

# Haematological and Hepatic Responses of the African Catfish *Clarias gariepinus* to Sublethal Exposure of Industrial Effluents from Ologe Lagoon Environs, Lagos, Nigeria

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**Abstract:** The present study is on the haematological and hepatic responses of the African catfish fingerlings *Clarias gariepinus* to sublethal toxicity of industrial effluents (IE) from the environment of Ologe Lagoon, Lagos, Nigeria. The fish were cultured in five concentrations of industrial effluents: 0% (control), 5%, 15%, 25%, and 35%. Trials were carried out in triplicates for twelve (12) weeks. The culture system was a static renewable bioassay and was carried out in the fisheries laboratory of the Lagos State University, Ojo-Lagos. Weekly physico-chemical parameters: Temperature ( $^{\circ}\text{C}$ ), pH, conductivity (ppm) and dissolved oxygen (DO in mg/L) were measured in each treatment tank. Haematological parameters: packed cell volume (PCV), red blood cells (RBC), white blood cell (WBC), neutrophil and lymphocytes etc., and hepatological alterations were measured after 12 weeks. The physico-chemical parameters showed that the pH ranged from  $7.82\pm 0.25$ – $8.07\pm 0.02$ . DO ranged from  $1.92\pm 0.66$ – $4.43\pm 1.24$  mg/L. The conductivity values increased with increase in concentration of IE. While the temperature difference remained insignificant with mean value range between  $26.08\pm 2.14$ – $26.38\pm 2.28$ . The DO showed significant differences at  $p < 0.05$ . Though survival was 100% during the sublethal study, haematological results showed that *C. gariepinus* had PCV ranging from  $13.0\pm 1.7$ – $27.7\pm 0.6$ , RBC ranged from  $4.7\pm 0.6$ – $9.1\pm 0.1$ , and neutrophil ranged from  $26.7\pm 4.6$ – $61.0\pm 1.0$  amongst others. The highest values of these parameters were obtained in the control and lowest at 35%. While the reverse effects were observed for WBC and lymphocytes, the liver shows normal liver cells in the control (0%), but at higher toxic levels, there were: vacuolation, destruction of the hepatic parenchyma, tissue becoming eosinophilic (i.e. tending towards Carcinogenicity) and severe disruption of the hepatic cord architecture. This study therefore shows that disposal of effluents into the aquatic environment affects the health of fishes by impairing normal hepatic functions and hindering vital physiological processes if exposure continues for a long period of time (sublethal effect).

**Key words:** Haematology, hepatology, sublethal toxicity, industrial effluents, *Clarias gariepinus*, Ologe Lagoon.

## 1. Introduction

Industrialization has led to huge waste generation over the decades; the absence of adequate facilities for treating such wastes in most developing nations has led to the discharge of effluents into the environment without proper treatment. Water pollution may be defined as any adulteration in its native characteristics by addition of anthropogenic contaminants to the

extent that it either cannot serve humans for drinking purposes and/or to support the biotic communities [1]. A change in the quality of water by the presence of toxins/contaminants makes it potentially harmful to life forms, instead of supporting them. The entry of toxicants into aquatic media may affect the water quality parameters, which in turn lead to changes in the haematological variables of fish and other aquatic lives due to close association with their external environment according to [2].

Alterations in the blood as biomarkers of exposure and the effect have been the subject of intense study

