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Antibiotics, algal evaluations and subacute effects of abattoir wastewater on liver function enzymes, genetic and haematologic biomarkers in the freshwater fish, *Clarias gariepinus*

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ABSTRACT

Abattoirs are positioned close to freshwaters in most developing countries where untreated wastewaters are discharged into with potential risk to aquatic life such as fish and ultimately human health. We assessed physicochemical parameters, antibiotics (oxytetracycline and diclofenac) and algal load of effluent collected from a major abattoir in Nigeria. Furthermore, liver function enzymes, genotoxic and haemotoxic effects of subacute concentration (10% of 96 hLC_{50} value) of the wastewater were evaluated over a period of 28 d in *Clarias gariepinus* (The African Sharptooth Catfish). The 96 hLC_{50} value of the abattoir wastewater against *C. gariepinus* was 154.14 mL/L (15.4%). Nitrates, phosphates, sulphates, chloride, ammonia, TDS, TSS, BOD_5 and heavy metals (Fe and Pb) in the wastewater were above permissible limits while diclofenac and oxytetracycline were below detection limit (BDL). Microalgae in the wastewater were mostly Bacilliariophyta (*Navicula* spp.) (45.64%) and euglenoids (*Euglena* and *Phacus* spp.) (49.48%). Liver function enzymes (LDH, AST, ALT) level were higher in exposed fishes except for ALP which was lower at day 28 compared to control levels. Erythrocytic genotoxic indices (nuclear abnormalities) were significantly higher ($p < 0.05$) in the exposed fishes particularly at day 28 compared to control. Haematologic indices level such as WBC, MCV MCH, MCHC increased significantly ($p < 0.05$) while lymphocytes, HGB, RBC, HCT levels decreased significantly ($p < 0.05$) in the exposed *C. gariepinus* by day 28 compared to control. These results demonstrate potential adverse effects posed to aquatic fish species in the Ogun River by the discharge of the abattoir effluent. The microalgae species identified in the effluent may be explored for pre-treatment of the effluent before discharge in order to prevent eutrophication and increased pollutant load in the River. The study results will contribute to evidence-based environmental risk management of the River which is relevant to the UN SDGs 6 (clean water and sanitation), 11 (sustainable cities and communities) and 14 (sustaining life below water).

1. Introduction

Livestock farming and animal processing activities such as the production of beef, hides and leather have over time adversely impacted both land and water (Sawalha et al., 2019). Abattoir wastewaters/effluents often contain one or more pharmaceuticals (tetracycline, oxytetracycline, diclofenac, among others (Wei et al., 2011; Sim et al., 2013)) used for the treatment of livestock diseases and endoparasites, pesticides from the treatment of livestock against ectoparasites and polycyclic aromatic hydrocarbons (PAHs) from anthropogenic activities particularly the processing of animal skin at the site of the slaughter (Olaniran et al., 2019). Most abattoirs in developing countries like Nigeria are sited close to flowing waters for easy disposal of waste (Osibanjo and Adie, 2007; Adeogun et al., 2013; Olaniran et al., 2019). Rivers that are contaminated by abattoir effluents and wastes contain high level of metals like cadmium, zinc, copper, iron, chromium and lead (Osibanjo and Adie, 2007) as well as high microbial loads (Sawalha et al., 2019). The discharge of these untreated wastewaters directly into the water bodies often increase the levels of SO_4^{2-}, PO_4^{3-}, NO^{3-} and heavy metals therein (Maria et al., 2009; Karadag et al., 2014). This in

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turn alters its characteristics such as appearance, smell and chemical composition causing impaired ecological functions in such water bodies (Maria et al., 2009; Karadag et al., 2014).

The effect(s) of these effluents on aquatic organisms can be assessed at subacute concentrations to evaluate the potential impact on resident fish species among other aquatic organisms in receiving water bodies (Adeogun et al., 2013; Olaniran et al., 2019; Sogbanmu et al., 2019). Subacute effects can be assessed using biomarkers of oxidative stress, genotoxic indices, histopathological changes, and changes in liver and kidney functions at organismal level (Alimba et al., 2015; Oladokun et al., 2020). Haematological parameters are one of the commonest and important biomarkers for diagnosing the structural and functional status of fishes exposed to effluents and pollutants (Faggio et al., 2014; Burgos Aceves et al., 2019). They are considered to be a reliable approach in the assessment of toxicity of different single chemicals and their mixtures to fish (Vosylienė et al., 2003). Changes in haematological parameters depend on the magnitude of the impact of contaminant (concentration), the duration of exposure, fish species, age and health status (Alimba et al., 2019). Disrupted haematological patterns appear very quickly and precede changes in fish behaviour and visible lesions (Bruck-a-Jastrzebska and Protasowicki, 2005). Alterations in white blood cell numbers might be regarded as a prognostic tool or an early-warning signal of the disturbance in homeostatic defense abilities of fish (Vosylienė, 1999). Further, exposure of fishes to abattoir effluents over a long period of time have been shown to induce cytotoxicity and genotoxicity through diverse mechanisms (Alimba et al., 2015). The African sharptooth catfish, *Clarias gariepinus* is a freshwater fish belonging to the clarid family which has been used as a bioindicator to assess several biomarkers such as cytogenotoxic (Alimba et al., 2015), haematological, histopathological and biochemical effects (Oladokun et al., 2020) of environmental toxicants and wastewaters/effluents.

