Organic Pesticides Concentration in Water, Sediments and Fish Tissues obtained from Ogbakiri River in the Niger-Delta Region, Nigeria and Its Health Implication on Human Consumption

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Abstract Organic pesticides concentrations in tissues of *Chrysichthys nigrodigitatus* (catfish) and *Liza falcipinis* (mullet) sampled from Ogbakiri River, Niger Delta, Nigeria were investigated to find out the extent of pesticide contamination in the river and its possible health implication on human consumption assessed. Pesticides were analyzed using gas chromatography coupled with electron capture detector (GC-ECD). The concentration of pesticides ranged from 0.0010 ± 0.0001 to 0.0095 ± 0.0002 µg/l and 0.0007 ± 0.0001 to 0.0110 ± 0.0002µg/g in water and sediments, respectively. Similarly, pesticides concentration in fish tissues ranged from 0.0002 ± 0.0001 to 0.0185 ± 0.0002µg/g and 0.0003 ± 0.0001 to 0.0098 ± 0.0002µg/g in liver and muscle respectively. Moreso, to assess health risk concerns, the measure concentrations of pesticide residues were compared with regulatory benchmarks. Risk hazard estimates of dichlorodiphenyltrichloroethanes, Chlordanes, hexachlorobenzene in the studied fishes showed no potential adverse effect to human consumption at the observed concentrations. The cancer risk was also evaluated according to the guideline as stipulated by USEPA and was within acceptable risk range of 10^{-4}. Thus exposed populations are not at cancer risk through consumption of fish from this river.

Keywords Organic Pesticides, GC-ECD, Risk Hazard Estimates, Cancer Risk

1. Introduction

In a bid to increase food production and attain food sufficiency, several pesticides have been used for agricultural purposes and thus led to the increase of these chemicals in our environment. Several other human activities such as forestry, urban development, mining and improper waste disposal occurring near aquatic bodies could lead to alterations in the water quality and also disruption in fish [1]. Pesticides such as dichlorodiphenyltrichloroethane and its metabolic products (DDTs), hexachlorobenzene (HCB) and several isomers of hexachlorocyclohexane (HCHs) are known global contaminants of the environment for years. Pesticide may be classified into rodenticides, insecticides, fungicides, herbicides and fumigants [2].Although, these pesticides may exhibit selective toxicity to these life forms, and they may be toxic to humans if pesticides-contaminated food is ingested. Several researches have established that pesticides are toxic to man [3]. Notable among the symptoms of pesticides poisoning include tremor, irritation, dizziness and chronic convulsion [4]. DDT in particular has been reported to block the influx of potassium across the membranes of nerve cells and leads to increased negative after potentials. DDT is also known for its ability to induce the mixed function oxidase system thereby interfering with the metabolism of xenobiotics and steroid hormones [3]. Organochlorine pesticides are among the pioneer class of pesticides in use and still in use in Nigeria despite them being banned in developed countries as a result of their associated health problems. The stability of these chemicals, lipophilicity and its adverse health effect on both humans and animals [5,6], has led global health policy regulators and researchers to be alarmed over their presence in the environment. Farmers, fishermen and other users have misused these pesticides in agricultural and fishing purposes. These pesticides are now found everywhere in the Nigerian environment and are being utilized in activities such as crop protection, weeds management, rodents control, animal husbandry and public health applications [7]. Chlorinated organic pesticides

exhibit high stability in all aquatic body systems and are highly resistant to photo degradation. They may leave the water body via secondary mechanisms such as, adsorption on sediments, biological breakdown by micro-organisms and bioaccumulation by fish through gills, skin and feeding. They are inadequately hydrolyzed and therefore biodegrades slowly in the environment [8]. Consequently these compounds persist in food chains and are easily bioaccumulated in animal tissues. Pesticide residues present severe problems in fish tissues, as seen by the elevated pesticides levels in the water and sediments [9]. Gills of fish come directly in contact with water and therefore reflect pesticide concentration in water where the fish live, whereas the concentrations of pesticides found in the liver reflects storage of pesticides in water [10]. Sediments acts as the ultimate sinks for several contaminants like pesticides and also play an important part in the remobilization of these contaminants in aquatic body systems. Fish samples are one of the most important indicators of pollution in aquatic systems and are therefore useful for the evaluation of pesticides pollution level [11]. Their use as bio-monitors is important in the assessment of bioaccumulation of contaminants within the aquatic ecosystem [10]. Several hazardous chemical elements, when released into the environment, can accumulate in the sediments of water bodies. Aquatic organisms lower in the food chain absorb and transfer these chemicals through the food chain to organisms in higher trophic levels, including fish. Under acidic conditions, free ions of some metals may be absorbed by the gills of fish directly from the water [10]. Consequently, concentrations of pesticides in the tissues of fish are determined basically by the level of pollution of the water and food under certain environmental conditions, chemicals accumulated in the silt and sediments of aquatic bodies can remobilize back into the water.

