PERSISTENCE AND ACCUMULATION OF ORGANOCHLORINE PESTICIDES INSELECTEDCOCOAFARMS IN SOUTHWESTERN NIGERIA

BY

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ABSTRACT

Organochlorine Pesticides (OCPs) are cyclic chlorinated hydrocarbons that are toxic and can bioaccumulate in the environment. Although, its usehas been prohibited in many countries including Nigeria; nevertheless they are still used extensively for pests control by cocoa (*Theobroma cacao*L.) farmers in Nigeria. In addition, OCPs residual concentrations among other factors in cocoa bean influences their price in the World market. Therefore, this study was designed toassess the persistence of OCPs in cocoa plants, soil, surface water, and sediments in selected cocoa farms in Southwestern Nigeria.

The cocoa farms selected for this study were classified as; farms where OCPs have been used and discontinued for 15 years [Cocoa Research Institute of Nigeria, Ibadan [(CRIN (I&II)]; for 3–5 years (Sabo, Ondo); still in use [(Igba, Ondo) and (Sore-Bale, Ogun)]. Sampling was carried out during wet and dry seasons between November, 2009 and September, 2011. A total of 480 samples were randomly collected comprising: 120 soils [(0-15 and 15-30 cm), (CRIN I&II-48; Sore-Bale-24; Sabo-24; Igba-24)]; 60surface water (CRIN I&II-24; Sore-Bale-12; Sabo-12; Igba-12); 60 sediments (CRIN I&II-24; Sore-Bale-12; Sabo-12; Igba-12); 60 each of leaves, bark, pods and seeds (CRIN I&II-24; Sore-Bale-12; Sabo-12; Igba-12, per matrix)for analysis. Control samples were collected 5.0 km from CRIN I, where OCPs were not used. Terrestrial Field Dissipation (TFD) and adsorption-desorption studies for OCP were done by EPA/OECD methods using endosulfan (6,7,8,9,10,10-Hexachloro-1,5,5a,6,9a-hexahydro-6,9-methano,2,4,3-benzodioxathiepin-3-oxide). Extractions and clean-up of OCPs were done using standard analytical methods. TheOCPs were determined using Gas Chromatography-Mass Spectrophotometric (GC-MS) technique. Data were compared with WHO/FAO limits and analysed using correlation and descriptive statistics.

Nineteen OCPs were detected in all the matrices. Total (∑) OCPs in vegetation (μgg^{-1}), soil (ngg^{-1}) ranged from <0.001 to 12.67 and 11.48 to 1,166.71, respectively. Amongst the Hexachlorocyclohexanes (HCHs), β-isomer was most predominant in CRIN. Bioaccumulation factors ranged from 0.1 to 658.0 in CRIN. The $\sum OCP_{0\text{-}15cm} < \sum OCP_{15\text{-}30cm}$ except in Sore-Bale and Igba, due to fresh input. Total Endosulfan was the most dominant, while α-/β-endosulfan and endosulfan sulfate/endosulfan ratios in Sore-Bale

were >1.7 (0-15 cm) and <0.3 (15-30 cm), respectively. Total OCPs residues in surface water ranged from 0.10-1.39 μgL^{-1} and 0.06-1.35 μgL^{-1} for dry and wet seasons, respectively. Igba and CRIN exceeded the WHO/FAO limit (1.0 μgL^{-1}). The ∑OCPs in sediments ranged from 0.88 to 9.85 μgg^{-1}, with γ-HCH (lindane) >0.01μgg^{-1}; these values exceeded consensus-based threshold effect concentrations and probable effect concentrations. The TFD studies, showed initial ∑endosulfan distribution trend as; leaves > bark > pods >seeds. Dissipation half-life ranged from 7.93 to 79.82 days. Adsorption rate constants for endosulfan sorption studies ranged from 6.91 to 11.52×10^{-3} min^{-1} for CRIN, Sore-Bale and Igba soils. The Freundlich adsorption constants for non-linear isotherm curve (≤0.5574) supports the mechanism of adsorption.

The presence of organochlorine pesticides in various matrices at Cocoa Research Institute of Nigeria and Sabo farms, established their persistence and accumulation. The pesticides were more adsorbed and persistent in the Cocoa Research Institute of Nigeria soil than other soils.

Keywords: Bioaccumulation of pesticides, Cocoa farm, Terrestrial field dissipation, Adsorption-desorption

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CERTIFICATION

I certify that this work was carried out by Mr E. N. VAIKOSEN in the Department of Chemistry, University of Ibadan

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ABBREVIATIONS

API	Atmospheric Pressure Ionization
ASE	Accelerated Solvent Extraction
ASTM	American Society for Testing and Materials
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	Bioaccumulation Factor
CB-PEC	Consensus-Based Probable Effect Concentrations
CB-TEC	Consensus-Based Threshold Effect Concentrations
CCME	Canadian Council of Ministers of the Environment
CI	Chemical Ionization
CNS	Central Nervous System
CRIN	Cocoa Research Institute of Nigeria
CSSV	Cocoa Swollen Shoot Virus
DCI	Desorption Chemical Ionization
DDD	dichlorodiphenyl dichloroethane
DDE	dichlorodiphenyl dichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DMDS	Dimethyldichlorosilane
ECD	Electron Capture Detector
EI	Electron Impact
EPA	Environmental Protection Agency
ES	Electrospray
ESD	Endosulfan diol
ESE	Endosulfan ether
FAB	Fast Atom Bombardment
FAO	Food and Agriculture Organization of the United Nations
FD	Field Desorption
FFRs	Fat/Fat Retainer Ratio
FI	Field Ionization
FID	Flame Ionization Detector
GC-MS	Gas Chromatography – Mass Spectrophotometer
GEMS	Global Environmental Monitoring System
GFEA	German Federal Environment Agency
GPC	Gel-Permeation Chromatography
GPS	Global Position System
GSC	Gas-Solid Chromatography
H^+	Hydrogen ion
HCH	Hexachlorocyclohexane
HEOD	Hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene
HHDN	hexahydro-1,4,5,8 dimethano-naphthaene
HI	Hysteresis Index
IARC	International Atomic Research Council
ICP	Inductively Coupled Plasma
IMP	Ion Microprobe
IUPAC	International Unit of Pure and Applied Chemistry

LD_{50}	Lethal Dose at 50%
LLE	Liquid-Liquid Extraction
LPME	Liquid-Phase Micro-Extraction
LRT	Long-Ranged Atmospheric Transport
MAE	Microwave-Assisted Extraction
MALDI	Matrix-Assisted Laser Desorption
MRL	Maximum Residue Limit
MSPD	Matrix Solid-Phase Dispersion
NAFDAC	National Agency for Food and Drug Administration
NAFTA	North American Free Trade Agreement
NCDC	National Cocoa Development Commission
ND	Not Detected/Not Detectible
NIST	National Institute of Standards and Technology
NRC	National Research Council
OCPs	Organo-chlorine Pesticides
OECD	Organization of Economic Co-operative and Development
OM	Organic Matter
PAH	Poly-Aromatic Hydrocarbon
PBBs	Polybrominated Biphenyl
PBI	Particle Beam Interface
PD	Plasma Desorption
PEC	Probability Effect Concentration
PLE	Pressurized Liquid Extraction
PLOT	Porous Layer Open-Tubular
POP	Persistent Organic Pollutants
PS	Plasmaspray
RT	Retention Times
SCOT	Support Coated Open-Tubular
SDME	Single-Drop Micro-Extraction
SEC	Size Exclusion Chromatography
SFE	Supercritical Fluid Extraction
SIMS	Secondary Ion Mass Spectrometry
SOP	Standard Operating Procedures
SPME	Solid-Phase Microextraction
SQGs	Sediment Quality Guidelines
SS	Spark Source
TCD	Thermal Conductivity Detector
TCM	Traditional Chinese Medicines
TEC	Threshold Effect Concentration
TETFUND	Tertiary Education Trust Fund
TFD	Terrestrial Field Dissipation
TOC	Total Organic Carbon
TSP	Thermospray
UK	United Kingdom
UNEP	United Nations Environmental Programme
USEPA	United States Environmental Protection Agency
UV	Ultra-violet

WCOT	Wall-coated open tubular
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1. PESTICIDES

According to Food and Agricultural Organisation (FAO) of the United Nations' definition – 'A pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal diseases, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs or substance which may be administered to animals for the control of insects, machines or other pest in or on their bodies. It also includes compounds used as plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit and substance applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport'(FAO, 2002).

Pesticides are classified according to their target pest or organism (e.g. insecticides – kills insects and arthropods; fungicides – kills fungi, including blights, mildews, molds, and rusts; herbicides - kills weeds and other plants growing where they are not wanted; rodenticides - controls mice and other rodents; avicides – kills birds; bactericides – kills and prevent growth of bacteria; algaecides - controls algae in water bodies like lakes, canals, swimming pools, water tanks, and other sites; nematicides - kills nematodes and microscopicworm-like organisms that feeds on plant roots; virucides – control growth of virus; molluscicides–kills snails and slugs) (Gilden *et al.*, 2010); Chemical structure (e.g. organic, inorganic, synthetic), biological (e.g., biopesticides) and physical state (e.g. gaseous fumigants) (AMA, 1997). Inorganic pesticides which contained elements like sulphur (S), arsenic (As), mercury (Hg), lead (Pb) etc. were used as pesticides around the 15^{th} century by man. Biopesticides are naturally occurring substances with pesticidal properties, derived from living organisms such as animals, plants and microbes. In recent

years, plant-derived pesticides or botanicals, such as the pyrethroid, rotenoids, nicotinoids and botanical rodenticides such as strychnine and scilliroside (Karim, 1997; Lamberth*et al.,* 2013) have come to the fore and on the increase.Also many pesticides can be grouped into chemical families. Notable insecticide families include organochlorines, organophosphates, carbamates and pyrethroids (synthesized pyrethrin). Organochlorines were the first generation insecticides with pesticidal activity, which largely is being replaced by organophosphates, carbamates and synthetic pyrethriods.

1.1.1. Organochlorine pesticides (OCPs) – Historical background

Organochlorine pesticides (OCPs) are chlorinated hydrocarbon of cyclic structure and high molecular weight. They are usually of low volatility in contrast to most hydrocarbon and fumigants. Since the discovery of the pesticidal property of dichloro-diphenyltrichloroethane (DDT), the organochlorine prototype by Muller in 1939, and later introduced in the 1940's for the control of pest in agriculture and vector organisms, many synthesized organochlorine pesticides (OCPs) have been produced(Karim, 1997). This had led to numerous challenges associated with their use. Dichloro-diphenyltrichloroethane had earlier been synthesized in 1874 by a German Chemist Zeilder. In the 1960s, it was discovered that DDT was preventing many fish- eating birds from reproducing which was a serious threat to bio-diversity. Public awareness and concern was aroused by Rachel Carson in the best-selling book – *Silent Spring (1962),* about biological magnification. This publication focused on the persistence of DDT and other OCPs. DDT was banned in many countries in the 1970sin response to public concern and scientific evidence linking DDT to destruction of wildlife from a number of monitored exercises.

Organochlorine pesticides (OCPs) are typical persistent organic pollutants (POPs), according to the United Nations Environment Programme (UNEP) Stockholm Convention of 2009 (UNEP, 2009). There has been great concern for OCPs due to their high persistence, bioaccumulation and toxicity (PBT) in the environment (Zhang *et al*. 2002; Wan *et al*. 2005). Furthermore, they are semi-volatile, long range transport species and have being reported found where they have not being used like the arctic and Antarctic regions, hence their ubiquitous nature.

OCPs have been reported virtually found in all compartments of the environment (such as soil, water, sediment, atmosphere and biotas) and trophic levels of our ecosystem (Cai *et al*. 2010; Liu *et al.* 2008). Soil is the major recipient of non-target applied OCPs. They are also primary accumulators from where plants take up OCPs through their roots and translocated to aerial parts (leaves, stem, bark and fruits) where they are bio-accumulated. Also OCPs in soils are leached during run-off to impact surface water or migrate to contaminate the aquifer with time. In the aquatic environment, low dose of OCPs might cause biologically adverse effects on aquatic life (Crisp *et al*. 1998) or lead to bioaccumulation and bio-magnification along the food chain in the ecosystem. The aforementioned is enhanced because of their lipophilic nature. Sedimentary system is the sink for OCPs in the aquatic environment and there is portioning and redistribution of OCPs amongst surface water, sediment, suspended particulate matter (SPM) and aquatic biotas (Liu *et al*. 2008). Contaminated edible aquatic organisms might be hazard to human beings when consumed. In man it has been reported to cause disruption of the endocrine system (Bretveld *et al*. 2006; Mnif *et al*. 2011)

The major sources of organochlorine pesticides introduction into the environment are by human activities - ie anthropogenic activities (Osibanjo *et al*. 1994). The use and application of OCPs could be done or carried out by simple spraying to control weeds, insect pests in small farms or aircraft spraying of large farms to check the menace of birds, army of insects (e.g., locust), and accidental spillage from an industrial or agricultural site, domestic and industrial effluents. Others sources of contamination includes run-offs (e.g. leachates) from solid wastes dump sites and gaseous emission due to incineration.

The numerous challenges facing cocoa farmers in the West Africa sub-region has necessitated the use of OCPs (such as Lindane, DDT, endosulfan, etc.) to improve their harvest. These problems include the presence of weeds and defoliating insects (grasshoppers, bettles, etc.) in young cocoa plants and also in principal crop production. They have been faced with mirid (capsid bug) problem for up to a century (Bateman 2003). For a long time, the decline in cocoa production in the West African sub-region has been attributed to the destructions caused by cocoa capsids (Heteroptera: Miridae; mostly *Sahlbergella singularis and Distantiella theobroma*) and diseases - such as swollen shoot,

caused by cocoa swollen shoot virus (CSSV) (transmitted by mealybug) and black pod caused by the fungi *Phytophthorapalmivora* and *P. megakarya* (Padi and Owusu, 1998; Bateman, 2008).

1.2. JUSTIFICATION OF STUDY

There is paucity of information on organochlorine pesticide residues in cocoa farms- vis-à-vis matrices associated with such farms; namely:- leaves, stem, pods, cocoa bean, roots, bark of the plant and as well as the immediate surrounding environment;- soil, water (surface and ground), sediments and atmosphere.

Due to their high lipophilic nature, they are easily stored in adipose tissues of mammals, fish, plants and other biotas - leading to bioaccumulation, bio-concentration and bio-magnification along the food chain -hence a potential health hazard. OCPs have been banned over four decades and there are series of report of their presence in our environment, even in places they were never used. They are highly persistent in the environment and found in all environment components – air, water (surface and ground), soil, sediment and biota.

Cocoa farms in Nigeria have been treated with OCPs since the sixties and about seventypercent (70%) of such farms are located in South-western Nigeria, with an annual production of 400 tonnesannually (Adeyeye, 2011). Cocoa bean is the major constituent of chocolate beverages and bars and Nigeria is a major player in the international cocoa market and industry. It is therefore necessary for this study to be carried out, not only to ascertain the levels of OCPs in the beans, but also on the adjoining environment.

1.3. OBJECTIVES OF STUDY

The major thrust of this research shall be:

I) To establish and estimate the extent/degree of the persistence of organochlorine pesticides in cocoa plants and its immediate surrounding environment (- soil, sediment and surface water) in cocoa farms in South Western Nigeria – by evaluating the residual concentrations of OCPs in the aforementioned matrices in farms where they were used in time past and also where they are still being used.

II) To establish the trend or order of OCP bioaccumulationand distribution in the various parts of cocoa plant (leaves, pods, seeds and bark) – to ascertain the part of the plant they tend to bio-accumulate most.

III) Evaluation of the risk level which farmers are exposed to in farming communities who depend on surrounding streams for domestic purposes by comparing residual values obtained with WHO permissible limits for surface water; And also to carry out an ecological risk assessment of sediments by comparing values obtained for sediments from streams in these cocoa farms with published numerical sediment quality guidelines (SQGs)

IV) Determine the fate of technical grade endosulfan pesticide in a cocoa farm environment - vegetation (fresh leaves, stem bark, pods and seeds) and immediate surrounding environment (soils and fell dry leaves) after a single treatment (T1) or application. And to enumerate the chemo-kinetic parameters and half-lives of the two-parent endosulfan enantiomers and the major metabolite – endosulfan sulphate.

V) Investigation of the interaction (adsorption-desorption studies) between commercial grade endosulfan (which comprise 70% α-endosulfan and 30% β-endosulfan) with cropped soils from cocoa farms in South-Western Nigeria. The adsorption-desorption mechanisms of endosulfan enantiomers in the topsoil profile (0-15cm) from these farms shall be enumerated.

1.4. SCOPE OF STUDY

1. This study shall cover cocoa farms on which OCPs were used and discontinued/abandoned as long as for 10-15 years; farms where OCPs applications were recently discontinued for 3-5 years and; thirdly, cocoa farms where OCPs are still being used. Cocoa vegetation (fresh leaves, stem bark, pods and seeds), surrounding soils, surface water and sediments where applicable shall be studied.
2. Field kinetic studies of sprayed technical grade endosulfan will be carried out on designated farm areas where OCPs have not been applied over a period of 60 days. Concentrations of isomeric parent endosulfans (α- and β-endosulfan) and major

metabolite (endosulfan sulfate) shall be monitored. Half-lives and other chemo-kinetic parameters of these chemicals shall be determined in plant (fresh leaves, bark, pods and seeds) and surrounding matrixes(fell dry leaves, soil, surface water and sediments).

3. Finally, soil reaction with technical grade endosulfan shall be carried out. This will involve the adsorption-desorption kinetic and isotherm studiesof endosulfan with aged and air-dried soils from three cocoa farms (CRIN, Sore Bale and Igba). Kinetic and Freundlich variables shall be determined for each farm soils.

CHAPTER TWO

LITERATURE REVIEW

2.1. CLASSIFICATION OF ORGANOCHLORINE PESTICIDES

Organochlorine pesticides (OCPs) could be classified into dichlorodiphenyl ethanes and cyclodiene (hexachlorocyclopentadiene), and other related compounds (Anon, 2014). Dichlorodiphenyl ethanes are made up of dichlorodiphenyl trichloroethane DDT, its metabolites such as dichlorodiphenyl dichloroethane (DDD), dichlorodiphenyl dichloroethylene (DDE) etc. and analogues like methoxychlor, bulan and dicofol. Cyclodieneconsist of OCPs such as aldrin, dieldrin, heptachlor (and its expoxides), endrin, endrin aldehyde, endrin ketone, chlordanes, endosulfans etc. Each cyclodiene is characterized by the presence of an endo–methylene bridge. Other classes are the Hexachlorocyclohexanes, with about eight isomers (only five isomers are of commercial significance; α-, β-, γ-, δ-, ε-HCH), Chlordecons (chlordecone, kelevan and mirex) and class Toxaphene. Most popular and toxic is the γ-HCH (lindane). Generally, they exert their pesticidal effect by disrupting the sodium/potassium balance of the nerve fiber, thereby causing the nerve to transmit continuously. They persist and readily bio-accumulates and their toxicities vary greatly (Atuma and Okor, 1985).

2.2. CHEMICAL STRUCTURE, PROPERTIES AND DEGREE OF TOXICITIES OF ORGANOCHLORINE PESTICIDES (OCPs)

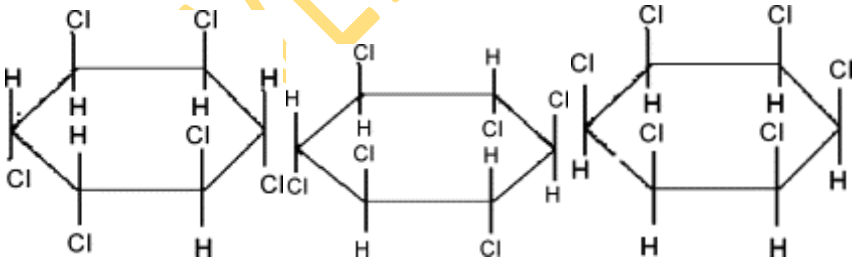
In most cases, the effective or activity of a chemical compound intended for use, as a drug, is greatly influenced by its structure. In likewise manner, the pesticidal activity of a compound used as a pesticide is predominantly associated with its structure (Kaushik and Kaushik, 2007). Also, the different moieties or substituent groups attached to parent compound and their spatial arrangements within the molecule and nature of such substituents,enhances direct or indirect the toxicity of the parent pesticidal compound. Other properties such as symmetry and asymmetry of molecules, the solubility, polarity

and sorption values, can also promote or reduce pesticidal effect of organochlorine compounds. Therefore it is imperative that their structure and chemical properties have a strong correlation to their toxicological effect.

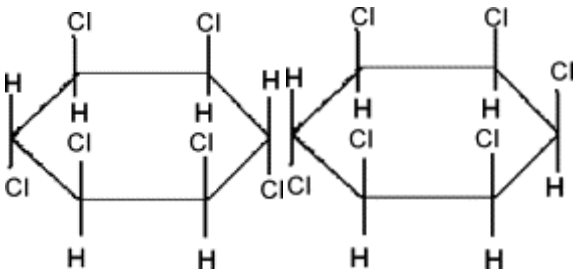
2.2.1. Chemical Structure and Properties of Some OCPs

2.2.1.1. Hexachlorocyclohexane (HCH)($C_6H_6Cl_6$)

Dupire and Raucourt (1945) and Slade (1945) independently discovered the insecticidal properties of hexachlorocyclohexane. It exists as multiple isomers. Sixteen (16) stereoisomers are possible, however eight (8) isomers are well known (Sullivan and Kriwger (2001). Amongst these are α-,β-, γ-, δ-, ε-isomers and onlyγ-HCH, α-HCH, β-HCH, and δ-HCH are of commercial importance andof environmental concern. Technical-grade HCH is a mixture of several isomers and its made up of approximately 60–70% α-HCH, 5–12% β-HCH, 10– 15% γ-HCH, 6–10% δ-HCH, and 3–4% ε-HCH (Kutz *et al*., 1991). However, only γ-HCH (lindane) has an effectual and pronounced insecticidal property. It is a colourless crystal with a melting point of 112.5^{o}C and the most soluble OCP in water. Lindane is stable to light, heat, and strong acid and has a saturated fatal dose of 150 mgkg^{-1} body weight (with an oral LD_{50} of 100mgkg^{-1} to rat) (Gaines 1969). It is marketed as Gammalin 20 or Gammazone. Other isomers such as β-HCH and ε-HCH are non-toxic, while α- and δ-isomers have LD_{50} > 1000 mgkg^{-1} to rat.



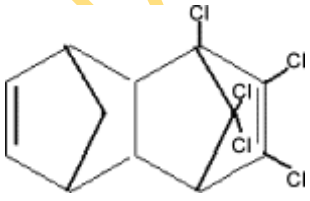
α-HCHβ-HCH δ-HCH



γ-HCH ε-HCH

2.2.1.2. Aldrin ($C_{12}H_8Cl_6$)

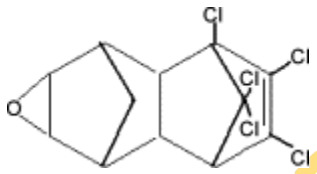
Aldrin {(1R,4S,4aS,5S,8R,8aR)-1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4 :5,8-dimethanonaphthalene}is derived from hexachlorocyclopentadiene as its parent compound and contains 95% w/w of 1,2,3,4,10,10-hexachloro,1,4,4a,5,8,8a-hexahydro-1,4,5,8 dimethano-naphthaene (HHDN) together with 5% w/w of other active related compounds. It is a colourless, white crystal, with a melting point of 104^{o}C. It is readily soluble in acetone, benzene and xylene. It is also stable to heat, alkali and mild acids. When heated to decomposition, it evolves highly toxic fumes of phosgene and hydrogen chloride. The insecticidal properties of aldrin were first reported by Kearns*et al.* (1945). The active ingredient is highly effective as a pesticide, however it residual life span is relatively short under field conditions at specified and normal applied concentrations.It has an LD_{50} of 39 $mgkg^{-1}$ to rat. Technical grade aldrin is approximately 82–85% pure and contains 1–3% each of polychlorinated hexahydro-dimethano-naphthalenes, di-adducts, bicycloheptadiene,Hexachlorocyclopentadiene, hexachlorobutadiene, hexachloroethane, and octachlorocyclopentene (Brooks, 1974a, 1974b). Aldrin is produced from the reaction of bicyclo (2.2.1) heptadiene (a condensation product of cyclopentadiene and acetylene) and hexachloro-cyclopentadiene (Zitko, 2003).



Aldrin

2.2.1.3. Dieldrin ($C_{12}H_8OCl_6$)

Dieldrin, 1,2,3,4,10,10-Hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene (HEOD) is another analogue from the hexachlorocyclopentadiene family and it is also insecticidal (Kearns *et al.* 1945). It is a light–tanned, flaky crystalline solid, without any distinct odour and melts at 175-176^{0}C. Technical grade dieldrin contains 85% of HEOD, while remaining 15% being chlorinated compound related to HEOD. It is stable to alkali and mild acids; practically insoluble in water. It is an epoxidized form of aldrin (Cannon and Bigger, 1958) and found to be highly effective against household pests and insects such as flies, fleas, ticks, ants, lice and mosquito larvae.It is one of the longest residually active chemical, with an LD_{50} 46 mgkg^{-1} to rat.It is manufactured from the epoxidation of aldrin, usually carried out by either in hydrogen peroxide in the presence of tungstic oxide as catalyst, in tert-butanol, dioxane, dimethyl formamide, or sulfolane, at 20–75 °C for 1–6 h. Alternatively, by simply dissolving aldrin in an organic solvent (likebenzene) and react it with peracid (like peracetic acid) in water (Sittig, 1977).

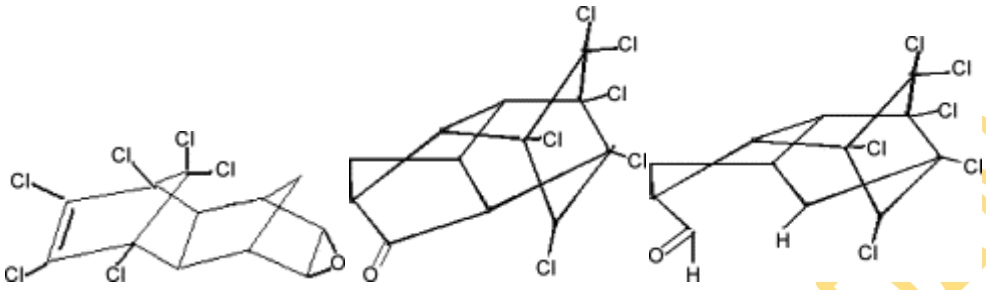


Dieldrin

2.2.1.4. Endrin ($C_{12}H_8OCl_6$)

Endrin, 1,2,3,4,10,10 Hexachloro 6,7 epoxy 1,4,4a,5,6,8,8a octahydro 1,4 endo, exo 5,8 dimethano–naphthalene is isomeric with dieldrin, except for the arrangement of two chlorine atoms at the endo-position (bridge). Endrin is the most toxic OCPs in the endo-form. Endrin is five times as toxic as dieldrin, Fifteen times as toxic as DDT to mammals, 30 times as poisonous to fish and 310 times as toxic to birds. Endrin is reported to have an oral LD_{50} of 7.5 -17.5 mg/kg to rats (Treon *et. al*. 1955) and most toxic of the aldrins.Hexachlorocylopentadiene is condensed with acetylene (at 150–175 °C, 2000–4000 psi) into an intermediate product which is further condensed with cyclopentadiene at 50–90 °C and atmospheric pressure; this is finally epoxidized by peracetic acid. The

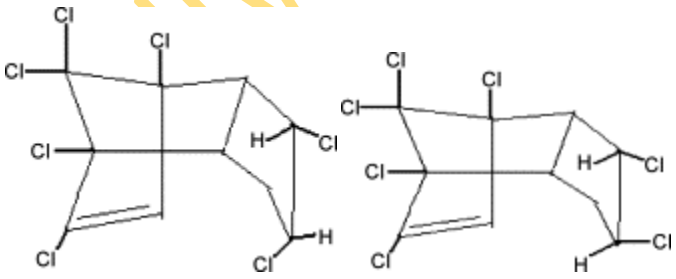
resulting mixture is steam-distilled to remove excess reagents and solvents and endrin is extracted with ether and dried (Sittig, 1977).Technical grade endrin is 96.6% pure, contains traces of aldrin, dieldrin, endrin half-cage ketone, and other minor impurities (Zitko, 2003).



Endrin Endrin ketone Endrin aldehyde

2.2.1.5. Chlordane ($C_{10}H_6Cl_8$)

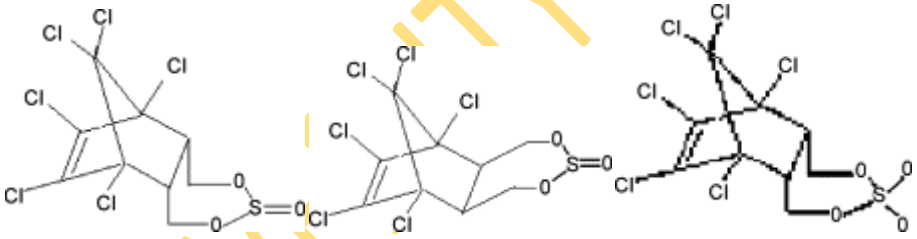
Chlordane is 1,2,4,5,6,7,8,8 – Octachloro 2,3,3a,4,7,7a hexahydro 4,7 methano-indene (IUPAC) and exist as cis – and trans-isomers. It is an amber viscous liquid, with a boiling point of 175^{o}C. Clordane is insoluble in water but soluble in most organic solvent, it is dechlorinated by alkali to form a non-toxic product by loss of hydrogen chloride.Chlordane is very stable in the environment – thus could persist for a long time. The oral LD_{50} ranged from 280 - 590 mgkg^{-1}and 83 mgkg $^{-1}$ (body weight of rat) for cis- and trans-isomers respectively (Gaines, 1969). In man its' acute lethal dose is estimated to be 25-50 mg/kg body weight. It was introduced as an insecticide in 1947, however its' pesticidal properties was first enumerated by Kearns *et al*. (1945).



cis-Chlordane trans-Chlordane

2.2.1.6. Endosulfan ($C_9H_6Cl_6SO_3$)

Endosulfan,6,7,8,9,10,10-Hexachloro 1,5,5a,6,9a hexahydro 6,9 – methano 2,3, benzo dioxathiepin -3-oxide (IUPAC name) usually consist of a mixture of two isomers (α- and β-isomers). It is a brown crystal. α-isomer, melts at 108-110^{o}C, while β-isomer at 208-210^{o}C.Finkenbrink (1956) first described the insecticidal properties of endosulfan in 1956. Endosulfan is a chlorinated hydrocarbon of the cyclodiene subgroup which acts as a contact poison for a wide variety of insects and mites. The slow conversion of the β-isomer to the α-isomer on storage has been reported by Hapeman *et al*. (1997) and Rice *et al.* (1997). α-Endosulfan isomer has greater toxico-insecticidal properties than β-endosulfan (Maier-Bode, 1968). Both isomers differ only in the spatial arrangement of the ring bearing the sulphite moiety. Endosulfan is metabolized in plants and animals to form the sulphate, which is much more toxic than its parent endosulfan. The oral LD_{50} in rats of endosulfan sulphate is reported to be 18mgkg^{-1}(Gaines, 1969), while α- and β-isomers are 76 mgkg^{-1} and 240 mgkg^{-1} respectively (Maier-Bode, 1967). The technical grade is composed of 70% α-isomer and β-isomer is about 30%. The solubility of α-and β-endosulfan and metabolite are 0.32 mgL^{-1}, 0.33mgL^{-1} and 0.48mgL^{-1}respectively (Extonet 1993 UniOregon). Endosulfan is stable to sunlight.



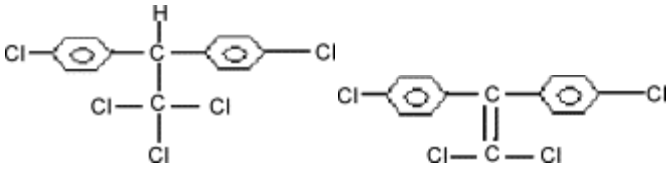
α-Endosulfan β-Endosulfan Endosulfan sulphate

2.2.1.7. Dichlorodiphenyltrichloroethane (DDT) ($C_{14}H_9Cl_5$)

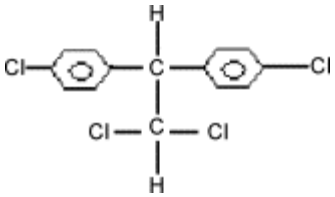
Dichlorodiphenyltrichloroethane (pp'-DDT), 1, 1, 1 Trichloro 2,2-bis (4-chlorophenyl) ethaneis a white or nearly white crystals, small granules, flakes or powder and exist in two isomeric forms:- op'- and pp'- DDT. pp'-DDT constitutes 70%, while remaining is made up of op'-DDT isomer and other related compounds. Melting point is 108^{o}C and it is insoluble in water. DDT biodegrades readily, transforming to dichlorodiphenyl dichloroethane (DDD) or dichlorodiphenyl dichloroethene (DDE) metabolites. DDT is the most toxic amongst its analogues and metabolites, with an LD_{50} of 300 mg/kg to rats

(Draize *et al*. 1944). DDE is highly persistent in the environment. It is the fore runner ofsynthetic OCPs and was referred to as a wonder chemical because of its effective pesticidal properties for years after its discovery.

DDT was first synthesized by Zeidler (1874), while its powerful insecticidal properties were discovered in 1939 by a Swiss entomologist, Muller (1955). At the time Muller discovered DDT, its major advantages that made it the wonder pesticide and most useful insecticide were its broad spectrum insecticidal activity, low cost, stability, low mammalian toxicity andits' relatively greater persistence.



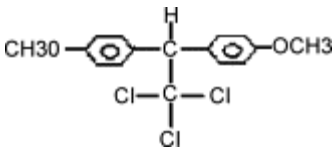
dichlorodiphenyl trichloroethane (DDT)dichlorodiphenyl dichloroethene (DDE)



dichlorodiphenyl dichloroethane (DDD)

2.2.1.8. Methoxychlor ($C_{16}H_{15}ClO_2$)

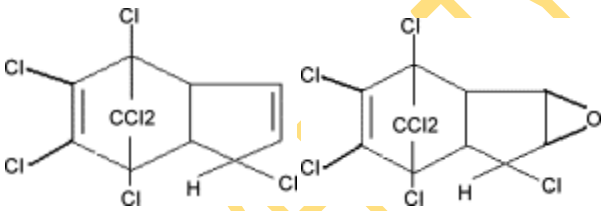
Methoxychlor, 1,1,1, trichloro-2-2 bis (4 methoxy phenyl) ethane is a grey flaky powder, insoluble in water. Technical grade contains 88% w/w of methoxychlor and about 12% of related isomers. The pure p.p'-isomer is a colourless crystal which melts at 89^{o}C. It is an analogue of DDT and similar to DDT in its physical and chemical properties, although less toxic than DDT. Methoxychlor has been reported to show little tendency to accumulate in body fat unlike most organochlorines, because it readily biodegrades by oxidative demethylation (Yim *et al.* 2008; Satsuma *et al.* 2012).



Methoxychlor

2.2.1.9. Heptachlor ($C_{10}H_5Cl_7$)

Heptachlor, 1,4,5,6,7,8,8a Heptachloro 3a,4,7,7a tetrahydro 4,7 endomethano-indene is a white crystalline solid which melts at 95-96^{o}C. It is insoluble in water and soluble in organic solvents. It is stable in acids and alkalis. It is converted to the epoxide form by animals, insects and microbes in the soil. Heptachlor is a very efficient stomach insecticide as well as exhibiting fumigant activity. The epoxide of heptachlor exhibit better insecticidal properties than parent heptachlor, as the poisoning symptoms appear parallel with the formation of the epoxide within the insect body (Perry *et al*.1958).



Heptachlor Heptachlor epoxide

2.2.2. Toxicity and Chemical Structure

Kaushik and Kaushik (2007) reviewed and assessed the correlation between chemical structure and toxicity of organochlorine pesticides. The authors concluded that the structures of OCPs have direct relationship with their toxicity and their mode of action in target organism is closely associated with the structure of pesticidal compound. Also, the parent molecule of pesticide compound is not only responsible for the activity but also the nature of substituents, presence of the epoxide ring, double–triple bond, conjugation,

aromaticity and the stereochemistry of the compound. Therefore an understanding of the structure of compounds and their correlation with toxicity to target organism is a very important parameter for developing better designed pesticidal compounds with tailored toxicidal properties on different pests.

The toxicological effects of OCPs on biota have been reported for a broad spectrum of species in the environment. They affect central nervous system (CNS) causing hyper-excitable state in brain, convulsions, tremor, hyper-reflexia and ataxia. Boyd and de Castro, (1996) reported on the relation of protein-deficient diet and DDT toxicity. Higher levels of OCPs stimulated the tissues to produce more of hepatic microsomal drug metabolizing enzymes. Effects in humans can be seen on prolonged and intensive exposure. Organochlorines may also interact with endocrine receptors like of estrogen and androgens, thereby disrupting their actions and causing hormonal in-balance. Their poisoning may cause various symptoms including headache, nausea, dizziness, vomiting, tremor, lack of co-ordination and mental confusion.

The ingestion of wheat treated with hexachlorobenzene (HCB) results in dermal toxicity, also known as porphyria cutanea tarda (Khayat *et al.* 2013; Andersen *et al.* 2015). They have also been reported to exhibit carcinogenic effects (Arrebola *et al*. 2015; Parada *et al*. 2015; Koutros *et al.* 2015). Respiratory depressions may also occur. The presences of OCPs in human breast milk have been reported in different studies (Shaw *et al.* 2000; Bouwan *et al*. 2006; Okonkwo *et al*. 2008). Organochlorine compounds have been found to have toxic effects on aquatic organisms (Zhou *et al*. 1999; Wang *et al.* 2011). The acute toxicity of endosulfan in juvenile rainbow trout showed severe focal necrosis in liver cells (Capkin *et al*. 2006),while in *Cichlasoma dimerus* toxicological effects like hyperplasia of inter-lamellar epithelium and mucous cells, blood congestion in secondary lamellae, hypertrophy in gills, pycnotic nuclei, testicular damage and hydropic degeneration in liver were seen (Da Cuna *et al.* 2011).

OCPs have been associated to the rapid decline in the population of the Mexican free tailed bats - *Tadarida brasiliensis* (Thies, *et al*. 1996). Studies also showed great chromosomal abberancy, with the male bat showing greater aberration than the female.

2.3. COCOA AND THE AFRICAN CONTINENT

Cocoa (*Theobroma cacao*) belongs to the natural order of *sterculiaceae* - a family of about 41 genera and 521 species planted in several parts of the world, especially in the warmer regions like Mexico, Brazil, Venezuela, Jamaica, Cuba etc.(TPD, 1996; Crane *et al*. 2009); However, production is concentrated most in West Africa, specifically Côte d'Ivoire which is the world's largest producer, Ghana, Nigeria and Cameroon – all four countries together represent more than 70% of world cocoa production (Abbott, 2013). Sierra Leone, Togo and Liberia produce small amounts of cocoa.Other cocoa-producing nations in Africa include Uganda, Tanzania, Madagascar, Equatorial Guinea and Sao Tome & Principe. These countries are highly renowned in the industry and among chocolate manufacturers for their cocoa's aromatic properties.

There is strong evidence that the cocoa tree, *Theobroma cacao*, originated from South America and its beans consumed as a drink by the Mayans and Aztecs, which covers the present day South American countries of – Honduras, El Salvador, Guatemala and Southern Mexico. It was introduced to Spain by Hernan Cortés in 1528 after his expedition to Aztecs. The Spaniards set up cocoa plantations in its West Indies colonies to meet demand and by the late 17th century, cocoa drinking spread across Europe - French, English, and Dutch territories.

Although there are indications that cocoa was introduced into Africa much earlier, but it was not until the late 19th century that production began on asignificant scale. The first large-scale production was in the 1880s by the Portuguese plantations on the islands of São Tomé andPrincipe (Clarence-Smith and Ruf, 1996). Also the Spanish island of Fernando Po'o (Bioko), off the Cameroonian coast was also one of the earliest cocoa-producing colonies in Africa. Production by Europeans and Creoles took off in the 1880s and 1890s respectively, largely triggered by the sharp downturn in prices for palm oil.However, this in contrast to the success story of African Smallholder farming in Ghana on the cultivation and expansion of cocoa production. The first successful introduction of cocoa is attributed to a Ghanaian, Tetteh Quarshie, who brought cocoa pods back from Fernando Po'o in 1876. From 1886 Ghanaian authorities also encouraged cocoa cultivation, providing some support including seed and training (Acquaah, 1999). Exports

startedin 1891 and within a space of 20 years Ghana was the world's largest cocoa producer - exporting almost 40,000 tonnes (t). The harvest rose further to over 200,000t in 1923, and 311,000t in 1936 – this record was unbroken until after independence, when a downturn was witnessed.

In Nigeria, early cocoa production was encouraged on a tripitate level by - the Christian Mission, the AfricanChurch, and the Coker family. It began in the Western Region and later spread to the southwest (Acquaah, 1999). The oil boom in the 1970s led to massive rural-urban migration in Nigeria which resulted in drastic fall in the production of cocoa.

Pesticides have been used in cocoa farms for over fifty years (50 years), with early independent researches carried out notably in Brazil, Cameroon, Cote d'voire, Indonesia, Malaysia, Togo and the former West African Cocoa Research Institute, now the Cocoa Research Institutes of Ghana and Nigeria.

2.3.1. Cocoa tree morphology, seed composition and uses

Cacao is native to lowlands of South America and grows wild and scattered in the tropical rain forest and it is a "handsome" evergreen tree of about 12-15 feet high and branches at the top. When cultivated it is not allowed to grow so high. The stem is erect, straight, 4 to 6 feet high. The wood is light and white, while the bark is thin, almost smooth and brown. The seeds are numerous, compressed, about 2.5 cm long, reddish brown externally, dark-brown internally and imbedded in a whitish, sweetish mucilage and buttery pulp. Cacao seeds are prepared for commerce either by simple drying, in which case their bitterness and astringency are retained or they are cured by a sweating process in which their bitter and astringent properties are much modified and the colour of the seed is changed. There are number of curing methods; however the best method is to allow the seeds to lie for a week in heaps covered with green leaves, such as plantain leaves, etc.(TPD, 1996).

Cocoa seeds is composed of 40 - 50% of fatty matter – this is also referred to as oil of cocoa or cocoa butter (or oleum theobromatis in some cases), 0.88 - 34% of the base theobromine($C_7H_8N_4O_2$), small quantities of caffeine - 0.05 to 0.36%, 1.3 to 7.5% starch, a red colouring matter (cacao red), albuminous matter (6-18%) (Ridenour, 1895). The presence of cacao-red is due to the decomposition of a glucoside under the influence of a

drastic fermentation, resulting in dextrose, cacao-red, theobromine and caffeine. Theobromine when absorbed acts powerfully as a diuretic and has a stimulant or exciting action. Cocoa beans contain up to10% of phenols and flavenoids which are antioxidants potentially inhibiting cancer or cardiovascular diseases, as well as potassium, magnesium, calcium and iron.

Chocolate bars and drinks are major cocoa products and the ingredients for chocolate – cocoa powder and cocoa butter (solids) – are prepared from fermented and roasted cocoa seeds. A typical bar of milk chocolate contains around 15% cocoa liquor and 20% cocoa powder. The distinctive flavour of chocolate develops during the fermentation process.

2.3.2. A Brief Overview on Nigeria's Cocoa Sector

The cocoa industry is one the most vibrant sub-sector in the Nigerian agricultural sector. In addition it is still a major source of revenue amongst the non-petroluem sector - providing employment for a teeming section of the Nigerian population. Cocoa contributed significantly to Nigeria's foreign exchange earnings in the sixties, however, at the turn of the nineties (1999), production level was at an abysmal - 170,000 metric tonne per annum (Ajao, 2006), from a peak production of 308,000 metric tonnes in 1971 before the oil boom of the seventies. Whereas cocoa provides 38 percent of agricultural value added in Côte d'Ivoire and 12 percent in Ghana (Abbott, 2013). In the last decade cocoa cultivation has witness tremendous changes in response to factors like increased demand and favourable price regime at the international market, besides, the Nigerian government has sensitized all stakeholders in the industry at all levels of governace, with policies aimed at improving the annual production of cocoa. In December 1999, the Federal government established the National Cocoa Development Commission (NCDC) which is made up of fourteen states - Abia, Adamawa, Akwa Ibom, Cross Rivers, Delta, Edo, Ekiti, Kogi, Kwara, Ogun, Ondo, Osun, Oyo and Taraba. The major trust and preoccupation of NCDC is to develop the produce through the provision of an enabling environment for its production, processing, packaging and export. Ten years after, the production level has increased to 370,000 metric tonnes (Ajayi *et al*. 2010). NCDC's goal is to increase Nigeria's production substantially to 600,000 tons in the nearest possible term.

2.4. USES OF ORGANOCHLORINE PESTICIDES (OCPs)

Oganochlorine pesticides are mainly used as insecticides. Mode of application varies widely - from pellet application in field crops to spraying for seed coating and grain storage. Some are applied to surfaces to kill insect that pitch there. Some OCPs – such as chlordane, heptachlors and pentachlorophenol – are used for wood treatment to prevent pest damage. Endosulfan was first used as insecticide and miticide in the U.S. in 1954. It was widely used to check control pest in vegetables, fruits, cereal group and cotton, as well as ornamental shrubs, trees, vines and plants.

Lindane has been used in the U.S. and some European countries to protect crop seeds from insects and pest control in forestry, livestock and households pest. And also as an active ingredient in the many medicated shampoos and soaps to control head lice and scabies.

In Africa most of the pesticides used are imported from Europe, America, China, India and Japan. Organochlorine pesticides such as DDT, toxaphene, endosulfan, lindane, dieldrin, heptachlor, and endrin are used in cotton plantations, timber protection, cocoa, coffee, maize, tobacco, bananas, sugar and rice (Osibanjo, 2003). Dieldrin and lindane are used to control desert locusts in countries like Sudan, Niger and Mali. Synthetic pesticides were introduced in the 1940s to the Nigerian market (Atuma and Okor, 1985). Although, there is a ban and restriction on organochlorine pesticides in Nigeria, farmers still use them in different formulations for the control of pests of cocoa, cotton, yams, cassava, groundnuts, cowpea, vegetables and stored products. DDT, Lindane, aldrin and chlordane are used prominently for controlling capsid disease in cocoa farms.

2.5. FATE OF ORGANOCHLORINE PESTICIDES IN OUR ENVIRONMENT

Organochlorine pesticides enter into the environment as a result of anthropogenic activities. They have almost become an integral part of our ecosystem, because of their persistence and unique physico-chemical properties. They are virtually found in all strata of our environment; soil, water, air and biota.

The application of OCPs for the control of pest in farms often leads to impact of the surrounding environment – since not all the applied pesticide reaches the supposed target site or organism. Part of the applied pesticide is lost to the atmosphere by drifting and

volatilization - thus may be transported further away from the point of use; some would drop on the surrounding soils; while others are washed away by surface runoff into receiving streams or water bodies (Plate 2.1). This could be called the first phase of the fate of pesticides in our environment and often referred to as initial distribution (Tiryaki and Temur, 2010).

With time, the pesticide is partitioned between these initial components in which they are distributed and their immediate surroundings, where they also persist and undergo several changes and reactions due to their properties and those of their surrounding environment. These changes or reactions involve their transformation and degradation into other compounds that may be more or less toxic.

The environmental fate or behaviour of a pesticide is largely influenced by the natural affinity of the chemical substance for any of the four major environmental compartments - solid matter (eg, soil), liquids (solubility in surface and ground water), gaseous form (volatilization) and biota (plants and animals).

This phenomenon is often referred to as "partitioning" and involves the determination of the soil sorption coefficient (K_{OC}), the n–octanol/water partition coefficient (K_{OW}) and as well as octanol/air partition coefficient (K_{OA}). These parameters are well known, for pesticides and it is used to predict the fate of pesticide in our environment.

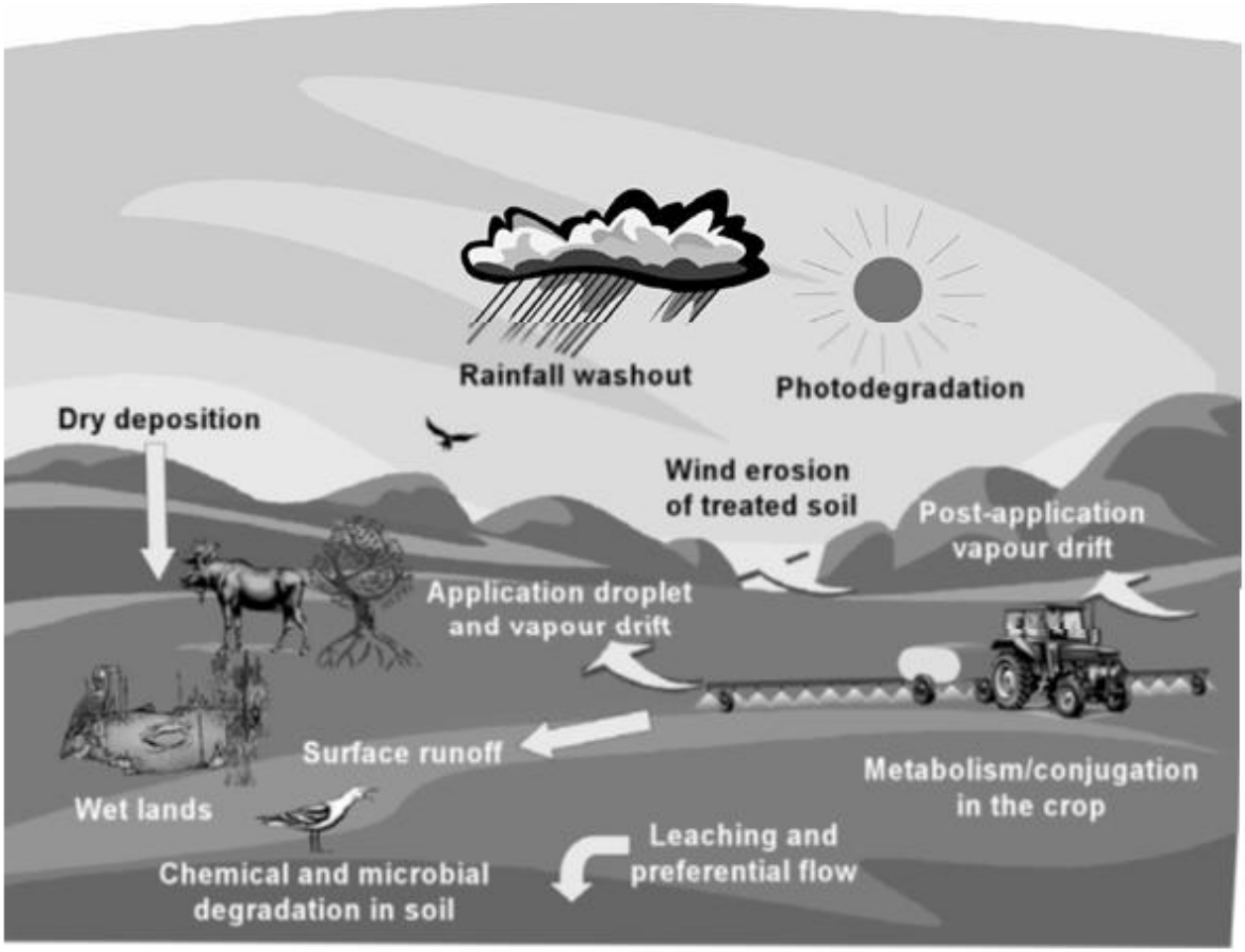


Plate 2.1. Routes of entry of pesticides into the atmosphere and into surface and ground waters and mechanisms of pesticide transformation in air, soil and plants

(Source: Cessna, *et al*. 2006).

Environmental persistence of organochlorine pesticides

Organochlorine pesticides are considered persistent because they are stable and difficult to break down to smaller organic units in the environment. Persistence of OCPs in soil, water, atmosphere and biota is as a result of their ability to resist photolytic, chemical and biological breakdown or changes (Imo *et al*. 2013), which is due to their physico-chemical properties; such as, their chemical structure (double bonds and aromaticity) and presences of carbon-chlorine bond. Also, the greater the number of chlorine substitution and/or functional group, the greater the resistant to biological and photolytic degradation (El-Shahawi *et al.* 2010). Organochlorine pesticides are highly lipophilic and this forms the basis of their persistence and bioaccumulation in fatty tissues ("lipids") of biotas (plants and animals) - which is the primary and ready ‘‘sink’’ for most pesticides.

The main difference between OCPs and other pollutants lies in their ability to persist (Levillain *et al*. 2012) in the environment. The United Nations Environment Program (UNEP) in 2001 listed twelve persistent organic pollutants (POPs) as dirty dozen at the Stockholm Convention, of which nine were OCPs. However, under the UNEP Stockholm convention of 2009 on POPs, fourteen (aldrin, chlordane, chlordecone, DDT, dieldrin, endrin, α-HCH, β-HCH, γ-HCH, HCB, heptachlor, mirex, pentachlorobenzene and toxaphene) of the twenty-one organic substances whose use is banned or restricted globally are OCPs (UNEP, 2009) and endosulfan that was recommended by the UNEP review committee has since being added by the Stockholm Convention at its Fifth meeting of the conference of May 2011, thus making a total of fifteen OCPs and constituting over 68% of POPs under the UNEP Stockholm convention (UNEP, 2011).

The persistence of organochlorine pesticides in the environment is measured by its half-life (which is the length of time required for one-half of the ambient or initial concentration to degrade).

2.5.1. Transport and Partitioning of OCPs in the environment

2.5.1.1. Organochlorine pesticides in the atmosphere

Generally, OCPs like most chemicals enters the atmosphere either by one or combination of these – application drift, post-application vapour losses from wind erosion of impacted soils. These OCPs in the atmosphere are prone to photo-degradation by ultra-violet light from the sun. The degraded products and their parent compounds (OCPs) are transported long distances from their point source. This long distance transport is terminated when they are deposited on the earth surface, water bodies or on any object by the process of atmospheric wet (precipitation) and dry (particulate) deposition (Cessna *et al.* 2006).

Application Drift

Organochlorine pesticides in most cases are applied in solution by spraying through nozzles, which are able to provide atomization, uniform flow rate and distribution of the pesticide-mix. The proportion of sprayed pesticide that reaches the target site and what is drifted away by air current is a function of the sizes of the pesticide droplets that passes through the nozzle as a result of the applied hydraulic pressure. Droplets below 150 µm in size are easily drifted or found to be prone to movement under windy air currents. The drift-prone percentage could be as high as 30% of total volume of droplets depending on the type of spray nozzle.

Post-application vapour losses

Conventionally, the use of crop pesticides in agriculture could either be by pre-emergence or post-emergence application. Pre-emergence applications (i.e., prior to the germination of crop) of pesticides tends to remain on soil surface if undisturbed, while in post-emergence application, pesticides are sprayed on the crop/plant from the canopy to the stem. Portions of the sprayed pesticide passes through the canopy and drop on the farm soil.

Wind-erosion of pesticide-treated soil

Pesticides adsorbed to soil surface are susceptible to wind erosion – whereby they are transported along with top soils that are detached due to abrupt and bustling increase of the wind carrying capacity (wind movement) and deposited at some distance from the initial point source. Detached OCP-soil particles could be transported by surface creeping, saltation and suspension – depending on size of particulate. This type of wind erosion involving these three processes is known as deflation (Plate 2.2).

2.5.1.2. Organochlorine pesticides in surface and ground water

Surface and Ground Water

Organochlorine pesticide enters surface water (like ponds, streams, rivers, etc.) through run-off from farms where they were applied. Generally,pesticides adsorbed to top soils (≤ 1 cm depth) are susceptible to been washed away during run-off into aforementioned receiving water bodies. Also, they can enter surface water through atmospheric deposition or precipitation (wet or dry) far away from source point (because of their ease to travel long distances). Other sources of entry are from wastewater discharge and OCP spillage (which could be accidental or deliberate) (see Plate 2.1).

Pesticides in the atmosphere are deposited or precipitated with rain, snow and hail and are transported during run-off into surface water (wet deposition). Also, during deflation process, some of the pesticides adsorbed to soil particulates are carried into drainages that are channelled into water bodies (dry deposition).

Organochlorine pesticides impact ground water when they migrate downwardsthrough the soil profile to reach the aquifer by leaching process. This process could take years and it is dependent on the physico-chemical properties of soil and pesticides involved. Water soluble pesticides and soils that are either loamy/sandy or low in TOC are prone to fast migration/mobility towards ground water. Contamination of OCP due to spillage at mixing and loading sites often witness is referred to as point-source pollution (ie specific identifiable point) , while non-point-source is as a result of the unrestricted movement of pesticides into non-targeted sites like, water bodies, atmosphere and plants.

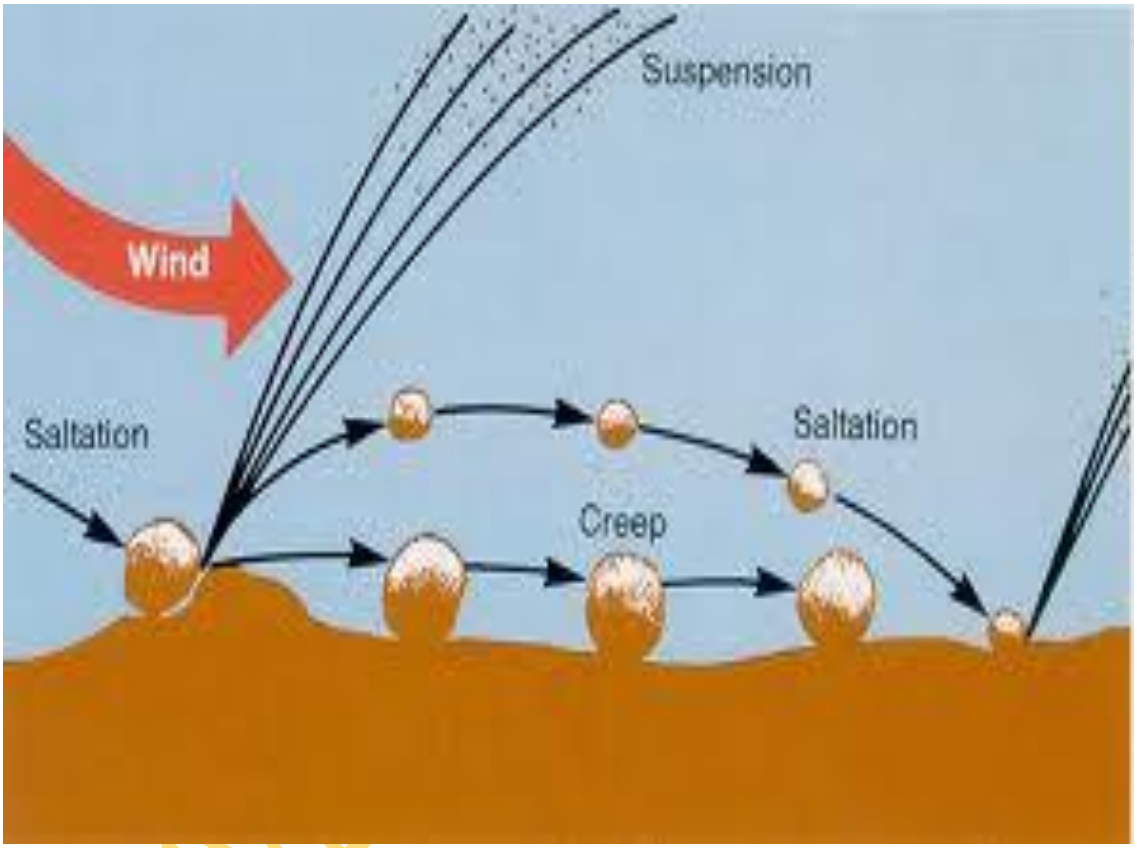


Plate 2.2. Deflation of pesticide treated soil by wind-erosion.

(Source: http://milford.nserl.purdue.edu/weppdocs/overview/wndersn.html)

2.5.1.3. Organochlorine pesticides in soil

Soil is the thin outer zone of the earth's crust that is capable of supporting rooted plants and it is the product of climatic effect and activities of living organisms on rock (McKone, 2005). True soils are mixtures of air, water, mineral, and organic components. They are basically heterogeneous.

A horizontal cross section of soil zone typically reveals several horizontal layers having different colours and textures. These layers are often divided into three major horizons (Plate 2.3);

◦The 'A' horizon, which encompasses the root zone and contains a high concentration of organicmatter;

◦The 'B' horizon, which is unsaturated, is below the roots of most plants, and contains much lower organic carbon content; and

◦The 'C' horizon, which is the unsaturated zone of weathered parent rock consisting ofbedrock, alluvial material, glacial material, and/or soil of an earlier geological period.

Ecologically, soils exist at the convergence of the atmosphere, the hydrosphere, the geosphere, and the biosphere. Thus, soil contaminants can impact human health and the environment through a complex web of interactions.

Agricultural soil is primarily the most impacted of all the three basic environmental components of man. It serves as primary/initial abode for sprayed pesticides as most applied pesticides fall to the soil, while the proportion that fell on plants are eventually washed down to the soil by rain and dew. Also, those that are dissipated by wind into the atmosphere from the point of application are later deposited (dry or wet) on the soil, plant or water. Redistribution and partitioning occurs from the soil to water bodies (run-off), plants (up-take by translocation) and to the atmosphere (Plate 2.4). Partitioning and redistribution of OCPs in soil is dependent on its adsorption capacity and some of its physico-chemical properties.

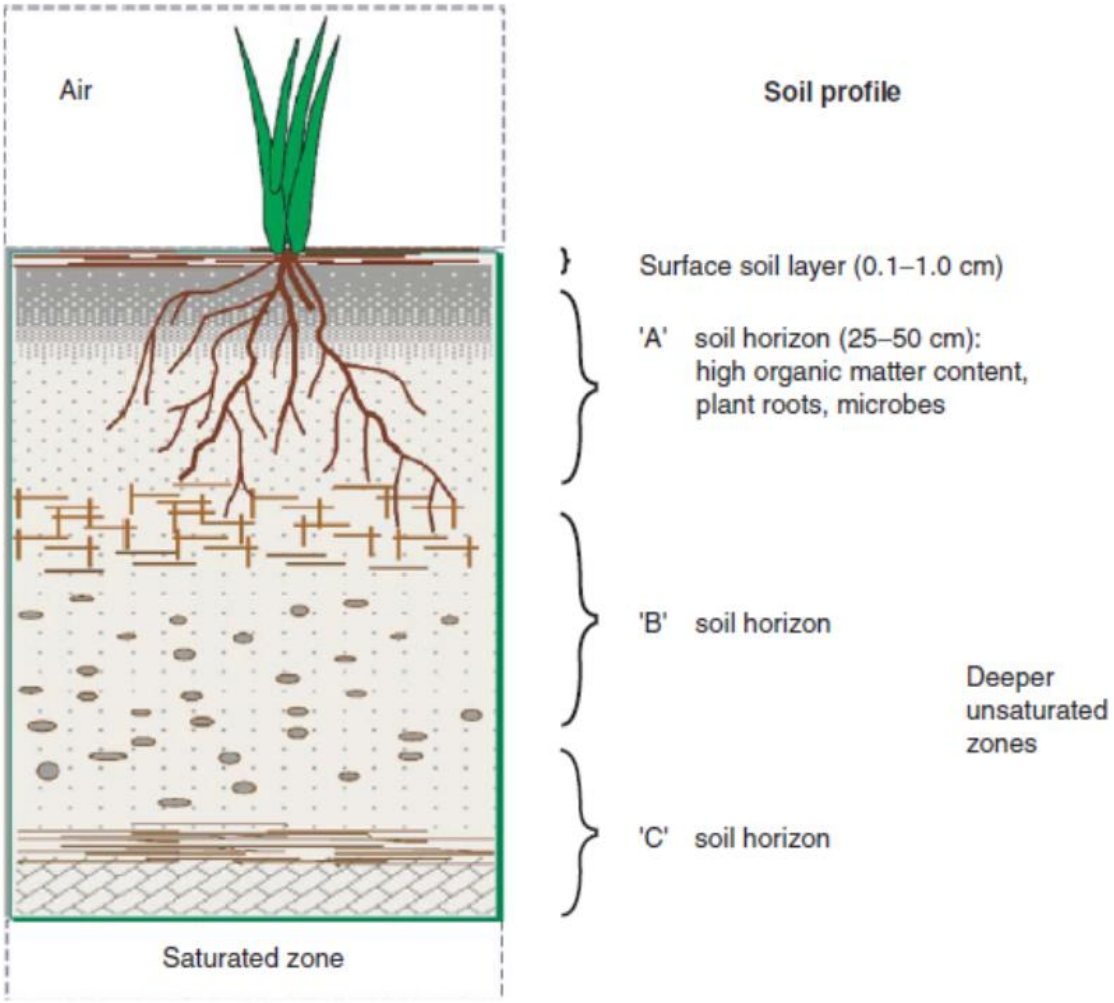


Plate 2.3.A typical horizontal profile and structure of soil in the unsaturated zone (Source:McKone, 2005).

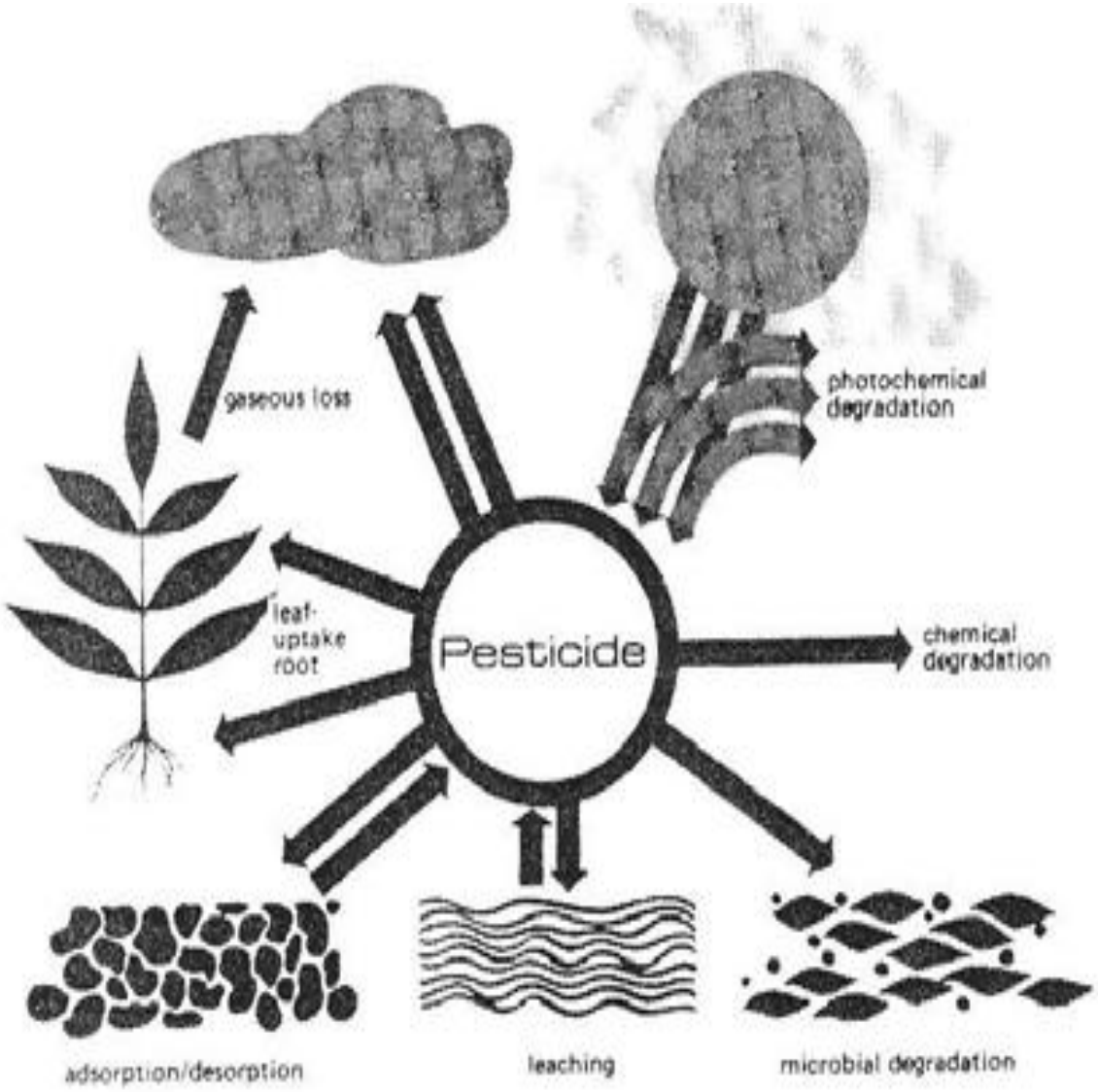


Plate 2.4. The fate of pesticides in the soil - plant system

(Source; Führ, 1991).

Chemo-dynamic processes of OCPs involving transportation, retention, degradation and bioaccumulation do influence their persistence, distribution and ultimately their fate in the environment. While transportation, retention and bioaccumulation are dependent on soil-pesticide interactions (i.e., sorption processes), degradation entails structural transformations or changes due to chemical and biological actions on the pesticides. Therefore, all the aforementioned chemo-dynamic processes could be classified into two major processes; sorption and degradation processes. These two could be said to have much relevance in field kinetic studies, whereby residual concentrations of OCPs are monitored over time after application in farms.

2.6. ORGANOCHLORINE PESTICIDE RESIDUE IN AGRICULTURAL SOILS

In the last decade, there have been various studies on residual levels of OCPs in cropped and uncropped soils across the seven continents of the world. Comparatively, high levels of contaminated agricultural soils have been reported for China and India (two major Asian countries) than any other part of the world. China has being a major producer and consumer of OCPs as an agricultural nation (Zhang *et al.* 2009, Jiang *et al.* 2009). High levels of OCPs in soils from Southeast China have been reported (Wang *et al.* 2005; Cao *et al.* 2007; Hao *et al.* 2008; Zhang *et al*. 2009; Yang *et al.* 2012). The spatial distribution of pesticide applied to crops in China regions following the order; southeast >central >northwest (Wang *et al.* 2005). In Shanghai, Southeast China, the residual concentrations and frequency of 24 OCPs detected showed that HCHs, DDTs, HCB and heptachlor epoxide were the most dominant (Jiang *et al.* 2009). Levels of HCHs, DDTs and heptachlors ranged between ND – 10.38 ngg^{-1}, 0.77–247.45 ngg^{-1} and 0.84–10.08 ngg^{-1} respectively, while total OCP concentrations ranged from 3.16 to 265.24 ngg^{-1}. Other Southeast regions with high consumption of pesticides include – Zhejiang, Fujian and Guangzhou, with a total percentage consumption of 36.7% (El-Shahawi *et al.* 2010). Residual concentrations in soils originated mainly from historical application. Other studies from Beijing, Northeast China, have also been reported to have high OCP contamination in soils (Xu, *et al*. 2005; Zhu *et al*. 2005; Wang *et al.* 2008; Yang *et al.* 2009).

Concentrations of HCH and DDT in 175 surface soil samples from different agricultural fields in the districts of Nagaon and Dibrugarh, Assam, India have been reported (Mishra *et al.* 2012)' . The mean concentrations of total HCH and total DDT ranged from 98 – 1945 ngg^{-1} and 166–2288 ngg^{-1} in district Nagaon, while in Dibrugarh, values ranged 178–1701 ngg^{-1} and 75–2296 ngg^{-1} respectively. The soils from paddy fields contained highest amounts of HCH and DDT residues. The total organic carbon was positively correlated to HCH and DDT residues. Ratios of DDT/(DDD+DDE) were 1.25 and 1.82, while that of α/γ-HCH were 2.78 and 2.51 for districts Dibrugarh and Nagaon, respectively. Historical source of pesticides revealed that soil residue levels have originated from long past and recent mixed source of technical HCH and Lindane for HCHs and mainly technical DDT for DDTs

OCP residues in the landlocked mountainous Republic of Tajikistan, South-western Asia have been reported for surface soils at different altitudes (570 – 4656 m) (Zhao *et al.* 2013). OCPs were detected in all samples (i.e., 100% detection frequency) with residual levels ranging between 52.83 – 247.98 ngg^{-1}. Aldrins, HCHs and endosulfans families were the most predominant.

In Europe, countries like Spain have reported high contamination of agricultural soils from Galicia area, Northwest, Spain, for hexachlorocyclohexane (HCH) (Pereira *et al.* 2010). The concentration of HCHs ranged from 4 to 2305 ngg^{-1}, while the α- and γ-isomers predominated, with residual concentrations range < 1 – 1404 ngg^{-1} and < 1 – 569 ngg^{-1} respectively, for 252 surface soils investigated.

Comparatively, there are dearths of information on OCPs studies on agricultural farms in Africa, in addition to its relatively lower residual levels reported in literature.

2.7. ORGANOCHLORINE IN SURFACE WATER AND SEDIMENTS

Organochlorine pesticides have continued to be a major global pollutant and of the three main components in man's environment and/or compartment for its accumulation, water is the most impacted by OCPs. OCPs enters into aquatic systems through discharge of domestic sewage and industrial wastewater, runoff from farms, direct dumping of wastes into the river systems, long-range atmospheric transportation (LRT), and leachate from

open dumps and landfills (Doong *et al*.2002; Osibanjo, 2003; Malik *et al.* 2009). In the river system, they are distributed or partitioned among the components of the river ecosystem, such as water, sediment and aquatic biota.

There are reports on global studies of OCPs in water, sediments and biota on rivers in developed and developing nations; however, these reports are grossly inadequate, especially on the African continent. UNEP (2003) reported that inspite of large-scale deposition from agricultural sprays and runoffs the information on pesticides in the whole water systems is scarce.

Generally, African inland waters are contaminated by a broad spectrum of OCPs (Osibanjo, 2003). The occurrence and levels of OCPs in nine (9) rivers in Ondo state, a major cocoa growing area in Nigeria was reported by Nwankwoala and Osibanjo (1992). OCPs detected were as follows, with mean in parenthesis; Lindane, ND-6.4, (2.4) ngL^{-1}; heptachlor, ND-5.0 (2.1) ngL^{-1}; endrin, ND-21 (5.1) ngL^{-1}; aldrin, ND-3.5 (1.0) ngL^{-1} and dieldrin, ND-2,150 (1,062) ngL^{-1}.

Considering OCPs levels in the continent, Lake Mariut in Egypt appears the most polluted water body, based on high value for γ-HCH (Lindane) - 1,310ngL^{-1} , pp'-DDE - 6,630 ngL^{-1} and total DDT with 21,440ngL^{-1} (Saad, 1981). Sediments from Inland waters in Africa continent recorded low residual values compared to those reported for developed countries (Eisenreich *et al.* 1979; Oliver and Charlton, 1984). Osibanjo *et al.* (1994) reported the presences of eleven (11) OCPs in 23 sediments from Lekki Lagoon in Lagos State. The concentration of OCPs ranged as follows (in ngg^{-1}, dry weight), with mean values in parenthesis – lindane, 0.11-4.9 (1.1); aldrin,ND-347 (56); pp'-DDE,11-555 (263); op'-DDD,ND–348 (88); endosulfan,7-1,155 (30);heptachlor,ND 1,845 (64); γ-HCH,ND-116 (18.6), HCB ND-3.3 (0.4); endrin,ND-129 (16.5),dieldrin,190-8,460 (4,560).

2.8. ORGANOCHLORINE PESTICIDE RESIDUES IN SOME AFRICAN FARMING COMMUNITIES

There have been few studies on OCP residues in African farming communities. However, Manirakiza *et al.* (2003), assessed the level of OCPs in Banjul (Gambia) and Darka

(Senegal) farming communities. Validated analytical procedures were applied in the assessment of 210 samples of water, soil and vegetable from nine (9) Sene-Gambian farms. The distribution of residual OCPs in water and neighboring soils and soil-plant transfer of these pesticides were discussed. The major contaminants were the DDTs, with a sum concentration (∑DDT) of 231.9 ngL^{-1}, 71.4ngg^{-1} and 5.03ngg^{-1} in water, soils and vegetables respectively. Bio-concentration factors for total HCHs, total DDTs and total endosulfans in 14 different vegetable types from six sampling location were obtained.

Oyekunle *et al*. (2011) evaluated the levels of OCPs in the farming soil of Oke-Osun farm settlement, Osogbo, Nigeria. The study revealed that the agricultural soils were contaminated, mainly due to the application of OCPs by the farmers. Higher levels were obtained in the dry season compared to the rainy season. Concentrations of OCPs in soils ranged from 13.09±21.66 μgkg^{-1} (α-HCH) to 42.01±17.50 μgkg^{-1} (pp'DDT) in the rainy season and from 30.74±17.38 μgkg^{-1} (β-HCH) to 82.88±32.24 μgkg^{-1} (pp'DDT) in the dry season.

Ntow (2001), investigated OCPs levels (in water, sediments, crops and human fluids) in a farming community in Akumadan area of Ghana. Lindane and endosulfan were found in water and sediment samples, while other OCPs - hexachlorobenzene (HCB), pp'DDE and heptachlor epoxide were in addition found in sediments. Heptachlor epoxide levels in tomato crops were appreciably high in crops. Mean values of HCB and p p''DDE in blood were 30μgkg^{-1} and 380μgkg^{-1} respectively, while in milk fats 40μgkg^{-1} (or 1.75μgkg^{-1} whole milk) and 490μgkg^{-1} fat (or 17.15μgkg^{-1} whole milk) respectively.

The presence of organochlorines (lindane, α-endosulfan and β-endosulfan), organophosphorus compounds (malathion and pirimiphos-methyl), synthetic pyrethroids (permethrin) and carbamates (carbufuran) residues in millet, maize, and cowpeas and their potential dietary risk to human on consumption were reported by Sonchieu *et al.* (2010) for eight farming communities in Northern Cameroon. The frequency of detection and residual concentration of OCPs were highest compared to other pesticides monitored in the grains. Of all pesticides, lindane recorded the highest concentration of 9.53 ± 4.00 $mgkg^{-1}$ in maize relative to what was obtained for other pesticides in the grains. Over 75% of samples contained residues above the WHO/FAO maximum residue limit (MRL).

The persistence of DDT in Western Zimbabwe has been reported (Matthiessen, 1985). This study was to access where there is accumulation of DDT in wildlife as a result of its use for tsetse fly control programme between 1982 and 1983 and possibly any deleterious biological effects. Result showed that DDT residues do not persist in the non-living environment (tree back, soil and riverine silt), but readily accumulates in insectivorous birds and bats (maxima of 32 μgg^{-1} and 20 μgg^{-1} wet weight total DDT in viscera respectively). These levels are sufficient to cause egg shell thinning in certain avian predators.

Inspite of the vast literature on organochlorine pesticide by numerous workers, there is dearth of literature on OCP residues in cocoa farms with respect to its distribution in different parts of cocoa plant and immediate environment – soil, water (surface and ground), atmosphere and sediments.

2.9. OCP RESIDUES IN FOODS

The OCP contamination of human foods - unprocessed or raw/natural and processed has continually remained a great source of concern to mankind. Although, pesticides are vital in modern agricultural practices, but due to their biocide activity and potential health risk to consumers, the control of the presence of pesticide residues in foods is a growing source of concern for the general population (Torres *et al.* 1996). There have been substantial studies on the residue levels of OCPs in various food products. The levels of OCP residues in different cereals have been reported. Cereals, such as - wheat grown in India (Riazuddin *et al*. 2011), in Serbia (Skrbic, 2007), in Koyon area of Turkey (Guler*et al*.2010), in Poland and Romania (Witczak and Abdel-Gawad, 2012; Alexa *et al*. 2009); rice, millet and maize in Cote D'ivoire (Alle *et al*. 2009) and Cameroon (Sonchieu *et al.*, 2010); sesame seeds from China (Wang *et al*. 2008) and pulses – green beans, soyabean (Shoiful *et al*.2013) and animal feeds (Tsiplakou *et al*. 2010). They were all found contaminated with OCPs.

Guler*et al.* (2010) and Witczak and Abdel-Gawad (2012) reported the presences of 25 and 13 OCPs respectively in wheat and rye grains. The residual concentration of OCPs in wheat from Konya region of Turkey ranged between 0.0002 $mgkg^{-1}$ to 0.0211 $mgkg^{-1}$.

Detection frequency of OCPs ranged between 42% and 100%. pp'- DDD, op - DDE, pp'- DDE and op - DDT recorded 97% frequency detection, while cis-chlordane and methoxychlor were found in all the 36 samples analyzed. Guler*et al.* (2010) concluded that the amount of organochlorine pesticides detected in wheat from Konya region has shown that the contamination of environment was due to the use of pesticides by farmers. Although most of the pesticide residues are below the EC MRLs, some residues exceed EC MRLs. Witczak and Abdel-Gawad (2012) compared the levels of OCP residue in wheat and rye grains from conventional and organic farming, with the soils from where they were harvested. The concentration of OCPs in wheat ranged from 0.07 ngg^{-1} to 16.69 ngg^{-1} and 0.05 ngg^{-1} to 6.44 ngg^{-1} respectively, with β-HCH having the highest concentration. The mean ∑OCP in wheat from both farming types were 45.64 ngg^{-1} (conventional farming) and 22.40 ngg^{-1} (organic farming), while their corresponding soils levels were 140.66 ngg^{-1} and 46.06 ngg^{-1} respectively. These values showed significant up-take of OCPs by the wheat plant and storage of the grains.

Of all crop produce, vegetables and fruits are most prone to OCPs contamination. Large quantities of fruits and vegetables are consumed annually; this is because of their health benefits which include reduction of heart disease, stroke, diabetes, bone loss, and various cancers. The global annual production is put at about 1.74 billion metric tons in 2013 (WFO, 2014). Vegetables such as cucumber (Lal *et al*. 2008; Kin and Huat, 2010; Shoiful *et al*. 2013), carrot (Guan *et al*., 2010;Witczak and Abdel-Gawad, 2012; Shoiful *et a l*. 2013), spring onion (Lal *et al*.2008; Barriada-Pereira *et al*.2010), pepper (Owago *et al*. 2009;Boadu *et al*. 2013), tomato (Barriada-Pereira *et al*.2010, Benson and Aruwajoye, 2011), while fruits like- kiwi fruit (Cho *et al*. 2007; Ucan *et al*., 2009), apple (Mladenova and Shtereva, 2009; Ucan *et al*. 2009; Dobrinas *et al*.2012), strawberry and papaya (Lal *et al*.2008; Bempah *et al*. 2011), banana and citus (Ucan *et al*. 2009; Bempah *et al*. 2012), spices (Manirakzia *et al*.2000) and fodders (Sharma *et al*. 2013) were reported to have various concentrations of OCP residues.

Adeyeye and Osibanjo (1999) reported the residual levels of OCPs in nine (9) raw fruits and fourteen (14) vegetables from markets in seven (7) cities (Ibadan, Ife, Osogbo, Akure, Ilesha, Ado-Ekiti and Ondo) in Southwestern Nigerian. OCP residues were detected in

91% and 92% of fruit and vegetable samples analysed respectively. The relative percentage occurrence of individual OCPs in all fruits samples were 77% for HCH, HCB 72%, aldrin 28% and ∑DDT 40%. The mean ∑DDT and aldrin were highest in guava (*Psidium guaja)* and banana (*Musa spp.*) respectively, while endosulfan was below detection limits in all fruit samples. In the vegetables, lindane, total-DDT, HCB, and aldrin were detected in all the samples, with percentage detection ranging from 50 – 95%. The mean residual levels of ∑DDT, aldrin and ∑HCH were highest in tomato (*Solanum lycopersicum),* cockscomb plant (*Celosia argentea*)and in eggplant (*Solanum gilo radii*) respectively. Residual levels of OCPs were generally higher in Akure, Ondo and Ife – which are major cocoa producing towns. However, residual levels were all low and below MRL for vegetables and fruits.

Natural products like honey have been reported to have high levels of OCPs, even above specified limits –MRL for European countries (Blasco *et al.* 2004; Wilczynska and Przybylowski, 2007; Wang *et al*. 2010; Amendola *et al.* 2011; Witczak and Ciemniak, 2012; Kujawski *et al*. 2012). Blasco *et al.* (2004) reported that of all forty-nine (49) honey samples from the Spanish (24 samples) and Portugese (25 samples) markets investigated, 14 Valencian samples (Spanish) and 23 Portugal samples were contaminated. Total HCH isomer showed the highest percentage frequency of detection of 48% and 91.6% in the Valencian and Portugese samples respectively, with corresponding mean concentrations and standard deviations of 0.579±0.747 mg/kg and 1.357±1.30 mg/kg. Aldrin, endrin, dieldrin, o,p-DDT, p,p-DDT, γ-HCH, o,p-methoxychlor and p,p-methoxychlor have been reported in 178 polish honey (Wilczynska and Przybylowski, 2007).

2.10. ENVIRONMENTAL AND ECOLOGICAL EFFECT

The application of pesticide (with the primary motive to improve agricultural productivity) has raised a number of environmental concerns, for example over 98% of sprayed insecticides and 95% of herbicides reaches destinations other than their target species, including non-target species, air, water and soil (Miller, 2004). Pesticide drift are bound to occur when pesticides suspended in the air as particles (after spraying) are carried by wind to other areas, where they are eventually deposited and therefore potentially contaminating

these areas. Pesticides are one of the causes of water pollution and they contribute to soil contamination after application.

Ecological impact by OCPs is mainly from contaminated water which is as a result of runoff, impacted soil or objects and the principal mechanisms by which the ecosystem is affected directly or indirectly is by the processes of bio-concentration and bio-magnification. In addition to ecological impacts in countries where they are used, OCPs and their metabolites that have long been banned in developed nations are consistently found in obscured areas such as the high artic because of their persistence and easy transportation due to their volatility. Chemicals that are applied in tropical and sub-tropical countries are transported over long distances by global air circulation (Ongley, 1996).

2.11. ORGANOCHLORINE PESTICIDES AND ENVIRONMENTAL PROCESSESS

There are several processes involved in the transformation of organochlorine pesticides that are released into the environment, whether in the gaseous or liquid or solid phase. These processes could either be physical, chemical and biological or a combination of two or all three processes. Metabolites transformed from parent OCPs through degradation process are in most cases structurally different as part(s) of the parent molecule is (are) lost. Some of these processes are enumerated below;

2.11.1. Degradation Process

Degradation of pesticides is the breakdown or chemical transformation of pesticide molecules into other forms or species. These newly formed products may not necessarily be simpler and/or less toxic compared to the parent molecule. In some situations, the bye products from degradation are even more toxic combined with pesticidal effects. For example, α-endosulfan, β-endosulfan and endosulfan sulphate exhibit an oral LD_{50} of 76 mg/kg, 240 mg/kg and 18 mg/kg to rats respectively (Maier-Bode, 1967 and Gaines 1969). Endosulfan sulphate is a metabolite of parent endosulfans. The rate of degradation of pesticides is usually measured in terms of half-life ($t_{1/2}$), which is the time required for the depletion of half (or 50%) of the amount of pesticide present initially. Degradation

processes could either be chemical or biological or both occurring at the same time. Chemical degradation generally occur in water or atmosphere and it follows one (or more) of these four reactions namely; oxidation, reduction, hydrolysis and photolysis. Also, biological degradation occurs mostly in soil and in living organisms and one (or more) of four reactions are utilized; oxidation, reduction, hydrolysis and conjugation. However, the inherent physico-chemical charateristics of pesticides and the nature of the environmental compartments in which it is hosted (i.e., water, soil, air and biota), determines the type of reaction they undergo.

Oxidation reaction of pesticides

Pesticide oxidation occurs when free oxygen in the environment reacts with pesticides. In addition, oxidation process can also take place by the presence of singlet oxygen, ozone, hydrogen peroxide, or other hydroxy radicals. Hydroxy radicals ($^{\cdot}OH$) are the primary agents that initiates or bring about chemical oxidation of pesticides in water or atmosphere. These radicals can be formed from either the pesticides or from other molecules present in the environment. *P,p`*-DDT for example undergoes both reduction as well as oxidation reactions in the soil under the aid of *Enterobacter aerogenes* microorganisms in the presence of UV light and/or iron catalyst to form reduced products; *p,p`*-DDE and *p,p`*-DDD as well as oxidized derivative which ultimately form *p,p`*-dichlorobenzophenone.

Reduction reaction of pesticides

Reduction of pesticides is a chemical reaction whereby the pesticide undergoes a reduction in oxidation state. A hydrogen ion or proton (H^+) is usually the reducing agent in the environment.

Hydrolysis reaction of pesticides

Hydrolytic reaction of pesticides occurs mostly in the presence water (i.e. H^+ and ^{-}OH) and it is pH dependent. It is one of the most common reactions that most pesticides undergo in the environment. Most organochlorines, organophosphates and carbamates have shown to undergo hydrolysis under alkaline condition. A pesticide that is very

soluble in water will tend not to favour retention in soil or accumulation in biota, because of its strong polar nature. This therefore suggests that it will degrade via hydrolytic process, since it is the reaction that is most favoured in water (Zacharia, 2011).

Photodegradation of pesticides

The breakdown or transformation of pesticides by the action of sunlight (or ultra-violet light) is referred to as photodegradation or photolysis. Chemical bonds in the pesticides are ruptured as a result of UV rays. Usually organic molecule absorbs photons and become excited with the ensuing release of electron thus changing the molecule. Photolytic reactions are important for degrading organic molecules in the upper atmosphere, in shallow aquatic environment, on foliage and on the surface of soils.

Biodegradation

Microbial action on pesticides usually leads to breakdown or transformation of these pesticides. Such bio-transformation and/or breakdown are often observed in water and soil environment where vast numbers of microorganism inhabits. The rate of biodegradation is dependent largely on a number of variables – namely; the amount and nature of pesticides present in the soil, the type of microbes and microbial population in the soil and soil conditions that favours microbial activities, such as warm temperature, favourable pH, adequate soil moisture, aeration and high organic matter content. The microorganisms involved in biodegradation include fungi, bacteria and other microorganisms that use pesticides as their substrate. Pyrethroids, organophosphates and some carbamates have been found to be more susceptible to biodegradation than OCPs. However, most OCPs have shown some high level of resistance to biodegradation due to the strength of C-Cl bond (Imo *et al.* 2013).

A good example of microbial degradation is the action of microbes on the endosulfan isomers (alpha- and beta-). The microbial degradation of endosulfan is known to follow different pathways depending on the types of microbes present. Also, an organisms can initiate a particular pathway of degradation (i.e., first step), but other organisms may carry out latter steps. Thus, it is pertinent to mention that some microbial enzymes are specific

to one isomer, or catalyze at different rates for each isomer (Kwon *et al.* 2005). For example, a *Mycobacterium tuberculosis* ESD enzyme degrades α- and β-endosulfan to form different metabolites. β-endosulfan is biotransformed by this microbial enzyme to form monoaldehyde and hydroxyether (depending on the reducing equivalent stoichiometry), but transforms α-endosulfan to the more toxic endosulfan sulphate (Sutherland *et al.* 2002a). However, oxidation of endosulfan or endosulfan sulphates by the monooxygenase encoded by *ESE* in *Arthrobacter sp*. KW yields endosulfan monoalcohol (Weir *et al*. 2006).Both *ESE* and *ESD* proteins are part of the two components Flavin Dependent Monooxygenase Family, which require reduced flavin. They are conditionally shown when none or very small amount of sulphate is available, and the availability of sulphur and oxygen atoms in endosulfan makes the *ESE* and *ESD* proteins to be expressed in these starved conditions (Sutherland *et al.* 2002b).

Alternatively, hydrolytic action on endosulfan by some bacteria e.g., *Pseudomonas aeruginosa* and *Burkholderia cepaeia* are reported to yield the less toxic metabolite endosulfan diol (Kumar *et al.* 2006). Endosulfan in alkaline conditions can hydrolyse spontaneously to the diol metabolite (Sutherland *et al.* 2002a); which in turn can be converted to endosulfan ether (Hussein *et al.* 2007) or endosulfan hydroxyether (Lee *et al*., 2003) and eventually to endosulfan lactone (Awasthi *et al*. 2003). Further hydrolysis of endosulfan lactone yields endosulfan hydroxycarboxylate (Walse *et al.* 2003) (see Plate 2.5).

These various bio-transformation and degradation of endosulfan often leads to the desulfurization of the parent compound, while leaving the chlorines intact, to exhibit the recalcitrants to bioremediation found in many halogenated aromatics.

Awasthi *et al*. (2000), Savadogo *et al.* (2009), Kataoka*et al.* (2011) and Tiwari and Guha, (2013) have reported the biodegradation of endosulfan by microbes and factors that influences their activity and subsequent breakdown of endosulfan. The bioremediation potentials of fungus - *Mortierella sp*. strain W8 (Kataoka*et al.* 2011) and two strains of bacteria - *Bacillus sp* (Awasthi *et al*. 2000) in endosulfan contaminated soils were reported. Various factors, such as increased presence of carbon sources, pH, moisture

content, concentration of endosulfan, and size of inoculum, influenced the degradation of α- and β-endosulfan. The degradation was found to be faster in wet soils than flooded soils (Awasthi *et al*. 2000). Kataoka*et al.* (2011) reported that the α-isomer was more susceptible to biodegradation, with β-isomer more persistent after 28 d of incubation in initial concentration of 3 $mgkg^{-1}$ (dry wet) in unsterilized soil, wheat bran and W8 strain of the fungus *Mortierella sp*. Percentage degradation were approximately 80% and 50% for α-endosulfan and β-endosulfan respectively.

2.11.2. Adsorption-desorption process

Adsorption-desorption is a term which encompasses two processes – adsorption and desorption. Adsorption is the process through which a substance, existing in a particular phase type (eg liquid), is transferred from that original phase to another phase type (eg solid), through an interface between both phases. In principle, this process is facilitated by the accumulation of the substance at the interface of both phases (ie, liquid-solid interface). In liquid-solid adsorption process, the substance in the liquid phase that is being depleted is referred to as the adsorbate, while the solid phase to which the substance is now adheres to, is the adsorbent. Desorption is the reserve process of adsorption. Substances (adsorbates) that were initially adsorbed to an adsorbent are leached into a surrounding medium - usually liquid (Site, 2000).

Adsorption is as a result of surface energy as observed during surface tension. Adsorption in soil arises due to bond deficiency of atoms in the inorganic and organic soil components. Thus, this lack energetically favours bonding with substances available to these deficient components in the soil. The bond type is dependent on the nature of the species involved (i.e., the adsorbent and adsorbate), however the adsorbate is generally classified as exhibiting physisorption or chemisorption.

Physisorptionor physical adsorption is the adherence of the adsorbate molecules to the adsorbent surface through van der Waals (weak intermolecular) interactions only; while chemisorptionis whereby adsorbate molecules adheres to adsorbent surface through the formation of a chemical bond (e.g, hydrogen bonding).

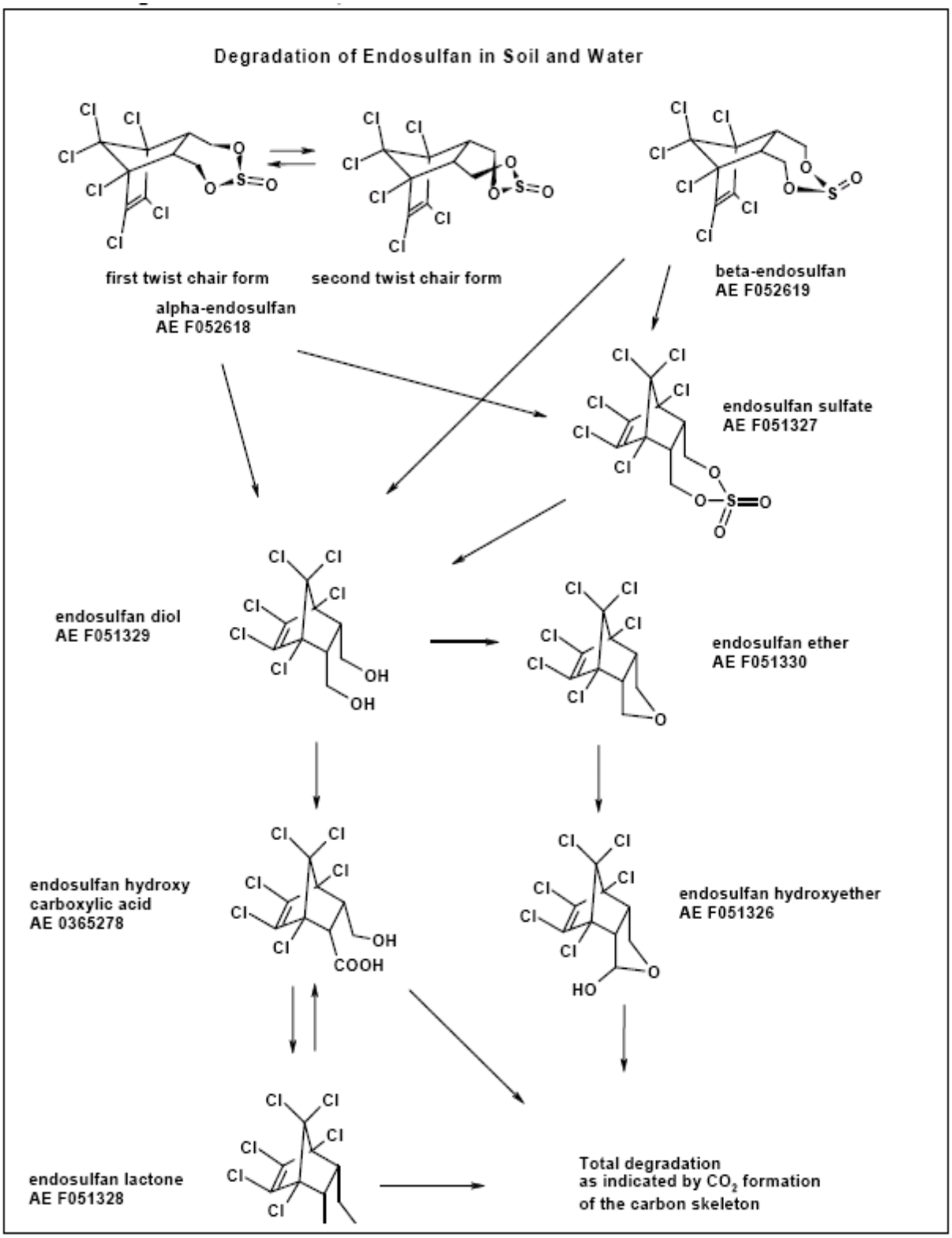


Plate 2.5. Routes of degradation of endosulfan in soil and water (Source:German Federal Environment Agency, CCME, 2010).

One of the major processes governing the fate of pesticides in the environment is the retention of pesticides by soils. A variety of mechanisms can be involved in the binding of pesticides to soils, including: London-van der Waals forces, hydrogen bonding, protonation, cation and water bridging, cation and anion exchanges, ligand exchange, covalent bonding, and physical trapping.

Soil sorption phenomena involving pesticides are of great tremendous importance from agricultural and environmental perspective. Pesticide sorption affects other processes like transport, degradation, volatilization, bioaccumulation, which influence the final fate of these compounds in the soil environment (Gao *et al.* 1998).

Moreover, soils are a heterogeneous mixture of several components, many of which are organic and inorganic compounds of varying composition and surface activity. They can bind pesticides and reduce the bioavailability (Torrents and Jayasundera, 1997). Thus, knowledge of the pesticide adsorption–desorption characteristics of soil is necessary for predicting their mobility, retention, disappearance and indeed to understand the soil-pesticide dynamics, whether bioremediation is a feasible option for the clean-up of contaminated soil.

Pesticide adsorption affects other processes determining the final fate of these compounds in the environment (transport, degradation, volatilization, living organism uptake) and also controls the biological effectively of the pesticide toward the target organism. Thus, knowledge of pesticide soil adsorption-desorption characteristics is useful in predicting mobility and partitioning in soil-water systems and in making herbicide recommendations. The chemical nature of the pesticide and the characteristics of soils are the most important factors affecting adsorption-desorption behaviour.

Adsorption is usually described through isotherms – this entails functions which relates the amount of adsorbate on the adsorbent, with its concentration (liquid phase) or pressure (gaseous phase). Some of the most frequently observed and sited models in literature - describing sorption of organic compounds in aqueous, vapour phase and natural sorbent are Freundlich isotherm, Langmuir isotherm, BET (Brunauer, Emmet, and Teller) isotherm, Gibb's isotherm etc. Freundlich and Langmuir isotherms are the most

commonly used models for solid – aqueous systems e.g., in activated carbon or natural sorbent (solid) in water and wastewater treatment (aqueous). Freundlich isotherm is an empirical equation. Langmuir isotherm has a rational basis

Langmuir Adsorption Isotherm:

The Langmuir model is based on the assumption that, at maximum adsorption only a monolayer adsorbate is formed on the outer surface of the adsorbent; and adsorbate molecules do not deposite on other already adsorbed adsorbates, primarily because they do not interact or transmigration in the plane of the adsorbent surface. Therefore, the Langmuir represents the equilibrium distribution of the adsorbate between the solid and liquid phases (Vermeulan *et al.*, 1966).

This isotherm model is only valid for adsorbent surfaces containing specific number of identical sites and the possession of uniform adsorption energies by all adsorbate molecules adsorbing the adsorbent surface. Based on these assumptions, Langmuir (1916) represented the following equation:

$$x/m = q_{max}\, b\, C_e / 1 + b\, C_e \qquad (2.1)$$

where, x/m is the quantity of adsorbed adsorbate ($\mu g g^{-1}$), C_e is the equilibrium concentration ($\mu g cm^{-3}$), q_{max} is the maximum adsorption capacity of adsorbent ($\mu g g^{-1}$), and b is the Langmuir constant.

Freundlich Adsorption Isotherm:

The Freundlich isotherm model is the earliest known adsorption isotherm for describing the relationship between a solid adsorbent and adsorbate in an aqueous system at equilibrium (Tempkin and Pyzhev, 1940; OECD, 2000).

This model to a large extent has been found satisfactory as an empirical isotherm and can be used in adsorption from diluted solutions. The Freundlich adsorption isotherm is generally expressed by the following equation (Freundlich, 1906);

$$C_s^{ads}(eq) = x/m = K_f^{ads} \times C_{aq}^{ads}\,(eq)^{1/n} \qquad (2.2)$$

Equation (2.2) can be expressed in a linearized form by taking the logarithm;

$$\text{Log } C_s^{ads}(eq) = \text{Log } (x/m)_s^{ads} = \text{Log } K_f^{ads}(eq) + 1/n \text{ Log } C_{aq}^{ads}(eq) \qquad (2.3)$$

where, C_s is the amount of adsorbate adsorbed on adsorbent ($\mu g g^{-1}$), C_{aq} is the equilibrium concentration in the solution ($\mu g cm^{-3}$), K_f ($cm^3 g^{-1}$) and $1/n$ are empirical constants, referred to as Freundlich adsorption coefficient and adsorption constant respectively. K_f and n are obtained by linear regression and characterizes the adsorbent and the adsorbate adsorbed.

Among the mathematical adsorption models proposed so far, the Freundlich isotherm is the one most frequently used to describe adsorption processes and characteristics – especially for heterogeneous surface, which is often observed in soils (Hutson and Yang, 2000; OECD 2000; USEPA, 2008).

Because of the great importance of adsorption-desorption phenomena of pesticide in the environment. Sorption of pesticide influences other processes such as transport, degradation, volatilization, persistence and bioaccumulation, which in turn determine their fate in heterogeneous environment like soil or sediment (Gao *et al.* 1998; Kumar and Philip, 2006). There are numerous reports available on the reaction of pesticides (Carbamates, organophosphates, organochlorines) with soil (Cox *et al*. 1993; Krishna and Philip, 2008; Kumar and Philip, 2008; Atasoy *et al*. 2009). Other hydrophobic organics like PAHs (Wang *et al*. 2012; Olu-Owolabi *et al.* 2014) and herbicides like atrazine, acetochlor and 2, 4-Dichlorophenoxyacetic acid (Prata *et al*. 2003; Boivin *et al*. 2005) have been reported.

Gao *et al.*(1998), studied the sediment adsorption–desorption of seven pesticides and metabolites. The pesticides were described by Freundlich isotherm ($1/n<1$). Pesticides adsorption was stronger and faster with increasing pesticide hydrophobicity (higher K_{ow}, lower solubility), while desorption was much less effective and incomplete even after a long equilibration time. This phenomenon (hysteresis) was due to both particle size and organic matter content of sediment - which also influenced the pesticide distribution to the particle-size fractions. In addition, the pH also exhibited a considerable influence on the adsorption-desorption behaviour of the pesticides.

Adsorption-desorption studies of endosulfan pesticide in four Indian soils classified according to ASTM (American Society for Testing and Materials) standards (Kumar and Philip, 2006). Sorption properties were evaluated with Langmuir and Freundlich models, while the binding characteristics of the functional groups were calculated using Scatchard plot. The adsorption capacities for different soils varied from 0.1- 0.45 mgg^{-1} and 0.0942 - 0.2722 mgg^{-1} for α-endosulfan and β-endosulfan respectively. The order of binding effect of functional groups with soils was clayey > composted > red > sandy, while the pH was directly proportional to adsorption of endosulfan to clayey soil.

Atasoy *et al.* (2009) also reported the sorption behaviour of the α- and β-endosulfan in a Vertisol from Southeast region of Turkey – a major cotton cultivating area with large irrigated lowlands. Both isomers were considerably adsorbed by natural sorbent (Vertisol), while endosulfan data fitted the Freundlich adsorption–desorption isotherms, with regression coefficient (R^2) > 0.98. Freundlich adsorption coefficients (K_f) for α-endosulfan ranged between 21.63 and 16.33 mLg^{-1}, while for the β-endosulfan they were between 14.01 and 17.98 mLg^{-1} for the Ap and Bw2 horizons. The high adsorption and desorption phenomena observed were attributed to poor bonding between the endosulfan molecule and the surfaces of fundamental soil particles.

