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Analysis of Organochlorine Pesticide Residues in the Igere River, Agbara Ado -Odo/Ota Local Government Area, Ogun State, Nigeria.

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Abstract: *This study intends to investigate the presence and levels of organochlorine pesticides (OCP) in fish, water, and sediment samples obtained from a river in Agbara, Ogun State, and suggest the associated consumption risks. Pesticide residues were extracted using ultrasound sonication and soxhlet extraction, followed by their quantification using gas chromatography with an electron capture detector. Supercritical fluid and liquid/liquid extraction methods were used to clean up the sediment and water samples. Analysis revealed the presence of 23 organochlorine pesticides, including endrin-ketone, endrin-aldehyde, p,p'-DDT, and heptachlor epoxides, with mean concentrations above WHO and FAO maximum residue limits. The water samples contained high levels of contaminants such as endrin-ketone (0.65 mg/L), endrin-aldehyde (0.30 mg/L), p,p'DDT (1.31 mg/L), and heptachlor epoxide (1.15 mg/L). These findings indicate the accumulation of hydrophilic substances in sediments and fatty tissues. While current fish consumption may not pose immediate health risks, long-term accumulation could be hazardous. This study highlights the importance of mitigating organochlorine pesticide contamination and calls for further research and effective pollution control strategies. The findings demonstrate the widespread presence of OCPs in all matrices, with concentrations in water samples notably surpassing permissible limits, highlighting significant environmental and potential health concerns.*

Keywords: Organochlorine pesticides, Gas chromatography with electron capture detector (GC-ECD), Dichlorodiphenyltrichloroethane (DDT), World Health Organization (WHO), Food and Agriculture Organization (FAO).

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Introduction

The widespread use of pesticides in agriculture and public health programs has led to significant environmental contamination, particularly of water bodies. Organochlorine pesticides (OCPs) are of particular concern due to their persistence, bioaccumulation, and potential adverse effects on human health and ecosystems. These compounds, once widely used for pest control, have been banned or restricted in many countries; however, they continue to persist in the environment due to their long half-lives.Pesticide usage at a global scale presents challenges due to the reliance on chemical-based pest management, particularly in African countries. Population growth and increased consumption are projected to triple agricultural demand by 2050, highlighting the need for sustainable approaches (Asiegbu *et al.*, 2022).

Distinguishing between natural and synthetic pesticides is crucial. While synthetic pesticides, like those used against insect pests, can lead to food toxins, naturally derived ones such as pyrethroids from Chrysanthemum flowers are generally considered safe (Dassanayake *et al.*,2021). Organochlorine pesticides (OCPs), like Dichlorodiphenyl-Trichloroethane (DDT), persist for decades (Fatunsin *et al*., 2020) and their presence in food is linked to health issues (Ingenbleek *et al.*, 2019). Nigeria grapples with complying with pesticide regulations, awareness, and effective monitoring of pesticide risks (Sosan *et al.*, 2020).

Rivers and other aquatic systems act as sinks for many environmental pollutants, including OCPs, which can accumulate in sediments and aquatic organisms. This poses a risk to aquatic life and, through the food chain, to human health. Fish, in particular, can accumulate significant levels of OCPs, which may then be transferred to humans through consumption.

Therefore, Water quality analysis is essential for ensuring public health. Various studies have assessed the physicochemical properties of water bodies, such as the Global Health Facility and Deoli Bhorus dam, to determine compliance with permissible limits (Aleem *et al*., 2018; Ofon *et al*., 2022). To address water pollution and pesticide residues, efficient technologies for pesticide removal and remediation are needed. Carbon nanotubes (CNTs) and nano-crystalline metal oxides (Fe2O4, Al2O3, ZnO) have shown promise in this regard (Sahitya *et al*., 2022). Previous studies have shown significant PCB contamination in fish species from the Ogun River and observed a causal relationship between endocrine disruption and contaminant burden (Ibor *et al*., 2016, 2017). Another study found higher pesticide levels in benthic fish species from the Owan River, with associated damage in reproductive tissues. Regular monitoring of OCP levels is crucial for effective mitigation efforts (Chukwuka *et al.*, 2019).

