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Occurrence of Phthalate Esters and Its Histopathological Effect on the Gills and Tissues of African Catfish *Clarias gariepinus* from Eleyele Lake in Ibadan, Oyo State

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ABSTRACT

The ubiquitous presence of synthetic chemicals in the environment poses significant challenges to both ecological integrity and human health. Fish can absorb PAEs through various routes, including gill respiration, ingestion of contaminated food, and dermal contact with water. African Catfish (30) were collected from Eleyele lake, 15 samples each collected in rainy and dry seasons, were investigated for PAEs. Extraction and clean-up of samples were done using established procedures. Histopathological analysis was done using standard procedures. Data were analyzed using Anova $\alpha_{0.05}$. Most dominant PAEs (DEP, DEHP, DBP, DiBP and DMP) were in African Catfish during rainy season, gills have higher concentrations (µg/kg) ranged from 0.404 to 2.1, muscle ranged from 0.25 to 1.040. Histopathology revealed atrophy of keratinocytes and degeneration of keratinocytes, respectively. The gills displayed necrosis of secondary lamellae and hyperplasia of secondary lamellae. Phthalate esters occurred in African catfish and were toxic to the fish species.

Key words:
Histopathology, African catfish, Phthalate esters

Introduction

The ubiquitous presence of synthetic chemicals in the environment poses significant challenges to both ecological integrity and human health. Phthalic acid esters (PAEs) have garnered increasing attention due to their widespread use in various industrial and consumer products. PAEs serve as plasticizers in plastics, adhesives, cosmetics, and other materials, making them prevalent contaminants in the environment (Savoca *et al.*, 2022; Wang *et al.*, 2023). PAEs are characterized by 1,2-benzenedicarboxylic acid and alkyl chains that affect their physicochemical properties, such as water solubility and biodegradation (Kong *et al.*, 2016). These plasticizers are present in the marine environment because they are released when plastics in the marine environment deteriorate (Fikarov´a *et al.*, 2019).

Fish can absorb PAEs through various routes, including gill respiration, ingestion of contaminated food, and dermal contact with water (Chen, *et al.,* 2023). Once absorbed, PAEs are distributed throughout the fish's body via the circulatory system. Bioaccumulation of PAEs in fish occurs when the rate of uptake exceeds the rate of elimination, leading to the accumulation of these contaminants in fish tissues over

time. This can be caused by the low vapor pressure of PAEs, which results in minimal volatilization loss in the water environment, and their strong adsorption and affinity for aquatic organisms (Chen and Li, 2021; Khisdost, *et al.,* 2023).

Histological analysis is the most effective and sensitive tool to determine cellular changes that may occur in vital organs due to toxicant exposure. Organs suitable for histological examination include skin, gills, liver, spleen and kidney (Datta, 1996). The histological changes in fish organs are useful biomarkers to indicate environmental pollution and health status of aquatic ecosystem and fish (Omitoyin *et al.,* 2021; Georgieva *et al.,* 2014).

This study aims to shed light on the histopathological effect of phthalate on African Catfish and contribute to the preservation of the environment.

Materials and Methods

Eleyele lake is a municipal water resource that supplies water to the Ibadan municipality and is located within the Eleyele catchment area in Ido local Government Area, Ibadan, Oyo State Southwest-Nigeria. It is located at an altitude of 125 m above sea level and lies within Latitude 7°25′–7°26′N and Longitude 3°51′–3°52′E (Fig**.** 1). The lake covers a distance of 62 km source to the dam site, is flood controlled, and has a total impoundment area of 5.46 km^2 and a maximum depth of 12 m during the flood season. At the time of creating the dam, the initial water storage capacity was 704 million liters (Imevbore, 1967), and this was later increased to 900 million liters to meet the increasing demand for domestic water supply to the Ibadan municipality.