2.11.3. Field application of pesticide

The field investigations of pesticides have been studied for a number of crops and plants. It is more reliable and preferred to laboratory studies for the determination of kinetic parameters of pesticides used in agriculture. Pesticides such as chlorpyrifos, thiamthoxam, endosulfan etc., have been reported for some farm crops (Ntow*et al*., 2007; Rosendahl *et al.* 2009; Duhana *et al.* 2011; Lu *et al.* 2014; Kumar e*t al.* 2014). Lu *et al.* (2014) reported the persistence and dissipation of chlorpyrifos in 6 vegetables - brassica chinensis (*Brassica chinensis L.*), lettuce (*Lactuca sativa spp*), pepper (*Capsicum annuum spp*), eggplant (*Solanum melongena L*), celery (*Apium graveolens*), and asparagus lettuce (*Asparagus Lettuce spp*) at a greenhouse in Nanjing, China. Initial concentration of chlorpyrifos on six plants ranged between 16.5 ± 0.87 $mgkg^{-1}$ (*Brassica chinensis*) and 74.0 ± 5.9 $mgkg^{-1}$ (pepper). The residual concentration on day 21 ranged from 1.47 ± 0.22 (asparagus lettuce) – 0.15 ± 0.01 (pepper), while their half-lives were; 0.91 d (pepper)

<3.0d (eggplant) < 3.9 d (asparagus lettuce) < 3.92 d (lettuce) < 5.46 d (celery) < 5.82 d (brassica chinensis). An average residual concentration of 14.9 ± 0.5 $mgkg^{-1}$ was reported for soil, with a half-life of 7.78 d.

Field dissipation of endosulfan in tomato (*Lycopersicon esculentus*) and eggplant (*Solanum macrocarpon L*.) grown in Akumadan, Ghana and Southern Benin, West Africa, were reported by Ntow*et al.*(2007) and Rosendahl *et al.* (2009) respectively. On plant surfaces dissipation was rapid with half-lives for *Solanum macrocarpon L* being 1.6 h, 6.7 h and 2.7 h for α-endosulfan, β-endosulfan and total endosulfan respectively (Rosendahl *et al.*, 2009), while the soil had a half-life of 74 d for total endosulfan. For *Lycopersicon esculentus* half-lives of α-, β-endosulfan and total endosulfan on foliar were 0.164 d, 0.921 d and 0.430 d respectively, with corresponding values of 4.31 d, 4.31 d and 6.30 d respectively in soil (Ntow *et al*. 2007).

Field studies on the fate of pesticide applied to farm crops are of great importance. The persistence of the applied pesticide vis-à-vis its disappearance could be asserted with time for all the farm components. Terrestrial field dissipation (TFD) studies - deals with the disappearance of applied pesticide over a period of time. The persistence of the applied pesticide is determined by its half-life, which is calculated kinetically assuming a first order reaction (OECD, 14; NAFTA, 2006). Also, Zhaoxh *et al*. (2011), Vanclooster *et al*. (2000) and von Gotz *et al.* (1999) reported that the degradation of pesticides was by first order.

The Lagergren first order rate equation – i.e, a pseudo-first order reaction is expressed as follows (Lagergren, 1898);

$$dc/dt = kt$$

where c is the concentration of substance, t is the time and k is the degradation rate constant for substance.

In studying field kinetics involving the application of pesticides in the farm, reduction in concentration over time, is not solely dependent on their degradation, but as well as their dissipation far away from point of initial application. Both biotic and abiotic factors are

involved in the cause of this reduction or disappearance. Hence, for pesticides kinetic field studies could be re-expressed as;

$$dp/dt = kt$$

where,*p* is the concentration of pesticide, *t* is the time and *k* is the disappearance rate constant for the pesticide.

Technical grade endosulfan is made up of α- and β-isomers in 7+3 formulation respectively. It is assumed that the formation of endosulfan sulphate and endosulfan diol metabolites occurs independently of the reaction of two isomers in our ecosystem.

2.12. INSTRUMENTATION TECHNIQUES OF ANALYSIS

2.12.1. Gas Chromatography

Gas chromatography (GC) is a commonly used analytical technique in many research and industrial laboratories. A broad variety of samples can be analyzed as long as the compounds are thermally stable and volatile enough. Like for all other chromatographic techniques, a mobile and a stationary phase are required. The mobile phase (carrier gas) is comprised of an inert gas e.g. helium, argon, nitrogen, etc. The stationary phase consists of a capillary column, which could either be packed with a solid support (solid stationary phase) or a solid support coated with an immobilized liquid polymer of high boiling point (liquid stationary phase).More commonly used in many instruments are capillary columns, where the stationary phase coats the walls of a small diameter tube directly. In gas chromatography different compounds are easily separated because of the way they interact with the stationary phase. The stronger the interaction is, the longer the compound remains attached to the stationary phase, and the more time it takes to go through the column (ie, longer retention time).

Usually separation is influenced by polarity of the stationary phase, temperature of the oven, length of capillary tube,flow rate of carrier gas and quantity of analyte injected. Polar compounds interact strongly with a polar stationary phase, hence have a longer retention time than non-polar columns (or vice-versa). Chiral stationary phases based on

amino acid derivatives, cyclodextrins, chiral silanes, etc are capable to separate enantiomers, because one form is slightly stronger bonded.

The higher the temperature of the oven, the greater the quantity of the compound in the gas phase and the interaction or bonding is reduced with the stationary phase, hence the retention time is shorter, but the quality of separation deteriorates (Christian, 2004).

Also high flow rate of carrier gas causes, the molecules of analyte not to have sufficient time to interact with the stationary phase, thus leading to poor resolution of chromatogram.

The longer the column is the better the separation usually is. The trade-off is that the retention time increases proportionally to the column length. There is also a significant broadening of peaks observed, because of increased back diffusion inside the column. Large quantity of injected analyte will lead to the tailing of chromatograms or peaks as result of poor separation.

2.12.1.1. Gas Chromatography Columns

There are two types of columns used in Gas Chromatography – packed columns and capillary columns. Packed columns were the first type and have been in use for many years. However, their use are now restricted to applications that do require high resolution or when increased capacity is needed (Christian, 2004). Capillary column are more commonly used today.

Packed Columns

They are usually made of glass, stainless steel or Teflon and could be coiled, U-shaped or W-shaped. Coiled tubes are mostly used and are 1 m – 10 m long and 0.2 cm – 0.6 cm in diameter. Short columns are made of glass, but longer ones are made of stainless steel because they are easily straightened during filling and packing of columns, however for purpose of inertness, glass columns are still preferred. The column is normally packed with small particles that often serves as stationary phase (adsorption chromatography) themselves or coated with a nonvolatile liquid phase of varying polarity (partition chromatography). In gas-solid chromatography (GSC) the stationary phase is packed with high surface inorganic materials such as alumina (Al_2O_3) or porous polymeric substances

(e.g., Chromosorb – a polyaromatic crosslinked resin with a rigid structure and a distinct pore size). Small gases species such as H_2, N_2, CO_2, CO, O_2, NH_2 and CH_4 and volatile hydrocarbons are easily separated using this type of solid stationary phase.

Solid support for liquid phase (i.e., gas-liquid chromatography) is made of high specific surface area that is of uniform size, chemically inert, thermally stable and also wettable by the liquid phase. Most commonly used supports are prepared from diatomaceous earth – a spongy siliceous material. They are sold under different trade names, for example, Chromsorb W and Chromosorb P.

Capillary Columns

The various effective and high resolution open-tubular column in use today is as result of Golay's (1957) work – which predicted that increase in plate number of a narrow open-tubular column with the stationary phase supported on the inner wall, will lead to the elimination of broad band due to multiple paths (eddy diffusion). Also, the rate of mass transfer will increase since molecules have small distances to diffuse.

Columns are made of thin fused silica (SiO_2) coated on the outside with a polyimide polymer for support and protection of the fragile silica capillary – thus making it to coil easily. The inner surface of the capillary is treated chemically with silane-type reagent – e.g., dimethyl dichlorosilane (DMDS), in order to minimize interaction between analyte and the silanol groups (Si-OH) on the tubing surface. Capillaries are also made of stainless steel and they are preferred to the fused silica type because they are more robust and are used for applications requiring very high temperatures.

Capillaries are normally 0.10 mm – 0.53 mm i.d., and 15 m – 100 m long (Plate 2.6). It can have several hundred thousand plates and even up to a million in some cases. This endows it with the advantage and characteristics of high resolution with narrow peaks, short analysis time and high sensitivity (with appropriate and modern detectors) (Christain, 2004).

Plate 2.6. Gas chromatography capillary columns
(Source;Agilent J & W GC columns)

2.12.1.2. Types of GC Detectors

Flame Ionization Detector (FID)

The FID is very sensitive towards organic molecules (10^{-12} g/s, linear range: 10^6 –10^7), but relative insensitive to a few small molecules e.g. N_2, NO_x, H_2S, CO, CO_2, H_2O. If proper amounts of hydrogen/air are mixed, the combustion does not afford any ions. If other components are introduced that contain carbon atoms cations are produced in the effluent stream. The more carbon atoms are in the molecule, the more fragments are formed and the more sensitive the detector is for this compound (ie great response factor). However, due to the fact that the sample is burnt (pyrolysis), this technique is not suitable for preparative GC. In addition, several gases are usually required to operate a FID such as hydrogen, oxygen (compressed air), and carrier gas.

Thermal Conductivity Detector (TCD)

This detector is less sensitive than the FID (10^{-5}-10^{-6}g/s, linear range: 10^3-10^4), but is well suited for preparative applications, because it is non-destructive. It is based on the comparison of two gas streams, one containing only the carrier gas, while the second stream has the carrier gas and the analytes. Naturally, a carrier gas with a high thermal conductivity e.g. helium or hydrogen is used in order to maximize the temperature difference (and therefore the difference in resistance) between two thin tungsten wires. The large surface-to-mass ratio permits a fast equilibration to a steady state. The temperature difference between the reference cell and the sample cell filaments is monitored by a Wheatstone bridge circuit.

Electron Capture Detector (ECD)

The detector consists of a cavity that contains two electrodes and a radiation source that emits β-radiation (e.g. ^{63}Ni, ^{3}H). The collision between electrons and the carrier gas (methane plus an inert gas) produces a plasma containing electrons and positive ions. If a compound is present that contains electronegative atoms, those electrons are “captured” and negative ions are formed, and the rate of electron collection decreases. The detector is extremely selective for compounds with atoms of high electron affinity (10^{-14} g/s), but has a relatively small linear range (~10^2-10^3). This detector is frequently used in the analysis

of chlorinated compounds e.g. pesticides, polychlorinated biphenyls, which show very high sensitivity.

2.12.2. Mass Spectrometry

This is a sophisticated instrumental technique that produces, separates and detects ions in the gas phase. The mass spectrometry experiments were performed about a century ago in 1910 by J. J. Thompson, who showed that neon consist of two different types of atoms (isotopes) and ions differing in mass by 1 part in 15 were resolved with this mass spectrometer. However between 1919 and 1920, F. W. Aston introduced electrostatic and magnetic focusing, thereby increasing the resolution to 1 part in 100. Today, a resolution of 125000 is easily obtained (Vogel, 2006). The instrument is made up of a high vacuum system to enable the conversion of molecules into gas phase ions capable to live a lifetime long enough to be measured. Usually at a vapour pressure of 10^{-4} to 10^{-7} torr (10 mPa to 1 µPa) and a temperature up to 300^{o}C are maintained during the processes. The sample is introduced via an inlet, which vaporizes first and then swept into the ionization source, where compounds are converted to ions.

2.12.2.1. Ionization Sources

Presently, there are over twelve (12) different ionization methods used in mass spectrometry. In literature, of all the ion sources listed in Table 2.1 above, electron impact (EI) and chemical ionization (CI) are often used as sources for ion production. However, electron impact is the most frequently used method, while chemical ionization is occasionally used as an alternative.

2.12.2.2. Electron (Impact) Ionization

In electron impact (EI), analyte molecules in gaseous phase are bombarded by a high-energy beam of electrons, usually at 70 electron volts (eV) energy.The electron beam is produced by a filament (rhenium or tungsten wire) and steered across the source chamber to the electron trap. A fixed magnet is placed, with opposite poles slightly off-axis, across the chamber to create a spiral in the electron beam.

Table 2.1. Ionization sources used in mass spectrometry.

Name	Abbreviation	Phase	Fragmentation
Election impact	EI	gas	M^+ and fragments
Chemical ionization	CI	gas	$(M + 1)^+$ and some fragments
Field ionization	FI	gas	M^+ or $(M + 1)^+$
Field desorption	FD	solid/liquid	M^+ or $(M + 1)^+$
Desorption chemical ionization	DCI	solid/liquid	$(M + 1)^+$
Fast atom bombardment	FAB	liquid	$(M – 1)^+$, $(M + 1)^+$, $(M + A)^+$
Plasma desorption	PD	liquid	$(M + 1)^+$,$(M + 2)^{2+}$, $(M + 3)^{3+}$
Matrix-assisted laser desorption	MALDI	liquid	$(M + 1)^+$,$(M + 2)^{2+}$, $(M + 3)^{3+}$
Secondary ion mass spectrometry	SIMS	solid	Element ion or M^+
Ion microprobe	IMP	solid	Element ion
Spark source	SS	solid	Element ion
Inductively coupled plasma	ICP	liquid	Element ion plus multiple charged ions
Atmospheric pressure ionization	API	gas/liquid	$(M + 1)^+$ and $(M + A)^+$
Thermospray	TSP	liquid	$(M + 1)^+$, $(M + A)^+$
Plasmaspray	PS	liquid	$(M + 1)^+$ plus fragments
Electrospray	ES	liquid	$(M + 1)^+$ to $(M + NH)^{n+}$n = 1- 60
Particle beam interface	PBI	liquid	EI or CI spectra

Note; $(M + 1)^+$ is a singly protonated and singly charged ion, i.e. MH^+; A = adduct.

This is to increase the chance of interactions between the beam and the analyte gas. There are no actual collisions between analyte molecules and electrons; ionisation is caused by electron ejection from the analyte or by analyte decomposition (Gates, 2005). A beam of electrons ionizes the sample molecules resulting in the formation of a molecular ion on losing an electron and is represented by $M^{+\cdot}$ (radical cation). When the resulting peak from this ion is seen in a mass spectrum, it gives the molecular weight of the compound. Due to the large amount of energy imparted to the molecular ion, it usually fragments producing further smaller ions with characteristic relative abundances that provide a 'fingerprint' for that molecular structure. Peaks are usually normalized to the one with the greatest abundance (relative abundance 100%). The largest peak is often referred to as the base peak. This information may then be used to identify compounds of interest and help elucidate the structure of unknown components of mixtures.

2.12.2.3. Chemical Ionization (CI)

It is often referred to as a ‘’softer’’ technique, in which the ion source generate less amount of fragmentation and the dominant fragment is usually the molecular ion. CI begins with the ionization of methane (or another suitable gas like iso-butane and ammonia), creating a radical which in turn will ionise the sample molecule to produce $[M+H]^+$ molecular ions. CI is a less energetic way of ionizing a molecule hence less fragmentation occurs with CI than with EI and also yields less information on structural details of the molecule. Sometimes the molecular ion cannot be detected using EI; hence the two methods complement one another (Gates, 2005).

The next component is a mass analyser (filter), which separates the positively charged ions according to various mass related properties depending upon the analyser used. Several types of analyser exist, such as; quadrupoles, ion traps, magnetic sector, time-of-flight, radio frequency, cyclotron resonance e.t.c. (McNair, 1997; Jennings *et al.* 1997) (Plate 2.7). The most common are quadrupoles and ion traps. After the ions are separated they enter a detector, the output from which is amplified to boost the signal. The detector sends information to a computer that records all of the data produced, converts the electrical impulses into visual and hard copy displays. In addtion, the computer also controls the operation of the mass spectrometer.

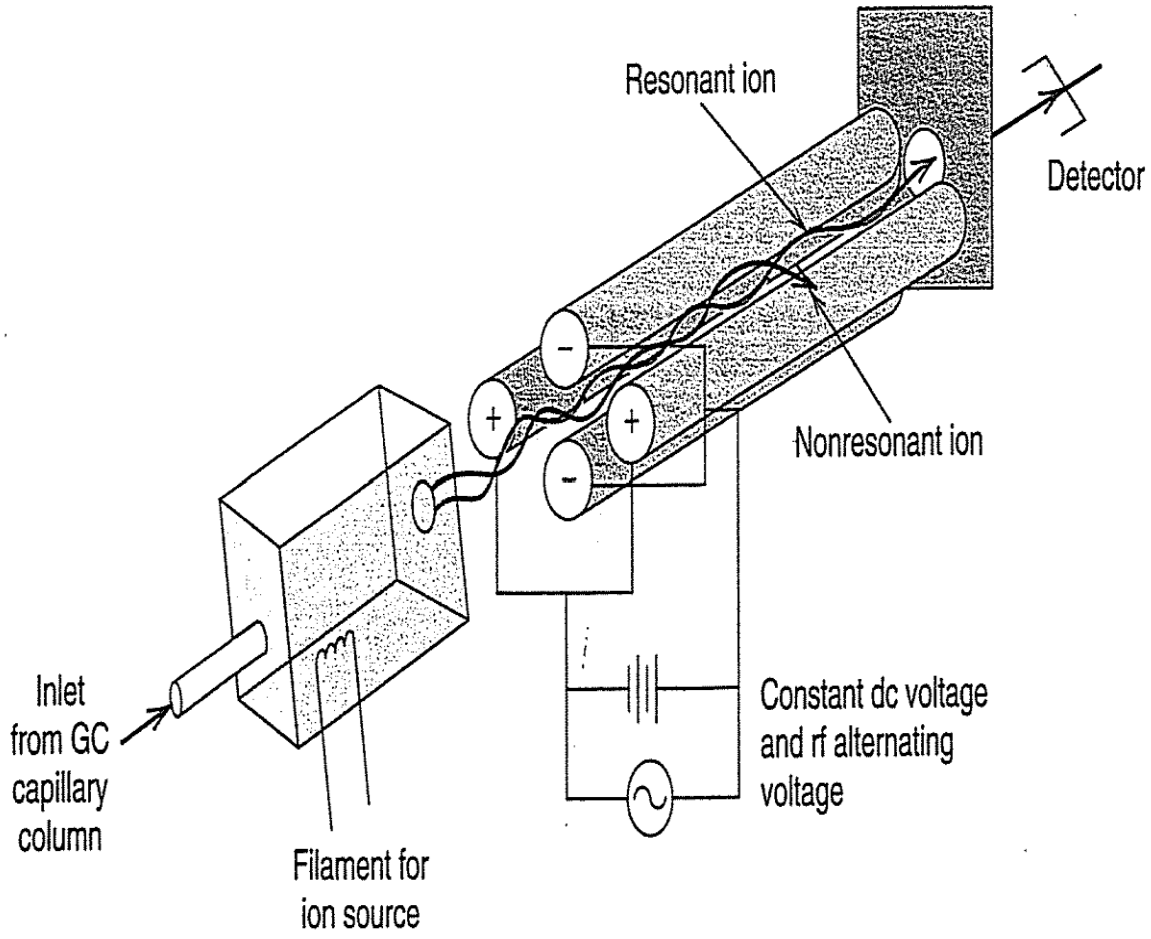


Plate 2.7. Quadrupole mass filter or analyser
(Source: Christain, 2004).

2.12.3. Gas Chromatography-Mass Spectrometry (GC/MS)

When a GC instrument is coupled with a mass spectrometer (GC-MS) the latter serves as a detector. Such combination is often referred to as a hyphenated technique and it is a good and effective analytical method. The GC separates the compounds from each other, while the mass spectrometer helps to identify them based on their fragmentation pattern.

Environmental analytes with complex components or mixtures of chemicals of low molecular weights are easily separated, identified and quantified. However, compound to be analysed by GC/MS must be sufficiently volatile and thermally stable. In addition, functionalised compounds may require chemical modification (derivatization) to eliminate undesirable adsorption effects that may affect the quality of the data obtained. Samples are usually analyzed in organic solutions consequently matrixes of interest (e.g. soils, sediments, tissues etc.) are solvent extracted prior to GC/MS analysis.

The sample solution is injected into the GC inlet where it is vaporized and swept by the carrier gas (usually helium) onto a chromatographic column. The sample comprising of different components of interest in gaseous phase are separated by virtue of their relative interaction with the coating of the column (stationary phase) and the carrier gas (mobile phase) as it flows through the column. The latter part of the column passes through a heated transfer line and ends at the entrance to ion source, where compounds eluting from the column are ionized to form singly charged molecular ions.

Inspite of the numerous methodologies and instrumental techniques applied by different workers for the determination of OCPs in various matrixes, no single technique or methodology is most preferred.

2.13. ANALYTICAL TECHNIQUES

2.13.1. Sample Preparation and Extraction Techniques

The measure of effectiveness, efficiency and accuracy of an analytical method is the technique involved in the sample preparation, which includes type and extent of pre-treatment of analyte and/or matrix, solvent type (polar and non-polar) and extraction

procedure used. To obtain a reliable analytical data for any analysis three major components are always involved:-

1. The system - a representative sample must be used/obtained (i.e., sampling should not be bias). This includes the analytes of interest and the matrix – in which the analyte is distributed and may interfere with the instrumentation.
2. The measuring instrument and
3. The Analyst.

Well over 50% of the time required for analysis is spent on sampling and sample preparation and they are men-related, hence most laborious and error-prone. Therefore extra-care should be taken during sampling and sample preparation.

2.13.2. Solvent System

Several multiresidue methods have been developed for organochlorine pesticides analysis in various matrices in the environment (Luke *et al.* 1975; Holland and Mcghie, 1983; Cook *et al.*1999; Adeyeye and Osibanjo 1999; Nur and Semra, 2004; Imo *et al.*2007; Aleksandra and Piotr 2007; Lal *et al.* 2008).

The solvent and solvent mixture types used for extraction in multi-residue OCPs analyses are of paramount interest since their extraction is a function of the polarity of the pesticides as well as the sample matrix type. OCPs are known to exhibit a wide range of polarity and solubility hence single neat solvent system cannot suffice and provide acceptable recoveries in OCPs analysis(Lal *et al.* 2008).

A number of solvents have been used for multiresidue OCPs extractions, some of the most commonly used in practice comprise – acetonitrile (Danis *et al.* 2002), acetone (Alvin and Lau 2003; Tagami *et al.* 2007; Lal *et al.* 2008), dichloromethane (GEMS ,1989; Polese *et al*. 1994; Aleksanda and Piotr 2007; Veljanoska-Saragiloska *et al*. 2010), ethyl acetate (Lal *et al*. 2008), hexane (Tagami *et al*. 2007) and cyclohexane (Alle *et al.* 2009).

Recently, Chen *et al.*, (2009), reported the use of solvent such as decane o-xylene, 1-octanol, p–xylene, and 1–hexanol for OCPs extraction. The overall best enrichment

solvent for the OCPs understudy - heptachlor, endosufan, p p'-DDE op' DDT and diedrin was in –hexanol.

Numerous single solvents and mixed solvents have been reported for extraction of OCPs from plants tissues. This ranging from non-polar solvents such as hexane, pentane, petroleum ether or spirit, through mixtures of non polar and polar solvent to very polar solvents like chloroform, acetone, methanol, acetonitrile, dichloromethane, etc.

Lal *et al*. (2008) investigated the use of different solvent mixtures like – acetone: ethyl acetate (10:90, v/v), ethyl acetate : hexane (90:10/10); acetone: ethyl acetate: hexane (10:80:10. v/v/v), acetone: ethyl acetate: hexane (20:70:10, v/v/v) and acetone: ethyl acetate: hexane (10:70:20, v/v/v) for the extraction of OCP residue in fruits and vegetables. They reported that the extraction mixture of, acetone: ethylacetate and hexane (10:80:10, v/v/v) exhibited the best recoveries for all the OCPs investigated.

2.13.3. Extraction Techniques

In this present era of 'green chemistry', sample preparation methods involving the use of large quantities of solvents (e.g liquid-liquid extraction) that are toxic are not justify for use in multi-residue determinations (Serodio and Nogueria, 2004). Modern approaches to sample preparation are mostly now geared towards environmentally friendly techniques in which smaller solvent volumes are used with corresponding reduced sample sizes or weights than previously used methods. Optimal sample preparation can reduce the time required for analysis and sources of error, and can enhance sensitivity, enable unequivocal identification, confirmation and quantification (Ridgway *et al.* 2007).

The analysis of OCPs in biota samples requires the use of extraction techniques, which allow the release of the analytes from the solid matrix, with optimum yield and selectivity, by using an appropriate and adequate solvent, such that minimal potential extraneous and interfering species are carried into the analytical separation stage. The solvents may be organic liquids, supercritical fluids, pressurised, or superheated liquids.

The most common extraction techniques for solid and semi-solid matrices include Soxhlet extraction, sonication-assisted extraction, supercritical fluid extraction (SFE), microwave-

assisted extraction (MAE), pressurized liquid extraction (PLE) and matrix solid-phase dispersion (MSPD).

2.13.3.1. Conventional Soxhlet Extraction

Soxhlet extraction is a general and well-established technique developed in 1879. The technique is based on exhaustive extraction of organic compounds (analytes) in a soxhlet system by an organic solvent, which is continuously refluxed through the sample placed in a porous thimble. The extracted analytes accumulate in a heated flask; therefore the analyte must be stable in the refluxing boiling solvent. Soxhlet extraction is the oldest technique used for the isolation of non-polar and semi-polar organic pollutants from different types of solid matrices, including biota samples (Barcelo, 1993;Luque deCastro and Gracia-Ayuso, 1998; Lopez-Avila, 1999).

Although the size of the system can vary, the more common procedures use 50–200 mL of organic solvent to extract the analytes from between 1 and 100 g of sample. It is essential to match the solvent polarity to the solute solubility and to thoroughly wet and permeate the sample matrix with the solvent used for extraction.

The advantages of soxhlet extraction include: it allows the use of large amount of sample (e.g. 1–100 g), no filtration is required after the extraction, the technique is not matrix dependent, and many soxhlet extractors can be set up to perform in unattended operation. Attempts to automate the technique were somewhat successful, and a few commercial systems are available in which several samples can be extracted in parallel with much shorter extraction times and less organic solvent than using conventional soxhlet (Luque deCastro and Gracia-Ayuso, 1998). The main disadvantages of soxhlet extraction are that it requires large amounts of solvent, the solvent must be evaporated to concentrate analytes before determination, the process takes several hours or days to complete the extraction, and it generates dirty extracts that require extensive clean-up ([Barcelo, 1993; Luque deCastro and Gracia-Ayuso, 1998). Therefore, this traditional method is being replaced by other new extraction techniques such as SFE, MAE and ASE with shortened extraction times, reduced organic solvent consumption and increased pollution prevention. However, soxhlet extraction is still an attractive option for routine analysis for its general

robustness and relatively low cost. Moreover, soxhlet extraction is widely used as a standard technique and reference for evaluating the performance of new extraction methods proposed.

2.13.3.2. Sonication-Assisted Extraction

The simplest solid–liquid extraction technique is to blend the solid sample with an appropriate organic solvent by ultrasonication. The process is carried out in discrete systems using an ultrasonic bath or a closed extractor fitted with a sonic probe. Sonication involves the use of sound waves to stir the sample immersed in the organic solvent use for extraction. Briefly, energy in the form of acoustic sound waves in the ultrasound region above 20 kHz, is used to accelerate mass transport and mechanical removal of analytes from the solid matrix surface by a process called "cavitation". This consists of the formation and implosion of vacuum bubbles trough the solvent, thus creating microenvironments with high temperatures and pressures (estimated up to 5000 °C and 100 MPa)(Priego-capote and Luque de castro,2003). This mechanical effect of ultrasound induces a greater penetration of solvent into solid materials and improves mass transfer leading to enhanced sample extraction efficiency. The sonication-assisted extraction is faster (5–30 min for sample) than the soxhlet mode and allows extraction of large amounts of sample with a relatively low cost. Unfortunately, it still uses about as much solvent as the soxhlet extraction, with filtration also required after extraction. In addition, it is labour intensive, since apart from the polarity of the solvent, the efficiency of the extraction is a dependent on the nature and homogeneity of the sample matrix, the ultrasound frequency and the sonication time used (Lopez-Avila,1999). Sonication-assisted extraction has been approved by EPA as method 3550B.

Ultrasonic-assisted extraction has been recently carried out using a dynamic extraction set-up (a flow system) which continuously supplies fresh extraction solvent to the extraction vessel. This approach may be considered as if it forces adsorbed analytes to partition continuously into new extraction solvent. A considerable reduction of extraction time, solvent consumption and sample handling, with respect to the extraction pattern, was reported (Priego-capote and Luque de castro,2004). Another feature of such dynamic arrangement is that the analytes are transferred out of the extraction apparatus as soon as

they are extracted, to avoid degradation of the analytes due to sonication or if analytes are thermo-labile and are extracted at higher temperatures and pressures. Domeño *et al*., (2006) used a dynamic sonication-assisted extraction procedure for extracting PAHs from lichens using hexane. The reported total extraction time was only 10 min as against 2 hours in the static extraction mode and 6 hours in Soxhlet extraction, while the PAHs relative recovery obtained by the three methods were similar.

Similar to soxhlet technique, only drying and homogenization is carried out before sonication-assisted extraction of biota samples. Drying the samples is performed by evaporation of water at room temperature (Blasco *et al*. 2006) or by grinding with sodium sulphate (Ratola *et al.*2006). Freeze-drying can also be used for sample drying (Barriada-Pereira *et al.* 2005).

2.13.3.3. Supercritical fluid extraction (SFE)

SFE is an extraction technique that uses a solvent in its supercritical state. Supercritical fluids have similar densities to liquids, but lower viscosities and so analytes show higher diffusion coefficients. This combination of properties results in a fluid that is more penetrating has a higher solvating power and may extract solutes faster and more efficiently than liquids (Smith, 2003; Lopez-Avila, 1999; Camel, 2001). In addition, the density (and therefore the solvent power of the fluid) may be adjusted by varying both the pressure and the temperature, affording the opportunity of theoretically performing highly selective extractions (Camel, 2001).

SFE utilises commercially available equipments where the fluid is pumped, at a pressure above its critical point, through the sample placed in an inert extraction cell (see Plate 2.8). The temperature of the cell is increased to overcome the critical value of the fluid. After depressurization, analytes are collected in a small volume of organic solvent or on a solid-phase filled cartridge (solid adsorbent trap).

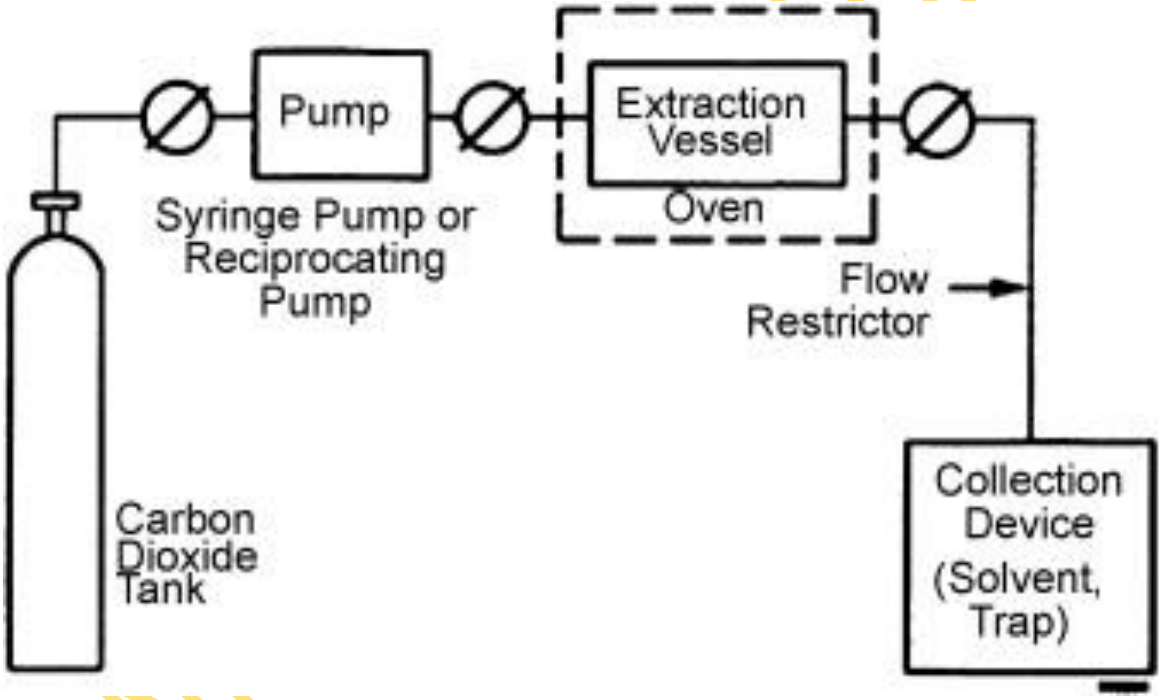


Plate 2.8. Schematic diagram of a SFE system

(Source: Vives and Grimalt, 2002).

Extraction can be performed in static, dynamic or recirculating mode: performing static extraction the cell containing the sample is filled with the supercritical fluid, pressurised and allowed to equilibrate; using a dynamic mode, the supercritical fluid is passed through the extraction cell continuously; finally in the recirculating mode the same fluid is repeatedly pumped through the sample and, after the required number of cycles, it is pumped out to the collection system.

Although many supercritical fluids have been investigated, the most commonly used is carbon dioxide (CO_2) because it reaches the supercritical state at a relatively low pressure (i.e. 7 MPa) and temperature (i.e. 31.3 °C), it is non-toxic, non-flammable, non-corrosive, chemically very inert, and affordable. Also, although CO_2 is non-polar, its polarity can be adjusted with modifiers such as acetone and methanol.

SFE efficiency is affected by a wide range of parameters such as supercritical fluid nature, temperature and pressure, extraction time, the shape of the extraction cell, the sample particle size, the matrix type, the moisture content of the matrix and the analyte collection system. Due to these numerous parameters affecting the extraction efficiencies, SFE affords a high degree of selectivity and the extracts are relatively cleaner (thus, they require only moderate additional clean-up). In fact, combined with solid adsorbent traps, SFE may provide a single-step extraction and clean-up. However, the need to control so many operating parameters makes SFE optimization tedious and difficult in practice. Other disadvantages of the SFE technique include: limited sample size and high cost of the equipment.

The use of SFE for extraction started in the mid-1980s, since then numerous applications of this technique in the analysis of environmental samples has been reported (Lopez-Avila, 1999; Camel, 2001;Smith, 2003). SFE was also used recently for extracting POPs from different plant materials (Ling *et al.* 1999; Zhu and Lee, 2002; Zuin, *et al.*2003; Quan *et al*. 2004). The plant samples were air dried at room temperature and chemical dried with anhydrous sodium sulphate or lyophilised before SFE. Ling *et al.* (1999) reported the extraction of several OCPs from Chinese herbal medicines using SFE with

CO_2 at 25 MPa and 50 °C (5 min static extraction time and 20 min dynamic extraction time) using Florisil as trapping sorbent. A similar procedure was used by Zuin *et al*. (2003) for the determination of OCPs and organophosphorus pesticides in Brazil's medicinal plants. Mild extraction conditions (pure CO_2; 10 MPa and 40 °C, 5 min static plus 10 min dynamic extraction time) and C_{18} as trapping adsorbent allowed for direct analysis of the extract by GC–ECD/Flame photometric detector (FPD) with no prior cleaning procedure. Quan *et al*.(2004) reported the extraction of OCPs from ginseng by SFE using CO_2 with 10 % ethanol–H_2O solutions as modifier at 30 MPa, 60 °C and C_{18} as trapping adsorbent

The use of SFE for extraction of POPs from different animal tissues has also been reported (Nerin *et al*. 2002; Antunes *et al*. 2003;Wolkers *et al*. 2006). Nerin *et al.* (2002) made a comparison between Soxhlet and SFE extraction for the determination of OCPs and some metabolites in frog tissues. The study highlighted the main advantages of SFE versus Soxhlet procedures, including efficiency (higher recoveries), time consumption, cost and more environmentally friendly. It must be kept in mind, however, that SFE requires an optimization in depth since the extraction behaviour is strongly affected by the type of sample. Similar to plant materials, animal tissue samples were desiccated with sodium sulphate before the extraction step to make the sample matrix more accessible to the supercritical fluid. SFE has been adopted by the EPA as a reference method for extracting PAHs (Method 3561) and POPs (Method 3562) from solid environmental matrices (http://www.epa.gov.).

2.13.3.4. Microwave-Assisted Extraction (MAE)

MAE uses microwave radiation (0.3–300 GHz) as the source of heating a solid sample–solvent mixture (Camel, 2000). Due to the particular effects of microwaves on matter (namely dipole rotation and ionic conductance) heating with microwaves is instantaneous and occurs in the heart of the sample, leading to very fast extraction. Heat generation in the sample by the microwaves field requires the presence of a dielectric compound. The greater the dielectric constant, the more thermal energy is released and the more rapid is the heating for a given frequency. Consequently, the effect of microwave energy is

strongly dependent on the nature of both the solvent and the solid matrix. Usually, the extraction solvent has a high dielectric constant, so that it strongly absorbs the microwave energy. However, in some cases (for thermo-labile compounds), the microwaves may be absorbed only by the matrix, resulting in heating of the sample and release of the solutes into the cold solvent. Therefore, the nature of the solvent is of great importance in MAE - it should selectively and efficiently solubilize the analytes in the sample, but at the same time, it should absorb the microwaves without leading to a strong heating (so as to avoid eventual degradation of the analyte compounds). Thus, it is common practice to use a binary mixture (e.g. hexane–acetone, 1:1) where only one of the solvent is absorbing microwaves. Other important parameters affecting the extraction process are the applied power, the temperature and the extraction time. Moreover, the water content of the sample needs to be carefully controlled to avoid excessive heating, allowing reproducible results.

The application of microwave energy to the samples may be performed either in closed vessels with pressure and temperature control (pressurised MAE) or in open vessels at atmospheric pressure (focused MAE) (Camel, 2001; Dean and Xiong, 2000). Whereas in focused MAE, the temperature is limited by the boiling point of the solvent at atmospheric pressure, in pressurised MAE the temperature may be elevated by simply applying adequate pressures.

Today MAE is considered a good alternative to traditional Soxhlet extraction for POPs in environmental solid samples because it reduces extraction time (e.g. 20–30 min per batch of as many as 12 samples), uses small amounts of solvents (30 mL in MAE versus 300 mL in Soxhlet extraction) and improves extraction yields. Consequently, several applications are reported (Eskilsson and Bjorklund, 2000) and an official EPA method 3546 (Microwave Extraction) has been approved for the extraction of organic compounds from solid environmental samples. However, inspite of MAE effiency it has also several drawbacks, such as (i) the extract must be filtered after extraction (ii) polar solvents are used (iii) clean-up of extracts is often needed because of co-extractives and (iv) the equipment is moderately expensive.

A number of recent studies have reported the use of MAE for extracting different POPs from plants (Cai *et al.* 2003; Barriada-Pereira *et al.* 2003; Barriada-Pereira *et al*. 2004; Barriada-Pereira *et al*. 2005) and animal tissues (Carro *et al*. 2000; Bayen *et al*. 2004; Bayen *et al.* 2005; Pen *et al*. 2006). Since the water content of the sample has a great effect on the extraction process, usually samples were air dried at room temperature, lyophilised (Barriada-Pereira *et al*. 2003, 2004 and 2005), freeze-dried (Carro *et al*. 2000) or chemically dried with anhydrous sodium sulphate before MAE. Cai *et al.* (2003) used MAE to extract OCPs from Chinese teas before solid-phase microextraction (SPME)–GC–ECD analysis. The recoveries of MAE were compared with those of ultrasonic extraction and results showed that MAE provided better recoveries (efficiencies) and shorter extraction times than ultrasonic extraction. Barriada-Pereira *et al*.(2003) carried out a comparative study between MAE and Soxhlet extraction of 21 OCPs from plants using *n*-hexane–acetone (1:1, v/v) as solvent in both cases. Both techniques showed similar recoveries but Soxhlet extraction was more laborious and required higher solvent consumption and longer extraction times than MAE. Barriada-Pereira *et al*.(2003 and 2004) used the developed MAE procedure for the determination of the same OCPs in tree leaves and five species of plants. The method was applied to determine POPs in a wide range of mangrove biota organisms(Bayen *et al*. 2005). Pena *et al*. (2006) reported on two types of fish samples representing low and high fat content (turbot and salmon) were considered for experimental optimization. Other samples of low and high fat content (mussel and lamprey) were also analysed to verify the applicability of the developed procedure. Accuracy validation using NIST SRM 2977 reference material was carried out and recoveries around 90% for the studied compounds were obtained.

2.13.3.5. Pressurised Liquid Extraction (PLE)

This technique, also named pressurized fluid extraction (PFE), was originally launched by Dionex Inc. in 1995 under the name accelerated solvent extraction (ASE) (Richer *et al*.,1996; Fitzpatrick *et al*. 2000). PLE is a solid-liquid extraction process performed in closed-vessels at relatively elevated temperatures, usually between 80 and 200 °C, and elevated pressures, between 10 and 20 MPa. Therefore, PLE is quite similar to SFE but

CO_2 is replaced by organic solvents to mitigate potential polarity troubles (Fitzpatrick *et al.*, 2000) (Plate 2.9).

Extraction is carried out under pressure to maintain the conventional organic solvents in its liquid state, but extracting at temperatures well above their atmospheric boiling points. Therefore, the solvent is still below its critical conditions during PLE but has enhanced solvation power and lower viscosities and hence allows higher diffusion rates for analytes. In this way the extraction efficiency increases, minimizing solvent needed and expediting the extraction process. Both static and flow-through extraction systems can be used (Bautz *et al*. 1998; Camel, 2001) - in the static extraction mode, the sample is loaded in an inert cell and pressurized with solvent heated above its boiling point during some time (then, the extract is automatically removed and transferred to a vial). The "flow-through extraction mode" uses fresh solvent continuously introduced to the sample. This improves the extraction efficiency but, of course, diluting the extract (Bautz *et. al*. 1998).

In PLE, the pressure is of comparatively minor importance because its role is just to maintain the solvent in its liquid state. This reduces the number of parameters that need to be optimized to achieve efficient extractions compared with SFE. The main parameters to consider now are temperature and time and so the time devoted to development and optimization of the extraction procedure can be reduced. Moreover, methodology is straightforward because the same solvent recommended in the official and routine Soxhlet methods can be used. Therefore, PLE is an attractive technique because it is fast (e.g. extraction time is approximately 15 min per sample), uses less solvent volume (15–40 mL), no filtration is required after extraction, the instrumentation allows extraction in unattended operation (at least 24 samples can be processed sequentially) and different sample sizes can be accommodated (e.g. 11, 22 and 33 mL vessels are available). The two main disadvantages of PLE include limited selectivity (so, it usually requires further clean-up of the extract obtained) and higher capital cost than SFE and MAE systems.

PLE has been accepted as an official EPA method (method 3545) for the determination of POPs in a variety of environmental solid samples.

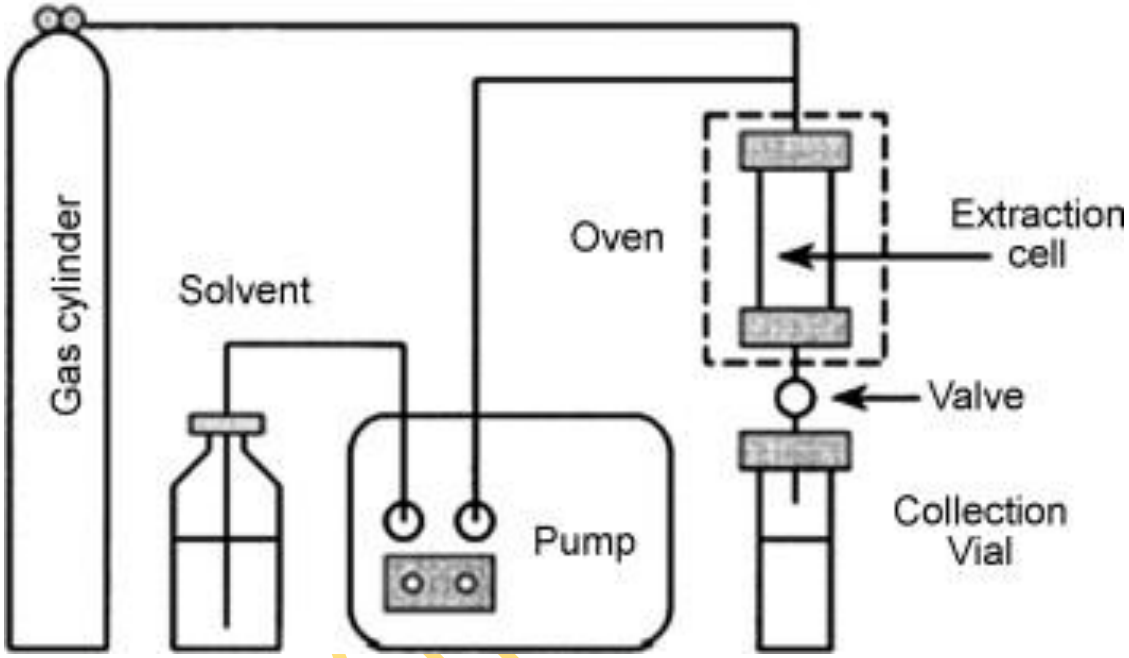


Plate 2.9. Schematic diagram of a PLE system.

(Source: Barcelo, 1993)

Numerous applications of PLE for the extraction of POPs from biota samples (vegetal and animal) have been reported (Adou *et al.*2001; Haib *et al.* 2003; Kitamura *et al*.2004;Tao *et al*. 2004; Johasson *et al.* 2006).In all the cited articles on PLE only dry samples were used - the absence of water in the samples makes the sample matrix more accessible and permeable to organic solvents and thus high extraction efficiency. Samples are often dried by grinding with sodium sulphate or hydromatrix, air-dried, freeze-dried or lyophilised before applying PLE.

Tao *et al*. (2004) applied PLE for extracting DDT and its metabolites from wheat with hexane/acetone (1:1, v/v) (pressure 101 MPa, temperature 120 °C). Moreno *et al*. (2006) investigated the extraction of 65 pesticides including OCPs from greasy vegetable matrices such as avocado using PLE with ethyl acetate–cyclohexane (1/1, v/v) at 120 °C and a pressure of 12 MPa. Adou *et al.*(2001) reported an analytical procedure based on PLE before GC–ECD or GC–FPD for the determination of different pesticides in fruits and vegetables. The recoveries were around 70% for almost all the compounds assayed. PLE was also used by Haib *et al*. (2003) for the extraction of OCPs from tobacco samples with acetone at 100 °C and 10 MPa of pressure.

When water is employed as the solvent for extraction in PLE, authors tend to use a different name and phases to highlight the fact that water is an environment-friendly solvent. Thus, terms such as pressurized hot water extraction, subcritical water extraction (SWE), superheated water extraction and high temperature water extraction are often found in literature (Ramos *et al*. 2002; Smith, 2003; Carabias-Martinez *et al*. 2005). Because the polarity of water decreases remarkably as the temperature is increased, superheated water at 100–200 °C, under a relatively low pressure, water can act as a medium to non-polar solvent (ethanol or acetone) and is an efficient extraction solvent for many analytes.

2.13.3.6. Matrix Solid-Phase Dispersion (MSPD)

MSPD is a process for the disruption and extraction of solid samples introduced in 1989 (Barker *et al*., 1989). MSPD combines aspects of several analytical techniques, performing sample disruption while dispersing the components of the sample on and into a

solid support. In this way a chromatographic material is generated that possesses unique character for the extraction of compounds from the dispersed sample (Kristenson *et al*., 2005).

In MSPD the sample is mixed (for liquid and semi-solid samples) or blended (for solid samples) with an appropiate sorbent until a homogeneous mixture is obtained (complete disruption and dispersion of the sample on the solid support).The resultant mixture is packed into an empty column, from which the analytes of interest are eluted with a suitable organic solvent while interfering matrix compounds are selectively retained on the column (Ramos *et al.* 2005). Another possibility is the elution of interfering compounds from the matrix in a washing step, after which, the target analytes are next eluted by a different solvent. Finally, eluants are either clean-up or analysed directly. Sometimes, the MSPD column is coupled on-line with a solid phase extraction (SPE) column or, as in several applications; the SPE sorbent is packed in the bottom part of the MSPD column to remove interfering matrix components (Barker *et al.*1989; Ramos *et al*. 2004).

MSPD can be regarded as a valid sample preparation technique, alternative to more classical methods, especially for solid and semisolid samples. It is simple, requires a small sample size, has a short extraction time, uses less solvent than conventional techniques, does not require preparation and maintenance of equipment and offers the possibility of simultaneously performing extraction and cleanup. However, the negative aspect is that MSPD is fairly labour intensive, requiring the sample to be ground up with the solid matrix and packed into a column for extraction, and quite a number of applications still use large volumes of solvents for extraction and clean-up.

The selectivity of a MSPD procedure depends on the sorbent/solvent combination used. Most methods reported to date use reverse-phase materials, such as C_8- and C_{18}-bonded silica as the solid support; silica, florisil and chemically-modified sorbents are used less frequently. For analyte extraction from animal tissues, C_{18}-bonded silica is by far the most popular sorbent while, for plant samples, both C_8- and C_{18}-bonded silica and also florisil are used extensively (Kristenson *et al*. 2006). The nature of the elution solvent is also important since the target analytes should be efficiently desorbed while the bulk of the remaining matrix components should be retained in the column. Most sorbents have been

tested in combination with a large variety of solvents, ranging from alkanes through toluene, dichloromethane and alcohols to water at elevated temperatures.

Most environmental applications of MSPD deal with extraction of pesticides from fruit, vegetables and animal tissue (Kristenson *et al.* 2006). However, there are also papers reporting the extraction of other POPs such as PAHs from fish tissue (Pensado *et al.* 2005) and pesticides, PCBs, PBDEs and polybrominated biphenyl (PBBs) from several marine species (Carro *et al.* 2005). Dry samples are most effectively and easily homogenised and dispersed with the solid support used in MSPD. Therefore, samples are usually dried with anhydrous sodium sulphate or freeze-dried before blending with the MSPD sorbent.

2.13.3.7. Clean-up of Extract

Whichever technique is used for extraction, various matrix components such as lipids, carotenoids, pigments and resins are often present as co-extractives and must be eliminated to permit a more definitive identification and quantification of lower levels of analyte and to minimize deterioration of chromatographic performance. Thus, the removal of co-extracted matrix components is critical and so different clean-up procedures have been developed to minimise their negative effects. Moreover, the clean-up step is usually necessary to remove not only the bulk of the co-extracted material, but also those compounds closely related to the analytes that could potentially interfere in the final determination. In this latter case adequate separation schemes or fractionation processes to allow for isolation of sub-groups of compounds (fractionation of the extract into different classes of compound) has to be carried out.

In the last few years there has been a lot of work on the miniaturization of sample preparation procedure using micro-extraction processes, especially in separation techniques. Solid phase micro-extraction (SPME), solvent micro-extraction (SME), and membrane- based liquid micro-extraction are recent examples of such development. A solvent-free SPME process developed by Pawliszyn and Arthur (1990) involved the simultaneous extraction and pre-concentration of analytes from aqueous samples. The principle is based on the partitioning of analytes between sample matrices and a polymer-coated stationary phase present on a silica fibre. Other related modes of SPME are

immersion (Zambonin *et al.* 2002), headspace (Lee *et al.* 1997; Lambropoulou*et al*. 2006b), and membrane-protected SPME (Basheer *et al*. 2002).

Liquid-phase micro-extraction (LPME) a miniaturized alternative to conventional liquid-liquid extraction (LLE) has been developed.

Also Blanco *et al*. (2003), compared single-drop micro-extraction (SDME) with solid phase extraction and solid phase micro-extraction (SPME) for determining α-endosulfan and β-endosulfan in water samples using GC-ECD. He reported that the limit of detection of the investigated pesticides were as follows -0.01 mg/kg, 0.02 mg/kg, 0.06 mg/kg for SDME, SPE and SPME respectively.

Xue *et al.* (2007), investigated and reported the use of concentrated sulphuric acid with water (90:10) for the cleaning of ten (10) Traditional Chinese Medicines (TCM) in the multi-residue analysis of 18 OCPs extracted using acetone-petroleum ether (1:1) solvent system. 1 mL aliquot of the organic phase was collected for GC-ECD analysis. The cleaned TCM extracts were found to allow determination without interference, with high recovery, lower limit of detection (LOD) and good reproducibility. This method could be considered satisfactory in routine analysis for monitoring protocols or exercises.

Some of the clean-up methods often used for extracts are as follows:-

2.13.3.8. Classical Liquid Adsorption Chromatography

Classical liquid adsorption chromatography is still the dominant technique for purification and fractionation of biota extracts. This classic technique is used in an “off-line” mode and involves passing extracts through several adsorbent columns prepared in the laboratory or through solid-phase extraction cartridges. Liquid adsorption chromatography can discriminate between the target compounds and the matrix components to a degree that depends on the selectivity of the sorbent (sorbents) used. Alumina, silica gel and florisil columns in different mesh sizes, levels of activity and column sizes (either separately or in combination) are widely used. Sometimes, an alkaline treatment (saponification) or a treatment with sulphuric acid is necessary prior to, or in conjunction with, adsorption columns to remove the bulk of co-extracted lipids. Official EPA Methods 3630C, 3610B

and 3620B (using silica gel, alumina and florisil Cleanup, respectively) have been approved for the purification of organic extracts from solid environmental samples (http://www/epa.gov/.).

Pena *et al*. (2006) reported the use of silica gel cartridges to clean-up fish tissue extracts containing PAHs after alkaline lipids digestion. Similarly, extracts of different vegetables and fruits (lettuce, tomato, cabbage, apple, grape and pear) were purified on a deactivated silica gel (15% water) column before PAHs determination (Camargo and Toledo, 2003). Crespo and Yusty (2005) also used a silica gel column to clean-up seaweed extracts in the determination of PCBs. Nerin *et al*. (2002), tested several adsorbents (3% deactivated silica, 5% deactivated Florisil) as well as their combinations and different elution solvents (in volume and nature) to clean-up frog extracts for the determination of OCPs. Best results were obtained with 3% deactivated silica and *n*-hexane as eluent.

Prado-Rosale *et al*. (2003) andYenisoy-Karakas, (2006) reported the clean-up of OCPs extracts in fresh vegetables and sunflower seeds respectively using florisil column. Also, plant extracts containing DDTs were purified with a florisil column after sulphuric treatment was reported by Tao *et al*. (2004).

On the other hand, Barriada-Pereira *et al.* (2004) compared cartridges filled with four different sorbents: Florisil, a tandem of florisil and alumina, silica, and carbon to clean-up tree leaves extracts prior to OCPs determinations. Carbon was found to be the sorbent that produced colourless eluates, cleaner chromatograms and low interferences. Similarly, florisil, silica and alumina cartridges as well as glass columns (filled with either florisil, silica or alumina) were also compared for pine needles extracts purification prior to GC determination of PAHs and alumina disposable cartridges were chosen as the most efficient.(Ratola *et al.* 2006). Silica gel, alumina, aminopropyl-silica, cyanopropyl-silica, florisil, graphitized nonporous carbon and silica gel–alumina mixture (3:1) were used for column chromatographic clean-up of PAHs, PCBs and DDTs in mussel tissue and krill samples in combination with modified supercritical CO_2 as the mobile phase (Fuoco *et al*.,2005). A silica gel–alumina (3:1) column was shown to offer the best performance in terms of clean-up efficiency.

Sometimes it may be necessary to use more than one column (adsorbent) to obtain adequate clean-up and/or fractionation of sample extracts. In this vein, extracts of cod liver containing several POPs were fractionated with florisil, activated carbon and basic alumina column chromatography (Sinkkonen and Paasivirta, 2000). Also clean-up column packed with florisil, followed by silica gel was used for extracts of tobacco containing low polar OCPs (Haib *et al.* 2003). 5% deactivated alumina was added to a florisil cartridge for purification of OCPs in different vegetation samples (grass, five species of plants) (Barriada-Pereira *et al*. 2004 and 2005).

2.13.3.9. Gel-Permeation Chromatography (GPC)

This technique is also referred to as size exclusion chromatography (SEC) and it is a size exclusion clean-up procedure that uses organic solvents for the mobile phase and a specialized column packed with divinyl benzene-styrene copolymer. The most typical packed phase uses Bio Beads SX-3 resin, packed into a glass column with mobile phase of interest. It is generally recommended for purifying extracts obtained from biological samples based on molecular size separation. GPC separation is used primarily to fractionate and remove lipidic material (>500 Å), which elute first from the column in biota matrices. GPC has several advantages over other methods currently being used for the clean-up of lipids, polymers, copolymers, proteins, natural resins, cellular components and other high molecular weight compounds from the analytes of interest, these includes i) it is non-destructive ii) it allows handling larger masses of lipids in each sample (e.g. compared to adsorption columns) and iii) GPC is more applicable when little information on the polarity or chemical properties of the molecule of an ‘‘unknown’’ contaminant isolate is available and iv) it can be fully automated.

Presently, an official EPA method (Method 3640A GPC Cleanup) has been approved for the purification of organic extracts from solid environmental samples (http://www/epa.gov/.), while Suchan *et al*. (2004) reported the purification of PCBs and OCPs in fish extracts by GPC on a S-X3 Biobeads column with cychlohexane-ethyl acetate (1:1, v/v) as mobile phase. One main disadvantage of the GPC system is that it is difficult to completely remove all traces of the lipids. Therefore, further clean-up steps are often necessary - by applying liquid adsorption chromatography columns. Smith *et*

al.(2006) used a column of alumina and silica gel before GPC on Bio-Beads S-X3 for the clean-up of PAHs in pasture vegetation extracts while Pan *et al.*, (2007) employed a multilayer silica column followed by a Bio-Beads S-X3 column and finally and alumina column for clean-up of POPs in extracts of mussel. OCPs in extracts from several biota (guillemot, mussels, green sea urchins,trout,salmon, sculpin and clams) were also reported purified using a Bio-Beads S-X3 column followed by a florisil column (Easton *et al*. 2002; Kuzyk *et al*. 2005; Ryan *et al.* 2005), while a Bio-Beads S-X3 column followed by a column of silica gel was also used for clean-up of PCBs and OCPs extracts in several biological matrices (Olsson *et al.* 1999; Vetter *et al*. 1999; Asmun *et al.*2004). Moreno *et al*. (2006) evaluated the comparative performance of Envirogel GPC columns (Manufactured by Waters) with C_{18}, Florisil and alumina cartridges for purification of pesticides in avocado. It was found that GPC offers the best results. Also, two Envirosep-ABC columns (Phenomenex) were connected in series for purification of pesticides in fatty matrices extracts, using acetate–cyclohexane (1:1, v/v) as mobile phase (Patel *et al.* 2005). This high performance GPC column was compared with the classical Bio-Beads S-X3 column for the purification of $MeSO_2$-PCBs and OCPs in extracts of marine mammal tissues (Herman *et al.* 2001). The best results were obtained when the classical GPC column was employed.

2.13.3.10. Solid-Phase Microextraction (SPME)

Solid-phase microextraction (SPME) is an extraction technique that has been used extensively by a number of researchers (Lord and Pawliszyn, 2000; Arthur and Pawliszyn 1990). This method is mainly suited for aqueous matrices and its' effectiveness is based on the partition of target analytes between a polymeric stationary phase coating a fused silica fiber, and the sample extract. During extraction the coated fiber is either directly immersed into the liquid extract or exposed to the headspace above the liquid. After extraction, the analytes, retained in the fiber, are thermally desorbed in the injector of a gas chromatograph for GC analysis.

Presently, SPME represents a more convenient alternative to the conventional extraction methods for liquid sample preparation [i.e. liquid-liquid extraction (LLE) and solid-phase extraction (SPE)]. SPME eliminates the use of organic solvents, is significantly quicker

and simpler than both LLE and SPE, because it integrates extraction, pre-concentration and sample introduction into a single step (Theodoridis *et al*. 2000).

Since its introduction in 1990 (Arthur and Pawliszyn, 1990) to analyse relatively volatile compounds in the environmental field, SPME has gained widespread applications for determination of organic pollutants, including POPs, in different types of samples (water, soil, food and biological fluids) as reported in several reviews (Penalver *et al.* 1999; Pawliszyn *et al.*2000*;* Alpendurada, 2000).

Water is by far the most widely analysed by SPME–GC. This is due to the fact that SPME application to more complex matrices, such as biota, is not straight forward. However, SPME can be used as a simple clean up/enrichment procedure for POPs determinations in vegetal and animal tissues after liquid–solid extraction, like soxhlet (Fidalgo-Used *et al.*2003); ultrasonic assisted extraction (Lambropoulou *et al*. 2006a and 2006b); MAE (Ho and Hsieh, 2001 and Cai *et al.* 2003); ASE (Wennrich *et al*. 2001) or SFE (Rodil *et al*. 2005).

Fidalgo-Used *et al*. (2003) developed a clean-up/enrichment procedure for OCPs in fish tissue samples based on Soxhlet extraction of the OCPs from the sample followed by SPME–GC–ECD over the corresponding organic extract. Ho and Hsieh (2001) reported SPME–GC in combination with MAE for the determination of OCPs in medicinal plants, while a novel fiber coating of polyphenylmethylsiloxane (PPMS) was also combined with MAE for the determination of OCPs in Chinese teas by Cai *et al*. (2003). The novel porous sol–gel PPMS fiber was reported to exhibit higher sensitivity and selectivity for OCPs compounds, higher thermal stability (up to 350 °C) and longer life time (adequate use for more than 150 times) than commercial polydimethylsiloxane (PDMS) fibers. Wennrich *et al*. (2001) used SPME–GC–MS for the determination of OCPs and chlorobenzenes in fruit and vegetables after a pre-extraction of analytes from the sample by ASE.

Finally, Rodil *et al.* (2005) have developed a new approach, based on simultaneous SFE-sample clean-up using SPE followed by SPME–GC–MS, for the determination of several POPs (OCPs, PCBs, PBBs and PBDs) in cultured marine species. The influence of several

parameters in the efficiency of the SPE/SPME combination was investigated by chemometrics approaches and the proposed procedure was validated with IAEA 406 reference material analysis (IAEA, 2000).

2.13.4. Integrated Extraction and Clean-up System

Several researchers have examined the suitability of integrating the clean-up step into supercritical fluid extraction (SFE) or pressurized liquid extraction (PLE) techniques by resorting to the use of sorbents in the extraction cell which would retain the matrix components (trapping sorbents). In the case of biota such sorbents are used mainly to retain the sample fat. However, there are few publications on work dealing with on-line combined extraction and clean-up procedures based on SFE (Ling *et al*. 1999;Jaremo *et al*. 2000; Wolkers *et al*. 2006) or PLE treatments (Bjorklund *et al*. 2001; Gomez-Ariza *et al*. 2002; Sporring *et al.* 2004; Eljarrat *et al.* 2004; Ligour *et al*. 2006). For instance, Ling *et al*. (1999) investigated different sorbents (e.g. Florisil, C_{18}, silica gel and neutral alumina) as "trapping" sorbents in SFE for the determination of OCPs in Chinese herbal medicine. Florisil was found to produce the most facile and effective integrated clean-up. Jaremo *et al*. (2000), investigated the use of basic alumina and florisil as fat retainers or sorbents for lipid-free extraction of PCBs from a model fatty sample using SFE, the basic alumina was finally preferred to selectively clean-up PCBs in the fat sample extract. Basic alumina was also used as fat retainer in SFE for the determination of OCPs, PCBs and PBDEs in seal tissue (Wolkers *et al*. 2006). Bjorklund *et al*. (2001) evaluated the ratios between fat and fat retainer to obtain fat-free extracts. They studied five different fat retainers, namely - florisil, basic alumina, neutral alumina, acidic alumina and sulphuric acid-impregnated silica in order to clean-up PCBs in fish using PLE and the use of sulphuric acid-impregnated silica provided the cleanest PCBs extracts. Sulphuric acid-impregnated silica as fat retainer in PLE was also successfully used for the determination of PCBs in several fat containing matrices including lard fat, pork fat, cod liver oil, fish meal, feed poultry and vegetable feedstuff. Four different fat/fat retainer ratios (FFRs) were tested (0.100, 0.075, 0.050 and 0.025) at 50 and 100 °C using *n*-pentane, *n*-hexane or *n*-heptane as extraction solvent. No fat was co-extracted when using a FFR ratio of 0.025.

2.13.5. Clean-up Techniques for OCPs

Most polar solvents are known to co-extract other components in addition to targeted OCPs. These co-extractives in most cases interfers with the performance of the instruments, thus their elimination is crucial in getting reliable analytical results. Generally, interfering substances can be removed by the following principles i) chromatography – column, TLC, gel-permeation and paper; (ii) liquid-liquid partitioning; (iii) chemical technique - acidification, saponification and oxidation, and (iv) distillation or volatilization. However, column chromatography is the most commonly used for OCP clean-up exercises and the adsorbents are florisil, silica gel, alumina, and activated charcoal (Al-Rashan and Helaleh, 2014). Luke *et al*. (1975), Stimac (1979) and Hsu *et al*. (1991) have developed rapid florisil clean-up procedures prior to OCP multi-residue GC analysis. Luke *et al*. (1975) extracted samples with acetone and eluated 31 pesticides with methylene chlorine–petroleum ether mixture. Holland and Mcghie (1983) reported a two-phase clean-up procedure for fruits based on methanol extraction. Water and highly water-soluble co-extractives were removed by initially partitioning the pesticides into toluene. Further clean-up was performed by carbon-cellulose-florisil before GC determination. High recoveries were obtained for the 30 pesticides studied. Holstege *et al*. (1994) reported the use of automated gel permeation chromatography, in which silica gel mini column or C18 solid phase extraction (SPE) were used for the clean-up of extracts of plant and animal tissues. 17 OCPs were clearly identified and quantified. Other OCP multi-residue clean-up methods reported in literature includes the gravity-fed SPE (Cook *et al*. 1999); SPE C18, C8,C2, CH and pH sorbent for the clean-up of melon, apple, cabbage, eggplant and green pepper (Odanaka *et al.* 1991); C18, carbon and amino-propyl cartridge SPE clean-up of 251 pesticide residues in fruits and vegetable samples (Fillion *et al*. 2000) and as well as the use of quaternary amine (SAX) and PSA for clean up by Sheridan and Meola (1999). Kuet and Seng (2002) investigated the use of SPE, SAX/NH_2 clean-up of 6 OCPs in acetone-dichloromethane extracts from carrot, cucumber and green mustard. Results obtained were comparable to those of silica gel clean-up method.The development of miniaturized SPE clean-up devices have led to the significant reduction in interference by co-extractives, quantity of solvents used and time spent on analysis and there is tremendous improvement in accuracy and precision.

CHAPTER THREE

MATERIAL AND METHODS

3.1. PERSISTENCE AND ACCUMULATION OF OCP IN COCOA FARMS

Five cocoa farms in Southwestern Nigeria which fell into the following categories - where OCPs were used and discontinued/abandoned for 10 - 15 years, 3 - 5 years and where they are still being usedwere identified with the assistance of CRIN's personnel.

3.1.1. Sampling Location

The sampling locations were as follows;

3.1.1.1. CRIN SITES I and II

These are located at Idi Ayunre town in Oluyole Local Government Area of Oyo state, and they are situated at latitude on $07^{0}12^{'}$ North and $003^{0}51^{'}$ East of the Greenwich Meridian. Organochlorine pesticides have not been used at the two sites in the last 15 years. On both farms mono cropping is being practiced and they are approximately 8 acres. A stream flows behind sampling site II (Figure 3.1).

3.1.1.2. SORE BALE FARM

The farming settlement is located in Owode Local Government Area of Ogun state and it is at latitude $07^{0}07^{'}$ North and $003^{0}43^{'}$ East of the Greenwich Meridian. Mix farming is being practiced and organochlorine pesticides are still being used in this farm (Figure 3.2). A streamwhich serves as their main source of waterflows through the farm and the community.

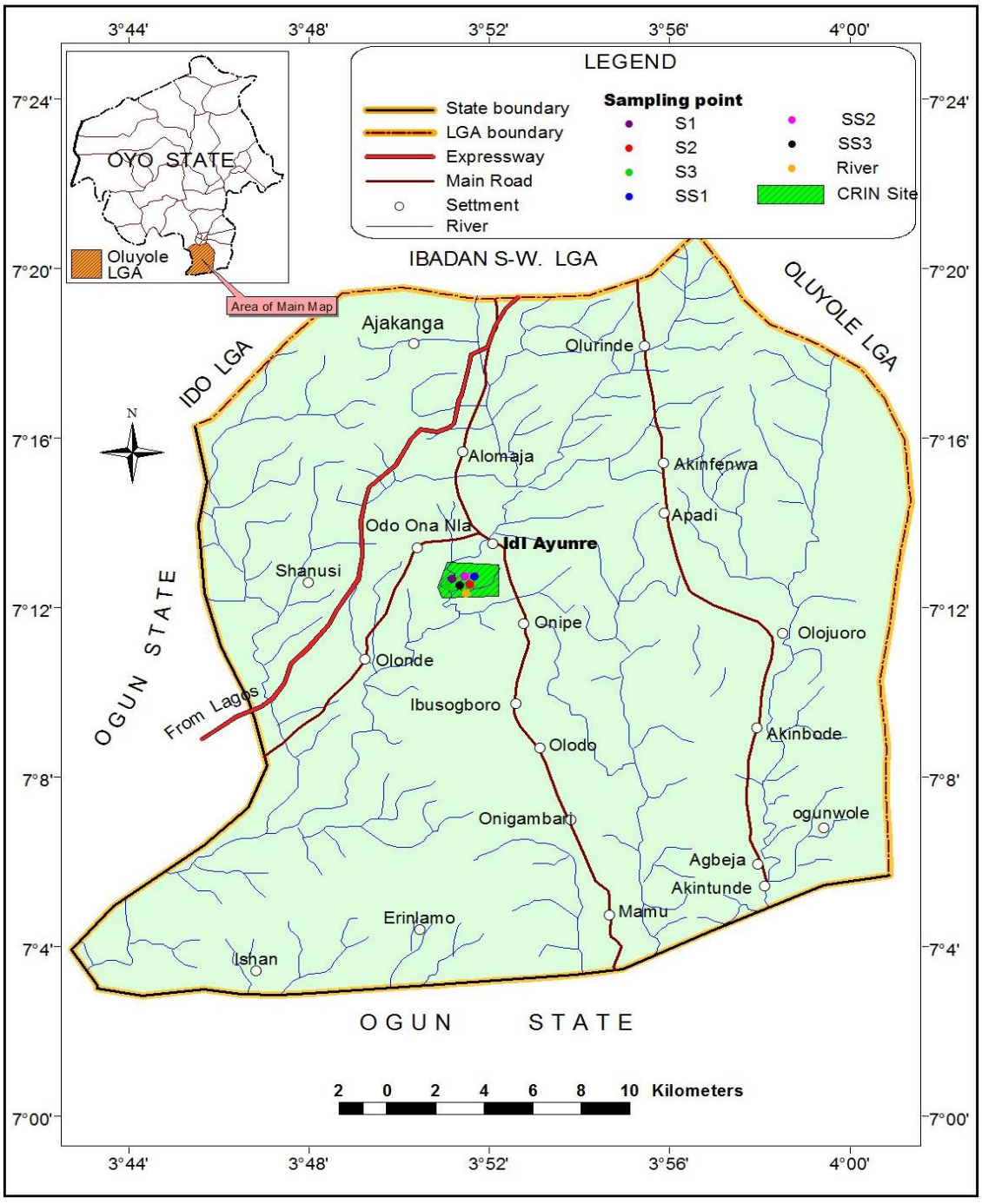


Figure 3.1. Map of CRIN 1 and CRIN 2 with sampling sites identified.

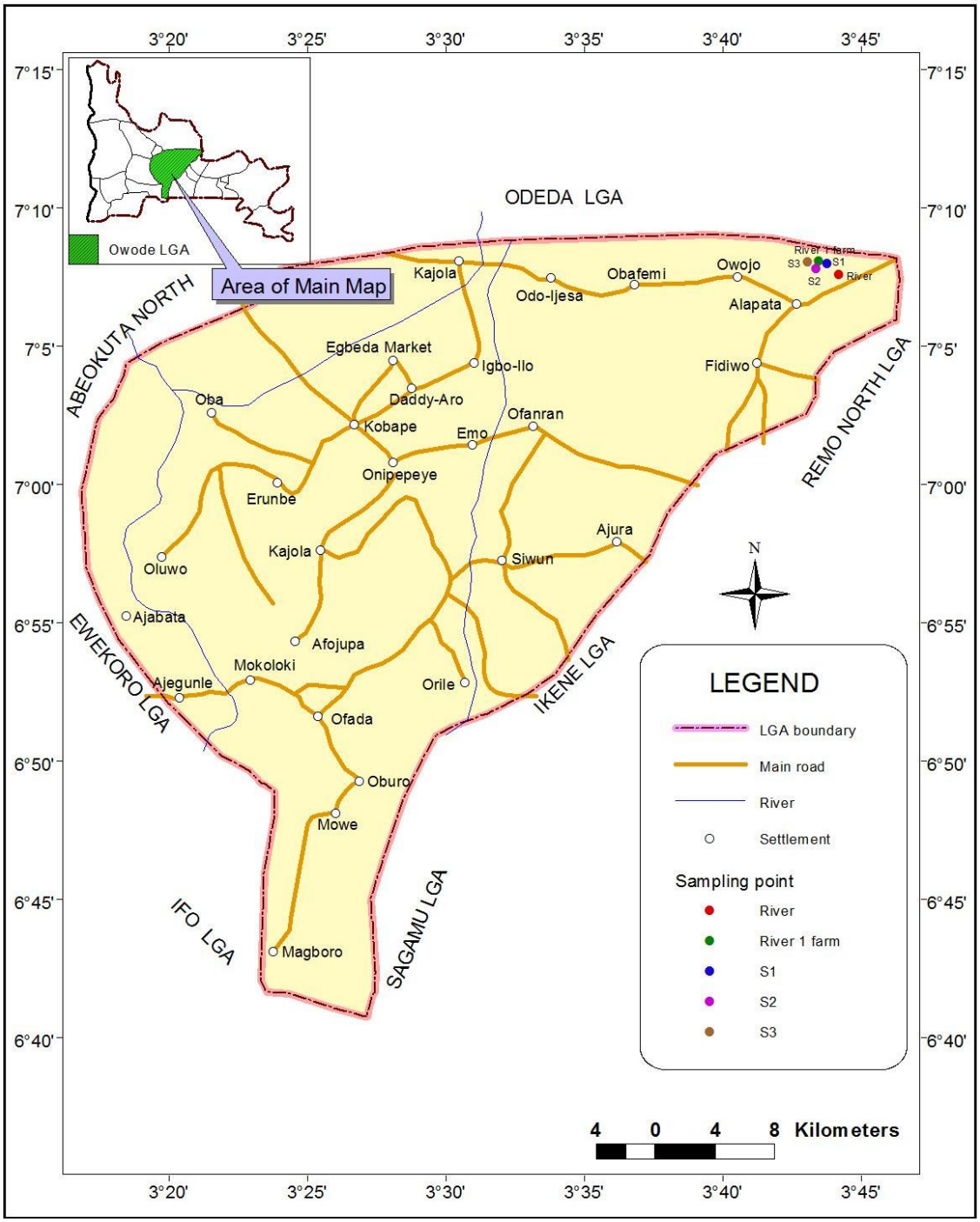


Figure 3.2. Map of Sore Bale with sampling sites identified.

3.1.1.3. ONDO I (Orimunlade Sabo)

The farm is located at latitude $07^{0}05'$ North and longitude $004^{0}48'$ East of the Greenwich meridian in Orimunlade Sabo area of Ondo town (Ondo West Local Government Area).The farm has been abandoned for about 3 years and organochlorine pesticides have not been used on this farm since the abandonment. Mix cropping is being practiced, kola nut trees are planted in the farm (Figure 3.3).

3.1.1.4. ONDO II (Igba)

The farm is located at Igba village in Ondo East Local Government Area of Ondo State at latitude $N07^{o}07'$ North and longitude $E004^{o}53$ East of the Greenwich meridian. Organochlorine pesticides are still being used in this farm (Figure 3.4). The farm is situated on a land of about 25 acres. There is a stream passing through the farm. Surface water and sediment samples were collected.

3.1.2. Sampling Coordinates, Sample Type and Sample Collection

A total of five hundred and four (504) samples comprisingfresh leaves, pods, bark, cocoa seeds, soil (0-15, 15 -30 cm), sediment and surface water were collected for two seasons (dry and wet) from the five sites.The coordinates and sample types collected are presented in Tables 3.1a and3.1b.

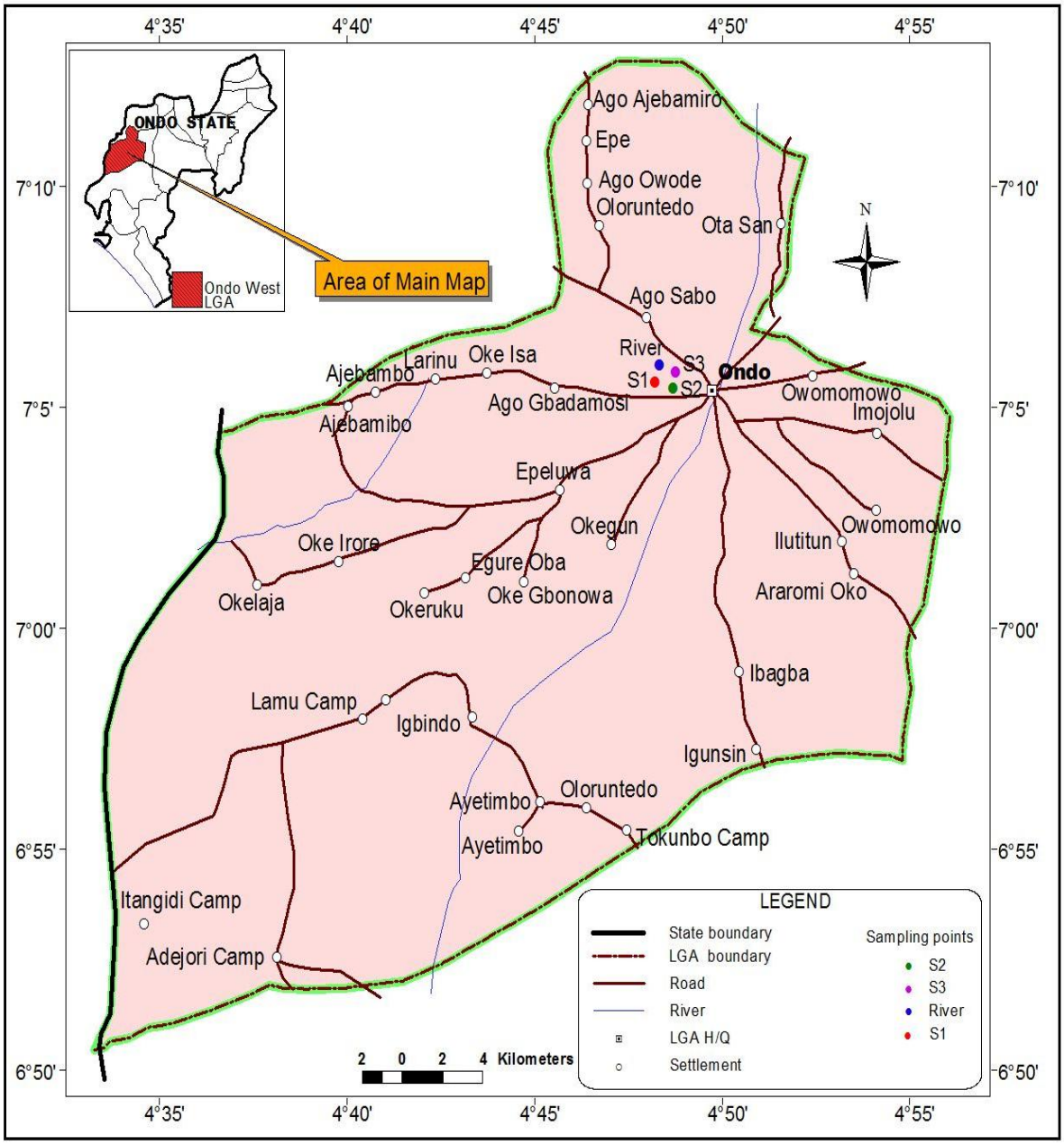


Figure 3.3. Map of Ondo 1 with sampling sites identified.

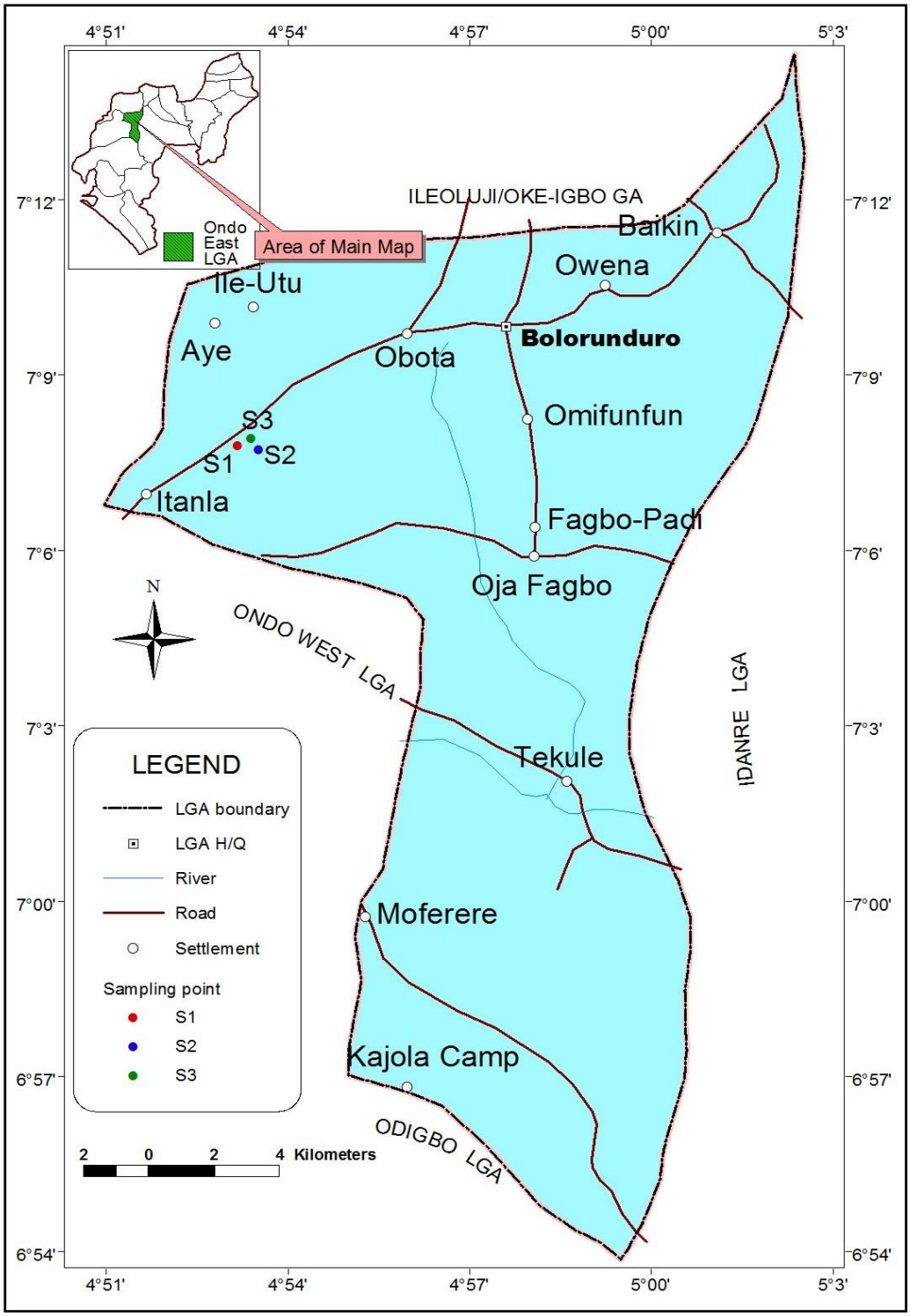


Figure 3.4. Map of Ondo II with sampling sites identified.

Table 3.1a. Sampling coordinates of soil samples for dry and wet seasons

Farm/Site	Sample collection 2009/10 seasonal Campaign Sampling Coordinates (Dry season)	Sample collection 2009/10 seasonal Campaign Sampling Coordinates (Wet season)	Sample collection 2011/12 seasonal Campaign Sampling Coordinates (Dry season)	Sample collection 2011/12 seasonal Campaign Sampling Coordinates (Wet season)
CRIN 1	P_{1DC1}N07°12'36.8",E003°51'37.6"	P_{1WC1}N07°12'37.1",E003°51'37.5"	P_{1DC1}N07°12'38.8",E003°51'39.0"	P_{1WC1}N07°12'37.1",E003°51'38.5"
	P_{2DC1}N07°12'37.6",E003°51'37.6"	P_{2WC1}N07°12'36.9",E003°5137.6"	P_{2DC1}N07°12'39.6",E003°51'38.7"	P_{2WC1}N07°12'39.9",E003°51'37.1"
	P_{3DC1} N07°12'36.5" E003°51'37.9"	P_{3WC1}N07°12'37.4" E003°51'37.7"	P_{3DC1}N07°12'37.5" E003°51'36.5"	P_{3WC1}N07°12'40.1" E003°51'37.0"
CRIN 2	P_{1DC2}N 07°12'35.9" E003°51'18.1"	P_{1WC2} N07°12'35.8" E003°51'16.2"	P_{1DC2}N 07°12'34.4" E003°51'18.1"	P_{1WC2}N07°12'35.6" E003°51'13.2"
	P_{2DC2}N07°12'35.2" E003°51'17.6"	P_{2WC1} N07°12'35.7" E003°51'17.5"	P_{2DC2}N07°12'35.9" E003°51'17.6"	P_{2WC1}N07°12'35.2" E003°51'16.5"
	P_{3DC2}N07°12"36.5" E003°51'17.6"	P_{3WC1} N07°12"34.4" E003°51'17.5"	P_{3DC2}N07°12"36.8" E003°51'17.6"	P_{3WC1}N07°12"35.0" E003°51'27.5"
SORE BALE	P_{1DSB}N07°08'01.8' E003°43'22.7'	P_{1WSB}N07°08'02.1' E003°43'21.2'	P_{1DSB}N07°08'01.4' E003°43'22.6'	P_{1WSB}N07°08'02.5' E003°43'21.0'
	P_{2DSB}N07°08'01.4' E003°43'22.7'	P_{2WSB}N07°08'01.9' E003°43'22.8'	P_{2DSB}N07°08'01.2' E003°43'22.5'	P_{2WSB}N07°08'01.8' E003°43'22.9'
	P_{3DSB}N07°08'0.1" E003°43'22.2'	P_{3WSB}N07°08'00.1" E003°43'21.4'	P_{3DSB}N07°08'01.4" E003°43'22.4'	P_{3WSB}N07°08'00.9" E003°43'21.5'
ONDOI(O. Sabo)	P_{1DOD1}N07°05'30.6"E004°48'11.1"	P_{1WOD1}N07°05'30.1" E004°48'11.3"	P_{1DOD1}N07°05'30.9" E004°48'11.3"	P_{1WOD1} N07°05'30.6" E004°48'11.4"
	P_{2DOD1}N07°05'30.6"E004°48'11.6"	P_{2WOD1}N07°05'30.6" E004°48'11.5"	P_{2DOD1}N 07°05'30.7" E004°48'11.2"	P_{2WOD1}N 07°05'30.7" E004°48'11.0"
	P_{3DOD1}N07°05'30.1"E004°48'11.1"	P_{3WOD1} N07°05'30.1"E004°48'11.2"	P_{3DOD1}N07°05'30.1"E004°48'11.9"	P_{3WOD1}N07°05'30.7"E004°48'11.9"
ONDO II (Igba)	P_{1DOD2} N07°07'57.6"E004°53'22.6"	P_{1WOD2} N07°07'57.5" E004°53'22.2"	P_{1DOD2}N07°07'57.6" E004°53'22.8"	P_{1WOD2} N07°07'57.4" E004°53'22.3"
	P_{2DOD2}N07°07'57.5"E004°53'22.9"	P_{2WOD2} N07°07'57.6" E004°53'23.2"	P_{2DOD2}N 07°07'57.7" E004°53'22.8"	P_{2WOD2} N 07°07'57.0" E004°53'23.9"
	P_{3DOD2}N07°07'56.9"E004°53'23.0"	P_{3WOD2}N07°07'56.4"E004°53'23.0"	P_{3DOD2}N07°07'56.8"E004°53'23.4"	P_{3WOD2} N07°07'56.6"E004°53'23.0"

Legend;P_{1D} - sampling point 1 for dry season; P_{1W} - sampling point 1 for wet season

subscripts; C1 - CRIN 1; C2-CRIN2; SB – Sore Bale; OD 1- Ondo I (Orimulade Sabo);OD 2 – Ondo II (Igba)

Table 3.1b. Sampled matrix and number of samples collected per season

Sample type/season	Farms/Samples collected in 2009/10 Sampling Campaign						Farms/Samples collected in 20011/12 Sampling Campaign						Total
	CRIN I	CRIN II	Sore Bale	Ondo I	Ondo II	Sub-total	CRIN I	CRIN II	Sore Bale	Ondo I	Ondo II	Sub-total	Dry+wet
Leaves													
Dry Season	3	3	3	3	3	**15**	3	3	3	3	3	**15**	**30**
Wet Season	3	3	3	3	3	**15**	3	3	3	3	3	**15**	**30**
Bark													
Dry Season	3	3	3	3	3	**15**	3	3	3	3	3	**15**	**30**
Wet Season	3	3	3	3	3	**15**	3	3	3	3	3	**15**	**30**
Pods													
Dry Season	3	3	3	3	3	**15**	3	3	3	3	3	**15**	**30**
Wet Season	3	3	3	3	3	**15**	3	3	3	3	3	**15**	**30**
Seeds													
Dry Season	3	3	3	3	3	**15**	3	3	3	3	3	**15**	**30**
Wet Season	3	3	3	3	3	**15**	3	3	3	3	3	**15**	**30**
Soil*													
Dry Season	6	6	6	6	6	**30**	6	6	6	6	6	**30**	**60**
Wet Season	6	6	6	6	6	**30**	6	6	6	6	6	**30**	**60**
Water													
Dry Season	3	3	6 (3+3)**	3	3	**18**	3	3	6 (3+3)**	3	3	**18**	**36**
Wet Season	3	3	6 (3+3)**	3	3	**18**	3	3	6 (3+3)**	3	3	**18**	**36**
Sediment													
Dry Season	3	3	6 (3+3)**	3	3	**18**	3	3	6 (3+3)**	3	3	**18**	**36**
Wet Season	3	3	6 (3+3)**	3	3	**18**	3	3	6 (3+3)**	3	3	**18**	**36**
Total													
∑dry season	**24**	**24**	**30**	**24**	**24**	**126**	**24**	**24**	**30**	**24**	**24**	**126**	**252**
∑wet season	**24**	**24**	**30**	**24**	**24**	**126**	**24**	**24**	**30**	**24**	**24**	**126**	**252**
TOTAL (gross)	**48**	**48**	**60**	**48**	**48**	**252**	**48**	**48**	**60**	**48**	**48**	**252**	**504**
Last applied (yr)	> 15	>15	in use	>3	in use		> 15	>15	in use	>3	in use		

Legend;* Soil samples were collected at 0-15 and 15-30cm depths

** Surface water and sediment samples were collected from Sore Bale farm and residential areas (3 samples each/season). This made total samples collected from farms alone to be 480.

3.2. FIELD KINETIC STUDIES

3.2.1. Description of Study area

The experimental site is located within the Cocoa Research Institute of Nigeria (CRIN), Idi-Ayunre, Oluyole Local Government Area, Oyo State, South-Western, Nigeria. It is about 12.5 kilometres from Ibadan metropolis and situated at latitude 7^0 14'N and longitude 3^0 52'E. This area exhibits the typical tropical climate with average high temperatures and high relative humidityof 72 – 77.6%, with two major seasons – rainy (March - October) and dry (November - February) seasons (Babalola, 2013). Temperatures usually range between 18.0 - 22.0 ^oC (minimum) and 26.0 – 33.0 ^oC (maximum). The temperatures are highest at the end of the harmattan period (which is usually from December (late Novermber in some cases) to mid-January). Rainfall figures ranges between 1200 mm and 1800 mm, with an annual average of 1500mm (Clayton, 1958; Aikpokpodion *et al.* 2010). The vegetation is rain forest, composed mainly of tall trees with large crowns, mixed with thick undergrowth.

3.2.2. Field study

The field experiment for this study was conducted from 18th of October to 18th December 2012, i.e. at the end of the wet season through the beginning of the dry season and harmattan period. The mean daily minimum temperature during this period was 20.7 ± 0.2^oC, while the daily maximum temperature was 32.2 ± 0.1^oC. No heavy rainfall was recorded during the study period. However, slight showers were observed on 5th of November 2012 and dew was also a common occurrence. There is no history of the use of OCPs in area marked for the study. The coordinates of the five plots and the sampling points for soils were recorded at each period to ensure collection of soil sample from the same point in the course of the study. The disappearance/dissipation of applied endosulfan pesticide from plant surfaces and impacted soils were studied on the same plots. Duplicate soil samples (0 -15 cm and 15 -30 cm) were collected with soil argur per plot, while plant surface samples were collected randomly. Composite samples were analysed per plot. Minimum distortion of the environment was ensured and disturbance of soil was minimal throughout the study, as dry fell foliar (leaves) which served as mulch were also sampled – fell dry leaves were not removed prior to spraying (Plate 3.1).

Plate 3.1.A typical *Theobroma cacao* tree in South-western Nigeria showing dry foliage on farm soil

Commercial endosulfan (usually comprising a mix of α – and β-isomers with a formulation of 7 + 3) was used in this study. Their basic properties are enumerated in Table 3.2. Endosulfan is not registered as an approved pesticide by NAFDAC for use in Nigeria – hence it was banned. Endosulfan (like most OCPs) is relatively cheap and effective for the control of pests; hence most cocoa farmers still use them. The pesticide was applied as water emulsion (water and commercial endosulfan, EC35) using a calibrated PB-10 knapsack hand-operated sprayer/dispenser.

3.2.3. Experimental Procedure

The experiment was conducted on an 8-hectare cocoa plantation plot at the Cocoa Research Institute of Nigeria (CRIN), Idi-Ayunre, Oluyole Local government area, Oyo State. South-Western, Nigeria.

1. Five sub-plots (0.00455 ha/plot), were condoned/quarantined and marked gold, green, lemon, peach and red for easy identification
2. Each of the plots had five matured cocoa trees, with cocoa pods matured enough for harvest
3. The marked plots were well spaced apart, by a distance of about 80 - 100 meters.
4. Prior to spraying, baseline characterization of these plots was carried out to ascertain background levels of endosulfan in soil (0-15cm, 15-30cm), fresh leaves from the cocoa trunk, dried leaves on the ground, bark, pods and seeds from each of the marked plots.
5. Each of the marked plots was sprayed with a single treatment (T1 method) of double dose - 1.4 kg ai/ha (0.5% ai) of the recommended dose concentration of 0.70kg ai/ha (0.25% ai) of technical grade endosulfan (Thiodan, 35 E) in water emulsion.
6. Cocoa tree trunks were sprayed from the bottom to the canopy, on both sides to achieve equal and adequate spread/coverage by pesticide(Plate 3.2).Heights of trees were> 12 feet.

Table 3.2 Physicochemical properties of endosulfan*

Parameters	α-endosulfan	β-endosulfan	Technical grade Endosulfan	Endosulfan SO_4
Melting point (^oC)	108-110	208-210	70-124	181-201
Solubility(mg/L @25 ^oC)	0.33	0.32	0.33	0.22
Vapour pressure(mm Hg, @25 ^oC)	$3.0x10^{-6}$	$7.2x10^{-7}$	$1.0x10^{-5}$	$8.3 x10^{-9}$ [b]
Bulk density (g/ml)			1.8 [c]	
Flammability		Not flammable [b]		
Henry's constant (atm m^3mol^{-1}@25^oC	$4.9x10^{-6}$ [d] $1.3x10^{-4}$ [b]	$1.2x10^{-6}$ [d] $2.1x10^{-5}$ [b]	$1.6x10^{-5}$	
Log K_{ow} (pH 5.1)	4.6-4.7 [b]	4.3-4.8 [b]	4.5-5.7	
K_{oc} (cm^3/g)	10600	13600	12,000 [a]	
Soil aerobic half-life (d)	19-33 [b]	42-58 [b]	31.6 [a]	100-150 [b]
Soil anaerobic half-life (d)			148 [a]	
Hydrolysis half-life (d)	11 (pH 7)	19 (pH 7)	14.8 [a]	
Photolytic half-life (d)			>200	

(**Source**: http://www.cdpr.ca.gov/docs/emon/pubs/tac/tacpdfs/endosulfan/endosulfan_fate)

[*] Data in this table are from USEPA, 2002 except for denoted ones.

[a] DPR chemical database (DPR, 2004).

[b] GFEA, 2004.

[c] Footprint, 2007.

[d] Calculated from vapor pressure and solubility.

Plate 3.2. Field Application of Endosulfan

7. Representative samples were collected in triplicate from the soil (0-15cm, 15-30cm), fresh leaves, fell dry leaves, stem bark, pods and seeds immediately after pesticide treatment from each of the designated plots (day zero samples) using aluminum foil first and then double-wrapped with cellophane. Sampling was quickly done on the first day (day 0) of treatment 30 minutes after spraying, while subsequent samples were collected at other set periods (days) – 7,14, 2, 28, 42and 60days.

Samples were immediately pre-treated, extracted and cleaned (see section 3.4.3 – 3.4.5). The residual concentrations of endosulfan and metabolite (endosulfan sulphate) were determined by GC-MS.

3.3. ADSORPTION/ DESORPTION STUDIES

3.3.1. METHODOLOGY

Top soil (0 -15 cm) profile were collected at three (3) geo-referenced locations from four (4) of the five (5) cocoa farms. The procedures enumerated below were adopted from the OECD (2000) and USEPA (2008) guidelines for testing chemicals for adsorption and desorption studies using batch isotherm.

3.3.1.1. Determination of soil/test solution ratio and equilibrium time

A preliminary investigation was carried out on two soil types (i.e, with highest and least organic carbon (OC) content) to ascertain the appropriate soil-test solution ratio and equilibrium time to be adopted for the sorption studies. The percentage OC of soil samples for study ranged from 1.2 to 2.18 for top soil and the following three soil/solution ratios were used;

- 50 g soil and 50 cm^3 aqueous solution of the test substance (ratio 1/1);
- 10 g soil and 50 cm^3 aqueous solution of the test substance (ratio 1/5);
- 2 g soil and 50 cm^3 aqueous solution of the test substance (ratio 1/25)

The air-dried soil samples were equilibrated by shaking with a minimum volume of 45 mL of 0.01 M $CaCl_2$ overnight (12h) before the day of the experiment. To the equilibrated mixture, a certain volume of the stock solution of the test substance was added and

adjusted to the final volume of 50 mL at the specified working concentration using the same 0.01 M $CaCl_2$.

One control sample with only the test substance in 0.01 M $CaCl_2$ solution (no soil) was subjected to precisely the same steps as the test systems, in order to check the stability of the test substance in $CaCl_2$ solution and its possible adsorption on the surfaces of the test vessels.

A blank run, with the same amount of soil and total volume of 50 mL 0.01 M $CaCl_2$ solution (without test substance) was also subjected to the same test procedure. This serves as a background control during the analysis to detect interfering compounds or contaminated soils. All the experiments, including controls and blanks, were performed in duplicate.

The pH of the aqueous phase was measured before and after contact with the soil.The mixture was shaken until adsorption equilibrium was reached. Aliquots of 5 mL were collected at intervals of 4, 8, 24 and 48 h and extracted with 10 mL of dichloromethane (DCM), followed by 2 × 5 mL of DCM. Combined extracts was evaporated to 1 mL using N_2 gas, then transfer quantitatively into a column of silica gel and elute with 10 mL of hexane:DCM (3:1 ratio) at a flow rate of 1mL/min. Eluate was concentrated to 1 mL,2 mL of n-hexane was added and concentrated to 1 mL. This was transferred into 1 mL graduated amber glass vial. All traces of DCM were replaced by n-hexane and store vial in the refrigerator for GC-MS analysis.

3.3.1.2. Adsorption Experiment

Kinetics study

Exactly 1g of soil sample was weighed into a calibrated 25mLcentrifuge tube. To the tube, 22.5 cm^3 of 0.01M $CaCl_2$ solution was added and shaken overnight (12hrs) to allow system to equilibrate. To this mixture 50µl of 2500 mgL^{-1} of technical grade endosulfan emulsion (stock solution) was added and adjusted to the final volume to 25 mL to give the concentration 5000 μgL^{-1} endosulfan (ie., 3500 μgL^{-1} and 1500 μgL^{-} of α- and β-endosulfan respectively), with a sorbent:test solution ratio of 1:25. The resultant mixture was shaken for 12 hrs with the aid of a GFL 3040 (end-to-end) mechanical shaker at a rate

of 22-24 rpm. At intervals of 15, 30, 45, 60, 90, 120, 180, 240, 360, 480 and 720 minutes, interactions/reactions between endosulfan and soil samples was stopped by centrifuging at 4500 rmp for 5 minutes with Beckmen Coulter, Allegra 21 digital centrifuge. An aliquot of 5cm^3 was transferred into a separatory funnel with Teflon stopper. Residual concentration (i.e unreacted/unadsorbed portion) of endosulfan was extracted, first with 10 mL DCM and then twice with 5 mL of DCM. Extracts was concentrated to 1 mLand cleaned by eluting with 10 mL of n-hexane:dichloromethane (DCM) mixture (3:1) on a silica gel adsorbent column, with a bed of anhydrous sodium sulphate on top. Elution was at a flow rate of 1 mL/min. Eluate was concentrated to 1 cm^3, 2 mL of n-hexane was then added and concentrated to 1 mL(to ensure that all traces of DCM was replaced by n-hexane) and thereafter transferred into an amber GC glass vial for GC-MS analysis.

Control

25 mL of 0.01 M $CaCl_2$was equilibrated over night (12h), without soil samples and made to mark giving the working concentrations of 5000 µgL^{-1} endosulfan. Aliquots were treated as in test samples.

Blank

A blank was carried out containing the same quantity of soil and total volume of 25 mL of0.01 M $CaCl_2$ solution (without the test sample) also subjected to the same test procedure.

3.3.1.3. Desorption Kinetics

The parallel method was adopted for desorption kinetics (OECD, 2000;USEPA, 2008). After the completion of the procedure for adsorption kinetics and the aqueous phases are removed as much as possible from the tubes. The decanted test solution was then replaced by an equal volume of 0.01 M $CaCl_2$ (without test substance) and the new mixtures were agitated again. At set intervals, sample tubes corresponding to 15, 30, 45, 60, 90, 120, 180, 240, 360, 480 and 720 minutes used in adsorption experiment were centrifuged and decanted. Aliquots of 5cm^3 were transferred into a separatory funnel and extracted with 10 cm^3 DCM, then with two more portions of 5 cm^3. Combine extracts and evaporate using N_2 gas in a hood to 1 cm^3. Residue extract was transferred into a silica gel column and eluted with 10 cm^3 of hexane:DCM (3:1) and evaporated to dryness. Cleaned extract was

re-dissolved with 1 cm^3 hexane and transferred into an amber GC vial for GC-MS analysis.

3.3.1.4. Adsorption Isotherms/Equilibrium Studies

Seven concentrations of 0.25, 0.50, 1.0, 1.50, 2.5, 5.0, 10.0, 15.0, 20.0 and 25.0 mgL^{-1} of endosulfan were prepared respectively by adding appropriate amount of technical grade endosulfan stock solution to pre-equilibrated soil samples in 20 mL of 0.01 M $CaCl_2$ solution. Mixture was then adjusted to 25 mLfinal volume to give soil/test solution ratio of 1:25 as in adsorption kinetic studies. Controls and blanks were also set-up (as in adsorption kinetics). Sample tubes were shaken for 12h to attain equilibrium. The phases were separated by centrifuging mixture at 4500 rmp for 5 minutes and decanting gently the aqueous phase. An aliquot of 5 mL of the supernatant was transferred into a separatory funnel and extracted with 10 mL DCM and then with two more portions of 5 mL. Extract was concentrated to 1 mL and cleaned by eluting with 10 mL of n-hexane:dichloromethane (DCM) mixture (3:1) on a silica gel adsorbent column, with a bed of anhydrous sodium sulphate on top. Elution was at a flow rate of 1 mL/min. Eluate was concentrated to 1 mL and 2 mL of n-hexane was added and then further concentrated to 1 mL(to ensure that all traces of DCM was replaced by n-hexane) and thereafter transferred into an amber GC glass vial for GC-MS analysis.

3.3.1.5. Desorption Isotherms/Equilibrium Studies

The parallel method was adopted for desorption kinetics (OECD, 2000;USEPA, 2008). After the completion of the procedure for adsorption isotherm/equilibrium and the aqueous phases are removed as much as possible from sample tubes (0.25, 0.50, 1.0, 1.50, 2.5, 5.0 and 10.0mgL^{-1} endosulfan). The decanted test solution was then replaced by an equal volume of 0.01 M $CaCl_2$ (without test substance) and the new mixtures were agitated again for 12h. The phases were separated by centrifuging mixture at 4500 rmp for 5 minutes and decanting gently the aqueous phase. An aliquot of 5 mL of the supernatant was immediately transferred into a separatory funnel and extracted with 10 cm^3 DCM and then with two more portions of 5 mL. Extract was concentrated to 1 mLand cleaned by eluting with 10 mL of n-hexane:dichloromethane (DCM) mixture (3:1) on a silica gel

adsorbent column, with a bed of anhydrous sodium sulphate on top. Elution was at a flow rate of 1 ml/min. The eluate was concentrated to 1 mL and 2 mL of n-hexane was added and then reconcentrated to 1 mL(to ensure complete elimination of all traces of DCM) and then transfered into 1 mL graduated amber glass vial for GC-MS analysis.

