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ORGANOCHLORINE PESTICIDE RESIDUES IN MUSCLE TISSUES OF *ETHMALOSA FIMBRIATA* AND *PSETTIAS SEBAE* FROM LAGOS LAGOON, NIGERIA

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ABSTRACT

Bonga fish (Ethmalosa fimbriata) and African Moony (Psettias sebae) were sampled from Lagos Lagoon and analysed for organochlorine pesticide residues namely aldrin, dieldrin, endrin, DDT, heptachlor, HCH, endosulfan, chlordane and methoxychlor. Sampling was conducted between December 2008 and September 2009 during the dry and wet seasons. The muscle tissues of the fishes were subjected to cold extraction with petroleum ether/acetone (1:1 v/v) mixture and clean-up on silica gel adsorbents. Gas chromatography was used to detect and determine the organochlorine pesticide residues. The residue levels were higher in Ethmalosa fimbriata during the dry season while Psettias sebae had higher levels during the wet season. The male fishes accumulated higher organochlorine pesticides than the female fishes. The total detectable organochlorines (wet weight) of the muscle tissues ranged from 5.72 ng/g in female Ethmalosa fimbriata during the wet season to 3005.35 ng/g in male Ethmalosa fimbriata. The dietary surveys indicated a mean value of 40 g/day as the amount of fish consumed daily. Except for endrin and heptachlor, the estimated daily intakes of the organochlorine residues were within the acceptable daily intakes. The concentrations of the residues in the fishes were within the permissible residue limits.

Key words: Organochlorine pesticide residues, muscle tissues, Lagos Lagoon, *Ethmalosa fimbriata, Psettias sebae*

1. INTRODUCTION

The contamination of the environment and food by organochlorine pesticides has become a topical issue of considerable concern in many parts of the world, and has led many researchers to investigate their occurrence, distribution and concentrations in several ecosystems (1-3). Nigerian fishes have been reported to contain all the commonly encountered pesticide residues (4-7). From these studies, coastal waters are as contaminated as freshwaters. Organochlorine pesticides (OCPs) have been used for agriculture and vector control purposes in Nigeria. The misuse of these chemicals for killing fishes is also practised. Being lipophilic, OCPs can be concentrated to harmful levels in the aquatic environment through bioaccumulation and biomagnification (8). Consequently, aquatic organisms that are commercially exploited for human food may pose a risk to man.

The occurrence of organochlorine pesticides in biological species, even at trace levels, is not desirable as they have toxic effects. Fishes are at the top of the aquatic food chain and do bioaccumulate these residues, leading to many diseases in man. OCPs contribute to many acute and chronic health effects, including cancer, neurological damage, birth defects, tremors, headache, dermal irritation, respiratory problems and dizziness. Long-term exposure to sub-lethal levels of OCPs and their metabolites through various pathways in the aquatic environment may cause far reaching ecological damage and health problems to man. OCPs act as central nervous system stimulants in aquatic fishes.

Pollution by persistent chemicals is potentially harmful to the organisms at higher trophic levels in the food chain. The aquatic organisms like fishes are able to accumulate several fold higher concentration of pesticide residues than the surrounding water (9). Research efforts indicate that more than 80% of the total intake of pesticide residues in human beings is through the food chain (10, 11). It has been reported that the consumption of contaminated fishes is one of the important pathways of human exposure to organochlorinated pesticides (12-14). Indeed, studies have related the presence of organochlorine residues in breast milk to the consumption of contaminated fishes (15). Data on the presence and distribution of OCPs in edible fishes are, therefore, important from the ecological and human health perspectives.

The monitoring of fishes serves as an important indicator of the water ecosystem where there is a vertical transport of OCPs leading to accumulation in the benthic organisms. Fishes were sampled because they are important foods commonly consumed by a cross section of Nigerians. This study was undertaken to investigate the occurrence and concentration of organochlorine pesticide residues in muscle tissues of two commonly consumed fishes in Nigeria: *Ethmalosa fimbriata* and *Psettias sebae*.

2. MATERIALS AND METHODS

Study area

The study area for the investigation is Lagos Lagoon which lies between latitude 6° 26' - 6° 37' N and longitude 3° 23' - 4° 20' E on the Western part of Nigeria. The lagoon empties into the Atlantic Ocean and is drained by Ogun, Agboyi, Majidun and Aye Rivers. Human activities associated with the lagoon include fishing.

