The effect of water soluble fraction of crude oil on the biochemical, hematological and enzymological changes in fed and starved clariid catfish juveniles.

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Abstract
Investigations on the effect of exposing fed (F_{1-5}) and unfed (U_{1-5}) catfish juveniles to water soluble fraction (WSF) of crude oil at varying LC_{50} were carried. Varying concentrations of 0, 12, 24, 36 and 48 mg/l of wsf were introduced into each of ten aquaria filled with 3L of borehole water containing ten *Clarias gariepinus* juveniles. 3H and 96H after exposure to the toxicant, the fish blood samples were collected and analysed. ANOVA showed that none of the haematological parameters (PCV, Hb, RBC, MCV, MCH and MCHC) of *C. gariepinus* exposed to different concentrations of WSF were significantly ($P > 0.05$) different with fed and unfed. WBC, MCV, MCH and MCHC had slightly higher mean with fed fish while PCV, Hb and RBc had higher mean values when starved. Biochemically, only Globulin, urea and alkaline phosphate were significantly different ($P < 0.05$) between fed and starved fish. As expected glucose level was higher with starved fish while AST increased with feeding and increase in LC_{50}. ALK increased with concentrations in both fed and unfed fish. Cholesterol decreased with increase in concentration in both but decreased more with unfed fish. ANOVA on percent increase or decrease over control showed significant differences with BCM and haematological parameters as well as LC_{50}. While Cholesterol, triglycerides, and globulin decreased in percent over control with increase in LC_{50}, total protein, albumin, urea, glucose, total bilirubin, conjugal bilirubin and ALK increased in percent with LC_{50} over control. In all, the deviation from the control shows the extent of damage to the liver and muscle of the fish due to toxic effect of wsf. WSF is toxic to *C. gariepinus* whether fed or starved and contributes to the environmental stressor resulting to abnormal condition in heamatological and biochemical parameters in fish.

Keywords: biochemical, haemotological, enzymological, toxicological, wsf, catfish

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INTRODUCTION

The degree of damage caused by oil spill event depends primarily upon the quality of oil spill, the chemistry and properties (types) of the oil and the sensitivity of the biological resources impacted, but the overall effect of crude oil on aquatic flora and fauna depend on a great number of factors acting separately or in concert (Adewoye, 2010). The effect of oil spill on aquatic lives are caused by either the physical nature of the oil (physical contamination and smothering) or by its chemical components (toxic effects) and accumulation leading to tainting. Aquatic lives may also be affected by cleanup operations or indirectly through physical damage to the habitats in which plants and animals live (Cooney, *et al.*, 2001).

Earlier report has shown that oil pollution impact negatively on fishery resources (Afolabi, *et al.*, 1985). Azed (2005) observed the eggs and young stages (fingerlings) of fish are especially vulnerable to the toxic effect of water soluble components of crude oil and its refined product. Oil pollution, one of the environmental consequences of crude oil exploration and exploitation activities produces aqua-toxicological effects, which are deleterious to aquatic life (Kori-Siakpere, 2000; Agbogidi, *et al.*, 2005; Zaki, *et al.*, 2014). A variety of pollutants including crude oil and its product are known to induce stress condition, which impair the health of fish (FEPA, 1991). Most often the degree of toxicity of pesticides on aquatic organisms is determined via the haematological, biochemical and enzymological parameters (Saravanan, *et al.*, 2011).

Enzyme activities have been used extensively as sensitive biochemical indicators prior to the occurrence of hazardous effects in aquatic organisms (Suvetha, *et al.*, 2015). The role of

phosphatase in the regulation of various metabolic processes and the attendant changes in acid and alkaline phosphatase activities in fish can be used as indices of growth, illness and spawning (Matusiewicz and Dabrowski, 1996) and also early warning of sensitive stress indicators (Suvetha, *et al.*, 2015). The action of cholinesterase on the nervous system of the fish and their measurement is extensively used as a marker of exposure to many xenobiotics (Whitacre and Nunes, 2011).

This research focuses on the effect of feeding and starvation on the biochemical, enzymological and hematological parameters of *Clarias gariepinus* (Catfish) exposed to water soluble fraction in the Laboratory.

MATERIALS AND METHODS

Preparation of water soluble fraction

Crude petroleum oil was obtained from the Department of Petroleum Resources (DPR) Port Harcourt, River State and was transported to Oceanographic Laboratory of the Institute of Oceanography, University of Calabar Cross River State.

