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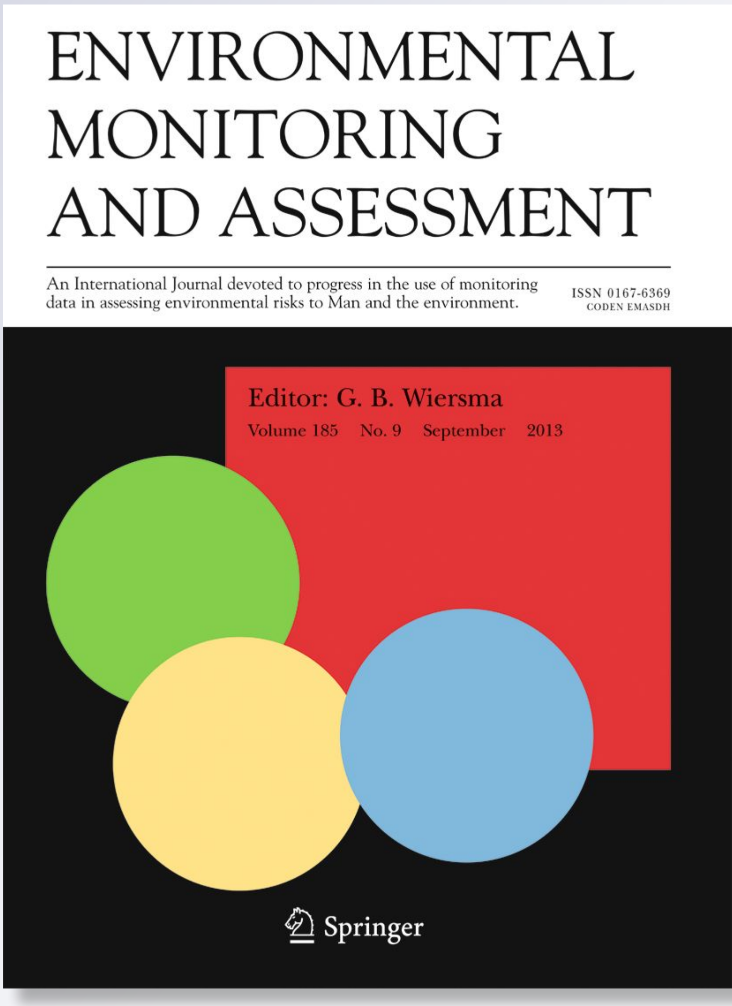


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# Biomonitoring, physico-chemical, and biomarker evaluations of abattoir effluent discharges into the Ogun River from Kara Market, Ogun State, Nigeria, using *Clarias gariepinus*

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**Abstract** The discharge of untreated effluents into aquatic ecosystems poses potential adverse effects to aquatic organisms. In this study, the physico-chemical characteristics of abattoir effluent from Kara Cow Market, Ogun State, Nigeria, surface water and sediments from the Ogun River were evaluated. Fish species and macrobenthic fauna diversity in the river were also examined. Acute toxicity and biochemical and histological studies were investigated in *Clarias gariepinus* exposed to sub-lethal concentrations of the effluent over a period of 28 days. Effluent physico-chemical parameters such as ammonia, conductivity, total dissolved solids, and total suspended solids were higher than set limits. Total polycyclic aromatic hydrocarbons (PAHs) in the effluent and sediment were 6.73 mg/L and 8.07 mg/kg, respectively. Tetracycline (an antibiotic administered to the cows at the market) levels in the effluent and surface water were 0.23 μg/mL and 0.85 μg/mL, respectively. Fish species diversity was lower at the test site compared to the reference site. *Chironomus* spp. and *Tubifex tubifex* dominated the benthic assemblage at the test site. There were significant changes ($p < 0.05$) in the biochemical indices but no histological alterations in exposed *C. gariepinus* after 28 days. The results demonstrate that the effluent poses potential risks to the aquatic organisms and ecosystem services provided by the river. We recommend that environmental regulatory agencies and stakeholders should establish effluent and solid wastes management systems at the market to prevent environmental and public health epidemics within the framework of the United Nations Sustainable Development Goals 6 (clean water and sanitation) and 14 (life below water).

**Keywords** African sharptooth catfish · Chronic toxicity · Freshwater species diversity · Priority and emerging pollutants · Slaughterhouse wastewater · Sustainable development goals

## Introduction

Since the rise of the industrial revolution and mass production in the 1900s to meet the demands of the ever-increasing population of humans around the globe, the state of the health of terrestrial and aquatic ecosystems have perpetually deteriorated leading to an environmental crisis. By-products from human activities are dispersed throughout the environment, and these enter the food chain having long-lasting consequences and, thereby, impacting the health of the planet Earth (Miller 2017). In recent times, improper management practices are being implicated as causes of the gross pollution of freshwater ecosystems resulting in a geometric increase in environmental issues and water-related diseases

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especially cholera, dysentery, diarrhea, and typhoid (Osibanjo and Adie 2007).

There are a number of slaughterhouses all over Nigeria which tend to the growing demand of the teeming population. One of such is the Kara Abattoir situated at the Kara Cow Market, located along the Lagos-Ibadan Expressway on the outskirts of Lagos, Nigeria. The abattoir is positioned along the course of the Ogun River, an important river in the South-West of Nigeria having its source at coordinates 8° 41″ N, 3° 28″ E, close to Shaki in Oyo State. It flows through Ogun State and discharges into the Ikorodu axis of the Lagos Lagoon at coordinates 6° 38″ N, 3° 22″ E (Ayoade et al. 2004). The market is quite important to the residents of Lagos and Ogun States because a large quantity of the livestock consumed daily in these states is obtained from the Kara Market. Like most slaughterhouses in Nigeria, the wastes generated from the Kara abattoir are improperly managed. The waste products and wastewaters hardly undergo any form of treatment before being released into the river (Adeyemo 2005). In Nigeria, local abattoirs which are the major meat processing industries are typically located close to water bodies so as to ease waste disposal (Osibanjo and Adie 2007). Discharges from this industry have increased the levels of sulfates, phosphates, and nitrates in water bodies and has altered properties such as color and odor (Adelegan 2002).

The continuous inputs of nitrogen and phosphorus compounds which encourage eutrophication may lead to ecological imbalance in such water bodies, the likely extinction of native species of organisms, introduction of invasive and opportunistic species, adverse health problems in humans, and the emergence of new diseases (Lopez-Lopez et al. 2011).

