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Seasonal Variation in Bioaccumulation of Organchlorine and Organophosphosphate Pesticides in Fish from River Owena, Nigeria and their Health Risk Appraisal

*[1]Ayodeji Oluwole Adegun, [2]Thompson Akinyele Akinnifesi, [1]Olumayowa Joseph Eluyeba, [1]Olubukola Oyebimpe Agboola, [1]Daniel Adeagbo, [3]Kayode Adeniyi Aseperi, [4]James Barker

[1]Department of Biological Sciences, Chemical Sciences unit, McPherson University, Seriki Sotayo, Ogun State, Nigeria

[2]Department of Chemical Sciences, Adekunle Ajasin University, Akungba Akoko, Nigeria

[3]Centre for Process Innovation, United Kingdom

[4]School of Life Sciences, Pharmacy and Chemistry, Kingston University, Kingston-upon-Thames, United Kingdom

*Corresponding Author: ayodeji2k15@gmail.com

ABSTRACT

The impact and human health risks of organochlorine (OC) and organophosphate (OP) pesticide residues washed into the River Owena, Nigeria via runoff from nearby cocoa farms on fish samples from the river was assessed in this study. Pesticide residues in fish samples were extracted using an ultrasonic bath extractor. The extracts were analysed with a gas chromatograph coupled to a mass spectrometry (GC-MS) detector. The total mean concentrations of the organochlorine pesticide residues in the fish samples ranged from 0.77±0.78 µg/g to 3.62±03.91µg/g and 0.07±0.06 µg/g to 0.36±0.14µg/g, for dry and wet seasons respectively, while the organophosphates varied from 0.91±0.04 µg/g to 1.29±0.58 µg/g and 0.16±0.03 µg/g to 0.26±0.04, for dry and wet seasons respectively. The concentrations of some of these contaminants in the fish samples were higher than 0.5g/µg/g and 0.8g/µg/g FAO/WHO maximum residue limits for OC and OP pesticides respectively. The health risk assessment indicated that some of the OC and OP pesticide residues constituted a health risk. This study concluded that the fish samples from the study area were contaminated with these chemicals. It is recommended that the consumption of contaminated fish from the river should be discouraged and that Government should enforce existing laws on the handling and usage of these chemicals for cocoa pesticide control.

KEYWORDS: Fish, organochlorine, organophosphates, pesticides, health risk

INTRODUCTION

River Owena runs along the major cocoa-producing area of Ondo State, Nigeria, where insecticides are frequently sprayed to control cocoa mirids (*Silbergella singularis* and

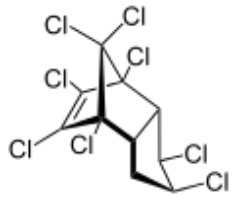
Distentiella theobramae). The infestation of cocoa plants by these mirids is one of the major problems facing cocoa farmers in the State as they cause diseases to cocoa plants [1-2]. Pesticide residues in soils may be discharged through runoff into ephemeral streams, as well as surface impoundments, and finally into the river [3]. There is paucity of information on the effects of these pesticide residues introduced on its aquatic organisms, such as fish, crabs, prawns and crustaceans consumed by the inhabitants of the study area

In spite of the benefits derived from the application of pesticides, the environmental consequences of the widespread use, handling and their disposal are of great concern [4-8]. Pesticides, especially organochlorine, have caused global concern due to their high toxicity, persistence, bioaccumulation, severe adverse effects on the ecosystem and human health through the food chain [9]. Effects of pesticides on human health are caused by inhalation, ingestion and through skin contact as well as through pesticides consumed in food, water and aquatic organisms [10]. Pesticides usage in Nigeria has continued to increase in commercial farming due to the need to intensify agricultural production [11]. Cocoa farmers in Nigeria used pesticides on the cocoa plants, which are vulnerable to attacks by a large variety of pests [12-13]. Annually, since 1957, farmers were faced with the problem of protecting cocoa plants against cocoa mirids or capsids (*Silbergella singularis and Distentiella theobromae*). Most of these pesticides have been banned locally and internationally because of their adverse effects on the environment, including the host plants and the soil properties, as well as human health [14-15].

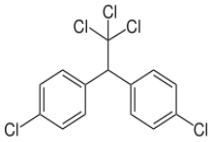
The growing interest in water pollution studies and control continues to increase in the past three decades in Nigeria [16]. Literature survey revealed that there is little or inadequate information, especially on organochlorine and organophosphate seasonal distribution levels in aquatic vertebrates and invertebrates and their health implication in the study area. Therefore, this study intends to fill the gap.

Organochlorine pesticides are known as the persistent organic pollutants (POPs) [17-18]. These pollutants are toxic chemicals that adversely affect human health and the environment [19-21]. The organochlorine pesticides include aldrin, chlordane, dichloro diphenyl trichloroethane (DDT), dieldrin, endrine, heptachlor, hexachlorobenzene, mirex, toxaphane and endosulfans (Figure 1). Exposure to OCP contamination leads to memory loss, loss of coordination, reduced speed of response to stimuli, reduced visual ability, asthma

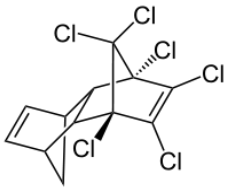
allergies and hypersensitivity, cancer, hormone disruption, problems of reproduction and fetal development [22-23]. Higher levels of organochlorine pesticides exposure cause a range of neurological health problems. OCP residues in mother's blood during pregnancy lead to poorer mental development in children at early age [24-25].



