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Assessment of Organochlorine Pesticide Residue Levels and Fat Content in Liver, Gill and Muscle Tissues of Catfish (*Clarias Spp)* and Tilapia (*Oreochromis Spp*) Obtained from River Kaduna and Fish Farms in Kaduna Metropolis, Nigeria.

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Abstract:
In this study, the levels of organochlorine (lindane, aldrin, endosulfan and p,p'-DDT) pesticide residues were assessed in the liver, gill and muscle tissues of catfish and tilapia obtained from three locations along River Kaduna, coded, A, B and C; and three Fish Farms in Kaduna Metropolis, coded D, E and F respectively. Aldrin and endosulfan were not present in any of the samples tested, however, lindane and p,p'-DDT were detected in varying concentrations ranging from 0.005 to 0.015 mg/kg. Lindane was more prevalent, as 23 of the 36 samples assessed were contaminated with this pesticide. Tilapia liver and muscle tissue obtained from sampling points A and C in River Kaduna had lindane concentrations of 0.015 ± 0.001 mg/kg and 0.050 ± 0.002mg/kg. Also, the gill and muscle tissue of catfish obtained from sampling point D showed concentrations of 0.013± 0.002 mg/kg and 0.011± 0.001 mg/kg respectively, which exceeded the European Union Maximum Residue Level (EU MRL) of 0.01 mg/kg. All other lindane containing samples had concentrations either equal to or less than the EU MRL. The concentration of p,p'-DDT ranged from 0.005 mg/kg to 0.01 mg/kg which were all within the EU MRL of 0.01 mg/kg. Lindane and p,p'-DDT showed strong positive correlations with fat in a significant number of samples, suggesting a possible influence of fat on the accumulation of these pesticides. Although, the residue concentrations were generally low, continuous attention needs to be paid to pesticide monitoring to eliminate or reduce to the barest minimum their levels in food and as well their implications on the health of humans and the environment.

***Key Word**: Organochlorine pesticides; fat content; catfish; tilapia; River Kaduna; fish farm.*

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I. Introduction

Modern agricultural productivity has been greatly improved by the use of pesticides.[1] However, this has come at a huge cost, as the concentration of the residues in food products and the environment has increased tremendously, with grave attendant health implications, including; cancer, neurological damage, diabetes, respiratory diseases, birth defects, endocrine disruptions and genetic disorders.[2,3] According to Bertolote *et al*[4], about three million cases of acute and severe pesticide poisoning occur annually, resulting in death of over 250,000 people.

Due to their lipophilicity, hydrophobicity, stability to photo-oxidation, and low chemical and biological degradation rates, pesticide tend to accumulate in biological tissues and therefore their concentrations magnify over time in organisms, progressing through the food chain.[5] The risk of chronic and acute toxic effects increases going up the food chain because the concentration of pesticides increases and therefore accumulates in the animals at the top of the pyramid, such as, fish, predatory birds and mammals.[6]

When used on farms, especially those with irrigation systems, these chemicals tend to find their way into water bodies, and since they are very stable in both fresh and salt water, and non-photodegradable, they persist.[7] However, their level in water reduces overtime through secondary mechanisms for instance, absorption on underwater soil sediment, biological breakdown by microflora and fauna, and absorption by fish through gills, skin and feeding.[7] Studies have shown that there is a positive relationship between the accumulation of chemicals and the lipid content of animals, and therefore, residue levels vary between individuals, species, and size groups depending on their lipid contents.[8,9]The accumulation of residues in the food chain can reach levels

toxic to predators and therefore pose a risk for human health. Therefore, assessing the bio-concentration and bio-magnification potential is an important issue for the environmental and human risk assessment of chemicals and one of the main features in environmental monitoring.[10]

The prevalence of pesticide residues in food substances raise serious public health concerns in countries across the world, both developed and developing. Organochlorine pesticides (OCPs) are among the first set of pesticides still in use in Nigeria even though they have been banned in developed countries, and also in Nigeria by the National Agency for Food and Drug Administration and Control (NAFDAC) due to the associated problems of indiscriminate potency and persistency.[11,7] Because of their high chemical stability and toxicity to humans and animals, their continued usage, especially by many ignorant people who deploy them for agricultural and fishing purposes, raises a lot of anxiety amongst researchers and government regulatory bodies.[11] Among all the pesticides extensively used in Nigeria for agricultural and other purposes, contaminations occurring from OCPs present a bigger challenge because of their slowness to biodegrade in the environment.[12]

Due to the continuous and extensive use of these pesticides in agronomical practices in Nigeria and their possible dispersion to water bodies and bioaccumulation in plants, animals and the environment, it is important to continue to monitor them closely. This research was therefore carried out to; assess the levels of organochlorine pesticide residues in the liver, gill and muscle tissues of catfish and talipia obtained from different sources in Kaduna State, Nigeria; compare the pesticide concentration levels against the European Maximum Residue Limits (EU MRLs) to establish their safety or otherwise for consumption; and also determine the extent of correlation between fat content and pesticide residue levels in the various species.

