Organochlorine Pesticides in Muscle of Varieties of Frozen Fish in Nigeria

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***Abstract*: Accumulation of toxic residues in water body affects all the living organism, and may possibly pose a high risk in organism that can accumulate and retain these toxic substances in their body such as the fishes. Fish consumption and demand is high that the local supply in Nigeria don't meet up, this gap is bridged by frozen fishes which are majorly imported into the country. This study looked into five (5) commonly sold frozen fishes sold in Nigeria, to determine the level of organochlorine pesticide residues in them, which will indicate the kind of water body they are coming from and its potential risk to human life. GC-ECD with GC mass spectrometric (GC/MS) was used in the analysis and a total of fifteen OCPs were detected in all the five fish samples. Hexachlorobenzene, β-HCH, δ-HCH, α-HCH, γ-HCH(lindane) were detected. α-HCH shows the highest wet concentration range of the HCH isomers with 1.10mg/kg in Herring fish sample to 4.00mg/kg in Atlantic mackerel. Endosulfan pesticide residue the highest concentration among all the OCPs quantified with a range of 3.16-4.18mg/kg. 12 OCPs were detected in sample F1 and F5 each and 14 were detected in F2, F3 and F4 each. The DDTs present suggest a possible recent use, which might raise a concern because these fishes are eaten in Nigeria almost every day and thus suggest a keen assessment of imported fishes into Nigeria, because of the bioaccumulation of pesticide residue in fishes thereby extending the effect to human health.**

***Keywords*: Organochlorine pesticides, fishes, GC-MS, HCH**

I. INTRODUCTION

Fishes had been in existence for millions of years and have been evolving even without human intervention, but like everything in existence human being has affected or touched every living organism directly or indirectly, even the aquatic life and its food chain. While fishes are been domesticated (for aesthetic and recreational) in aquariums, turned into laboratory animals for research purposes, or even farmed for consumption purposes, majority of the fishes commercially sold are still from their natural habitats. Fish and fish products are known worldwide to bean important part of diet because of their high nutritive quality and significance on improving human health. Fish is one of the most important animal protein foods available in the tropics(Eyo 2001) and its consumption has contributed to human development in every stage including infants and pregnancy(Akinbode and Dipeolu, 2012)

Agricultural production in Nigeria also involve fish farming as a major branch, but like every other part of agriculture in Nigeria, the domestic demand for fish is still higher than its production locally, thus importation has been filling the supply gap (Lenis*et al.* 2020, Akinbode and Dipeolu, 2012). Tilapia, catfish and carp are the major species farmed in Nigeria while catfish is the most cultured (Bradley*et al*, 2020).

Nigerian fishes have been reported to contain all the commonly encountered pesticide residues (Osibanjo *et al.*, 2002,Adeyemi *et al.*,2008, Williams 2013). While these could be attributed to washed pesticide residues into the water body, condensed pesticide particles into water or those who use it to kill them so as to harvest them easily.Generally, some agricultural practices (such as pesticide contamination) impede aquatic life (Loos *et al., 2009*, Devault*et al., 2009*) by affecting the fish health (Bony *et al.,* 2010, Gorbatiuk*et al.,* 2010) or accumulation in fish's edible tissue (Hu *et al.,* 2010), while resulting to a potential risk to their consumers (Sun*et al.,* 2006).

A major concern among these pesticidal residues are the persistence organic pollutants (POPs), such as the organochlorines.Organochlorine pesticides (OCPs) are in the hazardous class, and has been banned for agricultural and domestic uses in Europe, due to their environmental persistence and potential adverse effect on wildlife, animal and human health (Castillo *et al*., 2012). Organochlorines have long half live and bio-accumulates resulting to finding its residues in food products of animal origin such as fish, egg, meat, etc. especially in tissue and food high in fat (Ledoux*et al*., 2011). They are lipophilic compounds. Organochlorines gained their persistence in the ability of many of its compound not breaking down easily which means staying in the environment and the body of animals that consume it. This result to its accumulation in animals along the food chain, to which the highest predator will show the highest level, in the case of fishes, mostly human. This has been known as an important pathway of exposure to organochlorine pesticides in humans (Mwevura *et al.,* 2002; Zhou *et al., 2007, Muralidharan et al., 2008*).

Acute and Chronic health effect that can be induced by organochlorines include cancer, birth defects, tremors, neurological damages, dermal irritation, headache, dizziness, respiratory problems. (Fitzgerald *et al., 2001).*

This study looks into five (5) fishes, these include;

Table 1		
Fish varieties	Names of the fishes in Nigeria (South west)	Sample ID
Hake (*Merluccius specie*)	Panla	F1
Herring *(Clupea harengus*)	Shawa	F2
Horse Mackerel (*Trachurus trachurus*)	Kote	F3
Croaker fish *(Pseudotolithus senegalensis)*	Croaker	F4
Atlantic mackerel (*Scomber scombrus*)	Titus (from its English name)	F5

Keeping in view of public health significance of pesticide residues, safety of consumers, legal restrictions on export, quality of the water ecosystem and the presence of accumulation of organochlorine pesticides in edible fishes, the five varieties of fishes selected in this study are majorly harvested, packaged, frozen and imported to meet the demand. Therefore, this study aims to determine the OCPs in muscles of frozen fishes varieties in Nigeria especially in the south western part.

II. METHODOLOGY

2.1 Study area

Ado town is the capital of Ekiti- State South Western, Nigeria, it lies within the latitude of 7^0 40'N and longitude of 5^0 11' and 5^0 20E. It has the estimated population of about 580,781(World urbanization prospects; 2020)