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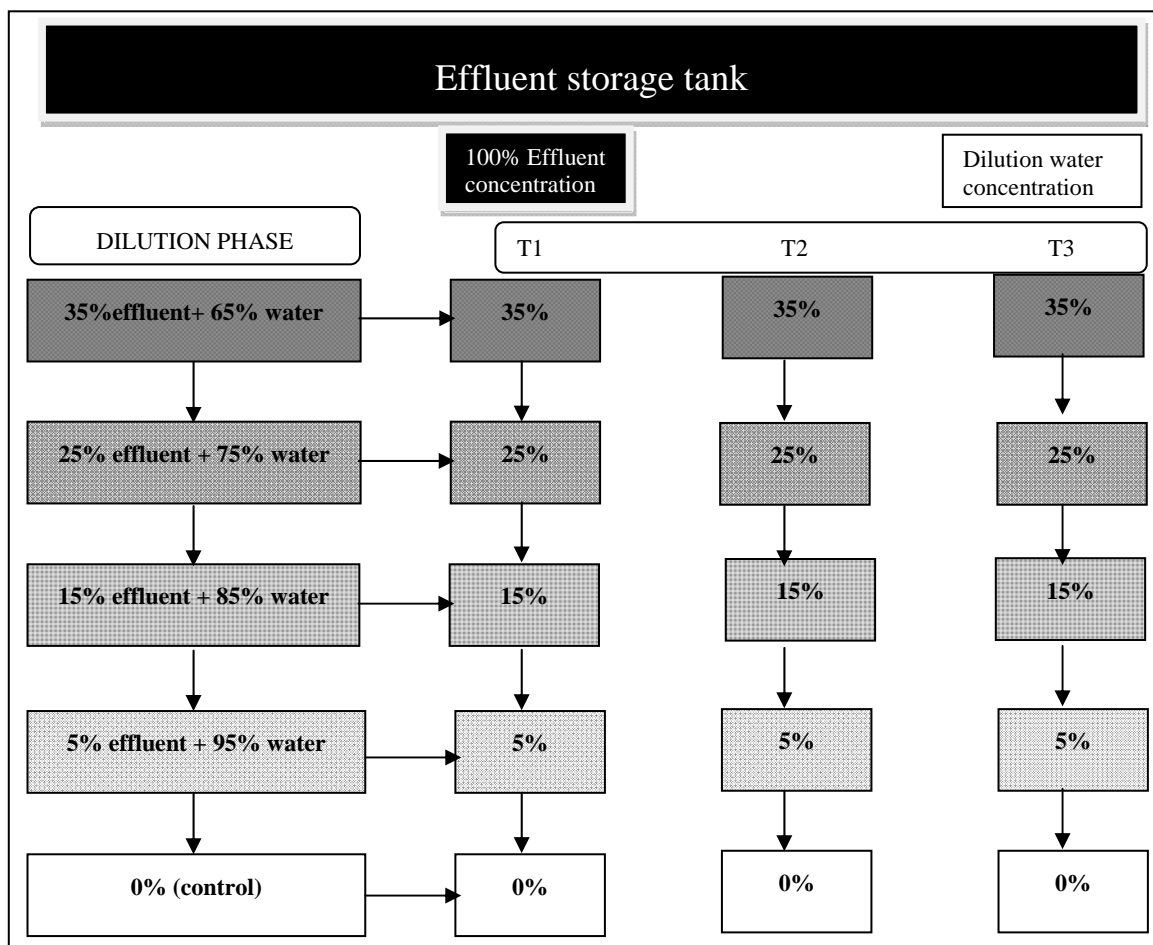
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over the past 20 years in toxicology. These have proven extremely useful in the early detection of low-doses chemical effects [3]. Several workers have investigated the toxicity and haematological changes of fish exposed to pollutants [4-6]. The use of haematological techniques in fisheries research is growing rapidly, and it is very important in environmental monitoring and predicting of fish health conditions [6]. Since fish are sensitive to the aquatic environment, the blood will reveal measurable physiological changes in the fish more rapidly than any physiological assessment parameters. One of the difficulties in assessing the state of health of natural fish population has been the paucity of reliable references of the normal condition. In pursuant of this goal, many fish physiologists have turned to the studies of haematology, probably because it has proved a valuable diagnostic tool in evaluating human health. Although fish haematology continues to offer the potentials of a valuable tool, progress in establishing normal range values for blood parameters has been slow and literature in this area is isolated and often incomplete. Perhaps, further confounding these data are variables such as age, sex, dietary state, and stress, all of which may alter blood values.

The hematological profile of some tropical African fish species has been reported, namely, *Clarias isheriensis* [7], *Clarias gariepinus*, *Heterobranchus longifilis* and *Clarias nigrodigitatus* [8, 9] *Oreochromis niloticus* [10], *Hemichromis fasciatus* and *Tilapia zillii* [11], *Sarotherodon melanotheron* [12] and *Heterobranchus bidorsalis* [13]. Meanwhile, there are no reports on the haematological profile of *Oreochromis niloticus* from industrially polluted aquatic ecosystem like Agbara industrial estate. Hence there is need to study the haematological profile to provide some useful information on aspect of the toxic effect of effluents on the health status of fish using changes in blood parameters as bio-indicators.

Histopathology of teleosts provides a sensitive indicator of stress induced by industrial effluents; and due to the central role of organs in the transformation of several chemical active compounds into aquatic environment, gills, kidney and liver has been the foci of toxicology studies [14]. Pathological changes resulting as consequences due to exposure to certain pollutants, especially Poly Aromatic Hydrocarbons (PAHs), have been included in the definitions of beneficial use impairment criteria. Fish that inhabit water bodies receiving high discharges of effluent from industries show a range of alterations related to physiological abnormalities [15]. These effects were attributed to various estrogenic chemicals present within treated or/and untreated industrial effluents. Chemicals contained in industrial effluents can induce many effects [16, 17]; and exposure to industrial effluents can inhibit the reproduction of fish [16, 18-20].

Ologe lagoon is used for fishery, waste disposal, sand mining and transportation; and as with other lagoons within the Lagos lagoon complex (Fig. 1), it is also regarded as the "large septic tank" in the region [21]. The main anthropogenic pressure on Ologe lagoon is from the adjacent Agbara industrial estate, where over 20 factories (food and beverages, pharmaceutical, breweries, metal finishing, chemical, pulp and paper industries) presently occupy the industrial area. The effluents of these industries are discharged in the lagoon all year round. Ologe lagoon drains into the Atlantic Ocean through the Badagry creek and Lagos harbour. This study determines the significance and severity of lesions observed in fish liver and changes in the blood profile of the fish; relative to the contaminated aquatic locations of the study area. The test species is the fingerlings of *Clarias gariepinus*, which was selected for use as a local sentinel species for the investigation on the basis of its market value in Nigeria.



## 2. Methodology

### 2.1 Sample Collection

The effluents (text materials) collected from each of the three stations were mixed together at ratio 1:1. The composite mixture was analyzed for physicochemical. Fish samples, fingerlings of *Clarias gariepinus* (test organism) were collected from Fisheries Hatchery/Farm of the Lagos State University, Lagos-Nigeria. Effluents were collected from three point sources: Guinness Nigeria Ltd, Nigerian bottling companies and a confluence from Hotels (as a domestic source). These were identified point of effluent entry into the lagoon. The effluents samples were collected once every month.

