controlled laboratory conditions. However, in summer some additional factors contribute towards the loss of the pesticide resulting into two different profiles. This would amount to the fact that the laboratory data cannot be always extended to the field studies.

This study cannot be rated as complete in itself but is definitely a pointer to predict the fate of malathion and methylparathion under field conditions. The result cited herein are not in agreement with those of Shanker [126] and Sharma [130] who have observed a complete decay of organophosphates in brinjal, potato, cabbage and tomato within seven days.

CHAPTER V

IDENTIFICATION OF METABOLITES OF MALATHION AND METHYLPARATHION.

It has already been pointed out earlier that the presence of pesticide residues in food has become an important environmental issue. The toxicity of a pesticide basically depends upon its chemical nature and persistence in the environment. But equally important is the toxicity of its decay products. It is a known fact that some times the metabolites are more toxic than the parent compound, therefore before accepting a pesticide for agricultural use the metabolites should be properly identified and their toxicity evaluated. The formation of the metabolites is susceptible to the nature of the medium and the prevailing environmental conditions. A study conducted on the identification of metabolites under laboratory conditions in a particular medium cannot be necessarily extrapolated to the field. Therefore for any meaningful conclusion on metabolites the laboratory studies should invariably be accompanied with investigations in various segments of the ecosystem. The information on the decay products can also be of great value in tracing the pathways of the pesticide. The requirement of generating a data bank of metabolites for forensic and epidemiological investigations is well known.

The widespread agricultural usage of the organophosphates and their potential mammalian toxicity dictate to carry out the identification of their metabolites. The most common organophosphorus pesticide, malathion, is used as a foliar spray and also used to kill mosquitoes on the stagnant water surfaces. Methylparathion is the second most widely used organophosphate

applied per acre in forestry and crop products but because of its acute toxicity it is not recommended for livestock use.

Biomonitoring exposure of malathion has been accomplished by determining dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP) [131-133] or the carboxylesterase metabolites, malathion α-monoacid (α-MMA) and malathion diacid (MDA)[134]. Umetsu *et al.*[135] observed the effects of impurities on the mammalian toxicity of technical malathion. Muan and Share [136] have described an anion exchange solid phase extraction procedure for the identification of malathion metabolites. Human exposures to malathion are monitored by the determination of malathion diacid in urine using an isotope dilution ion trap GC-MS[137]. Lores and Bradway [138] have extracted the organophosphate metabolites from urine. Photochemical degradation of a number of organophosphate pesticides including malathion and methylparathion has been studied by Dureja *et al.* [139]. They concluded that the rate of formation of metabolites is affected by many factors such as light intensity, wavelength of light, duration of irradiation, the state of chemicals (for example as a thin film or in solution), the kind of supporting medium or solvent, pH of the solution and the presence of water, air and photosensitizers. Laboratory studies on the metabolites of malathion have identified its half-ester, the dicarboxylic acid, dimethyl phosphorodithioate and dimethyl phosphorothioate[140]. Miles and Takashima [108] studied the fate of malathion and its by- product 0,0,0- trimethyl phosphorothioate in Hawaiian soil and water. Toia and coworkers [141] have studied the photolysis of malathion. They observed that after one hour on a glass surface the GC analysis indicated the formation of three new phosphorus containing compounds which eluted at 120^0, 141^0 and 157^0C. On the basis of comparison

of retention times with those of authentic materials the compounds eluting at 120^0 and 141^0C were assigned as 0,0,0,0-tetramethyl pyrophosphorodithioate and bis-(dimethoxyphosphinothioyl) sulfide, respectively. Malathion hydrolysis products, diethyl mercaptosuccinate and diethyl fumarate [120,97], were formed in malathion- Fenton reaction. Dowling and Lomley [142] have proposed the possible metabolic pathways of malathion and methylparathion in Fenton's reagent.

Oxidative activation of methylparathion and ethylparathion in vitro by NADP-glucose - phosphate fortified mouse and fish liver homogenates was measured. Fish liver homogenate did not differ in their ability to cleave both parathions to p-nitrophenol and their respective dialkyl phosphorothioates. In these studies enzymatic hydrolysis of the oxygen analogs was negligible [143]. Baker and Applegate [144] studied the effect of temperature and uv radiation on the persistence of methylparathion in soil. They concluded that after application of methylparathion to soil it undergoes oxidation to oxon. The metabolism of methylparathion in mice has been studied extensively [145,146]. In these studies the urinary dialkyl phosphates were recovered as the major metabolites of methylparathion metabolism. The data on human subjects suggest that dimethyl phosphate (DMP) is a major metabolite after oral exposure to methylparathion [147].

A closer look at the above literature on the metabolites of malathion and methylparathion indicates that the different group of workers have conducted studies in piecemeal confining to a particular aspect. A sizable fraction of the available information deals with the identification of possible metabolites in biological samples and those formed as a result of

photochemical degradation in laboratory. These investigations provide a clue about the formation of different metabolites but cannot present a clear picture of the contaminants in food sprayed with these pesticides. None of these studies reflect on the formation of different metabolites in various matrices with the passage of time. In order to arrive at any useful conclusion it becomes obligatory to look into the decay products of these two pesticides in water, soil and plant at different intervals of time and suggest possible pathways. The contents of this chapter include identification of metabolites of malathion and methylparathion in water (pH-5.5, 8.0), soil (pH-7.5) and in summer sample of radish. Out of the three seasons summer was chosen because of two distinct decay profiles observed in this season. No doubt this study would have been more revealing by collecting some additional data on metabolite formation under different conditions but the work was constrained because of the non availability of the GC-MS instrument on the campus. Moreover, the analysis involves lot of time and cost.

EXPERIMENTAL

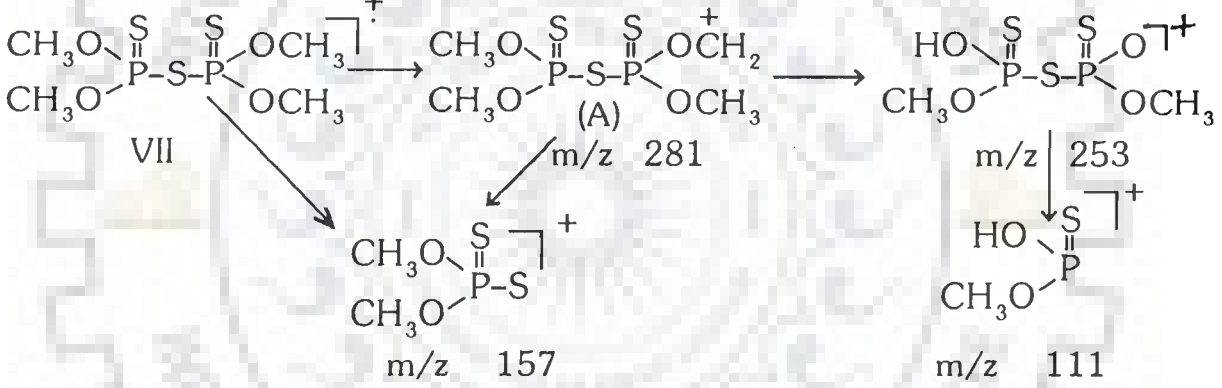
The details of GC-MS used for the identification of metabolites and its operating conditions are cited in chapter II. Metabolites were detected in the residue samples(chapter III and IV). Water (pH-5.5 and 8.0) and soil (Andhra Pradesh) samples ($20^{O}C$) collected after first half-life and on 30 th day were subjected to identification of the decay products. In radish the metabolites were identified in the samples collected on 3^{rd},15^{th}, 21^{st} and 30^{th} day. The MS signals have been reported as abundance or on 100 percent scale because of the change in the recorder.

RESULTS AND DISCUSSION

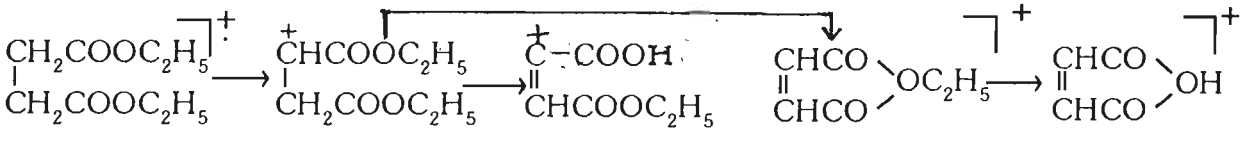
Identification of Metabolites of Malathion

The metabolic pathways of malathion in water, soil and radish are depicted in Flowsheet - 5.1.

The GC-MS of the 6th day water (pH-8.0) sample shows the formation of two products. The mass spectrum (Figure -5.2) of first product has a molecular ion peak at m/z 281corresponding to the structure(A)which arises from structure VII with the loss of H˙, alongwith fragment ion peaks at m/z 253, 207,157 and 111. It is tentatively identified as bis-(dimethoxy phosphinothioyl) sulfide (VII).



The mass spectrum (Figure - 5.3) of the second product has a molecular ion peak at m/z 254 (M^+) with fragment ion peaks at 207,157 and 111 and the product is identified as bis-(0-methoxy-0-hydroxyphosphinothioyl) sulfide (VIII). The 30th day sample shows a molecular ion peak at m/z 302(M^+) with fragment ion peaks at m/z 287,127, 125, 109, 99 and 93(Figure-5.4) and assigned as either α - or β– monoacid of malathion (II or III). Another product whose mass spectrum (Figure - 5.5) shows a molecular ion peak at m/z 174 (M^+) with fragment ion peaks at 173,158,143,127,99,93 and 79, has been identified as diethyl succinate (IX).



