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# Residues of Dichlorodiphenyltrichloroethane (DDT) and its Metabolites in Cocoa Beans from Three Cocoa Ecological Zones in Nigeria

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**Abstract:** The use of pesticides has been a long time practice among Nigerian cocoa farmers. The use of DDT on cocoa was banned many years ago due to its persistence in environment and hazard to human and non target organisms. This study was carried out to survey the level of DDT and its metabolites in Nigerian cocoa beans with a view to assess its safety for consumption. Ripe, matured cocoa pods were collected from selected cocoa plantations in Ondo, cross River and Ogun states. The pods were broken and cocoa beans were processed accordingly. Dried cocoa beans were processed, extracted and analyzed for pesticide residue with GC-MS. Results showed that, 70% of cocoa beans from Ondo State had DDT residue while 10% of cocoa beans from Cross River and 10% of cocoa from Ogun State had DDT residue. However, the concentration of DDT in most of the beans were below the maximum residue limit of DDT in cocoa beans set by the European Union.

**Key words:** DDT · DDE · DDD · Cocoa · Nigeria · Pesticide residue

## INTRODUCTION

The menace of pests and disease is a strong factor responsible for the dwindling production of cocoa in Nigeria. Among the various insect pests of *Theobroma cacao*, the brown Mirid, *Sahlbergilla singularis* Haglund is the most harmful insect pest of cocoa tree in Nigeria [1]. Mirid feeds by inserting its mouth parts into the plant and sucking the juices and at the same time, salivary secretions are injected into the tissue which results in plasmolysis of the cells. The cellular lysis results in necrosis, followed by the appearance of lesions [2]. Canker sores develop from lesions due to invasion by cryptogamous parasites causing weakness. The combination of tissue necrosis and cryptogamic attack results in wilting of the plant leading to very low productivity. Yield loss of about 30 – 70% has been attributed to Mirid infestation and damage [3, 4]. In order to combat the destructive activities of Mirid, Nigerian cocoa farmers use various brands of insecticides including organochlorine group. Orgnochlorine pesticides have been used extensively worldwide since the early 1950s [5, 6] until restriction were introduced in several developed and developing countries due to their persistence in the environment and growing evidence of adverse associated health implications. Many organochlorine pesticides pose substantial short and long-term health risks [7]. They are known to disturb the biological and physiological functions of erythrocytes and lymphocytes [8]. The adverse health effects include a series of chronic end-points including cancer [9, 10], neurotoxic [11], immunotoxic [12], developmental [13], endocrine [14], reproductive [15] and neuro- behavioral effects [16]. In recent time, many developed nations discovered an increase in the case of cancer among their citizens. This made the European Union to become conscious of the quality of food products meant for consumption among her citizens. In order to comply with the maximum residue limit regulation and produce high quality cocoa beans for exportation, it became expedient to assess organochlorine – DDT, DDE and DDD residue in cocoa beans from selected cocoa plantations in the three cocoa ecological zones in Nigeria. This will form the basis for sensitizing Nigerian cocoa farmers on the need to adopt Good Agricultural Practice (GAP) with a view of minimizing the use of pesticides.

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## MATERIALS AND METHODS

Selection of the cocoa farms used in this study was based on the density of cocoa production. In Ondo State, cocoa samples were collected from Idanre, Bamikemo, Afun and Owena while in Ogun State, cocoa samples were collected from Sora-Bale, Bodo, Sotiya and Ogunmakin. Ripe cocoa pods were collected from each cocoa farm in a randomized complete block design manner. The pods were transported to the fermentation section of Cocoa Research Institute of Nigeria, Ibadan, Nigeria where the pods were broken with wooden sticks and later fermented for six days. The fermented beans were sun - dried for seven days and later transferred into the oven to remove any absorbed moisture. The oven - drying was done at the temperature of 60°C for six hours until a constant weight was attained after which the samples were taken to IDAEA-CSIC Environmental Chemistry Laboratory, Barcelona, Spain.

