Supported by tetfund

Assessment of Organochlorine Pesticide residues in *Oreochromis niloticus* (Linnaeus, 1758), *Clarias gariepinus* (Burchell, 1822) and *Hepsetus odoe* (Bloch, 1794) in Eleyele Lake, Oyo State, Nigeria.

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Received: March 20, 2022 **Accepted:** June 18, 2022

Abstract: This study was carried out to determine the levels of Organochlorine pesticides in fish captured from Eleyele Lake. The Lake receives effluents discharged from cassava processing site, agricultural farm land, and waste water from domestic activities from the neighboring homes. *Oreochromis niloticus*, *Clarias gariepinus* and *Hepsetus odoe* were sampled from the Lake because of their abundance and economic importance. The levels of organochlorine pesticide residues were determined using Gas chromatography-mass spectrometry equipped with electron capture detector. The mean concentrations of organochlorine pesticide residues in the fish sampled were in this order *Oreochromis niloticus* > *Hepsetus odoe* > *Clarias gariepinus;* 27.50±8.71ng/g, 11.87±1.83ng/g and 5.81±4.90ng/g respectively. Among the organochlorine pesticides components examined, Endosulfan I had the highest concentrations in all the fish species. This study also revealed that, all the detected pesticide residues in the fish samples studied were above the Maximum Residues Limits and this can be an important process of transferring pesticides to humans. It also indicated that there is an extensive presence and usage of these pesticides in the study environment.

Keywords: Contamination, effluents, food fish, uptake

Introduction

Fish makes a vital contribution to the survival and health of a significant portion of the world’s population and fish industry has been threatened by natural and human activities (Tsadu *et al.,* 2006). Such human activities include farming, construction, mining, urban development and land pollution which occur near the watershed of a reservoir and could bring about water quality problems and disruption in fish.

In Nigeria, rapid urbanization, severe pest problems, weeds, rodents, locust grain eating birds have increased reliance on the use of pesticides. Organochlorine pesticides are a cheap form of insecticides and are widely used (Adeyemi *et al.,* 2008). Its availability in the environment can occur through spray drift, surface runoff and leaching (Chowdhury *et al.,* 2012; Smalling *et al.,* 2013; Mahboob *et al.,* 2015). Fish are often at the top of the aquatic food chain and are known for their ability to bio-concentrate pollutants in considerable amounts in the tissues and they are recognized as good accumulators of organic and inorganic pollutants (Ara *et al.,* 2014). Organochlorines have very low bio-degradation; get accumulated in environment causing serious problems (Ishan, 2018).

Eleyele Lake serves as a major source of potable water after purification in Ibadan and fishing is carried out daily on the river (Olaifa *et al.,* 2004). There is a relaxed control of effluent discharge from homes into the Lake. Therefore, this study was carried out to assess Organochlorine Pesticide residues in fish (*Oreochromis niloticus*, *Clarias gariepinus* and *Hepsetus odoe*) at Eleyele Lake, Oyo State, Nigeria. The choice of fish species was based on their economic importance, differences in biology and feeding habits.

Material and Methods

Sampling area

Eleyele Lake is an artificial lake constructed in 1942 which supports fish consumption and conservation in South-West, Nigeria. It is located at the North-Eastern part of Ibadan, Oyo State, Nigeria. The Lake is within the Longitude7°23’49” N and Latitude 3°52’2” E. It covers a distance of 62km from its source to the dam with a surface area of 152.76hectares, maximum depth 12m, and a mean depth of 6.5m. The Lake receives water during the rainy season principally from the River Ona and other associated small streams (Olubowale, 2002). The Lake receives effluents discharged from cassava processing site, agricultural farm land, automobile mechanical workshops and waste water from domestic activities from the neighboring homes.

Sample collection

Five samples each of *Oreochromis niloticus*, *Clarias gariepinus* and *Hepsetus odoe* were procured from the fishermen at the Landing sites of the Lake in May and June, 2019. The Fishes were identified using Field guide to Nigeria freshwater fishes by Olaosebikan and Raji (2013). The total and standard lengths were measured using meter rule, while the weight measurement was taken using sensitive weighing balance. The samples were labeled, wrapped with foil paper to avoid further contamination and transported to the laboratory on the same day.

