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SEASONAL VARIATION OF ORGANOCHLORINE PESTICIDES RESIDUE IN WATER AND SILVER CATFISH (*Bagrus bajad* FABRICIUS, 1775) FROM AJIWA RESERVOIR, KATSINA STATE

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ABSTRACT
Water is one of the major components of environmental resources that are under threat either from over exploitation or pollution exacerbated by human activities. Organochlorine pesticides (OCPs) are major contaminants of inland water bodies and could lead to pollution if they accumulate over time. This study was carried out to investigate seasonal variation in OCPs residue in water and its bioaccumulation in gills and muscles of the silver catfish (Bagrus bajad Fabricius, 1775) from Ajiwa reservoir, Katsina State, Nigeria, between February and September 2022. The fish and water samples were obtained from three major fishing landing sites near the reservoir; Kadaji, Gamji and Kundu-Waje. The organochlorine pesticide residue in the fish and water samples were extracted, clean up with Silica gel and analyzed with a gas chromatograph with Ni electron capture detector. The data obtained from the gas chromatography (GC) analysis were presented using descriptive statistics while T-test was used to compare each of the OCPs between the two seasons. The seasonal difference showed significantly higher amount of gamma-chlordane and endosulfan in gills; aldrin in muscles; methoxychlor and endosulfan in water in wet season compared to dry season. The results indicated that there is presence of some organochlorine pesticides in the water from the reservoir, gills and muscle of B. bajad at various concentrations. However, the concentrations are all below the recommended safety limits, nonetheless, continuous monitoring and discouragement of the use of OCPs in and around the reservoir is recommended to prevent it from getting to hazardous level.

Keywords: Ajiwa reservoir, organochlorines, pesticides, Silver catfish

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INTRODUCTION
Inland water bodies have been a great source of fish and the global contribution increased from 7.1 million tonnes in the 1990's to 12.1 million tonnes in 2019, representing 13.12% of the total captured fish and 6.82% of the global fish production (FAO, 2022). However, fish production from inland waters is threatened by so many anthropogenic activities, including pollution, habitat modification and degradation, sand mining, construction, river flow changes and overfishing. According to Mustafa *et al*. (2024), pollution of rivers is caused by toxic pollutants e.g. heavy metals, phenols, pesticides that have

detrimental effect on the aquatic biota. These pollutants also have an indirect effect in aquatic ecosystem due to bacterial action on them which resulted in lower dissolved oxygen content (Mustafa *et al*., 2024). Discharge of significant amounts of pollutants into water bodies can cause their accumulation in the water, sediments, and aquatic food chains, leading to sub lethal effects and mortality of fish populations (McGeer *et al*., 2000). Water pollution is one of the most serious environmental threats to humans, affecting their biological organizations at various levels (M'Anampiu, 2011) and are of great concern as it not only threatens public water supplies but also causes damage to aquatic life (Vinodhini and Narayanan, 2009).

Organochlorines pesticides (OCPs) are ubiquitous environmental contaminants that are widespread worldwide and have been detected in food, meat, drinking water, sediment, and even a variety of biota, including fish (Ize-Iyamu *et al*., 2007). Organochlorine pesticides are widely used by farmers because of their effectiveness and their broad-spectrum activities (Ashujohri *et al*., 2008). Organochlorine compounds are highly lipophilic in nature, they generally tend to accumulate in the adipose tissue of fish and mammals, leading to declines in fish populations due to reduced reproductive success and premature death of juveniles (M'Anampiu, 2011). OCPs include a wide range of chemicals containing carbon, chlorine, and sometimes several other elements such as herbicides, insecticides, and fungicides. Organochlorine pesticides are a type of non-polar toxic chemical compounds classified as dichlorodiphenylethanes, cyclodienes, and chlorinated benzenes (Ademoroti, 1996). Studies have shown that exposure to dieldrin, DDT, and its metabolites is one of the many endocrine-disrupting chemicals (EDCS) that exhibit estrogenic and anti-androgenic effects (Olisah *et al*., 2020). Ajiwa reservoir is located in the semi-arid region of Nigeria. It is a well-utilized reservoir for irrigation farming with high tendency of OCPs contamination. Artisanal fisheries of the Ajiwa reservoir is well reported and silver catfish is one of the dominant fishes in its captures (Sadauki *et al.,* 2022). Therefore, this study examined the seasonal variation of OCPs in water and fish (*Bagrus bajad*) in the Ajiwa reservoir, Katsina state, Nigeria.