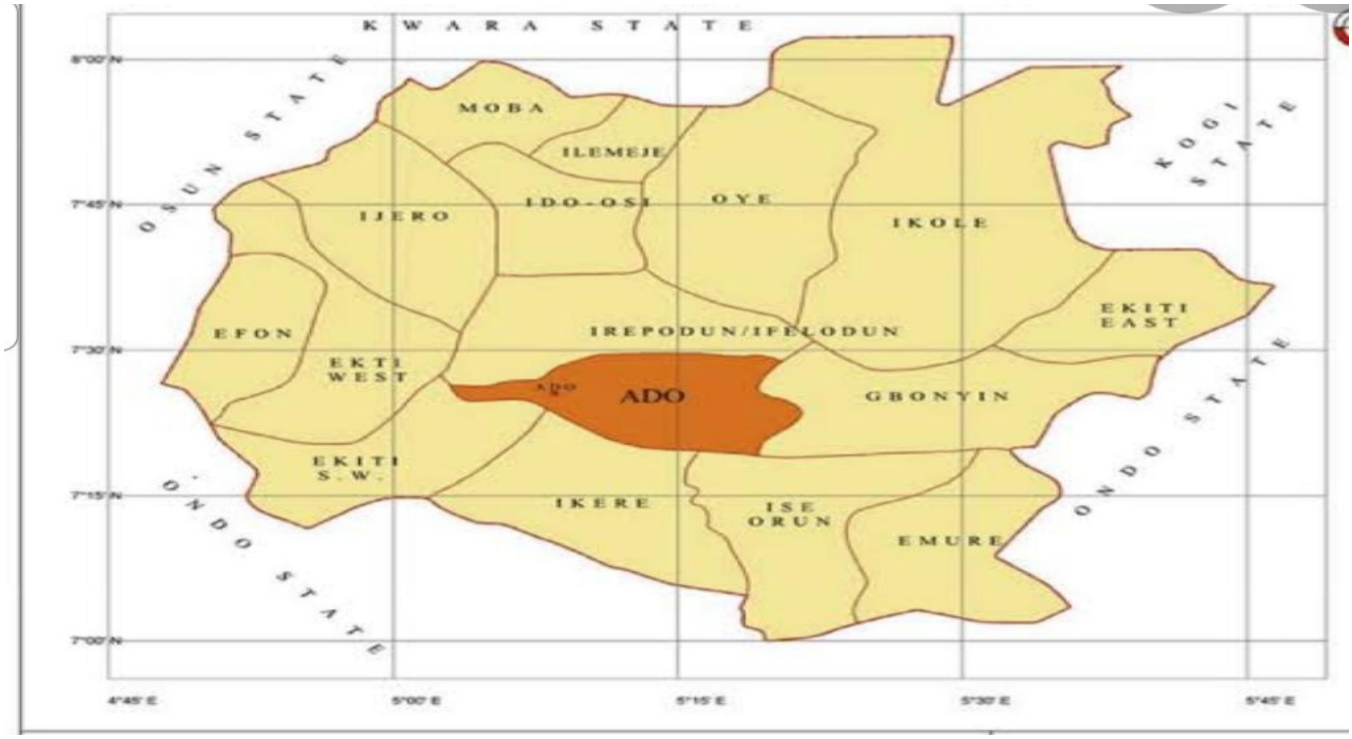


Fig 1: Shows the location from where the fish was bought

2.2 Sampling Collection

Five(5) variety of fishes were selected on the basisof their regular availability throughout the year as well as their commercial demand.

The five varieties of frozen fish (*Clupeaharengus, Trachurus trachurus,Scomber scombrus, Merluccius specie*and *Pseudotolithussenegalensis)*were purchase from different selling point at Oja-Oba market in Ado-Ekiti. Each sample was kept in a sterile plastic bag and was then taken to the laboratory as quickly as possible. The samples were oven dried at 105^0C, in which it was allowed to cool down at room temperature, after the dried tissues were extracted and was blended to a powdered form. The powdered sample was packed in a container and stored until further uses.

2.3 Preparation of fish samples

2.3.1 Sample extraction

- 20g portion of each powdered sample was weighed into a pre- extracted Whatman no 42 filter paper.
- The extraction was carried out in a Soxhlet extractor for 4hours using N- hexane as the extracting solvent. The extract was concentrated by distilling of the solvent (N- hexane) to about 2ml.
- The concentrated extract was cooled down to room temperature. The reduced extract was then preserved for clean-up. (modified version of Stoichev T. *et. al*, 2005 and Zhou *et al* 2007)

2.3.2 Sample clean- up

2.3.3 Clean-up procedure

A column of about 15 cm (length) × 1 cm (internal diameter) was packed with about 5 g activated silica gel prepared in a slurry form in n-hexane. About 5 g of anhydrous sodium sulphate was placed at the top of the column to absorb any water in the sample or the solvent. The column was pre-eluted with 15 mL of n-hexane without the exposure of the sodium sulphate layer to air. The reduced extract was placed in the column and allowed to sink below the sodium sulphate layer. Elution was done with 2 × 10 mL portions of the extracting solvent (N-hexane). The eluate was then collected, dried with anhydrous sodium sulphate and then evaporated to dryness under a stream of analytical grade nitrogen (99.999%). The dried eluate above was then reconstituted with 1mL spectra grade n-hexane and 0.5 mL of 20 mg/kg mixture of organochlorines pesticides we added as an internal standard. 1.0 µL of the mixture was injected into the GC-MS column for analysis. (modified version of Stoichev T. *et. al* , 2005 ; Zhou *et al* 2007)

2.4 OCP Analysis (Standard solutions)

A standard stock mixture containing nine OCP was prepared in n-hexane at a concentration of 400 µg mL-1 and stored at -18 °C until use. The solutions were stable for at least two months, although it was verified that 4,4'- DDT and endrin are not stable in solution for three months and their degradation products were observed in chromatograms obtained in the full scan mode. The working standard solutions were prepared daily at concentrations of 0.160 to 21.0 µg mL-1 by dilution of the standard stock solution with hexane.

2.5.1 Analytical curves

Determinations of the OCP were carried out by the external standard method using analytical curves with six concentration levels in six replicates over the range of 0.125 to 1.000 µg mL^{-}

2.6 Instrumentation (Gas Chromatograph Analysis)

Gas Chromatography (GC) was used for the determination of DDT and lindane using Varian 3800/4000 GC (Varian, Walnut, Greek, USA). The chromatographic separation was achieved using an HP-5MS capillary column (30 m ×250 µm and 0.25 µm film thickness). The carrier gas was nitrogen at a constant flow rate of 1.1 ml/min. The separation temperature program was initially set at 85 °C for 0.3 min, increased to 150 °C (hold for 4 min) at a rate of 30 °C/min, then to 185 °C at a rate of 2 °C/min, and finally to 290 °C (hold for 5 min) at a rate of 4 °C/min. The MSD was operated in electron impact ionization mode (EI) at 70 eV and temperature at 220 °C. EI spectra were monitored by scanning ions within the range of 50–500 amu. The target compounds were identified by their full scan mass spectra and retention time using the total ion current as a monitor to give a total ion chromatogram (ITC). The use of the full scan mode allows comparing the spectrum obtained for interested compounds with the EI-MS libraries. Besides, selective ion monitoring (SIM) mode was used for the identification and confirmation of tested compounds according to their selective specific ions for the compound of interest

The external standard method of analysis was used. The sample extract was made up to 1 mL with hexane and analyzed on the GC. The instrument was operated in the selective ion monitoring mode and the computer which controlled the system had an EI-MS library of standards for the target pesticides under the experimental conditions used. In addition, there was retention time match of analyte peak to standard peak for further confirmation of analyte identity. Data acquisition and processing was integrated with Agilent Chem Station, a GC workstation.

