International Journal of Ecotoxicology and Ecobiology
2017; 2(4): 150-157
http://www.sciencepublishinggroup.com/j/ijee
doi: 10.11648/j.ijee.20170204.13
ISSN: 2575-1727 (Print); ISSN: 2575-1735 (Online)

Evaluation of Metabolites of *Clarias gariepinus* Exposed to Sub-Lethal Concentrations of Oilfield Wastewater

Nedie Patience Akani[1], Adaobi Patricia Ugbomeh[2], Ugwemorubong Ujagwung Gabriel[3]

[1]Department of Microbiology, Rivers State University, Port Harcourt, Nigeria

[2]Department of Animal and Environmental Biology, Rivers State University, Port Harcourt, Nigeria

[3]Department of Fisheries and Aquatic Environment, Rivers State University, Port Harcourt, Nigeria

Email address:

nedieakani@yahoo.com (N. P. Akani), Ugbomeh.adaobi@ust.edu.ng (A. P. Ugbomeh), ugwem@yahoo.com (U. U. Gabriel)

To cite this article:

Nedie Patience Akani, Adaobi Patricia Ugbomeh, Ugwemorubong Ujagwung Gabriel. Evaluation of Metabolites of *Clarias gariepinus* Exposed to Sub-Lethal Concentrations of Oilfield Wastewater. *International Journal of Ecotoxicology and Ecobiology*. Vol. 2, No. 4, 2017, pp. 150-157. doi: 10.11648/j.ijee.20170204.13

Received: March 20, 2017; **Accepted**: October 31, 2017; **Published**: November 25, 2017

Abstract: Twenty-eight adult *Clarias gariepinus* (mean weight 205 ± 12.89g SD; mean length; 31.13 ± 3.82cm SD) were exposed to various concentrations (0. 10, 20, 30, 40, 50 and 60%) in quadruplicates of an oilfield wastewater and were investigated for its responses on metabolites in tissue (plasma, gill, liver, kidney and muscle) samples after 28 days. The result showed that in all the tissues tested the values of total protein, creatinin and total bilirubin in all the control (0%) were higher ($p \leq 0.05$) than at treatment level. Metabolites in the plasma differed ($P \leq 0.05$) between concentrations except for creatinine and total bilirubin. Albumin in the control and treated fish were similar ($p \geq 0.05$) except in plasma and liver which recorded lower values than their control ($p \leq 0.05$). Total urea recorded its highest value at the highest concentration (60%) ranging between 4.25±0.09 in kidney and 12.0±1.47 mmol/l in muscle. Generally, the values did not follow a particular trend except for albumin values that decreased with an increased concentration of the toxicant. Significant changes observed in the study suggest stress induced by the oilfield wastewater on *C. gariepinus* hence the advocating for proper treatment of the wastewater before discharge into the environment.

Keywords: *Clarias gariepinus*, Oilfield Wastewater, Metabolites, Tissues

1. Introduction

The clariids occur in natural and artificial freshwaters in Africa and the Middle East (1Gupta and Gupta, 2008)and *C. gariepinus*is a very important species in the fisheries of these countries and the most cultured species in Nigeria, next to the tilapine fishes [2]. The petroleum resources are the backbone of Nigeria's economy, accounting for many years over 90% of foreign exchange earnings and over 70% of total government revenue [3]. The continuous exploration, exploitation of petroleum products and associated activities such as waste disposal, processing of crude oils, transportation and marketing expose the environment to constant threat of oil pollution [4]. Oil reservoirs frequently contain large volumes of the wastewater, while gas reservoirs tend to produce only small quantities [5]. Hence, to achieve maximum oil recovery, additional water is usually injected into the reservoirs to help force the oil to the surface. Furthermore, the author reported that in depleted oil fieldsthe amount of produced water released along with the hydrocarbons increases. The produced water is separated from the hydrocarbons and then either discharged as oilfield wastewater into the environment or injected back into the wells [6]. Since oilfield produced water has variable chemical compositions, inorganic and organic constituents [7] and hydrocarbons [8], these impact theirbehaviour [9]. There is the great possibility that contamination of the aquatic environment with wastewater will impact negatively on the overall physiology (tissue and blood chemistry) of *C. gariepinus*. Such deliberate or accidental contaminations are commonplace in the Niger Delta region of Nigeria that has the highest level of petroleum related activities.

The evaluation of the biochemical status of fishes to assess environmental impacts is a routine and very important tool in clinical practices in aquaculture and wild fish populations.

These biomarkers have been effectively and extensively used to assess the impacts of a large number of environmental contaminants [10] on fishes generally, and also in the clariids [11, 12, 13, 14, 15, 16]. Very few reports are available on the effects of oilfield wastewaterson fishes which include impacts on the mycology [17], bacterial flora [18], haematology [19], organ indices [20] and enzymes [21] of *C. gariepinus*. However, there is scarcely any literature on the effects of oilfield wastewaters on the metabolites in *C. gariepinus*. This paper is a report on the impact of oilfield water on the metabolites (total protein, albumin, creatinine, total urea and total bilirubin) in the plasma, gill, liver, kidney and muscle tissues of *C. gariepinus* under laboratory conditions which has not hitherto been reported neither in the fields nor open water bodies.

