Contents

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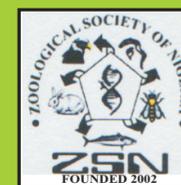
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Histological, microbiological, physicochemical and heavy metals evaluation of effluent from Kara Cow Market, Ogun State, Nigeria in guppy fish (*Poecilia reticulata*)

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Abstract

The Ogun River is a sink for untreated effluents discharged from the Kara Cow Market, Ogun State. In this study, microbiological, physicochemical and heavy metals evaluation were carried out on effluent from the market. Standard methods were utilised for the examination of physicochemical and microbiological parameters of the effluent. The histological effects of sublethal concentrations of the effluent were assessed in the gills, intestine and skin of *Poecilia reticulata* (guppy fish) over a period of 56 days following standard methods and international ethical guidelines. None of the physicochemical parameters were within the set limits by the National Environmental Standards and Enforcement Agency (NESREA) for effluent discharges into surface waters. Coliforms, pathogens and fungi were observed in the effluent with a total plate count of 2.14×10^7 cfu/mL. The median lethal concentration (96 h LC_{50}) of the effluent to *P. reticulata* was 71.50 mL/L (7.15%). Histological alterations such as distorted mucosal architecture with shortening and widening of intestinal villi were observed in the intestine of exposed groups of *P. reticulata* at day 56. The observed adverse physicochemical parameters, histological alterations and pathogenic microbes may pose ecological and public health risks to aquatic organisms and humans respectively. It is recommended that an effluent treatment plant should be installed at the market to reduce the pollutants load of the effluent before discharge into the Ogun River.

Keywords: Effluents; histological effects; Kara Market; Ogun River; physicochemical parameters; pathogenic microorganisms; *Poecilia reticulate.*

Introduction

Ogun River is a valuable resource to its surrounding inhabitants as it is a source of food such as fish and water for drinking and domestic uses. Furthermore, the river provides a suitable ecosystem for freshwater animals amongst other ecosystem services. However, it serves as a sink for the discharge of mostly untreated effluents from various industries and communities that are bound by it. The nature of the industries and other anthropogenic activities that discharge effluents into the river is of concern due to the increase in the ubiquity of emerging pollutants like pharmaceuticals, personal care products and endocrine disrupting compounds in aquatic ecosystems (Ibor *et al,* 2016).

A study by Alani *et al* (2014) had documented the impact of the effluents from the market on the physicochemical properties of Ogun River with major heavy metals exceeding the WHO and Federal Ministry of Environment (FMEnv) limits in the water and sediments. Effluents from abattoirs are a reservoir of pathogens (*Salmonella* spp.,

Escherichia coli, Campylobacter spp.) (Adebowale *et al* 2016; Ayoade and Olayioye, 2016), organic contaminants, persistent organic pollutants, antibiotics amongst others. The biological effects of these pollutants include endocrine disruption, increase in disease burden of fisheries and antibiotic resistance (Ibor *et al,* 2016). Biochemical, physiological and histological indices in fish can reflect the health of aquatic ecosystems (Athira and Jaya, 2018; Olaniran *et al,* 2019). Few studies have been conducted to evaluate the histological effects of the Kara Cow Market effluent in freshwater fishes like the guppy fish, *Poecilia reticulata*. Also known as million fish or rainbow fish, *P. reticulata* is a common freshwater aquarium fish. It has a transparent body covered with colourless scales and possesses ornamental dorsal and caudal fins (Mokhtar, 2015). They are model organisms commonly utilised in ecological, evolutionary and behavioural studies (Magurran and Phillip, 2008).

The pollution load and impact of the Kara Market Abattoir effluent on the physicochemical parameters of
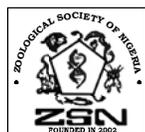
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the Ogun River has increased over the years (Udusoro, 1997; Oketola *et al* 2006; Adeogun *et al* 2011; Alani *et al* 2014). Hence, the objectives of this study were to assess physicochemical, heavy metals and microbiological parameters of the effluent from the abattoir at Kara Market as well as to determine the acute toxicity and histological effects of sublethal concentrations of the effluent on a model freshwater fish, *Poecilia reticulata* (Guppy fish).

