STUDIES OF THE DISSIPATION OF p,p'-DDT IN MTWAPA, KILIFI DISTRICT, OF THE COAST PROVINCE, KENYA.

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ABSTRACT.

The rate of disappearance of the insecticide, p,p'-DDT, [1,1,1-trichloro-2,2'-(4-chlorophenyl)ethane], in Mtwapa, Kilifi District, Coast Province, Kenya, was carried out using nuclear techniques. A plot was prepared by digging, and removing all weeds and stones. PVC cylinders (length: 15 cm; diameter: 10.4 cm), were driven into the soil and ^{14}C-p,p'-DDT was applied to the soil contained in the cylinders at rates of 3.78 ppm (total radioactivity, 5.88 µCi). Sampling was done weekly for the first month, biweekly for the second and third month, monthly for the fourth month and bimonthly for the rest of the nine month study period. The amount of ^{14}C-p,p'-DDT remaining was monitored by a Liquid Scintillation Counter (LSC), for the solvent extractable ^{14}C-p,p'-DDT equivalents (residues) and by a Biological Material Oxidiser (BMO), for the bound (unextractable ^{14}C-p,p'-DDT equivalents (residues).

The dissipation half-life (time taken for 50 % of the test compound to disappear) for the parent compound ^{14}-p,p'-DDT, was found to be 196.9 days assuming a 1st-order rate process. The p,p'-DDT was lost either by volatilisation or by conversion to p,p'-DDE. The dissipation curve indicated a biphasic pattern, i.e., an initial rapid rate of disappearance from 0-56 days, and a slower rate from 56-292 days. Bound residues rose to 5.7 % of the applied 14-C-p,p'-DDT showing a low rate of binding in this type of soil.

The degradation products were investigated using Electron-capture Gas Chromatography (GC-EC), a TLC Linear Analyser (TLC-LA), and a combined Gas Chromatograph/Mass Spectrometer. The initial analysis on GC-EC revealed the presence of three major compounds. These were identified as the parent compound p,p'-DDT, and the possible metabolites p,p'-DDE [1,1-dichloro-2,2'-(4-chlorophenyl)ethene, and p,p'-DDD [1,1-dichloro-2,2'-(4-chlorophenyl)ethane]. Identification was by comparison of their retention times with those of DDT, DDE, and DDD standards. However, analysis of the same sample solutions on the TLC-LA indicated the presence of only two components. To confirm the identity of components of the sample solution, structural determination was carried out using GC/MS. This revealed the presence of the parent compound DDT, with the main metabolite being DDE. The structure of the compound formerly identified as DDD (its retention time was similar to that of DDD) was that of 2,4- dimethyl-decane. Its presence in the sample solution was attributed to contamination, and not to the degradation of DDT.

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

PETER NG'ANG'A KAIGWARA

This thesis has been submitted for examination with our approval as university supervisors.

Prof. SHEM O. WANDIGA

Dr. GEOFFREY N. KAMAU

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I dedicate this to my parents, Mr. & Mrs. Kaigwara and to my brothers and sisters.

CONTENTS.

LIST OF TABLES.

LIST OF FIGURES.

CHAPTER ONE.

INTRODUCTION.

1.1 Background.

The rate of growth of the world's population presents a challenge to the agricultural community to provide sufficient food for the ever increasing population. Since there are only marginal possibilities for extending agricultural land, this can only be achieved by intensifying production on a given acreage. The challenge is even greater in developing countries such as Kenya whose economy largely depends on agriculture. The maximum yield potential of crops has to be secured against detrimental effects and hence the need for an affordable, relatively safe, and effective pesticide.

Transport, storage, transformation and biological effects of low level chemical contaminants is a major source of concern to health and to the environment. Pesticides hold a unique position among environmental contaminants, being present in the environment in such small quantities as compared to other contaminants such as industrial wastes and fertilizers. The major factor which accounts for public and scientific concern is their high biological activity and tendency to bio-accumulate. The break down products of some of the pesticides are more toxic to mammals than the parent compounds [O'Brien, 1967], e.g., dieldrin and paraoxon, metabolites of aldrin and parathion respectively. These pose a health hazard to farm

workers and consumers, who may come into contact with the residues unknowingly. The organochlorine (OC) pesticides are slowly metabolized and some of the metabolites (e.g., DDE, a metabolite of DDT) are extremely resistant to further degradation. These have also been found to persist for long periods of time in the environment, especially in temperate regions of the world. For example, DDT half-lives ranging from 2 to 16 years have been reported in temperate regions of the world [Edwards, 1966]. In addition, the solubility of OC compounds in water is very low, e.g., 1.0 x 10^{-5} g/100 cm^3 for p,p'-DDT. Thus they are more soluble in fat substances than in water. This causes them to be easily concentrated through food chains as they tend to partition into lipoidal biological materials in increasing concentrations, reaching potentially hazardous concentrations at the top of the food chain. DDD (a metabolite of DDT) concentrations in water of 0.02 ppm were found to be magnified 80,000 times in the visceral fat of the fish, bass, and the bird, grebe, through an aquatic food chain consisting of plankton ---> herbivore fish ---> predaceous fish ---> fish eating birds [Rudd, 1964]. Due to the persistence and the bioaccumulation of the OC pesticides, low levels have been detected in foodstuffs. This has led to accumulation in various organisms, including man. In Kenya, residues of dieldrin were detected in the brain and liver of non-target wild animals in Lambwe Valley after aerial spraying against tse-tse flies [Alsopp, 1978]. Low levels of DDT and other organochlorine pesticides have also been detected in human

adipose tissue and milk [Wasserman et al, 1972; Kanja, 1988; Wandiga and Mutere, 1988]. Ingestion of small quantities of toxic substances over a long period may bring about chronic toxicity. This may be dangerous, especially to fetus, as well as infants and young children, whose developing biological systems are vulnerable and immature. In temperate regions of the world, DDT and other organochlorine pesticides have been implicated in the thinning and subsequent breakage of the eggshells of various flesh-eating birds [Lockie et al, 1964]. This led to a crash in the population of these birds.

Environmental monitoring plays an essential role in the evaluation and management of pesticides. Without it, detection of serious environmental contamination and threats to human health caused by chemical pollutants may occur only after critical and irreversible damage has been done. In the U.S.A., following several years of large-scale applications of pesticides, birds, fish, and mammals have been found dead and continue to die. Environmental monitoring, together with laboratory experiments can act as indicators or warning signals for harmful effects of pesticides. One such laboratory experiment indicated adverse effects on the reproduction of raccoons fed on a diet containing 2 ppm dry weight or 0.67 ppm wet weight of dieldrin [Morris, 1968]. This level is frequently exceeded in food organisms prevalent in areas treated with aldrin or dieldrin for control of the white-fringed beetle, or the corn root-borer worm.

A lot of literature is available on the persistence of organochlorine pesticides in the environment of temperate zones [Edwards, 1966, Bierman et al, 1982, Cooke and Stringer, 1982]. The persistence of DDT and other OC pesticides and their subsequent bioaccumulation in the environment and in food chains led to their ban and restriction, especially in temperate regions of the world [Bevenue, 1976]. Organophosphorus, carbamates, and pyrethroids were the main substitutes. These have very short half lives, e.g., for organophosphorus pesticides have half lives ranging from 1 to 3 weeks [Voss and Geissbuller, 1971], while aldicarb, a carbamate, persists for 36 days [Ramaprasad and Joshi, 1974]. Pyrethroid compounds are well known for not leaving any residue in the environment after 24 hours [Charbonneau, 1989]. However, organophosphate, carbamate and pyrethroid pesticides have certain disadvantages when weighed against organochlorine pesticides. One, is that they are more expensive. Using them in the place of the organochlorine pesticides would increase the cost of coverage several fold, which the third world countries cannot afford [WHO, 1989]. Two, is that the organophosphate, carbamates and synthetic pyrethroids are much more toxic especially to mammals and ingestion of these compounds directly, as in the case of suicide, or accidentally, as in the mishandling of the pesticides by farm workers has a higher fatality rate than the organochlorine compounds [Hayes, 1976]. Table 1.1 shows the toxicity of some pesticides to rats. The measure of

toxicity is LD_{50}-lethal dose required to kill 50 % of the population of the test organism. It can be noted that the carbamate, aldicarb, and the organophosphorus compound azinphos-ethyl have a lower LD_{50} (i.e., are more toxic) than the organochlorine pesticides, alachlor and DDT.

Table 1.1: Toxicity to rats of some pesticides.

Compound	LD_{50} mg/kg
Alachlor	930
Aldicarb	0.93
Azinphos-ethyl	12.5-17.5
DDT	113-118

[Worthing and Walker, 1983]

Information on the degradation and dissipation of these pesticides under tropical and subtropical conditions is, however, limited. A few studies have indicated that DDT and lindane do not persist in soil under tropical conditions as they do in temperate conditions [El Zorgani, 1976; Wandiga and Natwaluma, 1984; and Mghenyi, 1988]. Except for its tendency to persist and produce resistance in some target pests, the economic viability and excellent residual activity of DDT in the control of agricultural pests makes its use attractive. Likewise, its high efficacy in the public health sector (e.g. control of malarial vectors, i.e. mosquitoes) also makes its use attractive. It

is therefore important that studies be conducted on the degradation and dissipation rates of DDT in tropical and subtropical environments to provide sufficient information, which would enable regulating agencies to advise on the use of this important pesticide.

Any xenobiotic added to food or the environment will always be present from that moment onwards, either as the original chemical compound or as its equivalent derivatives and breakdown products. Fortunately, most foreign chemicals undergo sufficiently rapid physical dispersion and/or chemical degradation so that the resulting residues are of little or no ecological or toxicological significance. However, in assessing the possible significance of such residues in food or the environment, it is essential to obtain a complete picture of the fate and biological activity, not only of the parent compound but of all its likely derivatives. It is not sufficient to study only the disappearance of the parent substance only. The metabolites may also be of significance in relation to possible concentration in food chains, chronic toxicity or long-term ecological effects.

Isotope tracer techniques have been found to be especially useful in basic studies of this kind and in the preparation of 'balance' sheets to account for the overall fate of the original substance. Another advantage of the tracer technique is that it also provides information on metabolites, derivatives, etc., which may not be recovered or extracted by the usual analytical extraction procedures,

especially from aged or stored samples [IAEA, 1972]. Thus this technique will be employed during the present study.

1.2 The insecticide DDT.

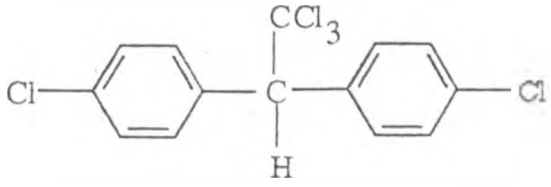


Fig. 1.1: The chemical structure of p,p'-DDT [1,1,1-trichloro-2,2'-(4-chlorophenyl)ethane].

Commercial formulations:

Gerasol, Neocid, Dicophane, Chlorphenathan, Gesapon

NB-These are the registered trademarks.

1.2.1 Laboratory preparation.

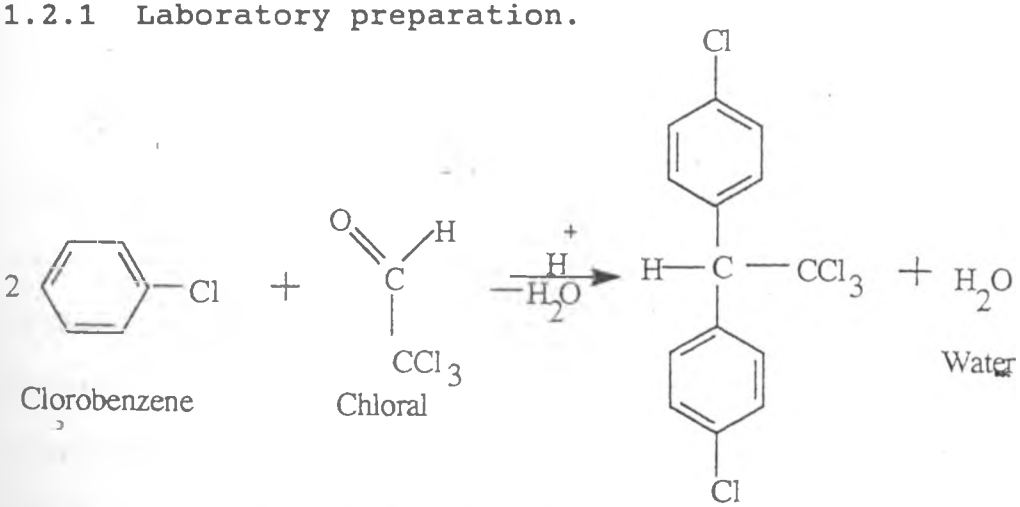


Fig.1.2: Synthesis of p,p'-DDT.

DDT was first synthesized by Zeigler in 1874. It is synthesized by the condensation of chloral with chlorobenzene in the presence of sulphuric acid (Fig. 1.2), [Cremlyn, 1978].

1.2.2 Physical and chemical properties of p,p'-DDT.

The structure of p,p'-DDT (Fig. 1.1), permits several different isomeric forms, an example of which is o,p'-DDT [1,1,1-trichloro-2-(2-chlorophenyl)-2'-(4-chlorophenyl)ethane]. The technical product of p,p'-DDT is a waxy product with a melting point at 89^{0}C. It contains $\leq$ 30 % o,p'-DDT which being of insecticidal value is not usually removed. Chemically, pure p,p'-DDT consists of white needles which melt at 108-109^{0}C and boils at 260^{0}C. Its vapour pressure is 1.5 x 10^{-9} mmHg at 20^{0}C and a solubility of 1.0 x 10^{-5} g/100 cm^3 in water; It is moderately soluble in polar organic solvents, petroleum oils; readily soluble in most aromatic or chlorinated solvents.

1.2.3 Uses of DDT.

After the first synthesis of DDT by Zeigler in 1874, its insecticidal properties were discovered by Paul Muller in 1939. At first, the entire production of DDT in the U.S.A. was used for the protection of troops against malaria, typhus, certain other vector borne diseases, or against biting flies or other insects that are merely pests. It was later released for commercial use in the U.S.A. in August 1945 and far greater amounts were used for the control of agricultural and forest pests.

DDT was found effective in controlling the paddy stem borer, Tryporyze oryzae Walker which was very destructive in certain parts of Japan. It was also used for the control of apple insects, particularly for the fruit-moths and the

leaf-rollers [Hidetsugu, 1972]. In the U.S.A., it was used to control bark beetles which transmit the Dutch elm disease-causing fungus. DDT has also been used for the control of raspberry pests, protecting glass-house crops, and against willow foliage pests among others [Matsumura, 1972].

Thermal vaporizers using gamma-BHC, DDT or a mixture of the two, were widely used against flies, stored food pests and a variety of others, in food premises, intensive poultry houses, cattle rearing houses, offices, shop-mills, warehouses, hospitals and in the home. However, this was stopped especially in living-room and similar situations [Brooks, 1972].

1.3 DDT alternatives.

DDT was widely used to protect cereals in the field and the only suitable alternative for DDT susceptible crops such as barley was aldrin (presently banned). Malathion, azinphos-methyl and carbaryl are as effective as DDT for some other uses, and systemic organophosphorus sprays provide better control of aphids than DDT. Azinphos-methyl and carbaryl, though being more expensive, are satisfactory alternatives for DDT for controlling pests which attack peas, such as moths. Malathion and azinphos-methyl are reliable DDT alternatives for the control of certain raspberry pests. DDT and gamma-BHC have been widely used against pests of glasshouse crops. Ornamentals and outdoor herbaceous plants, usually have a high cash value and,

although infestation may be infrequent, the damage done can be costly; gamma-BHC, malathion and trichlorfon are alternatives for several uses of DDT in this context. DDT has been used in formulations with gamma-BHC as spray against willow foliage pests, and in forestry, where for economic reasons, its persistence has been widely useful [Matsumura, 1972]. Malathion is a useful replacement of DDT. DDT is still used extensively for both malarial control in some tropical countries. Substituting DDT with malathion or propoxur would increase the cost of malaria control by approximately 3.4 or 8.5 fold, respectively, and this increase can not be supported by some countries without decreasing the coverage of their control programs [WHO, 1979].

1.4 DDT and its metabolites.

The initial isolation of metabolites from in-vivo (within living organisms) investigations is frequently tedious and exacting and the quantities available are so small that first evidence regarding structure comes from mass spectrometry. Thus, techniques which produce metabolites, or probable metabolites on a large scale have been used. Some methods of doing this are, the use of tissue extracts, ranging from crude whole homogenates through microsomal preparations to pure enzymes; model chemical systems simulating enzyme reactions; and exposure to various types of radiation.

The following discussion attempts to relate current

biological and chemical findings and offers some speculations regarding metabolic pathways of p,p'-DDT.

The reductive dechlorination of DDT[1] to DDD[4] is effected by dilute solutions of reduced iron-porphyrin complexes [Castro, 1964, Miskus et al, 1965]. In 1968, Rowland linked the conversion in live wheat germ with the anaerobic peroxidation of unsaturated fats by iron-porphyrin enzymes. The conversion of DDT[1] to DDD[4] has been demonstrated in yeast [Kallman and Andrews, 1963], rats [Datta et al, 1964], stagnant rumen fluid [Miskus et al, 1965] and in vertebrate liver [Bunyan et al, 1966, Walker, 1969]. This may involve the reduced cytochrome P450, either enzymatically or non-enzymatically. DDD[4] has also been found, along with DDE[5] in the leaves of cabbage and carrots treated with ^{14}C-DDT. In a study done by Johnson et al in 1967, the following 23 species of bacteria associated with plants, when incubated with DDT1 under anaerobic conditions, converted it to DDD[4]:

Pseudomonas, 6 spp.	Aerobacter aerogenes
Xanthomonas, 4 spp.	Agrobacterium tumefaciens
Erwinia, 4 spp.	Clostridium pasterianum
Bacillus, 3 spp.	Corynebacteriummichiganense
Achromobacter sp.	Kurthia zopfi

Systematic isolations from soils revealed 9 species of Pseudomonas and 3 of Bacillus, besides a Micrococcus and an arthrobacter, that could degrade DDT[1] to DDD[4] under aerobic

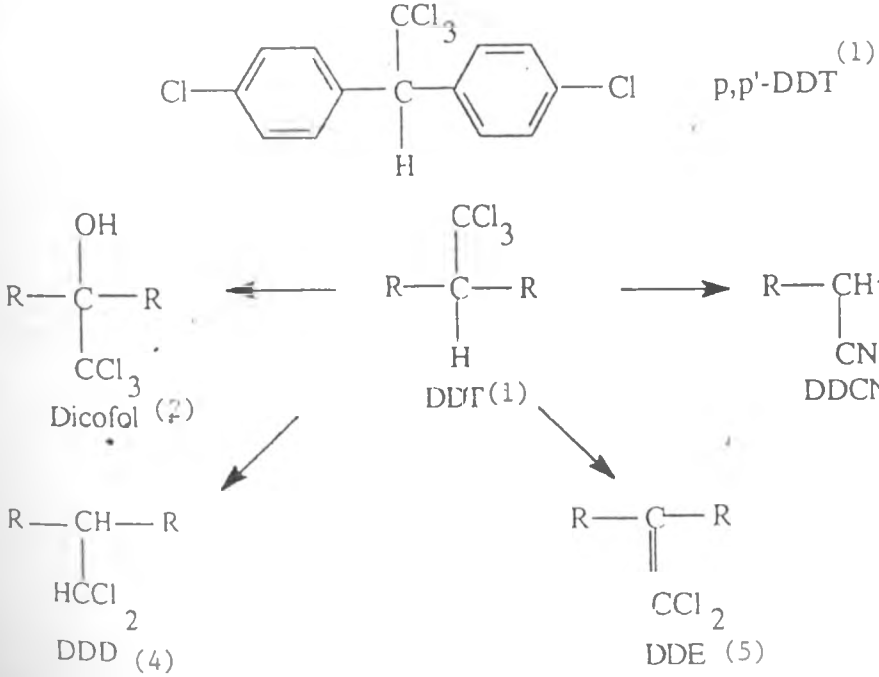
conditions; of these 14 isolates, 12 produced DDA[11] also, while 9 yielded a compound resembling dicofol[2] [Patil et al, 1970].

Chacko reported in 1966 that when 8 species of Streptomyces were incubated with DDT[1] without anaerobic measures, 5 of them degraded it to DDD[4], and so did a species of the actinomycete, Norcadia. Guenzi et al [1967], incubated ^{14}C-DDT anaerobically in silt loam soil for 4 weeks and found that 35 % degraded to DDE[5], while 19 % remained unchanged; only 0.25 % was converted to DDD[4]. However, the following metabolites each contained 0.6 % of the radioactivity: dicofol, DDA[11], DBP[14] and PCPA[15] (see Fig. 1.3). Aerobacter aerogenes, a bacteria associated with plants, when incubated with DDT[1] in broth was found to produce the metabolites DDMU[6], DDMS[7], DDNU[8] and DDOH [Wedemeyer, 1967a] and to degrade DDA through DDM[12] and DBH[13] to DBP[14] [Wedermeyer, 1967b]. Isolates of the common soil fungi were inactive whereas the actinomycetes were active [Chacko et al, 1966], but Matsumura and Boush [1968], showed that 12 out of 18 isolates of Trichoderma viride were able to metabolize DDT[1] under aerobic conditions, 8 producing DDD[4] plus dicofol (Fig. 1.3), 1 producing DDD[4] plus DDE[5], and 3 producing DDD[4] only; an isolate from an Ohio orchard degraded 90 % of the DDT[1] in 3 days. The fungus, Fusarium oxysporum, could produce DDD from either DDT or DDE, the metabolic pathway then passing through DDMU[6], DDOH[10] and DDA[11] to DBH[13] [Engst and Kujawa, 1967].

In soil, it is difficult to detect in any quantity the

metabolites beyond DDD[4] and DDE[5] possibly because the polar compounds cannot be recovered from soil samples [Ott and Gunther, 1965]. Whereas DDD[4] is further metabolized, DDE[5] is evidently an end product; Aerobacter for example, cannot degrade it under either aerobic or anaerobic conditions [Wedermeyer, 1967a].

In a study by Patil et al [1970], 20 microbial species failed to convert DDT[1] to DDE[5]. The presence of DDE[5] in the soil has been attributed to photodecomposition of DDT[1] by UV light from the sun. Extensive studies have shown that DDT[1] is readily degraded photochemically in organic solvents, water and air, giving DDE[5] as a prominent and early product [Zepp et al, 1977, Wandiga and Natwaluma, 1984]. Further investigation by Zepp et al revealed that further exposure of the DDE[5] solution to sunlight gave an isomer of DDE[5] whose spectral, chemical, and chromatographic properties were virtually identical to those of DDE5. This isomer was shown to be a mixture of cis- and trans-1-(p-chlorophenyl)-1-(2,4-dichlorophenyl)-2,2-dichloroethylene.



