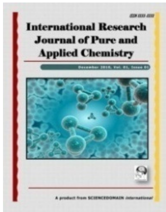

International Research Journal of Pure & Applied Chemistry
5(2): 150-159, 2015, Article no.IRJPAC.2015.014
ISSN: 2231-3443

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Level of Organochlorine Pesticides Residue in Fish, Water and Sediment in Biu Dam (Reservoir) Borno State Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DYS, ONM and JTB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IRJPAC/2015/13199
Editor(s):
(1) Wenzhong Shen, State Key Laboratory of Coal Conversion, Institute of Coal Chemistry, CAS, China.
Reviewers:
(1) Anonymous, Industrial Research Laboratories Complex Karachi, Pakistan.
(2) Anonymous, Ahvaz Jundishapur University of Medical Sciences Ahvaz, Iran.
(3) Anonymous, Universidade de São Paulo, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=807&id=7&aid=6835

Original Research Article

Received 7th August 2014
Accepted 14th October 2014
Published 6th November 2014

ABSTRACT

The health risks posed by organochlorine compounds to the total environment have continued to elicit concern among researchers in developing countries worldwide. Hence, this study focused on investigating the levels of Organochlorine pesticides residues in water sediment and fish samples in Biu dam reservoir. The pesticides residues in the fish and sediment samples were extracted by soxhlet extraction process using a mixture of n-hexane and acetone while water sample was extracted using dichloromethane liquid- liquid extraction method. The extracts were cleaned and analysed using a gas chromatograph mass spectrometer (GCMS) equipped with electron capture detector. Endrin, aldrin, dieldrin, endosulfan I, endosulfan II, alpha BHC and heptachlor were detected in the study area. While chlordane, methoxychlor, other isomers of BHC, DDT and its degradation products were not detected in all the samples analysed. Endrin had the highest value

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of 14.758 mg/Kg in catfish while the lowest endrin value of 0.017mg/Kg was found in sediment. The findings provide evidence of pollution of some the organochlorine pesticides residue in Biu dam reservoir which is slightly contaminated with organochlorines pesticide. Therefore serious need for the monitoring of these pesticide residues in water, sediments, fish and the environment, as this will go a long way towards preventing various environmental and public health hazards.

Keywords: Organochlorines; pesticides; sediments; reservoir; Biu dam; water.

1. INTRODUCTION

Pesticides are substance used to kill, repel, or control certain forms of plant or animal life that are considered to be pests. Pesticides include herbicides for destroying weeds and other unwanted plants, insecticides are used for controlling a wide variety of insects, fungicides used to kill or prevent the growth of fungus, antibacterial for preventing the spread of bacteria, and compounds used to vermin control. Because of the widespread use of agricultural chemicals in food and agricultural production, people are exposed to low levels of pesticide residues through their diets.

Pesticides are frequently applied in agricultural, forestry, and urban settings. There are tens of thousands of pesticides in use, many of which are synthetically produced. Pesticides will break down in the environment forming by-products, some of which are toxic whereas others are relatively non-toxic [1,2]. Fishes are suitable indicators for environmental pollution monitoring because they concentrate pollutants in their tissues directly from water and also through their diet [3]. Acute (immediate) toxic effects can influence the survival or reproduction of aquatic species leading to the disruption of predator-prey relationships and a loss of biodiversity. If aquatic organisms are not harmed immediately, they may accumulate chemicals from their environment into their tissues. This bio-concentration can lead to bio-magnification, a process in which the concentrations of pesticides and other chemicals are increasingly magnified in tissues and other organs as they are transferred up in the food chain. Terrestrial predators that feed on aquatic species may also be affected [2,4]. People are exposed to pesticides through aquatic systems either by ingesting fish or shellfish that have stored these compounds in their tissues or directly by drinking contaminated water. Reports have shown that pesticide exposure has been linked to cancer, neurological damage, immune system deficiencies, and problems with the endocrine system [4-7].