Sampling strategy

Sampling was undertaken between December 2008 and September 2009 during the dry and wet seasons.

Fish collection

Male and female bonga fish (*Ethmalosa fimbriata*) and African moony (*Psettias sebae*) were harvested with the aid of fishing net. The harvested fish samples were wrapped in aluminium foil, stored in ice-packed coolers and transferred to the laboratory where they were frozen, thawed, cleaned in distilled water and their scales sloughed off.

Sex determination

The fish samples were separated into males and females by examining their gonads.

Length measurement

The total and standard lengths of the fishes were measured using a ruler.

Percentage (%) dry matter

1.0 - 2.0 g of muscle tissue of each of the fresh fish samples was weighed and dried in an oven maintained at 105^{0}C for 8 hours. The dried fish samples were cooled in a desiccator and weighed in an analytical balance to constant weight. The percentage dry matter was calculated as follows:

$$\%\ Dry\ matter = \frac{dry\ weight}{fresh\ weight} \times 100$$

Condition factor (CF)

The condition factor (CF) which describes the physiological condition of the fishes (16) was calculated according to the equation (17):

$$CF = \frac{W}{L^3} \times 100$$

where, W = the fish wet weight (g)

L = the fish total length (cm)

Fish fat content

10 g of fish muscle tissue was homogenized with 10 g of anhydrous Na_2SO_4. Cold solvent extraction was carried out using 50 cm^3 petroleum ether/acetone (1:1 v/v) mixture in a reagent bottle. The mixture was well shaken and allowed to stand for 30 minutes and then filtered. After evaporating the solvent extracts with the aid of a rotary evaporator, the fat content of the muscle tissue was determined gravimetrically:

$$\%\ Fat = \frac{weight\ of\ \ fat}{weight\ of\ \ tissue} \times 100$$

Extraction of fish samples

10 g of muscle tissue of the fish samples was homogenized with 10 g of anhydrous granulated Na_2SO_4. Cold solvent extraction was performed. 50 cm^3 of the petroleum ether/acetone (1:1 v/v) mixture was introduced into a bottle containing the homogenized fish sample. The mixture was shaken and allowed to stand for 30 minutes and then filtered (18).

Pre-concentration of extracts

The solvent extracts were concentrated to 1 cm^3 using a rotary evaporator and clean-up.

Clean-up of extracts

Column chromatography was used to clean-up the extracts (19). The glass separating column was packed with activated silica gel (90% < 45 µm) and washed down with n-hexane to remove dirts. The extracts were demoisturized over 1 g of anhydrous granulated Na_2SO_4 and separated into two eluted fractions using mixtures of dichloromethane, hexane and acetonitrile as eluting solvents. For the first fraction, 30 cm^3 of a dichloromethane/ hexane (20/80) mixture was used, while 30 cm^3 of a dichloromethane/hexane/acetonitrile (50/49.5/0.5) mixture was used for the second fraction to ensure that the polar acetonitrile eluted any remaining residue. The fractions were combined, concentrated to 1 cm^3 and subsequently analysed.

Identification and determination of organochlorine pesticide residues

The cleaned-up extracts were dried and re-dissolved in 1.0 cm^3 isooctane (20). Organochlorine Pesticides II EPA Method 8081A was used for the analyses. The detection and determination of the residues were performed by injecting 1 µL of the 1.0 cm^3 purified extract into the injection port of a gas chromatograph with a ^{63}Ni electron capture detector (GC-µECD Agilent Technology 7890A) equipped with the ChemStation software. Identification of pesticide residues was accomplished using reference standards and relative retention time techniques, while the residues were determined by comparing the peak heights of the samples with the corresponding peak heights of the reference standards of known concentrations. The concentrations (ww) of the pesticide residues were calculated by the gas chromatograph after inputing the weight of the samples. The operating conditions of the gas chromatograph are as shown in Table 1.

Quality assurance

Standards were run to check for the column performance, peak height, resolution and the detection limit. The correlation coefficients of calibration curves of the pesticides were all higher than 0.998. The quality assurance measures included cleaning procedures, recovery of spiked standards and monitoring of detector response. Blank runs were made for background correction and performance of the system. The stock solution of the organochlorine pesticide standards was purchased from Restek Corporation, USA. It contained 1000 ppm in n-hexane and was serially diluted to obtain the desired concentrations of 10, 20 and 40 ng/mL.