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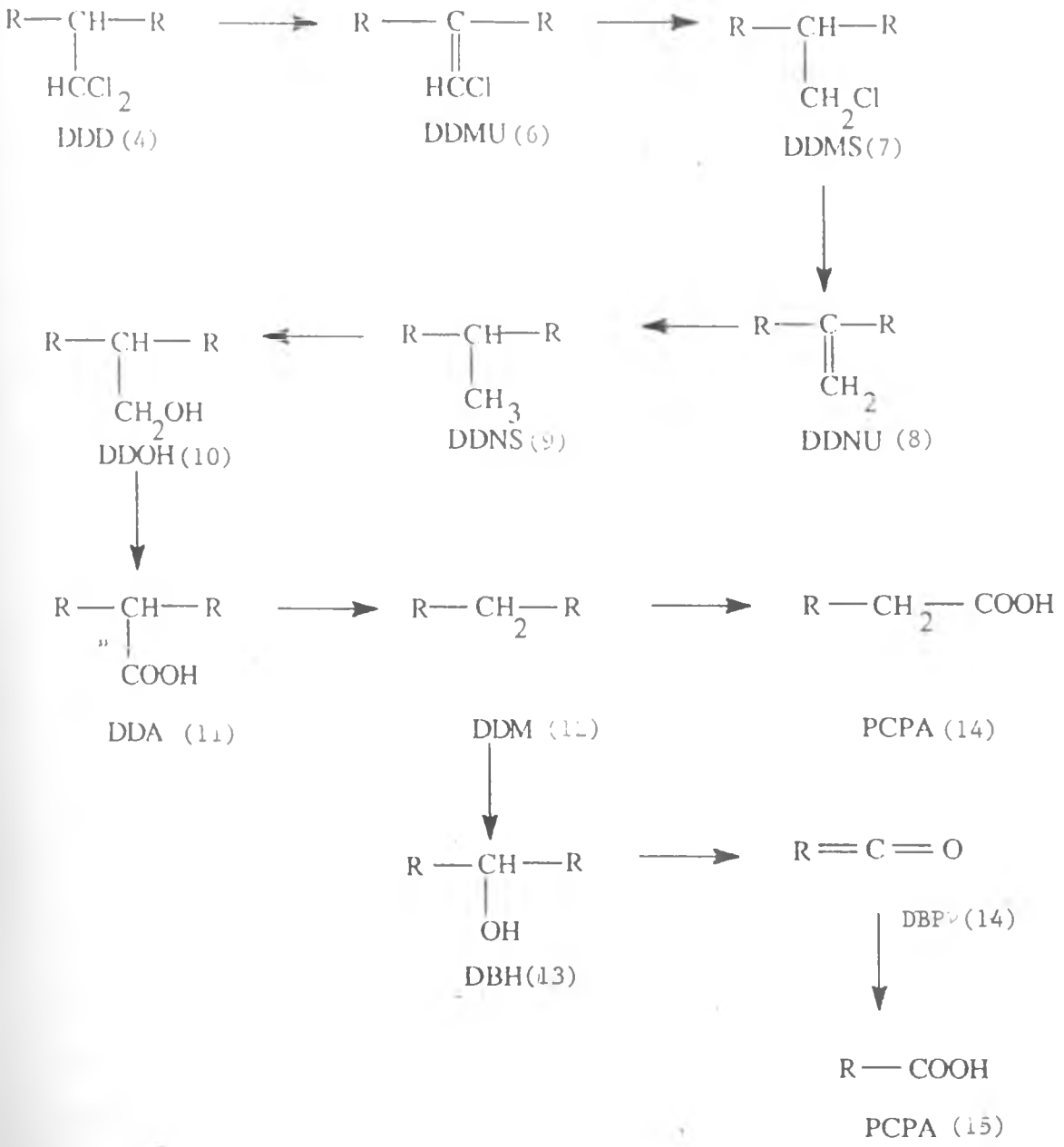

Fig. 1.3: The degradation of DDT in soils and in cultures of micro-organisms [Matsumura, 1975]

DDT	Dichlorodiphenyl trichloroethane	Dicofol	Dichlorodiphenyl trichloroethanol
DDCN	Dichlorodiphenyl acetonitrile	DDD	Dichlorodiphenyl dichloroethane
DDE	Dichlorodiphenyl dichloroethylene	DDMU	Dichlorodiphenyl chloroethylene
DDMS	Dichlorodiphenyl chloroethane	DDNU	Dichlorodiphenyl ethylene
DDNS	Dichlorodiphenyl ethane	DDOH	Dichlorodiphenyl ethanol
DDA	Dichlorodiphenyl acetic acid	DDM	Dichlorodiphenyl methane
DBH	Dichlorobenzohydrol	DBP	Dichlorobenzophenone
PCPA	p-Chlorophenyl acetic acid	PCBA	p-Chlorobenzoic acid

1.5 Motivating factors and problem statements.

Agriculture is the backbone of Kenya's economy. The potential areas for agricultural production are about one-fifth of the total land mass and this holds about 58.3 % of Kenya's human population and slightly less than 50 % of the livestock population. Urban areas carry 16.7 % of the human population and largely depend on the potential agricultural areas for food supply. The arid and semi-arid lands constitute about four-fifths of the country's total land surface and carry about 25 % of the total human population and slightly more than half of the livestock population [Ministry of Economic Planning and National Development, 1989-1993]. Thus, Kenya needs to intensify food production per acreage to meet the growing food demand.

DDT has been largely used in WHO programs for malaria control and was considered to be responsible for saving the lives of 1 billion people world wide. Its popularity stemmed from high insecticidal activity, low acute mammalian toxicity, wide spectrum of activity, simple manufacture and handling and hence cheap and long duration of activity. According to the Pesticides Control Product Board of Kenya, (PCPB), the body which regulates the importation, exportation, manufacture, distribution and use of products for the control of pests, DDT use in agriculture has been banned. However, it was retained for use in the public health sector for the control of malarial vectors. Unofficial reports tell of the use of DDT for agricultural purposes. Previously, DDT was used as one of

the main pesticides in combating tse-tse flies, cotton pests, the maize stalk borer and horticultural pests, among other agricultural pests.

By generating an appropriate data bank pertaining to the behavior of DDT in the Kenyan tropical soils, possibilities can be explored of either allowing restricted or full use of DDT in the Kenyan environment.

Although the types of pesticides that will be used in the future years will change, reliance will continue to be placed on chemical pest control because the technology is established and there is an ever-expanding world market. Constela (1988) reported that the demand for pesticides as of 1987 in Latin America exceeded US $ 2000 million in value. Up to 1990, the consumption was expected to rise by 7 to 12 %. Nevertheless, pests are responsible for the loss of 25 to 40 % of the potential harvests.

In contrast to certain restrictions on the use of some chlorinated hydrocarbons (mainly insecticides) in the industrialized countries, production and application in developing countries have increased. Recent studies made by the World Health Organization [Smith et al, 1984] and the Economic and Social Commission of Asia and the Pacific [Staring, 1985] confirm this fact and show the need for the extended use of insecticides such as DDT, dieldrin, lindane, aldrin and endosulfan. Reluctance to abandon DDT as a means of pest control is evident in studies by Vollner et al [1988], which were aimed at increasing the residual activity of DDT and other chlorinated insecticides widely

used in tse-tse fly eradication campaigns.

The main users of these chemicals are tropical countries where mosquitoes, ticks, termites, tse-tse flies etc. cause tremendous problems. On the other hand, excessive use of such chemicals may create severe hazards to the environment.

1.6 Degradation and mobility studies.

The dissipation of pesticides in the soil involves both degradation and transfer processes. Degradation may be chemical, photochemical or biological. Transfer processes include absorption, exudation and retention of unaltered pesticide by plants or other organisms; retention and release by soil particulate matter, movement of pesticide vapours from the soil to the atmosphere; movement of pesticides downwards through the soil in percolating water and via run-off into the surface waters [Weber, 1988].

1.7 Factors affecting the behavior of pesticides in the soil.

The behavior of a given pesticide is regulated by its chemical, physical and biological properties. This is influenced by the properties of the soil and the climatic conditions. Soils with a higher content of organic matter tend to hold pesticides longer than mineral (sandy or clay) soils [Lichtenstein and Schulz, 1959]. This is because soils high in organic matter tend to assume a colloidal form which causes the residues to be partitioned onto the

soil organic matter. In a muck soil, which is about 50 % organic matter, insecticide residues are partitioned onto the soil organic matter to such an extent that they are less effective against insects than in a sandy soil [Lichtenstein et al, 1960]. The same author reported in 1959 that crops absorb most readily from sandy soils and least readily from muck soils.

The higher the soil moisture content, the higher the vapourization and degradation. Lichtenstein (1964) reported that water seemed to cause aldrin to be displaced from soil particles, and much of the insecticide then vapourized and escaped from the soil. Parathion is decomposed and disappears through reaction with water (hydrolysis) or by being converted to its amino form by micro-organisms, whose action is enhanced by water.

Available data by Lichtenstein and Schulz [1959], suggests that rising soil temperature increases the rate at which pesticides in the soil vapourize and escape as well as the rate at which they decompose. These data was obtained by keeping soils containing pesticides at three different temperatures in the laboratory. The volatilization rate also increases with increasing wind speed because air movement and turbulence decrease the thickness of the stagnant air layer immediately at the surface of the soil [Hartely, 1969]. Vapourization from soil is also controlled by pesticide solubility and adsorption as well as by vapour pressure and environmental climatic conditions [Farmer et al, 1972, Spencer et al,

1975].

Cover crops reduce the rate of vapourization at the soil-air interface [Lichtenstein et al, 1962]. In one instance, two to three times more residue was recovered from the plots covered with alfalfa than from plots on which no crop was growing. Cultivation of soil increases the rate at which pesticides disappear. The amount of applied DDT lost from a field plot in four months was measured in a study in which part of the plot was cultivated regularly and part was left uncultivated. The amounts remaining were 55.9 % and 74.2 % respectively, [Lichtenstein, 1964].

The population and activity of micro-organisms which attack the various pesticides also affect the persistence of these pesticides, e.g., aldrin is oxidized to dieldrin by microbiological action. Aldrin persists longer in dry soil, in which micro-organisms are relatively inactive. It also persists longer in sandy soil which contain few micro-organisms, or in soil in which the population of the micro-organisms has been reduced by autoclaving (heating) [Lichtenstein et al, 1960].

1.8 Occurrence, behavior and effect of DDT residues in the environment.

DDT and other organochlorine residues have aroused great concern especially in developed countries. This has led to their banning. The major reason for this ban was the tendency of DDT and other organochlorines to persist in the

environment, distribute themselves and hence bio-accumulate in food chains.

In a study carried out by Cooke et al [1982] in Bristol, England, the amount of DDT and its breakdown products were investigated for a number of years (1972-79) after the use of the insecticide in the orchard had ceased in 1969. The time for 50 % decrease in the concentration was found to be 11.7 years for DDT and the breakdown of DDT to DDE was a significant feature of the persistence of DDT. It was concluded that there were no losses by volatilization. The long half-life can be explained by the fact that the temperatures were lower in this temperate region and hence little or no volatilization, photodecomposition or microbial degradation took place. The crop cover provided by the trees in the orchard inhibited loss of DDT by volatilization and processes of photodecomposition and microbial degradation. The losses were significantly slowed down due to the little sunlight reaching the soil.

The chemical structure of DDT is such that it is extremely resistant to degradation by light and oxidation in the environment, and hence it persists for long periods of time. Bacterial decomposition and chemical degradation take place very slowly [Plimmer et al, 1968]. Many micro-organisms convert DDT to DDD under anaerobic but not aerobic conditions [Ecobichon et al, 1967]. The prevailing conditions in the field were aerobic, thus little conversion occurred. Ecobichon et al (1967) also reported

that chemical reduction by reduced coenzymes, metalloporphyrins and metalloproteins could reduce aliphatic C-Cl bonds of DDT. Under the influence of UV light (e.g., from the sun), DDT is converted to DDE [Zepp et al, 1977, Wandiga and Natwaluma, 1984].

Volatilization and subsequent aerial transport is believed to be a major pathway of pesticide disappearance from application sites. In a study done in Ibadan, Nigeria on the fate of DDT applied to a crop of cowpea over a 4 year period by Perfect [1980], major loss of the applied DDT was attributed to volatilization. No literature is available documenting the amounts lost due to the process of volatilization. Aerial transport is the principal method of pesticide dispersion over wide areas and into bodies of water far removed from their sites of manufacture, use or disposal. Primary sources of pesticides are drift and evaporation during application and post-application volatilization from plant and soil surfaces. They may also evaporate from pesticide manufacturing sites and factories which use pesticides as their intermediates, e.g., in the manufacture of mothballs [Nicholson, 1967].

1.9 DDT in the aquatic environment.

DDT is a hydrophobic compound with low water solubility. It tends to adsorb strongly on particulate materials and can thus be transported during seasons of heavy rainfall and in rivers and streams. Its solubility in lipids is much higher than in water. This property leads to

the accumulation of DDT in various compartments in fish tissue. Shimizu et al (1978) reported results of a study in which fish, carp(Cyprinus caripio), were administered with ^{14}C-labelled DDT and parathion by bath exposure, topical application and oral injections. The gall bladder concentrated the insecticides through these treatments. Levels as low as 1 ng per litre of DDT have been shown to affect the hatching of coho salmon eggs, and DDT levels of up to 1 ppb are lethal to brown trout (Salmo trutta). Accumulation of DDT in fish is a potential source of contamination to man, birds and other fish eating animals due to its magnification through the following food chains.

DDT---> lake---> plankton---> fish---> bird (grebe)

DDT---> lake---> plankton--->small fish---> lake trout--> animal (or man)

In 1972, Koeman et al reported low concentrations of DDT, DDE, and dieldrin in the tissues of fish collected from Lake Nakuru. Greichus et al [1978], found slightly higher levels of DDE, DDD and dieldrin in the lake when he did his study later. Munga [1985], investigated the concentration of DDT, DDE, DDD and endosulfan in fish in the Tana river basin, Kenya. The pesticides DDT and endosulfan were being used for the control of cotton and maize pests. The four species studied Clarias mossambicus (Peters), Labeogregorii (Boulenger), Oreochromis mossambicus (Trewaves), and Tilapia zilli (Gervais) were

found to have the pesticide residues. Of the four species studied, C. mossambicus, a bottom feeding species had the highest concentration of the residues in muscle tissue. Lincer et al [1981], also found higher residues of DDE in the fish Labeo cylindricus (bottom feeding), than in Tilapia zilli (surface feeding) fish from Lake Baringo, Kenya.

1.10 DDT in birds.

Residues of DDT and other organochlorine insecticides have been found in birds. In a study done in Lucknow, India by Khaphalia et al (1981), DDT and other organochlorine residues were found in the internal body organs, depot fat and blood plasma of Indian wild birds. In another study done in Scotland, the levels of organochlorine residues in birds were found to differ according to their diets and environment.

In general, for the environment, the order was

Aquatic birds (flesh eating)> Terrestrial (flesh and plant eating)

With the diets, the order was as follows

Fish (Aquatic birds)> Flesh (Terrestrial birds)> Omnivorous (Terrestrial birds)> Plants (Terrestrial birds)> Plants (Aquatic birds)

The residues most commonly found were p,p'-DDE, p,p'-DDD, p,p'-DDT, dieldrin and BHC isomers. Residue concentrations in the body varied as follows

Fat tissue concentration> Breast muscle concentration

This was reported by Moore et al in 1964. DDT residues are thought to cause thinning of the eggshells of some birds and hence the reduction in the population of these birds [Cooke et al, 1976]. Bitman et al [1970], confirmed this in a study in which sparrow hawks, Japanese quail and mallards were fed with DDT. The content of calcium in the eggshell declined and reproduction was impaired. Residues stored in body fat may be a serious hazard to birds when their food reserves are mobilized during periods of starvation [Moore and Walker, 1964].

Various workers have reported on DDT in birds found in Kenya. In 1972, Koeman et al reported low concentrations of DDT, DDE, and dieldrin in tissues of birds collected from Lake Nakuru. Lincer et al (1981), studied the variation of DDE residue level between two bird species, the African cormorants (Phalacrocorax africanus), and the single white necked cormorant (Phalacrocorax carbo), collected in 1970 from Lake Nakuru. He found that the former had 15 times as much as the latter species. This suggested a difference in the diets of the two bird species. In 1981, the same authors found levels of DDE in the white pelican had doubled since 1970, indicating an increase in organochlorine contamination of the lake system.

1.11 DDT in animals.

Milk from dairy animals has been found to contain DDT residues and this has been attributed to intake of DDT through contaminated feed and to a lesser extent by skin

absorption following dermal treatment [Downey, 1972, Kalra et al, 1986]. In Kenya, Maitho [1978], found low levels of p,p'-DDT, p,p'-DDE, lindane, aldrin, and dieldrin in the fat and milk of cattle. However, high levels of DDT and dieldrin in eggs from Embu district were detected in a study by Kahunyo [1983]. Alsopp [1978], found low levels of Hexachloro-epoxy-octahydro-dimethanonaphthoxirene-HEOD(the active ingredient of dieldrin) in game animals after aerial spraying of dieldrin for tse-tse fly control in the Rift Valley. The rate of transfer of the pesticide to milk is influenced by the dosage level, the kind of feed consumed, the milk yield, the stage of lactation and the species of the animal. Different routes of exposure result in the excretion of DDT in different forms in milk [Witt et al, 1966].

DDT, β-HCH, dieldrin, and heptachlor epoxide have been detected in the adipose tissue of people in Kenya with DDT being the main contaminant [Wasserman et al, 1972]. Pesticide residues have also been detected in human milk. In a study by Wandiga and Mutere [1988] human milk procured from mothers attending clinic at a Nairobi hospital showed levels of $9*10^{-6}$ to 1.0 ppm of gamma-BHC. In a similar study done in Australia by Newton et al [1972], human milk samples contained DDT, DDE and HCB in concentrations ranging from 0.063 ppm to 0.139 ppm. Kanja (1988), investigated the presence of organochlorine pesticides in the milk of mothers collected from eight different areas of Kenya. Thirteen organochlorine pesticide residues were

detected in the following order of frequency: p,p'-DDT (100 %), p,p'-DDE (100 %), HCB (60 %), aldrin (35 %), lindane (30 %), β-HCH (27 %), dieldrin (20 %), α-HCH (8 %), transnonachlor (6%), heptachlor (4 %), endrin (4 %), heptaclor epoxide (0.4 %), and oxychlordane (0.4 %). Differences were observed in the level of these compounds from one region to another, e.g., the mean level of total DDT ranged from 1.69 mg/kg in milk fat of nomads from Loitokitok to 18.73 mg/kg milk fat in human milk from Rusinga Island. The mean ratio of p,p'-DDT to p,p'-DDE also varied with the area, e.g., 0.7 in Karatina to 4.4 in Turkana. The highest mean level of α-HCH, 10.3 µg/kg, was found in milk samples from Embu with corresponding high levels, 11.1 µg/kg and 22.1 µg/kg of β-HCH and γ-HCH (Lindane), respectively. The main source of DDT and other organochlorine pesticides in humans is through oral intake of foods, e.g., vegetables, beef and dairy milk containing these residues [Matsumura, 1972, Kanja, 1988]. Inhalation of pesticide vapours and consumption of contaminated water are other sources, especially in agricultural areas. However, not much is known of the dangers of low level contamination in humans. Chronic toxicity may occur on ingestion of small quantities of DDT over a long period of time. This may affect the immature developing systems of fetus and infants. DDT and other organochlorine pesticides have been shown to affect the reproduction of various test animals. However the effect varies with the different test animal, making extrapolation to humans difficult. In a

study done on the effect of DDT on humans, mild poisoning was produced in one volunteer who ingested 750 mg of DDT in oil [WHO, 1979].

1.12 Effect of DDT on the soil fauna and flora and its persistence in the soil.

Organochlorines do not affect microbial populations if applied at the recommended rates. Reduced nitrification, reduced ammonification, increased CO_2 evolution and reduced root nodulation of legumes occur if the recommended rates are exceeded [Brooks, 1972].

DDT has been found to upset the pest-natural enemy equilibrium. Soon after introduction of DDT into apple insect control in Japan, particularly for fruitmoths and leafrollers, abnormal increases of the wooly aphid, Eniosoma lanigerum Hausmann occurred. Its imported parasite, Aphelinus mali Haldeman, was badly affected. An abnormal increase of the apple leaf miner, Lithocolletis ringoniella, gradually became apparent because of the destruction of Hymenopterous parasite, Copidosoma sp., in orchards where DDT was employed. The introduction of DDT and parathion for apple insect control induced a buildup of red spider populations to the extent that acaricides were required [Hidetsugu, 1972].

Earthworms, which make a larger contribution to soil fertility were shown to be tolerant to most insecticides except chlordane, heptachlor, phorate and carbaryl. However, particularly in orchards where fruit trees were

sprayed with DDT, earthworms were shown to contain more than ten times the concentrations of organochlorines that are found in the surrounding soil [Edwards, 1969]. As a lower link in the food chain, they may be an important source of contamination for birds and higher animals. The flow chart below shows the process of uptake of DDT through the earthworm to birds.

DDT---> leaf (litter)---> earthworm---> bird (robin)

DDT is not known for its systemic properties (i.e., absorption through the roots into the plant). However, in a study done in Bombay, India, vegetables procured from different markets were found to contain residues of DDT among other organochlorine insecticides. The presence of the residues was attributed to injudicious and liberal use of the pesticides by farmers and or/storers [Khandekar et al, 1982].

The loss of pesticides through processes involving volatilization, photodecomposition and microbial degradation is expected to be more rapid under tropical and subtropical conditions. In a field test designed to investigate the rate of disappearance of DDT from soil near Lake Nakuru, Sleischer and Hopcraft [1984], found that the time for 50 % disappearance of the DDT was 110 days. In a similar study done by Wandiga and Natwaluma [1984] in Nairobi, surface deposited DDT had a half-life of 117 $\pm$ 10 days while soil incorporated DDT took 118 $\pm$ 13 days. In general, radioisotopes have been of great value in studying

pesticide dissipation and pesticide research. The extractability and formation of bound ^{14}C-labelled residues in an agricultural loam soil were investigated with "non-persistent" insecticides ^{14}C-methyl-parathion and ^{14}C-fonofos and with "persistent" insecticides ^{14}C-dieldrin and ^{14}C-p,p'-DDT [Lichtenstein, 1977]. These studies showed that the "persistent" insecticides had relatively lower binding rates and higher extractability from the soil than the "non-persistent" ones.