**Chemicals and Reagents:** The DDT (o,p and p,p'-DDT, DDE and DDD), were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Solvents (hexane and dichloromethane SupraSolv) were from Merck (Darmstadt, Germany). Florisil Solid Phase Extraction cartridges (5 g, 25 ml) were purchased from Waters (USA). Nitrogen for drying (99.995% purity) was from Air Liquid (Barcelona, Spain).

**Sample Preparation:** One g of sample was placed in centrifuge glass tubes with 30 ml hexane/dichloromethane (1:1) and samples were vortexed and sonicated for 10 minutes in a 360 W Selecta ultrasonic bath (J.P. Selecta, Barcelona, Spain). This procedure was repeated three times and sample was vortexed in between extractions. Samples were centrifuged at 3000 rpm for 10 min at 10°C. The supernatant was collected in a clean vial and reduced to less than 1 ml under a gentle stream of nitrogen (TurboVap) before clean-up. Then, SPE with florisil cartridge (5 g, 25 ml) was used, since florisil has a great capacity to eliminate the lipids that are present in the sample (in cocoa beans, the% fat is of 50%). Different Florisil cartrigdes, all of 5 g and 25 ml, were used: Waters (Waters, USA); Isolute, ENV (IST, England), Supelco (USA). Best performance was obtained with Florisil SPE cartridges from Waters and further this cartridge was used. Cartridges were conditioned with 30 ml hexane-dichloromethane (1:1) and pesticide elution was done with 30 ml hexane-dichloromethane (1:1). The resulting extract was collected in a clean vial and concentrated under a gentle stream of nitrogen (Turbovap) to circa 0.5 ml. The extract was transferred to a chromatographic vial and evaporated to almost dryness in a Reacti-Therm III from Pierce (Rockford, IL, USA) under a gentle nitrogen stream and finally reconstituted with 500 µl of hexane.

**Gas Chromatographic Determination:** Gas Chromatography coupled to Mass Spectrometry using electron ionization (GC-EI-MS) was performed on an Agilent 6890 gas chromatograph connected to an Agilent 5973 Network mass spectrometer (Santa Clara, CA, USA). An Agilent HP-5MS (30 m x 0.25 mm i.d., 0.25 µm film thickness) containing 5% phenyl methyl siloxane capillary column was used with helium as the carrier gas at 15 psi at initial flow of 1.1 ml/min. The temperature program was from 65°C (held for 1 min) to 160°C at 12°C/min, to 310°C at 8°C/min and to 325° at 10°C/min (held for 5 min). The total run time was of 34 min. Two µl of sample were injected in splitless mode at 16.41 psi, purge flow of 50 mL/min and purge time of 1 min. Injector, transfer line and ion source temperatures were 280, 250 and 230°C, respectively. Quadrupole temperature was of 150°C.

## RESULTS

Figure 1 presents the concentration of DDT and its metabolites in cocoa beans obtained from selected cocoa plantations in Ondo State. Results showed that ppDDE residue in cocoa beans ranged from non-detected to 0.49 mg $kg^{-1}$ with an average value of 0.17mg $kg^{-1}$. Among the samples with detectable DDE samples from Idanre 1, Bankemo 3 and Owena 2 had the minimum DDE residue while cocoa samples from Idanre 3 had the maximum DDE residue. Residue of o,p DDD in cocoa samples obtained from Ondo State ranged from non-detected to 0.50 mg/kg with an average value of 0.22 mg/kg. Sample from Owena 2 had the minimum o,p DDD among the samples with detectable residue while sample from Idanre 3 had the maximum DDD residue. p,pDDT residue ranged from non-detected to 0.12mg/kg with an average value of 0.08 mg/kg. Sample from Bankemo 2 had the minimum DDT residue among the samples with detectable DDT while cocoa samples from Idanre 3 had the highest concentration of DDT. The total DDT in cocoa beans obtained from selected cocoa plantations in Ondo State ranged from 0.25 to 1.11mg/kg with a mean of 0.44mg/kg. Samples from Owena 2 had the least value of total DDT while samples from Idanre 3 had the highest value for total DDT in the studied cocoa samples from Ondo State. However, cocoa samples obtained from Idanre 1, Owena 1 and Owena 3 had no detectable DDT and its metabolites.