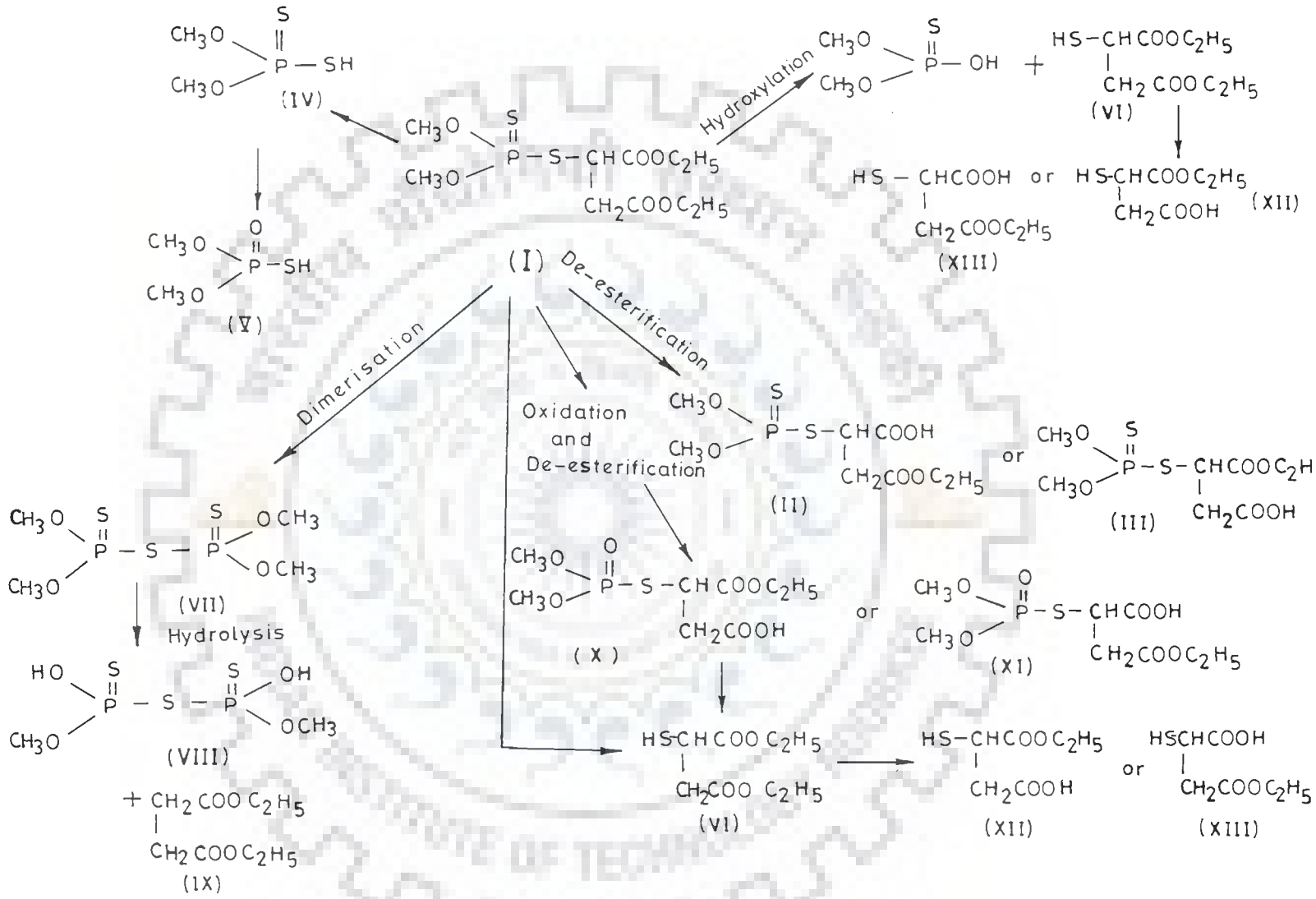


Figure 5·1 Metabolic Pathway of Malathion in Water, Soil and Radish

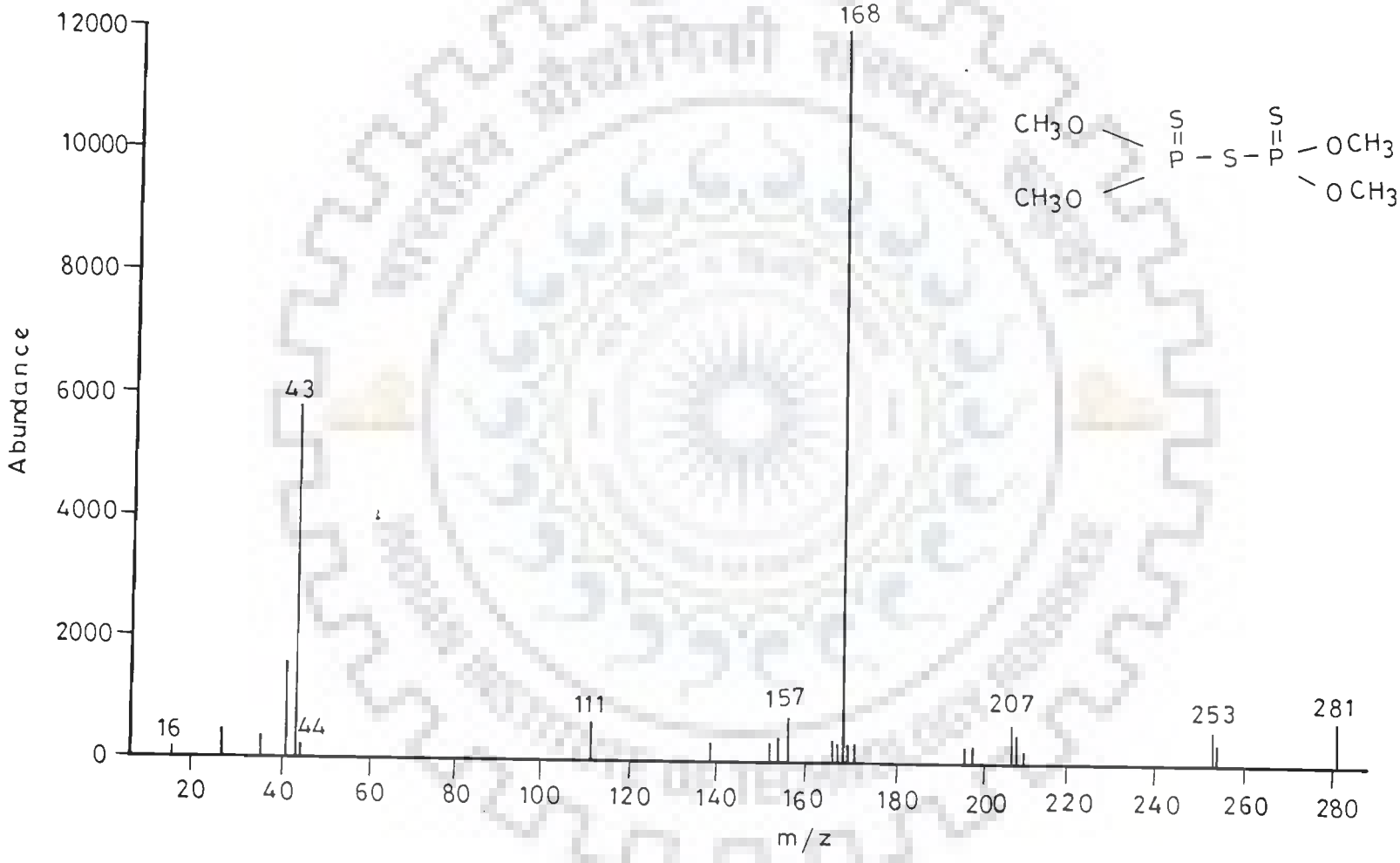


Figure 5·2 Mass Spectra of Bis-(Dimethoxy Phosphinothioyl) Sulfide (VII)

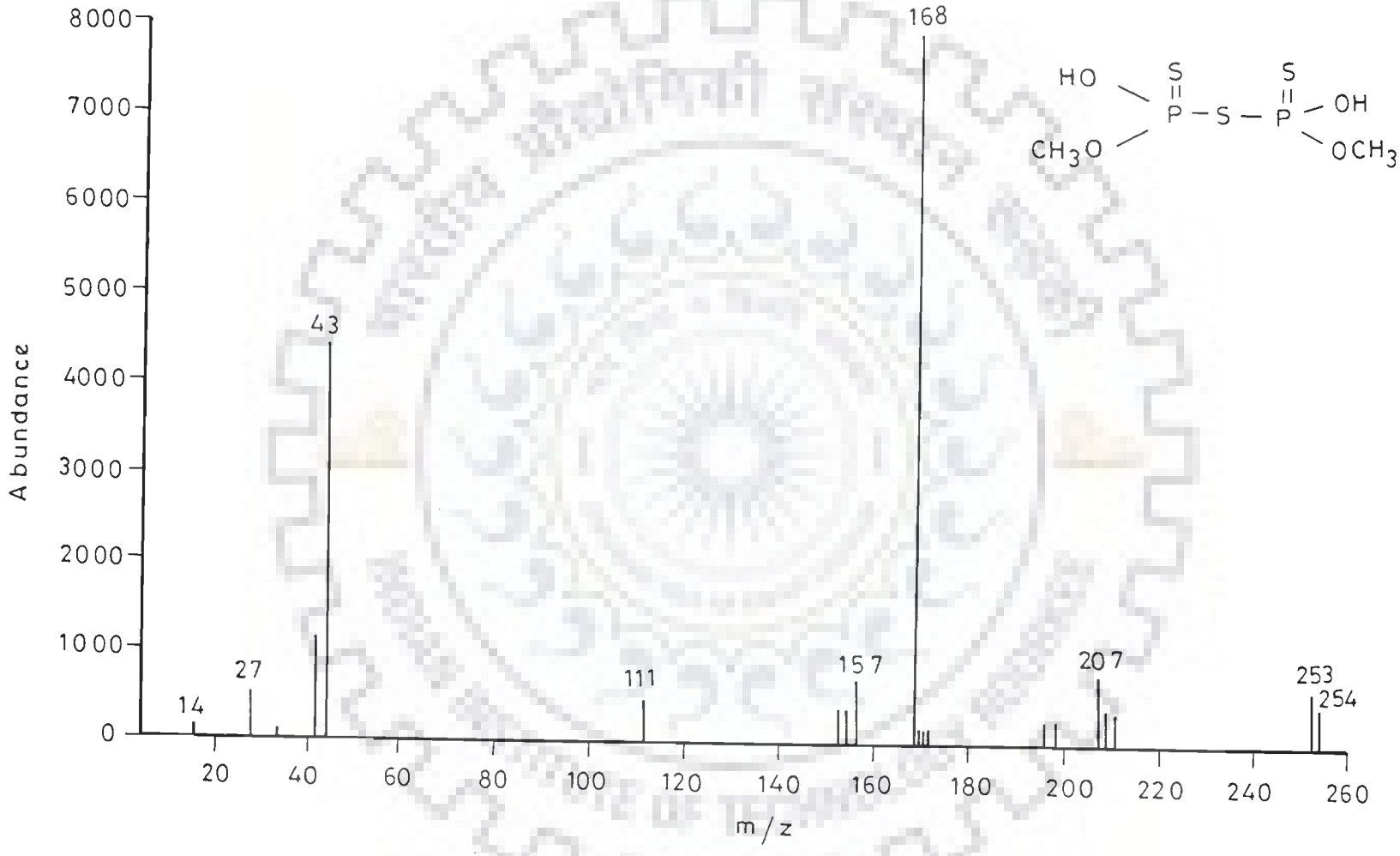


Figure 5·3 Mass Spectra of Bis – (O – Methoxy–O — Hydroxyphosphinothioyl) Sulfide (VIII)