The aim of this study was to determine the distribution of selected pesticides in water, sediment and biota of the Ogbakiri River. Very few studies in Nigeria have assessed the risk of pesticide occurrence to non-target organisms and humans. Therefore this study assesses pesticide levels in fish tissues and the health risk implications to humans as a result of the eventual consumption of pesticide contaminated fishes.

2. Materials and Methods

Site Description and Sampling

Ogbakiri is a community in Emuoha Local Government Area of Rivers State, Nigeria and is known for its fishing activity with the coordinates latitude 4.794007 and longitude 6.913886. The major landing beach for the fishermen is at the Egbelu water side. This community is known for agricultural activities where farmers constantly make use of agro chemicals to enhance their crop production. Majority of the inhabitants of this community are farmers, traders and fishermen. The Egbelu River otherwise known as the Calabar River connects Tombia and Ogbakiri. This water is a major means of transportation between these communities and a hub for fishing activities.

Water samples were obtained from surface parts of the river from three sites along the stretch of the river following US-EPA procedure. Samples were collected using a stainless steel bottle and transferred to cleaned aluminum jars, acidified with 0.1N hydrochloric acid, clearly labeled and stored in the dark at temperature between 0 and 4^oC. Sediment samples were obtained using a teflon coated spoon and immediately transferred into an aluminum foil and stored [12]. Three samples each of catfish and mullet were bought from local fishermen at the landing beach of the river and immediately stored in an ice-cooler at 4^oC, and transported to the laboratory for analysis.

Extraction of Pesticides in Water Samples

Extraction of pesticides was carried out using liquid-liquid extraction (LLE) as described in APHA [13], each surface water sample (800 ml) was filtered using Whatman filter paper (i.d. 70 mm) to get rid of the dirt and suspended materials and then transferred to a 2 liter separatory funnel. During the first LLE, the mixture of 100 ml n-hexane and dichloromethane (1:1 v/v) was added to the water samples and shaken vigorously for 2 min before phase separation. The water-phase was transferred from the separatory funnel into a 2000 ml beaker. The organic-phase through a 200 ml concentrator tube was cautiously poured into a glass funnel containing 20 g of anhydrous sodium sulfate. For the second and subsequent LLE, the water-phase was poured back into the separatory funnel to re-extract with 50 ml of the solvent. The extract was then concentrated to 2 ml volume using a rotary evaporator and then analyzed with Gas Chromatography coupled with Electron Capture Detector (GC-ECD) [14].

Extraction of Pesticides in fish and Sediment Samples

Fish tissues and sediment samples were oven dried at 70^oC for 72 hours and thereafter ground to powder using a blender to obtain a homogenous sample. Pesticide residues in sediments and fish samples were extracted using Soxhlet extractor apparatus [15]. Exactly 10 g of the homogenized sample was placed in a beaker containing 50 g anhydrous sodium sulfate and mixed thoroughly. The sample mixture was then transferred to an extraction thimble and placed in the Soxhlet extractor. The mixture was extracted with 150 ml of acetone: n-hexane (20:80 v/v) at 50°C for 4 hours. The extracts were filtered and concentrated to 1 ml using vacuum rotary evaporator. Extract clean up was carried out by dissolving the raw extract in 10 ml hexane and passed through pre-conditioned octadecyl C-18 columns at a rate

of 2 ml min G. The column was washed with 1 ml, 30% methanol followed by 1 ml ultrapure water and was allowed to dry. The extract sample which was trapped in the column was eluted for 5 times with 0.5 ml aliquots of hexane to recover the pesticide residues. Hexane in the sample was then allowed to evaporate off leaving the residue alone in the vial. Dried sample was dissolved in 1 ml portion of hexane, mixed thoroughly with a whirl mixer and then transferred to auto sampler vials ready for gas chromatography [16]. Statistical analyses were carried out by analysis of variance (ANOVA) using SPSS 20 software. Mean values were analyzed by the Duncan's test.