### 2.2 Experimental Feed

The fingerlings were fed with 45% protein

COPPENC feed. The ingredients composition and proximate analysis of the feed was ascertained, fixed feeding regime of 4% of the body weight per day was used [22].

### 2.3 Dilution Water

The uncontaminated ground water (bore hole) of the Lagos State University, Ojo laboratory was tested as part of the background study and used as dilution water for the experiment.

### 2.4 Sublethal Toxicity

Thirty (30) rectangular glass tanks of 50 liters capacity were constructed using glass of 5 mm thickness and silicone. And 600 healthy specimens of the species were obtained from Lagos State University fish farm and randomly distributed into the glass tanks. Fishes were fed with 56% protein commercial feed

(Coppens) at 4% body weight per day during the exposure (12 weeks). Twenty (20) specimens were randomly distributed into each tank and exposed to five toxicant concentrations and a control. Five dilutions of the toxicants were made in geometric progression in similar ratios in the following order: 0% (Control), 5%, 15%, 25%, and 35% [23, 24]. Healthy fishes were randomly selected and placed in appropriate sized tanks and covered with mosquito nets that were fastened with rubber band to prevent fish escape. Blood samples obtained from live specimens for haematological analyses. Water quality parameters of the test media (temperature, conductivity, pH and dissolved oxygen (DO)) were monitored weekly in replicates using standard methods [25].

#### 2.4 Haematological Examination

Blood was obtained from two fishes from each treatment by cardiac puncture. For the fingerlings, the blood was drawn out using insulin syringe and needle rinsed with ethylene diamine tetra-acetic acid disodium salt (EDTA), while 5 mL disposable syringe and 21 G needle rinsed with EDTA was used for the juvenile. The needle was inserted and slightly aspirated during penetration. It was then pushed gently down until blood started to enter as the needle punctures the heart [24]. The blood gotten was used for estimation of the red and white blood counts, differential counts and haematocrit.

##### 2.4.1 Blood Haematocrit (Packed Cell Volume)

The haematocrit was determined using the methods outlined by Refs. [26, 27] using micro-haematocrit centrifuge (SH 120-1). Blood was drawn into heparinized micro-haematocrit tube sealed at one end with plasticine. The blood is immediately centrifuged under standard conditions at 2,500 rpm for five minutes. The packed red blood cell volume was measured directly and expressed as a percentage of the total blood volume with a micro-haematocrit meter.

##### 2.4.2 Blood Cell Counts

The improved Neubauer counting chamber was

used in counting the Red Blood Counts (RBC) and White Blood Counts (WBC). Rees-Ecker solution was used as the diluting fluid using the method of Ref. [26]. Blood was drawn directly into RBC pipette just below the 1.0 mark, touching the tip with absorbent tissue to maintain exactly the 1.0 mark volume. This is dropped into the diluting fluid to make 1:100 dilutions and mixed for two minutes. The diluted blood was introduced into the counting chamber. Erythrocytes were counted in five 1 mm squares and leucocytes in four under an inverted microscope with  $\times 10$  objective. The eyepiece rate was giving a total magnification of  $\times 100$ . Two counts for each diluting pipette were taken and their mean values recorded.

##### 2.4.3 Blood Smear

Blood smears were made on two slides for each fish sample. A thin film of the blood was also made on each slide and stained with Fleischman stain according to Ref. [28]. A thin blood was dropped at one end of the slide and a cover slip was used to drag the blood to the other end. The blood was air dried for 3-5 minutes and fixed with absolute methyl alcohol for 5 min. This was later immersed in Fleishman stain for 3 min and rinsed off with pH 6.0 buffer. The smear was allowed to air dry overnight. The cells were observed under the microscope using oil immersion and photographed under  $\times 100$  objective lens.

##### 2.4.4 Differential Count

From the prepared blood smear, the relative leucocytes type (lymphocytes and neutrophil) in the peripheral blood was recorded and the results were expressed as a percentage of the total white blood cell population counted. A drop of oil immersion was applied to the dry thin film using  $\times 100$  objective lens, the film was examined systematically until a minimum of 100 cells are counted [29].

#### 2.5 Hepatological Examination

Live specimens of *C. gariepinus* were collected from the experimental tanks after 12 weeks of sublethal exposure. Fishes were weighed, the total and

standard lengths were recorded, dissected, and the liver, were excised. Liver samples of each specimen were fixed with 10% formalin in phosphate buffer for 36 hours. Fixed specimen were dehydrated in graded levels of ethanol and transferred into xylene for five minutes. Liver samples were then embedded in paraffin and histological sectioning subsequently done at 5 µm using a TBS® CUTTM rotary microtome. Sections were mounted on glass slides and air-dried prior to staining using H & E (30), viewed under a light microscope, this was done at the pathological Department of the University of Ibadan.

### 3. Results

#### 3.1 Water Quality Parameters of Effluent Treated tanks for *Clarias gariepinus* after 12 Weeks

The summary of data on the water quality parameters of the industrial effluent treated tanks with sub-lethal concentrations for *Clarias gariepinus* fingerlings and juveniles are presented in Tables 1 and 2 below.