The persistence of ^{14}C-labelled p,p'-DDT and lindane in Nairobi and Mombasa was investigated over a three month period by Mghenyi in 1988. He found that the time for 50 % dissipation of DDT in soil was 98 $\pm$ 17 days for Nairobi and 88 $\pm$ 5 days for Mombasa. Mombasa is in the coastal region of Kenya and is generally hotter and more humid than Nairobi. Nairobi is at a higher altitude. The author did not look at the metabolic profile of DDT in this two regions. One of the major objectives of the present study is to find the metabolic profile of DDT in the coastal region. The rate of dissipation of DDT in Mombasa will also be looked at using nuclear techniques. Lalah et al [1994a], found that ^{14}C-DDT had a half-life of 64.6 days under field conditions in Nairobi. He also found that DDE, a principal metabolite of DDT in the environment, had an over-all half-life of 145 days under-field conditions in Nairobi [Lalah et al, 1994b].

CHAPTER TWO.

EXPERIMENTAL SECTION.

2.1 INTRODUCTION.

2.1.1 Tracer methodology.

The tracer method is a technique used to investigate certain characteristics of a population of specific objects such as molecules, organisms or other entities by observing the behavior of the tracer [IAEA, 1991]. The substance to be traced is generally termed the tracer. The criteria for an ideal tracer are that it must be chemically and physically indistinguishable from the tracee at the tracer concentration level used, and that the introduction of the tracer must not disturb the system. Both these criteria are almost perfectly met by radioactive or stable isotopic tracers. Isotopes have similar chemical properties, and they can be obtained in high specific activity or tracer abundance. Therefore, the introduction of an isotope generally adds negligible mass to the system and does not disturb its kinetics.

In principle, a stable isotope can be used as a tracer just as well as a radioisotope. In practice, however, a few radioactive atoms are detectable, whereas many more atoms of a stable isotope constitute the smallest detectable amount. Conversely, the stable isotope is better than the radioisotope in two respects, namely (a) there is no radiation hazard and (b) the life of the tracer is infinite. Generally, these advantages do not outweigh the

sensitivity of radiotracer detection.

Isotopic tracers are most commonly used to follow the pathway of an entity in a chemical, physical or biological system [IAEA, 1991].

When the entity is, for example, an intact organism or an inorganic object, the radioactive label used may belong to any element. The choice of label will then be governed by, (a) the ease of incorporation and attachment of the label and its stability of attachment, (b) the ease of detection of its radiation, and (c) the half-life (in order to be able to follow the label over the desired period of experimentation, yet not allow unduly long-termed contamination of the environment after the experiment is finished).

When the entity is, for example, an organic material or compound, the radioactive label used may belong to one of the elements in the tracee. This often reduces the choice to ^{14}C, ^{3}H or ^{15}N, ^{32}P, ^{35}S, ^{36}Cl or ^{131}I. The label may be incorporated into the tracee through biological growth, chemical synthesis or exchange processes.

If the tracee is a mineral nutrient, the label should be an isotope of that element. In general, elements in the same chemical group e.g. alkali metals, have similar chemical properties, but not sufficiently so for an isotope of one element to serve in general as the tracer for another element in the same group.

If the tracer is introduced into the system, its identification in other parts of the system infers

electrical energy for counting by the spectrometer.

The bulk solvent must efficiently transfer energy to a scintillator molecule and be capable of dissolving the scintillator and the sample material. Aromatic solvents such as toluene or xylene are favoured because of their efficiency in energy transfer which is facilitated by the pi-electrons on their rings. When large amounts of water are involved, 1,4-dioxane is used; naphthalene is often added to improve the energy transfer process and reduce quenching. Sometimes, incorporation of aqueous sample solutions into a toluene based system is possible by adding a non-ionic surfactant such as Triton X-100. Glycol ethers and alcohols are also used as secondary solvents to improve water miscibility, and to allow counting at low temperatures.

The scintillator must be capable of absorbing light at one wavelength and reemitting it at a longer wavelength. Most scintillator spectrometers are sensitive to the fluorescent emission of a primary scintillator. However, if an older model is being used, it may be necessary to add a secondary scintillator. The latter absorbs the light emitted by the primary scintillator and emits it at a yet longer wavelength. When used, it is added to the extent of one-tenth or less of the primary scintillator. The most popular primary scintillator is 2,5-diphenyloxazole (PPO). The most widely used secondary scintillators are 2,2'-p-phenylene-bis(5-phenyloxazole), (POPOP) and 2,2'-p-phenylene-bis(4-methyl-5-phenyloxazole), (dimethyl-POPOP).

For the primary scintillator the fluorescence emission maximum lies in the range from 360 to 365 nm, whereas that for POPOP lies around 410-420nm [Willard et al, 1986]. As shown in Fig.2.1, the POPOP absorption spectrum overlaps well with the PPO emission spectrum, and since POPOP has a very large molar absorptivity in the overlap region, only a very small amount of POPOP is necessary for nearly complete absorption of the PPO fluorescence. Also the POPOP fluorescence closely matches the response of the blue-sensitive photomultiplier tubes.

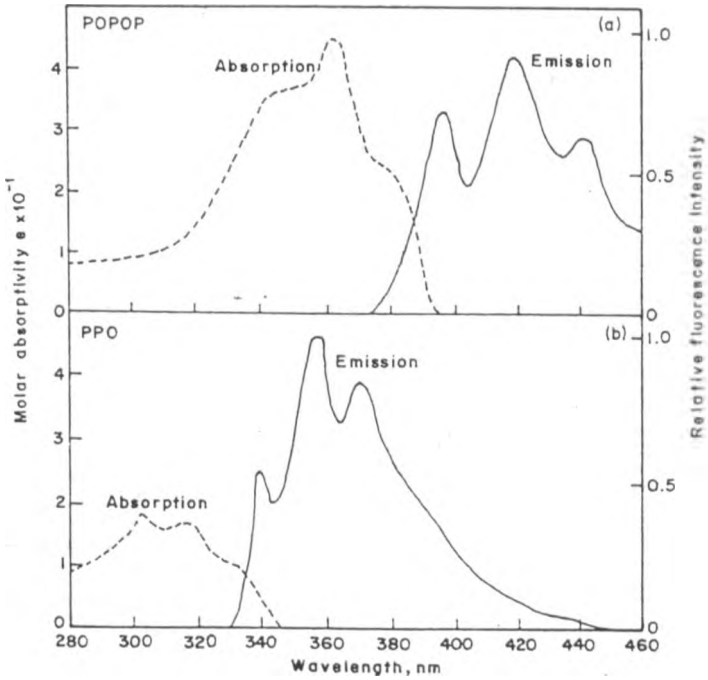

Fig. 2.1: (a) Absorption and fluorescence emission spectra of (a) POPOP and (b) PPO [Willard et al, 1986].

Liquid scintillation procedures can be extended to various samples. If radioactive carbon-dioxide is being

measured, the scintillator solution should contain a trapping agent, e.g., 1-amino-2-phenylethane (phenethylamine), or a base such as NaOH. Quartenary ammonium hydroxides in methanolic solution act as tissue solubilizers. For materials that cannot be solubilized, a suitable gelling agent is used to prevent settling; this technique is termed suspension counting. Finely divided amorphous silica is widely used as a gelling agent.

Figure 2.2 shows a block diagram of a liquid scintillation spectroscopy system. Usually, two

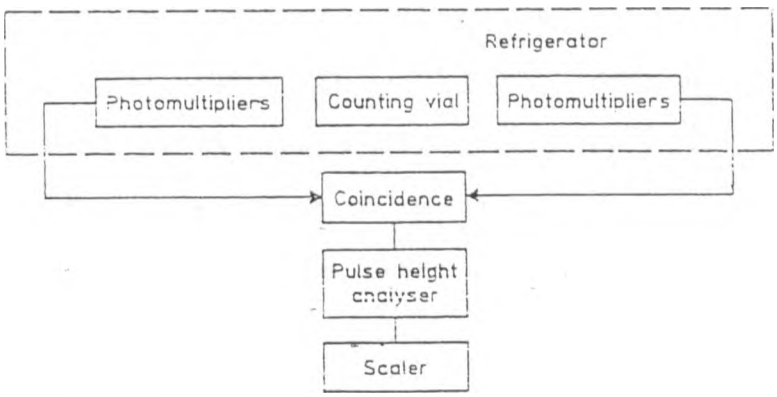

Fig. 2.2: Block diagram of a typical liquid scintillation counter [IAEA, 1991].

photomultiplier tubes are used to collect the light emitted from the scintillation vial. This is done to increase the ratio of sample counts to electronic noise. After each ionizing event, light photons will normally be registered at both photocathodes simultaneously. The coincidence circuit is designed to produce an output if it receives an

input pulse from each of the two photomultipliers simultaneously (within about 1 μsec), i.e. in coincidence. Electronic noise pulses in one photomultiplier tube will seldom be in coincidence with those in the other and will therefore be rejected. Thus the ratio of true count rate to electronic noise rate is increased, and thereby the sensitivity is increased [IAEA, 1991].

The size of the output pulse is proportional to the energy lost in the liquid scintillator and therefore pulse height analysis is possible and radiation spectroscopy can be carried out. Fig.2.2 shows a block diagram of a liquid scintillation spectroscopy system.

Chemical impurities and other compounds present in the solution to be measured may interfere with the transfer of energy from solvent to solute to produce chemical quenching or they may absorb the light emitted
from the solute molecules to produce colour quenching. Chemiluminescence will give yet a third change in the spectrum. Since quenching commonly occurs, it must always be considered.

The three most important methods of correction are listed below:

(1) Removal of coloured material. The solution may be filtered through activated charcoal or treated by an ion exchange to remove the quenching agent. Digestion of the solution with hydrogen peroxide or perchloric acid also reduces or eliminates colour quenching.

(2) Channel-ratio method. In general, the net loss due to

quenching is greatest for the most intense light flashes produced by the highest energy particles. Therefore, when quenching occurs, the output pulse spectrum is shifted towards lower energy. If, by discriminator settings, the ratio of a lower energy part (channel) to a higher energy part (channel) can be obtained, then it is possible to observe the relation between counting efficiency, E, and channel ratio. This is done using a set of standards with a known constant amount of activity and measuring with increasing amounts of chemical quenching (i.e. increasing the channel ratio). A standard curve of E versus channel ratio can then be prepared and subsequently used to correct sample measurements for any decrease in count rate due to quenching. However, this curve will not account for quenching due to coloured material.

(3) External standard technique. In some instruments, a standard source may be moved into position. Thus, the relative decrease in the standard count for each sample counting will be proportional to the amount of quenching material and will give the quench correction to be used for that vial count.

2.2.2 Biological material oxidizer.

This equipment is used to prepare biological samples for liquid scintillation counting. It combusts any biological and most organic materials (wet or dry) in a stream of oxygen gas at 900^{0}C [IAEA, 1991]. All biological materials contain carbon and hydrogen in any number and

variety of forms. When burned at 900°C in an oxygen stream, the bulk of the material is converted to carbon-dioxide and water. The combustion products are then passed through a

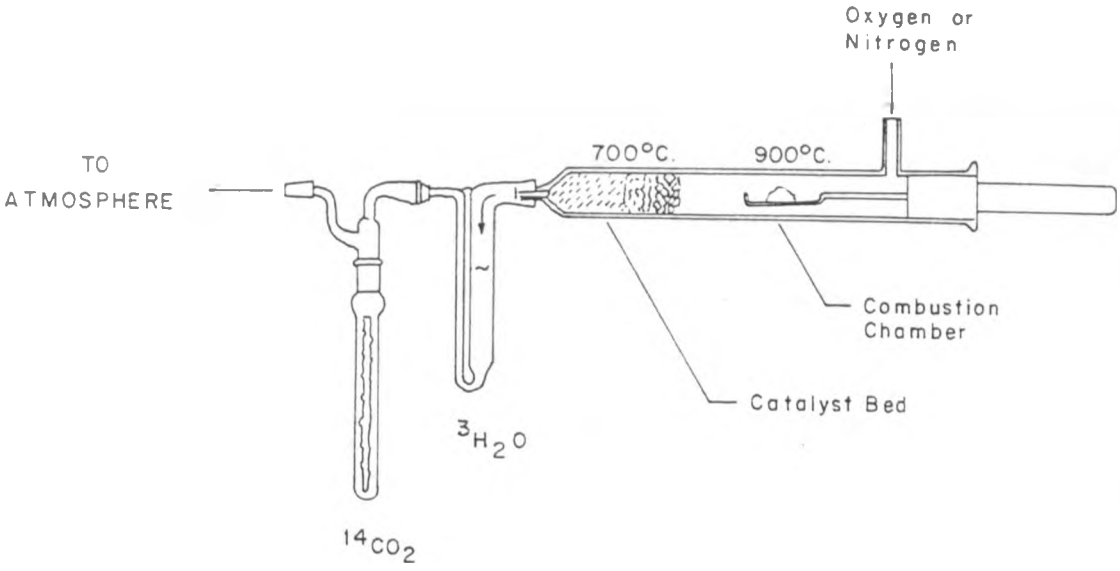

Fig. 2.3: Flow diagram of a biological material oxidizer.

series of catalysts at 680°C and the carbon-14 dioxide and /or tritiated water are trapped in external trap(s) which contain the trapping solution. The trapping solution is then taken for liquid scintillation counting.

2.2.3 Gas liquid chromatography.

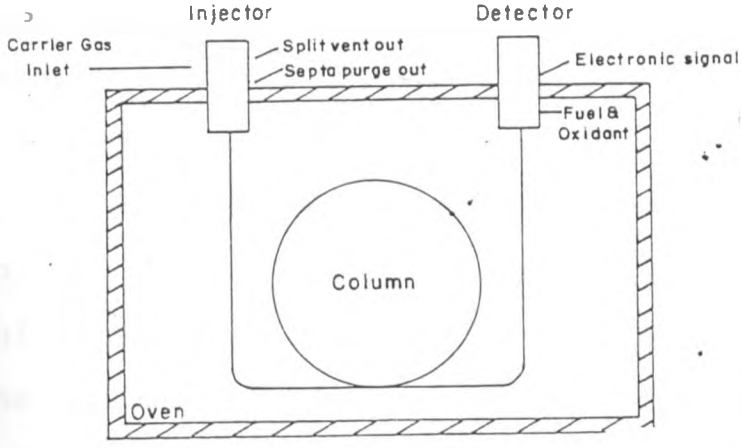

Fig. 2.4: Flow diagram of the GC system.

This is a very important technique in the determination of pesticide residues both qualitatively and quantitatively. A simple diagram of a GC system is shown on Fig. 2.4 above.

Gas-liquid chromatography is the process of separating the components of a mixture by making use of their partition coefficients between a gaseous moving phase and a liquid stationary phase [Pattison, 1973]. The stationary phase is deposited on an inert support material. In the chromatographic process a vapour A which is soluble in both phases would be extracted from the liquid stationary phase by the gaseous moving phase.

An inert gas such as nitrogen, argon, helium or hydrogen is used as the mobile phase and the stationary phase consists of a non-volatile organic liquid impregnated on an inert solid support material. This material is packed in a glass tube usually ca. 4 mm diameter and 1.8 m long. In addition, longer columns (as much as 50 m) of capillary tubing are used. (Capillary columns may be prepared by coating the inner walls with non-volatile organic liquids or by packing them with coated solids). The column is installed in an oven, whose temperature can be varied, and a stream of gas is passed through it at a constant rate.

A solution of the pesticides is injected into the hot column (via the injection port) at the end nearest the gas inlet. The pesticide(s) vapourize and are carried through the column by the gas at different rates which are influenced by differences in their partition coefficients

between the liquid stationary phase and the mobile gas phase. The separate chemicals emerge from the far end of the column at different times and pass through a detector which measures their concentration. The time taken between injection and detection is called the retention time. This time is characteristic for each pesticide under a specific set of operating conditions. Peak area is proportional to the amount of compound that has passed through the detector.

Detectors commonly used for GC include flame ionization (FID), thermal conductivity (TC), and electron capture (EC). FID responds only to organic samples and gives little response to H_2O and CS_2 . TC is universal and can also be used in the analysis of H_2O and inorganic samples. EC which is of interest in this work is selective towards molecules containing the electronegative atoms, N_2, O_2, S, and particularly halogens.

In the ECD, the effluent from the column is passed over a beta-emitter (a radioactive compound that emits electrons) such as Nickel-63 or tritium (adsorbed on platinum or titanium foil). An electron from the emitter causes ionization of the carrier gas and produces several electrons. In the absence of organic molecules that tend to capture electrons, a constant standing current between a pair of electrodes results from this ionization. However the current decreases in the presence of these organic molecules containing electron capturing groups such as the halogens, and this is recorded.

2.2.4 Thin Layer Chromatography Linear Analyzer.

The Linear Analysers' (TLC-LA) most important part is a position sensitive proportional counter (Fig. 2.5). The TLC plate is positioned as close as possible to the entrance window, the counter height being only 5 mm. Beta (ß) particles emitted from the chromatogram plate enter the counter from the bottom through a 250 mm long and 15 mm wide open entrance window. Therefore, the counter can detect very low energy beta particles, as e.g. ^{3}H and ^{125}I. The counting wire is stretched along the center line of the counter. As the detector operates as a proportional counter, the electron avalanche produced by gas amplification of the primary ionization is limited to locations above those points where the particles are emitted from the TLC plate.

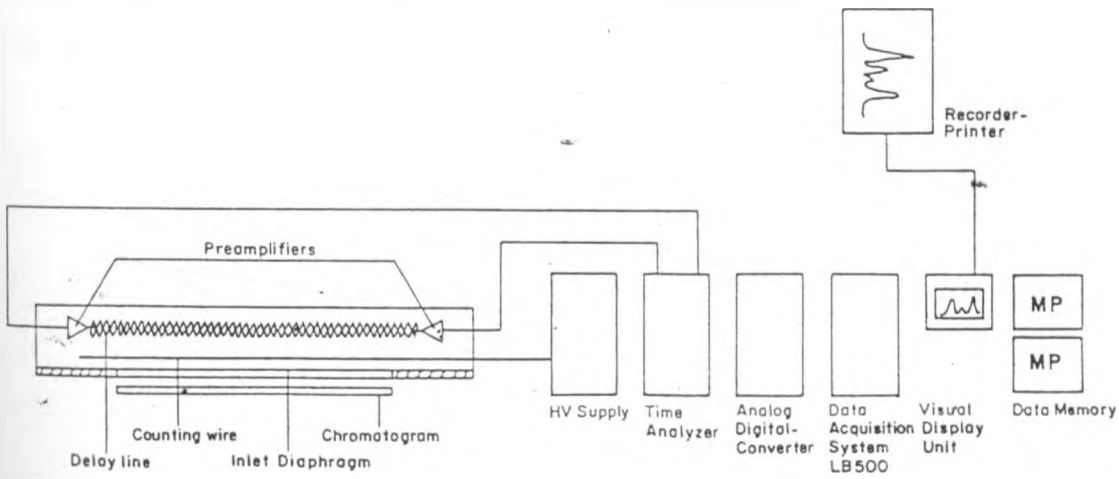

Fig. 2.5: Schematic diagram of a linear analyzer.

The count pulses, which are proportional to the electron avalanches and in time to the position of the incident particles are coupled electromagnetically into a delay line, which also runs over the full length of the counting chamber. These pulses pass along the delay line in both directions, and the arrival of the pulses at each end of the delay line is electronically recorded. The time difference between the arrival of two associated pulses is a direct measure of the location of an incident particle.

2.2.5 Mass Spectrometry.

Liquid samples are volatilized under vacuum in the heated reservoir (about 1 µg), and the vapour is leaked into the ionization chamber (Fig. 2.6). To facilitate volatilization, especially of samples with high boiling points, heating of the reservoir is necessary. Solid samples are introduced into the ionization chamber on the tip of an insertion probe.

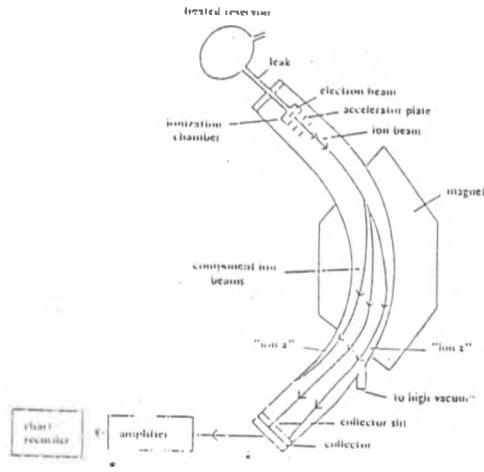

Fig. 2.6: Schematic diagram of a mass spectrometer [Creswell et al, 1972].

The sample is bombarded with a stream of electrons of 70 eV energy in the ion source. The energy absorbed by the molecules promotes ionization by loss of electrons from bonding and non-bonding orbitals. The ions formed by the removal of one electron from the original molecule are called molecular or parent ions. Some of the molecular ions fragment into smaller daughter ions and neutral fragments. Both positive and negative ions are formed, but only positively charged species are of concern. A small positive potential is used to repel the positive ions out of the ionization chamber. An acceleration plate with a positive potential of 2000 V is used to accelerate the positive ions down the tube into the magnetic field. The ions are deflected by the magnetic field depending on their mass/charge (m/e) ratio, e.g., ion $C_5H_{11}^+$ is deflected to a lesser extent than ion $C_3H_7^+$. The ion beam is thus split into component ion beams of different m/e ratio. Each ion beam in turn is made to pass through a collector slit and impinges on a collector plate. Each ion acquires an electron from the plate which utilizes the positive charge. A flow of current is produced in the collector circuit, amplified and recorded as a function of the m/e ratio. The size of each peak is a measure of the relative number of ions in each beam.

Combined gas chromatography/mass spectrometry (GC/MS), is a method of choice for analysis of organic mixtures. The ability to separate a complex mixture of organic compounds and identify the individual components, or to look for a

specific compound in a complex mixture are the two primary applications of GC/MS. The requirements for the use of GC/MS are that the mixture be amenable for analysis by gas chromatography, and that the compounds or their derivatives are volatile and not thermally labile.