Materials and methods

Study area and collection of effluent from Kara Cow Market, Ogun State

Kara Cow Market is located on the outskirts of Lagos, along Lagos-Ibadan Expressway near the Ojodu Berger Bus Stop. The area bound by the Ogun River is characterized by the presence of visible and foul-smelling abattoir effluent (Plate 1a), solid wastes near the river bank and the invasive plant, water hyacinth (*Eicchornia crassipes*) (Plate 1b). The effluent was collected once in January 2016 from the discharge point of the abattoir at the Kara Market into the Ogun River (6° 382 48.03 N, 3° 222 46.53 E) (Plate 1b, Plate 2) into a 5 L pre-cleaned plastic container and refrigerated in the laboratory at 4°C until use for bioassays (Adeogun and Chukwuka, 2011).
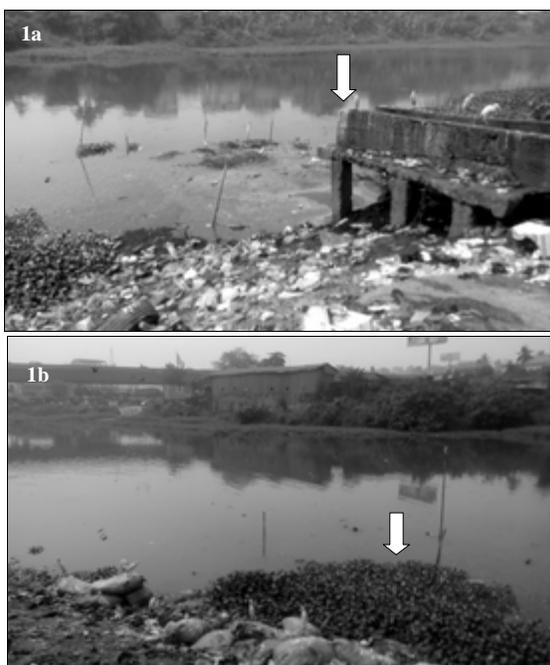
Plates 1a-b: The outfall/point of discharge (arrow) of effluent from Kara Cow Market into the Ogun River (1a), water hyacinth (*Eicchornia crassipes*) and solid wastes on the bank of the Ogun River (1b).

A portion of the effluent was transported to the Chemistry Laboratory at the University of Lagos for physicochemical analyses. For microbiological studies, the effluent was aseptically collected in a sterile container (Ayoade and Olayioye, 2016) and transported to the Microbiology Laboratory, University of Lagos, Lagos, Nigeria for analysis.

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Plate 2: Map of the study area at Kara Market showing the effluent discharge point into the Ogun River (*Source:* Google Maps, 2019).

Physicochemical and microbiological analyses of effluent from Kara Cow Market

The physicochemical and heavy metals analyses of the effluent were conducted according to APHA (2005). The physicochemical parameters analysed were pH, temperature, dissolved oxygen (DO), oil and grease, biological oxygen demand (BOD), chemical oxygen demand (COD) and total suspended solids (TSS). The heavy metals analysed using the Flame Atomic Absorption Spectrophotometer (Perkin Elmer A Analyst 200) were, lead; zinc; iron; copper; manganese; cobalt; cadmium and nickel. The microbiological evaluation of the effluent was conducted using the multiple tube technique. Coliforms, pathogens and fungi/yeast were identified according to Inglis and Cohen (2002) (Table 1).

Table 1: Media used for the microbiological evaluations of Kara Cow Market effluent.

Microorganism Group		Media
Coliforms		MacConkey Agar
		Eosin Methylene blue Agar
Pathogens	*Staphylococci*	Mannitol Salt Agar
	Clostridia	Reinforced Clostridial agar
	Salmonella-shigella	Salmonella-shigella agar
	Vibrio cholerae	TCBS Agar
Fungi/Yeast		Potato dextrose Agar
Total Plate Count		Nutrient Agar

Key: TCBS agar – Thiosulfate Citrate Bile Salts sucrose agar.