Recovery study

The recovery of organochlorine pesticide residues was carried out in replicate and was determined by spiking the previously analysed samples with the pesticide standard at concentrations similar to those expected in the samples. The recovery percentages were calculated from the chromatograms.

$$\% \text{ Recovery} = \frac{CS_2 - CS_1 \text{ x } 100}{CS}$$

where, CS_1 = concentration of pesticide residues in the sample
CS_2 = concentration of pesticide residues in the spiked sample
CS = concentration of added pesticide

Estimation of daily intakes (EDI) of organochlorine pesticide residues by humans

The daily intake of organochlorine pesticide residues by humans was estimated based on questionnaires and interviews conducted in 100 families. The respondents were consumers of the fishes harvested and were categorized into males and females. Information on preference of the fish species, ages and weights of the respondents and frequency of consumption was collated to estimate the daily dietary intakes. The average per capita consumption was estimated and compared with the acceptable daily intake. The dietary intake of the OCPs was calculated by multiplying concentrations measured in the muscle tissues of each fish by the per capita consumption. The estimated daily intake of the organochlorine pesticide was calculated:

$$\text{EDI} = \frac{FDC}{BW} \text{ x CC} \qquad (21)$$

where, FDC = fish daily consumption (g)
CC = contaminant concentration (ng/g)
BW = body weight (kg)
EDI = Estimated daily intake of OCPs (ng/kg body weight/day).

US EPA recommended values were used for daily intake calculations. 70 kg was taken as average body weight, 6.5 g as daily fish consumption and 70 years as exposure for a lifetime.

Analyses of data

The fish extracts were analysed for aldrin, dieldrin, endrin, DDT, heptachlor, HCH, endosulfan, chlordane and methoxychlor. Concentrations of OCP residues were calculated individually and as the sum of their isomeric forms. Description of data was performed using a Statgraphics Centurion XV statistical software, with the level of significance maintained at 95% for each test. The mean and standard deviation were calculated from the detectable values, and values below the detectable limit were considered not detected (ND). The mean was calculated from triplicate determinations.

3. RESULTS AND DISCUSSION

Table 1. Operating conditions of the gas chromatograph

Detector	Electron capture
Column	DB-5 fused silica capillary column (30 m length × 0.32 mm i.d. × 0.25 µm film thickness). HP – 5 5% Phenyl methyl siloxan
Carrier gas	Helium (99.9992) flowing at 20 mL/min
Carrier gas pressure	10.744 psi
Make-up gas	Nitrogen (99.9995%)
Injector temperature	250^0C
Injection	1 µL Splitless
Detector temperature	300^0C
Temperature programme	Initial temp 50^0C at a rate of 25^0C/min to 100^0C (held for 1 min), then at a rate of 5^0C/min to 300^0C

Table 2. Mean biometric data of *Ethmalosa fimbriata* and *Psettias sebae* in Lagos Lagoon during the dry season

Fish species	Feeding mode	Sex	Wet weight (g)	% Dry matter	% Fat	TL (cm)	SL (cm)	CF
E. fimbriata	Herbivorous	Male	65.8±0.3	17.1±0.3	0.6±0.1	17.0±0.3	13.0±0.2	1.3±0.2
		Female	69.9±0.6	18.9±0.6	4.0±0.2	18.5±0.5	14.0±0.6	1.1±0.5
P. sebae	*Carnivorous*	Male	25.4±0.6	20.6±0.5	0.8±0.2	13.0±0.5	10.0±0.5	1.2±0.4
		Female	85.2±0.5	28.3±0.4	1.8±0.1	20.0±0.5	16.0±0.5	1.1±0.4

TL = total length of wet fish; SL = standard length of wet fish; CF = condition factor of fish
The mean value was calculated from 3 fishes of each species

Table 3. Mean biometric data of Ethmalosa fimbriata and Psettias sebae in Lagos Lagoon during the wet season

Fish species	Feeding mode	Sex	Wet weight (g)	% Dry matter	% Fat	TL (cm)	SL (cm)	CF
E. fimbriata	Herbivorous	Male	65.6±0.4	17.1±0.3	0.6±0.1	17.0±0.3	13.0±0.3	1.3±0.3
		Female	69.0±0.3	18.9±0.3	4.0±0.1	18.4±0.3	13.9±0.3	1.1±0.1
P. sebae	*Carnivorous*	Male	25.0±0.3	20.5±0.3	0.8±0.1	13.0±0.3	10.0±0.3	1.1±0.2
		Female	75.2±0.7	27.2±0.7	1.8±0.2	19.0±0.6	16.0±0.6	1.1±0.3