Extraction of Organochlorine Pesticides (OCPs)

5g of homogenized fish sample was weighed into a beaker and 25mL of a mixture of acetone and hexane (1:1) was added. The beaker was sonicated for 10min in an ultrasonic bath and the extract was transferred to another beaker. The extraction was repeated with 10mL of the solvent mixture. The extracts were combined, concentrated to about 2.0mL

Clean-up of the OCPs extract

Clean-up of the extracts was done with a column chromatography loaded with silica gel, glass wool and anhydrous sodium sulphate and then eluted with 5mL of hexane. The purified extracts were then re-constituted in 2mL hexane.

Derivatization of the extract

1 mL each of the extract was transferred to 10mL centrifuge tubes, 20µL of 5 % K_2CO_3 solution was added until pH$\geq$10. 50 µL of tetrachloroethylene, 125 µL of acetic anhydride and 4 mL of deionized water were added. The tubes were shaken for 1 minute and centrifuged at 3,500 rpm for 2 min. 50 µL of organic solvent phase was transferred to a vial and made up to 1000 µL with n-hexane. 1 µL of the extract was injected into the GC–MS system.

Gas chromatography

An analysis was performed with Gas chromatography (Agilent Technologies 7890 A) equipped with 5975 Mass Spectrometry Detector (MSD). A low polar HP-5 column of

30 m length, 0.32mm i.d and 0.25 um film thickness was used. The injection method and volume were splitless mode and 1.0 µL respectively. Helium was used as a carrier gas at a flow rate 1.8 mL/min. The operating parameters were as follows: Injector temperature was set at 280°C for the detector, the MSD transfer line temperature was at 280°C. The quantitative determination of the pesticide components by Mass Spectrometry was done using Selected Ion Monitoring (SIM).

Statistical Analysis

Data collected were subjected to one-way analysis of variance (ANOVA) to assess whether pesticide residues varied significantly between fish species. Mean values that are less than 0.05 were significantly different. All statistical calculations were performed using SPSS 20.

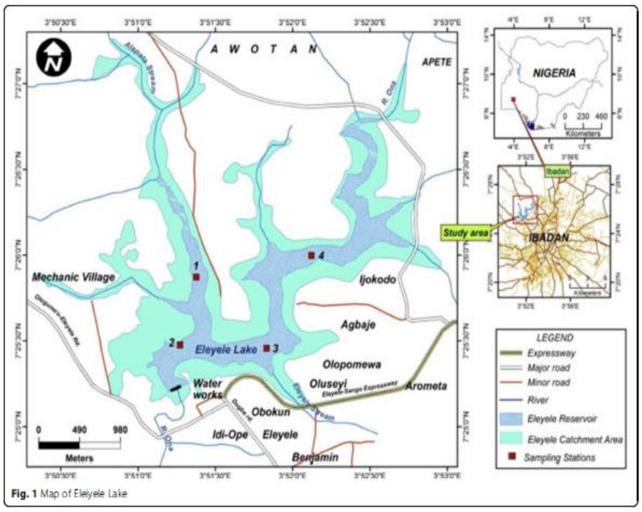

Figure 1: Map of Eleyele Lake
Source: Ogunola *et al.*, 2018

Results

The morphometric characteristics of fish and assessment of Organochlorine Pesticides residues was carried out in Eleyele Lake with three different fish species. The results were presented below.

Table 1: Morphometric characteristics of fish samples collected in May and June, 2019

Month	Fish species	Number of samples	Total Length (cm)	Standard Length (cm)	Weight (gram)
May	*Oreochromis*	5	16.92±1.94	13.96±1.66	117.5±38.8
	niloticus	5	34.00±2.24	29.80±1.92	350.58±64.67
	Clarias gariepinus	5	25.85±1.20	21.10±1.27	128.05±24.11
June	*Hepsetus odoe*	5	16.96±2.98	13.18±2.46	106.78±48.10
	Oreochromis niloticus				
	Clarias gariepinus	5	32.50±0.50	29.40±0.82	237.44±28.64
	Hepsetus odoe	5	26.25±2.36	21.25±2.02	139.07±40.72

Table 2: Organochlorine pesticides (OCPs) (ng/g) in the fish species for the month of May