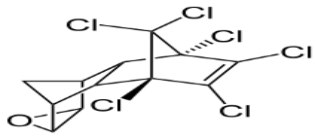
(a) Chlordane



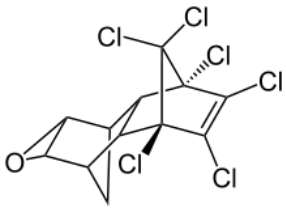
(b) DDT



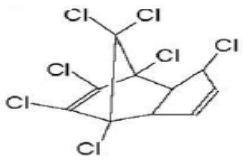
(c) Aldrin



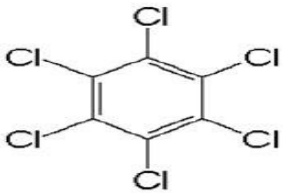
(d) Endrin



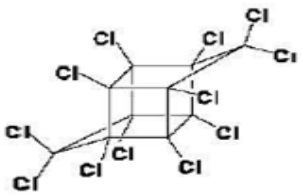
(e) Dieldrin



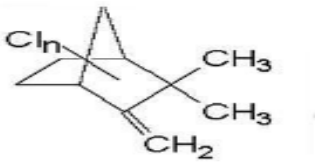
(f) Heptachlor



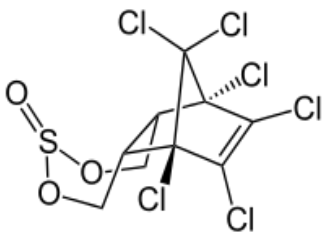
(g) Hexachlorobenzene



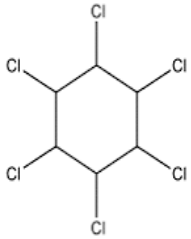
(h) mirex



(i) Toxaphane



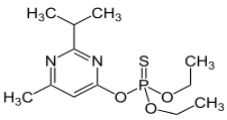
(j) Endosulfan



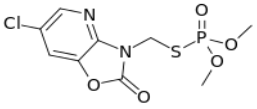
(k) Hexacyclochlorohexane

Figure 1(a-k): Chemical structures of some organochlorine pesticides

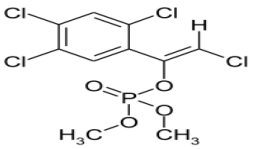
Organophosphates pesticides (OPP) are the basis of many insecticides, herbicides and poison nerve agents [26]. The United States Environmental Protection Agency lists organophosphates as very acutely toxic to bees, wildlife, and humans [27]. Organophosphates appeared in general use as a result of the development of resistance against organochlorine [28]. Organophosphorous pesticides have been the insecticides most commonly used by professional pest control bodies and home owners for the past three decades. They are toxic to mammals, but they are degraded in 2-4 weeks [29]. Organophosphates are degraded by general esterases such as cholinesterases, phosphomonoesterases, carboxylesterases and oxidases. Several of the organophosphates cause inhibition and elevation of cholinesterase due to the cumulative effect of pesticides. The symptoms of neurotoxicity, hepatotoxicity and kidney dysfunctions are shown on exposure to this pollutant [30]. Studies suggest a possible link to adverse effects in the neurobehavioral development of foetuses and children, at very low levels of exposure, to organophosphate poisoning. Organophosphates are widely used as solvents, plasticizers, and additives [31]. Headache, restlessness, convulsions, loss of consciousness, coma, rhinorrhoea, bronchorrhea, dyspnea, hyperpnea, bradypnea, and tachycardia are some of the symptoms due to exposure to organophosphate pesticides [24,32]. These OPP include: parathion malathion methyl parathion chlorpyrifos, diazinon, dichlorvos, phosmet, fenitrothion, tetrachlorvinphos, azamethiphos and azinphos-methyl (Figure 2).



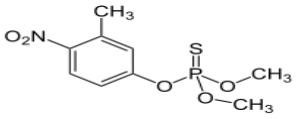
(a) Diazinon



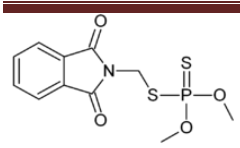
(b) Azamethiphos



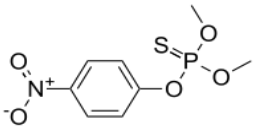
(c) Tetrachlorvinphos



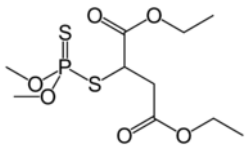
(d) Fenitrothion



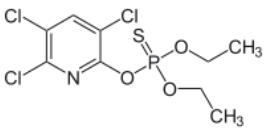
(e) Phosmet



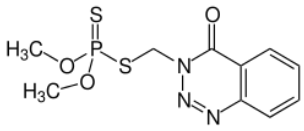
(f) Methyl Parathion



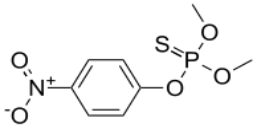
(g) Malathion



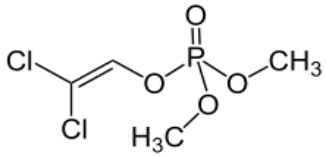
(h) Chlorpyrifos



(i) Azinphos-methyl



(j) Parathion



(k) Dichlorvos

Figure 2 (a-k) Chemical structures of organophosphate (OP) pesticides

Several research works have been carried out on pesticides globally for environmental monitoring. In Nigeria, Ayesanmi and Idowu [20], worked on organochlorine pesticide residues in soils of cocoa farms in Ondo State Central Senatorial District, Nigeria. The study reported that the soils of those selected farms were contaminated with organochlorine pesticides and that there was a correlation between these contaminants and soil organic matter. Akinnawo *et al.* [14] reported that the sediment samples of River Ilaje were contaminated with organophosphate pesticide residues in a study on the determination of the concentration of organophosphate pesticide residues in water and sediment samples from River Ilaje, Nigeria. Okoya *et al.* [12] reported the presence of some OCP pesticide residues in the water and sediment matrices of some cocoa producing areas of Ondo State South western, Nigeria. That

study reported higher concentrations of OCPs residues in the sediment samples than the water samples and the study further revealed that OCP level in water samples was significantly higher during the dry season than the wet season. Recent studies by Adegun *et al.* [3, 4, 6] on pesticides in selected environmental matrices in Ondo, Nigerain State also reported the presence of carbamates, pyrethroids and neonicotinod pollutants in the Owena river basin.

The hypothesis of this study is based on whether OC and OP pesticide residues washed into the Owena river from the nearby cocoa farms could be detected by monitoring of their fate in aquatic organisms, particularly fish. Therefore, the specific objectives of this study are (i) to assess the seasonal bioaccumulation potential of these pesticide residues in fish tissues, (ii) develop baseline data on their occurrence levels in the different fish species from the river and (iii) conduct a health risk evaluation in the study area.

MATERIALS AND METHODS

Study Area

The study area is the Owena River basin, which is located on geographical coordinates 7^0 15′ and 5^0 5′E in the Ondo East (Local Government Area) of Ondo State, Nigeria. River Owena is the major source of drinking water supply in Ondo Central Senatorial District in Nigeria, which comprises of six Local Government Areas (Akure North, Akure South, Ifedore, Idanre, Ondo East and Ondo West). These Local Government Areas constitute about 40% of the total population of Ondo State, Nigeria. This is an environmentally sensitive area, since fishes of the River Owena are consumed by people. This area is comprised of Ifedore, Akure North, Akure South, Ondo West, Ondo East an Idanre Local Government Areas. The villages around the study area are Pepeye, Kajola, Ijiziogba, Ago Elesin and Bolorunduro.This river basin is under the Benin-Owena River Basin Developmrnt authority.