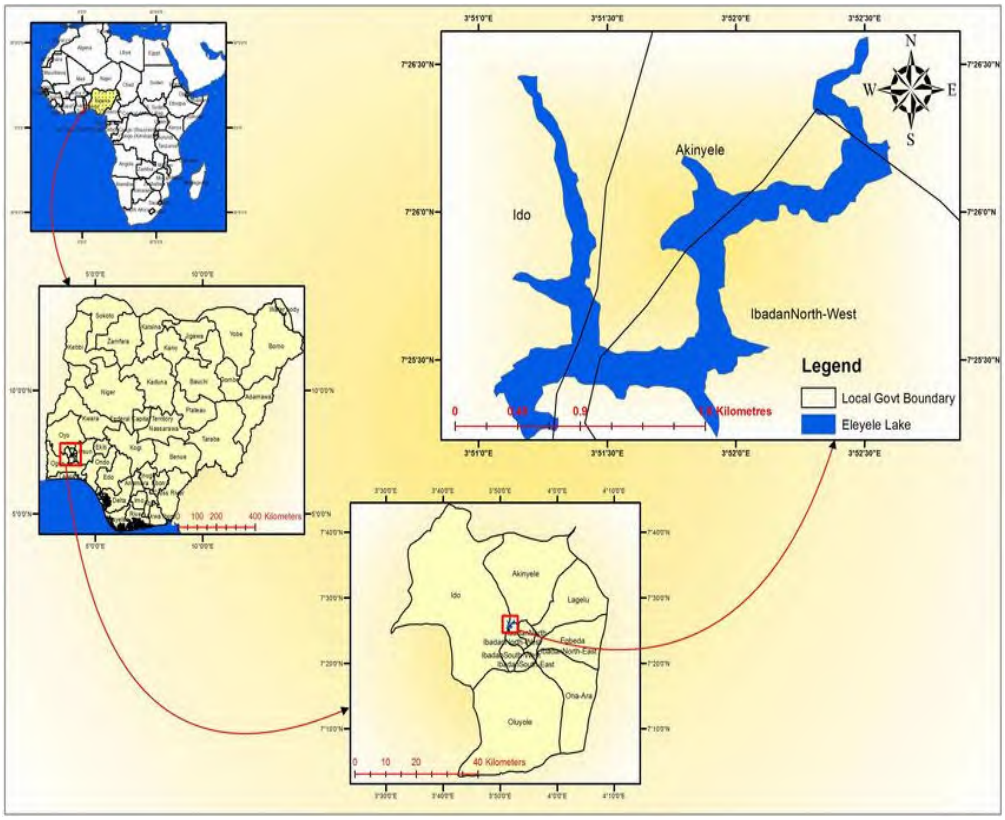


Figure 1: The map of Eleyele lake and sampling areas.
Source: Oludare *et al.,* 2016

The Experiment

This research involved the collection of *Clarias gariepinus* samples from Eleyele lake to assess the occurrence of phthalic acid esters in the gills and tissues of the fish. This was done in two seasons (rainy and dry season). The type of phthalates were determined after the characterization of the phthalates in the fish samples from the wild (Eleyele lake).

Sample collection

A total of 30 fish samples of African catfish (*Clarias gariepinus*) species were collected randomly from the entire length of Eleyele lake at various points with the aids of artisanal fishermen in two seasons (rainy and dry season) for PAEs analysis. For each season, 15 samples of African catfish (*Clarias gariepinus*) were collected. Overall, thirty gills (considering left and right gills as one sample) and thirty tissues were collected. Then, 2/15 gills and 2/15 tissues samples of African catfish was used for PAEs analysis for each season. The fish species is selected based on its feeding pattern as Omnivore. In addition, the fish species is one of the most important wild and cultured freshwater fish in Nigeria. The fish samples were then placed on ice and transported to the laboratory for analysis.

Sampling (Experimental Fish from Eleyele lake)

Fish samples were transported in a stainless container to the wet laboratory, washed with double-distilled water to remove external debris and other contaminants, the fish were labelled and their length and weight was recorded. A total of 30 fish samples were collected.