The lowest mean pH value of 7.81 was obtained in the 5% and 35% tank for fingerlings but 7.25 was obtained as lowest level for juveniles in 25% treatment tank. While the highest mean pH values of 8.06 and 7.86 were recorded in the control tanks (with no effluent introduced) for the fingerlings and juveniles respectively. This could be based on the effect of the increased effluent concentrations as a further decrease in the pH of the various tanks led to more acidity which will become harmful to the test organism. However, the difference among

concentrations was not significant at  $p < 0.05$ . Uniform temperature values were obtained in the tanks for the two life stages, which ranged between 26.07-26.36 in the fingerling tanks and 25.03-25.07 °C for juveniles. The temperature values did not also vary/differ significantly in the different concentrations, but differed slightly amongst life stages.

The dissolved oxygen concentration ranged from 1.97-4.43 mg/L for fingerlings and 2.89-4.72 mg/L for juveniles. The highest mean values were  $4.43 \pm 1.24$  and  $4.72 \pm 1.75$  mg/L for the respective early life stages and both values were obtained from the control tanks. While the lowest mean values of  $1.97 \pm 0.66$  mg/L and  $2.72 \pm 0.78$  mg/L were recorded in 35% and 25% respectively. There were considerable reductions in the mean dissolved oxygen (DO) values consistent with increase in concentrations. The decrease was significant throughout the concentrations with the control tank having the highest. This presupposes that the effluent is mainly an oxygen limiting toxicant with its evident impact on the health and well being of the fish. The highest mean conductivity values obtained were  $402 \pm 30.92$ ~ $486 \pm 45.81$  ppm for fingerlings and juveniles respectively. There were significant differences among various concentrations and also between the two life stages. Concentration of effluent at 0% and 5% in both age groups had the highest conductivity values and the lowest values were obtained from the 35% concentration ( $384 \pm 32.82$  and  $339 \pm 10.47$ ) throughout the period of the experiment/exposure (12 weeks each).

**Table 1** Some water quality parameters of medium during sub-lethal bioassay studies (12 weeks) for *C. gariepinus* fingerlings.

Water quality parameters				
Concentration (%)	pH	Temperature (°C)	Dissolved oxygen (mg/L)	Conductivity (ppm)
0	8.06±0.21 <sup>a</sup>	26.38±2.28 <sup>a</sup>	4.43±1.24 <sup>a</sup>	486±45.81 <sup>a</sup>
5	7.81±0.25 <sup>a</sup>	26.19±2.26 <sup>a</sup>	3.32±1.21 <sup>b</sup>	408±42.28 <sup>b</sup>
15	7.83±0.23 <sup>a</sup>	26.16±2.19 <sup>a</sup>	2.91±0.99 <sup>b</sup>	412±37.74 <sup>b</sup>
25	7.89±0.19 <sup>a</sup>	26.12±2.18 <sup>a</sup>	2.11±0.86 <sup>c</sup>	426±40.99 <sup>b</sup>
35	7.81±0.25 <sup>a</sup>	26.07±2.14 <sup>a</sup>	1.97±0.66 <sup>c</sup>	384±32.82 <sup>c</sup>

Values in each column with similar superscripts are not significantly different (at  $p > 0.05$ ).

**Table 2** Water quality parameters of medium during sub-lethal bioassay studies for *C. gariepinus* juveniles.

Water quality parameters				
Concentration (%)	pH	Temperature (°C)	Dissolved oxygen (mg/L)	Conductivity (ppm)
0	7.86±0.25 <sup>a</sup>	25.03±0.62 <sup>a</sup>	4.72±1.75 <sup>a</sup>	390.74±12.00 <sup>a</sup>
5	7.81±0.26 <sup>a</sup>	25.05±0.73 <sup>a</sup>	4.70±1.12 <sup>a</sup>	402.74±30.92 <sup>a</sup>
15	7.77±0.23 <sup>a</sup>	25.04±0.61 <sup>a</sup>	3.06±1.01 <sup>b</sup>	362.89±13.27 <sup>a</sup>
25	7.25±1.69 <sup>a</sup>	25.07±0.74 <sup>a</sup>	2.72±0.78 <sup>c</sup>	385.00±20.50 <sup>a</sup>
35	7.69±0.78 <sup>a</sup>	25.05±0.66 <sup>a</sup>	2.89±2.17 <sup>c</sup>	339.78±10.47 <sup>a</sup>

Values in each column with similar superscripts are not significantly different (at  $p > 0.05$ ).

**Table 3** Haematological changes of *C. gariepinus* exposed to various concentration of industrial effluent for 12 weeks.

