VARIATION OF PLANT p,p'-DDT UPTAKE AND ITS DISSIPATION WITH AGE, TEMPERATURE AND SOIL TYPE.

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BY

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A thesis submitted in partial fulfilment of the requirements for a degree of Masters of Science in chemistry, University of Nairobi

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree in any other University.

KIFLOM, G.W

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

Prof. G.N.KAMAU

Prof. S.O. WANDIGA

DEDICATION

To my dear sister TURUWERK

AKNOWLEDEMENT

I am deeply indebted to my sponsors the Deuche-Albert-Einestein-Fluechling-Initiative (DAFI) program and AREP- Foundation who awarded me the scholarship. I wish to express my appreciation to them for all the financial and moral assistance they provided me throughout my study and particular thanks are due to Mrs. U.Pandikow and Mr. Yohannes T. who were very understanding and co-operative to me regarding my requests.

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ABSTRACT

The extent to which insecticides may be absorbed and translocated from ^{14}C-p,p'-DDT contaminated soils into cowpeas plant tissues and the variation of uptake of p,p'-DDT by the plants in relation with the dissipation of p,p'-DDT in the soils was studied for three months (90 days) using a radio-nuclide tracer technique. Sampling was done biweekly and the residue levels were determined per dryweight of the plants and soil samples. Substantial absorption and accumulation of residue was observed for the cowpeas grown in two different sites namely,in the coastal province, Mombasa, Mtwapa, and highland region, Nairobi,chiromo campus. The degree of uptake also varied with soil type and growing conditions. A total residue level ranging from 0.945±0.040 μg/g to 7.765±0.211 μg/g were obtained for 2 weeks old to 12 weeks old Mombasa plants, respectively. For the Nairobi plants, the corresponding values fell in the range of 1.136±0.038 μg/g to 3.239±0.007 μg/g.

The extractable and non-extractable (bound) residue levels in the plants and soil samples with respect to time were also determined for the two sites. The Mombasa plants gave a range of residue levels from 0.800±0.065 μg/g to 6.110±0.038 μg/g and 0.084±0.001 μg/g to 1.390±0.003 μg/g for extractable and non-extractable residue, respectively. The corresponding values for Nairobi samples were 1.034±0.011 μg/g to 2.241±0.014 μg/g and 0.080±0.002 μg/g to 0.411±0.007 μg/g, respectively.

According to an experiment carried out for one Mombasa plant sample which has been analysed for the residue accumulation in different parts of the plant namely, the

leaves, stem and roots, the total amount of p,p'-DDT residue in the parts of the plant were in the order of stem < leaves < roots. This limited data showed also that the majority of the residue was found to be in the roots which actually accumulated five fold of either of the two parts. The percentage of extactable residue for the parts was also found to decrease in the order of leaves > stem > roots. However, the bound residue was found least in the leaves and highest in the roots.

A study on the effect of temperature on dissipation of p,p'-DDT from the soil samples with respect to the total, extractable and bound residues was also made separately for soil samples of the two sites. As a result temperature was found to have a significant effect on the variation of total, extractable and bound residue levels. For both Mombasa and Nairobi soils the total and extractable residue were found to decrease with increase in temperature and the bound residue increase with increase in temperature. The critical temperature was found to be 90°C in all cases. The halflife of p,p'-DDT in the two soil samples were also determined using first order kinetic analyses and the results gave 119.7 and 107.2 days for Mombasa and Nairobi, respectively.

CHAPTER ONE

1.1 INTRODUCTION

The astonishing advance in chemistry, physics and engineering in this era comprises the latest phase of an industrial revolution that has changed civilization. The mechanical revolution completely altered agricultural methods and now the chemical revolution is carrying onto a new height of efficiency.

A major component of the chemical revolution is the use of pesticides. By the extensive use of pesticides developed countries have become self-sufficient in terms of the total food needs. Not only has the quantity of food produced increased but there has been a consistent increase in quality. The steadily advancing demand for pesticides will continue to expand and so maintain their important contribution to increasing global food production and protection of health.

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DDT, the widely used organo-chlorine pesticide has been known as one of the most effective and economical pesticides. Before DDT fell from grace due to its persistence and toxicity, it ranked with penicillin as one of the great wonder drugs of modern chemistry (Friedman, 1992). In fact, the immediate source of heavy interest in insecticides is believed to have come from the dramatic success of DDT. Thus from the time it was learned that DDT

was effective and economical pesticide, through many years, millions of pounds of production and use continued as a pesticide without equal. This was due to its "permanent kill" characteristics, relatively low cost and ease of use (Miller, 1992).

The wide disclosure about the wide spread, dispersal and accumulation of DDT in animal tissues together with the irreparable damage that may already have been produced by chronic or delayed effect of DDT residue already in existence, has produced a strong body of opinion in favour of placing severe limitations on the use of DDT. The indiscriminate use of DDT itself in such a manner has now been reduced or suspended and even banned in many countries. Although DDT and most organochlorine pesticides are believed to have toxic effect and health hazards, it is the persistence rather than the toxicity which has resulted in attempt to phase them out wherever possible. A group of chlorinated pesticides called the cyclodienes (e.g. aldrin, dieldrin, chlordane and heptachlor) are among the most persistent of all pesticides, especially when applied to soils where they are absorbed to fine particles (Martin, 1969). The persistence of DDT is due to its low volatility,low water solubility and due to good stability though when in solution, it is readily decomposed by alkalies with loss of hydrochloric acid and insecticidal activity.

In contrast to this overriding public health consideration however, many countries of Africa and other developing countries still permit DDT to be applied regularly onto their farms. In Kenya considerable quantities of pesticides are used every year to combat human, livestock and agricultural pests. A record of import by Kenyan Government according to the report of pest control product board of Kenya shows that Kenya has been importing most of the major classes of pesticides like Insecticides, Acaricides, Herbicides, Fungicides and others (Lalah, 1993). According to the report the quantities of pesticide imported by Kenyan government in the years 1986 to 1990 were as follows.

Pesticide class	Amount (tonnes)
Insecticides and Acaricides	1076 - 1576
Herbicides	1129 - 1136
Fungicides	1330 - 6584
Others	808 - 857

Specifically, DDT, Malathion, carbofuran, Furadan and Carbonsufor were imported over the years although according to the fore-mentioned report,there has been no importation of DDT since 1985. The major use of Malathion in Kenya was against storage pests and in horticulture crops and DDT (before it was banned) as an insecticide for public health control of Malaria.

1.2 Statement of the Problem

There are many issues of concern as well as real problems that confront man in a society. Of all such problems the most pressing are those of increasing human population and food deficiency. One of the reasons for food deficiency is the crop losses caused by pests. Agricultural pests have co-existed with humans since the dawn of civilization and it is particularly difficult to separate pests from life affairs. Pests are African farmers major agricultural problems and their presence in the farm is exacerbated by poverty and other socio-economic problems. Humans in a desire for increased food and fibre production have constantly been trying to eliminate pests.Tremendous amounts of pesticides have been poured into the African environment in an attempt to combat weeds and other pests.

However, pesticides being toxic compounds have been found to have detrimental effect on the environment. Chlorinated hydrocarbon insecticides used extensively during the last decades have now presented major residue problems. Such accumulation of residues is believed to have detrimental effect on subsequent crops as well. Not only may roots be affected by those residues but some may be translocated into the crop tissues. This study was then done with a view of generating reliable experimental data as to how far and how much of p,p'-DDT may be accumulated in the widely cultivated crops around tropical African countries.

A wide range of grain legumes are grown in tropical countries like Kenya. The major grain legume crops in tropical Africa are field beans, cowpeas, ground nuts and pigeonpeas. In the present study cowpeas (*vigna unguiculata)* have been chosen because of their abundance in the farming practice of the two sites under consideration (Mombasa and Nairobi). The other major reason for their selection is that most of their parts including the leaves are edible. Cowpeas are grown as a sole crop by large scale farmers but the majority of the small scale farmers grow them as a component of inter-cropping system. Towards the end of the eighties, Nigeria had been the world's leading producer of cowpeas (Okubundo,1987).

This study was done with the consideration of the effect of inherent environmental factors such as temperature, soil type and climatic conditions. The result of this study is expected to lay out basic information supported by experimental results pertaining to the degree of possible intake of p,p'-DDT by cowpeas. The second part of this study i.e. the effect of temperature on dissipation and adsorption of p,p'-DDT was carried out with the intention of identifying the dominant environmental factors that enhance or decrease the degree of uptake by plants with regard to the fate of the pesticide in soil. This was also done considering various temperatures in a laboratory condition keeping all other factors to be constant. It is anticipated that the laboratory experiment could yield information about the actual environmental condition. The

various temperatures were taken as analogy to the varied actual temperatures where the pesticide is applied by farmers living in various zones of the earth with latitudinal variation such as the polar, tropical and temperate.

1.3 Objectives

The primary aim of this research was to bring together under one experiment the most significant environmental problem of p,p'-DDT and the uptake by plants. Persistence has been singled out as the most undesirable aspect of the compound. In this experiment attention was given to the inter-dependence among persistence, soil type, temperature and growing conditions of the plants.

Overall, the present work was done with the following objectives

1. To find out the extent to which p,p'-DDT may be absorbed or translocated by cowpeas plants

2. To analyze quantitatively the amount of p,p'-DDT taken up by cowpeas grown in two different sites; namely the coastal region,Mombasa and highland region, Nairobi.

3. To investigate how plant p,p'-DDT uptake varies with environmental factors with a view to establishing dominant parameters that affect the rate and degree of uptake

4. To compare and contrast the observations in the course of growing of the plants at the two sites.

5. To determine the amount of residue accumulated in the three different parts of the plants (leaves, stems and roots).

6. To study the effect of temperature on dissipation and adsorption of p,p'-DDT on soil particles in terms of the total ,bound and extractable residues.

CHAPTER TWO

2.1. Historical Development of Pesticides.

A pesticide is defined as a substance used for mitigation, control or elimination of plants or animals detrimental to human health and economy (MC-Graw-Hill, 1987). Any animal or plant out of context is regarded as a pest such that even volunteer cabbage plants growing with onions have to be regarded as weed pests. There are several ways of classification of pesticides but the commonly used basis of classification are:

a) chemical structure

b) types of target organisms they are applied on and

c) their mode of action.

Detailed explanation of the classification would take us far beyond the scope of this thesis. However, it becomes indispensable to mention few details of pesticides as related to the status of DDT.

Researchers from numerous disciplines including biochemistry, biology, soil science and engineering have contributed to the discovery and development of several types of pesticides. The old phase of man's attempt to use some inorganic chemicals like arsenic (As), lead arsenate [Pb_3 $(AsO4)_2$] had been found extremely poisonous to man and other non-target organisms. Besides, inorganic pesticides as a group lack both the high phytotoxicity and selectivity of many of the organic pesticide (Ware, 1975). For instance, inorganic herbicides are believed to generally

pose fewer environmental pollution problems than the organic herbicides. Any health hazard arising from the use of many of the inorganic herbicides is likely to be contact rather than systematic or physiological injury while such injuries are limited to the immediate users (Audus, 1976). For example, sodium chlorate has low mammalian toxicity but is a fire hazard. However, the discovery of organic pesticides has provided man with new and powerful weapons for his constant war against pests, vector-born diseases and weeds.

Considering the chemical structure and principles of pesticide actions, some classes of pesticides have been designed to have some general formulae. For example, organo-phosphates and carbamates were designed to have the general formulae shown in figure 1.1. The substitution of different alkyl groups in the structure give several synthetic organo-phosphates and carbamates.

On the other hand the exploration of chemical principles in this field resulted in the achievements of extended innovation and discovery of some biological control methods such as pheromones or sex-attractants. Pheromones originally are referred as ecto-hormones which are complex chemical compounds basically long chain hydrocarbons such as alcohols, esters, ketones, aldehydes and sometimes ethers. In most cases the formulation of chemical derivatives of pesticides is more or less a modification of the parent insecticide. Examples of pesticides in which derivatives have been developed are 2,4

phenoxy carboxylic derivatives and other phenoxy herbicides such as the benzoic acid herbicide, Trichloroacetate and glyphosphate herbicides (Hill,1987).

Figure 1.1 General structural formulae of organo-phpsphates and carbamates

Depending on the type of target organisms, pesticides are commonly classified as Herbicides (used to kill weeds), insecticides (used for killing harmful insects), Rodenticides (used to kill rodents), acaricides (used to kill ticks and mites), fungicides (used for the control of plant fungal diseases), nematicides, molluscides and bactericides (used to kill nematodes, molluscs and bacteria, respectively).

Lastly, pesticides have also been known in a number of names based on their modes of actions. These are;

1. Repellents - Pesticides that are designed to keep insects away.

2. Anti-feedants - chemicals that block part of the feeding response in some phytophagous insects.

3. Fumigants- volatile substances that vaporize and the toxic gases kill pests within enclosed containers, greenhouse or in soil.

4. Smokes - finely divided insecticidal powder mixed with a combustible material where the insecticide is dispersed as smoke only of use in green houses and other enclosed spaces.

5. Stomach poison- those pesticides that have to be injected by animals pests to be toxic.

6. Contact poisons - Pesticide which are usually absorbed directly through the cuticle and enter the body of the organism.

7. Systematic poisons- Pesticides which are watered into the soil,sprayed on to the plant or applied to the trunk.

2.2 Toxicity of Pesticides

All pesticides are by definition toxic to some form of living organisms but like many tools invented by human for their use, there are ways in which these chemicals can be used safely. The extent of toxicity depends on many reasons

including the mode of action of the chemical, its formulation and concentration, the dose taken, the method of intake, the state of health of the organism, its body weight and sex (Hutson, 1985). Toxicity of pesticides is expressed on the basis of Median lethal dose called LD_{50} . This is a statistical estimate of a chemical dose which when administered to test animals, will kill 50% of them under some stated conditions. This lethal dose is expressed in weight (Kg) of the test animal. The oral LD_{50}, dermal LD_{50} and vapour form of the chemical LC_{50} are some of the parameters used depending on the method of intake.

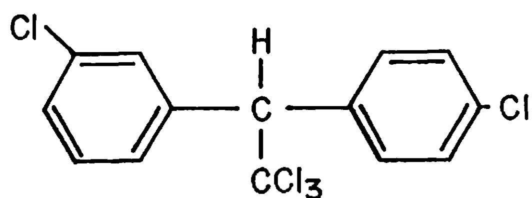
Pesticides, in general, are known to have harmful effect also on soil micro fauna and flora. Although DDT is believed to have an acute mammalian toxicity, the effect is relatively insignificant while the major problem is the tendency of DDT and its metabolites, especially DDE, to accumulate in the fatty tissue of mammals.

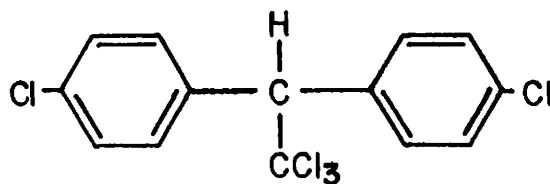
In order to have complete picture of the effect of any particular pesticide, it is of utmost importance that its actions at various levels ranging from those of molecules to whole animals be studied. In fact, DDT was found to vary with diet, race, age and undetermined individual differences. There is evidence that other pollutants may interfere with excretion of DDT. In one experiment, dogs exposed to DDT and aldrin at the same time stored twice as much DDT as dogs exposed to DDT alone (Miller, 1992). To understand the toxicological action on animals or humans it may not be enough to know the action at each level only but

the actions at various levels must be integrated to construct a picture of the toxic effect on the infant organism (Navahashi, et al, 1989)

2.3 Characteristics of DDT

The insecticide DDT has the following characteristics; Empirical formula $C_{14}H_9Cl_5$, Molecular weight 354.49, Structural formulae of the two isomers are,





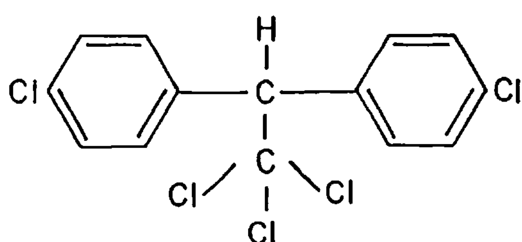
o,p'— DDT

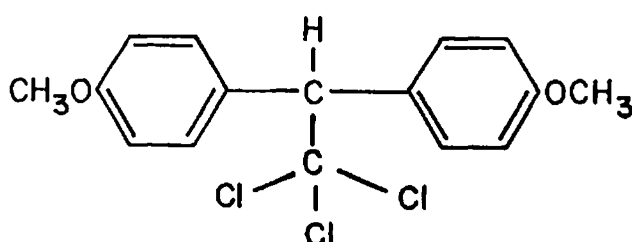
p,p'— DDT

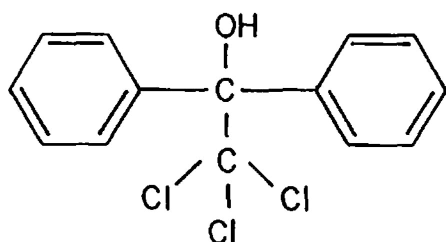
The I.U.P.A.C. name of p,p'-DDT is 1,1'-Bis (p-chlorophenyl)-2,2,2-trichloroethane. Registered trade marks of DDT in commerce are; Girasol, Noecid, Dicophane, Chloro-phenanthan and Gesopon.

Physical properties of p,p'-DDT;

It exists as white needles of melting point 108-109,°C, density = 1.6 kg m^{-3}, vapour pressure = 1.5 x 10^{7} mm Hg at 20°C, solubility =0.1 mg/l in water and moderately soluble in petroleum oils and readily soluble in most aromatic and chlorinated solvents.







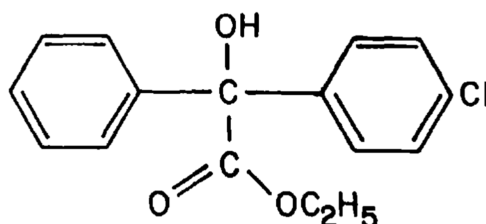
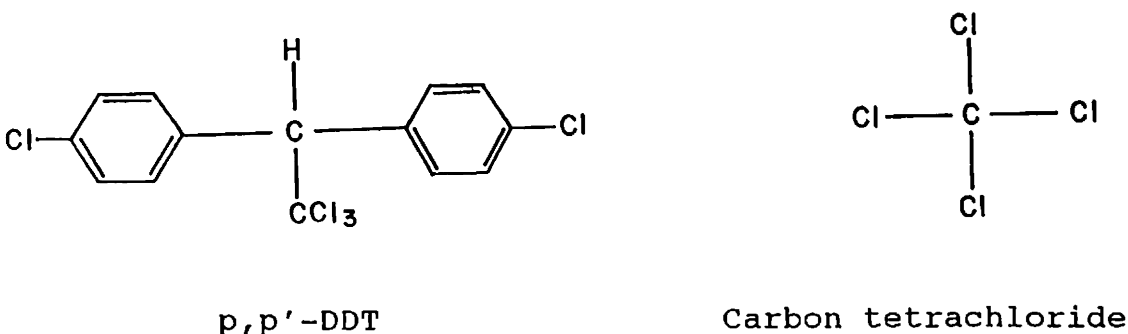


Figure 1.2 Analogs of p,p'-DDT

The p,p'-isomer of DDT forms a colourless crystal practically insoluble in water. By comparison of its structure to that of CCl_4 (carbon tetrachloride), one might predict DDT would be soluble in fats. Figure 1.3 shows the comparison of structure of DDT with CCl_4. The other analogs of DDT which have similar structures as that of DDT are shown in figure 1.2.