MATERIAL AND METHODS

Study Area

The study area is Ajiwa reservoir (Figure 1) located on the latitude 12°98'N and longitude 7°75' E, in Batagarawa Local Government Area, Katsina State. The main purpose of the reservoir is irrigation farming and public water supply to Katsina, Batagarawa, Mashi and Mani LGAs of Katsina state. The capacity of the water is nearly 22,730,000 m^3 (Parkman and Haskoning, 1996). Its fisheries serve as source of revenue for the neighboring villages; Masabu, Kunturu, Watsa, Danku, Kadaji, Kunduwaje among others, all in Batagarawa LGA of Katsina state, Nigeria.

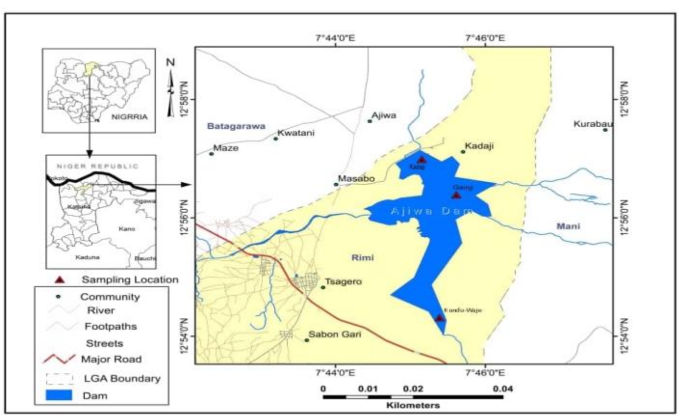

Figure1: Map of Ajiwa reservoir showing sampling stations

Fish and Water Sample Collection

Fish and water samples were collected monthly from three selected landing sites, Kadaji, Gamji and Kundu-waje (Figure 1). Water was conveyed using empty (50 cL) bottles (between February to September 2022.

Extraction of Organochlorine Pesticides from Water Samples

To assess the pesticide residues, a liquid-liquid extraction method was used according to the method described by Pandit *et al*. (2006). 50 mL of n-hexane was added to a 2L separator funnel along with 1L of filtered water, manually shaken for 5 min, and allowed to settle. After complete separation, the organic phase was drained into a 250 mL conical flask, while the aqueous phase was re-extracted twice with 50 mL of n-hexane. The three extracted organic phases were combined and dried by passing through a glass funnel containing anhydrous sodium sulfate. The organic fraction was concentrated using a rotary evaporator, and the samples were stored in a refrigerator at 4 °C for gas chromatography (GC) analysis.

Extraction of Organochlorine Pesticides from Fish Muscles and Gills Samples

Fish samples (muscles and gills) were extracted using the method described by Hladik and McWayne (2012). The sample (20 g) was weighed into a 150 mL conical flask, followed by the addition of 20 g of anhydrous sodium sulfate and 5 g of sodium bicarbonate. 1: 1 (v/v) 100 ml of the ethyl acetate/dichloromethane mixture was transferred to the 20 g of fish sample and the conical flask was shaken until the mixture was completely dissolved while adding 20 g of corked anhydrous sodium sulfate to the contents of the conical flask followed by addition of 20 g of sodium hydrogen carbonate. The conical flask was tightly capped and the mixture was thoroughly shaken for 10 minutes. The contents were allowed to stand for 3 hours. The organic layer was decanted into a 200 mL round bottom flask and rotary evaporated at 40°C. The pesticide in the rotating flask was dissolved, collected with 2 mL of ethyl acetate, and transferred to a 2 mL vial ready for cleaning.

