

## Haematological, Biochemical and Histopathological Responses of African Catfish *Clarias Gariepinus* Juveniles to Sublethal Concentrations of Crude Oil in Water

Flora E. Olaifa, Ibukun Omotola Akinrele, Jacob Somorhine Ewutanure

Department of Aquaculture and Fisheries Management

Faculty of Renewable Natural Resources

University of Ibadan

Ibadan Nigeria.

E-mail: [floraolaifa@yahoo.com](mailto:floraolaifa@yahoo.com)

### ABSTRACT

The spread of constituents of crude oil in the bio-physical environment is a major concern, causing aquatic pollution, toxicity, increased susceptibility to diseases, death of fish, absorption and accumulation of trace metals in biological tissues. This study was carried out to evaluate the response of *Clarias gariepinus* juveniles exposed to varying sublethal concentrations of crude oil in water. Biochemical and histological investigations were undertaken to assess the effect of crude oil-polluted water on the African catfish (*C. gariepinus*) under laboratory conditions for 7 days. A total of 192 juvenile catfishes (average weight 12 g) were grouped into seven treatments and control of 12 catfish per 20L Aquarium glass tank and held for 7 days in different mixtures of crude oil-polluted water (1.0%, 2.0%, 3.6%, 6.4%, 10.0%, 15.0%, and 20.0% oil-polluted water). Catfish in the control group were held in borehole water without oil contamination. At the end of 7 days, blood samples were drawn from the posterior caudal vein of the fishes for analysis. Livers and kidneys of fish samples were also assessed for histopathological changes. The haematological parameters obtained were in the following ranges: Pack Cell Volume  $43.50 \pm 2.12$  (T<sub>4</sub>) -  $51.50 \pm 0.71\%$  (T<sub>1</sub>), haemoglobin  $14.95 \pm 0.07$  (T<sub>5</sub>) -  $16.95 \pm 0.78$  g/dl (T<sub>1</sub>); Red blood cell  $7980.00 \pm 28.28$  (control) -  $1015.00 \pm 494.97$  (cells/mm<sup>3</sup>) (T<sub>1</sub>); Mean Corpuscular Haemoglobin  $1.67 \pm 0.00$  (T<sub>1</sub>) -  $1.90 \pm 0.09$  Pg (control); Mean Cell Haemoglobin Concentration  $3.22 \pm 0.12$  (T<sub>2</sub>) -  $3.55 \pm 0.04$  g/dl (T<sub>4</sub>). Control fish had higher Mean cell volume value than the fish in T<sub>1</sub>-T<sub>7</sub>. Sodium, potassium, plasma protein and albumin levels were not significantly different across the treatments ( $P > 0.05$ ). Chlorine concentration in the control and T<sub>2</sub> were similar  $105.00 \pm 0.00$ , however, the highest concentration was obtained in T<sub>1</sub>. Bicarbonate concentration was highest in T<sub>7</sub>,  $25.00 \pm 1.41$  and similar levels were observed in the control and T<sub>3</sub>. Urea concentration was lowest in T<sub>5</sub>,  $27.50 \pm 2.12$  and highest in T<sub>4</sub>. The lowest creatinine level was  $0.55 \pm 0.07$  (T<sub>5</sub>) and the highest  $0.75 \pm 0.07$  (T<sub>1</sub> and T<sub>4</sub>). Globulin concentrations ranged from  $2.95 \pm 0.07$  (T<sub>5</sub>) -  $3.35 \pm 0.07$  g/dl (T<sub>4</sub>). The lowest Aspartate Aminotransferase (AST) was obtained in T<sub>3</sub>, while the highest level was in T<sub>5</sub>. Similarly, alanine aminotransferase (ALT) was highest in T<sub>5</sub> and lowest in the control. Alkaline phosphatase (ALP) ranged from  $29.00 \pm 1.41$  (T<sub>2</sub>) -  $46.00 \pm 4.24$  (T<sub>4</sub>) (iu/l). Glucose levels ranged from  $53.50 \pm 0.71$  (T<sub>2</sub>) to  $67.50 \pm 3.54$  (T<sub>6</sub>). Histological changes in livers and kidneys of fish included vacuolar degeneration of hepatocytes and tubular degeneration. Crude oil affects the haematological, biochemical and histological profiles of fish. The alterations of these parameters can serve as suitable biomarkers in monitoring of crude oil pollution in the aquatic environment and to protect aquatic life.