Biochemically DDT is nerve poison because it concentrates in the fat like brain tissue and interferes with Calcium metabolism essential to the formation of healthy bones and teeth (Hill, 1972). The main metabolite

of DDT is DDE [1.1(dichloro-ethylene)], which is formed by dehydro-chlorination under alkaline conditions. Vigorous hydrolysis yields 4-chloro α-(4-chlorophenyl) benzene



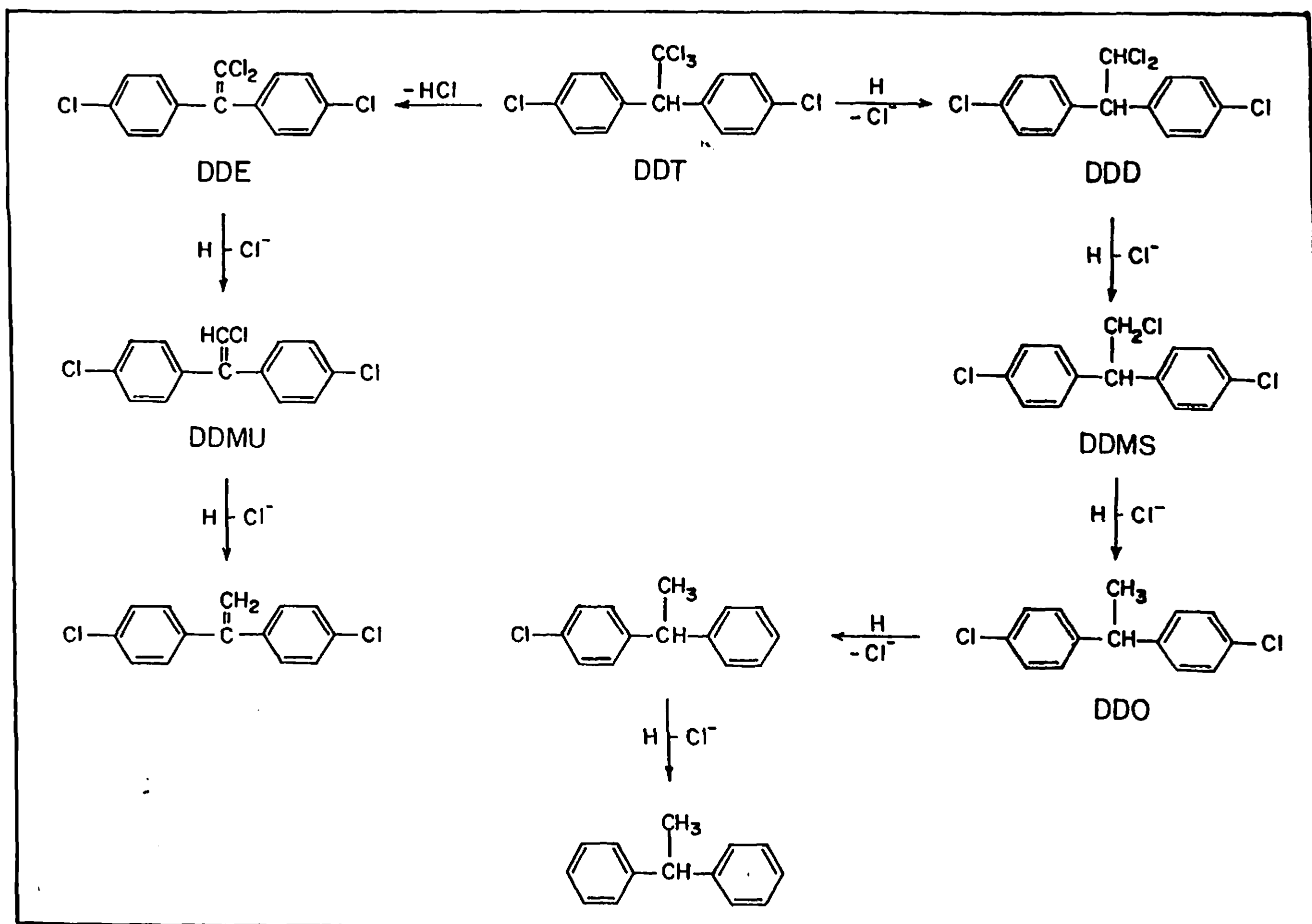
p,p'-DDT Carbon tetrachloride

Figure. 1.3 Comparison of structure of p,p'-DDT with that of CCl_4

acetic acid, DDA, which is usually excreted in urine of contaminated mammals (Hill, 1983). 1,1'-(2,2-Dichloro ethylidene) bis (4 chloro benzene), DDD, is a further degradation product which is a primary metabolite and has been detected in insects and mammalian organisms. The other common metabolites and reaction pathways are shown on figures 1.4 and 1.6. DDMS and DDMU are secondary metabolites of DDD and DDE, respectively.

2.4 Synthesis of DDT

DDT was first synthesized by O. Zeidler in 1874 but its insecticidal properties were not discovered until 1939 by Paul Muller (Fine, 1992). The Zeidler synthesis is described as a condensation reaction of chloral with chloro



Abreviations

DDD - Dichloro diphenyl dichloro ethane
DDE - Dichloro diphenyl dichloroethylene
DDMU - Dichloro diphenyl chloro ethylene
DDMS - Dichloro diphenyl chloro ethane
DDO - Dichloro diphenyl ethane
DDT - Dichloro diphenyl tri chloro ethane

Figure 1.4 Metabolic and degradation pathways of DDT

benzene in the presence of sulphuric acid as shown in figure 1.5.

The abbreviation DDT refers to Dichloro Diphenyl Trichloro ethane. The technical product as a result of the the reaction indicated (figure 1.5) is a mixture of compounds and may contain up to 30% of the o,p'-isomer (ortho-para isomer). The specification universally accepted for DDT and approved by W.H.O. calls for a content of at least 70% p,p'-isomer (para-para isomer) which is the more effective insecticide (Hill, 1978). The factors responsible for the phenomenal success of DDT in the years subsequent to its synthesis and discovery of insecticidal

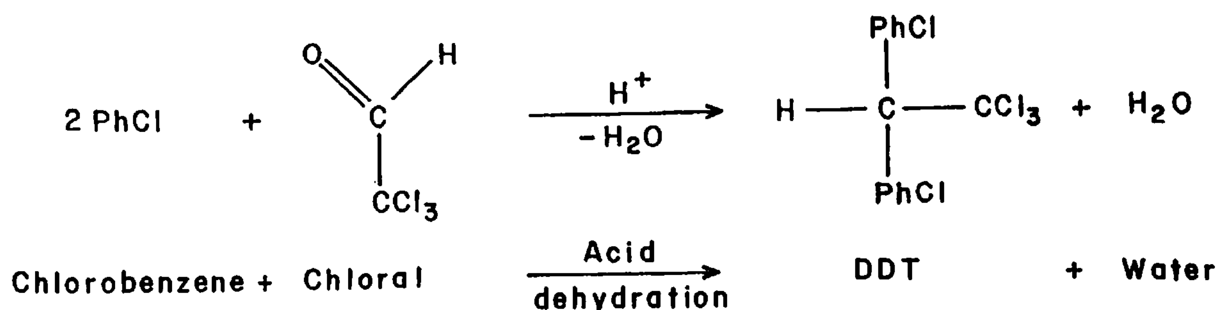


Figure 1.5 Synthesis of DDT

activity are:

i) High insecticidal activity and broad spectrum.

ii) Low price

iii) Simple manufacturing and handling process

iv) Long duration of activity and low acute mammalian toxicity.

2.5 Pesticidal Activity of DDT

The wide-spectrum of DDT allows it to be applicable in almost all sectors of pest control activities. Before it was banned, DDT had been used as a powerful antidote

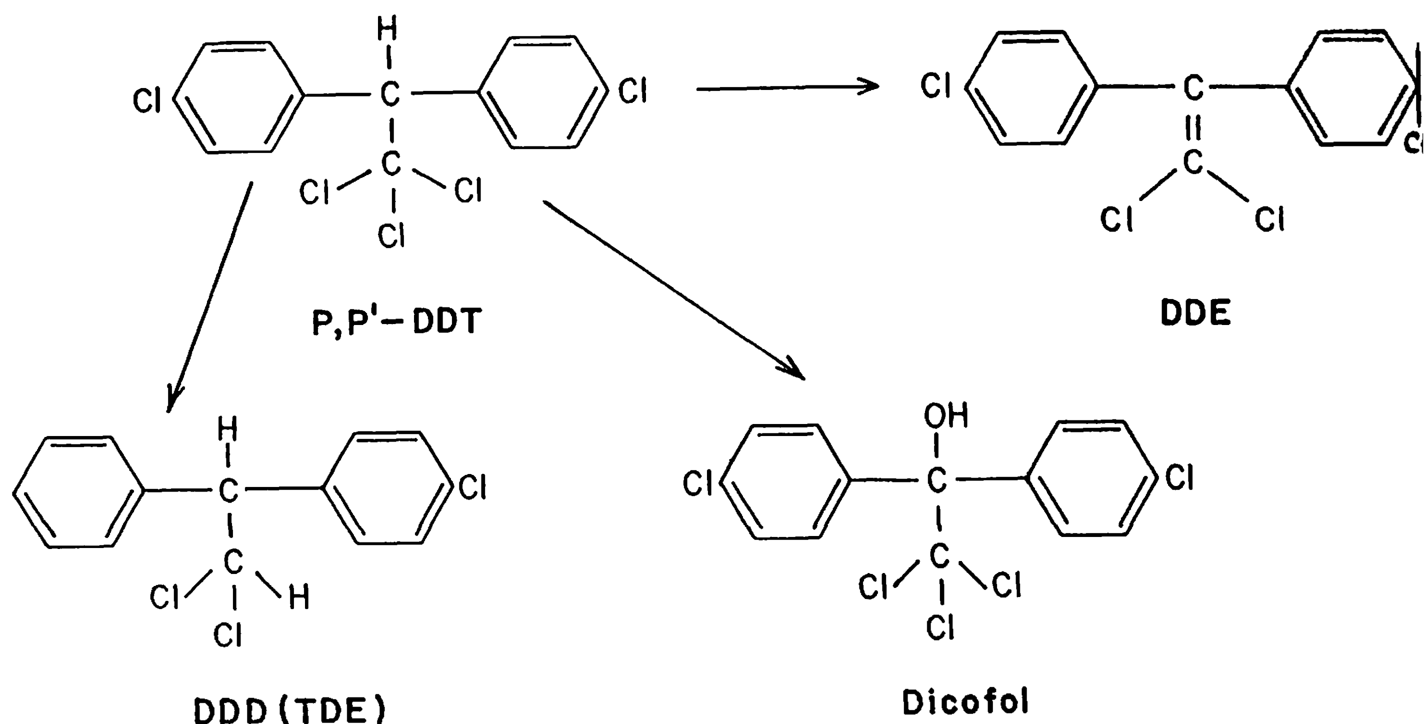


Figure 1.6 Metabolism products of DDT and their structures.

of most insect species. DDT is both stomach poison and residual contact insecticide, which even a walking insect can retain lethal dose of it when it comes in contact with sprayed leaves. Insect species Dipteral (flies, gnats) and chewing insects (beetles, caterpillars) are very susceptible to DDT, whereas aphids are only slightly susceptible to DDT and spider mites not at all. The pronounced contact activity of DDT is due to the highly lipophilic characteristic of the compound, which enables it

to penetrate the insect cuticle. DDT coating on solid surfaces has a considerable duration of activity such that a mixture of DDT with pyrethrum in household spray guarantees a rapid knockdown effect. Little is known about the possibility of chronic poisoning by DDT. However, it is believed that DDT acts by disturbing the sodium balance of the nervous membrane and the enzyme catalysed conversion into inactive DDE (Hill, 1978).

2.6 LITERATURE REVIEW

2.6.1 DDT Residue in the Environment

Once DDT finds its way into the soil, water bodies and non-target organisms, the very same properties such as persistence and toxicity to wide variety of insects, that makes it a valuable agricultural chemical, starts imposing some undesirable long term and short term effects. This phenomena is related to the slow degradation and dissipation rates of DDT. This aspect in relation to the residue accumulation has been studied extensively in soil, animals and plants.

It has been estimated that less than 0.1% of the pesticides applied to crops reach the target organisms; thus, more than 99% of the applied pesticides have the potential impact on non-target organisms and to become widely dispersed in the environment (Tardiff, 1992). The same report has revealed that approximately 5 million tons

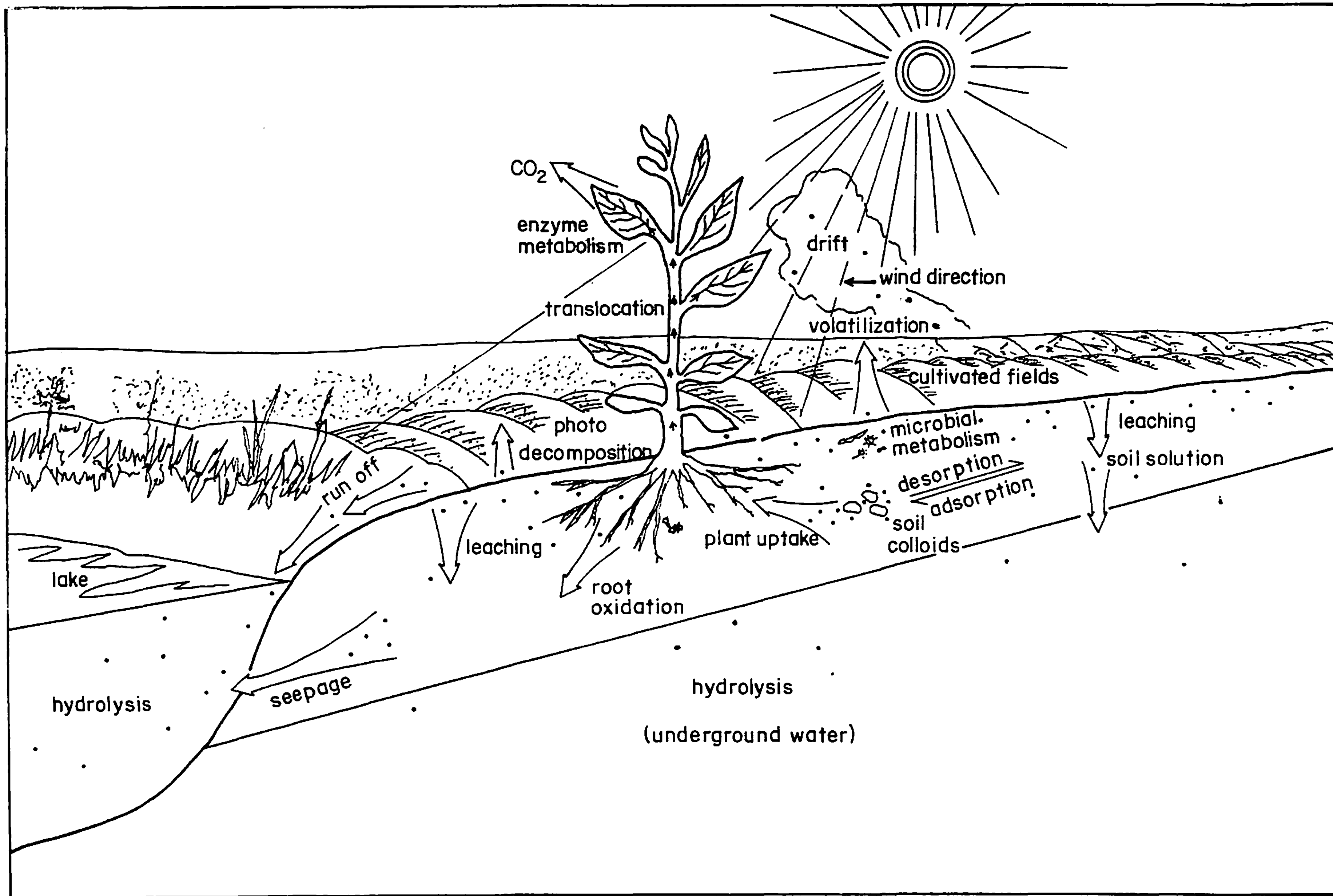


Figure 1.7 Major processes affecting the fate of pesticides in the environment.

of pesticides, perhaps 70% herbicides and only 5% insecticides are applied annually in the world of which about 70% is used for agriculture and the remainder by public health agencies and government agencies for vector control by home owners. Likewise some of the DDT sprayed from planes is carried into the upper atmosphere, to come down eventually in rain water in even the most remote places. These and others are the main factors that enhance the chance of making the environment rich in residue of several pesticides. The major processes affecting the fate of pesticides in the environment are shown diagramatically in figure 1.7.

2.6.2 DDT Residue in Soil

Soil is a complex mixture of minerals, organic matter, water and air. It is a natural medium for the growth of land plants and is a dynamic natural body on the surface of the earth in which physical, chemical and biological processes occur. Extensive studies have been made on the rate of degradation and dissipation of DDT in soils. For example, to help explain low concentration of DDT found in some birds of Kenya, a field test of the rate of disappearance of DDT from tropical soil was conducted by Siecher and Hopcraft (1978) in the Department of Chemistry University of Nairobi. This study which was conducted at a place near Lake Nakuru, gave a halflife of 110 day for DDT. Their data suggested that the DDT sublimed directly

without prior degradation to DDE and the results showed that sublimation alone can account for the disappearance of pesticides of low volatility even if they are strongly adsorbed on soils.

According to another experiment conducted by L. Vollner on soil in 1993, DDT was found to decompose and evaporate in a semi-closed system during 55 days up to 20%. The rate of evaporation and degradation in an open system was found to be higher than 80% during the same period of exposure. Similarly, a study on the degradation and dissipation of ^{14}C-p,p'-DDT in tropical soils of Nairobi under field and laboratory conditions for a period of six months by Lalah (1993) showed that DDT dissipated more rapidly from the soil under field condition of tropical region than reported for temperate region. An overall halflife of 64.6 days was found for Nairobi while researches done in most tropical countries showed longer half lives of more than 80 days. Moreover, in the same study the effect of solar radiation on the dissipation of ^{14}C-p,p'-DDT from non-sterile soil was also studied under laboratory condition and it revealed that the major dissipation process involved in the degradation of p,p'-DDT was volatilization.

Similar study on dissipation and degradation under field condition of ^{14}C-p,p'-DDT in Mtwapa, at Kenya coast over a period of 168 days as reported by Ng'ang'a (1994) showed that p,p'-DDT dissipates in a biphasic pattern i.e. a rapid phase from 57-168 days with an over all halflife of

270.2 days over the entire 168 days period. Another report of the second FAO/IAEA research co-ordination meeting on radio-tracer studies of behaviour of DDT in tropical environment has also shown that the disappearance of the radioactivity and extractable p,p'-DDT generally followed a biphasic curve with initial curve being rapid followed by slower phase. According to this report, the dissipation of ^{14}C-p,p'-DDT from soil was studied under field conditions for one year in nine countries. Volatilisation and microbial degradation were apparently found to constitute the main mechanism by which DDT dissipates from soil. Among the nine countries, in Indonesian soil about 2/3 of the applied dose dissipated during 36 weeks and bound residue was only 2%. In Nigerian soil, only 26% of the applied dose dissipated during 32 weeks and binding to soil was relatively high (10%).In Hawaii, the halflife of DDT was estimated to be 105 days. In Egypt, DDE showed a higher rate of dissipation than DDT and only 10% of the applied dose could be recovered from a 14 cm column after 3 months. Furthermore, all experiments indicated a relatively high percentage of dissipation of DDE from soil,largely due to volatilisation.

Persistence of some chlorinated hydrocarbons and p,p'-DDT have also been found to depend on soil type, rate of application and temperature. A field and laboratory studies conducted to determine the effect of soil type, rate of application and temperature on the persistence of DDT, Lindane and Aldrin have shown that soil temperature is an

important factor in the persistence of insecticides (Liechtenstein,1958). According to this study, no insecticide loss was found in frozen soil. At a temperature of 6°C (16% to 27%) of aldrin and heptachlor were lost during a period of 56 days and only 2% to 14% of the initial insecticides were found after 56 days when held at a temperature of 46°C. DDT was found to be the most persistent of the three insecticides investigated and the difference in the rate of loss as influenced by muck and loam soil was the least noticeable. The depletion curve obtained for DDT in those two soil types were nearly parallel after the initial loss during the first summer season. Besides, the amount of DDT recovered from muck soil was 1.4 times more than that recovered from Miami silt loam six months after treatment and the same ratio was obtained when both soil types were analyzed for DDT during the following three years.

The influence of other factors like concentration, air flow and vapour pressure were also studied by several researchers. Volatilization of pesticides from soil surfaces and subsequent dispersion in the atmosphere is one means by which pesticides may rapidly spread throughout the environment. Therefore, it is important to know how various soil properties influence volatilization. Farmer (1972), have studied the influence of these factors and concluded that the vapour density of the soil-applied pesticide was the main factor controlling volatilization. According to this report the rate of volatilization of

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dieldrin concentration from Gila silt loam increased with increasing soil pesticide concentration until the soil dieldrin reached 25 μg/ g. An increase in temperature of 10°c increased the rate of volatilization approximately four fold. This study showed also that increasing the rate of air movement over the soil surface from 0.005 to 0.018 mile/hr increased the rate of volatilization two fold and the order of volatilization of the three insecticides was lindane > dieldrin > DDT. The same order was observed for increasing vapour pressure although the increase in the rate of volatilization of the three insecticides was less than the increase in saturation vapour density of the pure compound. Generally, the volatilization rate decreased rapidly as soil pesticide concentration decreased.