System performance check was conducted by standard solution containing a mixture of 500 ng mL^{-1} each, of dichlorvos, pp-DDT, op-DDT; lindane and, 500 mg mL^{-1} plant oil was prepared in the elution solution. The recoveries for the individual pesticides were determined from the analysis ofsample blank matrix spiked at 2 levels with analytes; 0.3 and 1.5mgL^{-1}. The run-to-run (intra-assay) precision was established by replicate injections of the sample blank spiked at both levels. The day-to-day (inter assay) precision was determined by the analysis of 6 replicates each day on 3 different days. Mixed pesticides standard solutions at 4 concentration levels (0.125, 0250, 0500 and 1.000 µgmL^{-1}) were analyzed to obtain standard curves for all the analytes of interest using external standard method. The instrument detection and quantitation limits were based on a signal-to-noise ratio of 3:1 and 10:1, respectively.

III. RESULTS AND DISCUSSION

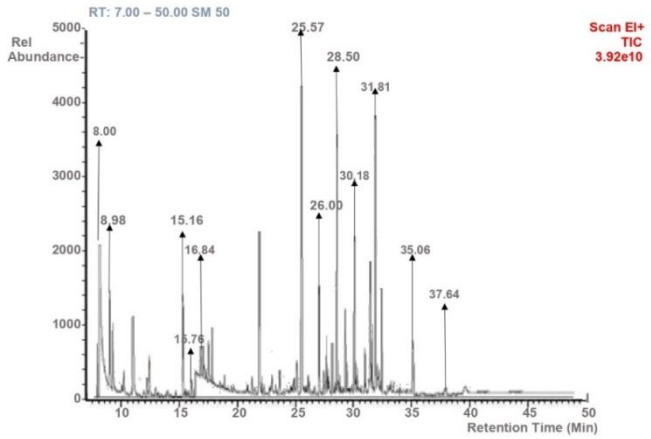


Fig 2 (Sample 01) *Merluccius specie(Panla fish)*

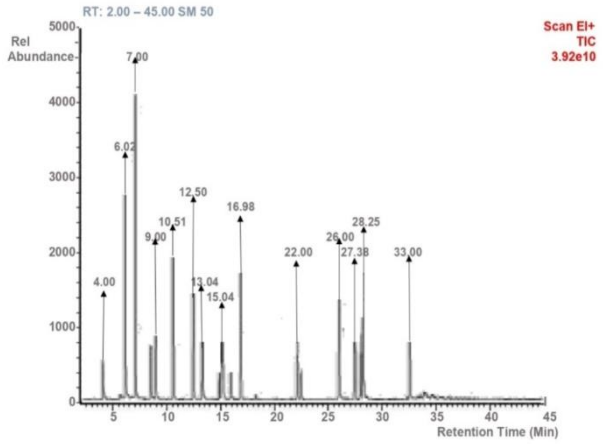


Fig 3. (Sample 02): *CLUPEA HARENGUS (Shawa fish*

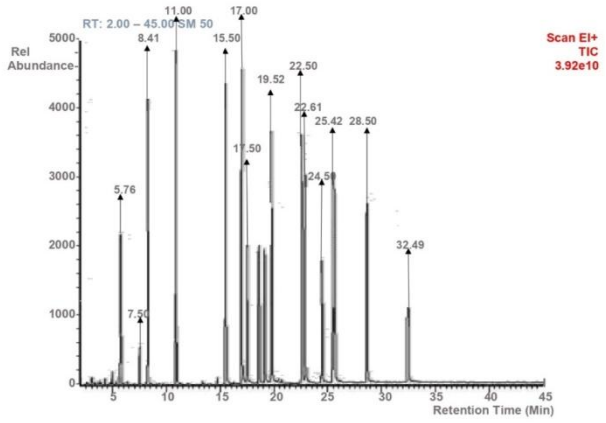


Fig 4. (Sample 03)*TRACHURUS TRACHURUS (Kote fish)*

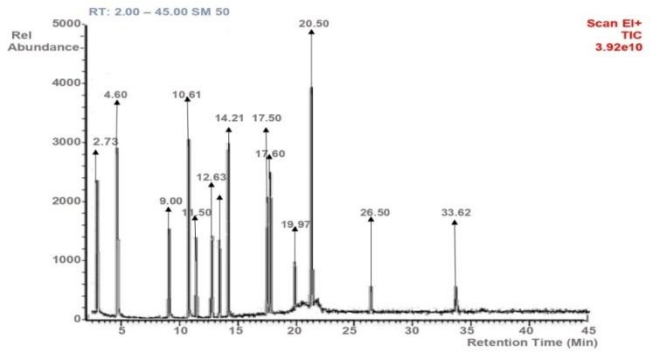


Fig 5. (Sample 04): *Pseyddotolithus senegalensis (Croaker fish)*

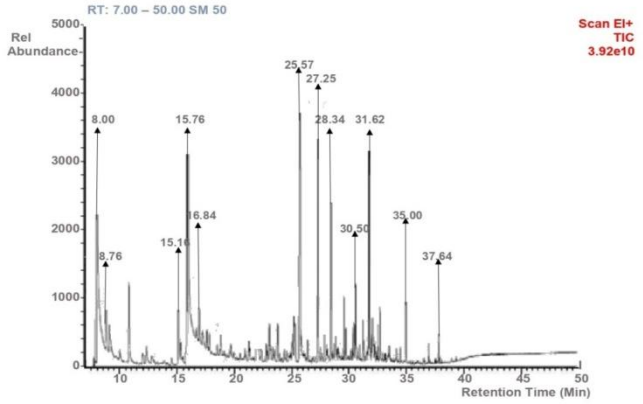
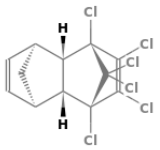
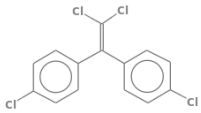
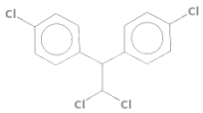
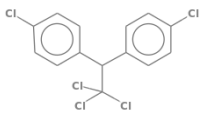
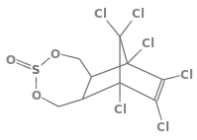
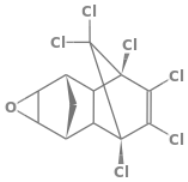
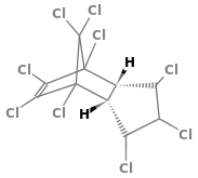