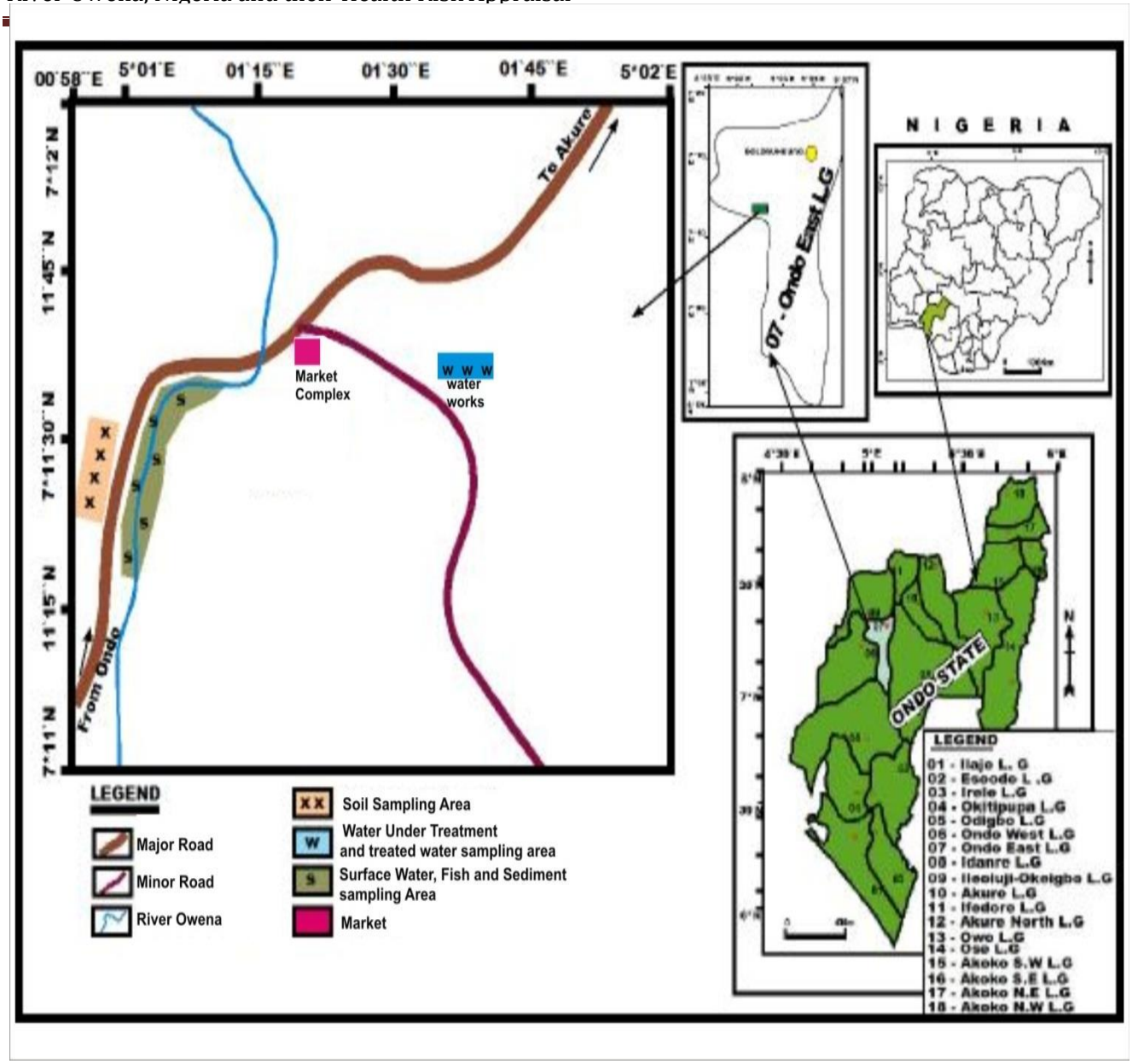


Figure 3: Map of Ondo State, Nigeria, showing the sampling sites

Sample Collection, Preservation and Preparation

Twenty-four fish samples of six species and sixteen fish samples of four species were caught with gill nets from Owena River in wet and dry seasons respectively. The fish samples were transported to the laboratory the same day in a glass container with water. The length and weight of these fishes ranged between 65 cm -100 cm and 60 g-150 g respectively. They were identified at the Department of Fisheries and Aqua Culture, Adekunle Ajasin University, as Nile tilapia (*Oreochromis niloticus*), African sharp tooth cat fish (*Clarias gariepinus*), African knife fish (*Gvmnarchusni niloticus)* and mango tilapia (*Sarotherodon galileous*). The fish

samples were frozen until analysis. The fish samples' skin was removed and the muscle tissues were dissected and homogenized with a meat grinder. The mixing was repeated until the composite sample appeared to be homogenized; and then kept frozen until extraction [2].

Extraction of Pesticide Residues from the Fish Samples

Pesticide residues were extracted from the fish samples by EPA method 3550c [33]. A Super Scientific-100005 (50 W, 45 KHz) ultrasonic bath was used. Ten grams of the fish samples were weighed into a 50 mL glass beaker containing 5 g of Na_2SO_4, mixed and then covered with aluminium foil to prevent spilling. Twenty millilitres of the extracting solvent mixture (acetone/hexane 1:1v/v) were used. The temperature of the ultrasonic bath was set at 25 ^oC and the extraction time was set at 30 minutes. A glass beaker was placed on the ultrasonic bath and sufficient water quantity was deposited into the bath to cover three quarters of the beaker (the water in the bath was above the volume of the solvent in the beaker). This condition was remained constant during the experiments. The extractions were carried out thrice and the extracts were combined and filtered prior to the clean-up step **[**2].

Clean-Up of the Extracts

The filtrates of the extracts were evaporated to 20 mL volume with a gentle stream of N_2 current. Ten millilitres of 0.1 mL Na_2CO_3 solution were added and shaken for 10 minutes. The aqueous phase, which contained free fatty acids was discarded [14]. The organic phase was separated by a chromatographic clean-up technique with 10 g of alumina as adsorbent placed in the chromatographic column. The column was tapped to settle the alumina and 2 g of anhydrous Na_2SO_4 was added to the top of the column. The column was then pre-eluted with 40 mL of hexane at an elution rate of 2 mL/min. Two mL sample extracts were quantitatively transferred into the column using additional 2 mL of hexane to complete the transfer. The column was finally eluted with 140 mL ethylether/hexane (1/4) (v/v) and concentrated [33].

