



## Multi-residue Analysis of Organochlorine Pesticides in Cocoa Beans (*Theobroma cacao*) and Soils from Ondo, Nigeria

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### Abstract

Organochlorine pesticides (OCPs) residues were determined in a total of 160 samples comprising of cocoa beans and soil collected from four major cocoa farms in Ondo, Nigeria. The residues were extracted with dichloromethane, cleaned up by column chromatography and analysed with Gas Chromatography-Electron Capture Detector (GC-ECD). In the analysed cocoa samples, endrin and endosulfan I had the highest incidence of occurrence of 93% and 89%, respectively while lindane and heptachlor had the least occurrence of 19%. High incidences of occurrence in the soil samples were observed in endrin (93%) and endosulfan I (94%) while the least occurrence was  $\alpha$ -HCH (13%). The total residual concentrations of the OCPs were higher in the cocoa samples compared to the soil samples. The mean residual levels of HCHs ( $\alpha$ ,  $\beta$ ,  $\delta$ ) aldrin, dieldrin, endrin and endosulfan I and cis-permethrin in the cocoa samples were above Maximum Residual Limits (MRL) established by WHO/FAO while aldrin, dieldrin, endrin, endosulfans in the soil sample analysed were above MRL established by Netherlands. Bioaccumulation factor > 1 was recorded in most of the cocoa samples from the different farms especially for endrin, dieldrin, endosulfan I and *trans*-permethrin. This gives cause for concern considering the adverse health hazards pose by these pesticides.

**Keywords:** Organochlorine, Pesticide, Residues, Cocoa, Soil, Bioaccumulation factor

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### Introduction

Cocoa is an important tropical tree crop which serves as a foreign exchange earner for many West African countries like Nigeria. Before 1960, exportation of cocoa accounted mainly for the agricultural export, which made over 80% of the Gross National Product (GNP) of the Nigerian economy [1]. In recent years, as a result of the oil boom experienced in Nigeria, cocoa dropped to about 38% of agricultural export [2]. Consequently, cocoa farming was left mostly to smallholder farmers, most of who are geographically isolated, illiterate, poorly informed and have very limited resources for proper crop management.

Another major contributor to the decline were pests as 25-30% loss in yield of cocoa was attributed to the cocoa mired, *Sahlbergella singularis* while about 17% was lost through the feeding of the cocoa pod borer *Characoma strictigrapta* [3,4]. The collective efforts of minor pests (such as the shield bug, *Bathycoelia thalassina*, the pod miner, *Mamara* species, the root-feeding termites, *Macrotermes bellicosus*, *Mesohomotoma tessmanni* and the cacao thrips, *Selenothrips rubrocinctus*) have become significant especially under suitable conditions in young cocoa or ageing cocoa plantations.

Several concerted research efforts have been made to develop various environmentally friendly control techniques (such as cultural, biological and chemical) which could be adopted for integrated management of the major and minor pests of cocoa in Nigeria. Despite the various mechanisms developed for pest management, the farmers rely greatly on the use of pesticides because it provides immediate and quicker remedy in the periods of serious pest outbreaks [4]. In Nigeria, cocoa farmers use different insecticide formulations including the very notorious organochlorine types. Due to their bioaccumulation in the food chain and prolonged persistence in the environment coupled with numerous associated health risk, the National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria, placed ban on the use of most pesticides especially those with organochlorine formulations in line with the new European Union Legislation on pesticide use [5].

Despite, the ban on these chemicals, most farmers are only interested in protecting their crops from pests in order to get good harvest and maximize profit but have little concern for the detrimental effects of these pesticides on the soil, environment and public health [6].

The intensive use of pesticides leads to environmental problems such as contamination of soil, farm produce and underground water. On application of pesticides to destroy pests and pathogens, only 15% of the applied amount hits the targeted pests, with the remaining 85% being distributed in the soil and air [7]. The soil is the main matrix for pesticide disposition and the bulk of pesticide residues are generally confined to the upper 20 cm of the top soil [6]. Pesticide residue in the soil can move from the surface when they dissolve in runoff water, or percolate down through the soil, and

eventually reach the groundwater. Plants take up pesticides dissolved in water and are distributed in most part of the plants especially in fruits rich in fat such as cocoa beans. In view of this, the study was carried out to assess the levels of organochlorine pesticides residues in cocoa and soil from four major cocoa plantations in Ondo state, Nigeria.

## Materials and Methods

### Study Area

Four large cocoa plantations were carefully selected for this study: two of the plantations were located in Ile-Oluji (tagged Ile-Oluji-1 and Ile-Oluji-2) and one each from Owena Town (tagged Owena) and Idanre (tagged Idanre). The selected farms are located on the plain so as to eliminate the influence of sloppy topography on the results. Fig. 1 shows the map of the study area. The GPS location of the study area are as follows: Ile-Oluji ( $7^{\circ} 13' 0''$  North,  $4^{\circ} 52' 0''$  East), Owena, ( $7^{\circ} 12' 0''$  North,  $5^{\circ} 1' 0''$  East) and Idanre ( $6^{\circ} 43' 0''$  North,  $5^{\circ} 6' 0''$  East). To elicit information about the farms from the farmers, well-structured questionnaire was designed and distributed to the farmers where sampling was carried out (Table1).