Biomarkers (biological responses elicited in organisms as a result of exposure and/or effect of stressors) at various levels of biological organization can be used in assessing the health of ecosystems (Maria et al. 2009; Karadag et al. 2014; Sogbanmu et al. 2018). The biomarkers induce in aquatic organisms has been proposed as biomarkers of contaminants or seasonally mediated oxidative stress in response to pollutants (Borković et al. 2005; Karadag et al. 2014). Also, histological changes in fish exposed to pollutants have been reported as sensitive biomarkers of effect (Pandey et al. 2008; Saliu et al. 2017). Furthermore, fish and macrobenthic fauna species diversity can serve as indicators of aquatic ecosystem health (Ajagbe et al. 2012). Since fish and fishery products are a major source of animal protein in developing countries such as Nigeria (NSPFS 2005), contaminated fish and fisheries can be potentially hazardous for humans at the top of the food chain who consume them. The African sharptooth catfish, *Clarias gariepinus*, is a species of the catfish family, Clariidae, which are mainly found in rivers, lakes, and swamps throughout Africa and the Middle East. *Clarias gariepinus* is one of the most consumed fish species in Sub-Saharan Africa. Typically, it has black or dark gray coloration at its back which fades to a white belly and has an eel-like structure. The genus *Clarias* are distinctive for their ability to survive a wide range of environmental extremes and also have a highly efficient air breathing organ which enables them to survive in oxygen-depleted waters (De Graaf and Janssen 1996).

The potential and extent of environmental and human health risks of anthropogenic activities are usually unknown or little known to stakeholders. These are persons/industries that discharge their effluents untreated into the aquatic environment and/or regulators who are involved in the monitoring and development of policies for the management of these ecologically and economically important aquatic ecosystems. There are insufficient data to establish the effects of the Kara Cow Market effluent on species diversity and sublethal effects to aquatic organisms in the river. Therefore, the aim of this study was to assess the effects of the abattoir effluent discharged from the Kara Cow Market on species diversity and biomarkers in a typical freshwater fish, *Clarias gariepinus*, in the Ogun River. Our hypotheses are that the effluent has adverse impacts on the water quality of the river and animal species diversity across the gradient of the river close to the effluent discharge point and has probable sublethal effects on aquatic organisms. The potential outcomes of this study are the provision of evidence-based information for development of appropriate effluent treatment intervention, environmental risks advocacy to the market stakeholders, and holistic environmental management at the market. This will support the achievement of the United Nations Sustainable Development Goals 6 (clean water and sanitation) and 14 (life below water) particularly in developing countries like Nigeria.

## Materials and methods

### Study area and sampling operations

The study area is located along the Ojodu/Isheri axis of the Ogun River stretching from the Kara Market axis of the river at coordinates 6° 38′ 48.0″ N, 3° 22′ 46.5″ E to the Ikosi Ketu axis of the Ogun River at coordinates 6° 37′ 18.2″ N, 3° 24′ 14.2″ E (Fig. 1). The nature and description of anthropogenic activities at the study area are shown in Fig. 2 and Supplementary Material (SM) 1. Samples were randomly collected at three sampling points along the Kara axis of the Ogun River at coordinates 6° 38′ 48.0″ N, 3° 22′ 46.5″ E, the outfall which is at the end of the drainage discharge channel from the abattoir, 6° 38′ 44.1″ N, 3° 22′ 50.8″ E downstream from the effluent source point, and at 6° 37′ 18.2″ N, 3° 24′ 14.2″ E, a randomly selected control point upstream (Figs. 1 and 2).

### Samples collection, quality assurance, and quality control

Effluent from the abattoir and surface water samples from the delineated sampling points in the Ogun River were collected into properly labeled 2.5-L plastic bottles for physico-chemical analysis and 500-mL amber glass bottles for the analysis of oil and grease content, polycyclic aromatic hydrocarbons (PAHs), and tetracycline. The physico-chemical parameters analyzed were dissolved oxygen, biochemical oxygen demand, total dissolved solids, nitrates, sulfates, and phosphates. The sediment samples were collected at the same sampling points for surface water in the Ogun River using Van Veen Sediment Grab Sampler. They were wrapped in aluminum foil, properly labeled and stored at temperature below 4 °C prior to extraction. The plastic bottles were initially washed with dilute nitric acid, then with distilled water and air dried. Thereafter, they were rinsed twice with the sample to be collected. Samples were taken from the effluent discharge outlet between the hours of 7.00 and 7.30 am when cows were being slaughtered. It takes about two (2) min for the effluent to flow from the slaughter slabs to the effluent discharge outlet. All samples were appropriately labeled with the date and time of collection and transported to the laboratory in ice-packed coolers for further analysis.

### Sample analysis for physico-chemical parameters, PAHs, and tetracycline

Parameters assessed in situ using hand-held probes (Horiba multi water Sampler-Model U50) in the surface water of the Ogun River were temperature, pH, dissolved oxygen (DO), salinity, oxidation reduction potential (ORP), conductivity, total dissolved solids (TDS), and turbidity. PAHs, acidity, alkalinity, biochemical oxygen demand (BOD), nitrate, and phosphate analyses were conducted at the Environmental Resource Managers Laboratory, Lagos, Nigeria, following standard procedures (APHA/AWWA/WPCF 2005).

### Sample preparation, extraction, and analysis of PAHs

For water samples, 100 mL of the sample was extracted thrice with 20 mL of hexane:acetone (ratio 1:1) in a 250-mL separatory funnel, giving approximately 60 mL of the final extracting solvent. The content in the separatory funnel was vigorously shaken for 5 min while periodically venting the funnel to release excess pressure buildup. The sample organic extract layer was carefully decanted, dried with sodium sulfate, and washed using silica gel column. For sediment samples, 10 g ± 0.05 g of homogenized sediment sample was weighed into 250-mL Teflon bottle, after thawing at room temperature. About one to three spatulas full of activated sodium sulfate were added to the samples in the Teflon bottles in order to eliminate water/aqueous portions. Twenty milliliters of 1:1 acetone:hexane was used for extraction thrice, giving approximately 60 mL of final extracting solvent. The covered Teflon bottles were then sonicated in an ultrasonic bath at 70 °C for 30 min (Banjoo and Nelson 2005). The organic layer was decanted into a clean beaker/round-bottom flask, further dried with sodium sulfate, and cleaned up using silica gel column. Thereafter, the sample (surface water and sediments) extracts were concentrated to ~ 2 mL using a rotary evaporator prior to PAH analysis using a gas chromatograph coupled with flame ionization detector (GC-FID) (Wang et al. 2000).