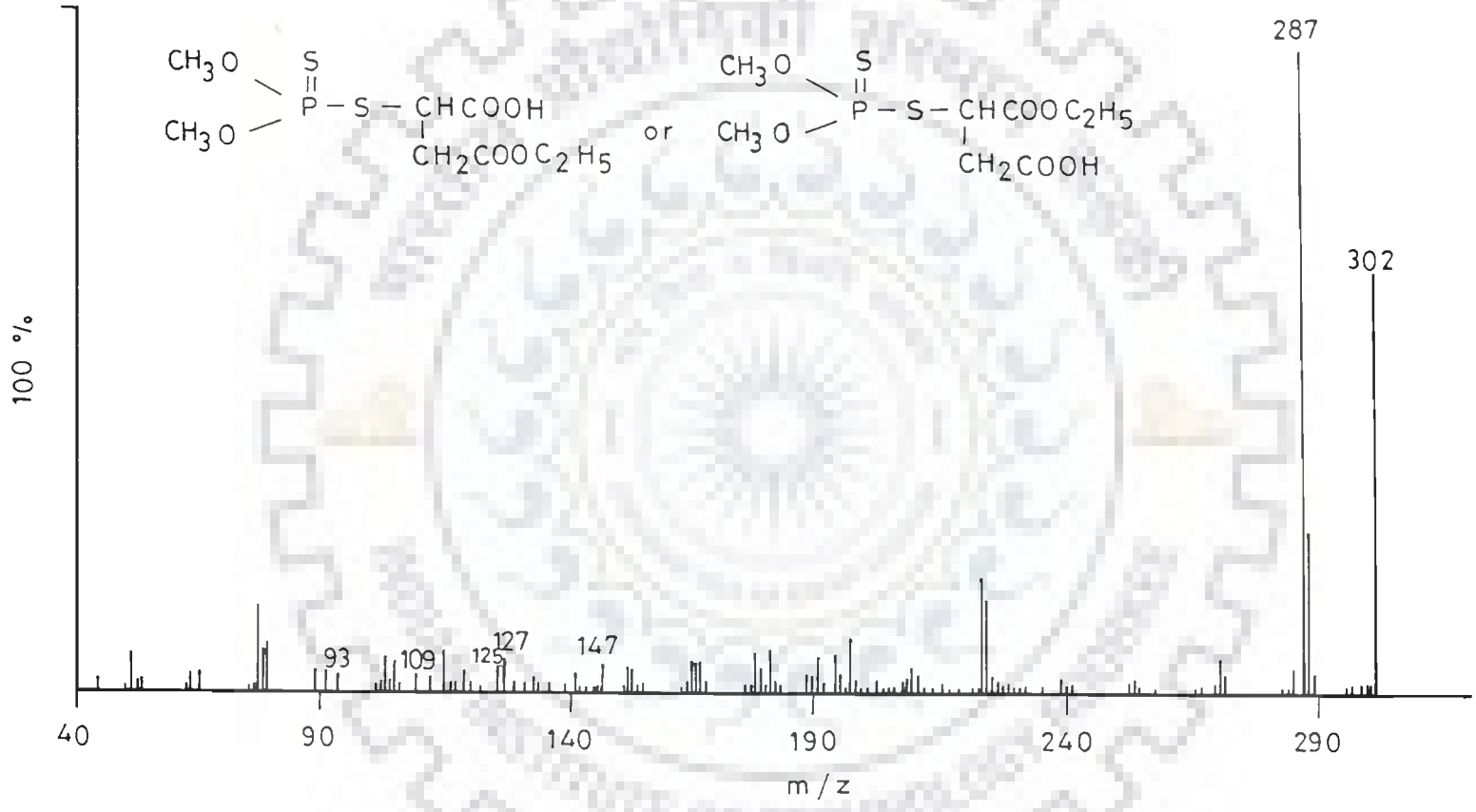


Figure 5·4 Mass Spectra of ∝ – or β – Monoacid of Malathion (II or III)

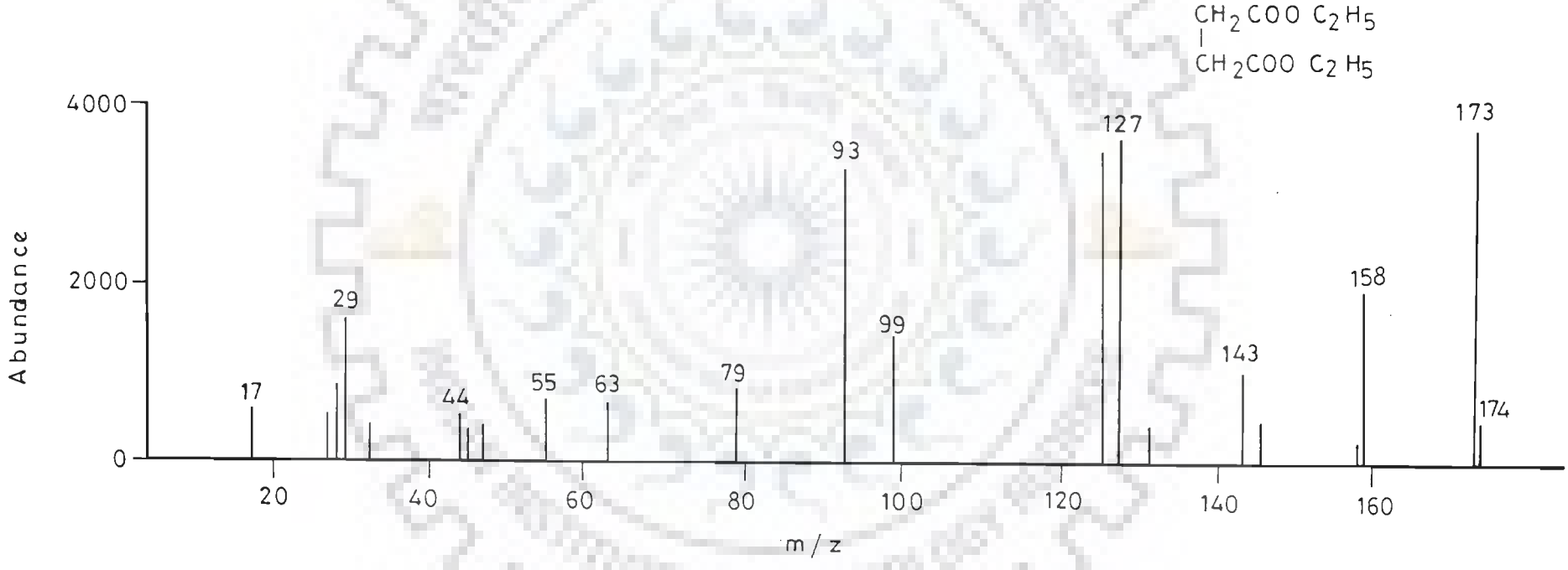
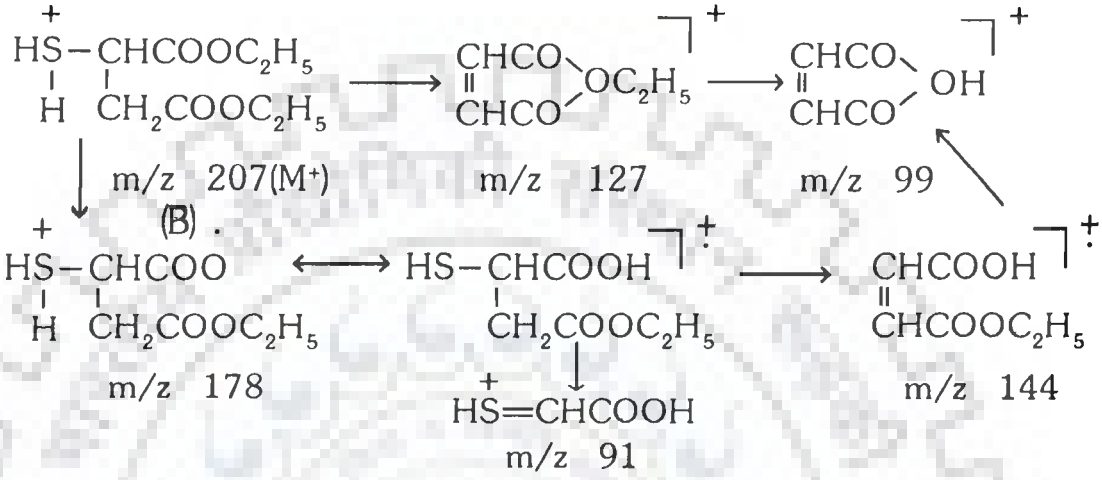


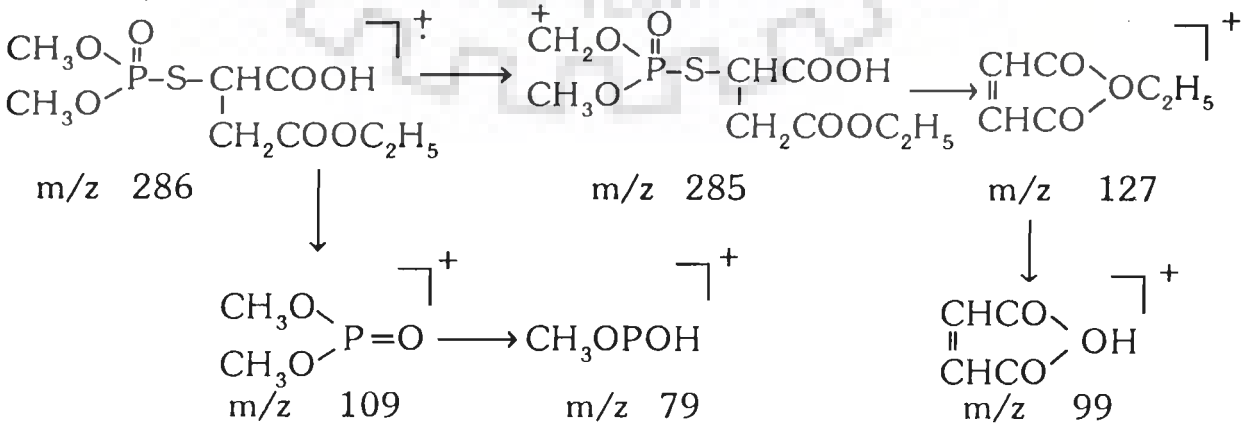
Figure 5·5 Mass Spectra of Diethyl Succinate (IX)

The 7th day water sample (pH-5.5) shows the formation of VI with a molecular ion peak at m/z 207 (M^+) and fragment ion peaks at m/z 178,144,127, 99, 91 (Figure - 5.6). It is assigned the structure of diethyl mercaptosuccinate. Under acidic condition diethyl mercaptosuccinate gets protonated giving corresponding cation (B) which show M^+ ion peak at 207.