2.6.3 DDT Residue in Animals and Humans

Findings of many studies have shown that DDT residue accumulation in humans and animals is also very common. This results from the intake of residues through several circumstances such as ingestion, feeding on DDT residue containing plants and body contact between DDT and the body of the organism. Once it gets into the body of the organism it may generate immediate or long term toxic effect on the organism. DDT is known to have harmful effect on bees, fish and livestock. Chlorinated hydrocarbons show high affinity for fats and are concentrated in fatty tissue of animals (Martin, 1969). Several reports have shown that DDT is

stored in the body fats of birds and animals and excreted in the milk of mammals. Once stored, most are readily excreted which has led to a negative impact on some non-target organisms. According to what was reported by Stecher and Hopcraft another study on chlorinated hydrocarbon residues at lake Nakuru was made by Koeman, *et al.*, in 1970, samples from several species of birds and fish were collected and analyzed for DDT, DDE, DDD and dieldrin and endrin. The results which were gratifyingly low, ranged from less than 0.001 to 0.064 ppm. The low level of pesticide residues found in this study suggested that these insecticides disappear more rapidly at lake Nakuru than in temperate climates where the disappearance is caused by sublimation, chemical degradation, bacterial degradation and transportation by wind erosion of surface dust.

Chlorinated hydrocarbons at low concentration have been especially harmful to some fish and birds species owing to the tendency of these chemical to become concentrated in fatty tissues and disrupt normal calcium-transport in cells. DDE, the toxic metabolite was an early suspect in egg shell thinning in pelicans and decline of bald eagles, osprey, and peregrine in the U.S.A. This has been generally attributed to wide spread use of insecticides (Blus *et, al.*, 1971). An evidence in favour of this was also found showing partial recovery of some of the metabolites from birds following the ban on most DDT use in the U.S.A. Soon after the introduction of DDT it was noted that there was a general decline in arthropods

variety in forested ecosystem (Haffman, 1967).

Increased storage of organo-chlorine pesticides has been found in people suffering from chore disease such as partial cirrhosis, carcinoma and hypertension (Liechtenstein, 1978). In another study by Ehrlich, 1972, in high doses, DDT was shown to increase the incidence of cancer specially liver cancer in mice. This indicates that DDT might also be carcinogenic in human being. At concentrations of roughly 10 ppm, DDT in rats has been shown to induce abnormally high levels of certain enzymes that breakdown many prescribed drugs and rendered them ineffective. It also increases the weight of uterus and deposition of dextrose in the uterus. The same study has suggested also that DDT affects the sex hormones of rats and birds and may produce sterility in rats. Rats reproductive physiology shows many similarity to humans but much is not known whether human changes occur or what their effects are if they do.

Findings of other studies have also shown the correlation between DDT levels in infant tissues and cause of death. Concentrations of DDT and its breakdown products, DDE and DDD as well as dieldrin were significantly higher in the fat of patients who died of softening of the brain, cerebral haemorrhage, hypertension, portal cirrhosis of the liver and various cancers than in groups of patients who died of infectious diseases (Ehrlich, 1972). The history of the patients in the study showed that concentration of DDT and its breakdown products in their fats were strongly

correlated with home use of pesticides, heavy users, having much higher concentrations than light or moderate users. According to the same report the mean concentration of DDT in human population was found to vary from one geographic location to another, both within and among countries analyzed.

2.6.4 DDT Residue in Plants

As soil containing insecticidal residues are used in agriculture, the insecticides might be translocated into various plant parts and consumed by humans and animals. To obtain more information as to the extent to which insecticides are translocated from contaminated soils into plant tissue, the relationships between the absorbance, insecticidal residues, soil type and crops were studied by many researchers out of which the present work is one.

Studies done by researchers as early as 1964 (Miles and Haris,1964) on organochlorine insecticides revealed that pesticides applied on 15 farms in north Ontario, Canada were consistently found in all plants and were the highest in the order orchards > vegetables > tobacco > other field crops. Comparison made by the same study indicated no significant accumulation of residues in mineral soils used for production of fruits, tobacco and other field crops. Vegetable samples collected from Bombay markets were found to contain residues of organochlorines in a three years study by Khanderkar *et al.*,(1982). An

experiment carried out by Shahamet (1980) also showed that soil bound residues of DDT were absorbed by oat plants grown in organic soil treated with radio-labelled DDT. The oats were found to contain more extractable residues (75%) with nearly 51% on the shoots.

To answer the question as to which of the two forms of soil residues (bound or extractable) interact with the environment and are likely to be taken up by plants and animals, the following example could be cited.The study on data release of an extractable residue of methyl parathion and potential pick up of the radio-labelled residue by earth worms and oat plants indicated that soil bound pesticides are not entirely excluded from environmental interaction (Fuhreman, *et al.*, 1978). According to this study once they had pre-concentrated into animal or plant tissues, they were translocated and found partially in an extractable and bound forms. 58 to 66% of the pesticide taken up by the earth worms became bound in the bodies and 82-95% of the residue in oat plants were found extractable.

Some examples could also be cited concerning the studies on absorption of chlorinated hydrocarbon insecticides from soil into various crops. A study which was made of the extent to which insecticides may be absorbed and translocated from contaminated soils into plant tissues, and the relationship among absorbance of insecticidal residues, soil type and crops for three insecticides namely DDT, lindane and aldrin was done by Liechtenstein (1959) and the pesticides were found to have

been absorbed into crops, the degree being dependent on the type of the crop, the soil type in which the crop had grown, the concentration of the pesticide within the soil and the type of pesticide. Various soil types such as sandy loam soil, Miami silt soil and muck soils were taken as soil receiving the pesticides and plants like carrot, potatoes, peas, beans, cucumbers, tomatoes and cabbages were taken as variety of plants. Of all these, carrots were found to have not only absorbed more insecticides than any other crops but in the case of lindane accumulated greater quantities of the chemicals than occurred in the soil. Moreover, the insecticides were most readily absorbed from a sandy loam and least from muck soil. Carrots were found to have contained 7.7 times more insecticide residue and was accumulated with mostly in the edible part. The amounts absorbed by the same crop from the same type of soil were not in direct proportion to the concentration of the insecticide recovered from the soil and relatively less insecticide was absorbed from soils in which the insecticide was most concentrated. Crops grown in aldrin treated soils contained within their tissues both aldrin and dieldrin. From this experiment it was concluded that the soil type itself seems , in most cases, to have a remarkable influence on the absorption of lindane into crops.

2.6.5 Degradation of DDT by Soil Microorganisms

As a matter of natural ecological process chlorinated hydrocarbon pesticides which have found their way into the soil are expected also to undergo biodegradation by the micro organisms found in soils. Usually it is the top few centimetres of the soil in which most of biological activities take place. Considerable evidences exist, which show that these pesticides are decomposed by micro-organisms (Patil *et* al. and Pilmer *et al.*). Twenty microbial cultures which had been shown to degrade dieldrin were tested by Patil *et al*,(1970),to determine their ability to degrade DDT, endrin and other pesticides. The results showed that all isolates were able to degrade DDT and endrin. Moreover, the DDT metabolites were tentatively identified and the majority of the microorganisms tested were capable of converting DDT to DDD and none of the cultures produced DDE.

Further more, in another study carried out by Guenze and Beard (1984) on anaerobic biodegradation of DDT to DDD in soil, the result showed that a considerable amount of DDT was converted rather rapidly to DDD with only 57% of the initial DDT recovered in identifiable products after 4 weeks of incubation. According to this study seven possible decomposition products were separated by thin layer chromatography which showed that the DDT was dechlorinated by soil micro-organisms to DDD and only traces of the other degradation products were detected and

no degradation of DDT was detected in sterilized soil.

Although the DDT-DDE conversion has been reported in a number of systems, the exact mechanism of the reaction is still obscure. Some have been carried out on specific micro-organisms as well. The experiment of Kallman and Andrew (1963) on the conversion of DDT to DDD by yeast demonstrated that the DDE is not altered by this organism. It seemed unlikely that dehydro-chlorination occurred. In this experiment it was also confirmed that incubating DDT and DDE together, the rate of production of DDD did not exceed that from DDT alone and the recovery of DDE was the same as that of DDE incubated alone. The trichloro methyl moiety which appears in many organic pesticides, in many respects is one of the most difficult group to degrade from a metabolic standpoint. Under anaerobic condition, however, DDT is apparently susceptible to comparatively rapid reductive dechlorination by numerous species of micro-organisms. The result of another experiment conducted by Guenze, *et al.*(1984) has also shown that during anaerobic decomposition, DDT was converted directly to DDD and further breakdown did not result in an appreciable build up of the other identified product. Generally, the loss of pesticides through processes involving volatilization, photo-decomposition and microbial decomposition is expected to be more rapid under tropical and subtropical conditions (Hill, 1978). DDE is the major DDT-derived residue normally found in DDT contaminated systems. It is produced by dehydro-chlorination of DDT by

most insects, birds and mammals. The development of resistance to DDT in some species of houseflies and mosquitoes is closely correlated with greater capacity of resistant organisms to convert DDT to less insecticidal DDE. DDE is particularly effective at inducing microsomal enzyme in mammalian liver. This is not necessarily a harmful effect and does not alter the body's capacity to metabolise many types of foreign compounds including pollutants and medical drugs (Hutson, 1985). Generally, dissipation of pesticides in soil involves both degradation and transfer processes. Degradation may be chemical, photo chemical or biological. Transfer processes include, adsorption, oxidation and retention of the unaltered pesticide by plants or other organism; retention and release by particular matter movement of pesticide, vapours from the solid to the atmosphere; movement of pesticides downward through the soil in percolating water and movement of the soil into the surface waters.

CHAPTER THREE

3.0 EXPERIMENTAL SECTION

3.1 Introductory

3.1.1. Basic theory of Radioactivity.

With the discovery of radioactivity by Becquerel (Ensenbud,1987) at the end of 19th century and further discoveries on the nature of radioactivity in this century, various achievements have been made to exploit the chemistry of radioactivity. The study of radioactivity may be described as the investigation of radiation emitted in the spontaneous disintegration of certain atomic nuclei. Nuclear dis-integration of atoms is related to the stability of a nucleus and the stability of nucleus may often be inferred from the ratio of number of neutrons to protons which increases with atomic number.

The decay of a radioisotope is frequently accompanied by emission of one or more of the following radiations: Alpha (α), Beta (β) and photons (x-rays and gamma-rays). The rate of decay for all these radiations obey the law,

$$\frac{dN}{dt} = -\alpha N \quad \text{.........Eqn(1)}$$

where α is the decay constant, t is the time of the radioactive decay, N is the number of radioactive atoms of

halflife t.

Alpha (α) particles are emitted almost exclusively by elements above Lead (Pb) in atomic number. The energy is usually between 4-6 MeV and is dissipated in a short distance giving a high specific ionization. Beta (β) particles are emitted by many nuclides and the determination of the absolute disintegration rates of beta emitters is relatively easy. Moreover, they take few nano seconds to dissipate all their energies and the range may be a meter or more in air and several millimetres in aluminium. Thus beta detectors often absorb a part of beta particles energy. Generally, nuclear decay event produce approximately 10 protons per k.eV of energy. The energy dissipated in a period of time is on the order of $5x10^{-9}$ seconds. The process of nuclear decay is however a highly complex process involving the laws of relativity so this description is obviously an over simplification of radioactivity.

3.1.2 Measurement, Quantification and Interpretation of Radioactivity.

Radiation from a radionuclide can be detected and measured in many ways. The best method to employ in any particular situation depends upon the nature of the radiation and the energy of the radiation or particle involved. However, the random nature of nuclear events requires that a large number of individual events be

observed to obtain precise values of the count rate.

Several factors must be considered when attempting to measure any activity. Activity could be expressed using several units of measurements. It is usually quantified in terms of becquerel which is a measure of disintegrations per second (dps), curie, where 1 curie (ci) is 3.700 x 10^{10} dps, specific activity, the activity per unit quantity of radioactive sample which is expressed in a variety of ways by disintegrations per second per unit weight or volume. Units such as micro-curie or milli-curie per milli litre, per gram or per milli-mole are also widely used. For labelled compounds, the last unit of specific activity is preferably used.

Most old model liquid scintillation counters, like the one used in the present work give a readout of counts of radioactivity in terms of counts per minute (cpm). This, however, has to be converted into disintegrations per minute (dpm), which is of a practical meaning to quantify the amount of radioactivity. Mathematically, the (cpm) and (dpm) values are related by the equation

$$dpm = \frac{cpm}{\%Eff.} x100 \qquad ....Eqn(2)$$

The percentage efficiency which is determined from the calibration curve (see section 3.2.5) that takes care of the correction factor for any loss of counts due to

interfering factors such as quenching effect

3.1.3 The Tracer Methodology

Tracer methodology is an offspring of nuclear science which has provided essential support for the ever-widening and deepening knowledge of structure and function in physical, chemical and biological systems. The tracer method is defined as a technique used to investigate certain characteristics of a population of specific objects such as molecules, organisms or other entities by observing the behaviour of the tracer (IAEA,1991). In principle, a stable isotope can be used as a tracer just as well as a radioisotope, but generally, isotopic tracers are most commonly used to follow the path ways of entity in a chemical, physical or biological systems.

The choice of the radioactive label to be used may vary depending on the nature of the entity. For instance, for an intact organism or an inorganic object, the radioactive label used may belong to any element. That is, the radioactive label used may belong to one of the elements in the tracer. In most cases ^{14}C, ^{3}H, ^{15}N, ^{32}P, ^{35}S ^{36}Cl or ^{131}I are used in radioactive labelling of the compounds under consideration because of their abundance and efficiency of detection. The label may be incorporated into the tracer through biological growth, chemical synthesis or exchange processes. Some common ways of radio-labelling entities are described in the following section.

On the other hand if the tracer is a mineral nutrient the label should be an isotope of that element. The general elements in the same chemical group e.g. alkali metals, have similar chemical properties but not sufficiently so far an isotope of one element to serve in general as the tracer for another element in the same group. Nevertheless; ^{14}C, the long lived C-isotope is the most important single tool made available by tracer methodology because carbon occupies the central position in the chemistry of biological systems (Packard, 1985).

3.1.4 Preparation of Radio-labelled Compounds

Although the use of radioactive tracer technique is often an excellent way to study chemical reaction, follow process streams etc, less use is made of them than would be expected. This is due chiefly to difficulties in obtaining radioactive tracers in their proper chemical forms (Roth child, 1965). The introduction of a radio-labelled material into a sample system on a measurement of a natural or produced radioactivity of a system becomes very useful technique for rapid and economical method of analysis for the element or materials. Isotope dilution with radioactive tracers and labelled reagents, activation analysis on the use of radioactive tracers for procedure development have much use in analytical chemistry.

Novel means of introducing ^{3}H and ^{14}C into organic compounds have received considerable attention, and such

techniques have mainly made use of a hot-atom chemistry involving nuclear reactions. However, this procedure has serious disadvantages. Apart from the step by step conventional synthesis, with the availability of purified enzyme and enzyme systems, a great variety ^{14}C and ^{3}H labelled compounds of biological interest could be prepared with relative ease.

Some radio-labelling processes involve simple consideration reaction which provide good yields of radioactive products. However, prior to conducting the radioactive synthesis, one or more trial synthesis should be made with non-labelled material in order to gain experience and confidence in the procedure to be sure that the reagents are of acceptable quality. Analysis of the solution of the tracee by two dimensional TLC or autography and by liquid scintillation counting procedure to obtain an estimate radiochemical purity of the preparation and the identification as well as quantifying impurities is also important. In some cases if the purity is less than 95% additional purification by recrystallization or other methods may be needed before the preparation is used for experimentation. The radio-labelled ^{14}C-P P′ - DDT used in the present work, however, was not prepared locally but bought ready made from International Isotope, Munchen, Germany.

3.2 INSTRUMENTATION

3.2.1 The Radio-isotope Tracer Technique.

Although pesticides and their environmental impact are mainly analyzed by GLC, HPLC and TLC, the use of nuclear techniques and in particular radio-isotope tracer technique is a widely used vital part of pesticide research. Generally, nuclear techniques are based on the use of a source of radiation and detection of radiation. Being a nuclear technique, therefore, radioisotope technique also makes use of the advantage of traceable radioisotope elements which emit radiation and by virtue of their systematic detection and estimation of radiation,qualitative and quantitative information could be obtained.

In dealing with a radio-isotope tracer technique, instruments which estimate the radioactivity in various ways are used. The general requirement for laboratory counting equipment include, high sensitivity to the radiation being measured, high counting efficiency, low background counting rates and stability. The usual instrumentation for counting consists of a detector, pre amplifier, amplifier, power supply and count recording equipment (see Figure 2.1). The auxiliary equipment such as shields, timers and printing devices may also be required. Pulse height analyzers either simple or multi-channel may be used with the proper detectors to give qualitative and quantitative information on several

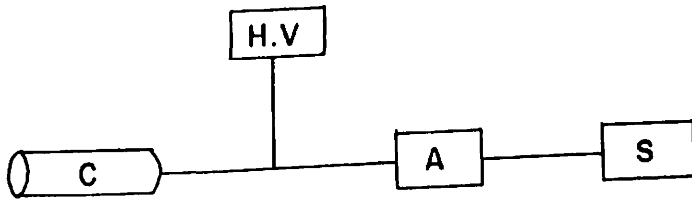
constituents in the sample.

Generally, three main types of counting equipments are known.

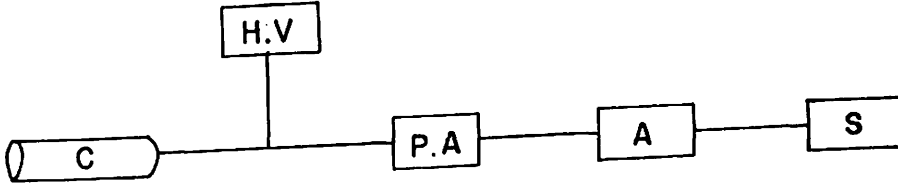
1. Geiger-Muller (G.M.) counters
2. Proportional counters
3. Scintillation counters.

The scintillation counting method is the one used throughout the present work. Its general principle of operation and description has been given in (section 3. 2.2). However, a brief description of the other two methods may also give us an over all view of the principle of radio-isotope tracer technique.

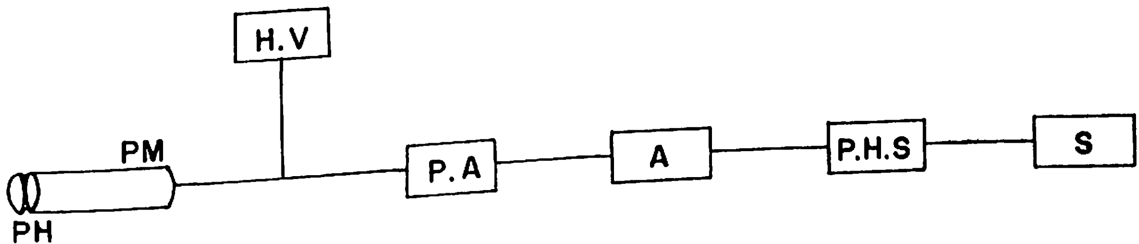
Geiger-Muller counters are used most widely for the detection and measurement of beta particles. G.M. counters operate at a reduced gas pressure and contain certain amount of quenching gases like alcohol or halogens. The efficiency of G.M. counters is determined mainly by the performance of the G.M. tubes which are used as detectors. For gamma rays they are not very effective because most of the photons will penetrate the gas without any interaction. Although energetic beta particles, electrons and gamma or x-photons emitted by radioactive liquids may be counted by G.M. counters, it is not possible to count low energy beta particles because of a problem that arises from the adsorption on the wall of G.M. tubes. Alternatively proportional counters are used to count and detect low energy beta particles such as from Carbon 14 isotope. In this technique the radioactive samples are placed inside



Geiger - Muller counter.



Proportional counter



Scintillation counter

Abreviation

A - Amplifier
P.H.- Scintillation Phosphor
P.M.- Photo-Multiplier
S - Scaler

P.A. - Pre-Amplifier
H.V. - High Voltage Power Supply
P.H.S. - Pulse Height Analyzer
C - Counting

Figure 2.1: Schematic block diagram of radio-activity counting equipments

the detector which will be transformed by a gas at atmospheric pressure.

Scintillation counters make use of a substance called scintillators for the absorption and remission of light. A scintillator is a substance which emits a small flash of light when stuck by fast charged particles. An example of a solid scintillator is (Ag)ZnS hit by an alpha ray. Depending on the type of scintillator used two types of scintillators are known, the solid and liquid scintillator.