Stage	Conc.	PCV (%)	RBC/mm <sup>3</sup>	WBC/mm <sup>3</sup>	Differential WBC counts (%)	
					Neutrophil	Lymphocyte
Fingerlings	0	27.7±0.6 <sup>a</sup>	9.1±0.1 <sup>a</sup>	7,100±100.0 <sup>a</sup>	61.0±1.0 <sup>a</sup>	34.0±4.6 <sup>a</sup>
	5	19.0±1.0 <sup>b</sup>	6.4±0.3 <sup>b</sup>	6,333±115.5 <sup>b</sup>	50.7±2.3 <sup>b</sup>	36.3±3.5 <sup>a</sup>
	15	17.3±1.2 <sup>b</sup>	5.7±0.4 <sup>c</sup>	8,133±208.2 <sup>c</sup>	25.0±4.0 <sup>c</sup>	63.0±1.0 <sup>b</sup>
	25	14.7±3.1 <sup>c</sup>	5.0±1.0 <sup>c</sup>	9,133±115.5 <sup>d</sup>	32.7±3.1 <sup>c</sup>	55.7±1.5 <sup>b</sup>
	35	13.0±1.7 <sup>c</sup>	4.7±0.6 <sup>d</sup>	10,800±200.0 <sup>e</sup>	26.7±1.5 <sup>c</sup>	63.0±1.90 <sup>b</sup>
Juveniles	0	92.97±1.66 <sup>a</sup>	9.4±0.4	6,300.0±10.0 <sup>a</sup>	175.0±5.0 <sup>a</sup>	88.4±2.1 <sup>a</sup>
	5	115.43±3.4 <sup>b</sup>	7.8±0.4	12,533.3±39.1 <sup>b</sup>	102.9±20.5 <sup>b</sup>	92.43±1.4 <sup>b</sup>
	15	108.73±8.2 <sup>b</sup>	8.07±1.4	23,800.0±18.5 <sup>b</sup>	54.33±0.31 <sup>c</sup>	94.63±2.2 <sup>b</sup>
	25	115.9±0.9 <sup>b</sup>	7.5±0.15	82,000.0±30.0 <sup>c</sup>	96.9±1.85 <sup>d</sup>	96.7±1.5 <sup>b</sup>
	35	102.07±2.0 <sup>b</sup>	7.5±1.23	97,330.3±50.2 <sup>d</sup>	64.97±3.0 <sup>e</sup>	96.2±1.1 <sup>b</sup>

The dissimilar letters as superscripts indicate that the mean values at the different concentrations differ significantly ( $p < 0.05$ ). PCV = packed cell volume (%), RBC = red blood cell (RBC/mm<sup>3</sup>), and WBC = white blood cells (mm<sup>-3</sup>).

### 3.2 Haematology of *Clarias gariepinus* after Sub-lethal Exposure

Results of the mean haematological parameters studied are presented in Table 3. It showed that at the different concentrations, the haematological parameters reduced as the concentrations increased, with exception of the WBC values and differential white blood cell count which increased with increase in concentration. Haematological results also present some remarkable observations, the highest PCV (27.7 ± 0.6~13.0 ± 1.7 and 92.97 ± 1.66~115.9 ± 0.9), RBC (4.7 ± 0.6~9.1 ± 0.1 and 7.5 ± 0.15~9.4 ± 0.4 RBC/mm<sup>3</sup>), and Neutrophil (26.7 ± 4.6~61.0 ± 1.0 and 64.97 ± 1.1~175.0 ± 100.0) for *C. gariepinus* fingerlings and juveniles respectively were obtained in fishes blood in the control treatment tanks and these values decreased with increase in effluent concentration (E.C), while, the highest WBC and lymphocytes values were obtained fish blood in 35%

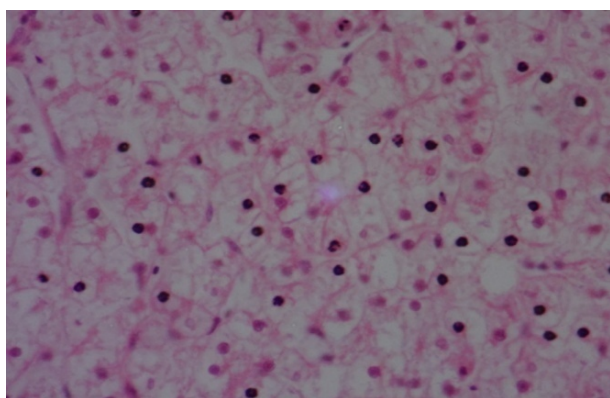
E.C. tanks. This reversal is a responsive systemic action by these white blood cell series to defend the organs against the toxicant. The statistical analysis (ANOVA) showed that the white blood cells differ significantly between the control and the other concentrations ( $p < 0.05$ ).

### 3.3 Histology of Liver of *Clarias gariepinus* Exposed to Sublethal Concentration of Industrial Effluents for 12 Weeks

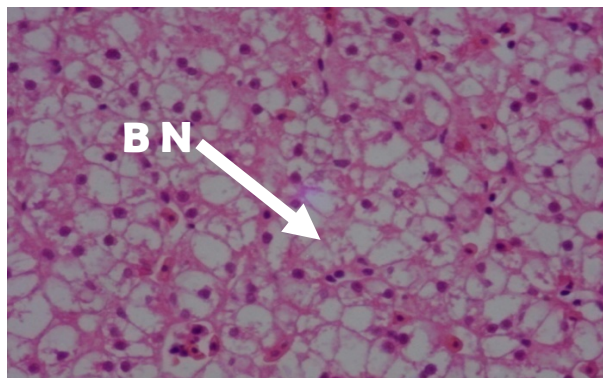
The histological results of the liver show mild to severe lesions in the liver. However, the degree of lesions reduces with sublethal exposure, showing some form of adaptations. The results are shown in Figs. 1-10 below. The histology of the livers of *C. gariepinus* in the control (0%) treatment tanks showed that the livers were intact and had no lesions; it revealed normal liver cells, hepatic cord is normal in both the fingerlings and juveniles. At 5% effluent concentration (E.C) (Fig. 2), the nuclear are becoming

disoriented and fusing together to form bi-nucleated cells (showing slight toxic condition). In Fig. 3, the parenchyma staining is becoming deeper and slightly vacuolated in the two life stages (fingerlings and juveniles). Also in Fig. 3, at 15% E.C; there was progressive increase in vacoulation and micro-vesicle formation (usually fluid-filled cells, membrane bound sac within the cytoplasm). Onset of fatty degeneration and the cell are becoming more damaged.