2. Materials and Methods

2.1. Collection and Preparation of Samples

Twenty-eight adult *C. gariepinus* (mean weight 205 ± 12.89g SD; mean length; 31.13 ± 3.82cm SD) were obtained from the African Regional Aquaculture Center (ARAC) at Aluu in Ikwerre Local government area of Rivers State, Nigeria. This fish was chosen because of its availability all the year round, ease of maintenance in laboratory conditions and relative sensitivity (high level of tolerance) to petroleum products. The experimental fish was transported in 50l aquarium containing borehole water to the Department of Microbiology laboratory. The mouth of the aquarium was covered with a net to avoid escape of the fish. On arrival, the fish was acclimated individually in rectangular aquaria for two weeks. The top of the acclimation aquaria were covered to control escape of fish. The water was changed daily and the aquaria washed with a piece of foam. The fish was fed twice with a 35% crude protein diet at 1% biomass daily (8.00 a.m. and 5.00 p.m.). Mortality during acclimation was less than one percent.

The test toxicant (oilfield wastewater) was collected from Ebocha Oil Centre in Ogba/Egbema Local Government Area of Rivers State, coordinates N05 27' 26.7''E006 41' 38.9'' The Ebocha Oil Centre serves as a collection centre for seven oilfields. The effluent was collected in 50 litres plastic containers on three occasions. These represented different ranges of the discharge at the discharge point. The oilfield wastewater samples were immediately transported to the laboratory after collection. Preliminary investigation was conducted to determine the range of concentrations of oilfield wastewater that exhibited sub-lethal effect on the *C. gariepinus*. Five concentrations (10, 30, 50, 70 and 100) of the oilfield wastewater were prepared by serially diluting from each effluent sample on a volume to volume (v/v) ratio so that the percentage (%) concentration in each test solution was obtained as shown below

$$Concentration\ of\ effluent(\%) = \frac{V_E}{V_E+V_{DW}} \text{x } 100 \qquad (1)$$

Where, V_E = Volume of effluent

V_{DW} = Volume of dilution water [22]

The determined volume of effluent was added to the desired quantity of dilution (borehole) water and vigorously mixed.

The water was not changed for a period of one week. However, the fish were fed twice daily as in the acclimation period. The purpose of the preliminary investigation was to determine the range of concentration to be used for the definitive (main) test. Concentrations that caused death within one week were omitted from the definitive test [23]. Each concentration of the oilfield wastewater had a fish sample (*C. gariepinus*) exposed to it.

Based on the results of the preliminary investigation, six [0.00 (control), 10, 20, 30, 40, 50, and 60% v/v] test concentrations, each with four replicates were used. A fish was then introduced into each of these concentrations contained in an aquarium and incubated for a period of 28 days at room temperature (30±2°C). Fish was exposed to 15lof each of the concentrations and fish was fed as in the acclimation period. The test solution was renewed weekly after washing the aquarium to get fresh toxicant as the old one would have deteriorated with time.

2.2. Physicochemical Characteristics of Water

The following physicochemical properties of the various oilfield wastewater samples collected at various times were analyzed according to APHA [24]; they include temperature, pH, salinity, turbidity, electrical conductivity, total dissolved solids (TDS), total suspended solids (TSS), chloride, alkalinity total hydrocarbon content and heavy metal.

Salinity of oilfield wastewater was determined by the method described inSterling [25]

Determination of total hydrocarbon content was performed using the toluene extraction method [26]

2.3. Analysis of Metabolites

Fish were killed and dissected in order to collect samples of the gill, liver, kidney, and muscle tissue. Then, 0.5g each of the organ/tissues were macerated (grounded) with pestle and mortar. To each of the samples was added separately 5m1 of diluted perchloric acid. After addition of the diluent the samples were centrifuged at the rate of 3000rpm for 10 minutes. The supernatants were then removed and stored in plain bottles at -20°C for analysis. The following metabolites were determined in the tissue/organs of individual *C. gariepinus*; total protein, albumin, creatinine, total urea and total bilirubin as described by O'chei and Kolhatkar [27].

2.3.1. Total Protein

Two tubes were labeled, blank and sample. Then, 1ml of protein was pipette into both tubes. This was followed by the addition of 0.02 ml of sample to the tube labeled sample and distilled water added to that labeled blank. These were mixed and incubated for 30 mins at 25°C. Absorbance of standard test against blank was read at 540 nm wavelength in a spectrophotometer. Total protein was calculated as shown below:

Protein concentration (g^{-1}) = 190 x Absorbance of sample (2)