Phthalates extraction and purification

Extraction and purification for PAE analysis were performed according to the method described by Tsumura *et al.*, (2001), with minor modifications (Cirillo *et al.*, 2013a). No plastic equipment was used, to avoid external contamination of PAEs and glassware was preliminarily heated in a muffle and washed with acetone and n-hexane before use. For the extraction, 1 g and 0.5 g aliquot of muscle and gills, respectively, previously freeze-dried, was collected in a test tube to which 10 ml of n-hexane-dichloromethane (50:50) was added. The tube was mechanically shaken and placed in an ultrasonic bath for 20 min. Then, the sample was centrifugated at 2500 rpm for 10 min to collect the supernatant in a round-bottom flask. The supernatant was concentrated using Rotavapor at 45 ◦C and mixed with 5 ml of n-hexane. After, the sample was purified on a manually packed chromatographic column. The column was prepared with wadding (preconditioned with acetone and n-hexane and placed in an oven at 100 ◦C for 1 h), 2.0 g of Florisil, 0.5 g of Bondesil PSA, and 1 g of anhydrous sodium sulfate (Na_2SO_4). Then, the sample was eluted, mixed with 10 ml of acetone and hexane (4:1), and concentrated using Rotavapor. Finally, 2 ml of n-hexane was added to the sample collected in an amber glass vial (2 ml).

GC-MS analysis

A GC-MS (Agilent 7890A GC system coupled to an Agilent 5975C mass selective detector (MSD) (Agilent Technologies, Santa Clara, CA, USA)) was used for the characterization and quantification of PAEs. The parameters were set as follows: initial oven temperature: 60 ◦C (holding time 0.5 min); injector temperature: 280 ◦C. After the injection, the ramp rate was programmed from 60 ◦C to 240 ◦C at 10 ◦C/min and until 280 (holding for 18 min) at 15 ◦C/min. The transfer line of the GC-MS interface was held at 280 ◦C. Samples (2 μL) was injected in splitless mode into the capillary gas chromatography column. The carrier gas was high-purity helium (99.999%) at a 1.0 ml/min flow rate.

Quality assurance

The entire working surfaces were cleaned with ethanol prior to start of work. Glasswares used for sample preparation and extraction were washed thoroughly with water and detergent, rinsed with clean water, soaked in undiluted hydrochloric acid (HCL) for 30 minutes, then, removed and rinsed with double distilled water and dried in the oven before use (Mgbemena *et al.*, 2017). All solvents were re-distilled and filtered before use to ensure non contamination (Ilechukwu *et al.*, 2019). Samples were covered when not in use and filters carefully handled to ensure non-contamination by airborne particles. Sample blanks were also extracted alongside the samples to ensure non-contamination.

Table 1: General information about the samples used for rainy and dry seasons.

Sample	Season	Code
Muscle of catfish	Rainy season	RM1
Muscle of catfish	Rainy season	RM2
Muscle of catfish	Drying season	DM3
Muscle of catfish	Drying season	DM4
Gill of a catfish	Rainy season	RG5
Gill of a catfish	Rainy season	RG6
Gill of a catfish	Drying season	DG7
Gill of a catfish	Drying season	DG8

Statistical analysis

Data analysis and graph processing were performed using R Software version 3.6.0 and the following package: ggplot2 (R Core Team, 2019; Wickham, 2016). Data were tested for the homogeneity of the variances (Bartlett's test) and normality (Shapiro-Wilk's test). Probit analysis was used to determine the concentration at which 50% mortality (LC_{50}) occurred using SPSS version 21.0. Finally, a one-way analysis of variance with post hoc Tukey's test was performed to ascertain any statistically significant difference. The significance level for all statistical analysis within the study was set to $p < 0.05$. All the statistical analyses were performed using GraphPad prism software (version 9.5.1).

Results

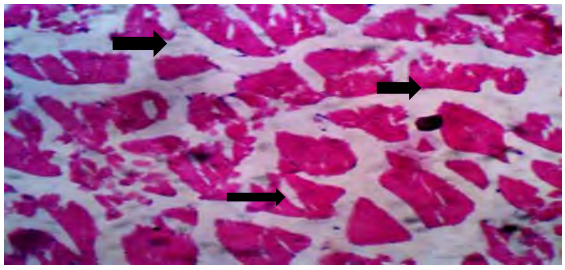
Histopathological observations

Histopathological observations in the skins and gills of *Clarias gariepinus* obtained from Eleyele lake.