In the liver histology of fishes exposed to 25% E. C (Fig. 4, and similar responses in Fig. 9 for responses at juvenile stage), the photomicrograph showed total destruction of the hepatic parenchyma (undifferentiated tissue cells frequently with air spaces between them), they are becoming more eosinophilic (i.e. its function involves regulation of allergic responses to destroy combat xenobiotics by producing enzymes tending towards being carcinogenic golgi apparatus activities). There were peri-nucleus halos and less viable nuclear. In the liver histology of fishes exposed to 35% effluent concentration tank in Figs. 5 and 10; for fingerlings and juveniles respectively, there was widespread vacuolar degeneration and severe disruption of the hepatic cord architecture (see arrows). Fusion of the hepatic cord, in both sizes, the staining properties of the nucleus are now carcinogenic.

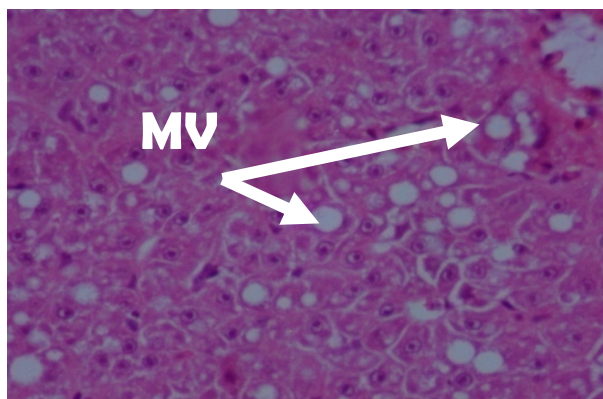


**Fig. 1** Photomicrograph histology of the liver of *Clarias gariepinus* fingerlings exposed to 0% sublethal concentration of industrial effluents for 12 weeks ( $\times 100$ ). It shows normal liver cells with no lesions; hepatic cord is normal.



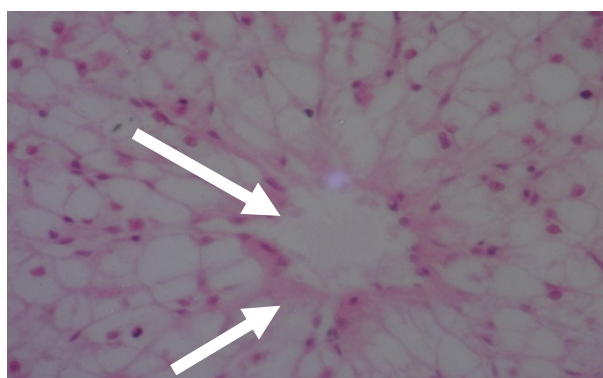
**Fig. 2** Photomicrograph histology of the liver of *Clarias gariepinus* fingerlings exposed to 5% sublethal concentration of industrial effluents for 12 weeks ( $\times 100$ ).

It shows that the nucleus are becoming disoriented and fusing together to form bi-nucleated (BN) cells (showing indications slight toxic condition).



**Fig. 3** Histology of the liver of *Clarias gariepinus* fingerlings ( $\times 100$ ).

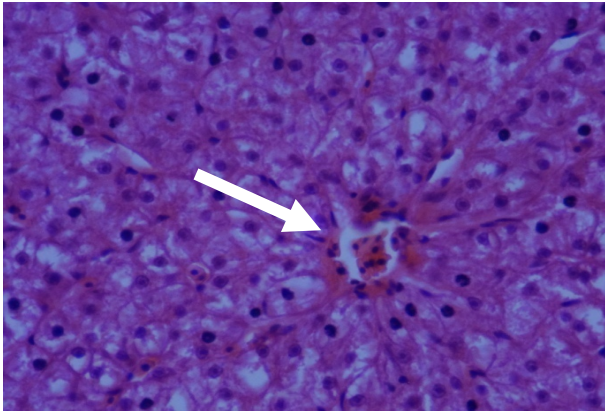
At 15% E.C, there was progressive increase in vacoulation and micro-vesicle formation (MV). Onset of fatty degeneration and the cell are becoming more damaged.



**Fig. 4** Histology of the liver of *Clarias gariepinus* fingerlings.

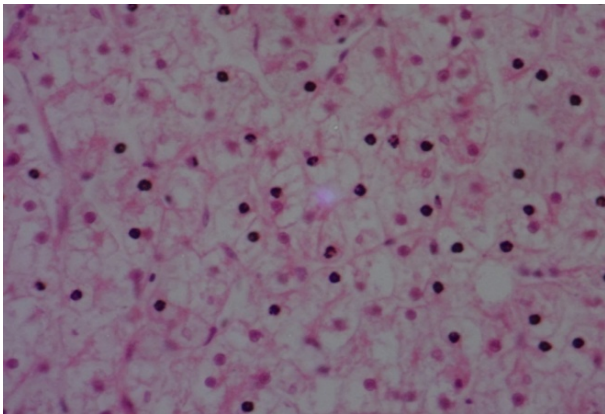
At 25% of industrial effluent, the photomicrograph showed total destruction of the hepatic cord and aggregation of cell towards severely damaged area (arrow).