Organochlorine pesticides (OCPs) are a class of non polar toxic chemical compounds classified as dichlorodiphenylethane cyclodienes and chlorinated benzenes [8]. OCPs are ubiquitous environmental contaminant which have spread globally and have been detected in food stuffs, meat, drinking water and sediments as well as wide range of biota including fish [9]. Numerous studies on both human and laboratory animals provide strong evidence of the toxic potential of the exposure to OCPs. The health effects associated with OCPs include reproductive failures, birth defect, endocrine disruption immune system dysfunction and cancer [10-12]. Other investigations confirmed that OCPs have strong potential to cross placental barriers even in minute concentration and cause serious neonatal damage [13].

OCPs are widely used by farmers because of their effectiveness and their broad-spectrum activities [14,15]. Moreover, OCPS showed very resistant characteristic to microbial degradation and are employed to control ecto-parasites of farm animals and pests. However, this chemical and several other chemical contaminants from the agricultural fields, comprising of pesticides and other agrochemicals have been reported in the drainage systems and are likely to jeopardise the quality of the water bodies that support the fishery industry, irrigation, industrial, domestic and human consumption. Africa has therefore been suggested as a highly relevant area for increased research activities to establish possible links between the increasing number of unexplained cancer cases and exposure to anthropogenic chemical pollutants in food, air, water and soil [16].

Organochlorine pesticides such as DDT are still frequently in use all over Africa both as a means for effective and cheap vector control health and for agricultural purposes [17]. One of the most controversial pesticides of all time, dichlorodiphenyltrichloroethane (DDT) is today even being introduced on a broader scale to defeat malaria, despite being banned in most countries of the world [18]. From an African perspective this might be understandable, since malaria still is a tremendous problem killing one child every 30 minutes in sub-Saharan Africa [19].

The contamination of the environment and food chain by chlorinated organic 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 commercially sold fruits, vegetables, milk, fish and water [20-24]. Organochlorine pesticides have been used extensively worldwide since the early 1950s [25,26]. Dichlorodiphenyltrichloroethane and HCH or BHC which had been produced for use in agriculture, forestry, and public health for more than four decades, are considered to be the most important OCPs in China [27]. Soil is likely the largest sink in the environment for OCPs and the release of these contaminants from soils continue to be a source to other environmental compartments [28]. Most of organochlorinated pesticides have been progressively restricted and then banned in the 1970s in most industrialized countries, since there uses have resulted to a widespread environmental pollution [29]. Rain water and irrigations can take away pesticides from its place application areas to the surface waters and even ground water. Organochlorinated pesticides solubility in water is limited, because they are mostly lipophilic compounds so they tend to connect in with the suspend matter, to precipitate in sediments, to accumulated and concentrated in biota of aquatic systems [30,31].

In Nigeria, particularly in the northeast zone, there is paucity of data on the concentrations of OCP residues in water and other environments. Therefore, there is a need for continuous monitoring of OCPs in the environments for occurrence, concentration levels, distribution and pathways of distributions and bioaccumulation especially water and in fish samples in Nigeria. Fish survives in water and some fish species feed on water sediments. This study was undertaken to determine the levels of organochlorine residues in water, sediment and two species of fish in a dam reservoir in Biu, Borno state, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

Biu dam is situated in Biu local government in Borno state and located on latitude 10°38'00" N and longitude 12°05'31'' E. The dam was constructed purposely to supply drinking water to Biu community being the second largest town in Borno state and the reservoir is to be used for irrigating the fertile agricultural land around the dam but these project was abandoned be successive government. The water from the dam reservoir is primarily used in irrigation, fishing and source of water large cattle farmer in the areas shown in Fig. 1.

2.2 Sample Collection

Samples of the environmental matrices-sediment, water and biota (fish) were collected at four locations from the study area for a period of July, 2013 - May, 2014 and prepared into five composite samples each during the dry and wet seasons. Five fish sample were selected at least and packed individually in plastic bags with eugenol, stored in a Styrofoam box with ice, and transported to the laboratory where they were stored at temperature of-20°C until ready for use. The fish species collected were *Tilapia zilli* (Tilapia) and *chrysichthys nigrodigitatus* (Catfish). They were identified by a specialist from Department of fisheries and Aquaculture Adamawa state university, Mubi.