Test animal collection and acclimatization

The freshwater fish, *P. reticulata* (Osteichthyes, Cyprinodontiformes, Poeciliidae) (weight: 0.20±0.05 g; length: 2.50±0.50 cm; *n*=100) were collected with a scoop net from an open drain at the back gate of the University of Lagos. They were transported to the laboratory in a plastic bucket half-filled with water from the point of collection. In the laboratory, they were transferred into two (2) holding tanks (28 x 51 x 29 cm) that were filled to one-quarter level with dechlorinated tap water and they were allowed to acclimatize to laboratory conditions (temperature: 26.0 ± 0.8 °C; relative humidity: 78 ± 2%; pH-7.0) for two weeks before using them in bioassays. The fish were fed twice daily *ad libitum* with commercial Coppens fish feed (size: 0.1-0.5 mm) according to Sogbanmu and Otitoloju (2014). The water in the holding tanks was replenished once every 48 h to prevent accumulation of wastes. Feeding was stopped 24 h prior to exposure of the fish to test media (Adeogun and Chukwuka, 2011).

Relative acute toxicity of effluent from Kara Market against Poecilia reticulata

Bioassay tanks used for acute toxicity were clean square-shaped glass tanks (volume – 3 L). Preliminary tests in which test animals were held in varying volumes of dechlorinated water showed that four (4) fish comfortably survived in 1 L of water for more than 4 days (96 h). Therefore, the test media were always made up to 1 L, holding 4 fishes in each bioassay tank. Four (4) active fish of similar sizes were randomly selected from the holding tanks with a plastic sieve and exposed in duplicates to varying concentrations (20, 40, 80, 120, 160 mL/L and untreated control) of the effluent in a static system for 96 h for the definitive test. The concentrations utilised for the definitive test were estimated following range finding tests which were conducted to establish the dose range for the effluent (Sogbanmu and Otitoloju, 2014). Standard procedures for bioassays were followed APHA/AWWA/WPCF (1995). Mortality was assessed once every 24 hours cumulatively for 4 days. A fish was considered dead when it failed to respond to mechanical touch (Jenyo-Oni, 2011).

Sublethal toxicity studies: experimental design and histological studies of Kara Cow Market effluent on Poecilia reticulata

In this experiment, *P. reticulata* were exposed to sublethal concentrations (1/100th and 1/10th of 96 h LC_{50} - 0.7 mL/L and 7.2 mL/L respectively) of the effluent for a period of 56 days in a static-renewal bioassay (Sogbanmu and Otitoloju, 2014). A total of twenty (20) fish were exposed in triplicates to the sublethal concentrations and untreated control. The test media were renewed every 48 h to avoid accumulation of excretory wastes in the medium.

At 56th day post exposure (after commencement of the experiment), fish from the test media were harvested and euthanized. The gills, intestine and skin were excised and immediately transferred into sample bottles containing the fixative – Bouin's fluid. Tissue processing was conducted according to Sogbanmu *et al* (2018). Briefly, tissues and organs were first dehydrated in increasing percentages of ethanol (50%, 70% and absolute) followed by clearing in xylene and embedding in paraffin wax. Thereafter, sections were cut using a rotary microtome at 4 μm thickness. Sections were then rehydrated in decreasing levels of ethanol and finally water. Following this was staining with haematoxylin and eosin (H and E) and mounting with DPX.

Ethical consideration

All applicable international guidelines (AVMA, 2013) for the care and use of animals were followed including guidelines for research involving fish or other aquatic animals (http://fisheries.org/policy-media/science-guidelines/guidelines-for-the-use-of-fishes-in-research/).

Data analysis

The dose-response data involving quantal response (mortality) from the acute toxicity studies were subjected to probit analysis (Finney, 1971) using SPSS version 20. Microsoft Excel Version 2010 was used to analyse the descriptive data obtained from the questionnaire.

Results

The physicochemical parameters results revealed that the effluent was slightly acidic (pH – 5.64) and low in dissolved oxygen (DO – 2 mg/L). The temperature was 26.70 ³. The BOD and COD were 37 and 64 mg/L respectively. The level of oil and grease was 6.32 mg/L while TSS was 3.99 mg/L. Heavy metals concentration ranged from 0.03 mg/L for nickel to 2.63 mg/L for zinc (Table 2).

Table 2. Physicochemical and Heavy metals Parameters of Kara Cow Market effluent.