Solid scintillator called flours are particularly suited for the detection of gamma rays, x-rays and annihilation radiations because of the high densities of certain solid crystals. The alkali halide, in particular NaI, has been the most useful. When a gamma ray photon is partially or totally absorbed in the scintillation crystal, at least one fast electron is liberated. These fast electrons cause excitation and ionization along their paths in the crystal. After counts have been collected for a period of time, the readout of the memory will be a gamma ray spectrum of the radiation absorbed by the scintillation detector. One of the main advantages of solid scintillation counters is the very short resolving time which enables high counting rates to be determined upto about 1000 counts/sec without the necessity of resolving time correction (IAEA,1983).

3.2.2 The Liquid Scintillation Counting (L.S.C.) and Its Principles

Like any other matter in motion, beta particle radiation dissipates its energy by collision in the medium in which it is released. In a liquid which is relatively dense medium, beta particles will travel only short distances before all their kinetic energy is dissipated. The energy is absorbed by the medium in three forms, heat, ionization and excitation of the molecules. Therefore liquid scintillation counting is an analytical technique which is defined by the incorporation of the radio-labelled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into nuclear photons (Packard, 1985). Besides its high sensitivity because of its being applicable to all forms of nuclear emissions (alpha, beta and gamma), liquid scintillation counting today is the most widely used technique for the detection and quantification of radioactivity. It offers many unique measurement advantages such as homogeneous sample geometry, no adsorption effect and maximum radio nuclide counting efficiency.

In most applications of liquid scintillation counting many researches involve the use of only one type of radio label tracer methodology. For example, the pesticide used in this research has been labelled with ^{14}C-radioisotope of carbon which emits low energy beta particles. In such cases

the basic objectives of the technique is to arrange for emitted beta particles to collide with the solvent molecules. The energy resulting from the collision excites the solvent molecules and is transferred to other molecules until it is finally transferred to the scintillator compound.

3.2.4 Scintillation Counting Aiding Reagents

In liquid scintillation counters, the solvents also involve in some effective absorption and remission of light. However, excited solvent molecules do not have the right wave length and are not readily recognized. Therefore, a scintillation solution (cocktail) which consists of a mixture of solvent and solute is used to aid the process of detection of the radio-activity. In this case, the bulk solvent must efficiently transfer energy to a scintillator molecule, and be capable of dissolving the scintillators and the sample material. Aromatic solvents such as toluene or xylene, are favoured because of their efficiency in energy transfer. 1, 4-Dioxane is employed when large amounts of water are involved; 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.

Two kinds of scintillators are commonly used in liquid scintillation counting techniques; the primary scintillators and secondary scintillators. A typical characteristic of a scintillator is its ability to absorb light at one wavelength and remit it at longer wavelength. Most scintillation spectrometers are sensitive to the fluorescent emission of primary scintillators. However, if an older model is used it may be necessary to add a secondary scintillator. Secondary scintillators absorb the light emitted by the primary scintillators and emits it at a yet higher wavelength. When used a secondary scintillator is added to the extent of one tenth or less of the primary scintillator.

The most popular primary scintillator is 2,5-diphenyl oxazole (PPO), and 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 fluorescent emission maximum lies in the range from 360 - 365nm whereas that for POPOP lies around 410 - 420nm. If a radioactive carbon dioxide is being measured, the scintillator solution should contain a trapping agent, 1-amino-2-phenylethene (Phenythylamine) called cocktail. Chemical impurities may interfere with the transfer of energy from solvent to solute to produce chemical quenching; or they absorb the light emitted from the solution molecules to produce colour quenching.

Chemiluminescence will give yet a third change in the spectrum. Colour quenching can be reduced or eliminated by digestion with hydrogen peroxide, perchloric acid or elution through activated charcoal. However, for ^{3}H and ^{14}C complete combustion of the samples to water and soluble carbonates produce a simple counting method.

3.2.5 Counting Interferences and Correction Procedures.

A number of counting interferences have been known to plague liquid scintillation counting users since the introduction of the technique. The quenching effect which is caused by several factors is one of the inherent problems in liquid scintillator process. The counting efficiency of the solvent solute system can be affected by many different types of quenching factors which may reduce detection efficiency. This may briefly be described as:

(1) Photon quenching which is complete transfer of beta particle energy to solvent molecules.

(2) Chemical quenching, sometimes called impurity quenching which causes energy loss in the transfer from solvent to solute and

(3) The colour quenching which is the attenuation of light photons in the solution.

As a result of these quenching problems the energy spectrum detected from the radionuclide appear to shift towards the lower energy (Figure 2.2). From the change in

energy distribution, it appears that the counting efficiency is dependent on the degree of quenching and thus on the nature of the sample; the scintillator used and the preparation method. It is therefore essential to monitor the counting efficiency in each sample for comparison with standards on other samples to be meaningful. In modern automatic scintillation counters, the counting efficiency is determined for each sample and the detected counts are converted to disintegrations to correct for the quenching effect.

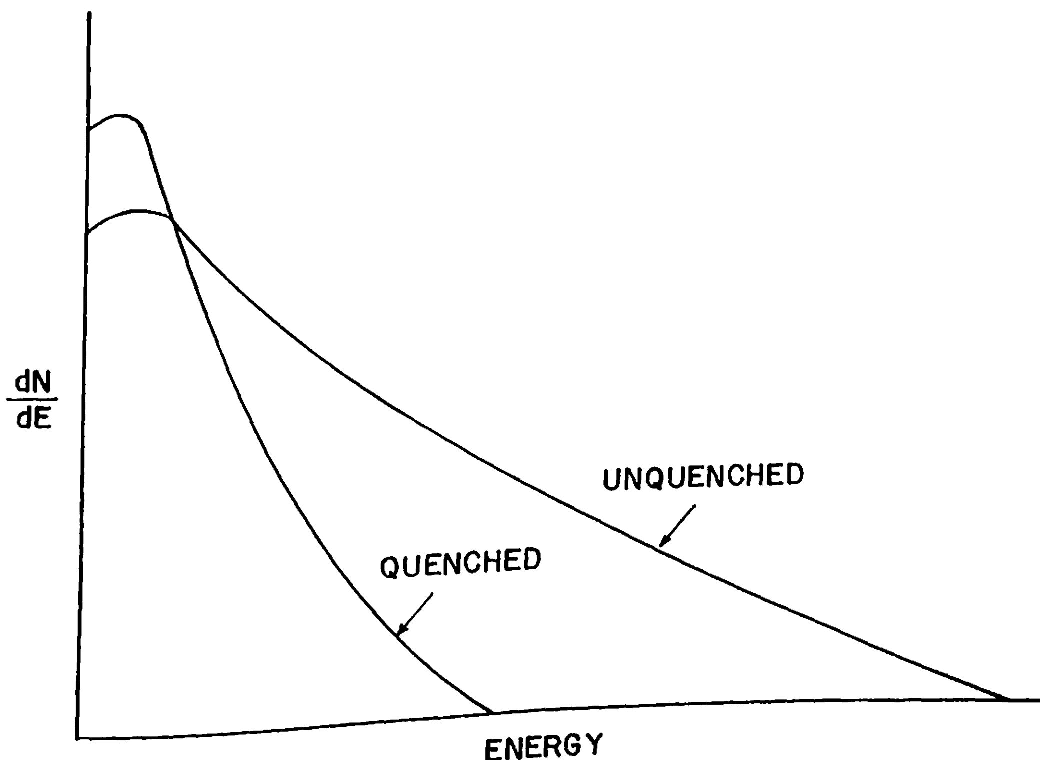


Figure 2.2 Shift of energy due to quenching

In the present work, however, the quenching level has been monitored considering one of the so called efficient and accurate parameters (Packard, 1985), Spectral index of

samples, SIS, to derive the quenching index. By doing so, a quenching curve (Calibration curve) has been prepared taking the SIS values of ten standards obtained from Canberra Company. These standards contains equal radioactivity of 130600 dpm but different quantity of quenching material. The SIS values for these standards which are given as a readout from the liquid scintillation counter, were then plotted against the percentage efficiency (Figure 2.3) as calculated using equation 3. From the calibration curve then, it has been possible to determine the percentage efficiencies for all the samples and hence the dpm values. All the data in this work have been reported in terms of ppm values and not dpm values for practical purposes. The conversion was made considering the dilution proportion of the hot and cold standards as well as the weight of the pesticide containing material (see appendix I). All values obtained in this way were converted to the corresponding equivalents of plant and soil samples in µg/g.

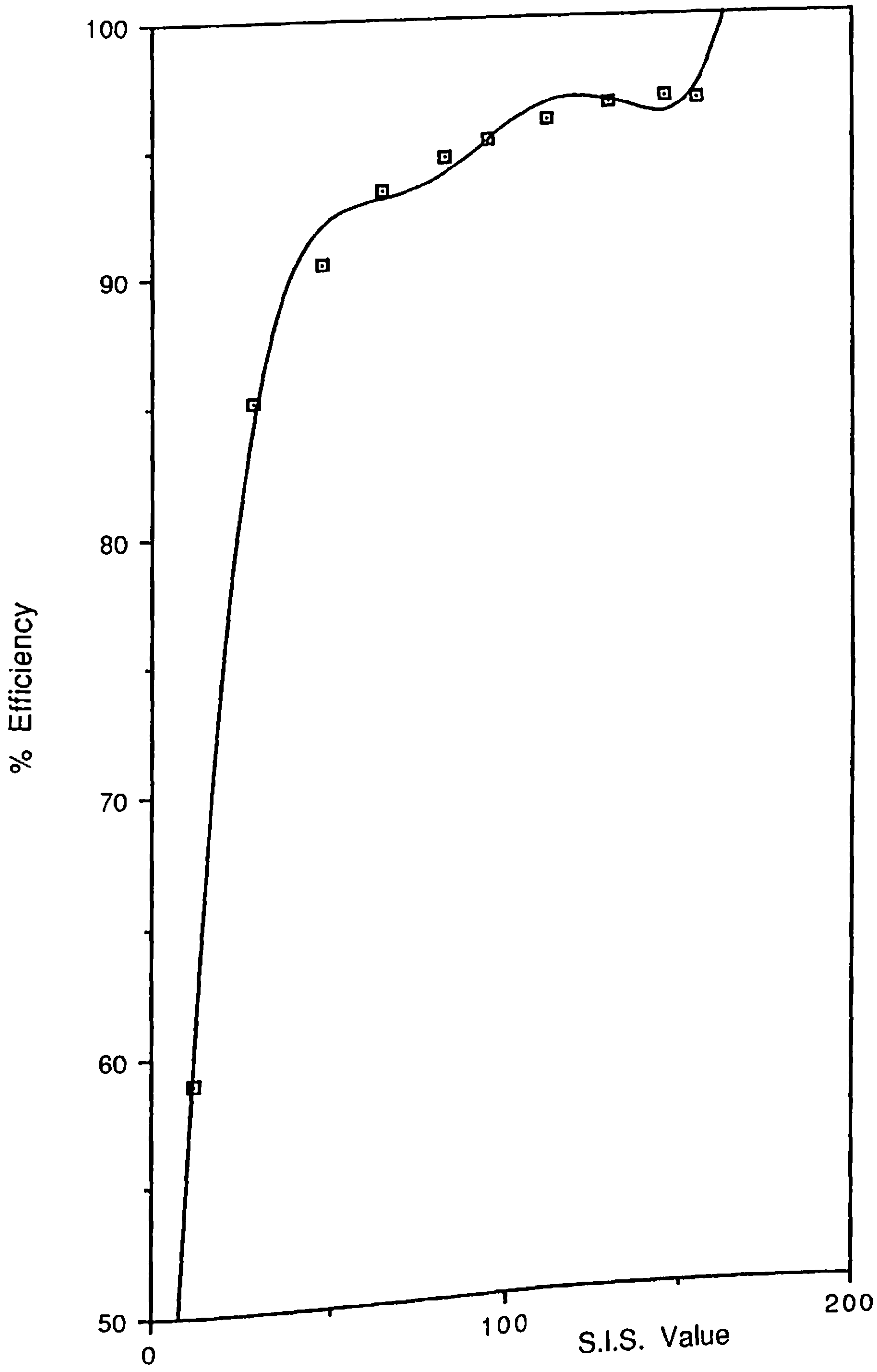


Figure 2.3 The Quenching Calibration Curve

3.2.6 The Biological Material Oxidizer

In the analysis of various samples known or suspected to contain residue of radioactive pesticides, it is not an usual to find that some of the radioactive material cannot be recovered via solvent extraction. These un-extractable residues may represent radioactive material bound on soil matrix or by biological tissues or in the later cases synthetic incorporation of radio-labelled fragments in the structure of natural products . When exhaustive extraction procedures fail to recover such bound materials the combustion method can be used to determine the concentration of the radio-labelled pesticides. Apart from this when further analyses of the extract is found undesirable, combustion of samples containing radioactive pesticides using combustion devices is one of the methods used to determine the radioactivity of samples.

Such devices are designed to prepare biological samples for scintillation counting. The substance to be combusted is placed in heat resistant boat and combusted in closed system inside the device at a very high temperature that can combust all the organic material in the sample. The oxidation process is also catalysed by a catalyst bed. The exhaust of the combustion process, the gaseous product is then made to pass through a trapping reagent(Figure 2.3). Through counting of the $^{14}CO_2$ that is liberated using liquid scintillation counter, quantitative information about the residues could be obtained. All biological

materials contain carbon and hydrogen in any number of variety of forms; although many forms of the halogens, sulphur and nitrogen might also occur. After combustion, metals, salts and materials with a very high melting point remain in the boat as ashes. The combustion side which initiates the oxidation of organic material converts the samples to a gaseous state.

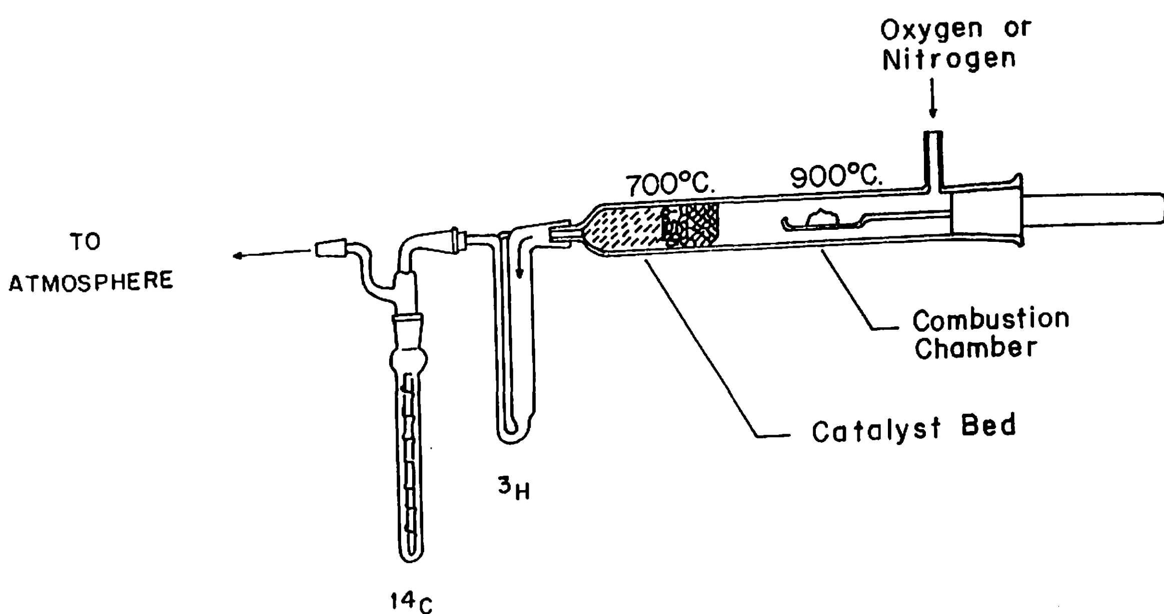


Figure 2.4 The Biological material oxidizer

The model ox-600 biological material oxidizer was the one used throughout the present work. This device combusts any biological and most organic materials (wet and/or dry) in a stream of oxygen gas at 900°C. The combusted products are then passed through a series of catalyst at 680°C and the $^{14}CO_2$ is trapped in external trap which contains the trapping solution. The procedure is illustrated in figure

2.4. The trapping solution is then taken for liquid scintillation counting.

3.3 EXPERIMENTAL METHODS

3.3.1 Selection and Preparation of the Experimental Sites.

Twenty pipes were laid for the study ,at one site, anticipating the sampling would be done biweekly, harvesting three replicates at a time and the plants would take three months to grow mature. Two sites were considered; one in the coastal region, Mombasa and another in a highland region, Nairobi. These were on a farm located at Mtwapa (altitude 21 m), Kilifi District and Chiromo campus, University of Nairobi, respectively. The Mombasa plot was 6 by 6 m. It was dug, all weeds and stones removed. Twenty P.V.C. cylinders (length: 20 cm ,diameter 10.4 cm) were driven into the soil. Any possibility of the pesticide being washed away by a running water had been taken care of by leaving 3 cm of the pipe protruding above the ground. The Nairobi pipes, twenty of them, however, were not buried in the field for some reasons of inconveniences but were kept in an open air site inside the department of Chemistry. Some of the practical problems that limited the Nairobi case were unavailability of appropriate farm conditions and vulnerability of the pre-supposed area. Nevertheless; several conditions have been adapted to assume the field conditions. One of the assumptions is that the soil beneath the pipes has

negligible contribution in the process of uptake of the pesticide because, practically, p,p'-DDT is not expected to leach down more than 15 cm from the top and the pipes are 20 cm long. In fact, the soil layer of 1 to 2 cm from the bottom of the pipes has always been removed to avoid unnecessary dilution of the residue. However, to avoid inhibition of extended growth of the roots beneath the pipes, some soil layer of approximately 3 cm has been made to bed the pipes covering the bottom part of the pipes.

3.3.2 Preparation and Application of The p,p'-DDT Solution

0.5g of the 98% pure non-labelled p,p'-DDT (cold standard) was placed in a 500 ml volumetric flask. Then 515 ml of toluene solution containing 150 µl of ^{14}C-p,p'-DDT was added into the volumetric flask. The flask was then topped with n-Hexane and thoroughly shaken. One week after the pipes were sunk 10ml of the solution was applied into each pipe. 10ml of the solution contains 3.0 µCi of ^{14}C-p,p'-DDT and 10mg of the cold standard. Finally, the cowpeas seeds were berried the same day after the solvent had evaporated from the soil.

3.3.3 Sampling and Handling the Plants and Soil Samples

For both the soil and plants sampling was done biweekly but in case of the soil harvesting was done also immediately after application for the determination of

initial concentration. The Nairobi plant samples were harvested by removing them from their respective pipes. The adhering soil mostly on the roots was then removed by washing the plant thoroughly with water and methanol. Then they were dried in an oven at 50°C. The respective soils were also labelled and air dried and preserved for residue analysis.

For the Mombasa plants, however, the removal of the plant samples was not done immediately after harvesting but after they reached the laboratory. This helped the plants stay fresh and be easily separated from the soil. Evidently, the same procedure was followed to prepare them for further analysis. The dried plant samples were then preserved for further analysis in a refrigerator at -20°C

3.3.4 Treatment of Plant Samples

The dried plant samples were crashed by mixing with acid washed sand and anhydrous $MgSO_4$. The aim of the addition of sand was mainly to aid the crashing process and the $MgSO_4$ to absorb any moisture that hinders the process of crashing. Depending on the size of the plant samples varied amounts were used. Table 2.1 shows the record of amounts added. Four sub-samples were weighed for the determination of the total residue and the rest of the crushed samples were put in the thimbles and extracted with 150 ml of distilled methanol at 70°C using heating mantles for about 2 1/2 hours (10 cycles). The extractable residues

were then determined by counting 1 ml of the aliquot of the extract after decolorising the green pigment with activated charcoal. The counts obtained in this way were multiplied by the total volume of the extract to determine the radioactivity of the extractable residue.

Table 2.1 Amounts of sand and anhydrous $MgSO_4$ mixed with the plant samples. (Mba = Mombasa, Nbi= Nairobi)

Age of the plant (days)	Dry Wt. of plants (g)		Amount of sand (g)		Amount of anhy. $MgSO_4$	
	Mba	Nbi	Mba	Nbi	Mba	Nbi
15	4.28	3.83	16	16	4	4
30	24.49	9.44	32	16	6	4
45	48.24	17.77	48	32	6	4
60	57.71	18.12	48	32	6	4
75	96.66	28.50	48	32	8	6
90	98.20	30.03	48	32	8	6

3.3.5 Treatment of the Soil Samples

The soil samples, after being air dried in the laboratory for one day, were ground with pestle and mortar and then thoroughly mixed by shaking inside a plastic bag for about 20 minutes. Three 50g replicates from each soil were placed in thimbles made of pre-extracted 18.5 cm

diameter filter paper. Pre-extraction of the filter paper was done using methanol to remove any substances that can possibly interfere with the further analysis of the extracts. The soil samples were then soxhlet
extracted for 2 1/2 hours (10 cycles) with 150 ml distilled methanol at 70°C using heating mantles. The extractable residues was then determined by counting 1 ml of aliquot of the extract after decolorising using activated charcoal. This was done before and after the pre-concentration of the extract. The pre-concentration was done using a rotary evaporator at 60°C to a volume of 10 ml. The counts obtained in this way were multiplied by the total volume of the extract before and after pre-concentration. Finally an average of the two reading was taken as a mean value of the radioactive reading.