Afolabi, *et al.*, (1988) procedure for the preparation of water soluble fraction (WSFs) was adopted. 250 ml of crude oil was gently mixed with 750 ml of borehole water in a wind Chester bottle and was shaken vigorously for two (2) hours and later poured into a glass separating funnel which was allowed for 24 hours to effect complete phase separation. Thereafter clear WSFs at the lower layer of the separating funnel was obtained in a flat bottom flask.

Test Animal

A total of one hundred (100) juveniles and sub adults *Clarias gariepinus* (catfish) were obtained from the University of Calabar Fish Farm and Hatchery Complex and transported to the Institute of Oceanography laboratory. Their length ranged from 9.1 – 19.7 cm and 5g to 15g in weight measured using a meter rule and an electronic weighing balance respectively. The fish

were randomly grouped into ten (10) different aquaria of 26.4 x 12.4 x 17.6 filled with three (3) liters of borehole water and acclimatized for 72 hours in duplicates. Groups F_1 & U_1 served as control (zero concentration), while F_2 U_2, F_3 U_3, F_4 U_4 and F_5 U_5 served as the experimental groups. Batches F_1, F_2, F_3, F_4 and F_5 were fed ad libitum with commercial feed (coppens) while U_1, U_2, U_3, U_4 and U_5 were starved.

THE EXPERIMENT AND TREATMENT

WSFs of crude petroleum oil was introduced into ten plastic containers filled with 3 liters of borehole water with a mixture of ten (10) juveniles and sub adults of *Clarias gariepinus* fish each at concentration of 0, 12, 24, 36 and 48 ml/l respectively. After 3 hours and 96 hours of exposure of sub adult *C. gariepinus* to the toxicant at five different concentrations, the blood samples were collected using sterilized scalpels to cut the fish in the musculature behind the opercula regions in the dorsal surfaces. The collected blood samples were kept in EDTA and Lithium Heparin labeled bottles to prevent the blood from clotting.

Biochemical and Enzymological study

The collected blood sera were used for further biochemical analysis of urea, sodium, potassium, chloride, Bicarbonate, Total Billirubin (CB), Albumin, Globulin, Cholesterol, Triglycerides, Uric Acid (UA), Creatimin, Glucose and Total protein (TP), as well as enzymological-Aspartate Amino Transferase (AAT/AST/AsAT/ASAT) aka serum glutamic oxaloacetic transaminase (SGOT) test for checking liver damage and Alkaline phosphatase (AP), using Glucose oxidized method. Blood samples of the fish at different concentrations were collected both from the starved fish and fed fish at 3H and 96H intervals using a sterilized scalpel to cut the fish in the musculature behind the opercula region in the dorsal surface and emptied into EDTA and Lithium Heparin bottle to prevent the blood from clotting. The results

were read spectrophotometrically at 505 nm using chemistry analysal Model 19C Cad Novel GMBH.

Haematological parameters

Haematological parameters examined were PCV (packed cell volume), Hb (Haemoglobin) content estimated by cyanmethaemoglobin method (Dacie and Lewis, 1968), RBC (red blood cells) and WBC counts were calculated using haemocytometer (Rusia and Sood 1992), and indices of erythrocytes- MCV (Corpuscular volume), MCH (mean corpuscular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration) using standard formulas (Dacie and Lewis, 1968).

Where,.

MCV (fl) = Hct (%) /RBC count in millions/ mm^3 x 10

MCH (pg) = Hb (g/dl) x RBC count in millions/ mm^3 x 10

MCHC (g/dl) = Hb (g/dl) /Hct (%) x 100

Statistical analysis

Single classification ANOVAs was used to compare individual parameters of fed and starved fish. The increase or decrease in percent of the mean values of concentrations over control for both the biochemical and haematological parameters were calculated as follows:

$$\% \text{ increase/decrease} = \frac{\text{Control } LC_{50} - LC_{50}\,(n_{i-j})}{\text{Control } LC_{50}} \times 100$$

Where,

n is the increase in LC_{50}.

Thereafter, they were subjected to two factor analysis of variance without replications using excel package.

RESULTS

The mean haematological parameters (Table 1) of fed and unfed Clarias gariepinus juveniles exposed to water soluble fraction (wsf) of crude oil for 96 hours decreased from the control with increase in concentrations for both fed and unfed fish. PCV had more decrease from the control in fed than unfed (29.00±1.00 to 34.00±1.00 fed and 32.5±0.50 to 34.00±0.400 unfed in %), WBC increased above the control when fed but generally below the control with unfed fish (5.16±0.15 to 7.24±0.68 fed and 6.19±1.18 to 7.06±0.06 unfed), Hb decreases over control were sharper and lower with fed but more gentle decreases with starved fish (9.75±0.40 to 11.40±0.10 fed and 10.80±0.40 to 11.45±1.45), RBC decreased with feed but increased when starved over control (4.90±0.10 to 5.50±0.20 fed and 5.15±0.15 to 5.80±0.20 unfed), MCV and MCH decreased with increase in concentration with both fed and unfed catfish while MCHC increased with increase in concentration though still below the control (Table 1). No mortality was recorded throughout the experiment.