**Keywords:** Haematology, serum biochemistry, histology, bioassay.

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## 1. INTRODUCTION

Crude oil and its products are the main sources of energy in modern society and account for 41% of the global energy demand, estimated at 8,677 million tons (International Energy Agency, 2012). Since the 1970s, oil has accounted for 80% of Nigeria's revenue and 95% of the export earnings. Crude oil is a naturally- occurring mixture, consisting predominantly of hydrocarbons, sulphur, nitrogen and metals (Olaifa, 2003; Yasin et al., 2013). Crude oil and its refined petroleum products contain several organic and inorganic substances including sulphur, nitrogen, oxygen, metals such as iron, vanadium, nickel and chromium (Olaifa, 2005; Olaifa, 2012, Incardona, 2017). Oil pollution occurs during extraction, storage or transportation of petroleum and its products (Ibigoni Clinton et al, 2009). It is the release of contaminants or pollutants associated with the extraction of crude oil into the environment. Oil exploration by seismic surveys involves clearing of seismic lines, dynamiting for geological excavation, which affects the aquatic environment. It causes mortality of fauna, turbidity in the water which leads to the blockage of gills of filter feeders among benthic fauna.

There is reduction of photosynthetic activity due to water turbidity caused by reduction in sunlight penetration. Annually, about 5 million metric tons of crude oil enters the aquatic environment from oil spills (Edwards et al 2003; Ali et al, 2014). Oil pollution contaminates water, air, and food crops with hydrocarbon and trace metals. The presence and quantity of these constituents can exert some acute and long-term adverse health effects on man. Fish are vulnerable and exposed to pollution with attendant detrimental effects (Authman et al., 2015; Mahboob et al., 2014; Saleh and Marie, 2014; Yarsan and Yipel, 2013). Some petroleum-derived hydrocarbons are toxic to a wide spectrum of marine animals because they preferentially accumulate in lipid compartments like cellular membrane (DiToro et al., 2001), disturbing the physicochemical and physiological membrane properties (Sikkema et al., 1994). The accumulation of soluble petroleum hydrocarbons in fish is extremely rapid. Biochemical, physiological and histological biomarkers, among others, have been used to determine the effects of petroleum derived hydrocarbons in aquatic biota (Simonato et al., 2008). Also, haematological studies are of ecological and physiological interest helping to understand the relationship of blood characteristics to the habitat and adaptability of the species to the environment (Ovuru and Ekweozor, 2004). Previous studies have shown that crude oil can have both lethal and sub-lethal effects on a wide range of organisms with drastic changes in liver enzyme activities of catfish (*C. gariepinus*) reported following exposure to crude oil (Sunmonu and Oloyede, 2006). Jack et al., (2005) reported increased levels of total hydrocarbons in shellfishes in crude oil- polluted stations in the Niger Delta area compared with unpolluted sites. The eco-physiological effects of crude oil on *Machaerium lunatus* was also reported (Bamidele and Agbogidi, 2006). This study was therefore aimed at evaluating the effects of dilutions of crude oil contaminated water on hematology, biochemical parameters and histology of juvenile *C. gariepinus*.

## 2. MATERIALS AND METHODS

### Sample Collection

Crude oil contaminated- water sample was collected from Gbalegbe River, in Ughelli North Local Government Area, Delta State, Nigeria in glass bottles, properly sealed and transported. Two hundred juveniles of *C. gariepinus* (mean weight 12g) were obtained and acclimatized for 14 days in a holding tank and fed twice a day (8:30am and 4:30pm) at 5% of body weight. All the test fish were observed to be in good condition before the onset of the experiment.