Risk Assessment

To evaluate the health risk associated with consumption of pesticide contaminated fish, the guidelines for potential risk drawn up by the USEPA were used. The oral reference dose (RfD) of each pollutant is the exposure level that is likely to be without an appreciable risk of deleterious adverse effects and was provided by the USEPA [17]. The estimated daily intake (EDI, with Eq. (1)), hazard quotients (HQ, with Eq. (2)) were calculated at both the 50th and 95th percentile to estimate the non-cancer risks. The cancer risk (Eq. (3)) was also calculated to estimate the likelihood that an individual will develop cancer in his lifetime as a result of exposure to these pesticides [18].

For this research, fish consumption rate was per day at 50th percentile was set at 4g/day and 31.9g/day at the 95th percentile. This range covers the FAO [19] per capita fish consumption rate (9kg) for Nigeria which was 24.7g/day, while body weight was set at 70kg.

$$ED\ I = \frac{Concentration\ of\ contaminant\ (mg/kg)\ \times\ Daily\ Intake\ Rate\ (g/day)}{Body\ weight\ (kg)} \qquad (1)$$

$$Hazard\ Quotient = \frac{Average\ Daily\ Intake\ (mg/kg/day)}{Oral\ Reference\ Dose\ (mg/kg/day)} \qquad (2)$$

$$Cancer\ Risk = \frac{Intake\ (mg/kg/day)}{cancer\ slope\ factor\ (mg/kg/day)} \qquad (3)$$

$$Intake = \frac{C\ \times\ IR\ \times\ EF\ \times\ ED\ \times\ CF}{BW\ \times\ AT} \qquad (4)$$

I = Intake – the level of chemical at exchange region (mg/kg/body weight/day)

C = Chemical concentration in contact over the period expose (mg/kg$_{fish}$)

IR = Ingestion Rate in g/day

EF = Exposure Frequency- describes how often exposure occurs (days/year).

ED = Exposure Duration - describes how long exposure occurs (year).

CF = Conversion Factor (kg/g).

AT = Averaging Time – period over which exposure is averaged (days).

3. Result

Table 1. Pesticides in liver and muscle of catfish and mullet from the study area

Pesticides (μg/g)	Catfish (Liver)	Catfish (Muscle)	Mullet (Liver)	Mullet (Muscle)	FDA/WHO
Glyphosate	0.0051±0.0001[a]	0.0047±0.0002[a]	BDL[ab]	BDL[ab]	0.25
γ-Chlordane	0.0013±0.0002[a]	0.0012±0.0001[a]	0.0038±0.0002[ab]	0.0026±0.0002[ab]	0.3
t-Nonaclor	0.0038±0.0001[a]	0.0036±0.0001[a]	0.0014±0.0001[ab]	0.0010±0.0002[ab]	0.3
α-HCH	0.0020±0.0001[a]	0.0018±0.0001[a]	0.0050±0.0001[ab]	0.0031±0.0001[ac]	0.3
γ-HCH	BDL[a]	BDL[a]	0.0002±0.0001[ab]	0.0003±0.0001[ab]	0.3
HCB	0.0067±0.0002[a]	0.0069±0.0000[a]	0.0076±0.0001[ab]	0.0060±0.0002[ab]	0.5
DDE	0.0010±0.0001[a]	0.0010±0.0001[a]	0.0006±0.0001[ab]	0.0006±0.0001[ab]	5.0
DDT	0.0095±0.0002[a]	0.0095±0.0002[a]	0.0185±0.0002[ab]	0.0098±0.0002[ac]	5.0
DDD	BDL[a]	BDL[a]	BDL[ab]	BDL[ab]	5.0
Total	**0.0294±0.0005[a]**	**0.0287±0.0001[a]**	**0.0372±0.0003[ab]**	**0.0234±0.0008[ac]**	

Values are expressed as mean± standard error of mean (SEM) of three replicates, (n=3). Values with different superscript in the same row are significantly different at the 0.05 level (p<0.05), while values with same superscript are not statistically significant. BDL implies below detection limit.