Table 1: Mean Hematological Parameters of Fed and Starved *Clarias gariepinus* exposed to Water Soluble Fraction (WSF) of Crude Oil for 96 hours

Parameters	0ml/L		12ml/L		24ml/L		36ml/L		48ml/L	
	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved
PCV (%)	36.00 ± 0.00	36.50 ± 0.50	31.50 ± 0.50	32.50 ± 0.50	34.00 ± 1.00	32.50 ± 0.50	31.00 ± 1.00	33.00 ± 1.00	29.00 ± 1.00	34.00 ± 4.00
WBC (10 $9L^{-1}$)	6.74 ± 0.07	6.48 ± 0.35	7.08 ± 0.64	6.43 ± 0.23	6.00 ± 0.01	5.77 ± 0.07	5.16 ± 0.15	7.06 ± 0.06	7.24 ± 0.68	6.19 ± 1.18
Hb (g/dl)	12.25 ± 0.05	12.45 ± 0.35	10.55 ± 0.25	10.80 ± 0.40	11.40 ± 0.10	11.25 ± 0.50	10.30 ± 0.00	11.15 ± 0.45	9.75 ± 0.40	11.45 ± 1.45
RBC (10 $12L^{-1}$)	5.50 ± 0.10	5.65 ± 0.50	4.90 ± 0.10	5.25 ± 0.25	5.30 ± 0.10	5.15 ± 0.15	5.20 ± 0.10	5.75 ± 0.25	5.50 ± 0.20	5.80 ± 0.20
MCV (Femtolitre)	65.50 ± 1.20	64.60 ± 0.30	64.30 ± 0.30	62.00 ± 2.00	64.15 ± 0.65	63.15 ± 0.85	59.60 ± 0.80	57.45 ± 0.75	52.70 ± 0.10	58.45 ± 4.85
MCH (Pg)	22.30 ± 0.50	22.05 ± 0.45	21.55 ± 0.05	20.60 ± 0.20	21.50 ± 0.20	20.90 ± 0.10	19.80 ± 0.40	19.40 ± 0.10	17.70 ± 0.20	19.70 ± 1.80
MCHC (g/dl)	34.05 ± 0.15	34.10 ± 0.50	33.50 ± 0.30	33.20 ± 0.70	33.55 ± 0.65	33.05 ± 0.25	33.25 ± 1.05	33.75 ± 0.35	33.60 ± 0.40	33.60 ± 0.30

- Alk (alanine aminotransferase), Ast/SGOT= aspartate aminotransferase/serum glutamic oxaloacetic transaminase, ALP=Alkaline phosphatase

Figure 1 shows the mean hematological parameters in percent of fed and starved *Clarias gariepinus* exposed to Water Soluble Fraction (WSF) of Crude Oil for 96 hours with decreases in percent over the control LC_{50} as LC_{50} increased. The following ranges were obtained: PCV (<5.6% to <19.44% decrease over control for fed and <6.80 % to <10.96% unfed), WBC ((<5.4% to <23.44% fed and <1% to <10.98% unfed), Hb (<6.9% to <20.46% fed and <6.8% to <13.25% unfed), RBC 0% to <10.91 fed and 2.7% to 8.8% unfed), MCV (<1.8% to <19.54% fed and <2.2% to <11.07% unfed), MCH (<3.4% to <20.63% fed and <5.2% to <12.02% unfed), MCHC (<1.3% to <2.3% and <1.03% to <3.08%).