### Experimental Protocol

The *C. gariepinus* juveniles were batch-weighed and distributed randomly with 12 fishes per tank. Seven Treatments (T<sub>1</sub>), (T<sub>2</sub>), (T<sub>3</sub>), (T<sub>4</sub>), (T<sub>5</sub>), (T<sub>6</sub>), (T<sub>7</sub>), and one Control were used for this study. The crude oil -polluted water samples were introduced at 400mL, 300ml, 200ml, 128ml, 72ml, 40ml, 20ml (representing 20-1% concentration of crude oil in water) and control (0 ml) in 20L of water. Each treatment had two (2) replicates. The fish samples in each treatment were exposed for 7 days. The glass tanks were covered with mosquito net to prevent fish from jumping out; there was no aeration, no water change throughout the test. Fish were monitored hourly for the first four hours, every 4 h for the next 24 h, and subsequently every 24 h, for the next 5 days erratic swimming, air gulping, loss of reflex and discoloration. The inability of fish to respond to external stimuli was used as an index of death. Water quality parameters such as Nitrates, Ammonium, Nitrites, Phosphate, Sulphate, Calcium, and Potassium were determined colorimetrically.

**Table 1: Dilutions of Crude oil- polluted water used for the study**

Percentage of crude oil - polluted water used	Required volume of crude oil- polluted (ml) in 20 L water	Volume of dilution water per 1000ml	Required volume of water for 20L
20	400	980.0	19,600
15	300	985.0	19,700
10	200	990.0	19,800
6.4	128	993.6	19,872
3.6	72	996.4	19,928
2.0	40	998.0	19,980
1.0	20	999.0	19,980
0.0	0	0	20,000

### Hematological Examination

Blood sample was drawn under pressure from the posterior caudal vein of *C.gariepinus* according to Schmitt et al., (1999), 2ml was decanted in heparinized bottles. Red blood cell count was estimated using modified hyme's dilution fluid (Jain, 1986) and the white blood cell according to the method of (Dacie and Lewis, 1975). The packed cell volume was measured using micro haematocrit reader (Blaxhall and Daisley 1973) and expressed as percentage. Haemoglobin concentration was evaluated using the cyanomethaemoglobin method (Schalm et al., 1975). The basic erythrocyte indices, mean cell/corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) were computed from red blood cell count, haemoglobin level and PCV values (Duncan et al., 1994). Differential counts (neutrophils and lymphocytes) were determined (Jain, 1986; Davies, 1994).

### Serum Biochemical analysis

The serum total protein was determined by the Biuret method (Reinhold, 1953) using a commercial kit (Randox Laboratories Ltd, U.K), while albumin value was obtained by bromocresol green method (Doumas et al., 1971). The globulin and albumin- globulin ratio was determined (Coles (1986). Biochemical indices determined included glucose (GLU), total proteins (TP), albumins (ALB), total globulins (GLOB), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), sodium (Na), potassium (K), inorganic phosphate (PHOS) and blood glucose.

### Statistical analysis

The data obtained were subjected to analysis of variance and the means compared using the Duncan's Multiple range test. A significant level of 0.05 was used. The experiments were all designed as a completely randomized design (CRD).

### 3. RESULTS

The results of the water quality analyses (Table 2) showed that the concentrations of ammonia, nitrate, nitrite and Sulphate were not detected in the control units. There were no statistical differences in the concentrations of sulphate recorded across the varying crude oil -containing water.