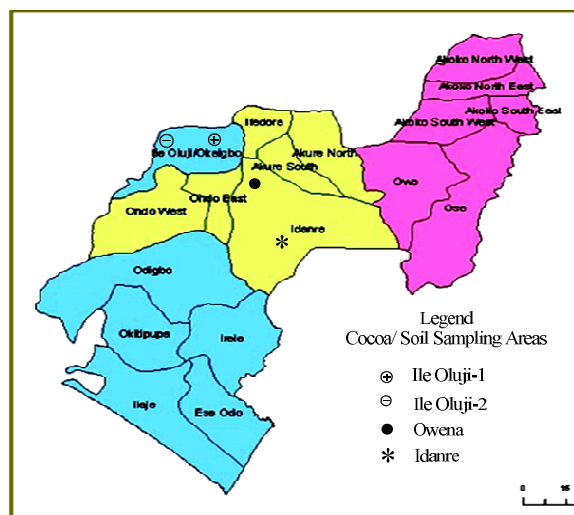


Figure 1. Map of Ondo state showing the study area

**Table 1.** Socio-economic characteristics of the farmers and farms in the study area.

Characteristic	Oluji-1	Oluji-2	Owena	Idanre
Sex	Male	Male	Male	Male
Age	Above 50	Above 40	Above 70	Above 50
Marital Status	Married	Married	Married	Married
Level of Education	Tertiary	Tertiary	Primary	Primary
Farm size (Hectares)	14	17	39	43
Age of Farm	15	10	110	103
Av. turn-out/annual (tonnes)	4.8	5.1	11.3	13.2
Knowledge of Pesticides	Yes	Yes	Yes	Yes
Pesticide Application	Yes	Yes	Yes	Yes
No. of years of pesticide application	9	7	67	71
Pesticide Applied Often	Pest-off (Dichlorvos)	Cypermethrin (Cypermethrin)	Chlorpyrifos (Chlorpyrifos)	Chlorpyrifos (Chlorpyrifos)
Freq. of application/yr	2-3 times	2-3 times	2-3 times	2-3 times
Safety wear on application	Yes	No	No	No
Health Hazard occurrence	Yes	Yes	Yes	Yes

### **Sample Collection and Processing**

Each farm was divided into four zones. Five composite samples were collected from each zone making a total of twenty soil samples from each farm. Each composite sample comprises of four subsamples randomly collected (0-20 cm) with the aid of clean garden hand trowel. The collected surface soil were mixed together on a clean aluminum foil material and reduced by quartering method to 500 g. The soil samples were packed into well labeled, clean and dried aluminum container. In the laboratory, soil samples were disaggregated and dried. Stones and debris were removed from each quartered composite sample, passed through 2.0 mm sieve and stored until extraction and clean up. Ripe cocoa pods were harvested from the four zones in each farm in the same manner as the soil samples. The harvested pods were thoroughly mixed together and reduced by quartering methods to a convenient size. The

pods were packed into well labeled clean and dried aluminum container. The pods were broken to obtain the cocoa beans which were fermented for six days. After the fermentation, the beans were air dried in dust free and clean environment and later oven dried at a temperature of 60°C to constant weight.

### **Extraction of Residues from Cocoa Beans and Soil Samples**

The method employed for the extraction process was that of Environmental Protection Agency (EPA 3570) with slight modifications [8]. The dried cocoa beans samples were milled twice to powdery form. Three grams of milled cocoa sample and equal grams of anhydrous sodium sulphate were weighed into a porcelain mortar and homogenized with a pestle. The mixture was carefully transferred to a pre-cleaned and well labelled Polytetrafluoroethylene (PTFE) extraction tube with a PTFE screw cap. 40 mL of dichloromethane was added to the 100 mL PTFE extraction tube; the extraction tube was tightly capped and placed on a mechanical shaker for 30 min after which it was allowed to stand for 20 min to ensure complete permeation of the added solvent to the matrix. More sodium sulphate was added and mixed as necessary to produce free-flowing, finely divided slurry. Care was taken to release pressure by opening and closing the tube at intervals. The solids were allowed to settle for some minutes after which the solvent layer was filtered through a small glass funnel containing a layer of anhydrous sodium sulphate over a plug of glass wool into a receiving cleaned clearly labelled conical flask. The sodium sulphate was thoroughly pre-wetted with dichloromethane (DCM) before sample filtration. The sodium sulphate was rinsed with 2 mL of dichloromethane as soon as the surface was exposed. The top of the sodium sulphate layer was not allowed to go dry to prevent binding of the targeted

pesticide residues to the layer. The filtered cocoa sample was extracted twice more by adding 40 mL of dichloromethane to the sample. At the end, all the extracts are combined and poured into the round bottom flask of a rotary evaporator. The round bottom flask of the rotary evaporator was placed in a constant temperature water bath maintained at 40°C. The evaporation continued until the sample volume was reduced to approximately 1.0 mL. The same process was employed in the extraction of the soil samples except that the soil samples were not milled since they were sieved earlier through 2 mm sieve [9].

### ***Clean-up of Residues from Cocoa Beans and Soil Samples***

For the clean-up process, a 600 mm x 19 mm internal diameter (id) clean-up column was prepared by blocking the hole with glass wool and adding 3 g of activated silica gel (60 mesh), calcined at 450 °C for 4 h, and then stored at 120 °C until use. The column was topped with 1cm of preheated Na<sub>2</sub>SO<sub>4</sub> (previously heated at 650°C for 18 h), and stored in a clean bottle in a desiccator. The column was rinsed by eluting with 20 mL n-hexane twice. Each concentrated extract in n-hexane was transferred to the column and eluted with 50 mL of (1:1) DCM/n-hexane (v/v ratio). The eluent was collected in a 100 mL round bottom flask. This fraction contained OCPs. The volume of the cleaned n-hexane extract was reduced to 1 mL using rotary evaporator. The same process was employed in the clean-up of the soil samples. The cleaned extracts were taken for gas chromatography identification and quantification.