Fig 6 (Sample 05) SCOMBER *SCOMBRUS (Titus fish)*

Table 2:Merluccius specie (F1)

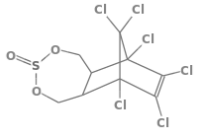
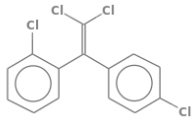
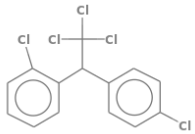
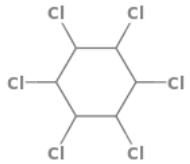
Peak	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp mg/kg wet weight	m/z	Structures
1	8.00	hexachlorobenzene	C_6Cl_6	284	9.19	0.73	107, 142, 284	
2	8.98	β-Hexachlorocyclohexane	$C_6H_6Cl_6$	290	7.44	0.52	109, 181, 290	

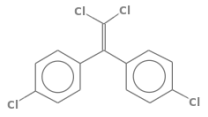
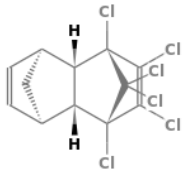
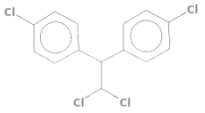
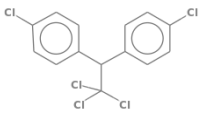
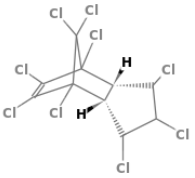
3	15.16	Aldrin	$C_{12}H_8Cl_6$	364	7.41	2.50	66, 263, 364	
4	15.76	Lindane	$C_6H_6Cl_6$	256	1.36	BDL	181, 219, 256	
5	16.84	p,p'-DDE	$C_{14}H_8Cl_4$	318	3.06	0.07	176, 246, 318	
6	25.57	α-Lindane	$C_6H_6Cl_6$	290	18.38	3.02	109, 183, 290	
7	26.00	1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane	$C_{14}H_{10}Cl_4$	320	7.00	0.91	165, 235, 320	
8	28.50	δ-Lindane	$C_6H_6Cl_6$	290	15.75	2.98	109, 219, 290	
9	30.18	p,p'-DDT	$C_{14}H_9Cl_5$	354	9.63	0.21	165, 235, 354	
10	31.81	Endosulfan	$C_9H_6Cl_6O_3S$	406	16.51	4.04	195, 241, 406	
11	35.00	Dieldrin	$C_{12}H_8Cl_6O$	380	3.90	0.02	79, 263, 380	

12	37.64	trans-Nonachlor	$C_{10}H_5Cl_9$	444	0.75	0.01	237, 409, 444	

The table shows the individual organochlorines, their respective concentrations and the total residue load in the muscles of the fish species.

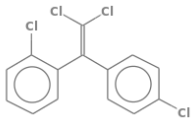
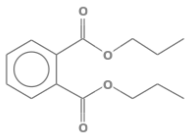
Table 3 (F2): *CLUPEA HARENGUS (Shawa fish)*

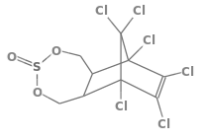
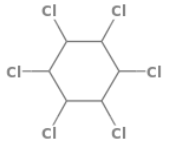
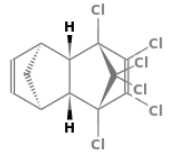
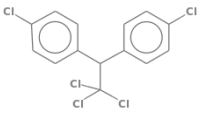
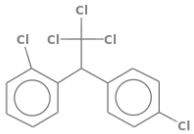
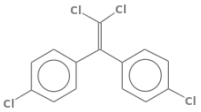
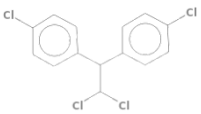
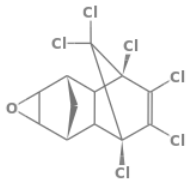
Peak	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp mg/kg wet wt	m/z	Structures
1	4.00	hexachlorobenzene	C_6Cl_6	284	3.05	0.04	107, 142, 284	
2	6.02	δ-Lindane	$C_6H_6Cl_6$	290	14.32	2.83	109, 219, 290	
3	7.00	Endosulfan	$C_9H_6Cl_6O_3S$	406	21.47	4.18	195, 241, 406	
4	9.00	o,p'-DDE	$C_{14}H_8Cl_4$	318	4.27	0.03	176, 245, 318	
5	10.51	o,p'-DDT	$C_{14}H_9Cl_5$	354	\	0.02	165, 235, 354	
6	12.50	β-Hexachlorocyclohexane	$C_6H_6Cl_6$	290	7.26	0.47	109, 181, 290	
7	13.04	Lindane	$C_6H_6Cl_6$	256	4.12	0.01	181, 219, 256	

8	15.04	p,p'-DDE	$C_{14}H_8Cl_4$	318	4.39	0.03	176, 246, 318	
9	16.98	Aldrin	$C_{12}H_8Cl_6$	364	9.15	2.76	66, 263, 364	
10	22.00	1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane	$C_{14}H_{10}Cl_4$	320	4.69	0.03	165, 235, 320	
11	26.00	α-Lindane	$C_6H_6Cl_6$	290	7.21	1.10	109, 183, 290	
12	27.38	Dieldrin	$C_{12}H_8Cl_6O$	380	4.27	0.02	79, 263, 380	
13	28.25	p,p'-DDT	$C_{14}H_9Cl_5$	354	6.18	0.16	165, 235, 354	
14	33.00	trans-Nonachlor	$C_{10}H_5Cl_9$	444	4.53	0.03	237, 409, 444	

The table shows 14 organochlorines present in F2, their respective concentrations The Table 2also shows the total residue load in the muscles of the fish species.