**Table 2: Water quality parameters (nutrients) in the experimental units**

Parameter	Control	T1	T2	T3	T4	T5	T6	T7
Calcium	1.99±0.0 1 <sup>b</sup>	1.80±0.2 8 <sup>b</sup>	2.08±0.29 b	2.01±0.3 3 <sup>b</sup>	2.30±0.35 <sup>b</sup>	2.21±0.2 5 <sup>b</sup>	2.78±0.25 ab	3.77±1.5 1 <sup>a</sup>
Potassium	10.89±0.0 01 <sup>b</sup>	24.90±3.0 25 <sup>a</sup>	20.40±1.7 0 <sup>ab</sup>	24.10±3.0 25 <sup>a</sup>	25.30±10.0 32 <sup>a</sup>	23.55±0.0 49 <sup>a</sup>	16.65±0.2 1 <sup>ab</sup>	20.85±0.0 21 <sup>b</sup>
Phosphate	0.08±0.0 0 <sup>b</sup>	0.81±0.5 5 <sup>ab</sup>	0.46±0.11 ab	1.04±0.2 3 <sup>a</sup>	0.43±0.16 <sup>a</sup> b	0.27±0.3 2 <sup>ab</sup>	0.71±0.69 ab	0.16±0.0 2 <sup>b</sup>
Nitrate	ND	1.23±1.5 6 <sup>ab</sup>	2.25±0.35 b	1.48±1.3 1 <sup>ab</sup>	2.36±0.00 <sup>a</sup>	2.16±0.5 3 <sup>a</sup>	2.41±0.33 a	2.72±0.2 3 <sup>a</sup>
Nitrite	ND	0.74±0.9 3 <sup>ab</sup>	1.35±0.21 a	0.89±0.7 8 <sup>ab</sup>	1.41±0.00 <sup>a</sup>	1.31±0.3 0 <sup>a</sup>	1.45±0.19 a	1.63±0.1 3 <sup>a</sup>
Ammonia	ND	0.26±0.3 2 <sup>ab</sup>	0.46±0.07 a	0.30±0.2 7 <sup>ab</sup>	0.48±0.00 <sup>a</sup>	0.45±0.1 1 <sup>a</sup>	0.50±0.06 a	0.56±0.0 4 <sup>a</sup>
Sulphate	ND	3.13±1.3 9 <sup>a</sup>	3.83±0.71 a	3.31±0.9 5 <sup>a</sup>	3.42±0.28 <sup>a</sup>	3.24±0.2 5 <sup>a</sup>	3.21±0.04 a	3.24±0.0 8 <sup>a</sup>

Mean values with different alphabet superscript on the same row are significantly different. ND-Not detected

**Table 3: Haematological profile of the juvenile *C. gariepinus* (African Catfish) exposed to varying dilutions of crude oil- polluted water**

Parameters	Treatments							
	Control	T1	T2	T3	T4	T5	T6	T7
PCV (%)	44.00±1.41 <sup>a</sup>	51.50±0.71 <sup>a</sup>	49.50±2.12 <sup>b</sup>	48.00±1.41 <sup>bc</sup>	43.50±2.12 <sup>d</sup>	45.00±1.41 <sup>cd</sup>	47.00±1.41 <sup>cd</sup>	49.50±0.71 <sup>bd</sup>
Hb (gm/l)	15.15±0.78 <sup>bc</sup>	16.95±0.78 <sup>a</sup>	15.950.07 <sup>abc</sup>	15.80±0.57 <sup>abc</sup>	15.45±0.92 <sup>bc</sup>	14.95±0.07 <sup>c</sup>	15.95±0.07 <sup>abc</sup>	16.50±0.14 <sup>bd</sup>
RBC (cells/mm <sup>3</sup> )	7980.00±28.28 <sup>d</sup>	10150.00±494.97 <sup>a</sup>	9330.00±890.95 <sup>abc</sup>	8825.00±247.49 <sup>bcd</sup>	8290.00±862.67 <sup>cd</sup>	8375.00±332.34 <sup>cd</sup>	8890.00±155.56 <sup>bcd</sup>	9675.00±233.35 <sup>a</sup>
WBC (cells/mm <sup>3</sup> )	7675.00±1873.83 <sup>a</sup>	8650.00±70.71 <sup>a</sup>	8005.00±134.35 <sup>a</sup>	8445.00±1689.99 <sup>a</sup>	8380.00±381.84 <sup>a</sup>	7055.00±219.20 <sup>a</sup>	7882.50±456.08 <sup>a</sup>	8029.50±123.74 <sup>a</sup>
N (%)	70.50±3.54 <sup>a</sup>	66.50±3.54 <sup>a</sup>	64.50±9.19 <sup>a</sup>	69.00±4.24 <sup>a</sup>	58.50±3.54 <sup>a</sup>	67.50±3.54 <sup>a</sup>	58.50±10.61 <sup>a</sup>	65.50±3.54 <sup>a</sup>
L (%)	29.00±2.83 <sup>a</sup>	33.00±4.24 <sup>a</sup>	34.50±7.78 <sup>a</sup>	30.00±2.83 <sup>a</sup>	40.50±2.12 <sup>a</sup>	31.00±2.83 <sup>a</sup>	40.00±8.49 <sup>a</sup>	34.50±3.54 <sup>a</sup>
M (%)	0.50±0.71 <sup>a</sup>	0.50±0.71 <sup>a</sup>	1.00±1.41 <sup>a</sup>	1.00±1.41 <sup>a</sup>	1.00±1.41 <sup>a</sup>	0.50±0.71 <sup>a</sup>	1.50±2.12 <sup>a</sup>	ND
MCV (fl)	5.51±0.20 <sup>a</sup>	5.08±0.18 <sup>a</sup>	5.32±0.28 <sup>a</sup>	5.44±0.01 <sup>a</sup>	5.26±0.29 <sup>a</sup>	5.37±0.04 <sup>a</sup>	5.29±0.25 <sup>a</sup>	5.12±0.20 <sup>a</sup>
MCH (pg)	1.90±0.09 <sup>a</sup>	1.67±0.00 <sup>c</sup>	1.72±0.16 <sup>abc</sup>	1.79±0.01 <sup>abc</sup>	1.87±0.08 <sup>ab</sup>	1.79±0.06 <sup>abc</sup>	1.79±0.04 <sup>abc</sup>	1.71±0.03 <sup>bcd</sup>
MCHC (g/dl)	3.45±0.29 <sup>ab</sup>	3.29±0.11 <sup>ab</sup>	3.22±0.12 <sup>b</sup>	3.29±0.02 <sup>ab</sup>	3.55±0.04 <sup>a</sup>	3.32±0.09 <sup>ab</sup>	3.39±0.09 <sup>ab</sup>	3.33±0.08 <sup>ab</sup>