Table 2. Pesticides in sediment from the study area

Pesticides (µg/g)	Ogbakiri (Sediment)
Glyphosate	BDL[a]
γ-Chlordane	0.0044±0.0002[b]
t-Nonaclor	0.0026±0.0002[a]
α-HCH	0.0055±0.0002[a]
γ-HCH	0.0007±0.0001[b]
HCB	0.0076±0.0002[a]
DDE	0.0010±0.0000[a]
DDT	0.0110±0.0002[b]
DDD	BDL[a]
Total	**0.0328±0.0003[b]**

Values are expressed as mean± standard error of mean (SEM) of three replicates, (n=3). Values with different superscript in the same row are significantly different at the 0.05 level (p<0.05), while values with same superscript are not statistically significant. BDL implies below detection limit.

Table 3. Pesticides in water from the study area

Pesticides (µg/mL)	Ogbakiri (Water)	FDA (µg/mL)
Glyphosate	0.0065±0.0002[b]	0.7
γ-Chlordane	0.0012±0.0001[a]	0.002
t-Nonaclor	0.0020±0.0001[a]	-
α-HCH	0.0019±0.0001[a]	0.0002
γ-HCH	BDL[a]	0.0002
HCB	0.0069±0.0001[a]	0.002
DDE	0.0010±0.0001[a]	-
DDT	0.0095±0.0002[b]	0.00036
DDD	BDL[a]	-
Total	**0.0290±0.0003[b]**	

Values are expressed as mean± standard error of mean (SEM) of three replicates, (n=3). Values with different superscript in the same row are significantly different at the 0.05 level (p<0.05), while values with same superscript are not statistically significant. BDL implies below detection limit.

Table 4. Bioaccumulation factors of contaminants in catfish and mullet from the study area

Contaminants	Catfish (liver)	Catfish (muscle)	Mullet (liver)	Mullet (muscle)
∑DDTs	1.00	1.00	1.82	0.99
HCB	0.97	1.00	1.10	0.87
∑HCHs	1.05	0.95	2.73	1.79
∑Chlordanes	1.60	1.50	1.63	1.13
Glyphosate	0.78	0.72	0.00	0.00

∑DDTs = Summation of Dichlorodiphenyltrichloroethanes; **∑CHLs** = Summation of chlordanes; **∑HCH**= Summation of Hexachlorocyclohexane

Table 5. Estimated daily intake (µg/g) and hazard quotient values of contaminants in catfish and mullet

Contaminants	Oral RfD (mg/kg/day)	50th (95th) Estimated Daily Intake(µg/g/day) (10⁻⁴)		50th (95th) Hazard Quotient		
		Catfish	Mullet	Catfish	Mullet	ADI (mg/kg/day)
∑DDTs	0.0005	0.006(0.05)	0.006(0.05)	0.001(0.01)	0.001(0.01)	0.02
∑CHLs	0.00006	0.0007(0.005)	0.002(0.02)	0.001(0.008)	0.003(0.03)	0.0005
HCB	0.0008	0.004(0.03)	0.003(0.03)	0.0005(0.004)	0.0004(0.004)	0.0005
∑HCH	0.0003	0.001(0.008)	0.002(0.02)	0.0003(0.003)	0.0006(0.005)	0.003
Glyphosate	0.1	0.0027(0.021)	BDL	0.000003(0.00002)	BDL	0.3

Oral Reference Dose (RfD) was obtained from USEPA's Integrated Risk Information System [22]. Acceptable Daily Intake (ADI) values were obtained from FAO/WHO [23]. ∑DDTs = Summation of Diclorodiphenyltrichloroethanes; ∑CHLs = Summation of chlordanes; ∑HCH= Summation of Hexachlorocyclohexane

Table 6. Cancer risk estimates for contaminants in catfish and mullet from the study area

Contaminants	Cancer Slope (mg/kg/day)	50th(90th) percentile Cancer Risk Estimates	
		Catfish	Mullet
∑DDTs	0.34	$3.0 \times 10^{-7}(8.0 \times 10^{-6})$	$2.9 \times 10^{-7}(7.9 \times 10^{-6})$
∑CHLs	0.35	$2.0 \times 10^{-7}(5.2 \times 10^{-6})$	$5.9 \times 10^{-7}(1.6 \times 10^{-5})$
HCB	1.6	$4.2 \times 10^{-8}(1.1 \times 10^{-6})$	$3.7 \times 10^{-8}(9.8 \times 10^{-7})$

Cancer Slope Factors were obtained from USEPA's IRIS System [22].