Table 4 (F3): *TRACHURUS TRACHURUS (Kote fish)*

Peak	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp mg/kg wet weight	m/z	Structures
1	5.76	o,p'-DDE	$C_{14}H_8Cl_4$	318	5.29	0.03	176, 245, 318	
2	7.50	Dipropylphthalate (DPrP)	$C_{14}H_{18}O_4$	250	1.38	ND	76, 149, 250	
3	8.41	δ-Lindane	$C_6H_6Cl_6$	290	10.06	2.25	109, 219, 290	

4	11.00	Endosulfan	$C_9H_6Cl_6O_3S$	406	11.74	3.16	195, 241, 406	
5	15.50	β-Hexachlorocyclohexane	$C_6H_6Cl_6$	290	10.35	0.53	109, 181, 290	
6	17.00	Aldrin	$C_{12}H_8Cl_6$	364	11.07	2.81	66, 263, 364	
7	17.50	Lindane	$C_6H_6Cl_6$	256	4.61	0.01	181, 219, 256	
8	19.52	α-Lindane	$C_6H_6Cl_6$	290	8.69	1.26	109, 183, 290	
9	22.50	p,p'-DDT	$C_{14}H_9Cl_5$	354	8.66	1.04	165, 235, 354	
10	22.61	o,p'-DDT	$C_{14}H_9Cl_5$	354	7.22	0.05	165, 235, 354	
11	24.50	p,p'-DDE	$C_{14}H_8Cl_4$	318	4.14	0.03	176, 246, 318	
12	25.42	1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane	$C_{14}H_{10}Cl_4$	320	7.58	1.22	165, 235, 320	
13	28.50	Dieldrin	$C_{12}H_8Cl_6O$	380	6.30	1.06	79, 263, 380	

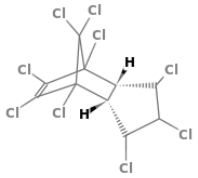
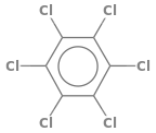
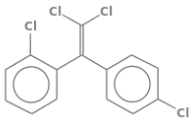
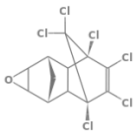
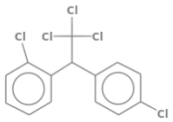
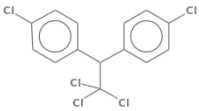
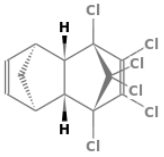
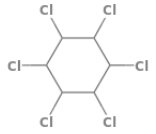
14	32.49	trans-Nonachlor	$C_{10}H_5Cl_9$	444	2.90	0.01	237, 409, 444	

Table 4: The table shows the individual organochlorines, their respective concentrations The Table also shows the total residue load in the muscles of the fish species.

Table 5 (F4): *Pseyddotolithus senegalensis (Croaker fish)*

Peak	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp mg/kg wet weight	m/z	Structures
1	2.73	hexachlorobenzene	C_6Cl_6	284	9.26	1.02	107,142, 284	
2	4.60	δ-Lindane	$C_6H_6Cl_6$	290	11.47	2.31	109, 219, 290	
3	9.00	o,p'-DDE	$C_{14}H_8Cl_4$	318	6.18	0.04	176, 245, 318	
4	10.61	Dieldrin	$C_{12}H_8Cl_6O$	380	8.11	0.21	79, 263, 380	
5	11.50	o,p'-DDT	$C_{14}H_9Cl_5$	354	5.41	0.02	165, 235, 354	
6	12.50	Lindane	$C_6H_6Cl_6$	256	1.62	ND	181, 219, 256	
7	12.63	p,p'-DDT	$C_{14}H_9Cl_5$	354	5.83	0.13	165, 235, 354	
8	14.21	Aldrin	$C_{12}H_8Cl_6$	364	11.59	3.00	66, 263, 364	
9	17.50	β-Hexachlorocyclohexane	$C_6H_6Cl_6$	290	8.11	0.56	109, 181, 290	

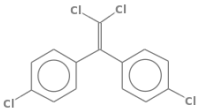
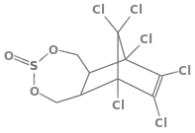
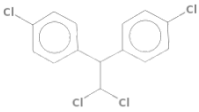
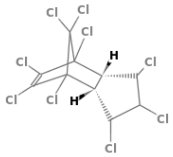
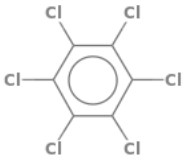
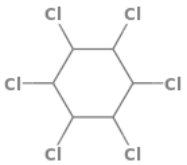
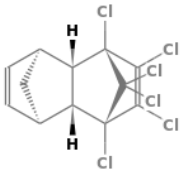
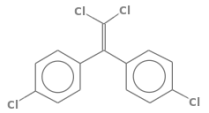
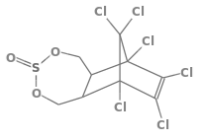
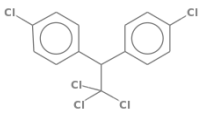
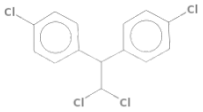
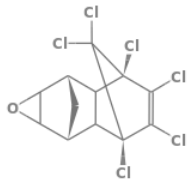
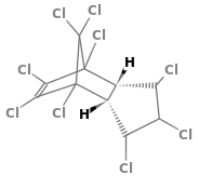
10	17.60	α-Lindane	$C_6H_6Cl_6$	290	9.66	1.14	109, 183, 290	
11	19.97	p,p'-DDE	$C_{14}H_8Cl_4$	318	3.52	0.01	176, 246, 318	
12	20.50	Endosulfan	$C_9H_6Cl_6O_3S$	406	15.51	3.20	195, 241, 406	
13	26.50	1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane	$C_{14}H_{10}Cl_4$	320	1.86	0.00	165, 235, 320	
14	33.62	trans-Nonachlor	$C_{10}H_5Cl_9$	444	1.85	ND	237, 409, 444	

Table 5: The table shows the individual organochlorines, their respective concentrations and the total residue load in the muscles of the fish species.

Table 6 (F5): *SCOMBER SCOMBRUS (Titus fish)*

Peak	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp mg/kg wet weight	m/z	Structures
1	8.00	hexachlorobenzene	C_6Cl_6	284	9.74	0.65	107, 142, 284	
2	8.76	β-Hexachlorocyclohexane	$C_6H_6Cl_6$	290	3.54	0.01	109, 181, 290	
3	15.16	Lindane	$C_6H_6Cl_6$	256	3.63	0.01	181, 219, 256	
4	15.76	Aldrin	$C_{12}H_8Cl_6$	364	13.73	2.39	66, 263, 364	

5	16.84	p,p'-DDE	$C_{14}H_8Cl_4$	318	3.72	0.02	176, 246, 318	
6	25.57	α-Lindane	$C_6H_6Cl_6$	290	15.94	4.00	109, 183, 290	
7	27.25	Endosulfan	$C_9H_6Cl_6O_3S$	406	15.05	3.93	195, 241, 406	
8	28.34	δ-Lindane	$C_6H_6Cl_6$	290	10.63	2.15	109, 219, 290	
9	30.50	p,p'-DDT	$C_{14}H_9Cl_5$	354	5.40	0.09	165, 235, 354	
10	31.62	1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane	$C_{14}H_{10}Cl_4$	320	13.29	2.11	165, 235, 320	
11	35.00	Dieldrin	$C_{12}H_8Cl_6O$	380	3.54	0.02	79, 263, 380	
12	37.64	trans-Nonachlor	$C_{10}H_5Cl_9$	444	1.77	ND	237, 409, 444	

The table shows the 12 organochlorines present in sample F5, their respective concentrations and the total residue load in the muscles of the fish species.