2.3.2. Albumin (Bromocresol Green Method)

Three sets of test tubes were labeled blank, standard and test. Reagent prepared included bromocresol green (BCG), albumin and Blank reagent. Bromocresol green reagent was prepared by adding the following 8.85 g, succinic acid; bromocresol green, 0.108 g; sodium azide, 0.100 g and Brij-35, 4.0 ml to 900 ml distilled water. The pH was adjusted to 4.1 and the final volume made to 1 litre. Albumin standard (4%) was prepared by dissolving 4.0 g of bovine albumin in 100 ml of normal saline. To this was added 0.1 g sodium azide as preservative. Blank reagent was prepared in the same way as BCG reagent without bromocresol green. Three milliliter of reagent was pipette into all the tubes. Then 0.01 ml of test and standard was added to appropriate tubes while distilled water was added to the tube labeled blank. These were mixed and incubated for 10 minutes at room temperature. The absorbance was read at 640 nm (red filter) setting the zero with blank. Albumin was calculated as shown below;

$$\text{Albumin (g/l)} = \frac{\text{Ab Test}}{\text{Ab Standard}} \text{x Concentration of Standard} \quad (3)$$

2.3.3. Creatinine

The measurement of creatinine level is a test of renal function and is based on the fact that creatinine reacts with alkaline picrate reagent to form an orange-red color which is measured in a spectrophotometer. The reagents were reconstituted in the required proportion of four parts picric acid to one part sodium hydroxide (NaOH). The samples were prepared for determination by deproteinizing them. This was achieved by pipetting 1.0 ml of sample into the tube and adding 0.5 ml of distilled water and 0.5 m1 of trichloroacetic acid (TCA). This was spurn for l0 minutes at 300 revolutions per minute to obtain the supernatant. The test tubes were now labeled as, blank, standard and samples, 1.0 ml of distilled water, standard and supernatants were pipette and dispensed into the labeled tubes accordingly. 1.0 ml of the prepared reagent was added. The mixture was shaken and allowed to stand for l0 minutes after which the absorbances of the sample and standard were read against the reagent blank at 490 nm.

2.3.4. Total Urea

Different test tubes were respectively labeled as blank, standard, sample and control. 10 µl of urease was dispensed into all the test tubes. The test tubes were incubated at 37°C for l0 minutes and 2.5 ml of diluted phenol was added to all the tubes. 2.5 ml of diluted sodium hypochlorite was also added. The content of each test tube was mixed, incubated at 37°C for l5 minutes. The contents were read against the content of the blank at 540 nm. The amount of the unknown was determined as shown below:

$$Unknown(mmol) = \frac{\text{Ab(Sample)}\times\text{(Standard)}}{\text{Ab (Standard)}} \quad (4)$$

2.3.5. Total Bilirubin

Two test tubes were set up for each test. Test tube 1 (sample blank), test tube 2 (sample) 0.2 ml of sulphanilic acid was dispensed into the tubes. 0.5 m1 of sodium nitrite was dispensed into the sample test tube. This was followed by addition of l ml each of 0.2 ml of caffeine to sample blank and test sample. 0.2 ml of the sample was added into the tubes, mixed and allowed to stand for l0 minutes at 20-25°C. One millilitre of tartarate was dispensed into the tubes, mixed and allowed to stand for 5-minutes at 20-25°C and the absorbance of the test sample against the sample blank was read spectrophotometrically at a wave length of 600 nm.

3. Results

The result of the physico-chemical properties of the constituted concentrations of the oilfield wastewater and their mean values are presented in Table 1. Generally, the values increased with increased concentration of the effluent: turbidity (1.5±0.05 – 3.5±0.0 NTU), conductivity (183.33±28.87 – 13666.67±288.68µs/cm), TDS (48.33±2.89 – 7316.67±275.3ppm), chloride (9.33±1.16 - 3726±64.29ppm), BOD (0.82±0.03 – 1.73±0.02ppm), and THC (0.0±0.00 - 11.81±3.12ppm). However, there was a decrease in DO (3.97±0.45 to 2.0±0.0ppm) with increased concentration of the effluent while alkalinity (9.33±1.16 - 32±0.0 ppm) increased with increased concentration of the toxicant but dropped to 14±0.0 ppm at 60% concentration.

Table 1. *Mean (± SD) levels of physicochemical characteristics of the constituent concentrations of oilfield wastewater (oww) used in the analysis.*