Mean biochemical parameters of fed and starved *C. gariepinus* after 96 hours of exposure to Water Soluble Fraction (WSF) of Crude Oil (Table 2) showed the following ranges of cholesterol (2.80±0.60 to 3.35±0.05 mmol/L fed, and 2.90±0.60 to 3.45±0.20 unfed), triglyceride (1.2±0.50 to 1.75±0.05 mmol/L fed and 1.15±0.45 to 1.9±0.0 unfed), Total Protein (58.00±0.05 to 60.00±0.00 g/L fed and 58.50±61.50±0.50 unfed), Albumin (29.6±0.50 to 35.50±0.50 g/L fed and 31.00±1.00 to 37.50±9.50 unfed), Globulin (24.50±0.50 to 28.5±0.50 g/L fed and 22.00±28.00±0.00 unfed), urea (2.20±0.60 to 9.15±0.85 mmol/L fed and 2.15±0.45 to 6.25±0.50 unfed), Total bilirubin (6.35±1.35 to 6.85±0.25 µmol/L fed and 6.15±1.25 to 7.30±0.20 unfed), AST (serum glutamic oxaloacetic transaminase) (28.50±1.10 to 33.96±0.65 iu/L fed and 29.00+1.10 to 32.85+1.15 unfed), Conjugate bilirubin (10.65+2.95 to 13.40±0.40 µmol/L fed and 10.85±2.85 to 13.15±0.85 unfed), the enzyme Alk (alanine aminotransferase) (15.40±0.40 to 46.90±23.20 iu/L fed and 15.40±0.10 to 47.05±20.65 unfed), and ALP (alkaline phosphatase) ranged from 48.6±1.70 to 52.25±1.15 iu/L when fed and 47.2±3.60 to 50.05±0.96 unfed.

Biochemical parameters (Figure 2) that decreased with increase in concentration in percentage with the control LC_{50} were cholesterol (2.98 % to 16.42% fed fish and 5.80% to

15.94% when starved), Triglycerides (14.29% to 31.43% with feed and 26.32 % to 39.47% when starved), Globulin (0 % to 14.4 fed and 0 % to 20 % unfed), AST (0.49% to 11.86% fed and 1.19% at 12 mg\l/L), and alkaline phosphatase (1.17 % fed to 4.6% fed and 0.1 % to 3.1 % unfed). Whereas the following increased above the control with the following ranges Total protein (fed fish 0 % to 3.4 % and 0 to 6.0 % unfed), albumin 6.8 % to 20.34 % and 0 to 5.1% unfed), urea (220.4% to 315.6% fed and 144.2% to 190.7% unfed), Glucose (11.5% to 14.16% fed and 0% to 20.35% unfed), Total bilirubin (0.78% to 7.9 % fed and <1.6% to >18.70% unfed), Conjugal bilirubin (3.29% to 25.82% fed and 1.38% to 21.2%). The enzymes AST (aspartate aminotransferase/serum glutamic oxaloacetic transaminase) increased with concentration above the control ((0.49% to 11.86% fed and 7.67% to 11.03% unfed) and Alk (alanine aminotransferase) had astronomical increases with concentrations above the control (46.45% to 205% fed and 69.80% to 206% unfed) and Alkaline phosphatase (1.17 % to 2.65 fed and 0.6 to 3.1% unfed). When the percentage compositions were subjected to analysis of variance there were highly significant different ($P<0.001$, $^{3}F_{23}$, amongst the parameters and $p<0.05$ with increase in concentrations.

ANOVA: two factor without replication

Source of Variance	SS	Df	MS	F	p-value	Fcrit
BCH	449883.8	23	19560.17	46.90708	2.3E-33	1.686897
$LC_{50}s$	3856.816	3	1285.605	3.083	0.032935	2.737492
Error	28772.87	69	416.9981			
Total	482513.95					

Table 2: Mean Biochemical Parameters of Fed and Starved *Clarias gariepinus* exposed to Water Soluble Fraction (WSF) of Crude Oil for 96 hours

