Assessment of the Level of Pesticide Residue in Cocoa Beans from Selected Mega Stores in Ado Metropolis, Ekiti State, Southwestern, Nigeria

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Abstract:

***Introduction*: Approximation of 30%-40% of cocoa produced are lost to either pest or disease hence the dependence on the use and application of pesticides to boost productivity.**

Objective: This study was carried out to assess the level of pesticide residue in cocoa beans from selected mega stores in Ado metropolis of Ekiti State, Nigeria.

***Methodology*: Dry cocoa beans were sampled from nine largest cocoa stores in Ado Metropolis using a composite sampling technique. Sample results were analyzed using AAS spectrometer. Data obtained were analyzed using T-test, ANOVA, simple percentage method, frequency table, charts and graphs.**

***Result:* Concentrations of the pesticide residues in each cocoa bean samples were calculated (in mg/kg). A total of nine (9) pesticide residues were detected in cocoa bean samples drawn, Pesticide residues identified in the cocoa beans were Lindane, Dieldrin, Aldrin, Gamma HCH, DDE, DDD, DDT, Beta HCH and Heptachlor.**

***Conclusion:* From this study it was concluded that heptachlor is the most widely used pesticides of all the chemicals analyzed in cocoa samples from Ado metropolis of Ekiti State. It is suggested that relevant agencies should educate Cocoa farmers on the approved a pesticides and safe application of pesticide in such a way that it will be within EU permissible level while also instituting a monitoring mechanism to ensure that**

***Keywords:* Pesticides, Cocoa Beans, Residue, AAS spectrometer, Maximum Permissible Limit**

I .INTRODUCTION

It has been reported (www.blog.worldcocoafounadtion) that approximately 30 to 40 percent of all potential cocoa production is lost to diseases and pests. In localities with exceptional disease and/or pest infestation, losses can exceed 80 percent. In dollar terms, annual losses total approximately $2 billion. While these losses have an impact throughout the supply chain, it is the cocoa farmer that feels the most immediate and direct impact on family income. Depending on tree variety and region, cocoa farmers can face a variety of fungal diseases and insect pests that attack the leaves, stems, trunks, or pods of their cocoa trees. Farmers therefore resolved at using pesticide at controlling pests and diseases attacks on their plantations to reduce loss. According to Bateman (2010), the term "pesticide" can be defined simply as any substance which is used to control a pest at any stage in crop production, storage or transport. It is now generally agreed that the term "pest" applies to any organisms that harm crops, be they insects, diseases, weeds, among others.

Pesticides have been used on cocoa for more than 50 years, with notable early research carried out independently in the former West African Cocoa Research Institute (now the research institutes of Ghana and Nigeria), Brazil, Cameroon, Costa Rica, Côte d'Ivoire, Indonesia, Malaysia and Togo. By the early 1970s a number of effective control techniques had become "established", and there was little incentive for change until environmental awareness increased in the 1990s. Most notable amongst these were concerns over the use of lindane for the control of cocoa insect pests; this was eventually phased out - but in some countries, not until the early 21st century. Many farmers believe that pesticides work, at least against some cocoa pest problems, and continue to use them - depending on the pest and country Cocoa has a relatively 'green image' and cultural methods (removal of diseased plant parts, *etc*.) are the most proven and cost effective first line of defence against diseases and insects.

However, pesticides are used on cocoa in certain circumstances (cases in category 1 of the table above are of greatest interest). Implementation by farmers of all control methods is often poor, and furthermore Cocoa (*Theobroma cacao* L.) is a perennial crop mainly cultivated in Africa, the Caribbean, South America and Asia (United Nations Development Programmes (UNDP), 2010) It is the leading cash crop in West Africa with over 70% of world cocoa production cultivated in the region (Afrane and Ntiamoah, 2011). Cocoa is cultivated either in agro-forestry systems in which some part of the natural forest is left in place or in newly cleared or converted land. This involves that the new land must be cleared under conditions which are ecologically not friendly (Asare, 2006). Cocoa grown under the canopy of original forest is considered the most environmentally friendly

form of production. Even though shade-grown cocoa has its attendant consequences such as loss of biodiversity, shade systems have been shown to have higher biodiversity than full-sun systems. Shade system requires less pesticide and this may contribute to higher levels of biodiversity which is associated with better pest control, pollination and more efficient nutrient cycling (Clay, 2014).

A pesticide is a toxic chemical substance or a mixture of substances or biological agents that are intentionally released into the environment in order to avert, deter, control and/or kill and destroy populations of insects, weeds, rodents, fungi or other harmful pests. Pesticides work by attracting, seducing and then destroying or mitigating the pests (Mahmood *et al.*, 2016). The pesticide used is dangerous to the environment and the health of the person applying the pesticide. Pesticides promise the effective mitigation of harmful bugs, but unfortunately, the risks associated with their use have surpassed their beneficial effects. Most of the pesticides reach a destination other than their target, nonselective pesticides kill non-target plants and animals along with the targeted ones (Mahmood *et al.*, 2016). Pesticide contaminates land and water when it escapes from production sites and storage tanks, when it runs off from fields, when it is discarded, when it is sprayed aerially and when it is sprayed into water to kill unwanted plants (Tashkent, 2008). Pesticide residue may enter streams through run-off and pose dangers to fish, birds, wild animals and plants in the aquatic habitat. Pesticides often are degraded in water (hydrolysis), by sunlight (photo degradation), and by soil and aquatic microorganisms (microbial degradation). Application rates and techniques have direct bearing on how a pesticide enters the environment. In addition, persistent pesticides such as DDT pesticide may bio-accumulate, move through the food chain and eventually be ingested by and adversely affect birds, wild animals and domestic livestock. Methyl bromide which is currently being replaced by phosphine for the fumigation of stored cocoa beans has been identified as an ozone-depleting substance (Oluromininyi and Emily, 2011).

Cocoa beans business in Nigeria is dated back to over 60 years ago before Nigeria got independence and before the country focused more on crude oil business. Cocoa business was not as booming as pro-independence era but it is still much in the market. Farmers embraced the use of organic chemicals and pesticides during and after plantation to enhance productivity. The over-dependence on the use of pesticides has become worrisome hence the need to find out and determine the level of pesticide residue in these cocoa products and identify their health effects on consumers. (Akanji et al 2016)

In the last several decades, many nations of the sub Saharan Africa region of West Africa have remained in the forefront of cocoa production far higher than other areas of the globe. With that have come larger export earnings due to rising demands and the reliance on cocoa in the manufacture of various products driving consumer demands in markets at local, regional and international levels. In as much as current cocoa production practices are shaped partly by pressures from transactions in the marketplace and regulatory frameworks in the West African region and beyond. (E. C. Merem et al 2020)

The growing activities of cocoa farming has in the past several years, left in its wake negative environmental liabilities that are now over stretching the capacity of natural areas in the zone. While there exists widespread use of agrochemicals to boost production along with the associated impacts of water pollution, in some places. The situation is now so critical that the expansion of cocoa plantations into vast forest landscapes known for their services as emission sinks, are now leading to ecosystem disturbances. Other risks from cocoa land use activities in West Africa involves the exposures to soil erosion, the flow of sediment loads onto local streams and the spreading of cocoa plant diseases which has emerged as a major issue to the detriment of communities and the surrounding ecology (E. C. Merem et al 2020).