II. Material and Methods

Study Area

River Kaduna which originates from the Kujama Hill near Vom, Plateau State, is the main tributary of River Niger in Central Nigeria. It flows for 210 km from its source before reaching Kaduna town, dividing the city of Kaduna into North and South. Beyond Kaduna, it flows south-westerly for about 100 km into the Shiroro Dam and then for another 100 km emptying into River Niger at the northern shores of Pategi.[13] The availability of water for irrigation makes dry season farming along the bank of River Kaduna attractive, and this exposes the river to pesticide contamination through leaching of remnants in the soil with rain water.

The fish farms used for the study are located south of River Kaduna in two Local Government Areas, namely; Kaduna South (Barnawa) and Chikun (Sabon Tasha and Anguwan Boro).

Sampling

Sampling was done using the method employed by Akan *et al*,[14] with slight modification. A total of 60 fishes of approximately the same weight (ranging from 550-600 g) were collected from 6 sampling locations (5 catfish and 5 tilapia per sampling location). The collection was made from three points along River Kaduna (where it traverses, Nasarawa, Kabala Doki and Unguwan Rimi areas of Kaduna Metropolis). These points were coded as Sampling Points A, B and C. Catfish and tilapia were also collected from three fish farms in Barnawa, Sabon Tasha and Unguwan Boro, which were also coded as Sampling Points D, E and F respectively. All sampling points are depicted in Figure 1.

The samples were placed separately in polythene bags, assigned unique identifier codes and put into ice chests for transportation. They were then removed from the chests and stored in a refrigerator at -20^{o}C until extraction was carried out. To extract the desired organs, the fishes were thawed and dissected to remove the liver, gill and muscle tissues. For each sampling point and fish species, the liver, gill and muscle tissues were extracted and each organ type was blended together to obtain homogenous composites of liver, gill and muscle tissue respectively. Each sampling point had a total of 6 composite samples of catfish (liver, gill and muscle tissue) and tilapia (liver, gill and muscle tissue) respectively, totaling 36 composite samples.

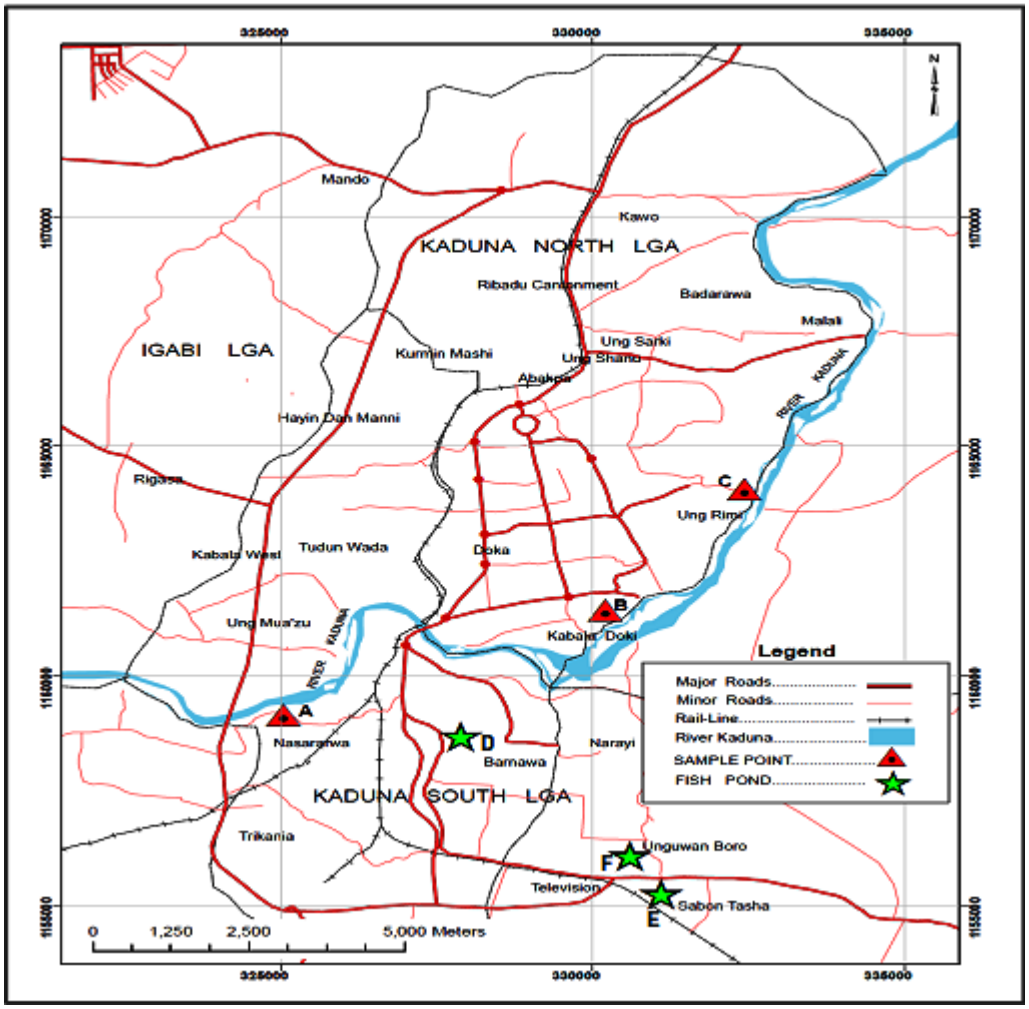

Figure 1: Map of Kaduna Metropolis showing sampling points.