Parameter (mg/L)	Result	NESREA Limit (for effluent discharges into surface water)
pH	5.64	6.5-8.5
Temperature (°C)	26.70	-
Dissolved Oxygen	2.00	>6
Biological Oxygen Demand	37.00	3
Chemical Oxygen Demand	64.00	30
Oil and Grease	6.32	0.01
Total Suspended Solids	3.99	0.25
Heavy Metals (mg/L)	Result	NESREA Limit
Lead	0.04	0.01
Zinc	2.63	0.01
Iron	1.37	0.05
Copper	1.67	0.001
Manganese	0.73	-
Cobalt	0.14	-
Cadmium	0.07	0.005
Nickel	0.03	0.01

Key: NESREA (National Environmental Standards and Regulations Enforcement Agency), 2011 – Fisheries and recreation water quality criteria standards effluent discharges into surface water.

The microbiological characterization of the effluent showed the presence of coliforms (*Escherichia coli* and *Kliebsiella* spp. – 1.60 x 10^3 cfu/mL), pathogens (*Staphylococcus aureus* (1.40×10^2) and *Shigella* spp. (1.10 x10^2 cfu/mL) and fungi/yeast (*Aspergillus niger*, *Fusarium* spp. and *Trichoderma* spp. – 10.00 x 10^3 cfu/mL). The total plate count comprising *Bacillus* spp., *Lactobacillus* spp., *Pseudomonas aeruginosa* and *Micrococcus* spp. was 2.14 x 10^7 cfu/mL (Table 3).

Table 3. Microbiological characteristics of Kara Cow Market effluent.

Microbial Group	Microorganisms Isolated	Results (cfu/mL)	NESREA
Coliforms	*Eschericia coli* *Klebsiella* spp.	1.60×10^3	
Pathogens	*Staphylococcus aureus* *Shigella* spp.	1.40×10^2 1.10×10^2	Must be absent
Fungi / Yeast	*Aspergillus niger* *Fusarium* spp. *Trichoderma* spp.	10.00×10^3	
Total Plate Count	*Bacillus* spp. *Lactobacillus* spp. *Pseudomonas aeruginosa* *Micrococcus* spp.	2.14×10^7	

The result of the relative acute toxicity of the effluent against *Poecilia reticulata* was 71.50 mL/L (7.15%) (Table 4).

Table 4. Acute toxicity results of effluent from Kara Market against *Poecilia reticulata*.

Treatment (mL)	96 h LC_{50} (95% C.L.)	Slope ± S.E.	Probit Line Equa.	d.f.
Kara Cow Market Effluent	71.50 (57.41– 92.78)	6.24 ± 1.85	$y = -11.57 + 6.24x$	3

Key: C.L.: Confidence Limit, S.E: Standard Error, D.F: Degree of Freedom, LC_{50}: median lethal concentration of test chemical to test organisms.

There were no histological alterations in gills of *P. reticulata* in the control and exposed groups by day 56 (Plates 3a-c).

In the intestine of *P. reticulata* exposed to sublethal concentrations of the effluent, distorted mucosal architecture with shortening and widening of the intestinal villi were observed at day 56 (Plates 4a-c).

The skin of *P. reticulata* in control and exposed groups were observed to be normal at day 56 (Plates 5a-c).
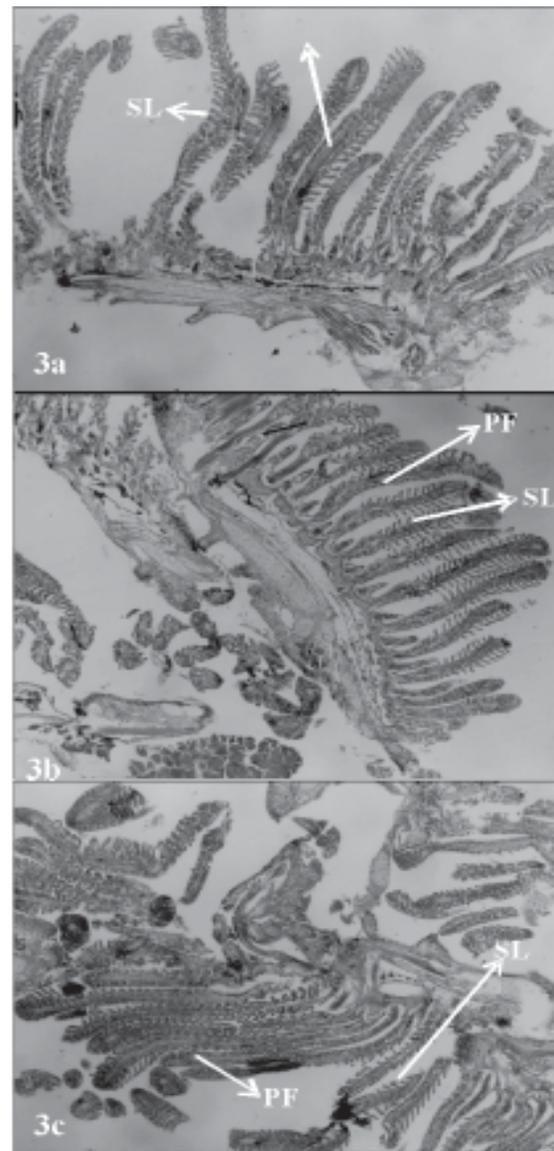
Plates 3a-c: Photomicrographs of sections through the gills of *Poecilia reticuata* exposed to sublethal concentrations of effluent from Kara Cow Market, Ogun state and the control at day 56. **Notes**: PF – Primary Gill Filament, SL – Secondary Lamella.