Table 4. Mean concentrations (ng/g) of OCP residues in the muscle tissues of male and female *Ethmalosa fimbriata* during the dry and wet seasons in Lagos Lagoon

OCPs	Lagos Lagoon			
	Dry season		Wet season	
	Male	Female	Male	Female
Alpha-BHC	35.09±2.25	49.47±4.06	ND	0.63±0.15
Beta-BHC	179.07±9.16	199.56±9.10	0.61±0.30	0.27±0.26
Lindane	33.91±2.53	29.11±5.72	0.81±0.28	0.76±0.14
Delta-BHC	121.88±8.78	62.41±12.18	ND	ND
Σ BHC	369.95±22.72	340.55±31.06	1.42±0.58	1.66±0.55
Heptachlor	74.29±7.43	56.25±6.91	1.08±1.11	1.08±1.26
Heptachlor-epoxide (B)	108.19±4.52	96.82±1.24	0.63±0.25	0.59±0.32
Aldrin	21.21±9.06	30.13±2.36	1.16±1.01	1.21±1.52
Dieldrin	40.74±2.15	69.45±3.45	0.70±0.28	ND
Endrin	154.62±6.86	131.69±4.84	0.45±0.30	0.48±0.29
Endrin aldehyde	665.6±16.27	665.6±16.27	ND	ND
Endrin ketone	678.37±4.65	559.51±23.05	ND	ND
Cis-Chlordane	66.01±5.23	45.00±7.23	0.58±0.18	ND

Trans-Chlordane	93.01±2.14	56.38±6.64	0.49±0.14	ND
Endosulfan 1	77.25±6.76	84.54±2.06	0.78±0.20	0.70±0.31
Endosulfan 11	34.92±9.92	39.85±5.73	ND	ND
Endosulfan sulphate	83.93±2.54	70.05±4.46	ND	ND
Methoxychlor	198.98±9.25	106.84±6.82	ND	ND
p,p´-DDE	67.53±0.45	37.36±4.25	0.80±0.25	ND
p,p´-DDD	79.30±0.24	66.03±5.54	ND	ND
p,p´-DDT	191.45±0.63	164.38±8.33	ND	ND
ΣDDT	338.28±1.32	267.78±18.12	0.80±0.25	ND
ΣOCPs	3005.35±110.82	2270.67±127.10	8.08±5.66	5.72±4.2 5

Table 5. Mean concentrations (ng/g) of OCP residues in the muscle tissues of male and female *Psettias sebae* during the dry and wet seasons in Lagos Lagoon

OCPs	Lagos Lagoon			
	Dry season		Wet season	
	Male	Female	Male	Female
Alpha-BHC	6.10±4.54	8.63±2.63	0.81±1.53	0.94±1.72
Beta-BHC	36.55±3.26	33.48±4.32	0.79±0.49	0.31±0.12
Lindane	11.02±8.62	9.63±6.56	1.36±1.85	0.56±0.10
Delta-BHC	18.06±5.21	2.07±1.81	1.40±1.41	1.81±2.32
Σ BHC	71.73±21.63	53.81±15.32	4.36±5.28	3.62±4.26
Heptachlor	37.52±4.65	106.35±8.34	1.17±1.25	1.63±1.93
Heptachlor-epoxide (B)	38.85±9.33	5.38±2.23	0.64±0.37	0.66±0.55
Aldrin	11.89±8.96	20.67±2.70	1.44±1.42	1.47±1.61
Dieldrin	14.41±7.32	7.74±1.83	ND	ND
Endrin	16.59±3.41	20.47±1.21	0.54±0.19	0.49±0.30
Endrin aldehyde	111.99±8.04	24.56±2.28	ND	ND
Endrin ketone	261.94±7.52	88.41±10.94	0	0
Cis-Chlordane	31.19±2.25	28.05±2.62	ND	ND
Trans-Chlordane	78.71±2.13	12.39±2.19	ND	ND
Endosulfan 1	28.93±1.72	6.55±1.24	0.82±0.58	0.73±0.45
Endosulfan 11	33.06±6.35	9.02±3.64	ND	ND
Endosulfan sulphate	61.15±9.54	20.59±4.32	ND	ND
Methoxychlor	35.66±5.16	10.54±3.71	0	0
p,p´-DDE	36.23±6.52	ND	0.68±0.30	0.66±1.32
p,p´-DDD	51.84±4.03	6.09±5.64	ND	ND
p,p´-DDT	78.56±5.15	5.71±4.09	ND	ND
ΣDDT	166.64±15.70	11.80±9.73	0.68±0.30	0.66±1.32
ΣOCPs	1000.27±92.08	426.32±56.98	9.65±9.39	9.28±10.42