The Igere River in Agbara, Ogun State, Nigeria, is a critical water source for local communities, supporting fishing, agriculture, and domestic needs. Despite its importance, there has been limited research on the contamination levels of OCPs in this river and the potential health risks associated with their presence.

This study aims to fill this gap by determining the presence and levels of organochlorine pesticide (OCP) in fish, water, and sediment samples from the Igere River. By employing advanced extraction and quantification techniques, this research provides a comprehensive assessment of the contamination status and evaluates the potential health risks associated with the consumption of fish from this river.The results will inform local authorities and the public, contributing to better environmental management and pollution control strategies in the region.

Materials and Methods

***Sampling*:** Three different water samples (A, B, and C) were collected from the Igere River on August 20th, 2023, along with sediment samples and fish samples (silver catfish, sole fish, and tilapia) at three different geographic points using GPS coordinates. Nine samples (three of each type) were stored in clean containers until analyzed for organochlorine pesticide residues.

***Equipment/Materials*:**

➡ BUCHI R215 Rotary Evaporator (Switzerland)
➡ Clean 120-HD Ultrasonic Bath (China)
➡ Agilent 7820A GC with 5975 Inert MSD (US)
➡ Agilent HP 5 Column (Length: 30m, ID: 0.32mm, Phase Thickness: 0.25µm)
➡ ADAM AAA250LE Weighing Balance (UK)
➡ N Hexane and Acetone from Merck (US)

Extraction of Water, Sediment, and Fish samples for Organochlorine Pesticides

Water:

Pesticide residue in the water samples was extracted via liquid-liquid extraction. N-hexane (50 mL) was mixed with 1 L of filter water in a separating funnel, shaken, and separated. The organic phase was collected and dried with sodium sulfate. The concentrated organic fraction was obtained using a

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rotary evaporator. Acetonitrile and sodium chloride were added to the concentrated organic extract to clean it up and induce phase separation between the

organic solvent layer and the water layer. The organic layer was carefully removed and transferred to a new vial. The cleaned-up organic solvent was evaporated under reduced pressure and reconstituted in n-hexane for gas chromatography analysis.

***Sediment*:**
Dry sediment samples (10 g) were extracted using 150 mL of a 4:1 n-hexane mixture in a soxhlet extractor for 8 hours. The extract was evaporated at 40 °C and cleaned up after being dissolved in 10 mL of n-hexane and transferred into a florisil mini-column. The pesticides were eluted with 30% diethyl ether in n-hexane, and their residues were dissolved in ethyl acetate for gas chromatography analysis.

***Fish*:**
Weighed fish samples (20 g) were mixed with anhydrous sodium sulfate (20 g) and sodium hydrogen carbonate (5 g), then shaken with 100 mL of 1:1 ethyl acetate/dichloromethane for 10 minutes. After standing for 3 hours, the organic layer was collected, evaporated, and transferred for cleanup. Extracted Fish samples were packed with 10 g of deactivated silica gel and 3 g of anhydrous sodium sulfate. The column was wetted and rinsed with 10 mL of a 1:1 (v/v) mixture of ethyl acetate and dichloromethane. The extracted residue, which contained water and fish, was transferred to the column and rinsed three times with 2 mL of ethyl acetate. The column was eluted with 80 mL of ethyl acetate/dichloromethane (5 ml/min) into a conical flask as the first fraction, followed by a second elution with a 50 ml portion of the same solvent mixture. The fractions were combined and concentrated to dryness using a rotary evaporator at 40 °C. The resulting residue was dissolved in 2 mL of ethyl acetate for gas chromatography analysis to identify and quantify the organochlorine pesticides present in the sample.

GC analysis for OCP
A standard solution containing 17 organochlorine pesticide (OCP) components at 2000 ppm was used to prepare five-point serial dilution calibration standards for Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Before calibration, the MS was auto-tuned to perfluorotributylamine (PFTBA), and instrument optimal and sensitivity conditions were checked. The sample was analyzed using GC-MS in selective ion monitoring (SIM) and scan mode on an HP-5 capillary column coated with 5% phenyl methyl siloxane. Quality control measures were ensured through the analysis of solvent and procedure blanks. The results of the analysis showed the pesticide determinations were accurate.