Physicochemical characteristics	Constituted concentrations (%)						
	0	10	20	30	40	50	60
Temperature (°C)	26.17±0.29	26.07±0.4	26.5±0.5	25.0±0.0	26.83±0.29	27.33±0.29	27.0±0.0
pH	7.1±0.1	7.47±0.31	7.67±0.12	7.23±0.25	7.47±0.31	8.0±0.0	8.0±0.0
Salinity (ppm)	0.0±0.0	47.67±2.52	517.33±28.31	1500±50	2443.33±309.25	3233.33±251.66	4556±51.07
Turbidity (NTU)	1.5±0.5	5.33±0.58	13.33±4.16	16±1.0	23.33±3.06	32.0±2.0	35±0.0
Conductivity (us/cm)	183.33±28.8	3766.67±251.6	1060±121.6	2166.67±288.6	4216.67±225.5	8433.33±404.2	13666.67±288.7
TDS (ppm)	48.33±2.89	976.67±25.17	1620±158.75	2966.67±152.75	4783.33±256.58	6146.67±128.58	7316.67±275.38
Chloride (ppm)	9.33±1.16	1760±52.92	1926.67±110.15	2316.67±189.29	2726.67±253.25	3472.67±25.33	3726.67±64.29
DO (ppm)	3.97±0.451	3.4±0.2	3.3±0.1	2.43±0.15	2.33±0.12	2.3±0.0	2.2±0.0
BOD (ppm)	0.82±0.03	1.02±0.03	1.18±0.06	1.52±0.06	1.58±0.02	1.64±0.0	1.73±0.02
Alkalinity (ppm)	9.33±1.16	20.0±2.0	23.5±0.5	26.67±3.06	31.33±1.16	32±0.0	14±0.0
THC (ppm)	0.0±0.0	1.98±0.082	3.09±0.085	3.49±0.445	6.17±0.651	7.45±0.135	11.81±3.118

The exposure of *C. gariepinus* to varying concentration of oilfield wastewater resulted in significant changes of metabolites in the plasma which differed (P≤0.05) between concentrations (Table 2) except for creatinine and total bilirubin. The values did not follow any particular trend. In the gills, the values of total protein, creatinine, total urea and total bilirubin in all the control (0%) were higher (p≤0.05) than at treatment level (Table 3); whereas albumin in the control and treated fish were similar (p≥0.05). Total urea recorded its highest value at the highest concentration (60%).

In the liver a difference (p≤0.05) was observed in the values of total protein and urea in the control fish and the treated group (Table 4), but the albumin and creatinine in the control fish were higher (p≤0.05) than the treated group. Responses of metabolites in the kidney showed that the values of albumin (1.60±0.00 g/l) and total bilirubin (18.50±0.00 µmol/l) were higher in the control (Table 5). At the other concentrations, total protein and creatinine did not follow any particular trend. The value of urea was generally raised with increase in the concentration of the effluent with a peak at 4.25±0.29 mmol/l.

In addition, metabolites in the muscle of *C. gariepinus* exposed to oilfield wastewater as presented in Table 6 differed (P≤0.05) at all concentration tested. Higher values of the metabolites were recorded for total protein, albumin and creatinine in the control (0%) than at other concentrations. Level of total urea at 60% was double its value at the control while total bilirubin had its highest value recorded at 30%. Generally these values did not follow a particular trend except for albumin that showed a decrease in values as the concentration of the toxicant increased.

Table 2. *Metabolites in the Plasma of C. gariepinus Exposed to Different Concentrations of an Oilfield Wastewater after 28 days*

Conc. of oww (%)	Metabolites				
	Total protein(g/l)	Albumin (g/l)	Creatinine (mmol/l)	Total Urea (mmol/l)	Total bilirubin (µmol/l)
0	40.50±1.29^{ab}	3.20±0.64^{b}	4.50±0.58^{b}	2.25±0.13^{b}	6.50±3.07^{a}
10	42.75±0.96^{abc}	2.13±0.55^{a}	3.00±1.16^{ab}	1.40±0.18^{a}	6.53±2.98^{a}
20	46.50±1.29^{bc}	4.10±0.18^{c}	2.00±0.00^{a}	1.95±0.41^{b}	5.55±0.06^{a}
30	47.50±2.38^{c}	2.00±0.18^{a}	2.50±0.58^{ab}	2.13±0.61^{b}	3.70±0.00^{a}
40	41.75±0.50^{abc}	2.30±0.52^{ab}	2.00±1.16^{a}	2.35±0.35^{b}	7.35±2.02^{a}
50	39.50±1.73^{a}	2.65±0.58^{ab}	3.00±0.00^{ab}	2.90±0.48^{c}	8.48±4.71^{a}
60	46.00±7.53^{abc}	2.63±0.38^{ab}	3.50±1.73^{ab}	4.70±0.08^{d}	5.55±2.14^{a}
Level of sig (P≤0.05)	0.008	0.001	0.015	0.001	0.296

*Means with the same superscript in the column are not significantly different at P≤0.05

Table 3. *Metabolites in the Gills of C. gariepinus Exposed to Different Concentrations of an Oilfield Wastewater after 28 days.*

Conc. of oww (%)	Metabolites				
	Total protein (g/l)	Albumin (g/l)	Creatinine (mmol/l)	Total Urea (mmol/l)	Total bilirubin (µmol/l)
0	51.75±15.31^{b}	1.613±0.91^{a}	37.50±2.89^{b}	2.75±0.29^{a}	82.50±2.89^{c}
10	33.63±16.32^{a}	1.20±0.46^{a}	17.50±8.66^{a}	4.50±1.73^{b}	14.00±5.19^{a}
20	19.50±0.58^{a}	0.80±0.00^{a}	10.00±0.00^{a}	1.50±0.00^{a}	9.88±0.25^{a}
30	19.00±0.00^{a}	0.80±0.00^{a}	12.50±2.89^{a}	1.25±0.29^{a}	10.00±0.00^{a}
40	19.00±0.00^{a}	0.80±0.00^{a}	12.50±2.89^{a}	1.25±0.29^{a}	10.00±0.00^{a}
50	20.00±0.00^{a}	0.80±0.00^{a}	12.50±2.97^{a}	6.00±0.00^{c}	36.75±10.41^{b}
60	23.75±5.49^{a}	0.80±0.00^{a}	12.50±2.89^{a}	9.75±1.76^{d}	18.50±0.00^{a}
Level of sig(P≤0.05)	0.001	0.04	0.001	0.001	0.001