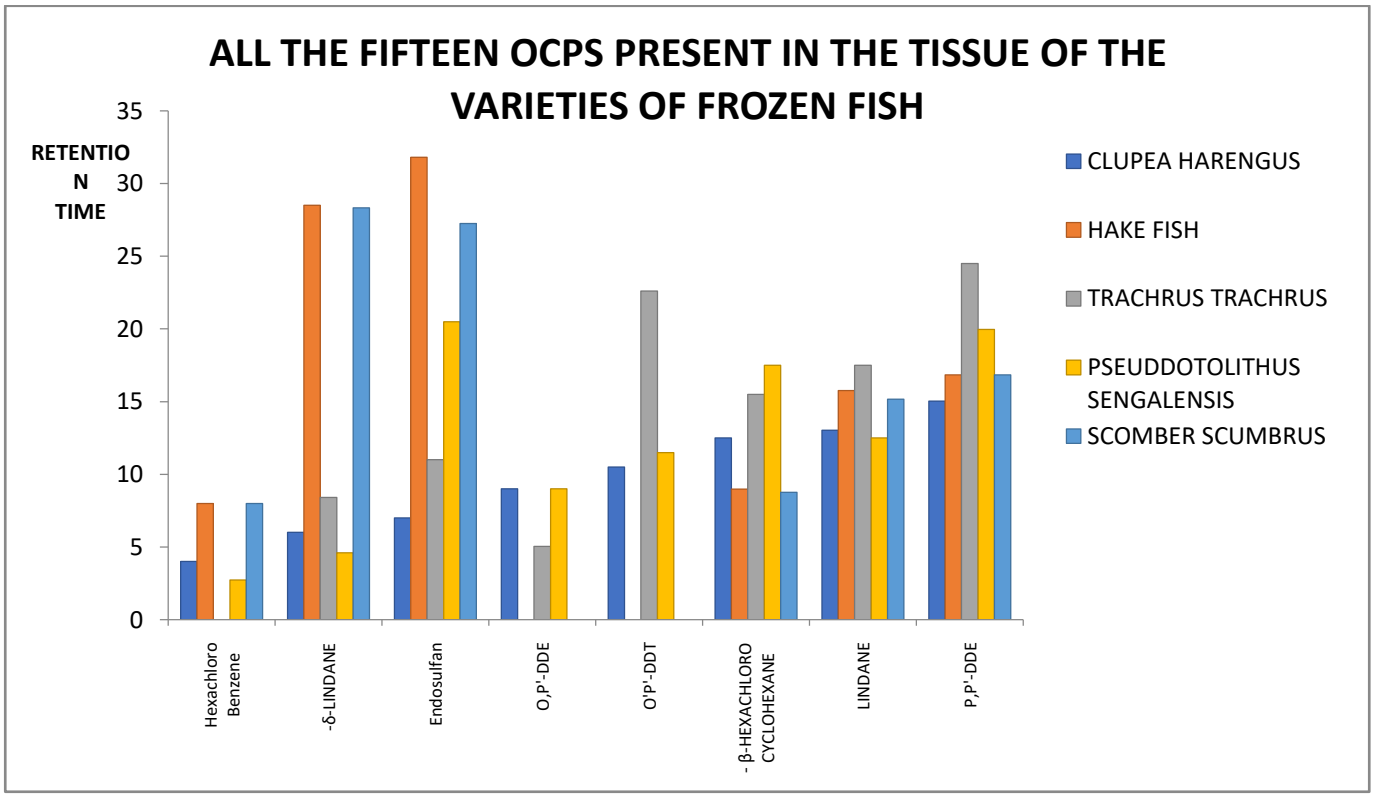


Fig 7a

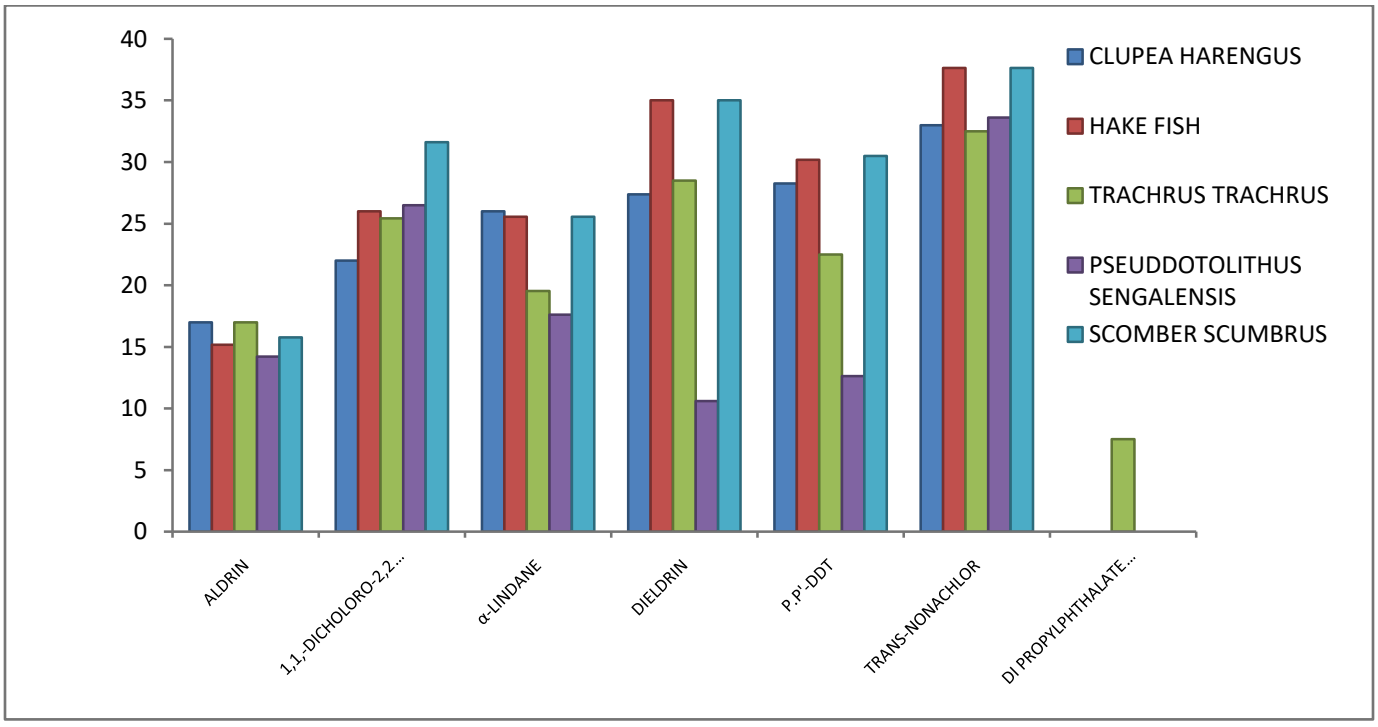


Fig7B

Fig 7a and 7b are Organochlorine pesticides compounds

Table 7. MRL levels of some OCPs	
Organochlorine pesticides	Maximum residue level in fish tissue
HCB	5 μg/Kg[1,2]
Lindane(γ-HCH), δ-HCH, β-HCH, α-HCH	5 μg/Kg [2]
Dieldrin	200 μg/Kg[1], 0.3μg/Kg[3], 5 μg/Kg [2]
Endosulfan	5 μg/Kg[2]
p, p DDE, o,p'-DDE, o,p'-DDT, p,p'-DDT	5 μg/Kg[2]
Aldrin	0.3mg/kg[3], 5 μg/Kg[2]
trans-Nonachlor	5 μg/Kg[2]
Diedrin	5 μg/Kg[2]
[1] EC 2005, [2] USGS 2016, [3]US FDA 2016	

Ratio

Table 8 ratio in fishes						
	Ratio	F1	F2	F3	F4	F5
DDT ratio	Opddt : ppddt		0.125	0.04808	0.154	
	p,p'-DDT: p,p'-DDE	3	5.3	34.6	13	4.5
HCH ratio						
	α: γ-HCH		110	126		400

4.1 Instrumentation's analysis

GC-ECD positive results were confirmed with GC mass spectrometric (GC/MS) detection. The HP 3800 gas chromatography interfaced to a HP4000 mass selective detector, and a HP-5 MS column, 30 m x 0.25 mm ID, the run time for the Hake fish (F1) sample was 50:00, for F2 was 45, while F3 and F4 was also 45, and F5 has the runtime of 50 . Identification was made on the basis of matching the mass spectrum and the retention time of the compound to that of a known standard.

The column chromatography method allowed the separation of the contaminants of interest in fractions. For the first scheme of sample F1, there were recoveries of 12 OCPs with Dieldrin having the highest retention time (RT) at 35.00 while the lowest retention time at 8.00 and 8.98 in hexachlorobenzene and β-Hexachlorocyclohexane (fig2), for the solvent systems was not polar enough for the first two compounds. The recoveries were improved significantly has the retention time increased for sample F1. The recovery results in Sample ID: DCA/CRL/GCMS/062021/110574/01 showed that the polarity index of the solvents influenced how complete the elution of the target compounds was. The second scheme (F2) involves the recoveries of 14 OCPs with trans-Nonachlor having the highest retention time at 33.00, while the lowest retention time at 4.00 and 6.02 inhexachlorobenzene and β-Hexachlorocyclohexane (fig3).The recovery results in Sample ID: DCA/CRL/GCMS/062021/110575/02 showed that the polarity index of the solvents influenced how complete the elution of the target compounds was, For the solvent systems was not polar enough for the compounds hexachlorobenzene, δ-Lindane, the recoveries were improved significantly and has the retention time increased for sample F2. 