PAH standard, 1000 ppm (catalog number: H-QME-01) containing 23 environmental PAH components was purchased from AccuStandard. Five (5)-point serial dilution calibration standards (1.00, 5.00, 10.00, 50.00, 100.00 ppm) were prepared from the stock and used to calibrate the GC-FID. The determination of the levels of PAHs in the samples was carried out using Agilent

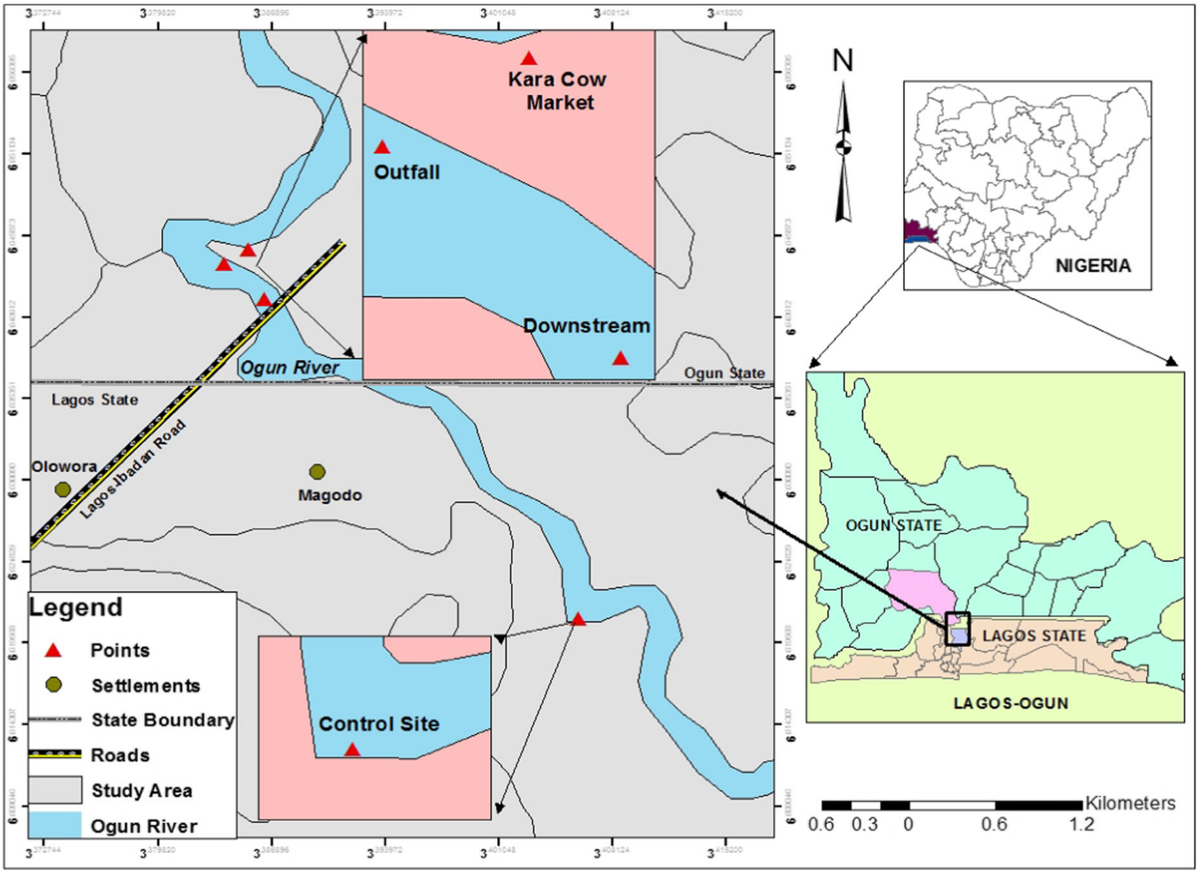



**Fig. 1** Map of study area at Kara Cow Market, Ogun State, Nigeria

7890B GC-FID. The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% phenylmethylsiloxane (30 m length × 0.32 mm diameter × 0.25-μm film thickness) (Agilent Technologies). One microliter of the samples was injected in splitless mode at an injection temperature of 300 °C, at a pressure of 13.74 psi and a total flow of 21.364 mL/min. Purge flow to split vent was set at 15 mL/min at 0.75 min. Oven was initially programmed at 40 °C/min then ramped at 12 °C/min to 300 °C (10 min). FID temperature was 300 °C with hydrogen, air flow at 300 mL/min, nitrogen was used as makeup gas at a flow of 22 mL/min. After calibration, the samples were analyzed and corresponding PAH concentration was obtained (Bi et al. 2009; Çok et al. 2012).

*Quality control*

The instruments for analysis were calibrated with 1 μL of the calibration standard. A five-point calibration curve was prepared using the calibration standard that was commercially obtained, and $R^2$ value was ≥ 0.995. The response factor (RF) for each analyte/components in the calibration standard using the area response, and the amount of standard material was calculated. Also, the relative standard deviation percentage (%RSD) of the Rf for each analyte across the calibration curve was calculated and the value was less than 15%. The average response factor for the weight ranges were calculated and used for sample quantification. Laboratory reagent blank was run to account for any interferences or contaminant in solvent, reagent, glass wares, and other sample processing could result to elevated baselines observed by GC/FID detection. A procedural blank was run after every 20 samples, or with every sample set. AnalaR grade reagents were used and obtained from the vendor with their certificate of analysis. Storage and handling of all reagents and standards were strictly in compliance with the safety precautions necessary as indicated in the MSDS of each respective reagent/standard.


**Fig. 2** **a**–**d** Photographs of the study area at Kara Cow Market, Ogun State, Nigeria showing outfall of the effluent discharge (arrow) into the Ogun River (Fig. 2a), burning activities (arrow) in close proximity to the Ogun River (Fig. 2b), cows (arrow) being sold at the market (Fig. 2c), the market adjoining the Ogun River (arrows) (Fig. 2d)

*Sample preparation, extraction, and analysis of tetracycline*

Samples were refrigerated and transported to the laboratory in amber bottles. Five milliliters of 0.1 M disodium-EDTA solution followed by 10 mL Mcllvaine buffer (pH 4) was added to 1 L of the water sample and stored frozen at − 20 °C until analysis. Before extraction, samples were thawed and filtered with cotton wool and filter paper of diameter 18 cm to remove suspended particles. Some of the samples were filtered twice or thrice depending on how much suspended particles were present.

HyperSep Retain Polar Enhanced Polymer (PEP) SPE cartridges (200 mg, 6 mL) were used for extraction and concentration (× 1000) of surface water and effluent samples on a Supelco Visiprep SPE Vacuum Manifold (standard, 24 port model). Cartridges were first conditioned with 4 mL methanol, followed by 4 mL water. A 500 mL aliquot of surface water and 200 mL of effluent were loaded into cartridges followed by a wash step of 4 mL water to further remove possible traces of EDTA and any unwanted interferences. The cartridges were allowed to dry. Bound actives were subsequently eluted with 4 mL (2 × 2 mL) methanol into salinized amber glass tube. Eluted extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted in 0.5 mL of methanol for surface water and 0.2 mL for effluent. Extract solutions were stored in amber glass vials at 4 °C and in the dark for high-performance liquid chromatography (HPLC) analysis. Glassware used for extraction of effluent samples was salinized to reduce analyte adsorption to glass surfaces. This procedure included an initial rinse with 50:50 (*v*/*v*) methanol:water before triplicate rinses with dichloromethane.