GC-MS Analysis

The cleaned extracts were analysed in triplicate on a gas chromatograph from Agilent USA hyphenated to a mass spectrometer (5975C) with triple axis detector (equipped with an auto 10 µL syringe) and helium was used as carrier gas. All the chromatographic separations were performed on a capillary column having specification: length 30 m, internal diameter 0.2 µm,

film thickness 250 µm, treated with phenyl methylsiloxane. Other GC-MS conditions were ion source temperature (EI) 250 °C, interface temperature 230 °C, pressure 16.2 PSI, 1µL injector in split mode with split ratio 1:50 and injection temperature of 300 °C. The column temperature started with 35 °C for 5 minutes and changed to 150 °C at a rate of 4 °C/min. This was raised to 250 °C at a rate of 20 °C/min and held for 5 minutes with a solvent delay of 5 minutes. The mass spectra obtained were compared with those of the standard mass spectra from NIST library for spectral interpretation and identification.

Quantification of Pesticide Residues

The concentration of the analytes was calculated using the external standard calibration method as described by US Environmental protection Agency [33].

$$\text{Concentration } (\mu g/g) \quad = \frac{(Ax)(Vt)(D)}{(CF)(Vi)(Ws)}$$

Where: Ax = peak area for the analyte in the sample

Vt = total volume of the concentrated extract (µL)

D = dilution factor = 1 (No dilution was made)

CF=Calibration Factor

Vi = volume injected

Ws = weight of fish sample

Quality Control/Assurance

All reagents used were analytical grade and all the solvents are HPLC or pesticide grade. Glassware was washed thoroughly to eliminate cross-contamination. Each sample was analysed in triplicate and mean concentrations were calculated. Other quality control measures include analysis of solvent blanks and procedure blanks.

RESULTS AND DISCUSSION

Total Mean Concentration of Organochlorine Pesticide Residues in Fish Species

The total mean concentration of OCP in the fish matrices is presented in Table 1. For the dry season, the identified fish species were *Oreochromis niloticus, Gvmnarchus niloticus, S. galileous* and *Claria gariepinus,* their mean OCP concentrations were: 0.77±0.78µg/g, 0.81±0.5µg/g, 1.32±0.99µg/g and 3.62±03.91µg/g respectively. Their total mean OCP concentration in the wet season was: 0.17±0.02 µg/g, 0.07±0.06 µg/g, 0.13±0.06 µg/g,

0.24±0.17µg/g, 0.26±0.2 5µg/g and 0.36±0.14µg/g for *Oreochromis niloticus, Gvmnarchus niloticus, S. galileous*, *Claria gariepinus*, *Clarias anguillaris* and *Parachanna obscura* respectively. The detected residues of OCP in all the fish samples in the dry season were from aldrin, lindane, α endosulfan, β endosulfan and hexachlorobenzene (HCB). HCB was not detected in the wet season. The presence of these contaminants could be due to their persistence in aquatic ecosystem [34, 35] or their recent usage in the study area. Additionally, since most of these OCP have higher specific gravities, they tend to sink into the sediments where they harm benthic organisms and bioaccumulate in aquatic tissues. The dry season concentration of these pollutants in descending order was *Claria gariepinus* > *S. galieous* > *Gvmnarchus niloticus* > *Oreochromis niloticus*, while that of the wet season was *Parachanna obscura* > *Clarias anguillaris* > *Claria gariepinus* > *S. galieous* > *Gvmnarchus niloticus* > *Gvmnarchus niloticus* (Table 1).

The presence of lindane in the fish samples could be attributed to the practice of using it to kill fishes in the river, apart from the runoff source. They could be poured directly into the river during fishing. The use of this insecticide for fishing was confirmed by conversations with some of the fishermen in the study area. S. *galileous* is an herbivorous fish and could bioaccumulate more of these pollutants from water plants that they feed on. The total mean concentration of OCP in *Claria gariepinus* was the highest among all of the fish studied, probably because of its carnivorous nature [17].

Generally, the different levels of OCP bioaccumulation in the fish species could be due to their different feeding habits. Carnivorous species such as *Gvmnarchus niloticus*, *Claria gariepinus, Parachanna obscura* and *Clarias anguillaris* may bioaccumulate these pollutants by eating other smaller contaminated fishes, since they are higher in trophic level and more prone to biomagnification than *S. galieous*, an herbivorous fish which feeds primarily on water phytoplankton and algae. It was observed that more OCP residues were detected in *Oreochromis niloticus*, an omnivorous fish which feeds on aquatic plants and insects. The frequency of occurrence of more OCP residues in carnivorous fishes is proof that they bioaccumlate more of these pollutants through eating contaminated smaller fishes. Therefore, the concentration of these pollutants increases and biomagnifies to top predators, including man [36]. Studies have shown that species-specific differences and feeding habit of fishes also influence the variation in accumulation of OCP residues among specie [37-41].

This study observed a lower mean level of OCP in the dry season than in a study by Kalfizadeh *et al.* [40] on the determination of organochlorine pesticide residues in water, sediments and fish from Lake Parishan, Iran in which 4.86 µg/g OCP residues was reported in the muscle tissue of fish studied.

This study also reported a higher total mean concentration (0.07-3.62 µg/g) of OCP in dry season than 0.027-0.310 µg/g reported by Akoto *et al.*, [17] in a study of pesticide residues in water, sediment and fish samples from Tono reservoir, Ghana. This study further reported a higher contamination dry season OCP level when compared with a study in *China* by Shu Rui *et al.*,[42] which reported a mean value of 0.02803µg/g. The level of OCP residue contamination from this study in the dry season was higher than the wet season (Figure 4). This could be as a result of mass movement of these pollutants in aquatic ecosystem in the wet season. This study also observed higher OCP contamination levels than 0.00268 – 0.876.4 µg/g reported by Gbasem *et al.* [43] in a study on the determination of organochlorine pesticide residues in water, sediment, and fish samples from the Meriç Delta, Turkey. The total mean concentration of OCP pesticide residues in the fish samples in the dry season was above the 0.5 µg/g FAO MRL [33].