The 30th day water sample shows a product with a molecular ion peak at m/z 178 (M^+) alongwith fragment ion peaks at m/z 127 and 99 (Figure - 5.7). The product has been tentatively identified as α-or β–ethyl mercaptosuccinate (XII or XIII).

The metabolites in the soil were identified in the 5th and 30th day samples. The GC-MS result of the 5th day soil sample shows a molecular ion peak at m/z 286 (M^+) with fragment ion peaks at m/z 285, 173, 158, 143, 127, 125, 109, 99, 93, 79 (Figure - 5.8). The product is identified as α - or β– monoacid of malaoxon (X or XI).



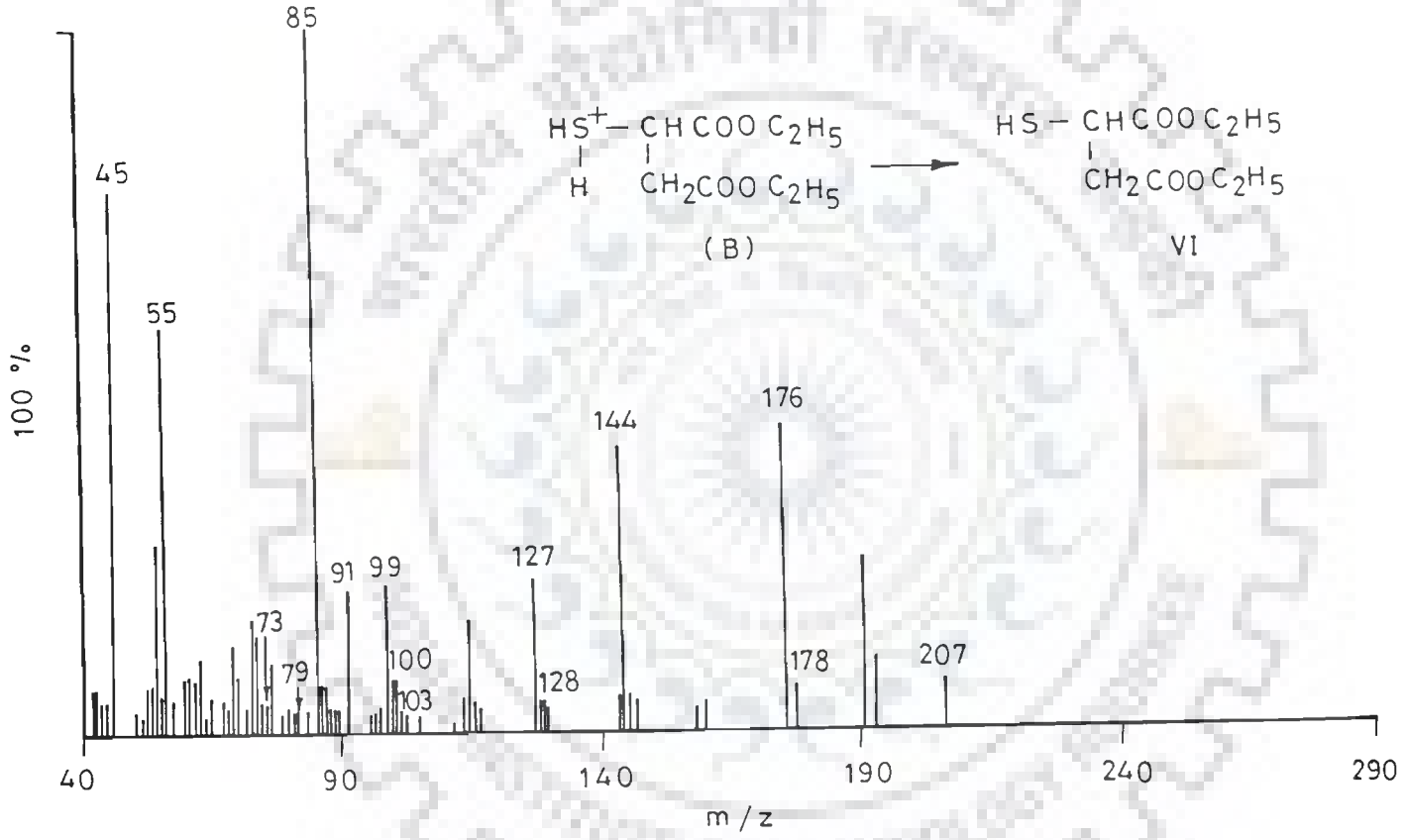


Figure 5·6 Mass Spectra of Diethyl Mercaptosuccinate (VI)

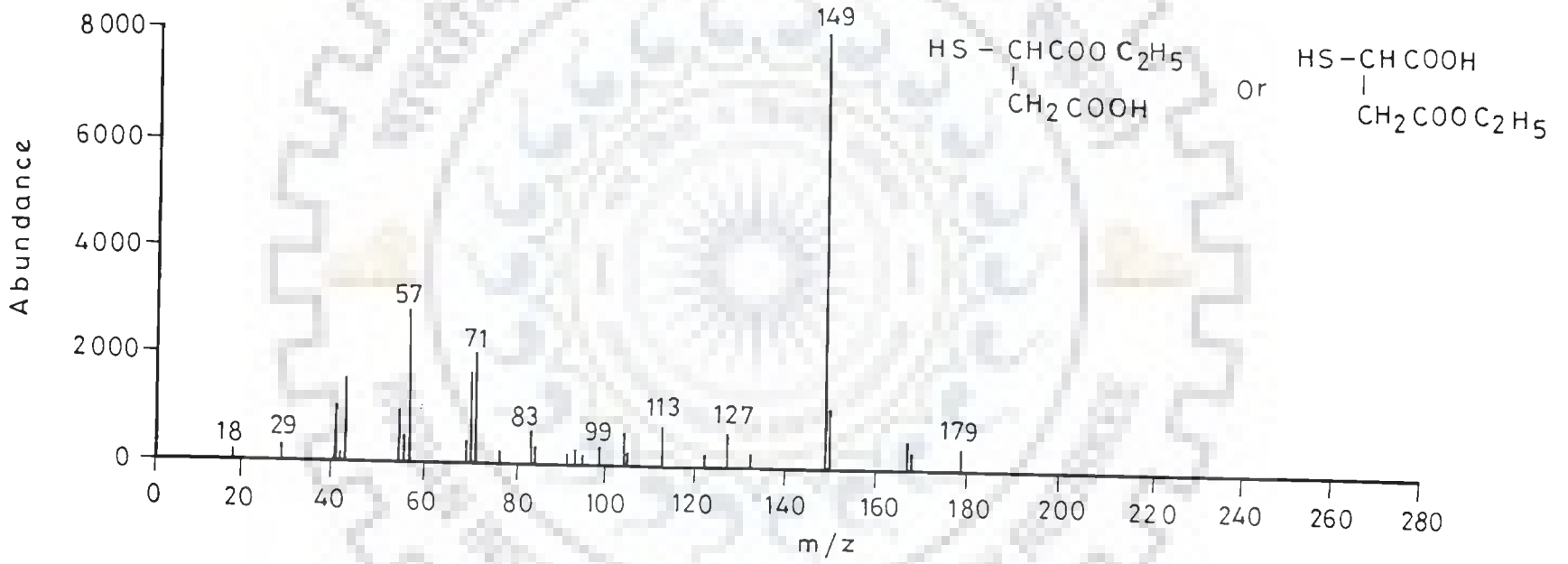


Figure 5·7 Mass Spectra of α–or β–Ethyl Mercaptosuccinate (XII or XIII)