3.4. QUALITY CONTROL/QUALITY ASSURANCE

All necessary quality assurance precautionary measures for field sampling and analytical protocols were strictly observed in order to eliminate or reduce any likely error.

3.4.1. Quality control during field sampling

Sampling materials and equipment were properly cleaned with appropriate solvents before being used.

3.4.2. Quality control in the laboratory

1. All glass wares were thoroughly washed with detergent, rinsed twice with distilled water and dry in the oven. Dried glassware were re-rinsed with hexane to ensure that they are organochlorine pesticides free or free of other possible contaminants
2. Soil and sediment samples were air dried in the laboratory at room temperature, where there are no chlorinated compounds and use of insecticides
3. All reagents and organochlorine pesticide reference standards were of high purity. Spectroscopic/Analytical grade of reagents and chemicals were used.
4. All laboratory instruments were cleaned and well calibrated and their standard procedures (SOP) were strictly followed.

3.5. ANALYTICAL PROCEDURE

Often, the analytical procedures used for the determination of conventional and emerging POPs in environmental samples (water, soil, sediment and biota) are known to involve a number of basic and very relevant steps, such as sampling, sample preparation, extraction and separation, identification and quantification of target compounds and data handling. The separation and detection steps (identification and quantification of analytes) is predominately associated with instrumental technique using gas chromatography (GC)

coupled to sensitive and specific detection systems, such as the electron capture detector (ECD) and mass spectrometry detection (MS) (Santos and Galceran, 2002).

3.5.1. Sample Collection

3.5.1.1. Cocoa leaves, pods and bark

Fresh leaves were collected from cocoa trees at three different points at each sampling site. The pods and bark of the cocoa plants were collected and later composited. Collected samples were properly wrapped with aluminum foil and marked for easy identification.

3.5.1.2. Soil

Soil samples were collected using a soil auger with the dimension - 2.5 cm (diameter) x 15 cm (deep). Cores of soil samples were collected at three (3) random points corresponding to where leaf samples were collected. Samples were collected at 0 – 15 cm and 15 – 30 cm depths respectively and wrapped with aluminum foil.

3.5.1.3. Sediment

Samples were collected using a Van Veen grab sampler of approximately 2000 cm^3 capacity. The grab sampler was lowered into stream and each grab was hauled out of the stream and collected in an aluminum foil. This was allowed to drain for some few minutes before being wrapped and marked.

3.5.1.4. Water

Water samples were collected into amber containers with Teflon caps. Containers were filled to the brim and transferred into a cooler with ice-pack. Samples were transferred into a refrigerator in the laboratory until analyzed.

3.5.2. Sample Handling

All samples were collected in appropriate containers or materials at the site or field and all sampling points were geo-referenced using a Global Positioning System (GPS). Samples were transported to the laboratory for analysis.

3.5.3. Pre-treatment of Samples

3.5.3.1. Leaves, pods, seed and bark

Fresh leaves were blended and homogenized using a Kenwood blender, before extraction. The bark was chopped into bits before being blended, while the pods were peeled before blending.

3.5.3.2. Soil and Sediment

Soil and sediment samples were allowed to air dry in a room that was well screened from direct sunlight and also insecticide-free. The dried samples were sieved (with 2mm mesh) to eliminate extraneous materials before extraction.

3.5.4. Sample Extraction and Clean Up

3.5.4.1. Extraction procedures

Leaves, pods, seed and bark

Methods by USEPA - method 3570 (USEPA, 2002) and Yeboah *et al*. (2003), were adopted for the extraction of OCPs in cocoa plant.

Ten (10) grams of blended leaf tissue was transferred into an amber extraction flask. Two (2) grams of anhydrous sodium sulphate was added and mixed with leaf tissue. Sixty (60) mililiters of a mixture of ethyl acetate: petroleum spirit (3:2) was added and intermittently shaken vigorously for 30 minutes. This was allowed to stand for 5 minutes in a fume hood for solvent to separate from solid matrix. The supernatant solvent was carefully decanted through a filter paper (Whatman no. 3) containing 2g of anhydrous sodium sulphate into a well graduated 50 mL pyrex glass measuring cylinder to obtain a 30 mL filtrate (equivalent to 5 g of sample). The filtrate was evaporated in a round-bottom flask, using a rotary evaporator to 5 mL. The concentrated residue was transferred into a 20 mL beaker. The flask was rinsed five times with 2 mL of hexane into the same 20 mL beaker and was further evaporated using air current to 2 mL residue. The resultant residue was kept in the refrigerator for clean-up.

Soil and Sediments

The USEPA, method 3570 (USEPA, 2002) was adopted for the extraction of soil and sediment samples.Twenty (20) grams of air dried and pre-sieved soil/sediment samples were transferred into a quick-fit extraction flask with a firmly fitted stopper. This was homogenized with 5 grams of anhydrous sodium sulphate. Fifty (50) mililiters of acetone: petroleum spirit (1:1) was added and swirled/shaken gently and intermittently for 60 minutes in a fume hood. The mixture was allowed to stand for 5 - 10 minutes for proper separation and sedimentation. The solvent was then carefully decanted through a filter paper plugged with cotton wool-impregnated anhydrous sodium sulphate (to prevent extraneous materials and remove water) into a well graduated 50 mL glass (pyrex) measuring cylinder. An aliquot of 25 mL of the filtered extract was collected (this is equivalent to 10 g of sample) into a round-bottom flask and reduced to 5 mL with the aid of a rotary evaporator. The 5 mL residual extract was transferred to a 20 mL beaker and the flask was rinsed four times with 2mL of hexane. This was further reduced to 2 mL with the aid of air current. The resultant residue was kept in the refrigerator for clean-up exercise.

Water

The UNEP/WHO (2000) Global Environmental Monitoring System (GEMS) water operational guide for water was adopted.

Fifty (50) milliliters of dichloromethane (CH_2Cl_2) was added to 1 litre of water sample in its original sample bottle in a fume hood. The bottle was tightly closed with an aluminum-lined cap after carefully dropping into it a Teflon coated stirring bar. The mixture was stirred on a magnetic stirrer and the vortex formed was ensured to reach the surface from the bottom of the bottle. After 30 minutes of stirring the content of the bottle was transferred carefully into a litre separatory funnel and allowed to stand in the fume hood for a while (10 – 15 minutes) for the organic and aqueous phases to separate. The organic phase was transferred into a 500 mL separatory funnel, while the aqueous layer was returned to sample bottle. The 1 litre separatory funnel was rinsed twice with 30 mL and 20 mL of dichloromethane (CH_2Cl_2), each was transferred into the sample bottle and the process of stirring, separation and rinsing described above was repeated. These processes

were repeated a third time and the water sample was discarded. After each separation, the organic layer was added to the initial dichloromethane extract in the 500 mL separatory funnel. The 1 litre separatory funnel was finally rinsed as before and added to the content of the 500 mL separatory funnel. This was then shaken for 2 minutes and allowed to separate for 10 minutes. About 10 g of anhydrous sodium sulphate was placed in a 125 mL sintered glass funnel and the CH_2Cl_2 combined extracts was gently filtered through the sintered glass funnel into a 500 mL round-bottom flask. To the small quantity of aqueous phase left in the separatory funnel, 50 mLof dichloromethane (CH_2Cl_2), was added, shaken for 2 minutes and allowed to separate for 10 minutes. The organic layer was then drained through the sodium sulphate (anhydrous) into the same round-bottom flask. The aqueous phase was discarded and the separatory funnel was rinsed twice with 25 mL of dichloromethane (CH_2Cl_2) and passed through the sodium sulphate (anhydrous) in the funnel. The anhydrous sodium sulphate filtration column was washed with 50 mL dichloromethane (CH_2Cl_2) under a vacuum. 3 mL of iso-octane was added to the 500 mL round-bottom flask after disconnecting the sintered funnel. The combined sample extracts was concentrated by evaporation under vacuum by a rotary evaporator at 30^o C – 35^o C to 5 mL which was transferred into a 20 mL beaker and the 500 mL round-bottom flask was then rinsed five times with 2 mL into the beaker. This was further concentrated to 3 mL with aid of air current. The residual extract was kept in the refrigerator for clean-up exercise (UNEP/WHO, 2000).

3.5.4.2. Clean-up of Extracts

Preparation of Silica Gel

Exactly 100 g of silica gel (60 -120 mesh) was calcined at 650^o C for 3 hours, cooled in a dessicator and packed in an air tight plastic container.

Packing of Column and elution

3.5 g of activated silica gel (60 – 120 mesh) previously mixed with 10%(w/w) of distilled water was packed into a 200 mm x 12 mm (i.d) clean-up pyrex glass column. This was followed by a bed of 0.5 g of anhydrous sodium sulphate. The packed column was first eluted with 50 mL of hexane and discarded. Concentrates of extracts were carefully

transferred into column with several hexane rinsings and thereafter eluted first with 50 mL of dichloromethane: hexane (1:4) and then with 50 mL of dichloromethane: hexane: acetonitrile (50:49.65:0.35) at a flow rate of 1 mL/min. The combined 100 mL eluate was collected into a round-bottom flask; 1 mL of iso-octane was added and reduced to 2 mL using a rotary evaporator. The 2 mL residue was transferred into a graduated tube and the flask was rinsed four times with 2 mL hexane into graduated tube and further concentrated to 1 mL. It was ensured that all traces of CH_2Cl_2 were replaced by hexane. This was transferred into an amber glass vial and kept in the refrigerator for Gas Chromatography-Mass Spectroscopy (GC-MS) analysis.

3.5.5. Control Sample

Control samples (leaves, bark, pods, seeds and soil) were collected from a cocoa farm within CRIN, where OCPs had never been applied (pristine farm). The sampled area was about 5 kilometers from CRIN 1.

3.6. INSTRUMENTATION

3.6.1. Global Position System (GPS)

All the sampling points were geo-referenced using a portable/handheld GPS - model map 60 (map navigator), manufactured by Garmin International, Inc., Kansas, U.S.A. The instrument was first powered, allowed to initialize to receive Satellite signals from at least three (3) satellites before the display of accurate position and location coordinates.

3.6.2. Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The hexane reconstituted clean up extract from leaves, soils, sediments and surface water was analysed with a Shimadzu QP – 2010 gas chromatography-mass spectrometer (GC/MS). Chromatographic separation was carried out on a 30 m x 0.25 mm id HP-5MS capillary column with a film thickness of 0.25 µm. The oven temperature was programmed and it was initially held at 80^{o}C for 1 min and was increased to 200^{o}C at a rate of 20^{o}C/min, held for 1 min and then raised to 280^{o}C at a rate of 10^{o}C /min and held for 2 min. The flow rate of the carrier gas (helium, 99.99% purity) was kept constant at 1.18 mL/min. Splitless injection mode at an injection temperature of 250^{o}C was carried

out at a pressure of 79.5 kPa. The linear velocity, purge flow and total flow were 40.0 cm/sec, 3.0 mL/min and 32.7 mL/min respectively. The interface line and ion source temperatures were 260^{o}C and 250^{o}C respectively.

Samples from field kinetic and adsorption-desorption studies were analysed using a Finnigan Ultra Trace gas chromatography-mass spectrometer (GC/MS). Chromatographic separation was carried out on a 30 m x 0.25 mm id HP-5MS capillary column with a film thickness of 0.25 µm. The oven temperature was programmed, which was initially held at 80^{o}C for 5 mins, and was raised to 200^{o}C at a rate of 20^{o}C/min, held for 5 min and then increased to 280^{o}C at a rate of 10^{o}C /min and held for 2 min. The flow rate of the carrier gas (helium, 99.99% purity) was kept constant at 1.18 mL/min. Splitless injection mode at an injection temperature of 250^{o}C was carried out at a pressure of 79.5 kPa. The linear velocity and total flow were 10.0 cm/sec and 32.7 mL/min respectively. The interface line and ion source temperatures were 260^{o}C and 250^{o}C respectively

3.6.3. Standard Calibration Curve

Standard Calibration graph using mixed standard of twenty organochlorine pesticides

A five point calibration curve was performed using a mixed standard containing twenty (20) organochlorine pesticides. The standards were co-mixed in iso-octane and each concentration contained all the pesticides being analyzed (0.063, 0.125, 0.250, 0.500, and 1.00 mgL^{-1}). Calibration curves for each organochlorine pesticide showed good coefficient of regression (r^2) between 0.9958 and 0.9999. All standards were used as external standards for the identification and quantification of corresponding OCPs present in various sample types.

Standard Calibration graph of α- and β-endosulfan for kinetic and adsorption-desorption studies

A five point calibration curve was obtained for standards by preparing α- and β-endosulfan in iso-propyl alcohol and hexane mixture (1:1). Working concentrations for α- and β-endosulfan standards were 200, 400, 600, 800, 1000 and 1200 µgL^{-1}, while for endosulfan sulphate concentrations were 200, 250, 300, 350, 400 and 450µgL^{-1}.

Calibration graph showed good coefficient of regression (r^2), values of 0.9993, 0.9989 and 0.9990 for α- and β-endosulfan and endosulfan sulphate respectively.

3.7. EVALUATION OF ANALYTICAL METHODOLOGY

Analyte recovery is one the most frequently used techniques for validation of analytical methods and results. To validate methodology, portions of cocoa vegetation (fresh leaves, bark, pods, seeds, fell dry leaves) and soil (0 - 15 cm, 15 - 30cm) samples collected for baseline determination (during field kinetic studies) were used for recovery experiments. Baseline samples were fortified at 125 and 500 $\mu g g^{-1}$ concentrations with α- and β-endosulfan and endosulfan sulphate standards. Each fortification was replicated thrice. Percent recoveries were calculated from the quantity of analytes recovered from matrixes using the expression:

$$\%R = Amount\ of\ the\ Analyte\ recovered / Amount\ of\ the\ analyte\ added \times 100$$

where;

% R = Percent recovery

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. STANDARD CALIBRATION CURVE AND IDENTIFICATION OF SPECTRA

4.1.1. Standard Calibration graph using mixed standard of twenty organochlorine pesticides (using Shimazu QP 2010 GC-MS)

The observed retention times (RT) for standards and corresponding regression coefficient for calibration graphs are shown in Table 4.1;

4.1.2. Calibration graph for standard α-, β-endosulfan and endosulfan sulphate for field kinetics and adsorption-desorption studies (using Finnigan Ultra Trace GC-MS)

The observed retention times (RTs) for α-, β-endosulfan and endosulfan sulphate standards, with corresponding regression coefficient for calibration graphs are shown in Table 4.2;

The identified peaks at RTs 18.62, 20.25 and 21.18 mins for α-endosulfan, β-endosulfan and endosulfan sulphate respectively (Figure B-1 in appendix), were confirmed by selected molecular ion peaks at m/z values using the National Institute of Standards and Technology (NIST) search library and they were as follows; α-endosulfan - 338.92, 277.12, 207.16, 195.12; β-endosulfan – 338.95, 277.03, 241.14, 207.06, 195.04, 160.08; endosulfan sulphate 441.83, 386.90, 288.99, 239.20 and 271.95.

4.2. PERSISTENCE AND ACCUMULATION OF OCPs IN COCOA FARMS

The mean values of twenty (20) OCPs estimated in various matrices - parts of cocoa plant (leaves, bark, pods and seeds), soils, sediments and surface water for five (5) farms for dry and wet seasons are presented in Tables 4.3 – 4.19.

Table 4.1. Retention time of OCPs and regression coefficients for calibration graphs

S/N	OCPs	Retention Time (min)	R^2
1	α-HCH	8.069	0.9997
2	γ-HCH	9.419	0.9996
3	β-HCH	10.048	0.9997
4	δ-HCH	10.631	1.0000
5	Heptachlor	11.197	0.9994
6	Aldrin	11.822	1.0000
7	Heptachlor epoxide	12.715	0.9998
8	cis-Chlordane	13.351	0.9978
9	trans-Chlordane	13.446	0.9977
10	α-endosulfan	13.496	0.9998
11	pp'-DDE	13.820	0.9996
12	Dieldrin	14.003	0.9999
13	Endrin	14.438	0.9999
14	op'-DDD	14.831	0.9998
15	β-endosulfan	14.980	0.9989
16	Endrin Aldehyde	15.197	0.9970
17	pp'-DDT	15.418	0.9998
18	Endosulfan sulfate	15.755	0.9971
19	Methoxychlor***	16.333	0.9992
20	Endrin Ketone	16.561	0.9946

Table 4.2. Retention times of endosulfan and regression coefficients for calibration graphs

S/N	OCPs	Retention Time (min)	R^2
1	α-endosulfan	18.62	0.9993
2	β-endosulfan	20.25	0.9989
3	Endosulfan sulphate	21.18	0.9990

4.2.2. Distribution of OCPs in cocoa plant

Tables 4.3- 4.7 showed the different distribution pattern of OCPs in various parts of cocoa plant studied (leaves, pods, bark and seeds) and surrounding environment of the five sites for both seasons.

Total OCPs (∑OCPs) in all vegetationmatrices (i.e., various parts of cocoa plants) ranged from not detected (ND or <0.001 μgg^{-1}) in cocoa seeds to 12.67 ±10.69 μgg^{-1} in fresh leaves during the dry season and ND (cocoa seeds and pods) to 7.12±5.96 μgg^{-1} (fresh leaves) in the wet season. The order of ∑OCPs distribution in cocoa plant parts was leaves >stem bark > pods > seeds in all the sites for both seasons, except in CRIN I where the order was leaves > pods >bark > seeds in the wet season (Figures 4.1 and 4.2).The OCP preference to persist and bioaccumulate in cocoa leaves relative to other plant tissues may have been due to its epicuticular waxy nature. Plant leaves are reported to consist of mainly long-chain polyesters that can accumulate lipophilic compounds like OCPs (Reischl *et al.* 1989; Calamari *et al*. 1991).

In the dry season, mean total OCPs (∑OCPs) in cocoa fresh leaves ranged between 2.82± 1.24 μgg^{-1} in CRIN I and 12.67 ±10.69 μgg^{-1} in Ondo II, while from 2.46±1.64 μgg^{-1} to 7.12±5.96μgg^{-1} in the wet season. The highest and least ∑OCPs in the leaves were found at Ondo II and CRIN I farms respectively for both seasons. OCPs are still in use in Ondo II, while it was last applied over 15 years ago in CRIN I and II. Their presencein cocoa plants in both farms signified bioaccumulation and/ or uptake, while detection in CRIN portrayed persistence.

Amongst the individual OCP families detected in the fresh leaves – the HCHs, aldrins and endosulfans were the most predominant in all the farms and at both seasons (Figures 4.3 and 4.4). Percentage contributions of the HCHs, aldrins and endosulfans to ∑OCPs ranged from 9.80 - 64.80%, 25.33 - 60.91% and 10.06 -55.01% respectively in the dry season, while in the wet season, 11.33-67.90%, 10.61-53.17% and 17.71-60.08% respectively.

4.2.2.1. Detection frequency

A range of 10to19 of the 20 OCPs monitored were detected in fresh leaves in the five cocoa farms in both seasons; the least number was detected in Sore Bale during the dry season, while the highest was found in Ondo II during the dry season – representing 45% and 95% of OCPs respectively. However, in CRIN I, 12 (60%) and (11)(55%) OCPs were detected in the dry and wet seasons respectively (with percentage of OCPs detected in parentheses).

During both seasons, heptachlor and its metabolite heptachlor epoxide, chlordanes (cis- and trans-isomers), pp'-DDE, op'-DDD, endrin aldehyde and methoxychlor were not detected in the analysed plant tissues of CRIN I, while at CRIN II, almost the same trend was observed, except the non-detection of endrin ketone in addition to those mentioned in CRIN I (Tables 4.3 and 4.4).

However, the number of OCPs detected in dry and wet seasons were; 11 (55%) each in CRIN II;11(55%) and 10(50%) respectively in Sore Bale;18(90%) and 14(70%) in Ondo I and 18 (95%) and 16(80%) in Ondo II, with percentage detection of total OCPs monitored during dry and wet seasons in parentheses (Tables 4.8 and 4.9). Ondo II cocoa farm vegetation recorded the highest number of detected OCPs, followed by Ondo I farm. This trend implied that farmers in Ondo area may have treated their cocoa vegetation with vast range of OCPs continuously over the years.

The least number of OCP types was recorded in CRIN I, CRIN II and Sore Bale farms. The low percentage detection of OCPs in CRIN farms may be due to their application over 15 years ago and in addition as a Research Institute, choice of OCPs may have played a prominent role in restricting the different types of OCP that may have been used in the past.

Table 4.3. Concentrations of OCPs (μgg^{-1} [ww]) distribution in cocoa plant during dry and wet seasons - CRIN I

OCPs	DRY SEASON				WET SEASON			
	Fresh leaves		Bark		Fresh leaves		Bark	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	0.13 ± 0.02	0.11 - 0.14	ND	-	0.10 ± 0.08	ND - 0.21	ND	-
βHCH	0.44 ± 0.09	0.32 - 0.55	ND	-	0.36 ± 0.17	0.19 - 0.52	ND	-
γHCH	0.10 ± 0.05	0.08 - 0.17	ND	-	0.12 ± 0.11	ND - 0.06	ND	-
δHCH	0.28 ± 0.14	0.10 - 0.44	ND	-	0.25 ±0 .17	0.06 - 0.46	ND	-
∑HCH	**0.95 ± 0.30**		**ND**		**0.83 ± 0.53**		**ND**	
pp'DDT	0.01 ±0.01	ND-0.02	ND	-	ND	-	ND	-
pp'DDE	ND	-	ND	-	ND	-	ND	-
opDDD	ND	-	ND	-	ND	-	ND	-
∑DDT	**0.01 ±0.01**		**ND**	-	**ND**	-	**ND**	-
Heptachlor	ND	-	ND	-	ND	-	ND	-
Heptachlor Epoxide	ND	-	ND	-	ND	-	ND	-
∑Heptachlor	**ND**		**ND**		**ND**		**ND**	
αEndosulfan	0.11 ± 0.04	0.06 - 0.15	0.07 ± 0.04	0.017-0097	0.23 ± 0.19	ND - 0.46	ND	-
βEndosulfan	0.37 ± 0.19	0.11 - 0.56	ND	-	0.28 ± 0.20	0.10 - 0.56	ND	-
Endosulfan SO_4	0.11 ± 0.10		0.05 ± 0.04	ND- 0.094	0.54 ± 0.34	0.21 - 1.01	ND	-
∑Endosulfan	**0.59 ± 0.33**		**0.12 ±0.08**		**1.05 ± 0.73**		**ND**	
Aldrin	0.44 ± 0.11	0.28 - 0.54	0.05 ± 0.05	0.002-0.123	0.22 ± 0.21	ND - 0.70	ND	-
Dieldrin	0.32 ± 0.32	0.06 - 0.77	ND	-	0.43 ± 0.31	0.40 - 0.85	ND	-
Endrin	0.51 ± 0.15	0.30 - 0.65	0.10 ± 0.08	ND-0.204	0.28 ± 0.22	ND - 0.25	ND	-
Endrin Aldehyde	ND	-	ND	-	ND	-	ND	-
Endrin Ketone	0.01 ±0.02	ND - 0.04	ND	-	0.04 ± 0.05	-	ND	-
∑Aldrin	**1.28 ± 0.60**		**0.15 ± 0.13**		**0.97 ± 0.79**		**ND**	
Cis- Chlordane	ND	-	ND	-	ND	-	ND	-
Trans- Chlordane	ND	-	ND	-	ND	-	ND	-
∑Chlordane	**ND**		**ND**		**ND**	-	ND	-
Methoxychlor	ND	-	ND	-	ND	-	ND	-
∑OCPs	**2.82 ± 1.24**		**0.27 ± 0.21**		**2.84 ±1.97**		**ND**	

NOTE: ND – Not detected (<0.001 μg/g)

Table 4.4. Concentrations of OCPs (µgg^{-1} [ww]) distribution in cocoa plant during dry and wet seasons - CRIN II

OCPs	DRY SEASON				WET SEASON			
	Fresh leaves		Bark		Fresh leaves		Bark	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	0.44 ±0.34	0.100-0.870	ND	-	0.50±0.33	0.092 - 0.507	ND	-
βHCH	1.57±1.12	0.368-2.342	ND	-	1.60±1.22	0.112 - 3.089	ND	-
γHCH	0.95±1.54	0.049-3.619	ND	-	0.21±0.30	ND - 0.633	ND	-
δHCH	0.52±0.13	0.368-0.582	0.07±0.07	ND-0.168	0.25±0.35	ND-0.745	ND	-
∑HCH	**3.48±3.11**		**0.07±0.07**		**2.56±2.20**		**ND**	
								-
pp'DDT	ND	-	0.02±0.00	0.011-0.018	0.01±0.01	ND – 0.011	ND	-
pp'DDE	ND	-	ND	-	ND	-	ND	-
opDDD	ND	-	ND	-	ND	-	ND	-
∑DDT	ND		**0.02±0.00**		**0.01±0.01**	-	**ND**	-
								-
Heptachlor	ND	-	ND	-	ND	-	ND	
Heptachlor Epoxide	ND	-	ND	-	ND	-	ND	-
∑Heptachlor	ND		**ND**		**ND**		**ND**	-
αEndosulfan	0.28±0.13	0.140-0.497	0.01±0.01	ND-0.016	0.16±0.19	ND - 0.42	ND	ND - 0.01
βEndosulfan	0.19±0.04	0.161-0.253	ND	-	0.28±0.21	0.10 - 0.57	ND	-
Endosulfan SO_4	0.07±0.06	0.029-0.159	ND	-	0.37±0.39	ND - 0.91	ND	-
∑Endosulfan	**0.54±0.23**		**0.01±0.01**		**0.80±0.79**		**ND**	
Aldrin	0.36±0.32	0.120-0.913	0.04±0.01	0.224-0.546	0.21±0.19	0.01- 0.46	ND	-
Dieldrin	0.12±0.04	0.071-0.173	ND	-	0.11±0.14	ND - 0.31	0.03±0.04	ND – 0.090
Endrin	0.79±0.21	0.571-1.002	ND	-	0.08±0.12	ND - 0.25	ND	-
Endrin Aldehyde	0.08±0.10	ND	ND	-	ND	-	ND	-
Endrin Ketone	ND	ND	ND	-	ND	-		-
∑Aldrin	**1.36±0.68**		**0.04±0.01**		**0.40±0.44**		**0.03±0.04**	
Cis- Chlordane	ND	ND	ND	-	ND	-	ND	-
Trans- Chlordane	ND	ND	ND	-	ND	-	ND	-
∑Chlordane	**ND**		**ND**		**ND**		ND	
Methoxychlor	ND	ND	ND	-	ND	-	ND	-
∑OCPs	**5.37±4.02**		**0.13±0.10**	-	**3.77±3.44**		**0.03±0.04**	

NOTE: ND – Not detected (<0.001 µgg^{-1})

Table 4.5. Concentrations of OCPs (µgg^{-1} [ww]) distribution in cocoa plant during dry and wet seasons – Sore Bale

OCPs	DRY SEASON				WET SEASON			
	Fresh leaves		Bark		Fresh leaves		Bark	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	0.52±0.40	0.098-1.157	ND	-	0.62±0.35	0.248-1.092	ND	-
βHCH	1.08±0.78	0.093-1.856	ND	-	1.13±0.67	0.212- 1.792	ND	-
γHCH	0.05±0.03	0.025-0.094	ND	-	0.11±0.03	0.063- 0.147	ND	-
δHCH	0.27±0.25	0.032-0.690	ND	-	0.31±0.30	0.001- 0.709	ND	-
∑HCH	**1.93±1.47**		**ND**		**2.17±1.35**			
pp'DDT	ND	-	0.01±0.02	ND-0.042	ND	ND - 0.002	ND	-
pp'DDE	ND	-	ND	-	ND	-	ND	-
opDDD	ND	-	ND	-	ND	-	ND	-
∑DDT	**ND**		**0.01±0.02**		**ND**		**ND**	-
Heptachlor	ND	-	ND	-	ND	-	ND	-
Heptachlor Epoxide	ND	-	ND	-	ND	-	ND	-
∑Heptachlor	**ND**		**ND**		**ND**		**ND**	
αEndosulfan	0.11±0.16	ND-0.395	0.04±0.05	ND-0.103	0.20±0.10	0.063 - 0.289	0.22±0.13	0.045 -0.335
βEndosulfan	0.29±0.44	ND-1.056	0.27±0.14	0.124-0.462	0.19±0.14	0.027- 0.368	0.11±0.06	0.027-0.268
Endosulfan SO_4	0.02±0.03	ND-0.075	0.03±0.03	ND-0.059	0.29±0.14	0.095- 0.409	0.18±0.03	0.195-0.271
∑Endosulfan	**0.42±0.64**		**0.34±0.21**		**0.68±0.38**		**0.51±0.22**	
Aldrin	0.40±0.36	0.126-1.009	0.13±0.08	0.127-0.220	0.22±0.19	ND - 0.459	0.07±0.05	ND-0.109
Dieldrin	0.062±0.06	ND-0.144	ND	ND	0.11±0.14	ND - 0.305	0.14±0.12	0.028-0.305
Endrin	0.80±0.74	0.041-1.850	0.84±0.43	0.445-1.439	0.66±0.51	ND -1.246	ND	-
Endrin Aldehyde	ND	-	ND	-	ND	-	ND	-
Endrin Ketone	ND	-	ND	-	ND	-	ND	-
∑Aldrin	**1.26±1.16**		**0.97±0.51**		**0.99±0.84**		**0.21±0.17**	
Cis- Chlordane	ND	-	ND	-	ND	-	ND	-
Trans- Chlordane	ND	-	ND	-	ND	-	ND	-
∑Chlordane	**ND**		**ND**		**ND**		**ND**	
Methoxychlor	ND	-	ND	-	ND	-	ND	-
∑OCPs	**3.61±3.26**		**1.32±0.74**		**3.84±2.51**		**0.72±0.38**	

NOTE: ND – Not detected (<0.001 µgg^{-1})

Table 4.6. Concentrations of OCPs (μgg^{-1} [ww]) distribution in cocoa plant during dry and wet seasons – ONDO I

OCPs	DRY SEASON				WET SEASON			
	Fresh leaves		Bark		Fresh leaves		Bark	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	0.03±0.04	ND-0.079	ND	-	0.14±0.10	ND - 0.248	0.01±0.00	ND -0.009
βHCH	0.33±0.30	0.080-0.747	ND	-	0.28±0.22	0.002 - 0.541	0.07±0.05	0.012 -0.121
γHCH	0.29±0.23	0.022-0.571	ND	-	0.11±0.11	ND-0.259	0.00±0.00	ND -0.005
δHCH	0.24±0.21	0.037-0.521	ND	-	0.22±0.30	ND-0.648	ND	
∑HCH	**0.89±0.78**		**ND**		**0.75±0.73**		**0.08±0.05**	
pp'DDT	0.14±0.18	ND-0.393	0.01±0.01	ND-0.019	0.07±0.07	ND - 0.172	ND	-
pp'DDE	0.07±0.10	ND-0.203	ND	-	0.07±0.08	ND - 0.191	ND	-
opDDD	0.00±0.00	ND-0.003	ND	-	ND		ND	-
∑DDT	**0.21±0.28**		**0.01±0.01**		**0.14±0.15**		**ND**	-
Heptachlor	0.02±0.01	ND-0.023	ND	-	ND	-	ND	-
Heptachlor Epoxide	ND	-	ND	-	ND	-	ND	-
∑Heptachlor	**0.02±0.01**		**ND**		**ND**		**ND**	
αEndosulfan	0.13±0.17	0.010-0.378	0.05±0.04	ND-0.092	0.15±0.08	0.063-0.248	ND	ND -0.009
βEndosulfan	0.04±0.06	ND-0.128	ND	ND	0.06±0.03	0.027-0.101	0.03±0.03	ND -0.061
Endosulfan SO_4	2.23±1.73	-	0.01±0.01	ND-0.025	2.00±1.71	0.405-4.371	0.07±0.10	ND -0.209
∑Endosulfan	**2.40±1.96**		**0.06±0.05**		**2.21±1.82**		**0.10±0.13**	
Aldrin	0.65±0.75	0.023-1.712	0.19±0.18	ND-0.423	0.36±0.19	0.103-0.552	0.07±0.10	ND -0.209
Dieldrin	0.40±0.27	0.067-0.722	ND	-	0.27±0.12	0.095-0.371	0.03±0.04	ND - 0.078
Endrin	1.72±1.39	0.585-3.680	ND	-	1.26±1.74	ND-3.721	ND	-
Endrin Aldehyde	1.11±0.81	ND-1.919	ND	-	0.76±0.83	ND-1.921	ND	-
Endrin Ketone	1.59±1.67	0.104-3.931	ND	-	0.87±0.66	0.273-1.782	ND	-
∑Aldrin	**5.47±4.99**		**0.19±0.18**		**3.52±3.54**		**0.10±0.14**	-
Cis- Chlordane	0.00±0.00	ND-0.004	ND	ND	ND	-	ND	-
Trans- Chlordane	0.01±0.01	ND-0.011	ND	ND	ND	-	ND	-
∑Chlordane	0.01±0.01		**ND**		**ND**	-	**ND**	
Methoxychlor	ND	-	ND	-	ND	-	ND	-
∑OCPs	**8.98±7.92**		**0.25±0.24**		**6.62±6.25**		**0.28±0.31**	

NOTE: ND – Not detected (<0.001 μgg^{-1})

Table 4.7. Concentrations of OCPs (μgg^{-1} [ww]) distribution in cocoa plant during dry and wet seasons - ONDO II

OCPs	DRY SEASON				WET SEASON			
	Fresh leaves		Bark		Fresh leaves		Bark	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	0.34±0.21	ND-0.572	ND	ND	0.43±0.34	0.108 - 0.892	0.03±0.04	ND - 0.089
βHCH	0.83±0.80	0.049-2.056	0.07±0.05	0.001-0.131	0.84±1.00	0.059 - 2.241	0.09±0.01	0.079-0.112
γHCH	0.05±0.03	0.013-0.089	ND	-	0.05±0.05	0.008 - 0.110	0.09±0.02	0.063-0.109
δHCH	0.28±0.14	0.134-0.497	0.17±0.09	0.061-0.283	0.24±0.19	ND - 0.258	ND	-
∑HCH	**1.50±1.18**		**0.24±0.14**		**1.55±1.57**		**0.21±0.07**	
pp'DDT	0.25±0.37	ND-0.899	0.01±0.02	ND-0.035	0.22±0.27	0.007 - 0.598	0.35±0.46	0.007-0.998
pp'DDE	0.01±0.01	ND-0.023	ND	-	0.03±0.05	ND - 0.101	ND	-
opDDD	0.01±0.01	ND-0.019	ND	-	0.01±0.01	ND - 0.023	ND	-
∑DDT	**0.27±0.39**		**0.01±0.02**		**0.26±0.33**		**0.35±0.46**	-
								-
Heptachlor	0.01±0.01	ND-0.013	ND	-	ND	-	ND	
Heptachlor Epoxide	0.00±0.00	ND-0.010	ND	-	ND	-	ND	-
∑Heptachlor	**0.01±0.010**		**ND**		**ND**	-	**ND**	-
αEndosulfan	0.68±0.68	0.131-1.845	0.10±0.07	0.012-0.183	0.32±0.09	0.248 - 0.451	0.20±0.10	0.063-0.289
βEndosulfan	2.44±2.10	0.340-5.556	ND	ND	1.25±0.71	0.427 - 2.161	0.12±0.07	0.027-0.168
Endosulfan SO_4	3.85±3.06	0.882-7.994	0.83±0.55	0.214-1.545	2.75±2.28	0.895 - 5.971	0.66±0.20	0.409- 0.895
∑Endosulfan	**6.97±5.84**		**0.93±0.62**		**4.32±3.08**		**0.98±0.36**	
Aldrin	1.13±0.46	0.415-1.672	ND	ND	0.37±0.39	ND - 0.909	ND	-
Dieldrin	0.09±0.06	0.023-0.182	ND	ND	0.09±0.09	0.005 - 0.207	ND	-
Endrin	0.83±0.44	0.369-1.557	1.57±0.78	0.607-2.524	0.40±0.31	ND - 0.752	ND	-
Endrin Aldehyde	0.04±0.07	ND-0.159	ND	ND	0.03±0.03	ND - 0.060	ND	-
Endrin Ketone	1.66±2.08	0.067-5.238	ND	ND	ND	-	ND	
∑Aldrin	**3.75±3.11**		**1.57±0.78**		**0.89±0.81**		**ND**	-
								-
Cis- Chlordane	0.04±0.04	ND-0.105	0.01±0.01	ND-0.022	0.01±0.00	ND - 0.009	**ND**	-
Trans- Chlordane	0.14±0.12	ND-0.296	ND		0.16±0.17		ND	-
∑Chlordane	**0.18±0.16**		**0.01±0.01**		**0.17±0.18**		**ND**	-
Methoxychlor	ND	ND	ND		ND		ND	-
∑OCPs	**12.67±10.69**		**2.75±1.57**		**7.19±5.96**		**1.54±0.90**	

NOTE: ND – Not detected (<0.001 μgg^{-1})

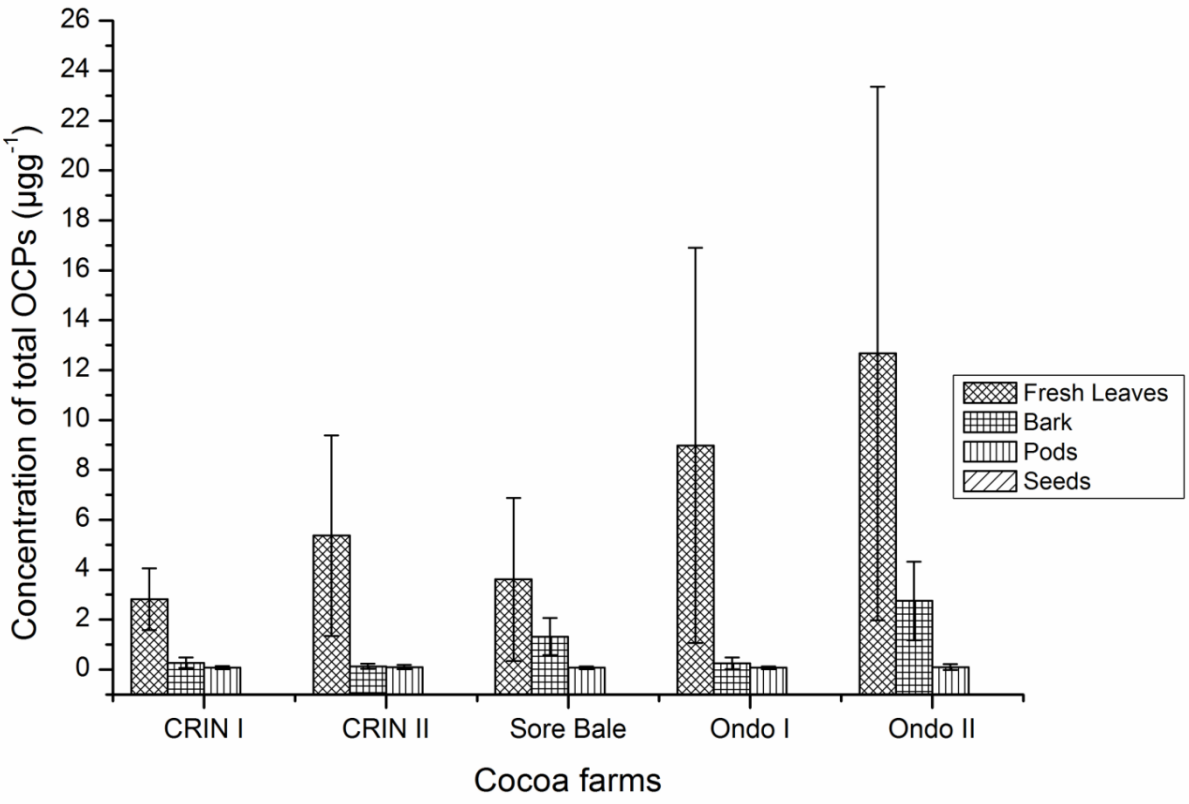


Figure 4.1. Distribution of ∑OCP in cocoa farm vegetation (dry season)

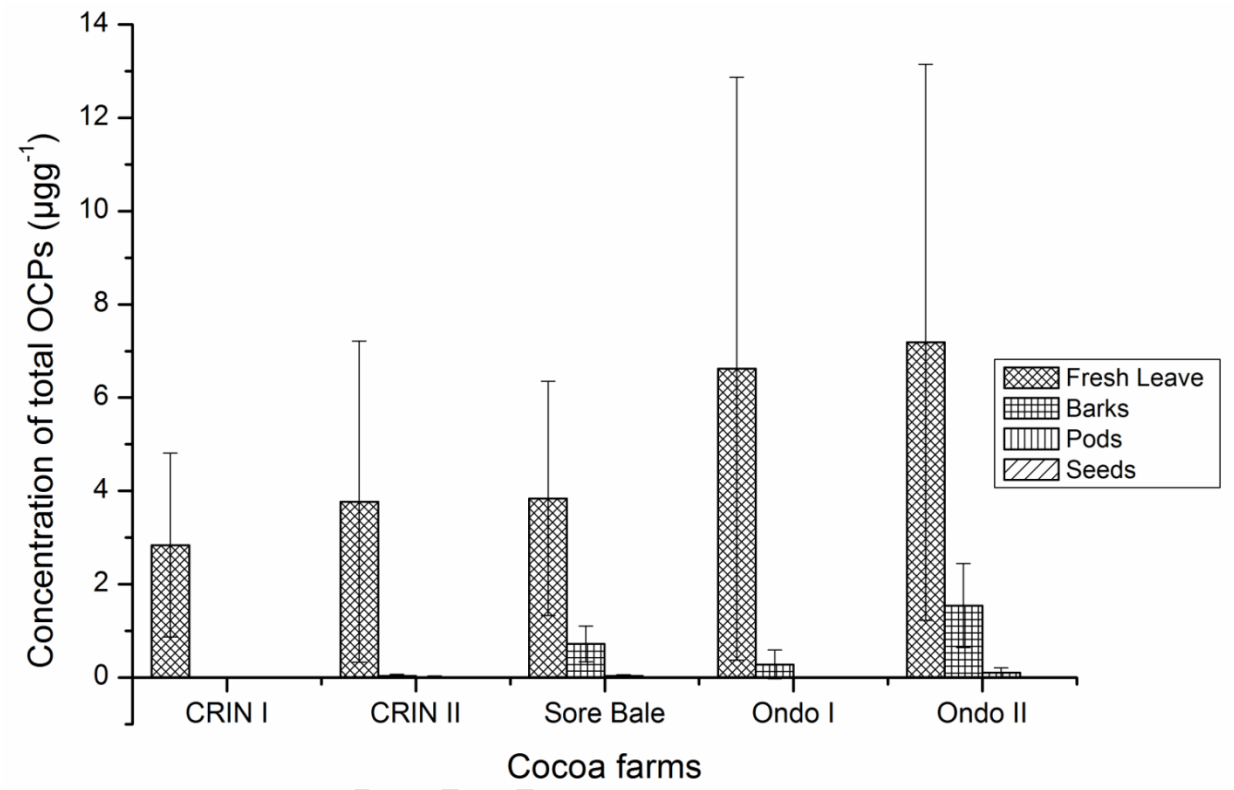


Figure 4.2. Distribution of ∑OCP in cocoa farm vegetation (wet season)

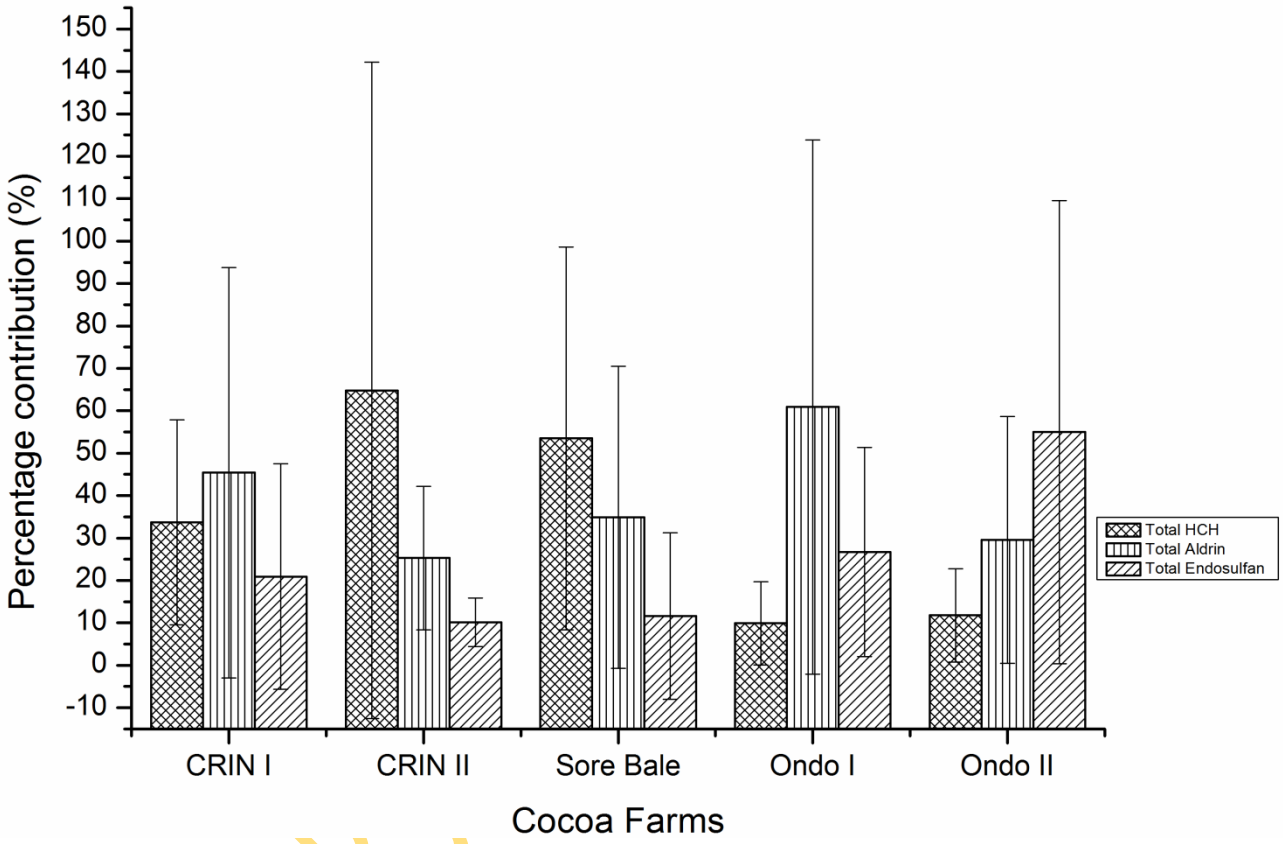


Figure 4.3. Percentage contribution of ∑HCHs, ∑Aldrins and ∑Endosulfans to ∑OCPs in dry season

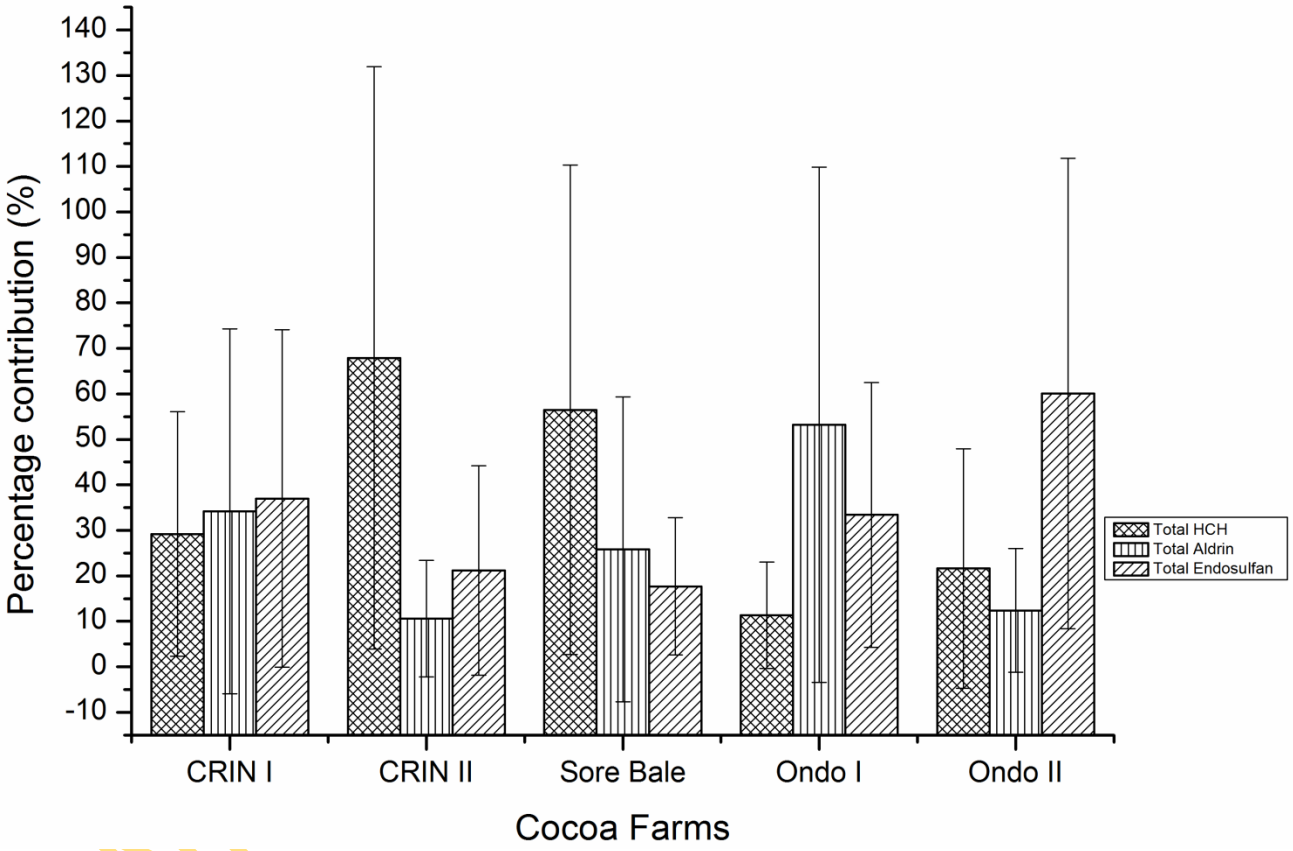


Figure 4.4. Percentage contribution of ∑HCHs, ∑Aldrins and ∑Endosulfans to ∑OCPs in wet season

The detection frequencies of individual OCPs belonging to the three most dominant families; HCHs, aldrins and endosulfans in fresh leaves ranged from 0% - 100.0% (Tables 4.8 and 4.9).

Amongst the HCHs, the detection frequency for both dry and wet seasons in the fresh leaves for CRIN I farm was 83.34%, 100%, 66.67% and 100% α-HCH, β-HCH, λ-HCH and δ-HCH respectively. Others were; CRIN II- α-HCH (100%), β-HCH (100%), λ-HCH (66.67%) and δ-HCH (50%); Sore Bale - α-HCH (100%), β-HCH (100%), λ-HCH (100%) and δ-HCH (50%); Ondo I - α-HCH (83.34%), β-HCH (100%), λ-HCH (83.34%) and δ-HCH (83.34%); Ondo II - α-HCH (87.5%), β-HCH (100%), λ-HCH (100%) and δ-HCH (83.34%). This implied that the β-HCH isomer was the most frequently detected and was found in all the fresh leaves analysed. The order of detection frequency was β-HCH > α-HCH > λ-HCH > δ-HCH in all the sites investigated. The β-isomer is relatively the most stable of all the HCHs (Owago *et al.,* 2009).

Dieldrin exhibited the highest detection frequency amongst the aldrins, with percentage (for combined seasons) ranging between 70.84% (Sore Bale) and 100% (CRIN I, Ondo I and Ondo II), while β-endosulfan was the most frequently detected member of the endosulfans in fresh leaves. Percentage detection frequency of the β-isomer ranged from 66.67% (CRIN I, CRIN II and Ondo I) – 100% (Ondo II).

The high detection frequency of β-endosulfan might be as a result of the fast volatilization of α-endosulfan from plant surfaces (Beard and Ware, 1969), its conversion to β-endosulfan in both soil and plants (Mukherjee and Gopal, 1994) and its photo-isomerization to β-endosulfan (Walia and Dureja, 1993) in the environment. The high occurrence of dieldrin in the leaf samples might be due to the readiness of aldrin to be metabolized through epoxidization to dieldrin in the presence of oxygen. This conversion may be favoured as air passes through stomata to enhance aldrin conversion to dieldrin (Matsumura, 1985).

Table 4.8.*Detection frequency of OCPs in fresh leaves and stem bark for both seasons

	FRESH LEAVES					BARK				
OCPs	**CRIN I**	**CRIN II**	**Sore Bale**	**ONDO I**	**ONDO II**	**CRIN I**	**CRIN II**	**Sore Bale**	**ONDO I**	**ONDO II**
αHCH	83.34	100	100	83.34	87.50	0.00	0.00	0.00	83.34	87.50
βHCH	100	100	100	100	100	0.00	0.00	0.00	100	100
γHCH	66.67	66.67	100	83.34	100	0.00	0.00	0.00	83.34	100
δHCH	100	50.00	50.00	83.34	83.34	0.00	33.34	0.00	83.34	83.34
pp'DDT	16.67	16.67	16.67	66.67	87.50	0.00	50.00	16.67	66.67	87.50
pp'DDE	0.00	0.00	0.00	33.34	58.34	0.00	0.00	0.00	33.34	58.34
opDDD	0.00	0.00	0.00	16.67	41.67	0.00	0.00	0.00	16.67	41.67
Heptachlor	0.00	0.00	0.00	33.34	12.50	0.00	0.00	0.00	33.34	12.50
Heptachlor Epoxide	0.00	0.00	0.00	0.00	12.50	0.00	0.00	0.00	0	12.50
αEndosulfan	83.34	66.65	87.5	100	100	83.34	50.00	83.34	100	100
βEndosulfan	100	100	75	66.67	100	16.67	0.00	100.00	66.67	100
Endosulfan SO_4	83.34	33.35	50	83.34	100	33.34	0.00	83.34	83.34	100
Aldrin	83.34	83.34	83.34	100	83.34	50.00	50.00	83.34	100	83.34
Dieldrin	100	83.34	70.84	100	100	0.00	16.67	50.00	100	100
Endrin	66.67	66.67	66.67	83.34	83.34	33.34	0.00	50.00	83.34	83.34
Endrin Aldehyde	0.00	25.00	0.00	66.67	45.84	0.00	0.00	0.00	66.67	45.84
Endrin Ketone	16.67	0.00	0.00	100	100	0.00	0.00	0.00	100	100
Cis- Chlordane	0.00	0.00	0.00	16.67	16.67	0.00	0.00	0.00	16.67	16.67
Trans- Chlordane	0.00	0.00	0.00	33.34	12.50	0.00	0.00	0.00	33.34	12.50
Methoxychlor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

***Mean detection frequencies of OCPs (%) = thenumberof times OCP is detected per sample matrix divided by total number of samples multipled by 100%

Table 4.9.* Detection frequency of OCPs in pods and seeds for both seasons

	PODS					SEEDS				
OCPs	**CRIN I**	**CRIN II**	**Sore Bale**	**ONDO I**	**ONDO II**	**CRIN I**	**CRIN II**	**Sore Bale**	**ONDO I**	**ONDO II**
αHCH	0.00	0.00	33.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00
βHCH	16.67	0.00	16.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
γHCH	0.00	0.00	16.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
δHCH	0.00	0.00	16.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pp'DDT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pp'DDE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
opDDD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heptachlor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heptachlor Epoxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
αEndosulfan	50.00	50.00	0.00	50.00	100.00	0.00	0.00	0.00	0.00	0.00
βEndosulfan	0.00	16.67	0.00	0.00	50.00	0.00	0.00	0.00	0.00	0.00
Endosulfan SO_4	33.34	16.67	0.00	16.67	50.00	0.00	0.00	0.00	0.00	0.00
Aldrin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dieldrin	0.00	0.00	0.00	0.00	50.00	0.00	0.00	0.00	0.00	0.00
Endrin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endrin Aldehyde	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Endrin Ketone	0.00	50.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cis- Chlordane	0.00	0.00	0.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00
Trans- Chlordane	0.00	0.00	0.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00
Methoxychlor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

***Mean detection frequencies of OCPs (%) = the number of times OCP is detected per sample matrix divided by total number of samples multipled by 100%

4.2.2.2. Residual concentrations and seasonal occurrence of OCPs in plant tissues

Endosulfan and its' metabolite

The mean concentrations of ∑endosulfan (sum of α-and β-endosulfan and endosulfan sulphate) recorded at all the sites in both dry and wet seasons for leaf tissues, bark and pods ranged from 0.42(0.64) - 6.97 (5.84) μgg^{-1}, ND - 0.98 (0.36) μgg^{-1} and ND - 0.10(0.11) μgg^{-1} respectively, with standard deviation values in parentheses. Parent endosulfan (α- and β-isomers) and its metabolite endosulfan sulphate were not detected (<0.001 μgg^{-1}) in cocoa seed samples collected from any of the five farms. Highest and least levels of ∑endosulfan in leaf tissues were found in Ondo II and Sore Bale in the dry season (Figures 4.5 and 4.6), Ondo II and CRIN I in cocoa tree bark in the wet season, while in pods - Ondo II and Sore Bale/Ondo I in the dry and dry/wet seasons respectively (Tables 4.3 - 4.12). Total residual concentrations in fresh leaves in CRIN I, CRIN II, Sore Bale, Ondo I and Ondo II were 0.58(0.32) μgg^{-1}, 0.54 (0.23)μgg^{-1}, 0.42 (0.64) μgg^{-1}, 2.41 (1.97) μgg^{-1} and 6.97 (5.84) μgg^{-1} in the dry season respectively, while in the wet season 1.05(0.72) μgg^{-1}, 0.80 (0.81) μgg^{-1}, 0.68 (0.38) μgg^{-1}, 2.21 (1.82) μgg^{-1} and 4.33(3.08) μgg^{-1}, with standard deviation in parentheses. The mean ∑endosulfan residue levels in Ondo I and Ondo II were greater than the WHO/FAO maximum residue limit (1984) of 2 $mgkg^{-1}$ (or 2 μgg^{-1}) for food vegetals. The order of ∑endosulfan residue or contamination was Ondo II >Ondo I > CRIN I > CRIN II > Sore Bale in both seasons. This trend implied that cocoa farmers in Ondo area used more endosulfan pesticides than farmers at other sites. This finding corroborated Aiyesanmi and Idowu, (2012), who reported that α- and β-endosulfan were the most frequently occurring with high residual concentrations in soils of nine (9) cocoa farms from Ondo State central Local Governments (Akure South, Ifedore and Idanre Local Government Areas).

Among the two isomers and metabolites of endosulfan (α-and β-endosulfan and endosulfan sulphate), the β-isomer was the most dominate and its percentage detection frequency was 100% in leaf tissues in CRIN I, CRIN II, Ondo II and stem bark from Sore Bale, Ondo II farms.

In Ondo II, residual concentrations for α-, β-endosulfan and endosulfan sulphate were 0.68 μgg^{-1}, 2.44 μgg^{-1}, 3.85 μgg^{-1} respectively in the dry season, with individual percentage contribution to ∑endosulfan being; α-endosulfan 9.7%, β-endosulfan 35.0% and endosulfan sulphate 55.3%. Individual levels in the wet season were 0.32 μgg^{-1} (7.4%), 1.25 μgg^{-1} (28.9%) and 2.76 μgg^{-1} (63.7%) for α-endosulfan, β-endosulfan and endosulfan sulphate respectively, with individual percentage contribution to ∑endosulfan in parentheses.

The mean concentration of ∑endosulfan residues in fresh leaves obtained from CRIN I, CRIN II and Sore Bale were comparable to values of BDL - 0.52 $mgkg^{-1}$(μgg^{-1}) reported for berseem (*Trifolium alexandrium*) - a green fodder in Haryana State, India (Sharma *et al*. 2013), while those of Ondo I and Ondo II were significantly higher (with residual range of 2.21 ± 1.82 to 6.97 ± 5.84 μgg^{-1}).Higher residual levels of endosulfan sulphate in plant tissues relative to parent isomers in Ondo I and Ondo II suggested the use of endosulfan pesticide in recent past. Ondo I cocoa farm has been abandoned for over three years, while Ondo II is still being cultivated – this is reflected in the residual amount of ∑endosulfan recorded (Figures 4.5 and 4.6).

The detection of α-endosulfan, β-endosulfan and metabolite - endosulfan sulphate in CRIN I, CRIN II and Ondo I cocoa vegetation showed their persistence and bioaccumulation especially in leaf tissues, since OCPs have not been used in these farms for over 3 years (Ondo I) and 15 years (CRIN I and CRIN II). However, in Sore Bale, the ratio of α-endosulfan + β-endosulfan/∑endosulfan was 0.95 and 0.57 for dry and wet seasons respectively, while the sum of residual concentrations of parent compound were higher than those of their metabolite (Table 4.5). This suggested that endosulfan may have being recently applied. OCPs are still being used in Sore Bale farms.

Technical grade endosulfan contains α–isomer and β-isomer in a 7:3 ratio, endosulfan is persistent and extremely toxic to fish and aquatic invertebrates (Antonius and Byers, 1997). Endosulfan sulphate is exclusively formed through biological oxidation in plant tissues and by soil microorganisms (Guerin and Kennedy, 1992) and colembolla (Kennedy *et al*. 2001) from endosulfan. Therefore, endosulfan sulphate residue observed in all samples is most likely to be a product of in-situ bio-transformation in the environment.

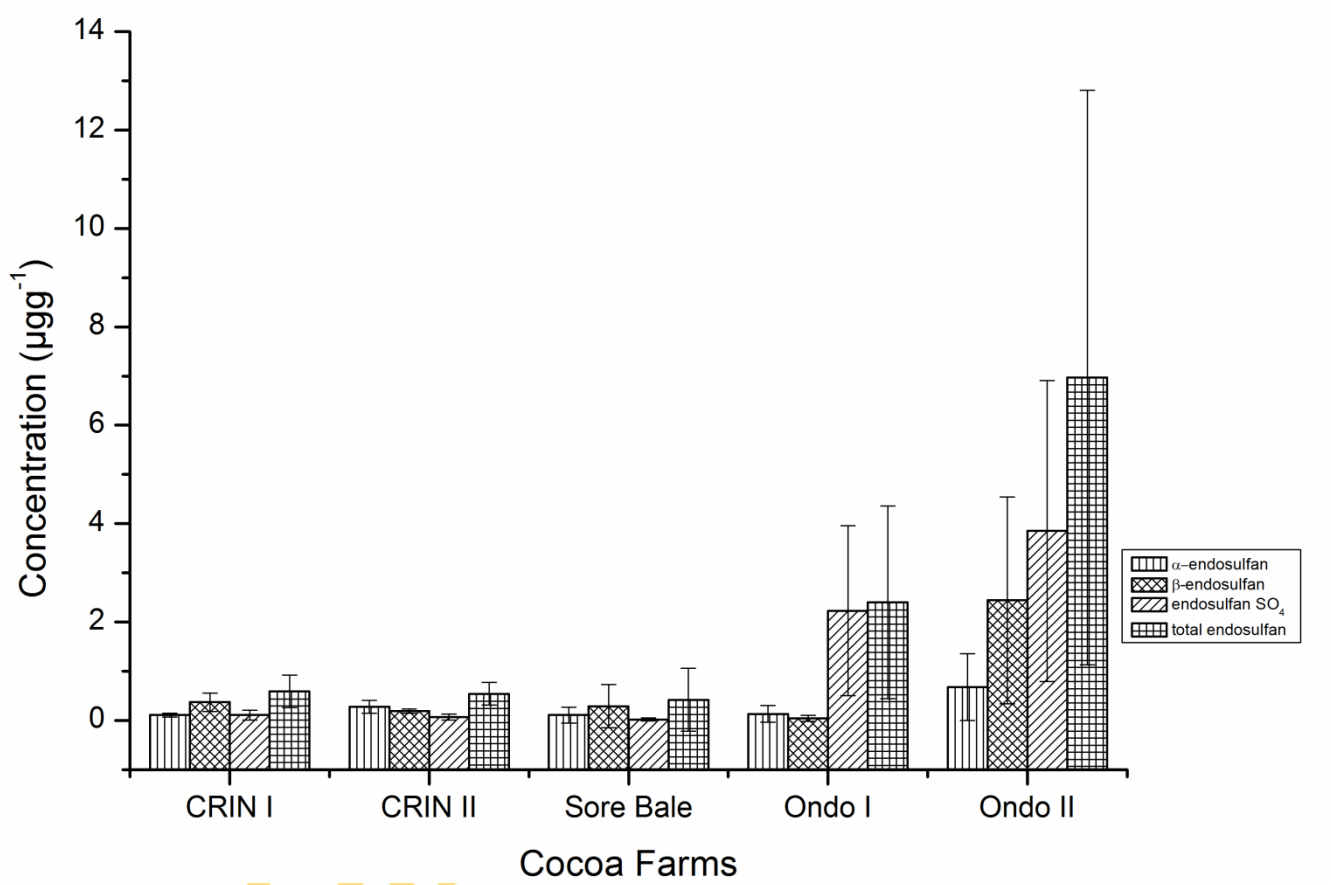


Figure 4.5. Distribution of ∑endosulfan, isomers and metabolite in fresh leaves (dry season)

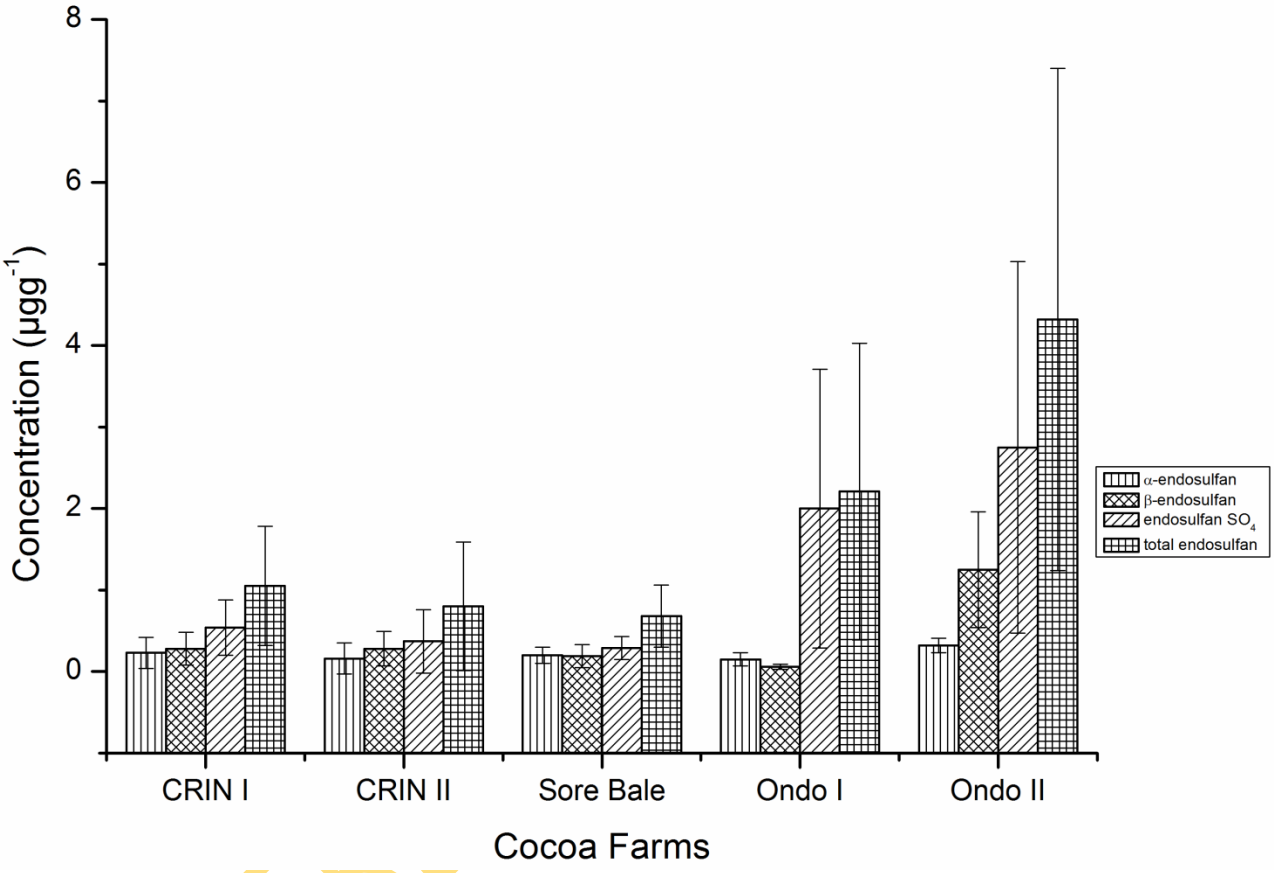


Figure 4.6. Distribution of ∑endosulfan, isomers and metabolite in fresh leaves (wet season)

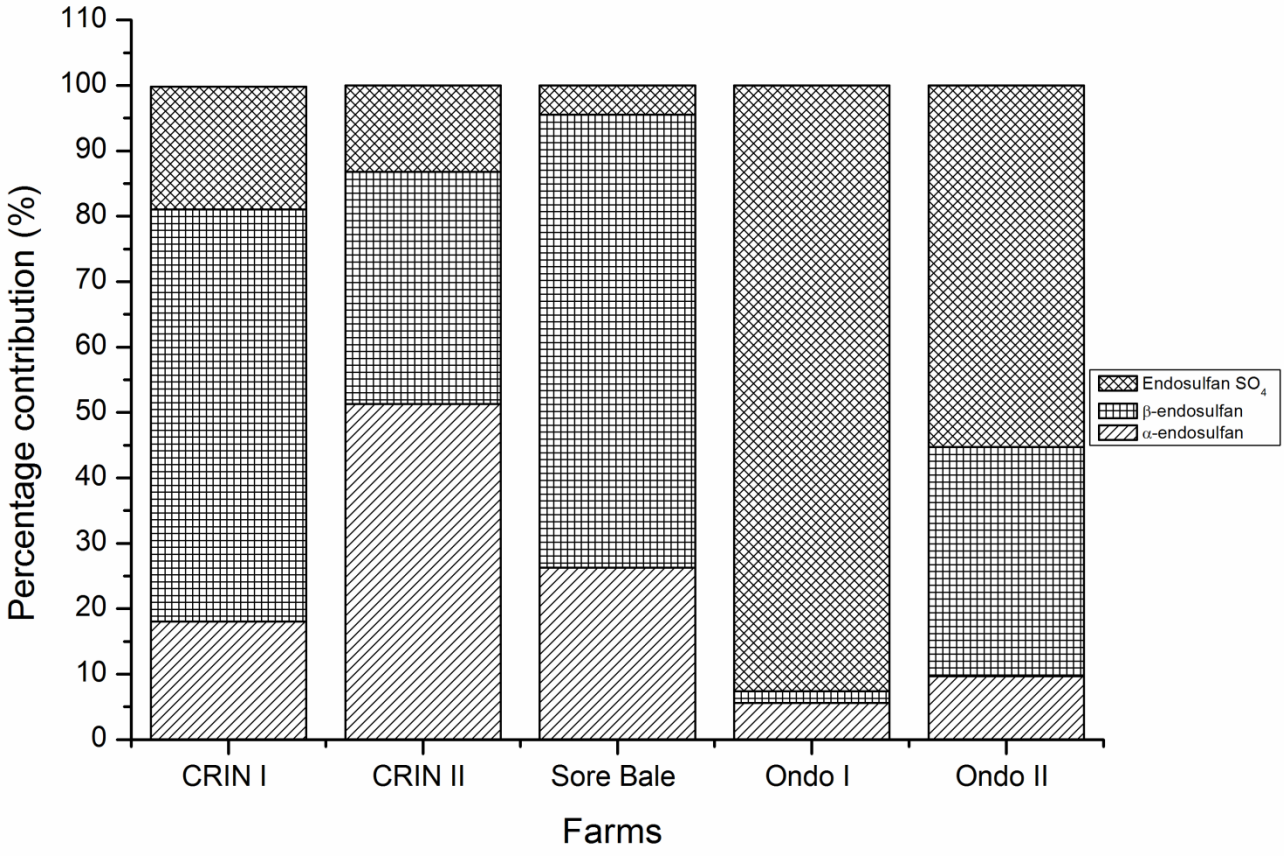


Figure 4.7. Percentage contributions of parent endosulfan and metabolite to ∑endosulfan in fresh leaves (dry season)

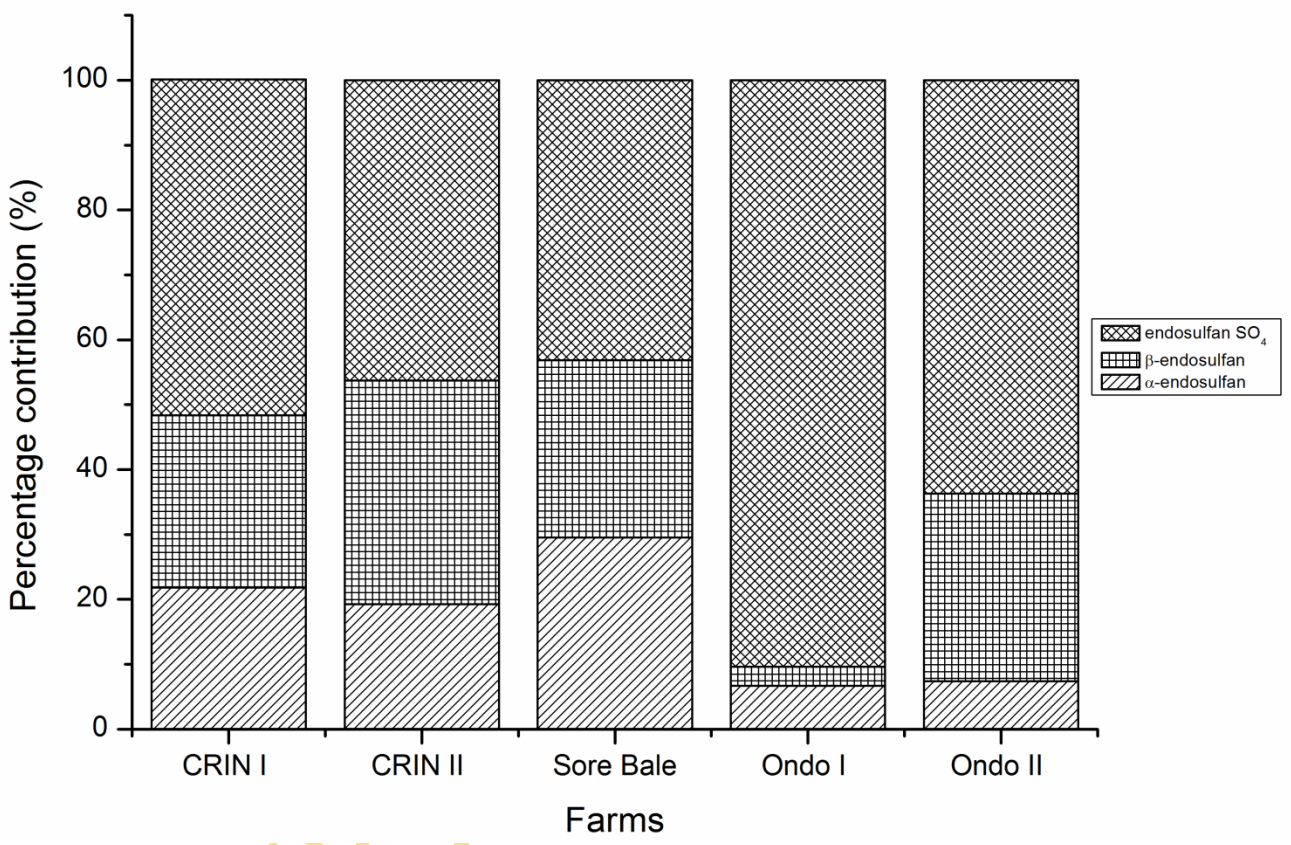


Figure 4.8. Percentage contributions of parent endosulfan and metabolite to ∑endosulfan in fresh leaves (wet season)

Aldrin and its' analogues

The combined detection frequencies in both seasons for aldrin and its major congeners – dieldrin and endrin in all five cocoa farms ranged from 83.3% - 100%, 70.84% - 100% and 66.7% - 83.3% respectively in cocoa fresh leaves; 50% - 100% (aldrin), 0% – 100% (dieldrin) and 0% – 83.3% (endrin) in cocoa tree bark, with pesticides in parentheses, while in pods aldrin recorded 0% - 16.7% and 0% for both dieldrin and endrin. None of the ''drins'' were detected in seeds (Tables 4.8 and 4.9).

The mean residual concentrations of ∑aldrin (aldrin + deildrin + endrin + endrin aldehyde + endrin ketone) for both seasons in fresh cocoa leaves, bark and pods ranged from 0.40-5.47 ugg^{-1}, ND -1.57 ugg^{-1} and ND-0.07 ugg^{-1} respectively, but was not detected in cocoa seeds (i.e., <0.001 ugg^{-1}) (Tables 4.3 – 4.7). The order of contamination was Ondo I > Ondo II > CRIN I > CRIN II >Sore Bale(Figures 4.9 and 4.10), while order of distribution of ∑aldrin within cocoa plant compartments was seeds < pods < bark < fresh leaves. In Ondo I, individual concentrations of member aldrins were 0.65ugg^{-1} (aldrin), 0.40 ugg^{-1} (dieldrin), 1.72ugg^{-1} (endrin), 1.12 ugg^{-1} (endrin aldehyde) and 1.59ugg^{-1} (endrin ketone).

Dieldrin is an epoxidized metabolic form of aldrin – it is converted to its epoxide analogue by mammals, soil microorganisms, plants and insects (Matsumuoa, 1985), while dieldrin and endrin are isomers. Endrin aldehyde and endrin ketone are metabolites of endrin. ∑endrin (sum of endrin and its metabolites) constituted 80.8% and 82.3% of ∑aldrin residue in cocoa fresh leaves in dry and wet seasons respectively in Ondo I. Percentage contribution to ∑aldrin by endrin and its metabolites in dry and wet seasons for Ondo II, Sore Bale, CRIN I and CRIN II were 67.5% and 57.9%; 63.6% and 46.5%; 41.0% and 33.0% and 64.3% and 48.37% respectively. High residual concentrations of dieldrin, endrin and its metabolites implied long time application of parent aldrin. The residual dieldrin/aldrin ratios in dry and wet seasons for fresh leaves were 0.73, 1.95 (CRIN I); 0.33, 0.52 (CRIN II); 0.15, 0.50 (Sore Bale); 0.61, 0.75 (Ondo I) and 0.08, 0.24 (Ondo II) respectively (Figure 4.11). Ratios > 0.5 are indicative of long time usage, while the presence aldrin, dieldrin, endrin and its metabolites also suggested persistence and bioaccumulation.

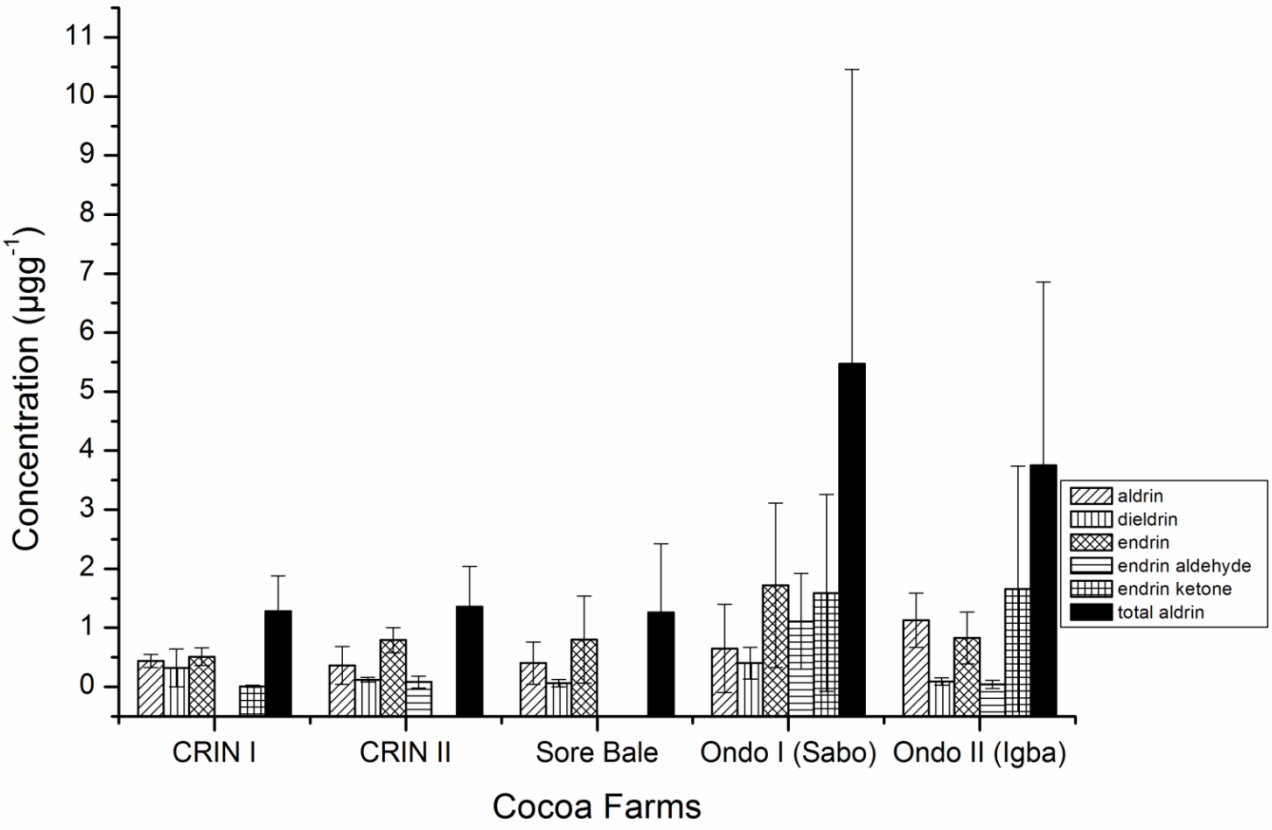


Figure 4.9. Distribution of ∑Aldrin, aldrin and analogues in fresh leaves of cocoa during the dry season

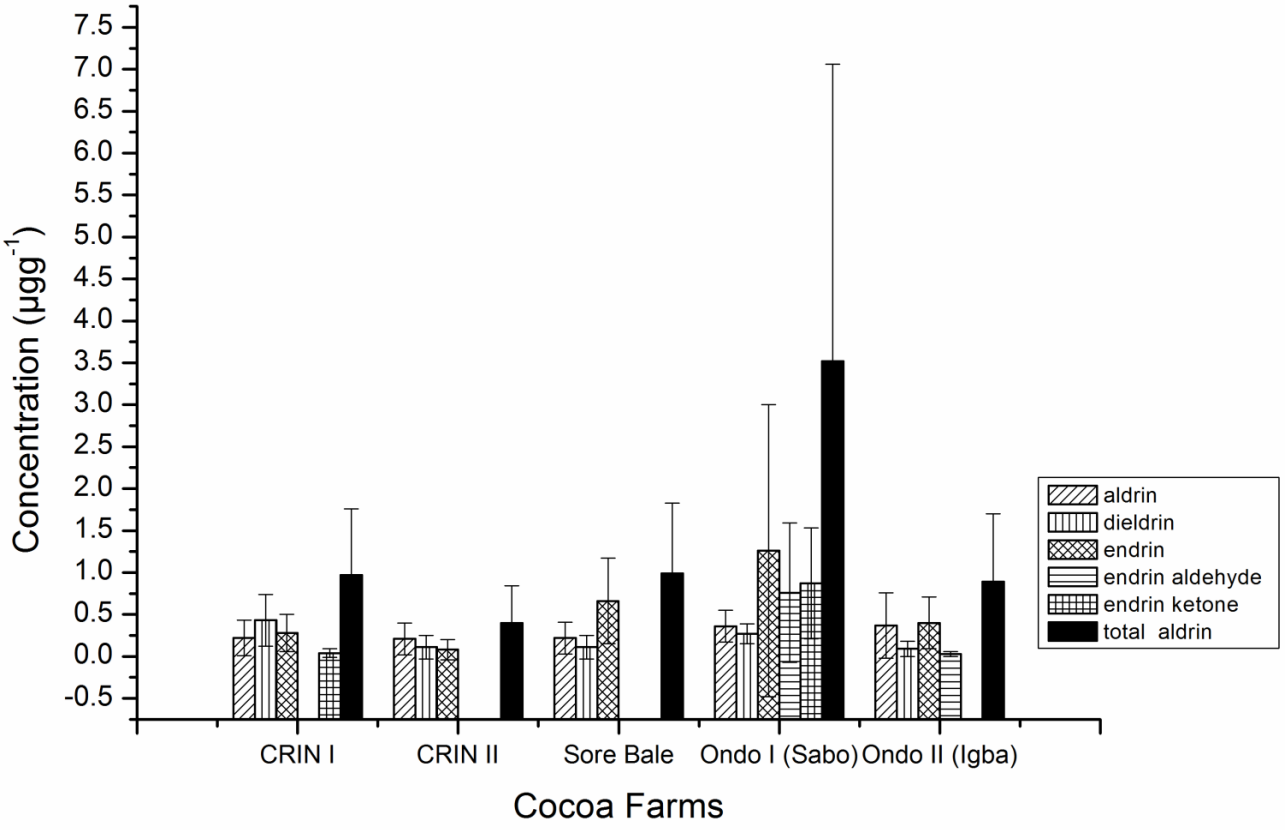


Figure 4.10. Distribution of ∑Aldrin, aldrin and analogues in fresh leaves of cocoa during the wet season

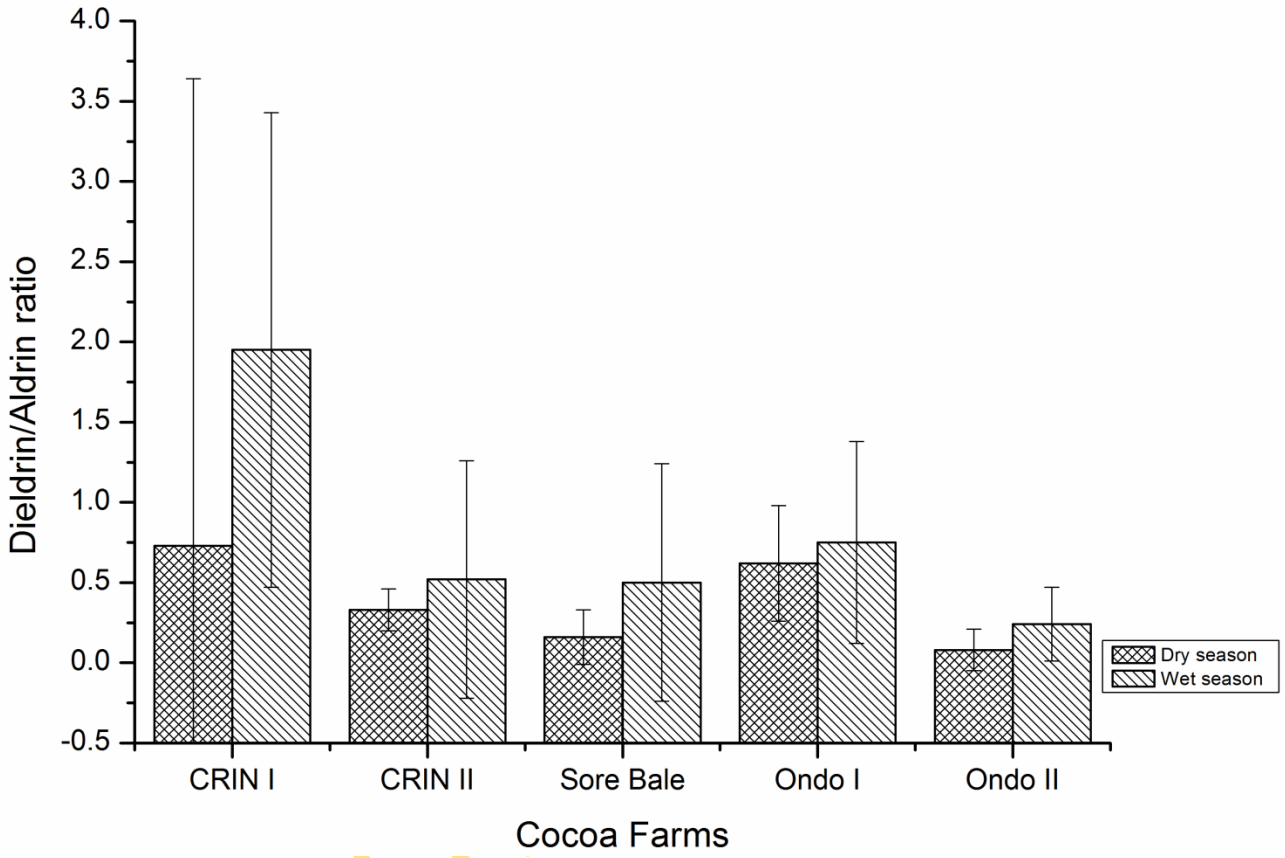


Figure 4.11. Dieldrin/Aldrin ratio in fresh foliage for dry and wet seasons

Hexachlorocyclohexane (HCH)

The mean concentration of ∑HCH residue in this study, ranged from 0.75 (0.73) -3.48 (3.11) μgg^{-1} for leaf tissues, ND – 0.24 (0.14) μgg^{-1} for cocoa bark and ND – 0.03 (0.03) μgg^{-1} for cocoa pods in dry and wet seasons (standard deviation in parentheses) (Tables 4.3 – 4.12). The highest ∑HCH residue was observed in CRIN II, while the least was obtained in Ondo I. The order of ∑HCH contamination in cocoa plant was CRIN II > Sore Bale > Ondo II > CRIN I > Ondo I in the dry and wet seasons (Figures 4.12 and 4.13), while the individual residual composition of each isomers in CRIN I was 13.7% α–HCH, 46.3% β-HCH, 10.5% γ-HCH and 29.5% δ-HCH in the dry season and in the wet season 12.0%, 43.4%, 14.5% and 30.1% respectively. β-isomer was most dominant and in terms of individual contribution to ∑HCH residues in the dry season for other farms; β-HCH contributed 45.1% (62.5%), 56.0% (52.1%), 37.5% (37.3%) and 55.3% (54.2%) in CRIN II, Sore Bale, Ondo I and Ondo II respectively, with percentages for wet season in parentheses (Figure 4.14). Percentage contribution and relative dominance of β-isomer in fresh leaves were comparable to values of 44%, 67% and 51.84% reported for other matrices like water and sediments (Zhang *et al*. 2002a, 2002b and 2003; Malik *et al*. 2009). The detection frequencies of HCH isomers in fresh leaves ranged between 50% and 100% (Table 4.8). β-isomer recorded a detection frequency of 100% in all the five sites – this implied that it was detected in all the samples analysed and also potrayed predominance and persistence (especially CRIN I and CRIN II). CRIN II was the most contaminated with respect to the HCHs. The mean residual concentrations of individual HCHs in the dry season was 0.44 (0.10 -0.87) μgg^{-1} α–HCH; 1.57 (0.37-2.34) μgg^{-1} β-HCH; 0.95 (0.05-3.62) μgg^{-1} γ-HCH and 0.52 (0.37-0.58) μgg^{-1}δ-HCH, with ranges in parenthesis, while 0.50 (0.09 - 0.91) μgg^{-1} α–HCH; 1.57 (0.11 -3.09) μgg^{-1} β-HCH; 0.21 (ND - 0.63) μgg^{-1} γ-HCH and 0.25 (ND - 0.75) μgg^{-1}δ-HCH were obtained in the wet season. The residual concentration for γ-HCH (lindane) for CRIN II (both seasons) exceeded significantly the WHO/FAO maximum residue limit (MRL) of 0.1 $mgkg^{-1}$ (or 0.1 μgg^{-1}) lindane in vegetables (WHO/FAO, 2006). The residual levels in CRIN I, Sore Bale, Ondo I and Ondo II also exceeded WHO/FAO maximum residue limit (MRL).

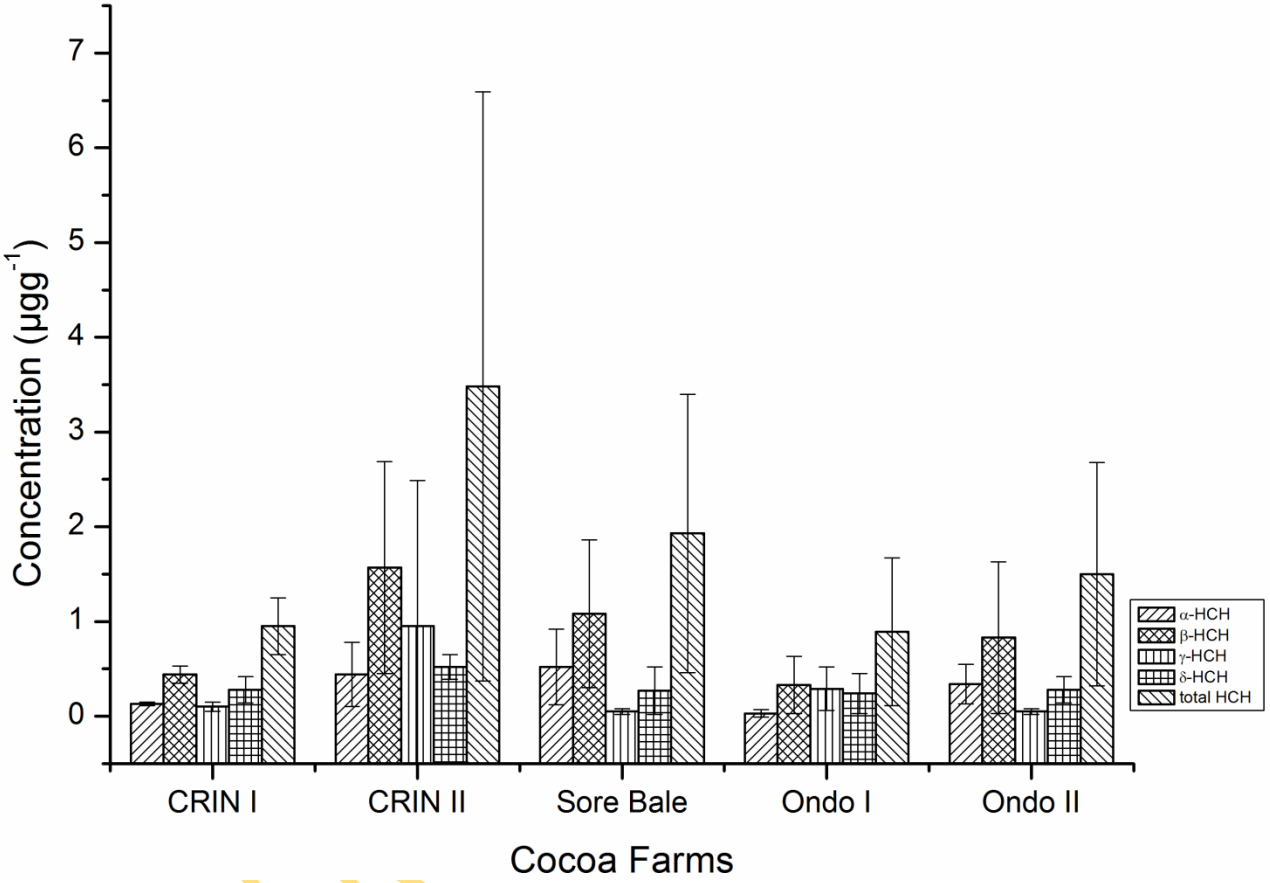


Figure 4.12. Distribution of ∑HCH and HCH isomers in fresh leaves of cocoa during the dry season

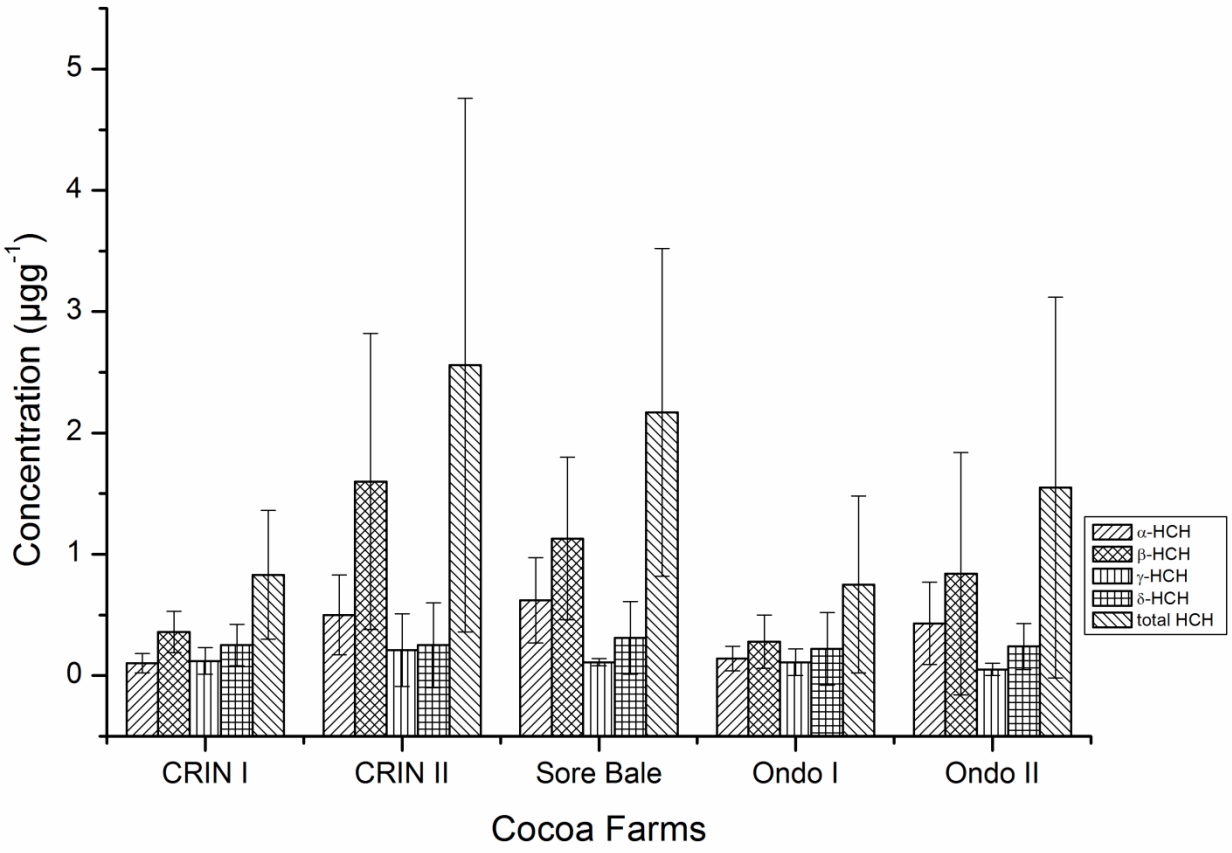


Figure 4.13. Distribution of ∑HCH and HCH isomers in fresh leaves of cocoa during the wet season

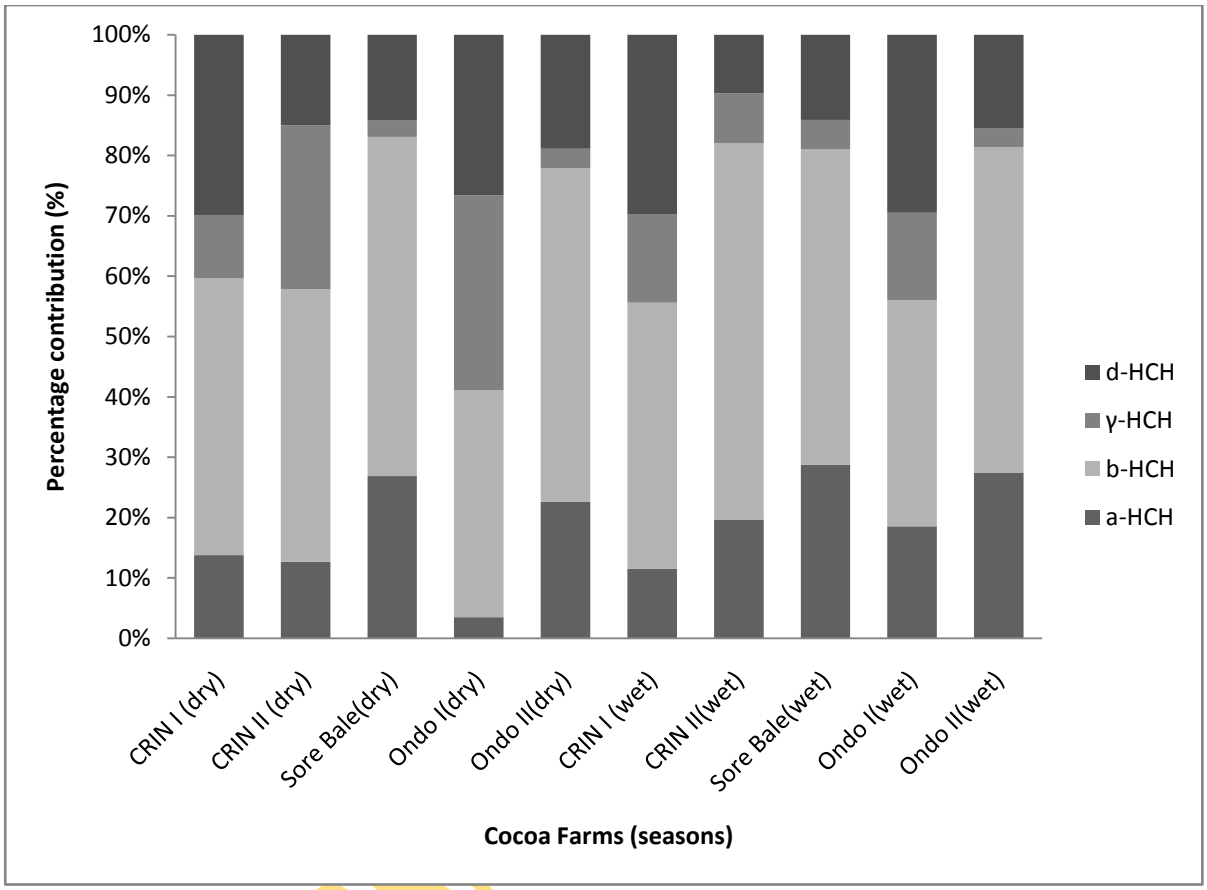


Figure 4.14. Percentage contribution of HCH isomers to total HCH in vegetation during the dry and wet seasons

The most common isomers of HCH in the environment are α-HCH, β-HCH, γ-HCH and δ-HCH (Malik *et al.* 2009). The order of predominance in whole plant for CRIN I, Sore Bale and Ondo II were the same in dry and wet seasons -the trend was as follows; CRIN I, β-HCH> δ-HCH > α-HCH > γ-HCH ; Sore Bale, β-HCH>α-HCH>δ-HCH>γ-HCH; and Ondo II, β-HCH>α-HCH>δ-HCH>γ-HCH. However, in the dry season the trend in CRIN II was β-HCH>γ-HCH > δ-HCH >α-HCH (β-HCH > α-HCH >δ-HCH > γ-HCH); Ondo I -β-HCH>γ-HCH>δ-HCH>α-HCH (β-HCH>δ-HCH>α-HCH>γ-HCH), with wet season trend in parentheses. The general trend of β-HCH>α-HCH>δ-HCH>γ-HCH agreed with those of Barriada-Pereira *et al.* (2005) and Owago *et al*. (2009). Owago*et al*. (2009) reported an overall average sequence of β-HCH > α-HCH ≈ γ-HCH >δ-HCH and β-HCH>δ-HCH >α-HCH ≈ γ-HCH for vegetable samples in Deyang and Yanting Areas Sichuan, China respectively, while Barriada-Pereira *et al.* (2005) reported that the most abundant pesticides were mainly α-HCH and β-HCH, and lower levels for γ-HCH and δ-HCH in Glaicia, Spain.

