Hazard Quotient and Bioaccumulation Assessment of 26 Organochlorine Pesticides in Water, Sediment and Fish (*Tilapia zilli*) Warri River, Niger Delta Region - Southern Nigeria

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Authors’ contributions

This work was carried out in collaboration among all authors. Author OE conceptualized the study in collaboration with authors JU and FEO. Author OE designed the study, wrote the protocol, carried out the literature review and wrote the first draft of the manuscript. Authors OE, JU and TG were responsible for the statistical analysis and converting the raw data to graphics. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJACR/2020/v6i130149
Editor(s):
(1) Dr. Olalekan David Adeniyi, Federal University of Technology, Nigeria.
Reviewers:
(1) Velber Xavier Nascimento, Centro Universitário Cesmac, Brazil.
(2) Jardel Nimet, Maringá State University, Brazil.
(3) Miguel Velazquez-Manzanares, Universidad Autónoma de Coahuila, Mexico.
Complete Peer review History: http://www.sdiarticle4.com/review-history/57603

Original Research Article

Received 05 April 2020
Accepted 10 June 2020
Published 18 June 2020

ABSTRACT

A pesticide is any substance or mixture of substances used to destroy, suppress or alter the life cycle of any pest. Organochlorine pesticides (OCPs) have been known to resist biodegradation, persist in the environment and thus can concentrate through food chains and produce a significant magnification that can pose severe toxic risk to human health. Bioaccumulation and concentration

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levels of 26 Organochlorine Pesticides were assessed in water, sediment and fish samples from the Warri River, Nigeria. Samples were collected across the two predominant seasons - rainy and the dry season. The results obtained reveal the presence of 22 of the investigated 26 OCPs in the dry season whereas 19 were detected in the rainy season. Results obtained revealed p,p'-DDT, γ-HCH, and aldrin as the most predominant pesticides detected in the water samples with concentrations of 0.588; 0.160 and 0.092 μg/L, respectively. γ-HCH, p'‐DDD and aldrin (29.00; 23.00 and 22.00 ng/g, respectively) for the sediment samples. The most predominant contaminants in the fish samples were HCB, o,p'-DDT, γ-HCH (60.00; 29.00 and 27.00 ng/g, respectively). The Hazard Quotient (HQ) analysis was carried out on the fish samples collected revealed factors of 1.34 to 3.17 indicating a moderate hazard while the Bioaccumulation Index (BI) was found to be below 1 indicating their suitability for consumption. However, the risk of toxicity and bioaccumulation arising from long-term consumption of the OCP-contaminated fish may still exist.

Keywords: Hazard quotient; water; fish; organochlorine pesticides; sediment.

1. INTRODUCTION

People interact with the ecosystem they live in and that supports their life. Therefore, any alteration or contamination of it, through pesticides for example, may in turn have an effect on the people. A pesticide is any substance or mixture of substances used to destroy, suppress or alter the life cycle of any pest. They can occur naturally in plants and microorganisms or be synthesized [1]. A subgroup of the pesticides are the OCPs, which consist of a wide range of chemicals that contain carbon, chlorine and several other elements. Their environmental effects and harmful nature together with the adverse health effects that they cause coupled with their stability and bioaccumulation properties led to the ban of these chemicals [1]. Organochlorines can be generated by industry on purpose or be formed unintentionally through chlorine combustion processes [1-7]. OCPs are very resistant to natural breakdown through physical or biological processes and are therefore extremely persistent [1-7]. The increasing application of pesticides in agriculture, industry and for domestic purposes has caused the pollution of air, soil and surface water [8-18]. The increase in industrial food production and the need for pest control has made pesticides application in agriculture inevitable [1-10,19-21]. OCPs such as hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethane (DDTs) have been of great global concern because of their environmental persistence and toxicity to non-target organisms [1-7]. OCPs are continuously being detected in water, air and soil, [8-10,19-21] although their use has been prohibited for years. Due to their biodegradation resistance, low polarity, low aqueous solubility and high lipophilicity they can be accumulated along the food chain. For example, the lipophilic DDT with a low solubility of 1.2μg/l in water can be concentrated to harmful levels in the aquatic environment through bioaccumulation, biomagnification and biogeochemical processes [7,22,23]. A significant magnification of the original concentration at the end of the food chain can occur [2,10,13-14]. This has been found to have a negative impact on the environment as well as human health due to direct exposure or consumption of contaminated food and drinking water [8,13,15-18,24-26].