3.3.6 Determination of The Total and Bound Residue

The extracted plant and soil samples were dried in the hood and then thoroughly mixed again. Replicate of 1.0 to 1.5g for the plants and exactly, 1.5g for soil samples were weighed. To the soil samples a piece of filter paper (cellulose), approximately 30 mg in weight was added. The samples were then taken to the biological material oxidizer OX-600 machine and combusted at 900°C in a stream of Oxygen. The CO_2 liberated as an exhaust was trapped in 7.5 ml of the harvey carbon 14 cocktail by the method described in section 2.4.2 and taken for counting with liquid

scintillation counter.

The determination of total residue for both the soil and plant samples was done in two ways. Directly, by combustion of the un extracted samples and indirectly from the sum of the bound and extractable residue. The former case was done by combustion of four sub-samples of un extracted sample and the later by an indirect method as discussed in the previous section. In all cases the sum of the extractable and bound residue has been found to be less than the amount obtained by direct combustion means. This is attributed to the loss of some counts during the process of extraction, concentration and decolorization. All the values of total residues indicated in the tables through out this thesis are the later ones.

3.3.7 Preparation of the liquid scintillation Cocktail

The liquid scintillation cocktail was prepared by dissolving 4g of PPO and 0.25g of dimethyl POPOP in one litre of Toluene. 1 ml aliquot of the pesticide containing methanol extract was placed in a 10 ml scintillation vial and 5 ml of the scintillation cocktail added. The remaining extract solution was reduced to 10 ml using a rotary evaporator. Again 1 ml of the concentrated extract was taken for liquid scintillation analysis. The remaining 9 ml of the extract was kept aside in vials for further analysis.

3.3.8 Chemicals, Materials and apparatus used.

3.3.8.1 Chemicals and Materials

A mixture of (98% pure by T.L.C) hot and cold p,p'-DDT standards, namely uniformly labelled ^{14} C-p,p'-DDT [1,1'-(2,2,2-trichloro ethylidene) bis(4-Chloro benzene)];specific activity 12mCi/m.mole and non-radio-labelled p,p'-DDT, respectively, which were purchased from International Isotope, Munchen, Germany were used to contaminate the soils. Analytical grade toluene from J.T.Baker Inc. U.S.A was used to dissolve the hot standard. General purpose grade methanol, n-Hexane,toluene, acetone obtained from Zeta suppliers in Nairobi were used for soxhlet extraction and other analytical works after distilling them using fractionating column.

Activated charcoal and anhydrous $MgSO_4$ from suppliers in Nairobi and Eastman Kodak Co. U.S.A., respectively, were used as decolorising and drying agents.Crystalline 2,5-Diphenyl Oxazole (PPO) from fisher chemical Co. U.S.A. and 2,2'-Phenyl bis (4-methyl-5-phenyl Oxazole),Dimethyl POPOP, from Eastman Kodak Co. U.S.A. were used in liquid scintillation spectrometry. Carbon 14 trapping cocktail from R.J. Harvey Instrument Co. U.S.A. was used as the trapping reagent and scintillators in the determination of bound and total residues by combustion method. Oxygen and white spot nitrogen from East African Oxygen limited were used as combustion and purging agents, respectively, during the process of combustion of plants and soil samples.

Watman 91 filter papers from Watman International limited were used to contain the samples in soxhlet extraction.

3.3.8.2 Apparatus

Soxhlet apparatus, rotary evaporators and separatory funnels were used during the process of soxhlet extraction and pre-concentration of methanol extracts. The liquid scintillation analysis was carried out using Tricarb 1000 TR liquid scintillation analyzer from Packard-Canberra Co..Bound residue samples were combusted using a biological material oxidizer from R.J. Harveys Instrument Corp. U.S.A..

P.V.C. cylinders were used to assume enclosed environment. A mortar and pestle along with acid washed sand were used for crashing and homogenisation of plant and soil samples. Glass vials of 20 cm^3 capacity were used to contain the samples to be counted. Adjustable micro-pipettes, pipette tips and pipette fillers were used accordingly to transfer and pipette out the radio-labelled chemicals into containers. Varied glassware including volumetric flasks, distillation flasks, fractionating column were used accordingly. An oven was used to regulate the elevated temperatures and heating mantles to heat the extracting solvent in the soxhlet extraction process. Deep freezer and Refrigerator were also used to keep soil samples at temperatures of 0°C and 20°C.

CHAPTER FOUR

4.0 RESULT AND DISCUSSION

4.1 The Study on Plant Uptake of ^{14}C-P, P'-DDT by Cowpeas

In this experiment, the following main factors were considered important enough to form basis for the discussion of the results.

(a) The variation of the plant uptake with the age of the plant.

(b) The variation of the plant p,p'-DDT uptake with the amount of p,p'-DDT residue remaining in the soil pipes.

(c) The variation of plant p,p'-DDT uptake with respect to the soil type and texture of the soils from two different sites. This was mainly with reference to their composition in terms of organic carbon content, water content and pH. The two types of soils were from Mombasa, Mtwapa, Coast province and Nairobi, chiromo campus.

(d) The variation of the plant p,p'-DDT uptake with respect to climatic conditions such as temperature, amount of rainfall and humidity

4.1.1 p,p'-DDT Uptake in Relation to and The Age of Cowpeas Plants

The results obtained from the follow up of the growth of the cowpeas, on harvesting of samples biweekly, have demonstrated that substantial amounts of p,p'-DDT is likely to be absorbed and translocated into plants grown in soils containing p,p'-DDT residues. This probably is not only as a result of absorption and translocation processes but also due to any kind of adsorption of the pesticide that might occur on the plants (especially the roots) internally and externally. Moreover, the results have produced a strong evidence that the amount of p,p'-DDT taken up by the plants with time increases with plant maturity (Tables 3.1 and 3.2), regardless of the soil type, climatic and other growing conditions. Furthermore, the analyses of the bound and extractable residues, in the plants, has also been done and the results showed that the percentage of bound residue increases with increase in the age of the plants. i.e. the older plants were found to retain greater percentage of the residues as non-extractable.

According to the graphs (Figures 3.1 and 3.2) the increase in the uptake of p,p'-DDT by the plants tends to be step wise, suggesting that within certain time duration the uptake remained the same. However, in all cases (total, bound, and extractable) there was a sharp increase in between 60 and 75 days of age. Though no more data points were collected beyond 90 days, the uptake of p,p'-

DDT by cowpeas tends to level off after 75 days. In fact at the age of 75 days, the plants gave signs of bud formation.

In the case of Nairobi plants the pattern is similar to that of Mombasa plants except the uptake is not as drastic as that observed from Mombasa (Table 3.2).

Pesticides applied to the soil go into solution in the soil water and may be absorbed by plant roots, emerging shoots that are under ground, under ground stems and by germinating seeds. A working knowledge of general

Table 3.1: Amounts of DDT residue equivalents recovered from dried Mombasa plants

Day of sampling	Total (μg/g)	Extractable (μg/g)	Bound (μg/g)
15	0.945 ± 0.040	0.800 ± 0.066	0.084 ± 0.008
30	1.532 ± 0.013	1.279 ± 0.019	0.179 ± 0.002
45	3.437 ± 0.110	2.856 ± 0.352	0.481 ± 0.011
60	3.785 ± 0.017	3.065 ± 0.135	0.591 ± 0.010
75	7.882 ± 0.244	6.235 ± 0.035	1.356 ± 0.014
90	7.765 ± 0.211	6.110 ± 0.038	1.390 ± 0.008

plant anatomy and physiology is necessary in order to fully understand the entry, movement and fate of pesticides in plants. However, roots are the primary organs for the absorption of nutrients and water from the soil. Pesticides

uptake from the soil also occurs primarily through the roots. Specifically, the root hairs play the most important part in the absorption of substances from the soil and substances migrate into the apparent free space of roots by mass flow. Therefore, the increase in the amount of total residue with increase in the age of the plant is expected as the plants grow bigger with time, at least because of the effect of increased ample contact time of the plants with the soil containing the p,p'-DDT. That is, the larger the plants grow the more likely they would accumulate p,p'-DDT residue in their tissues.

Table 3.2: Amounts of DDT residue equivalents recovered from dried Nairobi plants.

Day of sampling	Total (µg/g)	Extractable (µg/g)	Bound (µg/g)
15	1.136 ± 0.030	1.034 ± 0.011	0.080 ± 0.002
30	1.924 ± 0.017	1.728 ± 0.016	0.160 ± 0.002
45	2.298 ± 0.007	2.038 ± 0.010	0.214 ± 0.003
60	2.254 ± 0.011	1.961 ± 0.018	0.252 ± 0.003
75	3.098 ± 0.010	2.632 ± 0.012	0.366 ± 0.004
90	3.239 ± 0.007	2.741 ± 0.014	0.411 ± 0.007

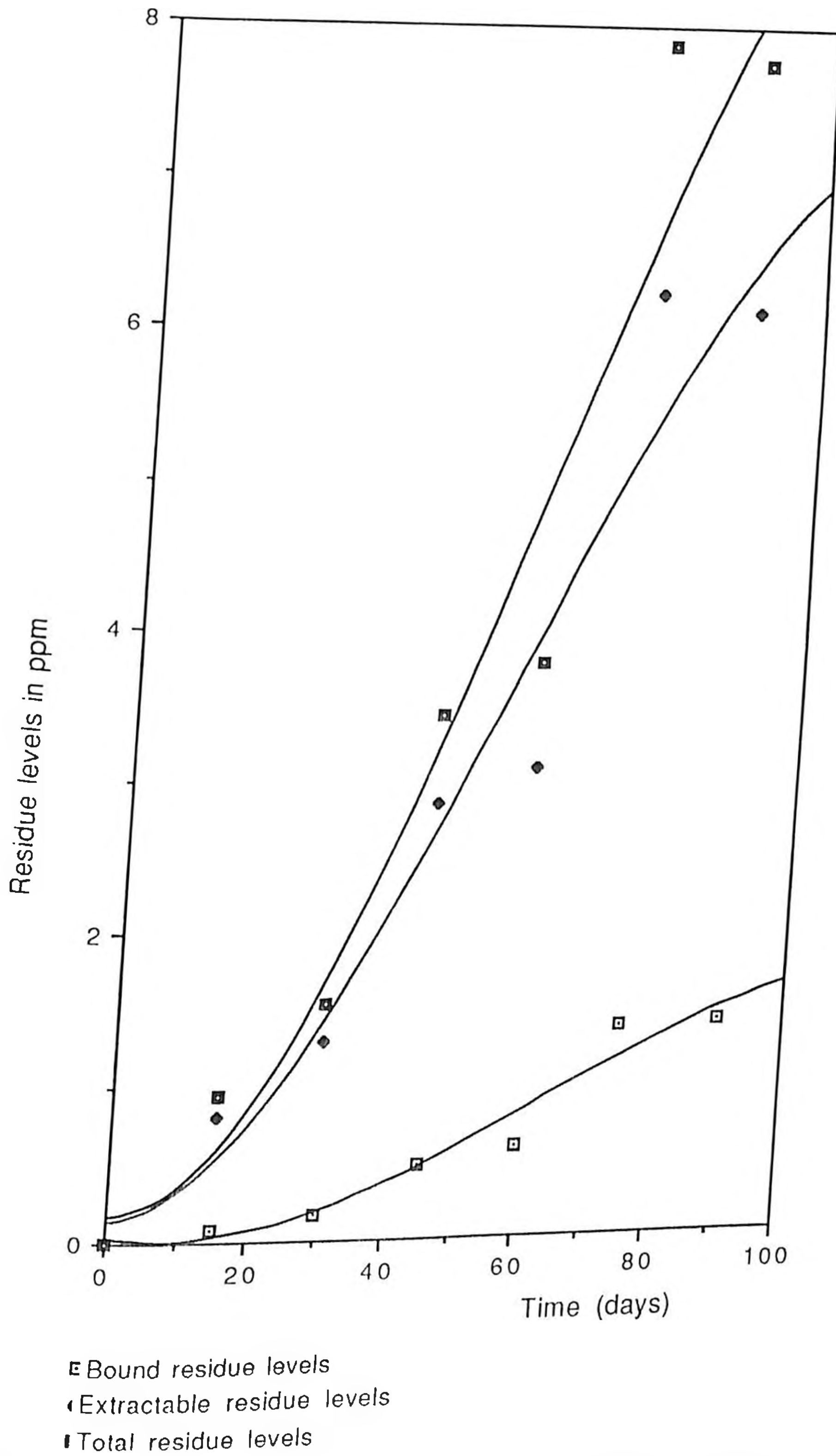


Figure 3.1. Variation of P,P'- DDT residue level in Mombasa plants vs. time

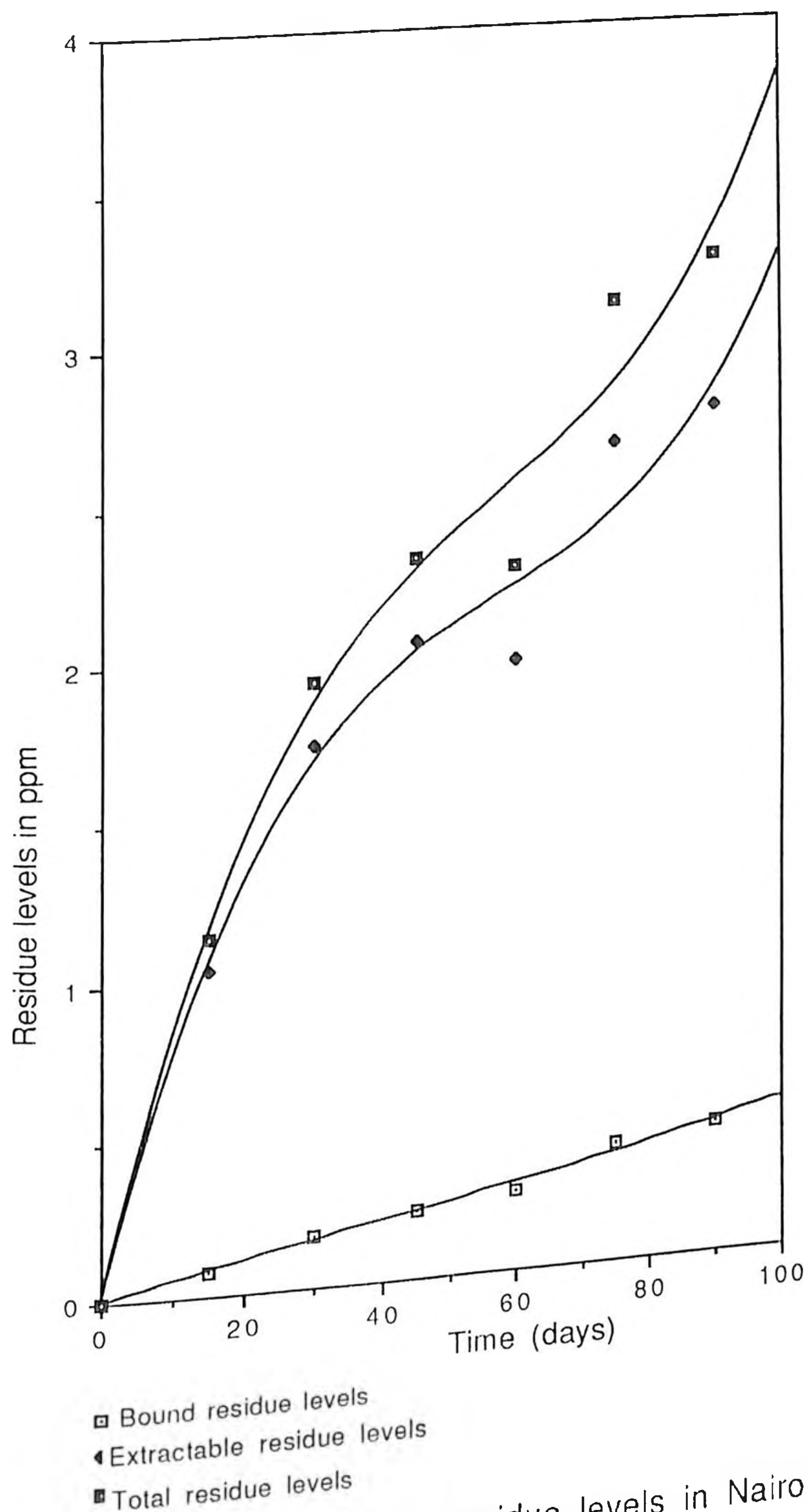


Figure 3.2 Variation of p,p'-DDT residue levels in Nairobi plants vs. time

Table 3.3 Percentages of bound and extractable residues for Mombasa and Nairobi plants

Day of Sampling	% Extractable		% Bound	
	Mba.	Nbi.	Mba.	Nbi.
15	85	91.0	9.0	7.0
30	83.5	89.5	11.7	8.3
45	83.1	88.7	14.0	9.3
60	81.0	87.0	15.6	11.2
75	79.0	85.0	17.2	12.0
90	78.7	84.6	17.9	12.7

Table 3.4 Percentages of bound and extractable residues for Mombasa and Nairobi Soils

Day of Sampling	% Extractable		% Bound	
	Mba.	Nbi.	Mba.	Nbi.
0	94.5	93.7	4.7	5.3
15	92.0	90.5	7.4	8.6
30	91.6	90.2	7.8	8.9
45	90.4	89.6	8.9	9.5
60	89.7	88.2	9.6	10.9
75	89.1	87.8	10.2	11.3
90	89.1	87.6	10.3	11.5

This fact was manifested in the plants of 15 to 30 days of age grown at both sites (Tables 3.1 and 3.2). That is, these plants which were observed to have similar size were also found to have accumulated close amounts of the pesticide. However, the decrease in the percentage of the extractable residue or in other words the increase in the percentage of the bound residue with increase in the age of the plants could be attributed to the increase in the complexity of the plant tissue as they grow older. That is, when they grow older, the residues get more chance to bind in the tissue of the plants at least because of the contact time. This is as expected in ordinary chemical reaction where contact time plays an important role. Moreover, as the plants grow older the degree of the metabolism of the pesticides already taken up would increase because larger plants are bound to have many leaves and hence increases availability of photosynthetic sites. For this reason the p,p'-DDT residue which has reached the leaves is likely to be metabolized into other forms becoming part of the biological structure of the plant making it difficult to extract. On the other hand, the fact that bound residue at both sites are statistically similar may show that incorporation of the pesticide follow the same route. Besides, the large extractable p,p'-DDT indicates that the compound moves along the plant food chain possibly unaltered. One thing worth mentioning here is that the percentage of bound residue obtained for the plants is even greater than the percentage of the

corresponding soil samples (Tables 3.3 and 3.4). This is probably because of the difference in the degree of binding and ease of extraction of the samples. Using similar argument on the basis of contact time, the trend for the bound residue is expected to be opposite of that for the extractable.

4.1.2 Variation of Plant Uptake with Growth Rate

The weather condition of Mombasa has been found favourable for cowpeas and as a result the plant grew much faster and flourished well as compared to the Nairobi plants. Consequently, the plants harvested from Mombasa were bulky and healthy. Moreover, there always appeared a significant increase in the size of the samples at every sampling time accompanied by increase in the accumulation of p,p′-DDT in their tissues.

Similarly, the plants grown in Nairobi though they did not show vigorous growth as the Mombasa plants, their uptake of p,p′-DDT also showed dependence on the growth rate like Mombasa plants. However, the corresponding data for the Mombasa plants were, in most cases, greater than that for Nairobi plants. As a result, the growth rate of the plants turns out to be one of the important factors that affected the results.

These results (Table 3.5) demonstrated that the higher the rate of growth the greater the tendency of the plants to the uptake of p,p′-DDT. From the gradients of the

Table 3.5 Weight of plants sample collected from Mombasa and Nairobi

Age of the plant(days)	Wt. of Mombasa plants (g)	Wt. of Nairobi plants (g)
15	4.28	3.83
30	24.49	9.44
45	48.24	17.77
60	57.71	18.12
75	96.66	28.50
90	98.20	30.03

graphs, it can easily be observed that there was an appreciable difference in the growth rate. Even among the plants of the same site there always was high uptake for high growth rate. The best example is the trend of residue accumulation observed for the plants aged more than 75 days.In both sites the residue levels tended to level off after 75 days where there was no much difference in the size of the plant samples.