2.3 MATERIALS AND METHODS.

2.3.1 Chemicals.

Uniformly labelled ^{14}C-p,p'-DDT, 1,1'-(2,2,2-trichloroethylidene)bis(4-chlorobenzene), specific activity 12 mCi/mmole was obtained from IAEA Vienna and non-labelled p,p'-DDT (97.2 % p,p'-DDT, and 2.8 % DDE on EC Gas analysis) from Greyhound Chromatography and Allied Chemicals, U.K. These were used in the preparation of the solution to be applied in the field. General purpose grade methanol, n-hexane, toluene and acetone were obtained from suppliers in Nairobi for use in Soxhlet extraction, liquid-liquid partitioning and analytical work. Crystalline 2,5-Diphenyloxazole (PPO) (Fisher Chemical, U.S.A.) and 2,2'-p-phenylbis(4-methyl-5-phenyloxazole) (Dimethyl POPOP) from Eastman Kodak Co. U.S.A. were used in liquid scintillation spectrometry.

Oxygen and white spot nitrogen (East-African Oxygen), were used as combustion and purging agents respectively, during bound residues determination. The Harvey C-14 cocktail, obtained from R.J. Harvey Instrument Co. U.S.A., was used as the trapping agent and scintillator in bound residues determination. Anhydrous Na_2SO_4 , analytical grade

acetone and toluene from J.T.Baker Inc. U.S.A. were used in other analytical work. Whatman 91 filter paper (Whatman International Ltd.), was used in Soxhlet extraction. Florisil for column chromatography 60-100 mesh from Machery Nagel Co., Germany was used during clean-up of samples. A chlorinated pesticide mixture (CPM) from Supelco Co. was used as the standard in GLC analysis.

2.3.2 Apparatus.

Hard PVC cylinders were used as the enclosed environment in the field. The chemical was applied by means of a pipette and a pipette filler. A mortar and pestle were used for soil sample homogenisation. General purpose grade solvents for extraction and other analysis were first distilled using a fractionating column. Soxhlet apparatus, a rotary evaporator and a separatory funnel were used for extraction, concentration and partitioning methanol and hexane extracts of the pesticide residues. A Gallenkamp oven was used for moisture content determination.

Liquid scintillation analysis was carried out using a Tricarb 1000 TR Liquid Scintillation Analyzer from Packard-Canberra Co. Bound residues samples for liquid scintillation analysis were combusted using a Biological Material Oxidizer OX-600 from R.J. Harvey Instrument Corp., U.S.A.

Glass microcolumns of internal diameter 1 cm and length 13 cm were used during cleanup. Other apparatus used were adjustable micropipette systems and glassware. Gas

chromatography was carried out using a Packard model 421 gas chromatograph equipped with a ^{63}Ni EC detector. Further investigations on the metabolic profile were done using a TLC Linear Analyzer LB 283 and a Combined Gas Chromatograph/Mass Spectrometer. The mass spectrometer was a Hewlett-Packard 5985B quadropole equipped with an RTE-VI data system. The system featured the Wiley and NBS mass spectral libraries which contain the mass spectra of 78,000 compounds.

2.4 METHODS.

2.4.1 Site of experimental plot.

The experimental plot was on a farm located in Mtwapa (altitude 21 m), Kilifi district of the Coast Province of Kenya. The analysis of the soil sample collected from the site gave the following properties pH 6.2; sand 90 %; silt 2 %; nitrogen 0.06 %; organic carbon 0.54 %; texture sandy.

2.4.2 Preparation of plot.

The plot was 5 m by 5 m and was dug and all weeds and stones removed. Thirty PVC cylinders (length:15 cm;diameter:10.4 cm) were driven into the soil and 2 cm left protruding above the soil surface to prevent runoff water. The pipes were spaced 40 cm apart with a margin of 40 cm left from the edge of the plot.

2.4.3 Preparation of solution.

About 0.19 g of non-radioactive p,p'-DDT was placed in a 250 ml volumetric flask. Then 420 µl of the toluene solution containing 6192.5 µg (210 µCi) of ^{14}C-p,p'-DDT, was added into the volumetric flask. Distilled n-hexane was added and made up to the mark and the solution thoroughly shaken.

2.4.4 Application of ^{14}C-p,p'-DDT in the field.

One week after the plot was prepared, the pipes were sunk and the pesticide applied. From the solution described in the above section, each pipe received 7 ml, i.e. 173.46 µg (5.88 µCi) of ^{14}C-p,p'-DDT and 5.26 mg of cold p,p'-DDT. Thus each pipe received 0.5437 mg (3.78 ppm) of p,p'-DDT amounting to 13,053,600 disintegrations per minute (dpm).

2.4.5 Sampling schedule.

Immediately after application, two tubes were sampled. This was followed by weekly sampling for the first month, biweekly for the second and third month, monthly for the fourth month and bimonthly for the rest of the nine month study period.

2.4.6 Sample treatment and analytical methods.

The samples were air dried in the laboratory for one day. The soil was ground with a mortar and pestle and then thoroughly mixed by shaking inside a plastic bag for about 20 min. Four 50 g replicates from each soil sample were

placed in thimbles made of pre-extracted 18.5 cm diameter filter papers. Pre-extraction with methanol of the filter papers was done to remove any substance that would interfere with the GC work. The soil samples were then extracted for 4 hours (5 cycles) using 150 ml distilled methanol.

2.4.7 Liquid scintillation counting.

The liquid scintillation cocktail was prepared by dissolving 4 g of PPO and 0.25 g of Dimethyl POPOP in 1.0 l of toluene. A 1 ml aliquot of the 150 ml pesticide containing methanol extracts was placed in a 20 ml scintillation vial and 10 ml of the scintillation cocktail added. The sample was now ready for counting. The remaining 149 ml was reduced to 10 ml using the rotary evaporator. A 1 ml aliquot was taken for liquid scintillation analysis. The remaining 9 ml of the extract was kept aside for liquid-liquid partitioning.

2.4.8 Bound residues determination.

The extracted soil samples were air-dried in the hood and then thoroughly mixed by shaking inside plastic bags for 20 min to ensure uniform distribution of the bound ^{14}C-p,p'-DDT. Replicates of between 1.0 - 1.5 g of soil were weighed and 25 - 50 mg of cellulose acetate or cellulose added. The sample was placed in the Biological Material Oxidizer OX-600 and combusted at 900°C in a stream of oxygen. The $^{14}CO_2$ was trapped in 7.5 ml of the Harvey

Carbon-14 cocktail which was then taken for counting.

2.4.9 Total ^{14}C-p,p'-DDT equivalents.

The total ^{14}C-p,p'-DDT equivalents were determined by combusting a known amount of unextracted soil sample and counting as described in Section 2.4.8. This was confirmed by adding the extractable and the bound ^{14}C-p,p'-DDT equivalents.

2.4.10 Liquid-liquid partitioning.

Ten ml of distilled water was added to the 9 ml methanol concentrate and mixed. This was then partitioned against three 15 ml portions of distilled n-hexane. The 45 ml hexane solution was then concentrated to 5 ml and dried with anhydrous Na_2SO_4 (one spatula). A 1 ml aliquot was counted. The percentage recovery of the ^{14}C-p,p'-DDT during partitioning was 73.91 $\pm$ 14.53 %.

2.4.11 Clean-up procedures.

Florisil 60-100 mesh was activated in the oven at 130^{o} C for at least 24 hrs prior to use. Two gms of the Florisil was then placed in the microcolumn and a small amount of anhydrous Na_2SO_4 added at the top of the column. The sample was then eluted with the following solvents.

10 ml	100 %	n-hexane
10 ml	95 %	n-hexane : 5 % acetone
10 ml	92 %	n-hexane : 8 % acetone
10 ml	90 %	n-hexane :10 % acetone

The recovery was approximately 70 %. Cleaning solvents were methanol and acetone. The eluting solvent was evaporated to dryness on a warm hot-plate and then 5 ml of distilled n-hexane was added. The samples were then stored at -4^0C for GC analysis.

2.4.12 Gas chromatographic analysis.

This was carried out using a Packard Model 428 Gas Chromatograph equipped with a ^{63}Ni EC detector kept at 250^0C. One µl of the hexane containing the pesticide was diluted 100-300 times depending on the sensitivity of the instrument at that time and the concentration of the solution. The carrier gas was N_2 flowing at 75 ml/min through a coiled glass column of length 2 m and diameter 1 mm. The stationary phase was GP 1.5 % SP™-2250/ 1.95 % SP 2401 coated on 100/120 mesh Supelcoport™. The column temperature was 210^0C.

The recorder was a Packard Model 621 and the chart speed was 10 mm/min. The various peaks were identified by comparing their retention times with those of the standards present in a chlorinated pesticide mixture Cat. No. 4-9150 from Supelco Co. The peaks were tall and narrow and quantitation was by peak height.

NB. TM - Trade-mark.

2.4.13 TLC Linear Analysis TLC-LA.

Quantities of the samples of radioactivity greater than 10,000 dpm were spotted on silica gel G UV_{254} TLC plates

and developed in a hexane:methanol (9:1) solvent system. The plates were then taken for analysis on the TLC-LA.

2.4.14 Gas Chromatography/Mass Spectrometry (GC/MS).

The sample solutions containing the pesticide residues, and the standards were taken for GC/MS. The chromatographic separation was done on a 15 m DB 1 fused silica capillary column with a 0.25 µm film. The injections were made in the splitless mode at an injector temperature of 300^{0}C. The temperature program used started at 160^{0}C, with a 2 minute hold, ramping to 280^{0}C at a rate of 10^{0}C/min. Helium carrier gas was flowing at a rate of 1 ml/min. The compounds of interest eluted in less than 10 minutes. They were dissolved in methanol before injection. The mass spectrometer was operated under standard electron ionization conditions and was scanned from 40 to 500 daltons.

2.4.15 Moisture content determination.

The moisture content of the air-dried soil was determined by weighing 5 gms of each sample into crucibles. These were kept over-night in an oven at 100-110^{0}C and then weighed again. The mass difference constituted the moisture content.

2.4.16 Preparation of calibration curves.

The unquenched standards were used for verification of instrument performance. The unquenched carbon-14 standard

was used to verify the carbon-14 counting region of interest for efficiency and reproducibility. The background standard was used to verify the instrument background which could be affected by radioactivity contamination, changes in environmental radiation, and electrical noise entering the counting channels. A series of quenched standards were used to establish a correlation between the counting efficiency in a region of interest and a quench indicating parameter (QIP). The QIP was the Spectral Index of Sample (SIS).

2.4.17 Weather data.

Weather data were obtained from the Kenya Meteorological Department, and were correlated with the results obtained. The data is shown in Figs. 3.17-3.19 (appendix).

CHAPTER THREE.

RESULTS AND DISCUSSION.

3.1 The Dissipation of ^{14}C-p,p'-DDT in Mtwapa, Kilifi District, Coast Province, Kenya.

3.1.1 Extractable, bound and total ^{14}C-p,p'-DDT equivalents in the soil.

The soil was extracted using methanol as described in Chapter Two, section 2.4.8. Total ^{14}C-p,p'-DDT equivalents were determined and confirmed as described in section 2.4.9. The results are shown in Table 3.1 below. The amount of the ^{14}C-p,p'-DDT applied on day zero, i.e. 3.78 ppm was taken as 100 %.

Table 3.1: Percentage ^{14}C-p,p'-DDT equivalents in soils.

Days after Treatment	Extractable µg/g	%	Bound µg/g	%	Total µg/g	%
0	3.66 ± 0.48	97.0	0.08 ± 0.01	2.0	3.74 ± 0.50	99.0
14	3.08 ± 0.55	81.4	0.16 ± 0.01	4.1	3.23 ± 0.56	85.5
21	3.03 ± 0.28	80.2	0.14 ± 0.07	3.7	3.17 ± 0.36	83.9
28	2.85 ± 0.26	75.4	0.17 ± 0.05	4.4	3.01 ± 0.31	79.8

Days after Treatment	Extractable µg/g	%	Bound µg/g	%	Total µg/g	%
42	2.70 ± 0.12	71.5	0.16 ± 0.02	4.2	2.86 ± 0.14	75.7
56	2.41 ± 0.30	63.9	0.15 ± 0.01	4.0	2.56 ± 0.31	67.9
84	2.43 ± 0.08	64.5	0.16 ± 0.02	4.1	2.59 ± 0.10	68.6
168	2.23 ± 0.06	59.1	0.22 ± 0.05	5.8	2.45 ± 0.11	65.0
245	2.05 ± 0.12	54.3	0.13 ± 0.03	3.5	2.18 ± 0.10	57.8
292	1.77 ± 0.16	46.8	0.21 ± 0.02	5.7	1.98 ± 0.18	52.0

The data given above are represented graphically by Fig. 3.1 (appendix). The total ^{14}C-p,p'-DDT equivalents, which is the sum of the extractable and bound ^{14}C-p,p'-DDT equivalents decreased to 52 % of the applied amount. The disappearance appears to be a combination of two major steps (Fig. 3.1): an initial rapid decrease, sometimes called the "dissipation" stage from day 0 to day 56; and the second slow decrease sometimes called the "persistence" stage from day 56 to day 292. This trend has been reported by various workers [Mghenyi, 1988; Pillai et al, 1988; Lalah et al, 1994a, 1994b]. Lalah et al [1994a] reported an

initial rapid dissipation phase from 0 to 37 days and a slower persistent phase from 51 to 172 days when working with ^{14}C-p,p'-DDT in Nairobi. The high initial rate of dissipation may have been favoured by the high rainfall recorded during the short rainy season (Fig. 3.17). The short rainy season starts from October and goes to December and part of January. However, this may not be the major route of dissipation as DDT is sparingly soluble in water. It can be a major factor if it adsorbs strongly to particulate materials and is carried along streams, etc. Again, this would be minimal since as in the present work, the DDT was contained in a pipe sunk into the ground and protruding 2 cm above soil surface to prevent run-off water. However, higher soil moisture content could have enhanced the rate of vapourization or co-distillation of the DDT with water vapour [Acree, 1963].

Table 3.2 below shows the percentage metabolic composition of the total residues as a percentage of the amount of p,p'-DDT applied initially. Kinetic analysis of the parent compound p,p'-DDT, (C), versus days after treatment, (t), assuming a first order process was done (see Appendix). Most workers have assumed first order processes for the total p,p'-DDT equivalents when discussing their data [Wandiga and Natwaluma, 1984,

Table 3.2: Percent metabolic composition of p,p'-DDT in Mtwapa soils.

Days after treatment	p,p'-DDE (%)	p,p'-DDT (%)
0	2.78	96.38
7	5.21	87.10
14	7.63	77.80
21	10.62	73.30
42	12.72	58.70
56	11.93	55.80
70	14.31	53.85
84	16.68	51.90
112	19.22	47.80
168	21.76	43.10
245	23.16	34.70
292	24.61	27.80

Mghenyi, 1988, Pillai et al, 1988, and Lalah et al, 1994a]. For purposes of comparison, the first order kinetic analysis results will be used to discuss the present work. The rate constants, (k), were obtained from the slope of the regression lines of natural logarithm of the ^{14}C-p,p'-DDT remaining, ln C vs days after treatment, t. The time

for 50 % loss t_{50} was calculated using the equation:

$$t_{50} = \ln 2/k \qquad \text{where } k = \text{rate constant}$$

The total duration of the experiment was divided into two periods of time; initial (0 to 56 days) and final (56 to 292 days). The respective rate constants and t_{50} values for these short periods along with the overall period were computed and are shown in Table 3.3 below.

Table 3.3: Partial and overall rate constants, k, halflifes, t_{50}, and correlation coefficients , r^2, for the dissipation of the total ^{14}C-p,p'-DDT equivalents in Mtwapa.

Period	Rate constants $k*10^3$ ($\pm$S.E.$*10^3$) (day^{-1})	t_{50} (days)	Regression equation	r^2
Initial	9.63 ($\pm$ 1.08)	72.0	$C=91.29e^{k.t}$	0.96
Final	2.89 ($\pm$ 0.31)	239.8	$C=68.03e^{k.t}$	0.97
Overall	3.52 ($\pm$ 0.39)	196.9	$C=77.48e^{k.t}$	0.92

S.E.= standard error of the regression of the slope

C= concentration or amount remaining, t= time

The zero order, first order, third order, and exponential regression analysis of the concentration of

p,p'-DDT were done and the results are presented in Figures 3.3, 3.4, 3.5, and 3.6 respectively (Appendix). Several processes take place during the dissipation of p,p'-DDT, for example, loss of p,p'-DDT by volatilisation and conversion of p,p'-DDT to p,p'-DDE. From Table 3.2, it is observed that the concentration of p,p'-DDE increases with time.

Relative to results obtained in temperate regions of the world, the t_{50} value given above (196.9 days) is short. In a study done in Bristol, England by Cooke et al [1982], a half life of 11.7 years (4270.5 days) was obtained for the dissipation of DDT in an apple orchard. The authors concluded that there were no losses by volatilization. This was favoured by several factors such as, the low temperatures prevailing in this region of the world which would favour low rates of vapourization [Lichtenstein and Schulz, 1959, El Zorgani, 1976, Wandiga and Natwaluma, 1984], and the crop cover given by the fruit trees which prevented the sunshine from reaching the soil [Lichstenstein et al, 1962].

Volatilization is a major route of dissipation of DDT in the subtropics and tropics [El Zorgani, 1976, Wandiga and Natwaluma, 1984] and was a major factor contributing to the loss of DDT in this study. The sunlight hours consisted of a daily average of 8.9 hours, with the soil temperatures (Fig. 3.10) averaging above $30^{0}C$ each day. Spencer and Cliath [1975], reported that the vapour pressure of DDT rises with increasing temperature. They found that at $20^{0}C$,

the vapour pressure of DDT was $1.52*10^{-7}$mm Hg and it rose to $33.2*10^{-7}$mm Hg at 40^{0}C. Lichtenstein and Schulz [1959], suggested that raising the soil temperature increased the rate at which pesticides in the soil vapourized and escaped. In the present study, there was no crop cover on the site of the experiment which would have favoured a longer t_{50}. Other workers in temperate regions have reported half-lives ranging from 2 to 16 years (730 to 5840 days) [Edwards, 1966].

Shorter periods for 50 % disappearance of DDT have been recorded for the tropical and subtropical climates. In a study conducted in Delhi, India in a sandy loam soil under field conditions, Pillai et al [1988], reported an overall time for 50 % dissipation of DDT as 90 days in the monsoon, 120 days in winter and 60 days in summer. The mean soil temperatures during the first 60 days of post-treatment in the monsoon, winter and summer treatments were 34 ($\pm$2), 11($\pm$3) and 36($\pm$2)^{0}C, respectively. The soil moisture content averaged about 6.7 % in monsoon, 8.0 % in winter and 8.2 % during summer. This is much higher than what was recorded in the present study (0.60 $\pm$ 0.09 %). The lower the moisture content of the soil, the longer the time for dissipation. Lichtenstein reported in 1964, that the higher the moisture content of the soil, the higher the rate of vapourization of the pesticide. This is because the water displaces the pesticide from the soil particles, causing much of it to vapourize.

In 1984, Wandiga and Natwaluma reported a half-life of

117±10 days for DDT which was surface deposited and 118±13 days for DDT which had been incorporated into the soil. This study was carried out in Nairobi which is at a higher altitude (1661 m above sea level) than Mombasa, Coast Province (0 m sea level), and experiences subtropical to tropical climate. Its soils are clay in texture and contain a higher amount of organic matter than in the coastal region. A similar field study was carried out by Sleischer and Hopcraft (1984) in Lake Nakuru. They found the time for 50 % dissipation of DDT to be 110 days. In their studies, Wandiga et al [1984] and Sleischer et al [1984] applied the pesticide by spraying over a plot of known area. The former workers sprayed a water emulsion of technical DDT solution. Thus, in the event of a subsurface flow of water, since DDT was not in a contained environment (e.g. as would be if it were applied into a hollow cylinder sunk into the ground), there would be a higher rate of loss of the DDT and this would favour a short time for 50 % disappearance by leaching. However, this route is not be pronounced due to the low solubility of DDT in water ($1.0*10^{-5}g/100cm^3$). Cooke and Stringer [1982], detected DDT residues from an orchard to a depth of 10 cm of the undisturbed soil profile. Mghenyi [1988], detected residues in the top 6.5 cm. Subsurface flow of water may increase the moisture content of the soil and thus increase the vapourization and the co-distillation rate of the DDT.

Mghenyi [1988], investigated the behavior of DDT in Mombasa and Nairobi for a 3 month period. The half-life was

88 (±5) days for Mombasa, Coast Province assuming a 1st order process. This is shorter than that reported in the present study (196.9 days). The location of his field experiment was 50 m from the sea-shore on the south coast of Kenya. In this case, the breeze from the sea would favour a higher rate of vapourization, and hence a shorter half-life. Hartely [1969], reported that the volatilization of pesticides increases with increasing wind speed because air movement and turbulence decrease the thickness of the stagnant air layer immediately at the surface of the soil. The experimental site of the present study was 2 to 3 km inland at an altitude of 21 m, and the area had tree cover which broke the wind coming from the ocean. Mghenyi [1988], also used hollow stainless steel cylinders. This would contribute to a higher rate of vapourization and hence a shorter half-life because of the higher heat build-up brought about by the steel which is a good conductor of heat. Hollow plastic PVC pipes were used for containing the environment in the present study and these are poor conductors of heat. The author also carried out the study for 122 days, which was enough time to record the initial rapid dissipation phase and a small part of the final slow dissipation phase. Thus the overall half-life was shorter than in the present study.

The dryness of the soil and its sandy nature could also favour a longer half-life. In 1960, Lichtenstein et al reported that aldrin persisted longer in dry soils, in which micro-organisms are relatively inactive and oxidized

little of the aldrin to dieldrin. He also found that aldrin persists longer in sandy soils, which contain few micro-organisms, or in soils in which the population of micro-organisms has been reduced by auto-claving (heating). As mentioned earlier the soil was sandy and had a moisture content of 0.60 ± 0.09 %.

3.1.2 Bound ^{14}C-p,p'-DDT equivalents.

As seen in Table 3.1 and Fig. 3.1, the bound residues go from 2.0 % on day zero to 5.6 % on day 292. This binding rate is slightly higher than that reported by Mghenyi (1988) who found the highest value to be 2.6 % of the initial value after 122 days. This was favoured by among others, the lower soil moisture content during the course of the study. Pillai et al [1988] found a value of 8 % of the initial dose after 365 days. In a study to investigate the rate of disappearance of DDT from Nairobi soils, Lalah et al [1994a], found that the bound residues rose from 1.3 % on day zero to 7.1 % on the 84th day which is a higher rate of binding than that found by Mghenyi (1988) and in the present study. The soil properties at the Nairobi site were: pH 6.3; sand 22 %; clay 46 %; silt 32 %; nitrogen 0.15 %; organic carbon 1.96 %; texture clay, while those of the Mtwapa experiment were: pH 6.2; sand 90 %; clay 8 %; silt 2 %; nitrogen 0.06 %; organic carbon 0.54 %; texture sandy. Lichtenstein [1959], reported that soils with a higher content of organic matter tend to hold pesticides longer than sandy soils. Lalah et al [1994b], in a study to

investigate the rate of disappearance of one of the metabolites of DDT, DDE, found that the DDE was bound more than DDT in the same soils in Nairobi. The highest value was 20 % after 189 days.