The sediment samples were collected using the triple sediment corer made by Uwitec fitted with plexiglas tubes of 50cm height. Five sediment samples were collected at 2 and 8 cm depths each from different location of the dam reservoir, mixed thoroughly to form a composite sample and brought to the laboratory as soon as possible under temperature-controlled conditions. In the laboratory, sediment cores were weighed dried at 40°C until constant dry weight samples were obtained. Samples were then homogenized using Agatha mortar and pestle and sieved with 0.5mm mesh sieves and the samples were stored at – 4°C until further analysis.

Five water samples from Biu dam reservior were collected using 2L amber glass bottles from different location, mixed thoroughly to form a composite sample. The composite water samples were pre-filtered through 0.45 lm fiber glass filters (whatman) to remove suspended material and then preserved by the adding of concentrated H_2SO_4 to prevent biological activity [32].

H_2SO_4 (aq) + H_2O (l) → H_3O^+(aq) + HSO_4^-(aq). The samples were preserved by refrigeration at -10ºC until analysis was undertaken.

2.2.1 Extraction of water samples

Liquid-liquid extraction method was used to extract organochlorine pesticides from the 2 L water samples earlier collected. About 500 mL of water sample was put into a 1 L separatory funnel at a time. This was extracted with 10 mL (3 portions) of dichloromethane DCM by vigorous shaking for 30 minutes for each of the triplicate extraction. The combined extracts were dried with anhydrous sodium sulphate $Na_2SO_{4\ (s)}$ and concentrated to about 2 mL under a pure stream of nitrogen of 99.999% percentage purity.

2.2.2 Extraction of sediment samples

Dry Sediment sample was extracted according to Darko et al. [32], with some slight modifications. About 10 g of sediment samples were weighed and transferred into extraction thimble that had been previously washed with n-hexane and acetone and oven dried. The sample was extracted using 100 mL of n-hexane acetone