Key:
3a: Section through the gills of *Poecilia reticulata* in control group showing normal gill architecture at day 56, H and E stain, x100.
3b: Section through the gills of *Poecilia reticulata* in exposed (0.7 mL/L) group with no histological alterations at day 56.
3c: Section through the gills of *Poecilia reticulata* in exposed (7.2 mL/L) group showing normal gill architecture at day 56, H and E stain, x100
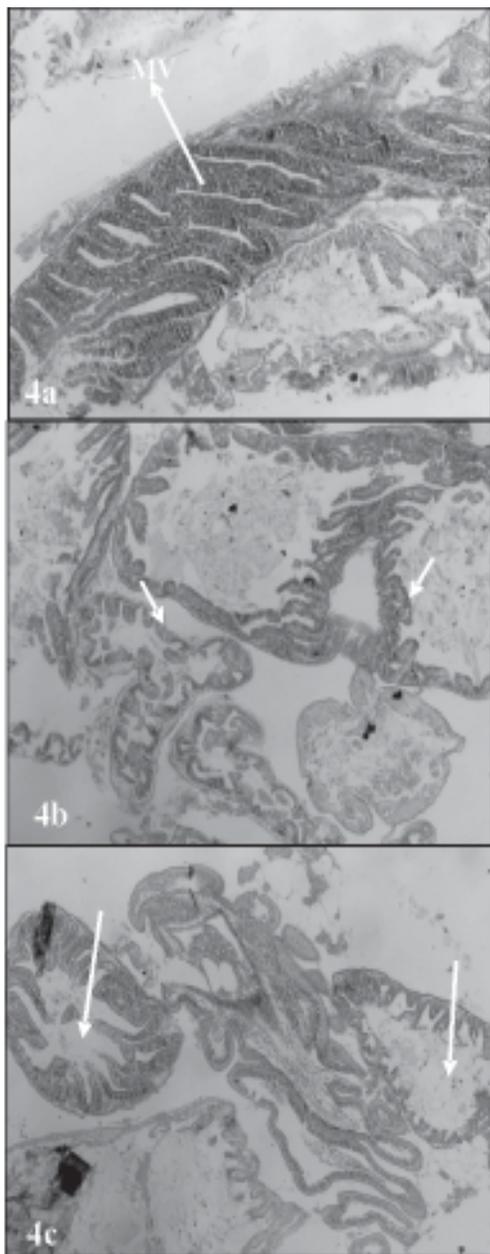
Plates 4a-c: Photomicrographs of sections through the intestine of *Poecilia reticuata* exposed to sublethal concentrations of effluent from Kara Cow Market, Ogun state and the control at day 56.