Fifty milligrams of tetracycline was weighed, and a stock solution of 1 mg/mL was prepared using HPLC-grade methanol. Lower concentrations of calibration standards (50, 10, 5, 1 μg/mL) were prepared from the stock solution and transferred to HPLC vials for analysis. An Agilent 1100 series HPLC with a quaternary pump, an auto-sampler, a thermostated column compartment, Variable Wavelength Detector (VWD) UV detector, and a Shimadzu DGU-14A online degasser was used for the analysis (Bečić et al. 2014).

## Biomonitoring studies

*Fish species sampling*

Fish samples were collected from two stations. A total of eighty-three (83) fishes were collected at the Kara axis of the Ogun River and sixty-eight (68) fishes at the control station upstream of the river (Fig. 1). The fishes were caught with cast net using the net throwing method (FAO 2002). Fish abundance was determined by monitoring and recording the fish catch data from both locations. The fish species caught were identified to taxonomic family level (Ayoola and Kuton 2009).

*Macrobenthic fauna sampling*

Sediment samples were collected using a Van Veen Grab, and the grab samples were sieved immediately after retrieval using a 1-mm mesh sieve. The macrobenthic fauna were sorted and identified (Saliu and Eruteya 2006; Tagliapietra and Sigovini 2010). Species diversity and abundance were determined using Shannon and Margalef indices (EGASPIN 2002). The biological monitoring working party (BMWP) score systems was used to identify sensitive and organic pollution tolerant species. The BMWP is based on the sensitivity/tolerance of aquatic invertebrates to organic pollution (that is, nutrient enrichment that can affect the availability of dissolved oxygen). The presence of mayflies or stoneflies, for instance, indicates the cleanest waterways and is given a tolerance score of 10. The lowest scoring invertebrates are worms (Oligochaeta) which score 1 (Hawkes 1998) (SM 2).

Bioassay studies

*Test animal collection and acclimatization*

Fingerlings (weight 3.25 ± 0.50 g) and juveniles (weight 9.85 ± 0.50 g) of *Clarias gariepinus* (Chordata, Osteichthyes, Siluriformes, Clariidae) were obtained from the Nigerian Institute of Oceanography and Marine Research (NIOMR), Lagos, Nigeria. The fish were transported to the Ecotoxicology laboratory in the morning in 50-L kegs containing 25 L of culture water. On arrival, the fish were distributed into large plastic tanks (20 in by 10 in) for acclimation. The plastic tanks contained the water used in transporting the fish. After the day of arrival of the fish, they were fed with 1.5-mm (fingerlings) and 3-mm (juveniles) Coppens feed. The fish were acclimatized to laboratory conditions (temperature, 26.0 ± 0.8 °C; relative humidity, 78 ± 2%; pH 7.0) for a period of 2 weeks prior the commencement of the study.

*Acute toxicity studies*

A range finding toxicity test was conducted to estimate values for the definitive toxicity tests. Four (4) *C. gariepinus* fingerlings were exposed in triplicates to differing concentrations of the effluents as follows: 40 mL/L, 80 mL/L, 160 mL/L, 240 mL/L, and 320 mL/L and control. Mortalities of the test organisms were recorded every 24 h for 4 days (96 h) (OECD 1992). A fish was considered dead when there was lack of opercula movement when prodded with a glass or plastic probe.

*Experimental design for sublethal toxicity studies*

Juveniles of *C. gariepinus* were exposed to a sublethal concentration (1/10th of the 96-h $LC_{50}$ value—12.6 mL/L) and control in duplicates. These series of bioassays went on for 28 days and the semi-static bioassay procedure was adopted (Sogbanmu and Otitoloju 2014). In the semi-static procedure, each test media was changed into a fresh solution of exactly the same concentration of test media or untreated control respectively once every 2 days, transferring the same exposed test animals into the freshly prepared test media over the 28-day period.

*Biochemical and histological evaluations in Clarias gariepinus*

For the biochemical studies, at the end of 28 days, blood samples were taken from exposed and control fishes through the caudal vasculature of the fish with a 1-mL syringe into plain red cap sample bottles. The samples were centrifuged at 3000 rpm for 10 min to obtain the supernatant. The supernatant was decanted and stored at − 21 °C until spectrophotometric determination of oxidative stress (malondialdehyde (MDA), an index of lipid peroxidation) and antioxidant enzymes (superoxide dismutase (SOD), reduced glutathione (GSH), glutathione-s-transferase (GST), and catalase (CAT) activity using UV-visible spectrophotometer (Habbu et al. 2008).

For histological evaluations, at the end of 28 days, fishes were harvested from the exposed and control media and euthanized. Gills and livers were excised and transferred into sample bottles containing Bouin's fluid, fixed for 24 h, washed in 70% ethanol, and dehydrated through a graded series of ethanol. Clearing was done in xylene followed by embedding in paraffin. They were sectioned at 4–5 μm, stained with hematoxylin and eosin and examined using light microscope (Sogbanmu et al. 2018). Slides imaging and descriptions of alterations were carried out by a pathologist.

### Statistical analysis

The results of the physico-chemical parameters were analyzed using Statistical Package for Social Sciences (SPSS) version 20. Species diversity analysis was evaluated with Margalef and Shannon-Wiener indices (Margalef 1951; Shannon and Weiner 1963) using Paleontological Statistics (PAST) software package for education and data analysis 1.97 (Hammer et al. 2001). The 96-h $LC_{50}$ value of the acute toxicity studies was computed using probit analysis (Finney 1971) on SPSS version 20. The data obtained for the sub-lethal toxicity test as mean ± standard deviation were subjected to one-way ANOVA to test for significant difference between means and difference in means were considered significant when $p < 0.05$.

## Results

### Physico-chemical parameters of abattoir effluent and from the Kara Cow Market

The dissolved oxygen level of the abattoir effluent was lower than the set limit by the National Environmental Standards and Regulations Enforcement Agency (NESREA) (Table 1). Furthermore, color, oil and grease, nitrate, ammonia, chloride, total suspended solids (TSS), and total dissolved solids (TDS) were higher than the set limits by NESREA and the Federal Environmental Protection Agency (FEPA). Other physico-chemical parameters were within set limits except for those without specified limits (Table 1).

### Physico-chemical parameters of surface water from the Ogun River near the Kara Cow Market

The pH of the surface water across the sapling points was generally acidic and lower than NESREA set limits with the lowest pH observed near the outfall of the effluent into the river (Table 2). Also, the dissolved oxygen of the surface water across the sampling points was lower than NESREA set limits. On the other hand, oil and grease, nitrate, and TSS were higher than NESREA set limits across the sampling points (Table 2).

### Physico-chemical parameters of sediments from the Ogun River near the Kara Cow Market

The values of total nitrogen, nitrate, nitrite, sulfate, ammonia, and total organic content of the sediments at the outfall and downstream of the test site were higher than the values for sediments collected from the control site (Table 3).