Table 1: Mean concentration (µg/g± sd) of organochlorine pesticide residues in fish samples from Owena River in dry and wet seasons

Pesticides	*Oreochromis niloticus*	*Gvmnarchus niloticus*	*S.galileous* mean±sd	*Clarias gariepinus*	*Clarias anguillaris*	*Parachanna obscura*
		Wet Season				
Aldrin	BDL	0.02± 0.01	0.09 ±0.03	0.06±0.01	BDL	BDL
Lindane	BDL	0.12±0.02	0.29±0.07	0.29±0.07	0.08±0.03	BDL
α Endosulfan	0.15± 0.09	BDL	BDL	0.38±0.24	0.43±0.02	0.22±0.15
β Endosulfan	00.18±0.06	BDL	BDL	BDL	BDL	0.50±0.09
HCB	BDL	BDL	BDL	BDL	BDL	BDL
Chlobycyclen	BDL	BDL	BDL	BDL	BDL	BDL
Cis-chlordane	BDL	BDL	BDL	BDL	BDL	BDL
Trans-chlordane	BDL	BDL	BDL	BDL	BDL	BDL
∑OCP	0.33	0.14	0.26	0.73	0.51	0.72
Total mean	0.17	0.07	0.13	0.24	0.26	0.36
SD	0.02	0.06	0.06	0.17	0.25	0.14
CV	11.77	85.71	46.15	70.83	96.15	38.89
ANOVA	P ≥ 0.05	P ≥ 0.05	P ≥ 0.05	P ≥ 0.05	P ≥ 0.05	P ≥ 0.05

REMARKS	NS	NS	NS	NS	NS	NS

		Dry Season		
Aldrin	BDL	BDL	2.02 ±0.10	8.49 ±3.26
Lindane	1.92 ±1.06	1.29 ±0.09	0.62±0.03	5.09±2.07
α Endosulfan	0.28 ±0.02	0.22 ±0.01	BDL	0.48 ±0.06
β Endosulfan	0.30 ±0.08	BDL	BDL	0.43 ±0.04
HCB	0.59 ±0.09	0.93 ±0.07	BDL	BDL
Chlobycyclen	BDL	BDL	BDL	BDL
Cis-chlordane	BDL	BDL	BDL	BDL
Trans-chlordane	BDL	BDL	BDL	BDL
∑OCP	3.09	2.44	2.64	14.49
Total mean	0.77	0.81	1.32	3.62
SD	0.78	0.54	0.99	3.91
CV	101.30	66.67	75	108.01
ANOVA	P ≥ 0.05	P ≥ 0.05	P ≥ 0.05	P ≥ 0.05
REMARKS	NS	NS	NS	NS

∑OCP = Total organochlorine pesticides residues; BDL=Below Detection Limit=0.00001 µg/g; SD = Standard deviation; ANOVA=Analysis of variance; NS = No significant difference; CV = Coefficient of variation, HCB=Hexachlorobenzene

Figure 4: Level of occurrence of organochlorine pesticide residues in fish samples during dry and wet seasons

Total Mean Concentration of Organophosphate Pesticides

Table 2 contains the total mean concentration of OPP residues as presented in dry and wet seasons. The mean OPP concentrations for fish species *Oreochromis niloticus*, *Gvmnarchus niloticus*, *S. galileous* and *Claria gariepinus* in the dry season were 0.96±0.12, 0.91±0.04, 0.90±0.04 and 1.29±0.58 respectively. Their total mean concentration in wet season were 0.16±0.03, 0.26±0.04, 0.18±0.04, 0.24±0.05, 0.19±0.06 and 0.17±0.04 for *Oreochromis niloticus*, *Gvmnarchus niloticus*,*S. galileous*, *Clarias anguillaris* and *Parachanna obscura* respectively. The two frequently occurring OPP residues in the fish samples were chlopyrifos and diazinion.

The results from this study indicate that pesticides containing chlopyrifos and diazinion could have been frequently used by cocoa farmers around the study area and might have been washed into the river through runoff.

The trend in the distribution of OPP residues in the fish samples in the dry season is in the descending order *Claria gariepinus* > *Oreochromis niloticus* > *Gvmnarchus niloticu* > *S. galileaus*. The order in the wet season is *Gvmnarchus niloticus* > *Claria gariepinus*> *Clarias anguillaris* > *S. galileous* > *Parachanna obscura* > *Oreochromis niloticus* (Table 2). The presence of OPP residues in all the fish samples could be due to their resistance to microbial degradation and their tendency to concentrate in lipid rich tissues of aquatic organisms. The hydrophobic nature of these contaminants in water could lead to their accumulation in sediment and bioaccumulation in fatty tissues [43]. *Claria gariepinus and Gvmnarchus niloticus* had the highest mean concentrations of OPP residues in both seasons because of their feeding habits as carnivorous fishes. This study is in agreement with a similar study by Akan *et al.* [27] which reported 0.77µg/g -2.22 µg/g values for the concentration range of chlopyrifos, an organophosphate. This study is also in agreement with a study by Akoto *et al.* [17] which reported 0.1607µg/g level for fish species in a study of organophosphate pesticide residues in water, sediment and fish from Tono reservoir, Ghana. The mean concentration of OPP pesticide residues in the dry season is higher than in the wet season (Figure 5). The dry season fish samples' contamination level was above the 0.8 µg/g FAO/WHO MRL [33] and this call for health concern.