Chemicals and Reagents

Analytical grade stock solution of organochlorine pesticide standard mix and pre-packed QuEChERS extraction salts containing; 6.0 g magnesium sulphate ($MgSO_4$) and 1.5 g anhydrous sodium acetate (CH_3COONa) were obtained from Sigma-Aldrich, UK. Pre-packed clean up tubes containing; 150 mg $MgSO_4$, 50 mg C_{18} sorbent, 7.5 mg graphitized carbon black (GCB) and 50 mg of primary secondary amines (PSA) were also obtained from Sigma-Aldrich, UK. Acetonitrile (CH_3CN), hexane (C_6H_{14}) and acetone (C_3H_6O) used were all of analytical grades and obtained from Sigma-Aldrich, Germany.

Sample Extraction and Dispersive Solid Phase Extraction (dSPE) Clean-up Processes

Sample extraction was done using modified AOAC QuEChERS Method 2007.01.[15] 10 g each of the homogenized samples were weighted into 50 mL centrifuge tubes. Two replicates of some of the samples (tilapia liver obtained from Sampling Point D, catfish gill obtained from Sampling Point E, catfish gill and muscle tissue obtained from sampling points B and C) were also placed in 50 mL centrifuge tubes, and 10 mL of cold distilled water was added to the samples and shaken to create the required aqueous environment, and left for about 30 mins. The replicate samples were then spiked with 0.1 ppm of mixed organochlorine pesticide standard containing the analytes of interest. 10 mL of acetonitrile (CH_3CN) containing 0.5% acetic acid (CH_3COOH) was added, followed by the addition of QuEChERS salt (6 g $MgSO_4$ + 1.5 g of CH_3COONa). The mixture was shaken and vortexed for 1 minute, centrifuged for 5 minutes at 5000 revolutions per minute (rpm), for phase separation.

Using a micro pipette, 6 mL of the clear upper acetonitrile extract was drawn and transferred into a 15 mL dSPE tube containing cleanup mixture of 150 mg $MgSO_4$, 50 mg PSA, 50 mg C_{18} sorbent and 7.5 mg GCB for further cleanup. The mixture was vortexed for 2 minutes and centrifuged for 5 minutes at 5000 rpm. 4 mL of the clear upper layer was carefully transferred into a 10 mL graduated glass test tube. 40 µL of 5% formic acid solution in acetonitrile was added, and then 2 mL of the extract was transferred into another 10 mL glass test

tube using a transfer pipette. The resulting solution was concentrated by evaporation at 40^0C with a constant flow of Nitrogen gas using a TurboVap Concentrator. The dried extract was reconstituted with 1 mL of hexane: acetone (4:1), and 1 mL of the reconstituted sample extract was carefully transferred into GC vials for analysis.[15]

Preparation of Calibration Curve

The calibration curve was prepared using the method described by Abdulhamid *et al*[16], with slight modification. Organochlorine pesticide standard stock solution was diluted to 200 ppm, by transferring 1 mL of the solution into a 10 mL volumetric flask and filled to mark with acetone. An intermediate solution of 10 ppm was prepared from the 200 ppm solution by transferring 500 µL into a 10 mL volumetric flask and filling up to mark with acetone. Five-point calibration concentrations of 1 ppm, 2 ppm, 3 ppm, 4 ppm and 5 ppm were then prepared using serial dilution.

GC-MS-MS Analytical Conditions

A Shimadzu TQ8040 triple quadrupole system equipped with column type Rxi-5Sil MS (30 m L x 0.25 mm I.D. x 0.25 µm) was deployed for the analysis according to the method of Bhone *et al*[17]. A portion of 1.0 µL of calibration standard solution was injected into the GC-MS-MS, followed by the injection of the reconstituted sample extract using the instrument's auto-sampler. Helium was used as the carrier gas with a flow rate of 1.69 mL/min, whereas Argon was used as a CID gas. The column temperature was programmed as follows: 80°C for a hold time of 1.5 min; 80 – 178°C at the rate of 30°C/min; 178 - 205°C at the rate of 3°C/min; 205°C for 4 min; 205-300°C at the rate of 30°C/min; and maintained at 300°C for 5 minutes.

Pesticide Identification and Quantification

The pesticide residues (analytes) were identified by comparing the retention time, peak area and peak height of the sample in respect to each pesticide reference standard. To determine the quantities of residues in the sample extracts, calibration curves were drawn from the chromatographic data obtained from the standard solutions. The concentrations of the unknown pesticide residues were calculated using regression analysis of the spiked calibration standards. Chromatograms were acquired using the computer-based GC software (GCMS Solution) supplied by Shimadzu Corporation. Quantification was based on 5 calibration levels for the organochlorine pesticide standard.[16]

Spike Recovery

The reliability of the method was tested according to the procedure specified by AOAC.[15] Some of the samples were fortified with known concentrations of the pesticide standards. For every batch, control samples spiked with 0.1 ppm of mixed organochlorine pesticide standard were run. Analysis of the spiked samples was carried out in the same manner as the unspiked samples, and the percentage spike recovery was calculated using the following formula;