The growing global concerns about the effects of the increasing use of agricultural chemicals by farmers, consumers of agricultural produce and the ecology require a re-examination of the issues related to their application and concentration in the cocoa products (Owusu et al., 2012)

In 2011, Aldrin was detected in cocoa bean sample from selected markets in Ondo State and the residue were discovered to be relatively high in some samples accounting for 40% of cocoa major market in Ondo State (Olusa 2011)

There is an increasing use of banned pesticides and their side effects in Nigeria. According to Okeke (2010, p. 142), cocoa farmers are the most affected because of the levels of chemical requirement in cocoa farming. It was observed by Okeke (2010, p.140) that cocoa pesticides constitute about 37% of total annual agro-chemical usage in Nigeria. However, the expanding use of these chemicals without proper regulations and controls has contributed to an increasing vulnerability of farmers in Nigeria, where;

- Farmers are exposed to these chemicals without appropriate safety measures.
- They are seriously affected by the impacts of these pesticides.
- There is a lack of availability and access to resources that could improve the adaptive capacity of farmers to pesticide hazards.
- Farmers lack effective adaptive strategies or the capacity to prepare, cope and recover from the impacts of pesticide hazards, and;
- There isn't an effective effort in mitigating the impacts of these chemicals on farmers.
- There is persistence of these chemicals in the environment.
- Residue of these chemicals is found in cocoa beans and Cocoa products.

Objectives

1. To determine the various pesticides found in Cocoa beans in Ado-Ekiti metropolis.
2. To identify the concentration level of pesticide found in Cocoa beans in the study area.
3. To compare the levels of the pesticides found in Cocoa beans in the study area with International Allowable Maximum Residue Levels (MRL).

Research Hypothesis

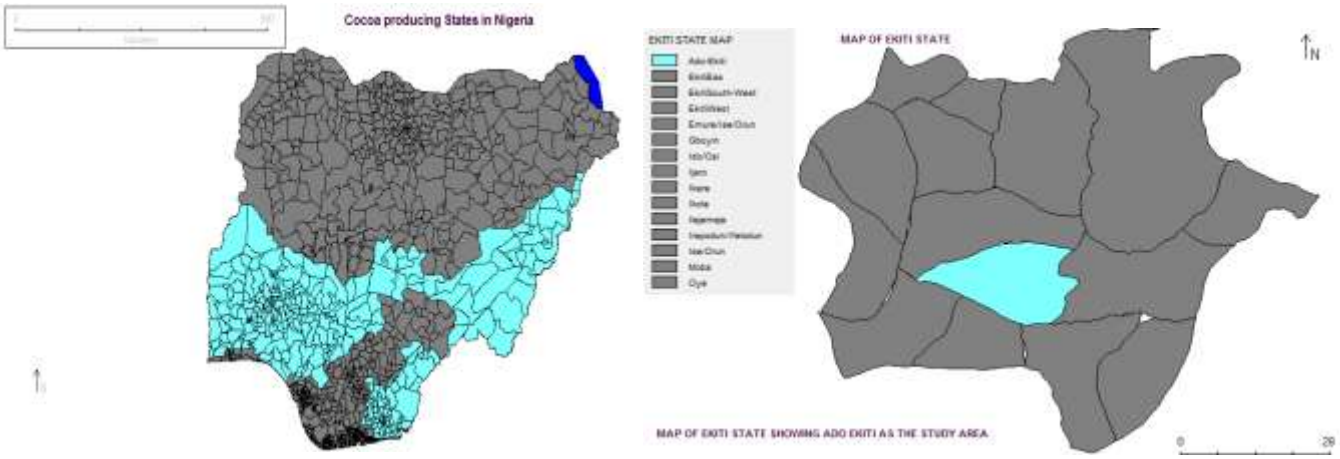
Ho: There is no significant difference in the level of pesticide residue in Cocoa beans in Ado Metropolis, Ekiti State.

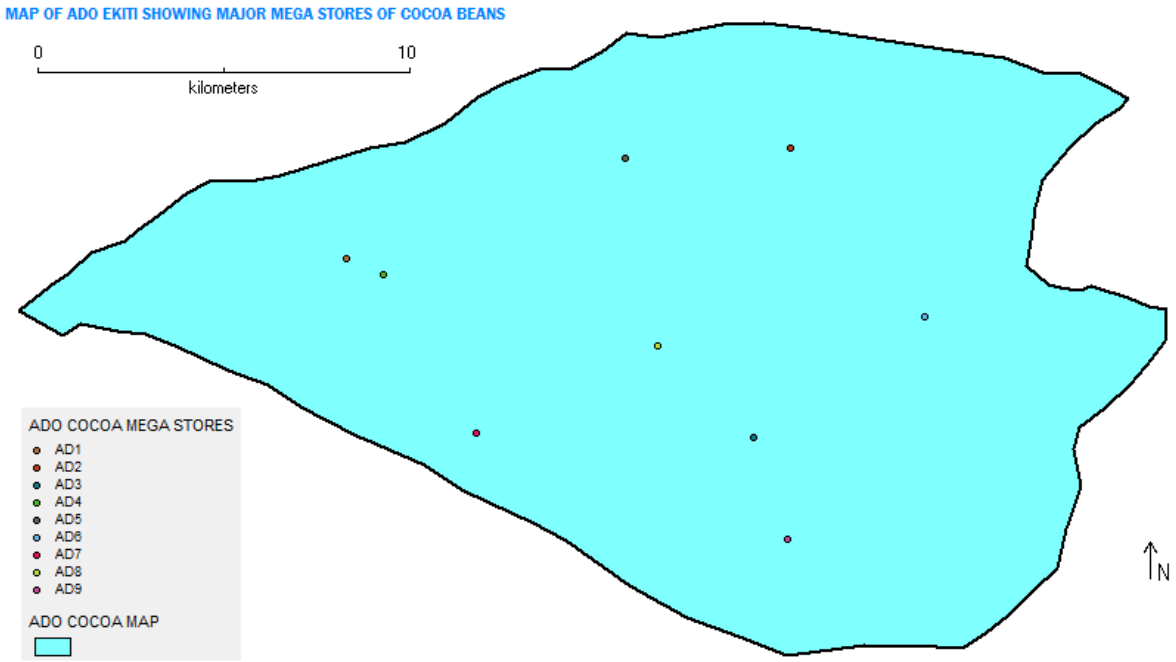
H1: There is significant difference in the level of pesticide residue in Cocoa beans in Ado Metropolis, Ekiti State.