The histopathological changes observed in the gills and tissues of *Clarias gariepinus* from Eleyele lake in rainy and dry seasons is shown in **Plate 1 - 4.**

Skin (A1): *Clarias gariepinus* obtained from Eleyele lake in rainy season, shows there is atrophy of keratinocytes (**plate 1**).
Skin (A2): *Clarias gariepinus* obtained from Eleyele lake in dry season, shows there is degeneration of keratinocytes (**Plate** 2).
Gills (B1): *Clarias gariepinus* obtained from Eleyele lake in rainy season, shows there is necrosis of secondary lamellae (**Plate** 3).
Gills (B2): *Clarias gariepinus* obtained from Eleyele lake in dry season, shows there is hyperplasia of secondary lamellae (**Plate** 4).

A1



A2

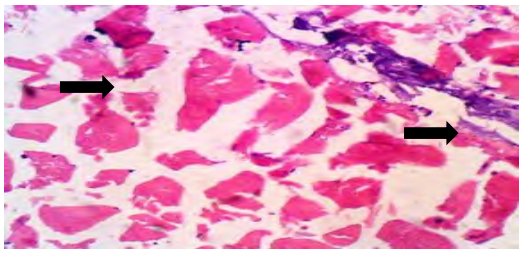
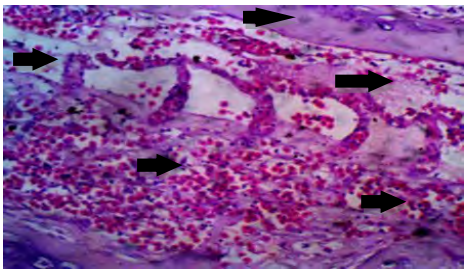


Plate 1: Skin of *Clarias gariepinus* obtained from Eleyele lake in rainy season, shows there is atrophy of keratinocytes (**A1**).

Plate 2: Skin of *Clarias gariepinus* obtained from Eleyele lake in dry season, shows there is degeneration of keratinocytes (**A2**).
Haematoxylin and Eosin magnificent (H and E) ×400)

B1



B2

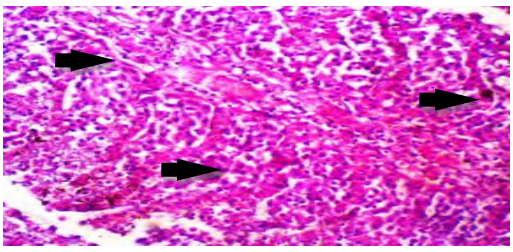


Plate 3: Gill of *Clarias gariepinus* obtained from Eleyele lake in rainy season, shows there is necrosis of secondary lamellae (**A3**).

Plate 4: Gill of *Clarias gariepinus* obtained from Eleyele lake in dry season, shows there is hyperplasia of secondary lamellae (**B2**).
(H and E ×400)

Levels of phthalic acid esters (PAEs).
The concentrations of PAEs determined from 4 fish samples of four gills and four tissues (8 samples) collected from Eleyele river, Ibadan, is presented in **Table 2**. The concentrations of ∑5PAEs in rainy season and dry season fish samples varied between 0.83 to 2.1µg/kg and 0.25 to 0.41µg/kg respectively. This result however indicated that the lowest ∑5PAEs concentration was obtained for the dry season fish samples while the highest ∑5PAEs concentration of 2.1µg/kg was obtained for the rainy season fish samples. Di-butyl phthalate (DBP) has the highest concentration of 0.960µg/kg while di-methyl phthalates have the lowest concentration of 0.040µg/kg among the fish samples collected in the rainy season. Among the fish collected in the drying season, di-(2-ethylhexyl) phthalate (DEHP) has the highest concentration of 0.240µg/kg while di-methyl phthalate has the lowest concentration of 0.004µg/kg.

Table 4.11: Concentrations of the five PAEs (µg/kg) congeners in catfish samples.