Further, wastewaters are often a source of nutrients to cells of microalgae which are ubiquitous plant species commonly found around heavily polluted wastewater (Boelee et al., 2011). Many algal genera have species that grow well in wastewater containing a high concentration of organic wastes (Mahapatra et al., 2013; Priya et al., 2014) Green algae *Chlamydomonas*, *Euglena*, diatoms, *Navicula*, *Synedra* and blue-green *Oscillatoria* and *Phormidium* are emphasised to tolerate organic pollution. At species level, *Euglena viridis* (Euglenophyta), *Nitzschia palea* (Bacillariophyta), *Oscillatoria limosa*, *O. tenuis*, *O. princeps* and *Phormidium uncinatum* (Cyanophyta) were reported to be present than any other species in organically polluted waters (Priya et al., 2014). These are important not only as bioindicators but for treatment of wastewaters (Mahapatra et al., 2013). There is a need to evaluate multiple biological effects of the effluent on indigenous fish species such as the African sharptooth catfish (*Clarias gariepinus*) which may be found in the adjoining freshwater body (the Ogun River) as well as to evaluate the effluent physicochemical and biological constituents to make evidence-informed decisions on its management. Therefore, the aim of this study was to assess the physicochemical parameters, selected pharmaceuticals and algal load in abattoir effluents from a livestock market in Nigeria as well as effects of the untreated abattoir effluent on biochemical, genotoxic and haematological parameters in the African sharptooth catfish. The study results will contribute to evidence-based environmental risk management of the abattoir as well as the freshwater ecosystem to which the untreated abattoir effluent is being discharged. Further, the microalgae identified in the effluent may be explored for low-cost and environmentally-friendly treatment of the livestock processing effluent (Mahapatra et al., 2013). Evidence-based recommendations from this study will support efforts towards achievement of the United Nations Sustainable Development Goals (UN SDGs) 6 (clean water and sanitation), 11 (sustainable cities and communities) and 14 (sustaining life below water) particularly in developing countries like Nigeria.

2. Materials and methods

2.1. Study area, sampling and physicochemical evaluation of Kara abattoir effluent

The Kara cow market (6° 38′ 48.0″ N, 3° 22′ 46.5″ E) is located along the Lagos-Ibadan expressway, Ogun state, Nigeria (Olaniran et al., 2019). It is one of the largest livestock markets in South-Western Nigeria. The market has an abattoir (see graphical abstract) where livestock (see graphical abstract) are butchered and wastewaters/effluent from the abattoir are channelled into the adjoining surface water, the Ogun River (see graphical abstract). Primary data on the use and frequency of pharmaceuticals on the livestock at Kara cow market were collected through the interview of herders, veterinarians and pharmacists who dispense medicines to the herders. Notes of the commonly used pharmaceuticals were taken and the effluent was screened for the two (2) commonly used pharmaceuticals (oxytetracycline and diclofenac).

Effluent samples were collected from the outfall of the abattoir into the Ogun River using pre-labelled 600 mL amber glass bottles for the analyses of physicochemical parameters, heavy metals, diclofenac and oxytetracycline. The physicochemical parameters analysed were nitrates, total suspended solids (TSS), dissolved oxygen (DO), biochemical oxygen demand (BOD_5), pH, total dissolved solids (TDS), chlorides, sulphates, phosphates and some heavy metals. The bottles were initially washed with dilute nitric acid, then with distilled water and air dried (Olaniran et al., 2019). Sample preparation and analysis of heavy metals were conducted as described in Elemile et al. (2019). Briefly, the effluent sample was thoroughly shaken together. Then, 100 mL of the sample was transferred into a beaker and 5 mL of concentrated nitric acid was added. The beaker was placed on a hot plate and evaporated to dryness. It was then cooled and another 5 mL concentrated nitric acid was added. Heating was continued until a light-coloured residue was observed. Then 1 mL concentrated nitric acid was added and the beaker was warmed slightly to dissolve the residue. The walls of the beaker were then washed with distilled water. The extract was filtered and iron and lead were determined in the filtrates using the Atomic Absorption Spectrophotometer (Elemile et al., 2019). The samples were properly labelled and transported in ice-packed coolers to the laboratory for analysis.