Silica Gel Clean-up of Fish Sample Extracts

Extracts of fish samples (muscles and gills) were cleaned using the method described by Hladik and McWayne (2012). Briefly, ten grams (10 g) of the deactivated silica gel was weighed and transferred to a 10 mm glass chromatography column, followed by the addition of 3 g of anhydrous sodium sulfate. The column was wetted and rinsed using ten mL (10 mL) of a 1: 1 (v/v) ethyl acetate/dichloromethane mixture. The extraction residue (muscle and gills) in 2 mL of ethyl acetate was transferred to the column and the extracted vial was rinsed with 2 mL of ethyl acetate (3 times). The column was eluted with 80 mL portions of ethyl acetate/dichloromethane at a rate of 5 mL/min and placed into conical flask as fraction one. For the second elution, the column was re-eluted with 50 mL of ethyl acetate/dichloromethane and added to the first extract. All fractions of each sample were evaporated to dryness at 40 °C using a rotary evaporator. Each residue was dissolved in 2 mL of ethyl acetate and collected, and the samples were stored in a refrigerator at 4 °C for GC analysis.

De-fattening of the Muscle Samples

De-fatting of muscle samples was performed according to the method described by Hladik and McWayne. (2012). Briefly, 50 mL of 1: 1 (v/v) hexane/acetonitrile solution was added to 2 mL of the pesticide extracted from the fish sample in a 100 mL separator funnel. The separator funnel was shaken gently for 3 minutes while releasing the gas pressure. The separatory funnel was left for 20 minutes to allow the organic solvent to phase separate. The acetonitrile fraction containing the pesticide was collected in a 50 mL beaker while the fat containing hexane solvent phase was discarded. The resulting acetonitrile solvent extract was further cleaned with 25 mL of pure hexane. The acetonitrile fraction was concentrated using a rotary evaporator at 40°C, and the contents of the flask were dissolved and collected in 2 mL of ethyl acetate in a 2 mL vial. Vials containing pesticide extracts were stored in a refrigerator at 4 °C for GC analysis.

Gas Chromatography Analysis

A gas chromatograph with a Ni electron capture detector (GC-µECD Agilent Technology 7890A) was used for the identification and determination of the OCP residues. The cleaned extracts were dried and re-dissolved in 1.0 cm^3 analytical grade isooctane before injecting 1 µL of the purified extract into the injection port of the gas chromatograph (Pandit *et al*., 2002).

Data Analysis

Data were presented with mean and standard error. After homogeneity of variance and normality distribution test, the data were subjected to T-test to compare seasonal variation for the OCPs in water samples, fish gills and fish muscles. Simple bar graph was used to present the classification of OCPs. All analysis was performed using IBM SPSS version 22 and Microsoft office excel 2016.

RESULTS

A total of 20 OCPS from three different groups namely dichlorodiphenyltrichloroethane (DDT) and its analogues, cyclodienes and hexachlorocyclohexanes (HCH) were observed in this study. DDT and its analogues were three, with four analogues from HCH group while cyclodienes dominated the OCPS with 13 analogues. The OCPS in the water, gills and muscle samples are shown in Table 1, 2 and 3. There was no significant seasonal difference (P> 0.05) in most of OCPS in the gill samples except gamma-chlordane and endosulfan which were significantly higher (P<0.05) in the wet season compared to the dry season (Table 1). OCPs in muscles showed that there was no significant difference (P>0.05) due to season except, for aldrin which was significantly higher (P<0.05) in wet season compared to dry season (Table 2). Likewise, OCPs in water showed no significant difference (P>0.05) except for methoxychlor ($5.15\pm0.60ngL^{-1}$) and endosulfan ($4.31\pm0.81ngL^{-1}$) which were significantly higher (P<0.05) in the wet season compared to the dry season (Table 3). The percentages of these classes of OCPs residues found in fish and water from the reservoir are shown in Figure 2-4 where, Cyclodienes concentration were highest in the gills (67.92%), muscle (70%), and water samples (65.45%), followed by HCH while DDT and its analogues were the lowest in the fish gills, muscle and water sample from the reservoir.

Table 1: Seasonal variation in organochlorine pesticide present in Silver catfish