Studies revealed that OCPs have a strong potential to cross placental barriers even in minute concentration and cause serious neonatal damage [3,27]. DDT in particular can block the potassium flux across the membranes of nerve fibres and cause further negative effects [3]. Moreover, DDT influences the mixed function oxidase system thereby altering the metabolism of xenobiotics and steroid hormones [3,11].

Most water bodies in Nigeria serve as disposal site for waste for about 2000 medium and large scale industries located in the metropolis [28]. This situation has become more and more worrisome as a result of increased urbanization and industrialization and the lack of enforcement of environmental regulations in developing countries [5,19,29]. OCPs are among the first set of pesticides used and still in use in Nigeria despite their ban in developed countries due to the associated problems of bioaccumulation and environmental persistence, and potency [3,22,29]. The chemical stability, high lipid solubility and toxicity to humans and animals have led governments and researchers to be concerned about their presence in the environment.
Although most of the pesticides used in Africa are imported, there are a few production facilities in some countries for OCPs, e.g. Nigeria, Senegal, South Africa, Côte d’Ivoire and Egypt [3]. It is estimated that about 25,000 tons of OCPs are in use in the region. The lack of scientific and ecotoxicological data on chemical pollutants, effective control of them and prevention of aquatic pollution has been recognized in Africa [30].

OCPs residues reach the aquatic environment through direct usage in agricultural lands, run-off, leaching, careless disposal of empty containers and washing of equipment. Nigeria has a strong and growing agricultural sector, which requires increased and wide use of pesticides. In Nigeria OCPs find applications for agricultural purposes and especially for the control of mosquitoes [31-34] with over 100 different brands of pesticides being imported into the country annually [35]. Meaningful development of management policies and regulatory framework for the protection of the aquatic environment in Nigeria can only be achieved on the availability of reliable and adequate scientific data generated in the region. Therefore, the unavailability of data has necessitated this research. Thus, this report will enhance the availability of OCP levels for Warri River in water and sediment as well as in fish where bio-accumulation occurs.

2. MATERIALS AND METHODS

2.1 Chemicals

Analytical grade pesticide standards (>97% purity) were obtained from EGT, Fluka, LGC, Promochem and Restek. PCB Nr. 209 and Tetramethylxylene (TMX) used as surrogates were obtained from Ehrenstorfer, Germany. Sodium sulphate from Fluka was used. Cyclohexane and acetone were obtained from Schalau, Spain. The SPE cartridges Isolute 1g FL 712-0100-C and Resprep 500 mg carboprep 90 were obtained from Biotage, Sweden and Restek, USA, respectively.

2.2 Extraction Methods

Extraction was done using a 1:1 mixture of cyclohexane and acetone with a recovery of 78% ± 8%. The water samples were spiked with 2.5 μL 1-Br-Dodecane as internal standard and 50 μL PCB Nr. 209 + 50 μL TMX were added as surrogates. Liquid/Liquid extraction (LLE) was carried out for the water samples, while a Liquid/Solid Extraction (LSE) was carried out for the sediment and fish samples. Clean up was done using a SPE cartridges (Florisil and Carboprep cartridges).

2.3 Study Area

The study area at Warri River is located on latitude 050 31’ 01.94” and longitude 0050 47’ 36.29” (sample station 1; rainy season) and latitude 050 31’ 12.13” and longitude 0050 46’ 35.41” (sample station 2; dry season) in Delta State- Niger Delta region of Nigeria which is the second largest delta in the world and the largest mangrove swamp in Africa [36]. It spans over 20,000 km². The Niger Delta region is located at latitudes 5°31’N and 5°33’N and longitudes 5°30’E and 5°32’E. It covers an area of 70,000 km² of marshland, creeks and tributaries that drains the River Niger into the gulf of Guinea in the Atlantic Ocean. The coastal region cuts across ten (10) states in southern Nigeria. This region has an estimated population of over 35 million people, with fishing and farming as the main source of livelihood and sustenance. Economic activities include oil and gas exploration and exploitation, fishing industries, shipping, agriculture and tourism.