*Means with the same superscript in the column are not significantly different at P≤0.05

Table 4. *Metabolite in the Liver of C. gariepinus exposed to different concentrations of an oilfield wastewater after 28 days.*

Conc. of oww (%)	Metabolites				
	Total protein (g/l)	Albumin (g/l)	Creatinine (mmol/l)	Total Urea (mmol/l)	Total bilirubin (µmol/l)
0	28.50±10.97^{a}	2.40±0.92^{b}	50.00±4.08^{b}	5.13±1.03^{ab}	14.25±4.91^{b}
10	19.00±0.00^{a}	1.20±0.46^{a}	22.50±2.89^{a}	4.50±1.73^{ab}	10.00±0.00^{a}
20	28.50±10.97^{a}	1.60±0.00^{ab}	22.50±8.66^{a}	3.50±0.91^{a}	10.00±0.00^{a}
30	14.25±5.49^{a}	1.60±0.00^{ab}	22.50±2.89^{a}	4.25±0.29^{ab}	18.50±0.00^{c}
40	28.50±10.97^{a}	1.60±0.00^{ab}	20.00±0.00^{a}	4.25±0.29^{ab}	10.00±0.00^{a}
50	23.75±5.49^{a}	1.60±0.00^{ab}	20.00±5.77^{a}	4.25±0.29^{ab}	18.50±0.00^{c}
60	19.00±13.44^{a}	1.60±0.00^{ab}	12.50±2.89^{a}	6.00±0.00^{b}	18.50±0.00^{c}
Level of sig(P≤0.05)	0.001	0.00	0.00	0.004	0.007

*Means with the same superscript in the column are not significantly different at P≤0.05

Table 5. *Metabolites in the Kidney of C. gariepinus exposed to different concentrations of an oilfield wastewater after 28 days.*

Conc. of oww (%)	Metabolites				
	Total protein (g/l)	Albumin (g/l)	Creatinine (mmol/l)	Total Urea (mmol/l)	Total bilirubin (µmol/l)
0	24.25±4.91^{bc}	1.60±0.00^{c}	10.00±0.00^{b}	1.00±0.00^{a}	18.50±0.00^{b}
10	29.00±10.39^{c}	1.20±0.46^{b}	5.00±0.00^{a}	1.50±0.00^{ab}	9.75±0.29^{a}
20	9.50±0.00^{a}	0.80±0.00^{a}	5.00±0.00^{a}	3.63±2.75^{abc}	14.25±4.91^{ab}
30	9.500±0.00^{a}	0.80±0.00^{a}	5.00±0.00^{a}	3.50±0.91^{bc}	14.25±4.91^{ab}
40	14.25±5.49^{ab}	0.80±0.00^{a}	5.00±0.00^{a}	2.75±0.29^{abc}	14.25±4.91^{ab}
50	19.00±10.97^{abc}	0.80±0.00^{a}	12.50±2.89^{c}	3.63±0.75^{bc}	10.00±0.00^{a}
60	9.50±0.00^{a}	0.80±0.00^{a}	5.00±0.00^{a}	4.25±0.29^{c}	10.00±0.00^{a}
Level of sig(P≤0.05)	0.001	0.001	0.001	0.004	0.007

*Means with the same superscript in the column are not significantly different at P≤0.05

Table 6. *Metabolites in the Muscle of C. gariepinus exposed to different concentrations of an oilfield wastewater after 28 days.*

Conc. of oww (%)	Metabolites				
	Total protein (g/l)	Albumin (g/l)	Creatinine (mmol/l)	Total Urea (mmol/l)	Total bilirubin (µmol/l)
0	107.50±6.46^{c}	4.93±0.97^{b}	37.50±2.89^{d}	6.00±0.00^{a}	18.50±0.00^{b}
10	42.75±16.45^{b}	2.50±0.00^{a}	25.00±5.77^{c}	4.50±1.73^{a}	10.00±0.00^{a}
20	9.50±0.00^{a}	2.50±0.00^{a}	12.50±2.89^{b}	5.13±1.03^{a}	10.00±0.00^{a}
30	23.75±16.45^{ab}	2.50±0.00^{a}	5.00±0.00^{a}	7.38±1.60^{a}	32.38±5.34^{c}
40	23.75±5.49^{ab}	2.05±0.52^{a}	10.00±0.00^{ab}	7.38±1.60^{a}	18.50±0.00^{b}
50	28.50±10.97^{ab}	1.60±0.00^{a}	20.00±5.77^{c}	6.63±0.75b^{a}	10.00±0.00^{a}
60	9.50±0.00^{a}	1.60±0.00^{a}	12.50±2.89^{b}	12.00±1.47^{b}	10.00±0.00^{a}
Level of sig(P≤0.05)	0.001	0.001	0.001	0.001	0.001