OCPs	*Oreochromis niloticus*	*Clarias gariepinus*	*Hepsetus odoe*
	Mean±SD	Mean± SD	Mean±SD
alpha-HCH	0.03±0.01^{a}	0.03±0.01^{a}	0.00±0.00^{a}
beta-HCH	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
gamma-HCH	0.16±0.02^{a}	0.06±0.02^{a}	0.16±0.02^{a}
Heptachlor	1.84±0.56^{a}	0.00±0.00^{a}	1.09±0.58^{a}
Aldrin	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Heptachlor epoxide	37.44±7.59^{a}	7.11±1.14^{b}	0.00±0.00^{c}
Edosulfan I	52.90±8.61^{a}	60.10±9.35^{a}	156.10±33.10^{b}
p,p' DDE	0.38±0.16^{a}	2.32±0.78^{a}	5.78±3.24^{b}
Dieldrin	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Endrin	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Endosulfan II	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
p,p'DDD	0.00±0.00^{a}	2.86±0.84^{b}	0.42±0.06^{a}
Endrin aldehyde	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
p,p'DDT	1.55±0.44^{a}	0.00±0.00^{b}	0.00±0.00^{b}
Methoxychlor	0.14±0.05^{a}	0.83±0.05^{a}	0.26±0.07^{a}

*Means of values with same superscript along rows are not significantly different ($p<0.05$)
SD = standard deviation

Table 3: Organochlorine pesticides (OCPs) (ng/g) in the fish species for the month of June

OCPs	*Oreochromis niloticus*	*Clarias gariepinus*	*Hepsetus odoe*
	Mean±SD	Mean± SD	Mean±SD
alpha-HCH	0.25±0.07^{a}	0.06±0.03^{a}	0.03±0.01^{a}
beta-HCH	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
gamma-HCH	0.53±0.23^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Heptachlor	3.81±0.70^{a}	1.09±0.58^{b}	3.65±0.25^{a}
Aldrin	1.51±0.37^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Heptachlor epoxide	0.00±0.00^{a}	10.37±3.07^{b}	0.00±0.00^{a}
Endosulfan I	309.01±67.69^{a}	0.00±0.00^{b}	0.00±0.00^{b}
p,p’ DDE	0.00±0.00^{a}	0.81±0.20^{a}	5.78±1.03^{b}
Dieldrin	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Endrin	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Endosulfan II	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
p,p’DDD	2.59±0.74^{a}	0.17±0.04^{b}	3.38±0.06^{a}
Endrin aldehyde	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
p,p’DDT	0.84±0.17^{a}	1.05±0.29^{a}	3.37±0.66^{b}
Methoxychlor	0.29±0.09^{a}	0.13±0.05^{a}	0.19±0.09^{a}

*Means of values with same superscript along rows are not significantly different ($p<0.05$)
SD = standard deviation

Table 4: Comparison of Organochlorine pesticides residue with the Maximum Residue Limit (MRL) (ng/g)

OCPs Component	Total OCPS	Total OCPS	Total OCPS	MRL (ng/g) (FAO/WHO, 2009)
	Oreochromis niloticus	*Clarias gariepinus*	*Hepsetus odoe*	
alpha-HCH	0.28±0.08	0.34±0.04	0.03±0.01	0.01
beta-HCH	0.00±0.00	0.00±0.00	0.00±0.00	0.01
gamma-HCH	0.69±0.25	0.06±0.02	0.16±0.02	0.01
Heptachlor	5.60±1.26	1.09±0.58	6.45±0.83	0.003
Heptachlor epoxide	37.44±7.59	17.81±4.21	0.00±0.00	0.003
Aldrin	1.51±0.37	0.00±0.00	0.00±0.00	0.03
Dieldrin	0.00±0.00	0.00±0.00	0.00±0.00	0.20
Edosulfan I	361.91±76.30	60.12±9.35	156.10±33.10	0.10
Endosulfan II	0.00±0.00	0.00±0.00	0.00±0.00	0.10
p,p’ DDE	0.38±0.16	3.13±0.98	11.44±4.27	1.00
p,p’DDT	1.66±0.17	1.05±0.29	3.37±0.66	1.00
p,p’DDD	2.59±0.74	3.03±0.88	3.80±0.12	1.00
Endrin	0.00±0.00	0.00±0.00	0.00±0.00	0.20
Endrin aldehyde	0.00±0.00	0.00±0.00	0.00±0.00	0.20
Methoxychlor	0.42±0.14	0.47±0.10	0.46±0.16	0.20
MRC	∑27.50±8.71	∑5.81±1.83	∑11.87±4.90	