II. METHODOLOGY

Description of the Study Area

The study area for this research work is Ekiti State, located in Southwest Nigeria sharing boundary with Ondo State, Kwara State and Osun state. Ado-Ekiti is located at Longitude 5.26660 & Latitude 7.58312 with Length 0.746254356298 and Area 0.026378179515. Ado-Ekiti has land mass for agriculture which includes but not limited to cultivation of yam, cocoa, cassava, vegetables, and cocoyam.





III. SAMPLING PROCEDURES, COLLECTION AND PREPARATION

The sampling technique used is a composite sampling technique. Dry cocoa beans were sampled from nine largest cocoa stores in Ado Metropolis. From the dried cocoa bags, a long tong was used to draw samples from each bags and bulked into a container. The tong holds about 20 -23 cocoa beans at once. The bulked samples was mixed together and divided into quarters. I purchased two quarters out of the four and rejected two quarter, the purchased quarter were packaged into mini-bags and this process was repeated in each of the major stores selected, making a total of eighteen 18 mini-bags of cocoa beans purchased from nine major store of cocoa beans in Ado Metropolis. After this process, the researchers mixed the samples from each stores together and picked 18 beans randomly. The 18 cocoa beans were divided into two mini-pack making up of nine (9) cocoa bean in each mini pack designated for each market stores hence, the researcher finally drew a sample bag from each store. The sample bags were then packaged and labeled using the market store codes (AD1, AD2, AD3, AD4, AD5, AD6, AD7, AD8 & AD9 and were sent to the laboratory for analysis.

Sample preparation

Stage 1	Stage 2	Stage 3
Samples were grinded	Sample were weighed in a jar of 150ml	10ml of distilled water was added and left for 10 mins
Stage 4	Stage 5	Stage 6
20ml of acetonitrile added and homogenized for 2mins	Sample centrifuged @2000rpm for 5mins	Filtered into a 50ml volumetric flask
Stage 7	Stage 8	Stage 9
10ml of sample was filtered and pipetted into a separating funnel	5g of NaCl and 10ml of 0.5mol/L phosphate buffer (pH 7.0) was added and shaken for 10-15 minutes	Aqueous layer removed and acetonitrile layer collected for clean up
Step 10	Step 11	Step 12
Sample loaded into cartridge and eluded with 10ml of acetonitrile	Sample transferred into 50ml RBF and evaporated at 30oC using a rotatory evaporator	5ml acetone was added to the flask and dissolved in 2ml ethyl acetate
Step 13		
Extract stored in a refrigerator until analysis		

Data Analysis

The results were analyzed using simple percentage, graphs, charts, mean, S.D, Variance, ANOVA, t-test @ 0.05 significant level using STATA MP12 and SPSS.

Sample Analysis

20 ml of sample was reduced to 2ml hence we have 202=10. Therefore, the concentration factor for the sample is ten. (F=10). 20ml was reduced from 100ml sample solution hence 10020=5

$$\{[CF] \times 5\}/M$$

Eq 1: used to calculate the concentration of pesticides in mg/kg

Where C = concentration in µg/ml

F = Concentration Factor (10)

M = mass in grams

IV. RECOVERY

The percentage recovery was calculated as: % Recovery= Amount of analyte recovered Amount of analyte spiked×100

Equation 2: used to calculate the recoveries of the samples

The amount of analyte recovered was calculated by subtracting the non spiked sample from the spiked sample.

Inclusion and exclusion Criteria

Inclusion Criteria

Only the nine selected mega stores of cocoa beans in Ado Metropolis are included which are Olaoluwa Cocoa Store, C-C cocoa room, Adesua Cocoa stores, Pa Anjorin Ccoca House, B&B Cocoa Store, Oyediran Cocoa Mall, Cash & Price Cocoa store, Ibidun cocoa Sale House and Big Mummy & Sons Cocoa Mega Store.

Exclusion Criteria

Not all Cocoa beans stores in Ado Metropolis were included; the mega stores were included while the small cocoa stores were excluded.

V. RESULTS & DISCUSSION

Table 1 Retention Times for Pesticides

S/N	STANDARDS	RETENTION TIMES [MINS]
1	BETA HCH	13.736
2	GAMMA HCH	13.867
3	HEPTACLOR	25.292
4	DIELDRIN	20.791
5	ALDRIN	17.563
6	DDD	21.990
7	DDT	23.348
8	DDE	22.540
9	LINDANE	15.567

For the Multi-residue analysis, mixed standard solutions in ethyl acetate were prepared from each pesticide standard by serial dilutions of the stock standard.

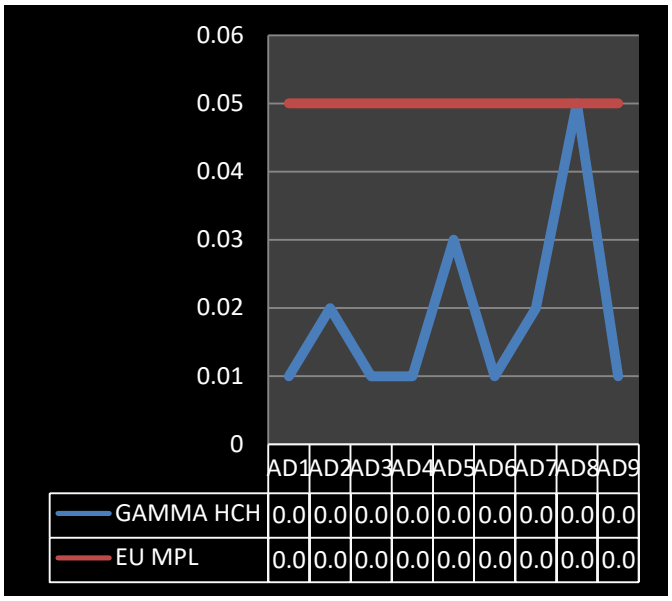
Table 2: Concentration of Pesticide Residue (Mg/Kg) In Cocoa Beans From Selected Major Stores In Ado Metropolis (Ad1 – Ad9)

SAMPLE	LINDANE	DIELDRIN	ALDRIN	GAMMA HCH	PpDDE	PpDDD	PpDDT	BETA HCH	HEPTACOR
AD1	ND	0.01	0.02	0.01	0.01	ND	0.05	0.03	0.02
AD2	0.01	0.01	0.01	0.02	0.02	ND	0.05	0.04	0.05
AD3	0.01	0.01	0.01	0.01	ND	ND	0.06	0.01	0.04
AD4	0.02	0.03	0.02	0.01	0.01	0.01	0.08	0.02	0.14
AD5	0.02	ND	ND	0.03	0.02	ND	0.08	0.04	0.15
AD6	0.02	0.02	0.01	0.01	0.03	ND	0.09	0.03	0.05
AD7	0.01	0.03	0.02	0.02	0.01	0.01	0.10	0.03	0.13
AD8	ND	0.02	0.04	0.05	0.02	ND	0.06	ND	0.04
AD9	0.02	0.01	0.02	0.01	ND	ND	0.07	0.01	0.04