### ***Gas Chromatographic Analysis***

The extracted and cleaned organochlorine pesticide residues from cocoa and soil were subjected to Gas Chromatography, Shimadzu GC-ECD-MS QP2010 (Japan) and capillary Column Type:

HP1MS (30 m x 0.25 µm x 0.25 mm id) (Japan) for identification and quantification of organochlorine pesticides (OCPs). Analytical standards (>98% purity) were used to prepare standard solutions. The optimal conditions of the GC are presented in the supplementary material (Suppl 1). Calibration was done using reference standards of organochlorine pesticides (Hexachlorocyclohexane: (α-HCH, β-HCH, γ-HCH), Heptachlor, Heptachlor-epoxide (B), Aldrin, Dieldrin, Endrin, Endosulfan (I, II and sulphate), Dichloro-Diphenyl-Trichloroethane (DDT), cis- and trans-Permethrin). These standards were run six times; to calculate the mean; range and then the standard deviation; and also to determine the peak column performance, peak height and resolutions. The column was flushed with the carrier gas, helium. The GC was equipped with ECD, <sup>63</sup>Ni-370 MBq radiation source from Shimadzu.

### ***Quality Assurance***

All reagents used were of pesticide residue grade (BDH, England). All apparatus and glassware were washed with detergent, rinsed with double distilled water and dried. OCPs Mix AB# 1 Certified standard obtained from Restek Corporation was used to carry out recovery analysis. The premixed certified standard was used to spike 3 g of milled cocoa beans samples. The spiked and un-spiked samples were subjected to the extraction and clean up procedures described earlier. The residues were quantified with GC-ECD and percentage recoveries were obtained (Table 2) using the following equation:

$$\% \text{ Recovery} = \frac{\text{Conc of spiked sample} - \text{Conc of unspiked sample}}{\text{Spikin Conc}} \times 100 \quad (1)$$

Detection limits of the method were determined by the signal-to-noise method [10]. A signal-to-noise ratio of three is generally accepted for estimating LOD. Blank analysis was also carried out to check interference on the sample.

Table 2. Recovery of OCPs in cocoa beans samples.

Compounds	Certified values (µg/mL)	Conc. of un-spiked (µg/mL)	Conc. of spiked (µg/mL)	% Recovered	Mean % Recovery $\pm$ SD
$\alpha$ -HCH	200.6	ND	200.0	99.70	99.73 $\pm$ 0.04
	200.6	ND	200.10	99.75	
$\beta$ -HCH	201.6	ND	200.70	99.55	99.82 $\pm$ 0.38
	201.6	1.11	202.90	100.09	
$\gamma$ -HCH	201.2	ND	201.60	100.19	100.12 $\pm$ 0.10
	201.2	ND	201.30	100.05	
$\delta$ -HCH	200.8	0.22	201.62	99.80	99.85 $\pm$ 0.07
	200.8	0.39	201.99	99.90	
Heptachlor	200.1	ND	199.80	99.85	99.68 $\pm$ 0.25
	200.1	ND	199.10	99.50	
Heptachlor epoxide	201.2	0.12	200.11	99.40	99.39 $\pm$ 0.02
	201.2	0.39	200.32	99.37	
Aldrin	200.4	0.68	200.89	99.91	99.96 $\pm$ 0.07
	200.4	0.60	201.20	100.01	
Dieldrin	200.3	0.18	199.37	99.45	99.48 $\pm$ 0.04
	200.3	0.30	199.60	99.50	
Endrin	200.7	0.92	201.02	99.70	99.75 $\pm$ 0.07
	200.7	1.55	201.85	99.80	
Endosulfan I	200.5	13.67	213.87	99.85	100.01 $\pm$ 0.23
	200.5	4.55	205.39	100.17	
Endosulfan II	201.4	0.08	200.47	99.50	99.58 $\pm$ 0.11
	201.4	0.17	200.87	99.65	
Endosulfan sulphate	200.6	ND	200.0	99.70	99.78 $\pm$ 0.11
	200.6	3.13	203.43	99.85	
p, p'DDT	201.1	ND	201.50	100.20	100.28 $\pm$ 0.11
	201.1	0.30	202.10	100.35	

### Determination of Soil Physicochemical Parameters

The organic carbon and total organic matter in the soil samples obtained were analyzed by modified Walkley-Black methods [11]. These methods operate on the basic principle of wet oxidation (digestion) of organic carbon in an acid dichromate solution followed by back titration of the remaining dichromate with ferrous ammonium sulphate or by photometric determination of  $\text{Cr}^{3+}$ . The particle size distribution in soil samples was determined by the Hydrometer method. The soil pH was determined with a pH meter.

### Bioaccumulation Factor

Bioaccumulation factor (BAF) was calculated as the ratio of the concentration of

OCPs in the cocoa beans to that in the corresponding soil in order to quantify the bioaccumulation effect of cocoa on the uptake OCPs from the soils [12]. The BAF was computed as:

$$\text{BAF} = \frac{C_c}{C_s} \quad (2)$$

where  $C_c$  and  $C_s$  represent the organochlorine concentrations in cocoa beans and soils, respectively.