Note: PCV (Packed cell volume), Hb (Haemoglobin concentration), RBC (Red Blood cell Count), WBC (White Blood cell Count), N (Neutrophil), L (Lymphocyte), M (Monocyte), MCV (Mean Corpuscular Volume,) MCH (Mean Corpuscular Haemoglobin), MCHC (Mean Corpuscular Haemoglobin Concentration).

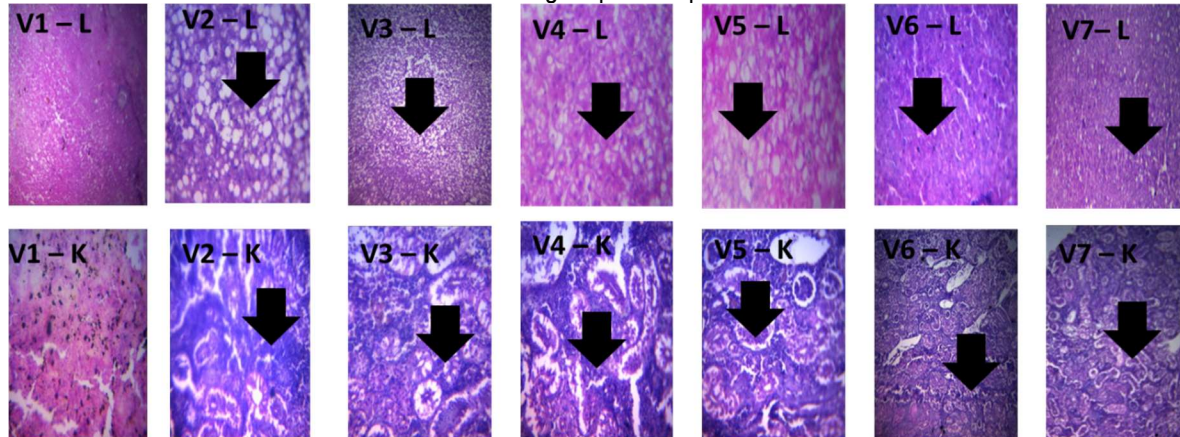
There were no significant differences in the WBC, Neutrophil, Lymphocytes, Monocyte, MCV counts across the crude oil-containing treatments. The control fish had higher MCV values than the fish treated with varying concentrations of crude oil in water, but the difference was not statistically significant.

**Table 4: Serum biochemical profile of juvenile *Clarias gariepinus* (African Catfish) exposed to dilutions of crude oil polluted water**

The results showed that the sodium, potassium, plasma protein and albumin levels were not significantly different across the treatment levels (P> 0.05).