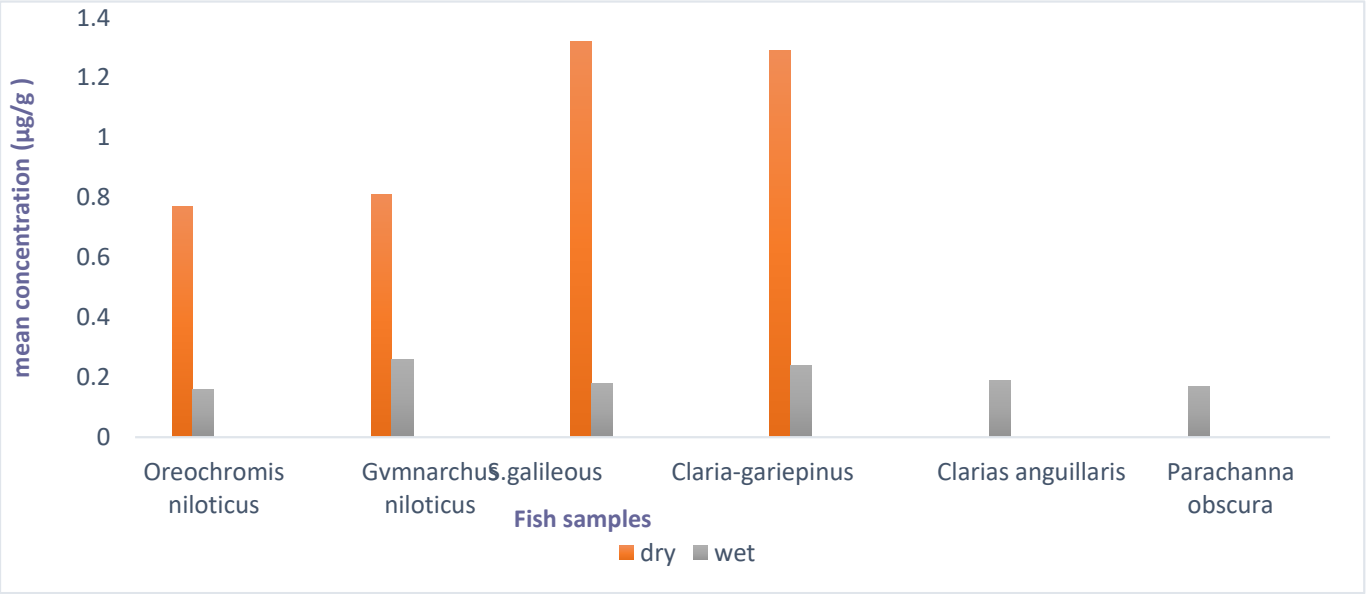


Figure 5: Level of occurrence organophosphate pesticide residues in fish samples during dry and wet seasons

Table 2: Mean concentration (µg/g) of organophosphate pesticide residues in fish samples from Owena River, Nigeria during wet seasons

Pesticides	*Oreochromisniloticus* mean±sd	*Gvmnarchus niloticus* mean±sd	*S.galileous* mean±sd	*Claria gariepinus* mean±sd	*Clarias anguillaris* mean±sd	*Parachanna obscura* mean±sd
		Wet Season				
Malathion	BDL	BDL	BDL	BDL	BDL	BDL
Chlopyrifos	0.30± 0.17	0.22±0.05	0.21 ±0.01	0.29±0.07	0.23±0.05	0.22± 0.02
Fenitrothion	BDL	BDL	BDL	BDL	BDL	BDL
Methyl parathion	BDL	BDL	BDL	BDL	BDL	BDL
Tetrachlorvinphos	BDL	BDL	BDL	BDL	BDL	BDL
Diazinion	0.02± 0.01	0.30 ±0.07	0.14± 0.01	0.19±0.02	0.15± 0.23	0.12± 0.02
Azinphos-methyl	BDL	BDL	BDL	BDL	BDL	BDL
Dichlorvos	BDL	BDL	BDL	BDL	BDL	BDL
∑OPP	0.32	0.52	0.35	0.48	0.38	0.34
Total mean	0.16	0.26	0.18	0.24	0.19	0.17
SD	0.03	0.04	0.04	0.05	0.06	0.04
CV	17.65	15.38	23.53	20.83	31.58	27.78
ANOVA	P ≥ 0.05	P ≥ 0.05	P ≥ 0.05	P ≥ 0.05	P ≥ 0.05	P ≥ 0.05

Remarks	NS	NS	NS	NS	NS	NS
		Dry Season				
Malathion		BDL	BDL	BDL	BDL	
Chlopyrifos		0.87 ± 0.05	0.89± 0.03	0.06± 0.01	0.88±0.06	
Fenitrothion		BDL	BDL	BDL	BDL	
Methyl parathion		BDL	BDL	BDL	BDL	
Tetrachlorvinphos		BDL	BDL	BDL	BDL	
Diazinion		1.04 ± 0.02	0.93± 0.01	0.89± 0.04	1.70±0.03	
Azinphos-methyl		BDL	BDL	BDL	BDL	
Dichlorvos		BDL	BDL	BDL	BDL	
∑OPP		1.91	1.82	1.81	2.58	
Total mean		0.96	0.91	0.90	1.29	
SD		0.12	0.04	0.04	0.58	
CV		12.5	4.40	4.44	44.96	
ANOVA		P ≥ 0.05	P ≥ 0.05	P ≥ 0.05	P ≥ 0.05	
Remarks		NS	NS	NS	NS	

∑OPP = Total organophosphates pesticides residues; BDL =Below Detection Limit=0.00001 µg/g; SD = Standard deviation; ANOVA=Analysis of variance; NS = No significant difference; CV = Coefficient of variation.