4GAMMA HCH

Gamma HCH residues was detected in all the cocoa beans sampled in the different locations in Ado metropolis. The concentration of Gamma HCH ranged from 0.01 mg/kg to 0.04 mg/kg in cocoa beans from Ado metropolis. Most of the cocoa beans sampled for Gamma HCH from Ado metropolis had concentrations below the MRL value of 0.05 established by EU with the exemption of sample AD8. This negates the findings of Olalekan 2014 who found out that all Gamma HCH residue found in all samples are (0.02–0.03 mg/kg). Cocoa beans sampled from the mega stores in Ado metropolis had concentrations of beta HCH which were below detection limit (0.05 mg/kg) for samples from all the major market stores. The highest concentration of 0.02 mg/kg was recorded for cocoa beans from AD1, AD2, AD6,AD9 while AD3, AD5, AD7 and AD8 had no residue of Beta HCH. The samples with residue concentrations are below EU MRL of 0.05 mg/kg. From literatures, it is common to find high levels of Gamma HCH residues in food samples especially in the tropics because the higher temperature conditions favour its photochemical degradation as compared with the temperate region (Stockholm Convention, 2007). Due to its persistence Gamma-HCH can still be detected at low levels in the environment. In a study conducted in 14 districts of Haryana in India 140 bovine milk samples collected between 1998 and 1999 were analysed for organochlorine pesticide residues. Four percent (4%) of the samples exceeded the MRL of 0.05 mg/kg as recommended by WHO for Gamma-HCH (Sharma *et al*., 2006). A monitoring study of 192 samples of cow's milk from Mexico revealed 0.001 - 0.201 mg/kg alpha-HCH (ATDSR, 2005) On the other hand the trend for the HCH isomers for cocoa beans sampled from Ado metropolis was gamma<beta. The concentration for Gamma HCH ranged from 0.01 mg/kg to 0.05 mg/kg. The lowest concentration of gamma HCH detected in cocoa beans was from AD1, AD3, AD4, AD6 and AD9 respectively with highest concentration found in cocoa sample from AD8 at 0.05mg/kg.

Fig 1 Concentrates Of Gamma HCH in Cocoa Samples



Sample AD8 recorded highest value of Gamma HCH detectable in samples which is within EU MPL at 0.05mg/kg

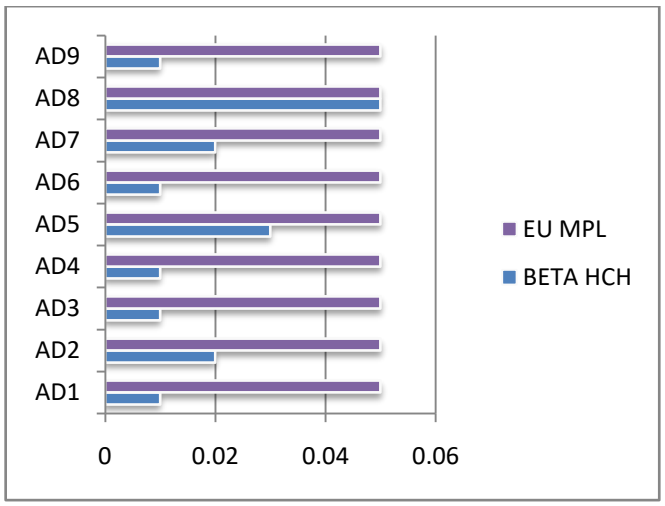
BETA HCH

The concentration of Beta HCH residues were within the EU allowable limits in cocoa beans.

Cocoa beans sampled from the mega stores in Ado metropolis had concentrations of beta HCH which were below detection limit (0.05 mg/kg) for samples from all the major market stores. The highest concentration of 0.02 mg/kg was recorded for cocoa beans from AD1, AD2, AD6,AD9 while AD3, AD5, AD7 and AD8 had no residue of Beta HCH. The samples with residue concentrations are below EU MRL of 0.05 mg/kg. All samples had concentration of Beta HCH which are within EU MPL despite samples from AD2 and AD5 recording the highest Beta HCH concentration at 0.04mg/kg except for samples from AD8 which has no concentration Beta HCH. The lowest concentration of gamma HCH was found in samples from AD3 and AD1.

This is different from the result of Olayinka et al 2017 who detected abundance of Beta HCH in most of cocoa samples and also stated that they are beyond maximum permissible limit.

Fig 2 Concentration of Beta Hch in Cocoa Samples



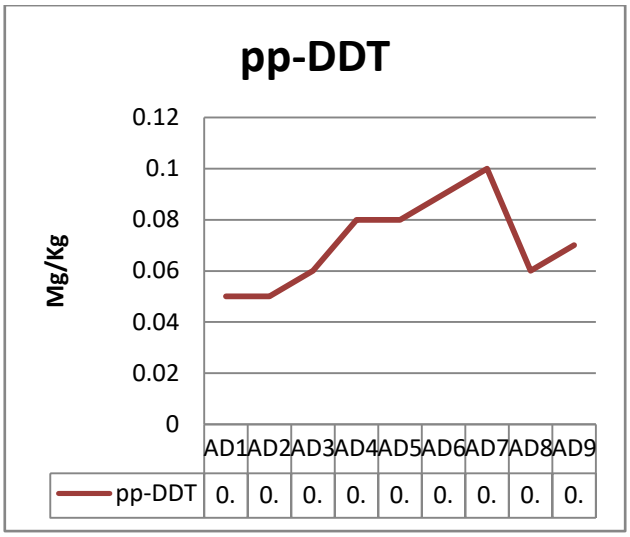
Concentrates of BETA HCH are found to be within the limit of EU MPL at 0.05mg/kg.

DDT

For DDT and its metabolites, DDT was detected in 100% of the analysed samples. It could be inferred from the trend that there is a continuous use of DDT in the area due to its lower cost and effectiveness as well as its broad spectrum activity despite its ban (Amoah *et al.*, 2006).