4. Discussion

Ogbakiri River is surrounded by farm lands where a significant amount of agro-chemicals (fertilizers and pesticides) are used by farmers to increase crop yield. These chemicals can enter aquatic bodies through surface run-off water and garbage and wastewaters poured in the wetland by inhabitants. These and other factors may lead to the contamination of this river.

Results from the study have shown in Table 1 that mullet accumulated the highest total pesticide residues (0.0372±0.0003 μg/g) when compared to that found in catfish. And this highest value was observed in the liver tissue which may be attributed to the fact that the liver in the major detoxification organ for xenobiotics as corroborated by Hinton and Lauren [24]. DDT was the predominant pesticide found in the tissues of both aquatic biota and contributed significantly to the total pesticide concentration. According to Kaur *et al.,* [25], fishes obtained from the Punjab region in North India showed that DDT was the predominant pesticide while other organochlorine pesticides were found at lower levels. The result as obtained in this research agrees with their findings. Rios *et al.,*[26], reported a higher concentration of DDT (7.1 μg/g) in fish tissues than those observed in this present study. However, DDD was not detected in the tissues of all the biota sampled. Also, glyphosate and γ-HCH were not detected in mullet and catfish respectively. When the levels of these pesticides were compared to the recommended levels as stipulated by FDA/WHO, they were found to be within the acceptable limits for human consumption. The concentration of pesticides in fish tissues was generally higher as compared to the levels observed in the sediments and water. This finding agrees with the study carried out by Charles *et al.,* [27] who asserted that fish are mobile and so may have been exposed to compounds in other region of the hydrologic system and also that the rate of pesticide accumulation and bioaccumulation in fish increases with increase in temperature. Kidwell *et al.,* [28] also added that the high accumulation of these pesticides in fish was as a result of its lipid content.

Results in **Table 2 and 3** show the pesticide residue concentration in the sediment and water obtained from the study area. It was observed that the concentration of total pesticide residues in the sediment (0.0328±0.0003 μg/g) was higher than the concentration in the water (0.0290±0.0003 μg/ml). This difference in pesticide concentration between the sediment and water may be as a result of the sediment acting as the ultimate sink for contaminants and as such have the tendency of accumulating pesticides [29]. Moreso, the lower level of pesticides in water recorded in this research may be attributed to the hydrophobic nature of pesticides that make its accurate determination difficult and therefore make their presence in water to be at a lower level [30]. This author also stated that the adsorption of these chemicals to sediment and particulate matter is an essential mechanism for their removal from aquatic bodies, consequently the sediments will accumulate higher amounts of this pesticides. The levels of most of the pesticides in fish were higher than those found in water. Pesticide residues in the river are likely to emanate from nonpoint sources through surface runoff, atmospheric deposition and leaching as a result of agricultural applications and pest control activities [31]. The river sediments act as a sink for most contaminants, which upon resuspension during the river's mixing may increase pesticide bioavailability and accumulation in fish tissues [32]. Pesticide pollution to the river is therefore, likely to pose a danger to both aquatic organisms and humans. It was observed that pesticide concentrations in water was higher than the guideline value of 0.0001 μg/mL in fresh water bodies as stipulated by European Union [33], thus water from these communities are not suitable for human and domestic use [34].

The bioaccumulation factors of contaminants in Catfish and mullet obtained at the study area are as shown in **Table 4**. DDTs had values of 1.00 in muscle and liver of catfish while mullet recorded a value of 1.82 and 0.99 for liver and muscle respectively. The highest value for HCB and HCHs were recorded for the liver of mullet. Glyphosate had values below unity in all samples while chlordanes had the lowest and highest values of 1.13 and 1.63 in muscle and liver of mullet respectively. This result show that most of the bioaccumulation values were comparatively elevated (>1) indicating that contaminant levels in the fish tissues were from either the water or sediments from the study sites [12]. Therefore the presence of contaminant residues implies a clear evidence of their bioaccumulation from the aquatic environment. The result also showed that there was biological magnification of contaminants in fish parts analyzed [35].