### Polycyclic aromatic hydrocarbons and tetracycline in abattoir effluent, surface water, and sediments from the Ogun River

The total PAH (sum of 24 individual PAHs) concentration in the effluents and sediments obtained from the outfall were 6.73 ± 0.01 mg/mL and 8.07 ± 0.01 mg/kg respectively (SM 3, SM 4, SM 5). The concentration of tetracycline was 0.23 ± 0.01 μg/mL in the effluent and 0.85 ± 0.06 μg/mL in the surface water obtained downstream (SM 6, SM 7).

### Fish species diversity in the Ogun River

Three species of catfish were found at the outfall of the effluent discharge point into the Ogun River (Fig. 3a–f). These were butter catfish, *Schilbe mystus*, bagrid catfish, *Chrysichthys nigrodigitatus*, and upside-down catfish, *Synodontis nigrata*. At the control site, four species of fish were found viz., sickle mullet, *Liza falcipinnis*, redbelly tilapia, *Tilapia zilli*, blackchin tilapia, *Sarotherodon melanotheron*, and butter catfish, *Schilbe mystus* (Fig. 3a–f).

The Margalef and Shannon-Weiner fish species diversity indices at the sampling points in the Ogun River were 0.45 and 1.09, respectively, compared to 0.71 and 1.29 for the control point. This indicates lower species richness and diversity at the Kara axis (Table 4).

### Macrobenthic fauna diversity in the Ogun River

Organic pollution tolerant species of macrobenthic organisms such as sludge worms, *Tubifex tubifex*, and midges, *Chironomus* sp., larvae were found at

**Table 1** Physico-chemical parameters of effluent from Kara Cow Market

| Parameters | Effluent | NESREA limit | FEPA limit |
|---|---|---|---|
| Color (Pt/Co) | 446.34 | NS | 7 |
| pH | 7.74 | 6.5–8.5 | 6–9 |
| Temperature (°C) | 26.5 | – | < 40 |
| Turbidity (NTU) | 104 | NS | – |
| Electrical conductivity (μS/cm) | 40140 | – | – |
| Total dissolved solids (mg/L) | 20492 | – | 2000 |
| Total suspended solids (mg/L) | 104 | 0.75 | 30 |
| Salinity (ppt) | 2.55 | – | – |
| Chloride (mg/L) | 1418.00 | 350 | 600 |
| Sulfate (mg/L) | 210.85 | 500 | 500 |
| Ammonia (mg/L) | 74.76 | 0.5 | – |
| Nitrate (mg/L) | 8.11 | 0.08 | 20 |
| Total phosphorus (mg/L) | 0.08 | – | – |
| Available phosphorus (mg/L) | BDL | – | – |
| Oil and grease (mg/L) | 193.67 | 0.1 | 10 |
| $BOD_5$ (mg/L) | 39 | 6 | 50 |
| Dissolved oxygen DO (mg/L) | 2.1 | > 4 | – |

*NS*, not specific; *BDL*, below detected level, FEPA 1991, NESREA 2011a

the outfall and downstream unlike at the control where periwinkle, *Tympanotonus fuscatus*, and freshwater snail, *Melanoides tuberculata*, were found (Table 5). Biological indices are shown in Table 5 with the control site having the highest Margalef index of 0.56 indicating higher species diversity.

**Table 2** Physico-chemical parameters of surface water from the Ogun River

| Parameters | Near outfall | Downstream | Control | Range | NESREA |
|---|---|---|---|---|---|
| Color (Pt/Co) | 386.68 | 353.02 | 467.75 | 353.02–467.75 | – |
| pH | 5.32 | 5.83 | 6.11 | 5.32–6.11 | 6.5–8.5 |
| EC (μS/cm) | 10980 | 10600 | 10550 | 10550–10980 | – |
| TDS (mg/L) | 5015 | 4899 | 4776 | 4776–5015 | – |
| TSS (mg/L) | 32.00 | 29.00 | 41.00 | 29–41 | 0.25 |
| Turbidity (NTU) | 45.50 | 48.50 | 48.00 | 45.5–48 | – |
| Salinity (ppt) | 0.06 | 0.03 | 0.19 | 0.03–0.19 | – |
| $BOD_5$ (mg/L) | 2.90 | 4.50 | 2.00 | 2.0–4.5 | 3.0 |
| Dissolved oxygen (mg/L) | 5.10 | 5.40 | 4.90 | 4.90–5.40 | > 6.0 |
| Chloride (mg/L) | 35.45 | 17.725 | 106.35 | 35.45–106.35 | 300 |
| Sulfate (mg/L) | 11.96 | 9.46 | 9.18 | 9.18–11.96 | 100 |
| Ammonia (mg/L) | 2.85 | 2.67 | 2.95 | 2.67–2.95 | – |
| Nitrate (mg/L) | 1.95 | 1.80 | 1.70 | 1.70–1.95 | 0.02 |
| Acidity (mg/L) | 14.00 | 20.00 | 16.00 | 14.00–20.00 | – |
| Alkalinity | 260.00 | 280.00 | 250.00 | 250.00–280.00 | – |
| Bicarbonate (mg/L) | 317.20 | 341.60 | 305.00 | 217.20–341.60 | – |
| Total phosphorus (mg/L) | 0.01 | 0.07 | 0.01 | 0.01–0.07 | – |
| Available phosphorus (mg/L) | BDL | BDL | BDL | BDL | – |
| Oil and grease (mg/L) | 4.17 | 4.17 | 4.85 | 4.17–4.85 | 0.01 |

*BDL*, below detected level, NESREA 2011b

**Table 3** Physico-chemical of sediments from the Ogun River

| Parameters | Outfall | Downstream | Control | Range |
|---|---|---|---|---|
| pH | 6.95 | 6.91 | 7.40 | 6.91–7.40 |
| Electrical conductivity (μS/cm) | 352.00 | 322.00 | 283.00 | 322.00–352.00 |
| $SO_4^{2-}$ (ppm) | 5.54 | 5.40 | 1.18 | 1.18–5.54 |
| Total nitrogen (%) | 0.41 | 0.31 | 0.27 | 0.27–0.41 |
| $NO_3$–N (ppm) | 1.25 | 1.37 | 1.20 | 1.20–1.37 |
| $NO_2$–N (ppm) | 0.13 | 0.09 | 0.08 | 0.08–0.13 |
| $NH_4$–N (ppm) | 1.24 | 1.06 | 0.93 | 0.93–1.24 |
| Total phosphorus (%) | 0.00 | 0.00 | 0.00 | 0.00 |
| Exchangeable acidity (Cmol/kg) | 1.10 | 1.09 | 0.80 | 0.80–1.10 |
| TOC (%) | 7.47 | 6.44 | 0.12 | 0.12–7.47 |
| Oil and grease (ppm) | 5.44 | 5.68 | 5.46 | 5.44–5.68 |

### Biochemical indices in *Clarias gariepinus* exposed to Kara Cow Market effluent

The derived 96-h $LC_{50}$ value of the abattoir effluent was 126.00 ml/L (12.6%) (Table 6). Malondialdehyde, glutathione-s-transferase, and superoxide dismutase values were significantly lower ($p < 0.05$) in the blood of effluent-treated catfish compared to the control group (Table 7) after 28 days of exposure to sublethal concentration (12.6 mL/L) of the effluent. Also, reduced glutathione and catalase values were lower in the blood of effluent-treated catfish compared to the control group but there were no significant differences between them ($p > 0.05$) (Table 7).