The higher concentrations could also be attributed to the enormous past uses and due to its longer slow degradation and persistence in the environment. For DDT, the lowest concentration was 0.05 mg/kg and was recorded for cocoa beans from AD1 and AD2 and the highest concentration was 0.10 mg/kg which was recorded for cocoa beans from AD9. This is similar to the result by Olalekan 2014 where in the most frequently found and abundant pesticide residue was the metabolite of DDT (p,p'-DDT) which occurred in 62.5 % of the samples though he stated that none of the detected pesticide mean residues recorded from the various study sites exceeded their European Union (EU) Maximum Residue Limits (MRLs) for cocoa beans whereas my findings revealed that all the samples showed concentration of DDT above Union (EU) Maximum Residue Limits (MRLs) for cocoa beans. Bate 2014 in his study also discovered DDT in Cocoa samples drawn from five major stores in South Africa while Atanda 2012 discovered residues of dichloro-diphenyltrichloroethane DDT in all samples drawn. DDT has been banned from agricultural use and restricted due to public health concern under the Stockholm convention in which Nigeria is a signatory (Akanni et al. 2010; Agbeve et al. 2014). Therefore, the occurrence of DDT and its metabolites in cocoa beans from the study area is an indication of the current illegal use of the pesticide by cocoa farmers in the study area. This is confirmed by the occurrence and high concentration of p,p'-DDT compared to its metabolites p,p'-DDE and p,p'-DDD, which is an indication that there might be recent input of DDT in the various cocoa plantations. The continued use of DDT products by the farmers could be due to their efficacy and lower price. It could also be that, new pesticides containing DDT as its active ingredient but un-familiar trade names were sold to the uninformed farmers. A similar observation was made by Aikpokpodion et al. (2012). Similarly, Adu-kumi et al. (2010) noted DDT contamination at some sampled sites in Ghana and attributed the presence to their recent use. However, the higher concentrations of DDT could also be attributed to the enormous past uses of the parent compound DDT and due to its longer half-life (i.e., slow degradation and its long term persistence in the environment).

Fig 3 Concentration of DDT in Cocoa Samples



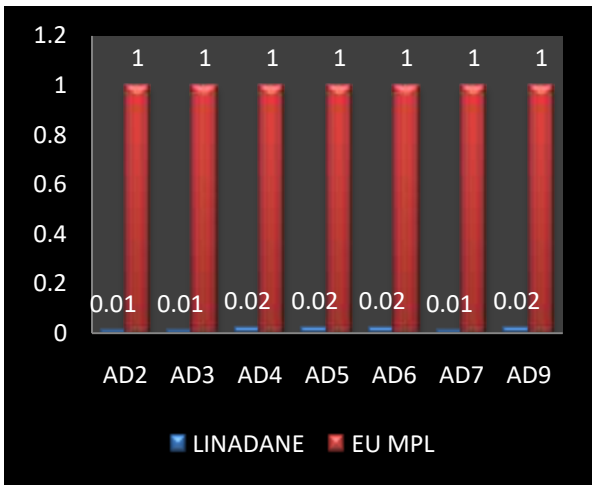
The concentration of DDT is significantly abundant in all the samples drawn from the study area with the highest concentration detected in AD7.

Lindane

Lindane concentration observed in this study was higher than the mean concentrations of 0.01 mg/kg, 0.01 mg/kg, 0.01 mg/kg and 0.0001 mg/kg reported by Botchway (2010), Frimpong et al. (2012), and Olayinka (2013) in cocoa bean samples, respectively. However, the mean lindane concentration observed in this study was lower than the concentration of 0.411 mg/kg reported by Apau and Dodoo (2010) in cocoa beans from the Central Region of Ghana. The findings of lindane in this study was contrary to that of Olayinka et al. (2010) which recorded no lindane residue in cocoa beans from selected farms in Ondo State. This could be due to none use of pesticides containing lindane as active ingredient in cocoa production in the state.

On the other hand, the concentration of lindane in cocoa sample from Ado metropolis is notable in most of the samples but in agreement with result of Bovi 2015 who stated that Lindane residue found in cocoa samples were below EU MPL with the highest concentration of lindane residue from samples in Ado metropolis at 0.02mg/kg.

Fig 4 Concentrates Of Lindane in Cocoa Samples

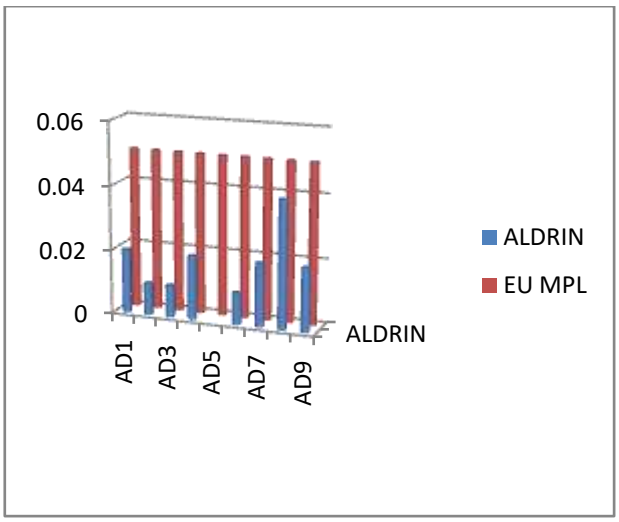


Lindane residues in all the samples are within EU MPL.

Aldrin

Aldrin is readily converted to dieldrin (once it enters either the environment or the body), which is considered one of the most persistent of all pesticides (Miles et al. Hogarh et al. 2014). These pesticides were used extensively in agriculture until their use was restricted. Although the use of aldrin and dieldrin is banned in many countries, these pesticides were detected in the cocoa bean samples analysed from the study area. Aldrin was detected in all the samples except sample from AD5 while the samples with concentration of aldrin residue ranged from 0.01mg/kg at AD2, AD3, AD6 to 0.04 mg/kg at AD8 with a mean value of 0.03 ± 0.01 mg/kg. This was very close to the one detected by Aminu F.0 2019 results which revealed that the concentrates of Aldrin residue was found in samples and they were significantly below EU MPL. The mean concentrations of aldrin recorded in this study were below the EU MRL of 0.05 mg/kg for cocoa beans. Aldrin is more volatile and readily degrades to dieldrin in the environment. However its metabolite was still present in cocoa beans because of its stability, lipophilicity and bioaccumulation in fats. More than 56% of the original weight of aldrin converts to dieldrin and about 19% of the original aldrin weight disappears (Osibanjo,2009). Levels of dieldrin in cocoa beans ranged from 0.03 mg/kg for samples from Brekum, Nkrankwanta, Sankore and Kukuom to 0.06 mg/kg for samples from Asumura. Concentrations of dieldrin in samples were within the EU allowable level in cocoa beans All the samples had levels below the Japanese MRL value of 0.10 mg/kg. A study conducted in 2009 by Adeyeye and Osibanjo revealed levels of aldrin in kolanut below detection limit. The result above is similar to the result of Olusa in (2011), who said that Aldrin was detected in cocoa bean sample from selected markets in Ondo State and the residue were discovered to be relatively high in some samples accounting for 40% of cocoa major market in Ondo.

Fig 5 Concentration of Aldrin in Cocoa Samples

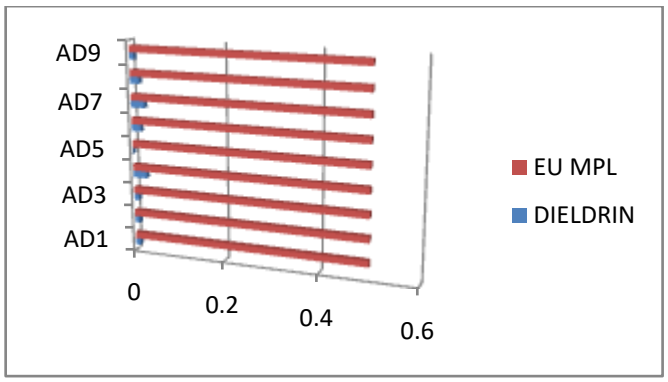


Showing concentrates of Aldrin detected are within EU MPL with the highest spike in sample from AD8 and not found in sample AD5.