Parameters	0ml/L		12ml/L		24ml/L		36ml/L		48ml/L	
	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved
Cholesterol (mmol/L)	3.35 ± 0.05	3.45 ± 0.05	3.00 ± .20	3.25 ± 0.25	2.90 ± 0.50	3.15 ± 0.35	3.25 ± 0.25	2.95 ±0.75	2.80 ± 0.60	2.90± 0.60
Triglyceride (mmol/L)	1.75 ± 0.05	1.90 ± 0.00	1.2± 0.50	1.40 ± 0.30	1.50 ± 0.70	1.30 ± 0.60	1.25 ± 0.45	1.30 ± 0.50	1.45 ± 0.55	1.15 ± 0.45
Tot Protein (g/L)	58.00± 0.50	58.50 ± 1.50	58.5 ±0.5	58.00 ± 0.00	60.00 ± 0.00	58.50 ± 1.50	60.00 ± 0.00	61.50 ± 0.50	58.00 ± 2.00	61.00 ± 2.00
Albumin (g/L)	29.50 ± 0.50	31.00 ± 1.00	31.5 ±1.5	36.00 ± 1.00	35.50 ± 0.50	36.00 ± 1.00	31.50 ± 0.50	33.50± 0.50	33.50 ± 2.50	37.50 ± 9.50
Globulin (g/L)	28.50 ± 0.50	27.50 ± 0.50	27.0 ±1.0	22.00 ± 1.00	24.50 ± 0.50	22.50 ± 2.50	28.50 ± 0.50	28.00 ± 0.00	24.50 ± 0.50	23.50 ± 7.50
Urea (mmol/L)	2.20 ± 0.60	2.15 ± 0.45	8.3± 0.60	5.25 ± 0.25	8.30 ± 0.40	6.25 ± 0.50	7.05 ± 0.75	5.80 ± 0.00	9.15 ± 0.85	5.85 ± 3.05
Glucose (mmol/L)	5.65 ± 0.05	5.65 ± 0.05	6.3± 0.60	6.55 ± 0.65	6.40 ± 0.60	6.35 ± 0.65	6.45 ± 0.65	6.65 ± 0.65	6.45 ± 0.75	6.80 ± 0.15
Tot Bilirubin (µmol/L)	6.35 ± 1.35	6.15 ± 1.25	6.40 ±0.7	7.30 ± 0.20	6.85 ± 0.25	7.00 ± 0.90	6.85 ± 0.50	6.05 ± 0.85	6.70 ± 0.60	6.65 ± 1.25
Con Bilirubin (µmol/L)	10.65 ± 2.95	10.85 ± 2.85	11.05 ± 1.75	13.15 ± 0.85	11.75 ± 1.55	12.75 ± 1.65	13.40 ± 0.40	11.00 ± 2.30	12.10 ± 1.80	12.35 ± 2.45
Ast (iu/L)	30.35 ± 0.95	29.35 ± 0.75	28.55 ± 1.25	29.00 ± 1.10	30.50 ± 0.60	31.60 ± 1.70	32.25 ± 0.55	32.05 ± 0.45	33.95 ± 0.65	32.85 ± 1.15
Alk (iu/L)	15.40 ± 0.40	15.40 ± 0.10	22.6 ± 6.2	26.15 ± 9.55	35.75 ±19.85	42.20 ± 26.20	42.60 ± 24.10	41.00 ± 20.30	46.90 ± 23.20	47.05 ± 20.65
Alka. Phosphate (iu/L)	50.95 ± 2.85	48.55 ± 1.25	48.6 ± 1.70	47.20 ± 3.60	52.25 ± 1.15	48.50 ± 1.40	49.05 ± 0.25	50.05 ± 0.95	51.55 ± 0.55	48.85 ± 0.45

- Alk (alanine aminotransferase), Ast/SGOT= aspartate aminotransferase/serum glutamic oxaloacetic transaminase, ALP=Alkaline phosphatase