Parameters	Treatments							
	Control	T1	T2	T3	T4	T5	T6	T7
Na <sup>+</sup>	140.00±1.41 <sup>a</sup>	140.00±0.00 <sup>a</sup>	139.50±2.12 <sup>a</sup>	137.50±0.71 <sup>a</sup>	141.0±1.41 <sup>a</sup>	139.00±0.00 <sup>a</sup>	770.50±89.25 <sup>a</sup>	138.00±1.41 <sup>a</sup>
K <sup>+</sup>	4.00±0.00 <sup>a</sup>	4.10±0.00 <sup>a</sup>	3.85±0.21 <sup>a</sup>	3.90±0.14 <sup>a</sup>	4.10±0.00 <sup>a</sup>	3.85± 0.07 <sup>a</sup>	4.10±0.14 <sup>a</sup>	4.00±0.28 <sup>a</sup>
Cl <sup>-</sup>	105.00±0.00 <sup>ab</sup>	110.00±0.00 <sup>a</sup>	105.00±0.00 <sup>abc</sup>	100.00±0.00 <sup>a</sup>	107.50±3.54 <sup>ab</sup>	102.50±3.54 <sup>ab</sup>	107.50±3.54 <sup>ab</sup>	102.50±3.54 <sup>ab</sup>
HCO <sub>3</sub> <sup>-</sup>	24.00±1.41 <sup>ab</sup>	21.50±0.71 <sup>bc</sup>	22.50±2.12 <sup>abc</sup>	24.00±1.41 <sup>ab</sup>	20.50±0.71 <sup>c</sup>	24.50±0.71 <sup>ab</sup>	21.50±0.71 <sup>bc</sup>	25.00±1.41 <sup>a</sup>
Urea(mg/dl)	28.50±2.12 <sup>b</sup>	32.00±1.41 <sup>ab</sup>	29.00±1.41 <sup>ab</sup>	29.00±1.41 <sup>ab</sup>	34.50±4.95 <sup>a</sup>	27.50±2.12 <sup>b</sup>	32.50±0.71 <sup>ab</sup>	28.50±2.12 <sup>b</sup>
Cr(mg/dl)	0.65±0.07 <sup>ab</sup>	0.75±0.07 <sup>a</sup>	0.65±0.07 <sup>ab</sup>	0.60±0.14 <sup>ab</sup>	0.75±0.07 <sup>a</sup>	0.55±0.07 <sup>b</sup>	0.70±0.00 <sup>ab</sup>	0.65±0.07 <sup>ab</sup>
TP(g/dl)	7.20±0.14 <sup>a</sup>	6.95±0.07 <sup>a</sup>	6.95±0.21 <sup>a</sup>	6.95±0.35 <sup>a</sup>	7.30±0.14 <sup>a</sup>	6.95±0.07 <sup>a</sup>	7.15±0.07 <sup>a</sup>	7.05±0.21 <sup>a</sup>
ALB(g/dl)	4.10±0.14 <sup>a</sup>	3.75±0.21 <sup>a</sup>	3.90±0.14 <sup>a</sup>	3.80±0.28 <sup>a</sup>	3.95±0.07 <sup>a</sup>	4.00±0.14 <sup>a</sup>	4.10±0.00 <sup>a</sup>	3.90±0.00 <sup>a</sup>
GLB(g/dl)	3.10±0.00 <sup>ab</sup>	3.20±0.28 <sup>ab</sup>	3.05±0.07 <sup>ab</sup>	3.15±0.07 <sup>ab</sup>	3.35±0.07 <sup>a</sup>	2.95±0.07 <sup>b</sup>	3.05±0.07 <sup>ab</sup>	3.15±0.21 <sup>ab</sup>
AST (iu/l)	11.00±1.41 <sup>ab</sup>	12.00±0.00 <sup>ab</sup>	12.00±2.83 <sup>ab</sup>	10.00±1.41 <sup>b</sup>	14.00±1.41 <sup>ab</sup>	15.00±1.41 <sup>a</sup>	11.00±1.41 <sup>ab</sup>	12.50±3.54 <sup>ab</sup>
ALT (iu/l)	6.50±0.7 <sup>c</sup>	9.50±0.71 <sup>abc</sup>	9.00±1.41 <sup>abc</sup>	7.50±0.71 <sup>c</sup>	11.00±1.41 <sup>ab</sup>	12.00±1.41 <sup>c</sup>	8.50±2.12 <sup>bc</sup>	9.50±0.71 <sup>abc</sup>
ALP (iu/l)	40.00±1.41 <sup>abc</sup>	32.00±4.24 <sup>bc</sup>	29.00±1.41 <sup>c</sup>	40.50±6.36 <sup>abc</sup>	46.00±4.24 <sup>a</sup>	40.50±3.54 <sup>abc</sup>	45.50±7.78 <sup>a</sup>	42.50±7.78 <sup>ab</sup>
GLU(mg/dl)	56.00±0.00 <sup>ab</sup>	63.50±7.78 <sup>ab</sup>	53.50±0.71 <sup>b</sup>	56.00±5.66 <sup>ab</sup>	59.00±2.83 <sup>ab</sup>	65.00±2.83 <sup>ab</sup>	67.50±3.54 <sup>a</sup>	60.50±9.19 <sup>ab</sup>