14 OCPs were recovered in sample F3 analysis, with trans-Nonachlor having the highest retention time (RT) at 32.49 while the lowest retention time at 5.76 in o,p'-DDE, and 7.50 in Dipropyl phthalate (DPrP) respectively. The recovery results (Sample ID: DCA/CRL/GCMS/062021/110576/03) showed that the polarity index of the solvents influenced how complete the elution of the target compounds was, because the solvent systems was not polar enough for the first two compounds o,p'-DDE and Dipropyl phthalate (DPrP), the recoveries were improved significantly and has the retention time increased for F3. The recoveries of F4 include 14 OCPs with trans-Nonachlor having the highest retention time at 33.62 while the lowest retention time was for hexachlorobenzene at 2.73 and δ-Lindane at 4.60 respectively. The recovery results in Sample ID: DCA/CRL/GCMS/062021/110576/04 also showed that the polarity index of the solvents influenced how complete the elution of the target compounds was due to the solvent systems not being polar enough for the first two compounds hexachlorobenzene and δ-Lindane. Lastly, the scheme for sample F5, involved the recoveries of 12 OCPs with trans-Nonachlor having the highest retention time at 37.64 while the lowest retention time hexachlorobenzene at 8.00 while β-Hexachlorocyclohexane at 8.76 respectively (fig 6). While its recovery results (Sample ID: DCA/CRL/GCMS/062021/110578/05) showed that the polarity index of the solvents influenced how complete the elution of the target compounds was, which was influenced by how the solvent systems was not polar enough for the first two compounds hexachlorobenzene and β-Hexachlorocyclohexane and thus, has the retention time increased for sample F5.

The concentration of Organochlorine pesticide residues in wet weight (mg/kg) of *Merluccius specie* (F1) is presented in table 2, where hexachlorobenzene, β-Hexachlorocyclohexane, Aldrin, p,p'-DDE, Lindane, α-Lindane, 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane, Endosulfan , Dieldrin and trans-Nonachlor residues are present in the muscle of the hake fish sample (making it a total of 12 compounds detected). Endosulfan has the highest residue concentration of 4.04mg/kg, followed by α-Lindane, δ-Lindane and aldrin with 3.02mg/kg, 2.98mg/kg and 2.50mg/kg respectively, while the rest falls below 1mg/kg. Sample F2 (Herring) shows 14 OCPs being detected constituting hexachlorobenzene, δ-Lindane, Endosulfan, o,p'-DDE, o,p'-DDT, β-Hexachlorocyclohexane, Lindane, p,p'-DDE, Aldrin, 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane, α-Lindane, Dieldrin, p,p'-DDT and trans-Nonachlor (Table 3). Endosulfan, δ-Lindane, aldrin and α-Lindane has the highest wet weight concentration of 4.18, 2.83, 2.76 and 1.10 respectively all in mg/kg, the rest detected falls below 0.5 with lindane having the least wet weight of 0.01mg/kg. The horse mackerel (F3) also has 14 OCPs present (Table 4), with Endosulfan, δ-