2.2. Sample preparation, extraction, analysis and quality control for oxytetracycline and diclofenac

The effluent samples were preserved in a refrigerator (at 4 ℃) until analysis. For the analysis (conducted at Hydrochrome laboratory, Lagos, Nigeria), the samples were defrosted, centrifuged and filtered twice to remove floating and suspended particles. After filtration, 25 g of sodium EDTA (Na_2EDTA), a metal complex forming agent, was added to 500 mL of the filtered sample. Waters Oasis HLB Cartridges (200 mg, 6 mL) were used for extraction and concentration (× 1000) of effluent samples on a Supelco Visiprep SPE Vacuum Manifold (standard, 24 port model) (Himmelbach and Buchberger, 2005). Cartridges were first conditioned with 6 mL methanol, followed by 6 mL water and then 6 mL of Na_2EDTA-McIlvaine consecutively.

100 mL of effluent were loaded into the cartridge followed by washing of the cartridge with 6 mL of water to further remove traces of EDTA and any unwanted interferences. The cartridges were allowed to dry. Bound actives were subsequently eluted with 6 mL (2 × 3 mL) methanol into salinized amber glass tubes. Eluted extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted in 0.1 mL of methanol. Extract solutions were stored in amber glass vials at 4 °C and in the dark for high-performance liquid chromatography (HPLC) analysis. Glasswares used for extraction of effluent samples were sanitized 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.

A 717 Plus autosampler, 600 controller, Waters 616 LC system, and Millennium 2010 chromatography software was used in quality control. Mass spectrometry was performed with a Finnigan LCQ ion trap (Thermoquest, San Jose, CA, USA) equipped with heated capillary interface, and electrospray ionization (ESI) source. Thermoquest Navigator software was employed to control the mass spectrometric conditions and Lcquan software was used for the quantification of oxytetracyclines and diclofenac. Isocratic separation at 508C was achieved using a 250 × 2 mm end-capped BetaBasic C 5 mm reversed-phase HPLC column (Keystone Scientific, Bellfonte, PA, USA). No equilibration time was needed between each analysis. Optimum separation occurred using a mixture of water, 5% formic acid, acetonitrile, and methanol (23:40:25:12) at a flowrate of 0.1 mL min. A 20 μL injection volume was used and calibration standards were analysed throughout each run. The curve of quantity versus relative response of analyte to internal standard exhibited good linearity and reproducibility (five replicates) over the calibration range for diclofenac and oxytetracycline (r^2= 0.9974, and 0.9999, respectively). Handling, storage and standard of all reagents used were in compliance with the safety precautions indicated in the MSDS (Olaniran et al., 2019). Additional details on the standard preparation are described in SI 1.

2.3. Algal evaluation of abattoir effluent

Microalgae which are bioindicators of organic pollution and sometimes useful for biological treatment of organics-rich wastewaters (Mahapatra et al., 2013) were evaluated in this study. 10 mL of concentrated abattoir effluent sample was corked with cotton wool to allow free passage of air (CO_2 intake from the environment while oxygen would be given off in the process of photosynthesis by the algae), avoid contamination (from microspores that may be present in the environment) and sieve dirt that may be present in the air. The mixture was allowed to stand for 24 h. Using a clean pipette, a drop of the diluted sample was placed on a slide and covered with a cover slide. Algae species found were viewed and counted. Species found were identified using keys (Biggs and Kilroy, 2000). The method of counting was adopted from Verlecar and Desai (2004).

2.4. Collection of test fish and acclimatisation

150 fingerlings (weight 3.05 ± 0.50 g, length 4.18 ± 0.14 cm) and 50 juveniles (weight 49.85 ± 0.50 g) of *Clarias gariepinus* (Chordata, Osteichthyes, Siluriformes, Clariidae) were obtained from the Department of Marine Sciences, University of Lagos, Nigeria. The fish were transported to the Ecotoxicology laboratory in the morning in large containers filled with 40 L of culture water. On arrival, the fish were distributed into large plastic tanks (20 in by 10 in) in which air (oxygen) was continuously bubbled into through an aerator for acclimation. The plastic tanks contained the water used in transporting the fish. At the laboratory (temperature: 26.4 ± 1.6 °C; pH: 7.0; light:dark cycles − 12 h:12 h), the fishes were fed with Coppens feed every morning and evening for the seven (7) days period of acclimatisation. The holding tanks were cleaned and water in the acclimatisation units was replaced with the fresh water daily to prevent accumulation of waste metabolites in the culture water (OECD (Organization for Economic Cooperation and Development), 1992).