Table 1:Data on performance of method

| Pesticides | $T_R$(min) | ions | a | B | Repititivity | $R^2$ | %Recovery |
|---|---|---|---|---|---|---|---|
| o,p DDE | 12.12 | 246-248-318 | 3.0E+06 | -59756 | 7 | 0.99 | 76 |
| P,p DDE | 12.80 | 246-248-319 | 3.0E+06 | -86025 | 11 | 0.99 | 80 |
| o,p DDD | 12.94 | 335-237-165 | 4.0E+06 | -14933 | 13 | 0.99 | 105 |
| p,p DDD | 13.65 | 235-237-165 | 6.0E+06 | -30654 | 11 | 0.99 | 109 |
| o,p DDT | 13.65 | 235-237-165 | 6.0E+06 | -30654 | 11 | 0.99 | 109 |
| p,p DDT | 14.39 | 235-237-165 | 1.0E+06 | -29426 | 6 | 0.99 | 81 |

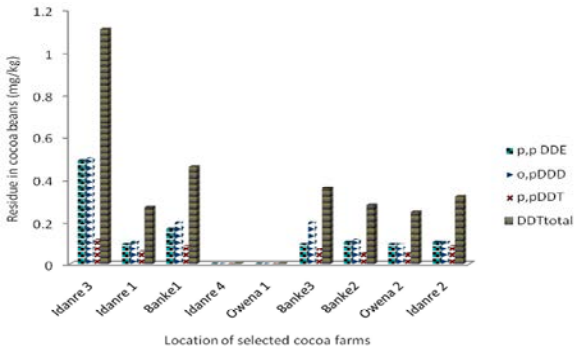


Fig. 1: Residues of DDT and its metabolites in cocoa from Ondo State

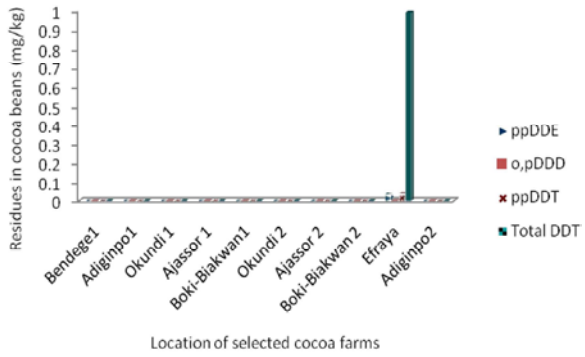


Fig. 2: Residues of DDT and its metabolites in cocoa sample from Cross Rivers State

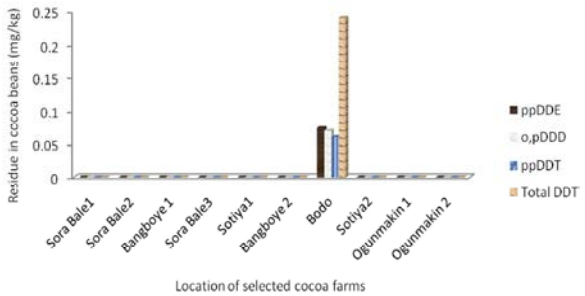


Fig. 3: Residues of DDT and its metabolites in cocoa samples from Ogun State

Figure 2 presents the concentration of DDT and its metabolites in cocoa beans collected from selected cocoa plantations in Cross Rivers State. Among the various