MRC = Mean Residue Concentration

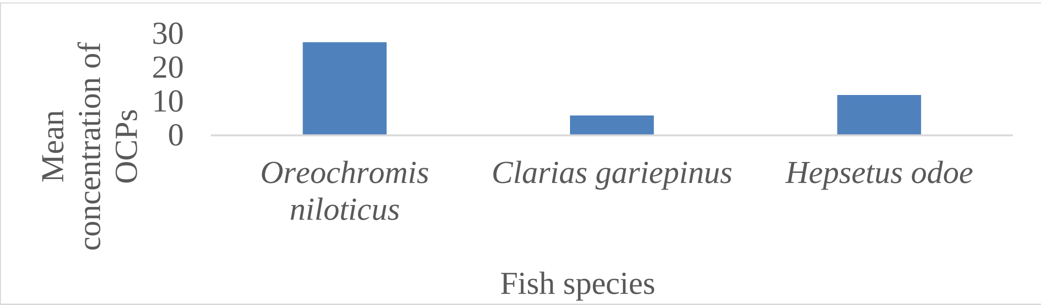

Figure 2: Mean concentration of Organochlorine pesticides residue in fish

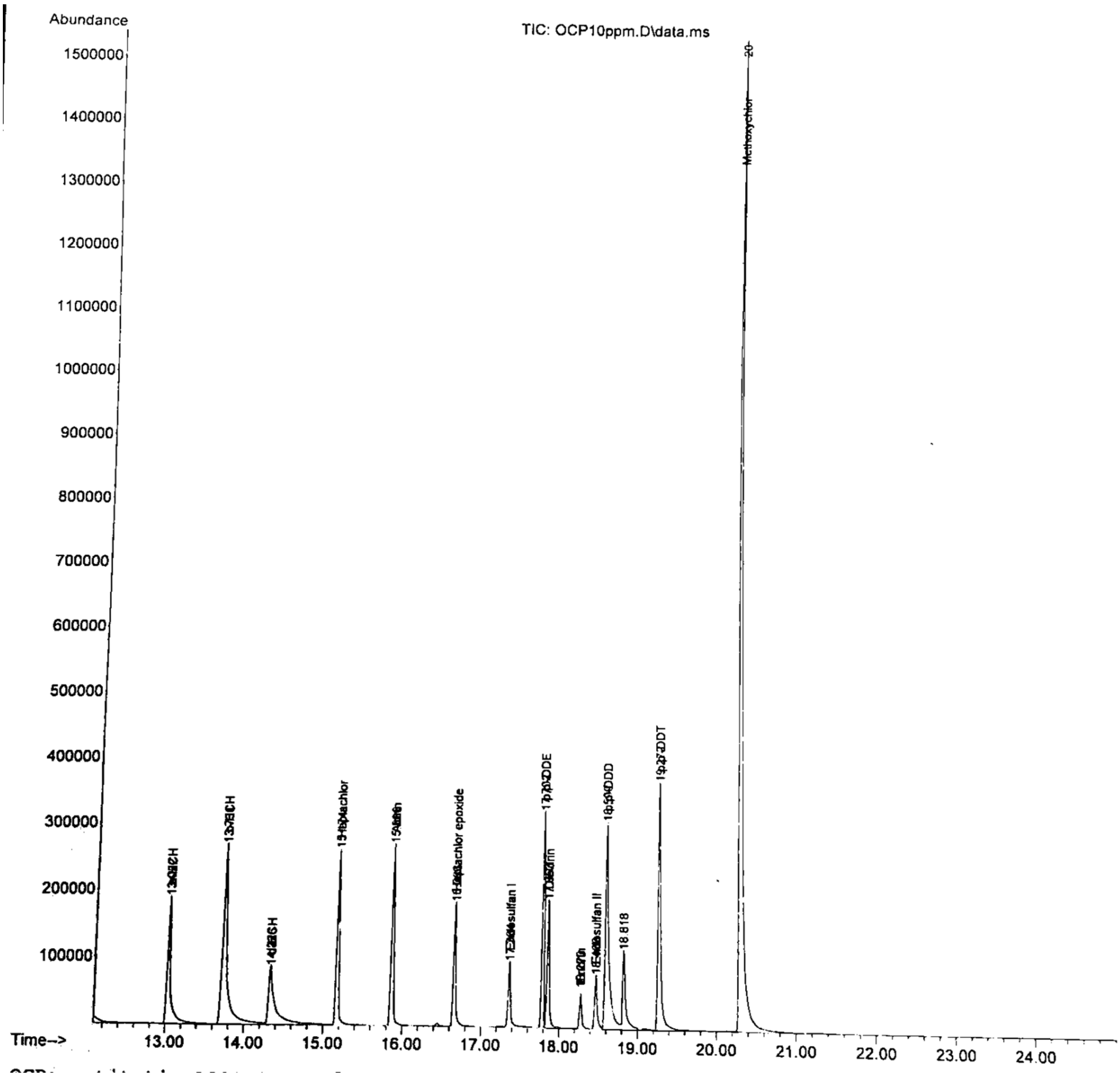

Figure 3: Chromatograph of Organochlorine pesticides

Morphometric characteristics of fish

The morphometric characteristics of fish were measured in the month of May and June (Table 1). The total lengths of fish ranged from 16.92±1.94 - 16.96±2.98cm (*O. niloticus*)*;* 32.50±0.50 -34.50±2.24cm (*C. gariepinus*); 25.85±1.20 - 26.25±2.36cm (*H. odoe*). The standard length ranged from 13.18±2.46 - 13.96±1.66cm (*O. niloticus*); 29.40±0.82 - 29.80±1.92cm (*C. gariepinus*); 21.10±1.27 - 21.25±1.27cm (*H. odoe*). The weight ranged from 106.76±48.10 - 117.5±38.8g (*O. niloticus*); 237.44±28.64 - 350.58±64.67g (*C. gariepinus*); 128.05±24.11 - 139.07±40.72g (*H. odoe*)

Concentration of Organochlorine pesticides residue

Table 2 shows the concentrations of pesticides residues in the month of May. The highest mean value of alpha-Hexachlorocyclohexane was recorded in *O. niloticus* and *C. gariepinus* (0.03±0.01ng/g). Alpha-Hexachlorocyclohexane was not detected in *H. odoe.* Gamma- Hexachlorocyclohexane had the highest in *O. niloticus* and *H. odoe* (0.16±0.02ng/g), while the lowest value was observed in *C. gariepinus* (0.06±0.02ng/g). Heptachlor and Heptachlor epoxide had similar trends where the mean values were recorded in *O. niloticus* (1.84±0.56 and 37.44±7.59ng/g) respectively. The lowest value was recorded in *C. gariepinus* (0.00ng/g for Heptachlor) and *H. odoe* (0.00ng/g