In a study to compare the extractability of ^{14}C-labelled residues in agricultural loam soil, Lichtenstein [1977], found that soil bound residues of ^{14}C-p,p'-DDT amounted to 25 % of the applied dose after 28 days. In the present study, the bound residues after 28 days amounted to 4.4 % (see table 3.1).

3.1.3 Soil moisture content.

Table 3.4 below shows the moisture content of the soil during the course of the field study.

The moisture content of the soil had an average value of about 0.60 $\pm$ 0.09 %. In his work, Mghenyi (1988) reported an average of 2.8 %. The higher the soil moisture content, the higher the rate of vapourization and codistillation of the DDT with the water vapour. Lalah et al [1994a], reported an average soil moisture content of 8.42 $\pm$ 0.50 % for the duration of the work in which they obtained a half-life of 64.6 days for ^{14}C-p,p'-DDT in Nairobi soils. This is shorter than that reported by Mghenyi [1988], and the present work, and is supported by the much higher moisture content. The soils in Nairobi have a high content of organic matter. This tends to retain moisture, resulting in high moisture content.

Table 3.4 Soil moisture content.

Days after treatment	Moisture Content(%)
0	0.64 ± 0.19
7	0.62 ± 0.05
14	0.22 ± 0.05
21	0.66 ± 0.14
28	0.70 ± 0.26
42	0.48 ± 0.06
56	0.57 ± 0.07
70	0.54 ± 0.07
84	0.48 ± 0.11
112	0.81 ± 0.03
168	0.86 ± 0.01
245	0.80 ± 0.04
292	0.37 ± 0.05

3.2 Metabolic profile of ^{14}C-p,p'-DDT in soils in Mtwapa.

3.2.1 Metabolite peak identification on EC-GC and GC/MS.

A chlorinated pesticide mixture (CPM), whose chromatogram is shown in Fig. 3.7, was used as the standard during electron capture gas analysis (EC/GC). Known quantities of the sample were injected and their retention times, Rt, compared with those of the CPM. Figure 3.8 shows a chromatogram of one of the samples. The chromatogram suggested the presence p,p'-DDE, p,p'-DDD and p,p'-DDT. The respective peaks were at Rt 2.70, 4.10, and 4.85 minutes.

The results of the TLC linear analyzer (TLC-LA), are shown on Fig. 3.12. On day 0 (Fig. 3.12a), a single peak was observed, and was probably due to ^{14}C-p,p'-DDT. On day 56 (Fig. 3.12b), a second peak is observed which grows larger during subsequent days until it is about the same size as the first peak on day 292 (Fig. 3.12e). It is noted that only two peaks are observed instead of the expected three, i.e., from the results obtained on the electron-capture gas chromatograph. This indicates there probably was only one metabolite from ^{14}C-p,p'-DDT. To shed more light on the identity of the second peak, i.e., whether it was DDD or DDE, analysis of the samples was done using combined gas chromatography/mass spectrometry, (GC/MS).

On the GC/MS, the gas chromatogram of the DDE, DDD and DDT standards is shown in fig. 3.13. Their retention times, Rt, were 5.7, 6.4 and 7.1 minutes respectively.

The mass spectra of p,p'-DDE (Fig. 3.14a), had a

parent peak at m/e 318 and the most abundant peak at m/e 246. Other peaks were at m/e 283, m/e 235, m/e 212, m/e 176, m/e 140, m/e 123, m/e 105, m/e 75 and m/e 44.

The mass spectra of p,p'-DDD (Fig. 3.14b), had a parent peak at m/e 318 and the most abundant peak at m/e 235. Other peaks were at m/e 237, m/e 199, m/e 165, m/e 137, m/e 123, m/e 101, m/e 75, and m/e 50.

For p,p'-DDT, the mass spectra (Fig. 3.14c), had a parent peak at m/e 352 and the most abundant peak at m/e 235. Other peaks were at m/e 281, m/e 212, m/e 199, m/e 165, m/e 123, m/e 106, m/e 75 and m/e 50.

The gas chromatograms of the samples had peaks at Rt 5.7, 6.4 and 7.1 minutes (Fig. 3.15). These suggested the presence of p,p'-DDE, p,p'-DDD and p,p'-DDT respectively. The mass spectra of the compounds eluted at Rt 5.7 and 7.1 showed that they were p,p'-DDE and p,p'-DDT respectively (Fig. 3.16a and Fig. 3.16b). Their respective probabilities of identification on the RTE VI data system were 98 % and 89 %. However, the mass spectra (Fig. 3.16c) of the compound eluted at Rt 6.4 was not of p,p'-DDT, but was of 2,4-dimethyl-decane. Its presence was brought about due to contamination. Other hydrocarbon contaminants were also detected in the samples.

The absence of p,p'-DDD is supported by the fact that p,p'-DDT is mainly converted to p,p'-DDD by micro-organisms under anaerobic conditions [Ecobichon et al, 1967]. Aerobic conditions prevail in the field. The soils on which the study was done were sandy. In such soils, the population

and activity of micro-organisms which attack various pesticides is low. The soils were also dry (average moisture content of 0.60 ± 0.09 %). These factors do not favour the conversion of p,p'-DDT to p,p'-DDD [Lichtenstein et al, 1960].

Lalah et al [1994a], detected the presence of p,p'-DDT, p,p'-DDE and p,p'-DDD during their work on the degradation of p,p'-DDT in Nairobi. Lalah et al [1994b], found that p,p'-DDE applied to the same soil during an experiment to determine its rate of disappearance, remained unchanged. Wandiga and Natwaluma [1984], reported the presence of DDE and 4-PCB during their study on the persistence of DDT in Nairobi. However, some of the chromatographic peaks obtained in their study were not identified. Cooke and Stringer [1982], in a study on the distribution of DDT in orchard soil in Bristol, England, found that residues of p,p'-DDT and o,p'-DDT decreased gradually while those of p,p'-DDE increased. o,p'-DDT was present as an impurity in the technical DDT applied at the beginning of the experiment. Eder et al [1976], and Agarwal [1983], have reported DDE to be the major metabolite of DDT in soils.

3.2.2 Percentage composition of the metabolites.

The results of the GC analysis of the samples are given in Table 3.2, section 3.1.1. These data is represented graphically on Fig. 3.9.

Lalah et al [1994a], had 90.4 % p,p'-DDT, 5.3 % p,p'-DDE, and 4.3 % p,p'-DDD in the starting material and ended with 79.1 %, 20.9 %, and 0.0 % respectively in Nairobi soils showing most of the DDT degraded to DDE. Regression analysis on the two main components (p,p'-DDT + p,p'-DDE) in a study on the breakdown of p,p'-DDT in orchard soil by Cooke et al [1982], showed that during the post spray era, the breakdown of p,p'-DDT to p,p'-DDE was a main feature of the persistence of p,p'-DDT in the orchard soils.

3.4 Conclusion and recommendations.

From the results obtained here and various other studies e.g. Sleischer and Hopcraft, 1984, Wandiga and Natwaluma, 1984, Mghenyi, 1988, Lalah et al, 1994a, it is clear that the rate of loss of DDT in the tropical regions is higher than that recorded in temperate regions of the world [Edwards, 1966, Cooke and Stringer, 1982]. The persistence is still considerable and residues of DDT may remain in the soil for some time after application even in tropical regions, thus only restricted use should be allowed. As it was noted in this study, DDT mainly breaks down to DDE which is thought to be more persistent than DDT itself. It has been suggested that DDE does not breakdown

further [Wedermeyer, 1967a]. To get an accurate picture of the behaviour of p,p'-DDE in the environment, the breakdown of p,p'-DDT should be studied for a longer period and the data treated with a more sophisticated statistical approach such as the Maximum Likelihood Programme developed at Rothamsted. This will take into account the various processes such as volatilisation, conversion of p,p'-DDT to p,p'-DDE and the subsequent slower loss of p,p'-DDE.

Several factors do favour the use of DDT in the tropics and are supported by the shorter half-life obtained in this and other studies. DDT has a low mammalian toxicity and in the case of residues occurring in the environment in low levels, no great danger of poisoning would be encountered. Developing countries will also have a cheap and effective insecticide for the control of such disease carrying vectors as mosquitoes and tse-tse flies which constitute a far greater threat to humans and livestock than low level DDT residues in tropical countries. However, to prevent the develcpment of DDT resistant strains of insects e.g. mosquitoes, and the accumulation of DDT residues in the environment, it should be used interchangeably with other pesticides.

This is a matter of interest for countries in temperate regions where the use of DDT has been banned, as the DDT which vapourizes from tropical areas may do so unchanged and be carried away to the temperate regions. Traces of DDT have been recovered from dust known to have drifted over 1000 km and in water melted from the Antarctic

snow [WHO, 1979]. Restricted use of DDT would prevent excessive amounts of DDT in the atmosphere.

Studies on the uptake of DDT by plants in the tropics would be of interest and should be under taken to give a complete picture of the behavior of DDT in these regions.

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APPENDICES.

A. First Order Processes.

First order rate processes are changes whose rates depend only on the first power of the concentration of the reacting specimen, and the general equation to describe a first order reaction is

$$\text{Rate} = -\,dC/dt = k.C \quad \ldots\ldots\ldots \quad (1)$$

where, C = concentration of the reacting species,

t = time after the start, and

k = rate constant.

Rearranging equation (1),

$$dC/C = -k.dt$$

On integration,

$$\ln C = -kt + I \quad \ldots\ldots\ldots \quad (2)$$

$$C = e^{(-kt + I)} \quad \ldots\ldots\ldots \quad (3)$$

where I= constant of integration.

Integrating equation (2) between zero time, t_0, and any time, t, with the corresponding values of concentration, C_0 and C respectively, we obtain

$$\ln C_0/C = kt \quad \ldots\ldots\ldots (4)$$

At $t = t_{1/2}$ or t_{50}, time when 50 % of the substance has disappeared,

$C = 1/2\ C_0$.

then equation (4) becomes

$$\ln 2 = k.\ t_{50}, \text{ and}$$

$$t_{50} = \ln 2/k \quad \ldots\ldots\ldots (5)$$

Example. Calculation of the time for 50 % dissipation of ^{14}C-p,p'-DDT in Mtwapa, Kilifi, Coastal region of Kenya.

For the initial (0-56 days) period,

First order rate constant, k = 0.00623 (± 0.000547),

then t_{50} = ln 2/0.00623

= 111.25 days.

For the final (56-292 days), k = 0.00112 (± 0.000317)

then t_{50} = ln 2/0.00112

= 618 days.

For the entire (0-292 days) period, k = 0.0020 ($\pm$0.000254)

then t_{50} = ln 2/0.002

= 346.57.

NB. First order rate analysis gives a good approximation of the time needed for 50 % of the DDT to disappear in the field. However, this disappearance involves many processes e.g., volatilisation, leaching, degradation, etc, and is not entirely chemical. Thus, first order kinetic analyses do not fit the perfect model.

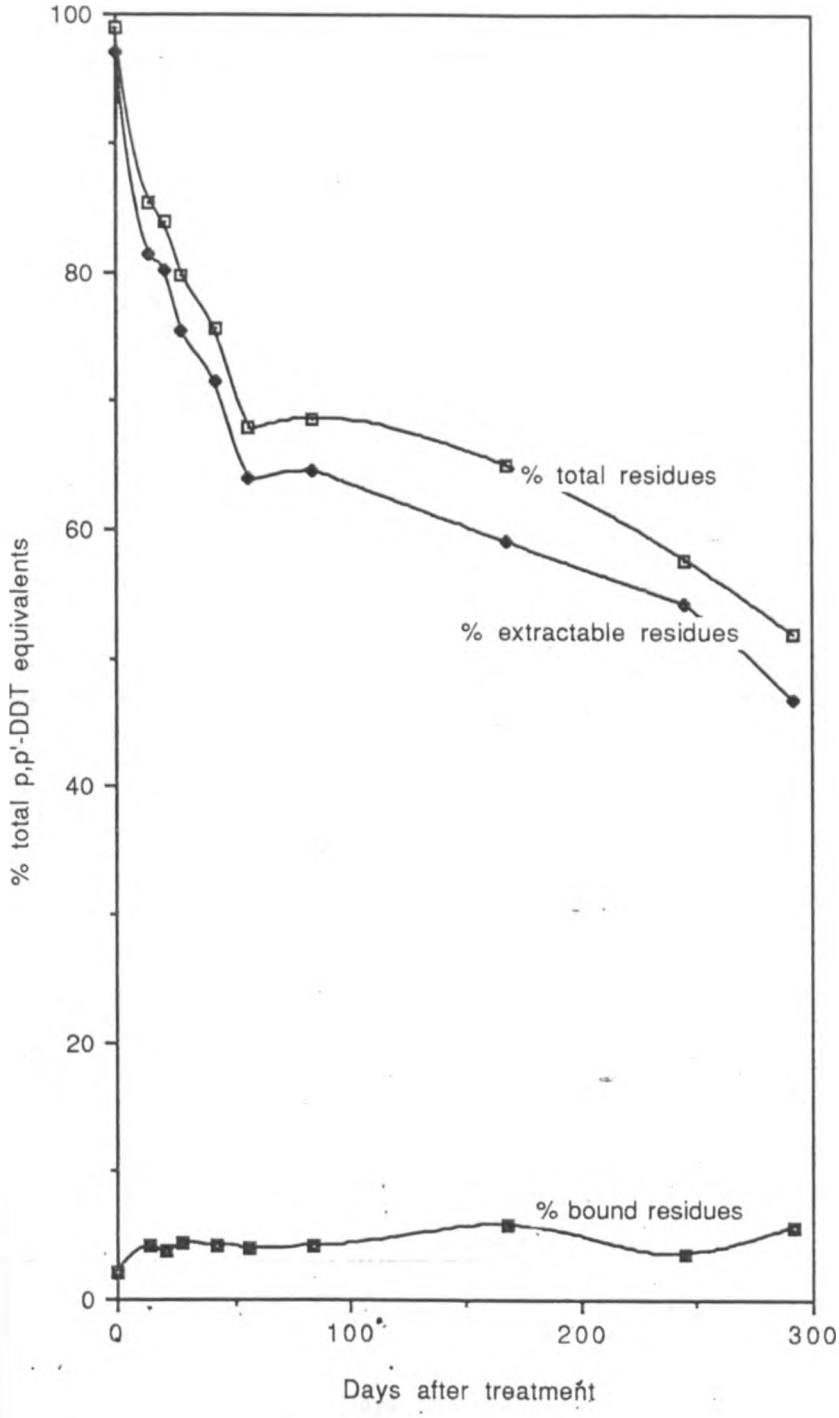


Fig. 3.1: The dissipation of the total p,p'-DDT in Mtwapa.

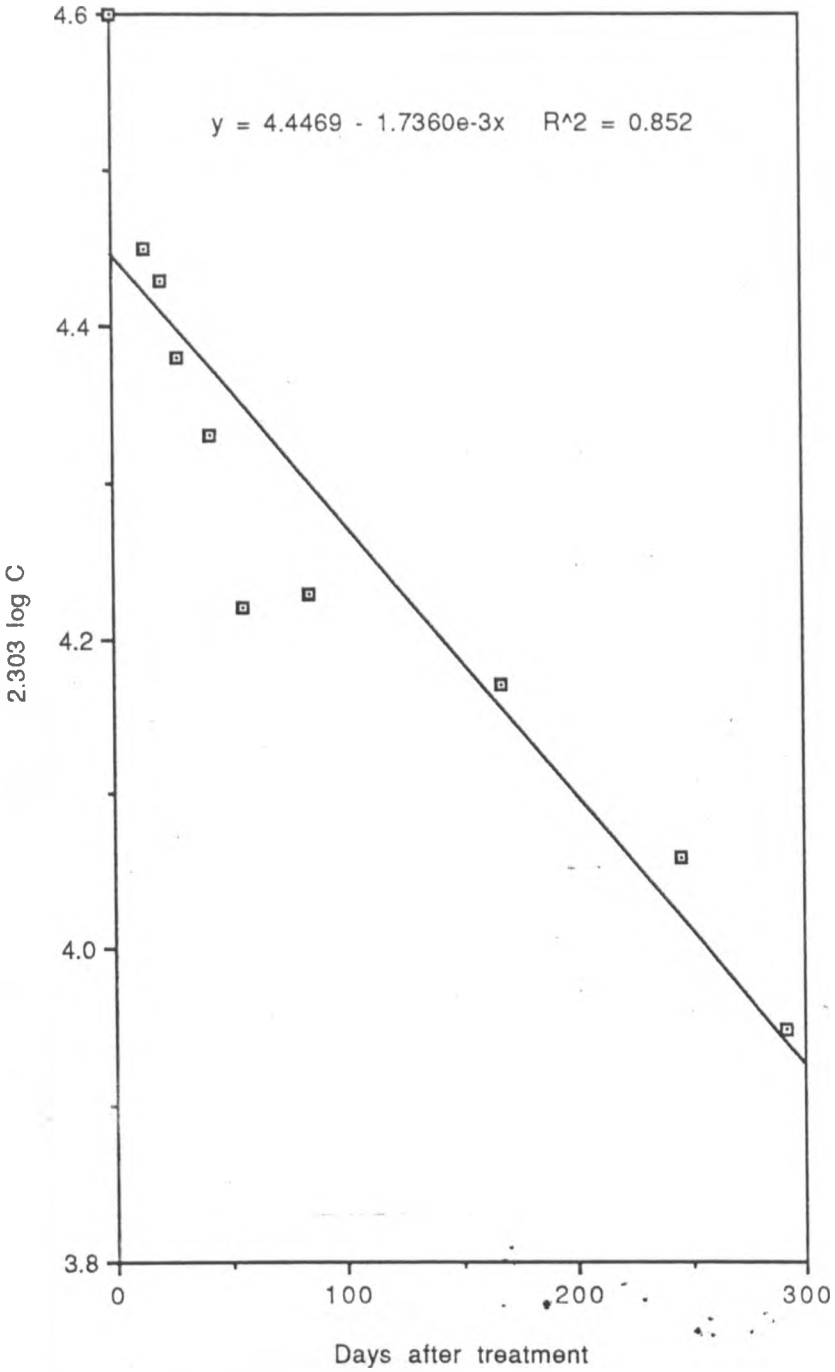


Fig. 3.2: First order linear regression of the total p,p'-DDT equivalents in soil.

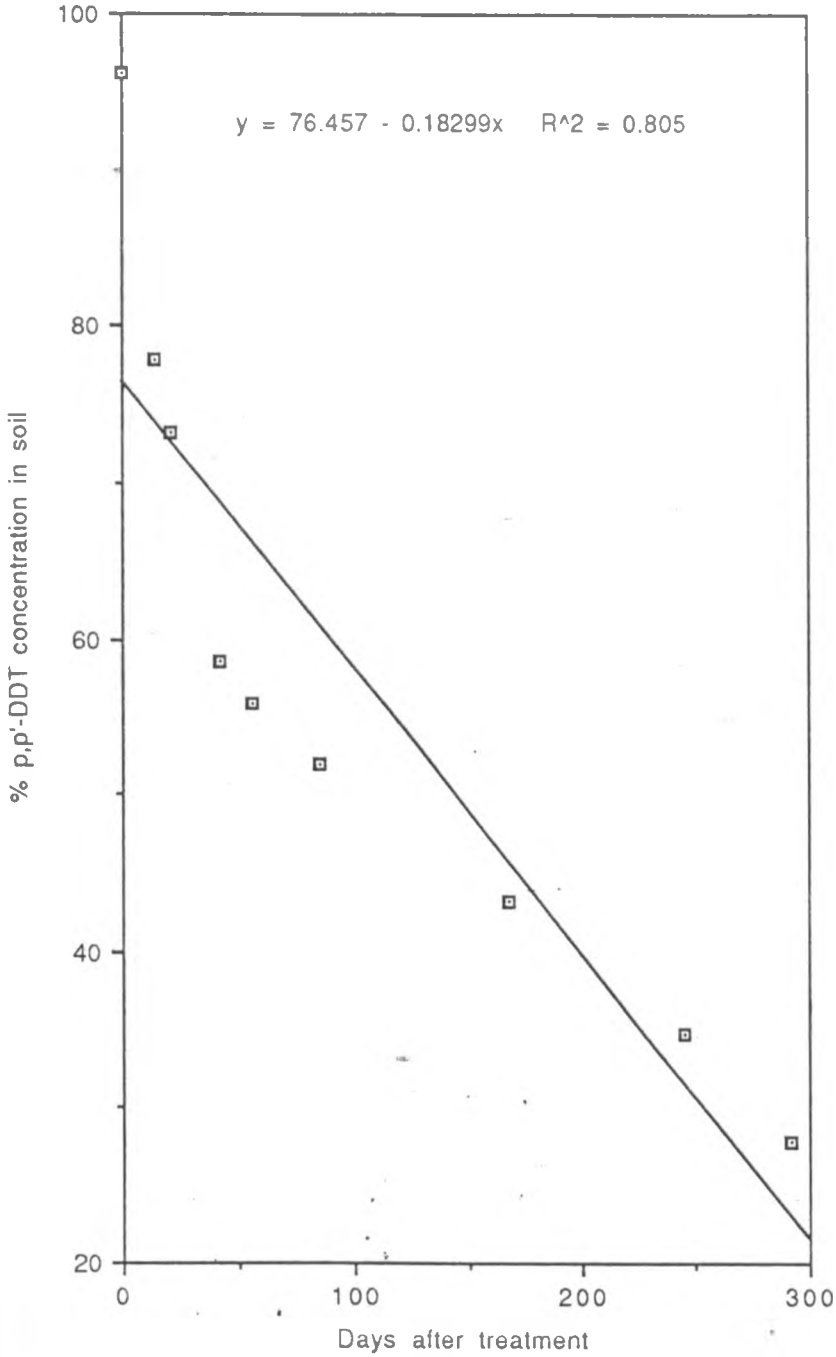


Fig. 3.3: Zero order regression of p,p'-DDT concentration in soil.