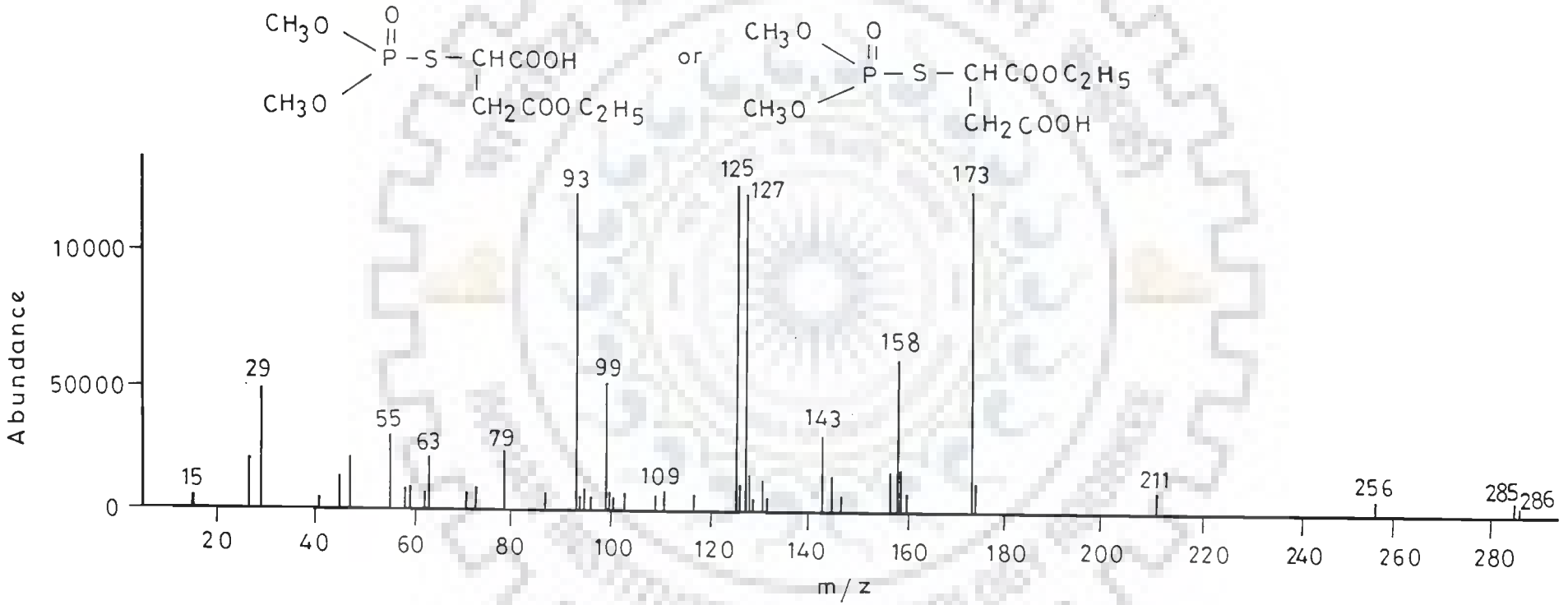
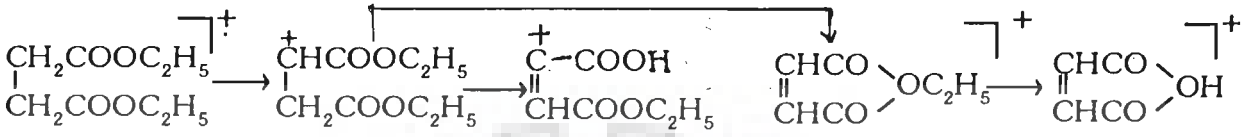


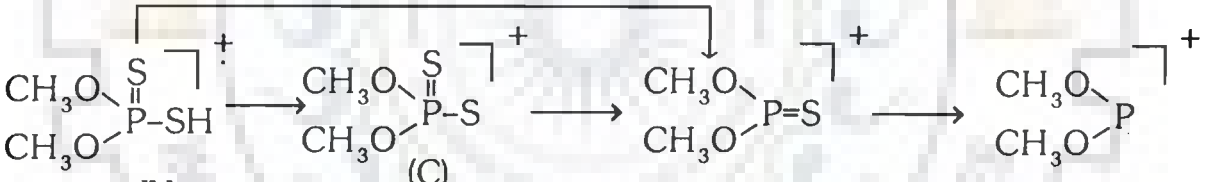
Figure 5·8 Mass Spectra of ∝ – or β – Monoacid of Malaoxon (X or XI)

The GC-MS of the 30th day soil sample shows the molecular ion peak at m/z 174 (M^+), with fragment ion peaks at m/z 173, 158, 143, 127 and 99 (Figure-5.5). The product is identified as diethyl succinate (IX).



m/z 174 m/z 173 m/z 143 m/z 127 m/z 99

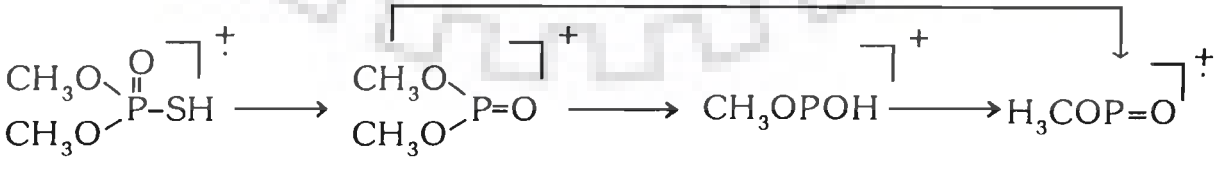
The GC-MS of third day sample of radish shows a formation of α - or β–monoacid of malathion (II or III) (Figure-5.4). The fifteen days sample of radish shows the presence of II or III alongwith another product.This product is identified as 0, 0-dimethyl phosphorodithioate (IV) by its mass spectrum which shows a molecular ion peak at m/z 157(M^+) corresponding to the structure (C) which arises from the structure IV by the loss of H· with fragment ion peaks at 125 and 93 (Figure- 5.9).



IV (C)

m/z 157 m/z 125 m/z 93

The 21st day old sample besides II or III, shows the presence of another product V instead of IV. The GC-MS of this new product shows a molecular ion peak at m/z 142(M^+) with fragment ion peaks at m/z 109 and 79 (Figure - 5.10). It is assigned the structure as 0,0 - dimethyl phosphorothioic acid.



m/z 142 m/z 109 m/z 79 m/z 78

The thirty day old sample shows II or III and VI(diethyl mercaptosuccinate)

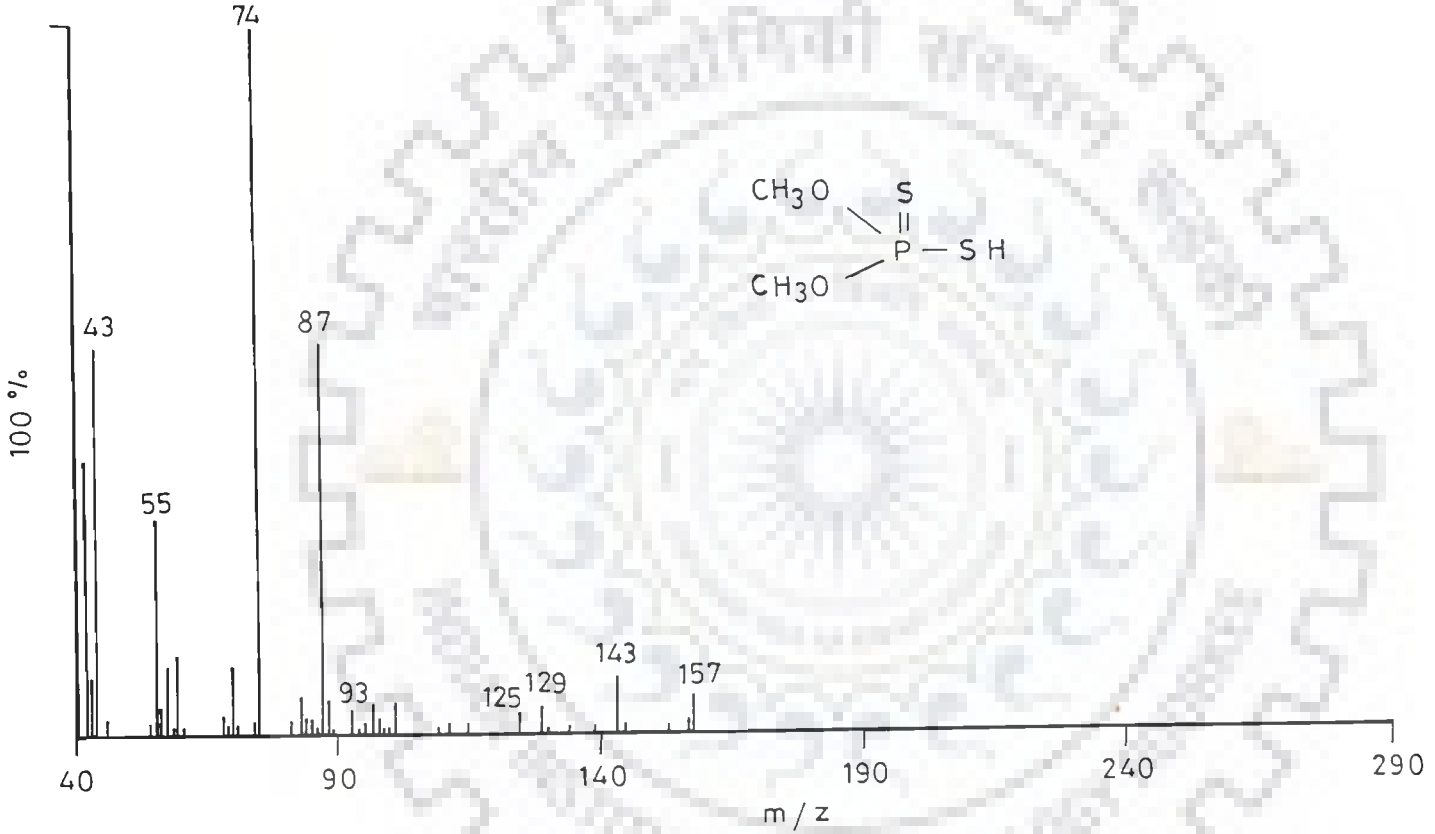


Figure 5·9 Mass Spectra of O,O Dimethyl Phosphorodithioate (IV)