% Recovery = (Conc. in spike sample – Conc. in blank sample)/Conc. of spiked × 100

Determination of Fat Content

Fat content of the samples was determined using the using the acid hydrolysis method AOAC.[18] Two grams each of wet homogenized composites of liver, gill and muscle tissue of catfish and tilapia were weighed into a clean dry test tube. Then 10 ml of distilled water and 10 ml of conc. HCl were added and placed in boiling water bath for 30 minutes for hydrolysis to occur. The hydrolyzed sample was cooled and transferred to the separating funnel. The test tube was then rinsed with 10 ml ethanol and added to the experimental separating funnel. To the experimental separating funnel, 30 ml of diethyl ether was added and swirled, then allowed to settle for separation to occur. A clean dry empty conical flask was weighed as W_1. The ether layer was collected in a pre-weighed conical flask. The aqueous layer was then re-extracted twice with 25 ml diethyl ether and the ether layers were added to the pre-weighed flask and the combined ether extract was evaporated over a boiling water bath. After the evaporation, the evaporated conical flask was placed in an oven maintained at 105 ^{o}C for 2 hours after which it was cooled in a desiccator and weighed again as W_2. The percentage fat in the various samples was calculated using the following
formula;

Percentage fat = weight of fat extracted / weight of sample × 100

$= W_2\text{-}W_1$/ weight of sample × 100

Statistical analysis

The results obtained from triplicate analysis were subjected to descriptive statistics (means and standard deviations), two-tailed Pearson's correlation of pesticide concentrations with fat content, to estimate the effect of fat on accumulation of the pesticides in the various organs of the fish species using SPSS software version 21.

III. Results

Concentration of Organochlorine Pesticide Residues in Fishes obtained from River Kaduna and Fish Farms

The results of analyses of four organochlorine pesticides (lindane, aldrin, endosulfan and p,p'-DDT) carried out on the liver, gill and muscle tissue of catfish and tilapia obtained from Sampling Points A, B, C, D, E and F respectively, are presented in mean ± standard deviation in Tables 1 and 2.

Table 1: Mean Concentration of OCPs in Catfish and Tilapia Obtained from River Kaduna

Sampling Point	Organochlorine Pesticide Residue	Concentration (mg/kg)						EU MRLs (mg/kg)
		Catfish Liver	Catfish Gill	Catfish Muscle Tissue	Tilapia Liver	Tilapia Gill	Tilapia Muscle Tissue	
Nasarawa (A)	Lindane	0.006 ± 0.002	-	0.007 ± 0.003	0.015 ± 0.001	-	0.007 ± 0.002	0.01
	Aldrin	-	-	-	-	-	-	0.01
	Endosulfan	-	-	-	-	-	-	0.01
	p,p'-DDT	-	-	-	-	0.007 ± 0.001	-	0.01
Kabala Doki (B)	Lindane	-	0.008 ± 0.002	0.006 ± 0.001	-	-	-	0.01
	Aldrin	-	-	-	-	-	-	0.01
	Endosulfan	-	-	-	-	-	-	0.01
	p,p'-DDT	-	0.010 ± 0.005	0.007 ± 0.003	0.002 ± 0.001	0.006 ± 0.002	-	0.01
Unguwan Rimi (C)	Lindane	0.008 ± 0.001	0.006 ± 0.002	0.007 ± 0.002	-	0.004 ± 0.001	0.050 ± 0.002	0.01
	Aldrin	-	-	-	-	-	-	0.01
	Endosulfan	-	-	-	-	-	-	0.01
	p,p'-DDT	0.010 ± 0.001	-	-	-	0.005 ± 0.001	0.008 ± 0.003	0.01

Means ± S.D, n=3, - = Not detected

Table 2: Mean Concentration of OCPs in Catfish and Tilapia Obtained from Fish Farms

Sampling Point	Organochlorine Pesticide Residue	Concentration (mg/kg)						EU MRLs (mg/kg)
		Catfish Liver	Catfish Gill	Catfish Muscle Tissue	Tilapia Liver	Tilapia Gill	Tilapia Muscle Tissue	
Barnawa (D)	Lindane	0.005 ± 0.001	0.013 ± 0.002	0.011 ± 0.003	0.010 ± 0.002	0.010 ± 0.001	-	0.01
	Aldrin	-	-	-	-	-	-	0.01
	Endosulfan	-	-	-	-	-	-	0.01
	p,p'-DDT	-	0.008 ± 0.002	-	-	0.008 ± 0.001	-	0.01
Sabon Tasha (E)	Lindane	-	0.007 ± 0.001	0.008 ± 0.002	-	-	0.004 ± 0.001	0.01
	Aldrin	-	-	-	-	-	-	0.01
	Endosulfan	-	-	-	-	-	-	0.01
	p,p'-DDT	0.009 ± 0.002	-	0.010 ± 0.003	-	-	0.001 ± 0.000	0.01
Unguwan Boro (F)	Lindane	-	-	0.006 ± 0.001	0.004 ± 0.002	0.009 ± 0.003	0.009 ± 0.001	0.01
	Aldrin	-	-	-	-	-	-	0.01
	Endosulfan	-	-	-	-	-	-	0.01
	p,p'-DDT	-	-	-	0.001 ± 0.000	-	-	0.01

Means ± S.D, n=3, - = Not detected

Spike Recovery

Table 3 shows the spike recovery values for all organochlorine pesticides tested which were in the range of 73-119, with a relative standard deviation of <20%.