*Means with the same superscript in the column are not significantly different at P≤0.05

4. Discussion

Water quality parameters such as temperature, dissolved oxygen, free carbon (IV) oxide, pH, alkalinity and conductivity are important and affect fish health, growth and reproduction [14, 28]. However, Richards [29] reported that the main cause of mortality in aquarium fish was the inadequate maintenance of the water environment. The characteristics of the oilfield wastewater used in this study have been evaluated by Akani and Gabriel [19]; the values agree with that of Wemedo *et al*. [30] but differ from that of Obire and Amusan [31]. Akani and Gabriel [21] reported that all the physico-chemical properties except temperature, DO, BOD and THC fell above acceptable limits of the Federal Environmental Protection Agency [32] of 35°C, 5.0 ppm, 50.0 ppm and 48.0 ppm respectively. The low levels of DO indicate that the environment is stressed [33]. *C. gariepinus* has been shown to have a wide tolerance for temperature ranges, low BOD, DO and salinity [34]. Akani and Daka [20] also reported that the constituted concentration of oilfield wastewater in their study gave a range of values of the constituent variables which were correlated with the indices of change in weight in the fish.

Total protein is an important constituent of all the cells and tissues which play vital role in the physiology of living organism [14]. Since the fishes have little carbohydrate, protein is used to meet the increase in the energy demand. Proteins are mainly involved in the architecture of the cell, which is the chief source of nitrogenous metabolism. During chronic periods of stress however, proteins are used as a source of energy [35]. The decrease in plasma total protein reported in this study was supported by the study of Das and Mukherjee [36] on *Labeo rohita* exposed to sublethal concentrations of cypermethrin. Adamu and Kori-Siakpere [14] also recorded similar findings when they exposed hybrid catfish (*Clarias gariepinus* and *Heterobranchus bidorsalis*) to sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust. The stress induced by the oilfield wastewater on protein synthesis probably caused depletion in the plasma protein as observed in this study. The decreases in plasma total protein level suggest high protein hydrolytic activity due to the elevation of protease activity. This agrees with the report of Tiwari and Singh [37] who recorded a decrease in serum total protein in snake head fish (*Channa punctatus*) exposed to sublethal concentrations of latex of *Euphorbia royleana.* Reeta *et al.* [38] reported inhibition in the total serum protein of an air breathing fish *Heteropneustes fossilis* after exposure to different pesticides (DDT, YBHC and Malathion). Ravichandran *et al.* [39] also reported depletion of protein due to proteolysis after exposing *Oreochromis mossambicus* to nominal concentrations of phenol. Bradbury *et al.* [40] pointed out that the decreased protein content might also be attributed to the destruction or necrosis of the cells and consequent impairment in protein synthesis machinery. The quantity of protein is dependent on the rate of protein synthesis, or on rate of its degradation. This may be affected by incorporating the impaired amino acids into polypeptide chains [41].

Albumin functions as transport proteins for several steroid hormones and for fatty acids from adipose tissue to muscle. It is responsible for about 80% of the total osmotic regulation [14]. Jee *et al.* [42] had reported decrease in serum albumin in Korean rock fish (*Sebastes schlegeli*) exposed to cypermethrin and suggested that it may impede its function of transportation. The significant ($p<0.05$) decrease in albumin as compared to the control in all the tissues tested

may have resulted from the inhibitory effect of the oilfield wastewater on protein hydrolytic activity due to elevation of protease activity and is also suggestive of some kidney problem [42]. Fafioye *et al*. [43] had also reported reduced albumin value in the kidney of *Oreochromis niloticus* exposed to sublethal concentrations of aqueous extract of *Raphia vinifera.* On the other hand, Adamu and Kori-Siakpere [14] observed increased value of liver albumin in fish exposed to *Nicotiana tobaccum*. They suggested that it could be as a result of accumulation of the protein in the liver impeding other functions of the liver.

Similarly, the decrease in creatinine levels in fish exposed to oilfield wastewater compared to the control as observed in this study indicates that creatinine was completely used up by the muscle as a result of the stress induced by the toxicant. Creatinine leaves the muscle and enters the blood where it is a waste product largely from the muscle breakdown. It is removed by filtration through the glomeruli of the kidney and is excreted as urine [14]. Therefore, it is frequently used in the diagnosis of renal function especially the glomeruli filtration rate [44]. Yakubu *et al*. [45] reported significant decrease in serum content of urea and creatinine throughout 15 days exposure period on some functional parameters of rat (200-250g) liver and kidney when exposed to yohimbe (14 mg/kg body weight) an aphrodisiac.