S/N and Classification	OCPs(ngg^{-1})	Dry	Wet
DDT and Its Analogues			
1	p,p'-dde	1.47 ± 0.25^a	2.36 ± 0.48^a
2	p,p'-ddd	1.13 ± 0.32^a	1.33 ± 0.67^a
3	p,p'-ddt	N.D	0.32 ± 0.32^a
Cyclodienes			
4	Heptachlor	2.10 ± 0.41^a	1.47 ± 0.74^a
5	Aldrin	2.70 ± 0.39^a	1.67 ± 0.20^a
6	Heptachlor-epoxide	2.32 ± 0.97^a	1.53 ± 0.43^a
7	Gamma-chlordane	1.36 ± 0.13^a	2.61 ± 0.26^b
8	Alpha-chlordane	1.19 ± 0.44^a	1.38 ± 0.41^a
9	Endusulfan I	1.32 ± 0.45^a	0.83 ± 0.19^a
10	Diedrin	1.12 ± 0.21^a	0.91 ± 0.80^a
11	Endrin	0.36 ± 0.23^a	1.69 ± 0.50^a
12	Endusulfan II	N.D	N.D
13	Endrin aldehyde	0.83 ± 0.69^a	1.33 ± 0.55^a
14	Endosulfan	0.53 ± 0.38^a	2.56 ± 0.32^b
15	Methoxychlor	0.34 ± 0.19^a	1.16 ± 0.30^a
16	Endrin ketone	1.60 ± 0.80^a	2.51 ± 0.22^a
HexachloroCyclohexanes (HCH)			
17	Alpha-bhc	3.09 ± 1.57^a	1.80 ± 0.20^a
18	Beta-bhc	2.13 ± 0.8^a	1.03 ± 1.03^a
19	Gamma-bhc	0.92 ± 0.46^a	0.41 ± 0.31^a
20	Delta-bhc	0.83 ± 0.48^a	0.94 ± 0.94^a

Different letters as superscripts across rows shows significant differences (P<0.05)

Table 2: Seasonal variation in organochlorine pesticide present in the Silver catfish

S/N and Classification	OCPs(ngg^{-1})	DRY	WET
DDT and Its Analogues			
1	p,p'-dde	1.25±0.58 [a]	1.55±0.71 [a]
2	p,p'-ddd	0.84±0.50 [a]	N.D
3	p,p'-ddt	N.D	N.D
Cyclodienes			
4	Heptachlor	0.35±0.13 [a]	1.35±1.05 [a]
5	Aldrin	1.72±0.32 [a]	2.81±0.18[b]
6	Heptachlor-epoxide	1.77±0.24 [a]	2.53±0.44 [a]
7	Gamma-chlordane	2.02±0.44 [a]	3.69±0.92 [a]
8	Alpha-chlordane	1.04±0.31 [a]	0.25±0.25 [a]
9	Endusulfan I	0.55±0.18 [a]	0.29±0.15 [a]
10	Diedrin	0.93±0.52 [a]	1.83±0.40 [a]
11	Endrin	2.33±1.66 [a]	1.37±0.31 [a]
12	Endusulfan II	N.D	N.D
13	Endrin aldehyde	1.97±1.06 [a]	4.19±0.71 [a]
14	Endosulfan	1.24±1.07 [a]	1.40±1.40 [a]
15	Methoxychlor	1.25±1.25 [a]	1.60±160 [a]
16	Endrin ketone	3.45±1.03 [a]	3.89±0.34 [a]
HexachloroCyclohexanes (HCH)			
17	Alpha-bhc	3.58±0.57 [a]	2.20±0.61 [a]
18	Beta-bhc	N.D	N.D
19	Gamma-bhc	0.67±0.67 [a]	0.55±0.28 [a]
20	Delta-bhc	0.36±0.27 [a]	1.06±1.73 [a]

Different letters as superscripts across rows shows significant differences (P<0.05)

Table 3: Seasonal variation in organochlorine pesticide present in the water

S/N and Classification	OCPs(ngL^{-1})	DRY	WET
DDT and Its Analogues			
1	p,p'-dde	2.61±0.35 [a]	2.85±0.16 [a]
2	p,p'-ddd	2.13±0.20 [a]	4.53±1.40 [a]
3	p,p'-ddt	N.D	0.21±0.21 [a]
Cyclodienes			
4	Heptachlor	1.94±0.46 [a]	2.72±1.14 [a]
5	Aldrin	1.94±0.98 [a]	4.17±0.62 [a]
6	Heptachlor-epoxide	3.31±0.48 [a]	3.22±0.78 [a]
7	Gamma-chlordane	2.18±0.37 [a]	2.80±0.72 [a]
8	Alpha-chlordane	1.90±0.14 [a]	3.30±0.94 [a]
9	Endusulfan I	2.34±0.19 [a]	1.92±0.87 [a]
10	Diedrin	1.83±0.40 [a]	2.77±1.58 [a]
11	Endrin	1.37±0.31 [a]	1.73±0.78 [a]
12	Endusulfan II	N.D	N.D
13	Endrin aldehyde	2.42±0.35 [a]	2.61±1.31 [a]
14	Endosulfan	1.44±0.63 [a]	4.31±0.81[b]
15	Methoxychlor	1.50±1.03 [a]	5.15±0.60[b]
16	Endrin ketone	2.54±0.62 [a]	4.59±0.78 [a]
HexachloroCyclohexanes (HCH)			
17	Alpha-bhc	3.69±0.61 [a]	4.10±1.14 [a]
18	Beta-bhc	0.47±0.24 [a]	3.92±0.71 [a]
19	Gamma-bhc	0.71±0.41 [a]	2.73±1.61 [a]
20	Delta-bhc	1.60±0.43 [a]	4.98±1.08 [a]