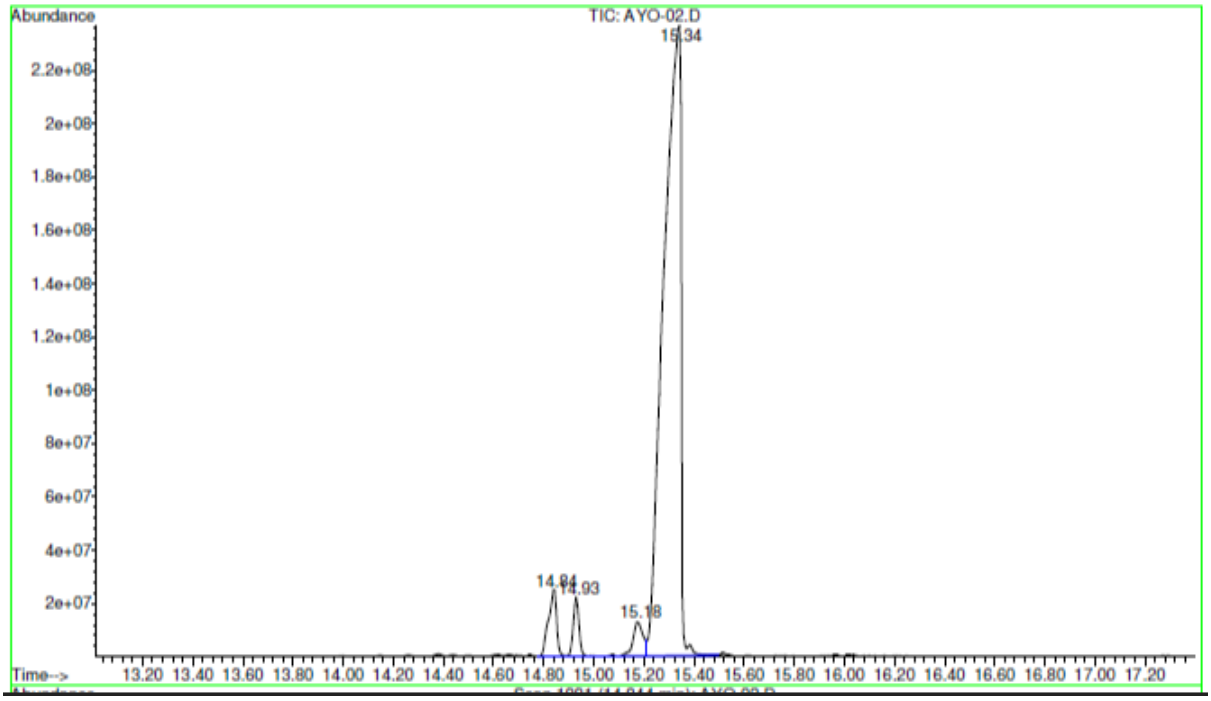


Figure 6: Representative Chromatographic Peak for Organochlorine Pesticides Residues

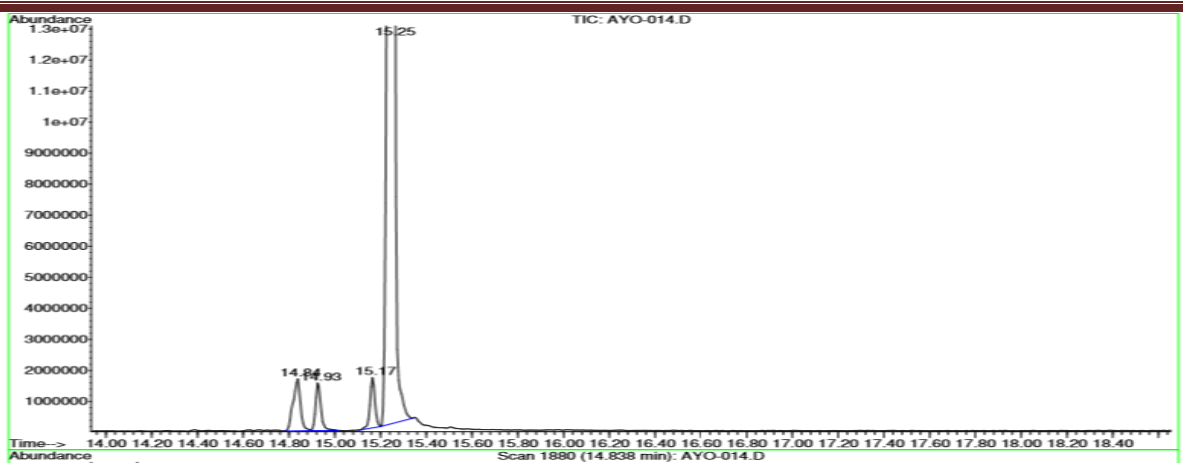


Figure 7: Representative Chromatographic Peak for Organophosphate Pesticide Residues

Estimation of Daily Intake of Organochlorine and Organophosphate Pesticides in Fish

The estimated daily intakes (EDIs) of the various pesticides in each fish species were determined by using equation:

EDI = CXD/B

where C, D and B represent the concentrations of pesticide residues in fish (µg/g) on wet weight basis, average daily intake of fish estimated at 65.5g/person/day for adults and average body weight considered to be 60 kg for adults [17].

The estimated HIs were obtained by dividing the EDI by their corresponding values of accepted daily intake (ADI) [44].

The Health Risk (HR) was calculated using the equation:

HR= EDI/ADI.

A Health Index (HI) >1 shows a health risk due to the consumption of contaminated fish species while HI < 1 shows no health risk based on their consumption.

Health Risk Index of Organochlorine and Organophosphate Pesticide Residues in Owena River Fish samples

The hazard indices associated with the consumption of fish contaminated with organochlorine pesticide residues in the study are shown in Table 3. This revealed that there is a health risk from the consumption of fish contaminated with hexachlorobenzene with an HI of 1.07 in

Oreochromis niloticus. However, α and β endosulfan with hazard indices- HI 0.05, HR < 1 constitutes no health risk due to the lower HI value. In *Gvmnarchus niloticus*, lindane with an HI of 2.82 also constitutes a health risk. In *S. galileous*, aldrin and lindane residues with HI values of 22.05 and 1.35 respectively constitute health risks. In *Claria gariepinus*, aldrin with HI values of 11.11 also constitutes an health risk in dry season. However, α and β endosulfan with hazard indices of 0.09 and 0.82 respectively constitute no health risks. There is no health risk associated with the consumption of any of the fish species contaminated with organochlorine pesticide residues in the wet season as their hazard indices ranged from 0.02 to 0.99.

There is also no health risk associated with the consumption of fish species contaminated with organophosphates pesticide residues in dry and wet seasons. The hazard indices ranged from 0.01 to 0.98 (Table 4).

Table 3: Health Risk Evaluation of Organochlorine Pesticide Residues in Fish Samples from River Owena, Nigeria.

Species of fish	Pesticide	mean±sd	Range	AD1	EDI	HI	HR
		Dry season					
Oreochromis niloticus							
	Lindane	1.92±1.06	1.89-1.96	0.0005	$2.10x10^{-3}$	0.42	No
	αEndosulfan	0.28±0.02	0.25 -0.32	0.006	$3.06x10^{-4}$	0.05	No
	βEndosulfan	0.30±0.08	0.28-0.36	0.006	$3.28x10^{-4}$	0.05	No
	HCB	0.59±0.09	0.55-0.64	0.0006	$6.44x10^{-4}$	1.07	Yes
Gvmnarchus niloticus	Lindane	1.29 ±0.09	1.27-1.34	0.0005	$1.41 x10^{-3}$	2.82	Yes
	αEndosulfan	0.22 ±0.01	0.18 - 0.26	0.006	$2.40x10^{-4}$	0.04	No
S.galileous	Aldrin	2.02 ±0.10	1.98 -2.06	0.0001	$2.21x10^{-3}$	22.05	Yes
	Lindane	0.62±0.03	0.58± 0.66	0.0005	$6.77x10^{-4}$	1.35	Yes
Claria gariepinus	Aldrin	8.49 ±3.26	4.96 - 5.45	0.0005	$5.56x10^{-3}$	11.11	Yes
	Lindane	5.09±2.07	0.42-0.52	0.006	$5.24x10^{-4}$	0.09	No
	αEndosulfan	0.48 ±0.06	0.39-0.44	0.006	$4.91x10^{-4}$	0.82	No
	βEndosulfan	0.43 ±0.04	0.30 -0.43	0.006	$3.98x10^{-4}$	0.79	No
		Wet Season					
Oreochromis niloticus	αEndosulfan	0.15± 0.09	1.89-1.96	0.006	$1.64x10^{-4}$	0.02	No
	βEndosulfan	0.18±0.06	0.21 -0.32	0.006	$1.97x10^{-4}$	0.03	No