From the aforementioned orders, the β- and γ-HCHs were the most and least predominant respectively in cocoa plant. Barriada-Pereira *et al.* (2005), Owago *et al.* (2009), Benson and Aruwajoye (2011), Olivella *et al*. (2012) and Sharma *et al*. (2013) reported β-HCH as most predominant and abundant isomer of the HCHs in vegetation from contaminated environments. The bioaccumulation of β-HCH may be due to its easy uptake by the cocoa plant from the soil through their roots and diffusion through the aerial portion of the plant – especially the leaves, because of its morphological nature, while its persistent and high residual concentration compared to other isomers is because of its physicochemical properties of relatively having the highest solubility in fats (most lypophilic) and vapour pressure, most stable and resistant to hydrolysis, microbial and/or environmental degradation (Willet *et al.,* 1998 and Malik *et al.*2008). In addition, its predominance could be due to the readily conversion of α-HCH to β-HCH in the environment (Wu *et al.* 1999; Walker, 1999); and as well as the ability of γ-isomer to bio-isomerize into α-HCH (Steinwandter, 1976; Waliszewski *et al.* 2004), hence high residual levels of the β-isomer is highly favoured. Technical grade HCH is expected to have a ratio of α-HCH/γ-HCH of 4-7 and 0.07-0.2 for β-HCH/α-HCH, since it principally consist of five (5) isomers in the

following proportion, α-HCH (60-70%), β-HCH (5-12%), γ-HCH (10-15%), δ-HCH (6-10%) and ε-HCH (3-4%) (Walker *et al*. 1999), while pure lindane contains approximately 99% γ-HCH (Iwata *et al*. 1993). The α-HCH/γ-HCH ratio for CRIN I, CRIN II, Sore Bale, Ondo I and Ondo II in the dry season was 1.30 (0.83), 0.46(2.38), 10.4 (5.64), 0.10 (1.27) and 6.80 (8.60) respectively, with ratio values for wet season in parentheses. These ratio values suggested that lindane must have been applied in the past to CRIN I, CRIN II and Ondo I(and not the technical grade HCH) since their α-HCH/γ-HCH ratios are less than 4, while technical grade HCH was recently or continuously being used in Sora Bale and Ondo II (ratios > 4). The β-HCH/α-HCH ratios were CRIN I 3.38 (3.60), CRIN II 3.57 (3.20), Sore Bale 2.08 (1.82), Ondo I 11.00(2.00) and Ondo II 2.44 (1.95) for dry and wet (in parentheses) seasons. These ratio values are 10-fold or more > 0.2, which supported the readily conversion of α-HCH to β-HCH in our environment and the application of the pesticide (technical grade and/or pure lindane) in the past. Besides the aforementioned, the epicuticular waxy nature of plant leaves, which consists mainly of long-chain polyesters, has been reported to accumulate lipophilic compounds such as organochlorine pesticides (Reischl *et al.* 1989; Calamari *et al*. 1991). Amongst the HCH isomers, β-HCH is the most fats soluble and will therefore readily persist and accumulate more than the other isomers in cocoa leaves.

These observed ratio values and trends are in consonance with the different categories which these farms represented. The CRIN I and CRIN II cocoa farms have not witness the use of OCPs in the last 15 years, while in Ondo I it has been discontinued for 3 years. OCPs are still being used in Sore Bale and Ondo II. These trends inferred the persistence of HCHs in cocoa plant tissues in CRIN I, CRIN II and Ondo I and bioaccumulation with respect to all five farms. Commercial Lindane is also marketed as Gammalin 20.

The residual concentration of β-HCH in all five cocoa farms ranged from 0.28 to 1.60 $\mu g g^{-1}$, which was significantly lower when compared to the range of 0.42 -40.26 mg kg^{-1} (or $\mu g g^{-1}$) reported for plant species in Galicia, Spain (Barriada-Pereira *et al.* 2005), 1.69 - 2.34 mg kg^{-1} (or $\mu g g^{-1}$) in *Capsicum annuum*Lfrom a retailer market at Ota, Ogun State, Nigeria (Benson and Aruwajoye, 2011). Also ∑HCH levels in the aforementioned studies in Spain and Nigeria were 1.7 - 62.5 $\mu g g^{-1}$ and 3.14 – 4.08 $\mu g g^{-1}$ respectively, while

residual concentrations range of 13 - 44 mgkg $^{-1}$ (μgg^{-1}) found in vegetation from contaminated area in North India (Abhilash *et al*. 2008) were much higher. However, ∑HCH residual concentrations reported for lettuce in an urban market, Ghana (Amoah *et al*. 2006) and vegetation in India (Sharm *et al.* 2013) were significantly lower than values obtained in this study.

Dichlorodiphenyl trichloroethane (DDT) and metabolites

The mean concentration of total DDT (∑DDT, i.e., sum of pp'-DDT and metabolites - op'-DDD and pp'-DDE) residue in the dry season for cocoa leaf tissues and tree bark for all farms ranged from ND - 0.27 ± 0.39μgg^{-1} (Ondo II) and ND - 0.02 μgg^{-1} (CRIN II) respectively (Tables 4.3, 4.5, 4.7, 4.9 and 4.11), while residual levels of ND-0.26 ±0.33μgg^{-1} (Ondo II) and 0.05 ±0.04 μgg^{-1} (Ondo II) in the wet season (Tables 4.3 - 4.7). However, no DDT and its metabolites were detected in cocoa pods and seeds in both seasons. Residual levels of DDTs were high in Ondo I and Ondo II, while very low levels were recorded in CRIN I, CRIN II and Sore Bale.

The residual concentration of ∑DDT in all five cocoa farms were significantly lower when compared with values reported in fodder grasses (Kaphalia and Seth, 1982) and *Mangifera indica* (Marco and Kishimba, 2007) from Nainital, Uttaranchal, India and Vikuge farm, Tanzania respectively – with corresponding residual concentrations ranging from 0.164 – 0.631 $mgkg^{-1}$(or ugg^{-1}) and 6.1 – 1242 ngg^{-1} (or 0.006 – 1.242 ugg^{-1}). However, comparable levels were reported in *Pennisetum americanum* (pearl millets) by Sharma *et al.*(2013), while ranges reported in vegetables from markets in the Greater Accra region, Ghana (Bempah *et al*. 2012) were significantly lower thanvalues obtained in Ondo I and Ondo II, but higher than values recorded for CRIN I, CRIN II and Sore Bale .

The frequency of detectionfor pp'-DDT, pp'-DDE and op'-DDD in fresh leaves ranged from 16.67% (CRIN I, CRIN II) to 87.5% (Ondo II), 0% (CRIN I, CRIN II, Sore Bale) to 58.34% (Ondo II) and 0% (CRIN I, CRIN II, Sore Bale) to 41.67% (Ondo II) respectively for combined seasons (Table 4.8); low percentage detection frequencies in CRIN I and CRIN II farms suggested long term application of pp'-DDT, while its presence depicted persistence.

Parent pp'DDT contributed 100% to ∑DDT in CRIN I (dry season) and CRIN II (wet season), while both parent compound and metabolites were not found in the wet season in CRIN I and CRIN II. Levels of pp'DDT in CRIN I (dry season) and CRIN II (wet season) were 0.01 $\mu g g^{-1}$each. In Ondo I, parent DDT contributed 66.7%, 50.0% to ∑DDT in the dry and wet seasons respectively, while for Ondo II percentage contribution was 92.6% and 84.6% respectively.

Gonzelaz *et al*. (2003) reported that ratios of DDT/DDE in biota are often used as indicators of recent application of DDT into the environment or as a measure of the extent of degradation (degradation efficiency). High ratios indicated recent application.

In fresh leaves, DDT and DDE were found in Ondo I and Ondo II in both seasons, while only the parent compound was detected in CRIN I (dry season) and CRIN II (wet season). The DDT/DDE ratio for fresh leaves ranged from 0.5 in Ondo I (wet season) to 25.0in Ondo II (dry season) where both compounds were found. These values implied that parent DDT was recently used in Ondo II farm, while it was used in recent past in Ondo I. DDT/DDE ratios < 1.0 are indicative of aging and weathering of parent DDT. High residual concentrations for parent DDT relative to metabolites in leaf tissues may have resulted from its passage/diffusion through stomata into plant tissues after its application. The detection of parent compoundswithout metabolites in CRIN I and CRIN II plant tissues (Tables 4.3 and 4.4) may have been due to deposition from the atmosphere and not due to previous uptake and translocation. OCPs are known for their long range/distance atmospheric transport (LRT) and deposition due to volatization and global distillation (Wania and Mackay, 1996, Waliszewski *et al*. 2004). OCPs have been detected in places, such as the artics - where they have never being used. Therefore the pp'-DDT detected in CRIN I, CRIN II and Ondo I must have been due to long-range atmospheric transport (LRT) and fresh application, since OCPs have not been used in these farms for 3 – 15 years.

Heptachlorand its' metabolite

Parent heptachlor and its metabolite – heptachlor epoxide were not detected in any of the five farms investigated in both seasons, except in Ondo I, where heptachlor was found in

the fresh leaf tissues during the dry season - heptachlor and its epoxide were not found in the tree bark, pods and seeds at any of the farms.Total heptachlor (∑heptachlor, i.e., heptachlor + heptachlor epoxide) in Ondo I was 0.02 ±0.01 μgg^{-1}(Tables 4.6). The presence of parent compound alone (i.e., 100% contribution to ∑heptachlor) is suggestive of recent application of heptachlor to Ondo I, since no metabolite was found. This mean value (Ondo I) is comparable to concentration reported in *solanum lycopersicum* in Accra, Ghana (Bempah and Donkor, 2010), however, they were incomparable to an astronomical concentration of 1,650 μgg^{-1} found from Akumadan vegetable farming community in Ghana (Ntow, 2001). The value of heptachlor epoxide found in this community might be as a result of oxidation of heptachlor because commercial heptachlor epoxide is not available (WHO 2004).

Total Chlordanein cocoa vegetation

Chlordane (cis-chlordane and trans-chlordane) was not recorded in CRIN I, CRIN II and Sore Bale in both seasons, however, ∑chlordane in Ondo I and Ondo II were 0.01 ±0.01 μgg^{-1} (<0.01 μgg^{-1}, 0.01 μgg^{-1}) and 0.18 ±0.16μgg^{-1} (0.04, 0.14 μgg^{-1}) respectively in the dry season, with residual levels of cis-chlordane and trans-chlordane in parenthesis (Tables 4.6 and 4.7). In the wet season, chlordane isomers were not detected in Ondo I, while in Ondo II, ∑chlordane mean concentrations was 0.17 ± 0.18 μgg^{-1}, with individual concentrations of cis-chlordane and trans-chlordane being 0.01 (ND- 0.02) μgg^{-1} and 0.16(ND-0.40) μgg^{-1}, detected ranges in parentheses (Table 4.7). The cis/trans-chlordane ratios were 0 and 0.29 for Ondo I and Ondo II respectively in the dry season, while the ratio obtained in the wet season for Ondo II was 0. 06. These ratios showed that a pure commercial grade trans-chlordane or technical grade heptachlor may have been recently used in these farms, since residual levels of the cis-isomer were very low. Commercial grade chlordane may contain 15% each of cis- and trans-isomers (Dearth and Hites, 1991) or 19% and 24% respectively (Rostad, 1997) in addition to other 140 organic compounds, while technical grade heptachlor contains about 72% heptachlor and 28% related compounds (20–22% *trans*-chlordane and 4–8% nonachlor) (IARC, 2001). Heptachlor was also found in this environment. In addition the trans-isomer is reported to be more persistent and stable than the cis-isomer in the environment (Malik *et al*. 2009); therefore

it implied that that pure chlordane was applied in the past to these farms. However if the origin of chlordane in these farms was from the use of heptachlor, then the presence of the cis-isomer must have resulted from biotransformation of the trans-isomer.

4.2.3. Concentration Profile of OCPs in Soil

4.2.3.1. Distribution of OCPs in cocoa farm soils

Total mean OCPs (∑ OCPs) in soil samples (dry wt.-dw) for all five cocoa farms ranged from 64.60±40.75- 615.83±732.08 ngg^{-1} and 51.45 ±46.59 - 1,166.71± 1,052.62 ngg^{-1} for 0-15 cm and 15-30 cm depths respectively in the dry season, while wet season residual concentrations ranged from 11.48±14.17 ngg^{-1} - 287.21±317.85 ngg^{-1} at depth 0-15cm and 50.35±43.80 - 1066.78±475.01ngg^{-1} for 15-30 cm depth (Tables 4.15 – 4.19). Maximum ∑OCP was found in CRIN I farm soil at both depths during the dry and wet seasons, while the least ∑OCP values for top and bottom soils were observed at Ondo II in the dry season; however corresponding minima in the wet season were recorded at CRIN II and Ondo II for depth 0 - 15 cm and 15 - 30 cm respectively - thus making the CRIN I soil the most contaminated.

The order of contribution to ∑OCPs in CRIN I topsoil (0-15cm) and sub-surface soil (15-30cm) in the dry was ∑ endosulfan > ∑aldrin > ∑ HCH> ∑DDT>∑ heptachlor>∑ chlordane (Tables 4.10, 4.12 and 4.13), while trend in the wet season at both depths was ∑ endosulfan > ∑aldrin > ∑ HCH> ∑DDT>∑ heptachlor ≈ ∑ chlordane and ∑ endosulfan > ∑aldrin > ∑ HCH> ∑DDT ≈ ∑ heptachlor ≈ ∑ chlordane respectively (Tables 4.11, 4.12 and 4.14). The aforementioned trend showed that amongst the individual OCP families detected in the soil profiles – the endosulfans, aldrins and HCHs were the most predominant in CRIN I. Contribution of ∑endosulfan, ∑aldrins and ∑HCHs to ∑OCPs in top and bottom soils ranged from 0.9 - 74.4%, 0.20 -89.0% and 0.05 – 60.1% respectively during both seasons. The highest percentage contributions by HCHs and aldrins to ∑OCPs were observed at the 15 -30 cm depthduring the wet season, while ∑endosulfan was found at the topsoil in the wet season. This high residual concentrations of OCPs in CRIN I after 15 years of abandonment, depicted their persistence in the environment.

Table 4.10. Concentrations of OCPs (ngg^{-1}[dw]) distribution in cocoa farm soil during dry season - CRIN I & CRIN II

OCPs	CRIN I (0 -15 cm)		CRIN I (15 -30cm)		CRIN II (0 -15 cm)		CRIN II (15 -30cm)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	2.35±3.28	0.002-6.989	1.25±1.17	0.235-2.896	1.37±1.88	ND-3.990	3.99±2.21	0.951-7.523
βHCH	24.98±28.57	0.089-64.986	139.75±158.73	25.345-364.212	9.50±5.92	0.792-2.965	10.99±8.38	2.326-22.326
γHCH	3.25±1.50	1.468-5.126	4.25±3.31	0.224-8.321	0.13±0.11	-	4.47±4.54	0.052-10.712
δHCH	4.02±1.81	2.570-6.569	11.50±12.77	1.756-29.538	10.01±7.81	ND-36.009	9.35±7.04	1.674-18.674
∑HCH	**34.60±35.16**		**156.75±175.98**		**21.01±15.62**		**28.80±22.67**	
pp'DDT	14.00±19.80	ND-42.001	18.19±15.01	36.191-186.239	ND	-	ND	-
pp'DDE	5.25±5.94	ND-13.557	39.29±40.43	-	ND	-	ND	-
opDDD	0.40±0.43	ND-1.001	34.77±31.09	-	ND	-	ND	-
∑DDT	**19.65±26.17**		**92.25±86.53**		**ND**		ND	
Heptachlor	3.75±4.00	0.342-9.360	2.00±2.45	0.051-5.461	ND	-	ND	-
Heptachlor Epoxide	0.83±0.91	ND-2.092	1.99±1.76	ND-4.442	ND	-	ND	-
∑Heptachlor	**4.58±4.90**		**3.99±4.21**		**ND**		ND	
αEndosulfan	23.00±32.46	0.009-68.901	10.00±4.08	5.012-14.998	0.30±0.10	0.186-0.419	0.11±0.09	ND-0.254
βEndosulfan	92.51±120.30	0.025-262.414	125.75±92.63	19.387-245.152	1.88±1.30	0.128-3.215	1.56±1.16	0.067-2.863
Endosulfan SO_4	316.75±396.22	19.631-876.731	443.55±447.01	0.001-1055.411	2.82±2.14	0.624-5.724	7.00±3.27	2.891-10.879
∑Endosulfan	**432.26±548.97**		**579.30±543.71**		**5.00±3.53**		**8.67±4.52**	
Aldrin	25.75±31.97	0.538-70.857	3.25±3.85	0.044-8.667	ND	-	ND	-
Dieldrin	22.50±25.03	2.659-57.805	1.50±1.29	0.201-3.265	ND	-	ND	-
Endrin	46.75±32.61	12.758-90.745	17.01±22.649	ND-49.016	37.33±33.75	8.889-84.751	41.67±48.56	0.008-109.770
Endrin Aldehyde	19.50±13.10	4.489-36.401	255.42±182.18	96.349-510.473	ND	-	ND	-
Endrin Ketone	10.25±14.17	0.098-30.282	57.25±32.21	24.567-101.069	ND	-	ND	-
∑Aldrin	**124.75±116.87**		**334.43±242.19**		**37.33±33.75**		**41.67±48.56**	
Cis- Chlordane	ND	-	ND	-	2.00±2.83	ND-6.001	11.00±7.63	5.313-21.789
Trans- Chlordane	ND	-	ND	-	2.33±2.10	0.009-5.093	7.00±5.07	2.886-14.145
∑Chlordane	**ND**				**4.33±4.93**		**18.00±12.70**	
Methoxychlor	ND	-	ND	-	ND	-	ND	-
∑OCPs	**615.84±732.08**		**1166.72±1052.62**		**67.67±57.93**		**97.14±88.44**	

NOTE: ND – Not detected (<0.001 ng/g)

Table 4.11. Concentrations of OCPs (ngg^{-1} [dw]) distribution in cocoa farm soil during wet season - CRIN I & CRIN II

OCPs	CRIN I (0 -15 cm)		CRIN I (15 -30cm)		CRIN II (0 -15 cm)		CRIN II (15 -30cm)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	0.59±0.43	ND - 1.005	6.67±4.11	2.080-12.060	1.61±2.08	0.080 -4.552	3.43±2.52	0.670-6.754
βHCH	19.03±13.20	0.412 - 29.591	151.70±28.45	120.312-189.189	2.50±3.19	0.189 - 7.008	9.70±8.90	0.189-21.591
γHCH	1.29±1.21	ND - 2.91	2.12±1.36	0.363-3.670	0.04±0.05	ND -0.108	3.94±2.90	ND- 6.892
δHCH	ND	-	1.15±1.32	0.091-3.000	0.09±0.12	-	7.84±7.58	0.298-18.217
∑HCH	**20.91±14.84**		**161.64±35.24**		**4.24±5.44**		**24.91±21.90**	
pp'DDT	5.61±3.99	ND - 9.001	1.79±2.53	-	ND	-	ND	-
pp'DDE	5.52±5.77	0.127- 13.520	3.92±3.92	-	ND	-	0.97±1.37	ND-2.911
opDDD	1.19±1.18	ND - 2.801	7.64±6.16	-	ND	-	ND	-
∑DDT	**12.32±10.94**		**13.35±12.61**		**ND**		**0.97±1.37**	
Heptachlor	ND	-	1.75±1.33	-	ND	-	ND	-
Heptachlor Epoxide	ND	-	0.21±0.16	-	ND	-	ND	-
∑Heptachlor	**ND**		**1.96±1.49**		**ND**		**ND**	
αEndosulfan	0.23±0.19	ND - 0.463	62.612±8.95	50.102 - 70.561	ND		ND	
βEndosulfan	10.28±14.07	0.102 - 30.174	126.56±17.37	105.980 - 148.463	0.71±1.00	ND - 2.125	2.05±2.24	0.061-5.174
Endosulfan SO_4	203.18±233.61	0.212 - 530.410	357.54±111.80	243.009 - 509.212	4.10±5.65	ND - 12.089	4.54±4.66	0.212 -11.029
∑Endosulfan	**213.69±247.87**		**546.72±138.12**		**4.81±6.65**		**6.59±6.90**	
Aldrin	14.96±15.11	0.152 - 35.701	3.91±3.56	0.152 - 8.701	ND		ND	
Dieldrin	10.56±8.35	0.399 - 20.851	5.23±4.11	0.399 - 10.438	ND		ND	
Endrin	3.05±4.14		12.28±7.28	1.980-17.249	0.67±0.94	ND -2.002	14.87±13.18	ND -32.043
Endrin Aldehyde	ND		262.04±188.22	28.110 - 488.98	0.03±0.05	ND - 0.098	1.82±1.97	ND -4.561
Endrin Ketone	11.73±16.59	ND -35.190	59.67±84.38	ND - 179.001	0.000		ND	
∑Aldrin	**40.30±44.19**		**343.13±287.55**		**0.70±0.99**		**16.69±15.15**	
Cis- Chlordane	ND	-	ND	-	0.71±0.28	0.312 - 0.951	10.70±7.75	ND-18.095
Trans- Chlordane	ND	-	ND	-	1.02±0.81	ND - 1.987	6.34±5.56	1.003-14.003
∑Chlordane	**ND**		**ND**	**-**	**1.73±1.09**		**17.04±13.31**	
Methoxychlor	**ND**	**-**	**ND**	**-**	**ND**	**-**	**ND**	**-**
∑OCPs	**287.22±317.84**		**1066.80±475.01**		**11.48±14.17**		**66.20±58.62**	

NOTE: ND – Not detected (<0.001 ngg^{-1})

Table 4.12. Concentrations of OCPs (ngg^{-1} [dw]) distribution in cocoa farm soil during dry &wet seasons - Sore Bale

OCPs	Sore Bale (dry season) (0 -15 cm)		Sore Bale (dry season) (15 -30cm)		Sore Bale (wet season) (0 -15 cm)		Sore Bale (wet season) (15 -30cm)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	6.34±4.52	ND-10.224	0.95±0.77	ND-1.892	7.67±4.13	0.028 -5.986	13.33±7.59	5.428-13.328
βHCH	16.99±6.30	10.031-25.291	1.41±0.83	0.409-2.429	6.88±4.53	15.189 - 31.591	12.63±7.26	3.816-21.592
γHCH	ND	-	0.57±0.16	0.366-0.762	22.36±6.85	0.363 - 23.245	8.88±4.92	4.368-15.725
δHCH	ND	-	ND	-	ND	-	ND	-
∑HCH	**23.33±10.82**		**2.92±1.76**		**36.91±15.52**		**34.84±19.77**	
pp'DDT	216.86±331.44	43.099-783.244	53.66±70.52	0.014-165.324	43.02±58.68	0.002 - 125.987	28.36±35.90	ND - 78.998
pp'DDE	127.47±19.81	ND-45.965	4.01±5.67	ND-12.029	45.58±40.68	ND - 98.760	ND	
opDDD	ND	ND	ND	ND	0.66±0.93	ND - 1.980	14.00±19.80	ND - 42.005
∑DDT	**344.33±351.25**		**57.67±76.19**		**89.26±100.29**		**42.36±55.70**	
Heptachlor	31.00±43.14	0.002-92.011	ND	ND	12.53±8.36	2.438-22.902	ND	-
Heptachlor Epoxide	ND	ND	ND	ND	0.43±0.42	0.098-1.032	ND	-
∑Heptachlor	**31.00±43.14**		ND		**12.96±8.78**		**ND**	
αEndosulfan	2.95±2.20	0.142-5.498	1.27±0.74	0.231-1.818	0.73±0.83	0.101-1.907	0.23±0.19	ND - 0.463
βEndosulfan	1.38±1.15	ND-2.819	2.18±1.55	0.716-4.316	0.43±0.42	0.031-1.002	0.28±0.20	0.102 - 0.561
Endosulfan SO_4	0.67±0.66	0.002-1.561	3.22±1.13	2.386-4.809	0.39±0.51	ND -1.102	0.54±0.34	0.212 - 1.009
∑Endosulfan	**5.00±4.01**		**6.67±3.41**		**1.54±1.76**		**1.05±0.73**	
Aldrin	ND	ND	ND	ND	ND	-	ND	
Dieldrin	ND	ND	ND	ND	ND	-	ND	
Endrin	114.00±136.80	7.749-307.139	51.67±44.74	0.349-109.375	8.99±10.93	0.249 - 24.398	0.18±0.05	0.126 - 0.249
Endrin Aldehyde	ND	ND	ND	ND	12.47±13.93	0.432 - 32.001	ND	-
Endrin Ketone	ND	ND	ND	ND	4.28±2.86	0.915 - 7.912	ND	-
∑Aldrin	**114.00±136.80**		**51.67±44.74**		**25.74±27.72**		**0.18±0.05**	
Cis- Chlordane	2.38±2.24	0.294-5.483	5.33±5.03	0.109-3.759	0.15±0.11	ND-0.245	5.65±1.98	3.107 - 7.923
Trans- Chlordane	4.62±4.17	0.316-10.269	5.00±4.37	0.015-10.666	0.19±0.13	0.008-0.297	5.49±4.48	ND - 10.980
∑Chlordane	**7.00±6.41**		**10.33±9.40**		**0.34±0.24**		**11.13±6.46**	
Methoxychlor	ND	ND	ND	ND	ND	-	ND	-
∑OCPs	**524.66±552.42**		**129.27±135.50**		**166.75±154.31**		**89.56±82.71**	

NOTE: ND – Not detected (<0.001 ngg^{-1})

Table 4.13. Concentrations of OCPs (ngg^{-1} [dw]) distribution in cocoa farm soil during dry season - ONDO I & ONDO II

OCPs	ONDO I (0 -15 cm)		ONDO I (15 -30cm)		ONDO II (0 -15 cm)		ONDO II (15 -30cm)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	ND	-	ND	-	15.72±5.57	8.018-20.983	1.30±0.93	ND-2.141
βHCH	0.09±0.02	0.056-0.116	37.36±27.66	ND-66.091	14.86±8.50	4.589-25.392	11.88±8.11	0.598-19.282
γHCH	ND	-	ND	-	2.38±4.15	1.982-12.059	2.87±3.47	ND-7.750
δHCH	0.06±0.04	ND-0.102	31.00±30.26	2.193-72.804	4.16±2.34	0.927-5.122	4.35±3.94	0.270-9.681
∑HCH	**0.15±0.06**		**68.36±57.92**		**42.12±20.55**		**20.41±16.45**	
pp'DDT	112.67±121.10	ND-400.723	76.64±83.86	ND-193.340	1.05±0.88	0.027-1.045	1.67±1.66	0.375-4.007
pp'DDE	26.67±16.25	-	20.03±15.30	-	0.34±0.45	ND-0.967	0.34±0.26	0.007-0.631
opDDD	13.33±15.67	-	ND	-	0.04±0.04	ND-0.078	0.41±0.31	ND-0.752
∑DDT	**152.67±153.02**		**96.67±99.16**		**1.43±1.36**		**1.67±1.66**	
Heptachlor	3.33±4.72	ND-10.003	ND	-	ND	-	0.21±0.26	ND-0.572
Heptachlor Epoxide	ND	-	ND	-	ND	-	0.36±0.22	-
∑Heptachlor	**3.33±4.72**		**ND**		**ND**	-	**0.58±0.48**	
αEndosulfan	8.00±3.46	3.397-11.733	6.67±4.62	1.011-12.331	0.40±0.41	0.020-0.978	1.27±1.42	ND-3.253
βEndosulfan	ND	-	53.30±36.32	2.598-74.736	0.69±0.40	0.132-1.067	3.04±4.02	0.028-8.813
Endosulfan SO_4	ND	-	99.30±33.71	58.732-97.909	0.95±0.63	0.165-1.709	5.08±6.75	0.082-14.620
∑Endosulfan	**8.00±3.46**		**159.27±74.65**		**2.04±1.45**		**9.38±12.19**	
Aldrin	ND	-	ND	-	5.67±4.73	0.011-11.581	2.33±2.52	0.529-5.898
Dieldrin	ND	-	ND	-	10.33±9.31	2.625-23.433	14.00±10.42	1.532-27.027
Endrin	98.67±48.85	32.332-148.525	75.33±20.23	51.209-100.722	1.34±1.88	ND-3.99	1.67±1.84	0.234-4.266
Endrin Aldehyde	ND	-	ND	-	1.00±0.93	ND-2.231	ND	-
Endrin Ketone	ND	-	ND	-	0.67±0.55	ND-1.341	ND	-
∑Aldrin	**98.67±48.85**		**75.33±20.23**		**19.01±17.39**		**18.01±14.78**	
Cis- Chlordane	5.33±2.93	ND-7.971	7.66±2.59	4.883-6.124	ND	-	0.67±0.48	0.077-1.245
Trans- Chlordane	7.67±2.65	5.023-17.272	9.00±4.38	4.893-19.069	ND	-	ND	
∑Chlordane	**13.00±5.58**		**16.66±6.97**		**ND**		**0.67±0.48**	
Methoxychlor	ND	-	ND	-	ND	-	ND	-
∑OCPs	**275.82±219.03**		**416.29±258.93**		**64.60±40.75**		**51.450±46.594**	

NOTE: ND – Not detected (<0.001 ngg^{-1})

Table 4.14. Concentrations of OCPs (ngg^{-1} [dw]) distribution in cocoa farm soil during wet season - ONDO I & ONDO II

OCPs	ONDO I (0 -15 cm)		ONDO I (15 -30cm)		ONDO II (0 -15 cm)		ONDO II (15 -30cm)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	0.08±0.06	ND-0.155	0.11±0.10	ND – 0.200	14.01±7.78	4.018 -22.983	1.43±1.38	0.341-3.710
βHCH	0.15±0.17	0.014-0.378	3.66±3.60	0.089 -8.591	14.03±1.12	12.591 - 15.312	11.84±10.44	0.452-25.770
γHCH	0.03±0.04	ND-0.089	0.06±0.09	-	6.72±3.44	1.982 - 10.059	2.20±1.96	ND-4.753
δHCH	0.07±0.09	ND-0.198	0.34±0.39	-	4.08±1.55	1.923 - 5.502	3.50±4.37	0.142-9.681
∑HCH	**0.32±0.36**		**4.17±4.18**		**38.83±13.88**		**19.47±18.21**	
pp'DDT	ND	-	37.08±29.53	0.005-72.272	0.33±0.47	ND - 1.002	2.03±2.15	ND - 5.001
pp'DDE	ND	-	33.40±23.80	0.074-54.143	0.26±0.18	0.091-0.512	0.000	
opDDD	ND	-	9.02±12.75	ND-27.045	ND		0.33±0.47	ND - 0.998
∑DDT	**ND**		**79.49±66.08**		**0.59±0.65**		**2.36±2.62**	
Heptachlor	ND	-	ND	-	ND	-	0.02±0.02	ND-0.045
Heptachlor Epoxide	ND	-	ND	-	ND	-	0.04±0.04	0.002-0.091
∑Heptachlor	**ND**		**ND**		**ND**		**0.06+0.06**	
αEndosulfan	0.28±0.20	0.932 - 7.076	3.56±4.54	0.223 - 9.980	5.86±5.08	0.095 - 12.463	2.31±1.92	ND - 4.693
βEndosulfan	4.16±2.52	0.102 - 0.561	54.61±13.50	38.102 - 71.174	5.80±3.82	0.561 -9.572	2.17±1.52	0.561 -4.212
Endosulfan SO_4	1.54±1.75	0.212 - 4.009	62.54±29.81	21.212 - 90.410	3.22±1.72	3.023 - 5.417	6.05±4.90	0.212 - 12.209
∑Endosulfan	**5.98±4.46**		**120.71±47.86**		**14.87±10.63**		**10.53±8.34**	
Aldrin	ND	-	0.05±0.07	ND - 0.152	1.08±0.79	0.089 - 2.008	1.95±2.17	0.005 - 0.701
Dieldrin	ND	-	0.28±0.200	ND - 0.438	5.62±3.62	1.399 - 10.241	14.90±11.44	0.438 - 28.399
Endrin	43.39±28.30	8.932 -78.249	8.18±5.61	0.249 - 12.281	0.13±0.09	0.019 - 0.249	0.42±0.43	ND - 0.249
Endrin Aldehyde	9.04±6.50	ND - 15.021	14.41±6.21	9.019 - 23.102	2.44±2.06	0.211- 5.181	0.000	
Endrin Ketone	2.66±2.06	ND - 5.012	0.60±0.43	0.011 - 1.009	1.00±1.415	ND - 3.0015	0.07±0.10	ND - 0.210
∑Aldrin	**55.09±36.86**		**23.52±12.51**		**10.27±7.98**		**17.34±14.13**	
Cis- Chlordane	0.32±0.18	0.100 - 0.529	0.44±0.28	0.109 - 0.782	ND	-	0.45±0.34	ND-1.048
Trans- Chlordane	0.18±0.13	ND - 0.321	0.44±0.42	ND - 1.006	ND	-	0.15±0.11	-
∑Chlordane	**0.50±0.31**		**0.88±0.70**		**ND**		**0.59±0.45**	
Methoxychlor	0.00±0.00	ND - 0.008	ND		ND	-	ND	-
∑OCPs	**61.90±42.00**		**228.77±131.32**		**64.57±33.14**		**50.35±43.80**	

NOTE: ND – Not detected (<0.001 ngg^{-1})

In addition,their continuous detection and high residues in CRIN I and CRIN II may have resulted from over burdening of the farm soils with OCPs from previous applications.

In the dry season, ∑OCPs in top soil (0-15cm) and sub-surface soil (15-30cm) were615.83 ngg^{-1} and 1,166.71 ngg^{-1} respectively for CRIN I, others were as follows, 67.66 ngg^{-1} and 97.14 ngg^{-1} (CRIN II), 524.66 ngg^{-1} and 129.27ngg^{-1} (Sore Bale); 275.82 and 416.70 ngg^{-1} (Ondo I); 64.60 ngg^{-1} and 51.45 ngg^{-1}(Ondo II); while values in topsoil (0-15cm) and sub-surface soil (15-30cm) in the wet season were 287.21$ngg^{-1}$and 1066.80ngg^{-1}(CRIN I), 11.48 ngg^{-1} and 66.20 ngg^{-1} (CRIN II), 166.75 ngg^{-1} and 89.56 ngg^{-1} (Sore Bale); 61.90 and 228.77 ngg^{-1} (Ondo I); 64.57 ngg^{-1} and 50.35 ngg^{-1}(Ondo II). Residual concentrations for ∑OCPs recorded in these farms were comparable with values of 72.97 - 1281.67 μgkg^{-1} (or ngg^{-1}) reported for five sites within the Oke-Osun farm settlement, Osogbo, Osun State, Nigeria (Oyekunle *et al*., 2011), while they were significantly lower when compared to values of ND- 262.184 $mgkg^{-1}$ (or $\times 10^3$ ngg^{-1}), 0.041-352.65 $mgkg^{-1}$ (or $\times 10^3$ ngg^{-1}) and 0.012-282.64 $mgkg^{-1}$ (or $\times 10^3$ ngg^{-1}) reported for cocoa farms in Akure South, Ifedore and Idanre Local Government Areas respectively, in Ondo State (Aiyesanmi and Idowu 2012). The total OCP residue levels for all the sites were below the maximum limit of 50 $mgkg^{-1}$ (or 50,000 ngg^{-1}) recommended by the Victorian EPA (EPA 658/90) for contaminated soils (EPA South Australia 2009). The sub-surface soil (15-30 cm) was more contaminated as $\sum OCP_{0\text{-}15cm} < \sum OCP_{15\text{-}30cm}$ in all the cocoa farms except Sore Bale (dry and wet seasons) and Ondo II (wet season) (Tables 4.12 and 4.14). These exceptions may be due to fresh applications or continuous use of pesticides, physiochemical characteristics of the soil - which may differ significantly from farm to farm. In soil, parameter such as particle size distribution, pH, TOC, and moisture content affect the extent of retention of OCPs in the soil. Sarka *et al.*(1997) reported that fine particles can retain large amount of organics and pose high pollution potency in the environment. The ratio of $\sum OCP_{15\text{-}30cm}$ to $\sum OCP_{0\text{-}15cm}$ in farm soils can be used as an index to measure the extent of OCP distribution between both soil profiles and the migration of OCPs from top soil (0-15cm) to sub-surface soil (15-30cm). The $\sum OCP_{15\text{-}30cm}/\sum OCP_{0\text{-}15cm}$ residual ratios in the dry and wet seasons were CRIN I - 1.89 and 3.76, CRIN II - 1.05 and 3.83, Sore Bale - 0.24 and 0.58, Ondo I - 1.51 and 2.40, Ondo II - 2.02 and 0.62 respectively. Soil profile ratios > 1 indicated greater

distribution of OCPs in the sub-surface soil (15-30cm). Most OCPs are almost insoluble in water, therefore such high ratios is suggestive of long period of OCP usage and gradual migration from top soil (0-15cm) to sub-surface soil (15-30cm) over time - (this certainly is a serious threat to the aquifer or ground water as it may be contaminated by OCPs with time). The latter is corroborated as CRIN I, CRIN II and Ondo I farms recorded ratios > 1 in both seasons, while Sore Bale (dry and wet seasons) and Ondo II (wet season) had ratios < 1. Very low ratio values obtained in Sore Bale farm may be as a result of fresh input of pesticide - thus making $\sum OCP_{0\text{-}15cm}$ residue much higher than $\sum OCP_{15\text{-}30cm}$. OCPs are still being used in Sore Bale and Ondo II farms, while CRIN I, CRIN II and Ondo I farms have not been treated with fresh inputs for 3-15 years.

Hexachlorocyclohexane (HCH) in soil

Total HCH (∑HCH) residues in soil profilesfor all five sites in the dry season ranged from 0.15 ± 0.06 ngg^{-1}(Ondo I) to 42.12 ± 20.55 ngg^{-1} (Ondo II) for topsoil (Table 4.13), while for sub-surface soil - 2.92±1.76 ngg^{-1} (Sore Bale) to 156.76 ± 175.98 ngg^{-1} (CRIN I) (Tables 4.12 and 4.10 respectively). In the wet season residual range was 0.32 ± 0.36 ngg^{-1} (Ondo I) to 38.83 ± 13.88 ngg^{-1} (Ondo II) (Table 4.14) and 4.17 ± 4.18 ngg^{-1} (Ondo I) to 161.65 ± 35.24 ngg^{-1} (CRIN I) for top and bottom soils respectively.The maximum and minimum residual concentrations of ∑HCH in topsoils were observed in Ondo II and Ondo I respectively in both seasons, while corresponding residual levels at 15-30 cm depth were obtained in CRIN I and Sore Bale in the dry season and CRIN I and Ondo I wet season.

Among the HCH isomers, the order of dominance was β-HCH>δ-HCH > γ-HCH >α-HCH. The β-isomer was the most dominant contaminant at both depths (0 –15 cm; 15 -30 cm) in CRIN I, CRIN II and Ondo I in both seasons (Figures 4.15 and 4.16). However, it was only dominant in Sore Bale (0-15, 15-30cm) in the dry season and Ondo II (15-30cm) in wet and dry seasons. In CRIN I, the mean concentrations of HCH isomers - α-,β-, γ-, δ-HCH were 2.35 (0.00 - 6.99) ngg^{-1}, 24.98 (0.09 - 64.99) ngg^{-1}, 3.25 (1.47 - 5.13) ngg^{-1} and 4.02 (2.57-6.57) ngg^{-1} respectively in the dry season for 0-15cm depth, with ranges in parentheses; the individual contribution to ∑HCH was 6.79% α-HCH, 72.20% β-HCH, 9.39% γ-HCH and 11.62% δ-HCH (Figure 4.15).

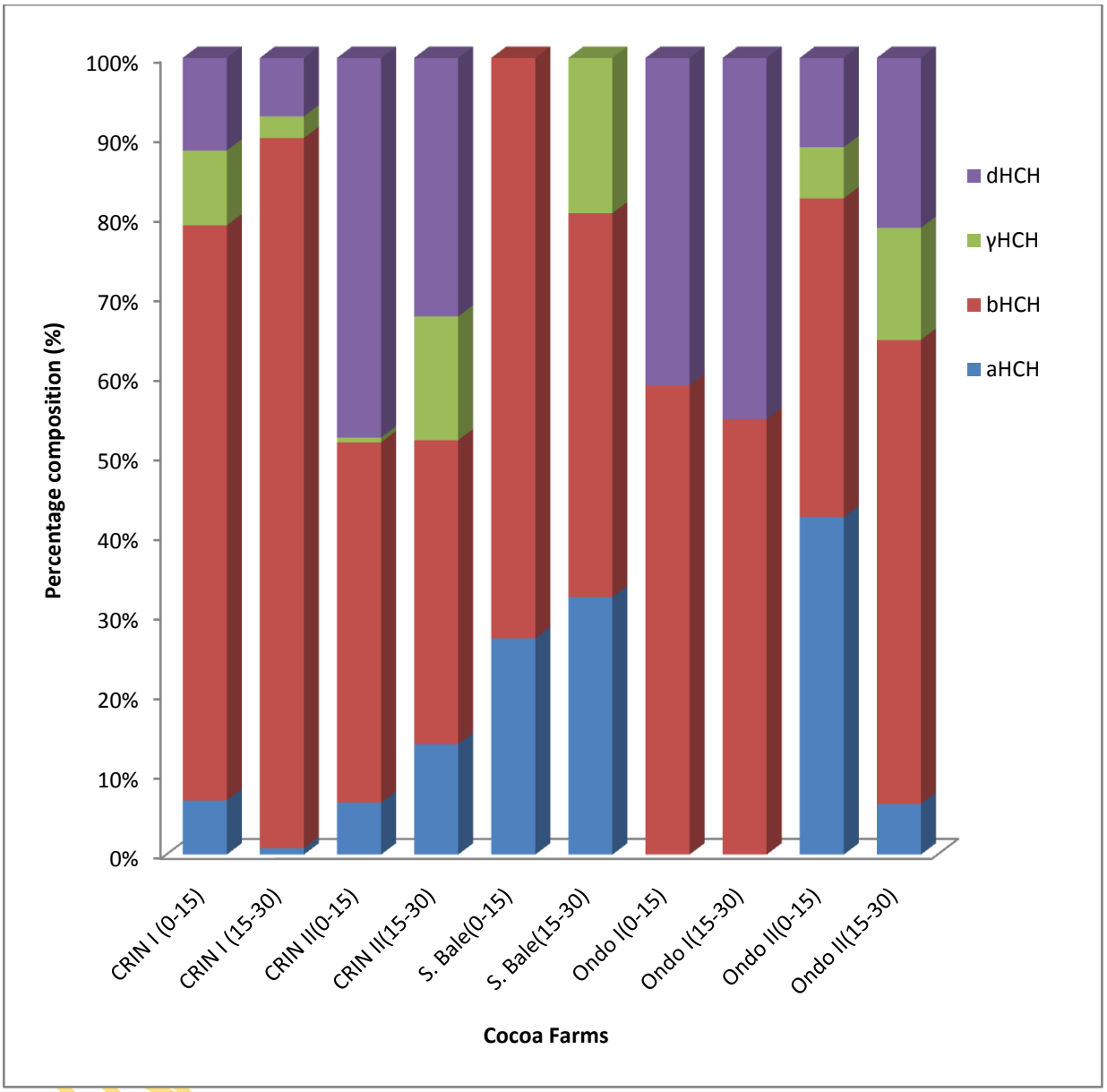


Figure 4.15. Composition of HCH isomers in soil profiles in the dry season

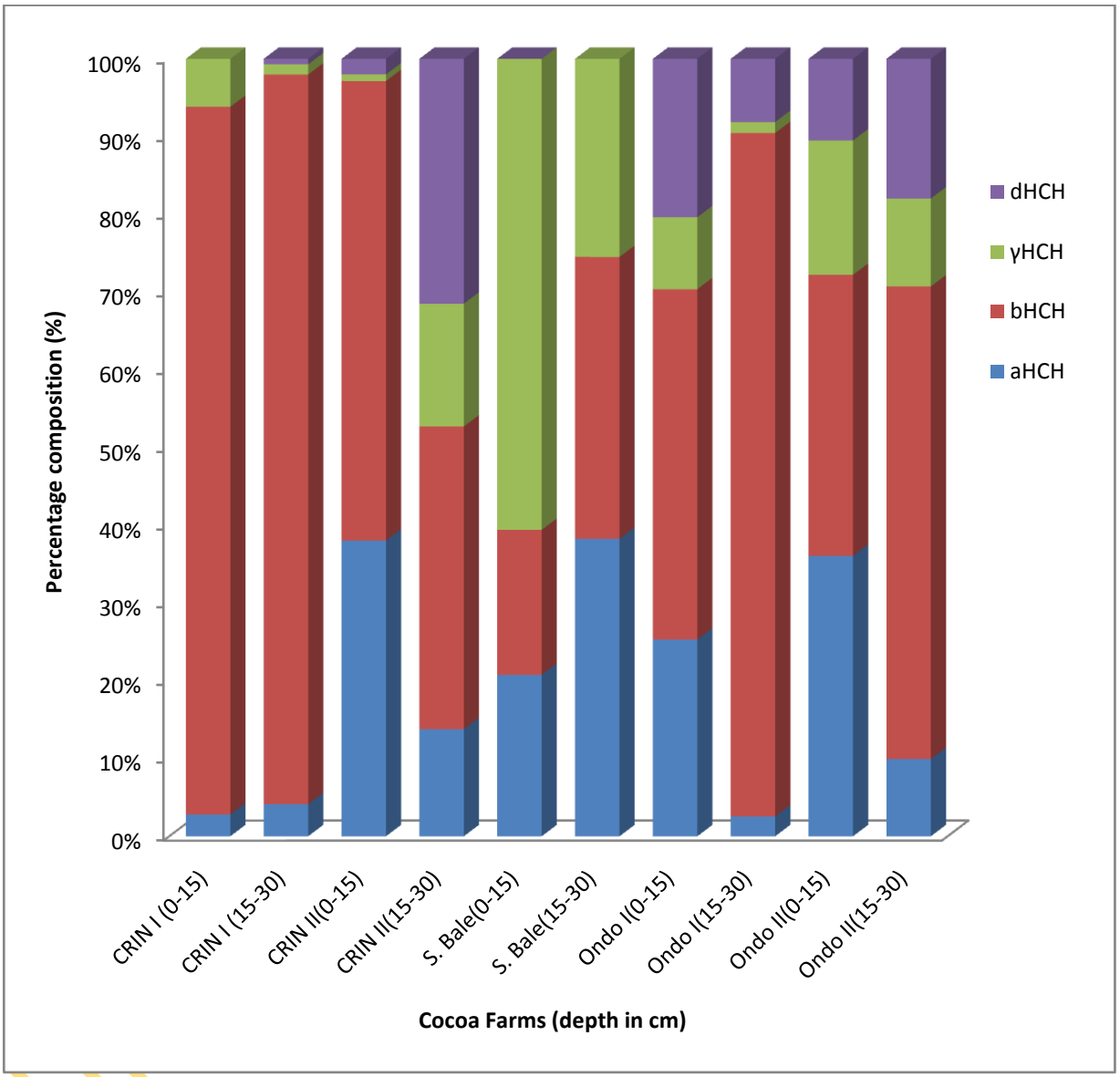


Figure 4.16. Composition of HCH isomers in soil profiles in the wet season

In CRIN II, Sore Bale, Ondo I and Ondo II, β-isomer contributed 42.86%, 72.85%, 58.90% and 35.28% respectively to ∑HCH at top soil during the dry season, while at 15-30cm depth profile, β-HCH contribution was 89.15%, 38.17%, 48.19%, 54.65% and 58.23% to∑HCH for CRIN I, CRIN II, Sore Bale, Ondo I and Ondo II respectively.

In the wet season, percentage contribution of β-HCH at 0-15cm and 15-30cm depths were CRIN I (91.01%, 93.85%), CRIN II (59.12%, 38.93%), Sore Bale (18.63%, 36.26%), Ondo I (45.20%, 97.52%), Ondo II (36.14%, 60.78%) respectively. Relatively low percentage contribution in Sore Bale top soil may have being due to fresh application of the pesticide, since OCPs are still being used in this farm. Residual levels of β-HCH were more predominant and distributed in the bottom profile than in the top soil. This is in agreement with the findings ofWitter *et al.* (2003), Gotz *et al.* (2006) and Kiersch *et al.* (2010) in which β-HCH isomer had the largest concentration at both depths (0 – 10 cm and 10 – 20 cm). Also residual levels reported were comparable to values obtained in this study. Residual concentrations range of < 1 - 92 ngg^{-1} for 0 -10 cm depth (Witter *et al.* 2003); < 1 - 170 $ngg^{-1}$for depth 10 – 20 cm (Gotz *et al.* 2006); 1.2 - 17 ngg^{-1} and 0.23 - 25 ngg^{-1} for 0-10cm and 10-20cm respectively (Kiersch *et al.* 2010). The persistence and dominance of β-HCH and δ-HCH may be due to their physicochemical properties. β-HCH is known among the HCHs to have the lowest water solubility and vapour pressure, and most stable and resistant to microbial degradation, while δ-HCH has the longest half-life of the HCH isomers (Willet *et al.* 1998), they could also exist in the environment for several years or longer (Simmonich and Hites, 1995). Also, the γ-isomer is known to bio-isomerize to α-isomer in the environment (Steinwandter, 1976; Waliszewski *et al.* 2004), which in turn can be converted to β-isomer (i.e., a transformation trend represented thus; γ → α → β) and in addition β-HCH is resistant to hydrolysis and environmental degradation (Wu *et al*, 1999; Walker*et al.* 1999). All aforementioned properties would tend to cause the β- isomer to be more predominate and persistent in the environment relative to other isomers.

The residual concentration of ∑HCH for top and bottom soils in all five (5) cocoa farms were comparable to HCH value of 37.30±2.85 ngg^{-1} reported by Kumar *et al.* (2011) for contaminated agricultural soils in Bhupander, North India, except for CRIN

I, where bottom soil samples were 156.756 and 161.646 $ngg^{-1}$for dry and wet seasons respectively(Tables 4.10 and 4.11). However, these values were significantly lower, when compared to values (30.19±20.30 ngg^{-1} - 141.23±84.53 ngg^{-1}) reported in topsoils (0 – 15 cm) for Oke-Osun farm settlement, Osogbo, Osun State, Nigeria for dry and wet seasons (Oyekunle *et al*. 2010), from cocoa farms in three local Government Areas in Ondo State (Aiyesanmi and Idowu, 2012), in agricultural soils from A Coruna (336.8 ±418 ngg^{-1}) and Lugo (110.3 ± 97.2 ngg^{-1}) provinces of North West Spain (Pereira *et al*., 2010).Also Osibanjo (2003), Waliszewski *et al.*(2004) and Manirakiza *et al.* (2003) reported HCH residue of 8.7 ngg^{-1}, 2.5 ± 3.3 $ngg^{-1}$and 0.7 ± 0.4 - 19.6 ± 9.3 ngg^{-1} in some Nigerian, Mexican and nine (9) Sene-Gambian farm soils respectively. The ∑HCH residues obtained for topsoils in CRIN II (wet season), Ondo I (both seasons) were comparable, while others wererelatively higher.

Technical-grade HCH is composed of α-HCH (60-70%), β-HCH (5-12%), γ-HCH (10-15%), δ-HCH (6-10%) and ε-HCH (3-4%) (Walker *et al*. 1999) - this implies that the ratio of αHCH/γ-HCH is 4-7, while pure lindane contains approximately 99% γ-HCH (Iwata *et al*. 1993). Therefore, αHCH/γ-HCH ratio could be used to predict the type and time of application (i.e., historical use) of this pesticide.

Considering CRIN I and Ondo II, where all HCH isomers were detected (top and bottom soils), α-HCH/γ-HCH ratio ranged between 0.29 - 3.14 and 0.45 - 6.60 repectively in both seasons. These ratios ranges showed that both lindane and technical grade may have been applied in both farms in time past. The α-HCH/γ-HCH ratio of 0.29 recorded at CRIN I, 15-30cm depth - implied higher residual concentration of the γ-isomer at that profile (which favours the application of lindane); this may be due to its high solubility in water and therefore would migrate faster to the bottom soil than the α-isomer. Also the high ratio of 6.60 at Ondo II, implied the predominance of the α-isomer, which may be as a result of the application of technical grade HCH and bio-transformation (or –isomerism) of γ-isomer to α-isomer (Steinwandter, 1976; Waliszewski *et al*. 2004). The detection of δ-HCH also implied that technical grade HCH was used in the past.

Endosulfan distribution in farm soils

Endosulfans were detected in all five cocoa farm soils in both seasons. Total endosulfan (∑endosulfan) for both depths and seasons ranged from 1.05±0.73 ngg^{-1} (Sore Bale, 15-30 cm, wet season) to 579.30 ±543.71ngg^{-1} (CRIN I, 15 -30 cm, dry season) (Tables 4.10 - 4.14). In the dry season, residual concentration of ∑endosulfan in soil profiles (0-15cm, 15-30cm) were CRIN I (432.25ngg^{-1}, 579.30 ngg^{-1}), CRIN II (5.00 ngg^{-1}, 8.67 ngg^{-1}), Sore Bale (5.00 ngg^{-1}, 6.67 ngg^{-1}), Ondo I (8.00 ngg^{-1}, 159.67 ngg^{-1}) and Ondo II (2.04 ngg^{-1}, 9.38ngg^{-1}). CRIN I farm soil recorded the highest residual concentration of ∑endosulfan, followed by Ondo I, while Ondo II was the least contaminated (Tables 4.10 and 4.13). In CRIN I, residual concentrations of individual parent compounds and metabolite were 23.00 (0.01 -68.90) ngg^{-1}, 92.51 (0.03 – 262.41) $ngg^{-1}$and 316.75 (19.63-876.73) ngg^{-1} for α-endosulfan, β-endosulfan and endosulfan sulphate respectively for 0-15cm depth, while corresponding values for 15-30cm depth were, 10.00(5.01- 15.00) ngg^{-1}, 125.75 (19.39 -245.15) ngg^{-1} and 443.55 (0.001 – 1055.41) ngg^{-1} with ranges in parentheses. The percentage contribution to ∑endosulfan at top- and sub-surface soils were, α-endosulfan 5.3%, 1.7%; β-endosulfan 21.4%, 21.7 and endosulfan sulfate 73.3%, 76.6% respectively (Figure 4.17), while endosulfan sulphate/endosulfan ratios were >2.7. The relatively lower concentrations of parent endosulfan to its metabolite - endosulfan sulphate suggested that source of contaminant was due to the usage of technical grade endosulfan in the past. Almost the same trend was observed in CRIN I (wet season), with endosulfan sulphate contributing 95.1% and 65.4% at 0-15 and 15-30cm respectively (Figure 4.18). Manirakiza *et al.,* (2002) reported the same trend for cultivated soils in seven (7) of nine (9) farms in Sene-Gambia.

The compositional percentage of endosulfan sulphate in the dry season at 0-15 and 15-30cm depths for other farms were; CRIN II - 56.4%, 80.8%; Sore Bale - 13.3%, 48.3%; Ondo I - 0%, 62.2%; and Ondo II - 46.5%, 66.7% (Figure 4.15), while corresponding endosulfan sulphate/endosulfan ratios were 1.3, 4.2; 0.2, 0.9; 0, 1.7; and 0.9, 1.2 respectively. The sub-surface soil recorded compositional values> 48.0% for endosulfan sulphate in all the farms – these high percentages may have being due to migrationand aging of the applied parent endosulfan especially in CRIN I, CRIN II and Ondo I.

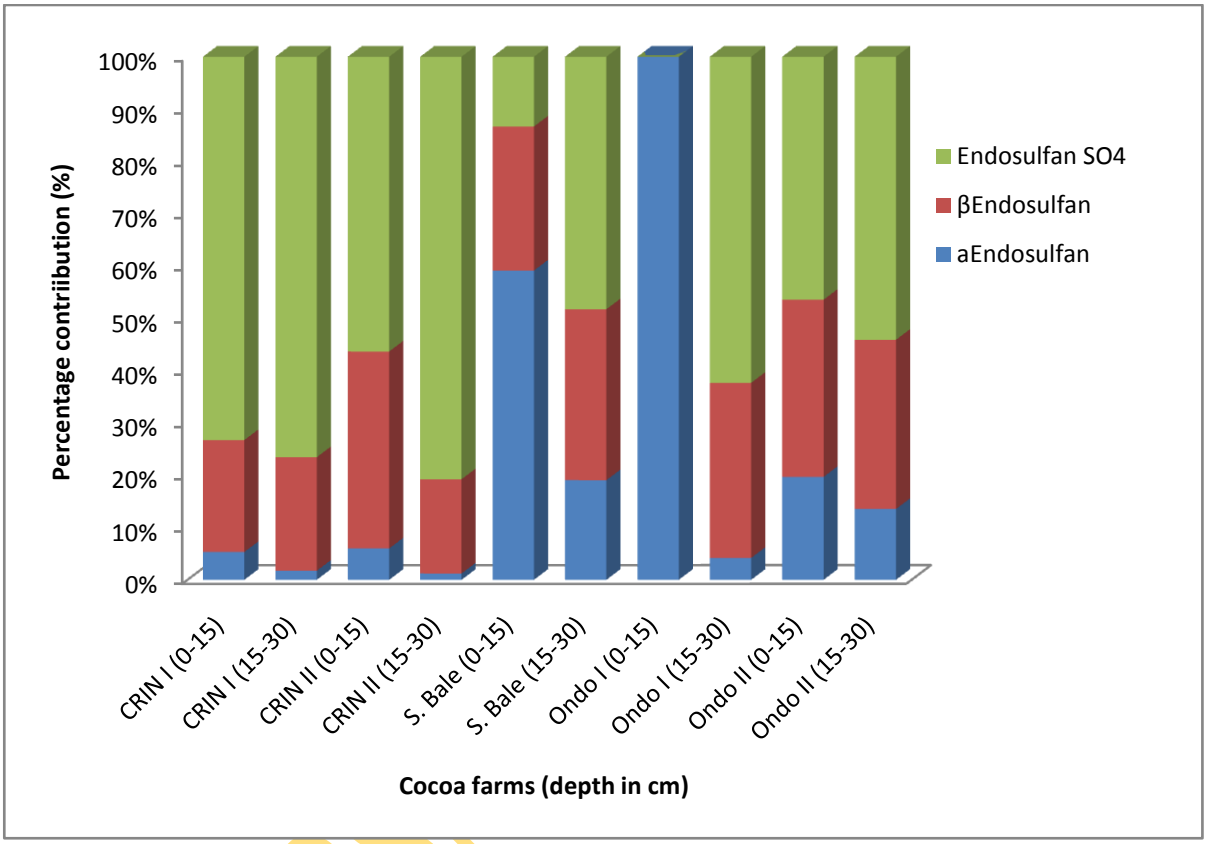


Figure 4.17. Distribution of endosulfans in cocoa farm soils at 0-15 and 15-30cm depths during the dry season

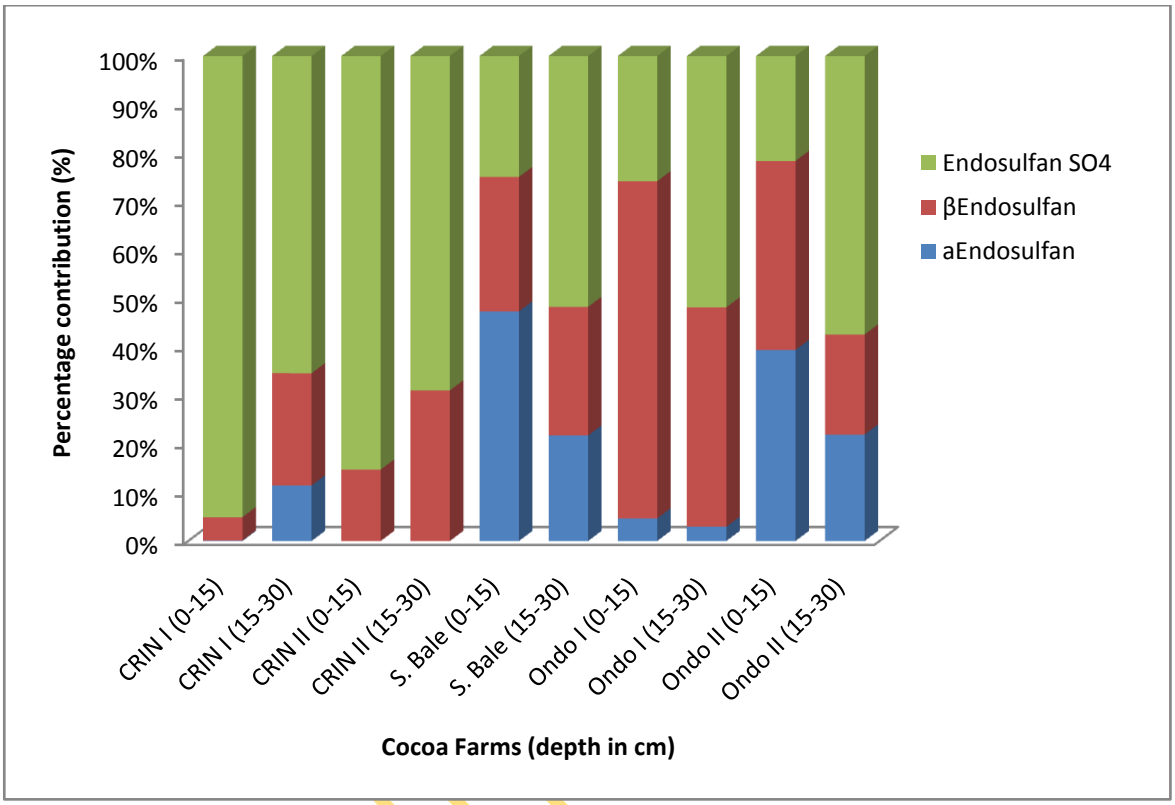


Figure 4.18. Distribution of endosulfans in cocoa farm soils at 0-15 and 15-30cm depths during the wet season

Low percentage composition of the metabolite at the top profile especially at Sore Bale (13.3%) and Ondo I (0%), implied high residual concentration of parent endosulfan, which indicated recent application of parent endosulfan to the environment. In addition, the endosulfan sulphate/endosulfan ratios for bottom soils were all >1, except at Sore Bale (0.9), which also corroborated the likely migration of endosulfan to the bottom soil due to long term application in the other farms and fresh use at Sore Bale. Technical grade endosulfan is composed of 7:3 ratio of α- and β-endosulfan respectively. The α- and β-endosulfan ratios for residual parent endosulfan in Sore Bale were 2.14 and 0.58 for top- and sub-surface soils respectively, with corresponding endosulfan sulphate/endosufan ratios being 0.2 and 0.9. The α-/β-endosulfan and endosulfan sulphate/endosufan ratios obtained for top soils in this farm portrayed a recent application, while the latter ratio pairs for the sub-surface soilindicated old usage.

In the wet season, residual concentration for ∑endosulfan in 0-15 cm depth ranged between 4.81 ± 6.65 $ngg^{-1}$and 213.69 ± 247.87 ngg^{-1}, with standard deviation in parenthesis, while a range from 1.05 ± 0.73 $ngg^{-1}$to 546.71 ± 138.13 ngg^{-1} was obtained for 15-30cm depth profile. CRIN I recorded the highest residual concentrations at depths 0-15 and 15 -30 cm, while the least contamination was observed in CRIN II and Sore Bale at 0-15 and 15 -30 cm depths respectively. Individual contribution of α-endosulfan, β-endosulfan and endosulfan sulphate to ∑endosulfan in CRIN I top soil was 0.23 ngg^{-1}(0.2%), 10.28 ngg^{-1} (4.8%), 203.18 ngg^{-} (95.1%) respectively, with percentage contribution to ∑endosulfan residue in parenthesis, while in bottom soil 62.61 ngg^{-1} (11.45%), 126.56 ngg^{-1} (23.2%), 357.54 ngg^{-1} (65.4%) (Figure 4.18).

The endosulfan sulphate/endosulfan ratio for both soil depths was > 1.9. The high percentage contribution of endosulfan sulphate to ∑endosulfan and endosulfan sulphate/endosulfan ratios obtained implied the dominance of the metabolite over the parent isomers and this is suggestive of long time usage of endosulfan and microbial action or in-situ bio-transformation of endosulfan isomers (Guerin and Kennedy, 1992; Kennedy *et al*. 2001).

Residual concentrations of ∑endosulfan in top and bottom soils in CRIN I were significantly higher than values reported for Oke-Osun farm Osun State, Nigeria

(Oyekunle *et al*. 2011), however, values obtained in CRIN II, Sore Bale, Ondo I and Ondo II were lower. Getenga *et al.*(2004), Westbom *et al*. (2008) and Aiyesanmi and Idowu, (2012) reported residual levels much higher for contaminated farm soils in Upper Awash Agro Farm, Ethiopia; Nyando Farm soil, Lake Victoria, Kenya and cocoa farms in Ondo State, Nigeria respectively.

The 15-30cm soils for CRIN I, CRIN II, Sore Bale and Ondo I were more contaminated as $[\sum endosulfan]_{15\text{-}30cm} > [\sum endosulfan]_{0\text{-}15cm}$, this may have resulted mainly from the migration of endosulfan deposited on the soils from the top soil with time. The detection of parent endosulfan and metabolites in CRIN I, CRIN II and Ondo I depicted persistence of endosulfan pesticide, since OCPs were applied over 3 years ago in these farms.

Conentration of aldrin and its analogues in farm soils

∑Aldrin (sum of aldrin and its analogues – dieldrin, endrin, endrin aldehyde and endrin ketone) was one of the most dominant pesticides detected in the soils of the five farms assessed (Tables 4.10 - 4.14). ∑Aldrin was the most dominant in CRIN II (0-15cm and 15-30cm, dry season) (Table 4.10), Ondo I (0-15 cm, wet season) (Table 4.14), while it was the second most dominant group in CRIN I (at both depths and seasons) (Tables 4.10 and 4.11), Sore Bale (0-15cm and 15-30cm, dry season) (Table 4.12), Ondo I (0-15 cm, dry season; 15-30cm, wet season) (Tables 4.13 and 4.14), Ondo II (0-15, dry season; 15-30 cm, dry/wet seasons) (Tables 4.13 and 4.14). ∑ Aldrin at 0-15cm depth ranged between 0.70 ±0.99 ngg^{-1} and 124.75±116.87 ngg^{-1}, while from 0.18 ±0.05 ngg^{-1} to 343.12±287.55 ngg^{-1} for 15-30cm depth. CRIN I top-soil (dry season) and sub-surface soil (wet season) were the most contaminated, while CRIN II top soil (wet season) and Sore Bale sub-surface soil (wet season) were the least contaminated. This implied that CRIN I and Sore Bale soils recorded the highest and least levels of ∑aldrin contaminant in considering both depths and seasons together. These values were significantly lower than ∑aldrin residue of 652 ngg^{-1} reported by Otani and Seike (2006) for a farm in Japanand below the South Australian EPA maximum limit of 2 $mgkg^{-1}$ (2000 ngg^{-1}) for contaminated soils (EPA,South Australia, 2009).In CRIN I, during the dry season aldrin and all its analogues were detected at both depths. The residual concentrations in 0-15cm depth for aldrin, dieldrin, endrin, endrin aldehyde and endrin ketone were 25.75 ±31.97 ngg^{-1} (20.6%), 22.50 ±25.03

ngg^{-1}(18.0%), 46.75±32.61 ngg^{-1} (37.5%), 19.50±13.10 ngg^{-1} (15.6%) and 10.25± 14.17 ngg^{-1} (8.2%) respectively (Tables 4.10, 4.12 and 4.13), with percentage contribution to ∑aldrin in parentheses; while thecorresponding values at 15 – 30 cm depth were 3.25 ± 3.85 ngg^{-1} (1.0%), 1.50 ± 1.29 ngg^{-1} (0.4%), 17.01 ± 22.65 ngg^{-1} (5.1%), 255.42 ± 182.18 ngg^{-1} (76.4%), 57.25 ± 32.21 ngg^{-1} (17.1%) respectively, – making endrin (and its metabolites) the most dominant in CRIN I (Figures 4.19 and 4.20). The high residual presence of endrin aldehyde (a metabolite of endrin) at the bottom layer indicated long term usage of aldrin (or dieldrin) in this farm and its predominance in the sub-surface soil may have been due to its vertical migration or leaching from topsoil to sub-surface soil. Endrin is an isomer of dieldrin and the latter is formed by the epoxidation of aldrin (Zitko, 2003).

Endrin was the only OCP detected in soil samples in all the five sites studied (Tables 4.10 – 4.14), with values ranging between 0.13 ± 0.09 ngg^{-1} (Ondo II, 0-15cm, wet season) and 114.00 ± 136.80 ngg^{-1} (Sore Bale, 0-15 cm, dry season). These ranges were comparable with values reported for Oke-Osun farm settlement, where residual concentration of endrin ranged from 14.79 – 120.78 ngg^{-1} and ND – 96.43 ngg^{-1} for dry and wet seasons respectively (Oyekunle *et al.* 2010), but were significantly higher than those reported by Manirakiza *et al.* (2003) in soils from farms in Senegal and Gambia (ND - 1.0 ± 1.6 ngg^{-1}).

Among the 'drins', endrin was the most dominant, with detection frequencies ranging from 33.3% to 100% and 66.7% to 100% for top and bottoms during both seasons (Table 4.8). Aldrin, dieldrin and the metabolites of endrin (endrin ketone and endrin aldehyde) were not detected in Sore Bale at both soil depths in the dry season and bottom layer in the wet seasons. Other sites where the same trend (i.e., endrin alone found) was observed were; CRIN II (0 -15 cm, 15-30 cm, dry season) and Ondo I (0-15 cm, 15 -30 cm, dry season).

However, low levels of endrin aldehyde (0.03 ngg^{-1} /top soil, 1.82 ngg^{-1} /sub-surface soil) were detected in CRIN II during the wet season in addition to endrin. The detection of only endrin in these sites suggested recent application of endrin pesticide or epoxidization of aldrin which resulted into endrin only (without dieldrin).

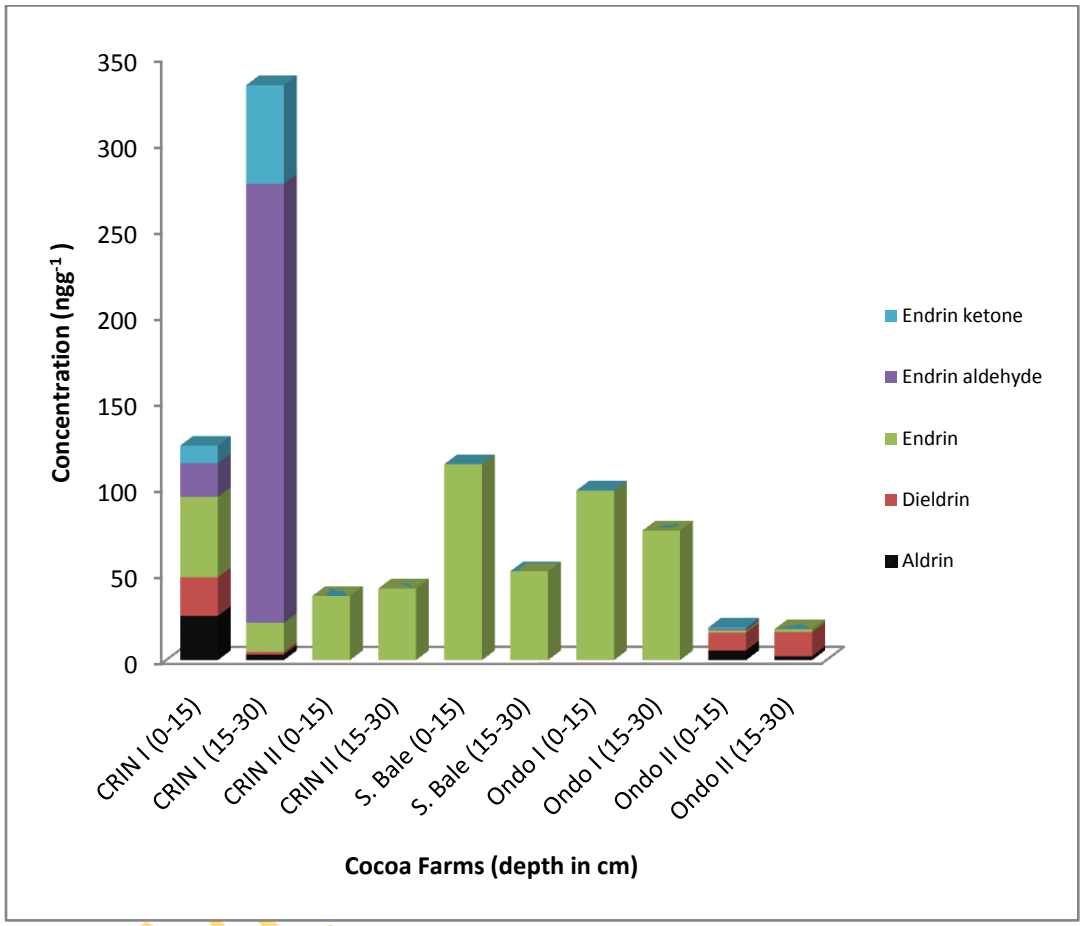


Figure 4.19. Distribution of Aldrin analogues in cocoa farm soils during the dry season

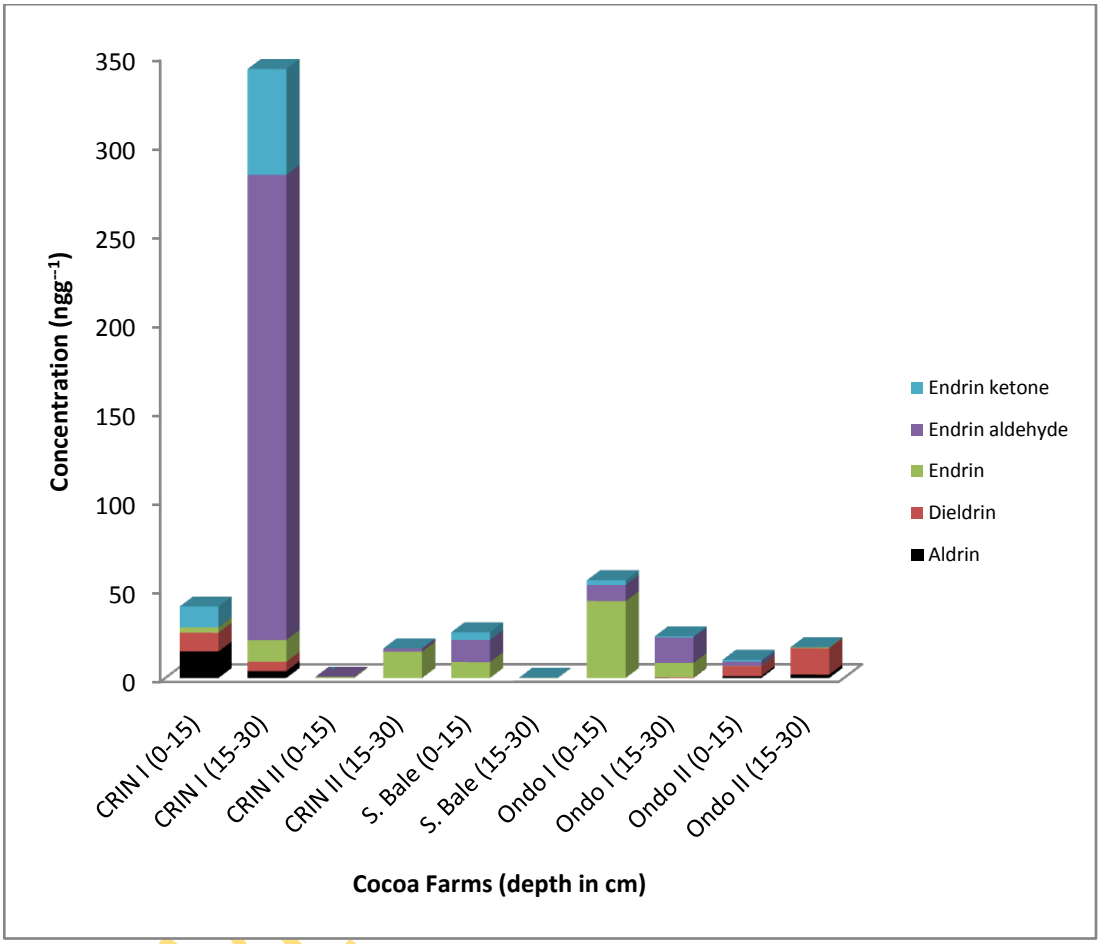


Figure 4.20. Distribution of Aldrin analogues in cocoa farm soils during the wet season

Endrin and dieldrin are isomers and they are epoxidized form of aldrin (Zitko, 2003). Furthermore, there may have been absence of metabolic action on endrin by micro-organisms or photo-isomerization in the soils as none of its metabolites - endrin ketone and endrin aldehyde were detected in the aforementioned sites; especially at depth 15-30cm due to absence of UV- light and endrin degrading microorganism. However, the high residual concentrations of 255.42 ±182.18ngg^{-1} (dry season) and 262.04 ±188.21 ngg^{-1} (wet season) recorded for endrin aldehyde at 15-30 cm depth in CRIN I soil, suggested anaerobic activity/photolytic action on endrin. The compositional percentage of endrin aldehyde was 76.4% (dry and wet seasons), while endrin ketone 17.1%, 17.4% to ∑aldrin at this depth - both metabolites of endrin contributed> 93% to total aldrin. In all the cocoa farms in both seasons and depths, endrin and its metabolites contributed between 2.8 % (Ondo II, 15-30cm, wet season) and 98.6% (Ondo I, 15-30cm, wet season), where other parent 'drins' were detected (Figure 4.19 and 4.20) .

The mean residual concentrations for other individual parent members at both soil depthsrangedfrom ND - 25.75 ± 31.97 ngg^{-1} (CRIN I, dry season) and ND - 22.50 ± 25.03 ngg^{-1} (CRIN I, dry season) aldrin and dieldrin respectively. These values were lower when compared to values reported in farm soils in some African countries (Getenga *et al.* 2004; Oyekunle *et al.* 2012; Idowu *et al*. 2013). Getenga *et al.* 2004 reported residual concentrations of aldrin and dieldrin in soil along (a sugar belt region) Lake Victoria Basin, Kenya, however, levels obtained in this study were significantly higher than in soils reported for Sene-Gambia farms (Manirakiza *et al.* 2003) and forest reserve in South-Western Uganda (Ssebugere *et al.* 2010).

Dichlorodiphenyl trichloroethane (DDT)residual concentration in soil

Parent pp'-DDT and metabolites (pp'-DDE and op'-DDD) were detected at both depths (0-15cm and 15-30cm) in all the cocoa farms during both seasons (Tables 4.10 - 4.14), except in CRIN II, where only pp'-DDE was detected at 15-30 cm depth in the wet season (Table 4.16). The residual levels of ∑DDT in all farms at the top-soil and sub-surface soil ranged from ND - 344.33±351.25 ngg^{-1} (Sore Bale, dry season) and ND - 92.25±86.53 ngg^{-1} (CRIN I, dry season) respectively. Sore Bale farm soil was the most contaminated with respect to ∑DDT (sum of pp'DDT, pp'DDE, and op'DDD). In the dry season, concentrations of pp'DDT, pp'DDE, and op'DDD in Sore Bale at 0-15cm depth were 316.86 (43.10 - 783.24) ngg^{-1}, 27.47 (ND- 45.97) ngg^{-1} and ND

respectively, with ranges in parentheses, while at 15-30cm profile corresponding values were 53.66(0.01 - 153.29) ngg^{-1}, 4.01 (ND-12.03) ngg^{-1} and ND. Total DDT was predominately due to parent pp'DDT, with a compositional contribution >62% at both depths, while the pp'DDE+op'DDD/pp'DDT ratio was < 0.6 for both soil depths (Figure 4.21). The relative concentrations of the parent DDT compounds to its metabolites can be used as indices for assessing the origin of the contaminant and likely period of application (Doong *et al.* 2002). These values - compositional contribution to ∑DDT and pp'DDE+op'DDD/pp'DDT ratio portrayed recent treatment of the Sore Bale farm with parent pp'DDT. The ratio of inverse (or reciprocal) pp'DDT to its metabolites has been used to infer sources and judge adequately the age of contaminant residues in soil (Zhu *et al.* 2005; Wesbom *et al.* 2008). A vast range of varying ratios from soil samples have been reported in previous studies: 2.3 - 2.8 (Popp *et al.* 1997), 0.03 (Fitzpratrick *et al.* 2000), 5.78 (Hubert *et al.* 2000), 0.006 - 63.9 (Zhu *et al.* 2005), 0.008 and 0.007 (Hussen *et al.* 2006) and 0.008 - 0.14 (Wesbom *et al.* 2008). In this study the ratio ranged from 0.09 (Sore Bale) to 0.40 (CRIN I) and 0.07(Sore Bale) to 4.07 (CRIN I) for topsoil and sub-surface soilrespectively in the dry season (excluding where either both metabolites or parent DDT were not found) (Figure 4.22)

Other sites with ratio values > 0.50 at both depths and seasons were CRIN I 1.20 (0-15cm, wet season), 6.45 (15-30cm, wet season), Sore Bale 1.08 (0-15cm, wet season). pp'DDE+op'DDD/pp'DDT ratios > 0.5 indicated long-term weathering. The ratios obtained at the aforementioned top soils at CRIN I and Sore Bale, inferred the usage of parent pp'DDT in the recent past, while much higher ratio at the lower depth (CRIN I) depicted that samples originated from old sources or usage. Based on the application history, the lower ratio observed for CRIN I topsoil may have been due to aerial deposition or precipitation rather than direct or fresh application, while the sub-surface soilmust have being due to old usage and downward migration of pesticide with time. OCPs are known for their long range/distance atmospheric transport (LRT) and deposition as a result of volatization and global distillation (Wania and Mackay, 1996; Waliszewski *et al.* 2004) from initial point source (or of usage). OCPs have been detected in places, such as the artics - where they have never being used (Weber *et al.* 2010).

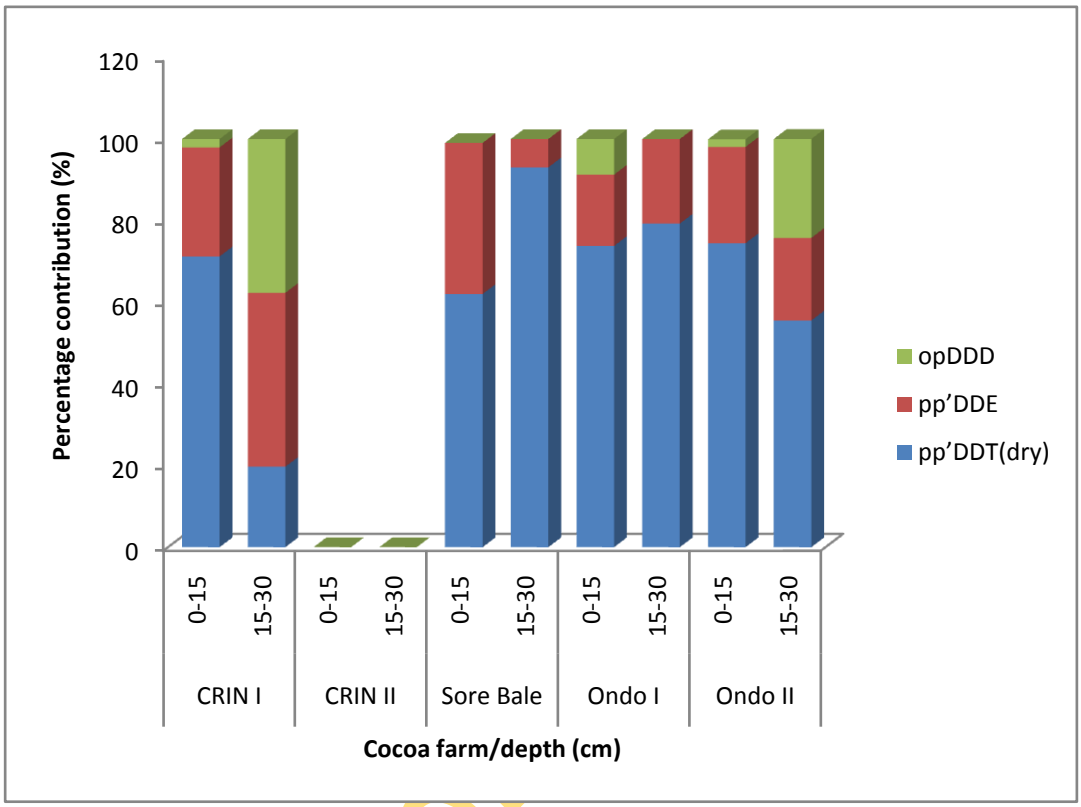


Figure 4.21. Composition of pp,DDT and its analogues in cocoa farm soils during the dry season

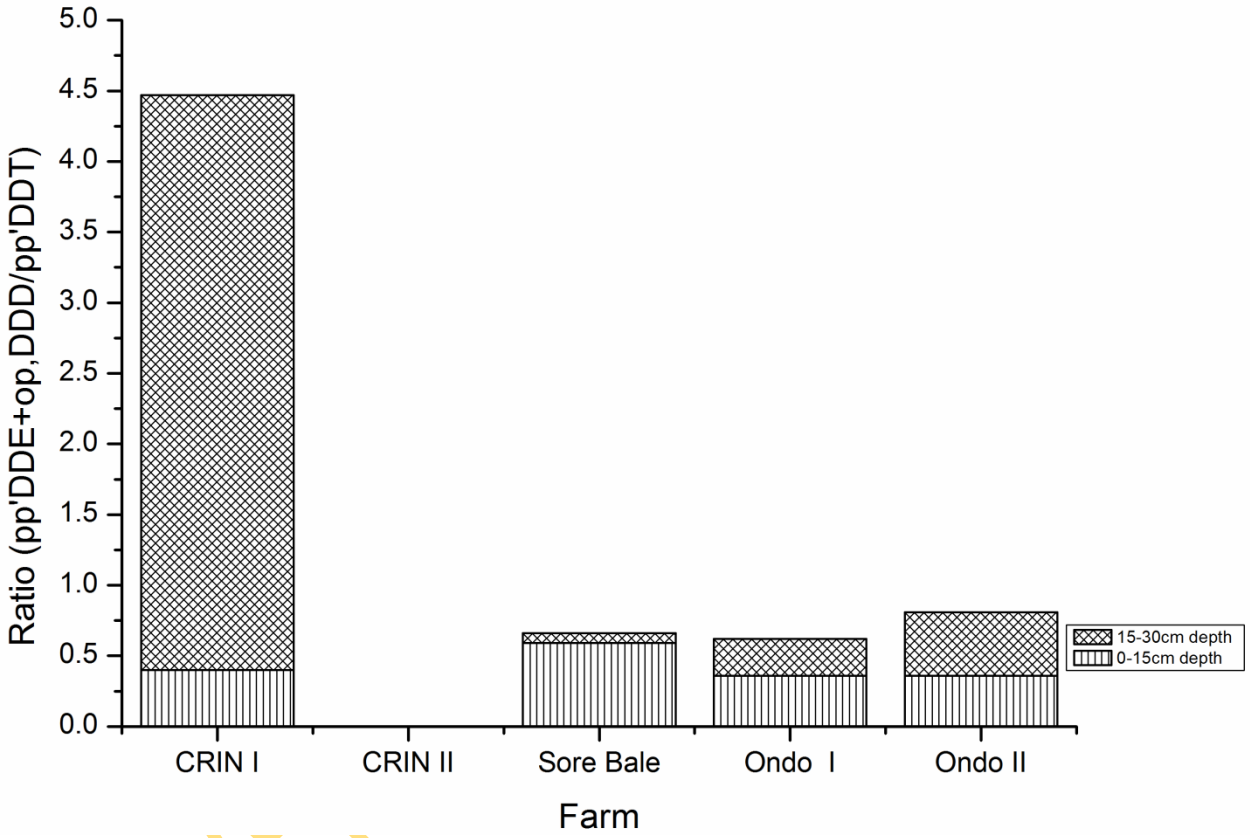


Figure 4.22. Ratio of parent DDT (pp,DDT) to the sum of metabolites (pp'DDE+op'DDD) in cocoa farm soils during the dry season

Also, a comparative residual concentration of DDT metabolites is key to decipher the type of biodegrading microbes present or process vis-à-vis if environment is anoxic or not. Biodegradation of DDT in an aerobic environment is reported to favour the formation of pp'DDE, while anaerobic conditions tend to lead the op'DDD. In comparing the residual concentrations of both metabolites, it was observed that the average percentage dominance at 0-15cm were 90% and 10% for pp'DDE and op'DDD respectively, while at depth 15-30cm, op'DDD was slightly higher, with an average percentage dominance of 53%. This therefore implied that the cultivated top soils were more and well aerated, while the 15-30cm depth may have been slightly more anoxic.

In the wet season, concentrations of residual ∑DDT at 0-15cm depth ranged from ND (CRIN II, Ondo I) - 89.26 ± 100.29 ngg^{-1} (Sore Bale) and 0.97 ±1.37 ngg^{-1} (CRIN II) - 79.49±66.08 ngg^{-1} (Ondo I), at depth 15-30 cm (Tables 4.11, 4.12 and 4.14). Sore Bale and Ondo I farm soils were the most contaminated at the top and bottom layers respectively.

The individual percentage contribution of parent DDT, pp'DDE and op'DDD in Sore Bale farm soil to ∑DDT at 0-15 cm depth was 48.2%, 51.1% and 0.7% respectively (Figure 4.23), while the reciprocal residual concentration ratio of parent DDT to its metabolites was 1.07 (Figure 4.24). These values inferred the application of the pesticide in recent past – since ratio > 1 is suggestive of aged and weathered DDT, while with respect to both metabolites the Sore Bale environment favoured the presence of aerobic biodegrading micro-organisms (Heberer and Dunnbier, 1999; Gao *et al.* 2008).

At the lower layer (15 -30 cm), Ondo I recorded individual residual concentrations of 37.08 (0.01 -72.27) ngg^{-1}, 33.40 (0.07-54.14) ngg^{-1} and 9.02 (ND-27.05) ngg^{-1} for pp'DDT, pp'DDE and op'DDD respectively, with ranges in parentheses, while corresponding individual percentage contribution to ∑DDT was 46.7%, 42.0% and 11.3% (Figure 4.23). Although, pp'DDT was the most dominant individual component, its concentration inverse ratio to those of its metabolites pp'DDE and op'DDD was > 1, indicating 'old usage' or aging and weathering of parent DDT.

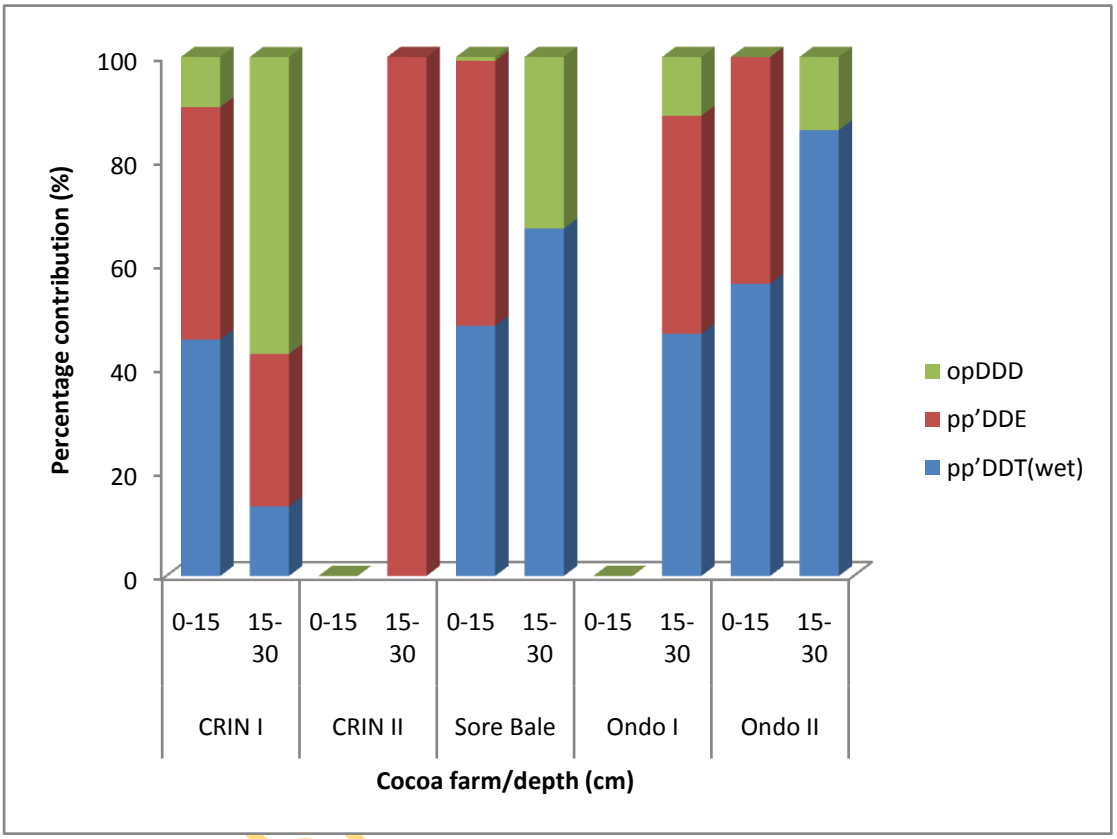


Figure 4.23. Composition of pp,DDT and its analogues in cocoa farm soils during the wet season

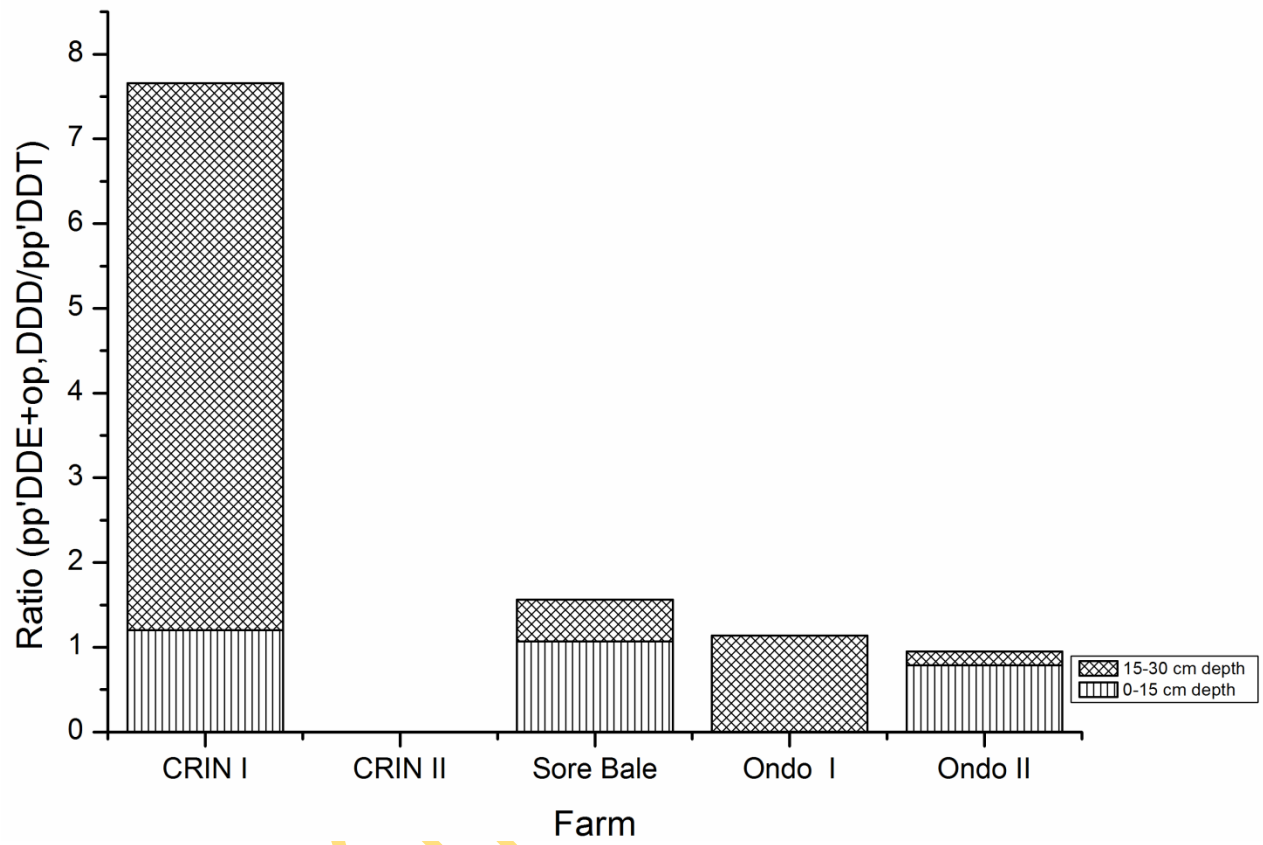


Figure 4.24. Ratio of parent DDT (pp,DDT) to the sum of metabolites (pp'DDE+op'DDD) in cocoa farm soils (wet season)

This is in agreement with the history of OCP application in the Ondo I farm environment where they were last applied over 3 years ago. Again, the metabolite ratio of pp'DDE to op'DDD ratio was 3.71, which inferred a more aerated sub-soil environment for aerobic microbial activity.

The sum of pp'DDT and its metabolites recorded at both depths in CRIN I, CRIN II, Ondo I and Ondo II farms were comparable to previous residual concentrations reported in other African agricultural soils, such as 1.5 - 71.4 ngg^{-1}in Sene-Gambia, West Africa (Manirakiza *et al.* 2003), 1.4 – 180ngg^{-1} in Tanzania, East Africa (Kishimba *et al.* 2004) and 120 ngg^{-1} in Brazzaville, Congo (Ngabe and Bidleman, 2006), however, the mean value of 344.330 ngg-1 obtained in Sore Bale (dry season, 0-15cm) was significantly higher than the aforementioned, but were comparable to concentrations in Oke-Osun farm settlement, Nigeria (Oyekunle *et al.* 2010) and in Dehradun rice farm, India (Babu *et al*. 2003). Also Lei *et al.* (2010) and Waliszewski *et al.*(2004), reported ∑ DDT residues of 51.26 ± 2.70 ngg^{-1} and 54.2± 21.3 ngg^{-1} for farm soils in Japan and Mexico respectively. The residual concentrations recorded for the five sites were all below the maximum allowable limit (MAL) of 2 $mgkg^{-1}$ (2000 ngg^{-1}) for contaminated soils in South Austrialia region (South Austrialia EPA, 2009).

There were strong positive relationships between parent pp'DDT and the sum of its metabolites (∑pp'DDE+op'DDD) in both seasons and depths (Figures 4.25 and 4.26). The correlation coefficient (r) obtained for all sites and both depths for dry and wet seasons were 0.8096 and 0.9377 respectively ($p < 0.005$), while seasonal relationship between dry and wet seasons for the ratios of metabolites to parent DDT was 0.0.9610 (Figure 4.27). However, seasonal relationship between dry and wet seasons for the sum of metabolites was moderate ($r < 0.5$, $p < 0.005$) (Figure 4.28).

Heptachlor concentrations in farm soils

Total heptachlor (i.e., sum of heptachlor and its' metabolite heptachlor epoxide, ∑heptachlor) in all the sites ranged from ND – 31.00 ± 43.14 ngg^{-1} and ND - 3.99 ± 4.21 ngg^{-1}at depths 0-15 cm and 15-30cm respectively, in the dry season (Tables 4.10, 4.12 and 4.13).