Dieldrin

Similarly, its breakdown product, dieldrin, was recorded in all of the cocoa bean samples analysed except for sample AD5 with a mean value of 0.02 ± 0.00 mg/kg, which ranged from 0.01mg/kg at AD1, AD2,AD3 to 0.03 mg/kg at AD4 and AD7 respectively. Similarly, there were no significant differences ($p > 0.05$) in mean values of dieldrin among the sampled sites. The mean concentrations of dieldrin recorded in this study were below the EU MRL of 0.50 mg/kg for cocoa beans. The occurrence of aldrin and dieldrin in the cocoa bean samples analysed suggests the continual use of the pesticide (pesticides with aldrin and dieldrin as their active ingredient) by cocoa farmers in the study area, and/ or previous use of the chemicals, since their use for agricultural purposes which is very close to the one detected by Afful et al.2010; Hogarh et al. 2014). In addition, the occurrence of dieldrin in the samples confirmed the possible degradation and/or metabolism of aldrin to dieldrin (which is stable, lipophilic and bioaccumulate in fats) in the environment. A similar observation was made by Boakye (2012). However, the relatively high percentage of cocoa beans (43.8 %) with detectable aldrin compared to samples (37.5 %) with detectable dieldrin, might also suggest a more or high input of aldrin at present than their degradation to dieldrin. The mean value of aldrin recorded in this study was higher than the mean values of 0.01 mg/kg and 0.01 mg/kg reported by Frimpong et al. (2012a) and Frimpong et al. (2012b), respectively, but lower than the 0.11 mg/kg reported by Daanu (2011) respectively. This could be due to the differences in the sampling methods used (in that, cocoa bean samples for this study was taken from cocoa market stores while samples used in the other studies were taken from cocoa farms.

Fig 6 Concentrates Of Dieldrin in Cocoa Samples

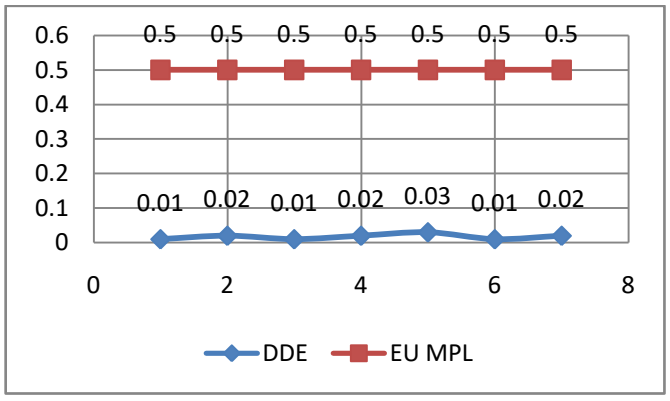


All samples are far below EU MPL.

DDE

p,p'-DDE was detected in all the samples except AD3 and AD9 while other samples with detectable level of DDE residue has mean concentration of 0.02 ± 0.00 mg/kg. The measured concentrations ranged from 0.03 mg/kg at AD6 and 0.01 mg/kg at AD1, AD4 and AD7. However, the differences in means were not statistically significant ($p > 0.05$). p,p'-DDE, one of the metabolites of DDT is formed by the loss of hydrogen chloride (dehydrohalogenation) in DDT. They are fat soluble and have the capacity to build up in the fat of animals and humans, and are rarely excreted from the body (Akinola et al. 2012). Studies have showed that exposure to p,p'- DDE can cause endocrine disruptions, oxidative stress, Alzheimer's and Parkinson's disease, contributes to breast cancer and damage the brain's dopaminergic system (Akinola et al. 2012). The mean concentration of p,p'- DDE recorded in this study was lower than the mean value of 0.04 mg/kg reported by Akinola et al. (2012) in cocoa bean samples from three cocoa ecological zones in Nigeria, but higher than the 0.001 mg/kg reported by Frimpong et al. (2012a) in cocoa beans ready for export in Ghana. Also, the mean value of p,p'-DDE observed in this study was similar to the 0.02 mg/kg reported by Nnamdi(2011). This study revealed that pp DDE residue in cocoa samples from Ado metropolis is below EU MPL.

Fig 7 Concentrates Of DDE in Cocoa Samples



Concentrates of DDE found in cocoa samples were within EU MPL.

DDD

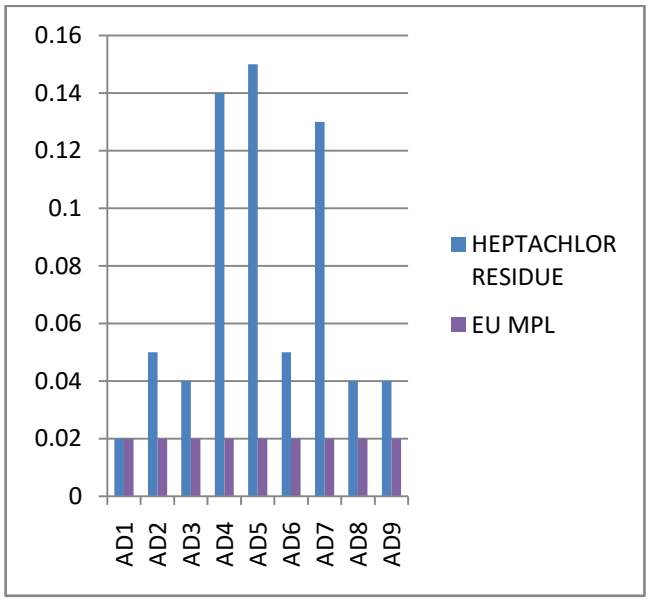
p,p'-DDD residue was detected in sample AD4 and AD7 while the rest of the sample has no record of pp-DDD residue. The mean value of the sample is 50 % of 0.00 ± 0.00 mg/kg with both samples showing the presence of pp-DDD residue at 0.01mg/kg. There were no significant ($p > 0.05$) differences in mean values of p,p'-DDD among the sampled stores. The mean p,p'-DDD residue observed in this study was lower than the mean value of 0.15 mg/kgthe mean values of 0.01 mg/kg and 0.001 mg/kg reported by Daanu (2011) and Frimpong et al. (2012a), respectively. p,p'-DDT was the most frequently detected residue in the samples analysed. The study reveals that the residue found in the two samples were far below the EU MPL which contradicts the result by Akanni 2014 in his results revealing the abundance of DDD in cocoa samples drawn from farms in Osun State. This may be attributed to distribution and availability of DDD in Osun state markets as against markets in Ekiti State. Generally the concentrations of DDD were lower than the parent compound DDT. This is because there are fresh inputs of DDT into the cocoa beans. In this study the presence of both DDT and its metabolite DDD in the samples may be due to the persistence and long range transport nature of DDT and its metabolite DDD (Finns *et al.*, 2005) even though they have been banned since 1985 (EPA 2008). The concentration of 4,4-DDT was higher than that of its metabolite p,p'-DDD. This may be that most of the DDT massively used in the past is in the metabolite state and fresh input of DDT in the environment is increasing in Ado metropolis. DDD residues were not detected in cocoa beans sampled from AD1,AD2,AD3,AD5,AD6, AD8 and AD9. The other two samples were below the EU value of 0.50 mg/kg. Conversely all the samples analysed were below the EU permissible level of 0.50 mg/kg.