Different letters as superscripts across rows shows significant differences (P<0.05)

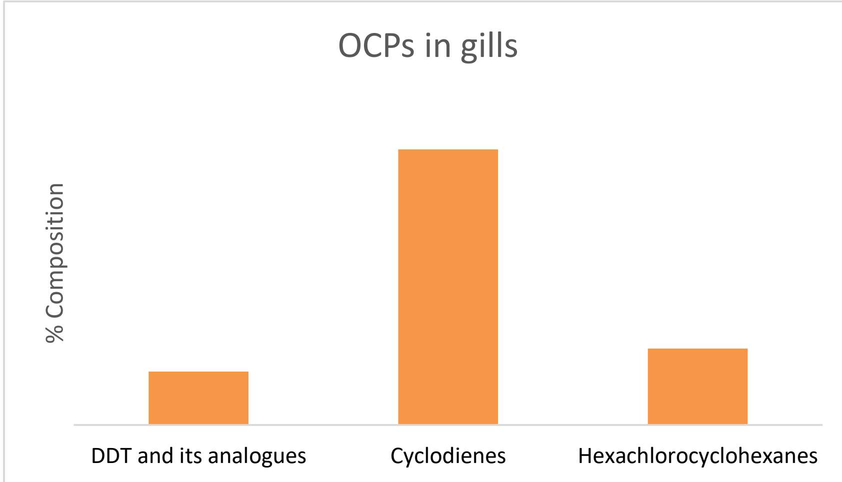

Figure 2: Classification of Organochlorine pesticides residues in gills

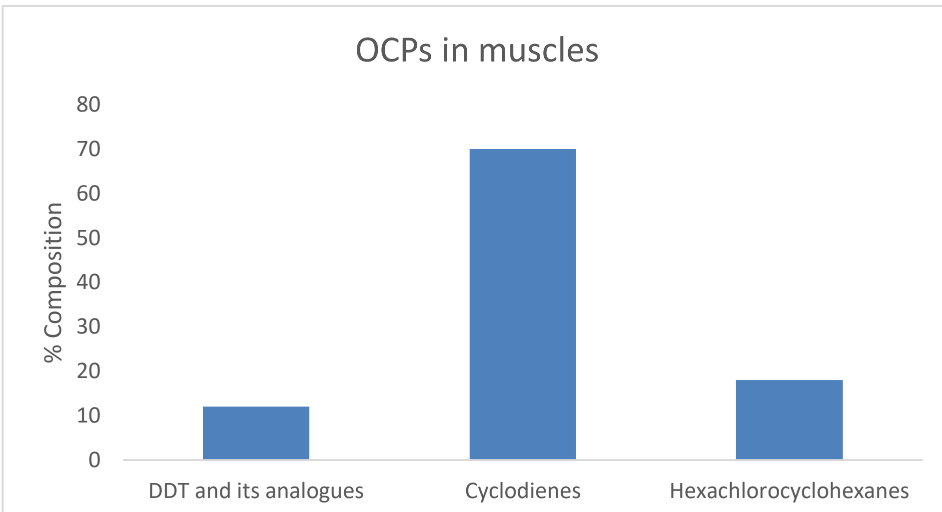

Figure 3: Classification of Organochlorine pesticides residues in muscles

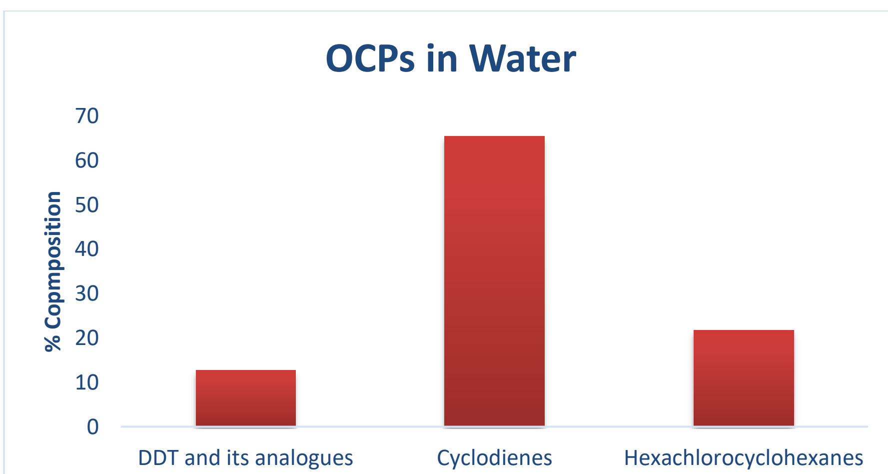

Figure 4: Classification of Organochlorine pesticides residues in Water

DISCUSSION

Twenty organochlorine pesticides were detected in the gills, water and muscles and this is in line with the findings of Akinsanya *et al*. (2020) who reported 18 OCPs in African snakehead fish sample from Lekki Lagoon in Lagos. The presence of 20 organochlorine pesticides indicates that there is wide use of pesticide in the reservoir, even though most of them were banned. Large amounts of OCPs accumulate in water systems through agricultural runoff, leaching from contaminated soil, droplet drift from pesticide spraying on food crops and careless disposal of pesticide containers (Sathish Kumar *et al*., 2024). Some of the OCPs were higher in the wet season in the water, gills and muscle compared to dry season. This could be due to increased farming activities in the wet season, since farming is one of the primary occupations of the people that live around the water body. Sadauki *et al*. (2022), earlier noted that the water body is used for irrigation of farmland. Therefore, it could be highly prone to OCPs contamination due to run off from farmland, with possible increase during the wet and farming season. High levels of HCB and δ-BHC were reported in water from Buffalo River, an important freshwater resource for domestic use by host communities in the United States of America (Olisah *et al*., 2020). A similar study conducted in South Africa also detected high levels of δ-BCH and heptachlor in agricultural runoff (Fatoki and Awofolu, 2004). Henry and Kishimba (2003) detected DDT, HCH, and endosulfan in surface water from nine districts in southern Lake Victoria, Tanzania. Adeyemi *et al*. (2011) reported chlordane, heptachlor, methoxychlor, HCB, endosulfan, DDT, dieldrin and aldrin in water samples from Lagos Lagoon, Nigeria.