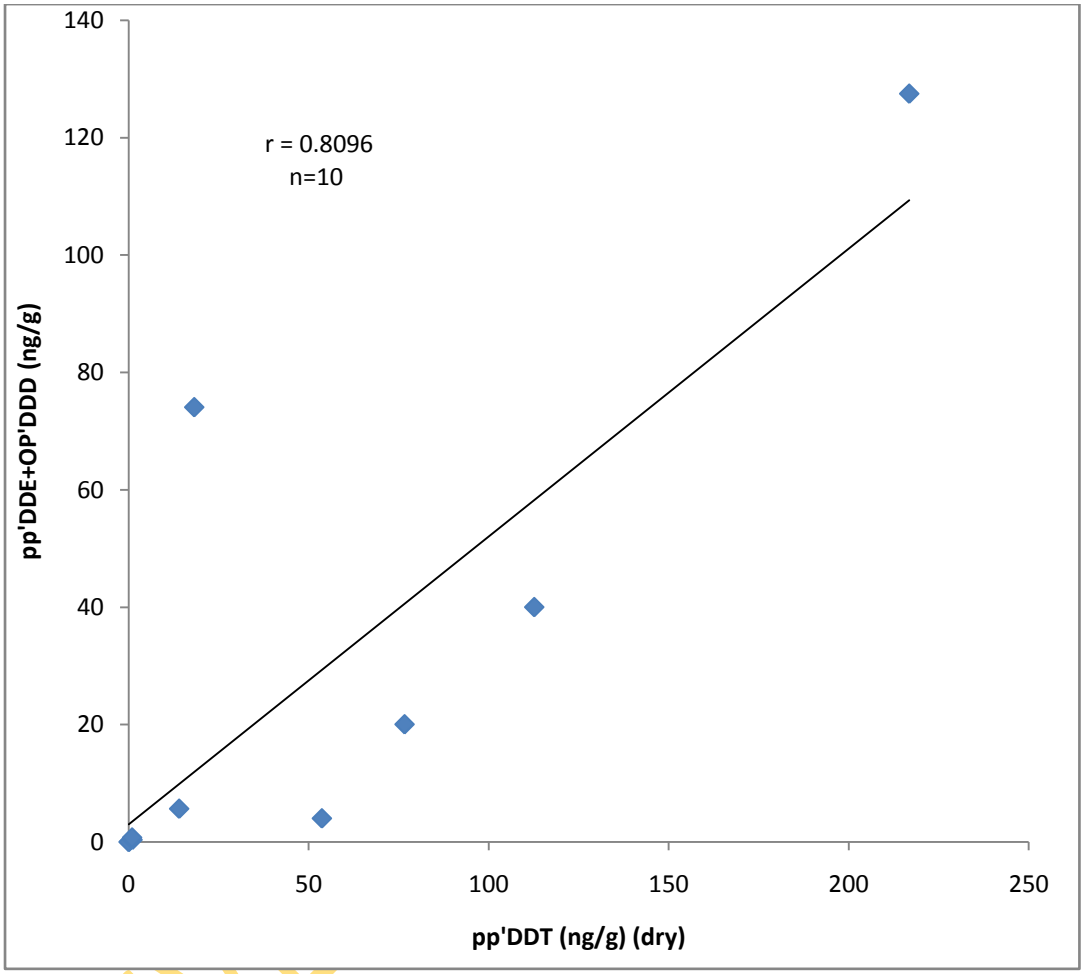


Figure 4.25. Relationship between parent pp'DDT and sum of metabolites (pp'DDE+op'DDD) in top and bottom soils (dry season)

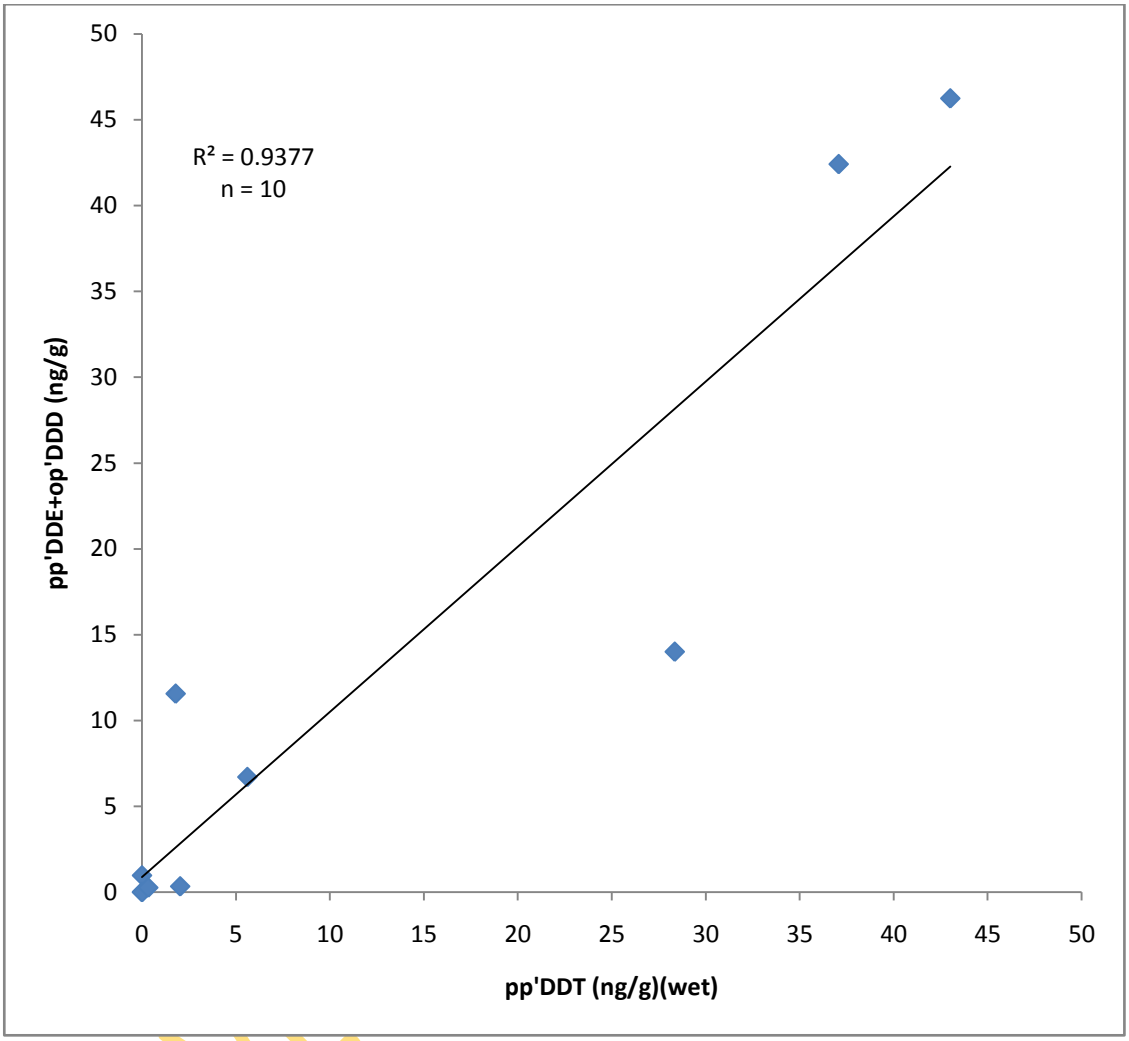


Figure 4.26. Relationship between parent pp'DDT and sum of metabolites (pp'DDE+op'DDD) in top and bottom soils (wet season)]

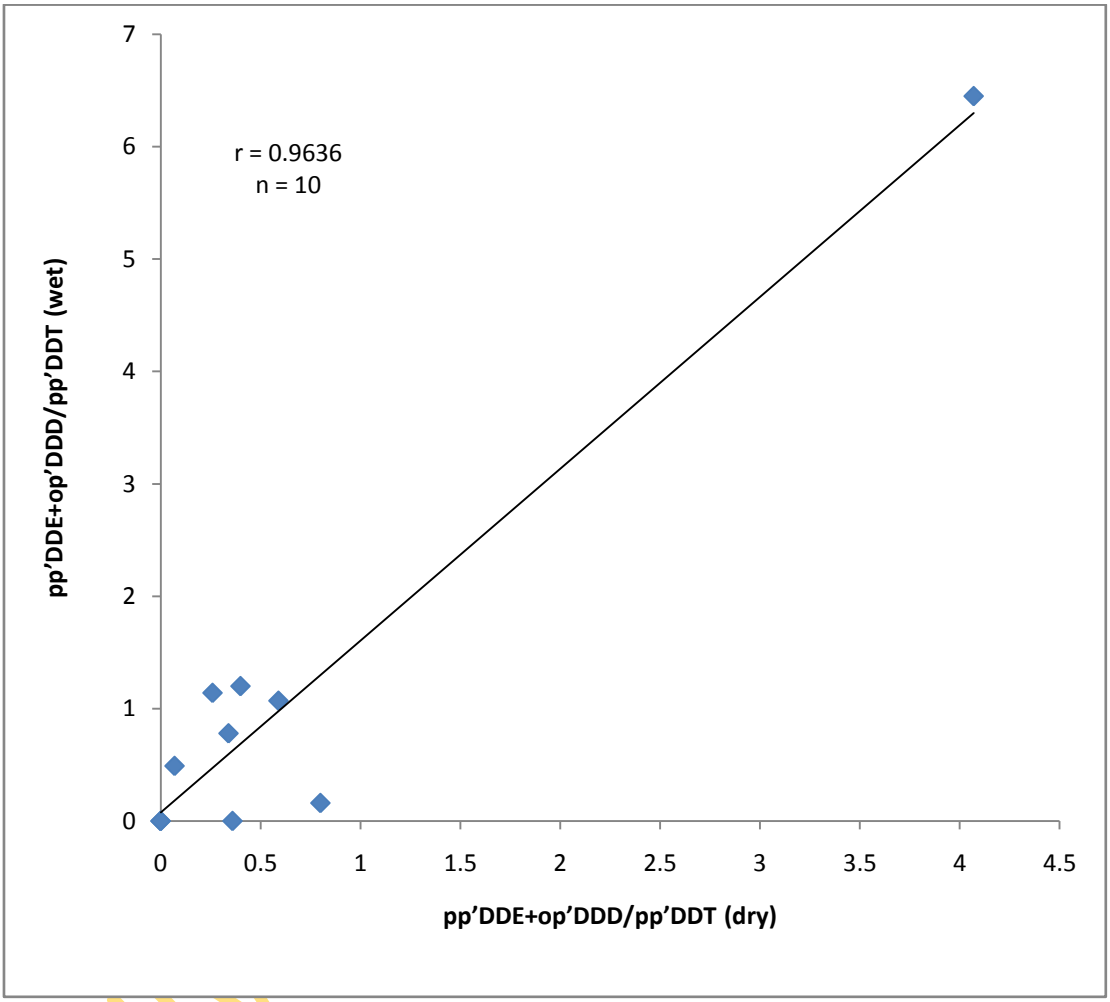


Figure 4.27. Seasonal relationship between dry and wet seasons for pp'DDE+op'DDD/pp'DDT ratio

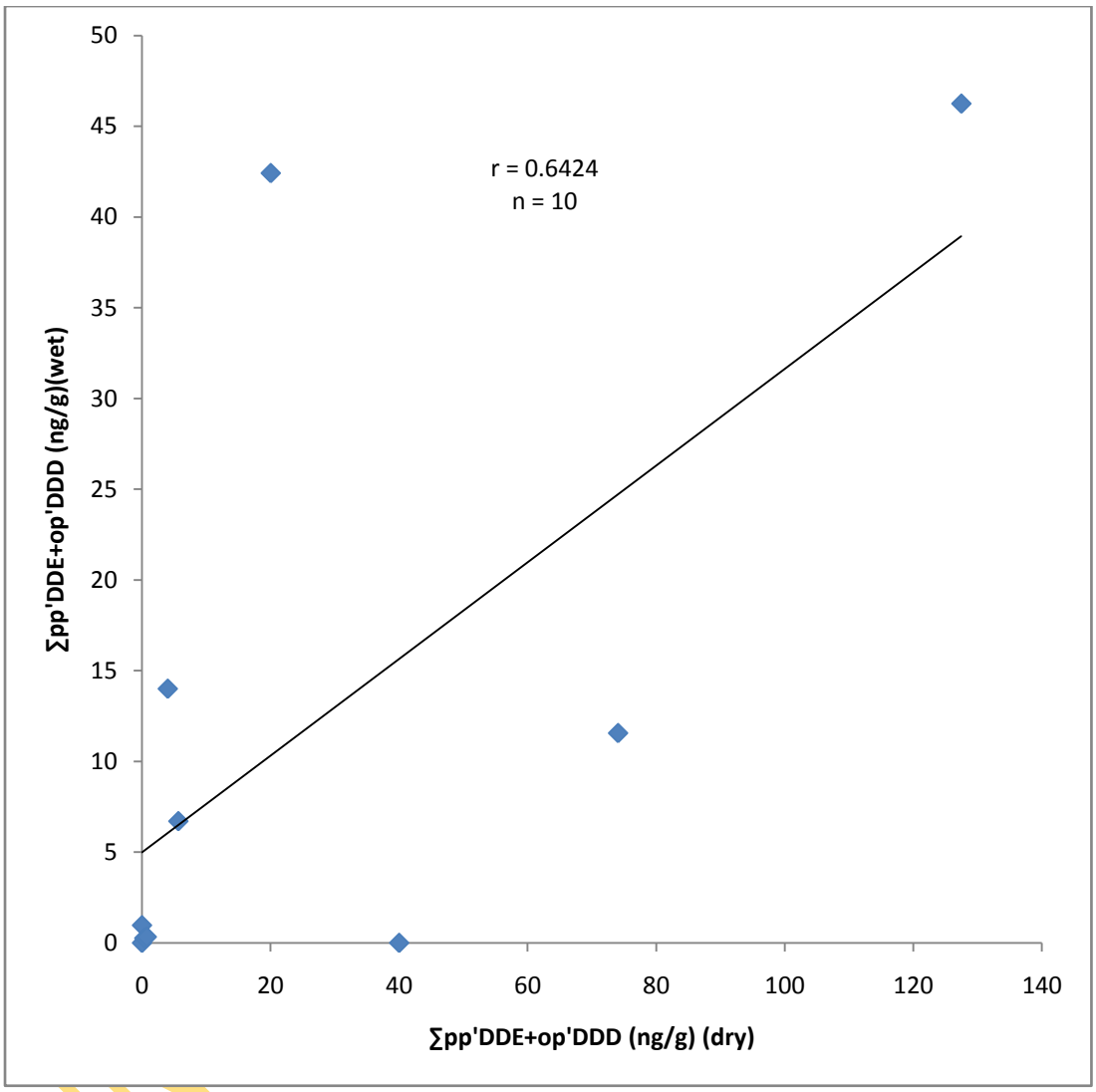


Figure 4.28. Seasonal relationship between dry and wet seasons for pp'DDE+op'DDD

The order of heptachlor contamination at 0-15 cm depth was Sore Bale > CRIN I > Ondo I > CRIN II ≈ Ondo II, while at 15-30 cm depth, CRIN I > Ondo II > CRIN II ≈ Sore Bale ≈ Ondo I. Sore Bale and CRIN I were the most contaminated with ∑heptachlor at topsoil and sub-surface soil respectively, while neither of both contaminants were detected in CRIN II and Ondo II at top soils and CRIN II, Sore Bale, Ondo I at the bottom soil. In Sore Bale farm soil, parent compound and metabolite were not detected at 15-30cm depth, while at depth 0-15cm ∑heptachlor was due to parent heptachlor, no epoxide was detected.

The presence ofthe metabolite heptachlor epoxide could be used as an index to judge the source and age of heptachlor. Technical-grade heptachlor contains about 72% heptachlor and 28% related compounds (20–22% *trans*-chlordane and 4–8% nonachlor) (IARC, 2001). The absence of heptachlor epoxide implied recent application of the pesticide to this farm – this is in consonance with the history of OCP usage in the Sore Bale farm, where OCPs are still being used.

CRIN I was the second most contaminated with ∑heptachlor concentration of 4.58±4.90 ngg^{-1} at the top soil. Residual concentrations of heptachlor and heptachlor epoxide were 3.75± 4.00 ngg^{-1} (81.8%) and 0.83±0.91 ngg^{-1} (18.2%), with compositional contribution in parentheses, while heptachlor epoxide/heptachlor ratio was 0.22. The reciprocal ratio of parent heptachlor to metabolite heptachlor epoxide could be used adequately to decipher source and time of its in-put to an environment. Heptachlor epoxide/heptachlor ratios <0.5 depicted recent application. The compositional distribution and ratio obtained at top soil was not in consonance with the history of OCP usage in CRIN I farm – which has not been treated with OCPs for over 15 years. Therefore, presence of heptachlor may not be due to recent application, but may be due to deposition or precipitation as a result of OCPs high susceptibility for long range/distance atmospheric transport (LRT). At the 15 -30 cm depth, CRIN I farm soil was the most contaminated with heptachlor, residual levels were 2.00±2.45 ngg^{-1} (50.2%) and 1.99 ±1.76 ngg^{-1} (49.8%) for heptachlor and heptachlor epoxide respectively (percentage contribution to ∑heptachlor in parentheses), with heptachlor epoxide/heptachlor ratio almost at unity (~ 1.0) (Table 4.10). The aforementioned values are suggestive of long-term application and confirmed the persistence of heptachlor and its metabolite. Heptachlor and chlordane have been reported to exhibit very low volatility and are essentially insoluble in water and their biodegradation in

soil is very slow, with halftimes measured in decades (IARC, 2001). These characteristics would definitely enhance their persistence in the environment.

In the wet season, heptachlor and its metabolite were not detected in any of the cocoa farms at 0-15cm depth, except in Sore Bale, where ∑heptachlor was 12.96 ±8.78 ngg^{-1}, with individual concentrations of 12.53 (0.44 -22.90) ngg^{-1} and 0.43 (0.10 – 1.03) ngg^{-1} for heptachlor and heptachlor epoxide respectively (concentration ranges in parentheses) (Table 4.12). Compositional contribution were 96.7% and 3.3% for heptachlor and heptachlor epoxide respectively, while the inverse ratio of parent heptachlor to metabolite heptachlor epoxide was <0.1 – both indices depicted fresh in-put of parent heptachlor pesticide to the environment. ∑heptachlor in the 15-30 cm depth ranged from ND – 1.95±1.49 ngg^{-1}, with CRIN I sub-surface soil being the most contaminated by heptachlor (Table 4.11). The individual concentrations in CRIN I were, 1.75 (ND- 3.24) ngg^{-1} and 0.21 (0.01 – 0.40) ngg^{-1} for heptachlor and heptachlor epoxide respectively, concentration ranges in parentheses. Compositional contributions were 89.4% and 10.6% for heptachlor and heptachlor epoxide respectively, while the inverse ratio of parent heptachlor to metabolite heptachlor epoxide were < 0.1 - both indices depicted fresh in-put of parent heptachlor pesticide to the environment. The astronomical differential residual levels in favour of the parent compound at depth 15-30 cm did not portray the history of OCP usage with respect to CRIN I farm; hence the high residual concentration of heptachlor recorded must be due to the persistent nature of heptachlor.The sum of heptachlor and its metabolite in CRIN I, Sore Bale and Ondo II were comparable to those reported in nine (9) farm soils in the Gambia and Senegal by Manirakiza *et al.* (2003) and Osibanjo (2003) and Oyekunle *et al*. (2011) for some Nigerian soil farms. Heptachlor and heptachlor epoxide residues ranged from 0.2– 1.5 ngg^{-1} and ND – 9.4 ngg^{-1} in the Sene-Gambia farms, while values reported for the Nigerian farms ranged from 3 – 43 ngg^{-1} (Osibanjo, 2003)and ND -51.03 ngg^{-1} (Oyekunle *et al*. 2011). Also, residual concentrations were significantly lower when compared to concentration range of ND - 2.493 $mgkg^{-1}$ (ND - 2493ngg^{-1}) for some farms in Ondo State, Nigeria (Aiyesanmi and Idowu, 2012).

Chlordanes in farm soils

Residual concentration of ∑chlordane (sum of cis- and trans- isomers) in top and subsurface soils for the five cocoa farms ranged from ND - 13.00 ±5.58 ngg^{-1} and ND - 18.00±12.70 ngg^{-1} respectively in the dry season (Tables 4.10, 4.12 and 4.13). Chlordane was not detected in CRIN I (top and subsurface soils) and Ondo II (top soil), while the highest ∑chlordane residue at 0 -15 cm and 15 -30 cm depths were recorded in Ondo I and CRIN II farms respectively. The order of contamination with respect to chlordane was Ondo I> Sore Bale > CRIN II > CRIN I ≈ Ondo II at depth 0-15cm, while at 15-30 cm depth the order was CRIN II > Ondo I > Sore Bale > Ondo II > CRIN I.

In Ondo I, residual levels of cis- and trans-isomers at 0-15cm depth were 5.33 ±2.93 ngg^{-1} (41.0%) and 7.67 ±2.65ngg^{-1} (59.0%) respectively, with percentage contribution to ∑chlordane in parentheses, while at 15-30cm depth, 7.66± 2.59 ngg^{-1} (46.0%) and 9.00 ±4.38 ngg^{-1} (54.0%). The trans-/cis-chlordane ratios were 1.44and 1.18 for top and bottom profiles respectively. Other trans-/cis-chlordane ratios in the dry season for top and bottom soils were 1.16, 0.64 in CRIN II and 1.94, 0.94 in Sore Bale farm soils respectively. There are several formulations of technical grade chlordane reported in literature (IARC, 2001; Jantunen *et al*. 2000; Zhang *et al*. 2006), however, a trans-/cis-chlordane ratio range of 1.17 - 1.41 for technical grade (Hinckley *et al.* 1990; Shen *et al.* 2005) was used as index for assessment of chlordane with respect to source, history and age/weathering in soil samples. Ratio values obtained for Ondo I (top- and sub-surface soils) and Sore Bale (top soil) suggested the recent use of chlordane, while CRIN II top and lower soil layers suggested application in the recent past and long-term respectively. In addition, the high residual concentrations of trans-isomer in Ondo I and Sore Bale soils may also be attributed to the fresh in-put of heptachlor into these environments; heptachlor was detected in these farms. Technical grade heptachlor is reported to contain 72% heptachlor, 20-22% trans-chlordane and 4-8% nonachlor (IARC, 2001). The presence of chlordane in Ondo I and CRIN II farms, buttressed the persistence of chlordane in the environment, since OCPs have not been applied in both farms for over 3years and 15 years respectively. Chlordane has been reported to be extremely persistent in the environment and could persist for more than 20 years after application in some soils (ASTDR, 2000). Lichtenstein and Schulz (1959), Nash and

Woolson (1967), Stewart and Chisholm (1971), Stewart and Fox (1971),Bennett *et al.* (1974) and Beeman and Matsumura (1981), have all reported residual chlordane of more than 10% of the initial level applied, 10 years or more after its use for treatment. This persistence may be as a result of very few microorganisms that are capable of degrading chlordane. In addition the presence of chlordane in CRIN II top soil may likely due to long range transport (LRT) rather than solely the persistent nature of chlordane after application.

In the wet season chlordane was not detected in CRIN I (top and sub-surface soils) and Ondo II (topsoil), however, mean concentration of ∑chlordane were 1.73(1.10) ngg^{-1} and 16.70(13.31) ngg^{-1} in top- and sub-surface soil layers respectively in CRIN II, with standard deviation in parentheses, while corresponding levels in Sore Bale, Ondo I and Ondo II were 0.34(0.24) ngg^{-1}, 11.13(6.46) ngg^{-1} ; 0.50 (0.31) ngg^{-1}, 0.88 (0.70) ngg^{-1} and ND, 0.59(0.45) ngg^{-1} respectively. The order of contamination at 0 – 15 cm and 15 -30 cm depths were CRIN II > Ondo I > Sore Bale > Ondo II ≈ CRIN I and CRIN II > Sore Bale > Ondo I > Ondo II >CRIN I respectively. The trans-/cis-isomer ratio at 0-15cm and 15-30 cm in CRIN II, Sore Bale, Ondo I and Ondo II were 0.70, 0.59; 1.27, 0.97; 0.56, 1.0and nil, 0.33 respectively (none of the isomers were detected at the topsoil in Ondo II). All ratios were ≤1.0, except for Sore Bale top soil with a trans-/cis-chlordane ratio that indicated recent application of technical grade chlordane and old usage in the other farms at both depths.trans-/cis-chlordane ratio < 1.0 is generally suggestive of weathered/aged chlordane. The trans-/cis-chlordane ratios in this study ranged from 0 - 1.94 (0 -15 cm) and 0 -1.18 (15 – 30 cm) in the dry season, while in the wet season it ranged from 0 - 1.27 (0 -15 cm) and 0.33 - 1.00 (15 - 30 cm). These ranges were comparable with ratios reported in top soils in South China (Li *et al*. 2006; Zhang *et al*. 2009), Northeastern China (Wang Xu *et al.* 2009) and from the mountains of Tajikistan (Zhao *et al*. 2013).

The highest residual concentration of 18.00±26.18 ngg^{-1} for ∑chlordane (15-30 cm) recorded in CRIN II was significantly below the EPA (South Australia, 2009) limit of 5 mg/kg (or 5000 ng/g) for contaminated soil.

4.2.3.2. Bioaccumulation factor of OCPs in Theobroma cacao vegetation

The bioaccumulation factors (BCFs) calculated for OCPs found in cocoa vegetation and surrounding soils are presented in Table 4.15.

Table 4.15. Bioaccumulation factor (BCF) for OCPs in CRIN I and CRIN II cocoa farms

	BIOACCUMULATION FACTOR					
	CRIN I			CRIN II		
OCPS	Whole plant (μgg^{-1})	Bulk soil (ngg^{-1})	BCF*	Whole plant (μgg^{-1})	Bulk soil (ngg^{-1})	BCF*
α-HCH	0.029	2.715	10.6	0.117	2.600	45.2
β-HCH	0.10	83.865	1.2	0.396	8.172	48.5
γ-HCH	0.028	2.727	10.1	0.145	2.145	67.6
δ-HCH	0.066	4.167	15.9	0.105	6.822	15.4
pp'DDT	<0.001	9.898	0	0.004	0	0
pp'DDE	<0.001	13.495	0	<0.001	0.243	0
opDDD	<0.001	11	0	<0.001	<0.001	0
Heptachlor	<0.001	1.875	0	<0.001	<0.001	0
Heptachlor Epoxide	<0.001	0.757	0	<0.001	<0.001	0
α-Endosulfan	0.061	23.96	2.6	0.067	0.102	658.5
β-Endosulfan	0.081	88.775	0.9	0.059	1.550	37.9
Endosulfan SO_4	0.087	330.255	0.3	0.055	4.615	11.9
Aldrin	0.089	11.967	7.4	0.077	0	0
Dieldrin	0.094	9.947	9.4	0.032	0	0
Endrin	0.111	19.772	5.6	0.109	23.635	4.6
Endrin Aldehyde	<0.001	134.240	0	0.01	0.463	21.6
Endrin Ketone	0.005	34.725	0.1	0	0	0
Cis- Chlordane	<0.001	<0.001	0	0.001	6.103	0.2
Trans- Chlordane	<0.001	<0.001	0	<0.001	4.173	0
Methoxychlor	<0.001	<0.001	0	<0.001	<0.001	0

*BCF calculated as – (Concentration in whole plant/Concentration in bulk soil) × 1000

Whole plant – mean concentration pesticide of leaves, bark, pods and seed in both seasons

Bulk soil – mean concentration of 0-15 and 15-3cm depths in both seasons

Values were determined for CRIN I and II farms. BCF ranged from 0.1 to 658 – only where OCPs were detected in *Threobroma cacao* tissues and surrounding soils. High BCF values are indicative of increased up-take by plant tissues with corresponding low residues in soil.

4.2.4. Concentrations of OCPs in water and sediments

The results obtained for surface water and sediments of studied locations for dry and wet seasons are presented in Tables 4.16-4.19. Sediment samples were analyzed in dry weights (DW).

4.2.4.1. Water Contamination profile

Total mean of OCP residues in surface water for dry season in all the farms ranged between 0.10 μgL^{-1} and 1.39μgL^{-1}, while in the wet season values ranged from 0.06μgL^{-1} to 1.35 μgL^{-1}. The order of contamination was Sore Bale II < Ondo I < Sore Bale I < CRIN < Ondo II and Sore Bale II < CRIN < Sore Bale I < Ondo I < Ondo II for dry and wetseasons respectively (Tables 4.16and 4.17). The stream by Ondo II cocoa farm recorded the highest total OCP load for both seasons, while Sore Bale II (residential) had the least residual total OCP concentration. Usually surface water and sediments are impacted as a result of run-off from OCP-treated farm land.

Distribution of aldrins in cocoa farm surface water

In the dry season, total aldrin (aldrin +dieldrin + endrin+ endrin metabolites) in surface water was 0.18 ±0.10μgL^{-1}, 0.27 ± 0.09 μgL^{-1} and 0.33 ±0.19μgL^{-1} in CRIN, Sore Bale I (farm) and Ondo I respectively, while aldrin was not detected in Sore Bale II (residential) and Ondo II (Table 4.16). ∑aldrin contributed 42.21%, 41.80% and 64.05% to the ∑OCPs in CRIN, Sore Bale I (farm area) and Ondo I surface water. In CRIN, ∑aldrin was due to residual concentrations of aldrin (0.08±0.03 μgL^{-1}) and dieldrin (0.10 ±0.07μgL^{-1}), while endrin and its metabolites were not detected. In Sore Bale I (farm area), aldrin contributed 100% to ∑aldrin residue of surface water in the dry season, while ∑aldrin in Ondo I (Sabo) farm was solely due to endrin ketone – a metabolite of endrin. The absence of endrin and its metabolites (in CRIN surface water), coupled with the predominance of dieldrin compared to aldrin (13:1 residual concentration ratio) suggested either recent application of technical dieldrin or aging and weathering due to previous use of aldrin on or around CRIN I and II farms,

Table 4.16. Concentrations of OCPs (µgL^{-1}) in surface water of cocoa farms during dry season

OCPs	CRIN		SORE BALE (Farm)		SORE BALE (Residence)		ONDO I		ONDO II	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	ND	-	ND	-	ND	-	ND	-	0.07±0.02	-
βHCH	ND	-	ND	-	ND	-	ND	-	0.07±0.02	-
γHCH	ND	-	ND	-	ND	-	0.11±0.03	0.074-0.142	0.04±0.02	-
δHCH	0.15±0.04	0.101-0.206	ND	-	0.10±0.02	0.066- 0.126	ND	-	0.05±0.02	-
∑HCH	**0.15±0.04**		**ND**		**0.10±0.02**		**0.11±0.03**		**0.22±0.08**	
pp'DDT	0.09±0.07	0.008 - 0.169	ND	-	ND	-	0.05±0.06	0.002-0.127	0.10±0.08	0.021-0.207
pp'DDE	ND	-	ND	-	ND	-	0.01±0.01	ND-0.030	ND	
opDDD	ND	-	ND	-	ND	-	ND	-	ND	
∑DDT	**0.09±0.07**		**ND**	-	**ND**	-	**0.06±0.07**		**0.10±0.08**	
Heptachlor	ND	-	ND	-	ND	-	ND		ND	-
Heptachlor Epoxide	ND	-	ND	-	ND	-	ND		ND	-
∑Heptachlor	**ND**		**ND**		**ND**		**ND**		**ND**	
αEndosulfan	ND	-	0.09 ±0.06	0.027 - 0.167	ND	-	ND	-	0.24±0.05	0.188-0.299
βEndosulfan	ND	-	0.09±0.04	0.045-0.134	ND	-	ND	-	0.12±0.05	0.072-0.206
Endosulfan SO_4	ND	-	0.19±0.11	0.049-0.326	ND	-	ND	-	0.71±0.35	0.409-1.201
∑Endosulfan	**ND**		**0.37±0.21**		**ND**		**ND**		**1.07±0.45**	
Aldrin	0.08±0.03	0.038-0.118	0.27±0.09	0.185-0.385	ND	-	ND	-	ND	-
Dieldrin	1.00±0.73	0.397- 2.203	ND	-	ND	-	ND	-	ND	-
Endrin	ND	-	ND	-	ND	-	ND	-	ND	-
Endrin Aldehyde	ND	-	ND	-	ND	-	ND	-	ND	-
Endrin Ketone	ND	-	ND	-	ND	-	0.33±0.19	0.132-0.589	ND	-
∑Aldrin	**1.08±0.76**		**0.27±0.09**		**ND**		**0.33±0.19**		**ND**	
Cis- Chlordane	ND	-	ND	-	ND	-	0.01±0.00	0.00-0.009	ND	-
Trans- Chlordane	ND	-	ND	-	ND	-	0.01±0.01	0.002 - 0.014	ND	-
∑Chlordane	**ND**		**ND**		ND	-	**0.02±0.01**		**ND**	-
Methoxychlor	ND	-	ND	-	ND	-	ND	-	ND	-
∑OCPs	**1.32±0.87**		**0.64±0.30**		**0.10±0.02**		**0.52±0.30**		**1.39±0.61**	

NOTE: ND – Not detected (<0.001 µgL^{-1})

Table 4.17. Concentrations of OCPs (µgL^{-1}) in surface water of cocoa farms during wet season

OCPs	CRIN		SORE BALE (Farm)		SORE BALE (Residence)		ONDO I		ONDO II	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	0.03±0.03	0.008 - 0.075	ND	-	0.02±0.02	ND-0.042	0.01±0.01	ND-0.024	ND	-
βHCH	0.06±0.07	ND - 0.157	ND	-	0.03±0.02	ND-0.045	0.02±0.03	ND -0.052	ND	-
γHCH	0.00±0.00	ND - 0.009	ND	-	ND	-	ND	-	ND	-
δHCH	0.01±0.01	ND - 0.021	ND	-	ND	-	ND	-	ND	-
∑HCH	**0.10±0.11**		**ND**		**0.05±0.04**		**0.03±0.03**		**ND**	
pp'DDT	0.05±0.03	0.009 - 0.088	0.01±0.01	ND -0.016	ND	-	0.03±0.04	0.001-0.091	0.11±0.13	0.001-0.291
pp'DDE	0.02±0.02	0.008 - 0.045	ND		ND	-	ND	-	0.03±0.02	ND-0.054
opDDD	ND	-	ND		ND	-	ND	-	ND	
∑DDT	**0.07±0.05**		**0.01±0.01**		**ND**	-	**0.03±0.04**		**0.14±0.15**	
Heptachlor	ND	-	0.01±0.01	-	ND	-	ND		ND	-
Heptachlor Epoxide	ND	-	ND	-	ND	-	0.01±0.01	ND - 0.011	ND	-
∑Heptachlor	**ND**		**0.01±0.01**		**ND**		**0.01±0.01**		**ND**	
αEndosulfan	ND		0.03±0.03	0.089 - 0.163	0.00±0.00	ND-0.007	0.01±0.01	ND - 0.021	0.33±0.10	0.223-0.468
βEndosulfan	ND		0.02±0.02	0.002-0.161	0.00±0.00	ND-0.009	0.01±0.01	0.001-0.011	0.28±0.20	0.102-0.561
Endosulfan SO_4	ND		0.06±0.03	0.212-1.010	0.01±0.01	ND-0.018	0.21±0.16	0.009-0.410	0.52±0.31	0.212-0.949
∑Endosulfan	**ND**		**0.10±0.08**		**0.01±0.02**		**0.22±0.18**		**1.14±0.61**	
Aldrin	ND	-	ND	-	ND	-	ND	-	ND	-
Dieldrin	0.03±0.01	0.009 – 0.040	ND	-	ND	-	ND	-	ND	-
Endrin	ND	ND - 0.007	0.04±0.05	ND-0.105	ND	-	ND	-	ND	-
Endrin Aldehyde	ND	-	0.000		ND	-	ND	-	ND	-
Endrin Ketone	ND	-	0.00±0.00	ND -0.010	ND	-	ND	-	0.07±0.10	ND-0.210
∑Aldrin	**0.03±0.02**		**0.04±0.05**		**ND**		**ND**		**0.07±0.10**	
Cis- Chlordane	ND	-	0.02±0.03	ND -0.083	ND	-	0.01±0.01	0.001-0.014	ND	-
Trans- Chlordane	ND	-	0.03±0.04	ND - 0.054	ND	-	0.01±0.01	ND - 0.021	ND	-
∑Chlordane	**ND**		**0.05±0.06**		**ND**	-	**0.02±0.02**		**ND**	-
Methoxychlor	ND	-	0.000	-	ND	-	0.00±0.00	ND - 0.008	ND	-
∑OCPs	**0.20±0.18**		**0.21±0.21**		**0.06±0.05**		**0.30±0.28**		**1.35±0.86**	

NOTE: ND – Not detected (<0.001 µgL^{-1})

since dieldrin is formed as a result of the epoxidation of aldrin, while endrin is isomeric to dieldrin.

Technical grade dieldrin is 83% pure, with impurities (like carbonyl compound and traces of benzene and acetic acid) that are usually present in technical-grade aldrin and it is normally manufactured by the epoxidation of aldrin using either H_2O_2 (in the presence of tungstic oxide) or by peracid, such as peracetic acid (Sittig, 1977; Zitko, 2003). Technical grade aldrin is approximately 82 - 85% pure and contains in addition of 1-3% each of polychlorinated hexahydrodimethanonaphthalenes, di-adducts, bicycloheptadiene, hexachlorocyclopentadiene, hexachlorobutadiene, hexachloroethane, and octachlorocyclopentene (Brooks 1974a,b).

The level of ∑aldrin in the wet season in five farms ranged from ND - 0.07 ± 0.10 μgL^{-1} (Table 4.17). The highest value was recorded in Ondo II farm (0.07 ± 0.10 μgL^{-1}), followed by Sore Bale I (0.04 ± 0.05 μgL^{-1}) and CRIN (0.03 ± 0.02 μgL^{-1}), while none of the 'drins' were detected in Sore Bale II (residential area) and Ondo I.Total aldrin in CRIN was solely due to dieldrin (0.03 μgL^{-1}), with aldrin, endrin, endrin ketone and endrin aldehyde not detected, while in Sore Bale I, only endrin was found.

The percentage contribution or composition of OCPs including some key metabolites is a ready tool for depth understanding of the origin and history of input to the environment and possible degradation pathways involved (Iwata *et al.* 1993). Total aldrin in Ondo II was solely due to endrin ketone residue; this implied the use of endrin or dieldrin and aldrin in the past. Residual concentrations of dieldrin and endrin in CRIN suggested recent past application of dieldrin pesticide, while the relatively low concentration of endrin may have resulted from bio-isomeration of dieldrin into endrin.

The mean value range obtained for dieldrin (ND - 0.10 μgL^{-1}) for both seasons in the streams associated with the cocoa farms, were relatively lower when compared with those reported by Nwankwoala and Osibanjo, (1992) and Okoya *et al*. (2013) in streams and rivers in Ibadan (Oyo State) and Ondo State. Concentrations of dieldrin in Ibadan and Ondo State streams were between 0.018 - 0.657μgL^{-1} and ND - 2.150 μgL^{-1} respectively (Nwankwoala and Osibanjo, 1992), while rivers Ala (dry season), Oluwa (dry season) and Osun (wet season) in Ondo State were 1.07±0.06 μgL^{-1}, 1.51±0.05 μgL^{-1} and 0.25±0.04 μgL^{-1} respectively (Okoya *et al*. 2013). However, higher residual

mean concentration range was recorded for aldrin (ND - 0.27 ±0.09 μgL^{-1}) compared to values reported for Ibadan (ND - 0.040 μgL^{-1}) and Ondo (ND - 0.004 μgL^{-1}) streams (Nwankwoala and Osibanjo, 1992).

The residual concentrations recorded for aldrin and dieldrin in CRIN and Sore Bale I were significantly higher (except where not detected) than the WHO maximum residue levels (MRLs) of 0.030 μgL^{-1} for aldrin and dieldrin (UNEP/FAO/WHO 1989). The ratio of aldrin to dieldrin CRIN surface water was 0.80 in the dry season, while no aldrin was detected in the wet season compared to 0.03 ±0.01 μgL^{-1} of dieldrin; these portrayed that aldrin was not recently applied (Gonzalez*et al*. 2003), and most of the aldrin must have been metabolized to dieldrin (since dieldrin is an epoxidized form of aldrin) and continued to persist even after 15 years in the CRIN environment. Also, the presence of aldrin in Sore Bale I (farm area) surface water and the non-detection of dieldrin - its' metabolite and other 'drins'- endrin, endrin ketone and endrin aldehyde suggested that aldrin was recently applied to the farm. Endrin is an isomer of dieldrin and undergoes photolytic degradation to endrin ketone and aldehyde (ATSDR, 1996). OCPs are still in use in Sore Bale cocoa farm.

Endosulfan isomers and major metabolite endosulfan sulphate in surface water

Mean concentrations of ∑endosulfan in the dry season ranged from ND (CRIN, Sore Bale II and Ondo I) to 1.07 ± 0.44 μgL^{-1} (Ondo II), while in the wet season it ranged between ND (CRIN) and 1.14 ± 0.61 μgL^{-1} (Ondo II) (Tables 4.16 and 4.17). In the dry season, Ondo II recorded the highest level of ∑endosulfan, followed by Sore Bale I, while none of the parent isomers and metabolite were detected in CRIN, Ondo I and Sore Bale II. The presence of α-endosulfan, β-endosulfan and endosulfan sulphate in Ondo II and Sore Bale I surface water, may have been due to the continuous application of OCPs in these farms, while its non-detection in CRIN and Ondo I may be due to its not being used for over 15 years and 3-5 years respectively. Endosulfan was detected in all surface water except CRIN in the wet season, however its presence in Ondo I may have been due to run-off from the farm soil or from a nearby farm where it was applied.

The residual concentrations of endosulfan (α-,β-isomers) and endosulfan sulphate in Sore Bale I stream (dry season) were almost at unity, with percentage contribution to ∑endosulfan as follows; α-endosulfan 25.20%, β-endosulfan 23.85% and endosulfan

sulphate 50.95%. The percentage contribution ratio of α-/β-isomers (or α-/β-isomers residual concentration ratio) and endosulfan/endosulfan sulphate ratio were 1.06 and 0.96 respectively (Tables 4.16). Technical-grade endosulfan is made-up of 70% α-endosulfan and 30% β-endosulfan, therefore, these ratios were significantly low and favoured the application of endosulfan isomers in recent past. In Ondo II (dry season) concentrations of α-,β-isomers and endosulfan sulphate in the stream were 0.24 ±0.05µgL^{-1}(22.15%), 0.12±0.07 µgL^{-1}(11.35%) and 0.71±0.35 µgL^{-1} (66.50%), with their individual percentage contribution to ∑endosulfan in parentheses. The endosulfan/endosulfan sulphate ratio (0.50) and α-/β-isomers ratio (1.95, in favour of α-endosulfan) suggested both past and recent application respectively of endosulfan isomers in Ondo II farm environment. The level of residual metabolite was almost twice that of the parent compound.Malik *et al.*(2009) reported that relatively lower levels of α- and β-isomers to the presence of metabolite (endosulfan sulphate) in river samples indicated long time usage of endosulfan in area studied. These ratios implied that there were run-offs and/or weathering from farm soils where endosulfan was used not too long ago and/or still being used. OCPs are still being used in Sore Bale and Ondo II farms.

The highest level of ∑endosulfan in the wet season was found in Ondo II, with individual mean concentrations ≥ 0.28 ±0.20 µgL^{-1} for α- and β-isomers, while their metabolite endosulfan sulphate was 0.52±0.31 µgL^{-1} (Table 4.17). These values were remarkably higher than any of the other streams; exceeding their levels with factors >11, >14 and >2.5 for α-endosulfan, β-endosulfan and endosulfan sulphate respectively. This indicated very high usage of endosulfan in Ondo II farm environment, relative to other farms. Endosulfan has been reported as the most predominately applied OCP in cocoa farms in Ondo state (Aiyesanmi and Idowu, 2012). Also, the endosulfan/endosulfan sulphate ratio was 1.17, while α-/β-isomers ratio was 1.20 in favour of β-endosulfan – both ratios suggested the application of endosulfan isomers to Ondo II farm environment in recent past. The second highest contaminated stream was Ondo I, which contained 0.01 ±0.01µgL^{-1} and 0.01 ±0.00 µgL^{-1} of α- and β-endosulfan respectively, while endosulfan sulphate recorded a concentration of 0.21 ±0.16 µgL^{-1}. Endosulfan sulphate concentration in this stream was more than 20-folds of either parent endosulfan or 10-folds with respect to combined parent endosulfan. Relatively higher levels of metabolite – endosulfan

sulphate to those of endosulfan isomers depicted long-term application of parent endosulfan. OCPs were last used in Ondo I farm for over 3 years and this corroborates the presence of high residual level of the metabolite compared to parent compound.

Considering studies on surface water from some African farms and rivers, the mean concentrations obtained in Sore Bale I (dry and wet seasons) and Ondo I (wet season) streams, were comparable with range values of ND - 0.026 μgL^{-1}, ND - 0.43 $\mu gL^{-1}$and ND - 0.185 μgL^{-1} reported for surface water in Sene-Gambian farms (Manirakiza *et al*. 2003), Nigerian farms (Nkwankwoala and Osibanjo 1992) and Densu river basin, Ghana (Kuranchie-Mensah *et al*. 2012) respectively, but significantly lower than Ondo II (dry and wet seasons). However, concentration of endosulfan observed in surface water from Warri river, Niger Delta region of Nigeria were significantly higher than values obtained in this study (0.01 -9.23 μgL^{-1}, Ezemonye *et al*. 2010).

Both endosulfan and endosulfan sulphate residues in Ondo II (dry and wet seasons) and Sore Bale (dry season) exceeded the WHO maximum residue levels (MRLs) of 0.10 μgL^{-1} for endosulfan and endosulfan sulphate (UNEP/FAO/WHO, 1989) in surface water, while only endosulfan sulphate was exceeded in Ondo I in the wet season. Other farms were all below WHO MRLs.

Dichlorodiphenyl trichloroethane (DDT)

Concentrations of ∑DDT ranged from ND – 0.10±0.08 μgL^{-1} (dry season) and ND – 0.14±0.15 μgL^{-1} (wet season) for all the farm streams (Tables 4.20 and 4.21). The order of contamination was Ondo II (0.10μgL^{-1}) > CRIN (0.09 μgL^{-1}) > Ondo I (0.06μgL^{-1}) > Sore Bale I ≈ Sore Bale II (ND) in the dry season, while in the wet season, Ondo II (0.14 μgL^{-1}) > CRIN (0.07μgL^{-1}) > Ondo I (0.03 μgL^{-1}) > Sore Bale I (0.01 μgL^{-1}) > Sore Bale II (ND).

In the dry season, ∑DDT in Ondo II and CRIN streams was due to the presence parent pp'DDT, with no metabolites detected. Ondo I recorded mean concentrations of 0.05 ±0.06 μgL^{-1} and 0.01±0.01 μgL^{-1} for pp'DDT and pp'DDE respectively. The presence of parent pp'DDT alone in Ondo II and CRIN suggested that the source of the contaminant was very recently applied (i.e., fresh application) and not a run-off from a farm due to weathering and age. Also the detection of pp'DDT and metabolite - pp'DDE in the Ondo I farm indicated past usage. pp'DDT is easily metabolized by

microbes into pp'DDE and op'DDD. The relative concentrations of the parent DDT compounds and its metabolites can be used as indices for assessing source of contamination (Malik *et al*. 2009; Jiang *et al.* 2009). The (DDE + DDD/∑DDT) ratio was 0.20 and indicated that source of DDT contamination was from recent past application.

OCPs were last applied in CRIN 15 years ago, besides, the CRIN stream is a free flowing one and hence the presence of fresh pp'DDT in-put must have been a run-off from a nearby farm where it was recently applied and not from CRIN. Also, the detection of parent pp'DDT and metabolite pp'DDE in Ondo I implied persistence (OCPs has not been used for over 3years).

Among DDT analogues in the wet season, only the pp'DDT and pp'DDE were found (op'DDD was not detected) and residual concentrations were 0.05±0.03 μgL^{-1} and 0.02±0.02 μgL^{-1} (CRIN); 0.01 ±0.01 μgL^{-1} and ND (Sore Bale I); 0.03±0.04 μgL^{-1} and ND (Ondo I) and 0.11 ±0.13 μgL^{-1} and 0.03 ±0.02 μgL^{-1} (Ondo II) respectively, while both parent pesticide and metabolites were not found in Sore Bale II. The relatively low concentrations of metabolites to parent pesticide and (pp'DDE+op'DDD)/∑DDT ratio of <0.30 in CRIN and Ondo II depicted not too long application of parent pp'DDT to both farms.

Residual concentrations for total DDT was comparable to 0.013 – 0.040 μgL^{-1} range reported for Densu river basin, Ghana (Kuranchie-Mensha *et al*. 2012), however, Manirakiza *et al*.(2003) reported higher concentration of 0.231±0.330 μgL^{-1} in Darka farms.

The residual concentrations of pp'DDT residues recorded in Ondo II (wet season) exceeded the WHO maximum residue level (MRL) of 0.100 μgL^{-1}(UNEP/FAO/WHO, 1989).

Hexachlorocyclohexane (HCH) in surface water of farms

The mean ∑HCH in streams in the dry season ranged from ND (Sore Bale I) to 0.22±0.07 μgL^{-1} (Ondo II) (Table 4.16).Only δ-HCH was found in CRIN (0.15μgL^{-1}) and Sore Bale II (0.10 μgL^{-1}), while γ-HCH alone was detected in Ondo I (0.11 μgL^{-1}). However, all four isomers α-, β-, γ- and δ-HCH were found in Ondo II, with mean concentrations of 0.07 ±0.02 μgL^{-1}, 0.07 ± 0.02 μgL^{-1}, 0.04 ±0.02 μgL^{-1} and 0.05±0.02

μgL^{-1} respectively. Commercial-grade HCH is composed of α-HCH (60-70%), β-HCH (5-12%), γ-HCH (10-15%), δ-HCH (6-10%) and ε-HCH (3-4%) (Walker *et al*. 1999), while pure lindane contains approximately 99% γ-HCH (Iwata *et al*. 1993). The detection of δ-HCH isomer alone in CRIN and Sore Bale II and four isomers in Ondo II suggested the use of commercial grade HCH around these environments. The absences of other isomers in the latter may have resulted from long-term application and degradation with time, while it was applied in recent past in Ondo II. Lindane (γ-HCH) may have been recently applied around Ondo I farm (no other isomers detected). The ratios of αHCH/β-HCH and α-HCH/γ-HCH in technical-grade HCH are 4 -15 and 4-7, while the value for corresponding ratios for residual concentrations in Ondo II were 0.94 and 1.51 respectively – which also implied that the source of contamination was due to old use of commercial-grade HCH.

The individual HCH isomers found in CRIN (wet season) were α-HCH (0.03±0.03 μgL^{-1}), β-HCH (0.06 ±0.07μgL^{-1}), γ-HCH (0.00±0.00 μgL^{-1}) and δ-HCH (0.01 ±0.01 μgL^{-1}) with mean residual concentrations in parenthesis (Table 4.17). The order of dominance was β-HCH (57%)> α-HCH (31%) > δ-HCH (9%) > γ-HCH (3%), with individual percentage contribution to ∑HCH in parenthesis. The percentage contribution of the β-isomer was comparable to value found in River Gomti (a tributary of Ganges River), India (Malik*et al.* 2009). Also, Zhang *et al*. (2002a, b and 2003) reported 44%, 44% and 67% contribution of β-HCH to ∑HCH concentration in River Pearl estuary, Wuchaun River and Minjiang River estuary respectively. The relative high residual levels of β-HCH obtained in most streams may be due to a number of reasons; firstly, β-HCH is reported to have high resistance to hydrolysis and environmental degradation (Willet *et al*. 1998); secondly, its' predominance could be due to the favourable conversion of α-HCH to β-HCH in the environment (Wu *et al*., 1997; Walker *et al*. 1999) and as well as the ability of γ-HCH to bio-isomerize into α-HCH readily – all of these may have inter-played in its favour and resulted to β-isomer being most persistent of the HCHs. The α-HCH/β-HCH (0.53) and α-HCH/γ-HCH (10.33) ratios in CRIN suggested the use of technical-grade HCH or Lindane in the environment long time ago. No OCPs have been used in this farm for 15 years, it therefore implied that the contamination source may have being from a run-off within the CRIN farm especially from the level of β-HCH present relative to other isomers.

Chlordane isomers in surface water of farms

No chlordane (cis-chlordane and trans-chlordane) was recorded in CRIN, Sore Bale (I and II) and Ondo II in the dry season, except in Ondo I, with cis- and trans-chlordane residual concentrations of 0.01 μgL^{-1}each, while in the wet season, ∑chlordane ranged from ND to 0.05 ± 0.06 μgL^{-1} (0.03 μgL^{-1}, 0.02 μgL^{-1}) with levels of cis-chlordane and trans-chlordane in parenthesis (Tables 4.16 and 4.17). There was no residual chlordane found in CRIN, Sore Bale II and Ondo II, while the highest residual level was obtained in Sore Bale I, this was followed by Ondo II stream. Commercial-grade chlordane contains more than 140 components, consisting mainly of C_{10} alicyclic chlorinated hydrocarbons, the most abundant being *cis*- and *trans*-chlordane (Royal Society of Chemistry, 1989; Dearth and Hites, 1991; Tomlin, 1999), with compositional percentage of 15% each (Dearth and Hites, 1991) or 19% and 24% respectively (Rostad, 1997).

Heptachlor and metabolite heptachlor epoxide in surface water

Heptachlor and its' metabolite (heptachlor epoxide) were not found in the dry season in any of the streams, however, in the wet season, ∑heptachlor (heptachlor + heptachlor epoxide) in Sore Bale I and Ondo I streams were 0.01 ±0.01 μgL^{-1} (0.01 μgL^{-1}, ND) and 0.01 ±0.01 μgL^{-1} (ND, 0.01 μgL^{-1}) respectively with the parent compound and metabolite in parenthesis. The presence of parent heptachlor alone in Sore Bale I stream implied that contaminant source was from very recent application. The reverse was the case for Ondo I, as no parent compound but heptachlor epoxide was detected. OCPs are still being used in Sore Bale farm, while it was last applied in Ondo I farm 3 years ago.

4.2.4.2. Charateristics of OCP Contamination in Sediments

In the dry season ∑OCPs in sediments ranged from 0.97 ±0.54 μgg^{-1} (Sore Bale II) to 9.85±7.78 μgg^{-1} (Ondo II), while in the wet season values ranged between 0.88±0.96 ngg^{-1} (Sore Bale II) and 9.70 ±9.73 μgg^{-1} (Ondo II) (Tables 4.18 and 4.19). The order of contamination was Ondo II > Ondo I > Sore Bale I > CRIN > Sore Bale II in both seasons.

Table 4.18. Concentrations of OCPs (µgg^{-1}) in sediments of streams in cocoa farms during dry season

OCPs	CRIN		SORE BALE (Farm)		SORE BALE (Residence)		ONDO I		ONDO II	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	0.32±0.25	0.018-0.638	0.04±0.06	ND-0.128	0.02±0.01	ND-0.029	0.04±0.01	0.093- 0.181	0.24±0.12	0.094-0.392
βHCH	0.41±0.16	0.227-0.617	0.03±0.04	ND-0.083	0.04±0.04	0.001-0.089	0.07±0.04	0.044-0.341	0.30±0.30	0.021-0.713
γHCH	0.05±0.05	0.003 - 0.128	0.16±0.14	0.011-0.345	0.01±0.01	ND-0.019	0.02±0.01	0.021-0.088	0.23±0.23	0.018-0.551
δHCH	ND	-	ND		0.01±0.01	ND-0.021	ND		0.06±0.08	ND-0.072
∑HCH	**0.79±0.46**		**0.23±0.24**		**0.07±0.07**		**0.13±0.06**		**0.83±0.73**	
pp'DDT	ND	-	ND		0.18±0.15	0.119-0.449	0.27±0.19	0.045-0.512	ND	-
pp'DDE	0.01±0.00	-	ND		ND	0.007-0.037	0.05±0.04	ND-0.090	0.01±0.01	ND-0.023
opDDD	0.01±0.01	-	ND		ND	ND-0.998	0.02±0.02	ND-0.038	ND	-
∑DDT	**0.02±0.01**		**ND**		**0.18±0.15**		**0.33±0.24**		**0.01±0.01**	
Heptachlor	ND	-	0.62±0.43	0.129-211	0.02±0.02	-	ND	-	ND	-
Heptachlor Epoxide	ND	-	ND	0.008-1.257	ND	-	ND	-	ND	-
∑Heptachlor	**ND**		**0.62±0.43**		**0.02±0.02**	**-**	**ND**	**-**	**ND**	**-**
αEndosulfan	0.03±0.02	0.001-0.057	0.04±0.04	0.003-0.087	ND	-	0.65±0.26	0.282-0.873	2.01±1.37	0.072-3.009
βEndosulfan	0.01±0.01	ND-0.024	0.02±0.01	ND-0.032	ND	-	0.28±0.12	0.168-0.445	1.50±1.69	0.098-3.879
Endosulfan SO_4	0.08±0.09	0.009-0.202	0.25±0.11	0.141-0.392	ND	-	1.45±0.77	0.832-2.529	4.74±3.70	0.112-9.178
∑Endosulfan	**0.12±0.12**		**0.31±0.15**		**ND**		**2.37±1.15**		**8.24±6.77**	
Aldrin	ND	-	ND	-	0.03±0.02	0.009-0.054	ND	-	0.02±0.02	ND-0.048
Dieldrin	ND	-	ND	-	0.01±0.01	0.007-0.026	ND	-	0.01±0.01	ND-0.012
Endrin	0.01±0.01	ND - 0.014	ND	-	0.09±0.07	0.012-0.184	0.07±0.05	0.001-0.111	ND	-
Endrin Aldehyde	0.02±0.02	ND- 0.045	ND	-	ND	-	ND	-	ND	-
Endrin Ketone	ND	-	ND	-	0.03±0.01	0.011-0.041	0.04±0.03	0.008-0.078	ND	-
∑Aldrin	**0.03±0.03**		**ND**		**0.16±0.11**		**0.11±0.08**		**0.03±0.03**	
Cis- Chlordane	ND	ND	ND	-	0.02±0.02	0.009-0.046	0.01±0.01	ND-0.021	ND	-
Trans- Chlordane	0.01±0.01	ND - 0.017	ND	-	0.03±0.02	0.014-0.054	0.02±0.01	0.012-0.025	ND	-
∑Chlordane	**0.01±0.01**		**ND**		**0.06±0.03**		**0.03±0.02**		**ND**	
Methoxychlor	ND	-	ND	-	ND	-	ND	-	ND	-
∑OCPs	**0.94±0.62**		**1.16±0.82**		**0.48±0.38**		**2.97±1.55**		**9.11±7.53**	

NOTE: ND – Not detected (<0.001 µgg^{-1})

Table 4.19. Concentrations of OCPs ($\mu g g^{-1}$) in sediments of streams in cocoa farms during wet season

OCPs	CRIN		SORE BALE (Farm)		SORE BALE (Residence)		ONDO I		ONDO II	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
αHCH	0.20±0.20	0.008-0.578	0.33±0.19	0.177-.591	0.01±0.01	ND-0.027	0.03±0.03	0.003-0.121	0.05±0.04	ND-0.104
βHCH	0.49±0.37	0.317-0.918	0.94±0.39	0.432-1.387	0.04±0.03	ND-0.076	0.07±0.06	0.009-0.156	0.22±0.09	0.137-0.336
γHCH	0.01±0.01	ND - 0.018	0.15±0.05	0.109-0.225	ND	-	0.01±0.00	ND-0.088	003±0.03	ND-0.061
δHCH	0.03±0.04	-	0.18±0.05	0.103-0.214	ND	-	0.04±0.04	ND-0.089	0.06±0.05	ND-0.107
∑HCH	**0.73±0.62**		**1.59±0.68**		**0.05±0.04**		**0.15±0.13**		**0.36±0.20**	
pp'DDT	ND	-	ND	ND-0.211	0.02±0.02	0.002-0.038	0.01±0.01	0.005-0.019	0.03±0.04	ND-0.078
pp'DDE	0.01±0.01	ND-0.012	0.02±0.01	0.005-0.124	0.01±0.01	ND-0.017	0.03±0.04	ND-0.090	0.00±0.00	ND-0.011
opDDD	ND	-	0.01±0.01	0.012-0.078	ND	-	0.09±0.13	ND-0.278	0.03±0.04	ND-0.081
∑DDT	**0.01±0.01**		**0.03±0.03**		**0.03±0.02**		**0.13±0.18**		**0.06±0.08**	
Heptachlor	ND	-	0.16±0.11	0.129-211	0.02±0.02	ND-0.045	ND	-	ND	-
Heptachlor Epoxide	ND	-	0.03±0.03	0.008-1.257	0.04-0.04	ND-0.098	ND	-	ND	-
∑Heptachlor	**ND**		**0.19±0.14**		**0.06±0.06**	**-**	**ND**	**-**	**ND**	**-**
αEndosulfan	0.09±0.12	ND-0.263	0.05±0.05	0.121-0.463	ND	-	0.57±0.27	0.275-0.932	1.58±0.77	0.497-2.253
βEndosulfan	0.03±0.03	0.102-0.261	0.03±0.04	0.102-0.561	ND	-	0.24±0.14	0.173-0.419	0.35±0.30	0.102-0.761
Endosulfan SO_4	0.07±0.05	0.212-1.009	0.08±0.04	0.407-1.594	0.02±0.02	-	1.79±0.99	0.718-1.541	4.47±4.70	0.212-11.009
∑Endosulfan	**0.19±0.20**		**0.15±0.13**		**0.02±0.02**		**2.60±1.41**		**6.39±5.76**	
Aldrin	ND	-	0.44±0.30	0.152-5.067	ND		ND	-	0.09±0.07	0.002-0.0.178
Dieldrin	0.00±0.01	-	0.01±0.01	-	0.03±0.02	0.004-0.141	ND	-	0.06±0.04	ND-0.107
Endrin	0.02±0.01	0.249 - 0.675	ND	ND-1.073	0.05±0.03	0.012-0.156	0.03±0.03	ND-0.071	0.01±0.01	ND-0.011
Endrin Aldehyde	ND	ND - 0.124	ND	-	0.01±0.01	ND-0.018	ND	-	ND	-
Endrin Ketone	0.03±0.04	ND-0.087	0.03±0.04	ND-0.219	0.01±0.01	0.001-0.021	0.04±0.05	ND-0.117	ND	-
∑Aldrin	**0.05±0.06**		**0.48±0.28**		**0.10±0.06**		**0.07±0.08**		**0.16±0.12**	
Cis- Chlordane	ND	-	ND	-	0.01±0.01	ND-0.015	ND	-	0.00±0.00	ND-0.008
Trans- Chlordane	0.06±0.05	ND - 0.111	ND	-	0.03±0.02	ND-0.054	ND	-	0.01±0.01	ND-0.014
∑Chlordane	**0.06±0.05**		**ND**		**0.03±0.03**	**ND-0.033**	**ND**		**0.01±0.01**	
Methoxychlor	ND	-	ND	-	ND	-	ND	-	ND	-
∑OCPs	**1.04±0.93**		**2.79±1.46**		**0.28±0.24**		**2.95±1.80**		**6.97±6.16**	

NOTE: ND – Not detected (<0.001 $\mu g g^{-1}$)

Sediments in Ondo II cocoa farm stream had the highest ∑OCPs load, while the least was found in Sore Bale II for both seasons. Sedimentary systems are reported to have high affinity for OCPs and it is the sink for them in the aquatic environment (Li *et al*. 2013).

Hexachlorocyclohexane (HCH) distribution in sediments

Hexachlorocyclohexane (HCH) residue was detected in all the sediments, except Ondo II in the dry season, while it was found in all the sediments in the wet season. ∑HCH in the dry season ranged from 0.07 ± 0.05 - 0.83 ± 0.73 μgg^{-1} (Table 4.18), while the wet season ranged between 0.05 ± 0.04 μgg^{-1} and 1.59 ± 0.68 μgg^{-1} (Table 4.19).

In the dry season, the highest mean value was found in Ondo II sediment, followed by CRIN (0.79 ± 0.47 μgg^{-1}). The concentration of HCH isomers in Ondo II sediments was α-HCH - 0.24 μgg^{-1} (0.09 - 0.39); β-HCH - 0.30 μgg^{-1} (0.02 - 0.71); γ-HCH - 0.23 μgg^{-1} (0.02 – 0.55); δ-HCH - 0.06 μgg^{-1} (ND - 0.17), with ranges in parenthesis. Isomeric predominance and percentage contribution to ∑HCH was in the order; β-HCH (36.4%) > α-HCH (29.0%) > γ-HCH (27.3%) > δ-HCH (7.3%). The range for ratios of α-/β-HCH and α-/γ-HCH in technical-grade HCH are 4 -15 and 4-7 respectively, while the value for corresponding ratios for residual concentrations in Ondo II sediments were 0.80 and 1.06. These ratios and presence of δ-HCH implied that the source of contamination was due to old use of commercial-grade HCH and to some extent the use of γ-HCH (lindane) in time past; pure lindane contains approximately 99% γ-HCH (Iwata *et al*. 1993; HSDB, 2009).

The latter deduction was as a result of the α/γ-HCH ratio that was almost at unity. If the range of α/γ-HCH ratio of the technical grade is 4-7 and previous studies showed that γ-HCH (Lindane) had the tendency of bio-isomerizing to α-isomer (Steinwandter 1976; Waliszewski *et al*.**,** 2004), it is therefore expected that the α/γ-HCH ratio should be ≥ 4 as more of α-isomer would be formed, hence pure lindane may also have been used in addition to technical grade in this environment. CRIN sediment was found to have the second highest amount of ∑HCH residue, mean concentrations of individual HCH isomers were; α-HCH - 0.32 μgg^{-1} (0.02 -0.64), β-HCH - 0.41 μgg^{-1} (0.23 -0.62), γ-HCH - 0.05 μgg^{-1} (0.003 – 0.13) and δ-HCH - ND (< 0.001 μgg^{-1}), with concentration ranges in parenthesis, while percentage contribution to ∑HCH was - 41.2%, 52.1%, 6.7% and 0% for α-, β-, γ- and δ-isomers respectively. β-isomer

contributed most to ∑HCH content in CRIN sediment. The percentage contribution of β-isomer to ∑HCH in the sediments investigated was 52.1% (CRIN), 12.6% (Sore Bale I), 67.6% (Sore Bale II), 53.3% (Ondo I) and 36.4% (Ondo II). The β-isomer was the most predominant of the HCHs in all the sediments except at Sore Bale I. β-isomer was reported to have contributed > 41% of individual components of HCHs at seven (7) of eight (8) sites sampled at Gomti river in India (Malik *et al.* 2009). The individual composition of HCHs at Sore Bale I was 0.04µgg^{-1} (α-HCH), 0.03 µgg^{-1} (β-HCH) and 0.16 µgg^{-1} (γ-HCH) corresponding to 18.6%, 12.6% and 68.8% contribution to ∑HCH respectively. The α/γ-HCH ratio of 0.27 and 68.8% contribution to ∑HCH (or γ-HCH/∑HCH ratio of 0.69) showed that the origin of contamination most have resulted from recent application of pure γ-HCH (lindane). It is pertinent to mention here that OCPs was last used in CRIN fifteen (15) years ago, while it is still being applied at Sore Bale I. The presence of HCH showed persistence in CRIN sediments and the order amongst the HCHs was β-HCH > α-HCH > γ-HCH > δ-HCH. The γ-isomer is known to bio-isomerize to α-isomer in the environment (Steinwandter, 1976; Waliszewski *et al.* 2004), which in turn can be converted to β-isomer (i.e., a transformation trend represented thus; $\gamma \leftrightarrow \alpha \rightarrow \beta$) and in addition β-HCH is resistant to hydrolysis and environmental degradation (Wu *et al*. 1999; Walker *et al*. 1999). All aforementioned properties would tend to cause the β- isomer to be more predominate and persistent in the environment relative to other isomers.

The results obtained for Ondo I, Sore Bale I and II sediments were comparable to values reported for ∑HCHs in Owena-Ondo (0.24 µgg^{-1}) and Ose Rivers (0.51 µgg^{-1}) in Ondo State (Okoya *et al.* 2013). However, Rivers Agoo, Ala, Ogbese, Aponmn and Oni recorded much higher concentrations for ∑HCHs ranging from 2.42 – 13.68µgg^{-1}.

Chlordane isomers in surface water sediments

Cis-Chlordane and trans-chlordane were detected in Sore Bale II and Ondo I, while only the trans-isomer was detected in CRIN and neither of the isomers was detected in Sore Bale I and Ondo II sediments in the dry season (Table 4.18). ∑Chlordane was 0.01 µgg^{-1} (CRIN), 0.06 µgg^{-1} (Sore Bale II), 0.03 µgg^{-1} (Ondo I) and ND (Sore Bale I and Ondo II). The trans-isomer constituted 100% (0), 58% (0.67) and 63% (0.50) of total residual chlordane in CRIN, Sore Bale II and Ondo I respectively, with cis/trans-isomer ratio in parenthesis. These percentages and ratios implied that the source of

contamination was from either technical grade chlordane or/and pure trans-chlordane (or heptachlor) which was previously or recently applied respectively around CRIN cocoa farm environment, while a technical-grade chlordane may have been used in Sore Bale and Ondo I farm areas in recent past. Pure trans-chlordane is not available commercially; therefore the presence of trans-chlordane alone in CRIN sediment may either be due to weathering or aging of technical grade chlordane, since the trans-isomer is more persistent and could be bio-isomerized from cis-isomer (Malik *et al.*, 2009) or as a result of run-off from a nearby farm that recently applied heptachlor. Commercial-grade heptachlor is composed heptachlor (72%), trans-chlordane (20 - 22%) and nonachlor (4-8%) (IARC, 2001) (no cis-isomer in the composition), while commercial-grade chlordane is predominately composed of 19% and 24% of cis- and trans-chlordane respectively (i.e., cis/trans ratio of 0.79). Sethunathan (1973) reported that chlordane does not degrade under anaerobic conditions in flooded soils; hence it is likely to persist in sedimentary environment. The presenceof chlordane in CRIN sediment thus depicted its persistence.

In the wet season residual concentrations of ∑chlordane ranged from ND (Sore Bale I, Ondo I) to 0.06 μgg^{-1} (CRIN) (Table 4.19). The individual percentage composition of cis- and trans-chlordane in ∑chlordane for CRIN and Ondo I sediments were 0% and 100%, Sore Bale II - 25% and 75% respectively. However, both isomers were not detected in Sore Bale I and Ondo I. The cis/trans-isomer ratios was 0 (zero)for CRIN and Ondo I (in favour of trans-chlordane), while 0.33 in Sore Bale II. Ratios< 0.79 implied the persistence of trans-chlordane and as well as strong indication that the origin of the contaminants was due to long-term usage of commercial-grade chlordane around these farms. The trans-isomer had relatively higher concentrations than the cis-isomer in all the sediments where they were detected and this signified the more persistent of the trans-isomer than cis-isomer(Malik *et al*. 2009).

Aldrin and its analogues in sediments

In the dry season, ∑aldrin (i.e., sum of aldrin and analogues, – aldrin analogues are dieldrin, endrin, endrin aldehydes and endrin ketone) ranged from ND (Sore Bale I) to 0.29 ±0.23 μgg^{-1} (Ondo II) (Table 4.18). The highest total aldrin was found in Ondo II, with residual composition of aldrin (70.73 %) and dieldrin (32.27%) only. Aldrin and dieldrin were not detected in CRIN, Sore Bale I and Ondo II sediments. However,

mean concentrations of aldrin were 0.03 μgg^{-1} (0.01 -0.05) and 0.20μgg^{-1} (0.02- 0.48) for Sore Bale II and Ondo II respectively, with ranges in parenthesis, while dieldrin was 0.01 μgg^{-1} (0.01 - 0.03) and 0.08μgg^{-1} (0.04- 0.12). The presence of both aldrin and dieldrin in Sore Bale II and Ondo II suggested that contamination source was from aldrin, which had been used in recent past in these environments. The ratio of residual concentrations of aldrin/dieldrin was > 2 for both Ondo II and Sore Bale II sediments. The second to the highest ∑aldrin was found in Sore Bale II, with the analogue endrin and its metabolite endrin ketone present. The percentage contribution of endrin and endrin ketone to ∑aldrin were 57.4% and 17.4% respectively (or 74.8% combined). This implied that the source contamination may likely have resulted from aging and long-term weathering of aldrin. Endrin is an isomer of dieldrin and undergoes photolytic degradation to endrin ketone and aldehyde (ATSDR, 1996), while dieldrin is formed from metabolic epoxidation of aldrin. The relative high level of endrin may have been due to isomeration of dieldrin, with source of contaminant being aldrin. Although, it could be argued that since commercial endrin existed, contamination source may be from the use of pure endrin. Commercial grade endrin is typically 96.6% pure and contains only traces of aldrin, dieldrin endrin ketone and other minor impurities (Zitko, 2003). The residual levels of aldrin (16.1% contribution) and dieldrin (9.1% contribution) in Sore Bale II sediment were not in trace amounts, but significant, hence aldrin was mostly likely to be the origin of contamination. Endrin ketone is the most important environmental metabolite of the aldrin family (Zitko, 2003). Total aldrin (0.04±0.04 μgg^{-1}) in CRIN sediment was due to endrin and endrin aldehyde, with residual composition of 0.01 μgg^{-1} (ND-0.01) and 0.03 μgg^{-1} (ND-0.08) respectively, ranges in parenthesis. The absence of aldrin and dieldrin inferred that contaminant may have resulted from weathering and aging of aldrin or recent application of endrin as pesticide. Organochlorine pesticides have not been applied in CRIN farms for over 15 years; hence historically, origin is either from aldrin or dieldrin.

Concentration of ∑aldrin in the wet season were as follows; 0.05± 0.06 μgg^{-1} (CRIN), 0.48±0.28 μgg^{-1} (Sore Bale I), 0.10 ±0.06 μgg^{-1} (Sore Bale II), 0.07±0.08 μgg^{-1} (Ondo I) and 0.16±0.12 μgg^{-1} (Ondo II) (Table 4.19). The highest value for ∑aldrin was recorded in Sore Bale I, with concentrations of aldrin, dieldrin and endrin ketone being 0.44 (0.14– 0.47)μgg^{-1}, 0.01 (ND - 0.03)$\mu gg^{-1}$and 0.03(ND – 0.09)μgg^{-1}respectively,

ranges in parenthesis. The compositional contribution of aldrin to ∑aldrin was > 90%, while epoxidized form – dieldrin and endrin ketone were 2.9% and 7.0% respectively. This implied that the Sore Bale I environment was recently treated with aldrin. The endrin ketone found may have resulted from previous application as no endrin was detected. The second highest level of ∑aldrin was recorded at Ondo II, followed by Sore Bale II, Ondo I and CRIN. In Ondo II individual percentage contribution to ∑aldrin was 59% aldrin (0.03- 0.18 μgg^{-1}), 37% dieldrin (ND - 0.10 μgg^{-1}) and endrin 4% (ND - 0.01 μgg^{-1}), with concentration ranges in parenthesis. This percentage composition inferred that origin of contamination may have been from aldrin applied in the past. CRIN recorded the least concentration for total aldrin - concentration of individual analogues were; dieldrin 0.00± 0.01 μgg^{-1} (ND - 0.01), endrin 0.02 ±0.01 μgg^{-1} (0.00 - 0.04) and endrin aldehyde 0.03± 0.04 μgg^{-1} (ND - 0.09), no aldrin was found. The absence of parent aldrin and relatively lower residual amounts of dieldrin and endrin compared to endrin aldehyde metabolite (with over 63% contribution to ∑aldrin) portrayed that aldrin may have been used in the CRIN environment long ago. OCPs have not been applied in the CRIN environment for 15 years. Their presence also depicted persistence.

Dichlorodiphenyl trichloroethane (DDT) and metabolites in sediments

The concentrations of total DDT (i.e., sum of pp'DDT, pp'DDE and op'DDD) ranged from ND (CRIN, Sore Bale II) to 0.33±0.24 μgg^{-1} (Ondo I) in the dry season (Table 4.18). The order of was Ondo I >Sore Bale II > Ondo II > CRIN ≈ Sore Bale I. In Ondo, where OCPs have not been applied for over 3 years, individual DDT analogues and metabolites found were; pp'DDT (0.27 μgg^{-1}, 0.05 – 0.51), pp'DDE (0.05 μgg^{-1}, ND – 0.09 μgg^{-1}) and op'DDD (0.05μgg^{-1}, 0.01 – 0.14), with mean and range concentrations in parenthesis. The relative concentrations of parent pesticides and its metabolites can be used as indicator for assessing the origin of the contaminant. DDT is biodegradable under aerobic and anaerobic conditions to DDE and DDD respectively and the ratio (DDE+DDD)/∑DDT > 0.5 is considered to have resulted from long-term weathering of parent DDT (Doong *et al*. 2002). However, the ratio of the sum of metabolites to total DDT [i.e., (DDE+DDD)/∑DDT] was 0.4, while parent pp'DDT contributed > 72% to ∑DDT. The very high residual concentration of pp'DDT and low ratio showed that DDT was recently applied, in addition to previous application which had resulted to the formation of metabolites. ∑DDT in Sore Bale II

and Ondo II sediments were solely due to pp'DDT and pp'DDE respectively, with corresponding (DDE+DDD)/∑DDT ratios of 0 (Sore Bale II) and 1.0 (Ondo II). The contaminants found in latter sediments may have been due to long-term weathering from pp'DDT application, while the former was from fresh application.

In the wet season, ∑DDT ranged between 0.01 ±0.01 μgg^{-1} and 0.13±0.18 μgg^{-1} (Table 4.19). The highest concentration was found in Ondo I sediments, followed by Ondo II, Sore Bale II, Sore Bale I and CRIN. Parent pp'DDT was detected in Ondo II (ND - 0.08 μgg^{-1}), OndoI (ND - 0.09μgg^{-1}) and Sore Bale II (0.00 - 0.04 μgg^{-1}), while metabolites (pp'DDE and op'DDD) were found in all sediment samples. The aforementioned indicated that all sites were contaminated (100% contamination). The presenceof metabolites depicted that parent compound must have been used in or around these farms. The ratios of (DDE+DDD)/∑DDT were 0 (CRIN, 100% pp'DDE), 1.0 (Sore Bale I), 0.3 (Sore Bale II), 0.9 (Ondo I) and 0.6 (Ondo II). 80% of the farm streams from where sediments were sampled together had ratios > 0.5 and 100% metabolite contamination – this suggested that contaminants were due to aged and weathered agricultural soils, with strong indications of recent application around Sore Bale farm environment. The presence of these contaminants in CRIN and Ondo I sediments showed persistence of DDT and its metabolites, since these farms were last treated with OCPs > 3 years ago. The DDE/DDD ratio in CRIN, Sore Bale I and Sore Bale II for both season and Ondo II (dry season) were either > 1 or I00% DDE metabolite, this implied that biological activity in these sedimentary environments were predominately aerobic, while activity was anaerobic in Ondo I (both seasons) and Ondo II (wet season) sediments. This observed trend may be due to the depth of the streams. It is pertinent to mention here that the streams in CRIN and Sore Bale farms are shallow and are expected to have comparatively high levels of dissolved oxygen and therefore well aerated sediments.

In comparing pp'DDT, pp'DDE and op'DDD values obtained in sediments with other reports, residual concentrations were relatively low when compared with pp'DDT concentrations of 40 -740 ngg^{-1} (or 0.040 – 0.740 μgg^{-1}) in Lake Kariba, Zimbabwe; 13 -740 ngg^{-1} (or 0.013 – 0.740 μgg^{-1}) in Voelvlei Dam, South Africa; pp'DDE concentration of 840 ngg^{-1} (0.840 μgg^{-1}) in Nozha Hydodrome, Cote d' Ivoire (Osibanjo *et al.*, 1994); and > 470 ngg^{-1} (0.470 μgg^{-1}) in Manzala Lake, Egypt (Yamashita *et al.*, 1997), while ∑DDT levels of 25 – 2428 ngg^{-1} in Eberie Lagoon,

Cote d' Ivoire, (Osibanjo *et al.* 1994), 1.3 – 513 ngg^{-1} in Baiyangdian Lake, North China (Hu *et al.* 2010); California Central Valleys, USA, < 1 – 384 ngg^{-1} (Leadprathom *et al.* 2009). Ullah *et al.* (2010) reported concentration range of 12636.58 – 22247.92 ngg^{-1} for sediment samples from Sialkot district of Pakistan. These values were astronomically higher than those obtained in this study.

Heptachlorand its metabolite – heptachlor epoxide in sediments

Heptachlor and its metabolite – heptachlor epoxide were not found in Ondo I and II sediments in both seasons, while they were detected in Sore Bale I and II in both season (Tables 4.18 and 4.19). However, in CRIN the metabolite was found only in the wet season. The highest concentration of total heptachlor (heptachlor + heptachlor epoxide) was obtained in Sore Bale I sediments and it was due to heptachlor, without the metabolite. The residual concentrations ranged from 0.20 - 1.21 μgg^{-1} (0.62), mean concentration in parenthesis. The absence heptachlor epoxide portrayed parent compound as source of contaminant and that application was freshly carried out. In Sore Bale II, contamination was solely heptachlor epoxide, with a mean concentration of 0.02 μgg^{-1} (ND - 0.05), indicating that sediment contamination was due to aging and weathering, since heptachlor epoxide is a metabolite of parent heptachlor.

In the wet season, parent heptachlor contributed 84% to ∑heptachlor in Sore Bale I sediments, while heptachlor epoxide contributed 63% in Sore Bale II. Also the heptachlor epoxide/heptachlor ratios were 0.19 and 2.0 for Sore Bale I and II sediments respectively. The very high percentage composition of heptachlor and low ratio in favour of heptachlor indicated that parent compound was recently used in Sore Bale I, while percentage contribution to total heptachlor by its metabolite and high ratio depicted long-term application in Sore Bale II (residential area). The stream depth around the residential area (Sore Bale II) is more than 8-fold that of the farm area (Sore Bale I) and therefore its sediment would support greater persistence and better sink for residual pesticides.

Endosulfans isomers and metabolite – endosulfan sulphate in sediments

Residual concentration of ∑endosulfan in sediments ranged from ND (Sore Bale II) - 8.44± 6.35 μgg^{-1} (Ondo II) in the dry season (Table 4.18). The order of contamination was Ondo II > Ondo I > Sore Bale I > CRIN > Sore Bale II. In Ondo II, the individual

concentrations of parent compound and metabolites were 2.01 μgg^{-1} (0.10 – 3.01), 1.70 μgg^{-1} (0.10 – 3.88) and 4.74 μgg^{-1} (0.91 – 9.18) for α-endosulfan, β-endosulfan and endosulfan sulphate respectively, with residual ranges in parentheses. The percentage contribution to ∑endosulfan was as follows; α-endosulfan 23.8%, β-endosulfan 20.1% and endosulfan sulphate 56.1%. The endosulfan sulphate/endosulfan ratio was >1, while theα-/β-endosulfan ratio was 1.34.Relatively lower concentrations of parent endosulfan to presence of endosulfan sulphate suggested that source of contaminant was the usage endosulfan in recent past. Almost the same trend was observed in other farm sediments where endosulfan was detected. The endosulfan sulphate contributed between 56.1% to 81.1% to ∑endosulfan, while the endosulfan sulphate/endosulfan ratios were 3.1, 4.4 and 1.6 for CRIN, Sore Bale I and Ondo I respectively. This implied that the source of contamination may have been due to long-term aging and weathering of parent endosulfan previous used in the CRIN and Sore Bale I farm environments, with a recent past application in Ondo I and Ondo II environments.

In the wet season, residual concentration for ∑endosulfan ranged between 0.02μgg^{-1} (0.02) and 6.39 μgg^{-1} (5.76), with standard deviation in parenthesis (Table 4.19). The highest residual concentration was found in Ondo II, while Sore Bale II had the least. Individual concentrations for Ondo II sediments was 1.58 μgg^{-1} (24.7%), 0.35 μgg^{-1} (5.5%) and 4.47 μgg^{-1} (69.8%) for α-endosulfan, β-endosulfan and endosulfan sulphate respectively, with percentage contribution to ∑endosulfan residue in parentheses. The concentration of α-isomer was more than 4-fold greater than the β-isomer (i.e., α/β ratio > 4.0), with relatively much higher presence of its metabolite endosulfan sulphate - endosulfan/endosulfan sulphate ratio was < 0.5. The α/β-isomer ratio in normal commercial grade endosulfan is 2.3. Therefore the aforementioned observations – relative high levels of endosulfan sulphate compared to parent compound and 4-fold α-isomer concentration relative to β-isomer, depicted both long-term and fresh application of endosulfan in the Ondo II farm environment. OCPs are still in use in this farm.

Residual concentrations of total endosulfan in all stream sediments were significantly higher than values obtained in River Densu (Kuranchie *et al.* 2012) and from a farming community (Ntow, 2001) in Ghana, but comparable values were reported by Okoya *et al.* (2013) for total parent endosulfan (endosulfan sulphate was not measured). Also, Idowu *et al.* (2013) reported much higher levels of 0.249 – 127.146 $mgkg^{-1}$ (or μgg^{-1})

in sediments from rivers around cocoa producing areas of Ondo State, while high levels of ND -37780 ngg^{-1} (Adeboyejo *et al*. 2012) and 17 -11155 ngg^{-1} (Osibanjo *et al.*1994) have also been found in sediments of Lagos and Lekki Lagoons respectively. ∑endosulfan recorded for CRIN, Sore Bale I and Sore Bale II sediments (ND – 0.31 μgg^{-1}) were comparable to levels reported in Agboya creek, Lagos (William, 2013), but lower than values obtained in California Central Valley, USA (1 – 571 μgkg^{-1} or 0.001 – 0.571 μgg^{-1}) (Weston *et al*. 2004).

4.2.5. Distribution of residual OCPs in Sore Bale farm and residential areas

The ∑OCPs in surface water and sediments were 0.63±0.29 μgL^{-1} and 1.16 ±0.82 μgg^{-1} (DW) respectively for Sore Bale I (farm area) in the dry season (Tables 4.15 and 4.17), while Sore Bale II (residential area) recorded 0.10 ±0.02 μgL^{-1} and 0.48±0.38 μgg^{-1} respectively (Tables 4.17 and 4.19). In the wet season, residual levels in Sore Bale I (farm area) were 0.21± 0.21 μgL^{-1} (surface water) and 2.79±1.46 μgg^{-1} (sediment), while 0.06 ±0.05μgL^{-1} (surface water) and 0.53±0.44 μgg^{-1} (sediment). The residual concentration of total OCPs in surface water and sediments in the farm area during the dry season were about seven-fold and two-fold respectively greater than corresponding levels at the residential area, while in the wet season – a four-fold and five-fold differential in favour of the farm area was also observed.

The contribution of individual OCPs to the total 'OCP-impact' to surface water in the residential environment during the dry season was δ-HCH (100%); while α-endosulfan (14.7%), β-endosulfan (13.9%), endosulfan sulphate (29.6%) and aldrin (41.8%) in the farm area, with percentage contribution to total OCPs in parentheses. Contaminants in surface water during the wet season were α-HCH, β-HCH, α-endosulfan, β-endosulfan, endosulfan sulphate in the residential area (5.2% - 43.1%), while nine individual OCPs were detected in the farm area, namely:- pp'DDT, heptachlor, α-endosulfan, β-endosulfan, endosulfan sulphate, endrin, endrin ketone, cis-chlordane and trans-chlordane (3.3% - 24.9%), percentage contribution range in parentheses. The maximum individual OCP contributions in the wet season were by β-HCH and endosulfan sulphate at the residential and farm areas respectively. Fewer individual OCPs were detected in the residential area compared to the farm area and where the same OCPs were detected in both areas, their levels were comparatively higher in the farm area. These trends may be due to the long distance between the farm area and the

residential area (about 2-3 kilometers apart), which had resulted to pesticide dispersion on the surface water as it moves away from the point source (farm area)– thus making the surface waterin the residential area less contaminated.

In the sedimentary environment, more individual OCPs were detected in the residential area than in the farm area. Percentage detection were 80.9% and 71.4% for residential and farm sediments respectively. Total OCPs in the residential area was slightly higher in the wet season than in the dry season. The detection of fewer OCPs in the farm sediment could be due to the shallow depth of stream at the farm area (< 60 cm) relative to that of the residential area (>180 cm). During heavy rain fall, OCPs clinging to organic matters and non-organic particulates (e.g, silts and clay) suspended in the surface water and on the sediment bed of the shallow stream in the farm area are easily swept along the stream towards the residential area of the Sore Bale stream as a result of high water current. The Sore Bale farm stream flows from the farm area (i.e., OCP point source) towards the residential area. It is therefore expected that OCPs-bound-particulates on sedimentary system or bed would reside longer in the residential area than the farm - which may eventually lead to accumulation and persistence if undisturbed.

Comparatively, the sedimentary environment and the surface water in the residential area has a much lower risk level to the farm, based on total OCPs obtained especially for the surface water – which serves as source of water for domestic purposes.

4.2.6. Ecological risks of organochlorine pesticides in sediments

There is no unified standard for ecological risk assessment of sediments;however, OCP values obtained from previous studies were compared to published numerical sediment quality guidelines (SQGs) (Long *et al*.1995;MacDonald *et al*. 2000;Tang *et al.* 2007; Qiu *et al*. 2009; Hu *et al*. 2010). However, in this study the potential risk of OCPs residues in sediments were evaluated by comparing residual levels in sediments from CRIN, Sore Bale (farm and residential areas), Ondo I and Ondo II to the consensus-based sediment quality guidelines (CB-SQG) for fresh water ecosystems (MacDonald *et al*. 2000; Yan Wang *et al*. 2013).

There are no consensus – based sediment quality guidelines (SQGs) for α-,β-,δ-,ε - HCH isomers (for freshwater sediments), except γ – HCH (Lindane) with threshold

effect concentration (CB-TEC) and probable effect concentration (CB-PEC) of 2.37 ngg^{-1} and 4.99 ngg^{-1} respectively. Threshold effect concentrations (TECs) are concentrations below which harmful or adverse effects are unlikely to be observed, while probable effect concentrations (PECs) are concentrations above which harmful effects are likely to be observed. The residual concentrations of γ-HCH (lindane) in all fivesediments ranged from 0.01 - 0.23 μgg^{-1} (10 - 230 ngg^{-1}) for both seasons; this range significantly exceeded the stipulated CB-TEC and CB-PEC limits for γ-HCH (lindane) (MacDonald *et al*. (2000). This therefore infers that the residual levels of γ-HCH in all sediments from study sites would have harmful and adverse biological effects on aquatic lives during both seasons.

All residual concentrations of chlordane in CRIN (dry and wet seasons), Sore Bale residential (dry and wet seasons), Ondo I (dry season) and Ondo II (wet season) exceeded the CB-TEC value of 3.24 ngg^{-1}, while CRIN (wet season) and Sore Bale residential area (dry and wet seasons) recorded levels above CB-PEC limit of 17.6ngg-1.

In the aldrin analogues only dieldrin and endrin have CB-SQGs, no consensus-based numerical sediment quality guideline (CB-SQGs) for aldrin, endrin aldehyde and endrin ketone. The CB-TEC limits for dieldrin and endrin are 1.90 and 2.22 ngg^{-1} respectively. All residual concentrations of dieldrin were above the stipulated CB-TEC limit, except where not detected - this represented about 60% and 80% of all sites in the dry and wet seasons respectively. Sediments from CRIN (dry and wet seasons), Sore Bale residential area (dry and wet seasons), Ondo I (dry and wet seasons) and Ondo II (wet season) have levels higher than the CB-TEC SQG limit for endrin. However, none exceeded the CB-PEC of 61.8 ngg^{-1} and 207 ngg^{-1} for dieldrin and endrin respectively (MacDonald *et al.*, 2000).

The residual levels of pp'DDT in Sore Bale (residence, both seasons), Ondo I (both seasons) and Ondo II (wet) exceeded the CB-TEC value of 4.16 ngg^{-1}, while value obtained for CRIN sediment was lower. CB-PEC for pp'DDT (62.9 ngg^{-1}) was below residual levels recorded in Sore Bale (residence, dry season) and Ondo I (dry season) (Table 4.21). Total DDT in all sediments (except for CRIN sediments during the wet season) exceeded the CB-TEC value of 5.28 ngg^{-1}, however they were all below the CB-PEC value of 572 ngg^{-1} for ∑DDT (MacDonald *et al.* 2000). This implied that all

sediments, except in CRIN (wet season) (and other sites where ∑DDT was not found), as shown in Tables 4.18 and 4.19, wouldnot have adverse biological effects on aquatic lives.

There is no consensus-based numerical sediment quality guideline (CB-SQGs) by MacDonald *et al.* (2000) for heptachlor; however, CB-TEC and CB-PEC for heptachlor epoxide are 2.47 ngg^{-1} and 16.00 ngg^{-1} respectively and except where heptachlor epoxide was not detected, all residual concentrations were above the CB-TEC level (CRIN, Sore Bale farm area, Sore Bale residential area), while sediments from Sore Bale farm and residential areas exceeded the CB-PEC limit – this suggesting that adverse biological effects might be expected, especially at Sore Bale residential area, where the highest residual concentration of 0.041μgg^{-1} (41 ngg^{-1}) heptachlor epoxide was recorded .

Finally, other OCPs such as endosulfans, aldrin, endrin aldehyde, endrin ketone and methoxychlor do not have consensus-based numerical sediment quality guidelines (SQGs) by MacDonald *et al*., (2000).

4.3. FIELD KINETICS – DEGRADATION OF ENDOSULFAN IN COCOA FARM

The results of GC-MS analysis for field kinetics over a period of 60 days on residual concentrations of ∑endosulfan (α-endosulfan, β-endosulfan and metabolite endosulfan sulphate) in cocoa plant (fresh leaves, bark, pods and seeds) and surrounding (soil and dry leaves) are shown in Figures 4.29 – 4.41 (and in Appendix Tables B-1 –B-8), while chemo-kinetic parameters are presented inTables 4.20.

4.3.1. Evaluation of analytical methods from recovery

The precision of results were evaluated from the standard deviation and relative standard deviation of three replicate measurements each for a given matrix using two concentrations of low (125μgg^{-1}) and high (500μgg^{-1}) for α-endosulfan, β-endosulfan and endosulfan sulphate standards.

The percent recovery for α-endosulfan, β-endosulfan and endosulfan sulphate with cocoa vegetation ranged from 88.6±2.3 - 105.6±3.8%, 87.7±1.8 - 102.2±1.7% and 89.1±1.4 - 100.8±1.5% respectively, while corresponding values with soils were

88.8±2.4 -104.7±3.0%; 89.4±3.4 - 103.3±2.4%; 89.2±2.3 -94.7±2.8% (Appendix B, Table B-8). The calculated %RSD for the three pesticides were ≤ 5.2 % for all matrixes (n = 42), while 85.7% of these replicate measurements had their %RSD in the range of 1.2 – 3.5 %. The aforementioned indicated a reasonable variability and high precision of the analytical methods adopted.

4.3.2. Theobroma cacao vegetation

4.3.2.1. Distribution of residual endosulfan concentration

The distribution of residual concentrations of ∑endosulfan on day 0 ranged between <0.001 and 97.01 $\mu g g^{-1}$ on cocoa plant (fresh leaves, stem bark, pods and seeds) (Figures 4.29 – 4.31).

Total endosulfan was due to parent compounds as no metabolite was found in leaves, bark, pods and seeds. The highest residual level of ∑endosulfan was recorded on fresh leaves, while initial levels of α- and β-endosulfan were 66.51±17.48 $\mu g g^{-1}$ and 30.50±8.24 $\mu g g^{-1}$ respectively in fresh foliage (Figure 4.29).

The order of ∑endosulfan residual concentrations was fresh leaves >bark >pods >seeds (Figure 4.32). This trend may be due to the exposed surface area, shape and position of each of these plant components on the cocoa tree; for example, the leaves have larger surface area, with flat surface horizontally positioned and may tend to accommodate more pesticide deposit than any other plant component after its application. In addition, the epicuticular waxy nature of plant leaves may have enhanced the initial distribution of endosulfan on the cocoa fresh leaves. Plant leaves are reported to contain predominately long-chain polyester that accumulates lipophilic substances such as OCPs (Reischl *et al.* 1989; Calamari *et al.* 1991).

Residual concentrations of α- and β-endosulfan isomers in fresh leaves decreased rapidly between day 0 and 7, while its metabolite - endosulfan sulphate was formed on day 7 (Figure 4.29). The percentage dissipation was 71.64% for ∑endosulfan, while parent isomers α- and β-isomers were 70.58% and 80.24%, respectively (Figure 4.33). These values agreed with the reports of Kennedy *et al.* (2001) and Ntow *et al.* (2007), who observed over 70% of dissipation of ∑endosulfan in foliar part of cotton and tomato plants respectively, after 7 days of initial treatment.

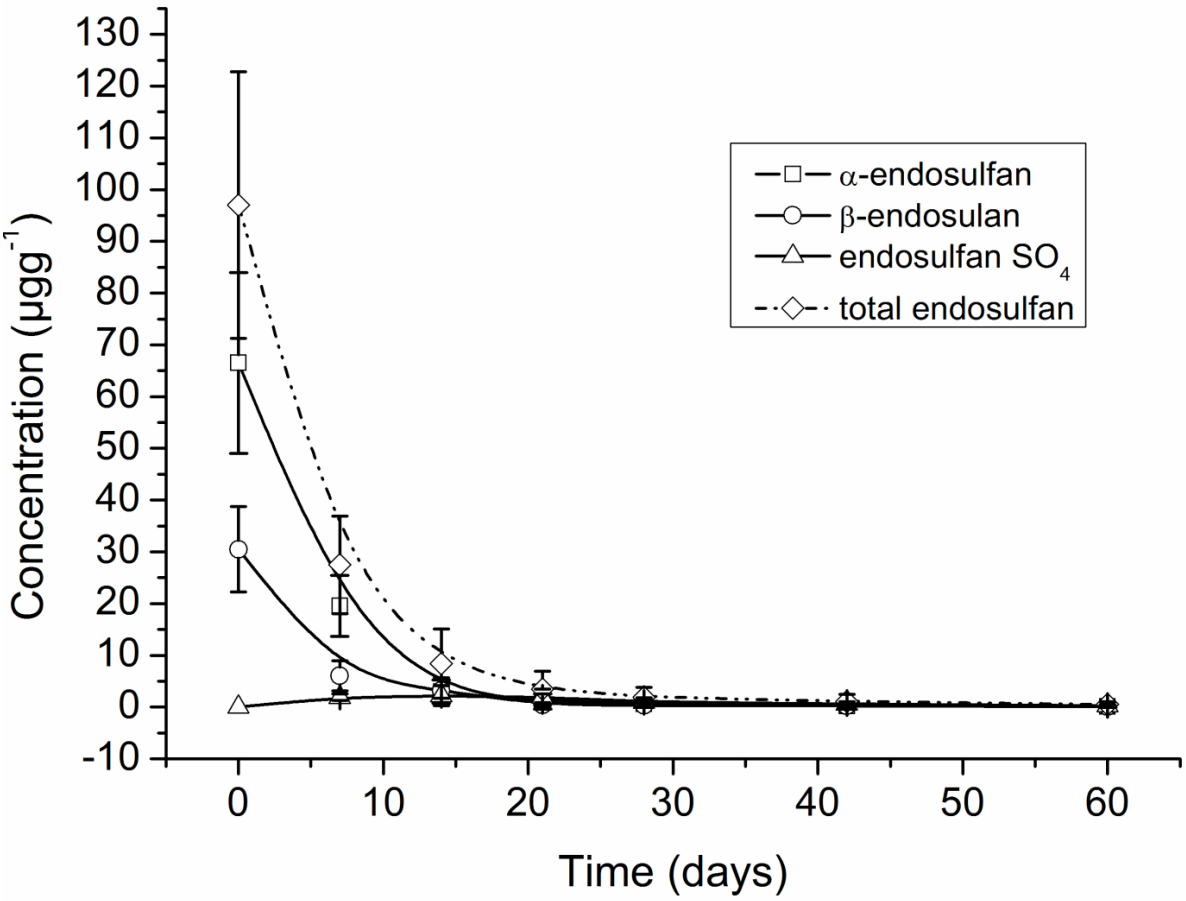


Figure 4.29. Residual concentration of α-, β-endosulfan, endosulfan SO_4 and total endosulfan in fresh cocoa leaves over 60 days

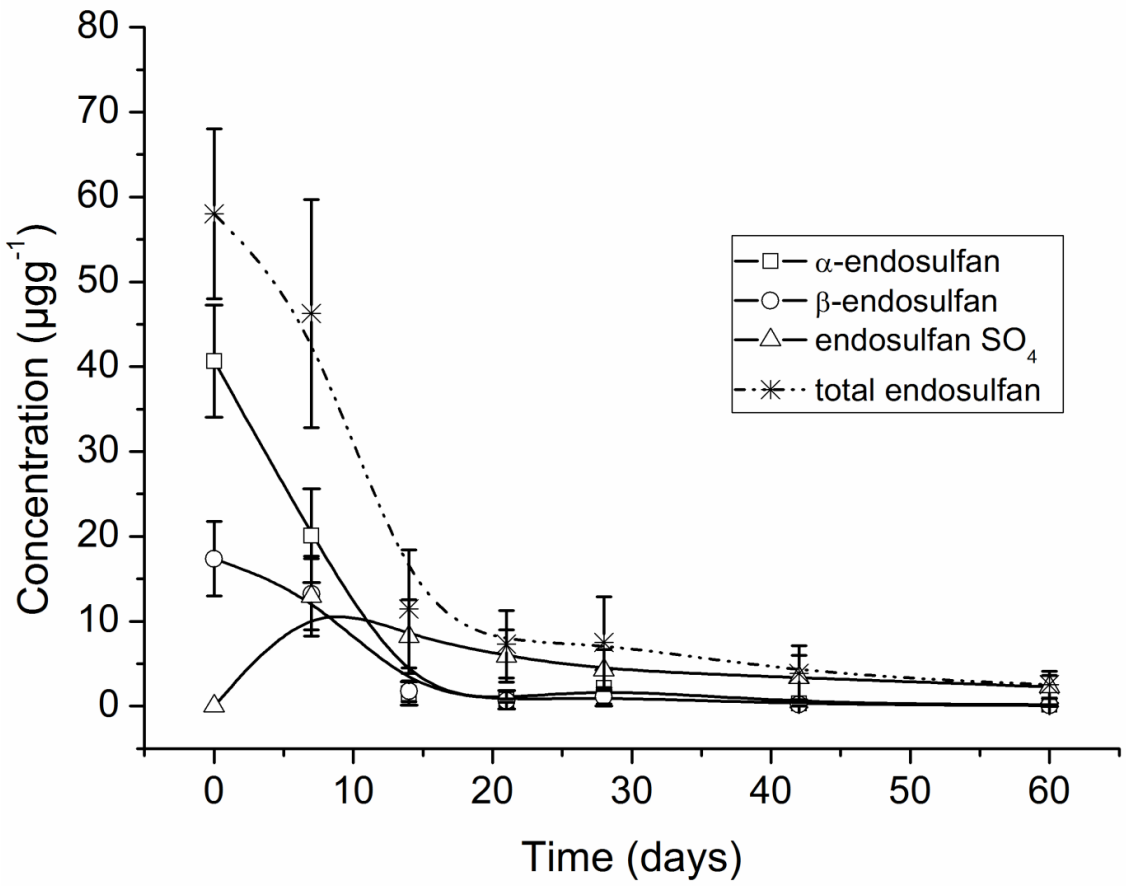


Figure 4.30. Residual concentration of α-, β-endosulfan, endosulfan SO_4 and total endosulfan in cocoa stem bark over 60 days.

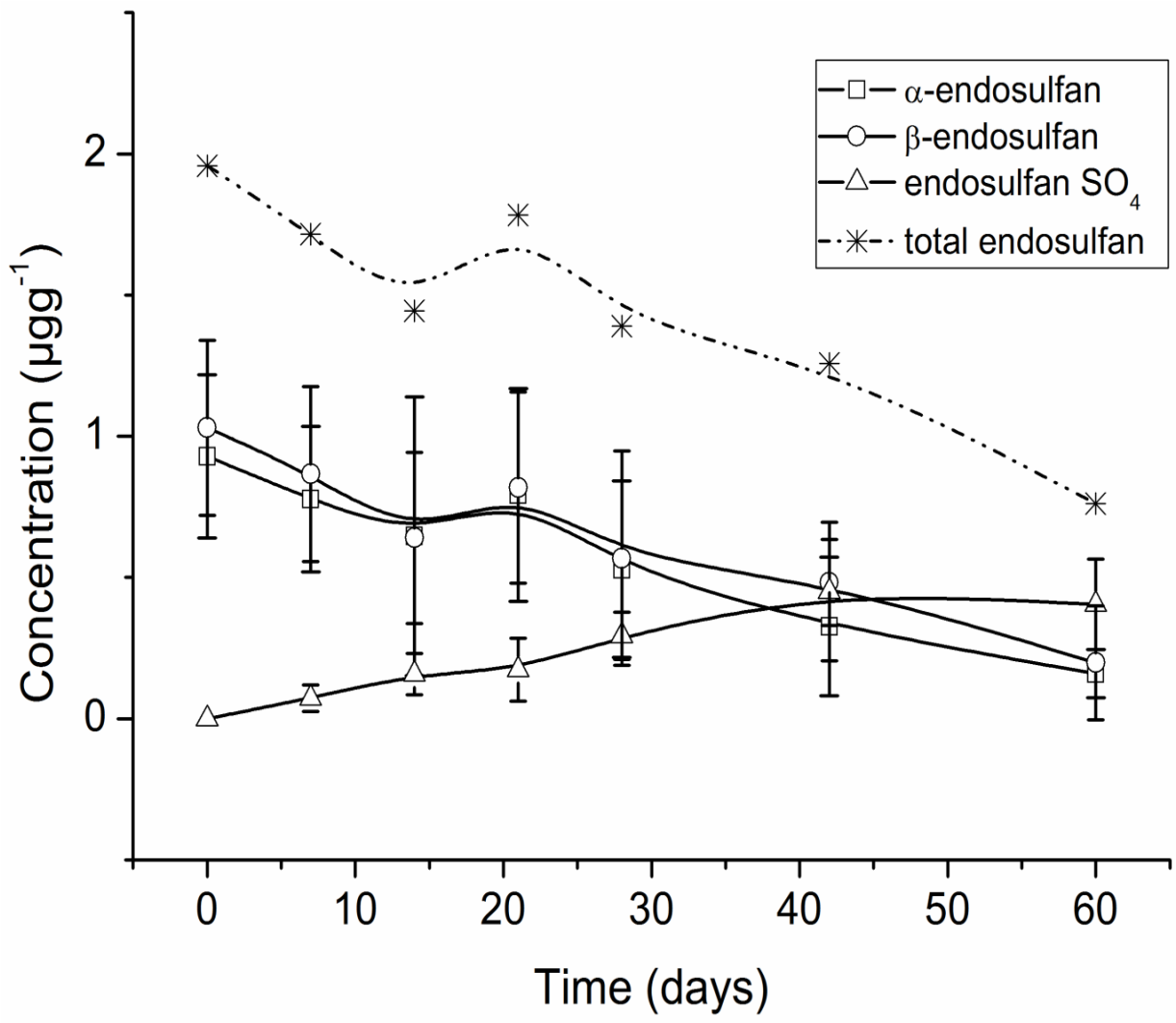


Figure 4.31. Residual concentration of α-, β-endosulfan, endosulfan SO_4 and total endosulfanin cocoa pods over 60 days.

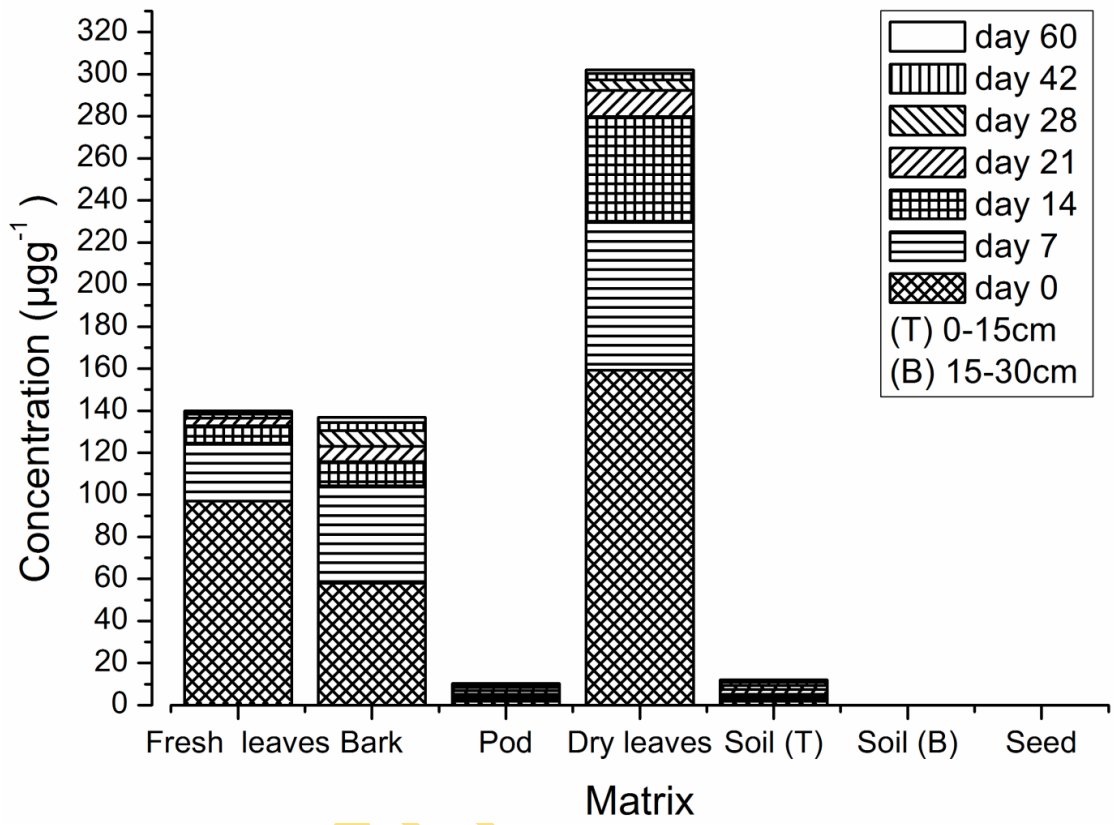


Figure 4.32. Residual concentration of total endosulfan over a period of 60 days in different matrixes

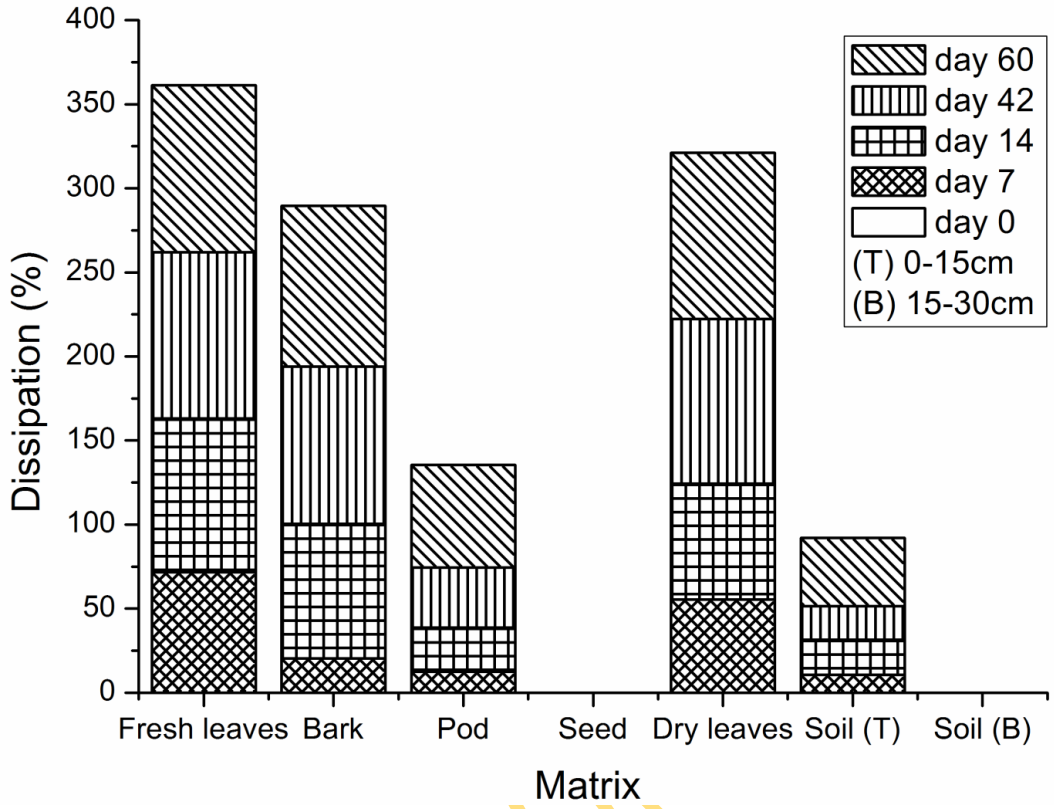


Figure 4.33. Percentage dissipation of total endosulfan in different matrixes on days 0, 7, 14, 42 and 60

The National Research Council, Canada (NRC) reported that in most fruits and vegetables, 50% of the parent residue is lost within 3 to 7 days after application (NRC, 1975). This rapid loss may be attributed to volatilization, although some level of degradation had occurred, since endosulfan sulphate – a metabolite was found at day 7. The higher percentage disappearance recorded for β-endosulfan compared to the α-isomer in fresh leaves may also be due to the conversion of the β-isomer to α-isomer (Rice *et al.* 1997; Hapeman *et al.* 1997;Schmidt *et al.* 2001).For other plant components like stem bark, the percentage dissipation-degradation for ∑endosulfan, α- and β-endosulfan was 20.26%, 50.55% and 24.01%, respectively, while pods values were 12.36%, 16.27% and 15.92%, respectively (Figure 4.33).