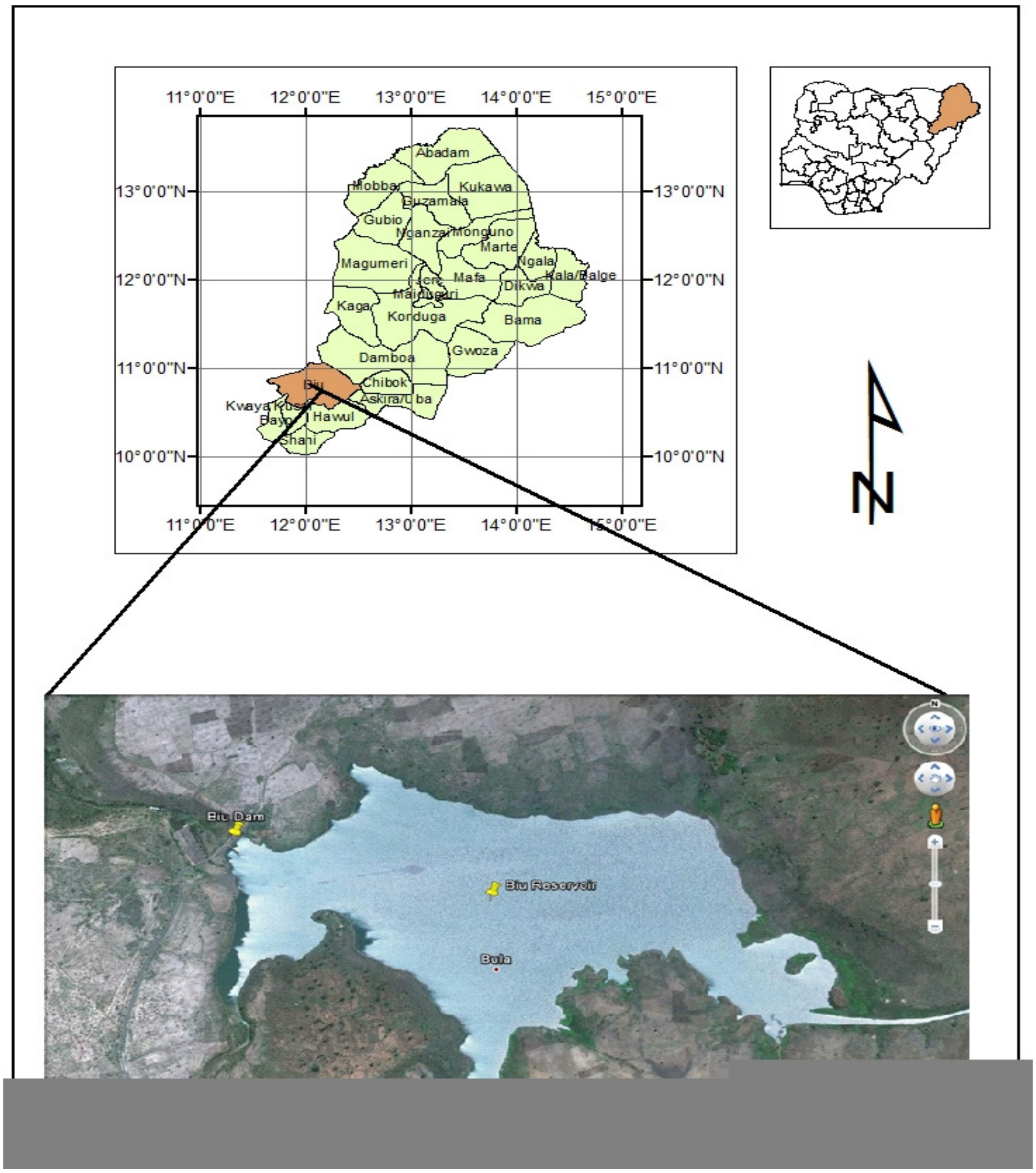

Fig. 1. Map of Borno state showing the location of Biu dam/reservoir

mixture 4:1 v/v for eight hours using soxhlet extractor. The extract was evaporated to dryness using a rotary evaporator at 40℃. Each extract was dissolved in 10 mL n-hexane and subjected to clean-up procedure.

2.2.3 Extraction of fish samples

The frozen composite whole-body of fish tissue samples were homogenized using an agate mortar and pestle [33,34]. Approximately 20 g of the properly chopped sample was further mixed with 10 g of anhydrous sodium sulphate. This was soxhlet extracted for eight hours using dichloromethane/n-hexane mixture. The extract was dried over anhydrous sodium sulphate and concentrated to about 2 mL as described earlier in readiness for the clean-up procedure.

2.2.4 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 (DCM). 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. Organochlorine Pesticide Mix AB #2 reference standard was obtained from Restek, USA. The Statistical analysis by ANOVA showed no significant differences ($P < 0.05$) between the pesticide residues as indicated in Tables 1 and 2. This suggests that, most of the indicated pesticides in Biu dam reservoir originate from agricultural runoff and probably mosquito control program may the sources of other pesticides detected in the study area.

Endrin is an organochlorine pesticide that was primarily used as an insecticide, as well as rodenticides. The compound endrin became infamous due to its persistence in the environment and for this reason it is banned in modern Shimadzu GC-MS QP-2010 was employed in analysing the standards and the calibration curve for each organochlorine compound was prepared automatically. The sample extracts were then analysed under the same conditions as for the standards, and in the Selective Ion Mode (SIM) with m/z values ranging from 65 to 410. Splitless injection mode was used with the injection temperature as 250℃ while the column oven temperature was ramped between 80 and 280℃. The GC-MS was operated at a pressure of 79.5 kPa and the flow rate was 1.18 ml/m. Method validation for this study maintained a RSD ±13% and percentage recoveries were in the range of 80 -110%.

Data obtained were subjected to analysis of variance (ANOVA) by SPSS version16 to determine the differences in the concentration of each pesticides residue.