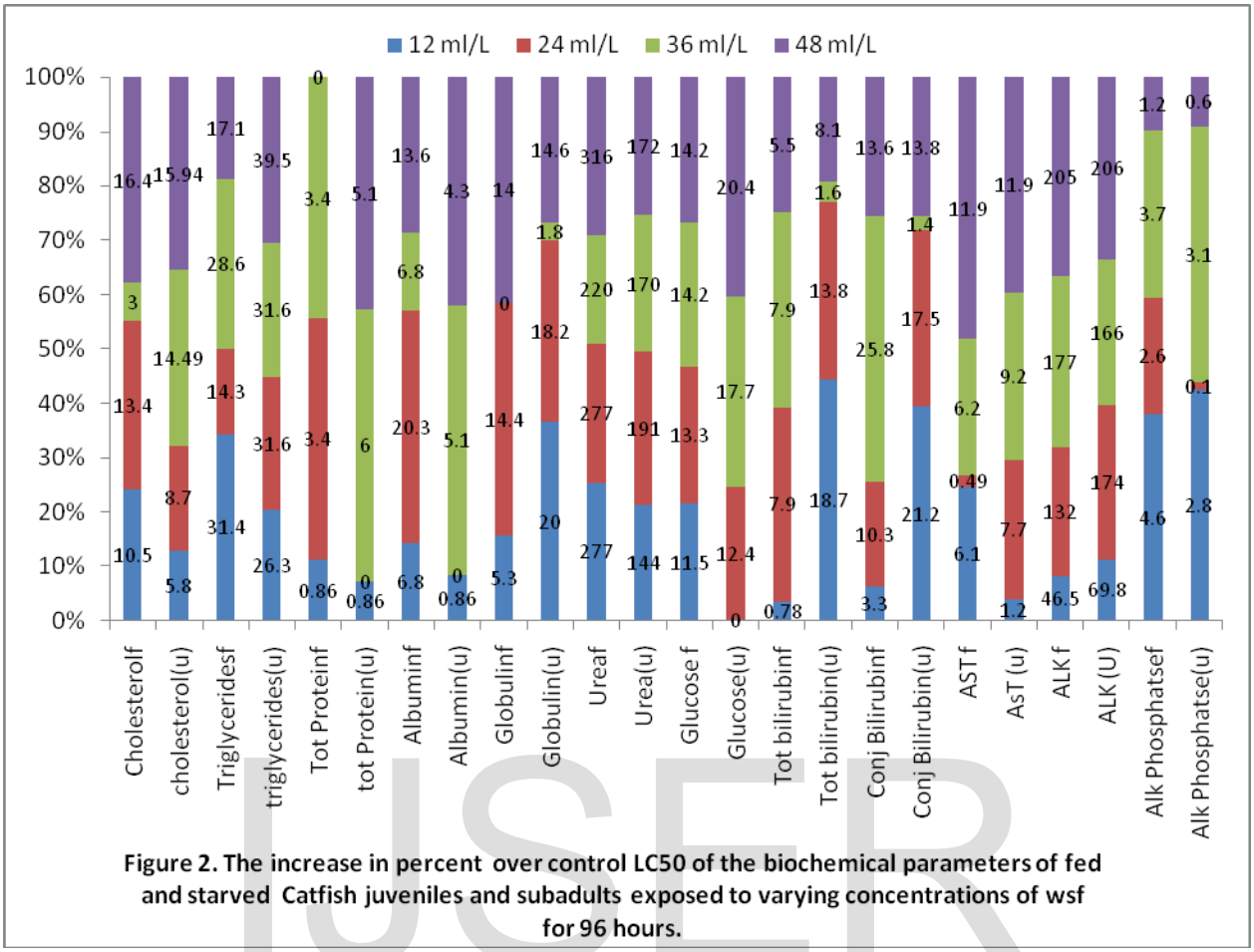

Figure 2. The increase in percent over control LC50 of the biochemical parameters of fed and starved Catfish juveniles and subadults exposed to varying concentrations of wsf for 96 hours.

Analysis of Variance of hematological and biochemical parameters of *Clarias gariepinus* exposed to different concentrations of water soluble fraction of crude oil for 96 hours showed that PCV, hemoglobin, RCB, MCV, MCH, urea and Ast were significant ($P<0.05$) at probability level of 0.05 whereas WBC, MCHC, cholesterol, triglyceride, total protein, albumin, globulin, glucose, total bilirubin, conjugate bilirubin, Alk and Alkaline phosphate were not significant ($P>0.05$).

DISCUSSION

In aquatic science, measurement of haematological and biochemical indices is commonly used as a diagnostic tool in aquatic toxicology and biomonitoring (Soimasue *et al.,* 1995; Jee and Kang 2005). Exposure of aquatic organisms to crude oil and its derivatives has been proven to induce a variety of toxic effects in exposed aquatic animals. Also, petroleum hydrocarbons can act as a mediator in generating free radical in fish that are capable of causing various side effects in their internal and external tissues (Davison *et al.,* 1993; Al-kindi *et al.,* 1996; Achuba and Osakwe, 2003; Khan, 2003; Zang *et al.,* 2004; Pachelo and Santos, 2016). Hematological parameters of fish can be used as very sensitive indicators of changes in ecophysiological condition (Vinodhni and Norayamn, 2009). According to Ubong, *et al.,* (2015) crude oil and other oil products concentrations of 0.01 mL/L are known to accelerate the death of fish in aquatic ecosystems. While no mortality was recorded in all the concentrations, Nwabueze and Agbogidi (2010) only recorded mortality at 50% and 100% WSF and corresponding reduction in growth with increase in concentrations.

The general reduction of the blood parameters indicates anaemic condition caused by exposure of *Clarias gariepinus* to toxicant over the given period. Changes in haematological parameters of fish such as *C. gariepinus* reported in this study which is due to stress induced by environmental pollutant (water soluble fraction of crude oil) have been reported by several researchers (Onusiriuka and Ufodike, 2000; Ezeri, 2001, Gabriel, *et al.,* 2001). These indices have been used in the effective monitoring of the responses of fish to the stressors including its health status under adverse conditions.