The order of loss was fresh leaves >bark >pods. The relatively higher residual ∑endosulfan contents (i.e, low loss or dissipation) in stem bark and pods compared to foliar part (fresh leaves), were likely due to the formation of the metabolite endosulfan sulphate and the screening from direct effect of sunlight that may have enhanced volatilization of parent compound.This is evident from the morphology of the cocoa plant, where the foliage and branches form a canopy, thereby covering the rest part of the plant from direct sunlight and wind (or air movement or current) – especially the stem. In addition, the foliage is favoured by the horizontal position of the lamina (Raha *et al.* 1993; Antonious *et al.* 1998) and its' remarkable advantage in wider surface area over other plant tissues or parts are likely to enhance greater volatilization – and thus higher dissipation.

4.3.2.2. Terminal concentrations of endosulfan and ratios

At day 60, percentage dissipation (due to volatilization-degradation) in *Theobroma cacao* vegetation ranged from 61.13 to 99.84% for ∑endosulfan, α- and β-endosulfan (Figure 4.33). Residual concentrations in cocoa foliage were 0.11 μgg^{-1} (99.84%), 0.12 μgg^{-1} (99.62%), and 0.51 μgg^{-1} (99.47%) for α-endosulfan, β-endosulfan and ∑endosulfan respectively (with percentage dissipation in parenthesis) on day 60 (Figures 4.29 and 4.33), while in stem bark residual amounts were 0.17μgg^{-1} (99.57%), 0.07 μgg^{-1} (99.58%) and 2.51 μgg^{-1} (95.67%) (Figures 4.30 and 4.33); pods were 0.16μgg^{-1} (80.78%), 0.20 μgg^{-1} (82.87%) and 0.76 μgg^{-1} (61.13%) for α-endosulfan, β-endosulfan and ∑endosulfan respectively (Figure 4.31 and 4.33).This implied that the

pod had higher residual levels of endosulfan than in the leaves. High carotenoid levels have been reported to be responsible for retention chlorinated hydrocarbons in the body and peel of vegetables (Miglioranza *et al.* 1999).

The residual concentrations of both isomers at day 60 were not significantly different in the foliage and pods (see Appendix Tables B-1and B-3); however, a significant differential was observed in the stem bark (see Appendix Table B-2). This was evident in the α/β ratio of ≈ 1, at day 60 (Figures 4.34 and 4.36). The α/β ratio of parent endosulfan and endosulfan sulphate/∑endosulfan ratio are often used as indicator to show its weathering or aging in the environment (Kennedy *et al.* 2001; Malik *et al.* 2009). The α/β ratio for stem bark was 2.37 compared to 0.90 and 0.80 for fresh foliar and pods respectively (Figures 4.34, 4.35 and 4.36). However, it is pertinent to mention that the initial concentration of α- and β-isomers in pods at day 0, were 0.93±0.30 μgg^{-1} and 1.03±0.31 μgg^{-1} respectively – this therefore gives an α/β ratio of 0.90 compared to 2.3 expected for technical grade endosulfan that was applied at the start of the experiment (Figures 4.31 and 4.36). The α/β ratio in fresh foliage and stem bark on day 0 were 2.18 and 2.34 respectively (Figures 4.34 and 4.35), these are comparable to the expected α/β-endosulfan ratio of the commercial endosulfan used in this study.This drastic deviation in the cocoa pods may have been due to an initial rapid enzymatic action on the pesticide (Wier *et al.* 2006; Ortiz-Hernandez *et al.* 2013), with the α-isomer being more susceptible to the enzymatic breakdown. However, the level of residual metabolite endosulfan sulphate was < 0.001 μgg^{-1} on day 0. This may be due to fast degradation of the endosulfan to other metabolites like endosulfan diol, endosulfan hydroxyl carboxylic acid, endosulfan ether, and endosulfan lactone (UNEP, 2009).

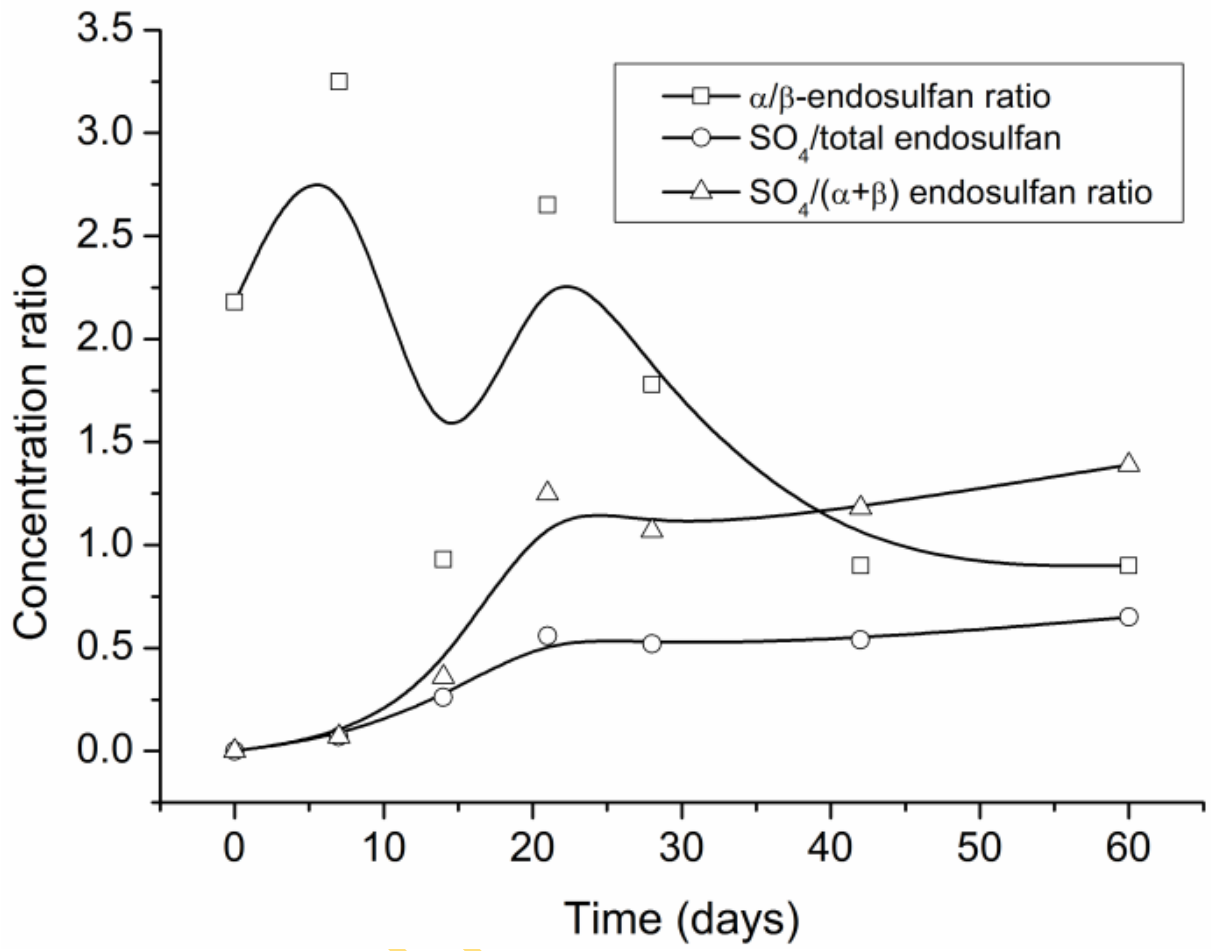


Figure 4.34. Ratios of α/β-endosulfan, endosulfan SO_4/total endosulfan and endosulfan SO_4/ (α+β) endosulfan on fresh leaves

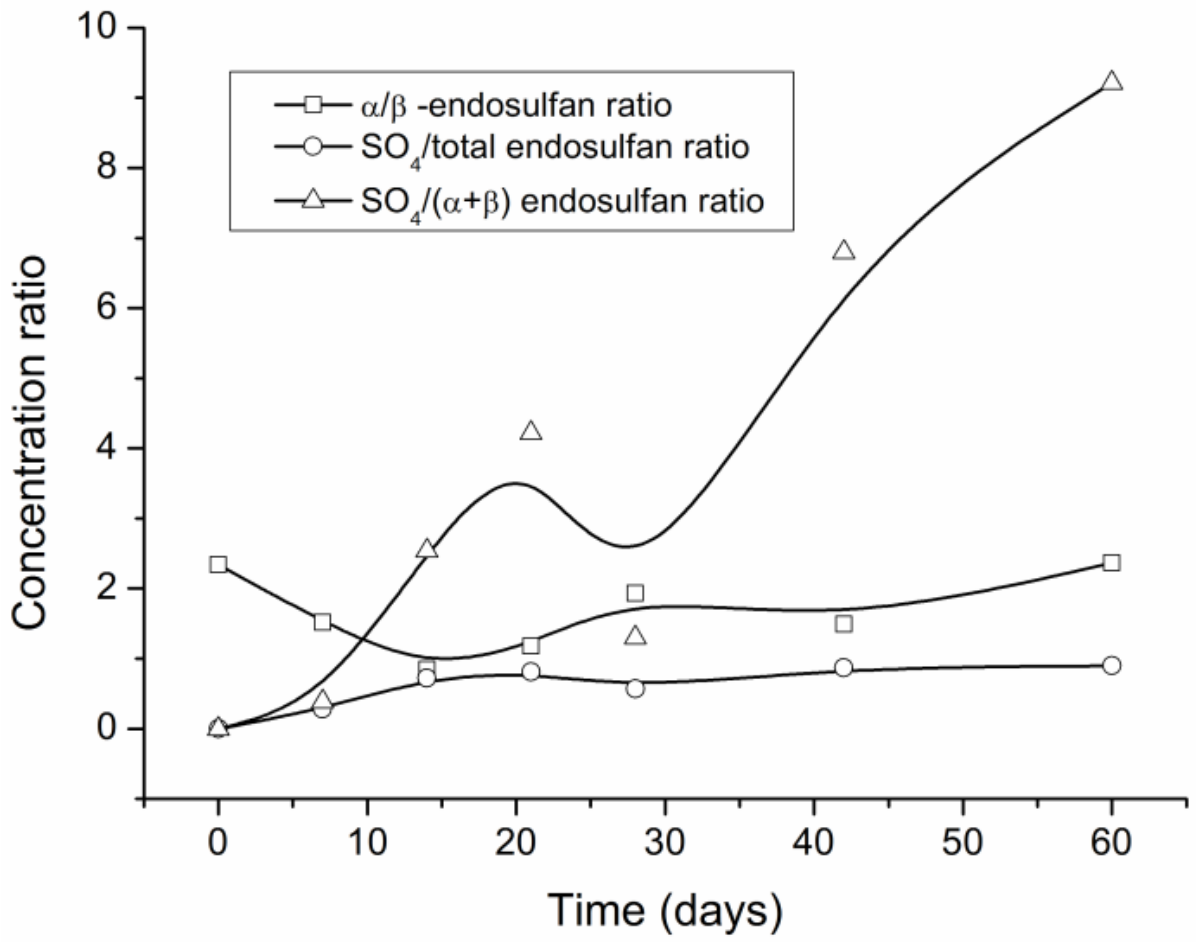


Figure 4.35. Ratios of α/β-endosulfan, endosulfan SO_4/total endo and endosulfan SO_4/ (α+β) endosulfan on stem bark

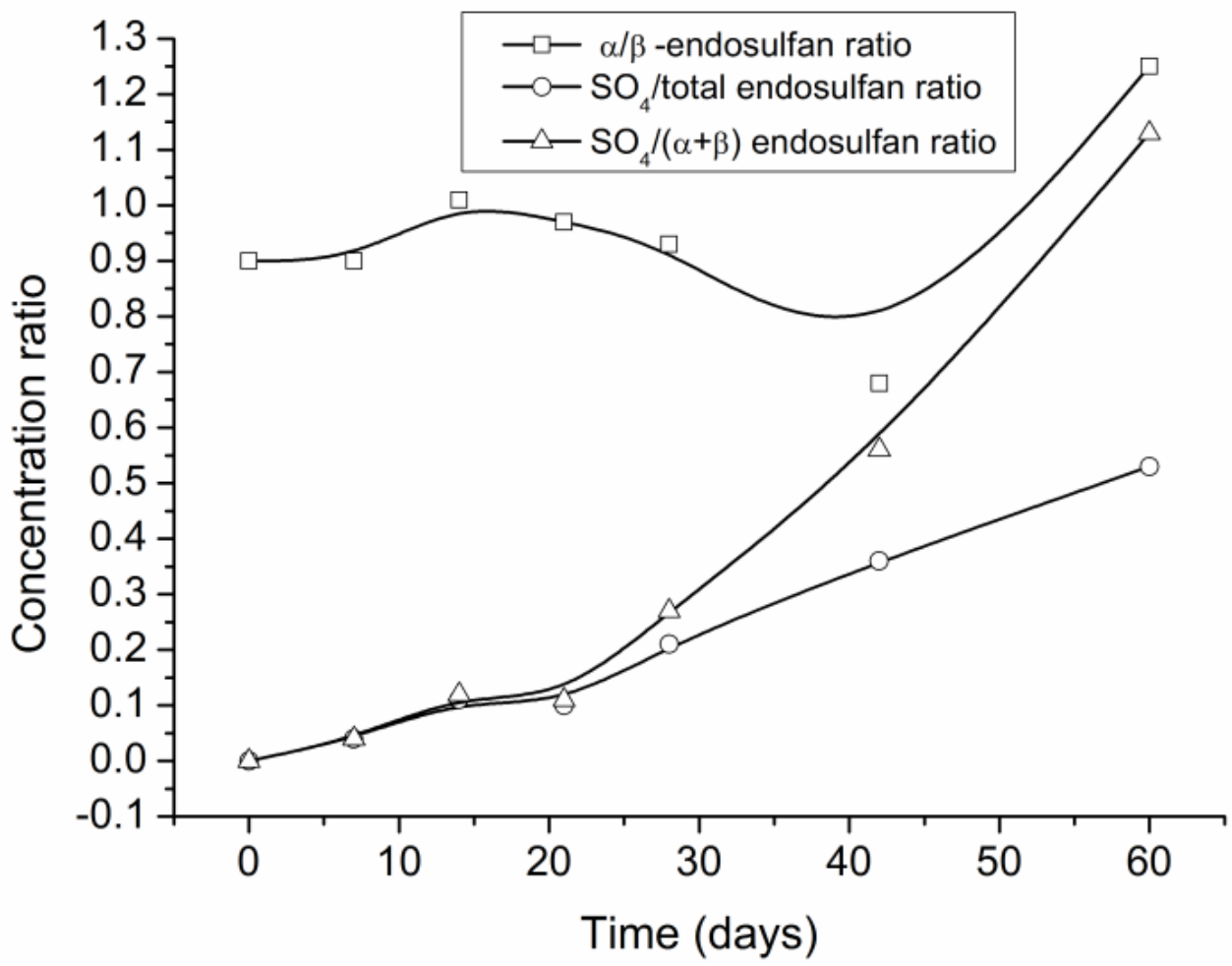


Figure 4.36. Ratios of α/β-endosulfan, endosulfan SO_4/total endosulfan and endosulfan SO_4/ (α+β) endosulfan on cocoa pods

In addition, in aqueous environment, endosulfan diol is the predominant metabolite of parent endosulfan and where the sulphate is formed it is further metabolized to endosulfan diol (USEPA, 2002). The moisture content of cocoa husk is about 14% (w/w), this can facilitate the hydrolysis of endosulfan in pod tissues to the diol metabolite. Besides, the presences of some endogenous biological enzymes such as lignin peroxidases and pectin methyl esterase (Tucker and Woods, 1990; Kullman and Matsumura, 1996) may have enhanced the rapid degradation of endosulfan observed on day 0. These other metabolites were not determined; endosulfan sulphateis reported to be the major metabolite of endosulfan - which also is an intermediary metabolite to the formation of other metabolites in plants and animals via the endosulfan diol route.

The relatively lower residual concentration of the α-isomer in the fresh leaves (with α/β ratio < 1.0) at day 60 and the rapid decline in residual concentration from day 0, may be due to the physiochemical properties of both isomers. The α-isomer has been found to be more volatile than the β-isomer, their vapour pressures at 20^{o}C are 0.006 mmHg and 0.003 mmHg respectively. This may account for the relative persistence of β-endosulfan in this environment. The rapid decline in the α/β-isomer ratio from day 7 (>3.0) to day 60 (<1.0), after an initial increase from 2.3 (day 0), depicted a faster rate of disappearance of α-isomer relative to the β-isomer – this also portrayed its' more persistence.The initial increase in the ratio between days 0 and 7, may be due to early conversion of the β-isomer to the α-isomer (Tiwari and Guha 2013).

It has been reported that residues of parent isomers are generally negligible after 2-3 weeks of application of 1.0 -100 mgkg^{-1} parent endosulfan, with α-isomer being less persistent than the β-isomer (NRC, 1975).

4.3.2.3. Formation and disappearance of metabolite - endosulfan sulphate

The levels of endosulfan sulphate were <0.001 µgg^{-1} on day 0, in all vegetation components assayed (Figures 4.29 – 4.31); however, various levels were recorded on day 7 in all the components. The concentrations of endosulfan sulphate were 1.92 ± 0.65 µgg^{-1}, 12.96 ± 3.70 µgg^{-1} and 0.07 ± 0.05 µgg^{-1}on fresh leaves, bark and pods respectively (Tables B-1, B-2 and B-3).

The levels of endosulfan sulphate was observed to have increased in almost all the components - due to build-up and persistence as time progresses, with significant

decline in the concentrations of the parent compound. Highest concentrations were observed on days 14 and 42 for fresh leaves and pods respectively, while on cocoa bark it was observed at day 7 and these persisted with slight decline through day 60 (Figures 4.29, 4.30 and 4.31). High level of metabolite in the bark may be due to the morphological nature of the stem bark which has crevices or small grooves that may have trapped the pesticides and also restricted oxidative action on endosulfan.

On day 7, about 1.98%, 22.31% and 3.73% of the initial contents of ∑endosulfan sprayed at the start of the experiment (i.e, day 0) was oxidized to endosulfan sulphate in the foliar, stem bark and pods respectively and this constituted 6.98% (foliar), 28.02% (bark) and 4.25% (pods) of ∑endosulfan on that period (Tables B-1, B-2 and B-3). Its contribution to ∑endosulfan increased steadily to 65.13%, 90.20% and 55.09% in cocoa leaves, stem bark and pods respectively at the end of the experiment (i.e., ratios of residual concentration of endosulfan sulphate to ∑endosulfan for vegetation matrices were > 1). Ratios of endosulfan sulphate/∑endosulfan and endosulfan sulphate/(α+β) endosulfan could be used as markers for weathering and degradation of applied technical grade endosulfan.The endosulfan sulphate/(α+β) endosulfan ratio curves for all vegetation components rose steadily/rapidly from day 7 through day 60 as more metabolite is being formed, with the stem bark exhibiting a more steep nature (Figures 4.35).

On plant surfaces endosulfan is oxidized to endosulfan sulphate (Antonious *et al.* 1998; Ntow *et al.* 2007). In most plant residue studies, endosulfan sulphate residue levels tend to increase relative to the parent isomers and other metabolites, thereby exhibiting more persistence (Coleman and Dolinger, 1982).Reports of field studies on endosulfan involving plants have shown that the sulphate metabolite is more persistent than the parent compound - accounting for 90% of the ∑endosulfan residue by week 11 after application (NCR, 1975). α-, β-isomers and metabolite endosulfan sulphate were not detected in the seeds during this study. The almost 100% disappearance of the parent endosulfan and metabolite at day 60, showed that the residual concentrations with respect to fresh leaves, pod and bark were due to the topical treatment and not due to root uptake from the soil and translocation to aerial parts.

4.4. SURROUNDING DRY FOLIAGE AND SOILS

4.4.1. Distribution of residual endosulfan on dry foliage and soils

The mean concentration of ∑endosulfan on fell dry foliage and topsoil (0-15cm) samples at day 0, were 159.25 ± 37.89 μgg^{-1} and 1.88 ± 1.05 μgg^{-1} respectively (Figures 4.37 and 4.38),while level in bottom soil (15-30 cm) was <0.001μgg^{-1} (Appendix Table B-7). The initial levels of α-and β-endosulfan were 108.77 ± 25.72 μgg^{-1} and 50.48 ± 12.16 μgg^{-1} respectively, while topsoil (0-15cm) values were 1.24 ± 0.65 μgg^{-1} and 0.64 ± 0.40 μgg^{-1} for α- and β-endosulfan respectively. The higher concentration of ∑endosulfan of more than eighty-fold magnitude for dry foliage compared to surrounding soil on day 0, was as a result of fell dry leaves covering the topsoil in the cocoa farm (see Figure 3.5).

As a normal practice, such dry leaves are left on cocoa farm soils to serve as mulch especially in the tropics. The dry leaves became initial receiving surface for non-target sprayed endosulfan, around the cocoa tree, thereby restricting large amount of pesticide reaching the top soil after its application on day 0. This was evident in the very high levels of the isomers (and indeed ∑endosulfan) on the dry leaves at day 0, relative to values obtained in soil samples. The levels of metabolite - endosulfan sulphate on both matrices were < 0.001 μgg^{-1} on day 0.

Between day 0 and day 7, residual concentrations of α-, β-endosulfan and ∑endosulfan, on dry foliage decreased rapidly, with 64.51%, 46.71% and 55.50% percentage losses respectively (Figure 4.37). These losses were mainly due to volatilization as amount of ∑endosulfan accounted for as residue was 44.45% (i.e., of initial concentration), with endosulfan sulphate constituting only 7.86% of the residual concentration on day 7 (and 3.50% with respect to day 0 concentration) (see Appendix Table B-5).

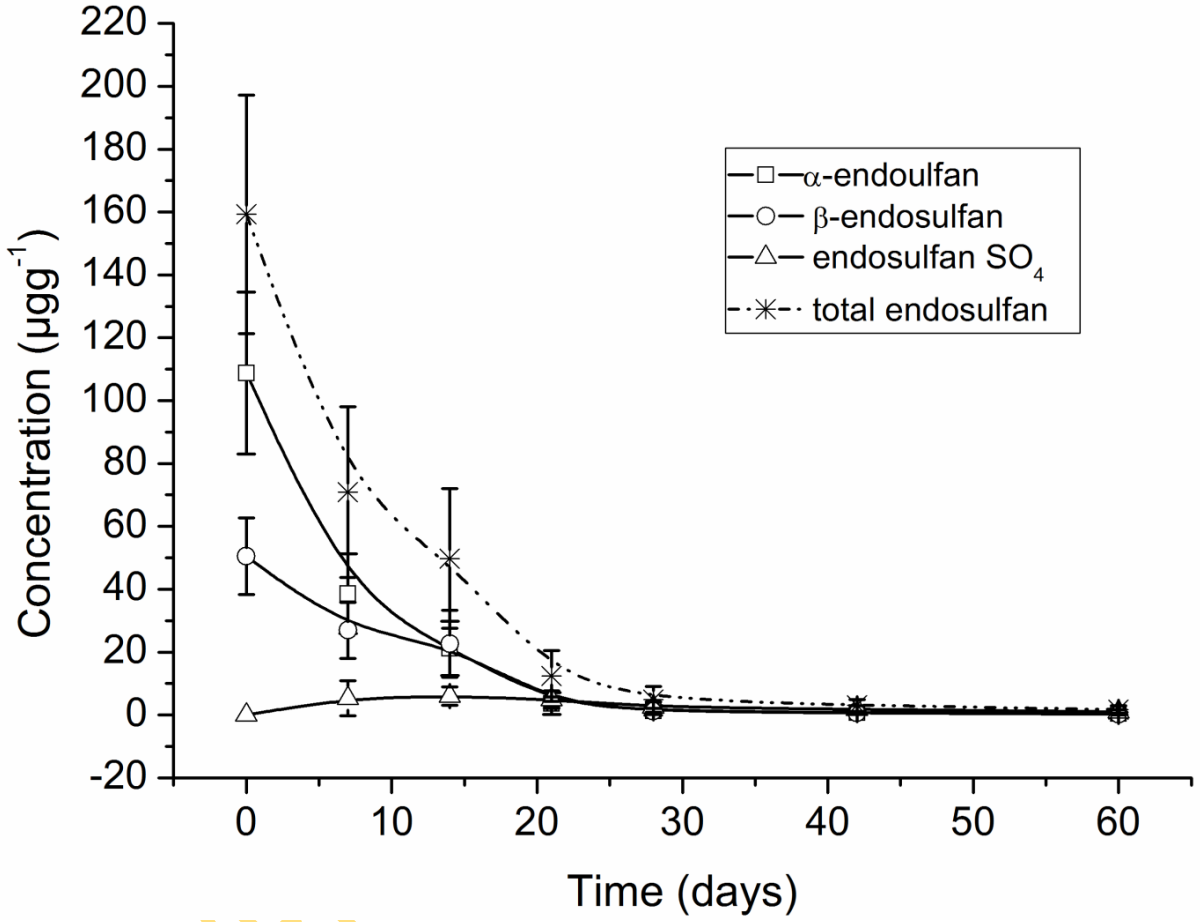


Figure 4.37. Residual concentration of α-, β-endosulfan, endosulfan SO_4 and total endosulfan on dry leaves over 60 days.

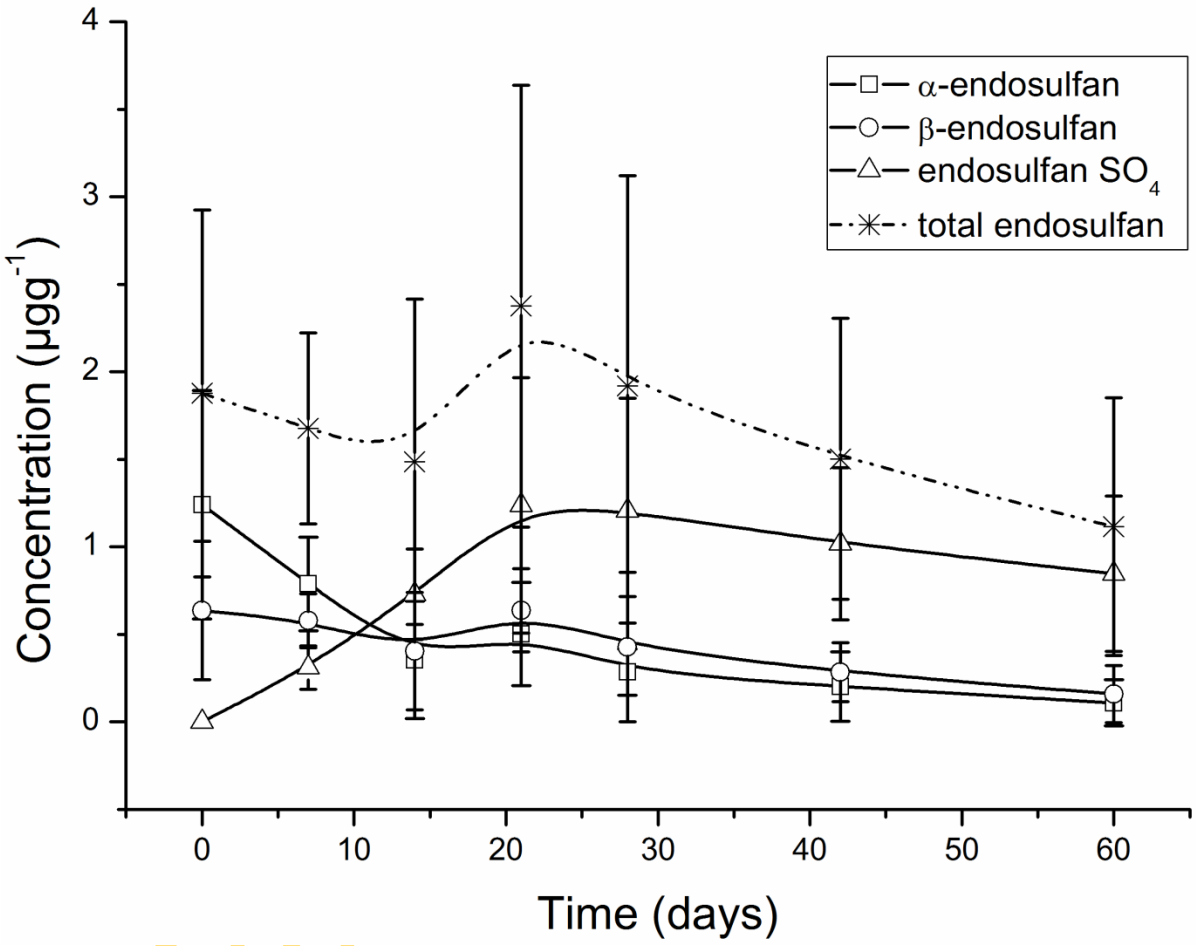


Figure 4.38. Residual concentration of α-, β-endosulfan, endosulfan SO_4 and total endosulfan in soil (0-15cm) over 60 days.

The parent compound was predominating. The vapour pressure of 0.83 mPa at 20°C for technical grade endosulfan indicates that it has an intermediate to high volatility under field conditions (Tomlin, 2000). The calculated Henry's law constants of 4.54 x 10^{-5} atm.m^3/mole and 4.39 x 10^{-5} atm.m^3/mol and the calculated 1/H values of 540 and 560, respectively, for α- and β-isomers indicated that both endosulfan isomers have the potential to volatilize from water or moist soil surfaces (Mackay *et al.* 1997). These physico-chemical properties must have accounted for the high dissipation-volatilization of endosulfan from dry leaves.

The percentage dissipation from the soil at depth 0-15cm was 36.50% (0.79 ± 0.27 μgg^{-1}), 9.11% (0.58 ± 0.16 μgg^{-1}) and 15.98% (1.58 ± 0.55 μgg^{-1}) for α-, β-endosulfan and ∑endosulfan respectively on day 7, with residual concentrations in parentheses (Figure 4.38). About 84.02% of ∑endosulfan at the start of the experiment was accounted for on day 7 by residual concentrations of α-, β-isomers and endosulfan sulphate (Table B-6). This suggested that a small proportion of initial concentration at the topsoil was lost or dissipated by volatilization and degradation. The low dissipation observed for topsoil may have been due to the use of fell dry cocoa leaves for mulching – which covered the soil from direct wind movement or current and heat energy from sunlight. In addition, some quantities of sprayed endosulfan from dry leaves and canopy may have drained into soil after collection of day 0 samples, thus replenishing the levels of the pesticide. The dissipation of ∑endosulfan from the soil exhibited a three-phase continuum process (Figure 4.38), with a gradual decrease from day 0 to day 14 (1.88 → 1.49 μgg^{-1}), followed by a rapid increase between days 14 and 21 (1.49 → 2.38 μgg^{-1}) and a gradual decline through day 60 (Appendix Table B-6). This implied that only 20.78% of ∑endosulfan disappeared after 14 days, with over 59.5% and 26.0% increase on day 21 with respect to days 14 and day 0 respectively. The abnormal trend where residual concentration on topsoil at day 21 was much higher than initial concentration may have resulted from dew and slight shower that fell during the week; thereby washing residues from the canopy (leaves, pods and stem) and dry foliage to the soil (Wauchope *et al.* 2004; Ciglasch *et al.* 2006). The order of individual contribution to ∑endosulfan was endosulfan sulphate (52.06%) > β-isomer (26.81%) >α-isomer (21.23%). A rapid decrease was observed between days 21 and 28 for α- and β-endosulfans. This sharp decline in α- and β-isomers contents may have resulted from increased microbial activity and hydrolytic action resulting from the

slight rain and dew that was observed during week 3 (Tiwari and Guha, 2013). Percentage disappearance/degradation between days 21 and 28 was 43.43% and 32.31% for α- and β-isomers respectively, while endosulfan sulphate recorded 2.43% - this suggested greater persistence of the metabolite when compared to parent compounds (NRC, 1975). These declines were mainly due to biodegradation, with minimum volatilization caused by air current or movement on loose soils as a result of mulching. The order of persistence was α-isomer< β-isomer <endosulfan sulphate. A slow reduction in residual concentrations was observed for α-, β-isomers and endosulfan sulphate from day 28 to day 60. Final residual contents in cropped soil was 0.11 μgg^{-1}, 0.16 μgg^{-1}, 0.85 μgg^{-1} and 1.12 μgg^{-1} for α-, β-isomers, endosulfan sulphate and ∑endosulfan respectively, with endosulfan sulphate contributing 75.96% to ∑endosulfan residue at end of the study (Appendix Table B-6). The level of endosulfan sulphate metabolite formed by oxidative, photolytic, hydrolytic and microbial actions on parent endosulfan in topsoil was 0.31 μgg^{-1} on day 7 - reaching a peak concentration of 1.24 μgg^{-1} on day 21 and 0.85 μgg^{-1} at terminal (day 60) (Figure 4.38). A moderate percentage degradation/disappearance of 31.53% was observed for endosulfan sulphate between peak concentration (day 21) and final concentration (day 60) over a period of 40 days - this again depicted persistence when compared to parent isomers. The ratios of endosulfan isomers and its metabolite are key in assessing the fate of technical grade endosulfan in the environment, which also is dependent on their individual physicochemical properties in soil. The α/β-endosulfan ratio on day 0 was ~ 2.0 (Figure 4.40). This dropped rapidly to < 1.0 on day 14, followed by a gradual decline to < 0.70 on day 60. This portrayed the β-isomer being more persistent in the soil. The α-isomer is reported to be more susceptible to microbial and hydrolytic degradation (Ghadiri and Rose, 2001), while β-isomer has more adsorptive and less volatile properties (Rice *et al.* 2002; USEPA, 2002).The endosulfan sulphate/(α+β)-endosulfan residual ratios increased rapidly from day 0 to day 60 – giving almost a linear trend, with a value > 3.0 at terminal day (Figure 4.40). This implied that metabolite was more predominate and persistent than parent compound. The endosulfan sulphate/∑endosulfan ratio ranged between 0.13 (day 7) and 0.76 (day 60), which intermittently revealed the amount of metabolite being contributed to ∑endosulfan as dissipation progresses. At terminal period endosulfan sulphate metabolite was the dominant compound – contributing 76% to ∑endosulfan in soil (Appendix-Table B-6).

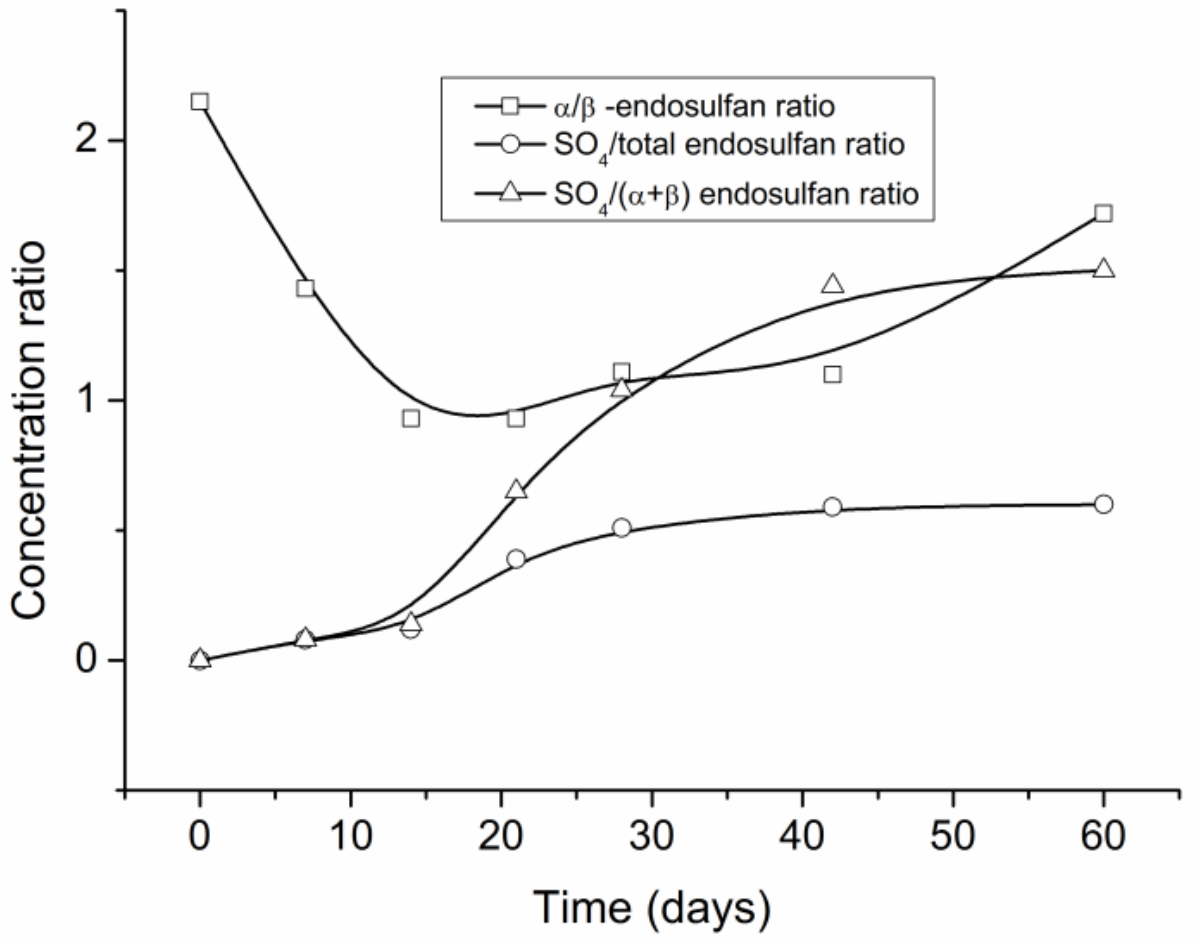


Figure 4.39. Ratios of α/β-endosulfan, endosulfan SO_4/total endosulfan and endosulfan SO_4/ (α+β) endosulfan on dry leaves

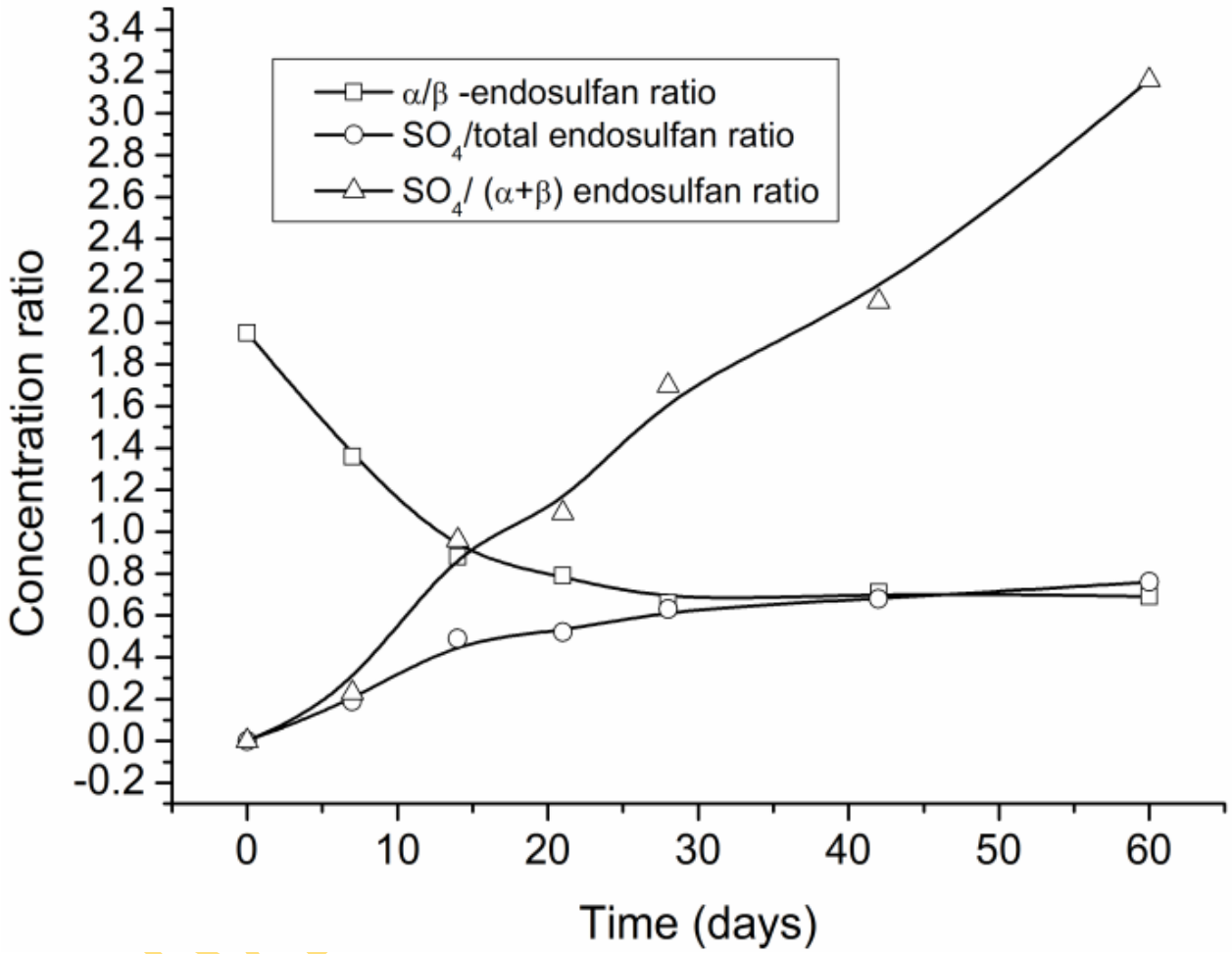


Figure 4.40. Ratios of α/β-endosulfan, endosulfan SO_4/total endosulfan and endosulfan SO_4/ (α+β) endosulfan in soil 0-15cm

The order of percentage dissipation of ∑endosulfan from all field matrixes at day 60, with respect to initial concentrations after treatment were 99.47% (0.51µgg^{-1}), 98.92% (1.71 µgg^{-1}), 95.67% (2.51µgg^{-1}), 61.13% (0.76ugg^{-1}), 40.60% (1.12 µgg^{-1}) for fresh foliage, dry foliage, stem bark, pods and soil respectively, with residual concentrations in parentheses (Figure 4.33).

4.5. CHEMO-KINETIC PARAMETERS–DISSIPATION RATE CONSTANT AND TERRESTRIAL FIELD HALF-LIFE

The kinetics of pesticide dissipation-degradation under terrestrial field application is mostly described as first-order reactions (Kumar *et al.* 2007; Tiwari and Guha, 2013;OECD, 2014). The rate of dissipation (degradation) (k) and field half-life (DT_{50}) for ∑endosulfan, α-endosulfan, β-endosulfan and endosulfan sulphate in vegetation (fresh leaves, bark and pods) and surrounding matrices (dry foliage and soil) were determined iteratively - taking successive residual concentrations from day 0 (initial) through each sampling days (7, 14, 21, 28, 42 and 60) (k' and and DT'_{50}) into consideration; and between days 0 (initial concentration) and 60 (final residual concentration) (*k and* DT_{50}).

4.5.1. Kinetic variables for vegetation

The dissipation rate constant (k') of ∑endosulfan in fresh foliage, bark and pods were 0.107 d^{-1}, 0.073 d^{-1} and 0.023 d^{-1} respectively, with corresponding DT'_{50} (field half-life) values as follows; 6.48 d, 9.49 d and 30.13 d (Table 4.20). The order of DT'_{50} values in cocoa vegetation was fresh foliage <stem bark< pods. The order is likely due to the greater loss of endosulfan from the leaves, which may be attributed to greater exposure to direct air movement (or wind), heat from sunlight, surface wash-off by rain or dew and oxidation due to availability of oxygen, compared to the pods and stem bark, which are often shaded by the trees' canopy. In addition to the aforementioned, cocoa stem barks are rough, often with shallow crevices and grooves (hence further shielding), this may have accounted for the relatively higher DT'_{50}value recorded in the bark.

Table 4.20. Chemo-kinetic parameters for endosulfan in *Theobroma cacao*vegetation and farm soils

Matrix	∑endosulfan			α-endosulfan			β-endosulfan			Endosulfan sulphate		
chemo-kinetic variables calculated iteratively from $d_0 \rightarrow d_{60}$												
	k'(d^{-1})	DT'_{50}(d)	R^2	k' (d^{-1})	DT'_{50}(d)	R^2	k' (d^{-1})	DT'_{50} (d)	R^2	k' (d^{-1})	DT'_{50} (d)	R^2
Fresh foliage	0.107	6.48	0.884	0.167	4.15	0.883	0.160	4.33	0.813	0.052	13.36	0.939
Bark	0.073	9.49	0.855	0.138	5.02	0.793	0.109	6.34	0.904	0.049	14.09	0.930
Pods	0.023	30.13	0.858	0.036	19.25	0.924	0.036	19.22	0.892	0.032	21.67	0.780
Seed	-	-	-	-		-	-	-	-	-	-	-
Dry leaves	0.094	7.37	0.905	0.133	5.21	0.879	0.100	6.95	0.922	0.031	19.25	0.937
Soil (0-15cm)	0.019	36.47	0.341	0.057	12.16	0.915	0.041	16.75	0.840	0.026	26.30	0.215
Soil (15-30cm)	-	-	-	-	-	-	-	-	-	-	-	-
chemo-kinetic variables calculated from d_0 & d_{60}												
	k(d^{-1})	DT_{50}(d)	R^2	k (d^{-1})	DT_{50}(d)	R^2	k (d^{-1})	DT_{50} (d)	R^2	k (d^{-1})	DT_{50} (d)	R^2
Fresh foliage	0.087	7.97	-	0.108	6.42	-	0.093	7.45	-	0.036	19.25	-
Bark	0.052	13.33	-	0.091	7.61	-	0.091	7.61	-	0.033	21.00	-
Pods	0.016	43.31	-	0.029	23.90	-	0.028	24.75	-	0.032	21.66	-
Seed	-		-	-		-	-		-	-		-
Dry leaves	0.076	9.12	-	0.092	7.53	-	0.088	7.87	-	0.032	21.66	-
Soil (0-15)	0.009	77.00	-	0.041	16.90	-	0.023	30.13	-	0.019	36.47	-
Soil (15-30cm)	-	-	-	-	-		-	-	-	-	-	-

Legend: $\boldsymbol{k}'$ – dissipation rate constant calculated iteratively on sampling days ($d_0 \rightarrow d_i \rightarrow d_{60}$); $\boldsymbol{k}$ – dissipation rate constant calculated using initial and final concentrations (d_0 & d_{60}); DT'_{50} –terrestrial field half-life (iteratively); DT_{50}– terrestrial field half-life (initial-final); R^2 – regression coefficient or correlation coefficient

Total endosulfan seemed to persist most in the cocoa pods (highest DT'_{50}). This may be as a result of a possible diffusion of the pesticide into the soft tissues of the pods. Ghadiri *et al*. (1995), reported the half-life of endosulfan in most fruits and vegetables is to be three to seven days. The dissipation rate constant k' for all vegetation components (fresh leaves, bark and pods, except cocoa seeds) ranged from 0.036 – 0.167 d^{-1}, 0.036 – 0.160 d^{-1} and 0.032– 0.049 d^{-1} for α-endosulfan, β-endosulfan and endosulfan sulphate respectively, with corresponding field half-lives from 4.15–19.25 d, 4.33 – 19.22 d and 13.36 –21.67 d. The field half-lives, DT'_{50} obtained for α-endosulfan on fresh foliage, bark and pods were 4.15, 5.02 and 19.25 d respectively, with corresponding values for β-endosulfan being 4.33, 6.34 and 19.22 d (Table 4.20). The difference in half-lives between both isomers was almost insignificant; however, the β-isomer showed more persistence in fresh foliage and stem bark, while almost the same in cocoa pods. This small differential in half-lives between both isomers must have been due to the slight difference in their vapour pressure and action of volatilization. The α-isomer is reported to be more volatile and dissipative (Siddique *et al*. 2003), while the β-isomer exhibits relatively more persistent character and their vapour pressures at 20^{o}C are 0.006 mmHg and 0.003 mmHg respectively. This volatility property enhances the long-range transport of endosulfan, while its adsorption tendency makes it to be persistent for much longer period in different environmental matrices or compartments. Adsorptiontends to restrict pesticide mobility.

There is a divide, from literature on preferential degradation of both isomers (Tiwari and Guha, 2013). Kwon *et al*. 2002 and Sethunathan *et al*. 2004 reported faster degradation for the α-isomer, while β-isomer was reported to exhibit faster rate by Walse *et al*., 2003. The observed half-life values (DT_{50}) in stem bark and fresh foliage may be due to fast dissipation of the α-isomer, followed by a very slow conversion of β-isomer to α-isomer (Rice *et al.* 1997; Schmidt *et al*. 2001), while there may have been an inter-conversion between α- and β-isomers in the pods. The conversion of α-endosulfan to β-endosulfan under field/crop conditions has been reported (Mukherjee and Gopal, 1994). However, it seems the α-isomer was favoured in the inter-conversion between both isomers in pods (Figure 4.36). The high field half-lives recorded in pods compared to other plant tissues may be due to possible diffusion of the applied pesticide into the soft tissues of the pods, thus leading to accumulation of

formed endosulfan sulphate metabolite (from the hydrolysis of parent endosulfan) and its subsequent persistence. Parent endosulfan and metabolites - endosulfan diol, ether and sulphate have been found to penetrate plant tissues and translocated from leaves to the roots of bean and sugar beet plants (Beard and Ware, 1969). Also, endosulfan is reported to have a log K_{ow} value of 3.55 (Mackay *et al*. 1997), thereby having a high potential to be bioaccumulated in biota (CCME, 2010).

The half-live values obtained in this study for *Theobroma cacao* vegetation (fresh foliar, stem bark and cocoa pods) were significantly higher than values reported in field grown tomato (*Lycopersicon esculentum*) at Akumadan, Ghana (Ntow*et al*.2007) and eggplant (*Solanum macrocarpon L*.) grown in Southern Benin, West Africa (Rosendahl *et al*.2009). Half-lives of α-, β-endosulfan and total endosulfan on foliar were 0.164 d, 0.921 d and 0.430 d respectively for *Lycopersicon esculentum*, while, 1.6 h (0.07 d), 6.7 h (0.28 d) and 2.7 h (0.11 d) in *Solanum macrocarpon L*respectively. The wide difference may be due mainly to their morphological differences, for example, wider surface leaf laminar, canopy and greater waxy leaf cuticle in *Theobroma cacao,* cultivation practiseand as well as higher dose concentration for crop treatment – all of these are likely to favour higher foliar half-live in *Theobroma cacao*.

4.5.2. Kinetic variables for surrounding matrixes

In soil (0-15cm), half-lives were 12.16 d (α-endosulfan), 16.75 d (β-endosulfan), 26.30 d (endosulfan sulphate) and 36.47 d (∑endosulfan), with the β-isomer being more persistent than α-isomer. The order of persistence amongst the parent compound and metabolite was α-endosulfan <β-endosulfan <endosulfan sulphate in this study. Endosulfan sulphate is reported more persistent and toxic than parent compound, with half-life two or more times longer than its parent isomers, while estimated half-lives for the combined residues – total endosulfan, ranged from 9 months to 6 years (USEPA, 2002). The DT'_{50} calculated for endosulfan sulphate was >2 and >1.5 times longer than values obtained for α- and β-endosulfan respectively, while ∑endosulfan was 36.47 d (Table 4.20). Previous studies have shown that the two isomers have different degradation times in soil. Half-lives of 35 d and 150 d have been reported for α- and β-endosulfan respectively, under neutral conditions (NRC, 1975), while under acidic environments they tend to persist longer (Howard, 1991; CCME, 2010).

Degradation rate in soil is pH dependent; alkaline conditions favour degradation, whereas acidic conditions slow down the process (Ghadiri *et al*., 1995).

The soil pH of the *Theobroma cacao* farm used in this study were alkaline (8.04 and 7.95 for top (0-15cm) and bottom (15-30cm) layers respectively (see Table 4.21), this may have accounted for lower DT'_{50} values obtained in this study in addition to the tropical environment. The DT_{50} values reported by NRC (1975), Howard (1991) and CCME (2010) were for studies carried out in temperate region, however, temperature tend to influence rate of degradation, thus lower half-life values may be expected in the tropics. Lower half-lives for α-, β-endosulfan and ∑endosulfan were reported in tropical West African farms soils at Akumadan, Ghana (Ntow*et al*. 2007) – with DT_{50} being 4.31 d, 4.31 d and 6.30 d respectively. However, DT_{50} values obtained from another field study in southern Republic of Benin, West African were comparable with values recorded in this study. The dissipation of endosulfan compounds proceeded faster for α-isomer than for β-isomer with DT_{50} ranges of 4.8 -13 d and 11 – 64 d respectively, while 17 – 74 d range for ∑endosulfan in horticultural soils (Acrisol and Arenosol) (Rosendahl *et al*. 2009). There is no previous terrestrial field dissipation (TFD) study reported for endosulfan on cropped soil in the Nigerian environment. However, there are DT_{50} values reported for other OCPs such as DDT (8.7 w or 60.9 d), aldrin (3.5 w or 24.5 d), and lindane (7.1 w or 49.7 d) (Osibanjo, 2003), with half-life values in parentheses.

In field studies, pesticide degradation rates in soils are known to vary in order of magnitude depending on soil characteristics amongst other environmental conditions. USEPA, (2001) carried out three comparative studies on terrestrial dissipation with or without crop cultivation in Georgia and California. Half-lives ranged from 6- 71 d for α-isomer, 23- 106 d for β-isomer, 41- 93 d for combined α-and β-isomers and 97-172 d for total endosulfan residues. Kennedy*et al*. (2001), found that endosulfan dissipation from both foliage and soil was best explained by a two-phase process. Half-lives of total endosulfan toxic residues (α- and β-endosulfan and the sulphate product) in the first phase were 1.6 d in foliage and 7.1 d in soil, mainly due to the rapid volatilization of the parent isomers in the first 5 d. In the second phase, half-lives were 9.5 d in foliage and 82 din soil, mostly due to the persistence of the degradation product, endosulfan sulphate.

Finally, no DT_{50} was computed for bottom soil (15-30cm), since no residual parent compound and metabolite were detected (all < 0.001 $\mu g g^{-1}$). This observation is in consonance with Kathpal *et al.* (1997), who reported that parent endosulfan and metabolites were confined to 0-10 cm depth in a terrestrial field study on bare cotton soil under sub-tropical conditions in Northern India.

4.5.3. Comparative field half-life

The DT'_{50} (computed iteratively) values for ∑endosulfan were 6.48 d , 9.49 d, 30.13 d, in fresh foliage bark and pods respectively, while corresponding DT_{50} (computed with t_0 and t_{60}) and *k* values were 7.97 d, 13.33 d and 43.31 d. The DT'_{50} and DT_{50} values for α-endosulfan in fresh foliage, bark and pods were 4.15 d and 6.42 d; 5.02 d and 7.61 d; 19.25d and 23.90 d respectively, while corresponding β-endosulfan half-lives in cocoa vegetation were 4.33 d and 7.45 d; 6.34 d and 7.61 d; 19.22 d and 24.75 d for DT'_{50} and DT_{50} respectively. The DT'_{50} values of both isomers for cocoa vegetation were all significantly lower than DT_{50} (Table 4.25). The same trend was observed for the surrounding matrices (topsoil and fell dry foliage).

It is pertinent that due consideration should be given to residual concentrations of parent compound, the formation and subsequent degradation of endosulfan sulfate metabolite as the reaction progresses from day 0 through days 7, 14, 21, 28, 42 and 60 and the interaction between the pesticide and its' environmental components. The consideration of initial and final concentrations (i.e, at t_{0d} and t_{60d}) only, would not adequately account or capture the phenomenal changes that would have occurred at intervals between successive sampling amplitudes or daysfrom day 0 through the terminal day, hence kinetic variables should be determined iteratively for field kinetic studies. However, statistically, there was strong correlation between DT'_{50} and DT_{50} values (R^2 = 0.9073; n = 28) (Figure 4.41), while there was no significant difference between both methods of calculation of half-lives for parent isomers and metabolite (*p* = 0.05, n = 28) using the paired t-test.

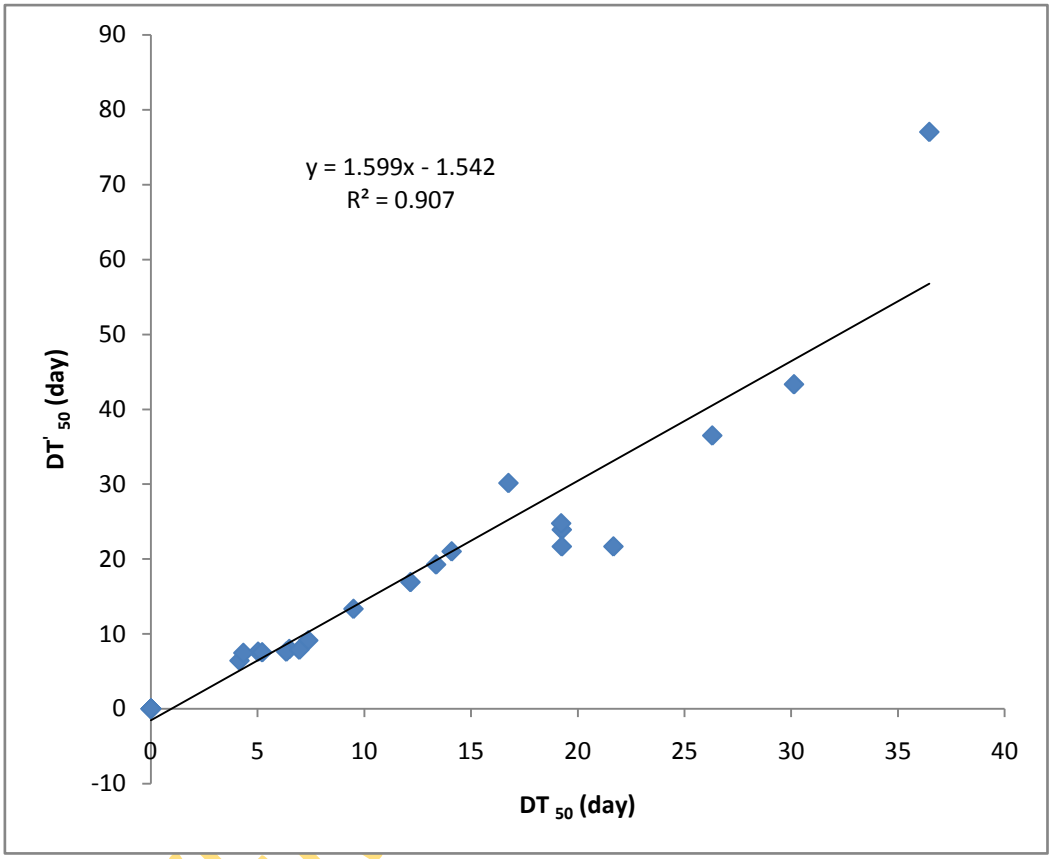


Figure 4.41. Correlation coefficient between two half-lives calculated iteratively (DT'_{50}) and using d_0 and d_{60} (DT_{50})

4.5.4. Assessment of Persistence

Persistence of pesticides is assessed based on half-lives and classified namely into; non-persistent (< 30 d), moderately persistent (30-100 d) and persistent (> 100 d) (EXTOXNET, 1993; Kerle *et al.* 2007). In this study endosulfan was non-persistent in fresh leaves, dry foliage and stem bark (DT_{50} of ∑endosulfan, parent endosulfan and metabolite endosulfan sulfate were < 30 d). It was moderately persistent in cocoa pod and soil (0-15cm) with respect to ∑endosulfan, but non-persistence for parent compound and metabolite. The implication of the former is two-fold. Firstly, persistence in soil may lead to residual build-up of endosulfan concentration over time and subsequently uptake by the roots, followed by translocated through the phloem and bioaccumulation in various parts of the cocoa plant. Secondly, there is a high probability of residual endosulfan in the cocoa pod being transferred to the seed during harvest (cross contamination). This may be the major source of OCPs contamination of cocoa beans which affected the quality and rating of Nigerian cocoa in the world market in the 1980s and 1990s.

4.6. ADSORPTION AND DESORPTION STUDIES: INTERACTION OF ENDOSULFAN WITH SOILS FROM THREE COCOA FARMS

Results obtained from the interaction of endosulfan pesticide withsoils from three different cocoa (*Theobroma cacao*) farms–CRIN, Igba (Ondo II) and Sore Bale farms are presented in Figures 4.42 - 4.53. Adsorption-desorptionphenomenon of hydrophobic and non-ionic organics like endosulfan is best assessed by applying kinetic and isotherm models. The OECD (2000) and EPA (2008) guidelines for testing chemicals by batch method was used in this study.

4.6.1. Soil samples

Some selected properties of the soil samples used in this study are presented in Table 4.21. The pHs of soils from the cocoa farms werepredominately basic as values ranged between 7.1 and 8.33 for top and bottom soils. Cocoa trees are known to thrive better in slightly basic soil. The TOC values ranged from 0.70 – 2.03 %, with the order of distribution being CRIN > Igba > Sore Bale in the topsoil of samples used for adsorption-desorption studies. The topsoils had higher TOC content more than the bottom soils except in Igba farm, where sub-surface soil was slightly higher than the topsoil by 0.02%.

Table 4.21. Physicochemical properties of soil samples used for adsorption-desorption studies.

PARAMETERS	CRIN*		SORE BALE		IGBA	
	0-15cm	15-30cm	0-15cm	15-30cm	0-15cm	15-30cm
pH	8.26	7.95	8.31	8.33	7.1	7.46
Total organic carbon (%)	2.03	1.72	1.32	0.70	1.72	1.74
Total nitrogen (%)	0.14	0.11	0.11	0.07	0.14	0.14
C:N ratio	15:1	16:1	12:1	10:1	12:1	12:1
Total organic matter (%)	3.61	3.06	2.35	1.25	3.06	3.10
C. E. C (meq/100g)	25.27	22.92	17.04	11.99	23.23	22.51
Particle size distribution						
% clay	13.65	13.61	10.92	11.28	19.11	20.15
% silt	16.84	17.59	14.87	18.45	25.53	22.26
% sand	69.51	68.80	74.21	70.27	55.36	57.59

**Mean values for CRIN 1 and CRIN 2 soil samples*

The total organic matter (TOM) also followed the same trend, with values ranging 1.25% to 3.61%, this is considered significantly high to influence physico-chemical properties of most soils. The C:N ratio for topsoil was 15:1 for CRIN, while Igba and Sore Bale recorded a ratio of 12:1. Generally, C:N ratio for most soils ranged from 8:1 to 15:1. The cation exchange capacity (CEC) ranged 11.99 meq/100g to 25.27 meq/100g for top (0-15cm) and bottom soils (15-30cm). Particle size distribution for topsoil in CRIN, Sore Bale, and Igba showed that they are loamy in texture (Table 4.26).

4.6.2. Adsorption-desorption kinetics of α- and β-endosulfan

Adsorption kinetics

The adsorption kinetics of α- and β-endosulfan exhibited a rapid and immediate adsorption using a nominal equilibration concentration of 5 μgmL^{-1}endosulfan pesticide (with α- and β-isomers in 70 + 30 % ratio), in which about 23.94 – 43.36% of the equilibration concentration for the α- and β-isomers were adsorbed by the three different soils under investigation. However, about 75.64 – 78.78% and 76.53 – 82.21% of the maximum adsorption capacities for α- and β-endosulfan respectively, were adsorbed within 60 minutes in all the three cocoa farm soils (Figures 4.42 and 4.43). This rapid adsorption was followed by a slow adsorption of the two adsorbate isomers to the different adsorbent samples (soils) – a bicontinuum phenomenon (Brusseau *et al*. 1991; Locke, 1992). The initial rapid adsorption observed is a surface phenomenon and due to the non-ionic and hydrophobic nature of endosulfan, vacant sites available in adsorbent samples were quickly occupied (von Oepen *et al*. 1991; Kumar and Philip, 2009). The sudden decrease in the rate of adsorption exhibited by the endosulfan isomers is as a result of their slow and gradual migration and diffusion into the organic matter matrix and clay mineral interlayers and surfaces (Gao *et al.*, 1998; Atasoy *et al*. 2009). The same trend was reported by Parkpian *et al*. (1998), while studying Thai's Rangsit lowland and Phrabat upland soils; Kumar and Philip, (2006) and Atasoy *et al*.(2009) for four Indian soils and Vertisol soils from Turkey respectively.

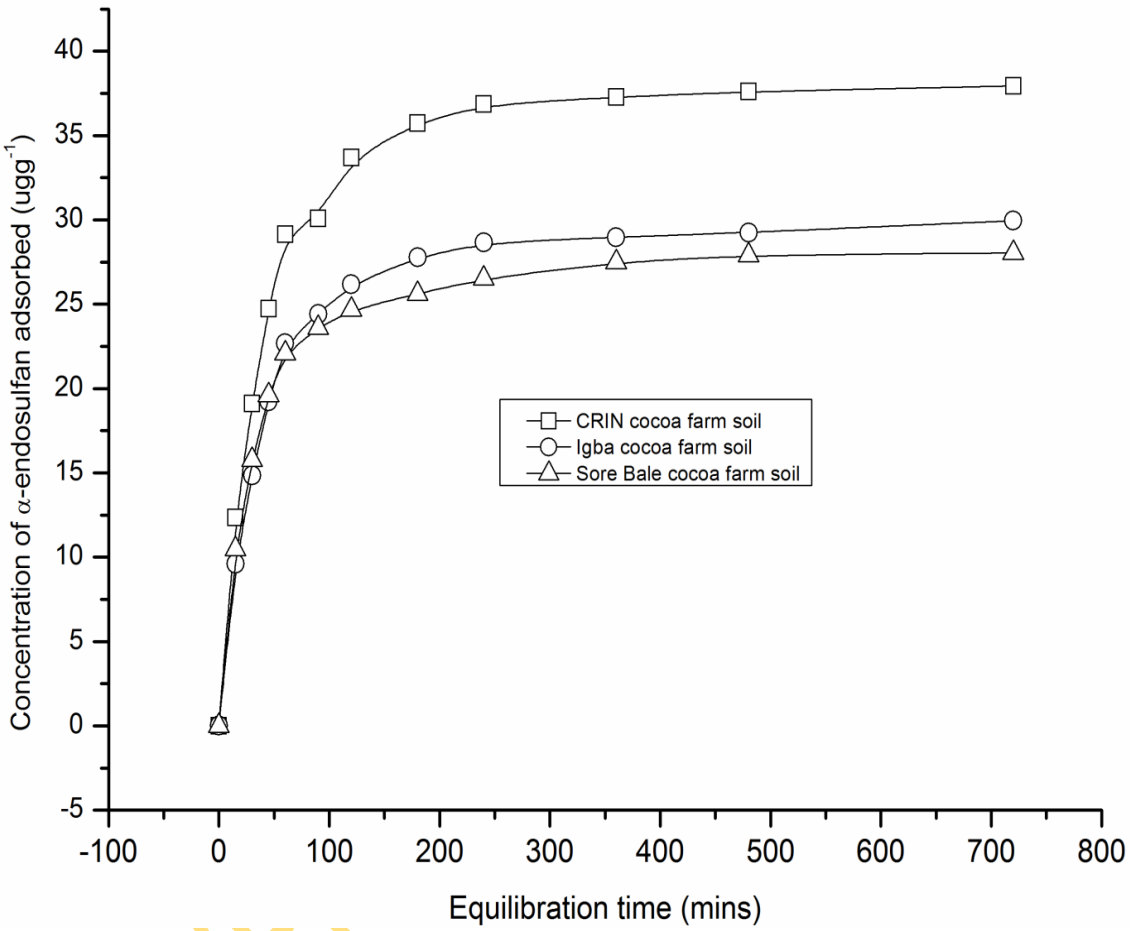


Figure 4.42. Adsorption kinetics of α-endosulfan on three different cocoa farm soils

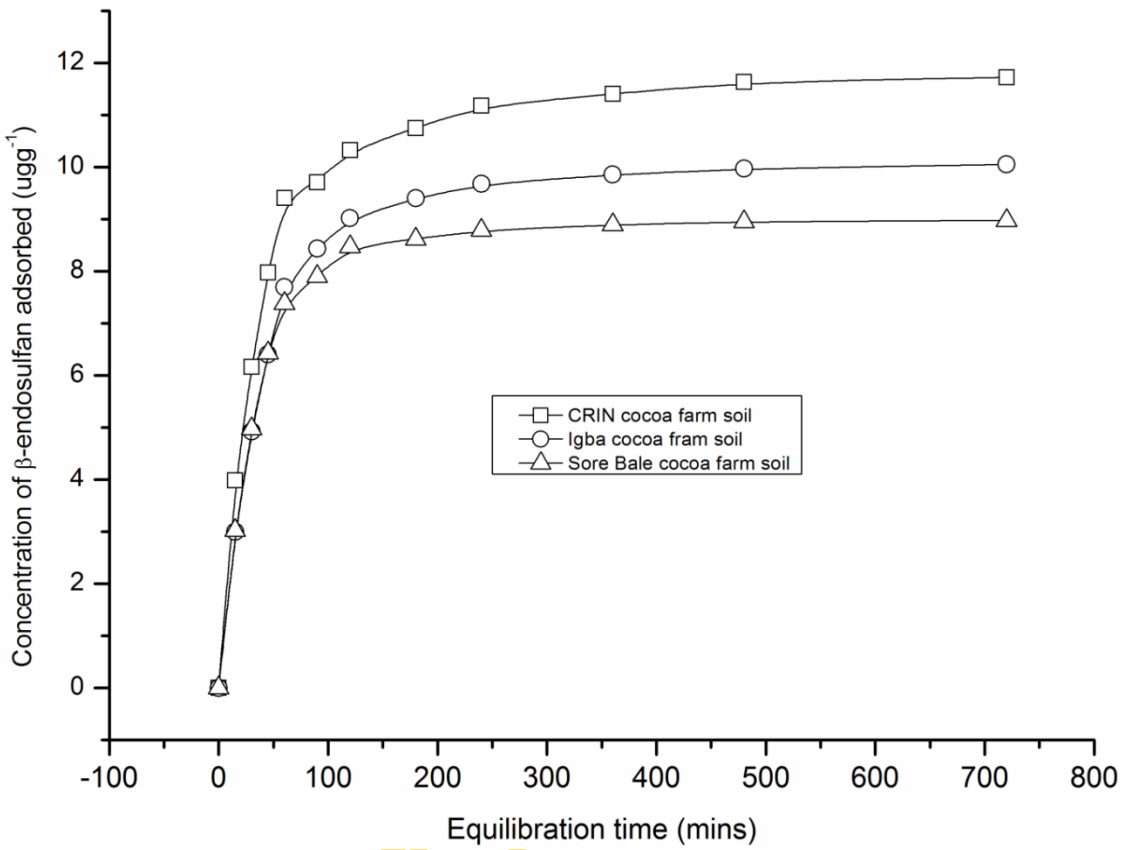


Figure 4.43. Adsorption kinetics of β-endosulfan on three different cocoafarm soils

It is pertinent to mention that, pseudo equilibria were attained by α-endosulfan, with formation of plateaus after 120, 180 and 240 minutes for Sore Bale, Igba and CRIN soils respectively, while for the β-isomer, plateaus were formed after 120 minutes with Sore Bale soil and 240 minutes with CRIN and Igba soils (see Tables C-1, C-5, C-9). The pseudo equilibrium time range obtained in this study (120 – 240 minutes) is comparable with the 120 minutes and 180 minutes reported by Parkpian *et al*.(1998) and Atasoy *et al.* (2009) respectively for both isomers in topsoil. It was observed that less than 3% variation of the adsorbate concentration (α- and β-endosulfan) that was remaining in the aqueous phase was observed, even at the termination of the adsorption kinetics at 720 minutes. This percentage variation of residual adsorbates after the pseudo equilibrium had been attained was comparable with the less than 2% reported by Kumar and Philip (2009).

The attainment of pseudo equilibrium and plateau formation by the three adsorbent-soil samples was in the order – SoreBale < Igba < CRIN. The relative ease of attaining pseudo equilibrium and plateau by Sore Bale soil was due to its relatively low adsorption capacity and percentage adsorption caused by the presence of fewer vacant sites for the adsorbate-isomers to occupy.In addition, the range obtained for percentage adsorption in this study was comparable with the range reported for the sorption studies of seven pesticides with sediment (Gao *et al*. 1998).Working on sediment of Teufelseiher pond (southern Germany), with equilibration concentrations ranging from 0.04 - 5 μgmL^{-1}, Gao *et al*. (1998), reported the rapid sorption of seven pesticides (desethylatrazine, atrazine, triadimenol, dimethoxyanilazine, terbutylazine, bifenox and fluroxyp) by which about 30 – 70% of the added pesticide amount were adsorbed within 4 h, followed by a slow sorption over a long period

In another study, using endosulfan about 72 - 78% of the added endosulfan was adsorbed within 1 h (Atasoy *et al*. 2009), with a working nominal concentrations ranging from 0.020 – 0.144 μgmL^{-1} (α-endosulfan) and 0.01 – 0.075 μgmL^{-1} (β-endosulfan). The incomparable adsorption percentage ranges between this study and the latter, may have been due to the large differential in working concentrations.

The partition coefficient (K_d) at equilibrium ranged from 11.792 - 19.139 mLg^{-1} and 7.869 – 11.684mLg^{-1} for α- and β-endosulfan respectively (Table 4.22). The same trend observed for percentage adsorption was also exhibited, with the order of partitioning as follows; CRIN > Igba > Sore Bale. High equilibrium K_d value signifies greater adsorption tendency of the adsorbate to the adsorbent and low tendency of the adsorbate to remain in the aqueous phase.

Desorption kinetics of α- and β-endosulfan

The degree of mobility of a chemical compound in soil can be assessed from desorption studies. The kinetic desorption of endosulfan was conducted to determine the extent to which both isomers could be leached into the aqueous phase with time.

Desorption of α- and β-endosulfan from soils followed a reversal trend as reported for adsorption kinetics, which was immediate and rapid in the inital stage, with the loss of both isomers from the adsorbents (soils) into the aqueous 0.01M $CaCl_2$.

The α- and β-endosulfan exhibited a rapid desorption kinetics by which about 34.68 – 50.09% and 45.83 – 58.82% of the maximum desorption capacities for α- and β-endosulfan were desorbed respectively after 30 minutes (Figure 4.44).

The relative ease of endosulfan desorption from the soils at this initial rapid stage was in the order of Sore Bale > CRIN > Igba. This trend may be as a result of the particle size distribution of the soils. Krishna and Philip (2008) reported 50% desorption of pesticides (lindane, carbofuran and methyl parathion) within 15 minutes from a sandy soil. The percentage of sand in topsoils was 71.21, 69.51 and 55.36% for Sore Bale, CRIN and Igba respectively.

The rapid loss or release of the endosulfan isomers by the soils continued until a pseudo equilibrium was reached, which was followed by a slow desorption process of the two adsorbate isomers.

Table 4.22. Adsorption-desorption kinetics parameter of α- and β-endosulfan for CRIN, Igba and Sore Bale farm soils

Adsorption kinetics - parameters						
	A_{cp} (µgg^{-1})	$A_{t\%}$	K_d (mLg^{-1})	K_{om}(mLg^{-1})	k_{ads} (mins^{-1})	R^2
CRIN farm soil						
α-endosulfan	37.941	43.36	19.139	462.300	9.67×10^{-3}	0.933
β-endosulfan	11.944	31.85	11.684	497.200	6.91×10^{-3}	0.875
Igba farm soil						
α-endosulfan	29.957	34.24	13.015	425.330	7.60×10^{-3}	0.867
β-endosulfan	10.049	26.80	9.159	299.304	9.67×10^{-3}	0.937
Sore Bale farm soil						
α-endosulfan	28.044	32.05	11.792	501.779	9.91×10^{-3}	0.964
β-endosulfan	8.978	23.94	7.869	334.843	11.52×10^{-3}	0.936
Desorption kinetics - parameters						
	D_{cp}(µgcm^{-3})	$D_{t\%}$	K_{des} (mLg^{-1})	K_{om} (mLg^{-1})	k_{des}(mins^{-1})	R^2
CRIN farm soil						
α-endosulfan	0.102	7.92	290.657	7020.700	1.16×10^{-4}	0.470
β-endosulfan	0.060	1489	142.898	3451.643	2.24×10^{-4}	0.444
Igba farm soil						
α-endosulfan	0.122	10.15	221.305	7232.190	1.49×10^{-4}	0.467
β-endosulfan	0.066	16.32	128.186	4189.085	2.48×10^{-4}	0.421
Sore Bale farm soil						
α-endosulfan	0.136	11.25	197.222	8392.426	1.70×10^{-4}	0.593
β-endosulfan	0.074	18.53	109.989	4680.383	2.85×10^{-4}	0.446

Where;

A_{cp} = Adsorption capacity at 720 mins equilibrium time.; $A_{\%}$ = Adsorption percentage at equilibrium; $D_{\%}$ = Desorption percentage at equilibrium; K_d = Distribution coefficient at adsorption equilibrium; K_{des} = Distribution coefficient at desorption equilibrium; K_{oc} = Distribution coefficient due to organic carbon; k_{ads} = adsorption rate constant; k_{des} = desorption rate constant; R^2 = correlation coefficient

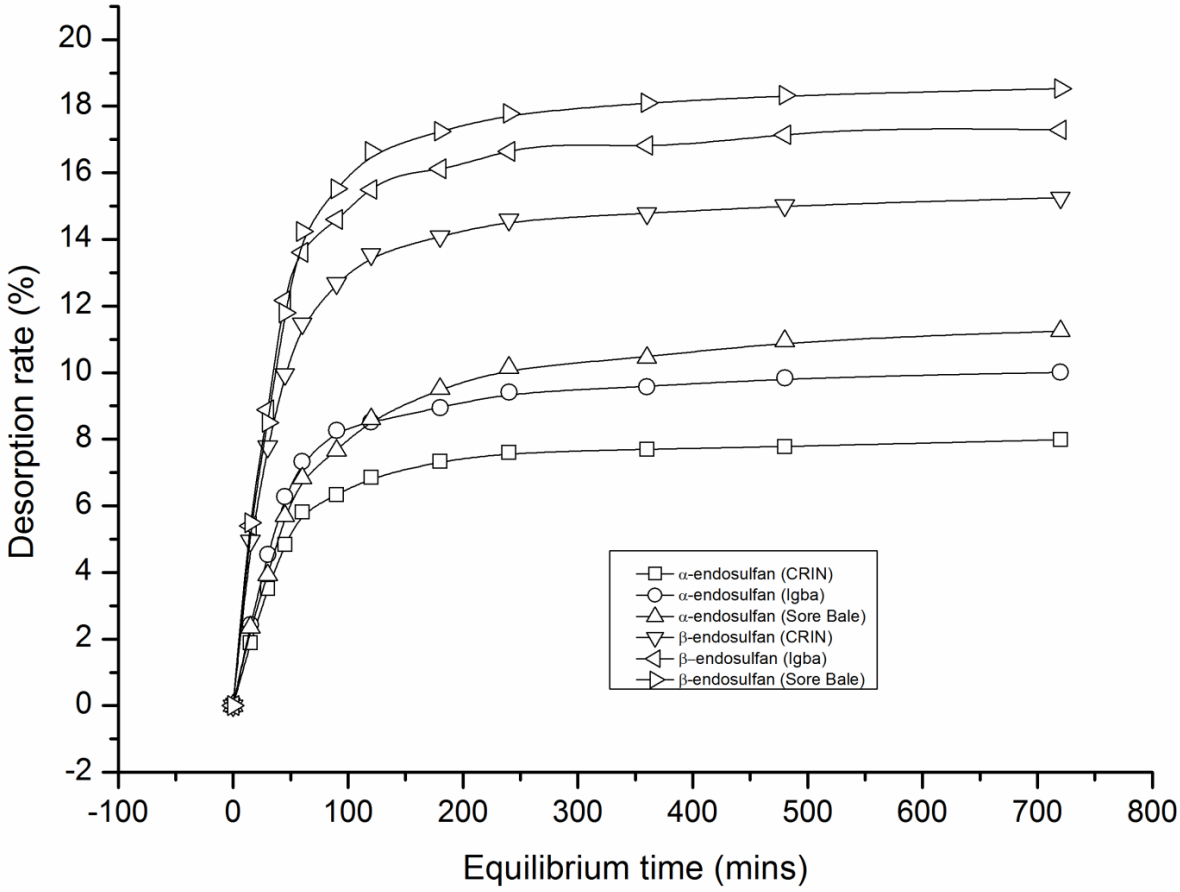


Figure 4.44. Percentage desorption of α- and β-endosulfan in CRIN, Igba and Sore Bale farm soils

Pseudo desorption equilibria in CRIN, Igba and Sore Bale soils for α- and β-endosulfan were attained within 180 - 240 minutes and 120 - 180 minutes respectively (Figures 4.45 and 4.46) as the β-isomer seem to show a greater tendency towards desorption than the α-isomer, hence exhibiting a faster desorption rate.

The apparent desorption capacities of α-endosulfan at terminal equilibrium (720 minutes) from CRIN, Igba and Sore Bale soils were 0.102 (7.92%), 0.122 (10.15%) and 0.133 µgmL (11.00%) respectively (with percentage desorption in parenthsis); while β-isomer was 0.060 (14.89), 0.066 (16.32%) and 0.074 µgmL (18.53%) respectively. The desorption equilibrium of some pesticides such as bentazone, dichlorporp and propiconazole were reportedly achieved immediately (Thorstensen *et al*., 2001). Atasoy *et al*. (2009) reported an attainment of 15% maximum desorption for α- and β-endosulfan within 60 minutes.

Sorption rate constant

The specific adsorption rate constants (k_{ads}) of α- and β-endosulfan for the different cocoa farm soils were determined by applying the Lagergren pseudo-first order sorption kinetics equation

$$\text{Log } (q_e - q_t) = \text{Log } q_e - k_{ads}t/2.303$$

Values for log $(q_e - q_t)$ were calculated for each time interval for both isomers at a temperature range of 24.0 – 26.0 ^{o}C. Where q_e and q_t represent the quantity of adsorbate adsorbed at equilibrium and time *t* intervals.

The adsorption (k_{ads}) and desorption (k_{des}) rate constants are presented in Table 4.21. The adsorption rate constants (k_{ads}) for α- and β-endosulfan evaluated from Lagergren plot varied from 7.60×10^{-3} to 9.91×10^{-3} mins^{-1} and 6.91×10^{-3} to 11.52×10^{-3} mins^{-1} respectively. The regression coefficient (R^2) for all the plots ranged between 0.867 and 0.964. The order for k_{ads} values were Igba < CRIN < Sore Bale and CRIN < Igba < Sore Bale for α- and β-endosulfan respectively. From this trend it is clear that the Sore Bale soil would attain equilibrium faster than the CRIN and Igba soils, this may have been due to its relatively low adsorption capacity, which was as a result of its low percentage clay and organic carbon contents.

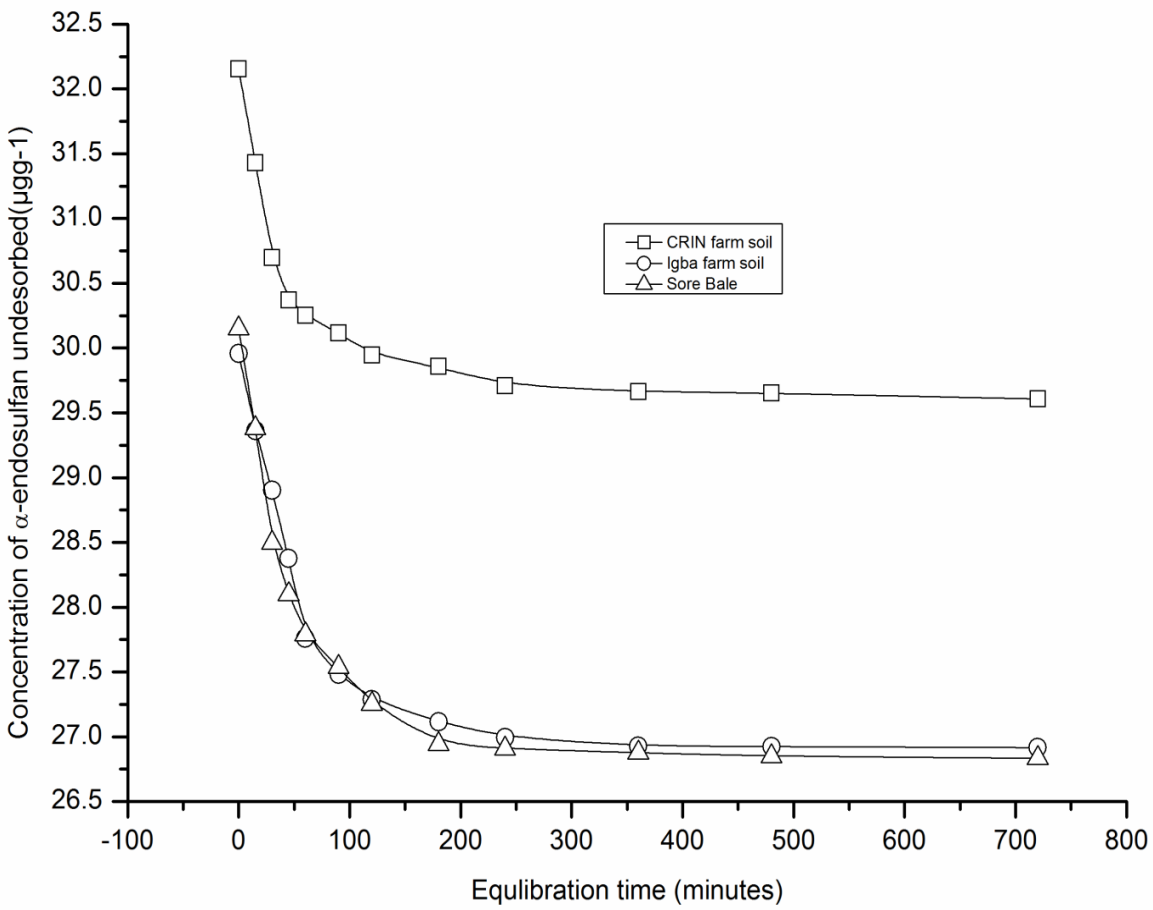


Figure 4.45. Desorption kinetics of α-endosulfan for CRIN, Igba and Sore Bale farm soils

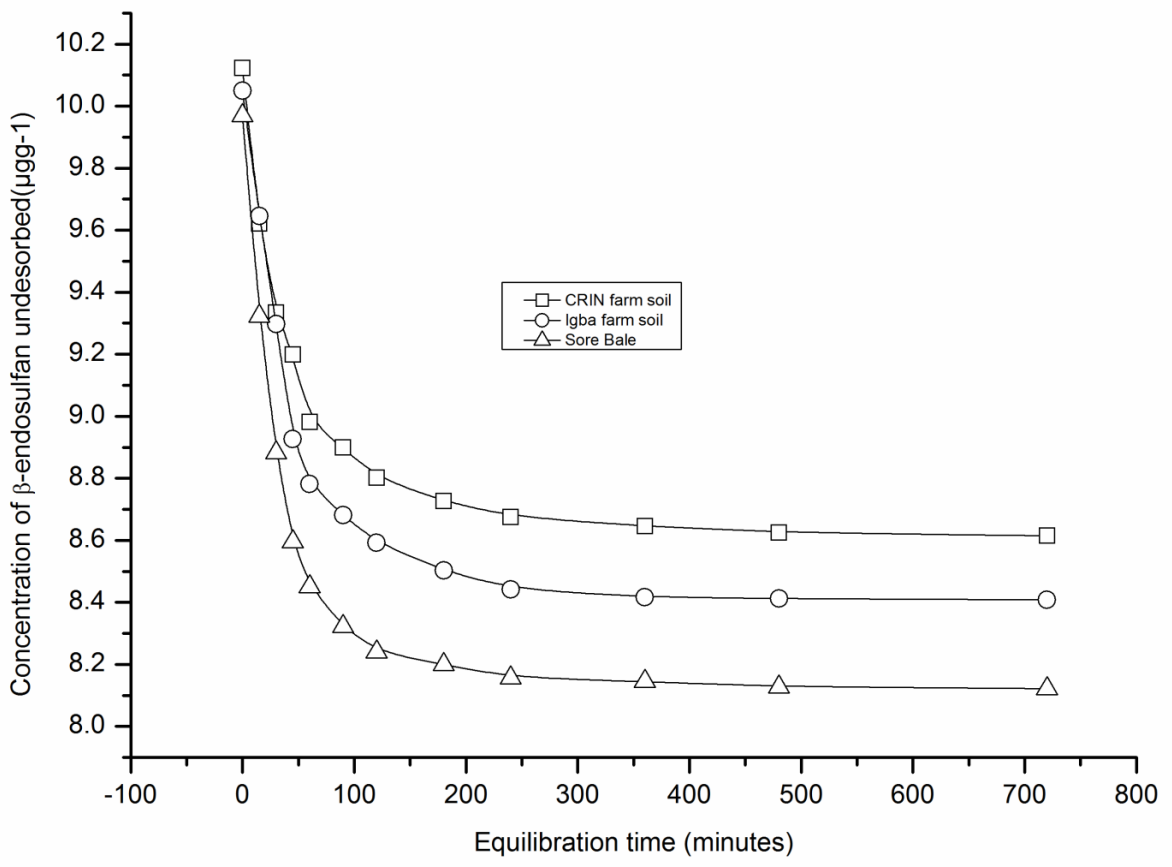


Figure 4.46. Desorption kinetics of β-endosulfan for CRIN, Igba and Sore Bale farm soils

The k_{des} for α- and β-endosulfan ranged from 1.16 – 1.70×10^{-4} $mins^{-1}$and 2.24 – 2.85×10^{-4} $mins^{-1}$ respectively, the order being CRIN < Igba < Sore Bale for both isomers. The calculated desorption rate constants (k_{des}) for the β-isomer were generally higher than those of the α-isomer, when compared to their corresponding soils. This implies that the β-isomer would have a greater tendency to be leached from soil during run-off and also great ease to migrate downward to contaminate the aquifer

These kinetic ranges are comparable with values obtained for endosulfan isomers in four Indian soils (Kumar and Philip, 2006). However, the attainment of pseudo-equilibria by both isomers seems to be slower when compared to that of adsorption for each soil samples. This indicated that adsorption of endosulfan seems to be faster than the desorption exercise, thuswill enhance its persistence in the cocoa farm soils.

4.6.3. Adsorption-desorption Isotherm of α- and β-endosulfan

Adsorption-desorption studies were conducted separately for the three cocoa farm soils using equilibration time of 480 minutes (from kinetic studies). The working concentrations for isotherm study ranged from 0.25 (0.175, 0.075) μgmL^{-1} to 25 (17.5, 7.5) μgmL^{-1}, with α- and β-endosulfan concentrations in parentheses (with α- and β-endosulfan concentration ratio of 70 + 30 % respectively) (see Tables C-3, C-4, C-7, C-8, C-11 and C-12). All data obtained from the experiments for each concentrations of endosulfan were subjected to the Freundlich model equation.The adsorption-desorption isotherms curves and values obtained for important variables in the linearized Freundlich equation for α- and β-endosulfan in soils are presented in Table 4.23 and Figures 4.47 – 4.50.

4.6.3.1. Adsorption Isotherm

The data obtained in adsorption equilibrium study for α- and β-isomers at temperature range of 25 ± 1^0C with the soils, fitted adequately the Freundlich isotherm - with regression coefficient ≥ 0.9921 for both isomers and soils. All isotherm plots were non-linear. The classification of the isotherm curves obtained in the Freundlich adsorption plot was type L (Giles *et al*. 1960) for both isomers, which is typical for adsorbents with high affinity for adsorbates. Endosulfan is hydrophobic by nature and would readily have strong and increased attraction to soil surfaces that are highly heterogeneous in an aqueous environment.

Table 4.23. Adsorption-desorption isotherm constants for α- and β-endosulfan in cocoa farm soils

Freundlich constants for adsorption isotherm								
	K_{fads}(mLg^{-1})	$1/n_{ads}$	K_{oc}(mLg^{-1})	R^2	LogK_f	K_{om}(mLg^{-1})	n_{ads}	LogK_{oc}
CRIN farm soil								
α-endosulfan	9.228	0.535	454.571	0.998	0.965	255.377	1.869	2.658
β-endosulfan	4.781	0.525	235.507	0.998	0.680	132.308	1.906	2.372
Igba farm soil								
α-endosulfan	7.471	0.519	434.384	0.999	0.873	244.036	1.928	2.634
β-endosulfan	4.376	0.503	254.430	0.997	0.641	142.938	1.989	2.406
Sore Bale farm soil								
α-endosulfan	4.748	0.557	359.689	0.992	0.677	202.073	1.794	2.556
β-endosulfan	2.738	0.547	207.409	0.994	0.437	116.522	1.829	2.317
Freundlich constants for desorption isotherm								
	K_{fdes}(mLg^{-1})	$1/n_{des}$	K_{oc}(mLg^{-1})	R^2	LogK_f	K_{om}(mLg^{-1})	n_{des}	LogK_{oc}
CRIN farm soil								
α-endosulfan	9.881	1.299	486.749	0.998	0.995	273.454	0.770	2.687
β-endosulfan	6.882	1.229	339.005	0.991	0.8377	190.452	0.814	2.529
Igba farm soil								
α-endosulfan	7.880	1.176	458.111	0.995	0.897	257.365	0.851	2.661
β-endosulfan	6.479	1.168	376.680	0.999	0.812	211.618	0.856	2.576
Sore Bale farm soil								
α-endosulfan	7.157	1.063	542.159	0.994	0.855	304.584	0.941	2.734
β-endosulfan	5.150	1.070	390.144	0.997	0.712	219.182	0.934	2.591

Where;

K_{fads}= Freundlich adsorption coefficient or capacity; K_{fdes}= Freundlich desorption coefficient or capacity; $1/n_{ads}$ = Freundlich exponent due to adsorption; $1/n_{des}$= Freundlich exponent due to desorption; n_{ads}= Adsorption intensity (reciprocal of Freundlich exponent due adsorption); n_{des} = Desorption intensity(reciprocal of Freundlich exponent due desorption); K_{oc} = organic carbon normalized adsorption coefficient; K_{om} = organic matter normalized distribution coefficient; LogK_{oc} = Lograthim of Freundlich coefficient due to organic carbon; LogK_f = Lograthim of Freundlich adsorption/desorption coeffiecient or capacity; R^2 = coefficient of determination

The values of $1/n_{ads}$ (i.e., strength of adsorption) for both isomers ranged between 0.503 and 0.557 (i.e., < 1.0) for all the soils – which depicted non-linearity of adsorption data and indeed the isotherm curve. The adsorption strength of α-isomer was greater than that of the β-isomer for all three soils. The low $1/n_{ads}$values in this study also indicated that endosulfan adsorption was normal and not cooperative (Mohan and Karthikeyan, 1997). The smaller the $1/n_{ads}$, the greater the expected heterogeneity of the adsorbent – which is characteristic of soil matrix. Low $1/n_{ads}$ values for some pesticides (such as bifenox, terbutylazine and anilazine) have also been associated with sediments with high organic matters (Singh and Saxena, 1986; Weber *et al*. 1992). The organic matter content in the three cocoa farm soils was significantly high (2.35 – 3.61%), while the n_{ads} (reciprocal of $1/n_{ads}$) values evaluated were all ranged from 1.794 to 1.928 and 1.829 to 1.989 for α- and β-endosulfan respectively. These ranges were considered comparable to ranges reported for α-isomer (1.4630 – 2.0010) and β-isomer (1.4065 – 1.7864) in four Indian soils (Kumar and Philip, 2006), with working concentrations and organic matter of soils ranging from 0.15 mgL^{-1} to 100 mgL^{-1} and 0.575 to 9.51% respectively.Calculated n_{ads}values lying between 1.0 and 10.0 are suggestive of a favourable adsorption process (Goldberg 2005) – the calculated n_{ads} values were ≥ 1.794.

The maximum adsorption (Cq_{max}) for α- and β-endosulfan at low and high concentrations was observed in CRIN soil, followed by Igba soil and Sore Bale (i.e., CRIN > Igba > Sore Bale) (Appendix Table C-3, C-7 and C-11). There was a relative and gradual decrease between successive maximum adsorption as the concentration of both isomers increases. This was distinctly reflected by a continuous and gradual decrease in percentage adsorption as working concentration move from low to high concentrations (Figures 4.47 and 4.48). This trend is as a result of more adsorbate (endosulfan) molecules available at higher concentrations competing to fill fixed number of available vacant sites in the adsorbents (soils). The separate plots of α- and β-endosulfan equilibrium concentration (μgmL^{-1}, at 480 minutes equilibrium time) against the adsorbed concentration (μgg^{-1}) showed a steep rise at low concentration ranges (0.175 – 1.050 μgmL^{-1} for α-endosulfan; 0.075 – 0.450 μgmL^{-1} for β-endosulfan), followed by a parabolic increase at higher concentration range (1.750 – 17.500 μgmL^{-1} for α-endosulfan; 0.750 – 7.500 μgmL^{-1} for β-endosulfan) in all the soil sample investigated (Figures 4.49 and 4.50).

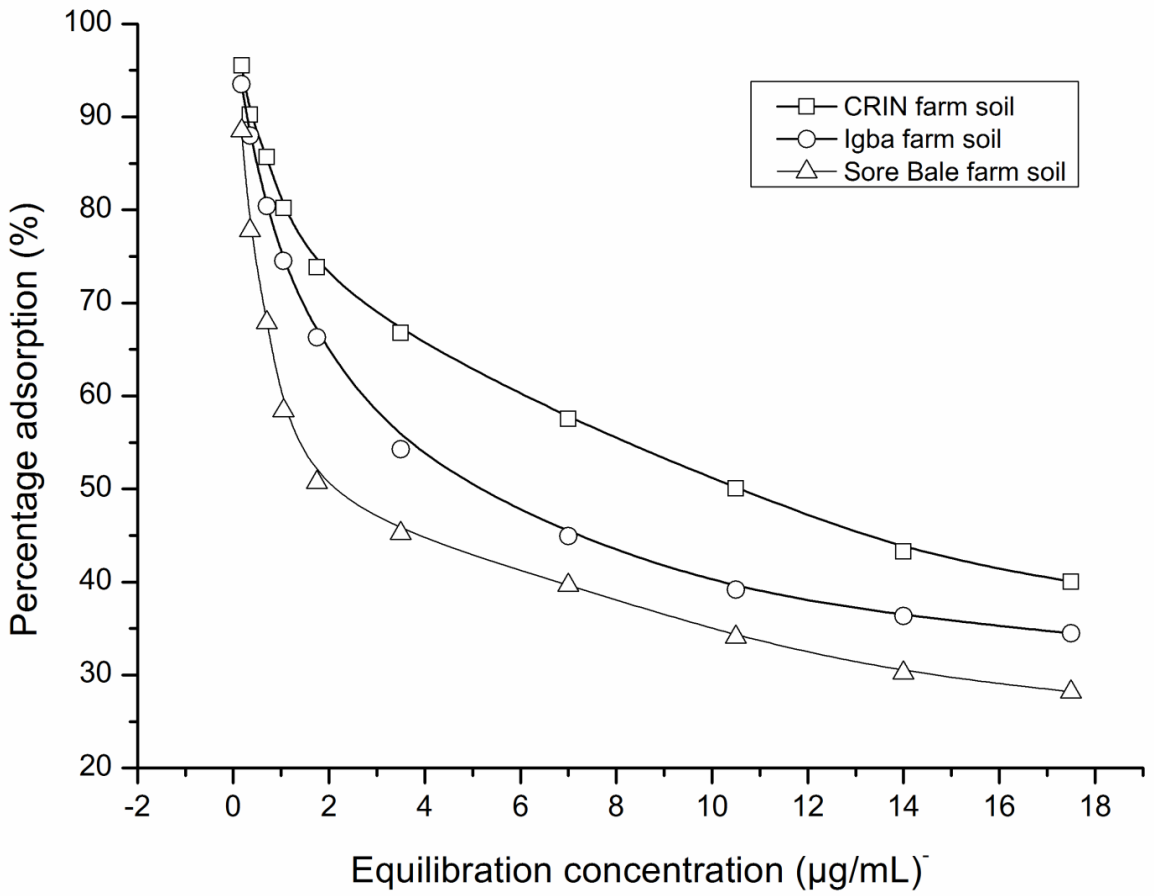


Figure 4.47. Percentage adsorption of α-endosulfan for CRIN, Igba and Sore Bale farm soils

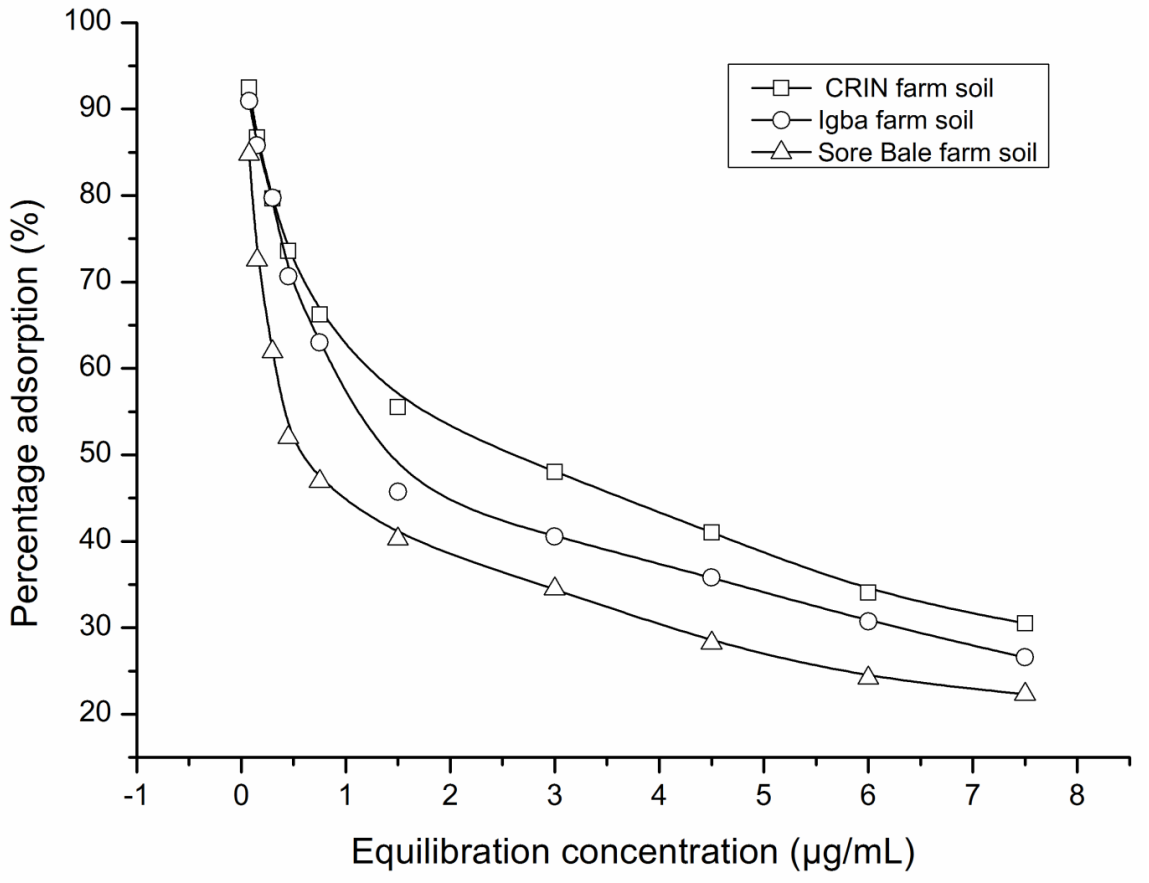


Figure 4.48. Percentage adsorption of β-endosulfan for CRIN, Igba and Sore Bale farm soils

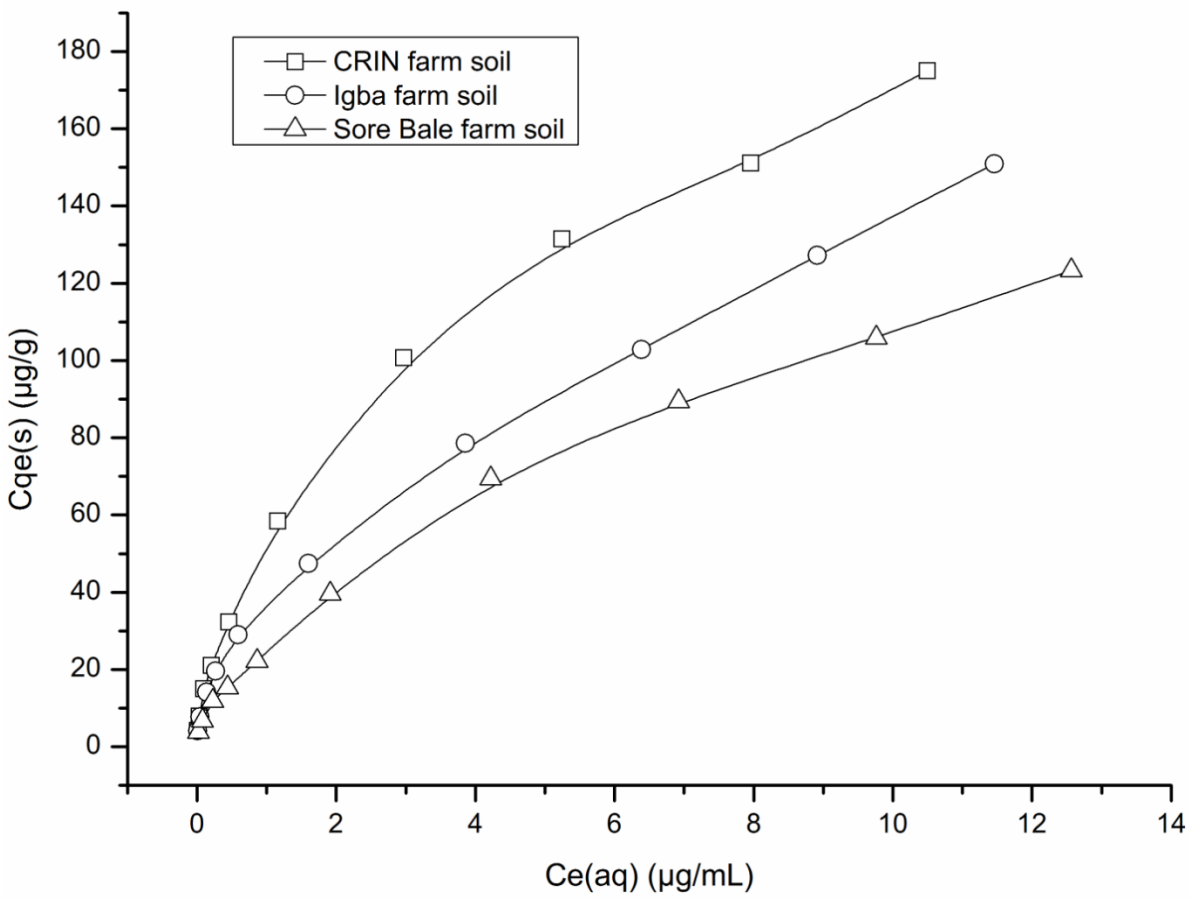


Figure 4.49. Adsorption isotherm of α-endosulfan for CRIN, Igba and Sore Bale soils

Where;

Cqe(s) = Concetration of α-endosulfan adsorbed to soil at equilibrium (µg/g)

Ce(aq) = Concetration of α-endosulfan remaining into 0.01M $CaCl_2$ at equilibrium (µg/mL)

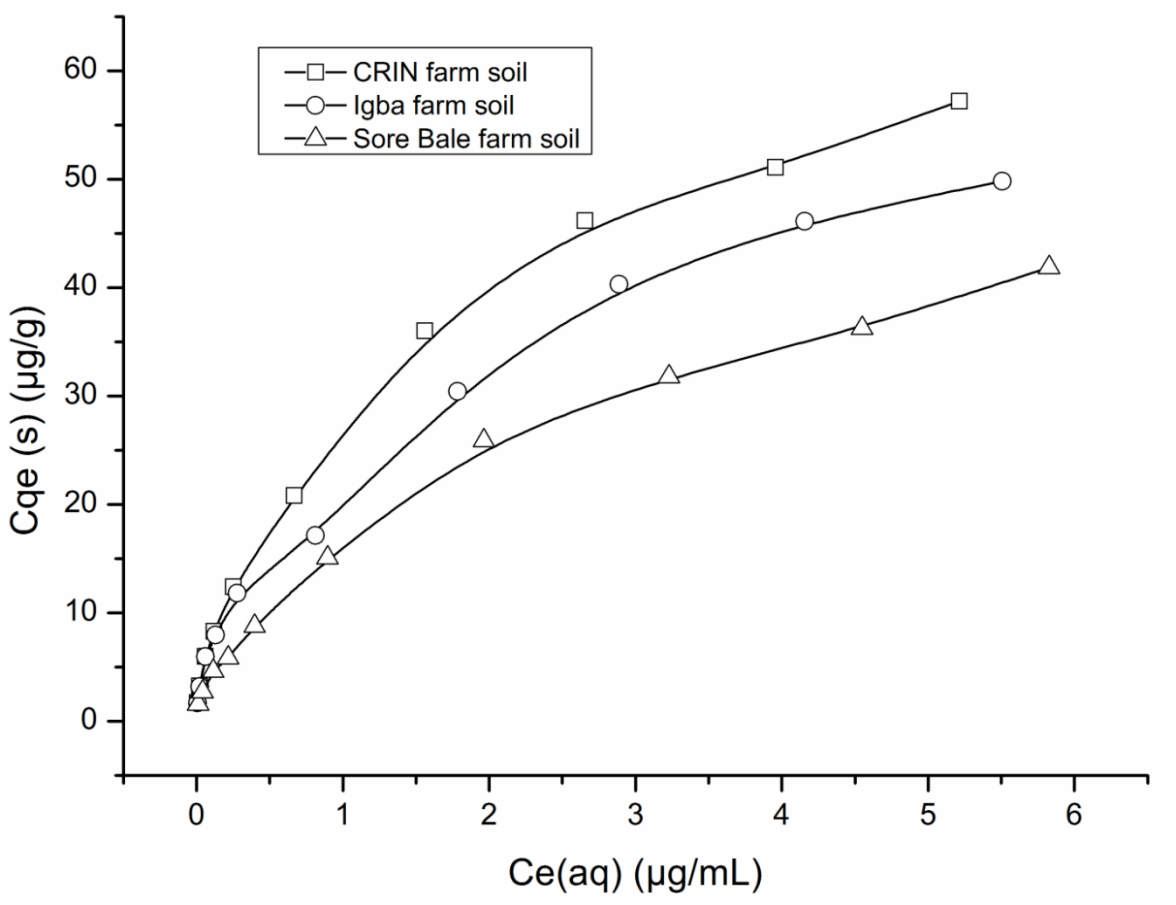


Figure 4.50. Adsorption isotherm of β-endosulfan for CRIN, Igba and Sore Bale soils

Where;

Cqe(s) = Concetration of β-endosulfan adsorbed to soil at equilibrium (µg/g)

Ce(aq) = Concetration of β-endosulfan remaining into 0.01M $CaCl_2$ at equilibrium (µg/mL)

This initial trend at low concentration may be due to the availability of abundant vacant sites in the adsorbent material (soil samples) being competed for by small quantities of adsorbate (α- and β-endosulfan) in the aqueous phase; while the parabola may have resulted from the gradual reduction in the number of vacant site available for adsorption by endosulfan as the concentration is increased. The behaviour of both isomers was almost similar in all the soil samples. However, the slight difference observed in the more 'parabolic shaped' adsorption isotherm curve for β-endosulfan at high concentration range, may likely be due to the more competitive nature of the α-isomer over the β. The α-endosulfan may have been favoured because of its rapid rate of adsorption and as well as having a relative higher concentration or proportion in commercial endosulfan (70% + 30 %) used in this study. Alpha endosulfan has been reported to have greater adsorption capacity over β-endosulfan (Kumar and Philip, 2006). The Freundlich adsorption coefficient (K_{fads}) obtained for α-endosulfan was highest in CRIN (9.228 mLg^{-1}), followed by Igba (7.471 mLg^{-1}) and Sore Bale (4.748 mLg^{-1}), with values in parenthesis, while the same trend was exhibited by the β-isomer (i.e., CRIN>Igba>Sore Bale), values ranged between 2.738 and 4.781 mLg^{-1} (Table 4.23).