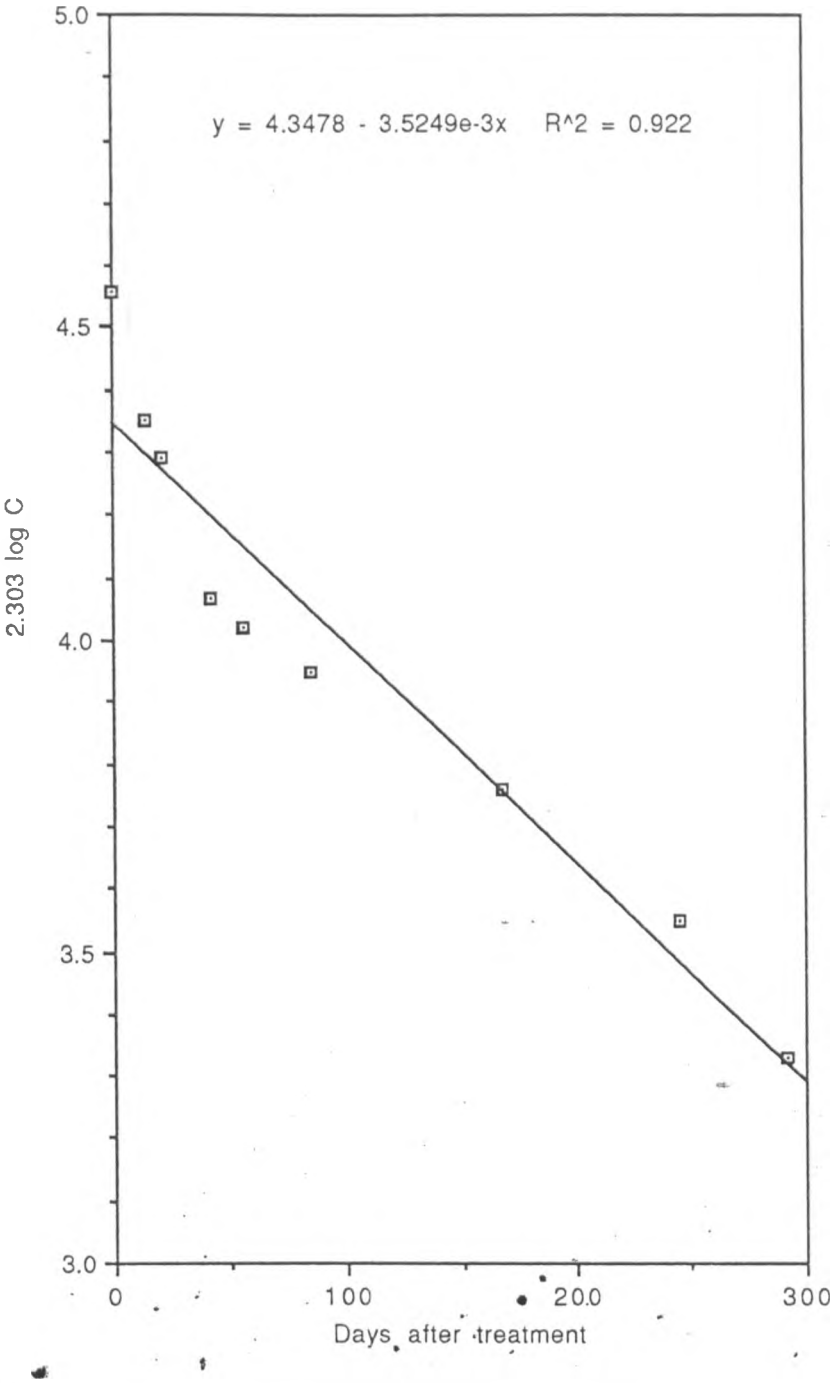


Fig. 3.4: First order linear regression of p,p'-DDT concentration in soil.

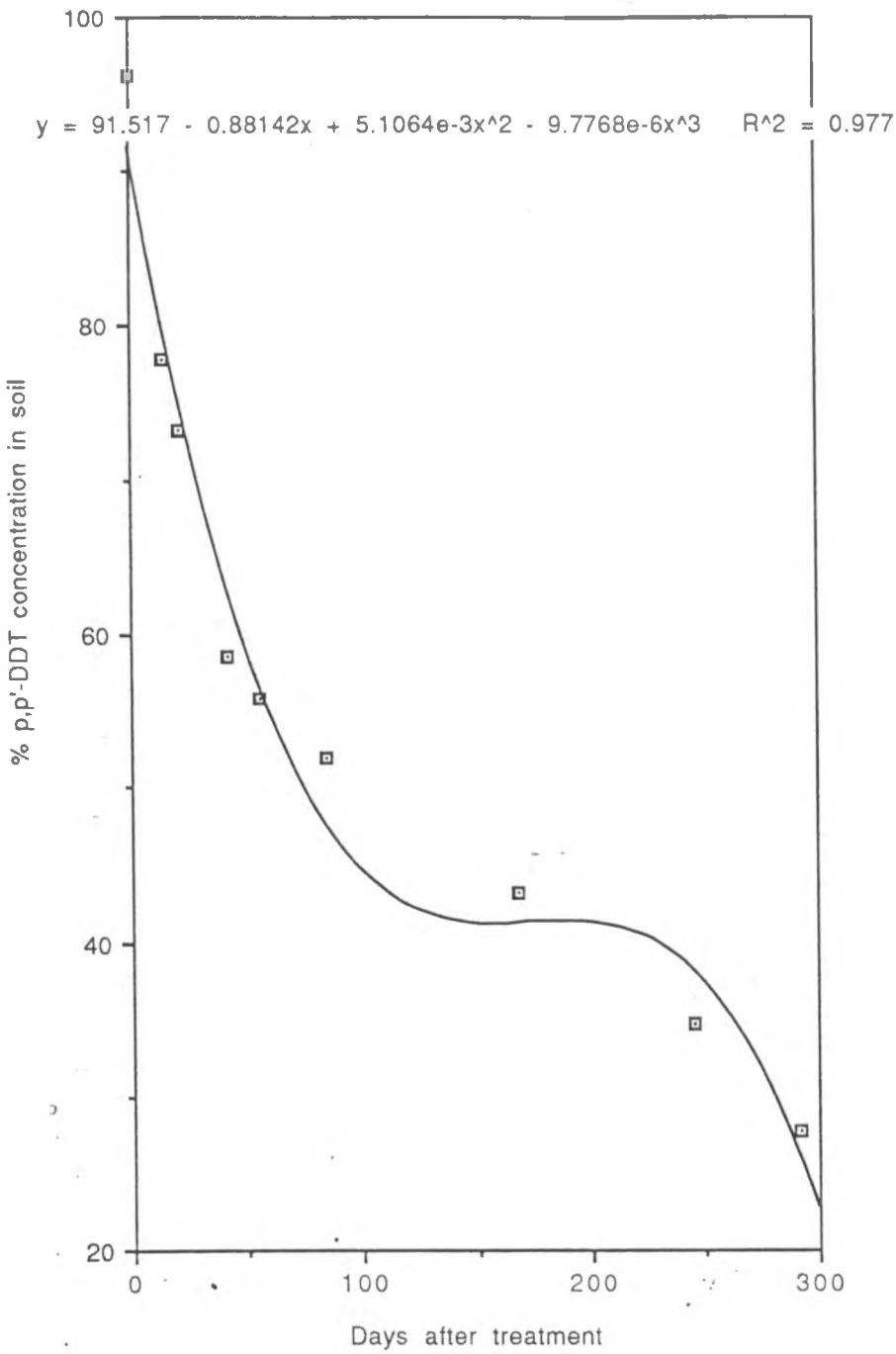


Fig. 3.5: Third order polynomial regression of p,p'-DDT concentration in soil

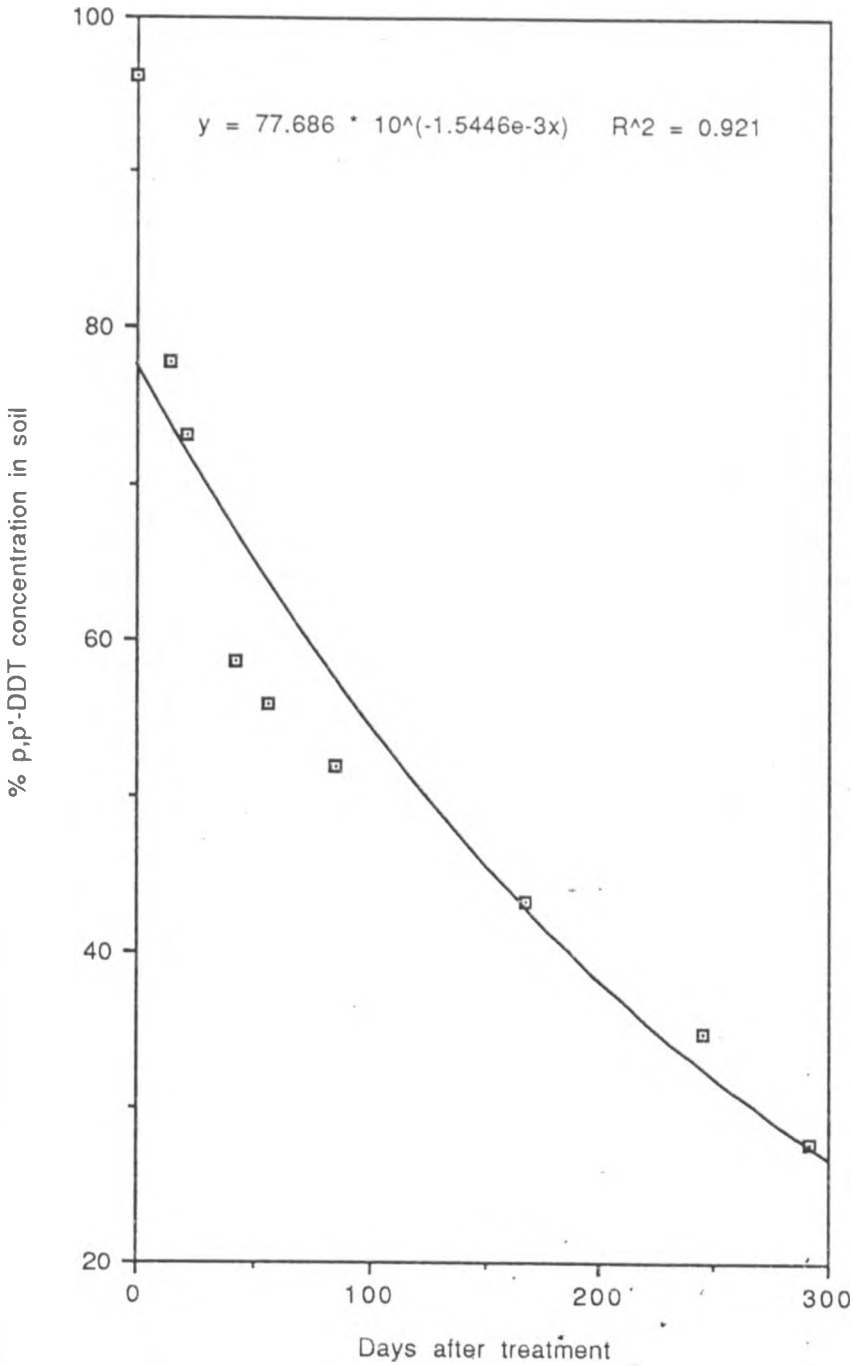
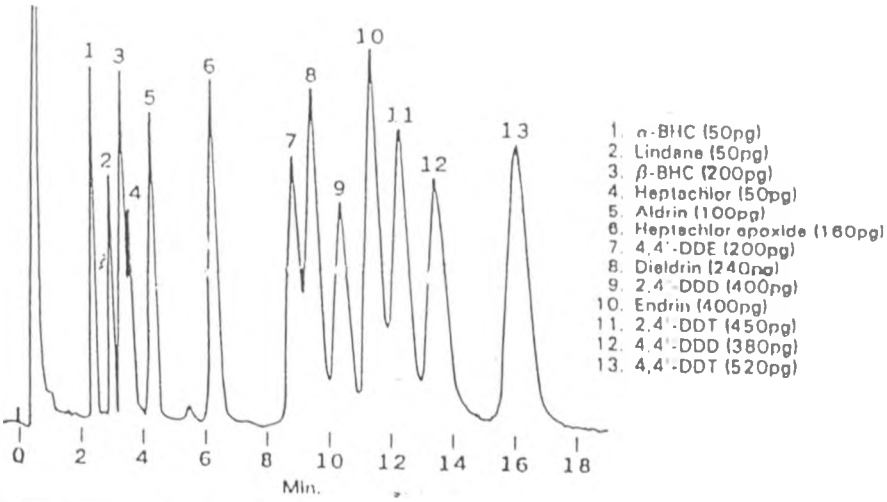


Fig. 3.6: Exponential analysis os p,p'-DDT concentration in soil.



1.5% SP™-2250/1.95% SP-2401 on 100/120 Supelcoport™, 2.0m x 4mm ID TightSpec™ glass, Col. Temp.: 200°C, Flow Rate: 80ml/min., N_2, Det.: ECD, ^{63}Ni, Sample: 2μl of Cat. No. 4-9160.

Fig. 3.7: Chromatogram of the chlorinated pesticide mixture (CPM) used as the standard.

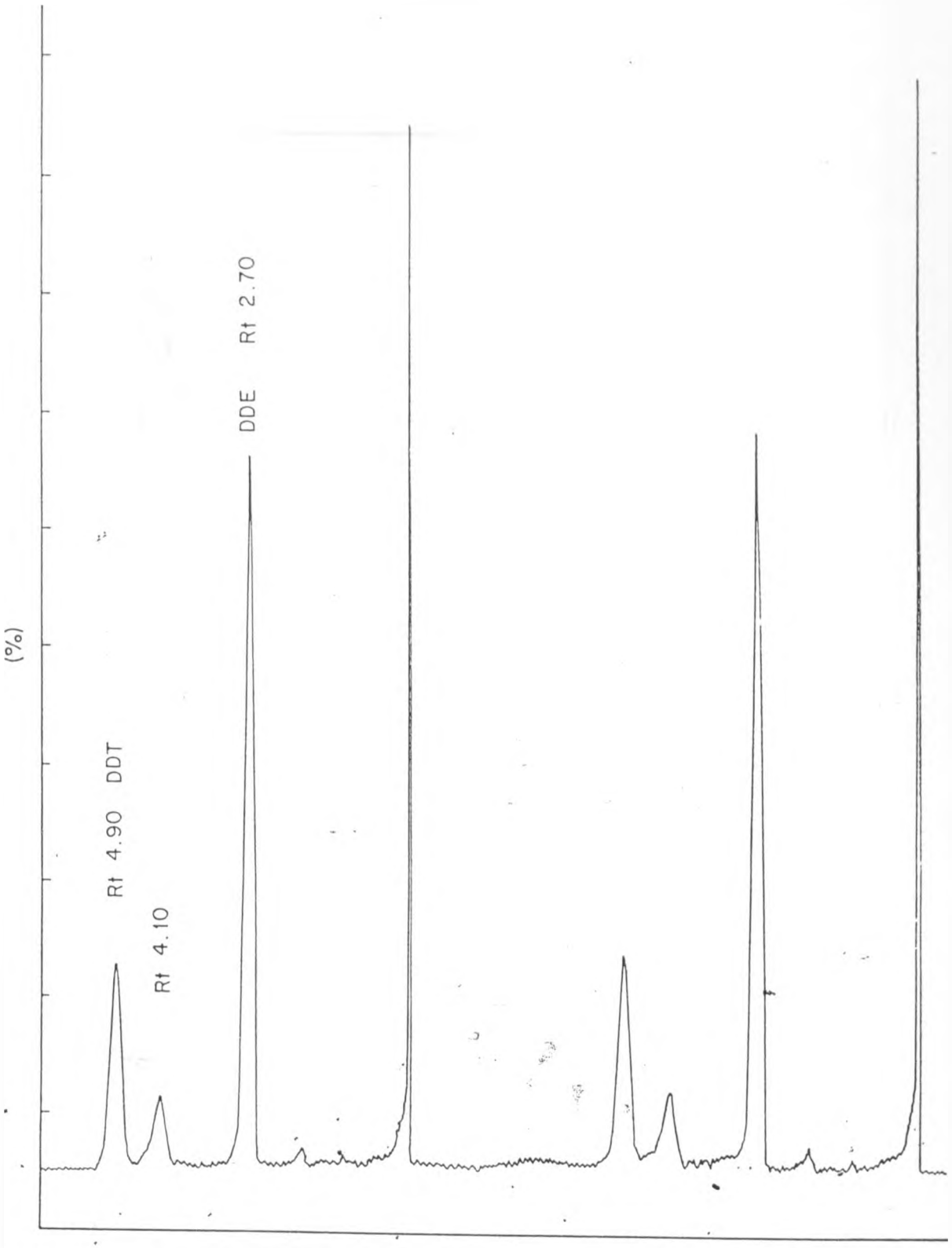


Fig. 3.8 : Chromatogram of sample collected on Day 292..

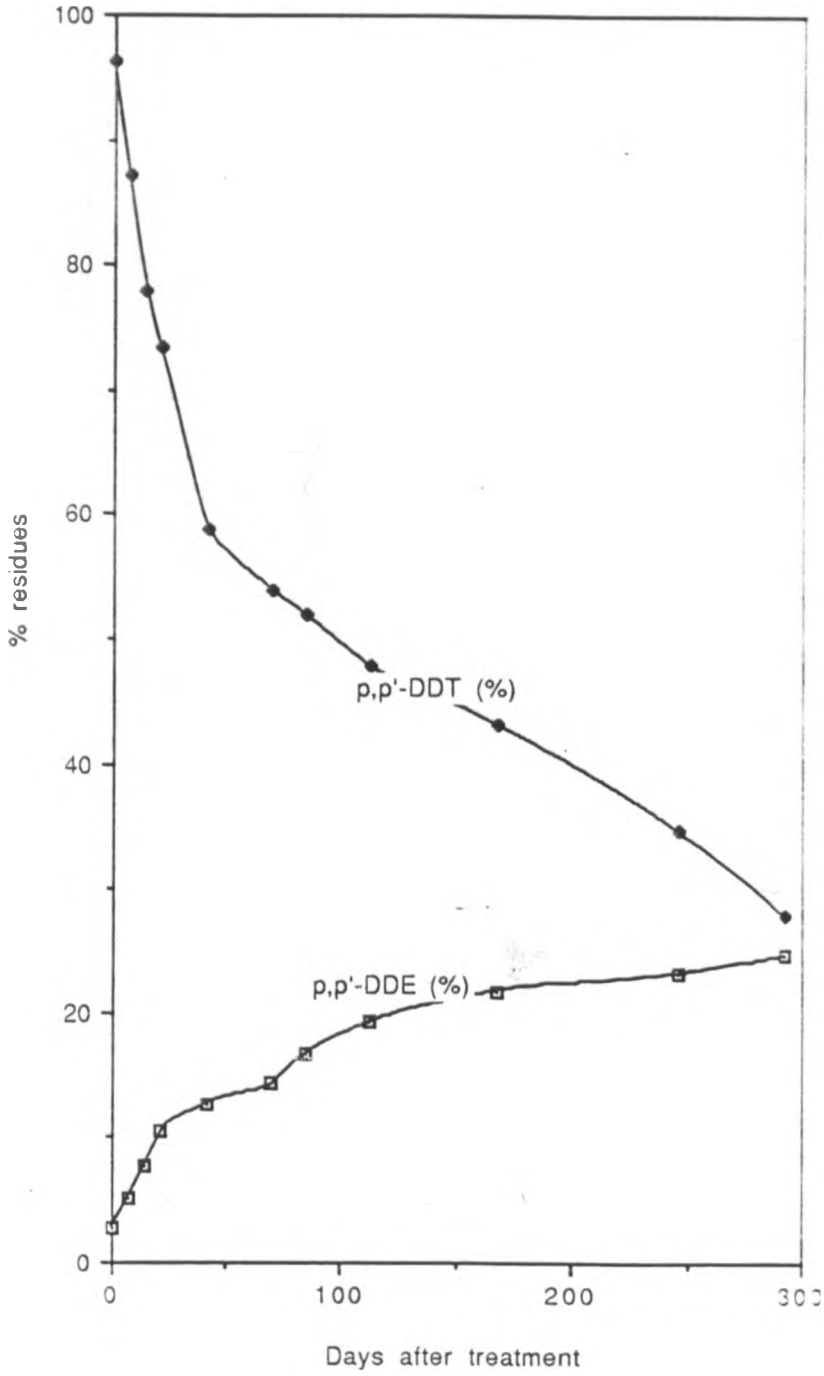


Fig. 3.9: The metabolic profile of p,p'-DDT in Mtwapa soil.