Heptachlor

Heptachlor was present in all the cocoa beans. Concentrations of heptachlor were detected in all the cocoa samples drawn from the major stores in Ado metropolis. This could be due to the incomplete breakdown of heptachlor which can be attributed to unfavourable degradation conditions and persistence in the environment though it is among the list of banned pesticides by Stockholm Convention for use on food (Afful *et al*, 2010). Concentrations of heptachlor ranged from 0.15 mg/kg for cocoa beans from AD5 to 0.02 mg/kg at AD1. All cocoa beans samples drawn were above the EU MRL value of 0.02 mg/kg. All the cocoa beans sampled from the study area had higher values than the EU MRL of 0.02 mg/kg except from AD1. It was observed that the concentration of heptachlor was higher than its metabolite. Generally concentrations of Heptachlor from AD4,AD5 and AD7 were significantly higher than those from AD1,AD2,AD3,AD6,AD8 and AD9 respectively. This is in agreement with the result revealed by Adetoun 2011 wherein Heptachlor was found to be present in sample 1,4,7,13 in concentration above WHO MPL. The result of this study is also substantiated with the result of Joel Kweku B.P 2017 who

in his study revealed that cocoa farmers used both registered and unapproved chemicals on their farms which show significant use organo-chlorides and heptachlors. The concentration of heptachlor in cocoa product is harmful to consumer health and dangerous to the user (farmers) This was again supported by Aminu F.O 2019 who revealed that a high number of the respondents experiencing head aches and burning sensations in the face after chemical application. Heptachlor is highly toxic to human and can be absorbed through the skin, lungs and gastrointestinal tract, and as well damage to the liver. Symptoms of poisoning observed in laboratory animals include lethargy, tremors, convulsions, stomach cramps or pain, coma and, death in severe cases as results of respiratory failure. Humans with convulsive disorders or liver damage are at increased risk from exposure and this was also in agreement with US EPA 2008.

Fig. 8 Concentration Of Heptachlor In Cocoa Samples



Heptachlor residue above EU MPL at 0.02mg/kg with the highest concentration of heptachlor found in sample AD4, AD5 and AD7

Table 3: Maximum Permissible Limit for Pesticide in Cocoa

S/N	PESTICIDE	MINIMUM PERMISSIBLE LIMIT
1	DIELDRIN	0.50
2	ALDRIN	0.05
3	HEPTACHOR	0.02
4	DDT	BANNED
5	DDD	BANNED
6	BETA HCH	0.05
7	DDE	0.50
8	LINDANE	1.00
9	GAMMA HCH	0.05

Hypothesis

Table 4: t-test statistics for comparison

Group	N	Mean	Std. Error	Std. Deviation	Df	T-value	p-value	Remark
Experimental	20	44.0	1.1120	8.2210	39	0.672	0.331	Rejected
Control	20	42.0	1.0624	7.1022				

Table 5: ANOVA statistic for comparison

Variations	Sum of squares	Df	Mean square	Square value	F-value	p-value	Remark
Within groups	1921.322	21	1.1120	82.104	0.684		Rejected
Between groups	1024.022	14	1.0624	61.241	.	0.610	
Total	2945.344	35					

Hypothesis testing for Level of Pesticide residue in Cocoa

Ho: There is no significant difference in the level of pesticide residue in Cocoa beans in Ado Metropolis, Ekiti State.

H1: There is significant difference in the level of pesticide residue in Cocoa beans in Ado Metropolis, Ekiti State.

The hypothesis (Ho): the result of the t-test in table 4 above shows that the Cal. Value is 0.672 is greater than the Tab. 0.331 hence, we reject the null hypothesis.

ANOVA was used in table 5 and it was revealed in the statistical result that F-value 0.684 is greater than P-value 0.610 as such, we reject Ho. Therefore, the result from both tests shows that there is significant difference in the level of pesticide residue in Cocoa beans in Ado Metropolis, Ekiti State.

VI. CONCLUSION

From this study it can be concluded that heptachlor is the most widely used pesticides of all the chemicals analyzed in cocoa samples from Ado metropolis of Ekiti State and that could be attributed to proliferation of the brand in Ekiti markets and its residue were found to be above EU permissible levels. There is also continual use of DDT and its composites despite ban. Among the pesticides detected it was found that heptachlor recorded the highest residual concentrations of 0.15 mg/kg for samples from AD5 and 0.14mg/kg from AD4 and 0.13mg/kg for samples from AD7. The lowest pesticide residual concentration was recorded for DDD with just two samples out of all the samples showing 0.01mg/kg. Dieldrin, Aldrin, Lindane, Gamma HCH and Beta HCH are found to be within EU permissible limit while heptachlor was used in abundance

exceeding EU permissible limit. DDT and its composites (DDE and DDD) were still in use despite their ban. The use of pesticides in order to improve agriculture has not only affected the crop, it has also altered the food chain and the ecosystem. These chemicals not only affect the crop in a specific area but also badly affect the ecosystem balance. Pesticides are causes of high morbidity and mortality.

VII. RECOMMENDATION

- Future work should concentrate on the processed cocoa (chocolate, Beverages) in order to compare levels of pesticide residues.
- Work should be done on different methodologies for extraction of pesticide residues to compare the recovery efficiencies.
- Work should be done on fresh and fermented cocoa beans
- Enforcing agencies (Ministry of Agriculture and Ekiti State Cocoa Regulation Board EKCOD) and the State Environmental Protection Agency should be empowered to provide and enforce more stringent measures to restrict the use of banned and obsolete chemicals on cocoa farms or crops or the use of any form of dangerous chemicals in treatment and pest control and ban importation of obsolete chemicals.
- Educate Cocoa farmers on the approved a pesticides and safe application of pesticide in such a way that it will be within EU permissible level while also instituting a monitoring mechanism to ensure that farmers who privately spray their farms do so with approved pesticides.

REFERENCES

[1] Aagbeve et al (2014). Bioremediation of malathion from environment for pollution control. Res.*J. Environ. Toxicol.,* 4: 147-150.

[2] Abdelgadirand, E. H. and Adam, S. E. I. (2011). Effect of various levels of dietary malathion on wistar rats. *J. Pharmacol. Toxicol*., 6: 69-75.