Effect of organic matter and clay content on adsorption

Endosulfan is non-ionic and hydrophobic and would tend to sorb strongly to organic components present in soil (Parkpian *et al*. 1998). The organic matter content obtained for the three soils was 2.35 % (Sore Bale), 3.06 % (Igba) and 3.61% (CRIN) (see Table 4.21). This soil property or variable can be used to predict the adsorption capacity of soils, especially when they are of heavy texture and contain not less than 1% organic matter. The order of adsorption capcity was CRIN>Igba>Sore Bale. The Freundlich adsorption capacity (K_{ads}) for both endosulfan isomers was highest with CRIN soil, followed by Igba and Sore Bale soils (Table 4.23). The K_{ads} values of a chemical with different sorbents are directly proportional to their organic matter or organic carbon content (Site, 2000). The adsorption capacities exhibited by α- and β-endosulfan in this study would negatively influence their migration and leaching within these soil profiles. It is therefore expected that the order of ease of mobility and leaching of endosulfan will be CRIN < Igba < Sore Bale. Si *et al*. (2006) reported that the leaching potential of a pesticide is inversely related to adsorption, while their mobility in soil is controlled by sorption. In heterogenous adsorbents like soil, the sorption of non-ionic

and hydrophobic organics are dependent on the quantity of organic matter and to some extent the clay mineralogy of each soil (Monkiedje and Spiteller, 2002;Olu-Owolabi *et al*. 2014) and these influences their mobility (Murphy *et al*. 1992; Pierzynski *et al.* 1994).

The order of clay content was Igba > CRIN > Sore Bale, while the order of organic matter was CRIN > Igba > Sore Bale. The latter trend was observed when the adsorption capacities of the three cocoa farm soils for α- and β-endosulfan were considered, thus suggesting greater affinity of endosulfan to organic matter than clay. Although, Torrents and Jayasundera (1997) in their sorption study of non-ionic pesticides reported that the intensity of sorption was a function of herbicide and clay content.

The differential in the clay and organic matter components, between Igba (19.11%)(3.06%) and CRIN (13.65%)(3.61%) top soils was 5.46% and 0.55% for clay and organic matter content respectively. Inspite of the minimal difference in organic matter content in favour of CRIN soil, a much greater adsorption capacity was recorded by the CRIN soil compared to Igba soil. This affirmed the findings of Krishna and Philip (2008), who reported that organic matter seems to have high affinity to lindane, carbofuran and methyl parathion pesticides as compared to clay. In addition, Gao *et al*. (2000) reported that if the organic matter of sediment and soil is less than 0.01%, clay and silt content are mainly responsible for adsorption of pesticides.Therefore, the contribution of the organic matter of the soils to the sorption of endosulfan was much more relevant than the contribution of clay and other components.

The K_{om} values of α- and β-endosulfan (calculated from Freundlich adsorption coefficient, K_{fads}) (Seybold and Mersie, 1996; USEPA, 2008) were 255.377 and 132.308 mLg^{-1}; 244.036 and 142.938 mLg^{-1} and 202.073 and 116.522 mLg^{-1} for CRIN, Igba and Sore Bale soils respectively. The K_{om} of α-isomer were higher than those of β-isomer for all the three soils. The order was CRIN > Igba > Sore Bale for α-endosulfan, while for β-endosulfan Igba > CRIN > Sore Bale.

The K_{om} values of pesticides with soils have been used to predict their mobility andaccording to Swann *et al*., (1983), pesticides with K_{om}values < 500 are considered as mobile with respect to leaching. The highest K_{om} value obtained in this study was

255.377 mLg^{-1} (α-isomer, CRIN); this therefore means that endosulfan have very high potential to contaminateground water in the three cocoa farm soils and β-isomer is expected to have a greater potential to contaminate ground water over α-isomer. In addition, it also implied that high levels of organic content in soils could be used to restrict the migration and mobility of endosulfan in soils, thereby reducing the possibility of surface and groundwater contamination.

4.6.3.2. Desorption Isotherm

The Freundlich desorption rate for α- and β-endosulfan in the farm soils are expressed in percentages with respect to adsorption capacity after 480 hours equilibration time (for concentrations ranging from 0.175 to 17.5 μgmL^{-1} and 0.075 to 7.5 μgmL^{-1}forα- and β-endosulfan respectively). The rate or extent of desorptionfor α-endosulfan in CRIN, Igba and Sore Bale farm soils ranged between 5.29% and 13.77%, while for β-endosulfan values ranged from 8.61% to 18.10% respectively (Figure 4.51). The percentage desorption range of 5.29 % - 18.10%, exhibited by both isomers for concentration range of 0.075 - 17.5 μgmL^{-1} is a clear indication of the mobility and migration of endosulfan in the soils being studied. The order of desorption rate was CRIN < Igba < Sore Bale for both α- and β-isomers.This trend could be attributed to the bonding of the endosulfan to the organic matter and nature of soil type. High adsorption and desorption behaviour of soils in most cases is due to poor physical bonding (as van der Waals force) between endosulfan isomers and the soil particles. Sore Bale farm soil had the highest desorption rate mainly due to it relatively low level of organic matter and higher level of sand content when compared to CRIN and Igba farm soils (See Table 4.21). Since adsorption of hydrophobic organics to sandy soil is predominately by physical forces (Kumar and Philip, 2006), it is expected therefore that desorption and leaching (or mobility) of compounds such as endosulfan will easily occur in sandy soil compared to clayey soil as observed in this study. CRIN farm soil had the least desorption rate over Igba farm soil, although having a higher percent of clay content of 19.11%, compared to 13.65% obtained for CRIN. However, CRIN soil sample have higher organic matter content. The organic matter content in topsoil was 2.35%, 3.06% and 3.61% for Sore Bale, Igba and CRIN soils respectively.

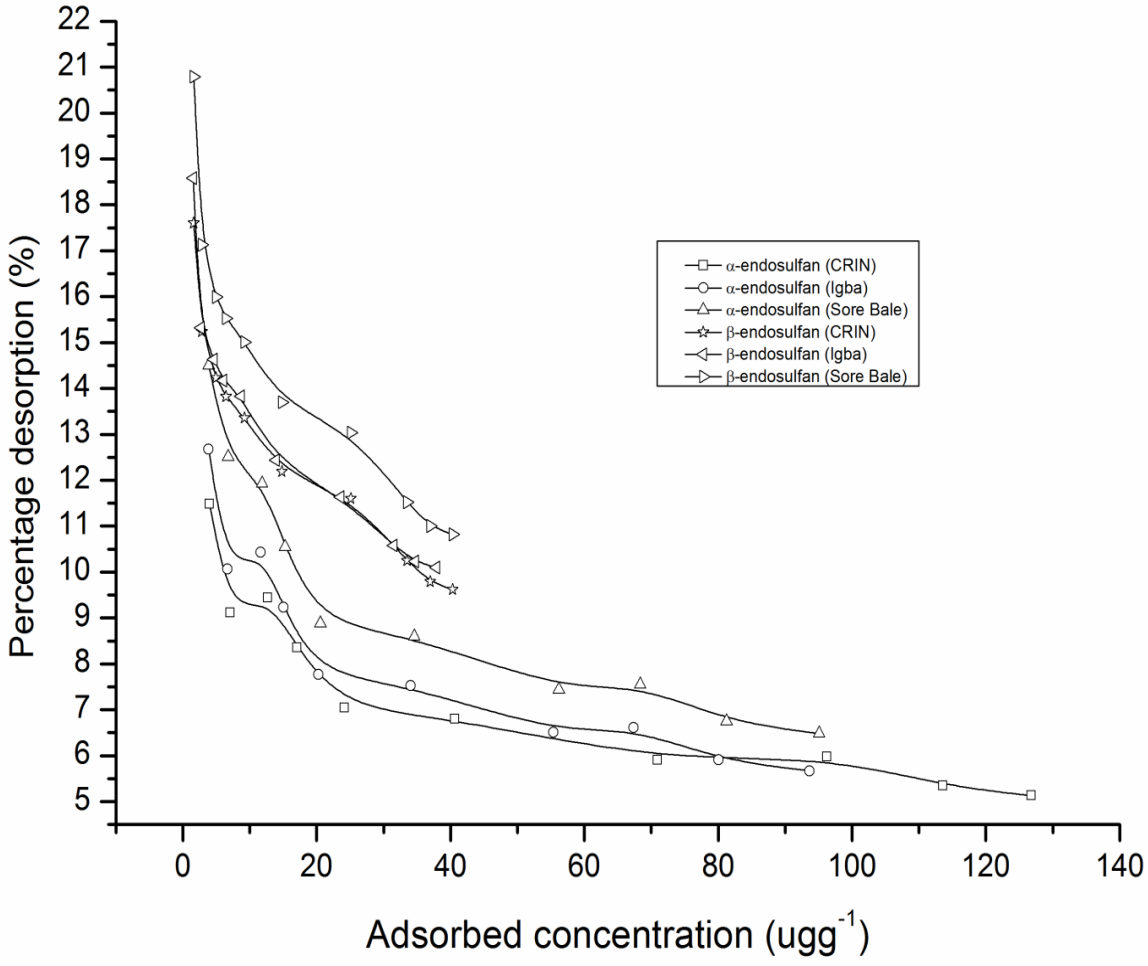


Figure 4.51. Percentage desorption isotherm of α- and β-endosulfan in farm soils

The relatively low percentage desorption exhibited by CRIN soil over Igba soil must have being due to its' higher organic matter content binding strongly to endosulfan. Desorption process is the reversal of adsorption and like adsorption, particle size distribution and organic matter content have being reported to play prominent role in desorption of hydrophobic organics in soil and sediment systems (Gao *et al*. 1998; Olu-Owolabi *et al*. 2014).Organic matter have been reported to have high affinity to pesticides when compared to clay on percentage basis (Schwarzenbach and Westall, 1981; Banerjee *et al*. 1985; Krishna and Philip, 2008) and this may have been responsible for the low desorption rate obtained for CRIN soil relative to Igba soil with higher clay content.

Greater desorption rate was observed for β-endosulfan than the α-isomer. This may have been due to the strong affinity of α-isomer to clayey soil and organic matter and greater amount of α-isomer adsorbed because of its relative dominant initial concentration (2.3:1 concentration ratio).

The Freundlich desorption parameters obtained for α and β-endosulfan are shown in Table 4.23, while all isotherm equilibrium plots of desorbed concentrations (Ce_{des}) against undesorbed concentrations (Cqe_{uds}) of both isomers were non-linear and fitted the Freundlich model (Figures 4.52and 4.53).Coefficients of determination, R^2 for Freundlich equation were ≥ 0.992 (Table 4.23). Desorption isotherm curves for both α- and β-isomers were of S-shape (sub-group 1) (Giles *et al.*, 1960) on all three farm soils, with a slight concavity at the middle stage of the isotherm. The concavity was more pronounced with the β-isomer, which exhibited greater rate of desorption than the α-isomer. This marked difference may be due to β-endosulfan being slightly more soluble than α-endosulfan in aqueous medium (Tomlin, 1994) at pH and temperature of 7.2 and 22^{o} C respectively. As the equilibration concentration used for adsorption process increases, the amount of desorbed concentration did not follow a proportional increase, but rather there was retarded and non-proportionate increase in the desorbed endosulfan with respect to increased undesorbed concentration from the adsorbed endosulfan. Graphical representation of Freundlich isotherm model with such concave curvature (S-shape) as obtained for the desorption process, were reported to have $1/n_{des}$>1 (Weber, 1972; Adamson, 1976).

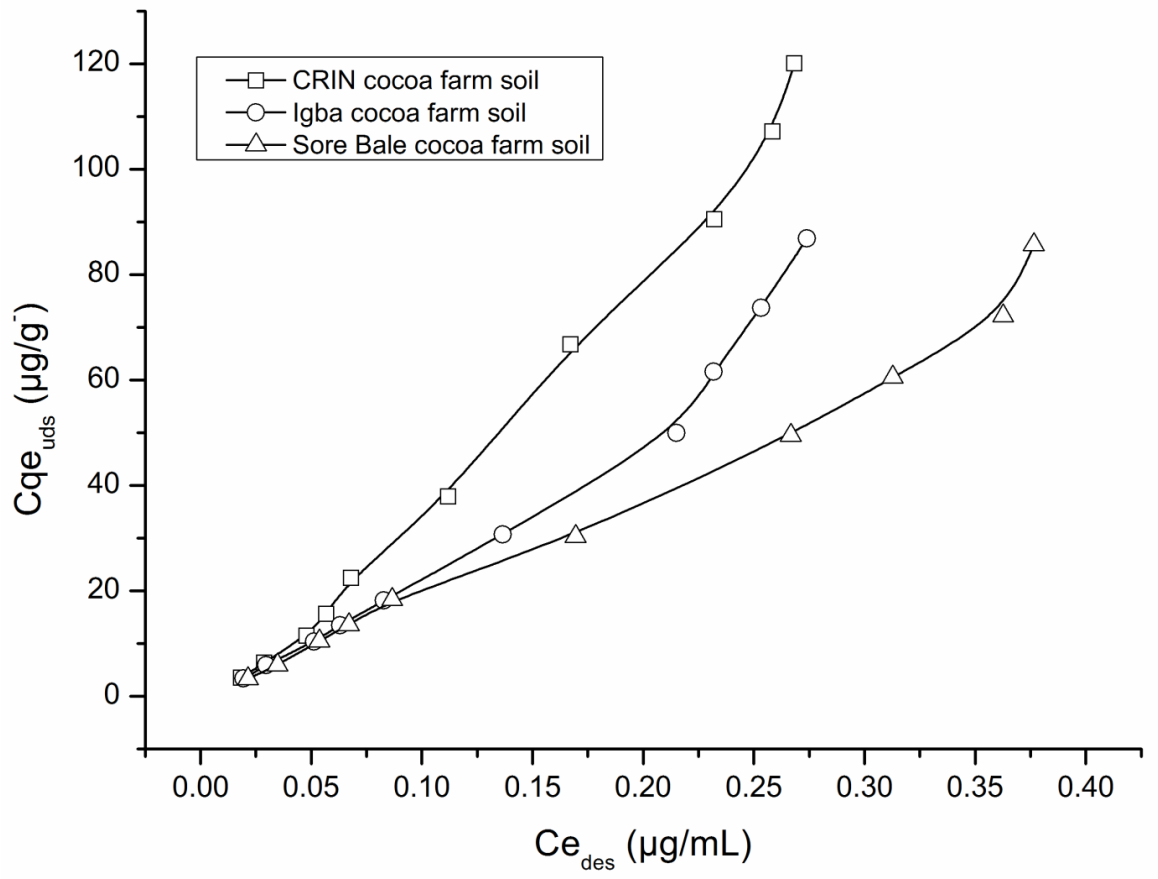


Figure 4.52. Desorption isotherm of α-endosulfan for CRIN, Igba and Sore Bale farm soils

Where;

Cqe_{uds} = Concetration of α-endosulfan undesorbed at equilibrium (µg/g)

$Ce_{des}(aq)$ = Concetration of α-endosulfan desorbed into 0.01M $CaCl_2$ at equilibrium (µg/mL)

\

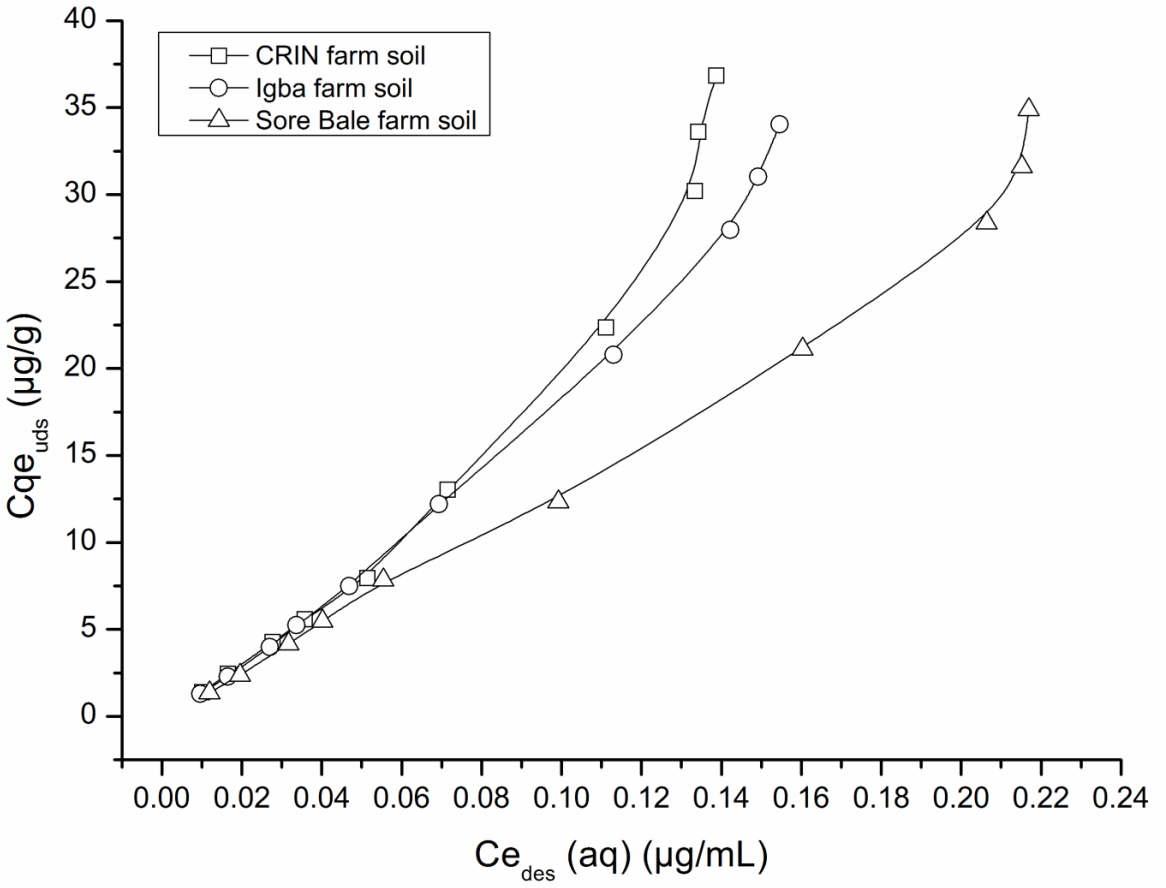


Figure 4.53. Desorption isotherm of β-endosulfan for CRIN, Igba and Sore Bale farm soils

Where;

Cqe_{uds} = Concetration of β-endosulfan undesorbed at equilibrium (µg/g)

$Ce_{des}(aq)$ = Concetration of β-endosulfan desorbed into 0.01M $CaCl_2$ at equilibrium (µg/mL)

The values for $1/n_{des}$ (i.e., strength of desorption) for α- and β-endosulfan were 1.299 and 1.229 for CRIN soil; 1.176 and 1.168 for Igba soil, while 1.063 and 1.070 for Sore Bale soil respectively – these values depicted non-linearity of the desorption data and indeed the nature of the isotherm curve. These values tend to agree with those reported by Atasoy *et al.* (2009), in which $1/n_{fdes}$values ranged between 1.15 and 1.29 for both isomers in Vertisol soil from southeast region of Turkey.

A significant variation was observed between the adsorption and desorption isotherms over the working concentration range. This remarkable difference is due to hysteresis - changes that occurred in the soil (Krishna and Philip, 2008). The Freundlich adsorption equilibrium constant ($1/n_{ads}$) values obtained for both α- and β-endosulfan for all soils (0.503 - 0.557) were relatively lower than those for Freundlich desorption isotherm ($1/n_{des}$) (1.063 – 1.299). Also, the Freundlich desorption coefficient values (K_{fdes}) were significantly higher than corresponding adsorption coefficient values (K_{fads}) for all soils and both isomers (Table 4.23). CRIN soil recorded the maximum K_{fdes}value 9.881 mLg^{-1}, followed by Igba and Sore Bale farm soils with the values of 7.880 mLg^{-1} and 7.157 mLg^{-1} respectively. The high K_{fdes}values in CRIN and Igba soils were probably due to the moderately high levels of organic matter and clay contents respectively, which have strong affinity for pesticides. Sore Bale farm soil was the least because of its high sandy nature andrelatively low organic matter content.

Adsorption – desorption hysteresis

Desorption is critical in assessing the extent to which pesticides are released from soil – that is the reversibility of adsorbed pesticide. The reversibility of adsorbed pesticides could be evaluated from Freundlich adsorption coefficient (K_{fads}) and desorption coefficient (K_{fdes}) or Freundlich adsorption constant ($1/n_{ads}$) and desorption contant ($1/n_{des}$). Adsorption-desorption hysteresis is often observed in pesticide sorption studies (Bowman and Sans, 1985; Koskinen and Harper, 1990; Roy and Krapac, 1994; Gao *et al.*, 1998). Positive hysteresis occurred for α- and β-endosulfan in all the three soils, i.e., $K_{fads}<K_{fdes}$ and $1/n_{ads}< 1/n_{des}$. The differential between the slopes for the Freundlich adsorption and desorption isotherms for α- and β-endosulfan for CRIN, Igba and Sore Bale soils were remarkable (Table 4.23). Hysteresis is exhibited when there is an increase in the difference between the adsorption and desorption isotherm slopes (Prata *et al*. 2003; Yang *et al*. 2009;Lima *et al*. 2010).The magnitude or degree

of hysteresis (ω) is often expressed as the hysteresis index (HI) (O'Connor *et al.*, 1980; Seybold and Mersie, 1996). According to Seybold and Mersie (1996), it is the ratio of Freundlich adsorption and desorption exponents $1/n_{ads}$ and $1/n_{des}$.

$$\omega = \{[1/n_{ads} : 1/n_{des}] \times 100\}$$

The HI for α-endosulfan ranged between 41.19% and 52.46%, while 42.69% and 51.08% for β-endosulfan (Table 4.24). Among the three soils, CRIN had the minimum HI for both isomers - with the order being, Sore Bale > Igba > CRIN. Desorption of sorbed endosulfan from soil samples was most difficult with CRIN soil, followed by Igba and Sore Bale soils. This order is reflective in the differences observed in the type of isotherm curves and slopes between sorption and desorption processes(Figures 4.39, 4.40, 4.42 and 4.43 and Table 4.23) in this study. The varying isotherms exhibited by the adsorbate (endosulfan)may bedue to the following; extent of its binding with OM, hydrogen bond formation, charge transfer, ionic bonds, cation bridges, hydrophobic interactions and physical diffusion into humic substances in the different soils (Prata *et al*. 2003; Boivin *et al*. 2005). The hysteresitic order was directly proportional to the level of organic matter in these three soils; thus making organic matter the prime factor in the hysteresis of endosulfan over clay content. Other factors such as hydrophobic interactions, physical diffusion into humic substances and charge transfer (clay mineralogy) are of less influence.The smaller the HI value, the greater the difficulty for adsorbed analyte (or adsorbate) to be desorbed from an adsorbent (Chefetz *et al*. 2004; Drori *et al*. 2005). The difference in HI between α- and β-isomers for each of the three soils was almost insignificant or minimal (Table 4.24). However, β-endosulfan exhibited higher hysteresis over α-endosulfan in CRIN, while the reserve was observed for Sore Bale and Igba soils; where α-endosulfan exhibited slightly higher hysteresis than β-endosulfan.

Table 4.24. Hysteresis Index (HI) for CRIN, Igba and Sore Bale soils

α-isomer	$1/n_{ads}$	$1/n_{des}$	$1/n_{ads}$:$1/n_{des}$*	$[1/n_{ads}:1/n_{des}]\times100$**
CRIN	0.5351	1.299	0.4119	41.19
Igba	0.5186	1.1758	0.4411	44.11
Sore Bale	0.5574	1.0626	0.5246	52.46
β-isomer				
CRIN	0.5248	1.2292	0.4269	42.69
Igba	0.5028	1.1677	0.4306	43.06
Sore Bale	0.5467	1.0703	0.5108	51.08

HI was calculated according to;

*O'Connor *et al.*, (1980)

**Seybold & Mersie, (1996)

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

5.1.1. Field assessment

Organochlorine pesticides were detected in all plant components except in cocoa seed. The order of distribution and bioaccumulation was leaves > bark > pods > seeds in all the sites for both seasons. The ease of persistence and bioaccumulation in cocoa leaves may have been due to its epicuticular waxy nature, which consists mainly of long-chain polyesters that can easily accumulate lipophilic compounds like OCPs.

Presence of OCPs in cocoa vegetation and other components in the CRIN environment indicated their bioaccumulation and persistence. Amongst the OCP families - the HCHs, aldrins and endosulfans were the most predominant, while dominant individual members were β-HCH, dieldrin and β-endosulfan respectively; amongst the HCHs, the β-isomer is the most stable and persistent. OCPs were not found in cocoa seeds, inspite of its high fatty content. Cocoa bean seeds are protected by the pod and mucilage mass. Therefore, reported detection of OCPs in seeds by Scientist/Reseachers may be predominately due to cross contamination during harvest. OCPs are still in use in Ondo II and Sore Bale.

Percentage detection of OCPs was lowest in the CRIN farms compared to other farms – this may have resulted from the restriction on the use and choice of OCPs applied to farms in the past as a Research Institute.Cocoa farms in Ondo were the most contaminated with endosulfan, while farmers in Sore Bale and Ondo were observed to have treated their farms with vast range of OCPs.

CRIN I soil was the most contaminated and $\sum OCP_{15\text{-}30cm}$ tend to be greater than $\sum OCP_{0\text{-}15cm}$ especially in soil where its application has been discontinued (CRIN I, CRIN II and Ondo I).Ondo II surface water was the most contaminated, while all

sediments were contaminated with γ-HCH and exceeded stipulated CB-TEC and CB-PEC limits based on SQGs.

Strong positiverelationship existed between parent pp'DDT and the sum of its metabolites (pp'DDE+op'DDD) at both depth and seasons (R was 0.8096 and 0.9377 respectively) and between seasons ratios of sum of metabolites (pp'DDE+op'DDD) and parent pp'DDT (R = 0.9610) at both depths, while a moderate positive seasonal relationship existed between sum of metabolites (R = 0.6424).

5.1.2. Field kinetics

Distribution of residual endosulfan in field kinetic studies, corroborated the general trend in vegetation - fresh leaves > bark > pods > seeds recorded in field assessment study involving fivecocoa (*Theobroma cacao)* farms. The percentage volatilization-degradation (dissipation) was >70.58% for parent isomers and ∑endosulfan after 7 days of application. Loss at the first period was predominately due to volatilization, degradation was minimal.

Over 99% of parent endosulfan in vegetation disappeared at day 60after initial treatment. Residual concentrations in *Theobroma cacao* vegetation over 60 days was due to topical application and not uptake from the soil, followed by translocation to various parts of the plant. Persistence of pesticide in *Theobroma cacao*vegetation was due to its diffusion into plant tissues. No stomata-uptake by the leaves and bioaccumulation after spraying was investigated.

Dissipation trend over a period of 60 days was distinctly biphasic and continuum for fresh and dry foliars, while tri-phasic and tri-continuum in pods and soil. This reflected a rapid disappearance phase due to volatilization and followed by a combination of degradation and volatilization.

The persistence of α- and β-endosulfan in fresh foliage, stem bark and dry foliage were almost the same, DT_{50} values for both isomers in the aforementioned showed slight differentials; with the β-endosulfan showing higher persistence in all matrixes especially in pods and soil (0-15cm). Persistence of both isomers in pods was three-fold greater than in fresh foliage, bark and dry foliage.

Rapid enzymatic actions in the pods must have been responsible forconcentrations of α- and β-isomers being almost at par (0.93 μgg^{-1}, 1.03 μgg^{-1} respectively) on day 0, after application of technical-grade endosulfan (Thiodan E35). No other metabolite except endosulfan sulphatewas determined.

Therefore from this study, α-endosulfan and β-endosulfan were non-persistent (DT_{50}<30d), with respect to fresh leaves, stem bark and dry leaves. Endosulfan sulphate and ∑endosulfan were moderately persistent in soil, while in cocoa pods ∑endosulfan was moderately persistent. There is high risk of cross contamination of cocoa seeds from pods.

5.1.3. Adsorption-desorption studies

The adsorption kinetics of α- and β-endosulfan was rapid, with >75.64% of the adsorption capacities of each soils achieved within 60 minutes. Adsorption was biphasic and bicontinuum. Pseudo equilibria were attained by α-endosulfan and β- with formation of plateaus in < 240 minutes for all soils.

The order of soil affinity for endosulfan pesticide was CRIN > Igba > Sore Bale soil, with the β-isomer exhibiting greater desorptive charater.

Endosulfan showed a greater affinity towards organic matter (OM) than clay conent, while the binding effect on soil was due to the presence of functional groups. Therefore, both soil properties could be used for the mitigation of endosulfan impacted soil.

All soils exhibited positive hyteresis, with $1/n_{ads} < 1/n_{des.}$The adsorption strength of α-isomer was greater than that of the β-isomer for all three soils.High percentage desorption exhibited by both isomers is indicative of their mobility and migration, thereby having great potential to impact both surface water and ground water (aquifer). In addition, the K_{om} values for all farm soils were <500. Comparatively, the β-isomer exhibited lower $K_{om.}$ HI and $1/n_{ads}$ to α-isomers from this study and all of these will enhance the mobility and leaching of the β-isomer in these farms.

5.2. Recommendations

The results obtained from this study showed that all major environmental compartments monitored in cocoa farms (vegetation, soils, surface water and

sediments) were contaminated with OCPs. In addition, previous investigations reported by other researchers (Oduwole, 2001; Asogwa and Dongo 2007) clearly suggested that majority of the cocoa farmers had limited awareness about safe pesticide management and undesirable practice. In addition, where such scientific technical knowledge has been acquired, some degree of inefficiency in pest management and control has been exhibited. The major reasons for the latter demeanour could be due to; financial constraints, poor techniques, inappropriate equipment for applying the pesticide, ill timing of application, inadequate understanding of how to use and impending consequences of careless use of pesticides, type and quantity of pesticide to be used to obtained the desired results.

In order to ameliorate the aforementioned environmental challenges posed by these findings the following suggestions are recommended:

i) The Federal Government of Nigeria through designated regulatory agencies should develop good and effective mechanism for monitoring and enforcement of laws and regulations for the management and use of pesticides (especially OCPs) by adopting UNPE/WHO/FAO guidelines

ii) Raising the awareness of local cocoa farmers on the implication of the continuous use of OCPs and design ways of solving existing challenges that have resulted from improper application.

iii) The Federal Government should disseminate scientific reports from research and development (R & D) programmes on the use of biopesticides, best available techniques (BAT) and other eco-friendly measures to cocoa farmers.This could be done effectively through training (Town and Gown synergy). Training should include good agricultural practises (GAPs) especially in the areas handling of pesticides, emergency respond when there are spills, appropriate dosage concentrations and application regiments and proper disposal of empty pesticide containers. Environmental contamination/pollution will greatly be minimized through this approach.

iv) Government policies on pesticide management and control should jointly encompass all key ministries and agencies like National Agency for Food and Drug Administration and Control (NAFDAC), Ministries of Environment, Agriculture and State Environmental Protection Agencies

(SEPAs) that are involved with its importation, regulation, application and its attended environmental consequence.

v) Preventive measures should be of top priority.Continuous surveillance and training of local farmers must be carried out by the Ministry of Health through the Cocoa Research Institute of Nigeria (CRIN). This should involve regular monitoring of residual levels of pesticides in cocoa vegetation, farm soils, water (surface and ground), sediments and surrounding atmosphere

vi) Mitigation measures – Farmers should be encouraged to increase the total organic matter (TOM) in thetopsoil because this will bind/adsorb non-target pesticides from being readily available for plant uptake.Organic matter will also prevent their migration and leaching that might impact ground water and surface water respectively.

Bioremediation of pesticide/OCP-contaminated farm soils by utilizing plant-associated microbes, and cultivation of transgenic crops for phytoremediation should be encouraged.

5.3. Futher work

In continuation with field kinetic study of sprayed endosulfan in cocoa farm, further sampling should be carried out to ascertain residual concentration after 2-4 years. Further kinetic variables and bioaccumulation factor (BCF) shall be deduced.

The enzymatic actions on endosulfan in cocoa pod should be further investigated. The moresusceptibility of the α-isomer to enzymatic metabolism by enzyme(s) present in the cocoa pod should be confirmed.

The use of various OM and clay content in cocoa farms soils as mitigants should be further studied for the purpose of restricting plant uptake of endosulfan (or OCPs).

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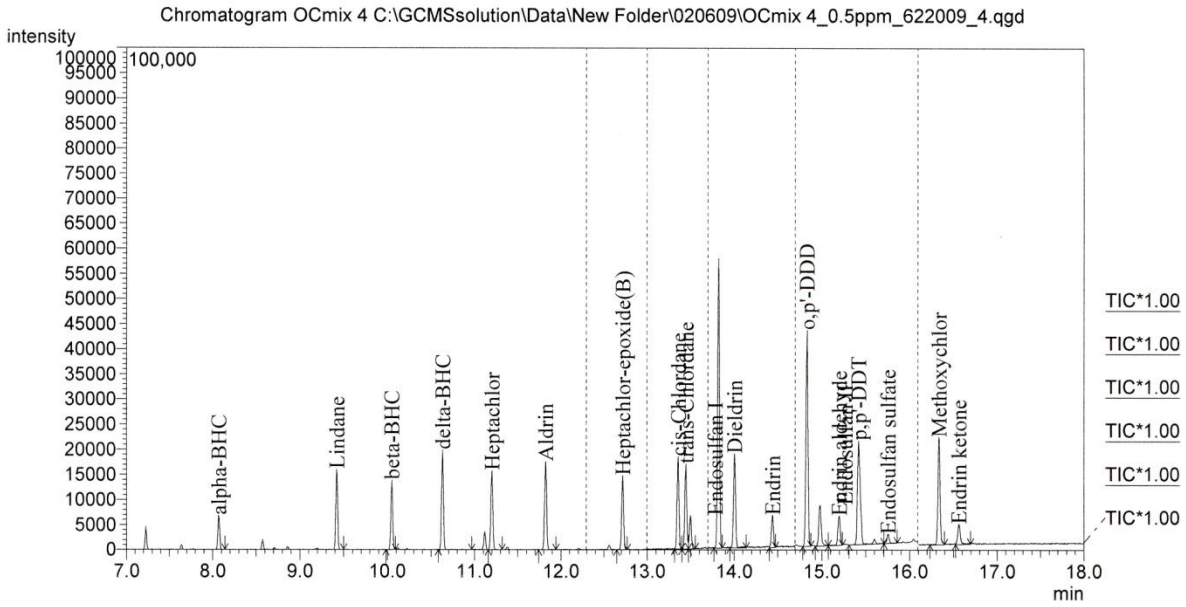
APPENDIX-A

FIELD ASSESSMENT

Sample Information

Analyzed	: 6/2/2009 7:27:08 PM
Sample Type	: Standard
Level #	: 4
Sample Name	: OCmix 4
Sample ID	: 0.5ppm
IS Amount	: [1]=1.000
Sample Amount	: 1.000
Dilution Factor	: 1.000
Vial #	: 4
Injection Volume	: 1.000
Data File	: C:\GCMSsolution\Data\New Folder\020609\OCmix 4_0.5ppm_622009_4.qgd
Org Data File	: C:\GCMSsolution\Data\020609\OCmix 4_0.5ppm_622009_4.qgd
Method File	: C:\GCMSsolution\Data\020609\ocpsim020609.qgm
Org Method File	: C:\GCMSsolution\Data\020609\ocpsim020609.qgm



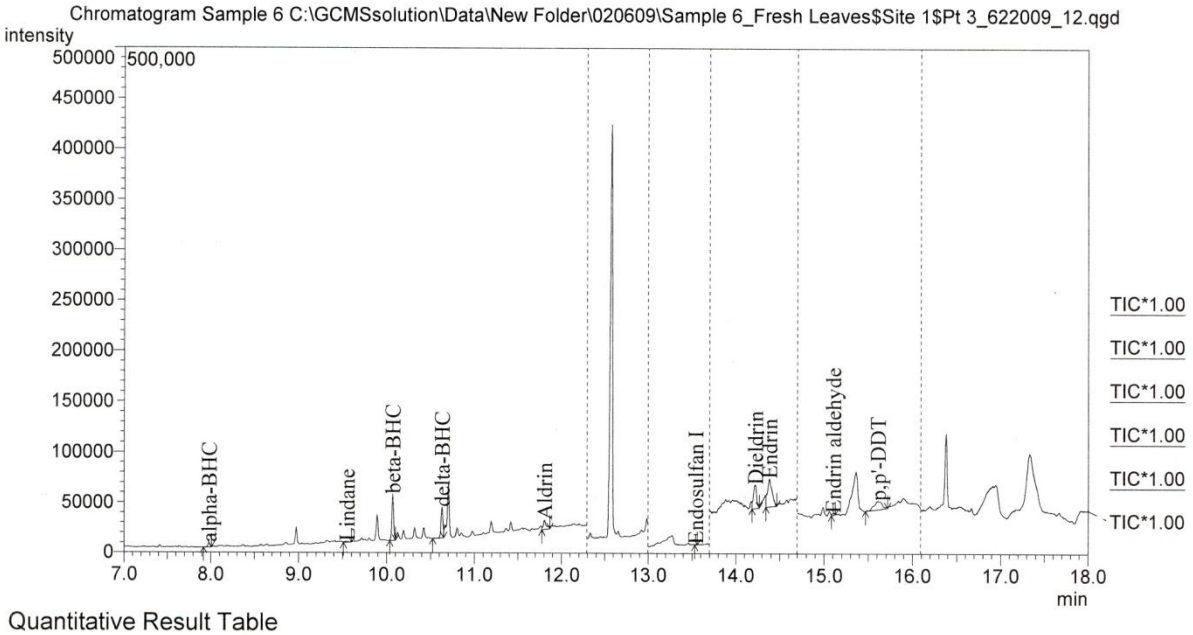
Quantitative Result Table

ID#	R.Time	m/z	Area	Height	Conc.	Conc.I	Recovery	Name
1	8.069	TIC	9708	6863	0.494 ppm		0.00	alpha-BHC
2	9.420	TIC	24657	16141	0.489 ppm		0.00	Lindane
3	10.049	TIC	20435	13916	0.487 ppm		0.00	beta-BHC
4	10.631	TIC	31017	19926	0.500 ppm		0.00	delta-BHC
5	11.199	TIC	24222	15724	0.484 ppm		0.00	Heptachlor
6	11.822	TIC	29463	17623	0.498 ppm		0.00	Aldrin
7	12.715	TIC	23601	14826	0.507 ppm		0.00	Heptachlor-epoxide(B)
8	13.356	TIC	29126	18426	0.527 ppm		0.00	cis-Chlordane
9	13.446	TIC	27541	16936	0.528 ppm		0.00	trans-Chlordane
10	13.496	TIC	10771	6698	0.508 ppm		0.00	Endosulfan I
11	13.820	TIC	87051	57615	0.512 ppm		0.00	p,p'-DDE
12	14.003	TIC	31172	18723	0.505 ppm		0.00	Dieldrin
13	14.439	TIC	10680	6361	0.493 ppm		0.00	Endrin
14	14.832	TIC	69605	42975	0.506 ppm		0.00	o,p'-DDD
15	14.979	TIC	15682	8096	0.514 ppm		0.00	Endosulfan II
16	15.197	TIC	11074	5839	0.522 ppm		0.00	Endrin aldehyde
17	15.418	TIC	46063	20662	0.493 ppm		0.00	p,p'-DDT
18	15.753	TIC	3624	1838	0.466 ppm		0.00	Endosulfan sulfate
19	16.334	TIC	36159	21325	0.488 ppm		0.00	Methoxychlor
20	16.561	TIC	8970	4043	0.521 ppm		0.00	Endrin ketone

Figure A-1. Chromatogram of organochlorine pesticide standard

Sample Information

Analyzed	: 6/2/2009 10:48:51 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: Sample 6
Sample ID	: Fresh Leaves/Site 1/Pt 3
IS Amount	: [1]=1.000
Sample Amount	: 6.880
Dilution Factor	: 1.000
Vial #	: 12
Injection Volume	: 1.000
Data File	: C:\GCMSsolution\Data\New Folder\020609\Sample 6_Fresh Leaves$Sit
Org Data File	: C:\GCMSsolution\Data\020609\Sample 6_Fresh Leaves$Site 1$Pt 3_62
Method File	: C:\GCMSsolution\Data\New Folder\020609\ocpsim020609.qgm
Org Method File	: C:\GCMSsolution\Data\020609\ocpsim020609.qgm



Quantitative Result Table

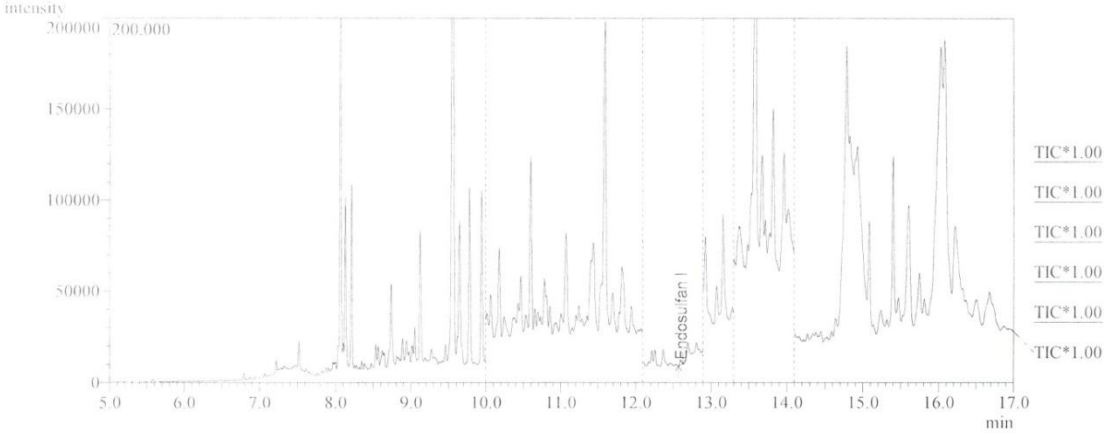
ID#	R.Time	m/z	Area	Height	Conc.	Conc.I	Recovery	Name
1	7.980	TIC	5171	3888	0.038 ppm		0.00	alpha-BHC
2	9.549	TIC	2932	869	0.010 ppm		0.00	Lindane
3	10.068	TIC	59496	44153	0.201 ppm		0.00	beta-BHC
4	10.629	TIC	37971	26073	0.089 ppm		0.00	delta-BHC
5	-	TIC	---	---	N.D.(Ref) ppm		-	Heptachlor
6	11.804	TIC	5260	5764	0.012 ppm		0.00	Aldrin
7	-	TIC	---	---	N.D.(Ref) ppm		-	Heptachlor-epoxide(B)
8	-	TIC	---	---	N.D.(Ref) ppm		-	cis-Chlordane
9	-	TIC	---	---	N.D.(Ref) ppm		-	trans-Chlordane
10	13.542	TIC	992	711	0.006 ppm		0.00	Endosulfan I
11	-	TIC	---	---	N.D.(Ref) ppm		-	p,p'-DDE
12	14.220	TIC	36884	18433	0.087 ppm		0.00	Dieldrin
13	14.380	TIC	60242	20352	0.408 ppm		0.00	Endrin
14	-	TIC	---	---	N.D.(Ref) ppm		-	o,p'-DDD
15	14.987	TIC	18410	9429	0.088 ppm		0.00	Endosulfan II
16	15.103	TIC	3738	2048	0.024 ppm		0.00	Endrin aldehyde
17	15.608	TIC	46093	7817	0.072 ppm		0.00	p,p'-DDT
18	-	TIC	---	---	N.D.(Ref) ppm		-	Endosulfan sulfate
19	-	TIC	---	---	N.D.(Ref) ppm		-	Methoxychlor
20	-	TIC	---	---	N.D.(Ref) ppm		-	Endrin ketone

Figure A-2. Chromatogram of organochlorine pesticide in CRIN cocoa fresh leaves

Sample Information

Analyzed by : Admin
Analyzed : 8/2/2009 9.15:08 PM
Level# : 1
Sample Name : 14
Sample ID : Bark 1/1
IS Amount : [1]=1.000
Sample Amount : 10.00
Dilution Factor : 1.000
Vial # : 26
Injection Volume : 1.000
Data File : C:\GCMSsolution \Data\New Folder\020609\Sample 14 Bark $ Site 1/1
Org Data File : C:\GCMSsolution \Data\020609\Sample 14 Bark $ Site 1/3
Method File : C:\GCMSsolution \Data\New Folder\ 020609\ocpsim020609.qgm
Org Method File : C:\GCMSsolution \Data\020609\ocpsim0206099.qgm

Chromatogram Sample 14 C\GCMSsolution\Data\New Folder\020609\Bark1$St1$Pt1CRIN _622009_25ggd



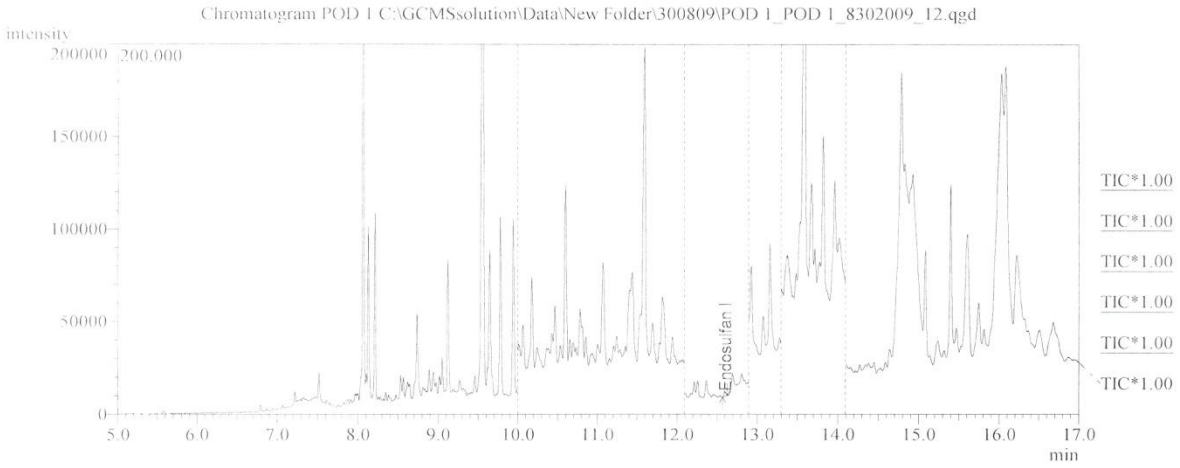
Quantitative Result Table

ID#	R.Time	m/z	Area	Height	Conc.	Conc.I	Recovery	Name
1	-	TIC	---	---	N.D.(Ref)	ppm	-	alpha-BHC
2	-	TIC	---	---	N.D.(Ref)	ppm	-	beta-BHC
3	-	TIC	---	---	N.D.(Ref)	ppm	-	Lindane
4	-	TIC	---	---	N.D.(Ref)	ppm	-	delta-BHC
5	-	TIC	---	---	N.D.(Ref)	ppm	-	Heptachlor
6	-	TIC	---	---	N.D.(Ref)	ppm	-	Aldrin
7	-	TIC	---	---	N.D.(Ref)	ppm	-	Heptachlor-epoxide(B)
8	-	TIC	---	---	N.D.(Ref)	ppm	-	cis-Chlordane
9	12.603	TIC	4547	2479	0.022	ppm	0.00	Endosulfan I
10	-	TIC	---	---	N.D.(Ref)	ppm	-	trans-Chlordane
11	-	TIC	---	---	N.D.(Ref)	ppm	-	o,p'-DDE
12	-	TIC	---	---	N.D.(Ref)	ppm	-	Dieldrin
13	-	TIC	---	---	N.D.(Ref)	ppm	-	Endrin
14	-	TIC	---	---	N.D.(Ref)	ppm	-	Endosulfan II
15	-	TIC	---	---	N.D.(Ref)	ppm	-	p,p'-DDD
16	-	TIC	---	---	N.D.(Ref)	ppm	-	Endrin aldehyde
17	-	TIC	---	---	N.D.(Ref)	ppm	-	Endosulfan sulfate
18	-	TIC	---	---	N.D.(Ref)	ppm	-	p,p'-DDT
19	-	TIC	---	---	N.D.(Ref)	ppm	-	Endrin ketone
20	-	TIC	---	---	N.D.(Ref)	ppm	-	Methoxychlor

Figure A-3.Chromatogram of organochlorine pesticide in CRIN cocoa stem bark

Sample Information

Analyzed by	: Admin
Analyzed	: 8/30/2009 7:51:17 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: POD 1
Sample ID	: POD 1
IS Amount	: [1]=1.000
Sample Amount	: 5.050
Dilution Factor	: 1.000
Vial #	: 12
Injection Volume	: 1.000



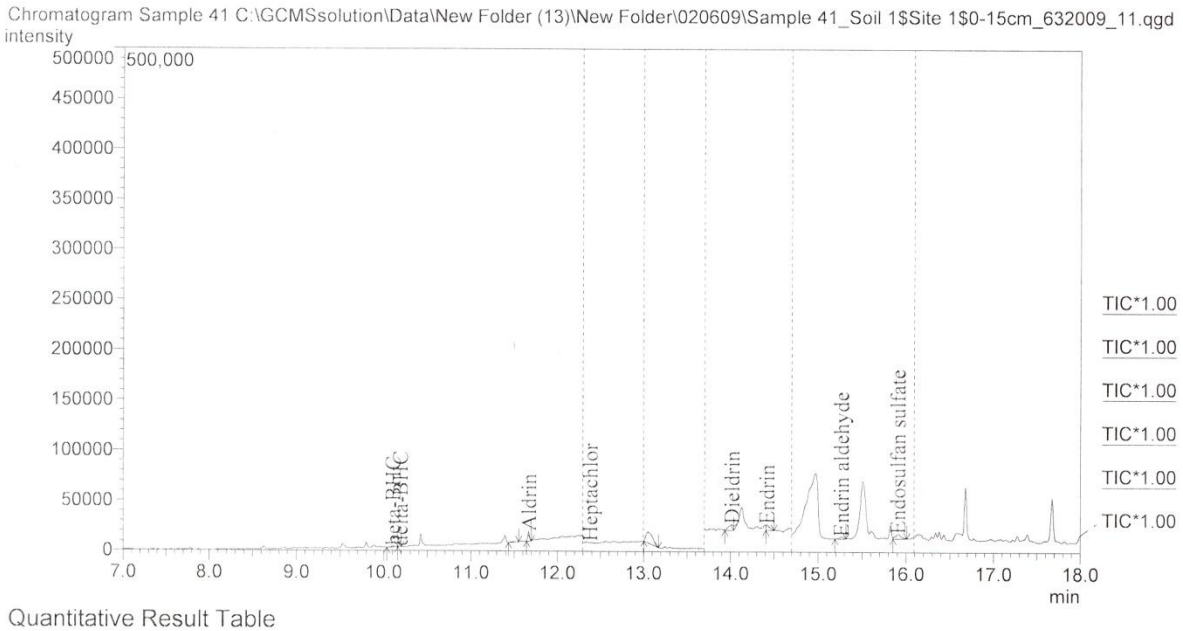
Quantitative Result Table

ID#	R.Time	m/z	Area	Height	Conc.	Conc.I	Recovery	Name
1	-	TIC	---	---	N.D.	(Ref) ppm	-	alpha-BHC
2	-	TIC	---	---	N.D.	(Ref) ppm	-	beta-BHC
3	-	TIC	---	---	N.D.	(Ref) ppm	-	Lindane
4	-	TIC	---	---	N.D.	(Ref) ppm	-	delta-BHC
5	-	TIC	---	---	N.D.	(Ref) ppm	-	Heptachlor
6	-	TIC	---	---	N.D.	(Ref) ppm	-	Aldrin
7	-	TIC	---	---	N.D.	(Ref) ppm	-	Heptachlor-epoxide(B)
8	-	TIC	---	---	N.D.	(Ref) ppm	-	cis-Chlordane
9	12.603	TIC	4547	2479	0.022	ppm	0.00	Endosulfan I
10	-	TIC	---	---	N.D.	(Ref) ppm	-	trans-Chlordane
11	-	TIC	---	---	N.D.	(Ref) ppm	-	o,p'-DDE
12	-	TIC	---	---	N.D.	(Ref) ppm	-	Dieldrin
13	-	TIC	---	---	N.D.	(Ref) ppm	-	Endrin
14	-	TIC	---	---	N.D.	(Ref) ppm	-	Endosulfan II
15	-	TIC	---	---	N.D.	(Ref) ppm	-	p,p'-DDD
16	-	TIC	---	---	N.D.	(Ref) ppm	-	Endrin aldehyde
17	-	TIC	---	---	N.D.	(Ref) ppm	-	Endosulfan sulfate
18	-	TIC	---	---	N.D.	(Ref) ppm	-	p,p'-DDT
19	-	TIC	---	---	N.D.	(Ref) ppm	-	Endrin ketone
20	-	TIC	---	---	N.D.	(Ref) ppm	-	Methoxychlor

Figure A-4. Chromatogram of organochlorine pesticide in CRIN cocoa pods

Sample Information

Analyzed	: 6/3/2009 8:35:30 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: Sample 41
Sample ID	: Soil 1/Site 1/0-15cm
IS Amount	: [1]=1.000
Sample Amount	: 10.230
Dilution Factor	: 1.000
Vial #	: 47
Injection Volume	: 1.000
Data File	: C:\GCMSsolution\Data\New Folder (13)\New Folder\020609\Sample 41_Soil 1$Site 1
Org Data File	: C:\GCMSsolution\Data\030609\Sample 41_Soil 1$Site 1$0-15cm_632009_11.qgd
Method File	: C:\GCMSsolution\Data\New Folder (13)\New Folder\020609\ocpsim020609.qgm
Org Method File	: C:\GCMSsolution\Data\030609\ocpsim020609.qgm



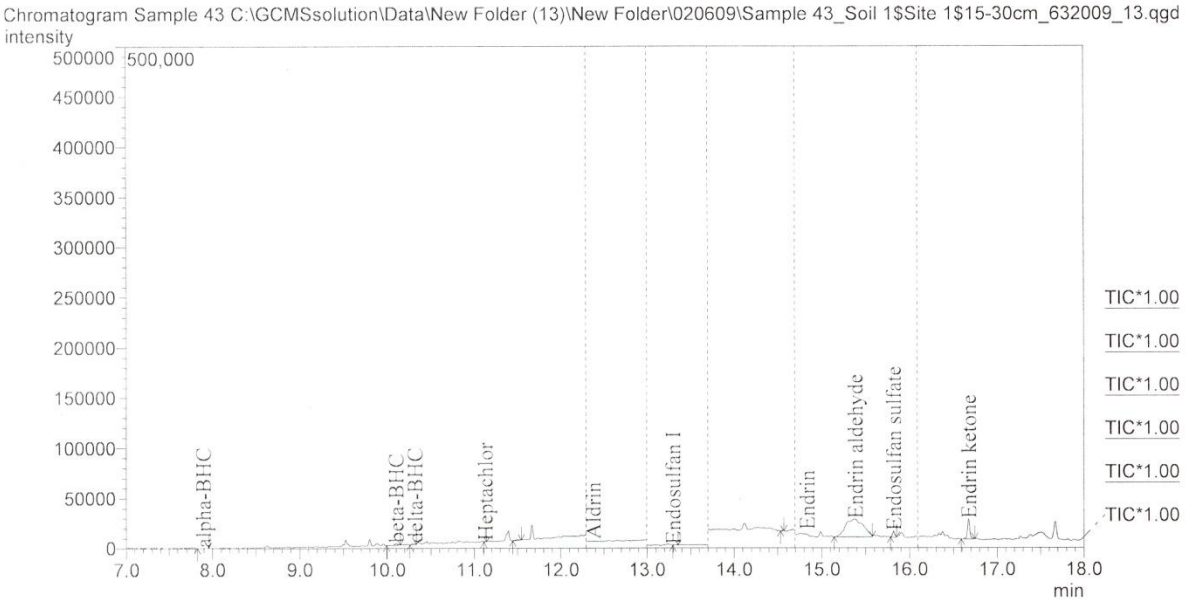
Quantitative Result Table

ID#	R.Time	m/z	Area	Height	Conc.	Conc.I	Recovery	Name
1	-	TIC	---	---	N.D.(Ref)	ppm	-	alpha-BHC
2	-	TIC	---	---	N.D.(Ref)	ppm	-	Lindane
3	10.100	TIC	4163	1521	0.011	ppm	0.00	beta-BHC
4	10.187	TIC	722	401	0.002	ppm	0.00	delta-BHC
5	11.523	TIC	3725	568	0.009	ppm	0.00	Heptachlor
6	11.666	TIC	13299	9531	0.022	ppm	0.00	Aldrin
7	-	TIC	---	---	N.D.(Ref)	ppm	-	Heptachlor-epoxide(B)
8	-	TIC	---	---	N.D.(Ref)	ppm	-	cis-Chlordane
9	-	TIC	---	---	N.D.(Ref)	ppm	-	trans-Chlordane
10	13.049	TIC	56467	10655	0.263	ppm	0.00	Endosulfan I
11	-	TIC	---	---	N.D.(Ref)	ppm	-	p,p'-DDE
12	14.013	TIC	23238	6039	0.036	ppm	0.00	Dieldrin
13	14.414	TIC	15850	5612	0.072	ppm	0.00	Endrin
14	-	TIC	---	---	N.D.(Ref)	ppm	-	o,p'-DDD
15	-	TIC	---	---	N.D.(Ref)	ppm	-	Endosulfan II
16	15.260	TIC	6255	1799	0.028	ppm	0.00	Endrin aldehyde
17	-	TIC	---	---	N.D.(Ref)	ppm	-	p,p'-DDT
18	15.904	TIC	18516	4329	0.231	ppm	0.00	Endosulfan sulfate
19	-	TIC	---	---	N.D.(Ref)	ppm	-	Methoxychlor
20	-	TIC	---	---	N.D.(Ref)	ppm	-	Endrin ketone

Figure A-5. Chromatogram of organochlorine pesticide in CRIN farm soil (0-15cm)

Sample Information

Analyzed	: 6/3/2009 9:26:01 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: Sample 43
Sample ID	: Soil 1/Site 1/15-30cm
IS Amount	: [1]=1.000
Sample Amount	: 10.300
Dilution Factor	: 1.000
Vial #	: 49
Injection Volume	: 1.000
Data File	: C:\GCMSsolution\Data\New Folder (13)\New Folder\020609\Sample 43_Soil 1$Site 1$
Org Data File	: C:\GCMSsolution\Data\030609\Sample 43_Soil 1$Site 1$15-30cm_632009_13.qgd
Method File	: C:\GCMSsolution\Data\New Folder (13)\New Folder\020609\ocpsim020609.qgm
Org Method File	: C:\GCMSsolution\Data\030609\ocpsim020609.qgm



Quantitative Result Table

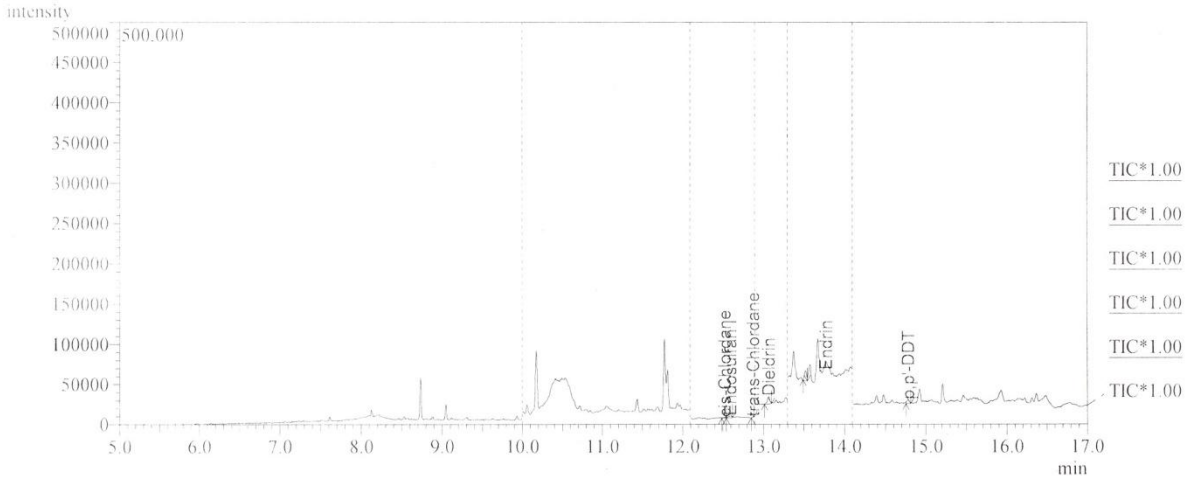
ID#	R.Time	m/z	Area	Height	Conc.	Conc.I	Recovery	Name
1	7.894	TIC	833	112	0.003 ppm		0.00	alpha-BHC
2	-	TIC	---	---	N.D.(Ref) ppm		-	Lindane
3	10.099	TIC	2783	1699	0.008 ppm		0.00	beta-BHC
4	10.316	TIC	4547	1308	0.008 ppm		0.00	delta-BHC
5	11.123	TIC	1802	1140	0.005 ppm		0.00	Heptachlor
6	11.520	TIC	4083	806	0.006 ppm		0.00	Aldrin
7	-	TIC	---	---	N.D.(Ref) ppm		-	Heptachlor-epoxide(B)
8	-	TIC	---	---	N.D.(Ref) ppm		-	cis-Chlordane
9	-	TIC	---	---	N.D.(Ref) ppm		-	trans-Chlordane
10	13.299	TIC	1368	640	0.006 ppm		0.00	Endosulfan I
11	-	TIC	---	---	N.D.(Ref) ppm		-	p,p'-DDE
12	-	TIC	---	---	N.D.(Ref) ppm		-	Dieldrin
13	14.549	TIC	1127	843	0.005 ppm		0.00	Endrin
14	-	TIC	---	---	N.D.(Ref) ppm		-	o,p'-DDD
15	-	TIC	---	---	N.D.(Ref) ppm		-	Endosulfan II
16	15.381	TIC	244711	17588	1.155 ppm		0.00	Endrin aldehyde
17	-	TIC	---	---	N.D.(Ref) ppm		-	p,p'-DDT
18	15.817	TIC	9442	5782	0.117 ppm		0.00	Endosulfan sulfate
19	-	TIC	---	---	N.D.(Ref) ppm		-	Methoxychlor
20	16.676	TIC	39346	19836	0.220 ppm		0.00	Endrin ketone

Figure A-6. Chromatogram of organochlorine pesticide in CRIN farm soil (15-30cm)

Sample Information

Analyzed by	: Admin
Analyzed	: 9/3/2009 9.15:08 PM
Level#	: 1
Sample Name	: 59
Sample ID	: Surface Water 1/3
IS Amount	: [1]=1.000
Sample Amount	: 10.00
Dilution Factor	: 1.000
Vial #	: 67
Injection Volume	: 1.000
Data File	: C:\GCMSsolution \Data\New Folder\020609\Sample 59 Surface water$ Site 1
Org Data File	: C:\GCMSsolution \Data\020609\Sample 59 Surface water$ Site 1/3
Method File	: C:\GCMSsolution \Data\New Folder\ 020609\ocpsim020609.qgm
Org Method File	: C:\GCMSsolution \Data\020609\ocpsim 020609.qgm

Chromatogram Sample 59 C:\GCMSsolution\Data\New Folder\020609\Sample 59 _Surface water$CRIN_622009_25ggd



Quantitative Result Table

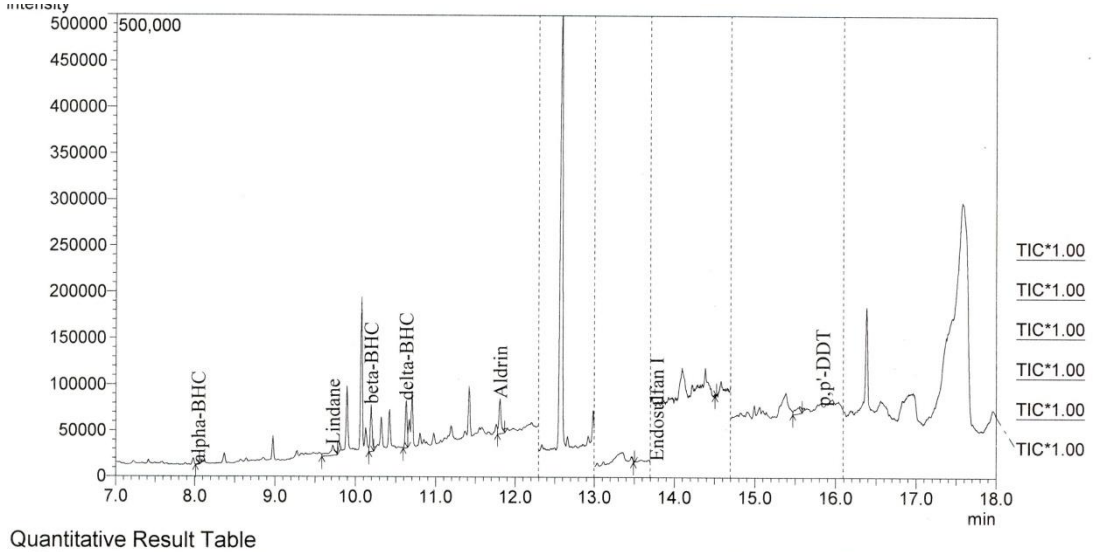
ID#	R.Time	m/z	Area	Height	Conc.	Conc.I	Recovery	Name
1	-	TIC	---	---	N.D.(Ref)	ppm	-	alpha-BHC
2	-	TIC	---	---	N.D.(Ref)	ppm	-	beta-BHC
3	-	TIC	---	---	N.D.(Ref)	ppm	-	Lindane
4	-	TIC	---	---	N.D.(Ref)	ppm	-	delta-BHC
5	-	TIC	---	---	N.D.(Ref)	ppm	-	Heptachlor
6	-	TIC	---	---	N.D.(Ref)	ppm	-	Aldrin
7	-	TIC	---	---	N.D.(Ref)	ppm	-	Heptachlor-epoxide(B)
8	12.522	TIC	4273	1380	0.009	ppm	0.00	cis-Chlordane
9	12.592	TIC	8256	2637	0.018	ppm	0.00	Endosulfan I
10	12.874	TIC	284	294	0.005	ppm	0.00	trans-Chlordane
11	-	TIC	---	---	N.D.(Ref)	ppm	-	o,p'-DDE
12	13.068	TIC	10940	7217	0.014	ppm	0.00	Dieldrin
13	13.521	TIC	23466	12428	0.082	ppm	0.00	Endrin
14	-	TIC	---	---	N.D.(Ref)	ppm	-	Endosulfan II
15	-	TIC	---	---	N.D.(Ref)	ppm	-	p,p'-DDD
16	-	TIC	---	---	N.D.(Ref)	ppm	-	Endrin aldehyde
17	-	TIC	---	---	N.D.(Ref)	ppm	-	Endosulfan sulfate
18	14.778	TIC	10918	4704	0.179	ppm	0.00	p,p'-DDT
19	-	TIC	---	---	N.D.(Ref)	ppm	-	Endrin ketone
20	-	TIC	---	---	N.D.(Ref)	ppm	-	Methoxychlor

Figure A-7. Chromatogram of organochlorine pesticide in CRIN surface water

Sample Information

Analyzed by	: Admin
Analyzed	: 9/1/2009 7.24:46 PM
Level#	: 1
Sample Name	: SED 1/2
Sample ID	: SED 1/2
IS Amount	: [1]=1.000
Sample Amount	: 10.00
Dilution Factor	: 1.000
Vial #	: 44
Injection Volume	: 1.000

Chromatogram Sample 44 C\GCMSsolution\Data\New Folder\020609\SED1&2_622009_25ggd



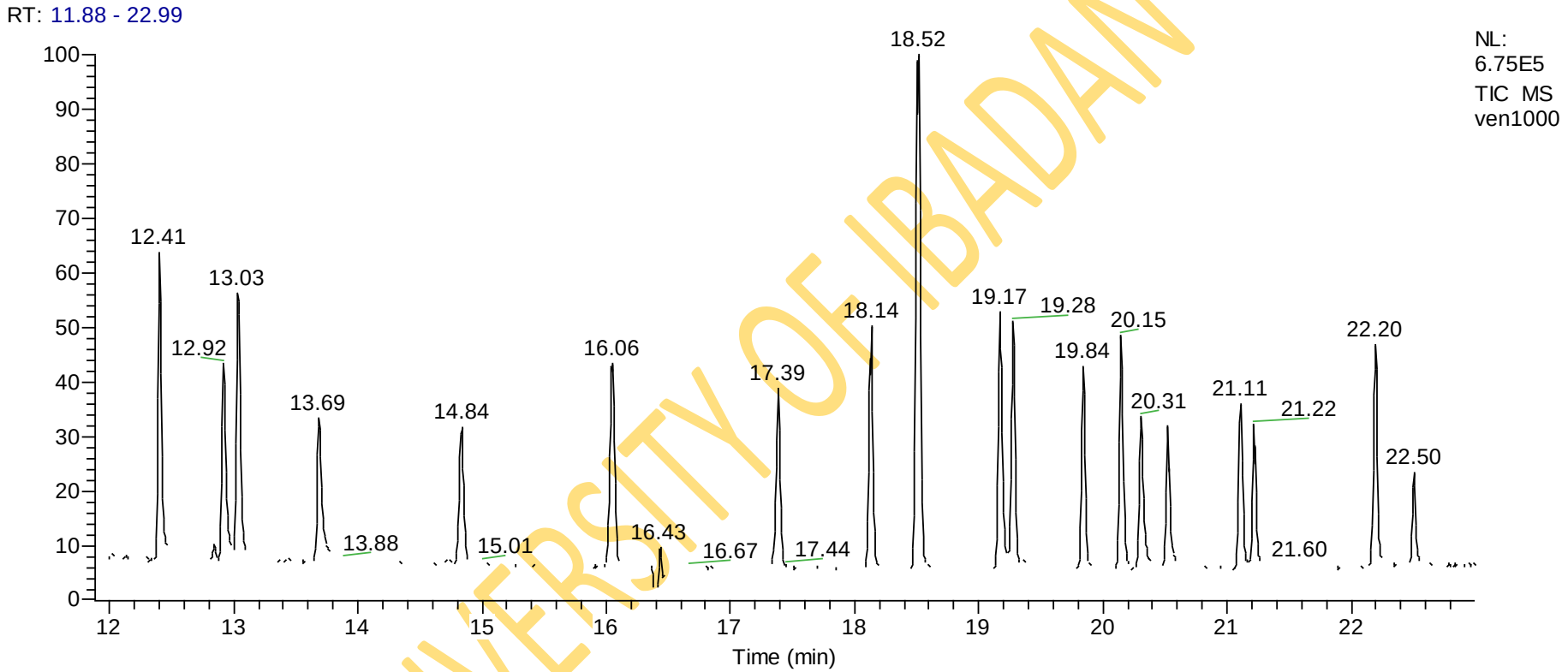
Quantitative Result Table

ID#	R.Time	m/z	Area	Height	Conc.	Conc.I	Recovery	Name
1	8.042	TIC	1047	636	0.009 ppm		0.00	alpha-BHC
2	9.718	TIC	10684	7356	0.043 ppm		0.00	Lindane
3	10.193	TIC	76046	49714	0.350 ppm		0.00	beta-BHC
4	10.630	TIC	83634	50786	0.265 ppm		0.00	delta-BHC
5	-	TIC	---	---	N.D.(Ref) ppm		-	Heptachlor
6	11.806	TIC	41163	30894	0.139 ppm		0.00	Aldrin
7	-	TIC	---	---	N.D.(Ref) ppm		-	Heptachlor-epoxide(B)
8	-	TIC	---	---	N.D.(Ref) ppm		-	cis-Chlordane
9	-	TIC	---	---	N.D.(Ref) ppm		-	trans-Chlordane
10	13.492	TIC	982	1486	0.008 ppm		0.00	Endosulfan I
11	-	TIC	---	---	N.D.(Ref) ppm		-	p,p'-DDE
12	14.096	TIC	91785	28900	0.299 ppm		0.00	Dieldrin
13	14.576	TIC	17909	11305	0.165 ppm		0.00	Endrin
14	-	TIC	---	---	N.D.(Ref) ppm		-	o,p'-DDD
15	15.058	TIC	29902	12570	0.198 ppm		0.00	Endosulfan II
16	-	TIC	---	---	N.D.(Ref) ppm		-	Endrin aldehyde
17	-	TIC	---	---	N.D.(Ref) ppm		-	p,p'-DDT
18	15.599	TIC	9120	5484	0.232 ppm		0.00	Endosulfan sulfate
19	-	TIC	---	---	N.D.(Ref) ppm		-	Methoxychlor
20	-	TIC	---	---	N.D.(Ref) ppm		-	Endrin ketone

Figure A-8. Chromatogram of organochlorine pesticide in CRIN sediment

APPENDIX-B

FIELD KINETIC STUDIES



12.41 –α-HCH; 12.92 – γ-HCH; 13.03 – β-HCH; 13.69-δ-HCH; 14.84-Heptachlor; 16.06-Aldrin; 17.37-heptachlor epoxide; 18.14-trans-chlordane; 18.52-α-endosulfan; 19.17-ppDDE; 19.28-Dieldrin; 19.84-Endrin; 20.15-β-endosulfan; 20.31-opDDD; 20.53-Endrin aldehyde; 21.11-Endosulfan sulphate; 21.22-ppDDT; 22.20-Endrin ketone; 22.50-Methoxychlor

Figure B-1. Chromatogram of OCPs mixed standard

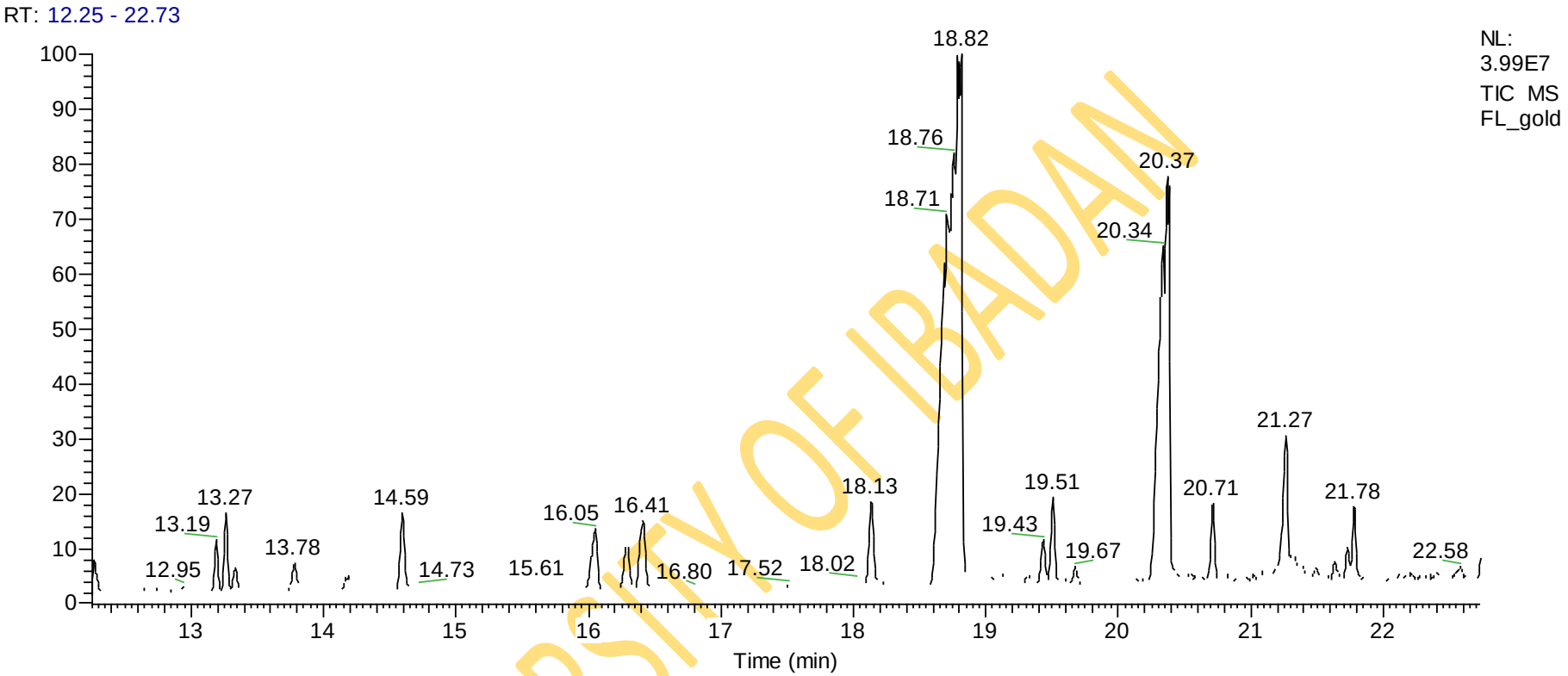


Figure B-2. Chromatogram of α-endosulfan (18.82mins) and β-endosulfan (20.37 mins) in fresh cocoa leaves at Day 0

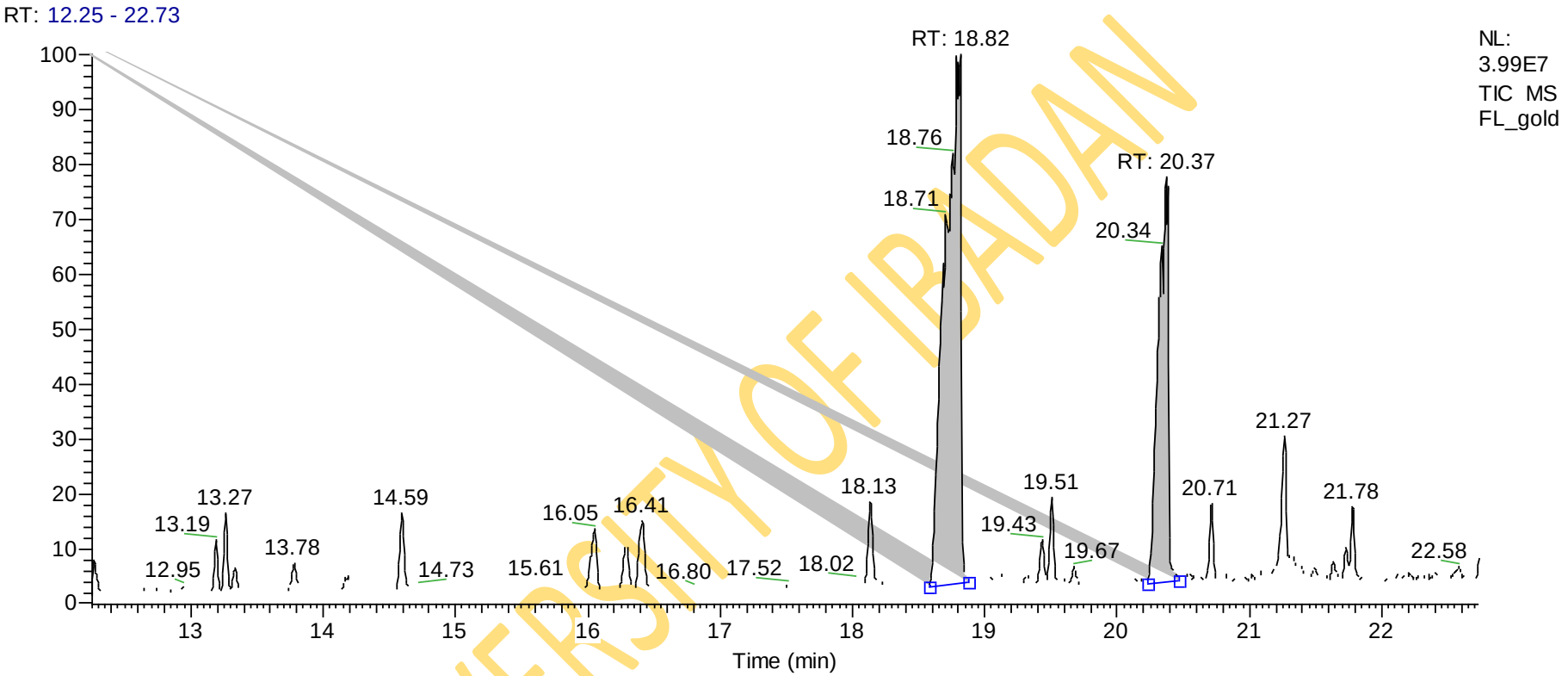


Figure B-2b. Chromatogram of α-endosulfan (18.82mins) and β-endosulfan (20.37 mins) in fresh cocoa leaves at Day 0

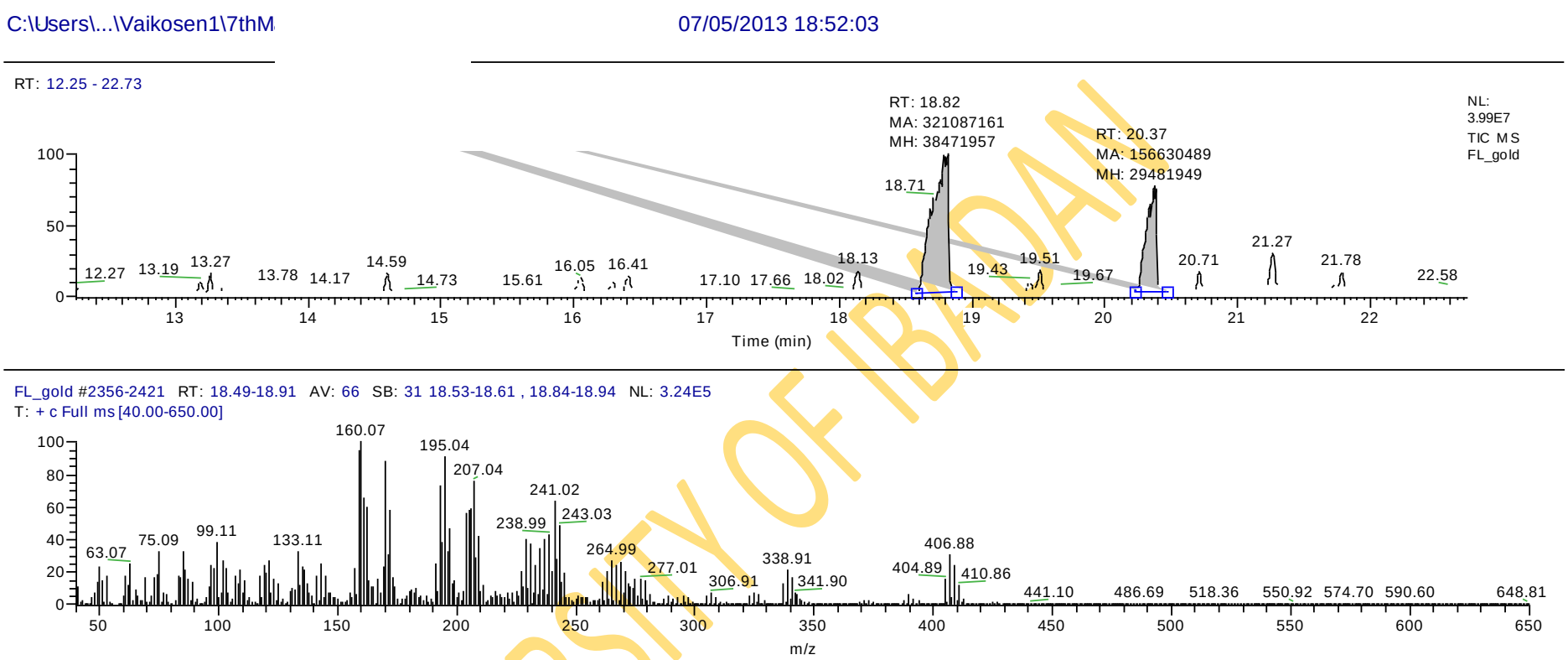


Figure B-2c: Chromatogram of α-endosulfan (18.82mins) and β-endosulfan (20.37 mins) in fresh cocoa leaves (above), with m/z spectrum below

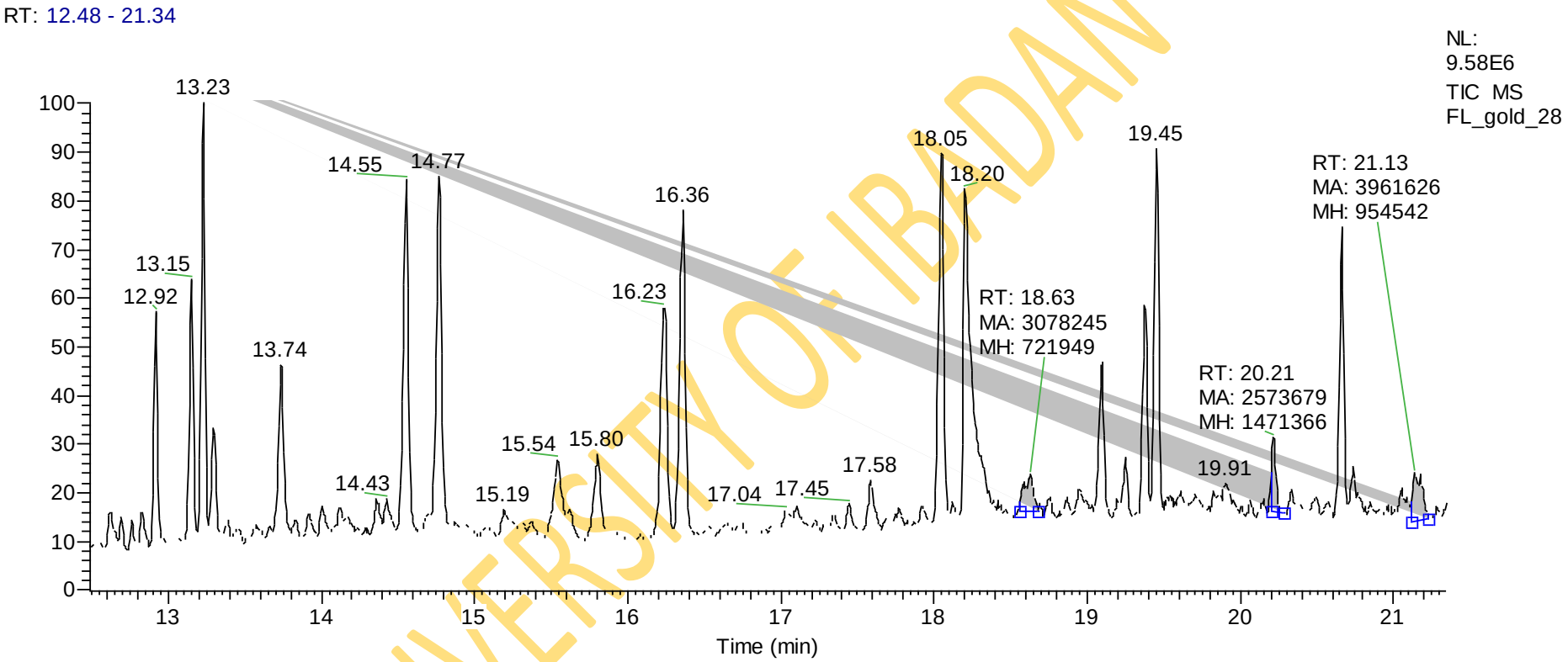


Figure B-3. Chromatogram of α-endosulfan (18.63mins), β-endosulfan (20.21 mins) and endosulfan sulphate (21.13) in fresh cocoa leaves at Day 28

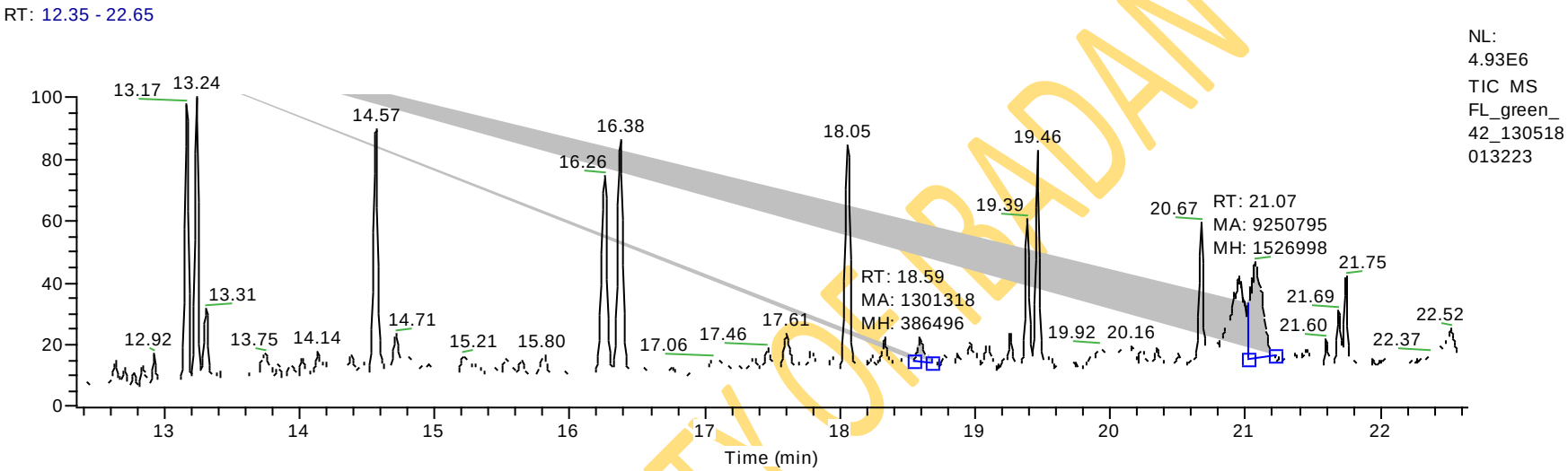


Figure B-4. Chromatogram of α-endosulfan (18.59mins) and endosulfan sulphate (21.07) in fresh cocoa leaves at Day 42

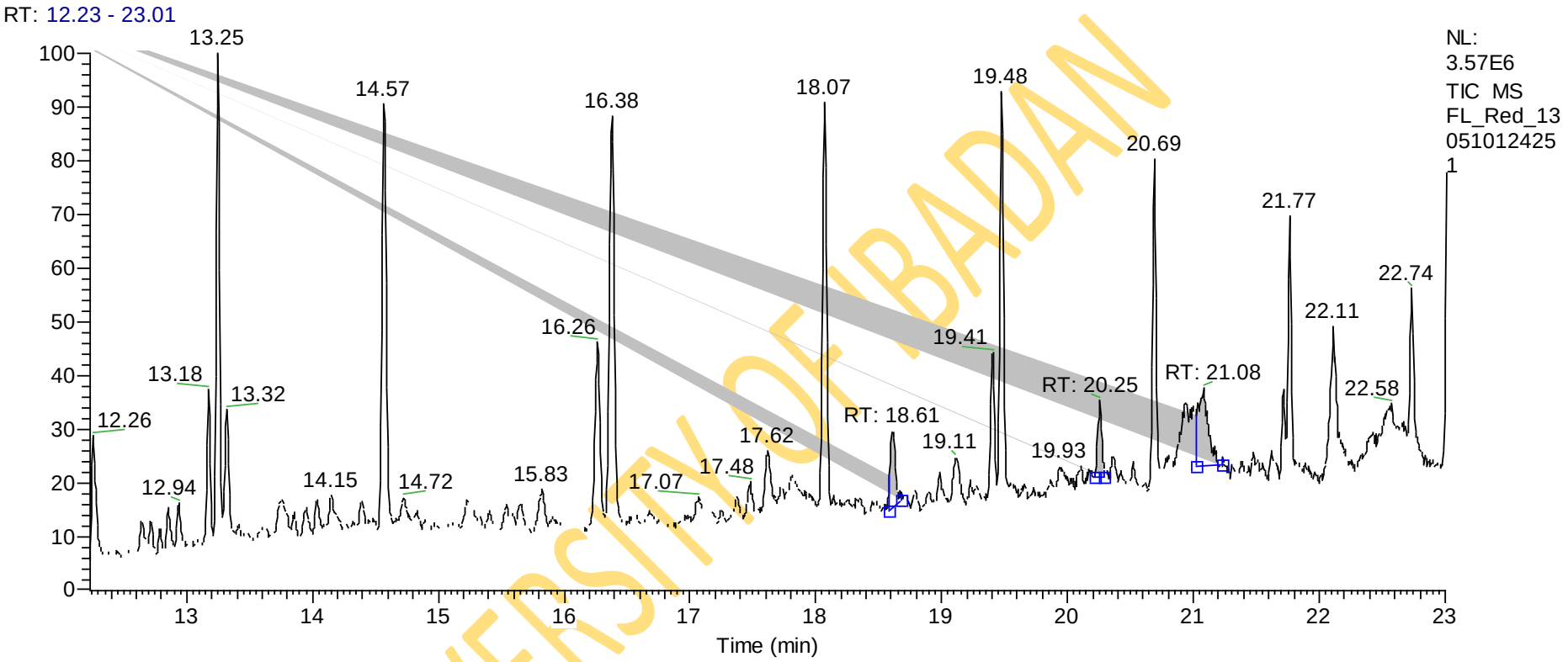


Figure B-4. Chromatogram of α-endosulfan (18.61mins), β-endosulfan (20.25 mins) and endosulfan sulphate (21.08 mins) in fresh cocoa leaves at Day 60

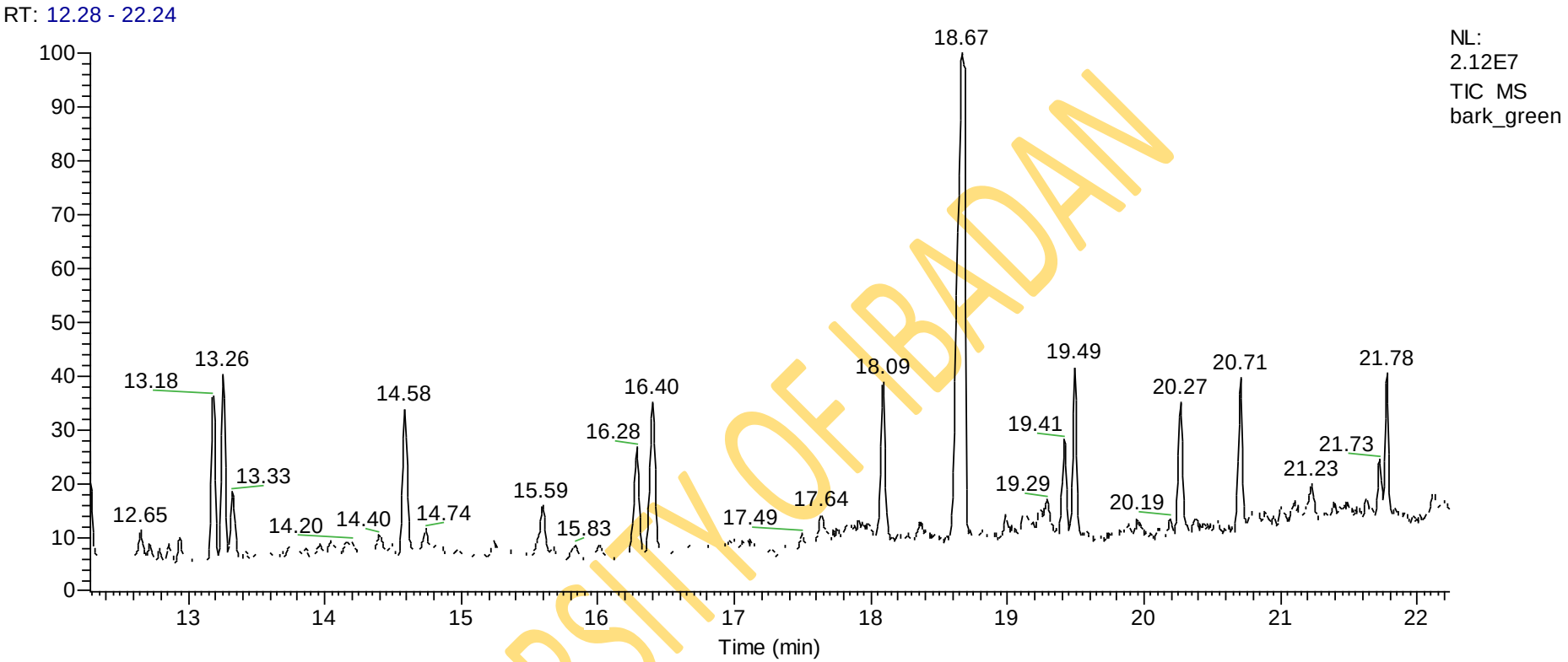


Figure B-5. Chromatogram of α-endosulfan (18.67mins) and β-endosulfan (20.27 mins) in stem bark at Day 0

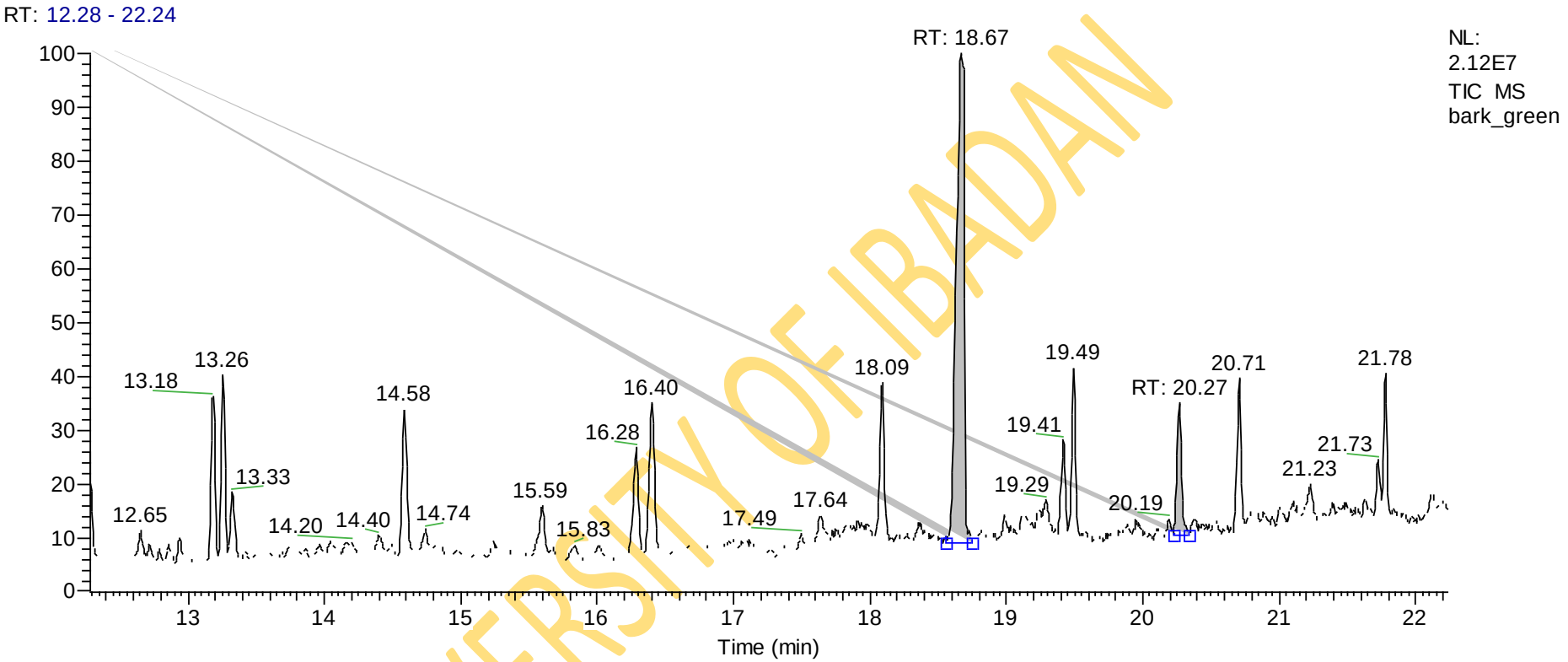


Figure B-5. Chromatogram of α-endosulfan (18.67mins) and β-endosulfan (20.25 mins) in stem bark at Day 0