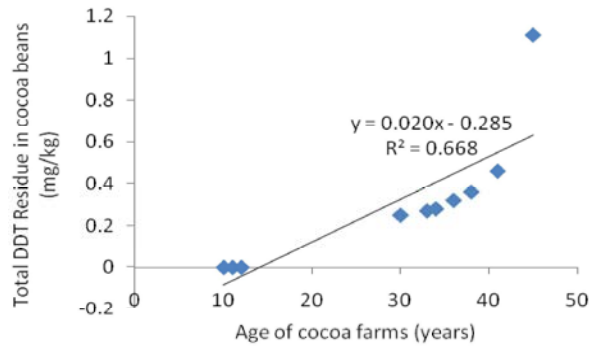


Fig. 4: Relationship between total DDT in cocoa beans and age of plantations

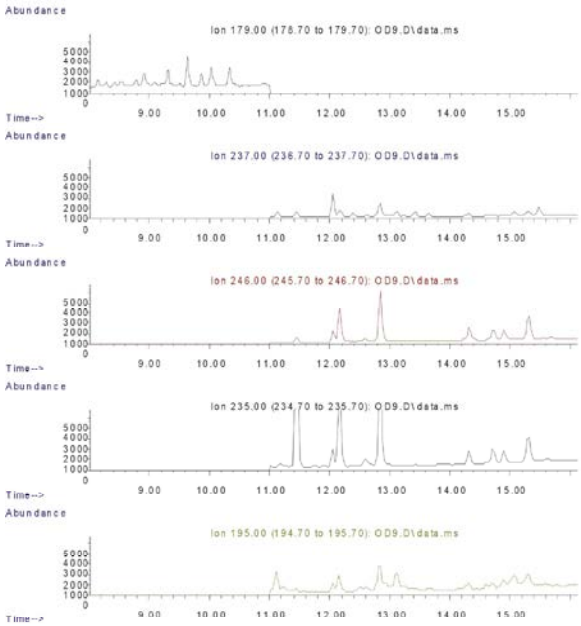


Fig. 5: Representative GC-MS chromatogram for cocoa samples

cocoa beans collected, only cocoa samples from Efraya had detectable p,p DDE (0.036mg/kg), p,pDDT (0.04mg/kg) and total DDT (0.99mg/kg) while the rest samples had no detectable DDT and its metabolites.

Figure 3 presents the residue of DDT and its metabolites in cocoa beans collected from selected plantations in Ogun State. Result showed that, of all the

collected samples, only cocoa beans from Bodo had detectable DDT and its metabolites. The samples contained p,p DDE (0.074 mg/kg), o,p DDD (0.07 mg/kg), p,p DDT (0.06 mg/kg) and total DDT (0.24 mg/kg)

Linear regression $R^2$ when used to determine the relationship between total DDT residue in cocoa beans and age of selected cocoa farms in Ondo State gave $R^2$ value of 0.668.