Key:
4a: Section through the intestine of *Poecilia reticulata* in control group showing normal architecture (arrow showing normal mucosal villi (MV) at day 56, H & E stain, x100.
4b: Section through the intestine of *Poecilia reticulata* in exposed (0.7 mL/L) group showing distorted mucosal architecture with shortening and widening of the intestinal villi (arrows) at day 56, H and E stain, x100.
4c: Section through the intestine of *Poecilia reticulata* in exposed (7.2 mL/L) group showing distorted mucosal architecture and widening of the intestinal villi (arrows) at day 56, H and E stain, x100.
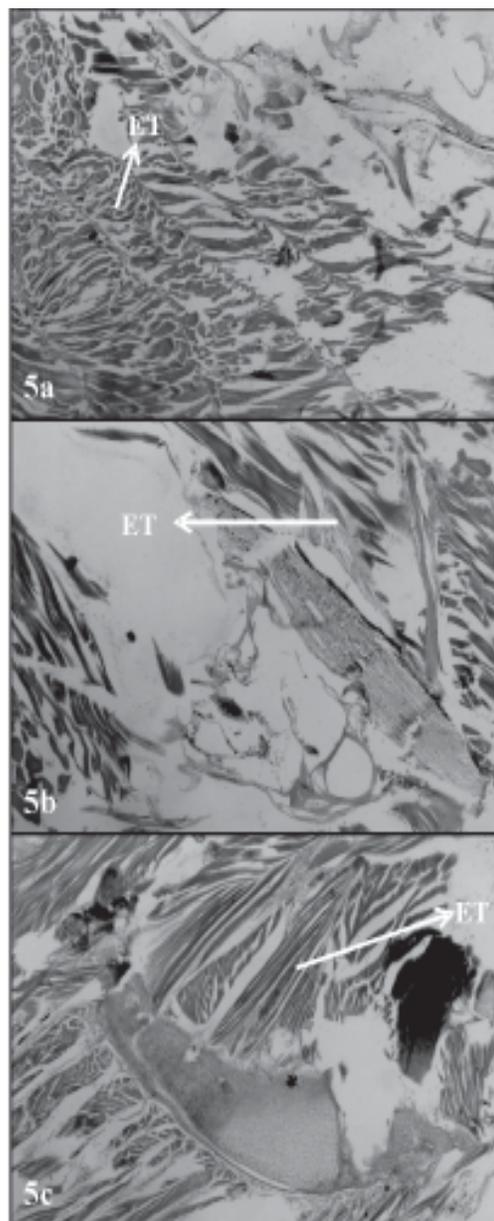
Plates 5a-c: Photomicrographs of sections through the skin of *Poecilia reticuata* exposed to sublethal concentrations of effluent from Kara cow market, Ogun state and the control at day 56.

Key:
5a: Section through the skin of *Poecilia reticulata* in control group showing normal skin architecture (ET – Epithelia tissue) at day 56, H and E stain, x100.
5b: Section through the skin of *Poecilia re.ticulata* in exposed (0.7 mL/L) group showing normal skin architecture (ET – Epithelia tissue) at day 56, H and E, x100.
5c: Section through the skin of *Poecilia reticulata* in exposed (7.2 mL/L) group showing normal skin architecture (ET – Epithelia tissue) at day 56, H and E, x100.

Discussion

In this study, the discharge of untreated effluent into the nearby Ogun River conforms to similar observations from studies in abattoirs in Nigeria (Adeyemo, 2002 and Adebowale *et al.,* 2016) and Ghana (Fearon *et al* 2014). The pH and DO levels in this study were below the NESREA limit (NESREA, 2011) while oil and grease, BOD, COD, TSS and the heavy metals exceeded the limits for effluent discharges into surface water to support the survival of fish. This is consistent with the findings of Alimba *et al* (2015) who assessed similar effluents from abattoirs in Bodija, Oyo State. These physicochemical properties have adverse impacts on the receiving waters (Ogun River) beyond the natural self-purification capacity of the Ogun River (Oketola *et al* 2006; Adeogun *et al* 2011). According to Bhatnagar and Devi (2013), optimum physiological activities for fish in surface waters is achieved with a BOD of 3-6 mg/L. Sublethal effects can be observed at levels of 6-12 mg/L and at levels higher than 12 mg/L, death of fishes can be expected due to suffocation. Solid wastes and effluents from abattoirs such as fats, blood, bone, and hairs are rich in organic matter and nutrients (Atuanya *et al* 2012) corresponding to a relatively high COD and BOD which are driven by microbial degradation of organic matter, nitrification and chemical oxidation (Alani *et al* 2014).