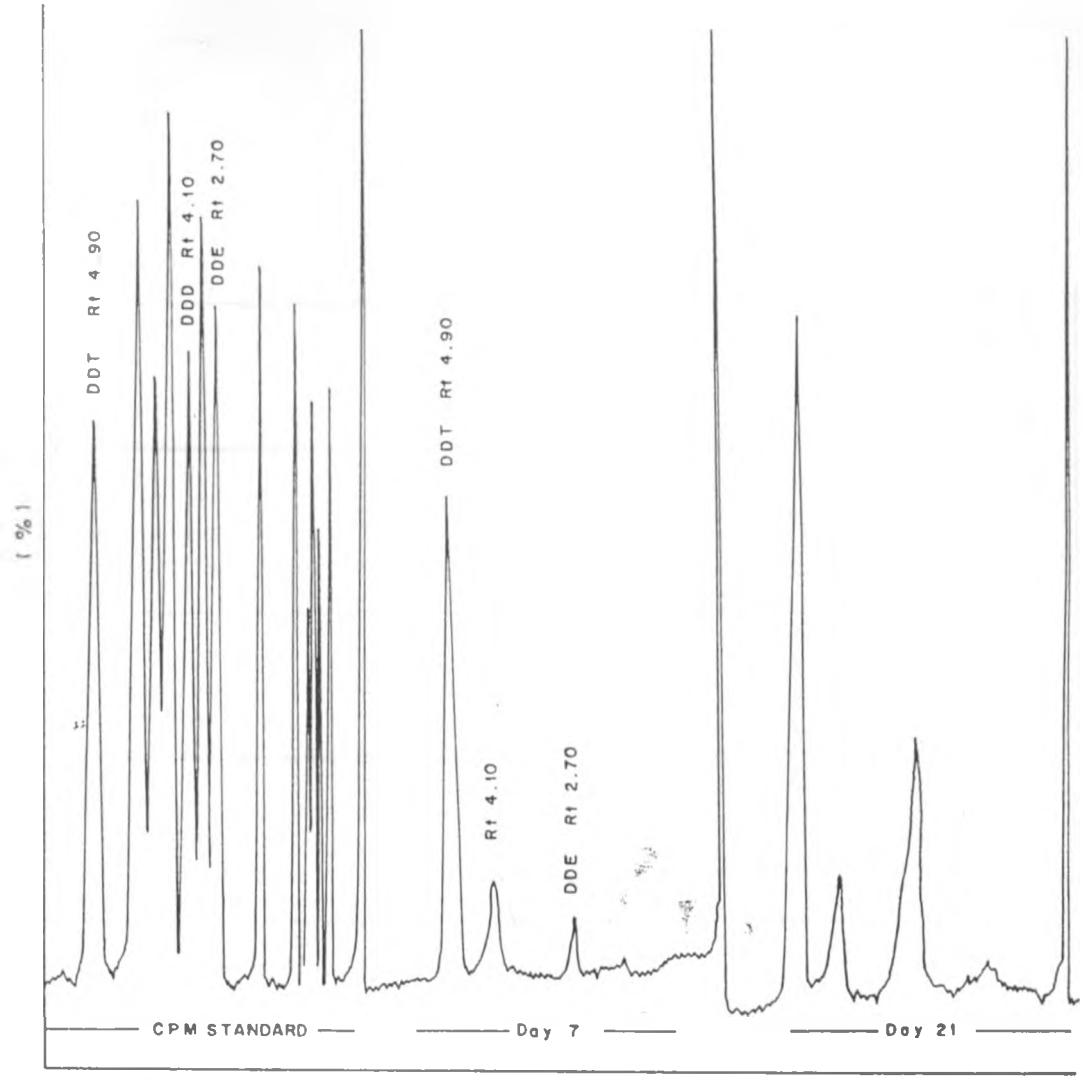


Fig. 3.10: Chromatogram of the CPM standard and samples collected on Days 7 and 21.

NB. Rt is in minutes.

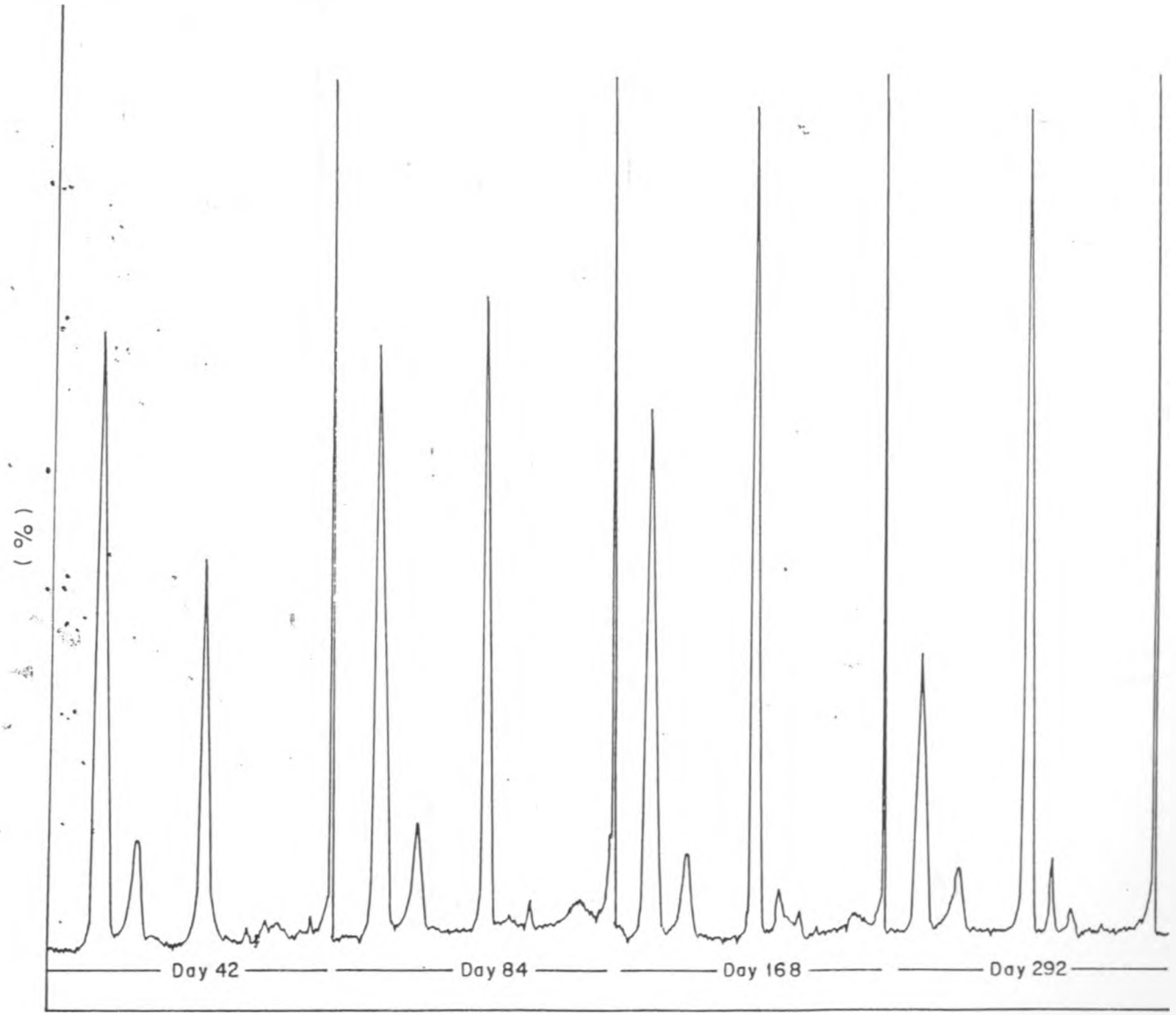


Fig.3.11: Chromatograms of samples collected on Days 42, 84, 168 and 292.

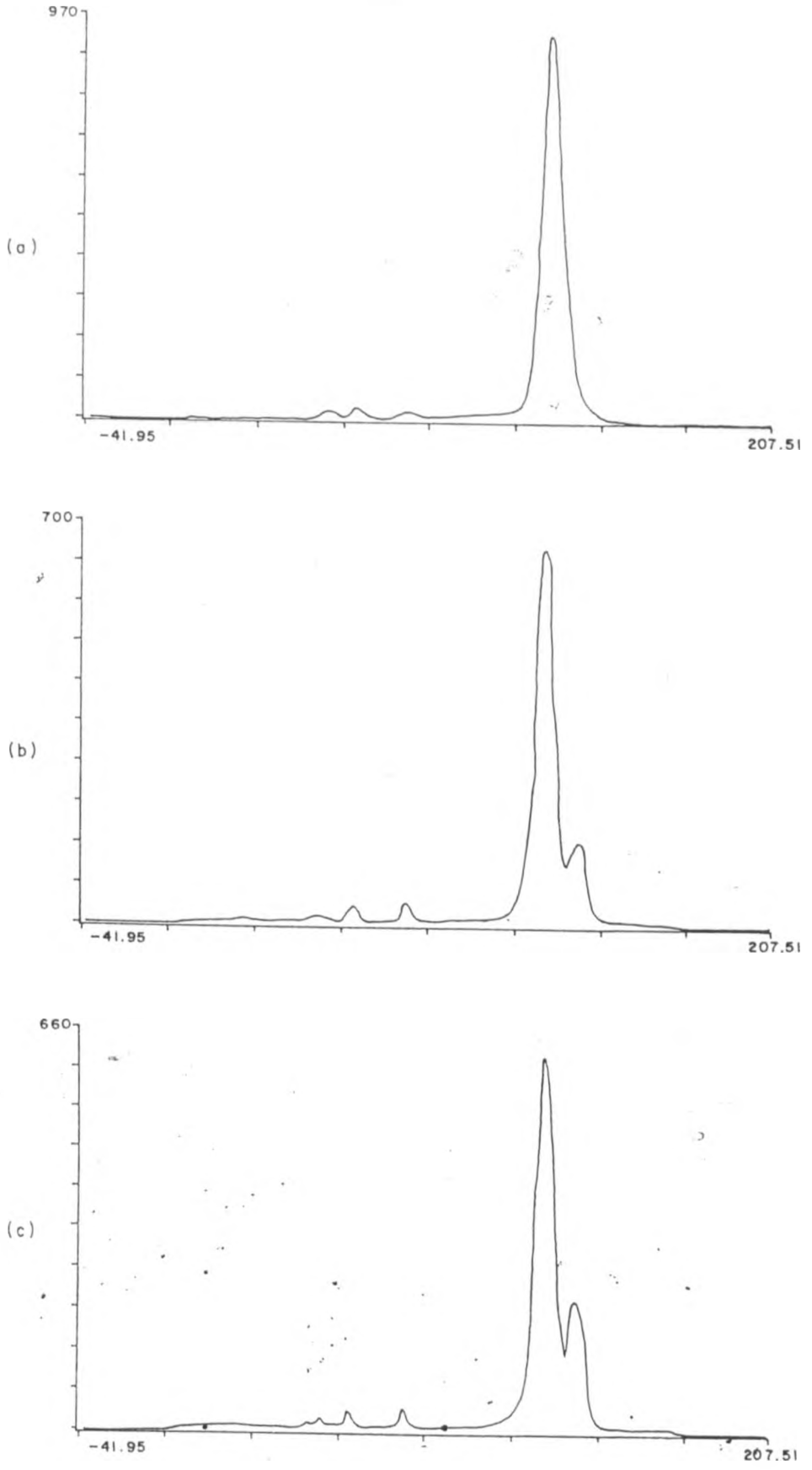


Fig. 3.12. TLC Linear chromatogram of samples collected on day (a) 0, (b) 56, (c) 84.

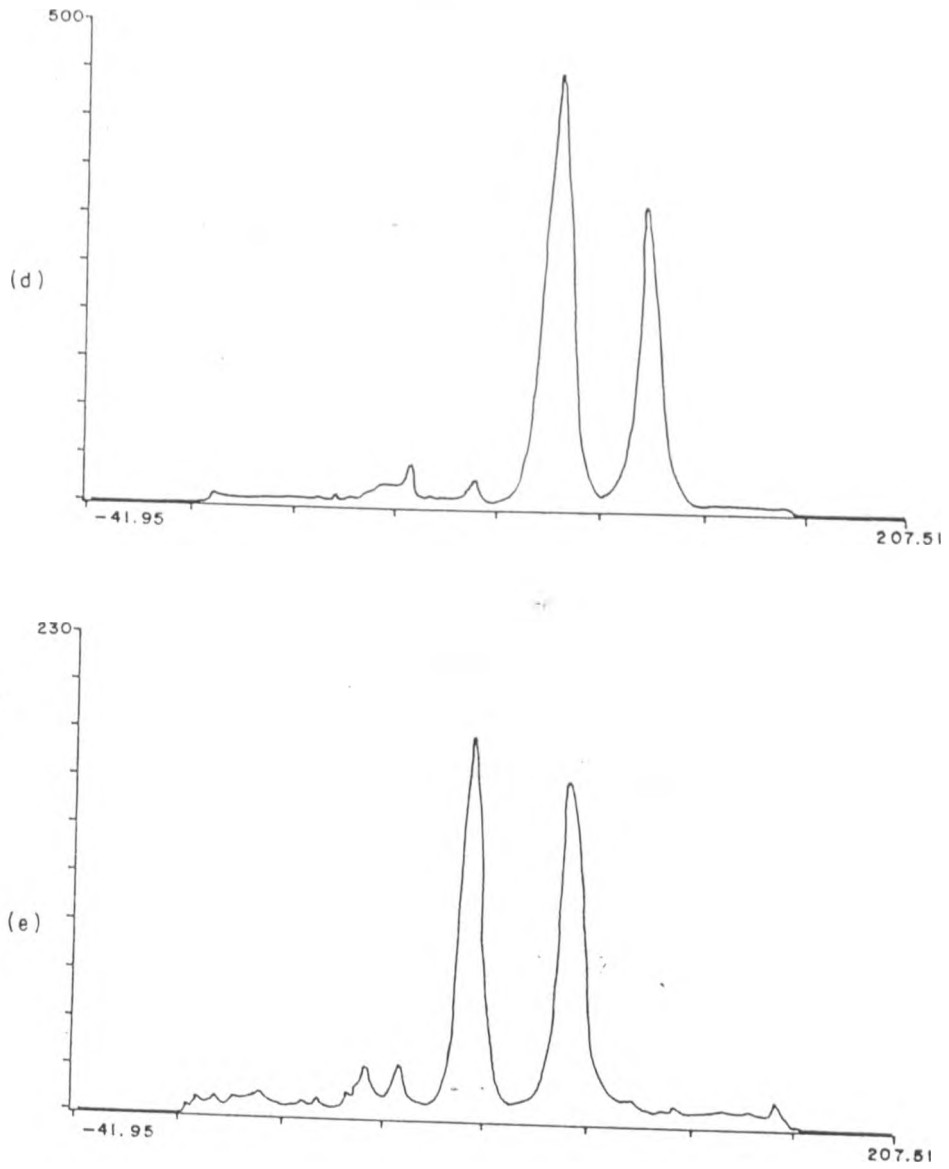


Fig. 3.12 : TLC Linear chromatogram of samples collected on day (d) 168, (e) 292 .

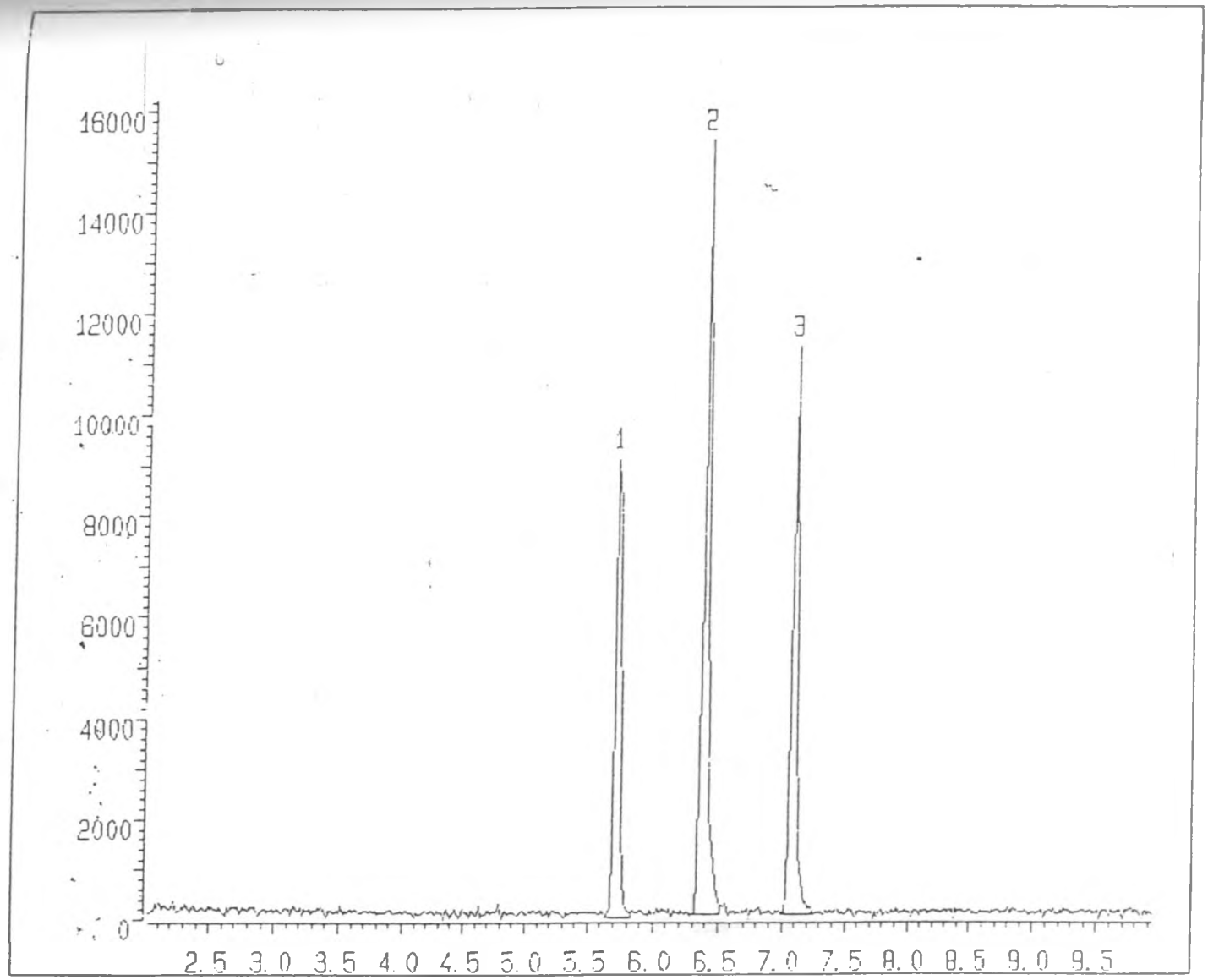
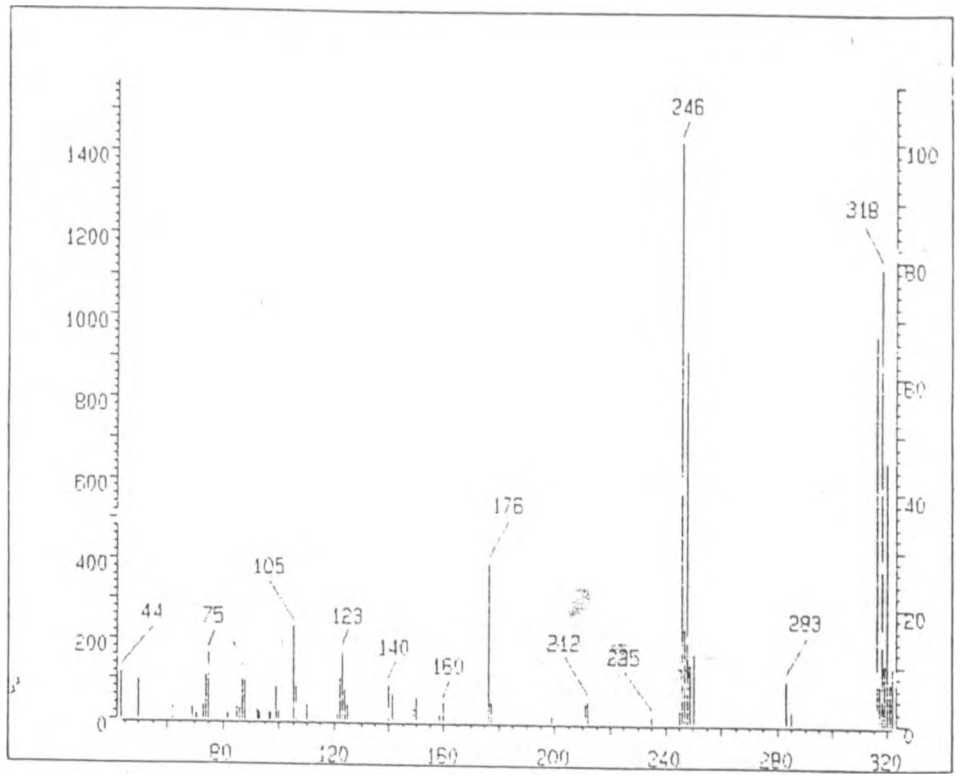


Fig. 3.13: GC/ms chromatogram of the standards (1) DDE, (2) DDD, (3) DDT

(a)



(b)

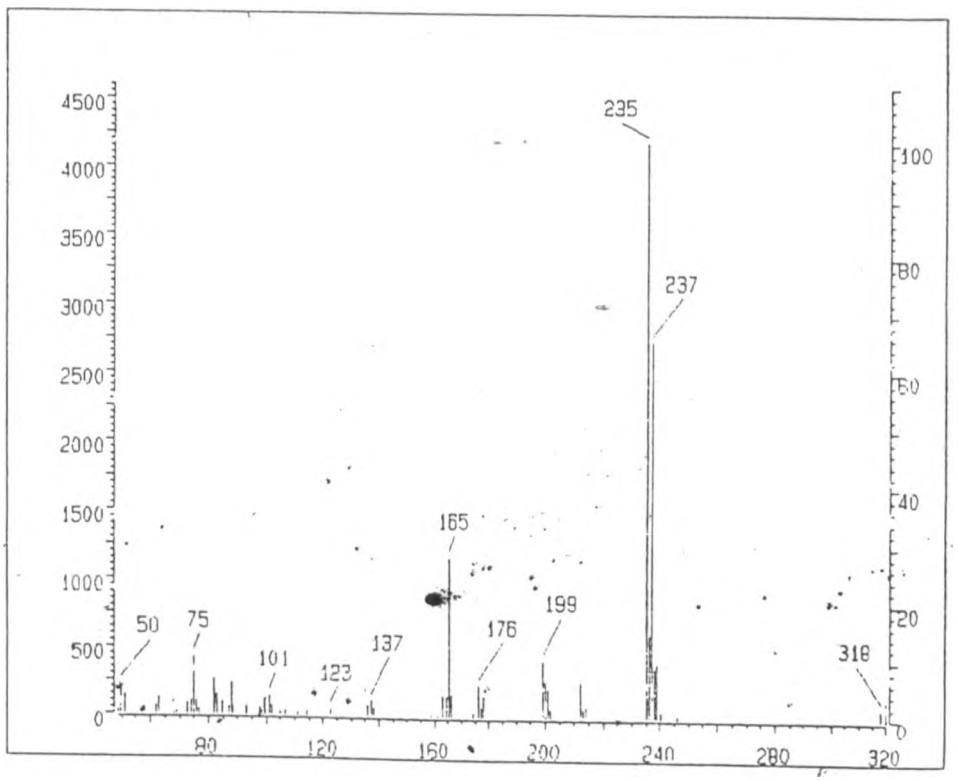


Fig. 3.14: Mass spectra of (a) DDE, (b) DDD, (c) DDT standards.