2.5. Acute toxicity studies with Clarias gariepinus exposed to abattoir effluent

Following concentrations utilised in a previous study for the same effluent (Olaniran et al., 2019), Four (4) fingerlings were exposed to various effluent concentrations in duplicates in order to estimate the range of concentrations (range finding tests) to be utilised for the definitive acute toxicity studies. In the definitive test, Five (5) fingerlings of *C. gariepinus* were exposed in duplicates to varying concentrations of the effluent − 100, 160, 220, 280, 340 mL/L and control (dechlorinated tap water). Mortalities of the test fish were recorded every 24 h for 4 days (96 h) (OECD (Organization for Economic Cooperation and Development), 1992). A fish was considered dead when there was lack of opercula movement when prodded with a glass or plastic probe.

2.6. Experimental design for sublethal toxicity studies with Clarias gariepinus exposed to abattoir effluent

Five (5) juvenile *C. gariepinus* were exposed in duplicates to a sublethal concentration (10% 96 hLC_{50} value – 15.41 mL/L (1.54%) for fingerlings) and control (Olaniran et al., 2019) in 5 L capacity exposure tanks. A static-renewal bioassay protocol was followed in which each test media was replaced with a fresh media once every 2 d for a period of 28 d. During this period, the animals were fed with Coppens feed every morning and evening while still in the test medium.

2.6.1. Liver function enzymes analyses with Clarias gariepinus exposed to abattoir effluent

After 14 and 28 d of exposure, one fish was selected from each replicate and fishes were euthanized according to AVMA (2013). Livers were extracted, weighed and homogenized in 0.5 mL KCL buffer. Homogenized tissues were centrifuged at 3000 rpm for 10 min and the supernatant was removed. Total protein was quantified by using biuret reagent. Aspartate aminotransferase (AST) and Alanine aminotransferase (L-ALT) activities in the plasma were determined at 37 ℃ by colorimetric method of Reitman and Frankel (1957). The activities of alkaline phosphatase (ALP) were determined by the colorimetric method of Plummer (1978) using phenolphthalein monophosphate as substrate while LDH was determined by the methods of Vassault (1983). The enzymes were assayed using reagents obtained from enzyme assay kits (Randox).

2.6.2. Erythrocytic genotoxic evaluation in Clarias gariepinus exposed to abattoir effluent

At the end of each exposure time of 14 and 28 d respectively, a fish was selected from each replicate and blood was collected from the caudal vein of each fish in the test and control groups for micronucleus analysis (Mumuni and Sogbanmu, 2018). A thin smear of the peripheral blood was made on three (3) pre-cleaned slides per fish. The slides were air dried, fixed in absolute methanol for 30 mins and counterstained with 10% May-Grunwald which was allowed to stay for 10 mins before staining with 5% Giemsa (Alimba et al., 2015). 3000 erythrocytes per fish were scored for micronucleus induction at a magnification of × 1000. Nuclear abnormalities (NAs) were also scored as cytotoxic parameters (Carrasco et al., 1990) at the same magnification.

2.6.3. Haematological indices in Clarias gariepinus exposed to abattoir effluent

At 14 and 28 d post-exposure, a fish was selected from each replicate. Blood samples were collected using a 2 mL syringe from the caudal artery at the peduncle and transferred into EDTA bottles. The blood samples were analysed for haematological indices (white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT)) using an automated haematology analyser (SysmexR KX-21) (Amaeze et al., 2020).

2.7. Statistical analysis

The results obtained were computed using MS Excel 2013. The 96 hLC_{50} value of the acute toxicity studies was computed using probit analysis (Finney, 1971) on SPSS version 20. Experimental data for

erythrocytic genotoxic (nuclear abnormalities), liver function enzymes and haematological parameters were presented as mean±SE. Significant differences between the treatment and control groups were analysed using One-way analysis of variance (ANOVA) with Dunnett multiple post hoc test with significance set at $p < 0.05$.

3. Results

3.1. Physicochemical parameters, heavy metals and pharmaceuticals in abattoir effluent

The colour of the effluent was dark brown and the pH was slightly acidic (6.8) (Table 1). Electrical conductivity, ammonia, nitrates, BOD, COD, oil and grease, Fe and Pb of the effluent were above the set regulatory limits in Nigeria (Table 1). However, the dissolved oxygen level (0.5 mg/L) was below the limits. Diclofenac and oxytetracycline were below their respective detection limits in the effluent (Table 1).

3.2. Algal composition of abattoir effluent

Diatoms (Bacillariophyceae) and euglenoids dominated the algae composition of the effluent with *Navicula* sp. having the highest percentage composition of 45.64% (Table 2). The euglenoids (*Euglena* and *Phacus*) had a total composition of 49.48% (Table 2) while the cyanobacteria (*Rivularia* sp. and *Chroococcus* sp.) had the least percentage composition (4.87%).

3.3. Acute toxicity of abattoir effluent to Clarias gariepinus

The derived 96 hLC_{50} value of the abattoir effluent against *C. gariepinus* fingerlings was 154.14 mL/L (15.4%).