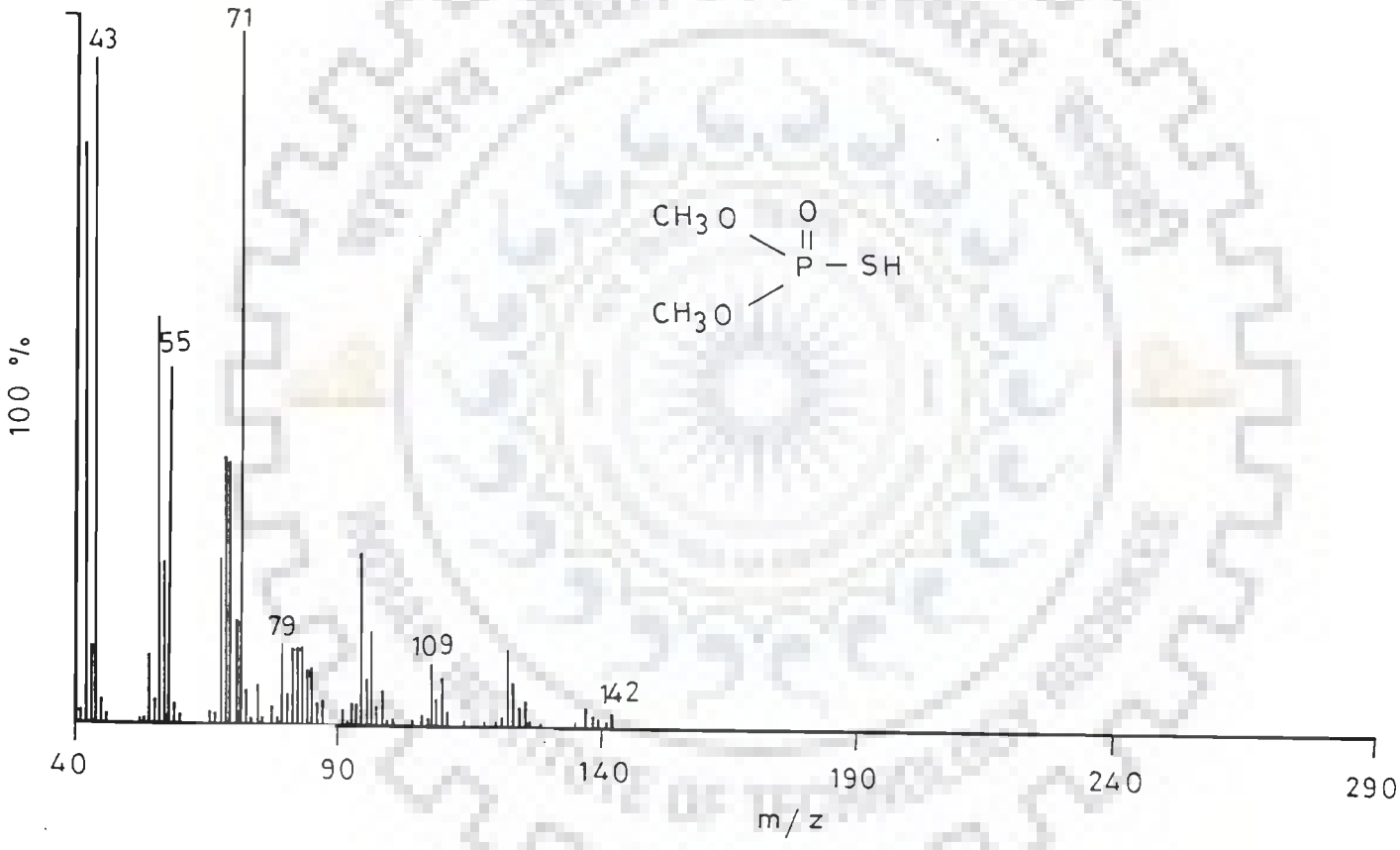


Figure 5·10 Mass Spectra of O, O–Dimethyl Phosphorothioic Acid (V)

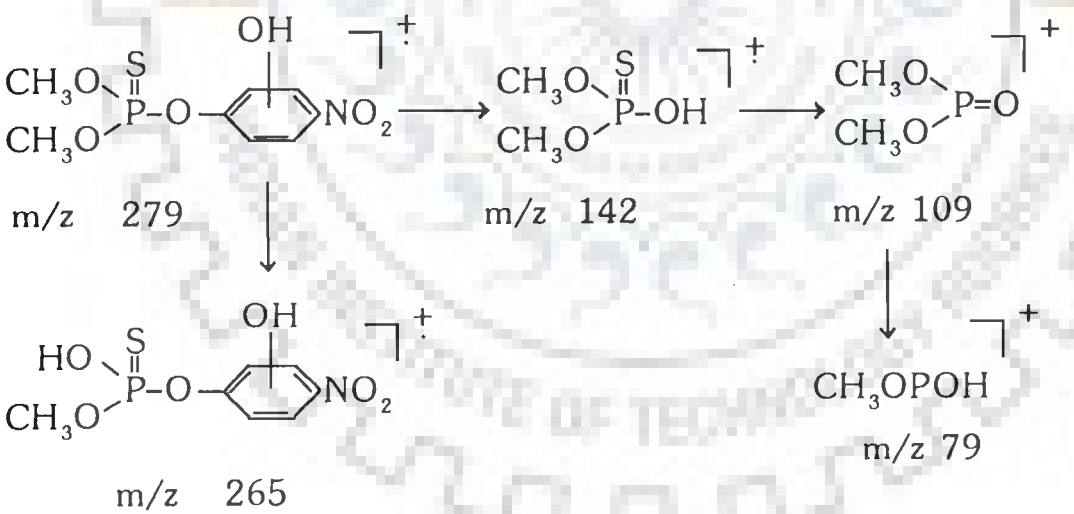
On the basis of above results it appears that the metabolite formation in water at both the (pH 5.5 and 8.0) is initiated by hydrolysis which takes place at different bonds. In the acidic range the rupture of P –S bond takes place resulting into the formation of diethyl mercapto succinate (VI) which subsequently undergoes de-esterification to yield α - or β– ethyl mercaptosuccinate (XII or XIII). At pH 8.0 the cleavage at C-S bond results in the formation of a dimer of dimethoxyphosphino sulfide (VII). This product has been detected as an impurity in technical malathion by earlier workers[135]. Subsequently the dimer undergoes hydrolysis to give bis- (O-methoxy-O-hydroxyphosphinothioyl) sulfide (VIII). In the 30th day sample bis-(O-methoxy-O-hydroxyphosphinothioyl sulfide is accompanied by diethyl succinate (IX).

The metabolism in soil follows a different pathway, initially malathion undergoes oxidation of P = S to P = 0 bond and this is followed by de-esterification to give α - or β– monoacid of malaoxon (X or XI). Further hydrolysis of monoacid gives diethyl succinate (IX). The product (IX) has been reported as a metabolite of malathion in soil and sea water [108]. The major route of metabolism in soil is by oxidation, de-esterification followed by hydrolysis. The pathway of metabolite formation in radish apparently seems to be complicated. This could be partly due to the fact that while following the decay route the samples have been analysed at shorter intervals of time. At first and second stage, de-esterification occurs and gives α - or β– monoacid (II or III), followed by hydrolysis to give 0,0-dimethyl phosphorodithioate (IV). The acid (II or III) persists in the 21st day sample but the oxidation of P = S to P=0 bond lead to the formation of one more compound which is identified as 0,0-dimethylphosphorothionic acid (V). In the 30th day sample the acids II or III and V remain but another compound diethyl mercaptosuccinate (VI) is formed by the rupture of P - S bond. It is interesting to note that α - or β– monoacid which is identified at the initial stages persists upto the end.

Identification of Metabolites of Methylparathion

The decay of methylparathion (Figure 5.11) seems to be simpler than malathion (Figure - 5.1). It may be important to point out that some of the molecular ion peaks observed in the different samples could not be identified.

The water (pH 5.5 and 8.0), soil and radish samples collected after first half-life show only the presence of product XV which is identified as 0,0-dimethyl 0-p-nitro - 2 or 3-hydroxyphenyl phosphorothioate. Its mass spectrum (Figure 5.12), shows a molecular peak at m/z 279 alongwith fragment ion peaks at m/z 265, 156, 142, 125, 109, 79, 65. In the 30th day sample of water (pH-8.0) and in soil only 0,0-dimethyl 0-p- nitro 2 or 3-hydroxyphenyl phosphorothioate (XV) is identified.



In the 30th day water sample (pH-5.5) a different product is identified (XVIII). The mass spectrum of product XVIII shows a molecular ion peaks at m/z 247 (M^+), alongwith fragment ion peaks at m/z 186, 138, 135, 109, 79 and 65