## DISCUSSION

Results showed that, 70%, 10% and 10% of the cocoa samples obtained from Ondo, Cross River and Ogun States respectively contained DDE (Dichlorodiphenyldichloroethylene). DDE- one of the metabolites of DDT is formed by the loss of hydrogen chloride (dehydrohalogenation) in DDT. DDE is fat soluble which has the capacity to builds up in the fat of animals. Due to its stability in fat, it is rarely excreted from the body and body levels tend to increase throughout life. The major exception is the excretion of DDE in breast milk, which delivers a substantial portion of the mother's DDE burden to the young animal or child. Some studies have indicated that DDE is an endocrine disruptor [17] and contributes to breast cancer, but more recent studies provide strong evidence that there is no relationship between DDE exposure and breast cancer [18]. What is clearer is that, DDE is a weak anti-androgen [19]. Other studies showed that exposure to DDE is linked to Alzheimer's and Parkinson's disease in humans. Animal studies showed that organochlorine pesticides such as DDE are neurotoxic, cause oxidative stress and damage the brain's dopaminergic system. The mean concentration of ppDDE in this work (0.17mg $kg^{-1}$) is much higher than the mean ppDDE (0.0085mg $kg^{-1}$) reported by Afful for organochlorine residue in fish samples from the Densu Basin. Essumang reported non- detectable level of DDE in fish from Fosu. The mean DDE in this work is also higher than 0.0094mg $kg^{-1}$ reported by El-Saeid [20] for residue in fish from Riyadh region and 0.058 mg $kg^{-1}$ in *S. lycopersicum* and 0.056 mg $kg^{-1}$ in *C. annuum* by Darko and Aruwajoye [21] reported mean value of 0.11mg $kg^{-1}$ and 0.031mg $kg^{-1}$ for ppDDE residue in beef fat from Kumasi and Buoho respectively. They also reported 0.042mg $kg^{-1}$ and 0.005mg $kg^{-1}$ for DDE residue in beef from Kumasi and Buoho abattoir respectively. DDD residue was detected in 70% and 10% of cocoa samples obtained from Ondo and Ogun States respectively. There was no DDD detected in any of cocoa beans from Cross River. DDD is a metabolite formed by reductive dechlorination of DDT. It is closely related chemically and similar in properties to DDT but considered to be less toxic to animals than DDT. DDD is in group B2 classification meaning that it is a probable human carcinogen. This is based on the increased incidence of lung tumors in male and female mice, liver tumor in female mice and thyroid tumors in male rats. The mean DDD residue in this work is higher than 0.0001mg $kg^{-1}$ reported by Essumang for DDD residue in fish from Fosu Lagoon in Ghana. El-Saeid [20] reported 0.00002mg $kg^{-1}$ DDD in fish from Riyadh region which is much lower than the mean value in this study. Result showed that, 70%, 10% and 10% of the studied cocoa beans obtained from Ondo, Cross River and Ogun States contained DDT residue respectively. Total DDT was calculated by the addition of p,pDDE, o,pDDD and p,pDDT residues in the beans. The mean p,pDDT in samples from Ondo State is higher than mean DDT residue (0.046mg) reported by Nsikak and Aruwajoye in *S. lycopersicum* but lower than mean p,pDDT in *C. annuum* (0.081mg $kg^{-1}$). The average total DDT in the study is also lower than the average total DDT (0.4mg $kg^{-1}$) detected in lettuce collected from Ghana's urban market [22]. The mean values of p,pDDT residue reported by Musa *et al*. [23] in Gymnarchus (0.0045mg $kg^{-1}$) and 0.0038mgkg-1 in Tilapia from North-Eastern Nigeria are lower than the mean value of p,p DDT in this work. The fact that 70% of the studied cocoa beans from Ondo State had residue of DDT and its metabolites is an indication that, DDT was widely used among cocoa farmers in Ondo State at a particular point in time. The concentration of the residues however, suggests that most of the farmers still used DDT products after its use on cocoa had been banned. The continuous use of DDT by the farmers could be due to addiction to the insecticide based on efficacy. It could also be that, new pesticides having DDT as its active ingredient but un-familiar name was sold to the unlearned farmers. However, the low concentration of p,pDDT compared to its metabolites p,pDDE and o,pDDD is an indication that there might not be recent input of DDT in the various cocoa plantation. The detection of p,p DDE in cocoa beans is an indication of photochemical degradation of p,p DDT [24]. Due to the chemical and physical nature of DDT and its metabolites, they magnify through food chain. They are lipophilic and are stored mainly in body fat. This might be the reason why DDT and is metabolites were still detected at high level in cocoa beans obtained from plantations where its use had not been made for so many years. Report of Darko and Acquaah [21] confirmed the lipophilic nature of DDT and its metabolites where DDT and its metabolites