3.4. Liver function enzyme activities in Clarias gariepinus exposed to abattoir effluent

Aspartate aminotransferase (AST) and Lactate dehydrogenase (LDH) activities were higher ($p > 0.05$) at days 14 and 28 compared to the control and higher at day 28 compared to day 14 in the effluent-treated fishes (Fig. 1A; SI 2). Alanine Transaminase (ALT) activity was significantly higher ($p < 0.05$) at day 14 and non-significantly higher at day 28 ($p > 0.05$) compared to the control though lower ($p > 0.05$) at day 28 compared to day 14 in the effluent-treated fishes (Fig. 1B; SI 2). Similarly, Alkaline Phosphatase (ALP) activity was significantly higher ($p < 0.05$) at day 14 and significantly lower ($p < 0.05$) by day 28 in the effluent-treated fishes compared to the controls (Fig. 1B; SI 2).

Table 1
Physicochemical parameters, heavy metals and pharmaceuticals level in abattoir effluent.

Parameters	Abattoir effluent	NESREA limits
Colour (Pt/Co)	Dark brown with particles	NS
pH	6.80	6–9
Temperature (°C)	25.0	–
Turbidity (FTU)	10.0	NS
Electrical conductivity (μS/cm)	8648.0	–
Total dissolved solids (mg/L)	4324.0	–
Total suspended solids (mg/L)	7.0	0.75
Salinity (ppt)	0.7	–
Chloride (mg/L)	352.00	350
Sulfate (mg/L)	500.00	500
Ammonia (mg/L)	6.5	0.5
Nitrate (mg/L)	36.0	0.08
Total phosphorus (mg/L)	7.0	–
Oil and grease (mg/L)	3.0	0.1
BOD_5 (mg/L)	95.00	6
COD (mg/L)	190	30
Dissolved oxygen DO (mg/L)	0.5	> 4
Heavy metals		
Fe (mg/L)	21.0	0.5
Pb (mg/L)	0.34	0.1
Pharmaceuticals		
Oxytetracycline	BDL	–
Diclofenac	BDL	–

Note: NESREA (National Environmental Standards and Regulations Enforcement Agency) (2011) (Nigeria); BDL – below detection limit; NS – not specified; BOD – biological oxygen demand; COD – chemical oxygen demand.

Table 2
Composition and abundance of algae in abattoir effluent.

Species	Division	Total count/mL	Percentage abundance
Navicula sp.	Bacillariophyta	262	45.64%
Phacus	Euglenophyta	144	25.09%
Euglena	Euglenophyta	140	24.39%
Rivularia sp.	Cyanobacteria	20	3.48%
Chroococcus sp.	Cyanobacteria	8	1.39%

3.5. Erythrocytic genotoxic indices in Clarias gariepinus exposed to abattoir effluent

There were higher ($p > 0.05$) frequencies of micronuclei ($1.22 \pm 0.32‰_0$) and binucleated cells ($3.67 \pm 1.91‰_0$) at day 14 and significantly higher ($p < 0.05$) by day 28 (MN: $2.33 \pm 1.11‰_0$; BN: $6.56 \pm 2.36‰_0$) in the effluent-treated fishes compared to the control (MN – day 14: $0.33 \pm 0.17‰_0$, day 28: $0.44 \pm 0.18‰_0$; BN – day 14:

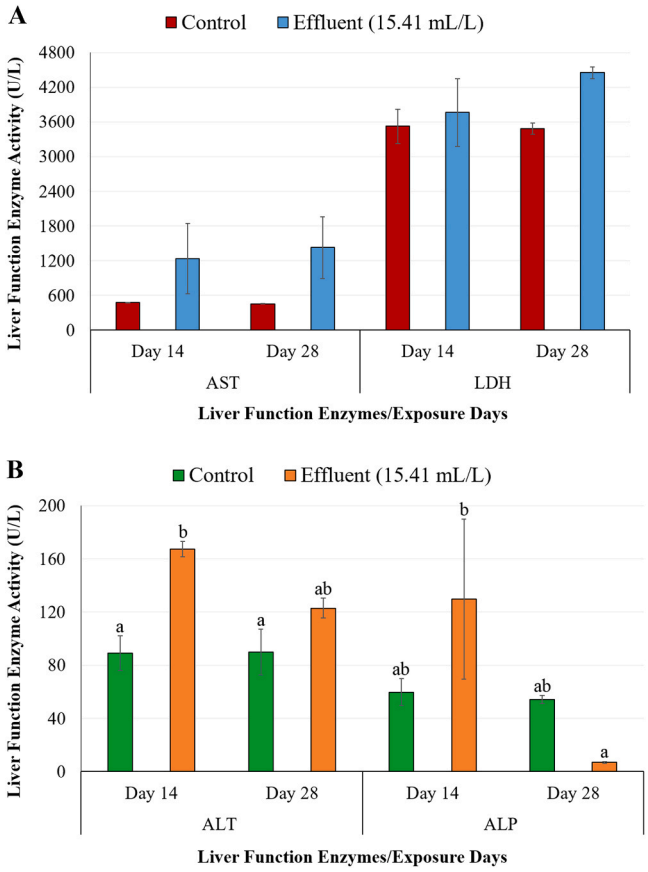

Fig. 1. Liver function enzymes activity over 28 days in *Clarias gariepinus* exposed to abattoir effluent. Key: 1A- AST and LDH activity; 1B- ALT and ALP activity; S.E – standard error. U – μmol/mL/min; ALT – Alanine Transaminase; ALP – Alkaline phosphatase; LDH – Lactate dehydrogenase; AST – Aspartate aminotransferase; n = 3; values are expressed as mean ± SE; significant differences analysed using ANOVA is set at $p < 0.05$; dissimilar superscript letters between treatment groups represents significant differences at $p < 0.05$.

0‰$_0$, day 28: 0‰$_0$) (Fig. 2; SI 3). Erythrocytic nuclear buds (Nbud) were significantly higher ($p < 0.05$) in the effluent-treated fishes at days 14 and 28 compared to the control though the frequency of Nbud decreased by day 28 compared to day 14 in the treatments (Fig. 2; SI 3). There were higher ($p > 0.05$) frequencies of lobed nuclei and empty cells at day 14 and significantly higher ($p < 0.05$) by day 28 in the effluent-treated fishes compared to the control (Fig. 2; SI 3).

3.6. Haematological indices in Clarias gariepinus exposed to abattoir effluent

There were non-significant increases ($p > 0.05$) in WBC, MCV, MCH, MCHC levels at day 14 in the effluent-treated fishes compared to controls and significant decreases ($p < 0.05$) by day 28 in the effluent-treated fishes compared to day 14 and the controls (Table 3). There were significant increases ($p < 0.05$) in % lymphocytes, HGB, RBC and HCT by day 28 in the effluent-treated fishes compared to day 14 and the controls (Table 3). PLT counts were higher ($p > 0.05$) in the effluent-treated fishes at days 14 and 28 compared to the controls with the counts being higher at day 28 compared to day 14 (Table 3).

4. Discussion

4.1. Physicochemical parameters, heavy metals and pharmaceuticals

The high levels of physicochemical parameters (electrical conductivity, TDS, ammonia, nitrates, BOD, COD, oil and grease) in the effluent compared to the set limits is characteristic of abattoir effluent (Sogbanmu et al., 2019; Olaniran et al., 2019) although, the parameters considered were generally lower than the levels reported by Olaniran et al. (2019). This variation may be due to difference in the season when the studies were conducted (Adeogun et al., 2013; Olaniran et al., 2019; Sogbanmu et al., 2019). Eutrophication potentially elicited by excessive inputs of organics, nitrates, phosphates and sulphates into the river from the abattoir may occur which will reduce the concentration of dissolved oxygen in the river because of the oxygen used in decomposition of organic matter (Rixen et al., 2010). The high electrical conductivity may be due to the presence of ions from blood, sulphates, phosphates and treatment chemicals used on the animals in the market (Ozturk and Yilmaz, 2019).

The non-detection of oxytetracycline and diclofenac in this study is in contrast to findings by Olaniran et al. (2019) who opined that pharmaceuticals such as tetracycline were detected in the wastewater from the Kara cow market abattoir. The absence of the pharmaceuticals in the effluent may be due to low usage as the herders alluded to an occasional use of pharmaceuticals. Some of the herders on the other hand do not treat their cattle at the market. The nomads only visit the market to trade. 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 and subtoxic but population-relevant effects in organisms (Brodin et al., 2013). Wei et al. (2011) reported a broad profile of veterinary antibiotic residues in animals, wastewater and surface water around large-scale livestock and poultry farms in Jiangsu Province of China.

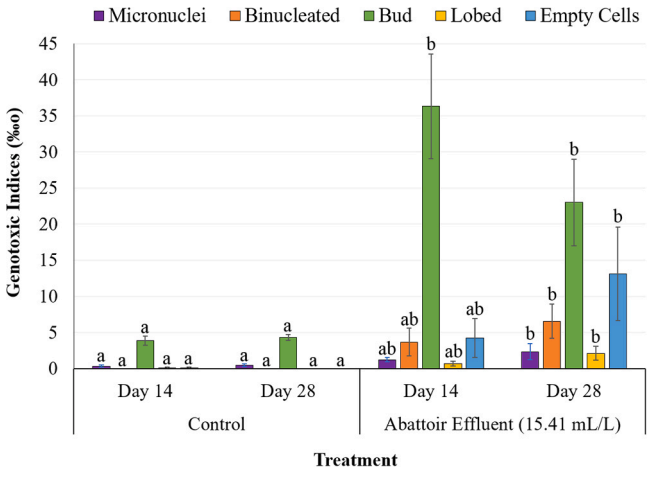

Fig. 2. Erythrocytic genotoxic indices in *Clarias gariepinus* exposed to abattoir effluent. Note: n = 9000 cells; Dissimilar letters represent significant differences in the means at $p < 0.05$ (ANOVA).