2.4 Sampling and Parameter Measurements

Sampling was carried out from October 2012 – September 2013. Samples were collected in duplicate (average value for the duplicate samples was taken as the final analytical value) for water, sediment, and fish during the sampling period for both seasons. Two sample for each matrix, making six (6) samples for separate seasons. Therefore giving a total of twelve (12) samples for the two seasons. Water, sediment and fish (T. zilli) samples were collected from Warri River during the predominant dry and rainy seasons. Grab water sampling technique was used to collect water at approximately 1 meter below the surface using a 1.0 L amber bottle. The pH of the water samples was measured in-situ using a portable pH-meter (Volcraft PH-410). Sediment samples were also collected using the soil auger sampling method. All samples were refrigerated at <4°C during the period of storage before analysis. Fresh Fish samples were captured by local fishermen at site and delivered at their arrival at shore to us (300g-910g/sample), we then wrapped with aluminum foil and kept frozen in ice during transportation and in the laboratory before analysis.
2.5 Analysis of Sediment Samples

Collected sediment samples were air dried and then 50 to 100 g of sample were measured with 20 ± 0.5 g sodium sulphate added. The samples were spiked with PCB Nr. 209 and TMX, subsequently, 200 mL of a 50% cyclohexane-acetone solution was then added. The samples were sonicated for about 20 minutes and then transferred to a mechanical shaker for about one hour. Aliquots of the extract were taken for clean up before analysis [37-40].

2.6 Analysis of Water Samples

1 L water sample was measured into an Erlenmeyer flask and 1 mL internal standard (ISTD) spike solution (2.5 µL 1-Br-Dodecane in 250 mL Cyclohexane), 50 µL PCB Nr. 209 and 50 µL TMX was added. This was then extracted with 5 mL cyclohexane, the organic layer was removed and the process repeated. The solvent extract was then transferred into a 20 mL vial and concentrated under a gentle stream of nitrogen gas (99%) to 1 mL [37,39,41,42].

2.7 Analysis of Fish Samples

Fish samples (Redbelly Tilapia (Tilapia zilli) GC) were collected during the sampling period. The gills, the stomach content and tissue in approximately equal ratio was removed from the fish and then homogenized together, with the aid of a Cryo mill grinder (Retsch Mill ZM200). About 20 g - 40 g of the samples was then weighed, to it Sodium sulphate (Fluka) 10 g – 20 g was added before being homogenized and then placed in an ultrasonic bath to sonicate for 20 minutes after the addition of PCB Nr. 209 and TMX as (surrogates). The sample was subsequently transferred to a shaking machine for two hours [38,43,44].

2.8 Sample Clean Up

Sample clean-up was carried out on the water sample extract using silica based Solid Phase Extraction (SPE) cartridges using a method previously reported by Maier [41]. The cartridges were pre-conditioned using ~10mL of a solution containing 1% acetone in cyclohexane under very low vacuum (~ 0.51 psi). 1 mL extract was
introduced directly onto the cartridge and eluted with ~50 mL of pre-conditioning solution. The collected eluent was then concentrated with the aid of a rotary evaporator (Buchi Syncore, SWISS) to ~3 mL and then concentrated under nitrogen gas stream to 1 mL.

Clean up on the fish sample extracts was done after the bulk lipid removal was performed using a centrifuge followed by the SPE cartridges methods as reported above.

2.9 Analytical Determination

Separation of the different analytes was carried out using a GC ECD (Agilent UK) coupled with two separate capillary columns, A and B. Column A: RTX-CL Pesticides 1. Column B: RTX-CL Pesticides 2, both columns were subjected to the same temperature programming and constant pressure flow. 2 µL extract was injected in split-splitless mode into the GC inlet with temperature of 200°C and a constant flow of 2.7 mL/min. A gradient oven temperature programme was used: 120°C, held for 1 min, then ramped to 320°C at a rate of 9°C/min., and held for 3 min. Please see Fig. 2 for an example of GC-ECD produced high sensitivity chromatogram. A quadratic seven point calibration using internal standard was prepared to determine the concentration of OCPs in the different samples. Laboratory and method blanks were analyzed simultaneously to ensure good QA/QC and to eliminate any background contamination [45].