Gvmnarchus niloticus	Aldrin	0.02 ±0.01	1.27-1.34	0.0001	2.18×10^{-5}	0.22	No
	Lindane	0.12 ±0.02	0.18 - 0.26	0.0005	1.31×10^{-4}	0.26	No
S. galileous	Aldrin	0.09 ±0.03	0.05-0.16	0.0001	9.83×10^{-5}	0.99	No
	Lindane	0.17±0.07	0.22-0.29	0.0005	1.86×10^{-4}	0.37	No
Claria gariepinus	Aldrin	0.06±0.01	0.04-0.12	0.0001	6.55×10^{-5}	0.66	No
	Lindane	0.29±0.07	0.24 - 0.42	0.0005	3.17×10^{-4}	0.63	No
Clarias anguillaris	AEndosulfan	0.38±0.24	0.29-0.56	0.006	4.15×10^{-4}	0.07	No
	Lindane	0.08±0.03	0.05-0.12	0.0005	8.73×10^{-5}	0.17	No
	αEndosulfan	0.43±0.02	0.39-0.45	0.006	4.69×10^{-4}	0.08	No
Parachanna obscura	αEndosulfan	0.22±0.15	0.18-0.29	0.006	2.40×10^{-4}	0.04	No
	βEndosulfan	0.50±0.09	0.47-0.58	0.006	5.46×10^{-4}	0.10	No

HI > 1 showed health risk due to the consumption of the studied fish species while HI < 1 showed no health risk due to the consumption of the fish studied

Table 4: Health Risk Evaluation of Organophosphates Pesticide Residues in Fish Samples from River Owena, Nigeria.

Species of fish	Pesticide	mean±sd	Range	AD1	EDI	HI	HR
		Dry season					
Oreochromis Niloticus	Chlopyrifos	0.87 ± 0.05	0.75-0.89	0.003	9.50×10^{-4}	0.32	No
	Diazinion	1.04 ± 0.02	0.95- 1.22	0.002	1.14×10^{-3}	0.57	No
Gvmnarchus Niloticus	Chlopyrifos	0.89± 0.03	0.82-1.34	0.003	1.03×10^{-3}	0.34	No
	Diazinion	0.93± 0.01	0.89 - 1.12	0.002	9.61×10^{-4}	0.48	No
S.galileous	Chlopyrifos	0.06± 0.01	0.02-0.11	0.003	1.00×10^{-3}	0.03	No
	Diazinion	0.89± 0.04	0.78-0.94	0.002	9.72×10^{-4}	0.49	No
Claria gariepinus	Chlopyrifos	0.88±0.06	0.82-0.98	0.003	9.61×10^{-4}	0.32	No
	Diazinion	1.70±0.03	1.66 - 1.85	0.002	1.95×10^{-3}	0.98	No
		Wet Season					
Oreochromis Niloticus	Chlopyrifos	0.30 ± 0.17	0.25-0.42	0.003	3.38×10^{-4}	0.11	No
	Diazinion	0.02 ± 0.01	0.00 -0.04	0.0002	2.18×10^{-5}	0.01	No
Gvmnarchus niloticus	Chlopyrifos	0.22± 0.05	0.17-1.32	0.003	2.40×10^{-4}	0.08	No
	Diazinion	0.30± 0.07	0.20-0.41	0.0002	3.28×10^{-4}	0.13	No
S.galileous	Chlopyrifos	0.21± 0.01	0.17-0.29	0.003	2.18×10^{-4}	0.03	No
	Diazinion	0.14± 0.01	0.09-0.24	0.0002	1.53×10^{-4}	0.08	No

Claria gariepinus	Chlopyrifos	0.29±0.07	0.24-0.36	0.003	$3.17x10^{-4}$	0.32	No
	Diazinion	0.19±0.02	0.14- 0.255	0.0002	$2.07x10^{-4}$	0.11	No
Clarias anguillaris	Chlopyrifos	0.23±0.05	0.19-0.29	0.003	$2.51x10^{-4}$	0.10	No
	Diazinion	0.15±0.23	0.12 –0.24	0.0002	$1.64x10^{-4}$	0.08	No
Parachanna obscura	Chlopyrifos	0.23±0.02	0.18-0.31	0.003	$2.40x10^{-4}$	0.01	No
	Diazinion	0.12±0.02	0.09 –0.18	0.0002	$1.31x10^{-4}$	0.07	No

HI > 1 showed health risk due to the consumption of the studied fish species while HI< 1 showed no health risk due to the consumption of the fish studied

STATISTICAL ANALYSIS

The ANOVA test for an individual class of pesticide residues in the fish samples showed that the p-value corresponding to the F-statistic value of all the studied fish species was higher than 0.05, suggesting that there was no significant difference in the analytes concentration. This was further confirmed by the Tukey HSD test, Scheffé multiple comparison tests as well as Bonferroni and Holm multiple comparison tests.

CONCLUSION

This study established that the investigated fish samples were contaminated with organochlorine and organophosphate pesticide residues from agricultural practices near the river. The presence of these contaminants in the fish samples indicated that the fish were sinks for the hydrophobic and lipophilic pesticides. This study revealed that the banned organochlorine pesticides at the Stockholm Convention are still being used, (probably under different trade names) in controlling mirrids, without proper control measures. Therefore, there is a health risk from the consumption of fish contaminated with OC and OP residues.

RECOMMENDATIONS

Nigerian government should domesticate the treaty signed at the Stockholm Convention in 2001. This action would lead to the systematic eradication of these pollutants, particularly, the persistent OC pesticides, from the environment when signed into law. Also, the consumption of contaminated fish from the study area should be discouraged since these contaminants biomagnify and can cause serious health concerns in man. Finally, farmers in the study area should be enlightened on the dangers of continuous application of these banned chemicals for cocoa pest control.

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