for Heptachlor epoxide). Edosulfan I residue was higher in *H. odoe* (156.10±33.10ng/g) and lower in *O. niloticus* (52.90±8.61ng/g). The highest mean value of DDT and its metabolites were recorded as follows: p,p' DDD (2.86±0.84ng/g in *C. gariepinus*), p,p' DDT (1.55±0.44ng/g in *O. niloticus*), p,p' DDE (5.78±3.24ng/g in *H. odoe*). The highest mean value of Methoxychlor was recorded in *C. gariepinus* (0.83±0.05ng/g). Lowest value was observed in *O. niloticus* (0.14±0.05ng/g)

Table 3 shows the concentration of Organochlorine Pesticides in the month of June. The highest mean value of alpha-Hexachlorocyclohexane was recorded in *O. niloticus* (0.25±0.07ng/g). The lowest alpha-Hexachlorocyclohexane was recorded in *H. odoe* (0.03±0.01ng/g). Gamma-Hexachlorocyclohexane had the highest in *O. niloticus* and lowest in *H. odoe* and *C. gariepinus* (0.00ng/g). The mean of Heptachlor was high in *O. niloticus* (3.81±0.70ng/g) and low in *C. gariepinus* (1.09±0.58ng/g). Heptachlor epoxide and Aldrin were only detected in *Clarias gariepinus* (10.37±3.07ng/g) *O. niloticus* (1.51±0.37ng/g) respectively. Edosulfan I residue was higher in *O. niloticus* (309.01±67.69ng/g) and lower *H. odoe* and *C. gariepinus* at 0.00ng/g. The highest mean value of DDT and its metabolites were recorded as follows; p,p' DDD (3.38±0.06ng/g in *H.*

odoe), p,p' DDT (3.37±0.66ng/g in *H. odoe*), p,p' DDE (5.78±1.03ng/g in *H. odoe*). The highest mean value of Methoxychlor was recorded in *O. niloticus* (0.29±0.09ng/g) while the lowest value was observed in *C. gariepinus* (0.13±0.05ng/g).