2.10 Hazard Quotient

A hazard quotient is the ratio of the potential exposure to a substance and the level at which no adverse effects are expected. Hazard quotient is thus calculated with the formula: [46]

\[
HQ = \frac{\text{Dose Rate}}{\text{Reference Dose}}
\]

Guidelines for interpreting HQ calculations are: [47]

HQ < 0.1 – no hazard exists
HQ = 0.1 – 1.0 – hazard is low
HQ = 1.1 – 10, hazard is moderate
HQ > 10, hazard is high

Fig. 2. QC-Standard chromatogram column A, with all 26 Organochlorines completely separated during integration and interpretation PA/IA (PA=peak area. IA=ISTD area)
2.11 Data Analysis and Statistics

Quantification was done using STAR Varian/Agilent Workstation software solution and was analyzed separately for each sample using descriptive statistics (means, range and histogram/charts). Statistical analysis was carried out using Excel and SPSS Version 19.0.

The description of the data was done in the form of mean (±) SD for quantitative data and frequency and proportion for qualitative data representation. On the environmental significance of the apparent elevated levels of OCPs in the Niger Delta region, evaluation was made in terms of bioaccumulation index and hazard quotient.

3. RESULTS AND DISCUSSION

The concentrations of the investigated 26 OCPs in water, sediment and fish in the Warri River during the rainy and the dry season are presented in (Fig. 3). Overall, 22 OCPs were detected. In the water samples p,p'-DDT (588 ng/L-1), γ-HCH (177 ng/L-1) and aldrin (123 ng/L-1) were most predominant OCPs, whereas γ-HCH (29.00 ng/g), p,p'-DDD (23.00 ng/g) and aldrin (22.00 ng/g) were mostly found in the sediment samples.

In the fish samples, HCB (60.00 ng/g), p,p'-DDT (29.00 ng/g) and γ-HCH (27.00 ng/g) were the predominant pesticides detected (Fig. 3). Of the 26 OCPs investigated, 19 OCPs were found to be present in the samples collected during the rainy season and 22 present the in dry season samples (Fig. 3). The phenomenon of fewer OCPs detected during the rainy season may be explained by the constant renewal of the water body by constant flow of the river and refreshing by rain fall. OCPs like γ-HCH, aldrin and 2,4-DDT were found to occur in all matrices and at considerable levels. A study conducted along the Niger River reported ΣOCPs levels higher during the dry season compared to the rainy season. This may be because the resident time of the sediment transported was higher during the dry season compared to the rainy season, which is characterized by storms, high current, and bottom scour. [48] Several other studies have reported similar findings, in Kilimanjaro, Tanzania, [15] in Kenya, [49] and in the Middle East and Eastern Europe [50].

The water samples collected during the rainy season shows the concentration of the most predominant analytes as: p,p'DDT (588 ng/L-1) > γ-HCH (160 ng/L-1) > aldrin (92 ng/L-1) > t-chlordane (14 ng/L-1) > p,p'DDD (10 ng/L-1), whereas for sediment samples, the order is γ-HCH (30.00 ng/g) > p,p'DDD (22.00 ng/g) > isodrin (17.50 ng/g) > HCB (10.00 ng/g) > p,p'-DDT (9.00 ng/g) and for the analysed fish γ-HCH (28.00 ng/L) > HCB and aldrin (22.00 ng/g) > o,p'-DDE and o,p'-DDT (18.00 ng/g). The sum of OCPs for the rainy season is Fish > Sediment > Water (Fig. 3).

The water samples collected during the dry season shows the most predominant analytes concentration in the following order: o,p'DDT (213 ng/L-1) > γ-HCH (177 ng/L-1) > aldrin (123 ng/L-1) > p,p'DDT (70 ng/L-1) > HCB and γ-heptachlor (60 ng/L-1) for water, whereas for sediment samples the order is γ-HCH (29.00 ng/g) > aldrin (22.00 ng/g) > isodrin (14.00 ng/g) > endrin (10.00 ng/g) > HCB (9.80 ng/g) and for the analysed fish HCB (60.00 ng/g) > p,p'DDT (29.00 ng/g) > o,p'DDT and δ-HCH (20.00 ng/g) > γ-HCH (19.80 ng/g) > o,p'DDE (12.00 ng/g). The sum of OCPs for the dry season is Fish > Sediment > Water (Fig. 3).