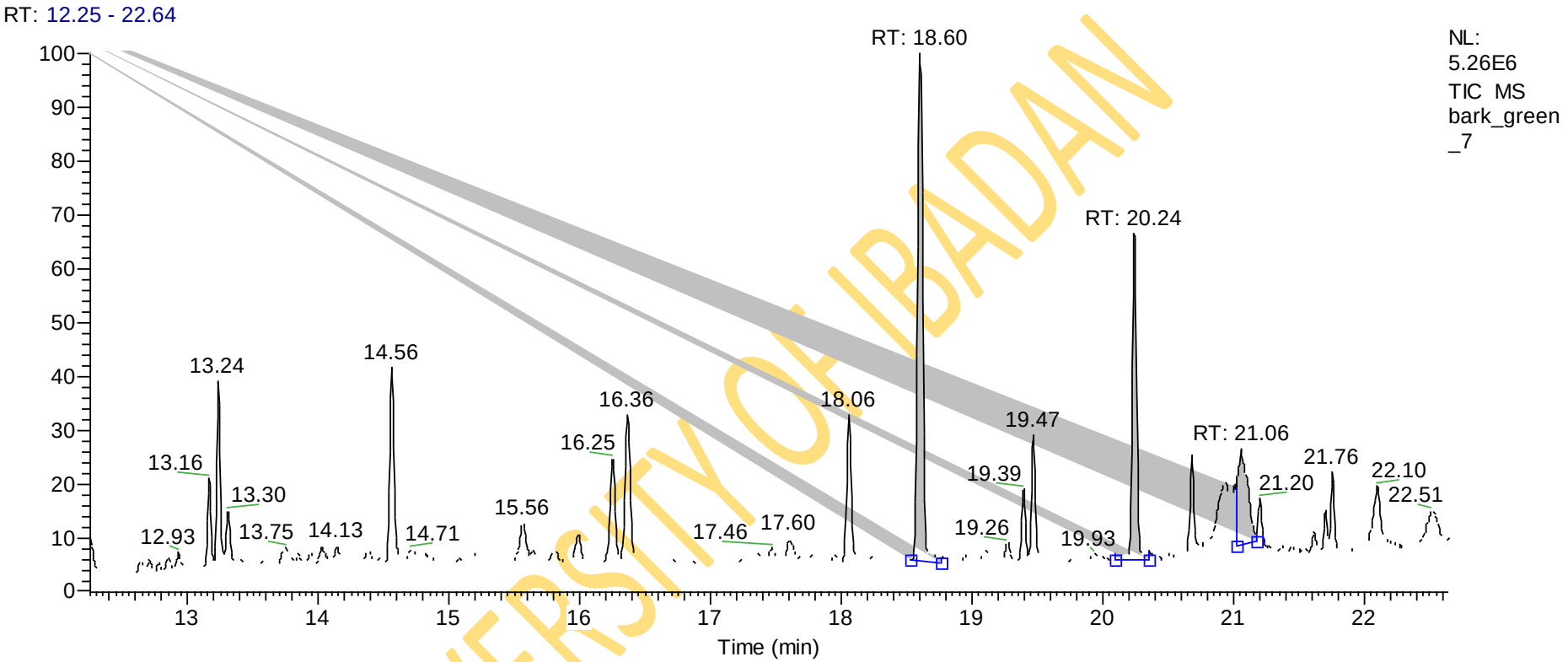


Figure B-7: Chromatogram of α-endosulfan (18.67mins) and β-endosulfan (20.25 mins) and endosulfan sulphate (21.06mins) in stem bark at Day 7

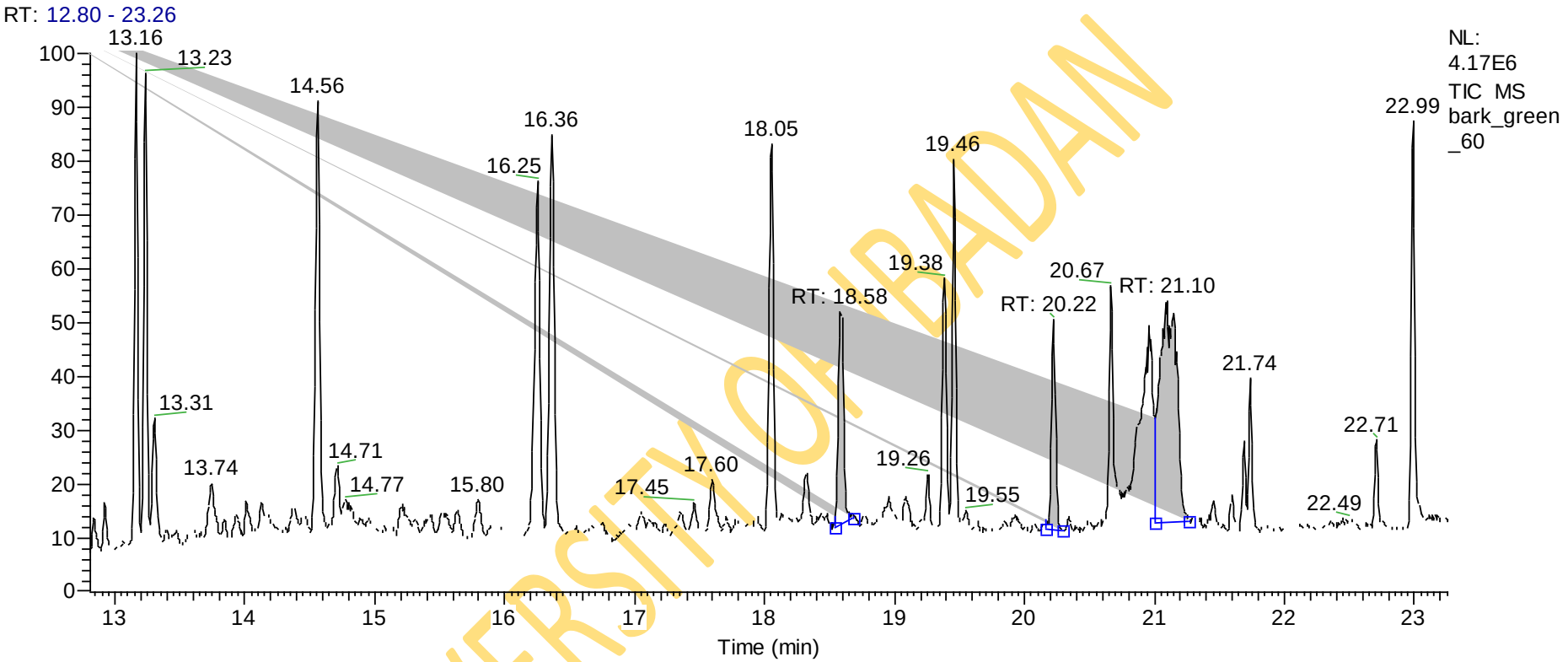


Figure B-8: Chromatogram of α-endosulfan (18.58mins), β-endosulfan (20.22 mins) and endosulfan sulphate(21.10 mins) in stem bark at Day 60

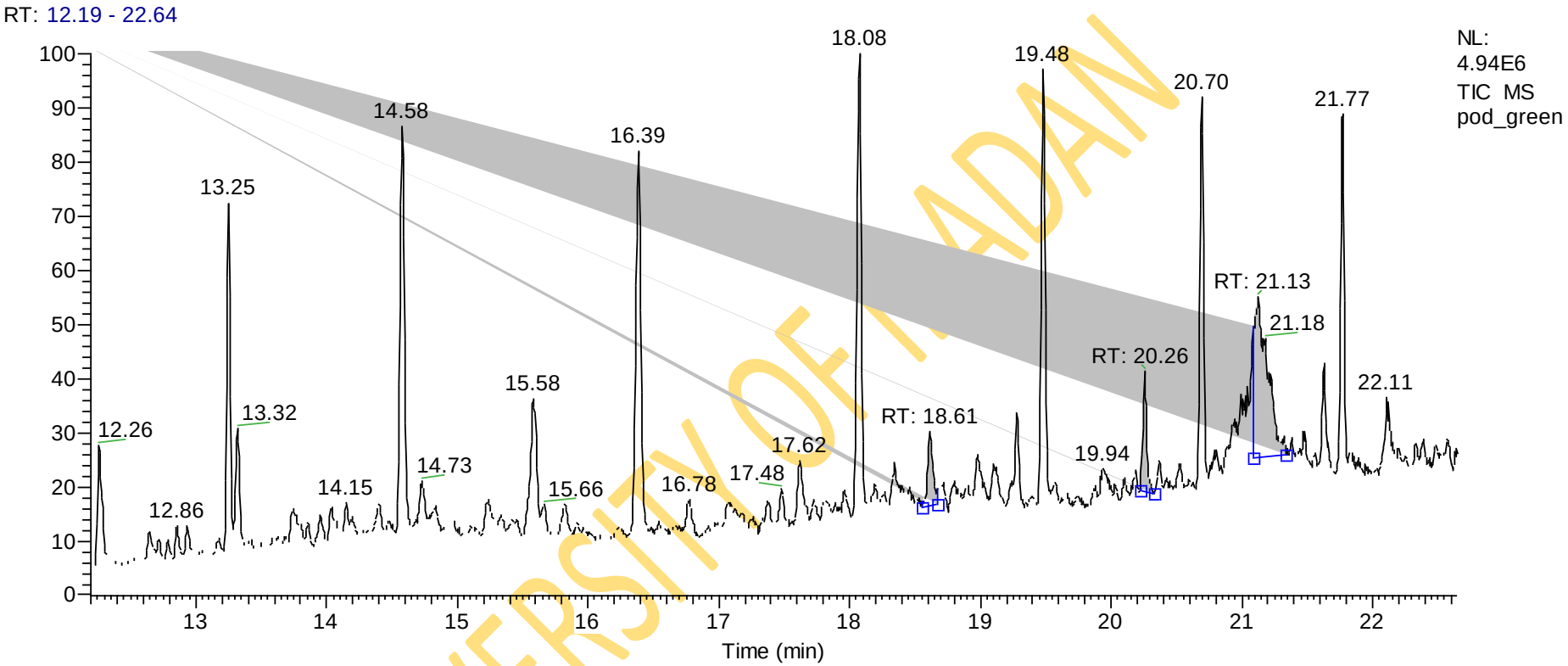


Figure B-9: Chromatogram of α-endosulfan (18.61mins), β-endosulfan (20.26 mins) and endosulfan sulphate(21.10 mins) in pods at Day 0

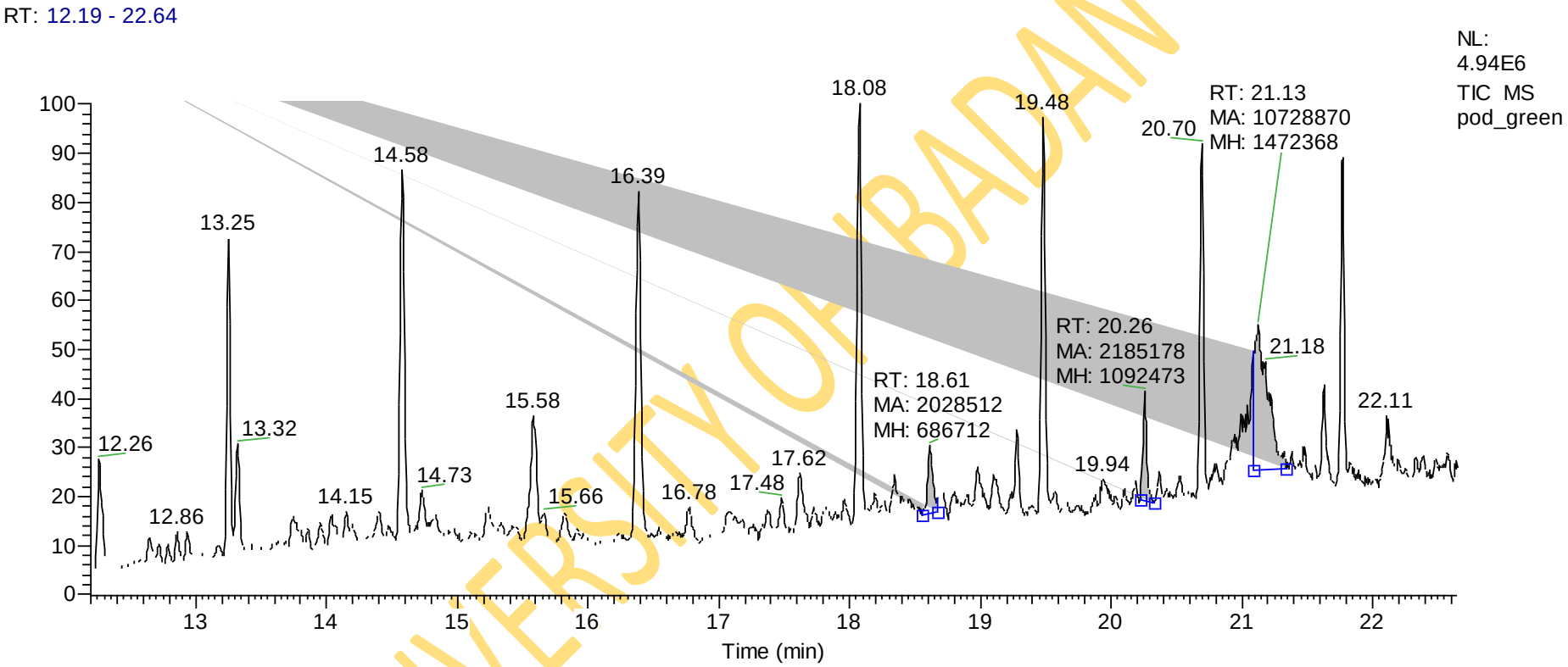


Figure B-10: Chromatogram of α-endosulfan (18.61mins), β-endosulfan (20.26 mins) and endosulfan sulphate(21.18 mins) in pods at Day 7

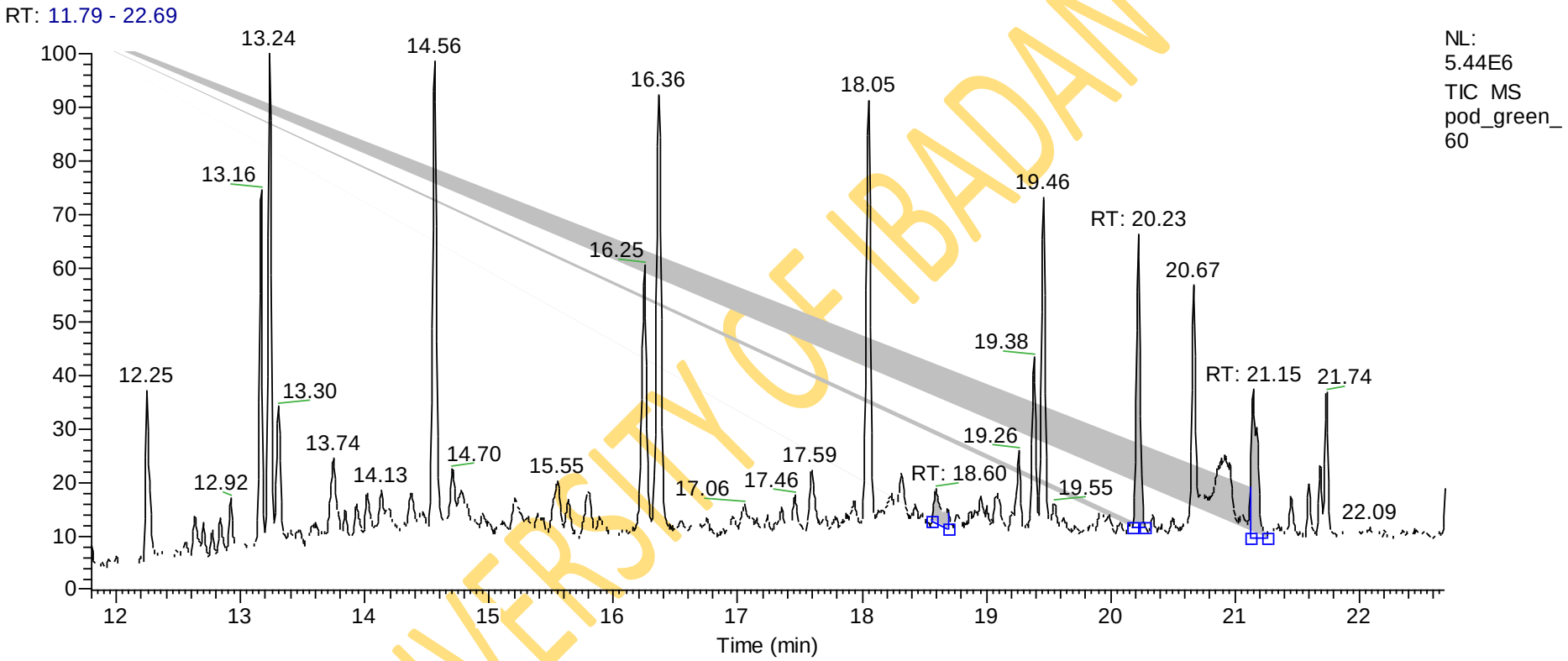


Figure B-11: Chromatogram of α-endosulfan (18.60mins), β-endosulfan (20.23 mins) and endosulfan sulphate(21.15 mins) in pods at Day 60

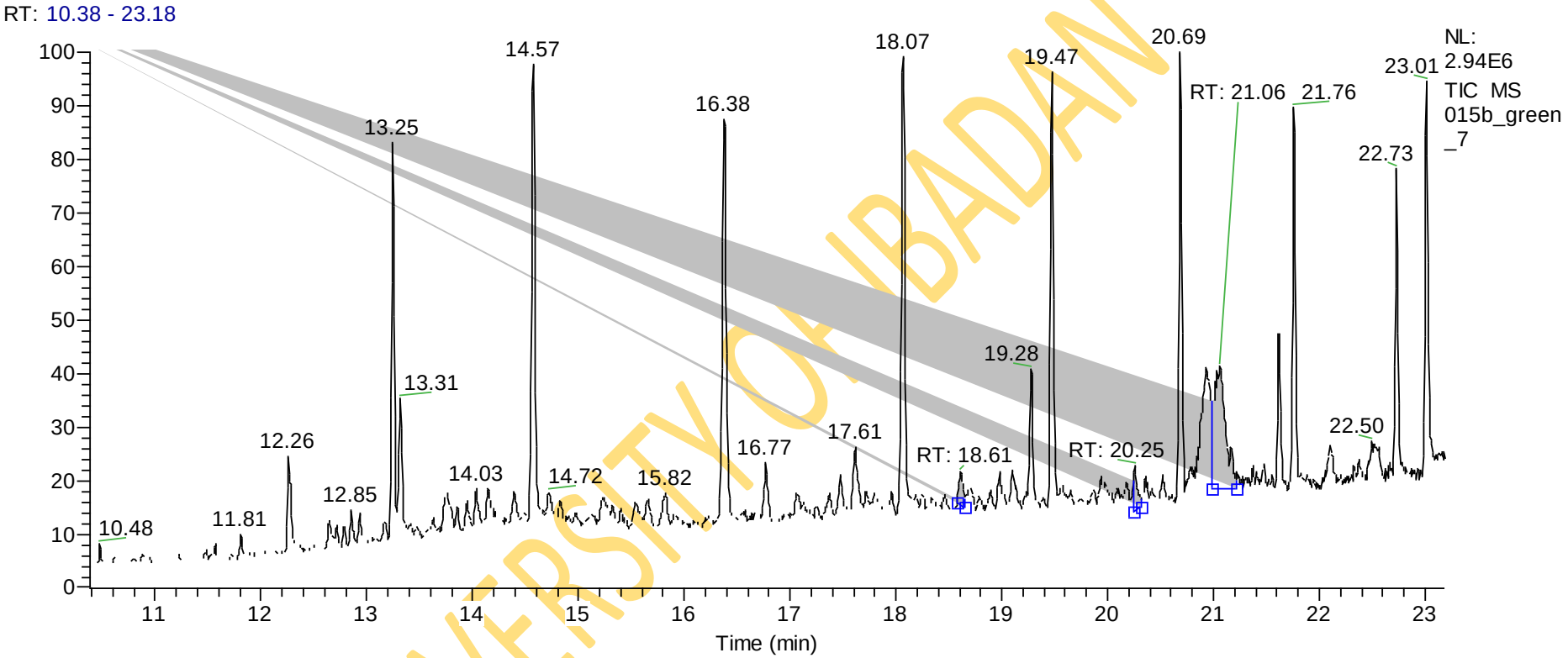


Figure B-12: Chromatogram of α-endosulfan (18.61mins), β-endosulfan (20.25 mins) and endosulfan sulphate(21.06 mins) in soil (0-15cm) at Day 7

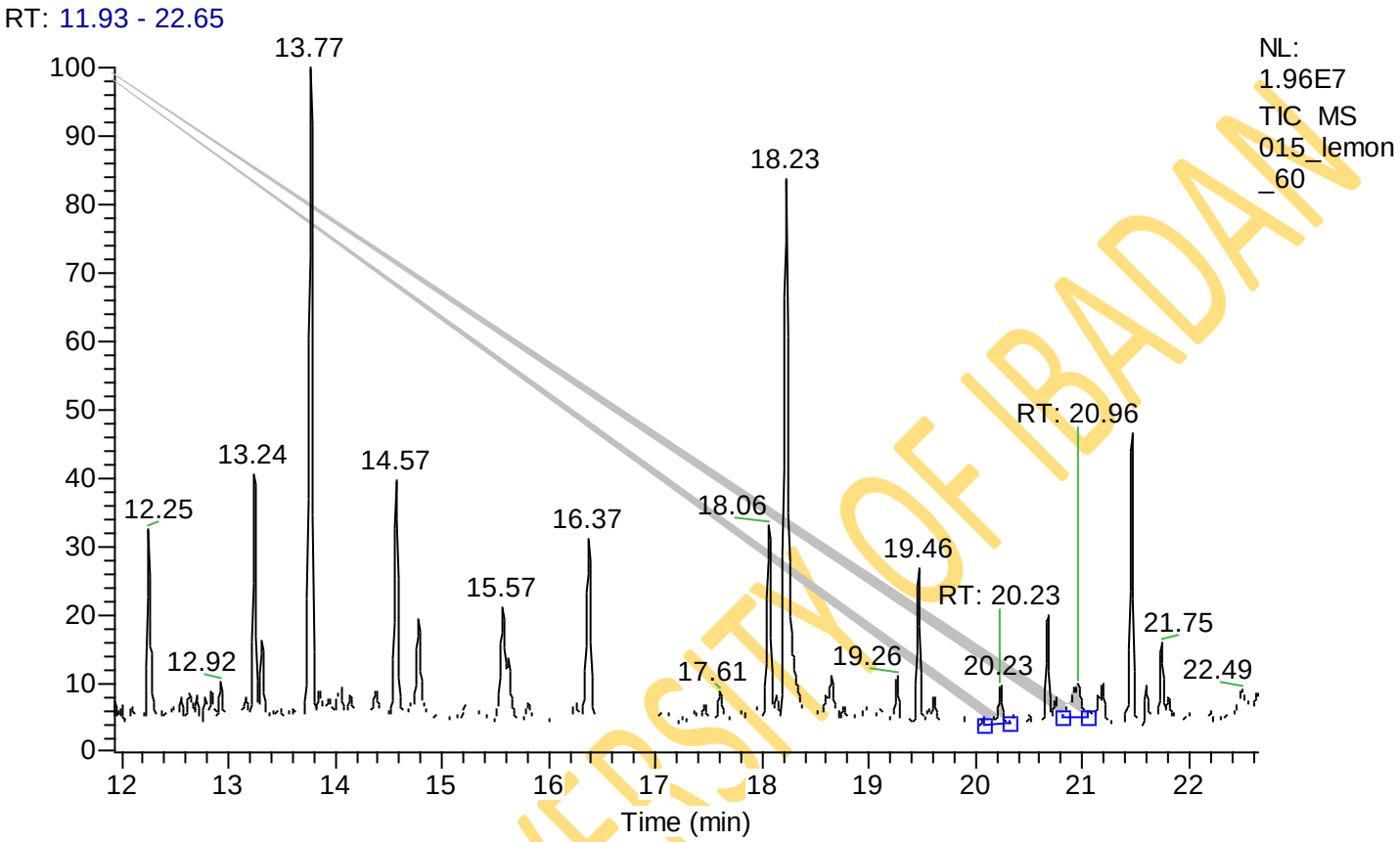


Figure B-13: Chromatogram of α-endosulfan (18.61mins), β-endosulfan (20.23 mins) and endosulfan sulphate(20.96 mins) in soil (0-15cm) at Day 60

Table B-1: Residual Concentrations of endosulfan isomers and metabolite in fresh leaves over a period of 60 days

Residual Concentration of endosulfan in fresh leaves (μgg^{-1})								
Time	α-endosulfan		β-endosulfan		Endosulfan SO_4		∑endosulfan	
(days)	mean	sd	mean	sd	mean	sd	mean	sd
0	66.512	17.482	30.497	8.242	0	0	97.009	25.724
7	19.567	5.879	6.025	2.911	1.919	0.646	27.511	9.436
14	2.976	2.275	3.2	2.45	2.219	1.965	8.395	6.69
21	1.125	1.467	0.424	0.461	1.937	1.546	3.486	3.474
28	0.59	0.67	0.331	0.396	0.982	0.861	1.903	1.927
42	0.238	0.414	0.263	0.443	0.591	0.494	1.092	1.351
60	0.105	0.113	0.117	0.095	0.291	0.29	0.513	0.498

Table B-2. Residual Concentrations of endosulfan isomers and metabolite in stem bark over a period of 60 days

Residual Concentration of endosulfan in stem bark (μgg^{-1})(ww)								
Time	**α-endosulfan**		**β-endosulfan**		**Endosulfan SO_4**		**∑endosulfan**	
(days)	**mean**	**sd**	**mean**	**sd**	**mean**	**sd**	**mean**	**sd**
0	40.643	6.607	17.367	3.391	<0.001	<0.001	58.01	9.998
7	20.098	5.537	13.198	4.198	12.964	3.699	46.26	13.434
14	1.482	1.387	1.75	1.241	8.214	4.333	11.446	6.961
21	0.756	1.073	0.643	0.205	5.908	3.086	7.307	4.364
28	2.151	2.118	1.117	0.834	4.255	2.401	7.523	5.353
42	0.296	0.439	0.199	0.202	3.368	2.632	3.863	3.273
60	0.173	0.185	0.073	0.093	2.265	1.331	2.511	1.609

Table B-3: Residual Concentrations of endosulfan isomers and metabolite in pods over a period of 60 days

Residual Concentration of endosulfan in pods (μgg^{-1})(ww)								
Time	**α-endosulfan**		**β-endosulfan**		**Endosulfan SO_4**		**∑endosulfan**	
(days)	mean	sd	mean	sd	mean	sd	mean	sd
0	0.928	0.289	1.03	0.31	<0.001	<0.001	1.958	0.599
7	0.777	0.257	0.866	0.409	0.073	0.047	1.716	0.713
14	0.646	0.494	0.64	0.303	0.157	0.073	1.443	0.870
21	0.792	0.377	0.818	0.338	0.173	0.112	1.783	0.827
28	0.529	0.312	0.568	0.379	0.293	0.084	1.39	0.775
42	0.326	0.245	0.482	0.153	0.45	0.146	1.258	0.544
60	0.159	0.085	0.198	0.202	0.404	0.160	0.761	0.447

Table B-4: Residual Concentrations of endosulfan isomers and metabolite in cocoa seeds over a period of 60 days

Residual Concentration of endosulfan in cocoa bean seeds (μgg^{-1})(ww)								
Time	α-endosulfan		β-endosulfan		Endosulfan SO_4		∑endosulfan	
(days)	mean	sd	mean	sd	mean	sd	mean	sd
0	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
7	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
14	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
21	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
28	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
42	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
60	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table B-5: Residual Concentrations of endosulfan isomers and metabolite in dry leaves over a period of 60 days

Residual Concentration of endosulfan in dry leaves (μgg^{-1})								
Time	α-endosulfan		β-endosulfan		Endosulfan SO_4		∑endosulfan	
(days)	mean	sd	mean	sd	mean	sd	mean	sd
0	108.768	25.722	50.484	12.171	<0.001	<0.001	159.252	37.893
7	38.598	12.687	26.901	8.932	5.367	5.569	70.866	27.188
14	21.146	8.618	22.641	10.658	6.011	2.876	49.798	22.152
21	3.639	3.537	3.905	1.795	4.92	2.74	12.464	8.072
28	1.282	1.443	1.153	0.954	2.521	1.682	4.956	4.079
42	0.649	0.303	0.59	0.413	1.782	1.205	3.021	1.921
60	0.434	0.415	0.252	0.166	1.028	0.610	1.714	1.191

Table B-6: Residual Concentrations of endosulfan isomers and metabolite in soil (0-15 cm) over a period of 60 days

Residual Concentration of endosulfan in soil (0-15cm) (μgg^{-1})(ww)								
Time	α-endosulfan		β-endosulfan		Endosulfan SO_4		∑endosulfan	
(days)	mean	sd	mean	sd	mean	sd	mean	sd
0	1.241	0.653	0.636	0.396	0	0	1.877	1.049
7	0.788	0.267	0.578	0.155	0.311	0.124	1.677	0.546
14	0.354	0.335	0.404	0.336	0.729	0.259	1.487	0.93
21	0.502	0.295	0.637	0.238	1.237	0.729	2.376	1.262
28	0.284	0.133	0.428	0.427	1.207	0.643	1.919	1.203
42	0.201	0.199	0.284	0.169	1.018	0.434	1.503	0.802
60	0.109	0.131	0.159	0.163	0.847	0.443	1.115	0.737

Table B-7: Residual Concentrations of endosulfan isomers and metabolite in soil (15 -30cm) over a period of 60 days

Residual Concentration of endosulfan in soil (15 -30cm) (μgg^{-1})(ww)								
Time	α-endosulfan		β-endosulfan		Endosulfan SO_4		∑endosulfan	
(days)	mean	sd	mean	sd	mean	sd	mean	sd
0	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
7	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
14	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
21	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
28	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
42	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
60	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table B-8a: Percentage Recovery for evaluation of methodology

α-endosulfan								
Matrix	% Recovery (125μgg^{-1})				% Recovery (500μgg^{-1})			
	1	2	3	mean	1	2	3	mean
Fresh leaves	89.8	90.7	87.9	89.5±1.2	102.7	101.6	104.6	103.0±1.2
Bark	90.8	85.5	89.5	88.6±2.3	90.5	98.2	95.4	94.7±3.2
Pods	89.9	85.7	92.8	89.5±2.9	99.4	94.9	104.8	99.7±4.0
Seeds	92.9	85.6	87.5	88.7±3.1	101.7	110.7	104.3	105.6±3.8
Dry leaves	101.8	102.7	99.8	101.4±1.2	90.9	100.7	89.8	93.8±4.9
Soil (0-15cm)	98	97.7	91.8	95.8±2.9	108.9	102.0	103.1	104.7±3.0
Soil (15-30cm)	85.9	91.7	88.7	88.8±2.4	101.0	99.1	97.9	99.3±1.3

β-endosulfan								
Matrix	% Recovery (125μgg^{-1})				% Recovery (500μgg^{-1})			
	1	2	3	mean	1	2	3	mean
Fresh leaves	87.1	90.1	86.2	87.8±1.7	101.1	100.9	104.6	102.2±1.7
Bark	98.8	96.9	99.9	98.5±1.2	100.5	98.2	95.4	98.0±2.1
Pods	89.9	95.7	92.8	92.8±2.4	99.4	94.9	104.8	99.7±4.0
Seeds	91.9	85.6	88.9	88.8±2.6	100.7	104.3	88.8	100.9±2.7
Dry leaves	101.8	102.7	99.8	101.4±1.2	88.9	90.7	92.8	90.8±1.6
Soil (0-15cm)	98.0	97.7	91.8	95.8±2.9	104.9	100	105.1	103.3±2.4
Soil (15-30cm)	87.4	94.2	86.7	89.4±3.4	91.9	87.1	88.9	89.3±2.0

	Endosulfan SO_4							
Matrix	% Recovery (125µgg^{-1})				% Recovery (500µgg^{-1})			
	1	2	3	mean	1	2	3	mean
Fresh leaves	89.1	90.9	87.4	89.1±1.4	92.1	89.3	87.2	89.5±2.0
Bark	97.4	98.9	100	98.8±1.1	100.5	98.2	95.4	98.0±2.1
Pods	89.7	94.7	92.7	92.4±2.1	99.4	94.9	99.8	98.0±2.2
Seeds	91.9	85.6	88.9	88.8±2.6	98.2	99.6	102.3	100.0±1.7
Dry leaves	101.8	98.7	101.8	100.8±1.5	91.8	99.7	97.8	96.4±3.4
Soil (0-15cm)	98.3	94.3	91.6	94.7±2.8	94.8	85.9	89.1	89.9±3.7
Soil (15-30cm)	88.2	92.4	86.9	89.2±2.3	89.9	95.2	97.9	94.3±3.3

Table B-8b: Relative standard deviation for percent recovery

Matrix	%RSD					
	125 µgg^{-1} concentration			500 µgg^{-1} concentration		
	α-isomer	β-isomer	Endosulfan SO_4	α-isomer	β-isomer	Endosulfan SO_4
Fresh leaves	1.3	2.1	1.6	1.2	1.7	2.2
Bark	2.5	1.3	1.1	3.4	2.1	2.1
Pods	3.3	2.6	2.2	4.1	4.1	2.3
Seeds	3.5	2.9	2.9	3.6	3.5	1.7
Dry leaves	1.2	1.2	1.5	5.2	1.8	5.1
Soil (0-15cm)	3.0	3.0	2.9	2.9	2.3	4.1
Soil (15-30cm)	2.7	3.8	2.6	1.3	3.2	3.5

$$\%RSD = Standard\ deviation/Mean\ value \times 100$$

APPENDIX-C

ADSORPTION-DESORPTION STUDIES

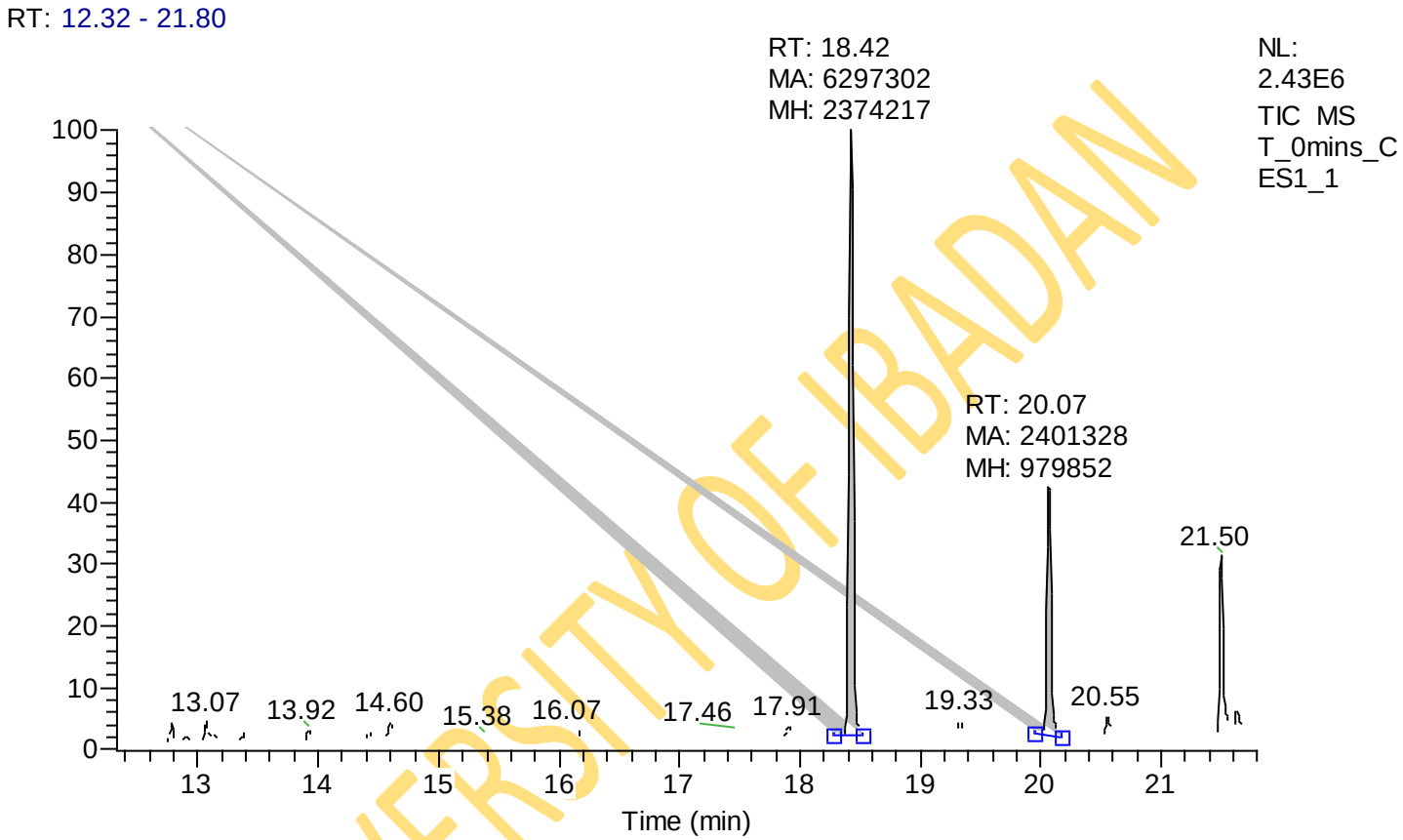


Figure C-1: Chromatogram of adsorption kinetics for α- and β-endosulfan at T_{0mins}

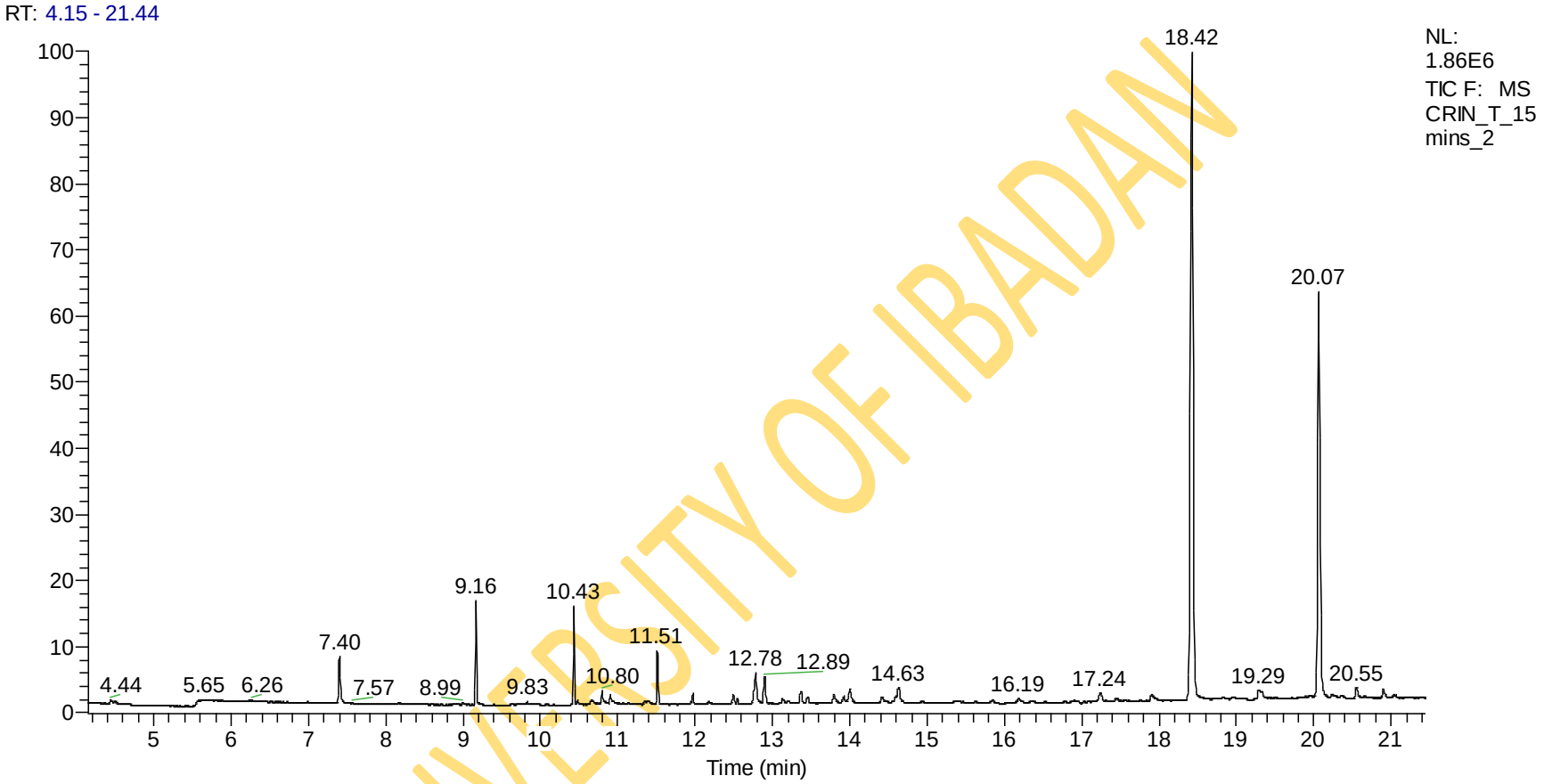


Figure C-2: Chromatogram of adsorption kinetics for α- and β-endosulfan at T_{15mins}

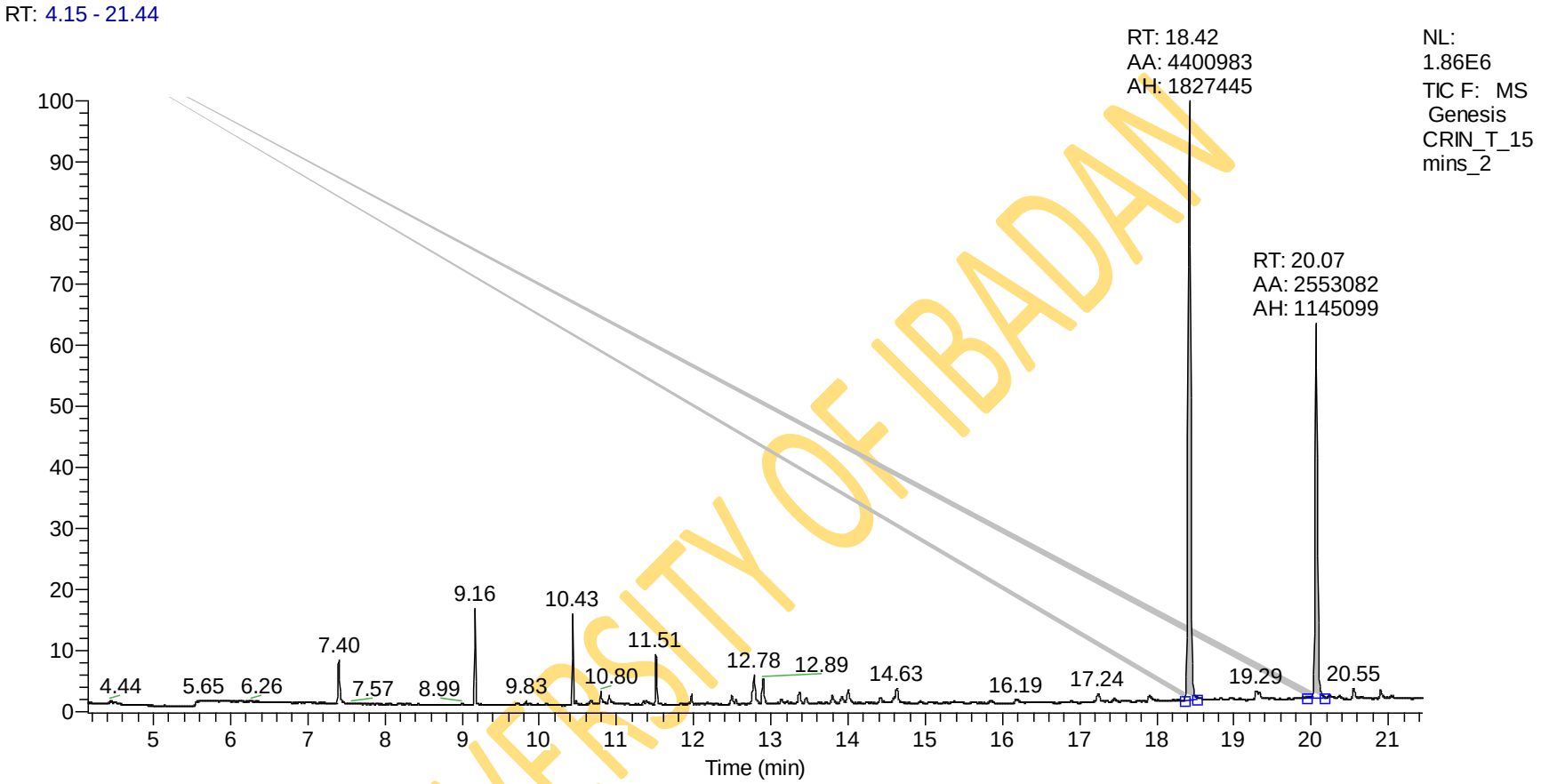


Figure C-2b:Chromatogram of adsorption kinetics for α- and β-endosulfan at T_{15}

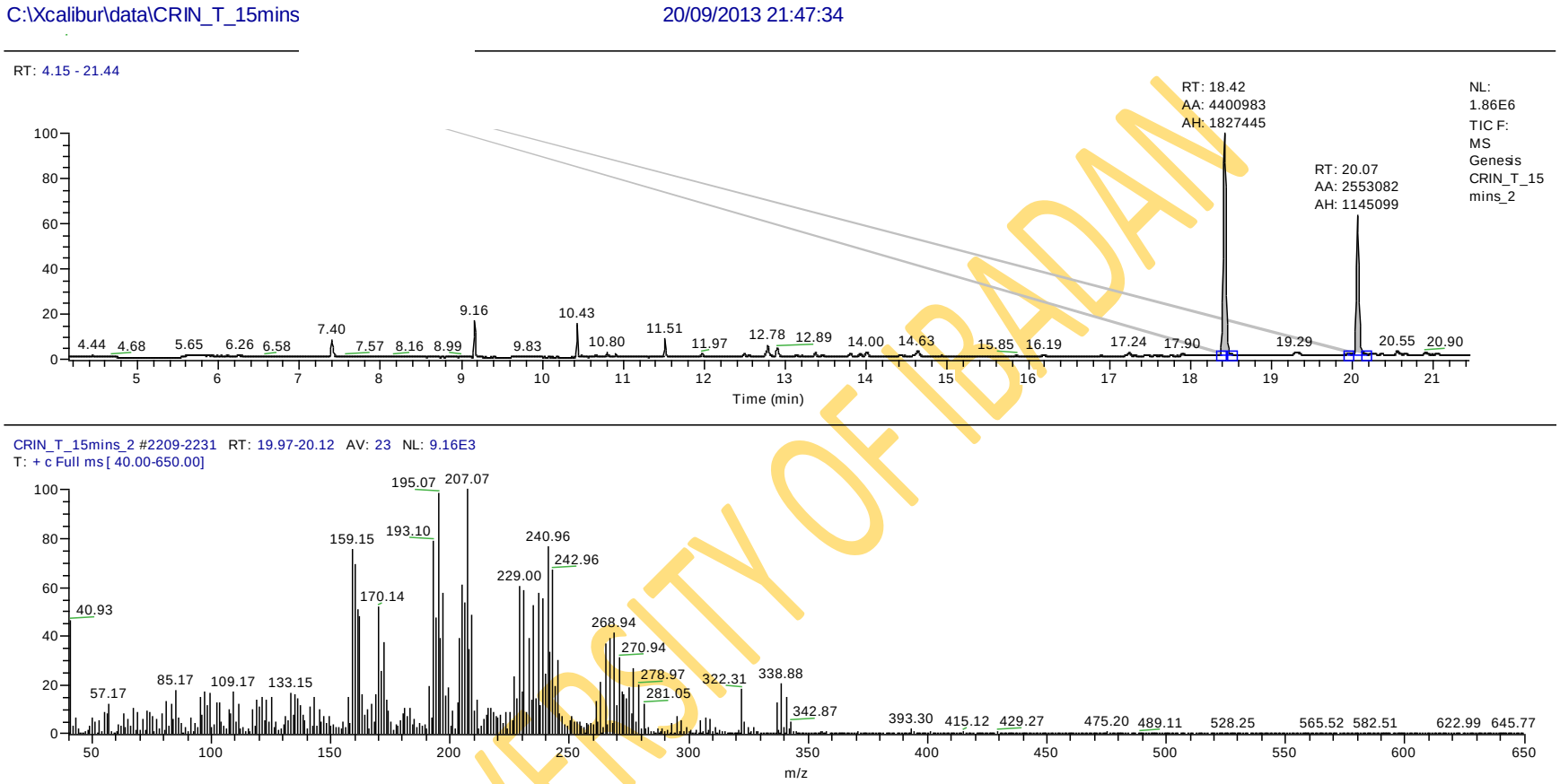


Figure C-2c:Chromatogram of adsorption kinetics for α- and β-endosulfan at T_{15} (with m⁄z spectrum below).

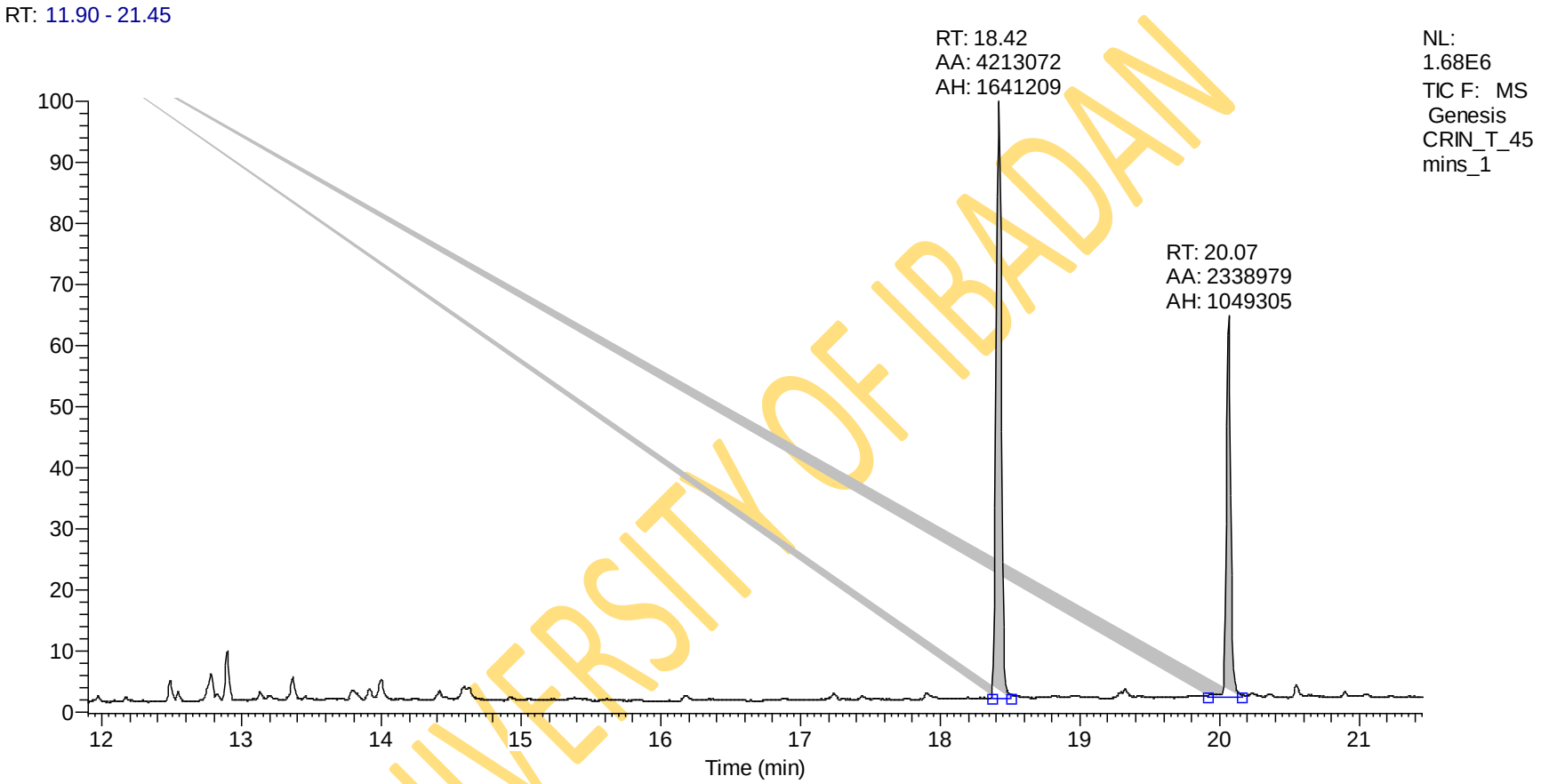


Figure C-3: Chromatogram of adsorption kinetics for α- and β-endosulfan at T_{45mins}

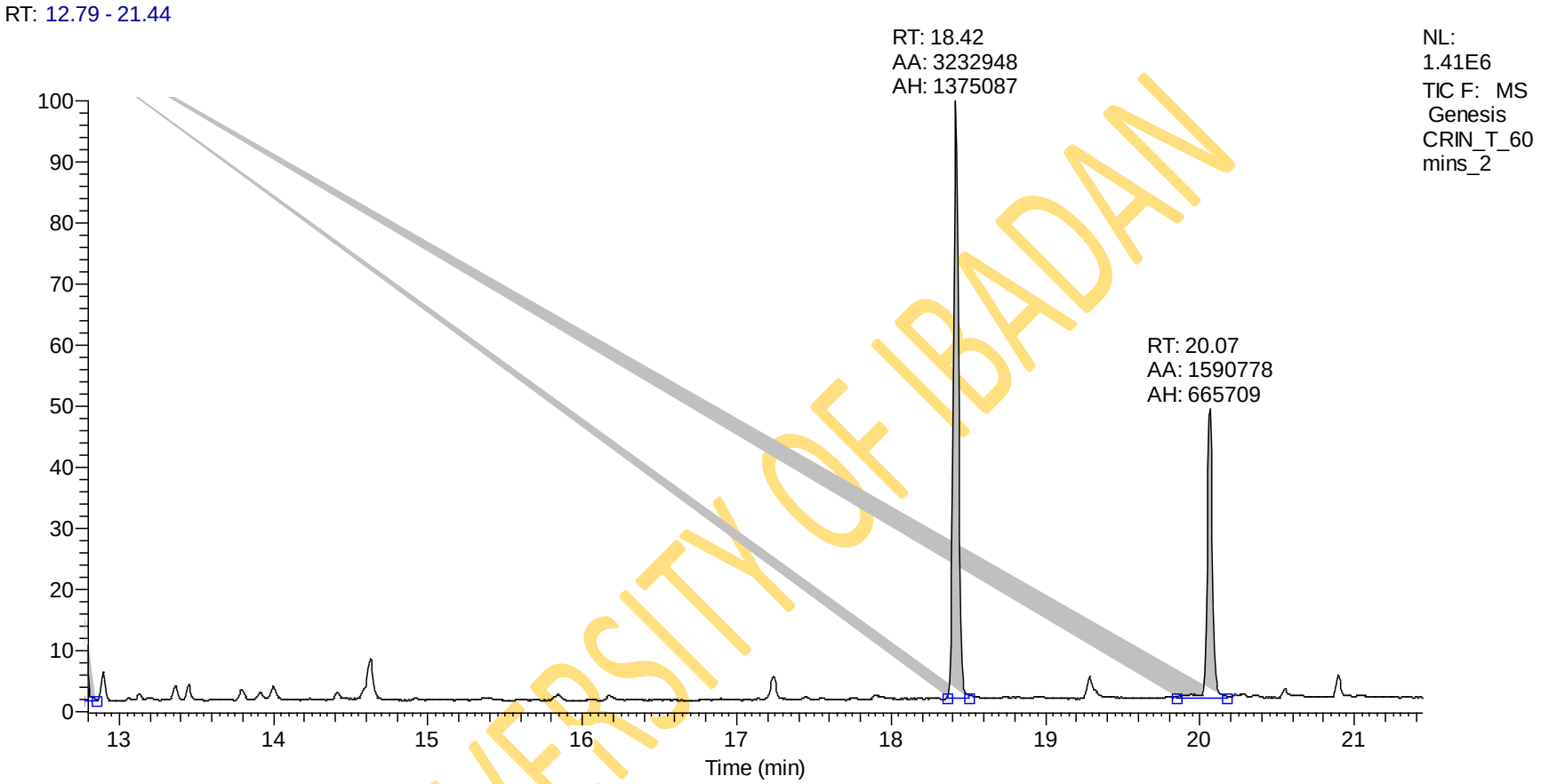


Figure C-4: Chromatogram of adsorption kinetics for α- and β-endosulfan at T_{60mins}

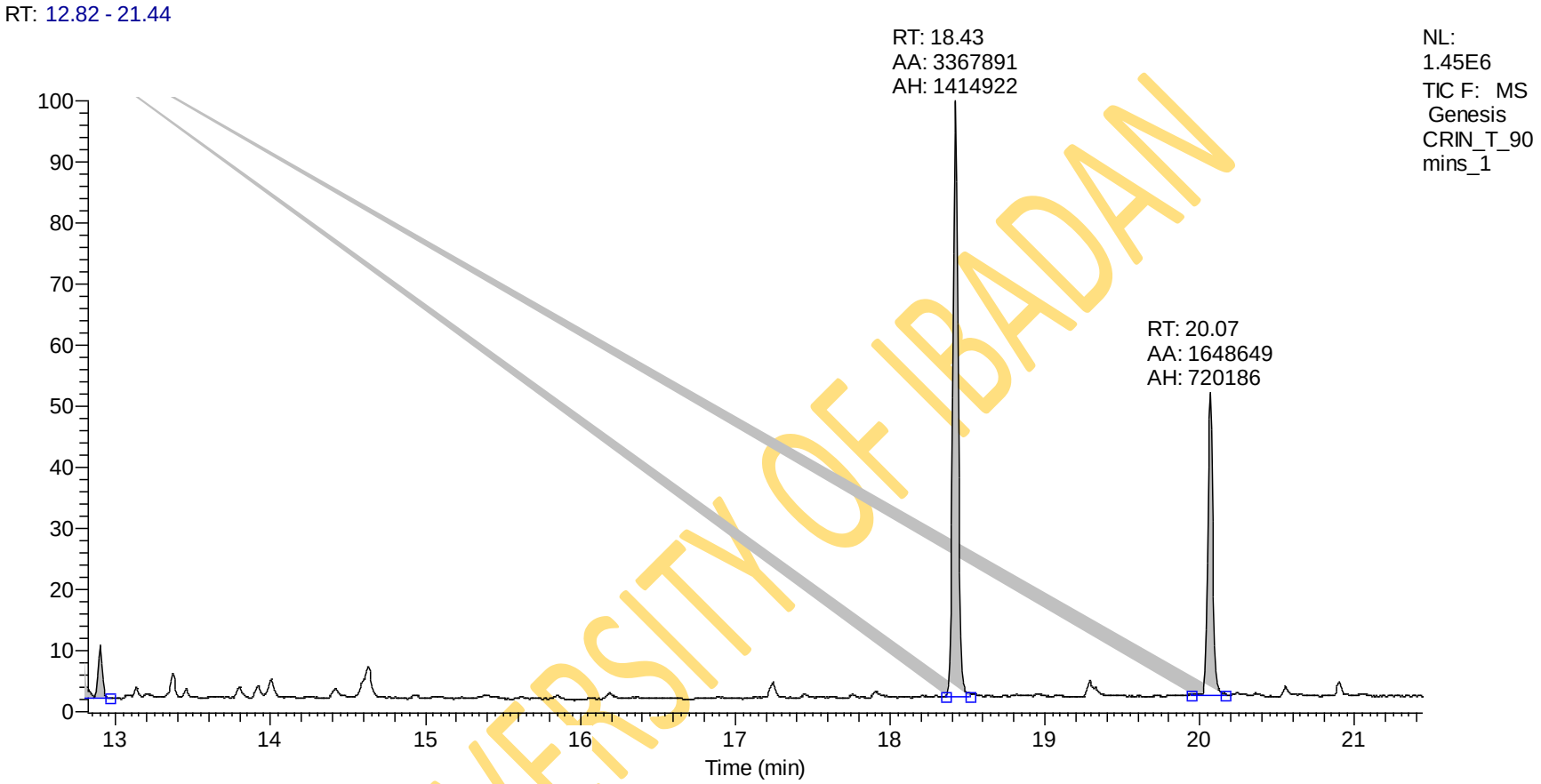


Figure C-5: Chromatogram of adsorption kinetics for α- and β-endosulfan at T_{90mins}

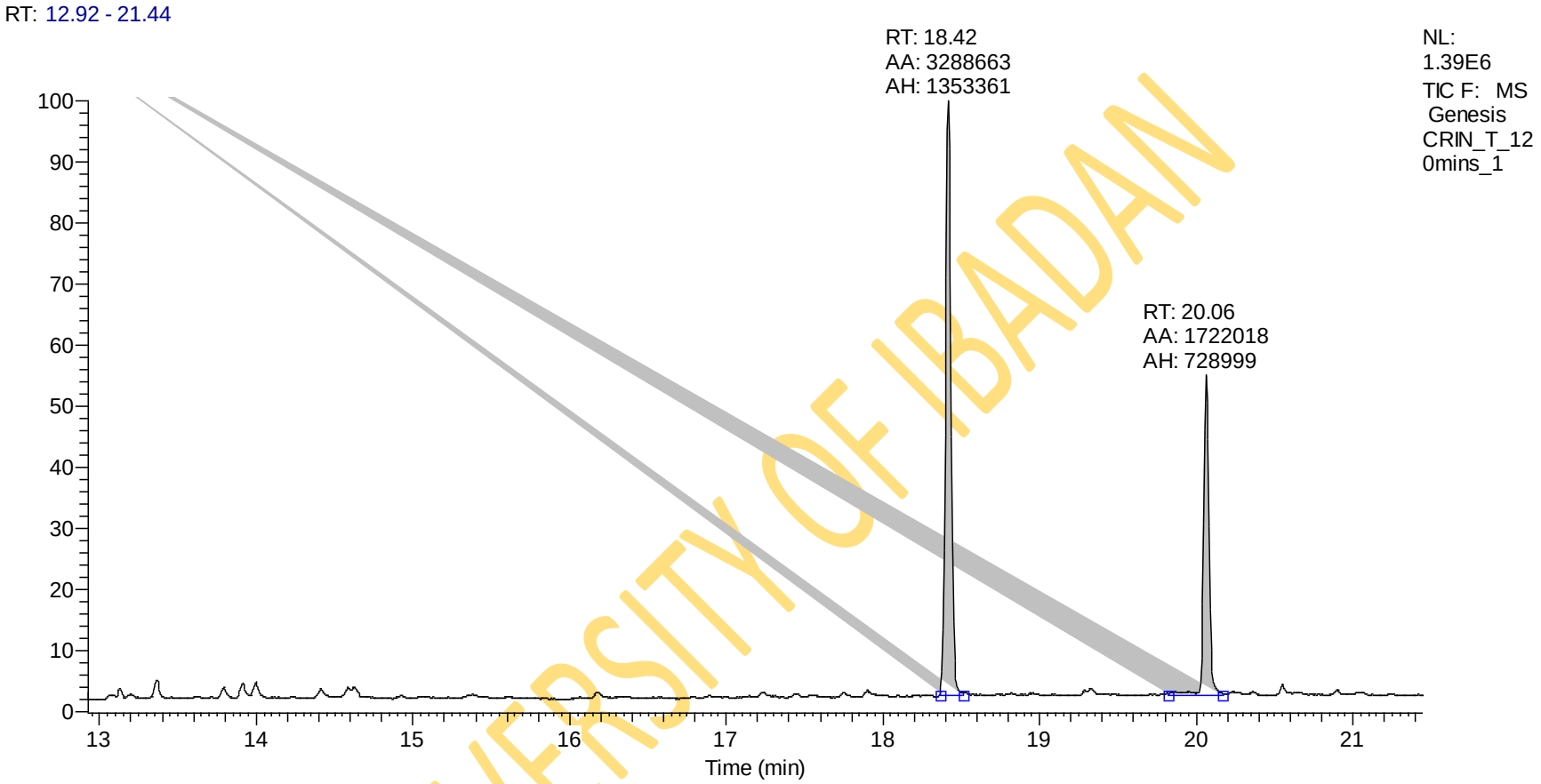


Figure C-6: Chromatogram of adsorption kinetics for α- and β-endosulfan at $T_{120mins}$

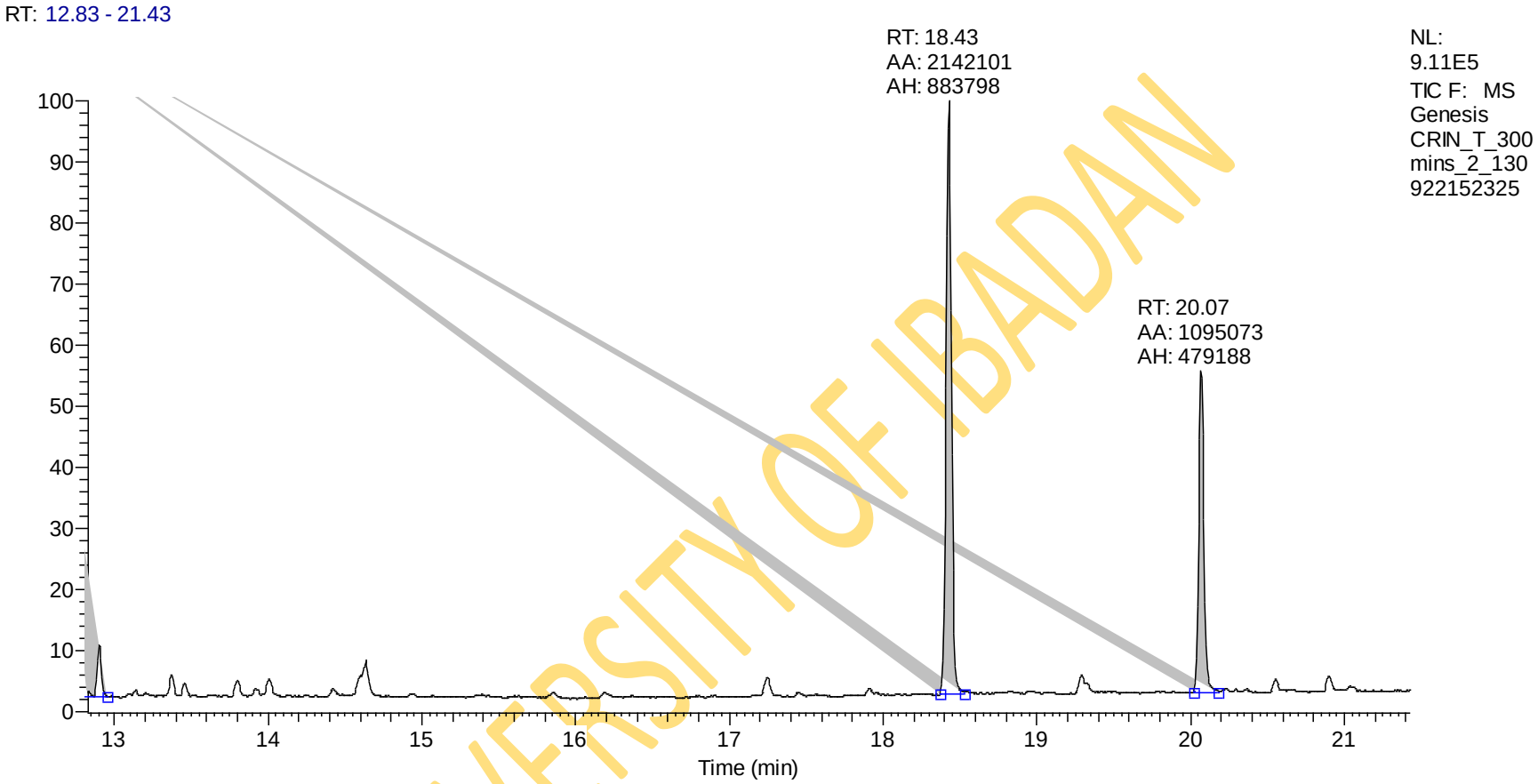


Figure C-7: Chromatogram of adsorption kinetics for α- and β-endosulfan at $T_{360mins}$

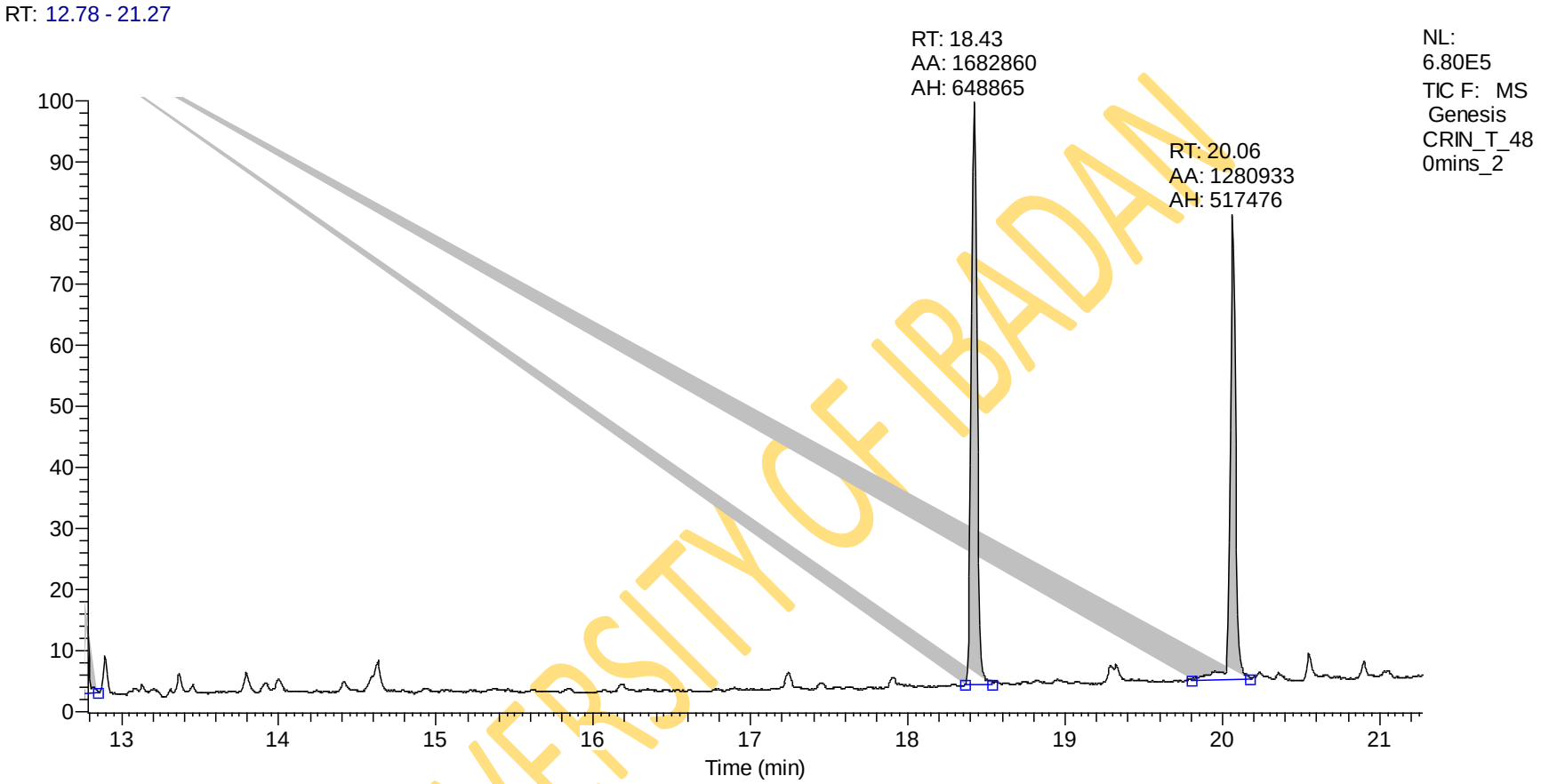


Figure C-8: Chromatogram of adsorption kinetics for α- and β-endosulfan at $T_{480mins}$

APPENDIX-C

Table C-1: Adsorption kinetics of α-and β-endosulfan for CRIN cocoa farm soil

α-endosulfan

Time (mins)	Ce(aq) µg/25cm^{-3}	Ce(aq) (µgcm^{-3})	Cqe (µgg^{-1})	K_{ads} (cm^{3}g^{-1})	Adsorption (%)	Control µg/25cm^{-3}	Blank µg/25cm^{-3}
0	87.5000	3.5000	0.0000	0.0000	0.00	87.5013	<0.000
15	75.1417	3.0057	12.3583	4.1117	14.12	87.4989	<0.000
30	68.3857	2.7354	19.1144	6.9877	21.84	87.5102	<0.000
45	62.7728	2.5109	24.7272	9.8479	28.26	87.5071	<0.000
60	58.3469	2.3339	29.1531	12.4913	33.32	87.4890	<0.000
90	57.4203	2.2968	30.0797	13.0963	34.38	87.8647	<0.000
120	53.8113	2.1525	33.6887	15.6513	38.50	87.5008	<0.000
180	51.7537	2.0701	35.7463	17.2675	40.85	87.4571	<0.000
240	50.6414	2.0257	36.8586	18.1959	42.12	87.5117	<0.000
360	50.2290	2.0092	37.2710	18.5505	42.60	87.4995	<0.000
480	49.8767	1.9951	37.6233	18.8581	43.00	87.5048	<0.000
720	49.5592	1.9824	37.9408	19.1392	43.36	86.9986	<0.000
					Mean	87.4870	
					Std	0.1720	

β-endosulfan

Time (mins)	Ce(aq) µg/25cm^{-3}	Ce(aq) (µgcm^{-3})	Cqe(s) (µgg^{-1})	K_{ads} (cm^{3}g^{-1})	Adsorption (%)	Control µg/25cm^{-3}	Blank µg/25cm^{-3}
0	37.5000	1.5000	0.0000	0.0000	0.00	37.4895	<0.000
15	33.5134	1.3405	3.9866	2.9739	10.63	37.5011	<0.000
30	31.3341	1.2534	6.1659	4.9195	16.44	37.5109	<0.000
45	29.5235	1.1809	7.9765	6.7544	21.27	37.5004	<0.000
60	28.0958	1.1238	9.4042	8.3680	25.08	37.3980	<0.000
90	27.7969	1.1119	9.7031	8.7268	25.87	37.6006	<0.000
120	27.1740	1.0870	10.3260	9.4999	27.54	37.501	<0.000
180	26.7526	1.0701	10.7474	10.0433	28.66	37.5001	<0.000
240	26.3208	1.0528	11.1792	10.6182	29.81	37.4555	<0.000
360	26.0966	1.0439	11.4034	10.9242	30.41	37.7019	<0.000
480	25.8692	1.0348	11.6308	11.2400	31.02	37.5507	<0.000
720	25.5559	1.0222	11.9441	11.6843	31.85	37.5002	<0.000
					mean	37.5175	
					Std	0.0693	

Note:

Ce(aq) – concentration of endosulfan in 0.01M CaCl solution at equilibrium time,$t_{i;}$
Cqe(s) – concentration of endosulfan adsorbed to soil at equilibrium time, $t_{i;}$ K_{ads} - distribution coefficient of endosulfan between soil and aqueous phases (adsorption)

Table C-2: Desorption kinetics of α-and β-endosulfan for CRIN cocoa farm soil

α-endosulfan						
Time (mins)	Desorbed µg/25ml	Desorbed µg/ml	Undesorbed µg/g	Desorption D_{ti} (%)	K_{des} cm^3g^{-1}	$k_{des\text{-}\alpha\text{-}endo}$ ($mins^{-1}$)
0	0.0000	0.0000	32.1532	0	0.0000	0.00000
15	0.7234	0.0289	31.4298	2.25	1086.111	0.001517
30	1.2701	0.0508	30.8831	3.95	607.9114	0.001344
45	1.7813	0.0713	30.3719	5.54	426.2635	0.001267
60	1.9003	0.0760	30.2529	5.91	398.0118	0.001015
90	2.0353	0.0814	30.1179	6.33	369.9447	0.000727
120	2.2057	0.0882	29.9475	6.86	339.4315	0.000592
180	2.2957	0.0918	29.8575	7.14	325.1401	0.000412
240	2.4436	0.0977	29.7096	7.6	303.9474	0.000329
360	2.4887	0.0995	29.6645	7.74	297.9974	0.000224
480	2.5015	0.1001	29.6517	7.78	296.3368	0.000169
720	2.5465	0.1019	29.6067	7.92	290.6566	0.000115
						0.000701

β-endosulfan						
Time (mins)	Desorbed µg/25ml	Desorbed µg/ml	Undesorbed µg/g	Desorption D_{ti} (%)	K_{des} Cm^3g^{-1}	$k_{des\text{-}\beta\text{-}end}$ ($mins^{-1}$)
0	0.0000	0.0000	10.1221	0	0	0
15	0.5010	0.0200	9.6211	4.95	480.0505	0.003385
30	0.7875	0.0315	9.3346	7.78	296.3368	0.002700
45	0.9231	0.0369	9.1990	9.12	249.1228	0.002125
60	1.1408	0.0456	8.9813	11.27	196.8279	0.001993
90	1.2227	0.0489	8.8994	12.08	181.9536	0.001431
120	1.3209	0.0528	8.8012	13.05	166.5709	0.001166
180	1.3958	0.0558	8.7263	13.79	156.2908	0.000825
240	1.4464	0.0579	8.6757	14.29	149.9475	0.000643
360	1.4758	0.0590	8.6463	14.58	146.4678	0.000438
480	1.4971	0.0599	8.6250	14.79	144.0331	0.000334
720	1.5072	0.0603	8.6149	14.89	142.8979	0.000224
						0.001388

Note:

$k_{des\text{-}\alpha\text{-}endo}$ - desorption rate constant of α-endosulfan

$k_{des\text{-}\beta\text{-}endo}$ - desorption rate constant of β-endosulfan

K_{des} - distribution coefficient of endosulfan between soil and aqueous phases (desorption)

Table C-3: Adsorption Isotherm of α- and β-endosulfan for CRIN farm soil

α-endosulfan						
Eq. Conc. $\mu g/25cm^3$	Cqe(s) μgg^{-1}	Ce(aq) $\mu g/25cm^3$	Ce(aq) μgcm^{-3}	LogCe(aq)	LogCqe(s)	A_{ti} (%)
4.375	4.1803	0.1947	0.0078	-0.7107	0.6212	95.55
8.75	7.8955	0.8545	0.0342	-0.0683	0.8974	90.23
17.5	14.997	2.5027	0.1001	0.3984	1.1760	85.70
26.25	21.0460	5.2040	0.2082	0.7163	1.3232	80.18
43.75	32.2988	11.4512	0.4580	1.0589	1.5092	73.83
87.5	58.4446	29.0554	1.1622	1.4632	1.7667	66.79
175	100.6775	74.3225	2.9729	1.8711	2.0029	57.53
262.5	131.4338	131.0663	5.2427	2.1175	2.1187	50.07
350	151.5500	198.4500	7.9590	2.2977	2.1806	43.30
437.5	175.0438	262.4563	10.4983	2.4191	2.2431	40.01

β-endosulfan						
Eq. Conc. $\mu g/25cm^3$	Cqe(s) μgg^{-1}	Ce(aq) $\mu g/25cm^3$	Ce(aq) μgcm^{-3}	LogCe(aq)	LogCqe(s)	A_{ti} (%)
1.875	1.7340	0.1410	0.0056	-0.8508	0.2390	92.48
3.750	3.2516	0.4984	0.0199	-0.3024	0.5121	86.71
7.500	5.9745	1.5255	0.0610	0.1834	0.7763	79.66
11.250	8.2789	2.9711	0.1188	0.4729	0.9180	73.59
18.750	12.4144	6.3356	0.2534	0.8018	1.0939	66.21
37.500	20.8200	16.6800	0.6672	1.2222	1.3185	55.52
75.000	36.0075	38.9925	1.5597	1.5910	1.5564	48.01
112.500	46.1588	66.3413	2.6537	1.8218	1.6643	41.03
150.000	51.0750	98.9250	3.9570	1.9953	1.7082	34.05
187.500	57.1875	130.3125	5.2125	2.1150	1.7573	30.50

Eq. conc. - Equilibrium concentration

Cqe(s) - Concentration of α-endosulfan adsorbed to soil at equilibrium (μgg^{-1})

Ce(aq) – Concentration of α-endosulfan remaining in 0.01M CaCl ($\mu g/25cm^3$) **or**

Ce(aq) – Concentration of α-endosulfan remaining in 0.01M CaCl (μgcm^{-3})

A_{ti} - Percentage adsorption of equilibrium concentrations at equilibrium time,t 480 minutes

Table C-4: Desorption Isotherm of α- and β-endosulfan for CRIN farm soil

α-endosulfan							
Eq. Conc. $\mu g/25cm^3$	Cqe(s) μgg^{-1}	$C_{des}(aq)$ $\mu g/25cm^3$	$Cqe_{uds}(s)$ μgg^{-1}	$C_{des}(aq)$ μgcm^{-3}	$LogC_{des}(aq)$	LogCqe(s)	D_{ti} (%)
4.375	4.0053	0.4598	3.5455	0.0184	-0.3374	0.5497	11.48
8.75	7.0955	0.7174	6.3781	0.0287	-0.1443	0.8047	10.11
17.5	12.6973	1.1986	11.4987	0.0479	0.0787	1.0606	9.44
26.25	17.046	1.4216	15.6244	0.0569	0.1528	1.1938	8.34
43.75	24.1876	1.7028	22.4848	0.0681	0.2312	1.3519	7.04
87.5	40.6546	2.7970	37.8576	0.1119	0.4467	1.5782	6.88
175	70.9275	4.1776	66.7499	0.1671	0.6209	1.8245	5.89
262.5	96.2588	5.8044	90.4544	0.2322	0.7638	1.9564	6.03
350	113.575	6.4624	107.1126	0.2585	0.8104	2.0298	5.69
437.5	126.7875	6.7071	120.0804	0.2683	0.8265	2.0795	5.29

β-endosulfan							
Eq. Conc. $\mu g/25cm^3$	Cqe(s) μgg^{-1}	$C_{des}(aq)$ $\mu g/25cm^3$	$Cqe_{uds}(s)$ μgg^{-1}	$C_{des}(aq)$ μgcm^{-3}	$LogC_{des}(aq)$	LogCqe(s)	D_{ti} (%)
1.875	1.6403	0.2513	1.3890	0.0101	-0.5998	0.1427	15.32
3.75	2.8575	0.4115	2.4460	0.0165	-0.3857	0.3885	14.40
7.5	4.9500	0.6930	4.2570	0.0277	-0.1593	0.6291	14.00
11.25	6.4688	0.8959	5.5728	0.0358	-0.0477	0.7461	13.85
18.75	9.2250	1.2869	7.9381	0.0515	0.1095	0.8997	13.95
37.5	14.8125	1.7879	13.0246	0.0715	0.2523	1.1148	12.07
75	25.1250	2.7788	22.3462	0.1112	0.4439	1.3492	11.06
112.5	33.5250	3.3693	30.1557	0.1348	0.5275	1.4794	10.05
150	36.9750	3.3573	33.6177	0.1343	0.5260	1.5267	9.08
187.5	40.3125	3.4709	36.8416	0.1388	0.5404	1.5663	8.61

Eq. conc. - Equilibrium concentrations of α-endosulfan

Cqe(s) - Concentration of α-endosulfan adsorbed to soil at equilibrium (μgg^{-1})

$Cqe_{uds}(s)$ – Concentration of α-endosulfan undesorbed at equilibrium (μgg^{-1})

$C_{des}(aq)$ - Concentration of α-endosulfan desorbed into 0.01M CaCl ($\mu g/25cm^3$) **or**

$C_{des}(aq)$ – Concentration of α-endosulfan desorbed into 0.01M CaCl (μgcm^{-3}) at equilibrium

D_{ti} - Percentage desorption of adsorbed α-endosulfan at equilibrium time,t 480 minutes

Table C-5: Adsorption kinetics of α-and β-endosulfan for Igba cocoa farm soil

α-endosulfan							
Time (mins)	Ce(aq) µg/25cm^{-3}	Ce(aq) (µgcm^{-3})	Cqe (µgg^{-1})	Kd (cm^{3}g^{-1})	Adsorption (%)	Control	Blank
0	87.5000	3.5000	0.0000	0.0000	0.00	87.5013	<0.000
15	77.8915	3.1157	9.6085	3.0839	10.98	87.4989	<0.000
30	72.6389	2.9056	14.8611	5.1147	16.98	87.5102	<0.000
45	68.2750	2.7310	19.2250	7.0395	21.97	87.5071	<0.000
60	64.8339	2.5934	22.6661	8.7401	25.90	87.4890	<0.000
90	63.0952	2.5238	24.4048	9.6698	27.89	87.8647	<0.000
120	61.3076	2.4523	26.1924	10.6807	29.93	87.5008	<0.000
180	59.7078	2.3883	27.7922	11.6368	31.76	87.4571	<0.000
240	58.8430	2.3537	28.6570	12.1752	32.75	87.5117	<0.000
360	58.5224	2.3409	28.9776	12.3788	33.12	87.4995	<0.000
480	58.2485	2.3299	29.251497	12.5546	33.43	87.5048	<0.000
720	57.5429	2.3017	29.9571	13.0151	34.24	86.9986	<0.000
					Mean	87.4870	
					Std	0.1720	

β-endosulfan							
Time (mins)	Ce(aq) µg/25cm^{-3}	Ce(aq) (µgcm^{-3})	Cqe(s) (µgg^{-1})	K_d (cm^{3}g^{-1})	Adsorption (%)	Control	Blank
0	37.5000	1.5	0	0	0	37.4895	<0.000
15	34.5036	1.38	2.9964	2.1713	7.99	37.5011	<0.000
30	32.5730	1.3028	4.9270	3.7819	13.14	37.5109	<0.000
45	31.09832	1.2436	6.4017	5.1477	17.07	37.5004	<0.000
60	29.8085	1.1912	7.6915	6.4570	20.51	37.3980	<0.000
90	29.0629	1.1612	8.4371	7.2658	22.50	37.6006	<0.000
120	28.4798	1.1384	9.0202	7.9235	24.05	37.501	<0.000
180	28.0994	1.1236	9.4006	8.3665	25.07	37.5001	<0.000
240	27.8245	1.1124	9.6755	8.6978	25.80	37.4555	<0.000
360	27.6446	1.1048	9.8554	8.9205	26.28	37.7019	<0.000
480	27.5299	1.1004	9.9701	9.0604	26.59	37.5507	<0.000
720	27.4511	1.0972	10.0489	9.1587	26.80	37.5002	<0.000
					Mean	37.5175	
					Std	0.0693	

Table C-6: Desorption kinetics of α-and β-endosulfan for Igba cocoa farm soil

α-endosulfan						
Time (mins)	Desorbed µg/25ml	Desorbed µg/ml	Undesorbed µg/g	Desorption D_{ti} (%)	K_{des} cm^3g^{-1}	$k_{des\text{-}\alpha\text{-}endo}$ ($mins^{-1}$)
0	0.0000	0.0000	29.9571	0	0.0000	0.0000
15	0.5932	0.0237	29.3639	1.98	1237.626	0.001333
30	1.0545	0.0422	28.9026	3.52	685.2273	0.001195
45	1.5817	0.0633	28.3754	5.28	448.4848	0.001206
60	2.1959	0.0878	27.7612	7.33	316.0641	0.001269
90	2.4745	0.0990	27.4826	8.26	277.6634	0.000958
120	2.6722	0.1069	27.2849	8.92	255.2691	0.000779
180	2.8429	0.1137	27.1142	9.49	238.4352	0.000554
240	2.9628	0.1185	26.9943	9.89	227.7806	0.000434
360	3.0287	0.1211	26.9284	10.11	222.2799	0.000296
480	3.0317	0.1213	26.9254	10.12	222.0356	0.000222
720	3.0406	0.1216	26.9165	10.15	221.3054	0.000149
					Mean	0.000763

β-endosulfan						
Time (mins)	Desorbed µg/25ml	Desorbed ug/ml	Undesorbed µg/g	Desorption D_{ti} (%)	K_{des} Cm^3g^{-1}	$k_{des\text{-}\beta\text{-}endo}$ ($mins^{-1}$)
0	0.0000	0.0000	10.0489	0	0.0000	0.0000
15	0.4030	0.0161	9.6459	4.01	598.4414	0.002729
30	0.7517	0.0301	9.2972	7.48	309.2246	0.002592
45	1.1225	0.0449	8.9264	11.17	198.8138	0.002633
60	1.2672	0.0507	8.7817	12.61	173.2554	0.002247
90	1.3677	0.0547	8.6812	13.61	158.6885	0.001626
120	1.4571	0.0583	8.5918	14.5	147.4138	0.001306
180	1.5465	0.0619	8.5024	15.39	137.4431	0.000929
240	1.6068	0.0643	8.4421	15.99	131.3477	0.000726
360	1.6319	0.0653	8.4170	16.24	128.9409	0.000492
480	1.6370	0.0655	8.4119	16.29	128.4684	0.000371
720	1.6400	0.0656	8.4089	16.32	128.1863	0.000248
						0.001445

Table C-7: Adsorption Isotherm of α- and β-endosulfan for Igba farm soil

α-endosulfan						
Eq. Conc. $\mu g/25cm^3$	Cqe(s) μgg^{-1}	Ce(aq) $\mu g/25cm^3$	Ce(aq) μgcm^{-3}	LogCe(aq)	LogCqe(s)	A_{ti} (%)
4.375	4.0906	0.2844	0.0114	-0.5461	0.6118	93.5
8.75	7.6991	1.0509	0.0420	0.0216	0.8864	87.99
17.5	14.0735	3.4265	0.1371	0.5349	1.1484	80.42
26.25	19.5615	6.6885	0.2675	0.8253	1.2914	74.52
43.75	28.9931	14.7569	0.5903	1.1690	1.4623	66.27
87.5	47.4688	40.0313	1.6013	1.6024	1.6764	54.25
175	78.5925	96.4075	3.8563	1.9841	1.8954	44.91
262.5	102.8475	159.6525	6.3861	2.2032	2.0122	39.18
350	127.1900	222.8100	8.9124	2.3479	2.1045	36.34
437.5	150.9375	286.5625	11.4625	2.4572	2.1788	34.50

β-endosulfan						
Eq. Conc. $\mu g/25cm^3$	Cqe(s) μgg^{-1}	Ce(aq) $\mu g/25cm^3$	Ce(aq) μgcm^{-3}	LogCe(aq)	LogCqe(s)	A_{ti} (%)
1.875	1.7046	0.1704	0.0068	-0.7684	0.2316	90.91
3.750	3.2183	0.5318	0.0213	-0.2743	0.5076	85.82
7.500	5.9798	1.5203	0.0608	0.1819	0.7767	79.73
11.2500	7.9481	3.3019	0.1321	0.5188	0.9003	70.65
18.750	11.8088	6.9413	0.2777	0.8414	1.0722	62.98
37.500	17.1563	20.3438	0.8138	1.3084	1.2344	45.75
75.000	30.4125	44.5875	1.7835	1.6492	1.4831	40.55
112.500	40.3088	72.1913	2.8877	1.8585	1.6054	35.83
150.000	46.0950	103.9050	4.1562	2.0166	1.6637	30.73
187.500	49.8188	137.6813	5.5073	2.1389	1.6974	26.57

Table C-8: Desorption Isotherm of α- and β-endosulfan for Igba farm soil

α-endosulfan							
Eq. Conc. $\mu g/25cm^3$	Cqe(s) μgg^{-1}	$C_{des}(aq)$ $\mu g/25cm^3$	$Cqe_{uds}(s)$ μgg^{-1}	$C_{des}(aq)$ μgcm^{-3}	$LogC_{des}(aq)$	LogCqe(s)	D_{ti} (%)
4.375	3.8373	0.4866	3.3507	0.0195	-0.3129	0.5251	12.68
8.75	6.6876	0.7403	5.9473	0.0296	-0.1306	0.7743	11.07
17.5	11.6654	1.2832	10.3822	0.0513	0.1083	1.0163	11.00
26.25	15.0543	1.5762	13.4781	0.0630	0.1976	1.1296	10.47
43.75	20.267	2.0733	18.1937	0.0829	0.3167	1.2599	10.23
87.5	34.0856	3.4154	30.6702	0.1366	0.5334	1.4867	10.02
175	55.3774	5.3771	50.0003	0.2151	0.7306	1.6990	9.71
262.5	67.3481	5.8121	61.5360	0.2325	0.7643	1.7891	8.63
350	80.0562	6.3405	73.7157	0.2536	0.8021	1.8676	7.92
437.5	93.6819	6.8481	86.8338	0.2739	0.8356	1.9387	7.31

β-endosulfan							
Eq. Conc. $\mu g/25cm^3$	Cqe(s) μgg^{-1}	$C_{des}(aq)$ $\mu g/25cm^3$	$Cqe_{uds}(s)$ μgg^{-1}	$C_{des}(aq)$ μgcm^{-3}	$LogC_{des}(aq)$	LogCqe(s)	D_{ti} (%)
1.875	1.5418	0.2402	1.3016	0.0096	-0.6194	0.1145	15.58
3.75	2.6861	0.4115	2.2745	0.0165	-0.3856	0.3569	15.32
7.5	4.6530	0.6761	3.9769	0.0270	-0.1700	0.5995	14.53
11.25	6.0806	0.8434	5.2372	0.0337	-0.0740	0.7191	13.87
18.75	8.6715	1.1733	7.4982	0.0469	0.0694	0.8750	13.53
37.5	13.9238	1.7321	12.1916	0.0693	0.2386	1.0861	12.44
75	23.6175	2.8247	20.7928	0.1130	0.4510	1.3179	11.96
112.5	31.5135	3.5547	27.9588	0.1422	0.5508	1.4465	11.28
150	34.7565	3.7294	31.0271	0.1492	0.5716	1.4917	10.73
187.5	37.8938	3.8652	34.0286	0.1546	0.5872	1.5318	10.20

Table C-9: Adsorption kinetics of α-and β-endosulfan for Sore Bale cocoa farm soil

α-endosulfan							
Time (mins)	Ce(aq) µg/25cm^{-3}	Ce(aq) (µgcm^{-3})	Cqe (µgg^{-1})	Kd (cm^{3}g^{-1})	Adsorption (%)	Control	Blank
0	87.5000	3.5000	0	0.0000	0.00	87.5013	<0.000
15	77.0175	3.0807	10.4825	3.4026	11.98	87.4989	<0.000
30	71.7413	2.8697	15.75875	5.4915	18.01	87.5102	<0.000
45	67.8825	2.7153	19.6175	7.2248	22.42	87.5071	<0.000
60	65.4063	2.6163	22.09375	8.4448	25.25	87.4890	<0.000
90	63.9100	2.5564	23.5900	9.2278	26.96	87.8647	<0.000
120	62.8338	2.5134	24.66625	9.8141	28.19	87.5008	<0.000
180	61.8713	2.4749	25.62875	10.3557	29.29	87.4571	<0.000
240	60.9788	2.4392	26.52125	10.8732	30.31	87.5117	<0.000
360	60.0075	2.4003	27.4925	11.4538	31.42	87.4995	<0.000
480	59.5875	2.3835	27.9125	11.7107	31.90	87.5048	<0.000
720	59.4563	2.3783	28.04375	11.7918	32.05	86.9986	<0.000
					Mean	87.4870	
					Std	0.1720	

β-endosulfan							
Time (mins)	Ce(aq) µg/25cm^{-3}	Ce(aq) (µgcm^{-3})	Cqe(s) (µgg^{-1})	K_d (cm^{3}g^{-1})	Adsorption (%)	Control	Blank
0	37.5000	1.5	0.0000	0	0	37.4895	<0.000
15	34.4738	1.3790	3.0263	2.1946	8.07	37.5011	<0.000
30	32.5238	1.3010	4.9763	3.8251	13.27	37.5109	<0.000
45	31.0725	1.2429	6.4275	5.1714	17.14	37.5004	<0.000
60	30.1200	1.2048	7.3800	6.1255	19.68	37.3980	<0.000
90	29.5950	1.1838	7.9050	6.6776	21.08	37.6006	<0.000
120	29.0325	1.1613	8.4675	7.2914	22.58	37.501	<0.000
180	28.8825	1.1553	8.6175	7.4591	22.98	37.5001	<0.000
240	28.7138	1.1486	8.7863	7.6499	23.43	37.4555	<0.000
360	28.6088	1.1444	8.8913	7.7697	23.71	37.7019	<0.000
480	28.5488	1.1420	8.9513	7.8386	23.87	37.5507	<0.000
720	28.5225	1.1409	8.9775	7.8688	23.94	37.5002	<0.000
						37.5175	
						0.0693	

Table C-10: Desorption kinetics of α-and β-endosulfan for Sore Bale cocoa farm soil

α-endosulfan						
Time (mins)	Desorbed µg/25ml	Desorbed ug/ml	Undesorbed µg/g	Desorption D_{ti} (%)	K_{des} Cm^3g^{-1}	$k_{des\text{-}\alpha\text{-}endo}$ ($mins^{-1}$)
0	0	0	30.1509	0	0.00	0.0000
15	0.768848	0.030754	29.38205	2.55	955.3922	0.001722
30	1.661315	0.066453	28.48959	5.51	428.7205	0.001890
45	2.050261	0.08201	28.10064	6.8	342.6471	0.001565
60	2.360815	0.094433	27.79008	7.83	294.2848	0.001359
90	2.611068	0.104443	27.53983	8.66	263.6836	0.001007
120	2.897501	0.1159	27.2534	9.61	235.1457	0.000842
180	3.208056	0.128322	26.94284	10.64	209.9624	0.000625
240	3.241222	0.129649	26.90968	10.75	207.5581	0.000474
360	3.274388	0.130976	26.87651	10.86	205.2026	0.000319
480	3.301524	0.132061	26.84938	10.95	203.3105	0.000242
720	3.316599	0.132664	26.8343	11	202.2727	0.000153
						0.000927

β-endosulfan						
Time (mins)	Desorbed µg/25ml	Desorbed ug/ml	Undesorbed µg/g	Desorption D_{ti} (%)	K_{des} Cm^3g^{-1}	$k_{des\text{-}\beta\text{-}endo}$ ($mins^{-1}$)
0	0	0.0000	9.9699	0	0.0000	0.0000
15	0.647047	0.0259	9.322853	6.49	360.208	0.004474
30	1.086719	0.0435	8.883181	10.9	204.3578	0.003848
45	1.374849	0.0550	8.595051	13.79	156.2908	0.003298
60	1.519413	0.0608	8.450487	15.24	139.042	0.002756
90	1.647027	0.0659	8.322873	16.52	126.3317	0.002007
120	1.729778	0.0692	8.240122	17.35	119.0922	0.001588
180	1.769657	0.0708	8.200243	17.75	115.8451	0.001086
240	1.812528	0.0725	8.157372	18.18	112.5138	0.000836
360	1.824492	0.0730	8.145408	18.3	111.612	0.000562
480	1.842438	0.0737	8.127462	18.48	110.2814	0.000426
720	1.847422	0.0739	8.122478	18.53	109.9164	0.000285
						0.001924

Table C-11: Adsorption isotherm of α-and β-endosulfan for Sore Bale cocoa farm soil

α-endosulfan						
Eq. Conc. $\mu g/25cm^3$	Cqe(s) μgg^{-1}	Ce(aq) $\mu g/25cm^3$	Ce(aq) μgcm^{-3}	LogCe(aq)	LogCqe(s)	A_{ti} (%)
4.375	3.8719	0.5031	0.0201	-0.2983	0.5879	88.50
8.75	6.8049	1.9451	0.0778	0.2889	0.8328	77.77
17.5	11.8790	5.6210	0.2248	0.7498	1.0748	67.88
26.25	15.3405	10.9095	0.4364	1.0378	1.1858	58.44
43.75	22.1900	21.5600	0.8624	1.3336	1.3462	50.72
87.5	39.5850	47.9150	1.9166	1.6805	1.5975	45.24
175	69.4225	105.5775	4.2231	2.0236	1.8415	39.67
262.5	89.4338	173.0663	6.9227	2.2382	1.9515	34.07
350	105.8750	244.1250	9.7650	2.3876	2.0248	30.25
437.5	123.3313	314.1688	12.5668	2.4972	2.0911	28.19

β-endosulfan						
Eq. Conc. $\mu g/25cm^3$	Cqe(s) μgg^{-1}	Ce(aq) $\mu g/25cm^3$	Ce(aq) μgcm^{-3}	LogCe(aq)	LogCqe(s)	A_{ti} (%)
1.875	1.5896	0.2854	0.0114	-0.5446	0.2013	84.78
3.75	2.7214	1.0286	0.0411	0.0123	0.4348	72.57
7.5	4.6463	2.8538	0.1142	0.4554	0.6671	61.95
11.25	5.8500	5.4000	0.2160	0.7324	0.7672	52
18.75	8.7994	9.9506	0.3980	0.9979	0.9445	46.93
37.5	15.1050	22.3950	0.8958	1.3502	1.1791	40.28
75	25.8825	49.1175	1.9647	1.6912	1.4130	34.51
112.5	31.7813	80.7188	3.2288	1.9070	1.5022	28.25
150	36.2700	113.7300	4.5492	2.0559	1.5595	24.18
187.5	41.8500	145.6500	5.8260	2.1633	1.6217	22.32

Table C-12: Desorption isotherm of α-and β-endosulfan for Sore Bale cocoa farm soil

α-endosulfan							
Eq. Conc. $\mu g/25cm^3$	Cqe(s) μgg^{-1}	C_{des}(aq) $\mu g/25cm^3$	Cqe_{uds}(s) μgg^{-1}	C_{des}(aq) μgcm^{-3}	$LogC_{des}$(aq)	LogCqe(s)	D_{ti} (%)
4.375	3.8949	0.5363	3.3586	0.0215	-0.2706	0.5262	13.77
8.75	6.7879	0.8709	5.9170	0.0348	-0.0600	0.7721	12.83
17.5	11.8404	1.3415	10.4989	0.0537	0.1276	1.0211	11.33
26.25	15.2801	1.6762	13.6039	0.0670	0.2243	1.1337	10.97
43.75	20.571	2.1641	18.4069	0.0866	0.3353	1.2650	10.52
87.5	34.5969	4.2381	30.3588	0.1695	0.6272	1.4823	12.25
175	56.2081	6.6663	49.5418	0.2667	0.8239	1.6950	11.86
262.5	68.3583	7.8202	60.5381	0.3128	0.8932	1.7820	11.44
350	81.257	9.0683	72.1887	0.3627	0.9575	1.8585	11.16
437.5	95.0871	9.4136	85.6735	0.3765	0.9738	1.9328	9.90

β-endosulfan							
Eq. Conc. $\mu g/25cm^3$	Cqe(s) μgg^{-1}	C_{des}(aq) $\mu g/25cm^3$	Cqe_{uds}(s) μgg^{-1}	C_{des}(aq) μgcm^{-3}	$LogC_{des}$(aq)	LogCqe(s)	D_{ti} (%)
1.875	1.6403	0.2969	1.3434	0.0119	-0.5274	0.1282	18.10
3.75	2.8575	0.4895	2.3680	0.0196	-0.3103	0.3744	17.13
7.5	4.9500	0.7915	4.1585	0.0317	-0.1015	0.6189	15.99
11.25	6.4688	1.0046	5.4642	0.0402	0.0020	0.7375	15.53
18.75	9.2250	1.3847	7.8403	0.0554	0.1413	0.8943	15.01
37.5	14.8125	2.4811	12.3314	0.0992	0.3946	1.0910	16.75
75	25.1250	4.0074	21.1176	0.1603	0.6029	1.3246	15.95
112.5	33.5250	5.1595	28.3655	0.2064	0.7126	1.4528	15.39
150	36.9750	5.3799	31.5951	0.2152	0.7308	1.4996	14.55
187.5	40.3125	5.4220	34.8905	0.2169	0.7342	1.5427	13.45