Lindane, aldrin, α-Lindane, p,p'-DDT, 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane and Dieldrin show wet weight greater than 1.0mg/kg. The fourteen OCPs present in sample F4 (croaker fish) include hexachlorobenzene, δ-Lindane, o,p'-DDE, Dieldrin, o,p'-DDT, Lindane, p,p'-DDT, Aldrin, β-Hexachlorocyclohexane, α-Lindane, p,p'-DDE, Endosulfan, 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane and trans-Nonachlor. While five of these OCPs are above 1.0mg/kg in wet weight concentration, endosulfan has the highest concentration of 3.20mg/kg. In contrast to the other four fish samples, sample F5shows α-Lindane having the highest weight wet concentration of 4.00mg/kg, followed by endosulfan, aldrin, δ-Lindane and 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane with 3.93mg/kg, 2.39mg/kg, 2.15mg/kg and 2.11mg/kg respectively as presented in Table 6. The table also shows 12 OCPs present in sample F5 in this study.

Hexachlorobenzene (HCB) ranges from 0.04mg/kg to 0.73mg/kg, while not present in F3, this is higher than what (El-Sayed *et al*., 2021) reported for tilapia fishes of 0.194 to 1.223µg/kgwhile (Edjere *et al*2020) on tilapia fish also reported 0.06mg/kg. HCB has the toxicity probability when combine with other polyhalogenated hydrocarbons (El-Sayed et al., 2021) or its acute level exhibit dioxin activity (El-Sayed *et al*., 2021). β-Hexachlorocyclohexane (β-HCH) ranges from 0.01mg/kg to 0.56mg/kgand other isomers of the HCH compound present in this study include lindane, α-Lindane and δ-Lindane. Lindane for F1 and F4 were below quantification, while value of 0.01mg/kg for sample F2, F3 and F5 were recorded each. Alpha lindane (α-HCH) ranges from 1.10mg/kg to 4.00mg/kg in four fish samples, and δ-HCH ranges from 2.15 to 2.98mg/kg in all the fish samples. α:γcan suggest the possible sources,either a technical HCH (α-HCH(60-70%),β-HCH(5-12%),γ-HCH (10-15%), and δ-HCH (6-10%),) input or lindane. Where a ratio close to 1 or less than one suggests lindane source whereas, if greater than 3 suggest a technical HCH input (Unyimadu *et al*, 2018). In this study, sample F2, F3 and F5 ratio are greater than 3, suggesting a technical HCH sources.β-HCH is the most stable and resistant to microbial degradation (willett*et al* 1998). The report of Zhou*et al*, 2007 of OCPs in several fishes has lower values of β-HCH compared to this study, while alpha lindane shows the highest concentration of HCH isomers.

Aldrin concentrations ranges from 2.39mg/kg in F5 to 3.00mg/kg in F4, these values are higher than Aldrin MRL for fish of 0.3mg/kg (Table 7) which suggest more assessment into fish importation because these fishes' consumption are at a high demand on a weekly basis in places like Nigeria and therefore may have higher human health risk. Aldrin is considered an extremely hazardous substance in the United Statesand potentially carcinogenic by Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health (Joshua, 2016). The correlation between dieldrin and aldrin (aldrin can easily metabolise to dieldrin;may suggest the reason for the low concentration in sample F1, F2 and F5. Whereas, the high values of F3 and F4, can point to the possibility of direct usage of dieldrin because of its environmental persistency (Botaro *et al*, 2011).

p,p'-DDE ranges in wet concentration from 0.01 to 0.07mg/kg, p,p'-DDT ranges from 0.09 to 1.04mg/kg while o,p'-DDE and o,p'-DDT ranges from 0.03 to 0.04mg/kg and 0.02 to 0.05mg/kg respectively. o,p'-DDE and o,p'-DDT are only detected in sample F2, F3 and F4. p,p'-DDT shows the highest concentration range among the four in this study. The DDT ratio for each fish samples may indicate a possible source (o,p'-DDT/ p,p'-DDT, Edjere *et al*, 2020) and the possible time of input (p,p'-DDT/ p,p'-DDE, Zhou et al, 2007). The ratio presented in Table 8, shows that p,p'-DDT/ p,p'-DDE ratio are greater than 1, suggesting a possible recent use of DDT (Zhou *et al*. 2007) where if less than 1 would have suggest an old use of DDT. DDT in the fish samples could also be as a result of metabolism of DDT to DDE, this is because DDT can be biodegraded to DDE and DDD under both aerobic and anaerobic conditions respectively (El-Sayed *et. al., 2021*, Edjere *et al.,* 2020).

Endosulfan ranges from 3.20 to 4.18mg/kg which is high in concentration and could pose challenges to human health.

IV. CONCLUSION

From the results obtained, the levels of Organochlorine pesticide (δ-lindane, aldrin, DDE, DDT and endosulfan) residues in some common varieties of frozen fish, Hake, Trachurustrachurus, Scombersrumbus, pseudotolithussenegalensis and Clupeaharengus herring(shawa) had been determined and information provided on the amount of the pesticides as well as the danger they may pose to consumers that depend on these imported fish as diet. In all, fifteen organochlorine pesticide (OCP) residues were detected in all of the five frozen species of fish muscles, the residue showing the highest concentration in the muscle of the frozen fishes were Endosulfan, delta lindane, alpha Lindane and Aldrin. These results show that the consumption of these fish species may have the tendency of elevating the overall levels of OCP residues in the individual since these residues have bio-accumulative effect. Lindane and aldrin were identified as the main organochlorine in the fish samples assessed. The mean concentrations of the trans-Nonachlor and Endosulfan studied in all the fish samples were found to be very high compared to their EPA maximum permissible limit. It was discovered that the mean concentrations of the pesticides in all the frozen fish samples analyzed were above permissible limits and this exposed the consumers to serious health risk such as cancers (breast and prostate cancers), hypertension, cardiovascular disorder, diabetes and other health related issues. The prolonged use of pesticides in agriculture has also caused serious health problems as these pesticides accumulate and affect the food chain. Organochlorine compounds are highly lipophilic and can accumulate in fat-rich food such as meat, fish, milk etc. because of these consumers should be aware of the high risk

in consuming high fatty foods and the danger it imposes on their health.

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