Data analysis
A total of 23 OCPs were detected and determined in the three matrices at one sample location (the Igere River). Concentrations of OCP residues were

calculated individually and as the sum of their isomeric forms. The means were calculated from the detectable values, and values below the detectable limit were considered not

detected (ND). The mean was calculated from triplicate determinations. There were values that were nil (i.e., non-detects). The results obtained were compared with FAO, WHO, and USEPA standards.

Statistical Analysis
Experimental data were summarised using descriptive statistics including mean, minimum and maximum values, and standard error of means. The Pearson moment correlation using SPSS 16.0 package was used to test the degree of relationship among the samples, while multivariate analysis was performed using R software version 3.6 to understand the relationship among the samples.

Results

Table I: Mean Concentrations of Organochlorine Pesticide Residues determined in the Igere river (water samples)

Organo chlorine Pesticides	Sample Concentrations (mg/L)			Mean conc.(mg/L)
	A	B	C	MEAN
alpha.-Lindane (a-BHC)	0.07	0.12	0.07	0.09
beta.-Lindane (b-BHC)	N.D	0.16	0.33	0.24
gamma.-Lindane (g-BHC)	0.05	0.26	0.15	0.15
delta.-Lindane (d-BHC)	0.05	0.18	0.09	0.11
Endrin	0.12	0.14	0.47	0.24
Heptachlor	N.D	N.D	0.56	0.56

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Aldrin	0.05	0.19	0.63	0.29
Isodrin	1.21	1.38	0.31	0.97
Heptachlor epoxide	0.61	1.19	1.65	1.15
a-Chlordane	N.D	N.D	N.D	Nil
Endosulfan I	0.15	0.09	0.26	0.17
p,p'-DDE (4,4'-DDE)	N.D	0.09	N.D	0.09
Endosulfan II	1.78	0.40	2.25	1.48
p,p'-DDD (4,4'-DDD)	N.D	0.08	N.D	0.08
trans-Nonachlor	1.03	0.30	0.86	0.73
cis-Nonachlor	N.D	N.D	N.D	Nil
Endrin aldehyde	1.45	1.05	1.40	1.30
Endosulfan sulfate	1.99	0.66	1.80	1.48
p,p'-DDT (4,4'-DDT)	2.22	0.57	1.14	1.31
Methoxychlor	0.40	1.46	0.03	0.63
Endrin ketone	0.79	0.40	0.75	0.65
g-Chlordane	1.19	1.44	0.93	1.19

Table II: Mean Concentrations of Organochlorine Pesticide Residues determined in Igere river (Sediment samples)

Organochlorine Pesticides	Sample Concentration (mg/L)			Mean conc (mg/L)
	A	B	C	MEAN
.alpha.-Lindane (a-BHC)	0.02	ND	ND	0.02
.beta.-Lindane (b-BHC)	0.04	ND	ND	0.04
.gamma.-Lindane (g-BHC)	0.03	ND	0.03	0.03
.delta.-Lindane (d-BHC)	N.D.	0.02	0.03	0.03
Endrin	0.02	0.01	0.01	0.01
Heptachlor	N.D.	ND	ND	Nil
Aldrin	0.02	0.04	0.02	0.03
Isodrin	1.00	0.02	0.21	0.41
Heptachlor epoxide (Is...	0.20	0.08	0.07	0.12
a-Chlordane (cis-Chlor...	N.D.	ND	ND	Nil
g-Chlordane (.gamma.-C...	N.D.	ND	ND	Nil
Endosulfan I	0.03	ND	0.03	0.03
p,p'-DDE (4,4'-DDE)	0.01	ND	ND	0.01
Endosulfan Sulfate	N.D.	ND	ND	Nil
Endosulfan II (.beta	0.09	ND	0.13	0.11
p,p'-DDD (4,4'-DDD)	N.D.	0.06	ND	0.06
trans-Nonachlor	N.D.	ND	0.09	0.09
cis-Nonachlor	N.D.	ND	ND	Nil
p,p'-DDT (4,4'-DDT)	N.D.	0.10	0.11	0.11
Methoxychlor	ND	ND	ND	Nil
Endrin ketone	0.25	0.08	0.29	0.21
Endrin aldehyde	0.08	ND	0.94	0.51

Abbreviations: ND, not detected; OCPs, organochlorine pesticides; p,p'-DDD, para, para dichloro diphenyl dichloroethane.