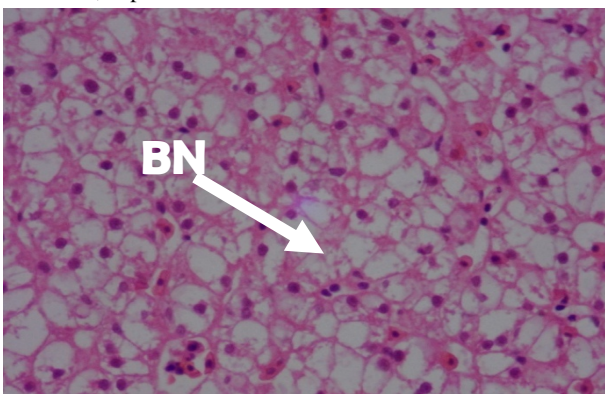
**Fig. 5** Histology of the liver of *Clarias gariepinus* fingerlings.

At 35% effluent concentration tanks, there was widespread vacuolar degeneration and severe disruption of the hepatic cord architecture (see arrows), fusion of the hepatic cord. Adaptive responses are observed.



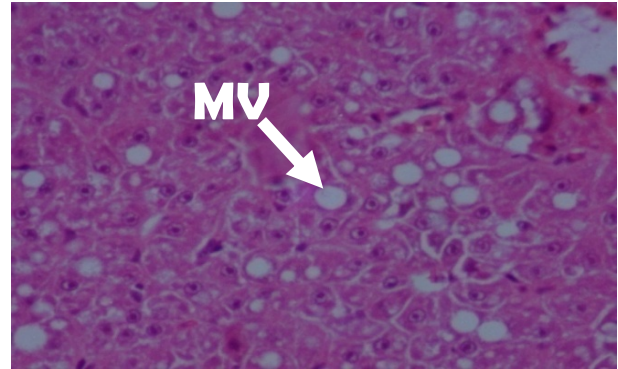
**Fig. 6** Photomicrograph histology of the liver of *Clarias gariepinus* Juveniles exposed to sublethal concentration of industrial effluents or 12 weeks ( $\times 100$ ).

At 0% effluent concentration, it shows normal liver cells with no lesions; hepatic cord is normal.



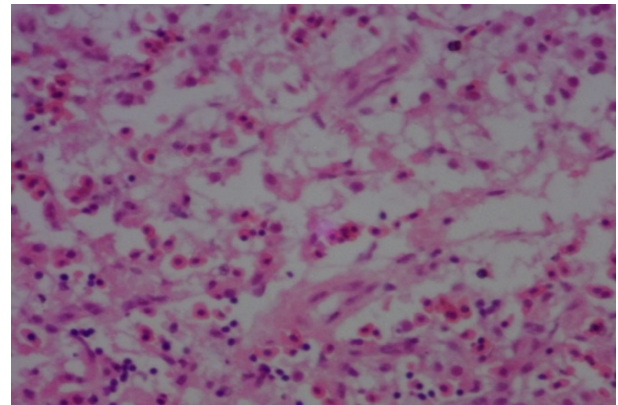
**Fig. 7** Photomicrograph histology of the liver of *Clarias gariepinus* Juveniles exposed to sublethal concentration of industrial effluents for 12 weeks ( $\times 100$ ) 5%.

The nuclear is becoming disoriented and fusing together to form bi-nucleated (BN) cells (showing slight toxic condition).



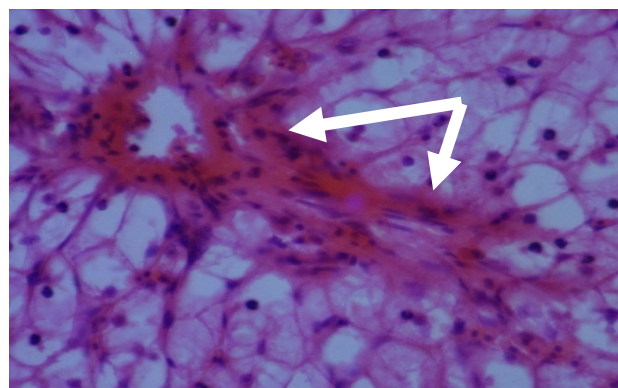
**Fig. 8** Photomicrograph histology of the liver of *Clarias gariepinus* Juveniles exposed to sublethal concentration of industrial effluents for 12 weeks ( $\times 100$ ) 15% industrial effluents.

There is progressive increase in vacuolation and micro-vesicle (MV) formation.



**Fig. 9** Photomicrograph histology of the liver of *Clarias gariepinus* Juveniles exposed to sublethal concentration of industrial effluents for 12 weeks ( $\times 100$ ) 25% industrial effluents.

There is total destruction of the hepatic parenchyma and necrosis.



**Fig. 10** Photomicrograph histology of the liver of *Clarias gariepinus* Juveniles at 35%.

There is total destruction of the hepatic parenchyma, the organ is becoming eosinophilic (i.e. tending towards Carcinogenic golgi apparatus activities), haemorrhage (bleeding) and necrosis of the areas shown by the arrows.



The chronic exposure of the industrial effluent causes significant disruption of the hepatic cord architecture and organ damages. In the liver of the fingerlings and juveniles, there is perivascular cuffing coupled with appearance of bi-nucleated cells and finally infiltration by the white blood cells series (Neutrophils).