The high concentrations of heavy metals in the effluent demonstrates the potential toxicity (such as induction of antioxidant enzymes in *P. reticulata* (Thamke and Kodam, 2016), cytotoxic, mutagenic and carcinogenic effects in animals (More *et al* 2003)) of the effluent to aquatic organisms in the river. This has potential implications for other ecosystem services provided by the river including its abstraction for drinking water. Furthermore, there is the potential for bioaccumulation of the heavy metals especially in benthic organisms which can be biomagnified along the food chain/web to organisms at high trophic levels including humans. Also, the cows were observed to drink water directly from the river showing the potential of bioaccumulation of these toxic metals to high levels in the animals with potential acute and sublethal effects (Alani *et al* 2014). Potential toxic effects in humans consuming heavy metals-contaminated cow meat are diarrhoea, stomach pains, vomiting, bone fracture, DNA damage, incidence of cancer, among others (Alimba *et al* 2015). The presence of pathogens and faecal coliforms in the effluent showed the potential public health and environmental risks to consumers of the river's resources including fishes (Gauri, 2004). Coliforms including *Escherichia coli* as observed in this study are major microbial contaminants in abattoir effluents (Adebowale *et al* 2010). Cattles are known reservoirs of *E. coli* which is a significant human enteric pathogen (LeJune *et al* 2004) causing life threatening haemolytic uremic syndrome in children and thrombocytopenia in the elderly (Padhye and Doyle, 1997). The microbial species identified in this study corroborates the report by Neboh *et al* (2013) who observed similar assemblages in abattoir effluent at Ijebu Igbo, Ogun State, Nigeria. Furthermore, long term health hazards ranging from temporary morbidity to mortality in high risk individuals could occur through transmission of these pathogens to humans who utilise the river water for recreation and fishing.

The median lethal concentration (7.15%) of the effluent in this study is seven (7) times lower than that reported by Adeogun *et al* (2013) in *Clarias gariepinus* exposed to a mixture of abattoir and saw mill effluent. This may be due to the relatively higher sensitivity of *P. reticulata* to pollution due to its small body size (thus, there is a larger body surface area for entry of pollutants into the organism) compared to *C. gariepinus*. Also, the difference in the nature of the effluent in both studies could contribute to the disparities.

In this study, the lack of histological alterations in the gills of exposed groups of *P. reticulata* at day 56 is at variance with observations in similar studies with *P. reticulata* exposed to lethal concentrations of textile dye industry effluent in which the gills showed enlargement of primary gill bar and detachment of secondary gill bars (Selvaraj *et al,* 2015). The variance observed may be attributed to the nature (abattoir effluent), concentration (sublethal) and the duration of exposure of the effluent used in this study (Selvaraj *et al,* 2015). Conversely, the alterations observed in the intestine of both exposed groups of *P. reticulata* in this study corroborate the findings of Selvaraj *et al* (2015). Since the gills and skin are the first point of contact for pollutants into the fish body, pollutants may be retained longer and at higher levels in the internal tissues and organs such as liver and intestine than the external tissues including gills and skin (Yancheva *et al* 2015). Consequently, the intestine could be a reservoir of the toxicants inherent in the effluent which may be the reason for the alterations observed in the intestine alone. In conclusion, the management of effluents discharged from industries and abattoirs in most developing countries such as Nigeria is a challenge (Adeyemi and Adeyemo, 2007). Sustainable environmental and public health management interventions are usually impeded by the ignorance of stakeholders about the adverse impacts of their activities. In this study, we demonstrate the potential adverse impacts of untreated abattoir effluent from the Kara Cow Market on fishes and surface water of the Ogun River. Consequently, we recommend the installation of an effluent treatment plant in the market to reduce the pollutants load of the effluent before discharge into the receiving surface water. Furthermore, we recommend that the relevant environmental and food safety regulatory agencies should conduct periodic advocacies and campaigns to inform stakeholders and the public about the adverse effects of indiscriminate effluent discharges into surface waters. Further studies should be conducted involving holistic evaluations of the fish diversity, surface water and sediment physicochemical and microbiological quality of the Ogun River as well as toxicological assessment of the whole effluent to aquatic organisms at lower levels of biological organization. These will support

the national efforts towards the achievement of the UN sustainable development goal 6 (clean water and sanitation) among other goals by 2030.

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