Table III: Mean Concentrations of Organochlorine Pesticide Residues determined in fishes in the Igere river (Tilapia fish, Sole fish, and Silver catfish sample)

Organochlorine Pesticides	Sample Concentration (mg/L)			Mean(mg/L)
	A (Tilapia)	B (Sole fish)	C (Silver catfish)	Mean

.alpha.-Lindane (a-BHC)	ND	ND	ND	Nil
.beta.-Lindane (b-BHC)	ND	ND	ND	Nil
.gamma.-Lindane (g-BHC)	ND	ND	ND	Nil
.delta.-Lindane (d-BHC)	N.D.	ND	ND	Nil
Endrin	0.02	0.01	ND	0.02
Heptachlor	N.D.	0.19	ND	0.19
Aldrin	0.02	ND	0.02	0.02
Isodrin	ND	ND	ND	Nil
Heptachlor epoxide (Is...	0.07	0.06	0.08	0.07
a-Chlordane (cis-Chlor...	N.D.	ND	ND	Nil
g-Chlordane (.gamma.-C...	N.D.	ND	ND	Nil
Endosulfan I	N.D	0.03	0.03	0.03
p,p'-DDE (4,4'-DDE)	N.D	ND	ND	Nil
Endosulfan Sulfate	N.D.	ND	ND	Nil
Endosulfan II (.beta	N.D	ND	0.11	0.11
p,p'-DDD (4,4'-DDD)	N.D.	ND	ND	Nil
trans-Nonachlor	N.D.	ND	ND	Nil
cis-Nonachlor	N.D.	ND	ND	Nil
Endrin aldehyde	3.06	2.71	0.84	2.20
Endosulfan sulfate	N.D	ND	ND	Nil
p,p'-DDT (4,4'-DDT)	0.10	ND	ND	0.10
Methoxychlor	N.D	ND	0.1	Nil
Endrin ketone	0.41	0.09	0.07	0.19

Abbreviations: ND- not-detected; OCP-OrganochlorinePesticides; p,p'-DDD-(para, para dichloro diphenyl dichloroethane)

Table IV: Criterion/Guideline value to protect freshwater aquatic life and drinking water quality as derived by US Environmental protection Agency(EPA) and World Health Organizations (WHO)

Organochlorine pesticides	USEPA/WHO Guideline value for freshwater/drinking water quality (mg/L)
. alpha.-Lindane (a-BHC)	0.002
.beta.-Lindane (b-BHC)	0.002
.gamma.-Lindane (g-BHC)	0.002
.delta.-Lindane (d-BHC)	0.002
Endrin	0.0006
Heptachlor	0.00052
Aldrin	0.0003
Isodrin	0.0003
Heptachlor epoxide (Is...	0.00052
a-Chlordane (cis-Chlor...	0.0024
g-Chlordane (.gamma.-C...	0.0024
Endosulfan I	0.02
p,p'-DDE (4,4'-DDE)	0.001
Endosulfan Sulfate	0.02
Endosulfan II (.beta	0.02
p,p'-DDD (4,4'-DDD)	0.001
trans-Nonachlor	0.0024
cis-Nonachlor	0.0024
Endrin aldehyde	0.0006
Endosulfan sulfate	0.02
p,p'-DDT (4,4'-DDT)	0.0011
Methoxychlor	0.02
Endrin ketone	0.0006

Table V: Common detected organochlorine pesticide (OCP) residues in water and associated toxicity data

OCP Residue	Maximum co	LC50 fish (µg/L)	ARR= Max conc/LC50	mean concentr	MCL(mg/L)	WHO Guide-line Value

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	nc. (mg/L			atio n		(mg/L)
Endosulfan sulfate	1.99	1.20	1.65	1.31	N/A	0.02
DDT	2.22	0.18	12.3	1.32	N/A	0.0011
Endrin aldehyde	1.45	1.18	1.22	1.30	N/A	0.0006
g-Chlordane	1.44	70	0.02	1.19	N/A	0.0024
Heptachlor epoxide	1.65	5.30	0.31	1.15	0.4	0.0005

Detected organochlorine pesticide (OCP) residues in water and associated toxicity data