3. RESULTS AND DISCUSSION

The distribution of various organochlorine pesticides in water, sediment and fish from Biu dam reservoir during dry and wet season is summarized in Tables 1 and 2 respectively. Organochlorine pesticides were detected in water, sediment and fish samples. Endrin had the highest residue value of 14.758 mg/Kg obtained in *Chrysichthys nigrodigitatus* (catfish) followed by aldrin residue value of 1.464 mg/Kg found in *Tilapia zilli (*Tilapia) while lowest pesticides residue value for endrin is found in sediment sample during dry season. Aldrin was detected in all the sample analysed except in cat fish while, chlordine, Methoxychlor, other isomers of HCH, DDT and its degradation products were not detected in all the samples analysed. In the rainy or wet season there's a significant decrease in the residue level of this pesticides, these decrease may be attributed to dilution of these chemicals in water, sediment and fish samples analysed as shown in Table 2. many countries. The majority of endrin (about 80%) was consumed as a spray to control insect pests of cotton by farmers in the study area before the banned on organochlorine pesticides in Nigeria. The remaining 20% was also used on rice and for seed treatment due to poor germination of seeds in the study area [35]. Cotton, maize and rice are commonly cultivated during wet and dry season in Biu and along the entire bank of the dam reservoir which may likely be the source of these pesticides detected in the study area. The levels of OCPs detected in the fish samples of the present study, were in most cases relatively higher than the values reported by Ogunfowokan [36], in freshwater and fish samples collected from various locations in Osogbo. Organochlorine pesticides tend to accumulate in living organisms especially in aquatic organisms and they substantially settle on the sediments [37]. Agricultural runoff is the primary source of these pesticides in aquatic ecosystems. Endosulfan I & II were detected in water and catfish respectively but not in sediment and Tilapia. Endosulfan has been shown to be highly toxic to fish and marine invertebrates and is readily absorbed in sediments. It therefore represents a potential hazard in the aquatic environment [38]. The value of endosulfan in this study 0.033mg/L in water is lower than study carried out by Idowu et al. [39].

Table 1. Shows the Mean±SD level of organochlorines (mg/kg) in water, sediment, and Fish found in Biu dam reservoir in dry season

Pesticides	Samples			
	Water (mg/L)	Sediment (mg/Kg)	Tilapia (mg/Kg)	Catfish(mg/Kg)
Gamma BHC	ND	ND	ND	ND
beta-BHC	ND	ND	ND	ND
alfa BHC	ND	ND	5.389±0.001^{a}	0.056±0.002^{b}
Endosulfan I	0.033±0.001^{a}	ND	ND	0.042±0.001^{a}
Endosulfan II	ND	ND	ND	0.122±0.012
Heptachlor	ND	0.036±0.001	ND	ND
Aldrin	0.183±0.003^{a}	0.090±0.001^{a}	1.464±0.001^{a}	ND
Dieldrin	ND	ND	0.044±0.000	ND
Endrin	ND	0.017±0.001^{a}	ND	14.758±0.001^{b}
Lambda-Cyhalothrin	ND	ND	0.147±0.002^{a}	0.010±0.000 ^{a}
Gamma-chlordane	ND	ND	ND	ND
p,p'-DDT	ND	ND	ND	ND
pp'-DDE	ND	ND	ND	ND
DDD	ND	ND	ND	ND
Methoxychlor	ND	ND	ND	ND

Note: All values represent Mean ±SD (Standard Deviation). Comparison was done across the period and values with different Superscripts are statistically different ($p < 0.05$). ND: Not Detected (at the limit of Detection $1x10^{-5}$ mg/kg)

Table 2. Shows the Mean±SD level of organochlorines (mg/kg) in water, sediment, and fish found in Biu dam reservoir in wet season

Pesticides	Samples			
	Water (mg/L)	Sediment (mg/Kg)	Tilapia(mg/Kg)	Catfish(mg/Kg)
Gamma BHC	ND	ND	ND	ND
beta-BHC	ND	ND	ND	ND
alfa BHC	ND	ND	2.389 ±0.001^{a}	0.026±0.000^{b}
Endosulfan I	0.033 ±0.004^{a}	ND	ND	0.032±0.002 a
Endosulfan II	ND	ND	ND	0.022±0.000
Heptachlor	ND	0.016 ±0.000	ND	ND
Aldrin	0.083 ±0.001^{a}	0.040 ±0.000 ^{a}	0.164 ±0.005^{a}	ND
Dieldrin	ND	ND	0.024 ±0.001	ND
Endrin	ND	0.010±0.001^{a}	ND	1.758±0.006^{b}
Lambda-cyhalothrin	ND	ND	0.347 ±0.005^{a}	0.030±0.015^{a}
Gamma-chlordane	ND	ND	ND	ND
p,p'-DDT	ND	ND	ND	ND
pp'-DDE	ND	ND	ND	ND
DDD	ND	ND	ND	ND
Methoxychlor	ND	ND	ND	ND