were more concentrated in beef fat than the tissue (meat). The detection of DDT in cow milk at high concentration was also an indication of the ability of the pesticide to associate with animal tissue [25]. This implies that, DDT can accumulate in human body and environment posing problem to human health. Through their persistence and lipophilicity, the pesticide may concentrate in the adipose tissues and in the blood serum of human. This was confirmed by the findings of *Sosan et al*. [26] who reported the detection of organochlorine residue in the serum of 42 out of 76 cocoa farmers in south Western Nigeria. The potential mechanisms of action on humans are genotoxic. DDT and DDE like other organochlorine have shown to have xenoestrogenic activity, meaning they are chemically similar enough to estrogens to trigger hormonal responses in animals. This endocrine disrupting activity has been observed in mice and rat toxicological studies and available epidemiological evidence indicates that, these effects may be occurring in human as a result of DDT exposure. The member of DDT family with the highest concentration in most of the samples examined is o,pDDD. This was however, not the case in cow milk as reported by Vesna *et al*. [25]. In which p,pDDE metabolite was the most prominent residue among the DDT family. The absence of DDT and its metabolites in cocoa beans obtained from Owena 1, Idanre 2 and Owena 3 suggests non application of DDT in the plantations. The statistics of the studied cocoa plantations showed that, the mentioned cocoa farms with no DDT were between the ages of ten and twelve compared with older cocoa farms whose ages ranged from 30 to 50 years. It is also an indication that, the use of DDT among cocoa farmers in Ondo State had become a thing of the past. However, quality assessment with respect to DDT residue in cocoa beans indicates that, only cocoa beans from Idanre 3 which happened to be the oldest cocoa plantation among the studied farms had total DDT residue above the maximum residue limit for DDT in cocoa beans. This suggests that, most of the cocoa beans produced from the studied area of Ondo State have DDT residue at concentrations that are not hazardous to human health. The presence of DDT and its metabolites in only 10% of cocoa beans obtained from Cross River and Ogun States suggests that, the use of DDT among cocoa farmers in the two States was uncommon. The presence of this banned pesticide in cocoa from Ondo State compared to Cross River and Ogun might be due to te fact that, cocoa cultivation was mostly popular in Ondo State compared to any other State in Nigeria in the 1960s when cocoa was the main source of foreign exchange in Nigeria. The cultivation later became popular in other States of the federation when the populate saw infrastructural, educational and social development achieved by the federal government with the profits made from the exportation of cocoa beans to the European nations. As a result, the use of cocoa pesticides must have been densely made in Ondo State compared to Cross River and Ogun State. This was supported by the linear regression $R^2$ which showed that, the concentration of DDT in cocoa beans increased with the age of plantations. On a general note, cocoa beans from the three cocoa ecologies studied in this work are still safe for consumption with respect to DDT residue. However, the wholesomeness of the cocoa from the studied cocoa producing zones is subject to the level of other contaminants that might be present in the cocoa beans.

## CONCLUSION

Most of the cocoa beans obtained from Ondo State had residues of DDT and its metabolites which is an indication that, DDT was widely used among cocoa farmers in Ondo State. The level of ppDDT in the samples showed that, its used among the farmers has been put to a stop while its use was unpopular among cocoa farmers in Cross River and Ogun States. The level of total DDT in the studied cocoa beans showed that, Nigerian cocoa is safe for consumption without the fear of health risk with respect to DDT toxicity.

## REFERENCES

1. Opeke, L.K., 1992. Tropical tree crops. Spectrum Books Ltd, Ibadan Nigeria, pp: 95-96.
2. Mariau, D., 1999. Integrated Pest Management of Tropical Perennial Crops. Science Publishers, Inc. USA, pp: 167.
3. Idowu, 1989. Control of Insect Pests of Cacao pp: 89-102 Res. Inst. Nigeria, Ibadan.
4. Ojelade, K.T.M., J.C. Anikwe and O.L. Idowu 2005. Comparative evaluation of miricidal efficacy of some insecticides for the control of the brown cocoa mirid *Sahlbergella singularis* in Nigeria. Journal of Applied Tropical Agriculture, 10: 46-53.
5. Kin, C.M., T.G. Huat and A. Kumari 2006. Method development for the determination of pesticide residues in vegetables and fruits by using solid phase microextraction. Malaysian J. Chem. B, 8(1): 67-71.