Urea is the principal end product of protein catabolism. It is a waste product metabolized in the liver and excreted by the kidney. Generally, level of urea in the tissues decreased slightly from the control and later increased with increasing concentration of the toxicant. This significant ($p>0.05$) increase observed especially in the kidney may be attributed to the inability of the kidney to filter this product and therefore a decrease in glomerular filterate rate (GFR). This may have been induced by the oilfield wastewater. This finding agrees with that of other researchers. Khleifat *et al.* [46] reported significant difference in blood urea of rats exposed to chronic effect of ethanolic extract of *Teuerium pollum* (20 mg/kg and 50 mg/kg) during the six weeks exposure period. Mahmoud *et al*. [47] also recorded a significant increase in urea and creatinine when they exposed *C. gariepinus* to mercury chloride. The amount of urea nitrogen in the blood plasma is an indicator of protein metabolism. The low value of plasma urea in this study may be attributed to the inability of the liver to metabolize protein as reported by Kori-Siakpere, [48]. The fluctuations observed in the level of total bilirubin in the tissues suggest that the liver may not have been affected by the toxicant.

5. Conclusion and Recommendation

This study which was intended to investigate the responses of *C. gariepinus* to different concentrations of oilfield wastewater observed that the metabolites either increased or decreased above their control values. The oilfield wastewater was also shown to contain TDS, salinity, conductivity and alkalinity that exceeded FEPA allowable limits. These chemicals can become potential hazards if discharged into the environment. Reduction in total protein and albumin as observed in this study is an indication of impaired synthesis of protein or loss of protein via excretion and is suggestive of kidney problem. Decrease in creatinine level in exposed fish is also suggestive of stress imposed on the fish by the toxicant and increased urea is an indication of the inability of the kidney to excrete excess waste. Fluctuations in total bilirubin in exposed fish suggest that the liver may not have been affected by the toxicant.

In view of these adverse effects imposed by the oilfield wastewater on *C. gariepinus,* it is imperative that the most effective oilfield wastewater treatment and disposal program be developed so as to reduce environmental issues associated with oilfield wastewater as observed in this study.

References

[1] S. K. Gupta, and P. C. Gupta,. General and applied Ichthyology (Fish and Fisheries). Chand and Company, Ram Nager, New Delhi, 2008, 739pp.

[2] FAO. Pattern of production of pulses and fish in Nigeria relative to the world (1965-1984). FAO, Rome, Italy. 1986, 74 pp.

[3] E. O. Aina. Keynote Address, Towards Environmentally Sustainable development in the petroleum industry. Proceedings of the International Seminar on Environmental. Impact Assessment and waste management in the Petroleum industry. Fairchild designs and prints, Ibadan, 1996, pp 1-6.

[4] P. G. C. Odeigah, O. Nuruddeen, and O. O. Amund. Genotoxicity of oilfield wastewater in Nigeria. Hereditas, 126, 161–167, 1997.

[5] J. Wills. A survey of offshore oilfield drilling wastes and disposal techniques to reduce the ecological impact of sea dumping, the effect of discharges of produced waters. Ekologicheaya Vahktan Sakhalina (Sakhalina Environmental Watch) 25th May, Sakhalina, London, 2000, pp. 1-5.

[6] H. J. Somerville, D. Benneth, J. N. Davenport, M. S. Holt, A. Lynes, A. Mahieu, B. Mccourt, J. G. Parker, R. R. Stephenson, R. J. Watkinson and T. G. Wilkinson. Environmental Effect of Produced Water from North Sea Oil Operations. Marine Pollution Bulletin, 18(10), 549-558. 1987.

[7] O. Obire, P. C. Adigwe, R. P. Ramesh and R. N. Okigbo. Effect of oilfield wastewater on microbiota of a freshwater in Nigeria. Journal of Pharmacy Research, 1(2), 264–270, 2008.

[8] C. B. Koons, C. D. Mcauliffe and F. T. Weiss. Environmental aspects of produced waters from oil and gas extraction operations in offshore and coastal waters. Journal of Petroleum Technology, 29, 723–729, 1977.

[9] O. O. James. Effect of Produced Water discharged from Imo River Flow Station SPDC (East) on Aquatic Environment. M. Sc. Thesis. Federal University of Technology, Owerri, 1999, pp 1–6.

[10] V. E. Forbes, A. Palmqvist and L. Bach, The use and misuse of biomarkers in ecotoxicology. Environ. Toxicol. Chem., 25(1), 272-280, 2006.

[11] V. Luskova, M. Svoboda and J. Kolarova. The effects of diazinon on blood plasma biochemistry in Carp (Cyprinus carpio L.). Acta Veterinaria Brno, 71, 117–123, 2002.

[12] H. O. Nwamba, C. E. Akinanu and K. C. Oyekwelu. Effect of crude oil and its products on bilirubin of African Catfish (Clarias gariepinus). Animal Research International, 3(3), 531–533, 2006.

[13] T. O. Sunmonu and O. B. Oloyede. Haematological Response of African Catfish (Clarias gariepinus) and Rat to Crude Oil Exposure. The Internet Journal of Hematology, 4, 1, 2008.