Note: All values represent Mean ±SD (Standard Deviation), Comparison was done across the period and values with different Superscripts are statistically different ($p < 0.05$), ND: Not Detected (at the limit of Detection 1x10-5 mg/kg)

The high level of endrin in catfish reported in this study shown in Tables 1 and 2, may be due to agricultural runoff which may be attributed to an unethical used of this pesticides spray on cotton to control cotton pest, since the used of these pesticides are banned in Nigeria. The catfish tend to live in the turbid and cloudy water, and furthermore it is exposed to different types of environmental contaminations rather than another fish types. Endrin in this study is higher than values reported in fish by Ogunfowokan et al. [36]. The level of endrin in sediment reported in this study is higher than those reported by Williams [40].

Aldrin was detected in all the samples analysed except in catfish while dieldrin was detected only in Tilapia. The presence of these pesticides agrees with Lanfranchi et al. [1] which reported that fish are suitable indicators for environmental pollution monitoring because they concentrate pollutants in their tissues directly from water and also through their diets. This study has clearly proved that this pesticides is still been used illegally in the study area since aldrin is used as a soil insecticide to control root worms, beetles, and termites which are common pest in in the study area over the years. Farmers in the Biu local government over the years were having problem of termite destroying their crops and affecting germination of cotton seed in the area. Dieldrin has been used in agriculture for the treatment of soil and seed and in public health to control disease vectors such as mosquitoes and tsetse flies [41]. The level of aldrin and dieldrin in this study is higher than those of fish reported in Allau dam reservoir by Akan, et al. [42]. The concentrations of aldrin and dieldrin in Tilapia fish sample were much higher than the WHO and FAO [43], set maximum residue limit (MRL) of 0.2 µg/kg and the Acceptable Daily Intake values (ADIs) of 0.0001µg/kg.

The commercial insecticide HCH sometimes referred to as BHC is a mixture of different isomers mainly α, β and γ-HCH (Lindane). Other isomers in the group are delta and epsilon. Lindane (γ -HCH) has been used as an insecticide and is the most toxic. β -HCH is the most symmetric and stable isomer; it is also the most persistent in nature. β -HCH is eliminated five times more slowly from the body than other isomers and has a much higher ability to accumulate in the fat tissue than lindane [44]. This may be the reason why some of this isomers were not detected in all the samples analysed except alpha BHC in Tilapia and Catfish since water and sediment have no fat tissue for the pesticides to bio-accumulates. The remaining two isomers β and γ -BHC were not detected in the study. The non detection of some of these pesticides shows that they are not in use in the surroundings of the dam reservoir which implies that the banned of these pesticides usage is being enforced in Nigeria. Lindane is also rapidly degraded in the environment [44], this explains the reason why lindane in all the samples analysis were not detected. Lindane degradation may be as a result of high temperature in the study area since temperature increase the rate of formation or disappearance of a substance. Non detection may also be due to continues monitoring of these banned and expired pesticide by regulatory agencies in Nigeria. Generally, the result of this study agrees with the study carried out by Idowu et al. [39], which reported the organochlorine pesticide residue levels in river water and sediment from cocoa-producing areas of Ondo State.

4. CONCLUSION

This study has shown that water, sediment and fish samples from Biu dam reservoir are contaminated with some organochlorine pesticides residue in varied degrees in both dry and wet seasons. Higher levels of OCPs were recorded in fish, however, higher levels of OCPs were found in the fish samples in the dry season than during the rainy season may be possible due bioaccumulation tendency of OCPs in the fish species during the dry season. Generally, the seasonal levels of OCPs in the fish samples were higher in most cases, than the recommended WHO Maximum Residue Levels (MRLs) in food items and should give cause for concern. The occurrence of the OPCs in fish sediments and water from Biu dam reservoir is consistent with the agricultural activities of the study area due to pesticide usage by the farmers. Biu dam is slightly contaminated with organochlorines pesticides, there is therefore serious need for the monitoring of these pesticide residues in water, sediments, fish and the environment, as this will go a long way towards preventing various environmental and public health hazards.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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