Table 3: Spike Recovery Values for Organochlorine Pesticides

Organochlorine Pesticide	Recovery (%)			
	Catfish gill obtained from sampling point B	Catfish muscle tissue obtained from sampling point C	Tilapia liver obtained from sampling point D	Catfish gill obtained from sampling point E
Lindane	101	92	102	92
Aldrin	109	107	116	98
Endosulfan	112	118	114	119
p,p'-DDT	98	102	93	73

Fat content in Catfish and Tilapia fish

The percentage fat content of all fish samples obtained from River Kaduna and fish farms within Kaduna Metropolis are presented in Table 4. The results are in mean ± standard deviation.

Table 4: Percentage Concentration of Fat in River and Farm Fish Samples

Sampling Point	Percentage Fat Concentration (%)					
	Catfish Liver	Catfish Gill	Catfish Muscle Tissue	Tilapia Liver	Tilapia Gill	Tilapia Muscle Tissue
Nasarawa (A)	3.20 ± 0.005	3.07 ± 0.714	4.93 ± 0.204	5.50 ± 0.898	0.23 ± 0.019	1.94 ± 0.572
Kabala Doki (B)	2.60 ± 0.032	3.37 ± 0.380	1.18 ± 0.199	1.42 ± 0.190	3.71 ± 0.369	2.46 ± 0.794
Unguwan Rimi (C)	3.98 ± 0.208	2.64 ± 0.221	2.85 ± 0.381	3.30 ± 0.593	1.09 ± 0.283	2.18 ± 0.209
Barnawa (D)	6.52 ± 0.002	6.31 ± 0.090	3.14 ± 0.968	4.05 ± 0.388	1.38 ± 0.477	3.42 ± 0.493
Sabon Tasha (E)	4.38 ± 0.219	2.18 ± 0.197	2.49 ± 0.413	3.51 ± 0.297	3.03 ± 0.695	4.35 ± 0.826
Unguwan Boro (F)	5.75 ± 0.121	2.54 ± 0.803	3.97 ± 0.607	3.98 ± 0.790	4.39 ± 0.903	1.94 ± 0.374

Means ± S.D, n=3

Correlation Matrix of Pesticide Residues and Fat in Catfish and Tilapia

The correlation Matrix for Pesticide Residues and Fat in Catfish and Tilapia Obtained from River Kaduna and Fish Farms in Kaduna Metropolis are presented in Tables 5 and 6 below.

Table 5: Correlation Matrix for Pesticide Residues and Fat in Catfish and Tilapia Obtained from River Kaduna

	Lindane_CL	Lindane_CG	Lindane_CMT	Lindane_TL	rLindane_TG	Lindane_TMT	p,p'-DT_CL	p,p'-DDT_CG	p,p'-DDT_CMT	p,p'-DDT_TL	p,p'-DDT_TG	p,p'-DDT_TMT	Fat_CL	Fat_CG	Fat_CMT	Fat_TL	Fat_TG	Fat_TMT
Lindane_CL	1.000																	
Lindane_CG	-0.500	1.000																
Lindane_CMT	0.971	-0.693	1.000															
Lindane_TL	0.277	-0.971	0.500	1.000														
Lindane_TG	0.693	0.277	0.500	-0.500	1.000													
Lindane_TMT	0.781	0.151	0.608	-0.384	0.992	1.000												
p,p'-DDT_CL	0.693	0.277	0.500	-0.500	1.000**	0.992	1.000											
p,p'-DDT_CG	-0.971	0.693	-1.000**	-.0500	-0.500	-0.608	-0.500	1.000										
p,p'-DDT_CMT	-0.971	0.693	-1.000**	-0.500	-0.500	-0.608	-0.500	1.000**	1.000									
p,p'-DDT_TL	-0.971	0.693	-1.000**	-0.500	-0.500	-0.608	-0.500	1.000**	1.000**	1.000								
p,p'-DDT_TG	-0.240	-0.721	0.000	0.866	-0.866	-0.794	-0.866	0.000	0.000	0.000	1.000							
p,p'-DDT_TMT	0.693	0.277	0.500	-0.500	1.000**	0.992	1.000**	0.500	-0.500	-0.500	-0.866	1.000						
Fat_CL	0.937	-0.167	0.826	-0.075	0.901	0.950	0.901	-0.826	-0.826	-0.826	-0.564	0.901	1.000					
Fat_CG	-0.927	0.140	-0.810	0.102	-0.913	-0.958	-0.913	0.810	0.810	0.810	0.586	-0.913	1.000**	1.000				
Fat_CMT	0.675	-0.976	0.833	0.896	-0.063	0.067	-0.063	-0.833	-0.833	-0.833	0.554	-0.063	0.576	-0.350	1.000			
Fat_TL	0.688	-0.972	0.843	0.888	-0.045	0.084	-0.045	-0.843	-0.843	-0.843	0.539	-0.045	0.592	-0.367	1.000**	1.000		
Fat_TG	-0.886	0.845	-0.971	-0.691	-0.280	-0.402	-0.280	0.971	0.971	0.971	-0.237	-0.280	-0.669	0.648	-0.940	-0.946	1.000	
Fat_TMT	-0.751	0.948	-0.887	-0.845	-0.044	-0.175	-0.044	0.887	0.887	0.887	-0.461	-0.044	-0.475	0.449	-0.994	-0.996	0.971	1.000

*Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Key: CL-Catfish Liver, CG-Catfish Gill, CMT-Catfish Muscle Tissue, TL-Tilapia Liver, TG-Tilapia Gill, TMT-Tilapia Muscle Tissue

Table 6: Correlation Matrix for Pesticide Residues and Fat in Catfish and Tilapia Obtained from Fish Farms

	Lindane_CL	Lindane_CG	Lindane_CMT	Lindane_TL	Lindane_TG	Lindane_TMT	p,p'-DT_CL	p,p'-DDT_CG	p,p'-DDT_CMT	p,p'-DDT_TL	p,p'-DDT_TG	p,p'-DDT_TMT	Fat_CL	Fat_CG	Fat_CMT	Fat_TL	Fat_TG	Fat_TMT
Lindane_CL	1.000																	
Lindane_CG	0.843	1.000																
Lindane_CMT	0.918	0.987	1.000															
Lindane_TL	0.918	0.560	0.684	1.000														
Lindane_TG	0.866	0.461	0.596	0.993	1.000													
Lindane_TMT	-0.832	-1.000**	-0.984	-0.543	0.444	1.000												
p,p'-DDT_CL	-0.500	0.044	-0.115	-0.803	-0.866	-0.064	1.000											
p,p'-DDT_CG	1.000**	0.843	0.918	0.918	0.866	-0.832	-0.500	1.000										
p,p'-DDT_CMT	-0.500	0.044	-0.115	-0.803	-0.866	-0.064	1.000**	-0.500	1.000									
p,p'-DDT_TL	-0.500	-0.887	-0.803	-0.115	0.000	0.896	-0.500	-0.500	-0.500	1.000								
p,p'-DDT_TG	1.000**	0.843	0.918	0.918	0.866	-0.832	-0.500	1.000**	-0.500	-0.500	1.000							
p,p'-DDT_TMT	-0.500	0.044	-0.115	-0.803	-0.866	-0.064	1.000**	-0.500	1.000**	-0.500	-0.500	1.000						
Fat_CL	0.775	0.313	0.460	0.962	0.987	-0.295	-0.935	0.775	-0.935	0.160	0.775	-0.935	1.000					
Fat_CG	0.997	0.798	0.884	0.946	0.903	-0.786	-0.567	0.997	-0.567	-0.430	0.997	-0.567	0.822	1.000				
Fat_CMT	-0.070	-0.596	-0.461	0.332	0.438	0.611	-0.829	-0.070	-0.829	0.899	-0.070	-0.829	0.576	0.009	1.000			
Fat_TL	0.600	0.075	0.232	0.868	0.919	-0.055	-0.993	0.600	0.993	0.393	0.600	-0.993	0.970	0.661	0.756	1.000		
Fat_TG	-0.892	-0.995	-0.998*	-0.640	-0.547	0.993	0.056	-0.892	0.056	0.837	-0.892	0.056	0.407	-0.854	0.513	-0.74	1.000	
Fat_TMT	0.131	0.643	0.514	-0.274	-0.383	-0.658	0.793	0.131	0.793	-0.924	0.131	0.793	-0.525	0.052	-0.998*	-0.715	-0.564	1.000

*Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

CL-Catfish Liver, CG-Catfish Gill, CMT-Catfish Muscle Tissue, TL-Tilapia Liver, TG-Tilapia Gill, TMT-Tilapia Muscle Tissue

IV. Discussion

Lindane in Liver, Gill and Muscle Tissues of Catfish and Tilapia

From Tables 1 and 2, it would be seen that endosulfan and aldrin were not detected in any of the samples analysed. This suggests that the ban on the sale and use of these pesticides by the National Agency for Food and Drug Administration and Control[11] of Nigeria is effective in the study areas. However, this does not agree with the study undertaken by Akan *et al*, [19] where endosulfan was found to have the highest concentration in all the samples investigated, with a mean concentration of 8.98 ± 0.02 µg/g in the liver of *Oreochromis niloticus*, while aldrin had concentrations of 1.23 ± 0.04 µg/g and 0.87 ± 0.07 µg/g in the liver and gill of *Tilapia zilli* respectively. Even though lindane and p,p'-DDT are also banned, they were found in some of the samples analysed, albeit in small concentrations, and only lindane slightly exceeded the European Union Maximum Residue Levels (EU MRLs) of 0.01 mg/kg for all pesticides in fish. [20] These low concentrations suggest appreciable levels of compliance with the ban imposed on their use. [11]. Additionally, the comparatively low concentration of p,p'-DDT may indicate a possible transformation to DDE or DDD, as this can happen under aerobic and anaerobic conditions respectively. [21] This is also corroborated by a study in which Bayen *et al* [22] observed that the metabolism of p,p′-DDT, which occurs mainly in the liver, resulted in the degradation of 2.5% of p,p′-DDT into p,p′-DDD. Another possible reason for the low concentration of the pesticide could be as a result of dilution over space and time. [23]