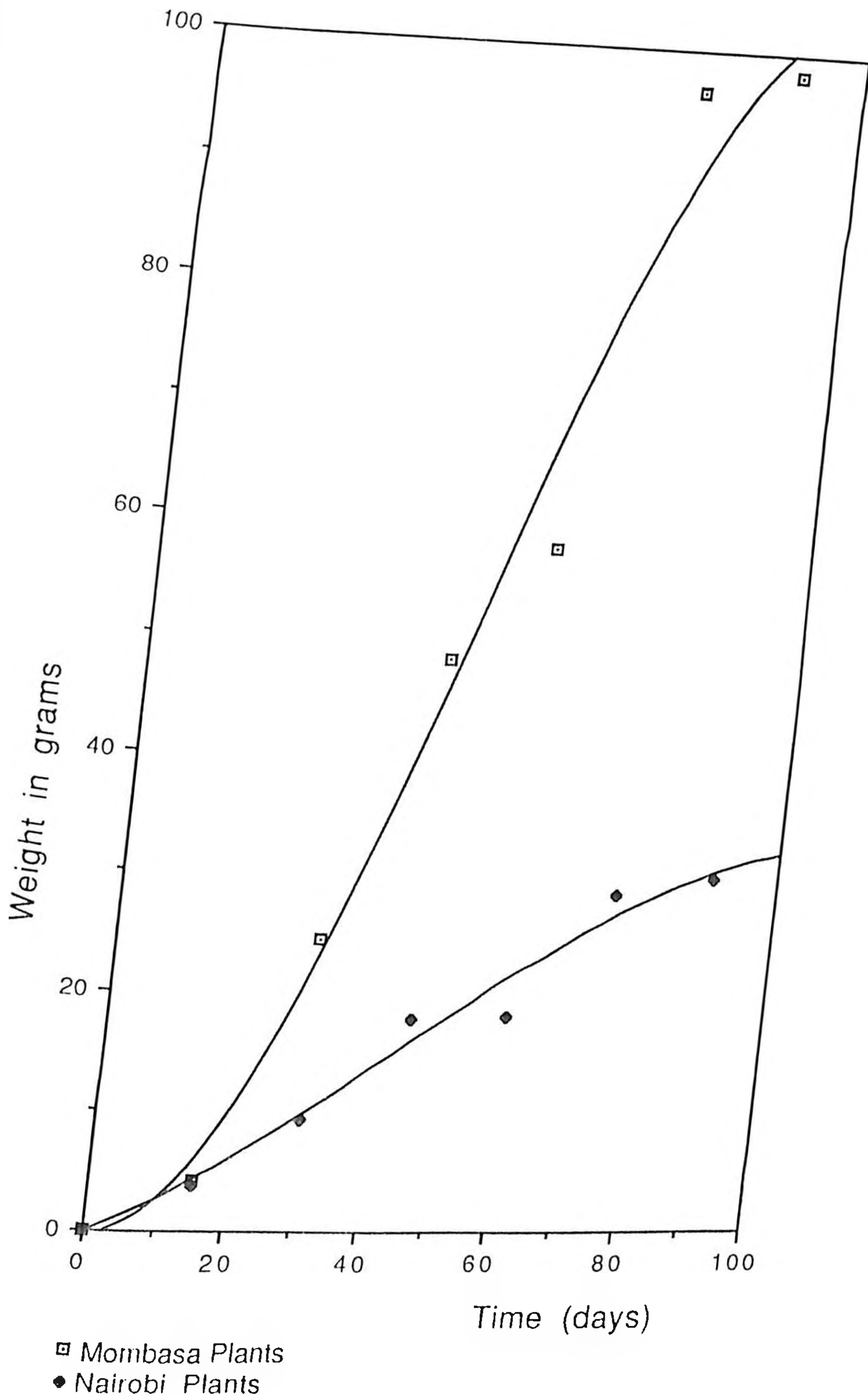


Figure 3.3 Variation of weights of plant samples collected from NBI & MBA

4.1.3 Dependence of Plant Uptake on Soil Residues

As a matter of fact, the pesticide applied into the pipe's cannot be expected to be all taken up by the plants. In fact, most of it is likely to remain in the soil as a residue without being affected or at times denatured by the environmental factors. For this reason the amount of p,p'-DDT which remains in the pipes has also been analyzed in the form of total, extractable and non-extractable residue as recovered from the respective pipes. The results (Tables 3.7 and 3.8) showed that the three forms of residues decrease with time due to the dissipation process that commonly affects p,p'-DDT residue in soils. This is again consistent with earlier reports.

As it was explained in section 4.1.3, in this experiment, the plant uptake of p,p'-DDT residue increased with time despite the decrease in the concentration of the residue in the soil. This implies that the decrease in the concentration of the residue in the soils has no net effect or has negligible effect on the plant uptake of p,p'-DDT. In other words it seems obvious that the concentration of p,p'-DDT remaining in the soil is still sufficiently high during the life span of the plants which is 15 to 90 days. During the life span of the plant the concentration of the soil residue remained within the range of 6.06 ppm to 3.217 ppm for Mombasa and 8.928 ppm to 4.281 ppm for Nairobi which implies that the plant uptake of residues by cowpeas at these ranges for the two sites is practically the same.

On the other hand, according to the experiment carried out for one sample which has been analyzed for the determination of p,p'-DDT residue in different parts of the plant, namely the leaves, the stems and the roots, the amount the three forms of residues obtained from the entire plants were as tabulated in table 3.6.

From the comparison the data (Table 3.6), the amounts of p,p'-DDT in different parts of the plant were found to be in the order, stem < leaves < roots. According to this limited data, the majority of the residue seems to be found in the roots which actually have accumulated five fold of either of the two parts (leaves and stems). This could be attributed to the fact that the degree of exposure of the roots to the pesticide is by far higher than the leaves and the stems because of the direct contact of the roots

Table 3.6: Amount of DDT residue equivalents recovered from different parts of a Mombasa plant sample

Part of the Plant	Total	Extractable		Bound	
	(µg/g)	(µg/g)	%	(µg/g)	%
Leaves	3.420 ± 0.012	3.115 ± 0.74	91	0.170 ± 0.008	5
Stems	3.273 ± 0.007	2.880 ± 0.123	88	0.262 ± 0.007	8
Roots	19.558 ± 0.007	16.820 ± 0.098	86	2.197 ± 0.009	11

with the soil as expected. Although the roots were washed thoroughly with distilled warm water and even rinsed with methanol before analyses, it seems obvious that apart from the amount of residue that finds its way into the plant tissues through similar biological process that apply to the leaves and stems, some residues could still have remained on the roots unremoved. Therefore, the relatively high amount of residues obtained from the roots must have come from the food adsorption process which include p,p'-DDT residue in the roots. Overall,the experimental and treatment condition for leaves, stems, and roots were the same.

The percentage of extractable residue for the parts of the plants has been found to decrease in the order leaves > stems > roots, because the efficiency of extraction decreases in the same order. This is consistent with what is expected due to the photosynthesis. Bound p,p'-DDT residue in leaves is incorporated through photosynthesis into plant tissues and the same process takes place in the stem. However, photosynthesis does not take place in the roots. Since extractable amounts are higher in leaves than roots it implies that there would be less incorporation of p,p'-DDT into the tissues of leaves and stems than roots. Cowpeas develop their fine roots before the above ground parts and the difference lies in the plant growth and physiology.

The bound residue was found least in the leaves and highest in the roots. This is expected as there is

increase in the complexity of the tissues as we go down
from leaves to the roots. Depending on the ease of
crashing of the three parts of the plant, the efficiency of
extraction may also vary because the leaves which were
crashed most finely are expected to be extracted most
effectively because of the surface area. the dried roots
were found relatively hard to crash which could lead to the
retention of more bound residue.

4.1.4 Halflife of p,p'-DDT in Mombasa and Nairobi soils

As it has been explained in section 4.1.3, the
dissipation of p,p'-DDT in the soils was also monitored for
the same duration of time the plants were being sampled.
The sampling was done bi-weekly along with the sampling of
the plants except that in the case of soil samples, zero
time sampling was done for the purpose of determination of
initial concentration. Tables 3.7 and 3.8 , show the record
of results obtained from the analyses of three forms of
residues. From these results the halflife of p,p'-DDT for
the Mombasa and Nairobi soil were determined by means of
kinetic analysis. The kinetic analysis of the total p,p'-
DDT equivalents (C), versus days after treatment (t),
assuming a first order process was done for the Mombasa and
Nairobi soils. The rate constants (K), were obtained for
the two sites from the slopes of the regression lines of
natural logarithm, ln C, of the p,p'-DDT remaining versus
day after treatment (t). The K values obtained for Mombasa

and Nairobi are 5.789 x 10^{-3} and 6.465 x 10^{-3}, respectively. The time for 50 % loss was calculated using the equation

$$t_{1/2}=\frac{Ln2}{K}=\frac{0.693}{K} \qquad ....Eqn(3)$$

For a period of three months a halflife of 119.7 days and 107.2 days were obtained for the Mombasa and Nairobi soils, respectively.

4.1.5 Variation of Plant p,p'-DDT Uptake with Soil Composition

The Nairobi soil which has been found to have higher organic carbon content than the Mombasa soil (Table 3.9) gave lower degree of uptake of p,p'-DDT by the plants. This observation seems compatible with what was reported by (Liechtenstein, 1958), about the phytotoxicity of lindane. According to the report organic rich soils were found to cause the least effect and it was directly related to the organic matter content of the soils. Moreover, in another study by the same people, it was suggested that pesticides dissolved in muck soil (organic rich soil) are likely to be less available for metabolism or pick up by plants. Therefore,this observation could suggest that the presence of high organic carbon content in the Nairobi soil may have decreased the availability of p,p'-DDT ready for the uptake by the plants.

This fact has also been manifested in the

difference observed in terms of the amount of extractable soil residue which has been found to be higher in the case of Nairobi soils. These observations may lead us to the generalization that soils with higher organic carbon content are likely to retain most of p,p'-DDT residue in them which is consistent with what has been reported

3.7: Amounts of DDT residue equivalents recovered from Mombasa soil samples.

Day of sampling	Total (μg/g)	Extractable (μg/g)	Bound (μg/g)
0	6.064 ± 0.007	5.731 ± 0.085	0.285 ± 0.011
15	5.862 ± 0.014	5.394 ± 0.184	0.434 ± 0.004
30	5.044 ± 0.016	4.621 ± 0.122	0.391 ± 0.012
45	5.033 ± 0.011	4.549 ± 0.183	0.484 ± 0.012
60	4.217 ± 0.009	3.781 ±0.137	0.404 ± 0.012
75	3.836 ± 0.007	3.417 ± 0.092	0.391 ± 0.012
90	3.217 ± 0.004	2.867 ± 0.180	0.332 ± 0.011

Table 3.8: Amounts of DDT residue equivalents recovered from Nairobi soil samples.

Day of sampling	Total (μg/g)	Extractable (μg/g)	Bound (μg/g)
0	8.928 ± 0.029	8.365 ± 0.060	0.437 ± 0.006
15	7.278 ± 0.015	6.587 ± 0.065	0.624 ± 0.009
30	5.664 ± 0.015	5.110 ± 0.126	0.501 ± 0.011
45	5.760 ± 0.031	5.161 ± 0.126	0.547 ± 0.011
60	5.392 ± 0.020	4.756 ± 0.146	0.588 ± 0.011
75	4.510 ± 0.013	3.960 ± 0.124	0.500 ± 0.008
90	4.281 ± 0.029	3.750 ± 0.088	0.491 ± 0.007

earlier by Liechtenstein *et al.*, 1958. That is the higher the soil organic carbon content, the greater the trapping power of DDT by the soil particles. This tendency of organic carbon rich soil makes p,p′-DDT unavailable for uptake by plants.

Table 3.9: Mombasa and Nairobi soil composition (source: soil department, Kabete, U.O.N)

Site	Texture	organic carbon content(%)	Nitrogen content(%)	pH in water
Nairobi	Clay	2.53	0.252	7.20
Mombasa	Sandy	0.68	0.088	6.65

4.1.6 Effect of pH on Plant Uptake of p,p'-DDT

There is not much known about the mechanism by which plants take up the pesticide. However, the mechanism of the chain of reaction by which p,p'-DDT degrades to its metabolites involves the process of acidification and dehydro chlorination (Hassan, 1990). This can give us a clue to suggest that the difference in the pH of the medium in which this reaction propagates will have significant effect in the process of degradation. Besides, there is also the possibility of exhibiting difference in the affinity of different species of plants to uptake of various metabolites of p,p'-DDT, for some plants may prefer to take a particular metabolite of p,p'-DDT. Furthermore, if by any chance, the uptake is related to the affinity of plants to take particular metabolite, the difference in the degree of uptake of p,p'-DDT by the plants in the two different soils could also be attributed to the difference in pH which could possibly cause significant difference in the abundance of the various metabolites. Thus, the residues found accumulated in the plants should not necessarily be expected to have come from the uptake of un-metabolized p,p'-DDT for the detection of radioactive carbon in the plant tissues simply means a radioactive carbon of p,p'-DDT origin has found its way into the plant tissues some how (Hutson,1985). Therefore, the slight difference in the pH of the soil of the two sites may be considered as one of the factors for the difference in the

plant uptake.

4.1.7 Effect of Climatic Conditions on Plant Uptake of p,p'-DDT

The climatic conditions like rainfall and humidity have rather an indirect effect on the rate of uptake of p,p'-DDT by plant. For instance, the presence of high rainfall can enhance the process of leaching of the pesticide in the soil pipes and hence decrease the availability of pesticide residue near the roots of the plant. This leads to the decrease in the likelihood of the uptake of the pesticide by the plants. On the other hand a humid atmosphere has been found to favour the growth of cowpeas in the present work. This effect may also lead to the accelerated rate of uptake of the pesticide by the plants, according to what has been found from the analysis of effect of growth rate on plant uptake. Moreover, high atmospheric humidity can also influence the volatility of the pesticide by influencing the soil water content (Igue *et al.*, 1971).

In this experiment, there was considerable difference in climatic conditions for the two sites (Tables 3.10 and 3.11). Overall, since the coastal region (Mombasa) has favoured not only the rapid growth of plants,but also enhanced p,p'-DDT uptake; to be more specific, it seems likely that high humidity, less soil organic content, as well as high temperature tends to favour the p,p'-DDT uptake by plants.

Table 3.10: Average climatic conditions of Mombasa,Mtwapa, during the days 15th of Jan. to 15th of Apr.,1994.

Duration (weeks)	Air temp.[°C]	Soil Temp.[°C]	R. Humid. (%)	Rain Fall [mm Hg]
0 - 2	27.8	35.0	73.0	0.5
2 - 4	25.4	33.0	71.2	3.7
4 - 6	26.0	34.4	68.0	2.8
6 - 8	29.0	37.4	71.0	1.0
8 - 10	27.8	38.4	79.0	5.2
10 - 12	26.0	40.6	82.0	8.4

Table 3.11: Average climatic conditions of Nairobi, chiromo campus, during the days 15th of Jan. to 15th of Apr.,1994.

Duration (weeks)	Air temp.[°C]	Soil Temp.[°C]	R. Humid. (%)	Rain Fall [mm Hg]
0 - 2	19.8	26.0	47.0	-
2 - 4	20.4	26.8	51.4	0.5
4 - 6	20.1	28.2	46.0	10.4
6 - 8	21.3	30.1	48.4	6.8
8 - 10	23.0	29.2	54.2	-
10 - 12	24.2	27.4	58.0	-

4.2 The study on the Effect of Temperature on Soil Residues

The experiment was conducted by taking two kinds of soils, one from Mombasa and the other from Nairobi (see section 2.4). The temperatures considered were -20°C, 0°C, room temperature (RT) of average 21°C, 40°C, 60°C, 90°C and 120°C. These samples were subjected at these temperatures for five days after which they were given time to cool or warm, accordingly, to room temperature. The results obtained from the study for the three forms of residues namely, the total, extractable and non-extractable (bound) residues, in soil showed that soil residues are affected significantly by temperature variation. The total and extractable residues were found to decrease with increase in temperature and the bound residues were found to increase with increase in temperature. Moreover, when the samples kept at temperatures above the room temperature were weighed after removal, losses of weight ranging from 9.93 g to 12.84 g were recorded (Table 3.12). On the other hand, for the samples kept at temperatures lower than the room temperature, gain in weight ranging from 0.43g to 1.49g were recorded. The decrease in weight was attributed mainly to loss of water content and evaporation of some volatile substances from the soil. However, condensation of water vapour could also contribute significantly to increase in weight at lower temperatures.

4.2.1 Variation of Total p,p'-DDT Residue with Temperature

Evaporation is one of the main factors that cause dissipation of pesticides. The decrease in the amount of total residues is attributed mainly to the evaporation of the pesticide due to high temperature and of course, the degree of evaporation will increase with increase in temperature.

Table 3.12: Amounts of mass lost and gained for soil samples at various temperatures.(+ gain, - loss)

Temp.	Wt. lost Mombasa (g)	Wt. lost Nairobi (g)
-20°C	+ 1.49 ± 0.04	+ 1.38 ± 0.07
0°C	+ 0.58 ± 0.07	+ 0.43 ± 0.03
R.T. (21°C)	- 4.85 ± 0.03	- 5.42 ± 0.04
40°C	- 10.05 ± 0.03	- 9.93 ± 0.04
60°C	- 11.02 ± 0.06	- 10.46 ± 0.05
90°C	- 11.36 ± 0.08	- 11.19 ± 0.06
120°C	- 12.84 ± 0.07	- 12.04 ± 0.08

According to the results obtained (Tables 3.13 and 3.14), the total residue for Mombasa soil was found to be

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maximum at room temperature and it decreased with both increase and decrease in temperature. This is in consistence with earlier reports which revealed that dissipation in tropical regions is greater than temperate regions (Lalah, 1993). The increase in dissipation of p,p'-DDT with increase in temperature accounts for the decrease in the total residue. However, the decrease in the total residue at 0°C and -20°C is attributed to the increase in the water content which indirectly affects the weight of soil samples that should be taken for the determination of concentration.

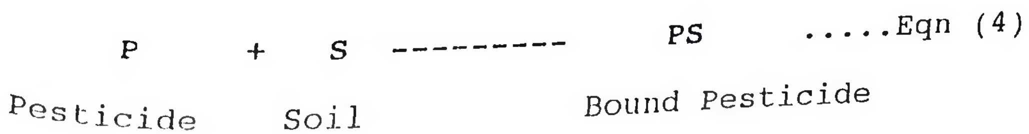
This experimental observation suggests that dissipation increases with increase in temperature regardless of the soil type until the total residue becomes almost equal to the bound residue. At high temperatures beyond 120 °C, little pesticide would possibly be extracted (see figure 3.1 and 3.2). These two graphs show that at an elevated temperature of 120°C, the total residue is close to the amount of bound residue and extrapolation of the graph is expected to give the convergence of the total and the bound residues, implying non-existence of extractable residue. On the other hand, the soil residue were found to be affected least at room temperature. This experiment was performed both in the absence and presence of added water (20%). Overall, the trend of dissipation versus temperature was similar.

For the Nairobi soil the maximum reading was obtained at 0°C. But the trend of variation for the total residue

was found the same as that of Mombasa soil. For the Nairobi soil which has been found to have a texture of clay soil, a temperature of 0°C seems suitable for retention of p,p'-DDT.

4.2.2 Variation of Extractable Residue with Temperature

The trend of variation of the extractable residue for the two types of soils is similar to that of the trend of variation of the total residue. That is, the extractable residue decreases with increase in temperature. This is because of the increase in the bound residue with temperature as expected. In this case, the rate of chemical reaction towards the formation of the product increases with temperature. If P is the pesticide, p,p'-DDT, and S is the soil particle then PS would be the result of the interaction due to adsorption of p,p'-DDT on to the soil particles as shown in Equation (4).



Thus chemical reaction between the pesticide and soil would be expected to increase with temperature. At very high temperatures of 120°C or more the extractable residue is even less than the bound residue (Figures 3.4 and 3.5).

Table 3.13: Amounts of p,p'-DDT residue equivalents at various temperatures (Mombasa).

Temp. (°C)	Total (μg/g)	Extractable (μg/g)	Bound (μg/g)
-20	7.166±0,007	6.779±0.151	0.298±0.008
0	7.284±0.006	6.876±0.138	0.306±0.009
(R.T)=21	7.307±0.010	6.869±0.120	0.344±0.007
40	7.021±0.010	6.249±0.127	0.675±0.008
60	6.833±0.004	5.674±0.105	1.055±0.008
90	6.751±0.007	5.055±0.113	1.608±0.007
120	4.522±0.011	2.303±0.145	2.122±0.011

Table 3.14 Amounts of p,p'-DDT residue equivalents at various temperatures (Nairobi).