4.2. Algae species in abattoir effluent

This study is the first report to the best of our knowledge of microalgae found in wastewater from the Kara cow market abattoir/slaughterhouse in Ogun state, Nigeria. The high organic enrichment of the effluent is a key reason for the high population of diatoms and euglenoids present in the effluent which may over time result in eutrophication (evidenced by sudden growth of *Eichhornia crassipes* at this end of the River in 2016 (Sogbanmu et al., 2019)) in the receiving water, Ogun River (Marella et al., 2018). Algae (such as *Euglena* sp.) found in organics-rich wastewater have been shown to be a low-cost method for renewable energy (biofuel production) (Mahapatra et al., 2013). Thus, the high population of euglenoids among other potential useful microalgae identified in this study may be considered as an environmental-friendly treatment option for the wastewater while recovering the microalgae for energy production.

4.3. Median lethal concentration of abattoir effluent

The median lethal concentration (96 hLC$_{50}$) of the abattoir effluent to *C. gariepinus* in this study is higher (less toxic) than that reported for the same species (*C. gariepinus*) (12.6%/126 mL/L) in Olaniran et al. (2019) and *Poecilia reticulata* (7.15%/71.5 mL/L) (Sogbanmu et al., 2019) exposed to effluent from the Kara Market abattoir. Similarly, 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. The disparity in the values could to be due to the differences in the physicochemical parameters of the effluents (such as presence and levels of pharmaceuticals and other pollutants), the size (frys, fingerlings, juveniles or adults), variant and species (*Poecilia reticulata*) of the fish used, time of sample collection (that is, peak of livestock processing or when not much processing was ongoing), and chemical interactions that may have occurred in the effluent (Adeogun et al., 2013).

4.4. Liver function enzyme activities

The higher ALT and AST activities in the liver of the treated fish compared to the control may suggest an impairment of the liver tissues as observed in Black-Jawed Tilapia (*Sarotherodon melanotheron)* exposed to industrial effluent (Nte et al., 2011). This higher activity may denote an increase in metabolic transport which may eventually result in a shift in biosynthesis and energy metabolism pathway of the exposed organism (Gagneten and Paggi, 2009). The significant decrease in ALP activity in the liver by day 28 reflects a possible decrease in biosynthetic activities and anaerobic capacity of *C. gariepinus* (Tripathi et al., 2003). Generally, higher LDH activity in the liver of the treated fish could be due to a decrease in the process of glycolysis which leads to a lowered metabolic rate in the presence of the effluent. It may also be due to the use of anaerobic respiration as an alternative, possibly due to the hypoxic environment as exhibited by the abattoir wastewater in this study. Asztalos et al. (1990) reported similar observations in *C. gariepinus* exposed to pesticides which caused a decrease in LDH in the tissues.

Table 3
Haematological indices in *Clarias gariepinus* exposed to abattoir effluent.

Parameters	Control		Abattoir effluent (15.41 mL/L)	
	Day 14	Day 28	Day 14	Day 28
White Blood Cell (WBC) $\times 10^3$/μL	72.13 ± 0.55^{ab}	70.03 ± 2.19^{b}	76.00 ± 0.66^{ab}	4.23 ± 0.44^{c}
Lymphocytes %	5.90 ± 0.58^{a}	5.90 ± 0.58^{a}	5.73 ± 0.09^{a}	45.93 ± 1.27^{b}
Haemoglobin (HGB) g/dL	6.93 ± 0.09^{a}	6.77 ± 0.24^{a}	7.30 ± 0.11^{a}	12.30 ± 1.19^{b}
Red Blood Cells (RBC) $\times 10^6$/μL	1.45 ± 0.01^{a}	1.45 ± 0.01^{a}	1.39 ± 0.08^{a}	4.54 ± 0.30^{b}
Haemocrit (HCT) %	21.40 ± 0.06^{a}	21.40 ± 0.06^{a}	22.53 ± 1.11^{a}	42.83 ± 3.98^{b}
Mean Corpuscular Volume (MCV) fL	147.00 ± 0.06^{a}	147.33 ± 0.34^{a}	163.00 ± 12.81^{a}	94.20 ± 2.54^{b}
Mean Corpuscular Haemoglobin (MCH) pg	47.87 ± 0.20^{bc}	46.50 ± 1.37^{b}	52.67 ± 3.21^{c}	26.97 ± 0.81^{a}
Mean Corpuscular Haemoglobin Concentration (MCHC) g/dL	32.33 ± 0.35^{a}	32.37 ± 0.33^{a}	32.47 ± 1.12^{a}	28.63 ± 0.13^{b}
Platelet (PLT) $\times 10^3$/μL	106.33 ± 1.45^{a}	107.00 ± 1.53^{a}	163.00 ± 5.03^{a}	168.67 ± 47.40^{a}

Note: n = 3; Dissimilar letters in superscripts represents significant differences in the means at $p < 0.05$.