PAEs	**Abbreviation**	**RM1**	**RM2**	**DM3**	**DM4**	**RG5**	**RG6**	**DG7**	**DG8**
di-(2-ethylhexyl) phthalate	DEHP	0.310	0.240	0.070	0.120	0.580	0.720	0.240	0.030
di-ethyl phthalate	DEP	0.180	0.140	0.030	0.040	0.080	0.140	0.010	0.090
di-butyl phthalate	DBP	0.360	0.140	0.020	0.060	0.960	0.260	0.030	0.070
di-methyl phthalate	DMP	0.040	0.070	0.010	0.007	0.050	0.070	0.004	0.050
di-isobutyl phthalate	DiBP	0.150	0.240	0.120	0.080	0.430	0.080	0.120	0.170
Σ5 PAEs		1.040	0.830	0.250	0.307	2.100	1.270	0.404	0.410

Discussion

Histopathological test.

In this study, PAEs caused histological damages to fish gills and tissue.
The histopathological results of the gills of the fish from Eleyele lake showed that there is necrosis of secondary lamellae and hyperplasia of secondary lamellae in rainy and dry seasons respectively. It also showed that in the muscle there is atrophy of keratinocytes and degeneration of keratinocytes in rainy and dry seasons respectively.
This result is in agreement with the report of Jiraungkoorskul *et al.*, (2002) in *Oreochromis niloticus* exposed to glyphosate, Devi and Mishra, (2013) in the gills of Channa punctatus exposed to Chlorpyrifos, Omitoyin *et al.*, (2021) in Heterobranchus bidorsalis exposed to zinc and copper and Lawrence and Temiotan, (2010) in *Clarias gariepinus* exposed to Gammalin 20.

In this study, the result however indicated that the lowest ∑5PAEs concentration was obtained from the dry season fish samples, while the highest ∑5PAEs concentration of was obtained from the rainy season fish samples from Eleyele lake. The high concentrations of PAEs detected in rainy season fish samples may be due to the facts that phthalate esters are commonly used in plastics and can be released into the environment through various sources like land runoff, weathering of products, leaching from plastics and industrial wastewater. This result is similar to the several reports by Liang, *et al.,* 2024; Cao *et al.,* 2022 and Jayaweera *et al.,* (2019), in the bioaccumulation and distribution of PAEs.
As regards the different bioaccumulation in the organs, a significantly higher concentration of PAEs was found in the gills than in the muscle ($p < 0.05$).

Occurrence of phthalic acid esters in *Clarias gariepinus*.

Widespread environmental and biota occurrence of PAEs is inevitable for three main reasons: (1) global annual production and usage of >5 million tonnes, primarily as additives to PVC plastics, industrial solvents, and components of several classes of consumer products (Huang *et al.,* 2013), (2) excessive usage of these chemicals in plastics, personal care products (PCP), and many other consumer products (Blount *et al.,* 2000; Koo and Lee, 2004), and (3) these compounds are not chemically bound to products in which they are used, and as result are gradually released into the environment (Kato *et al.,* 2005).
In this study, the result shows that phthalates occur in Eleyele lake. The gill of the fish samples collected from Eleyele lake, shows the highest PAEs concentrations of 2.1µg/kg and it was obtained in the sample RG5 collected in the rainy season, while the lowest PAEs concentrations of 0.250µg/kg was obtained in the muscle sample DM3 collected in the drying season. The high concentrations of PAEs detected in rainy season fish samples may be due to the facts that phthalate esters are commonly used in plastics and can be released into the environment through various sources like land runoff, weathering of products, atmospheric deposition, leaching from plastics and industrial wastewater. Additionally, changes in water quality and environmental conditions during the rainy season may also contribute to the higher concentration of phthalate esters in fish during this time. This is similar to the report of Squillante *et al.*, (2023).

The various histopathological lesions recorded in the gills and tissues of *Clarias gariepinus* fish exposed to PAEs in this study could be attributed to the oxidative stress induced by PAEs.

Conclusion

The occurrence and effect of PAEs in African catfish samples collected at Eleyele lake, Ibadan were investigated. This study shows that phthalate esters occur in Eleyele lake and posing significant threat on Clarias gariepinus species. All of the selected PAEs categories were present in all the samples, hence the need to mitigate the introduction of plastics into the lake and manage our waste properly.

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