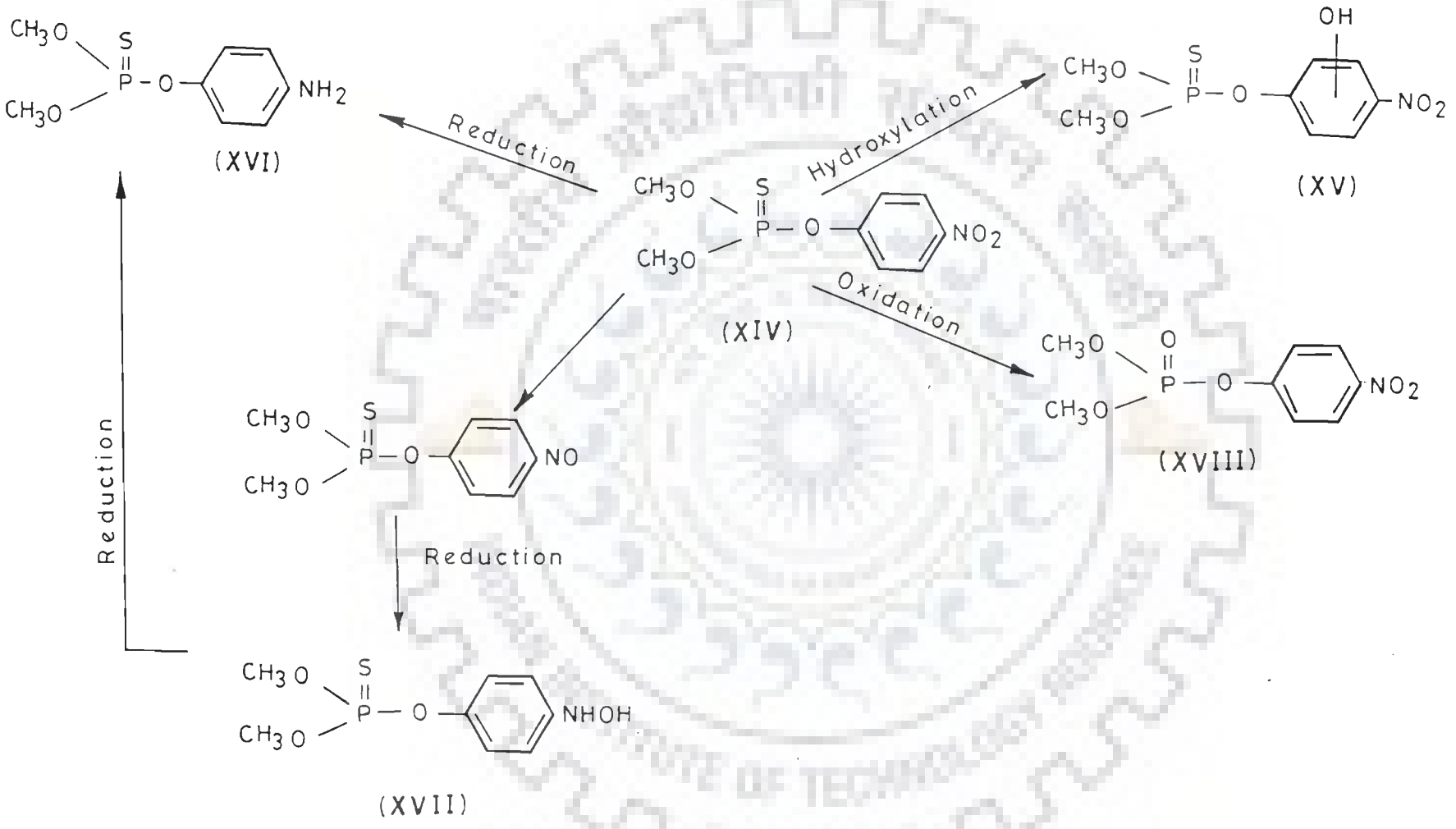


Figure 5·11 Metabolic Pathway of Methylparathion in Water, Soil and Radish

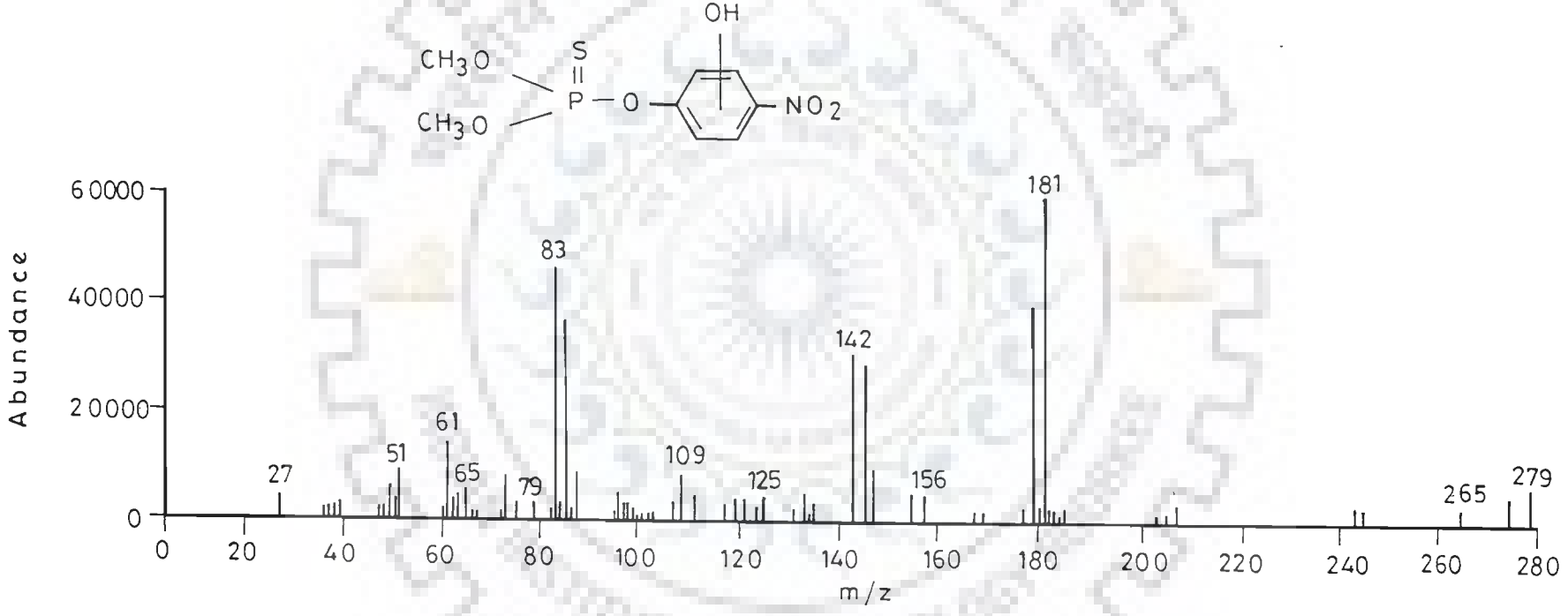
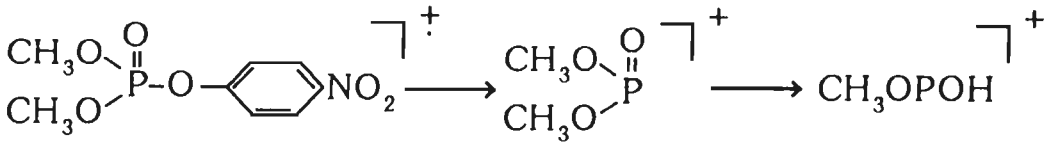


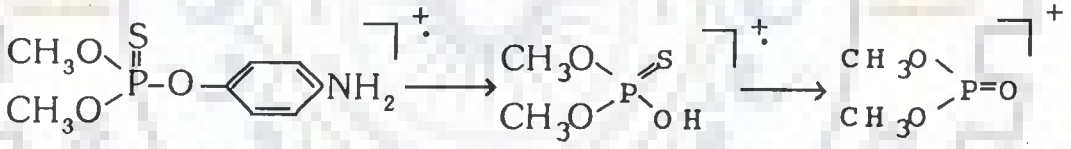
Figure 5·12 Mass Spectra of O, O–Dimethyl O-p-Nitro 2or3–Hydroxyphenyl Phosphorothioate(XV)

(Figure - 5.13). The product XVIII is identified as methylparaoxon.



m/z 247 m/z 109 m/z 79

In the radish samples of 15th and 21st day the product XV persists with some other compounds which could not be identified. The mass spectra of 30th day old radish sample, besides XV, shows the presence of two other products XVI and XVII. The mass spectrum of XVI (Figure- 5.14) shows a molecular ion peak at m/z 233 (M^+) with fragment ion peaks at m/z 142. and 109 and it is tentatively identified as 0,0 -dimethyl 0-p, aminophenyl phosphorothioate.



m /z 233 m /z 142 m /z 109

The mass spectrum of XVII (Figure 5.15) shows a molecular ion peak at m/z 249 (M^+) with fragment ion peaks at m/z 207, 167,93 and 79. It is tentatively identified as 0,0 - dimethyl 0-p- hydroxylaminophenyl phosphorothioate.

The above results indicate that in water, soil and radish OH radical is generated which hydroxylates the phenyl ring to give the product 0,0-dimethyl 0-p-nitro 2 or 3-hydroxyphenyl phosphorothioate (XV). The product XV persists upto 30th day in alkaline water and soil samples. However, in acidic water methylparathion is oxidized to give the product methylparaoxon (XVIII) which

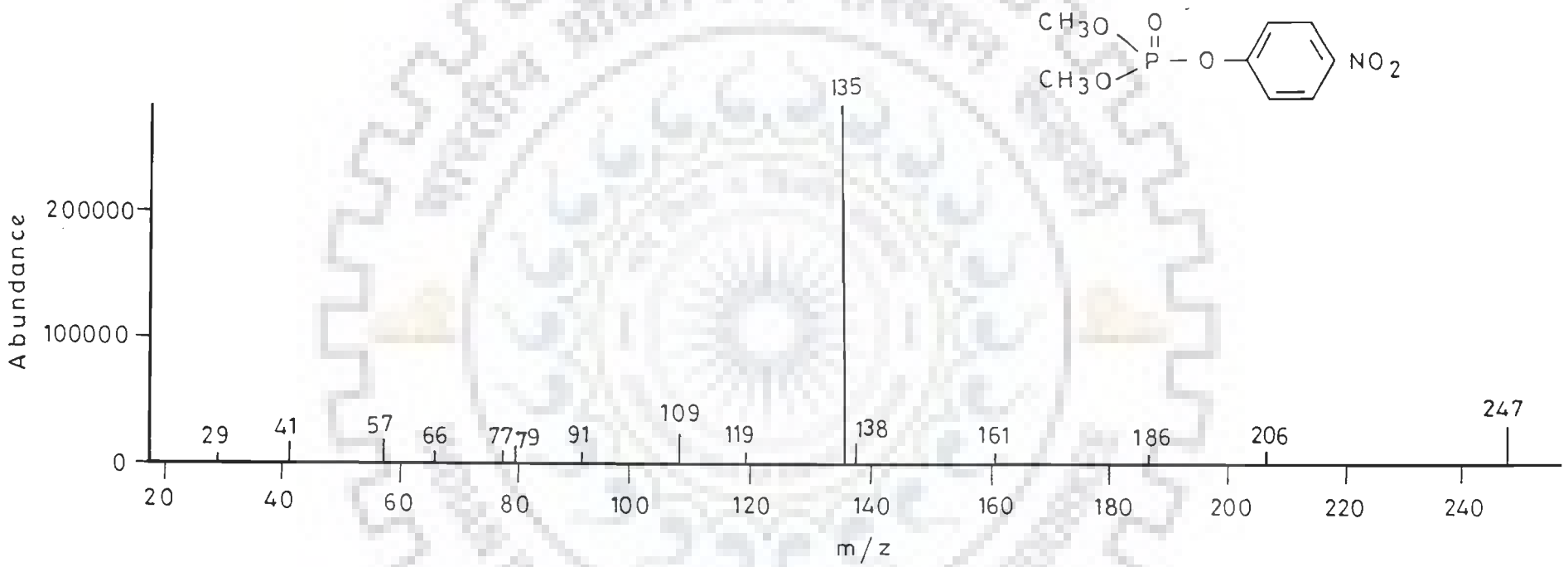


Figure 5·13 Mass Spectra of Methylparaoxn (XVIII)