**Fig. 3** **a**–**f** Pictures of fish species collected from the Ogun River showing (Fig. 3a) *Chrysichthys nigrodigitatus* (bagrid catfish); (Fig. 3b) *Schilbe mystus* (butter catfish); (Fig. 3c) *Synodontis nigrata* (upside down catfish) found at the Kara axis in the Ogun River; (Fig. 3d) *Liza falcipinnis* (sickle mullet), (Fig. 3e) *Sarotherodon melanotheron* (blackchin tilapia), (Fig. 3f) *Tilapia zilli* (redbelly tilapia) found at the control site in the Ogun River

**Table 4** Fish species diversity in the Ogun River

| Family | Species | Common name | Effluent outfall | Control | Total |
|---|---|---|---|---|---|
| Schilbeidae | *Schilbe mystus* | Butter catfish | 31 | 6 | 37 |
| Bagridae | *Chrysichthys nigrodigitatus* | Bagrid Catfish | 23 | 0 | 23 |
| Mochokidae | *Synodontis nigrata* | Upside down Catfish | 29 | 0 | 29 |
| Mugilidae | *Liza falcipinnis* | Sickle mullet | 0 | 20 | 20 |
| Cichlidae | *Tilapia zilli* | Redbelly tilapia | 0 | 17 | 17 |
| | *Sarotherodon melanotheron* | Blackchin tilapia | 0 | 25 | 25 |
| Total | | | 83 | 68 | 151 |
| Fish species diversity indices | | Effluent outfall | | Control | |
| Taxa_S | | 3 | | 4 | |
| Individuals | | 83 | | 68 | |
| Dominance_D | | 0.34 | | 0.29 | |
| Shannon_H | | 1.09 | | 1.29 | |
| Simpson_1-D | | 0.66 | | 0.71 | |
| Evenness_e^H/S | | 0.99 | | 0.91 | |
| Menhinick | | 0.33 | | 0.49 | |
| Margalef | | 0.45 | | 0.71 | |
| Equitability_J | | 0.99 | | 1.00 | |
| Fisher_alpha | | 0.61 | | 0.93 | |
| Berger-Parker | | 0.37 | | 0.37 | |

**Table 5** Macrobenthic fauna in the Ogun River

| Family | Species | Common name | Outfall | Downstream | Control | Total |
|---|---|---|---|---|---|---|
| Naididae | *Tubifex tubifex* | Sludge worm | 6 | 9 | – | 15 |
| Chironomidae | *Chironomus* sp. Larvae | Midges | 18 | 11 | – | 29 |
| Potamididae | *Tympanotonus fuscatus* | Periwinkle | – | – | 5 | 5 |
| Thiarida | *Melanoides tuberculata* | Freshwater snail | – | – | 1 | 1 |
| Total | | | 24 | 20 | 6 | 50 |
| Diversity indices for the macrobenthic fauna | | | Outfall | Downstream | Control | |
| Taxa_S | | | 2 | 2 | 2 | |
| Individuals | | | 22 | 20 | 6 | |
| Dominance_D | | | 0.60 | 0.51 | 0.72 | |
| Shannon_H | | | 0.59 | 0.69 | 0.45 | |
| Simpson_1-D | | | 0.40 | 0.50 | 0.28 | |
| Evenness_e^H/S | | | 0.90 | 1.00 | 0.78 | |
| Menhinick | | | 0.44 | 0.45 | 0.82 | |
| Margalef | | | 0.32 | 0.33 | 0.56 | |
| Equitability_J | | | 0.85 | 1.00 | 0.65 | |
| Fisher_alpha | | | 0.53 | 0.55 | 1.05 | |
| Berger-Parker | | | 0.73 | 0.55 | 0.83 | |

**Table 6** Acute toxicity results of effluent from Kara Market against *Clarias gariepinus*

| Treatment (mL/L) | 96-h $LC_{50}$ | Slope ± S.E. | Probit line equation |
|---|---|---|---|
| Kara Cow Market effluent | 126.00 (12.6%) | 4.97 ± 1.24 | $Y = 5.44 + 4.97x$ |

*C.L.* confidence limit, *S.E.* standard error, *D.F.* degree of freedom, $LC_{50}$ median lethal concentration of test chemical to test organisms

## Histological indices in *Clarias gariepinus* exposed to Kara Cow Market effluent

The photomicrographs of the gills and liver of the control and effluent-treated *C. gariepinus* showed no alterations in the tissues (Fig. 4a–d). After 28 days of exposure, normal primary lamella (PL) and secondary lamella (SL) were observed in the gills of both control and effluent-treated *C. gariepinus* (Fig. 4a, b) and also, normal hepatocytes were observed in the liver of both control and effluent-treated *C. gariepinus* (Fig. 4c, d).

## Discussion

A number of anthropogenic activities such as the large-scale processing of livestock that are carried out in abattoirs have created local and regional pollution challenges in most parts of Africa and the world (Adeogun 2012). The consequences of these are environmental degradation and ecosystem disruptions (Sainz et al. 2004).