Table VI: Mean, standard deviation, minimum, maximum, and range concentrations in water samples

Samples(mg/L)	Mean	Standard Deviation	Minimun	Maximum	Range
A	0.6743	0.6833	0.05	2.22	2.17
B	0.6524	0.5491	0.08	1.46	1.38
c	0.7981	0.7034	0.03	2.25	2.22

Table VII: Mean, standard deviation, minimum, maximum, and range concentrations in sediment samples

Samples(mg/L)	Mean	Standard Deviation	Minimun	Maximum	Range
A	0.1032	0.2505	0.01	1.00	0.99
B	0.0157	0.0283	0.01	0.10	0.09
c	0.0610	0.0882	0.01	0.29	0.28

Table VIII: Mean, standard deviation, minimum, maximum, and range concentrations in fish samples

Samples	Mean	Standard Deviation	Minimun	Maximum	Range
Tilapia	0.1079	0.5264	0.02	3.06	3.04
Solefish	0.2681	0.7966	0.01	2.71	2.70
Catfish	0.0970	0.2903	0.02	0.84	0.82

Table IX: Correlation coefficients between the concentrations of pesticides in the water,sediment and fish samples.

Water samples

Samples(mg/L)	A	B	C
A	1.000	0.366	0.744
B	O.366	1.000	0.211
C	0.744	0.211	1.000

Sediment samples

Samples(mg/L)	A	**B**	C
A	1.000	-0.216	0.074
B	-0.216	1.000	0.368
C	0.074	0.368	1.000

Fish samples

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Samples	Tilapia	Solefish	Silver catfish
Tilapia	1.000	0.995	0.994
Solefish	0.995	1.000	0.999
Catfish	0.994	0.999	1.000

Discussion

OCPs Analysis in Water Samples

Table I presents the mean concentrations of organochlorine pesticide (OCP) residues detected in water samples from the Igere River. The analysis revealed that out of 23 OCPs, significant concentrations were found for endosulfan, DDT and its metabolite, endrin aldehyde, chlordane, and heptachlor epoxide. The mean values for these contaminants (1.48 mg/L for endosulfan, 1.31 mg/L for DDT, 1.30 mg/L for endrin aldehyde, 1.19 mg/L for chlordane, and 1.15 mg/L for heptachlor epoxide) exceeded the World Health Organization (WHO) guideline values listed in Table IV. These findings align with previous studies, such as Okoya (2013), where similar exceedances of maximum residue limits (MRL) were observed in different rivers in Ondo State. The predominant detection of endosulfan suggests significant agricultural runoff into the river. Additionally, the presence of p,p'-DDD, a metabolite of DDT, indicates ongoing use or persistence of this pesticide in the environment. Sources of these residues include agricultural activities, household usage, leaching, and runoff during the rainy season, as well as improper disposal of pesticide containers and oil spills.

OCPs Analysis in Sediment Samples

Table II summarizes the concentrations of OCP residues in sediment samples from the Igere River. Higher concentrations were detected for endrin ketone, endrin aldehyde, isodrin, DDT and its metabolites, and endosulfan II, with mean values of 0.21 mg/L, 0.51 mg/L, 0.41 mg/L, 0.21 mg/L, and 0.11 mg/L, respectively. In contrast, heptachlor, chlordane, endosulfan sulfate, cis-nonachlor, and methoxychlor were not detected. Trace amounts of endrin were present with a mean value of 0.01 mg/L across samples A, B, and C. The detection of pp'-DDE in sample A and pp'-DDD in sample B, though absent in other samples, underscores the heterogeneous distribution of these compounds in sediments. This pattern suggests that OCPs in sediments result from historical usage and are influenced by factors such as sediment transport and deposition processes.