The comparison of the water samples for both seasons reveal that the OCP-contamination of ΣOCP 976 ng/L for the rainy season samples is slightly higher than the dry season samples with ΣOCP 855 ng/L. Similar trends were observed by Hellar-Kihampa [15], El Bouraie et al. [51], Hewitt and Harrison [52], Leadprathom et al. [53], Carabias et al. [54] and this observed trend was attributed to the fresh runoffs from agricultural farmlands. However, the reverse was the case for the sediment and fish samples. For the dry season, values of 114 ng/g dw and 183 ng/g were obtained for sediment and fish samples, respectively were higher than the rainy season values of 107 ng/g dw and 134 ng/g for fish and sediment samples, respectively. The concentrations of OCPs obtained in the sediment samples during the rainy season could be attributed to dilution, dispersion and transportation of the pollutants in the aquatic environment that is controlled by advection, mixing and diffusion [15, 52,55,56]. While the higher levels OCPs in fish samples during the dry season (Fig. 3). Generally could be because the fishes live in the water and feed on particulates and coarse vegetable matters that had accumulated the OCPs overtime [15,57 –59].
Fig. 3. OCP-Distribution in three matrices for two seasons with an assumed error of 25%
3.1 DDT and Its Metabolites – Assessment of Ratios

Summation of HCH and DDT were determined of the water, sediment and fish samples for both sampling seasons. For the water samples ΣHCH concentrations were found to be lower than the concentration reported in the Yellow River (1.10 µg/L) [15], Ebro River (3.38 µg/L) [16] and Illushi River (4.89 µg/L) [17]. Total DDT concentrations in the sediment samples from the study area ranged from below detectable limit to 29.00 ng/g dw, with an average of 1.32 ng/g dw.

Technical DDT is typically composed of 77.1% p, p’-DDT, 14.9% o, p’-DDT, 4% p, p’-DDE and some other trace impurities [4,59,60]. However, in this study, p, p’-DDT (60.00 ng/g) was the predominant metabolite obtained in the water samples, while γ-HCH the predominant metabolites obtained in the sediment (29.00 ng/g) and fish (29.00 ng/g) samples during the rainy season (Fig. 3). For the dry season, o,p’-DDT (22.00 ng/g) was the predominant analyte for the water sample, while γ-HCH (28.00 ng/g) was the OCP with the most occurrence in the sediment sample and fish sample had HCB (60.00 ng/g) as the predominant analyte. Generally, the o, p’-DDT/p, p’-DDT ratio ranged from 0.2 to 0.3 in technical DDTs and from 1.3 to 9.3 or higher in dicofol [61]. Therefore, sources of DDTs (from technical DDT or dicofol impurities or both) can be identified according to the ratio of o, p’-DDT/p, p’-DDT) [62]. In this study, the ratios of o, p’-DDT/p, p’-DDT in water, sediment and fish samples for the rainy season were 0.02, 0.22, and 9.00, respectively and 3.04, 1.50 and 0.69 for the water, sediment and fish samples for the dry season, respectively (Table 1), indicating that dicofol is the major source of DDT concentration reported [61].

DDT degrades to DDE and DDD under aerobic and anaerobic conditions, respectively [32]. In general, a ratio of (DDE + DDD)/DDT of more than 0.5 indicates long-term biotransformation of DDT to DDE and DDD, while a ratio of less than 0.5 may indicate recent input of DDT [63,64]. The ratios of (DDE + DDD)/DDT found in the Warri River for the rainy season was found to be 0.07, 2.82 and 1.25 for water, sediment and fish samples, respectively. For the dry season 0.18, 2.60 and 0.41 was found for water, sediment and fish samples, respectively (Table 1). The finding that all values except water samples for rainy season were higher than 0.5 indicates that the DDT compounds in Warri River may be mainly derived from DDT-treated aged and weathered agricultural sources as similarly reported by Li et al [63]. Moreover, a higher ratio of p, p-DDE/p, p-DDT can indicate the long range atmospheric transport of DDT compounds because p, p-DDE is transported more efficiently than p, p-DDT [63, 65]. This ratio can also be used to determine if the DDT emission occurred recently or in history [66 – 68].

The ratios of p, p-DDE/p, p-DDT varied from 0.03 to 2.00 for rainy season and 0.17 to 3.50 for dry season (Table 1) in the multiple environments of the river studied, indicating that the DDTs in this area may also come from atmospheric deposition [69]. Thus, the DDT-concentration found in the Warri River is as a result of the dicofol use for agricultural activities as well as atmospheric deposition. Furthermore, a p,p-DDE/DDT > 1 indicates old inputs of DDT whereas values < 1 indicate fresh input [70]. The relatively high detection ratios of DDT indicate that a small amount of DDT input may still occur in this area despite the ban (Fig. 3).