The variation in fish hematological parameters observed in this study may be due to physiological stress in the exposed fish. This finding is in agreement with findings of Rostem, and Soltani (2016) who observed variation in hematological parameters of juvenile Beluga

(*Husohuso*) exposed to water soluble fraction of crude oil. The variations in haematological indices observed in this study when exposed to different concentrations of WSF of crude oil are a defensive mechanism against crude oil toxicity through stimulation of erythropoiesis which corresponds with studies on *Sarotherodon melanotheron* (Oriakpono, *et al.*,, 2012), and *Tilapia guineensis* and eels (Hwang, *et al.*, 1989). Haemoglobin is known to be a sophisticated oxygen delivery system which provides the amount of oxygen to required by the tissues (Voet and Voet, 1990). According to Blaxhall and Daisley (2006), haemoglobin determination is a reliable or good indicator of anaemic conditions in fish. Generally, the hemoglobin value of fish depends on the oxygen carrying capacity of the blood (Larsson, Haux, and Sjobeck 1985).

In this study, the observed significant variation ($P<0.05$) in hemoglobin value of the exposed fish may be attributed to less oxygen content in the blood of the exposed fish. Moreover, lower Hemoglobin values are indication of shrinkage of cell due to toxicant stress on the erythropoietic tissue (Saravanan *et al*., 2011). Also, Rostem, and Soltani, (2016) attributed the significant variation in hemoglobin concentration of the fish exposed to crude oil to either an increase in the rate of hemoglobin destruction or a decrease in the rate of hemoglobin synthesis. The decreases in haemoglobin concentration indicates a considerable restriction in the fish's ability to provide oxygen sufficiently to the tissues resulting to a decrease of physical activity of the fish (Nussey, 1994).

According to Rostem, and Soltani, (2016), crude oil can affect RBC, causing a hemolysis by a disruptive effect on the erythropoietic tissues of spleen and kidney. In this study, RBC values were significantly different ($P<0.05$) in the exposed fish and this findings agrees with those of Rostem, and Soltani, (2016) for juvenile Beluga (*Husohuso*). This indicates that chronic exposure of *Clarias gariepinus* to water soluble fraction of crude oil can result in or stimulate

erythropoiesis in *C. gariepinus*. Erythrocytes are known to be produced in the haematopoietic tissue, situated in the spleen and head kidney of fish. It has been established that a reduction in the quantity and quality of erythrocytes as observed in this study will lead to a deteriorated oxygen supply (Kori-Siakpere, Martin and Ikomi 2009). This implies that fish exposed to crude oil toxicity will suffer from deteriorated oxygen supply which is very dangerous to fish health. Nasir and Hantoush (2010) observed a linear reduction in hemoglobin which suggests an anemic condition in the crude oil treated fishes. Sudakov (1992) reported that the toxic components especially those in crude oil are capable of changing blood chemistry thereby inducing anaemia by causing bone marrow hypoplasia and interference with platelet production in the exposed animals, hence resulting in the reduced values.

The reduction in the RBC may be attributed to the presence of environmental stressors which manifest in form of a change in the environment resulting to haemagglutination due to impaired osmoregulation (Rottman, Francis-Floyd and Durborow 1992) or erythropoiesis in the organs responsible for the production of RBC. According to Oriakpono *et al.,* (2012), packed cell volume (PCV) is an important haematological parameter that changes with fish activity and environmental stress. In this study, PCV values of the exposed fish for 96 hours varied ($P<0.05$) significantly with increasing concentration of water soluble fraction of crude oil which corroborates findings of Oriakpono *et al.,* (2012) for *Sarotherodon melanotheron* exposed to crude oil. Oriakpono, *et al.,* (2012) attributed these findings to changes in water balance, which could lead to a decrease in blood volume and an increase in the white blood cells resulting in reduced PCV. However, white blood cells (WBC) and mean corpuscular haemoglobin concentration (MCHC) of the exposed fish did not increase significantly ($P>0.05$) even after 3 hours and 96 hours comparison. This may indicate that water soluble fraction of crude oil did not

influence WBC values of *Clarias gariepinus* significantly. This result disagrees with the finding of David, *et al.,* (2002) who reported an increase in size and monocytes of *Tilapia guineensis* and *S. melanotheron* after exposure to industrial effluents. Ajani, *et al*., (2007) attributed the increase in WBC to recruitment of more cells to combat the stressor. Also, Anyanwu, *et al*., (2007) attributed the increase in WBC to non specific immune response to stress as a result of interaction of prolactin and cortisol hormones to restore ion balance in iso-osmotic salinity (and a stimulation of the immune system in response to toxicity of crude oil). The values of calculated haematological indices including MCHC, MCH, and MCV are crucial in the diagnosis of anaemia in most animals (Coles, 1986). The variations obtained in these haematological indices (MCV, MCH and MCHC) in the present study could be due to a defense against the toxic effect of crude oil through the stimulation of erythropoiesis (Mouse, 1999). According to Kori-Siakpere, *et al*., (2009), the decrease in MCV and low hemoglobin level indicate that the red blood cells have shrunk, either due to hypoxia or microcytic anaemia; microcytosis been due to the decrease in the haematocrit values. The fluctuation in the MCH values clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control over the exposure period, thus indicating an anaemic condition. The MCHC is a good indicator of red blood cell swelling (Wepener, *et al.,* 1992). The significant decreases in the MCHC values in the exposed fish are thus probably an indication of swelling of the red blood cells and/or a decrease in hemoglobin synthesis.