### Data Analysis

All values are presented as mean  $\pm$  SD. Data were subjected to one-way analysis of variance (ANOVA) to determine any significant difference in the concentrations of the OCPs in cocoa and soil samples from the various farms. All data analysis were performed using SPSS ver 16 for windows.

## Results and Discussion

### Soil Physicochemical Properties

Table 3 showed that soil pH of the selected farms ranged from 6.78-6.83 in Oluji-1, 7.15-7.25 in Oluji-2, 6.57-6.85 in Owena and 6.96-7.10 in Idanre. The pH value of Oluji-2 was tending towards alkalinity, an indication of presence of cations such as calcium and sodium ions in the soil. The soils of other selected farms were slightly acidic. This should be expected from this type of humid soils which are subject to leaching [13]. The pH values in the various farms in our study were mostly acidic compared to 7.34-8.46 reported in four cocoa farms in Ghana [14]. The result of the percentage particle size composition of the soil samples showed that the soils were characterized by high sand contents with overall range of 68.84-73.58%, the clay composition ranged from 14.56% to 16.64% and the silt content of the soils were in the range 11.86% to 15.62%. This showed

that soil samples from the selected farms were all sandy loam in nature [15]. With high sand contents, the soils might be susceptible to leaching which could cause poor retention of organic carbon and organic matter, consequently poor retention of pesticides [15]. The overall ranges of the organic carbon and the organic matter were 1.68-2.56% and 2.90-4.43%, respectively. The range of the percentage organic carbon content of the analyzed soil samples was higher than 0.44-0.9% reported by Bentum *et al.*, [15], who considered the result of their findings, in the top soils of some cocoa growing areas in five districts of the central region of Ghana, to be very low. However, our values were lower than 0.8-6.12% and 1.54-10.6% reported for organic carbon and organic matter in soils from a cocoa farm in Ghana [14].

Table 3. Mean results of the physicochemical properties of soil from cocoa farm.

Farm	pH	Sand %	Clay %	Slit %	Org. Carbon %	Org. Matter %
<b>Oluji-1</b>	6.80±0.03	72.47±1.06	15.06±0.47	12.48±0.82	2.26±0.08	3.91±0.14
<b>Oluji-2</b>	7.20±0.05	69.85±1.58	15.99±0.58	14.61±1.54	2.16±0.16	3.74±0.27
<b>Owena</b>	6.73±0.14	70.95±0.45	15.03±0.26	14.02±0.63	1.76±0.11	3.05±0.19
<b>Idanre</b>	7.04±0.07	70.25±0.54	16.24±0.29	13.15±0.82	2.42±0.15	4.18±0.25

### Organochlorine Pesticide in Cocoa Beans and Soil

Table 4 shows that fifteen different pesticide residues were detected in the cocoa samples while Table 5 presents the results of the OCPs in the soil. Among various organochlorines determined in the present study, endrin and endosulfan I are the most prominently detected compounds, as they were detected at a high incidence of > 90% in both cocoa and soil samples (Fig. 2). On the other hand, lindane and heptachlor were detected at a low incidence and they were only present

in less than 20% of the analyzed cocoa samples while  $\alpha$ -HCH was present in < 15% of soil samples. Generally, the incidence of contamination of the examined cocoa samples by the organochlorines followed the order: endrin > endosulfan I > trans-permethrin > dieldrin > cis-permethrin > heptachlor epoxide > aldrin >  $\alpha$ -HCH >  $\delta$ -HCH > endosulfan II > endosulfan sulphate >  $\beta$ -HCH > p, p' DDT > heptachlor = lindane (Fig. 2). For the soil samples, the order was: endosulfan I > endrin > trans-permethrin > dieldrin > endosulfan II > heptachlor epoxide > cis permethrin > aldrin > endosulfan sulphate >  $\alpha$ -HCH. Lindane, heptachlor,  $\beta$ -HCH,  $\delta$ -HCH and p, p' DDT were not detected in the soil samples.

Table 4. Mean Concentrations (mg/kg) of OCPs in cocoa beans of the selected farms.

OCPs	Oluji-1	Oluji-2	Owena	Idanre
$\alpha$ -HCH	ND	0.047±0.035	0.037±0.005	0.095±0.005
$\beta$ -HCH	ND	0.039±0.005	ND	0.369±0.025
Lindane ( $\gamma$ -HCH)	ND	0.033±0.010	ND	ND
$\delta$ -HCH	0.072±0.006	ND	0.037±0.004	0.128±0.006
Heptachlor	ND	0.269±0.006	ND	ND
Heptachlor-epoxide (B)	0.042±0.002	0.040±0.002	0.178±0.025	0.090±0.047
Aldrin	0.123±0.113	ND	0.043±0.003	0.105±0.066
Dieldrin	0.051±0.012	1.038±1.091	0.354±0.293	0.070±0.065
Endrin	0.292±0.081	1.505±1.099	0.691±0.575	1.252±1.242
Endosulfan I	10.943±12.125	1.988±2.099	1.720±1.689	4.777±4.918
Endosulfan II	0.028±0.003	0.034±0.003	ND	0.056±0.002
Endosulfan sulphate	0.499±0.002	0.686±0.002	ND	3.679±2.916
p, p'-DDT	ND	ND	0.086±0.002	0.101±0.002
cis-Permethrin	0.076±0.002	0.155 ±0.062	0.256±0.200	0.660±0.002
trans-Permethrin	0.082±0.020	0.086±0.010	0.093±0.003	0.120±0.035
$\Sigma$ (OCPs)	12.208	5.92	3.495	11.502

ND=not detected; Detection limit = 0.0001mg/kg

Table 5. Mean Concentrations (mg/kg) of OCPs in Soil samples of the selected farms.