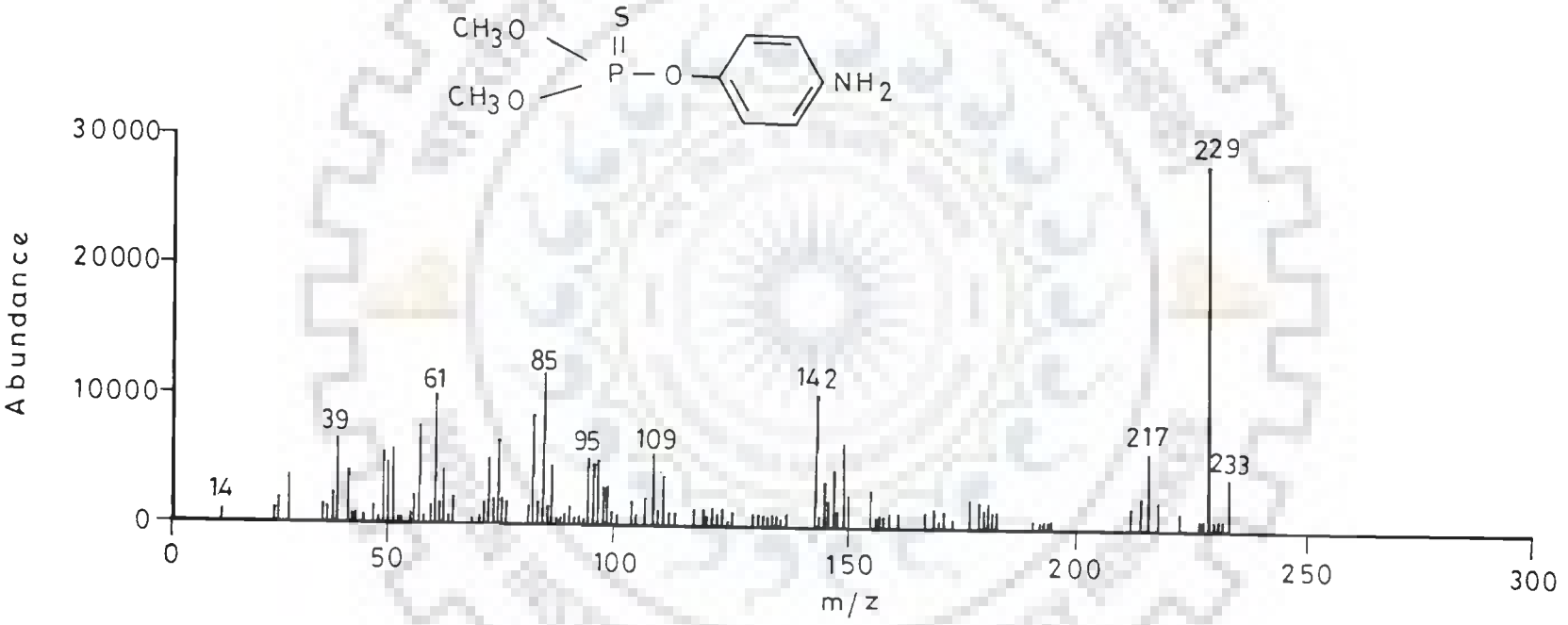


Figure 5·14 Mass Spectra of O,O Dimethyl O- p-Aminophenyl Phosphorothioate (XVI)

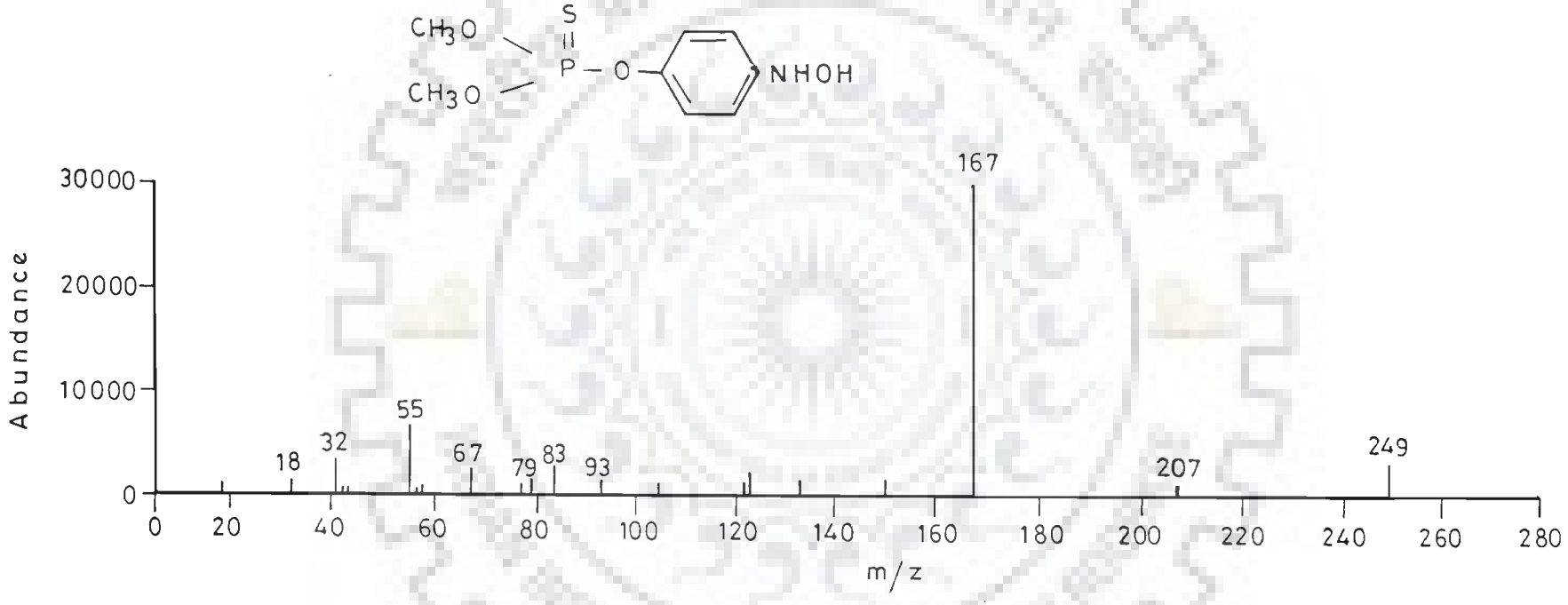


Figure 5·15 Mass Spectra of O,O – Dimethyl–O– p–Hydroxylaminophenyl Phosphorothioate (XVII)

has been reported by earlier workers in different media [142,144]. In the 30th day radish sample, besides the product XV, two new products 0, 0-dimethyl 0-p-aminophenyl phosphorothioate (XVI) and 0,0-dimethyl 0-p-hydroxylaminophenyl phosphorothioate (XVII) are identified.The product XVI can also be formed directly from methylparathion. The presence of the product XVII shows that methylparathion is initially converted into its nitroso derivative (not identified) which on reduction gives hydroxylaminoparathion (XVII).The hydroxylaminoparathion(XVII) on further reduction gives aminoparathion(XVI). The product (XVI) can also be formed directly from methylparathion by reduction.This route has been reported by Suzuki and Uchiyama[148] for parathion in Spinach homogenate.

This study indicates that the major pathways in the formation of different metabolites of malathion and methylparathion involve hydrolysis, oxidation, de-esterification and reduction. In water the cleavage occurs at C-S or P-S bond of malathion whereas hydroxylation of phenyl moiety occurs in methylparathion. In soil and radish both the pesticides follow different routes. Some of the metabolites identified herein have been reported by the earlier workers in different media. It may be interesting to point out here that in some cases the half-life of a pesticide in different media is nearly the same but the metabolites are not necessarily the same. This amounts to the fact that the half-life of a pesticide cannot be the sole criteria to evaluate the environmental hazard.

CONCLUSIONS

In today's agriculture pesticides have become an important tool for boosting production. No doubt several countries are reaching to a state of self-sufficiency in food grains because of widespread application of these agrochemicals but the darker side of their use envelopes the total human environment. There is an alarming rise in the number of deaths due to pesticide poisoning and it is feared that the future generation may also be affected. This is mainly attributed to the flooding of the market with an increasing number of these toxic chemicals without properly testing their persistence and toxicity. In the developing countries, the scenario in this regard is very gloomy. Despite restrictions and regulations on their use India accounts for one third of pesticide poisoning cases in the Third World. With the growing awareness about the pesticide pollution the studies on decay profiles and metabolites formation have assumed paramount significance. The present thesis is an endeavour in this direction and includes investigations on the parameters affecting the decay of malathion, methylparathion and endosulfan. Efforts have also been made to trace the metabolic pathways of the two organophosphates, malathion and methylparathion. Based on the studies the following conclusions have been highlighted.

Under controlled laboratory conditions the decay of all the three pesticides in water and soil increases with the increase in temperature and change in pH from acidic to alkaline range. The presence of humic acid enhances the decay rate of the two organophosphates but decreases the rate of

decay of endosulfan. The half-lives of malathion and methylparathion are comparable. However, endosulfan (α and β) persists longer in acidic water and soil. At 10°C endosulfan shows negligible decay in almost a month's time and it may be more injurious to use the same in cold climate.

In the field also malathion and methylparathion follow similar trends in decay in winter, summer and postmonsoon, both in plant and soil. The effect of temperature on the decay is apparent. The laboratory data are more or less similar to field results in winter and postmonsoon. However, in summer two decay profiles are observed. Initially the decay is faster due to higher temperature and surface loss but as the material is absorbed inside the surface loss becomes insignificant. In view of this a better way to compare the decay profile could be the time required for the 90% of the pesticide to decay ($t_{0.9}$) than $t_{0.5}$.

The study on the identification of metabolites of malathion and methylparathion reveals that they are formed as a result of hydrolysis, oxidation, reduction and/or de-esterification. The metabolic pathway in water is simpler than in plant and soil. A good number of metabolites identified during the decay cycle have been reported in different media by some earlier workers. The toxicity of many of these compounds could not be traced out and it is difficult to comment upon their toxicological aspects. At some stage during the decay cycle the oxons of malathion and methylparathion are formed which are more toxic than their parent compounds. The identification of metabolites of malathion in acidic and alkaline water confirm the proposed mechanisms of hydrolysis in the two media. The half-lives of malathion and methylparathion are nearly the same but their decay follows

different routes. The mass spectra of some of the products could not be interpreted due to lack of information.

The present study has been able to highlight some of the important aspects of the decay of malathion and methylparathion. The author is conscious of the fact that in order to correlate residual and metabolic findings some more data should have been collected. But there are some constraints which cannot be always overcome.

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