6. Kuet, A.C.L. and L. Seng 2004. Solid phase extraction clean-up for the fdetytermination of organochlorine pesticides in vegetables. Malaysian J. Chem. B, 8(1): 39-47.
7. World Health Organization (WHO) 1990. A public health impacts of pesticides used in Agriculture. World Health Organization, Geneva, pp: 1-128.
8. Banerjee, B.D., V. Seth, A. Bhattacharya, S.T. Pasha and A.K. Chakraborty, 1999. Biochemical effects of some pesticides on lipid peroxidation and free radical scavengers. Toxicol. Lett., 107(1-3): 32-47.
9. Settimi, L., A. Mashina, A. Andrion and O. Axelson, 2003. Prostate cancer and exposure to pesticides in agricultural settings. Int. J. Cancer, 104: 458-461.
10. Alavanja, M.C., J.A. Hopin and F. Kamel, 2004. Health effects of chronic pesticide exposure: Cancer and neurotoxicity. Ann . Rev. Public Health, 25: 155-197.
11. Kamel, F. and J.A. Hoppin, 2004. Association of pesticide exposure with neurologic dysfunction and disease. Environ. Health Perspect., 112(9): 950-958.
12. Galloway, T. and R. Handy 2003. Immunotoxicology of organophosphorus pesticides Ecotoxicology 12(1-4): 345-363.
13. Colborn, T., 2006. A case for revisiting the safety of pesticides: A closer look at neurodevelopment. Environ. Health Perspect., 114(1): 10-17.
14. Barlow, S.M., 2005. Agricultural chemicals and endocrine-mediated chronic toxicity or carcinogenicity. Scand. J. Work Environment Health 31(Suppl. 141-145): 119-122.
15. Garcia, A.M., T. Flectcher, F.G. Benavides and E. Ort 1999. Parental agricultural work and selected congenital malformations Am. J. Epidemiol., 149(1): 64-74.
16. Amr, M.M., M. Alam, A. Ossama, E. Balanouni, G. Samra and Z. Halim, 1993. Neurobehavioural changes among workers in some chemical industries in Egypt. Environ. Res., 63(2): 295-300.
17. Afful, S., A.K. Anim and Y. Serfor-Armah, 2010. Spectrum of organochlorine pesticide residues in fish samples from the Densu basin. Research Journal of Environmental and Earth Sciences, 2(3): 133-138.
18. Nsikak, U., Benson and O.I. Aruwajoye, 2011. Assessment of contamination by organochlorine pesticides in *Solanum lycopersicum* L. and Capsicum annuum: A market survey in Nigeria. African Journal of Environmental Science and Technology, 5(6): 437- 442.
19. Essumang, D.k., G.K. Togoh and L. Chokky, 2009. Pesticide residue in water and fish (Lagoon Tilapia) samples from lagoons in Ghana. Bull. Chem. Soc. Ethiopia, 23(1): 19-27.
20. El-Saeid, M.H., 2010. Monitoringof pesticide residues in Riyadh farm fish. World Applied Science Journal, 9(9): 1075-1080.
21. Darko, G. and S.O. Aruwajoye, 2007. Levels of organochlorine pesticide residues in meat. Int. J. Environ. Sci. Tech., 4(4): 521-524.
22. Amoah, P., P. Drechsel, R.C. Abaidoo and J. Ntow, 2005. Pesticide and pathogen contamination of vegetables in Ghana's urban market. Arch. Environ. Contam. Toxicol., 50: 1-6.
23. Musa, U., S.S. Hati, Y.I. Adamu and Mustapha, 2010. Pesticide residues in smoked fish samples from North-Eastern Nigeria. Journal of Applied Sci., 10: 975-980.
24. Wandiga, S.O., 1995. Organochlorine pesticide: Curse or blessing in tropical agriculture. Proceedings of the 6th International Chem. Conf. in Africa. Accra, Ghana, 6: 220-223.
25. Vesna, C., Z. Darinka and J. Jurij, 2000. Evidence of some trace elements, organochlorine pesticides and PCBs in Slovenian cow's milk.
26. Sosan, M.B., A.E. Akingbohungbe, I.A.O. Ojo and M.A. Durosinmi, 2008. Insecticide residues in the blood serum and domestic water source of cacao farmers in South Western Nigeria. Chemosphere, 72: 781-784.