Na<sup>+</sup> (Sodium), K<sup>+</sup> (Potassium) Cl<sup>-</sup> (Chlorine), HCO<sub>3</sub><sup>-</sup> (Bicarbonate), Cr (Creatinine) (mg/dl), TP (Plasma Protein) (mg/dl), ALB (Albumin) (g/dl), GLB (Globulin) (g/dl), AST (Aspartate aminotransferase) (iu/l), ALT (Alanine aminotransferase) (iu/l), ALP (Alkaline phosphatase) (iu/l), GLU (Glucose).

**HISTOPATHOLOGY of LIVER AND KIDNEY of *C.gariepinus* exposed to dilutions of crude oil-contaminated water.****Plate 1.** Liver and kidney of *C. gariepinus* juvenile subjected to toxicity of effluents from Gbalegbe River

Note: Magnification: X400; V1 to V7 = Treatments 1 – 7. V1 – L and V1 – K = Control while V2 – V7 are treatments for liver (L) and kidney (K) of *C. gariepinus* juvenile respectively. No visible lesions were seen in V1 – L and V1 – K; V2 – L and V3 – L showed severe vacuolar degeneration of hepatocytes, diffused degeneration of tubules with some tubular luminae containing protein casts; Periportal cellular infiltration and portal congestion were severe (V2 – L); Moderate diffused hydropic degeneration of hepatocytes occurred (V4 – L to V7 – L). V2 – K and V3 – K showed severe congestion in blood sinusoids; V2 – K to V5 – K showed severe and necrotic degeneration of vacuole in the renal tubules while V6 – K and V7 – K showed severe extensive renal congestion at the cortex.

**4. DISCUSSION**

Aquatic bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals (Olaifa et al., 2003). This study investigated the effects of different dilutions of crude oil-polluted water on some haematological parameters, serum biochemistry and histology of *Clarias gariepinus*. The fishes swam vertically about 6 hours after the introduction of the crude oil-polluted water. After 96hrs, mortality was recorded in two of the treatments; treatment 1 and Treatment 3. The intensity of the behavioral activities of the fish decreased with increasing concentration and duration of exposure.

The results obtained during the study are presented in Tables 2-4 and plate 1. Nutrients in the water used during the experiment are shown (Table 2) with Potassium dominating. Variations were observed in haematology of the *C.gariepinus* (Table 3) which tended to confirm that crude oil affects the blood profile of juvenile *C.gariepinus*. The haemoglobin counts were slightly higher than earlier reports (Ayotunde et al., 2010). The reduction of MCHC and the increase of MCH and MCV values from the control to the highest concentration of crude oil (20ml) in T<sub>7</sub> could be due to stress induced by crude oil pollution, and possibly from the shrinking cell size of erythrocytes (Akinrotimi et al., 2010). This finding is supported by Radoslav et al., (2013) who opined that decrease in MCHC values can be related to increase in erythrocyte volume (MCV) which is not followed with adequate increase of haemoglobin in them (MCH). However, slight increase of haemoglobin concentration indicates the beginning of its synthesis in mature erythrocytes. White blood cell (WBC) counts in juvenile *C. gariepinus* in the varying levels of crude oil treatments were higher than the control.