[3] Abou-Arab, A. A. K. (2009). Behaviour of pesticides in tomatoes during commercial and home preparation. *Journal of Food Chemistry*, 65: 509–514.

[4] ADM (Archer Daniels Midland Company). (2006). The De Zaan Cocoa Manual. pp 1-3. Available at viewer.zmags.com/publication.

[5] Adeyeye, A. and Osibanjo, O. (2009). Residues of organochlorine pesticides in fruits, vegetables and tubers from Nigerian markets. *The Science of the Total Environment* 231: 227 - 233.

[6] Adetoun (2010). Organochlorine pesticides, dioxin-like polychlorinated biphenyls polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in edible cocoa in Nigeria . *chemosphere* 81 : 675– 684

[7] Adu-kinni, C., Borrull, F. and Marce, R. M. (2010). Determination of organophosphorus pesticides in environmental water samples using GC-MS and two membrane extraction disks. Liquid Chromatography-Gas Chromatography. *Journal of Chromatography* 1214: 1048–1054.

[8] Afful et al (2010). Determination of pesticides in environmental waters by solid-phase extraction and gas chromatography with electron-capture and mass spectrometry detection. *Journal of Chromatography A.* 771 (2): 221–231.

[9] Appiah, M. R. (2004). Impact of cocoa research innovations on poverty alleviation in Ghana. *Ghana Academy of Arts and Sciences Publication*.1: 17–19

[10] Akanni et al (2010). The microbial ecology of cocoa bean fermentations in Indonesia. *Int. J. Food Microbiol.* 86: 87–99.

[11] Akinola et al (2012). The success story of cocoa processing and chocolate manufacturing in Ghana The success story that demolished a myth. 1–4, 33–50

[12] Aikpokpodion et al. (2012). Determination of selected polychlorinated biphenyls in soil by miniaturized ultrasonic solvent extraction and gas chromatography-mass selective detection, *Analytica Chimica Acta*. 577: 232-237. 70

[13] Amiah et al. (2006). Contamination levels of Organochlorine pesticides and farmers' knowledge, perception, practices in rural India – a case study. *Bulletin of Environmental Contamination and Toxicology* 73: 787–793.

[14] Aminu F.O (2019) Assessment of Cocoa residue in Northern Nigeria: *Journal of environmental Health and Assessment 62: 234-241*.

[15] Apau and Doodo (2010). Pyrethroids and pyrethrums . *Annu. Rev. Entomol*., 43, 1.

[16] Boakye (2012) A systematic review of the effect of pesticide residue in Cocoa products in Southwestern Nigeria. Springer, Food Science 216: 47-53

[17] Botchway (2010). Evaluation of organochlorine pesticide residues in human serum from an urban and two rural populations in Portugal. *Elsevier, Science of The Total Environment* 317 : 23–35 72

[18] Bovi (2015) Analysis of pesticide residue in cocoa beans in ashante province in Ghana pp. 14-19. Accessed July 2015

[19] Camu, N., De–Winter, T. Verbrugghe, K., Cleenwerck, I., Vandamme, P., Takrama, J. S., Vancanneyt, M. and De –Vuyst, L. (2007). Dynamics and biodiversity of populations 71 of lactic acid bacteria and acetic acid bacteria involved in spontaneous heap fermentation of cocoa beans in Ghana. *Appl. Environ. Microbiol*. 73: 1809–1824.

[20] Canatus, D. A. and Aikins, E. A. (2009). Reforming Ghana's cocoa sector. An evaluation of private participation in market. pp 1–2. Accessed on 20th June 2011 CDCP (Center for Environmental Health, Centers for Disease Control and Prevention).

[21] Cheesman, E. (1944). Notes on the nomenclature, classification and possible relationships of cocoa populations. *Tropical Agriculture* 21: 144–159.

[22] Daanu (2011) Gas chromatographic detectors in pesticides residue analysis (Training Manual). 4: 79 –81.

[23] Darko, G. and Acquaah, S. O. (2007). Levels of organochlorine pesticides residues in meat. *International Journal of Environmental Science and Technology* 4 (4): 521–524.

[24] Feo, M.L., Eljarrat, E., Barcelo', D. (2010). Determination of pyrethroid insecticides in environmental samples. *Trends in Analytical Chemistry* 29(7):692-705.

[25] Frimpong et al (2012). Parkinson's disease and brain levels of organochlorine pesticides. *Ann. Neurol*., 36: 100-103.

[26] Gallow, M. A and Lawryk, N. J. (1991).. Organic phosphorus pesticides. Handbook of Pesticide Toxicology, Classes of Pesticides. *Academic Press Inc*. 2:1 20–123 Ghana NIP (Ghana National Implementation Plan) (2007). Available at <http://www.pops.int

[27] Hashim, P. J., Selamat, S. K., Muhammad, S. and Ali, A. (1998). Effect of mass and turning time on free amino acid, peptide-N, sugar and pyrazine concentration during cocoa fermentation. *J. Sci. Food Agric*. 78:543–550.

[28] Hayes, W. J. and Laws E. R. (1990), Classes of Pesticides. Handbook of Pesticide Toxicology, *Academic Press, Inc.*, 3:15– 23

[29] Hogart and Miles (2014). Effects of incubation andpolyphenol oxidase enrichment on colour, fermentation index, procyanidinsand astringency of unfermented and partly fermented cocoa beans. *Int. J. Food Sci. Technol.* 38: 285–

[30] JMPR (Joint Meting on Pesticide Residues) (1999). Pesticides Residues in Food: Report of the 1999 Joint FAO/WHO Meeting of

Experts. Geneva, World Health Organization. Accessed on 13th May, 2010

[31] Kweku B.P (2017). The national cocoa diseases and pest control; Achievements and challenges. Available at myjoyonline.com.

[32] Lagunes, G. S., Loiseau, J., Paredes, L., Barel, M. and Guiraud. J. P. (2007). Study on the microflora and biochemistry cocoa fermentation in the Dominican Republic .*Int J .Food Microbiol* 114: 124 – 130

[33] Nnamdi (2011). Association between maternal serum concentration ofthe DDT metabolite DDE and preterm and small-for gestational- age babies at birth. *Lancet* 358: 110–114.

[34] Obatolu, C. R., Fashina, A. B. and Olaiya, A. O. (2003). Effects of Climatic changes on Cocoa Production in Nigeria. Proceeding of African Crop Science Conference, Lagos, Nigeria. *Springer-Verlag* 5: 957- 959

[35] Ojo, A. D and Sadiq, I. (2010). Effect of climate change on cocoa yield: a case of cocoa research institute farm. *Journal of Sustainable Development in Africa* 12(1): 12 – 20 81

[36] Olalekan (2014): Lindane and endosulfan residues in cocoa beans in Ondo state. Journal of Applied Sciences 10: pg 135–140

[37] Osibanjo, O. (2003). Persistent Organic Pollutants. The Handbook of Environmental Chemistry *Springer-Verlag* 3 : 5 –14