Furthermore, the alteration of these parameters can be used as health indicators of the aquatic environment and also provide early warning tools in monitoring environment quality

(Pimpao, *et al*., 2007). Moreover, an understanding on the adaptation and recovery is an important tool in the field of risk assessment (Du, *et al*., 2009; Wu, *et al*., 2005).

The release of very high AST and Alk into the blood of the fish at all the concentrations is an indication of injury to the liver or muscle due to wsf. Ast and Alk values were higher than the control and increased with increase in concentration and duration of experiment depicting damage to the liver and probably other organs such as the heart or kidney. ALP occurred in moderate amount showing less damage to liver or bone disorders. It also helps to break down protein in the body.

According to Nasir and Hantoush (2010) the degree of ecosystem contamination by toxicants can be evaluated by use of biochemical indices. Biochemical parameters of starved and fed *Clarias gariepinus* exposed to different concentrations of water soluble fraction of crude oil after 3 hours and 96 hours showed that urea and Ast were significantly different ($P<0.05$) cholesterol, triglyceride, total protein, albumin, globulin, glucose, total bilirubin, conjugate bilirubin, Alk and Alkaline phosphate were not significant ($P>0.05$). The very high urea content for both fed and starved fish with increase in concentration depicts very high compromise of the kidney function as a result of the toxic effect of wsf. Glucose level increased steadily above the normal in both fed and unfed fish at all levels of concentrations as a result of the toxic effect of wsf. The higher levels of total bilirubins formed with increased concentration is an indication of the breakdown of rbc in the fish due to the toxic effect of wsf. Proteins are naturally composed of fibrinogens, globulins and albumins which are responsible for the distribution of important materials from one part of the body to another during circulation. In living Organisms, protein is also known to possess or exhibit transporting, nutritive, protective, buffering and energetic properties or functions (Inyang, *et al*., 2010). In animal system, protein assessment is often used

as part of laboratory diagnosis to ascertain the extent of wellness of an organism (Edori, *et al.*, 2013). In this study, changes in total protein content of *C. gariepinus* after exposure to WSF of crude oil agrees with findings of Inyang, *et al*., (2010) and Edori, *et al*., (2013) who reported changes in protein content of *C. gariepinus* exposed to toxicants.

General variation was observed in the biochemical parameters of the exposed fish which was not time and concentration dependent and this observation is similar to findings of Edori, *et al*., (2013) for scallop (*Placopecten magellanicus*). According to Sambasiva Rao (1999), decrease in total protein content is caused by the degradation and utilization of degraded products for metabolic processes which results in increase in free amino acids due to the decreased incorporation of amino acids in protein synthesis. The variation observed in enzyme activities could be attributed to the crude oil toxicity and is similar to observation of Mahmoud, *Et al.*, (2011), Ramese, ., 2015) and Edori and Ekpeta (2014) who reported varying degrees or changes in enzyme activities on the mollusk (*P. magellanicus*) induced by crude oil. According to Gabriel, *et al.*, (2009), these enzymes (AST, ALP and ALP) are clinically important in the diagnosis of hepatic (hepatocellular) damage or disease. In the event of damage in the organs, especially those of hepatic importance such as the liver, spleen or the kidney, these enzymes leak from the organs to the blood and this alters the permeability of the cell membrane to an appreciable degree (Gad, 2007). The alteration of enzyme (AST, ALT and ALP) in any organism indicates a disturbance in the general physiological structure of important organs or tissues and the membrane transport (Roy, 2002). Also, the direct interference of crude oil with the tissues of *Clarias gariepinus* could be responsible in the alteration in the activities of these enzymes (AST, ALT and ALP) which resulted in the changes observed in the fish biochemistry.

Conclusion

Water soluble fraction of crude oil is an environmental stressor which leads to abnormal alteration in hematological and biochemical parameters in fishes. The alteration in some hematological and biochemical parameters is an indication that crude oil is toxic to *Clarias gariepinus* and its environment. This underscores the need for adequate and timely environmental check by government and other relevant agencies to protect the aquatic environment from the effect of crude oil toxicity since this fish is one of the most consumed and cheap protein source for the coastal inhabitants.

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