4.5. Erythrocytic nuclear abnormalities

The observed significant micronuclei and nuclear abnormalities in the effluent-treated fishes indicate increased genetic alterations which may enhance somatic mutation and cancer formation (Russo et al., 2004). This agrees with the findings of Alimba et al. (2015, 2019) and Amaeze et al. (2020) who reported significant time-dependent increase in erythrocytic nuclear abnormalities in *C. gariepinus* exposed to abattoir effluent, pharmaceutical industry effluent and pesticides respectively. Clastogens and cytotoxins have been indicated as potential cursors of the nuclear abnormalities in the abattoir-effluent-treated fishes (Alimba et al., 2015). This may lead to decreased embryonic viability, genetic disorders, reduced fitness, and biodiversity loss in aquatic biota (Kurelec, 1993). Higher concentration of nitrate in the effluent is attributed to the evisceration and slaughter processes which release undigested stomach content into the wastewater (Osibanjo and Adie, 2007). It is plausible that high nitrogenous compounds in the effluent formed reactive free nitric acid (NO), a cytotoxin, with mutagenic and carcinogenic properties, which increased the induction of micronucleated erythrocytes in the treated *C. gariepinus* (Burney et al., 1999).

4.6. Haematological indices

The significant reduction in the WBC count (Leukocytosis) is an indication of apoptosis in the leucocytes (Gogal et al., 1999; Shen et al., 2004). This may be due to a decrease in non-specific immunity of the fish as a result of heavy metal toxicity (Remya et al., 2015). Fishes exposed to heavy metal pollution are likely to have distortions in haematological parameters (Brucka-Jastrzebska and Protasowicki, 2005). The significant increase in % lymph agrees with the results of Alimba et al. (2019) on exposure of *C. gariepinus* to pharmaceutical effluent. The significant increase in HGB, RBC and HCT are an indication of significant concentration of iron which may have damaged the gills causing oxygen deficiency (Remya et al., 2015). These increases could be a compensatory effort of the fish to improve the transport of oxygen to deficient organs (Gaber et al., 2013). This result conforms with those of Amaeze et al. (2020) which showed significant increases in RBC, HCT and HGB in *C. gariepinus* exposed to nine (9) pesticides. However, it contrasts with the findings of Alimba et al. (2019) who reported a decrease in RBC, haematocrit and HGB on exposure of *C. gariepinus* to pharmaceutical effluents. They opined that the decreases were strong indication of anaemic condition which may be caused by the inhibition of erythropoiesis.

5. Conclusion

The physicochemical parameters of the effluent collected from the abattoir were not within the regulatory limits which promoted the growth of microalgae as observed in this study. This weight of evidence in concert with previous studies confirming the pollution status of the Ogun River at the Kara market end should be considered for strategic and contextual management of activities at the market. The observed liver function enzymes indices, nuclear abnormalities and haematological alterations in *C. gariepinus* portends ecological risk to freshwater fish species among other aquatic animal species in the River Ogun and potentially to humans who consume the fisheries. The presence and prevalence of euglenoids (microalgae tolerant of high organics-rich wastewater) confirmed an organically polluted environment. The microalgae species identified in the effluent may be explored for pretreatment of the effluent before discharge into the River to forestall biodiversity loss. The study results and recommendations are relevant to the UN SDGs 6 (clean water and sanitation), 11 (sustainable cities and communities) and 14 (sustaining life below water) as well as the African Union Agenda 2063 Goal 7 (environmentally sustainable and climate resilient economies and communities). Monitoring, environmental sensitization and advocacies by regulatory agencies to stakeholders including herders, Butchers, Veterinarians, pharmacists are recommended to promote knowledge of the environmental and ecological risk of discharging untreated abattoir wastewater into the River.

CRediT authorship contribution statement

Temitope Sogbanmu: Conceptualization, Formal analysis, Methodology, Project administration, Resources, Supervision, Visualization, Writing - review & editing. **Daniel Oyeniran:** Data curation, Formal analysis, Investigation, Project administration, Resources, Roles/Writing - original draft, Writing - review & editing. **Taofikat Adesalu:** Methodology, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.111982.

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