The levels of Lindane in the various organs of catfish which can be seen on Tables 1 and 2 were compared. Only the liver samples of catfish from Sampling Points A, C and D contained lindane, with concentrations of 0.006 ± 0.002 mg/kg, 0.008 ± 0.001 mg/kg and 0.005 ± 0.001 mg/kg respectively, representing 50% of the total catfish liver samples analysed for the contaminant. All catfish gills except those obtained from Sampling Points A and F contained lindane with concentrations of 0.008 ± 0.002 mg/kg, 0.006 ± 0.002 mg/kg, 0.013 ± 0.002 mg/kg and 0.007 ± 0.001 mg/kg respectively, and this represents 66.7% of the gills analysed for lindane residues. The results for the analysis of catfish muscle tissue revealed 100% contamination of all samples by lindane, with concentrations of 0.007 ± 0.003 mg/kg, 0.006 ± 0.001 mg/kg, 0.007 ± 0.002 mg/kg, 0.011 ± 0.003 mg/kg, 0.008 ± 0.002 mg/kg and 0.006 ± 0.001 mg/kg respectively. Residue levels in all the samples except catfish gill and muscle tissue obtained from Sampling Point D were below the EU MRL of 0.01 mg/kg. This is not in agreement with the work of Adeyemi and Abdulmalik[21] carried out on some fish species consumed within Kaduna Metropolis, in which lindane was found to have a mean concentration of 1.053±0.028 – 5.77±0.019 µg/g, far exceeding the fixed MRLs in.

The levels of lindane in the liver, gill and muscle tissue of tilapia obtained from River Kaduna and Fish Farms in Kaduna were also compared. From the result presented in Tables 1 and 2, lindane was present in the liver samples obtained from Sampling Points A, D and F, with concentrations of 0.015 ± 0.001 mg/kg, 0.010 ± 0.001 mg/kg and 0.004 ± 0.002 mg/kg respectively. In all tilapia liver samples tested for lindane residues, 50% were contaminated. Gill samples obtained from Sampling Points C, D and F, showed lindane concentrations of 0.004 ± 0.001 mg/kg, 0.010 ± 0.001 mg/kg and 0.009 ± 0.003 mg/kg. This also represents 50% contamination of gill samples analysed. The tilapia muscle tissues analysed contained lindane, with concentrations of 0.007 ± 0.002 mg/kg, 0.050 ± 0.002 mg/kg, 0.004 ± 0.001 mg/kg, and 0.009 ± 0.001 mg/kg respectively for samples obtained from Sampling Points A, C, E and F. This represents 66.7% contamination of the muscle tissues tested. This finding is in agreement with the work of Osibanjo and Bamgbose, [24] which reported that residues were found to concentrate more in the muscle tissues of fish. The tilapia liver sample obtained from Sampling Point A and muscle tissue from Sampling Point C exceeded the EU MRL of 0.01 mg/kg. Lindane residues in tilapia muscle tissue from Sampling Point C also showed the highest singular concentration of 0.050 ± 0.002 mg/kg among all the OCPs tested, which is five times the EU MRL of 0.01 mg/kg. From the discussion above, lindane concentration was more in the muscle tissues of both catfish and tilapia, with catfish muscle tissue having a higher rate of residue contamination. The high prevalence of lindane in the muscle tissues could be due to the tendency of chemical pollutants to selectively bind to fish muscle tissue.[25]

p,p'-DDT in Liver, Gill and Muscle Tissues of Catfish and Tilapia

The levels of p,p'-DDT in the liver, gill and muscle tissue of catfish in the results presented in Tables 1 and 2 were compared. It was observed that catfish liver samples obtained from Sampling Points C and E had concentrations of 0.010 ± 0.001 mg/kg and 0.009 ± 0.002 mg/kg of p,p'-DDT respectively. Only two of six catfish liver samples were contaminated with p,p'-DDT. Concentrations of 0.010 ± 0.005 mg/kg and 0.008 ± 0.002 mg/kg were observed in gills of catfish obtained from Sampling Points B and D respectively, while catfish muscle tissue samples obtained from Sampling Points B and E had concentrations of 0.007 ± 0.003 mg/kg and 0.010± 0.003 mg/kg. This shows that 33.3% of all gill and muscle tissue samples tested contained p,p'-DDT**.** The highest concentration of this pesticide in all contaminated samples was 0.01 mg/kg. This concentration is same as the EU MRL of 0.01 mg/kg, and this compares to the literature as reported by Unyimandu and Udochu[26] in a comparative analysis of fish samples obtained from River Saar, Germany, in which the concentration of PCBs ranged from 0.004 to 0.01 mg/kg. A comparison of p,p'-DDT levels in the liver, gill and