OCPs	Oluji-1	Oluji-2	Owena	Idanre
$\alpha$ -HCH	0.064 $\pm$ 0.003	ND	ND	ND
$\beta$ -HCH	ND	ND	ND	ND
Lindane ( $\gamma$ -HCH)	ND	ND	ND	ND
$\delta$ -HCH	ND	ND	ND	ND
Heptachlor	ND	ND	ND	ND
Heptachlor-epoxide (B)	0.318 $\pm$ 0.005	1.700 $\pm$ 1.029	0.105 $\pm$ 0.003	0.059 $\pm$ 0.038
Aldrin	0.349 $\pm$ 0.081	0.141 $\pm$ 0.004	ND	0.445 $\pm$ 0.025
Dieldrin	0.060 $\pm$ 0.008	0.041 $\pm$ 0.003	0.044 $\pm$ 0.003	0.064 $\pm$ 0.002
Endrin	0.282 $\pm$ 0.208	0.566 $\pm$ 0.569	0.131 $\pm$ 0.080	0.425 $\pm$ 0.284
Endosulfan I	1.320 $\pm$ 2.008	0.627 $\pm$ 0.879	3.295 $\pm$ 0.461	0.292 $\pm$ 0.384
Endosulfan II	0.098 $\pm$ 0.003	0.107 $\pm$ 0.097	0.035 $\pm$ 0.003	0.092 $\pm$ 0.005
Endosulfan sulphate	1.711 $\pm$ 0.003	1.219 $\pm$ 0.003	0.764 $\pm$ 0.003	ND
p,p'-DDT	ND	ND	ND	ND
cis-Permethrin	0.082 $\pm$ 0.007	0.279 $\pm$ 0.183	0.151 $\pm$ 0.004	0.077 $\pm$ 0.002
trans-Permethrin	0.095 $\pm$ 0.015	0.168 $\pm$ 0.078	0.079 $\pm$ 0.006	0.089 $\pm$ 0.013
$\Sigma$ (OCPs)	4.379	4.848	4.604	1.543

ND=not detected; Detection limit = 0.0001 mg/kg

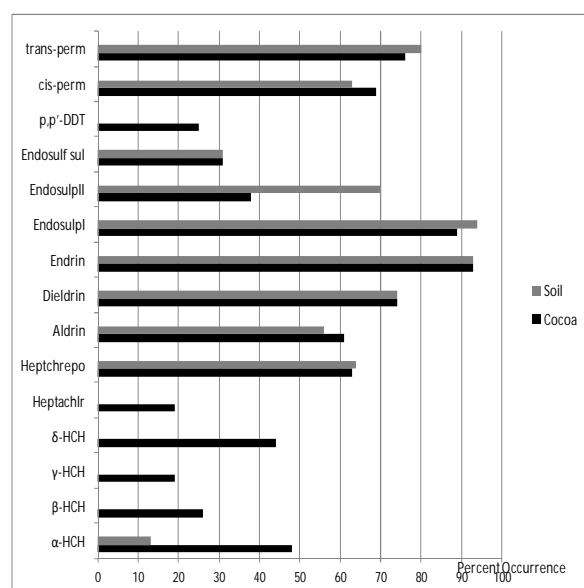


Figure 2. Percentage occurrence of pesticides found in cocoa and soil samples from all the four farms

### Hexachlorocyclohexane (HCH) and its Isomers

The  $\alpha$ -isomer of HCH was detected in 75% (15/20), 60% (12/20) and 55% (11/20) of cocoa beans with mean residual concentrations (mg/kg) 0.047, 0.037 and 0.095 from Oluji 2, Owena and Idanre farms respectively (Table 4). The highest mean residual concentration was recorded in Idanre farm (0.095 mg/kg). The  $\alpha$ -HCH was not detected in cocoa beans from Oluji 1 farm but was detected in 50% (10/20) of the soil samples from the farm. The  $\beta$ -HCH was only found in 50% (10/20) and 55% (11/20) of cocoa beans in Oluji 2 and Idanre farms, respectively. The highest mean residual concentration of  $\beta$ -HCH was recorded in Idanre farm. Lindane ( $\gamma$ -HCH) was only detected in 75% (15/20) of cocoa beans from Oluji 2 farm with a residual mean concentration of 0.033 mg/kg. This value is lower than 0.050 mg/kg and 1.000 mg/kg acceptable maximum residual limits (MRL) of FAO/WHO [16] and EU [17] for lindane but above 0.010 mg/kg MRL of France [18] for lindane. The  $\delta$ -HCH was detected in 50% (10/20), 75% (15/20) and 50% (10/20) of cocoa beans from Oluji 1, Owena and Idanre farms, respectively with the highest residual mean concentration (0.128 mg/kg) recorded in Idanre farm. The  $\beta$ ,  $\gamma$  and  $\delta$  HCHs were not detected in all the soil samples from the various farms while  $\alpha$ -HCH was only detected in soil from Oluji 1 farm. The mean residual concentrations  $\alpha$ ,  $\beta$  and  $\delta$ -HCHs in cocoa beans from all the farms were above MRL of 0.02 mg/kg established by European Union [17]. The mean residual concentrations of  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -HCH recorded in this study were similar to ( $\alpha$  = 0.040-0.850,  $\beta$  = ND-0.380,  $\gamma$  = ND-0.310 and  $\delta$  = ND-0.080) mg/kg reported by Boakye [19], for cocoa beans from Ashanti and Brong Ahafo regions of Ghana. However, our mean concentrations were much lower than 0.73-0.86, 0.13-0.47, 0.63-0.64, 0.65-