Temp. (°C)	Total (μg/g)	Extractable (μg/g)	Bound (μg/g)
-20	7.480±0.007	7.039±0.149	0.281±0.008
0	8.014±0.013	7.477±0.156	0.386±0.005
R.T=21	6.955±0.006	6.428±0.175	0.346±0.010
40	6.943±0.005	6.285±0.102	0.493±0.007
60	6.671±0.007	5.897±0.128	0.623±0.006
90	6.654±0.008	5.269±0.095	1.227±0.005
120	4.221±0.005	1.057±0.092	3.011±0.006

Extrapolation of the graph shows that the extractable residue approaches zero as temperature increases beyond 120°C.

The maximum amount of extractable residue was obtained at -20°C and 0°C for the Mombasa soil and the Nairobi soil, respectively. One possible reason that could contribute to the increase in the amount of extractable residue at these temperatures is the amount of water content because the presence of water in soil samples is known already to enhance efficiency of extraction (Igue, *et al.*, 1972), Therefore, the Mombasa soil may have higher water content at -20°C and the Nairobi soil at 0°C. However, this observation needs further investigation because this difference could have come from the experimental conditions for the samples were kept in a deep freezer.

Extrapolation of the result obtained for temperatures higher than 120°C is bound to give no extractable residue because the p,p'-DDT is expected to exist only as bound residue. According to the graph of temperature versus concentration (Figures 3.4 and 3.5), it seems apparent that the extractable residue curve and bound residue curve will meet at temperature slightly higher than 120°C for the Mombasa soil. For Nairobi soil, the curves cross over at 110°c. This result would imply that different types of soil have varying affinities for pesticide residues such that both the extractable and residues exhibit different behaviours especially with respect to temperature.

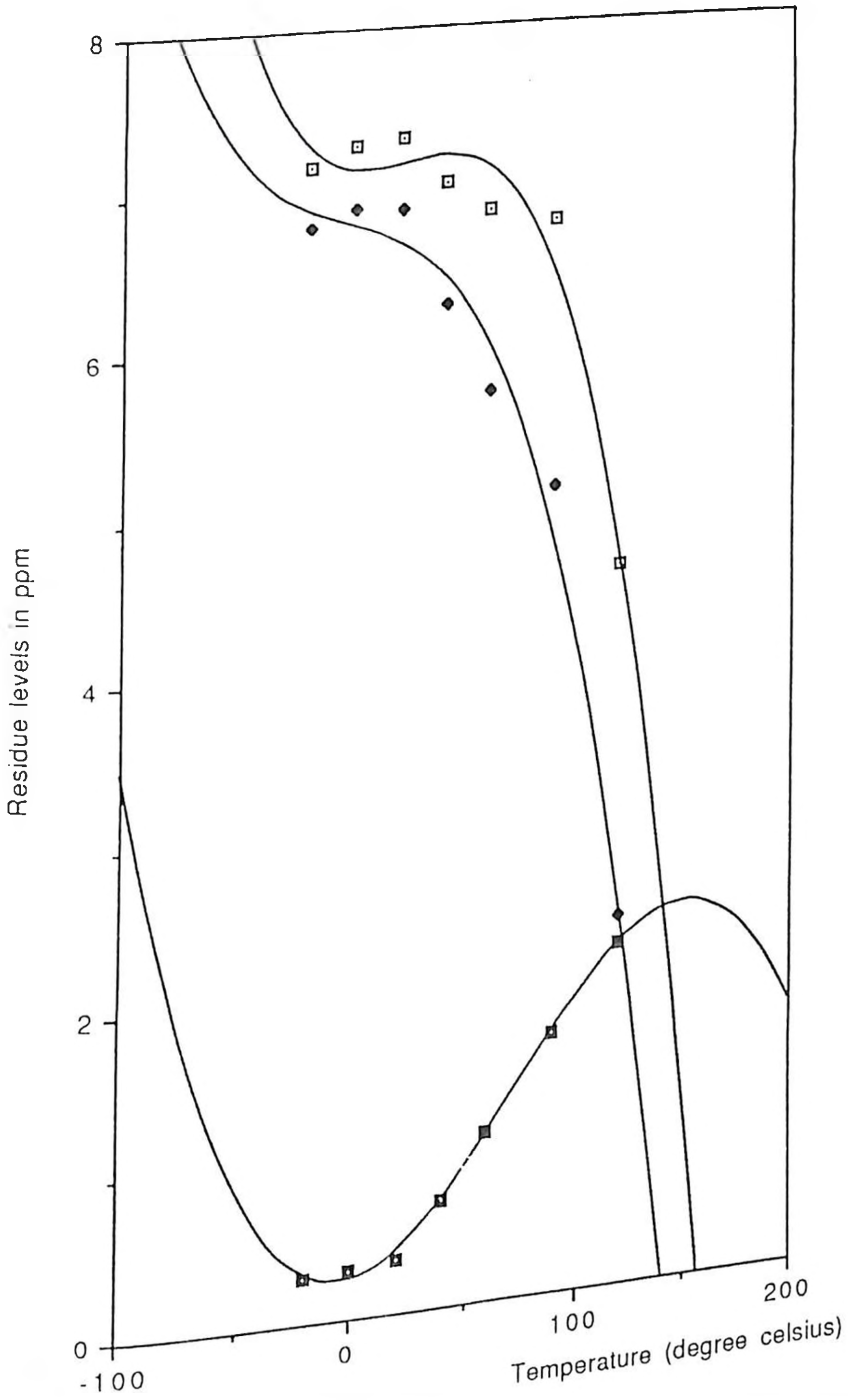


Figure 3.4. Variation of P,P'-DDT residue levels with temp. (MBA soil)

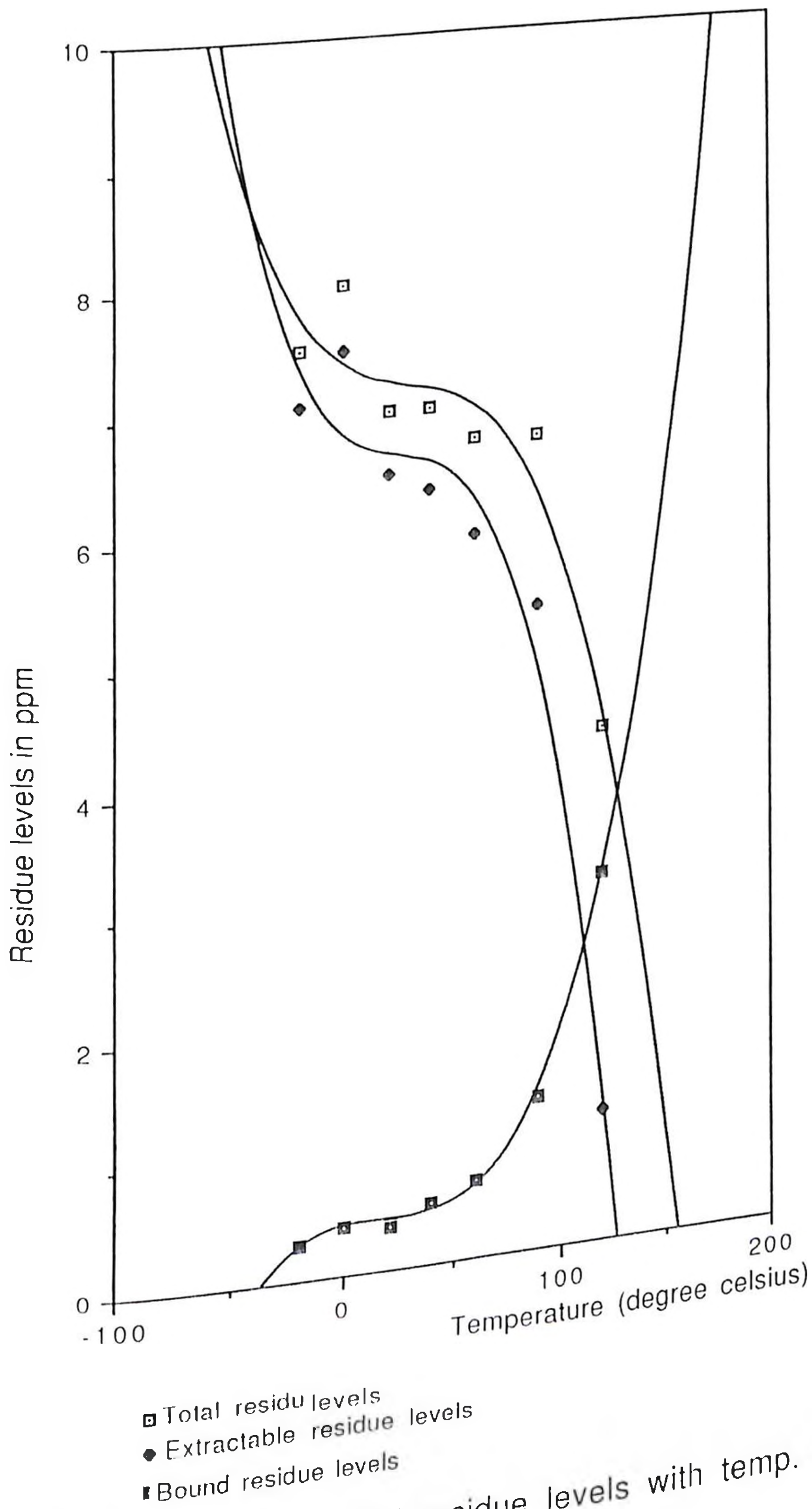


Figure 3.5 Variation of p,p'-DDT residue levels with temp. (Nbi. Soil)

4.2.3 Variation of Non-extractable (bound) Residue with Temperature

As opposed to the general trend of variation of the total and extractable residues, the bound residue was found to increase with increase in temperature. The amounts of bound residue obtained in this experiment were always small for the lower temperatures but slightly higher than 50% for the elevated temperatures. The percentage of extractable residue for the Mombasa soil ranges from 94.6% to 51% and for the Nairobi soil from 93.2% to 25%. Whereas the range of percentage of bound residues for Mombasa soil and Nairobi soil were 4.3% to 80.9% and 3.75% to 7.13%, respectively (Figure 3.6).

From the above observations it can be inferred that temperature not only has a significant effect on the dissipation of pesticides but also affects the degree of binding of the pesticide on the particles which as a result brings about the persistence of the pesticide in the environment, particularly in the soil. Many studies have shown that DDT dissipates by volatilization, chemical and biological degradation and binding to soil matrices. However, the high degree of binding of the pesticide on the soil particles is likely to cause the pesticides more persistent than when it is loose as extractable. Therefore, extrapolation of the laboratory experimental results to the field condition could suggest that high

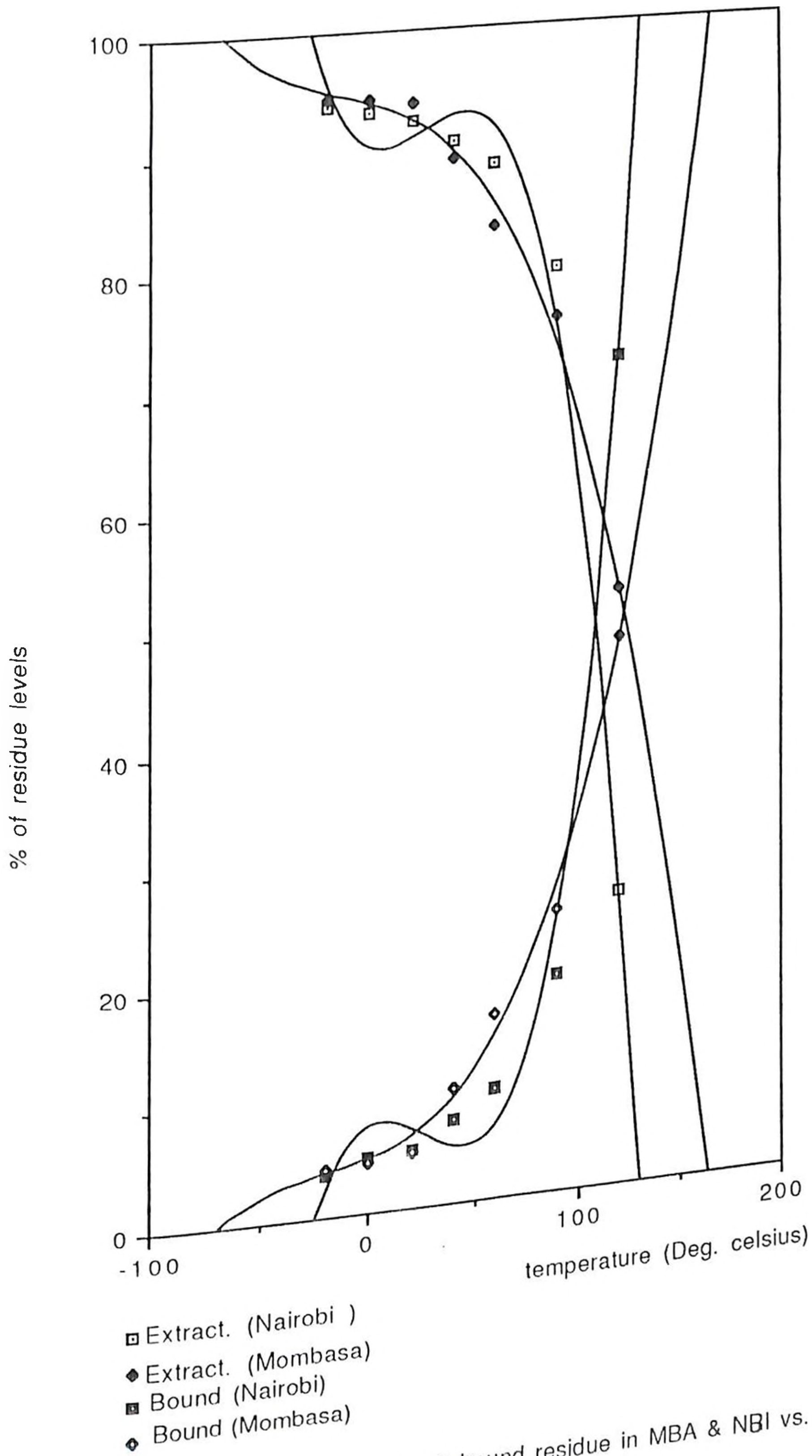


Figure 3.6. Variation of % extractable & bound residue in MBA & NBI vs. Temp.

temperature areas are likely to cause p,p'-DDT more persistent (as bound percentage would be higher). Moreover, assuming that plant can take up only the extractable residue available (Liechtenstein, 1958), it could also be concluded that plants grown in high temperature areas will take up lesser amount of pesticide than plant grown in colder areas.

4.3 Variation of the Effect of Temperature with Soil Composition

4.3.1 Water Content of Soil

Differences in the loss of p,p'-DDT for the soils(compare the two gradients of Nairobi and Mombasa) has something to tell us about the influence of soil type on the dissipation of p,p'-DDT. Figures 3.4 and 3.5, show that the loss gradient for Nairobi soil is higher than that of Mombasa. This could possibly be due to the difference in the variation of the water content with temperature because according to the observation obtained from the follow up of the loss of water content from all the samples (Table 3.12), the Nairobi soil which is clay in texture, was found to retain the water content more than the Mombasa soil which is sandy soil. Incidentally, this is consistent with the study made by Igue *et al.* 1970, on the volatilization of some organochlorine insecticides from soils which have shown that volatility is strongly affected by soil water content. Therefore, it follows that the higher the water retention by the soil the higher the

rate of evaporation for P,P-'DDT.

4.3.2 Organic carbon content

According to what was reported by Lichtentein *et al.*, 1958, on the effect of temperature on dissipation of aldrin from two types of soils, at high temperatures higher than 26°C, the loss of Aldrin was rapid for sandy soil (organic matter content 0.8%) than for loam soil(organic matter content 3.8%).However, the result obtained for p,p'-DDT in the present work demonstrated that the rate of p,p'-DDT dissipation with increase in temperature for clay soil, organic matter content 2.53% is faster than for sandy soil, organic matter content 0.68%. This implies that the presence of high organic carbon content does not have the same effect on all pesticide and organochlorines for that matter.

A look at the two factors (water content and organic matter content), above, and the effects on dissipation of p,p'-DDT residue in the soils has revealed that these two factors have opposite effects. However, the resultant effect obtained from the experiment show that the dissipation of p,p'-DDT from the soils is faster for Nairobi than for Mombasa. i.e.The Nairobi soil which has higher content of both water and organic matter exhibited an environmental behaviour dominated by water content.This implies that the effect of the high water content which increases the rate of dissipation has outweighed the

opposite effect caused by the presence of high organic matter content in the same type of soil.

CONCLUSION

DDT, because of its persistence in the environment is one of the severely limited pesticides in use. For instance in Kenya, there has not been any import of DDT since 1985. However, the fact that it persists in the environment makes its fate and behaviour in the environment worth studying. Extensive studies have been made on the degradation , rate and residue level in animal, birds, fish, soils and water bodies. In the present work the uptake of DDT by cowpeas plants(*VIGNA UNGUICULATA*) has been studied critically along with the fashion of its dissipation in the field condition.

The entire study was carried out using a radioisotope technique where a mixture of hot and cold standards, uniformly labelled ^{14}C-p,p'-DDT and 98% pure p,p'-DDT, respectively, were used to form the pesticide solution which was applied to PVC pipes buried in the soil of two sites. The two sites chosen were one from the highland region, Nairobi, Chiromo campus and the other from the coastal region, Mombasa, Kilifi district, Mtwapa. Composition analyses of soil samples has shown that the two sites have different soil type and different environmental and climatic conditions. Consequently, the plant grown in Mombasa which is mainly sandy soil were bulkier and healthier. On the other hand, the Nairobi soil which is a reddish clay soil, was found to be of higher organic matter content than the Mombasa soil and was also found to retain

water more. In light of all this, considerable differences in the rate of growth of the plants, degree of uptake of p,p'-DDT and rate of dissipation of p,p'-DDT residue were observed. Generally, substantial amounts of p,p'-DDT has been found accumulated in the tissues of the plants. A total residue level ranging from 0.945 ± 0.040 μg/g to 7.765 ± 0.211 μg/g were obtained for the two weeks to twelve weeks old Mombasa plants, respectively. The corresponding values for the Nairobi plants fell in the range of 1.136 ± 0.003μg/g to 3.239 ± 0.007μg/g. According to an experiment carried out for one Mombasa plant sample which has been analyzed for the residue accumulation in different parts of the plant namely, the leaves, stem and roots, the total amount of p,p'-DDT residue in the parts of the plant were in the order stem < leaves < roots. This limited data showed also that the majority of the residue was found to be in the roots which actually accumulated five fold of either of the two parts. The percentage of extractable residue for the parts was also found to decrease in the order leaves > stem > roots. Whereas the bound residue was found least in the leaves and highest in the roots.

The halflife of p,p'-DDT in the two sites were also calculated using first order kinetics. Halflife of p,p'-DDT

in Mombasa soil was found to be 119.7 days and in Nairobi soil 107.2 days.

The extractable and non-extractable (bound) residue levels for all the plants and soil samples of the two sites were also determined. The Mombasa plants gave a range of residue level from 0.800 ± 0.066μg/g to 6.110 ± 0.038μg/g and 0.084 ± 0.001μg/g to 1.390 ± 0.003μg/g for the extractable and bound residues, respectively. The corresponding values for Nairobi plant samples are 1.034 ± 0.011μg/g to 2.241 ± 0.014μg/g and 0.080 ± 0.002μg/g to 0.411 ± 0.007μg/g.

In consistence with many studies the plant p,p′-DDT uptake was found to vary with organic matter content, the higher the organic content the lower degree of uptake. The rate of growth of the plant was also one of the factors that affected the degree of uptake. Faster growth rate was found to enhance the degree of uptake and as the larger the plants grow the more p,p′-DDT residue they accumulated.

The second part of the study i.e. the effect of temperature on dissipation of p,p′-DDT from the soil samples with respect to the total, extractable and bound residues was also studied separately taking two soil samples of the two sites in a laboratory condition. This was carried out by subjecting soil samples at various temperatures for five days. As a result, the temperature was found to have a significant effect on the variation of the total, extractable and bound residue levels. For both Mombasa and Nairobi soils the total and extractable

residues were found to decrease with increase in temperature and the bound residues increased with increase in temperature. The critical temperature was found to be 90°C in both cases.

According to the results obtained the total residue for Mombasa soil was found to be maximum at room temperature of about 21°C and it decreased with both increase and decrease in temperature. The corresponding value for Nairobi soil was 0°C. Generally, evaporation of the pesticide could be regarded as one of the main factors that contributed to the dissipation of pesticides from soil. The amount of bound residues obtained in this experiment were always small for the lower temperatures and slightly higher than 50% for the elevated temperature.