Table 6. Estimated daily intake (EDI) of organochlorines (ng/g) by humans

OCPs	*Ethmalosa fimbriata*	*Psettias sebae*
BHC	34.35	6.66
Heptachlor	16.94	10.37
Aldrin	2.81	1.92
Dieldrin	6.45	1.34
Endrin	139.15	36.26
Chlordane	14.77	10.21
Endosulfan	18.21	123.15
Methoxychlor	18.48	3.31
DDT	31.41	15.47

Table 7. Acceptable daily intake (ng/kg body weight/day) of organochlorines in fish

Pesticides	FAO/WHO	Health Canada	USEPA (R_fD)
BHC	42000	18000	18000
Heptachlor	5000	-	-
Aldrin	7000	-	-
Dieldrin	-	-	-
Endrin	6000	-	-
Chlordane	-	3000	30000
Endosulfan	-	-	-
Methoxychlor	1200000	1200000	30000

Source: Oostdan et al. 1999; FAO/WHO 2005; USEPA 2006

The mean biometric data of Ethmalosa fimbriata and Psettias sebae are shown in Tables 2 and 3. There was a positive correlation between the total lengths and standard lengths of the fishes. Increase in fish lengths gave corresponding increase in fish weights. There was no correlation between the wet weights and % fat of the fishes. The fishes had condition factor more than 1 during the dry and wet seasons, signifying that they were healthy as the condition factor describes the physiological condition of fishes (16). The mean recoveries of the residues ranged from 88.45 to 98.42%, validating the methodology used.

The mean concentrations of organochlorine pesticide residues in muscle tissues of the male and female fishes are shown in Tables 4 and 5. A higher concentration of the residues was observed during the dry season. This is due to the higher contamination of the Lagos Lagoon and dilution effect that is witnessed during the wet season. The muscle tissues of the male fishes accumulated higher organochlorines than the muscle tissues of the female fishes. The residue levels were higher in Ethmalosa fimbriata during the dry season while Psettias sebae had higher levels during the wet season. The total detectable organochlorines (wet weight) of the muscle tissues ranged from 5.72 ng/g in female Ethmalosa fimbriata during the wet season to 3005.35 ng/g in male Ethmalosa fimbriata during the dry season and were of enhanced levels when compared to earlier studies in Ogun and Edo Rivers (6, 22) but with much reduced levels with respect to studies by Adeyemi et al. (7) in Lagos Lagoon.

The dietary surveys conducted in 100 families indicated that the amount of fishes consumed ranged from 20 to 200 g/day, with a mean value of 40 g/day. The mean consumption of fish in this study compared with the dietary surveys earlier conducted in China and Coimbatore city, India (14, 23). In this study, respondents were asked to give information about the amount of species of fish they consume because fish consumption represents an important pathway for exposure to organochlorines. Muscle tissue was used in determining the dietary intakes as muscle forms the major edible portion in a fish. The estimated daily intakes (EDI) of organochlorines by humans are shown in Table 6. ΣBHC, Σaldrin, Σendrin, Σchlordane, Σheptachlor and ΣDDT were used in estimating the daily intakes. Except for endrin and heptachlor, the estimated daily intakes of the pesticides were within the acceptable daily intakes. The appraisal of dietary intake was based on comparison of acceptable daily intakes established by the joint FAO/WHO expert committee, Health Canada and USEPA (Table 7). Acceptable daily intake (ADI) represents the daily concentration below which there is a high probability of no adverse health effect. It is an estimate of the residue that can be ingested by a person daily over an extended period of time without suffering deleterious effects. Levels of organochlorines in the fish species analysed were within the permissible limits (24-26).

4. CONCLUSIONS

A total of twenty three organochlorines were detected in the fish samples. A higher concentration of the residues was observed during the dry season. The residue levels were higher in Ethmalosa fimbriata during the dry season while Psettias sebae had higher levels during the wet season. The male fishes accumulated higher organochlorine pesticides than the female fishes.

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