2.37 mg/kg reported for  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  HCHs, respectively in stored cocoa bean in Ondo and Ile-Ife [20]. The mean residual concentration of  $\alpha$ -HCH in the soil samples was comparable to ND-15.150 mg/kg reported by Okoya *et al.*, [21] in the soil samples of some cocoa farms in Ondo state. It is worthy to note that Idanre farm which is one of the oldest farms recorded the highest mean residual concentrations of HCH isomers ( $\alpha$ ,  $\beta$  and  $\delta$ ) in cocoa compared to the other farms.

High levels of HCH isomers were observed in cocoa samples compared to levels detected in the soil samples. This showed that the soil has low capacity to retain HCH isomers. This could be due to the general lipophilic nature of pesticides, which made them to accumulate more in cocoa beans with higher fat content in comparison to soil. Also, most HCH isomers especially lindane has relatively high water solubility and tends to partition faster from a gas phase into the water phase [22]. This makes them to be easily leached by rain and run off and could contaminate surrounding surface water.

#### ***Heptachlor and Heptachlor Epoxide***

Heptachlor was only detected in 75% (15/20) of cocoa samples with a mean residual concentration of 0.269 mg/kg in Oluji 2 farm (Table 4) but was not detected in all the analysed soil samples from the four farms. However, its oxygenated derivative, heptachlor-epoxide was detected in 50% (10/20), 50% (10/20), 75% (15/20) and 75% (15/20) in cocoa samples from Oluji 1 and 2, Owena and Idanre respectively. The highest mean residual concentration (0.178 mg/kg) of heptachlor-epoxide in cocoa beans was recorded in Owena farm (Oldest farm) while the highest mean residual in soil samples (1.70 mg/kg) was recorded in Oluji 2 farm (Table 5). The non-detectable nature of heptachlor in most of the cocoa samples could

be as a result of its rapid oxidation to its epoxide, attributed to favourable conditions for photochemical and biological degradation processes in the environment. The mean residual concentration of heptachlor and its metabolite, heptachlor-epoxide in cocoa beans analysed were above 0.020 mg/kg acceptable MRLs stipulated by FAO/WHO [16], European Union [17] and 0.010 mg/kg acceptable MRL in France [18]. The residual mean concentration of heptachlor in this study was comparable (0.004-0.492 mg/kg) reported by Boakye [19] in cocoa beans from Ghana. Also, concentrations of heptachlor-epoxide in the cocoa beans from this study were comparable with 0.010-0.370 mg/kg reported by Boakye [19] for cocoa beans in Ghana. However, the concentrations of heptachlor and heptachlor-epoxide in this study were lower than values reported in stored cocoa beans in Ondo and Ile-Ife [20].

#### ***Aldrin, Dieldrin and Endrin***

Aldrin was detected in 75% (15/20), 70% (14/20) and 100% (20/20) in cocoa beans samples with mean residual concentration (mg/kg): 0.123, 0.043 and 0.105 from Oluji 1, Owena and Idanre farms respectively (Table 4). Its oxygenated metabolite, dieldrin was detected in 100% (20/20), 75% (15/20), 75% (15/20) and 45% (9/20) in cocoa bean samples with mean residual concentrations (mg/kg): 0.051, 1.038, 0.354 and 0.070 from Oluji 1, Oluji 2, Owena and Idanre, respectively. The stereoisomer of the metabolite (Endrin) was detected in all the cocoa samples (100%) from Oluji 1, Oluji 2 and Idanre with mean residual concentrations (mg/kg): 0.292-1.505, 0.691, respectively. However, only 75% of the cocoa bean samples from Owena farm was found to contain endrin with mean residual concentrations 1.252 mg/kg. The mean residual concentrations of aldrin in cocoa beans from Oluji-1, Owena and Idanre farms were above the established MRLs of 0.010



mg/kg in France [18] and 0.020 mg/kg by FAO/WHO [16] while the mean residual concentration of dieldrin and endrin in all the farms were above this limit. Generally, the mean residual concentrations of aldrin, dieldrin and endrin in the cocoa samples from some of the farms were comparable to values reported by Okoffo *et al.*, [23] and Boakye [19] but lower than values reported by Oyekunle *et al.*, [20]. High concentrations of aldrin in the cocoa samples in some of the farms might be due to prolonged exposure or recent application of the pesticide sold under an unsuspected different trade name to the farmers since its use has been banned. The concentration of metabolite (dieldrin) was higher than that of the parent (aldrin) in most of the farms, indicating high rate of metabolism of the parent in the environment due to favourable conditions. Most aldrin introduced into the environment is rapidly converted through epoxidation to dieldrin, which in turn is notably persistent in the environment due to its very low solubility in water and its extremely low volatility [24]. Dieldrin is extremely apolar, and thus displays high affinity for fat. This makes it to be highly accumulated in the cocoa beans. However, the concentration of endrin, a stereoisomer of dieldrin was found to be higher than the concentrations of aldrin and dieldrin in the analyzed cocoa samples.