From these observations and results, it could be inferred that temperature not only has a significant effect on the dissipation of p,p'-DDT but also affects the degree of binding of the pesticide on the soil particles which as a result brings about the persistence of the pesticide in the environment, particularly in the soil. Extrapolation of the laboratory condition to the field condition could suggest that higher soil temperature areas are likely to cause p,p'-DDT to be more persistent.

The difference of the composition of the soil at the two sites in terms of organic matter and water content were also studied in relation to the persistence of p,p'-DDT. A look at the two factors, water and organic matter content, and their effects on dissipation of p,p'-DDT residue in the

soil showed that these two factors have opposite effects. This is consistent with earlier reports i.e. the organic matter content decreases the degree of dissipation whereas water content increases. However, the resultant effect obtained from the soils showed that the dissipation is faster for Nairobi than for Mombasa. This implies that, even if the Nairobi soil has higher content of both organic matter and water the magnitude of the effect of the water content seemed to dominated the opposite effect of organic matter content.

Recommendation

Basic data on physico-chemical and biological properties is essential and should be known so that it can provide administrators, managers and field personnel with access to existing knowledge in the broad area of environmental pollution and its controls. Apparently most countries have environmental laws and regulations to provide at least minimum environmental standards for agricultural practices. While highlighting the danger of possible hazard due to the plant uptake of p,p'-DDT. This study, I believe, could generate a new regional and/or national initiative for control measures.

Increased controls and legislations on environmental contaminations during our time has been paralleled by a growing public awareness of environmental issue. In particular, attention has been focused on the use of

pesticides and their potential for contamination of crops, live stock, soil and water. However,field studies can be critically assessed only when the results of laboratory studies are known. Therefore, if a pesticide is shown to be translocated into plants, substantially, and is seen to vary with temperature and soil type as observed as a result of this research,then limited field work would be enough to confirm these findings and predictions.

It is recommended that any concerned local or national policy maker to consider the results of this research and related works when testing environmental hazards of DDT residue that already exist in the environment. Persuasive statements could also be placed to encourage policy makers to incorporate the result of this study into their knowledge when formulating precautious regulations about the possible risk of cultivating crops on soils containing DDT residues.

Finally, I recommend any one interested to work on this part of pesticide research to make further investigation on the degradation product of DDT as related to their accumulation in the plants. The accumulation of residue in the seeds could also be examined fully if adequate time is given to the plants under consideration until they bear seeds. Lastly, the study could also be made more informative by conducting it in soils contaminated with varied amounts of DDT solutions.

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APPENDICES

APPENDIX I. Conversion of Scintillation Counts to ppm Values

Given the radioactivity of a sample in dpm, the amount of total p,p'-DDT in grams found in the samples may be derived from the relation between the total weight of p,p'-DDT used in the mixture and the amount of radio-activity introduced into the mixture. But since the amount of radio-activity and weight of cold standard used are given values, the problem boils down to calculating the weight of p,p'-DDT that has been introduced in the form of the hot standard. If we let the radio-activity of the sample be R, W_c be the weight of the cold standard, W_h be the weight of the hot standard, W_t be the total weight , then,

$$W_t = W_c + W_h = 0.5g + W_h$$

but W_h can be calculated from the specific activity of the hot standard. This is done considering the amount of total radio-activity introduced into the mixture. A total of 150 uCi of hot standard has been mixed with 0.5g of the cold standard. The specific activity of the radio-labelled p,p'-DDT is

$$1.12 \text{ GBq/ m.mole} = 1.12 \times 10^{10} \text{dps/mole}$$
$$= 3.027 \times 10^{7} \ \mu\text{Ci / mole}$$
$$= 6.72 \times 10^{13} \text{ dpm / mole}$$

this implies that 3.027×10^7 μCi of radioactivity is found in one mole (354.45g) of p,p'-DDT

then W_h can be calculated from the relation

3.027×10^7 --------- 354.45g

150 μCi ---------- ? (W_h)

Where W_h =150 μCi x 354.45 /3.027 x 10^7 g = $\underline{1.756888 \times 10^{-3} g}$

The total weight of p,p'-DDT used to make the mixture will then be

$$0.5 + 1.756888 \times 10^{-3} \text{ g} = \underline{0.501756888 \text{ g}}.$$

but since the hot standard used has a radio-activity of 150 μCi = 3.33 x 10^8 dpm, corresponding to weight of 0.50176888 g, then the total weight of p,p'-DDT corresponding to R can be calculated using the relation

if 3.33 x 10^8 dpm -------- 0.501756888 g

R dpm --------- ? (W_t)

which implies that

W_t = 0.501756888 g x R dpm / 3.33x10^8 dpm = $\underline{1.506777 \times 10^{-9} R \text{ g}}$

but again since the quantity should be expressed in the conventional form of ppm values or specifically μg/g, then it becomes important to know the amount of radio-activity that corresponds to one gram of a plant or soil sample. i.e. if X is the weight of the sample utilized to give R counts, then the number of counts in 1 gram will be R/X.

This implies that

W_t (1 g of Sample) = 1.506777 x 10^{-9} R/g

W_t =1.506777 x 10^{-9} x 10^6 R/X μg/g = $\underline{1.5068 \times 10^{-3} R \text{ μg/g}}$

Figure A1. Arbitrary sample of scintillation counting print out for total residue.

19 AUG 94 10:04

Protocol #: 2
Time = 1.00
Radionuclide: C14
Region A: LL-UL= .0-156.0 Bkg= .00 %2 Sigma= .00 Div(K)=1.00
Region B: LL-UL= 4.0-156.0 Bkg= .00 %2 Sigma= .00 Div(K)=1.00
Region C: LL-UL= .0- .0 Bkg= .00
QIP = SIS

S#	TIME	CPMA/K	2S%A	CPMB/K	2S%B	SIS	FLAG
1	1.00	3631.00	3.32	2976.00	3.67	36.607	
2	1.00	4024.00	3.15	3317.00	3.47	36.412	
3	1.00	3902.00	3.20	3279.00	3.49	37.567	
4	1.00	4026.00	3.15	3288.00	3.49	36.036	
		3895.75	3.21	3215.00	3.53	36.656	A
5	1.00	4012.00	3.16	3348.00	3.46	36.997	
6	1.00	3601.00	3.33	2963.00	3.67	36.173	
7	1.00	3717.00	3.28	3044.00	3.62	35.912	
		3776.67	3.26	3118.33	3.59	36.361	A
(	1 missing vial)						
9	1.00	4454.00	3.00	3728.00	3.28	36.247	
10	1.00	4438.00	3.00	3702.00	3.29	37.561	
11	1.00	4538.00	2.97	3812.00	3.24	37.476	
		4476.67	2.99	3747.33	3.27	37.095	A
(	1 missing vial)						
13	1.00	4684.00	2.92	3877.00	3.21	36.572	
14	1.00	4439.00	3.00	3703.00	3.29	36.544	
15	1.00	4382.00	3.02	3656.00	3.31	36.915	
		4501.67	2.98	3745.33	3.27	36.677	A
(	1 missing vial)						
17	1.00	4188.00	3.09	3429.00	3.42	35.644	
18	1.00	4013.00	3.16	3319.00	3.47	35.692	
19	1.00	3813.00	3.24	3129.00	3.58	36.332	
		4004.67	3.16	3292.33	3.49	35.889	A
(	1 missing vial)						
21	1.00	4010.00	3.16	3280.00	3.49	36.539	
22	1.00	4336.00	3.04	3569.00	3.35	35.971	
23	1.00	4339.00	3.04	3539.00	3.36	35.343	
		4228.33	3.08	3462.67	3.40	35.951	A
(	1 missing vial)						
25	1.00	4243.00	3.07	3510.00	3.38	36.184	
26	1.00	4009.00	3.16	3319.00	3.47	35.951	
27	1.00	4198.00	3.09	3459.00	3.40	36.847	
28	1.00	4772.00	2.90	3952.00	3.18	35.377	
		4305.50	3.05	3560.00	3.36	36.090	A

Figure A2. Arbitrary sample of scintillation counting print out for bound residue.

24 AUG 94 11:13

Protocol #: 2
Time = 1.00
Radionuclide: C14
Region A: LL-UL = .0-156.0 Bkg= .00 %2 Sigma= .00 Div(K)=1.00
Region B: LL-UL = 4.0-156.0 Bkg= .00 %2 Sigma= .00 Div(K)=1.00
Region C: LL-UL = .0- .0 Bkg= .00
DIP = SIS

S#	TIME	CPMA/K	2S%A	CPMB/K	2S%B	SIS	FLAG
1	1.00	150.00	16.33	118.00	18.41	49.327	
2	1.00	155.00	16.06	129.00	17.61	51.981	
3	1.00	117.00	18.49	98.00	20.20	49.920	
		140.67	16.96	115.00	18.74	50.409	A
(1 missing vial)							
5	1.00	216.00	12.75	208.00	13.87	42.676	
6	1.00	241.00	12.88	192.00	14.43	54.943	
7	1.00	272.00	12.13	221.00	13.45	46.126	
		253.00	12.59	207.00	13.92	47.915	A
(1 missing vial)							
9	1.00	262.00	12.36	214.00	13.67	44.442	
10	1.00	260.00	12.40	206.00	13.93	52.940	
11	1.00	256.00	12.50	197.00	14.25	45.943	
12	1.00	431.00	9.63	350.00	10.69	40.959	
		302.25	11.72	241.75	13.14	46.071	A
13	1.00	414.00	9.83	335.00	10.93	39.954	
14	1.00	464.00	9.28	377.00	10.30	39.095	
15	1.00	434.00	9.60	343.00	10.80	37.277	
		437.33	9.57	351.67	10.68	38.776	A
(1 missing vial)							
17	1.00	602.00	8.15	494.00	9.00	37.105	
18	1.00	650.00	7.84	542.00	8.59	39.063	
19	1.00	596.00	8.19	492.00	9.02	38.334	
		616.00	8.06	509.33	8.87	38.167	A
(1 missing vial)							
21	1.00	1228.00	5.71	1021.00	6.26	38.810	
22	1.00	1351.00	5.44	1075.00	6.10	36.418	
23	1.00	1204.00	5.76	993.00	6.35	36.728	

Figure A3. Arbitrary sample of scintillation counting print out for the quenching standards.

24 FEB 94 09:34

Protocol #: 2
Time = 1.00
Radionuclide: C14
Region A: LL-UL= .0-156.0 Bkg= .00 %2 Sigma= .00 Div(K)=1.00 L
Region B: LL-UL= 4.0-156.0 Bkg= .00 %2 Sigma= .00 Div(K)=1.00 L
Region C: LL-UL= .0- .0 Bkg= .00
OIP = SIS

S#	TIME	CPMA/K	CPMB/K	SIS	FLAG
1	1.00	126710	122413	153.34	
2	1.00	126257	121602	138.07	
3	1.00	125408	120539	133.41	
		126125	121518	141.61	A
4	1.00	125954	120500	117.09	
5	1.00	124338	117743	98.598	
6	1.00	123792	116204	85.026	
		124694	118149	100.24	A
7	1.00	122031	112209	67.381	
8	1.00	119523	105766	50.000	
9	1.00	110797	86904.0	29.328	
		117450	101493	48.903	A
10	1.00	75146.0	23584.0	11.761	

Figure A4. Simple curves showing the variation of P,P'-DDT residue in Mombasa soil

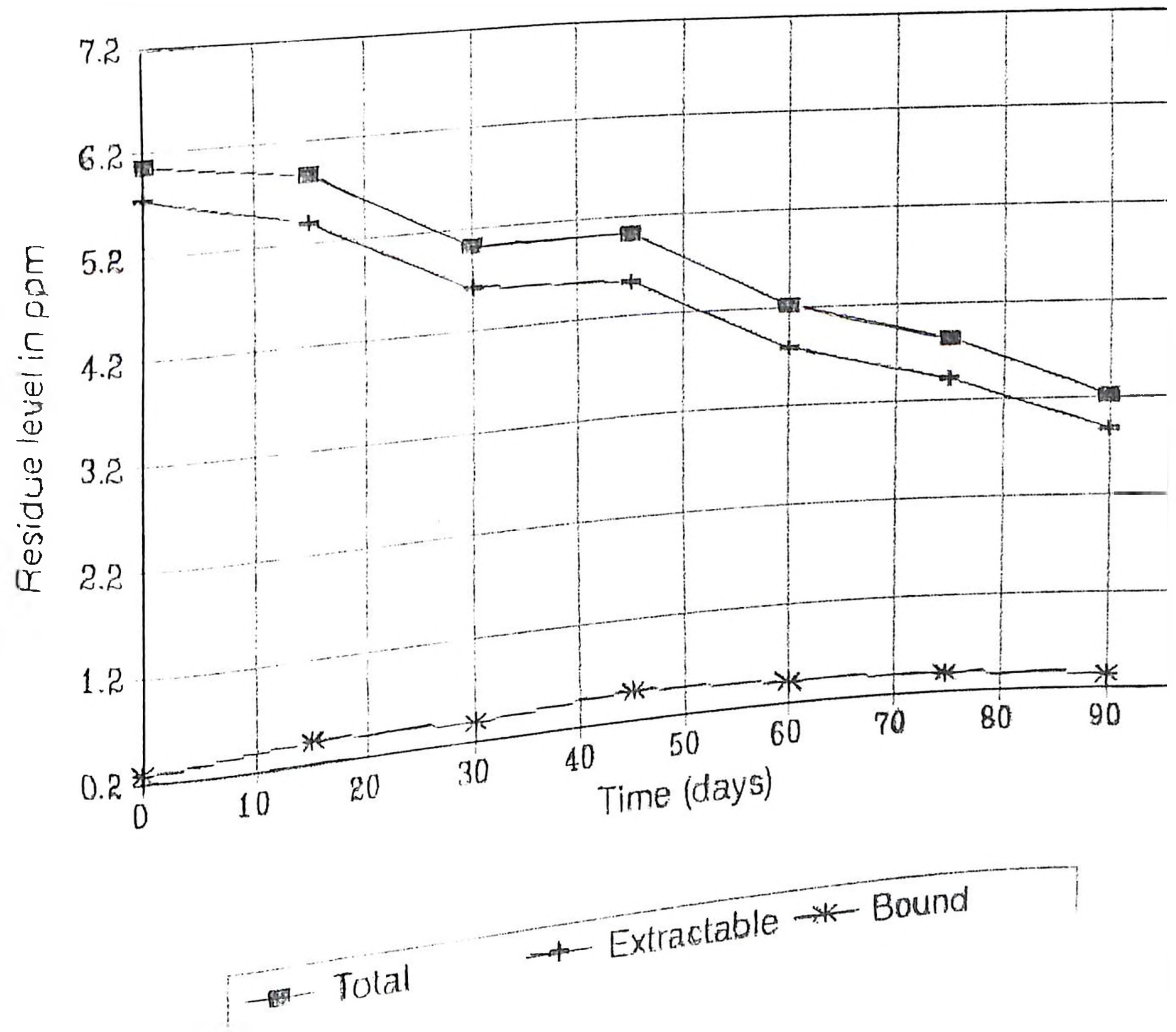

Figure A5. Simple curves showing the variation of P,P'-DDT residue in Nairobi soil

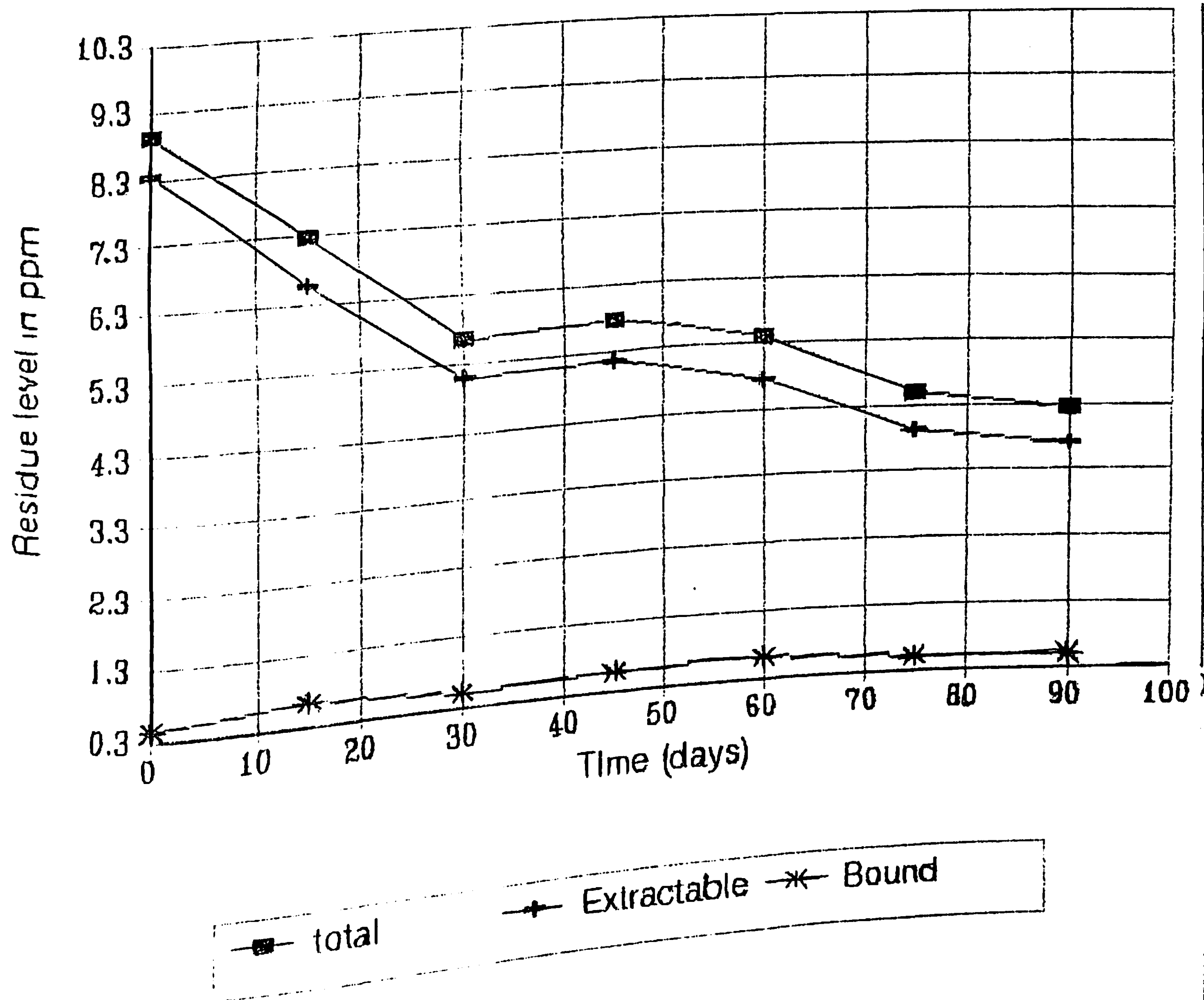

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Figure A6. Simple curves showing the variation of P,P'-DDT residue in Nairobi Plants

Residue level in ppm

4
3
2
1
0

0 10 20 30 40 50 60 70 80 90

Time (days)

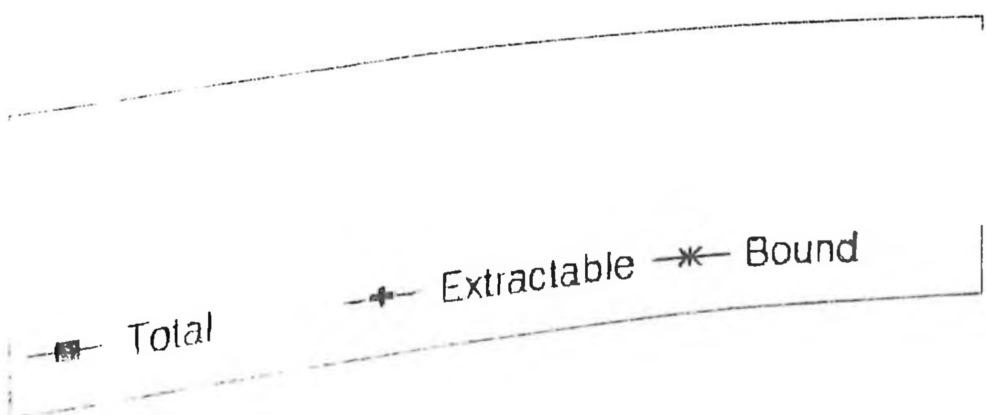

Figure A7. Simple curves showing the variation of P,P'-DDT residue in Mombasa Plants