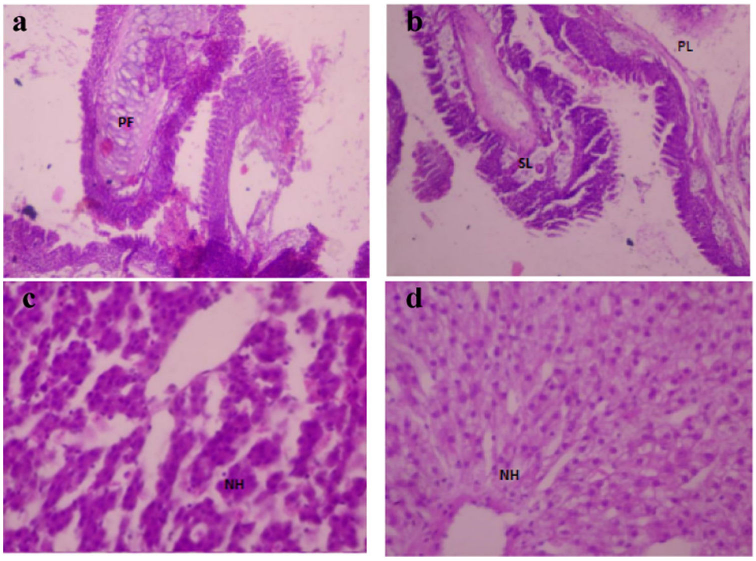
In this study, physico-chemical parameters of the Kara abattoir effluent such as total suspended solids, chloride, ammonia, nitrate, oil and grease, biochemical oxygen demand, and dissolved oxygen were not within the national regulatory limits for effluent discharges into surface waters (NESREA 2011a). Most of these parameters are characteristic of organic enrichment and agrees with Adeogun et al. (2013) who reported similar results with abattoir effluent released into the Upper Ogun River. High inputs of organic contaminants and nutrients can result in eutrophication of freshwater bodies and reduced dissolved oxygen due to the high level of oxygen required for the breakdown of organic components in the effluent. The high levels of chlorides in the effluent may be attributed to the detergents that are used in washing the slaughtered and roasted cows (Adeogun et al. 2013). Furthermore, pH, total suspended solids, oil and grease, DO, and nitrates were not within the national permissible limits for the support of fisheries and aquatic organisms in surface waters (NESREA 2011b). These results are similar to those reported by Alani and Ukoakonam (2014) in surface water from the Ogun River. The acidity of the surface water may be due to humic acid resulting from decaying organic matter from the effluents and organic-rich solid wastes discharged into the Ogun River from the Kara Cow Market. Furthermore, since the effluent is discharged untreated into the river with its pollutant-rich content, the surface water is consequently adversely impacted with deleterious effects on aquatic fauna in the river. These results thus justify the presence of the invasive plant, water hyacinth (*Eicchornia crassipes*) which thrives in nutrient and organic-rich water bodies (Rommens et al. 2003). This further impacts on the levels of DO and the BOD of the river as less oxygen is available for aquatic fauna to thrive in the river. In the sediments, TOC, nitrates, ammonia, and other organic contaminants were high at the test sites following the trends observed in the effluent and surface water. This result substantiates the reasons for the sudden overgrowth of *E. crassipes* in the Ogun River at the Kara Market axis as reported in June 2016 (National Daily Newspaper 2016).

The levels of total PAHs in this study were higher in the sediments than in the effluent. This agrees with

**Table 7** Biochemical biomarkers in the blood of *Clarias gariepinus* exposed to sublethal concentration of the Kara abattoir effluent over a period of 28 days

| Treatment (U/mg protein) | MDA | GSH | GST | SOD | CAT |
|---|---|---|---|---|---|
| Control | 0.07 ± 0.00 | 2.11 ± 0.00 | 1.76 ± 0.01 | 5.78 ± 0.00 | 26.41 ± 1.32 |
| Effluent (12.6 mL/L) | 0.04 ± 0.00* | 1.94 ± 0.09 | 1.63 ± 0.02* | 5.34 ± 0.08* | 24.39 ± 2.66 |

Each value represents mean ± S.D.; $n = 10$; *Significant difference in the means at $p < 0.05$; *U*, μmol/mL/min (GSH, SOD, CAT); μmol/ml (MDA, GST), *MDA*, malondialdehyde, *GSH* reduced glutathione, *GST* glutathione-s-transferase, *SOD* superoxide dismutase, *CAT* catalase




**Figs. 4 a-d** Photomicrograph of histological section through the gills and livers of *Clarias gariepinus* after 28 days in control and exposed media. **a** Gill in control fish showing normal primary filament (PF) (H & E × 100). **b** Gill in effluent-treated (12.6 mL/L) fish showing normal primary lamella (PL) an secondary lamella (SL). No areas of necrosis or inflammation are seen (H & E × 100). **c** Liver in control fish showing normal hepatocytes (NH) (H & E × 100). **d** Liver in effluent-treated (12.6 mL/L) fish showing radially arranged plates of normal hepatocytes (NH). No cytoplasmic fat vacuoles and no abnormalities were seen (H & E × 100)

Ogbonna and Ideriah (2014) who reported elevated levels of PAHs in soil samples around abattoirs than in the wastewaters. A reason for this could be the hydrophobic/lipophilic nature of PAHs as they are known to accumulate more in sediments than in the water column (Sogbanmu et al. 2016). Furthermore, the higher levels of PAHs in the sediments could be related to the high levels of TDS and TSS in the abattoir effluent and surface water of the Ogun River with final sink in the sediment. The detection of PAHs in this study is principally due to the burning activities at the abattoir during the slaughtering and roasting of cows for consumers. The charred materials are consequently washed off into the nearby river majorly during rainfall. The pollutants consequently bind to the organic-rich sediments where they accumulate and are potentially bioavailable to sediment-dwelling fauna, demersal fisheries, and detritus feeders (Daka and Ugbomeh 2013; Abdel-Shafy and Mansour 2016). The lipophilic nature of PAHs, particularly the high molecular weight PAHs which were mainly detected in this study, may result in biomagnification of the accumulated PAHs along the food chain and across food webs in the Ogun River.

The presence of pharmaceutical residues in wastewater is raising concerns because the wastewater such as that which is discharged from the Kara Cow Market is being released indiscriminately into water bodies. Although, there is not enough information on the fate of these emerging pollutants, scientists have discovered antibiotic-resistant strains of microorganisms in wastewaters containing these residues (Bougnom et al. 2019; Diallo et al. 2017). These pharmaceutical residues have been discovered in groundwater, which contaminate drinking water (Lapworth et al. 2012; Rivera-Utrilla et al. 2013). Pharmaceutical residues and/or their metabolites are usually detected in the environment at trace levels, but, even the low levels (ng/L or μg/L) have the potential to induce toxic effects (Hernando et al. 2006) and subtoxic but population-relevant effects in organisms (Brodin et al. 2013). In this study, the antibiotic (tetracycline) was detected at higher levels in the surface water ($0.85 \pm 0.06$ μg/mL) compared to the effluent ($0.23 \pm 0.01$ μg/mL). In studies conducted in China, antibiotic levels found in the effluent ranged between 0.009 and $2.054 \times 10^{-3}$ μg/mL (Xu et al. 2007a). Another study carried out on nine selected antibiotics present in the surface water of Victoria harbor, Hong Kong, and the Pearl River, Guangzhou, South China, had median concentrations ranging from 0.011 to $0.067 \times 10^{-3}$ μg/mL and 0.066 to $0.460 \times 10^{-3}$ μg/mL, respectively (Xu et al. 2007b). Wei et al. (2011) reported a broad profile of veterinary antibiotic residues in animal wastewater and surface water around large-scale livestock and poultry farms in Jiangsu Province of China.