Thirteen organochlorine compounds belonging to the cyclodiene group, four Hexachlorocyclo hexane and three DDTs were detected in the various samples in Ajiwa reservoir. The OCPs concentration in the water and fish samples showed a higher concentration of pesticides relative to what was obtained in Lagos lagoon by Olarinmoye (2011). Endrin, Endosulfan, Aldrin, Heptachlor, Chlordane, and metabolites of DDT were all detected and found to be lower than the recommended limit/guideline (10ng/l) for aquatic life. 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 (2012) in freshwater and fish samples collected from various locations in Osogbo, Osun State, Nigeria, irrespective of the season.

CONCLUSION

The study established a total of 20 OCPs in both the dry and wet seasons from the Silver Catfish (*Bagrus bajad*) gills and muscle, and from water in the Ajiwa reservoir. Some of the OCPs including Gamma-chlordane, Endosulfan, Aldrin and Methoxychlor were higher during the wet season compared to the dry season, although they are all lower than the recommended safety levels. The presence of OCPs in the fish and water of Ajiwa Reservoir is consistent with agricultural activities in the study area due to the use of pesticides by farmers. Ajiwa Reservoir is slightly contaminated with organochlorine pesticides, although, it is still safe for use considering the low concentrations. Therefore, the use of pesticides around the reservoir should be discouraged and there is a need for continuous monitoring of these pesticide residues in the reservoir water and fish as this will be in order to raise alarm when the concentration is rising and could lead to environmental hazards.

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