The values of Heptachlor epoxide**,** Endosulfan I, p,p'DDE, p,p'DDT and methoxychlor were significantly different at $p<0.05$ in all the three species of fish collected in May. Heptachlor and Heptachlor epoxide, Endosulfan I, p'DDE, p,p'DDT and p,p'DDD were also significantly different at $p<0.05$ across the species in the month of June.

Table 4 presented the total organochlorine pesticides in *O. niloticus*, *C. gariepinus* and *H. odoe.* The highest mean residue concentration was recorded in *O. niloticus* (27.50±8.71ng/g) while the lowest mean value was observed in *C. gariepinus* (5.81±4.90ng/g). All the detected Organochlorine residues were above maximum residue limit.

Discussion

In the present study, among the various isomers of Hexachlorocyclohexane (HCH), gamma-HCH was predominant followed by alpha-HCH, while belta-HCH was not detected throughout the study. The highest value of gamma-HCH was recorded in the muscle of *O. niloticus,* while the least value was recorded in the muscle of *C. gariepinus*. The concentrations of this pesticide in all the fish samples were much higher than the FAO/WHO (2009) set Maximum Residue Limit (MRLs) of 0.01 ng/g.

The highest levels of p,p'DDT and it metabolites in the present study was observed in the muscle of *Hepsetus odoe.* The detection of p,p'-DDE is an indication of photochemical degradation of p,p'-DDT. The concentrations of DDT and its metabolites in all the fish samples were much higher than the FAO/WHO (2009) set Maximum Residue Limit (MRL) of 1.0 ng/g.

Endosulfan was the highest in all the OCPs component examined in the month of May and June. This result is in agreement with the study carried out by (Afful *et al.*, 2010) which indicates high level of endosulfan residue in fish. The concentrations endosulfans in all the fish samples were much higher than the FAO/WHO (2009) set Maximum Residue Limit (MRL) 0.1 ng/g.

Dieldrin was not detected in all the fish species. Aldrin was only recorded in the Muscle of *O. niloticus*. The concentration of Aldrin and non-detection of Dieldrin may be that Aldrin has not undergone photolysis in the fish muscle. Aldrin has been reported to undergo photolysis to produce dieldrin as a metabolite (The United States Department of Health and Human services, 1993). The sale of dieldrin and Aldrin has been banned by National Agency for Food and Drug Administration and Control (NAFDAC) because of their toxicity and persistence in the environment which also posed imminent danger to human health (Akan *et al.,* 2013). The concentrations of aldrin in *O. niloticus* samples was much higher than the FAO/WHO (2009) set Maximum Residue Limit (MRL) of 0.2ng/g

The maximum concentration of methoxyclor was detected in muscle of *C. gariepinus*, while the least value was recorded in the muscle of *O. niloticus*. The amount of methoxychlor in the environment changes seasonally due to its use in farming. It does not dissolve readily in water - its degradation may take many months.

The concentration of heptachlor epoxide is higher than the concentration of heptachlor. Heptachlor epoxide is more stable in water and its bio-concentrate extensively in fish (Lu and Wang, 2002). The highest concentration of heptachlor epoxide was observed in *O. niloticus*. The concentrations of this pesticide in all the fish samples were much higher than the FAO/WHO (2009) set Maximum Residue Limit (MRLs) of 0.003ng/g.

O. niloticus has the highest mean concentration (Figure 2) which was also in line with Adeyemi *et al.* (2008). The lowest concentration was detected in *C. gariepinus*. The variation in concentration of OCPs detected in fish species could be as a result of the differences in feeding habit and metabolic characteristics of the fish species.

Conclusion

The study revealed that pesticide residue levels in the fish samples study were above the Maximum Residue Limits (MRLs) and this could be an important process of transferring pesticides to humans. It also indicated the extensive presence and usage of these pesticides in the studied environment, which include recent use of this pesticide for pest control. Thus, the use of these pesticides to control pests by farmers within the study area with little or no knowledge must be checked through adequate control of the trade and use of pesticides and the enforcement of appropriate sanctions.

Conflict of Interest

The authors declare No conflict of interest

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