The most detectable antibiotics found included sulfamethazine (75%), oxytetracycline (64%), tetracycline (60%), sulfadiazine (55%), and sulfamethoxazole (51%) with maximum concentration of 0.211 μg/mL for sulfamethazine and 0.0103 μg/mL for tetracycline. The higher levels of tetracycline detected in this study could be due to indiscriminate and non-regulated administration of this antibiotic to the cows in order to prevent diseases. These studies substantiate the presence and levels of these pharmaceutical residues in surface waters with potential risks to humans who utilize the water for various uses including as drinking water. Recent studies have proposed that antibiotics at sub-inhibitory concentrations are like signaling molecules that mediate numerous cell processes such as biofilm formation, quorum sensing, gene transcription, and expression (Sengupta et al. 2013; Andersson and Hughes 2014; Balcazar et al. 2015). Thus, antibiotics in the environment are an ecological factor that could potentially upset microbial communities. Some studies have discovered alterations of microbial community structure with the inclusion of antibiotics in soil and water environment. Some effects of these changes are phylogenetic structure alteration, resistance expansion, and ecological function disturbance (including disturbances in nitrogen transformation, methanogenesis, and sulfate reduction) in the micro-ecosystem (Ding and He 2010; Grenni et al. 2018). Furthermore, acute toxic effects including gill histological alterations and proxidative activity have been reported in the freshwater fish species, *Gambusia holbrooki*, exposed to tetracycline (Nunes et al. 2015). Similarly, chronic exposure to environmental levels and aquaculture doses of oxytetracycline and sulfamethoxazole causes physiological dysfunctions, impairment of nutritional metabolism, and fish immune system in the Nile tilapia (*Oreochromis niloticus*). A concomitant hazard risk quotient greater than 1 is implied in children who consume fish treated with legal aquaculture doses of oxytetracycline (Limbu et al. 2018).

Catfish are known to be able survive in adverse conditions. This is due to their ability to breathe through their skin and use their air bladder as an emergency lung by gulping surface air (Patra et al. 1983). The presence of the catfish family at the test site may be an indication of low oxygen availability at the Kara axis of the Ogun River which is as a result of the continuous discharge of the effluent into the river from the abattoir. In a study by Saliu and Eruteya (2006), *Chironomus* sp. and *Tubifex tubifex* were found in the gutters at the sample sites where the studies were conducted. These species of macrobenthic fauna are known to be tolerant to organic pollution. Likewise, these two species were found at the Kara axis of the Ogun River in this study suggestive of organic pollution at the test site. According to the Biological Monitoring Working Party BMWP Score table (Mason 2002), the pollution tolerant family, Chironomidae, which is a bioindicator of organic pollution has a score of 2 indicating the presence of organic pollutants. The low species diversity and presence of *Chironomus* sp. and *Tubifex tubifex* observed at the test site corroborates the presence of organic pollution at the Kara axis of the Ogun River. Consequently, the study reflects the poor condition of the river at the Kara Market axis, as the ecological integrity of the river is being compromised due to the continual discharge of the untreated abattoir effluent into it.

The median lethal concentration (12.6%/126 mL/L) of the abattoir effluent to *C. gariepinus* in this study is higher than that reported for *Poecilia reticulata* (7.15%/71.5 mL/L) exposed to effluent from the Kara Market abattoir (Sosanwo 2016). The disparity in the values could to be due to the difference and size of the fish species used in the two studies. Also, *C. gariepinus* being a more tolerant fish species compared to *P. reticulata* may be a reason for the higher median lethal concentration in it compared to *P. reticulata*. On the other hand, a more toxic median lethal concentration (6.28%/62.8 mL/L) was reported by Alimba et al. (2015) in *C. gariepinus* fingerlings exposed to Bodija abattoir wastewater. These variations may be due to the varying composition of toxicants in effluents from the two abattoirs (Kara and Bodija abattoir), time of sample collection, and chemical interactions that may have occurred in the effluent, which could produce synergistic, additive, or antagonistic effects in the test organism, thereby resulting in the inconsistency of the median lethal concentration.

Antioxidant enzymes such as glutathione peroxidase and superoxide dismutase prevent oxidative stress (Ognjanovic et al. 2008). In particular, the level of antioxidant enzyme is a satisfactory indicator for the impacts of pollutants that result in the formation of reactive oxygen species (Ates et al. 2008). In this study, the significant reductions in the levels of antioxidant enzymes, GST and SOD, compared to the control. This may be due to an elevation in their activities shortly following exposure to the effluent in response to the reactive oxygen species being produced and consequent depuration as the oxidative effects were being counteracted (Carney Almroth 2008). A similar result in which antioxidant enzymes revealed few changes in rainbow trout exposed to effluents from a sewage treatment plant has been reported (Carney Almroth et al. 2008). The reduced levels of GSH though not significant compared to the control may be in response to counteract the effects of reactive oxygen species due to oxidative stress in the blood of the fish species. The antioxidant responses are reflected in the significant reductions in MDA levels, an index of lipid peroxidation, in treated *C. gariepinus* compared to the control. Similarly, the lack of histological alterations may be attributed to the counteraction of oxidative stress in the fish species by the antioxidant enzymes as shown in this study.

## Conclusions

This study provides insights into the impacts of abattoir activities at the Kara Cow Market on the water quality and animal species diversity of the Ogun River. These activities have resulted in oxygen depletion of that part of the Ogun River, thereby making it unsuitable for the survival of a variety of aquatic organisms. In essence, the biodiversity of that part of the Ogun River is low and can only sustain aquatic species that are tolerant to organic pollution. Furthermore, the high input of nitrate, sulfates, phosphorus, and organic matter into the river results in enrichment of the river with these nutrients which encourages the recalcitrant growth of invasive species most especially water hyacinth, *Eichhornia crassipes*. In addition, various organisms play vital roles in sustaining the integrity of an ecosystem; thus, the absence of some sensitive/non-tolerant species in this part of the Ogun River may have effects on the stability of the ecosystem.

The results demonstrate that the effluent from the Kara Cow Market has potential adverse effects on aquatic organisms and ecosystem services provided by the river based on the species diversity and physicochemical parameters results. Though the sublethal effects study did not show significant biological effects in the test animal, acute exposure to high concentrations of the effluent has adverse impacts on fisheries as demonstrated in the acute toxicity results. We recommend further studies to evaluate acute and chronic biomarkers at lower levels of organization in sensitive fish and macrobenthic species. There is an urgent need for environmental risks communication to stakeholders at the market particularly concerning the use of antibiotics and environmental management. Environmental regulatory agencies and policy makers should implement effluent and solid wastes management systems at the market to prevent environmental and public health epidemics within the framework of the United Nations Sustainable Development Goals 6 (clean water and sanitation) and 14 (life below water).

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This study followed the principles in the Declaration of Helsinki on the humane treatment of animals used in research (http://www.wma.net/en/30publications/10policies/a18/) and the principles in the AVMA Guidelines for the euthanasia of animals (AVMA 2013).



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