#### **4. Discussion and Conclusion**

The mean temperature range of 25.03-25.07 °C obtained in this study was within range reported for tropical aquatic bodies; the minimum mean temperature of 25.03 °C was recorded for the organisms exposed to 0% effluent (control), while the maximum temperature of 25.07 °C was recorded for the same species exposed to 25% effluent. Dissolved oxygen (DO) had a marked difference in the exposure media. A remarkable trend was observed in the tanks, where the mean DO (mg/L) level in the control tank (0%) was  $4.72 \pm 1.75$ , but dropped drastically in the next level treatment (5%) to  $4.70 \pm 1.12$  as shown in Table 2, and continued to drop steadily to  $3.06 \pm 1.01$  in the 15% tank,  $2.72 \pm 1.01$  in 25% and had a slight increase in 35% to  $2.89 \pm 2.17$ . This presupposes that the effluent is mainly an oxygen limiting toxicant with its evident impact on the health and physiology of the fish.

The pH (hydrogen ion concentration) showed slight difference with a steady drop from alkalinity towards neutral from the highest concentration ( $7.86 \pm 0.25$ ) to the 0% (control) concentration. But in the 25% effluent treated tanks, the range was between (7.25-7.86) which is a deviation from the initial trend in the tanks. Conductivity levels showed no variations throughout the different concentrations and stages. The mean conductivity level remained between  $339.78 \pm 0.1$  and  $402.74 \pm 0.1$  ppm.

The whole effluent toxicity tests were carried out to determine the actual impacts of effluents on organisms residing in receiving waters where the effluents are discharged. For fingerlings and juvenile of the African

catfish, alterations in the blood as biomarkers of exposure and the effect have been the subject of intense study over the past 20 years in toxicology. These studies have delineated a number of chemical specific responses that have proven extremely useful in the early detection of low-doses chemical effects [31]. Several workers have investigated the toxicity and heamatological changes of pollutants in fish [4-6], and the use of hematological techniques in fisheries research is growing rapidly, as it is very important in toxicological research environmental monitoring and predicting of fish health conditions [32]. Haematological profiles of fishes are widely used to monitor the environmental pollution in aquatic ecosystems. These parameters are also used to detect the physiological status of animals and indicators of stress. The haematological parameters such as RBC, WBC, PCV, and other haematological indices like Neutrophil and lymphocytes are frequently used to assess the health status of fish [2]. The presence of toxicant in the aquatic media may affect the water quality, which in turn affects the values of haematological parameters of fish, due to its close association with the environment.

This study however, elicited various changes in the heamatological parameters in the test organisms after 12 weeks of sublethal exposure to industrial effluents. The mean values of hematological parameters in the treatment tanks can be seen in Table 3. The result of hematological parameters showed marked significant difference between control tanks and the treatments tanks, which is an indication of the deleterious effects of the chemical pollutant to the body fluid of *Clarias gariepinus*. Anaemic condition of *Clarias gariepinus* exposed to effluent is observed with reduction in the red blood cells (RBC) as a result of increase in the sublethal concentration of effluent, the same goes for the packed cell volume (PCV) during the trials which results in the low level of haemoglobin (Hb) in the test organisms. Inhibition of erythropoiesis due to effluent toxicity by its action on erythrocytes cell membrane

may be another possible reason (erythropoiesis is the formation and production of the red blood cells resulting in the release of matured red blood cells erythrocytes into circulation). Decrease in numbers of RBC in fish due to toxicant exposure has been reported by Refs. [33, 34]. The observed decrease in RBC in the present investigation, resulted from inhibition of RBC production or Hb synthesis by the toxicant. It might also be due to accumulation of effluents in the gill region which may have damaged the structure of the gill resulting in haemolysis (rupturing (lysis) of the red blood cells and the release of their content (cytoplasm) into surrounding fluid).

This investigation reports that there was significant ( $p < 0.05$ ) decrease in PCV, RBC, neutrophil and increase in the white blood cells and the lymphocytes during the exposure. This reverse response by the WBC and Neutrophils indicates some defensive action to protect the fish from further severe injuries. It is well known that the changes in Neutrophils counts after exposure to pollution may be associated with a decrease in non-specific immunity of the fish. Leucocytes and neutrophil (neutrophils are the first line of host cellular defense against attack or injuries) are involved in the regulation of immunological function in many organisms; also increase in WBC in stressed animals indicates a protective response to stress [35].

There are also significant increases in WBC during effluent treatment, which might have resulted in the stimulation of the immune system by the effluent and for protection of the fish against toxicity. The increase in WBC count during this study may be due to extended toxic effect of the effluent on the liver tissues, which are the primary sites of haematopoiesis, provoking immunosuppression. Another possible reason may be due to elevation of white blood cells maturation and the release from tissues reservoirs by the action of effluent to combat the stressor (36). Since fish are so intimately associated with the aqueous environment, the blood will reveal

measurable physiological changes in the fish more rapidly than any physiological assessment parameters.

The histological examination revealed several lesions as expressed by the livers. The histopathology of liver: control (0%) in figures 1 and 6, shows normal liver cells, at higher toxic level from figures 2-5 for fingerlings and figures 7-10 for juvenile of *C. gariepinus*, there were: vacuolation, destruction of the hepatic parenchyma, tissue becoming eosinophilic (i.e. tending towards Carcinogenicity) and severe disruption of the hepatic cord architecture. The study has shown that industrial effluents from the study area may affect fish health status and impair vital processes if exposure continues for a long period even at lower concentrations (sublethal).

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