3.2 Hazard Quotient Assessment

The Hazard Quotient (HQ) approach compares environmental concentrations of a contaminant with the measured effect or NO–effect level in test organisms [71].

Hazard Quotient assessment was carried out to ascertain the hazard level for the fish samples for both rainy and dry season. The results in (Table 2) show that the contaminated fish fall within moderate hazard. Evidently, this gives rise to health concerns for the inhabitants near the sampling region.

3.3 Bioaccumulation Assessment

The Bioaccumulation Index (BI) determined for the fish samples for both seasons in the sampled site indicates that the fish are suitable for consumption as the BI values were found to be less than 1. However, these BI values need to be closely monitored to avoid future health issues caused by the consumption of Tilapia zilli by the inhabitants around Warri river.
Table 1. Ratio of DDT and its metabolites in the different samples

<table>
<thead>
<tr>
<th></th>
<th>Rainy season</th>
<th>Dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water (µg/L)</td>
<td>Sediment (ng/g dw)</td>
</tr>
<tr>
<td>o, p – DDT</td>
<td>0.014</td>
<td>2.00</td>
</tr>
<tr>
<td>p, p’ – DDT</td>
<td>0.588</td>
<td>9.00</td>
</tr>
<tr>
<td>Ratio o, p’-DDT/p, p’-DDT</td>
<td>0.02</td>
<td>0.22</td>
</tr>
<tr>
<td>DDE + DDT</td>
<td>0.039</td>
<td>31.00</td>
</tr>
<tr>
<td>DDT</td>
<td>0.602</td>
<td>11.00</td>
</tr>
<tr>
<td>Ratio DDE + DDD/ DDT</td>
<td>0.07</td>
<td>2.82</td>
</tr>
<tr>
<td>p,p’-DDE</td>
<td>0.016</td>
<td>6.00</td>
</tr>
<tr>
<td>p,p’-DDT</td>
<td>0.588</td>
<td>9.00</td>
</tr>
<tr>
<td>Ratio p,p’-DDE/ p,p’-DDT</td>
<td>0.03</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Table 2. Hazard quotient for fish samples

<table>
<thead>
<tr>
<th>∑OCPs concentration (mg/kg)</th>
<th>HQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainy</td>
<td>1.34</td>
</tr>
<tr>
<td>Toxicty</td>
<td>0.134</td>
</tr>
<tr>
<td>Reference level</td>
<td>0.1</td>
</tr>
<tr>
<td>Dry</td>
<td>1.83</td>
</tr>
<tr>
<td>Toxicty</td>
<td>0.183</td>
</tr>
<tr>
<td>Reference level</td>
<td>0.1</td>
</tr>
<tr>
<td>Total</td>
<td>3.17</td>
</tr>
</tbody>
</table>

Table 3. Bioaccumulation index for fish samples

<table>
<thead>
<tr>
<th>∑OCPs concentration</th>
<th>Fish (mg/kg)</th>
<th>Water (µg/L)</th>
<th>BI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainy season</td>
<td>0.134</td>
<td>0.976</td>
<td>0.137</td>
</tr>
<tr>
<td>Dry season</td>
<td>0.183</td>
<td>0.855</td>
<td>0.214</td>
</tr>
<tr>
<td>Total</td>
<td>0.317</td>
<td>1.831</td>
<td>0.173</td>
</tr>
</tbody>
</table>

4. CONCLUSION

This study clearly revealed the presence of twenty-two OCPs at varying concentration in water, sediment and fish collected from Warri River. The fact that these OCPs were detected is consistent with usage of pesticides in agricultural and domestic activities within the study area. The toxicity and bioaccumulation properties of the OCPs may lead to future health issues at the top of the food chain. The study reveals that long-term consumption of such fish may be hazardous to health causing respiratory and reproductive issues as well as cancer. These results further stress the need for serious monitoring strategies to check the purchase, distribution, usage and disposal of OCPs in the environment. This may help to prevent various ecological, environmental and public health hazards as most of the population in the study area depends on the rivers as a source of their livelihood.

ACKNOWLEDGEMENTS

The authors would like to appreciate and thank all the Staff and management team at Bachema Analytical Laboratories Rutistrasse 22, Schlieren-Zurich, Switzerland for creating an enabling environment for the analysis of the samples presented in this publication.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/57603