Aldrin, dieldrin and endrin were detected in all the soil (100%) samples from Oluji 1 farm with mean residual concentrations (mg/kg): 0.349, 0.060 and 0.282, respectively (Table 5). Aldrin and dieldrin and endrin were detected in 50%, 50% and 70% of soil samples from Oluji 2 farm, respectively. Seventy five percent and 100% of the soil samples from Owena farm were found to contain dieldrin and endrin, respectively while 75%, 70% and 100% of the soil samples in Idanre farm contained aldrin, dieldrin and endrin, respectively. The highest

mean residual concentrations for aldrin (0.445 mg/kg) and dieldrin (0.064 mg/kg) were recorded in soils from Idanre farm while Oluji 2 soil samples recorded the highest mean residual concentration of endrin (0.566 mg/kg). It was observed that the concentrations of aldrin were higher than dieldrin in all the farms. This could be an indication of recent application of the pesticide in these farms as Table 5 shows that the ratios of aldrin to dieldrin concentrations were: 7:1, 6:1 and 3:1 in Idanre, Oluji-1 and Oluji-2, respectively. These ratios shows that the soil sample from these farms contains high percentage of aldrin: Idanre (88%), Oluji-1 (86%), Oluji-2 (75%)) in the soil since technical grade aldrin pesticide contains 90% of aldrin. The results of this study for aldrin, dieldrin and endrin content in the analysed soil samples were above 0.0025 mg/kg, 0.0005 mg/kg and 0.001 mg/kg MRLs established by the Netherlands for aldrin, dieldrin and endrin respectively [25]. However, they were within the range ND-0.450 mg/kg reported by Okoya *et al.*, [21] in soil samples of cocoa plantations in Ondo state. However, the mean concentrations of endrin in the soil from Oluji-1 and Owena farms were within the range ND-0.300 mg/kg reported by Okoya *et al.*, [21]. The mean residual concentrations of dieldrin in this study were higher than values reported in four cocoa farm soil in Ghana [14].

#### ***Endosulfan I, Endosulfan II and Endosulfan Sulphate***

Endosulfan I and II were detected in 100% and 50% of cocoa samples from Oluji 1 farm, respectively while 75%, 50% and 50% of the cocoa samples from Oluji 2 farm were found to contain endosulfan I, endosulfan I and endosulfan sulphate, respectively. Endosulfan I was detected in 80% of cocoa samples from Owena farm while endosulfan I, endosulfan II and endosulfan sulphate were detected in 100%, 50% and 75% of cocoa samples from

Idanre farm, respectively. All the analysed cocoa samples had high concentrations of endosulfan I while lower concentrations were observed in its metabolite endosulfan sulphate, the product of degradation of endosulfan I and II (Table 4). This could indicate the recent use of endosulfan I on the farms. The mean residual concentrations of endosulfan I in cocoa samples were above the acceptable MRLs (0.100 mg/kg) for FAO/WHO [16] and European Union [17] while the mean residual concentrations of endosulfan II were lower than this limit. The concentrations of endosulfan I and II in some of the cocoa samples analysed were lower than ND-0.120 mg/kg and 0.040-0.110 mg/kg reported by Boakye [19] for endosulfan I and II respectively. However, the values in this study were higher than ND-0.256 mg/kg reported for endosulfan I and II in cocoa beverages in Nigeria [26]. Endosulfan I, endosulfan II and endosulfan sulphate were detected in 100%, 75% and 45% of soil samples from Oluji 1 and 2, respectively. Seventy five percent, 55% and 35% of soil samples from Owena farm were found to contain endosulfan I, endosulfan II and endosulfan sulphate while 100% and 50% of the soil samples from Idanre farm contains endosulfan I and endosulfan II. The mean residual concentration of most of the endosulfans and its metabolite in this study (Table 5) were above 0.050 mg/kg MRL acceptable in the Netherlands [25]. The high concentrations of endosulfan I in this study should be a source of concern due to the danger associated with it [27]. Endosulfan was reported to have caused deformation in children and infertility, blood and liver cancers in adults in areas where it was used for prolonged duration in cashew plantations [28].

### *p, p' DDT*

*p, p'* DDT was only detected in 50% of cocoa sample from Owena and Idanre farms

with mean residual concentration 0.087 mg/kg and 0.100 mg/kg, respectively (Table 4). These results were comparable to 0.10 mg/kg MRL established by FAO/WHO [16] but above 0.050 mg/kg established by European Union [17] and France [18]. The levels of *p, p'* DDT in the cocoa samples in this study were comparable to 0.040-0.440 mg/kg and 0.010-0.110 mg/kg reported by Aikpokpodion [29] and Bempah *et al.*, [30] respectively. Also, similar values (0.03-0.118 mg/kg) has been reported in cocoa beverages in Nigeria [26].