The white blood cells are required to fight infections. The increase in WBC may indicate the fishes' reaction to the toxicant or an immune system disorder or stress (Abramson and Melton, 2000). Similar trend was observed in the lymphocyte count which was lowest in the control fish. Neutrophil levels were lower than the control in all treatments. The concentration of blood plasma protein is an indicator to general health condition of fish (Das et al., 2004). The serum biochemistry profile (Table 4) of the juvenile *C. gariepinus* showed that there was non-significant reduction in the plasma protein at exposure to higher concentrations of crude oil-polluted water compared to the control. A reduction in plasma protein is an indicator of the effect of toxins in the kidney, spleen and liver (Abdali et al., 2011). The decrease in protein level with higher levels of crude oil contamination may be attributed to the assertion by Singh and Singh (2002) as destruction or necrosis of cells, impairment of protein synthesis and mobilization of protein to meet energy requirements to sustain increased physiological activity (Martinez et al., 2004).

There was a significant increase in urea in the juvenile *C. gariepinus* compared to the control in T5 and 7. This could be as a result of possible tissue damage in the liver and kidney of the fish. Since urea is formed in the liver and excreted by kidneys (Andrade et al., 2014), urea analysis gives an indication of nitrogenous compound in the bloodstream. Serum creatinine levels were highest in T<sub>4</sub> and T<sub>1</sub> treated with 6.4ml and 20ml of crude oil in water suggesting a decrease in renal excretion and some degree of renal insufficiency.

Several soluble enzymes of blood serum have been considered as indicators of hepatic dysfunction and damage. Among the array of enzymes used are AST, ALT, ALP to detect the cellular damage caused by toxicants (Datta et al., 2007; Gad, 2007). There were significant differences ( $p < 0.05$ ) in ALP levels which is a marker enzyme for the plasma membrane and endoplasmic reticulum (Adeyemi and Muhammad, 2010). This could signify a lethal effect of the toxicant on juvenile *C. gariepinus* (Rao, 2006; Ruothalo, 2008). An increase in ALP level may carry the possibility of membrane damage, because ALP is a membrane-bound enzyme. There were significant differences ( $P < 0.05$ ) in the levels of AST and ALT in the serum biochemistry profile of the juvenile *C. gariepinus* exposed to sub lethal concentrations of crude oil. The tissue activities of the transaminase (AST and ALT) enzyme are markers for the functions and integrity of the heart and liver (USDA, 2006; Adeniyi et al., 2010). The significant increase in serum ALT activity in juvenile *C. gariepinus* indicates possible injury in hepatic tissue induced by the exposure since serum enzymatic activity of ALT is often used as a biomarker of hepatic toxicity (Andrade et al., 2014). Elevation of these enzymes in the serum have been reported to indicate cellular damage, tissue necrosis, and calculated risk for cardiovascular diseases and elevated myocardial infarction being attributed to elevation of ALT and AST respectively (Loannou et al., 2006).

The blood glucose levels significantly increased in the varying treatment levels except in T<sub>2</sub> above the control. The increase in blood glucose level agrees with the findings of Wegwu and Omeodu (2010) where they observed a significantly elevated plasma glucose concentration in flounder fish after 3-h exposing of fish to the crude oil water-soluble fraction, indicating occurrence of stress condition in fish. The hyperglycemic condition observed in many teleost fish under stress condition is mainly mediated by effect catecholamines on glucose release from liver (Min and Kang, 2008). The changes in the liver and kidney structure which may have affected their functions may be corroborated by the results of the histopathological examination (Plate 1) and earlier reports (Adams et al., 2010). It can be concluded that crude oil has a profound influence on the haematological and biochemical profiles of fish. The results confirmed that the alterations of these parameters can be used as suitable biomarkers in monitoring of crude oil toxicity in the aquatic environment and to protect aquatic life. With the realization of the tremendous adverse effects associated with crude oil and their wastes on humans and the environments, strict measures should be taken to prevent and reduce the occurrence of oil spillage in the Niger Delta and other places in Nigeria where there is crude oil exploration.

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