The estimated daily intake (EDI) and the hazard quotients (HQs) from consumption of pesticide contaminated food are as presented in **Table 5**. This

analysis estimated human exposure to various detected pesticide residues through fish consumption by evaluating data on contaminant concentration in fish, which is generally used to assess human risk [36]. According to the Food and Agriculture Organization (FAO) of the United Nations, Nigeria's per capita consumption of fish is estimated at 9 kg/year [19] which is equivalent to 24.7 g/day. Even though this might not be the exact figure for fish obtained from the Ogbakiri River to the Nigerian fish consumer, it is assumed to be the case in this current study. The concentration levels of contaminants in the present study were compared alongside existing international standards. The EDI was compared with the ADI as stipulated by the FAO/WHO Joint Meeting on Pesticide Residue [23]. To effectively assess the risk of exposure, the 50th and 95th percentile EDIs of contaminants for each species of fish were calculated. DDTs and HCHs at the 50th percentile and glyphosate at both the 50th and 95th percentile had their individual EDI values below the ADI while at the 95th percentile, DDTs, Chlordanes, HCB and HCH had their EDI values above the recommended ADI values. The EDI values for all the contaminants in mullet except glyphosate at the 95th percentile were higher than the recommended ADI value. There exist certain strategies that can be adopted to minimize the rate of exposure to pesticides through fish consumption. The number of times one consumes pesticide contaminated fish in a day can be reduced. Moreover, during preparation and prior to consumption certain fish parts such as the gills can be removed [37]. However, the hazard quotients for all contaminants analyzed were below unity and thus may not pose an adverse non-cancer effect to exposed population [38].

The criteria for public health screenings for carcinogens are set at cancer risk level of 10^{-6} as recommended by USEPA. The assessment of carcinogenic risks lower than 10^{-6} is regarded as acceptable while the cancer estimates greater than 10^{-4} are regarded as unacceptable [18]. A cancer risk evaluation for contaminants was done using the cancer risk estimates at both 50th and 95th percentile exposure levels. As presented in **Table 6**, DDTs and Chlordanes recorded the highest values of 8.0×10^{-6} and 1.6×10^{-5} in catfish and mullet respectively when compared to other contaminants. These values are within the acceptable limits for human health as stipulated by USEPA. Results in this study is in contrast to the study carried out by Jiang *et al.,* [39] who reported that daily exposure to contaminants (DDTs, PCBs, HCHs, HCB, Chlordane) due to fish consumption posed a lifetime cancer risk of greater than one in one million. High levels of pesticides in food samples suggest that these pesticides have been used indiscriminately and could be detrimental to human health as corroborated by Jallow *et al* [40]. However, the carcinogenic risk assessment as observed in our present study was low as compared to the study of Yohannes *et al.*[41]who reported a cancer risk estimates in the range of

0.7×10^{-4} to 36×10^{-4} in *O. niloticus* and *C. gariepinus*. Therefore result in this study suggests that consumption of fish at the observed concentrations of these pesticides will not pose a cancer risk to exposed consumers. Results in this study are in agreement with the study of Thompson, *et al.* [42].

5. Conclusions

Characterizing contaminant exposure level is an important step in the ecological risk assessment (ERA) process. This study has confirmed the presence of pesticides in Ogbakiri River from local agricultural activities and other anthropogenic sources. Results showed that aquatic biota in Ogbakiri River as well as humans are exposed to currently and non-currently used pesticides, including those banned decades ago. The observed concentrations in both surface water and sediment and their comparison with stipulated values indicated potential risks for aquatic organisms. The prevalence of DDT in all analyzed samples could also suggest the current use of this pesticide in this locality and could also present a risk to aquatic organisms and humans during their lifetime. Moreover, it is also pertinent to note that human health safety is dependent on the protection of natural resources. This study, as the first exposure characterization carried out in Ogbakiri River, constitutes valuable and useful information for the ERA process. Although this preliminary assessment of risks should be sustained through a weight-of-evidence approach, it had already unraveled health concerns in this ecosystem and the urgent need to preserve our water resources. In addition, it reechoes the importance of evaluating related aquatic bodies that are also affected by diffuse pollution. Therefore, pesticides use around the Ogbakiri River should be regulated so as to avoid the continuous build up of these chemicals in this water body and aquatic organisms inhabiting it.

Conflict of Interest

The authors declare no conflict of interest regarding this manuscript.

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