### *Permethrin*

Permethrin (*cis* and *trans*) isomers were detected in (50% and 100%) of the cocoa samples from Oluji 1, (100% and 75%) from Oluji 2, (75% and 55%) from Owena and (50% and 75%) from Idanre. Most of the analysed cocoa samples had levels of *cis*-permethrin above 0.10 mg/kg acceptable MRLs stipulated by European Union [17] while most samples had *trans*-permethrin below this limit. The levels of *cis* and *trans*-permethrin in this study were comparable to 0.190-0.600 mg/kg and 0.050-0.540 mg/kg reported by Boakye [19] for cocoa samples in Ghana respectively. Permethrin (*cis* and *trans*) isomers were detected in (95% and 100%) of soil samples from Oluji 1, (70% and 65%) from Oluji 2, (40% and 65%) from Owena and (45% and 90%) from Idanre. The ratios of mean concentrations of *cis*-permethrin to *trans*-permethrin in the soil samples analysed were approximately 1:1 in Oluji-1 and Idanre farms and 2:1 in Oluji-2 and Owena farms. The ratio of *cis*- to *trans*-permethrin in the soil showed that the *cis*-permethrin persisted more in the environment since the pesticide is formulated in ratio 1:3 (*cis*-permethrin: *trans*-permethrin). The high occurrence of permethrin in soil and cocoa samples is of great concern considering its stability and toxicity [31]. Permethrin is highly persistent in

soil, having a half- life 11.6 - 113 days in aerobic soils [31]. They have strong tendency to bind to soil and sediment and as such, not likely to leach through soil or move in the aqueous phase in runoff water [31]. Ingestion of permethrin may cause sore throat, abdominal pain, nausea, and vomiting [32] and US EPA has classified permethrin as likely to be carcinogenic to humans [31].

The total residual mean concentration of the OCPs in the various farms (Fig. 3) showed that the total OCPs were higher in cocoa samples than soil samples. It was also observed that Oluji 1 and Idanre farms have the highest total residual concentration in cocoa compared to Oluji 2 and Owena farms. However, Oluji 2 and Owena farms have the highest total OCPs concentration in soil samples. The results of ANOVA showed no significant difference between the mean concentrations of the pesticide residues in all the cocoa samples and soil samples from the various farms ( $p > 0.05$ ).

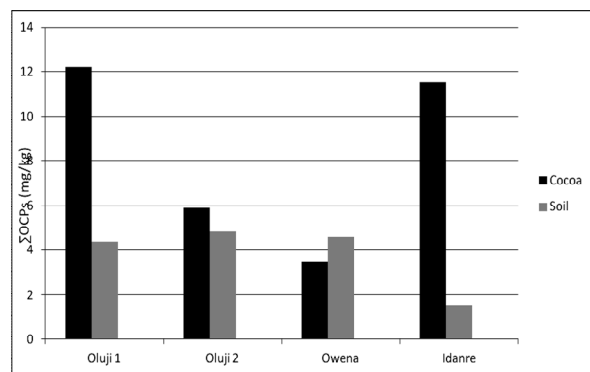


Figure 3. Total OCPs residues in cocoa and soil from the four farms

### Bioaccumulation Factor

Table 6 presents the bioaccumulation factor of the OCPs in cocoa from the different farms. In Oluji-1 farm, endosulfan I and endrin had bioaccumulation factor greater than 1 indicating that endosulfan I and endrin were not only absorbed but accumulated in the cocoa sample. In Oluji 2 farm, endosulfan I,

dieldrin and endrin were greater 1 with dieldrin having a much higher value (25.317). Owena and Idanre, the two oldest farms had much OCPs with BAFs greater than 1. Heptachlor epoxide, endosulfan sulphate, cis and trans-permethrin which had BAFs less than 1 in Oluji 1 and 2 were observed to have BAFs  $> 1$  in Owena and Idanre farms. The high bioaccumulation factors of the OCPs could also be attributed to their lipophilic nature which allowed them to be retained for long in the tissues. The bioaccumulation factors for heptachlor and HCHs isomers were very low in all the cocoa samples analysed.

Table 6. Bioaccumulation factors of OCPs residue in the cocoa samples from the selected farms in Ondo state.

OCPs	Oluji-1	Oluji-2	Owena	Idanre
$\alpha$ -HCH	-	0.047	0.037	0.095
$\beta$ -HCH	-	0.039	-	0.369
Lindane ( $\gamma$ -HCH)	-	0.033	-	-
$\delta$ -HCH	0.072	-	0.037	0.128
Heptachlor	-	0.269	-	-
Heptachlor-epoxide (B)	0.132	0.002	1.695	1.525
Aldrin	0.352	-	0.043	0.234
Dieldrin	0.850	25.317	8.045	1.094
Endrin	1.035	2.659	5.274	2.945
Endosulfan I	8.290	3.170	0.522	16.359
Endosulfan II	0.286	0.318	-	0.609
Endosulfan sulphate	0.292	0.563	-	3.679
p,p'-DDT	-	-	0.086	0.101
cis-Permethrin	0.927	0.555	1.695	1.348
trans-Permethrin	0.863	0.512	1.177	8.429

### Conclusion

The results of this study showed that the levels of pesticide residues were higher in cocoa beans than in top soil of the selected cocoa farms. This was attributable to the lipophilic nature of the pesticides. The results also showed that the ban imposed on HCHs isomers, DDT and heptachlor was effective as the levels of these pesticides were very low or non-detectable in both the cocoa and soil samples analysed from the selected farms. However, high concentrations of aldrin,

dieldrin, endrin and endosulfan in both cocoa and soil samples analysed indicated recent application of these pesticides on the selected farms despite the ban imposed on these pesticides. Considering the potential health and environmental hazards associated with high levels of the pesticides in cocoa beans and soil, stringent measure should be implemented to ensure compliance on the ban imposed on these pesticides. The paucity of data on the level of pesticide residues in cocoa beans and soil of cocoa farms made this study a suitable future reference on the assessment of pesticide residues on the cocoa beans and soil of cocoa farms in Nigeria and other regions where cocoa farming served as means of livelihood to farmers and viable source of foreign exchange. Similarly, the results of this study provided means of monitoring pesticide residues in the study area especially for the extension workers who disseminate information to the farmers.

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