muscle tissue of tilapia revealed that the liver was contaminated with 0.002 ± 0.001 mg/kg and 0.001 ± 0.000 mg/kg in samples obtained from point B and F; the gill had concentrations of 0.007±0.001 mg/kg, 0.006 ± 0.002 mg/kg, 0.005±0.001 mg/kg and 0.008± 0.001 mg/kg in samples from A, B, C and D; while muscle tissues had concentrations of 0.008 ± 0.003 mg/kg and 0.001 ± 0.000 in samples from points C and E respectively. 66.7% of tilapia gills tested were contaminated with p,p'-DDT, while 16.7% of muscle tissues sampled contained some residue of this pesticide. The finding of this study does not agree with that of Adeyemi *et al*[27] which recorded a mean DDT concentration of 0.18 mg/kg in tilapia fish, which was above the EU MRL of 0.01 mg/kg.

From the discussion above, it appears that p,p'-DDT concentrates more in the gill of tilapia. This finding agrees with Elnemaki and Abuzinadah[28] who showed that gills of *Oreochromis spilurus* (spp. of tilapia) were the most affected organ when fish was exposed to pesticides for variable periods of time. In catfish, all samples tested showed the same p,p'-DDT contamination level of 33.3%, suggesting similar level of exposure of the fishes to the pesticide. Out of 23 samples in which lindane was found, its concentration exceeded the EU MRL of 0.01 mg/kg slightly in 4, representing 11.1%, while that of p,p'-DDT was below the EU MRLs in all the samples. Despite these relatively low residue concentrations in the fishes, there is still a significant possibility of harm considering the rate of consumption, vis-à-vis accumulation in body tissues over time. [29]

Correlation Matrix of Pesticide Residue and Fat Content in Catfish and Tilapia

The correlation between pesticide residues and fat in the liver, gill and muscle tissues of catfish and tilapia obtained from River Kaduna and Fish Farms are presented in Tables 5 and 6 respectively. Lindane and p,p'-DDT were observed to be positively correlated with fat in various organs of the sampled fish species and this result corroborates the work of Danga *et al*,[30] which reported that bioaccumulation of certain types of contaminants in the tissues of animals is generally proportional to their lipid content. There was a very strong positive correlation between lindane and fat in catfish liver and muscle tissue, indicating a directly proportional relationship; hence, as fat increased, lindane accumulation in the liver and muscle tissue also increased. Generally, the liver recorded a higher average percentage fat content than the gill and muscle tissues in all the samples analysed as seen in Table 4. This is agrees with the work of Ando *et al*[31] in which five species of fish studied contained more lipid in the liver than muscle. This suggests that high fat content is an important contributory factor to lindane accumulation in liver and muscle tissue, also putting into considering its high overall prevalence rate of 63.8% in all samples tested. This is in agreement with the literature as reported by Helberg *et al*[5].

Conversely, the correlation that existed between lindane and fat in catfish gill was a very weak but positive one, suggesting that fat had very minimal influence on pesticide accumulation in the gill. In the tilapia samples, the correlation between lindane and fat was very strongly positive in the liver. This agrees with the work of Adeyemi *et al*, [32] in which polychlorinated biphenyls in fish samples from Lagos Lagoon were assessed. The correlation between p,p'-DDT and fat in the liver and gill of catfish were very strongly positive. This follows a similar trend with lindane, suggesting that both pesticides have strong affinity for lipids. [33] In tilapia, p,p'-DDT and fat had very strongly negative, weakly negative and moderately negative correlations in liver, gill and muscle tissue respectively.

The correlation matrix of samples obtained from fish farms is presented in Table 6. Lindane and fat had strong positive correlations in the liver and gill of catfish while in the muscle tissue it was a moderate negative correlation. In tilapia liver, the correlation was very strongly positive, while it was moderately and strongly negative in the gill and muscle tissue respectively. p,p'-DDT and fat had very strong negative correlation in the liver and muscle tissue of catfish, implying a strong inverse relationship between the two; hence as one increased, the other decreased and vice versa. On the other hand, p,p'-DDT had a strong positive correlation with fat in the gill, suggesting that its content increased proportionately to the level of fat content. In tilapia liver and muscle tissues, p,p'-DDT and fat had weak and strong positive correlations respectively, while they had a very strong negative correlation in the gill.

V. Conclusion

The results of this research showed that catfish and tilapia obtained from River Kaduna and fish farms within Kaduna Metropolis were contaminated with lindane and p,p'-DDT. However, only lindane was present with concentrations higher than the EU MRLs of 0.01 mg/kg in four of the samples tested. Lindane had the higher prevalence rate of 63.8%, being present in 23 of the 36 samples tested. The correlation between pesticide residue levels and fat content showed that fat had a substantial effect on the accumulation of lindane and p,p'-DDT. Considering the attendant health implications of pesticides, fishes containing these residues in excess of the permissible levels are unsafe for human consumption because they present an important channel for poisoning the human body. While most of the pesticides studied had zero to very low levels of contamination (equal to or below the EU MRLs), suggesting minimal use of these chemicals by farmers in the study area and

also the use of safe raw materials for feed production, there is still need for proper monitoring to ensure that contamination of water bodies and other sources is kept a bare minimum.

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