(c)

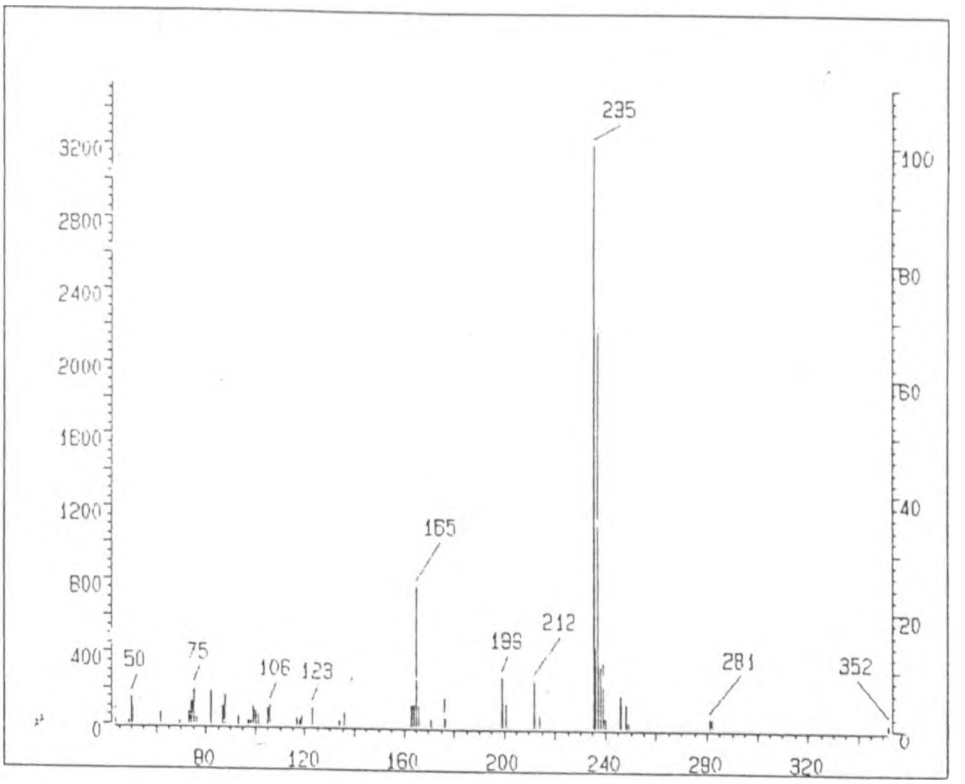


Fig. 3.14: Mass spectra of (c) DDT standards .

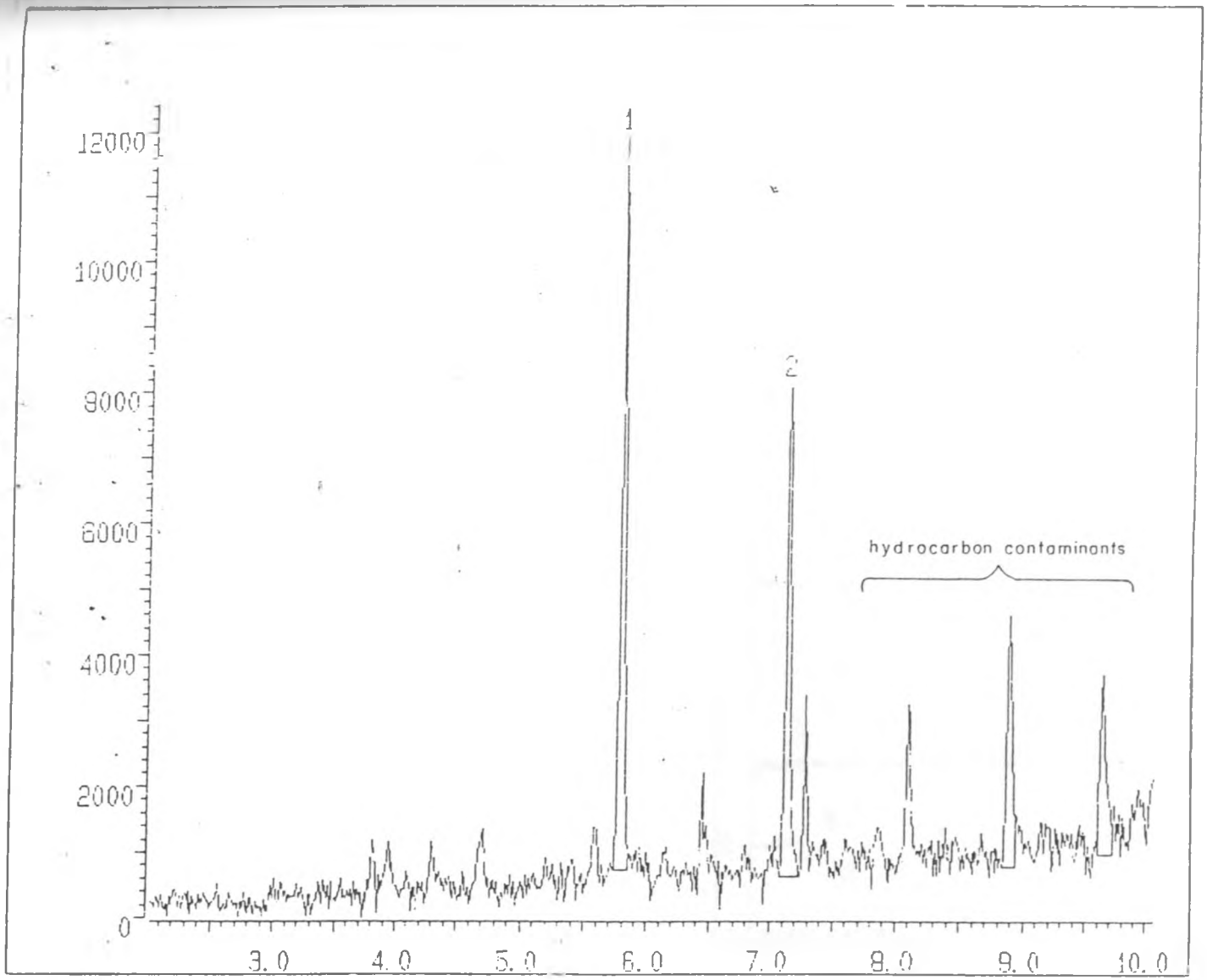
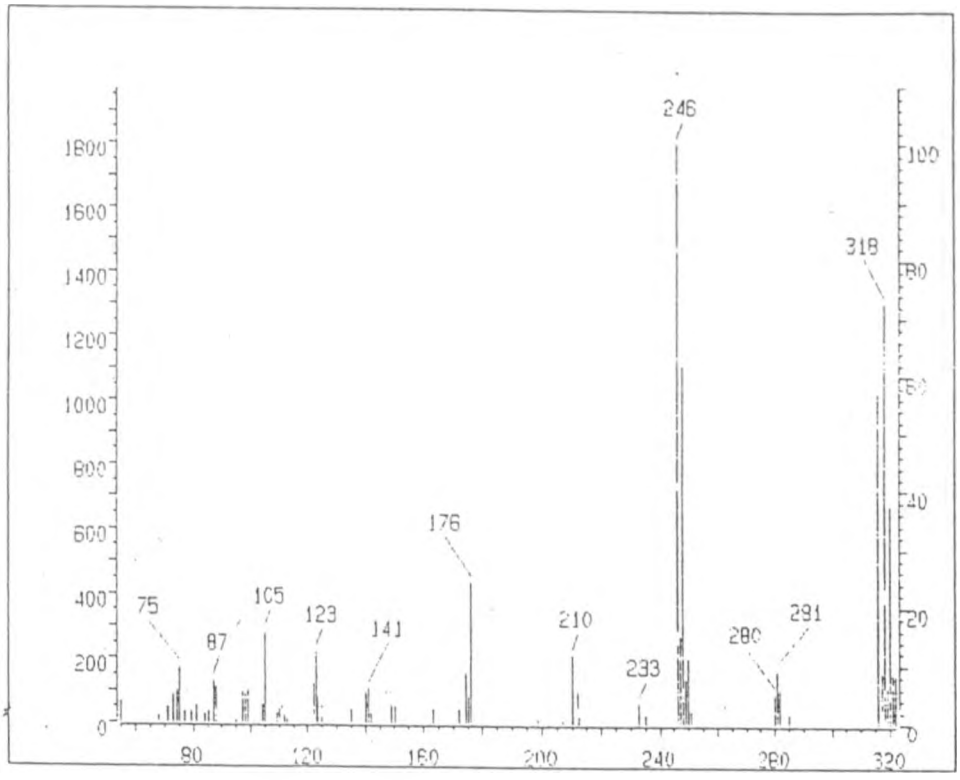


Fig. 3.15: GC/ms chromatogram of the sample collected on day 292 showing (1) DDE, (2) DDT

(a)



(b)

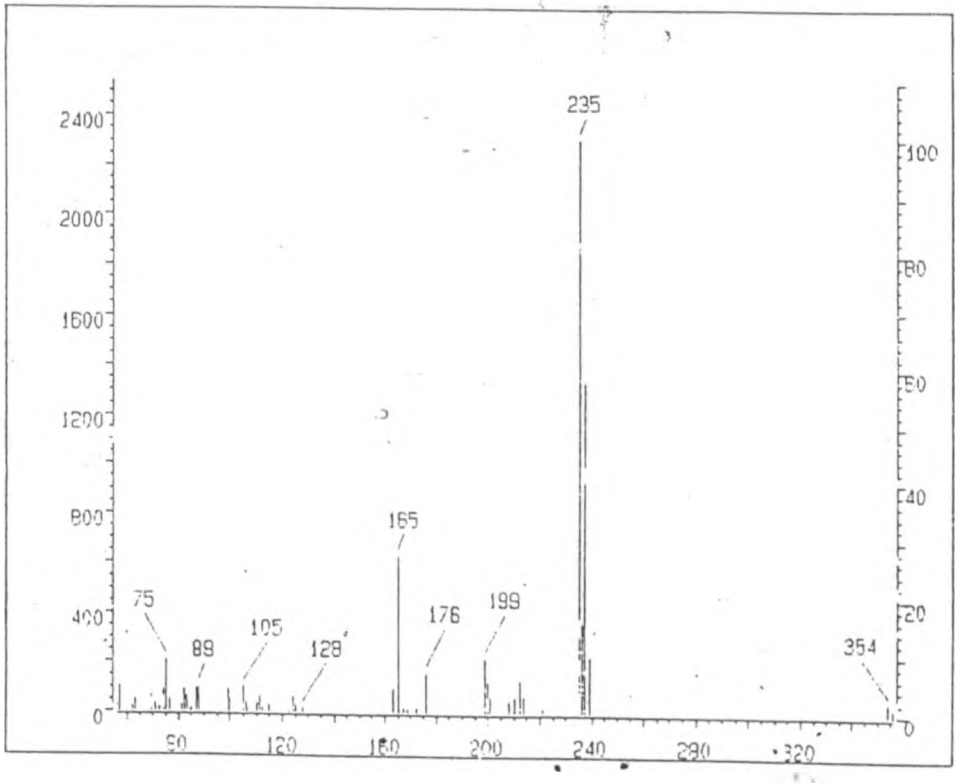


Fig. 3.16: Mass spectra of (a) DDE, (b) DDT in the samples.

(c)

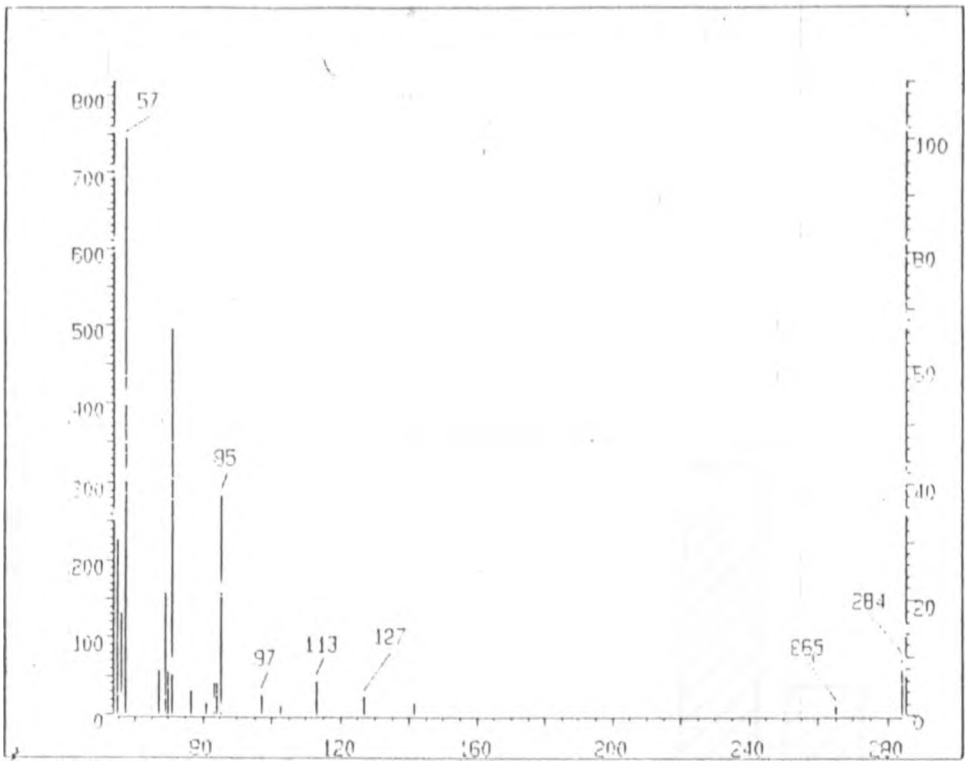


Fig. 3.16: Mass spectra of (c) 2, 4, dimethyl – decane, in the samples .

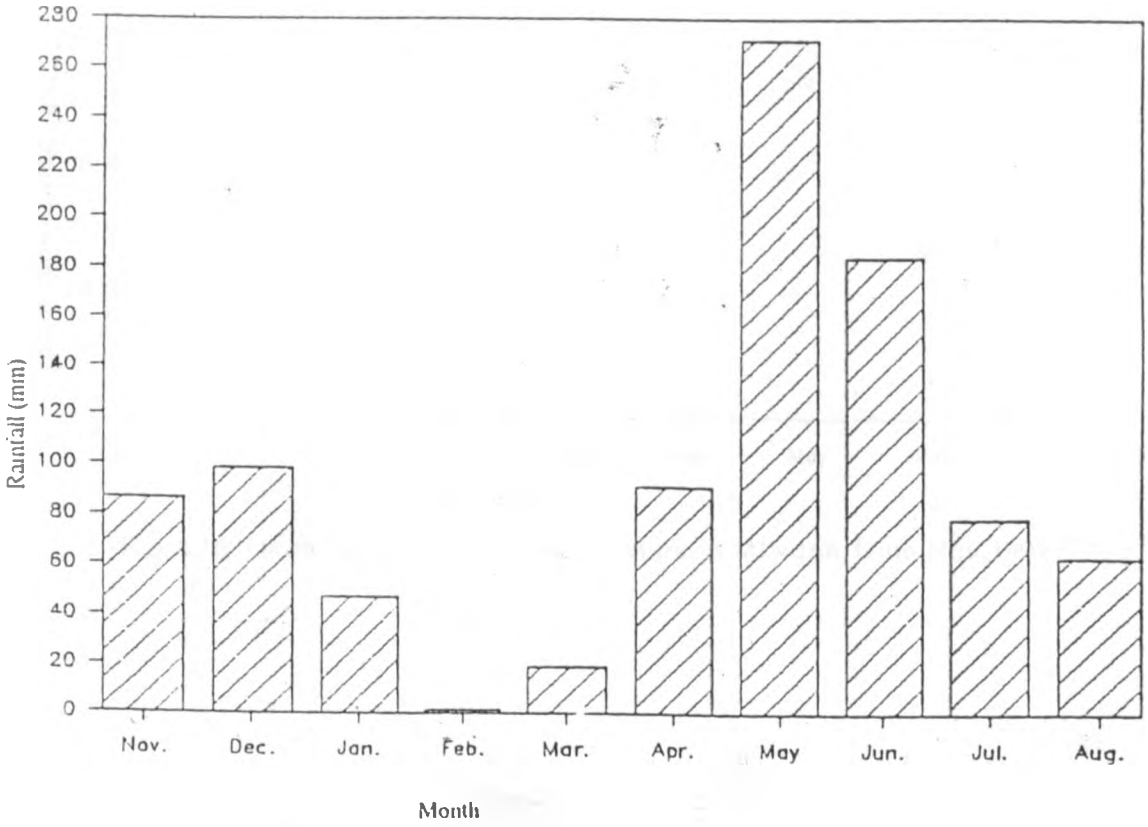


Fig. 3.17: Total monthly rainfall in Mtwapa from Nov. 1992 – Aug. 1993.

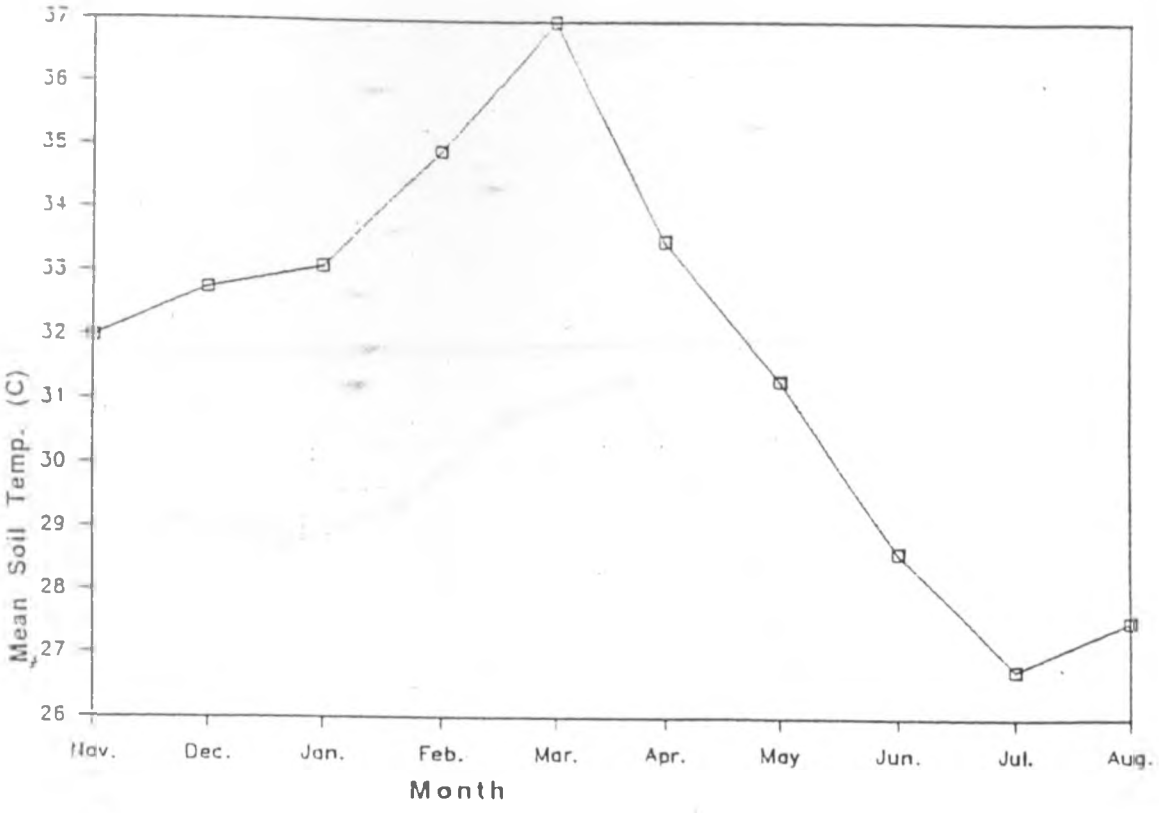


Fig.3.18: Mean monthly soil temperature in Mtwapa from Nov.1992—Aug.1993.

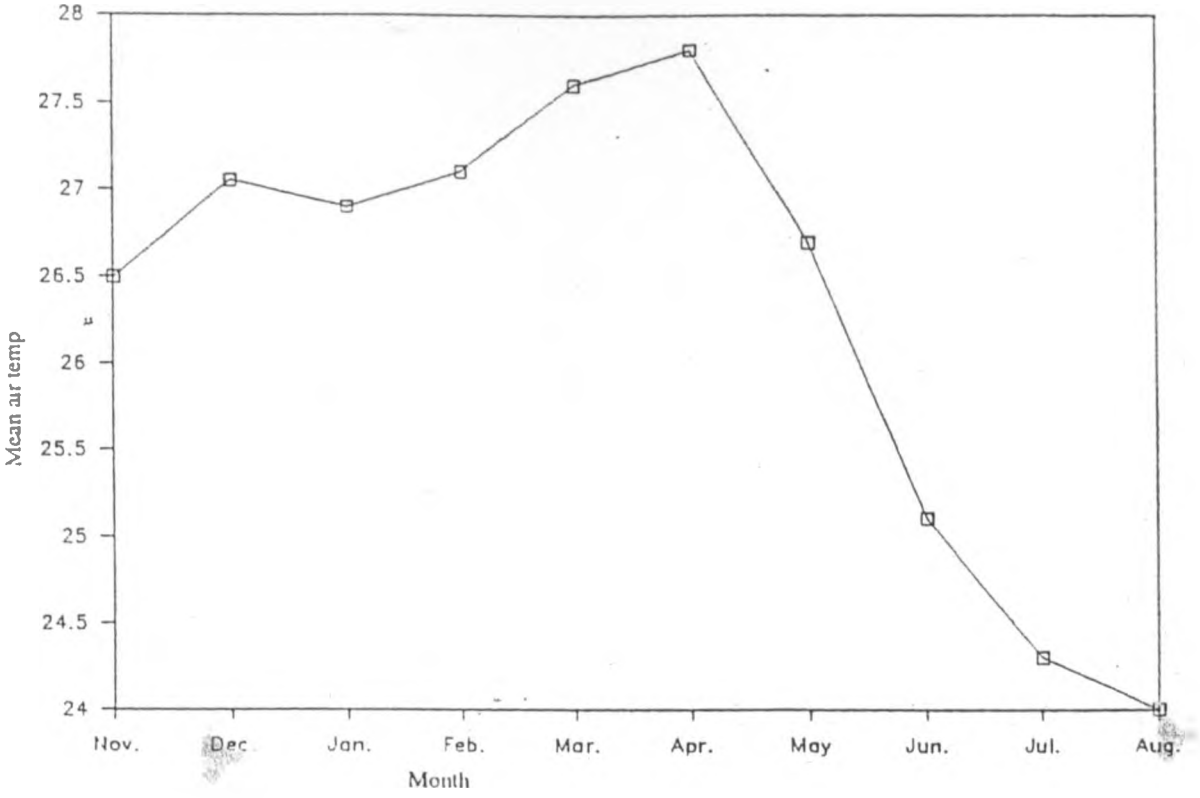


Fig. 3.19: Mean monthly air temperature in Mtwapa from Nov. 1992 - Aug. 1993.