OCPs Analysis in Fish Samples

Table III details the OCP residues found in fish samples (Tilapia, Sole fish, and Silver catfish) from the Igere River. Notably, a-BHC, b-BHC, g-BHC, d-BHC, isodrine, and chlordane were not detected in any fish samples. Endrin was detected at 0.02 mg/L in Tilapia and 0.01 mg/L in Sole fish, but not in Silver catfish. Heptachlor was detected only in Sole fish at 0.19 mg/L. Endosulfan II was found at a trace level of 0.11 mg/L in Silver catfish. Endrin aldehyde showed significantly high concentrations of 3.06 mg/L in Tilapia, 2.71 mg/L in Sole fish, and 0.84 mg/L in Silver catfish, exceeding WHO guidelines (Table IV). The lower OCP concentrations in fish compared to water and sediment samples suggest that these compounds tend to accumulate more in sediments and fatty tissues of organisms. However, the high level of endrin aldehyde in fish indicates a potential risk for bioaccumulation and biomagnification, posing a threat to fish-eating wildlife and humans. Uncontrolled discharge of endrin during its manufacture, formulation, and use can result in acute environmental problems associated with its high toxicity, and fish and fish-eating birds are at risk from surface runoff. To minimize exposure to OCP residues through tilapia consumption, the frequency of tilapia consumption can be reduced, and certain parts, such as the gills, can be removed before preparation and consumption. Tilapia consumers can also try to include other fish species with minimal OCP bioaccumulation and biomagnification factors in their diet to reduce their dependence on tilapia for fish protein

Statistical Analysis and Correlation relationship.

Table VI, VII, and VIII summarize the mean, standard deviation, minimum, maximum, and range concentrations of OCPs in water, sediment, and fish samples, respectively. Water samples showed the highest variability with a mean concentration of 0.6743 mg/L (sample A) and a range of 2.17 mg/L. Sediment samples had lower mean concentrations, indicating that OCPs are less hydrophilic and more likely to accumulate in sediments and organisms' tissues. Fish samples exhibited high variability in endrin aldehyde concentrations, highlighting the potential for certain OCPs to bioaccumulate in aquatic organisms.Table IX presents the correlation coefficients between pesticide concentrations in different samples. In water samples, there is a strong positive correlation (0.744) between samples A and C, suggesting consistent contamination patterns across these locations. Sediment samples showed weaker correlations, with the highest being between samples B and C (0.368). Fish samples demonstrated very strong correlations close to 1, indicating similar levels of contamination across different fish species.

Toxicity and Risk Assessment

Table V compares the maximum detected OCP concentrations in water with their respective LC50 values for fish. Endosulfan sulfate, DDT, and endrin aldehyde exhibit high ARR values, indicating significant toxicity risks to aquatic life. For instance, DDT's maximum concentration (2.22 mg/L) exceeds the LC50 for fish (0.18 µg/L) by a factor of 12.3, highlighting its potential for causing severe ecological harm.

Conclusion and Future Works

The study utilized supercritical fluid extraction (SFE) and liquid/liquid extraction (LLE) to clean up sediment and water samples for organochlorine pesticide (OCP) determination. Higher concentrations of contaminants in water samples suggest that OCPs, while generally accumulating in sediments and organisms' fatty tissues, may partition into the water column due to disturbances or specific environmental conditions. Although the OCP levels in fish were below WHO/FAO guideline values, continuous accumulation poses a future threat to human health and the environment. This study provides baseline data for future monitoring and comparison, and highlights the need for routine environmental surveillance and stricter control of pesticide usage to mitigate OCP pollution and its associated risks.The results revealed significant contamination of the Igere River ecosystem with various OCPs, including Endosulfan, DDT and its metabolites, Endrin aldehyde, Chlordane, and Heptachlor epoxide, which exceeded WHO guideline values. These high concentrations can be attributed to historical and ongoing agricultural practices, improper disposal of pesticide containers, and other anthropogenic activities. Water samples consistently showed higher concentrations of OCPs compared to sediment and fish samples. This suggests that while OCPs are hydrophobic and typically accumulate in sediments, certain environmental conditions or disturbances can cause them to enter the water column. Sediment samples, though containing lower concentrations, still had significant levels of several OCPs, indicating potential for long-term environmental persistence and bioaccumulation. Fish samples showed lower overall levels of OCPs, but the presence of Endrin aldehyde at concerning levels underscored the risks to aquatic life and potential health hazards to humans consuming fish from this river. The strong correlations between OCP concentrations in water and fish samples highlight the critical pathway of these contaminants through the aquatic food web. The study emphasizes the importance of regular monitoring of OCP residues to assess the effectiveness of regulatory measures and to mitigate potential health risks.

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