[14] K. M. Adamu and O. Kori-Siakpere. Effects of sub-lethal concentrations of tobacco (Nicotiana tobaccum) leaf dust on some biochemical parameters of Hybrid catfish (Clarias gariepinus and Heterobranchus bidorsalis). Brazilian Archives of Biology and Technology, 54(1), 183-196, 2011.

[15] S. M. Monteiro, J. M. Mancera, A. F. Fernandes and M. C. Sousa. Opper induced alterations of biochemical parameters of the gill and plasma of Oreochromis niloticus. Comparative Biochemistry and Physiology, 141, 375–383, 2005.

[16] I. R. Iyang, and S. Thomas. Toxicity of Fluazifop-p-butyl on blood cells and metabolites of a common African Catfish (Clarias gariepinus). Nigerian Journal of Agriculture, Food and Environment. 12(2), 128–132, 2016.

[17] N. P. Akani, and O. Obire. Mycological flora of Clarias gariepinus exposed to an oilfield wastewater in Nigeria. International Journal of Microbiology and Mycology. 3(1), 1–9. 2015.

[18] N. P. Akani, and O. Obire. Bacterial Populations of Clarias gariepinus (Burchell 1822) exposed to an oilfield wastewater in Rivers State, Nigeria. Asian. Journal of Biological Sciences 7(5): 208-216, 2014.

[19] N. P. Akani and U. U. Gabriel. Haematological responses of the African catfish Clarias gariepinus exposed to sublethal concentrations of an oilfield wastewater in Nigeria. Journal of Aquatic Sciences, 30(1B), 193–206, 2015.

[20] N. P. Akani and E. R. Daka. Evaluation of weight changes, condition factor and Organosomatic indices of Clarias gariepinus exposed to sub-lethal concentrations of an Oilfield wastewater. Current Studies in Comparative Education, Science and Technology, 2(2), 338–355, 2015.

[21] N. P. Akani and U. U. Gabriel. Enzymatic Responses of Clarias gariepinus (Burchell, 1822) exposed to sub-lethal concentrations of an oilfield wastewater. Journal of Applied Science and Biotechnology, 4(05), 026–032, 2016.

[22] FAO. Food and Agricultural Organization. Meeting on the toxicity and bioaccumulation of selected substances in marine organisms. FAO Fisheries Report, No 334, Rovinj, Yugoslavia, 5–9 Nov. 1984 FIR/R334.

[23] R. M. Gurure. Influence of two organo-chloride pesticides, thiodan and lindance on survival of fingerlings of O. niloticus and T. zilli. African Regional Aquaculture Centre Working Paper, 1978. ARAC/WP.6/87.

[24] APHA. American Public Health Association. Standard Methods for the Examination of Water and Wastewater. 20th edition, American Public Health Association, American Water Works Association and Water Environment Federation, Washington D. C., U.S.A. 1998.

[25] H. P. Sterling. Chemical and Biological Methods of Water Analysis for Aquaculturists. Pisces Press Limited Scotland, 1999.

[26] C. T. I. Odu, O. F. Esuruoso, L. G. Nwaboshi and J. A. Ogunwale. Environmental Study of the Nigerian Agip Oil Company operational Area. Soil and Fresh Water Vegetation. Union Grafts Publics. Milan, 1985.

[27] J. O'chei and A. Kolhatkar. Medical Laboratory Science: Theory and Practice. Tata Mcgraw Hill Publishing Co. Ltd. 2008, 1338pp.

[28] A. C. Camus, R. M. Burrow, W. G. Hemstreet, R. L. Thure and J. P. Hawke. Aeromonas Bacterial infections – Motile Aeromonas septicemia. Southern Regional Aquaculture Centre Publication No.478, 1998.

[29] R. Richards. Disease of Aquarium Fish The Veterinary Record, 101(9), 166–167, 1977.

[30] S. A. Wemedo, O. Obire and N. P. Akani. Bacterial population of an Oilfield Wastewater in Nigeria. Asian Journal of Biological Sciences, 5(10), 46-51, 2012.

[31] O. Obire and F. O. Amusan. The environmental Impact of Oilfield Formation Water on a Freshwater stream in Nigeria. Journal of Applied Science and Environmental Management, 7(1), 61–66, 2003.

[32] FEPA. Guidelines and Standards for Environmental Pollution Control in Nigeria. Federal Environmental Protection Agency. Abuja. Nigeria. 1991, Pp.54–58.

[33] R. B. Clerk. Marine Pollution. Clarandan Press, Oxford, 1986, 120-131.

[34] M. Ozmen, A. Güngördü, F. Z. Kucukbay and R. E. Gülar. Monitoring the effect of water pollution in Cyprinus carpio in Karakara Lake, Turkey. Ecotoxicology, 15, 157-169, 2006.

[35] B. L. Umminger. Relation of the whole blood sugar concentration in vertebrate to standard metabolic rate. Comparative Biochemistry and Physiology, 55, 457–460, 1977.

[36] B. K. Das and S. C. Mukherjee. Toxicity of cypermethrin in Labeo rohita fingerlings, Biochemical enzymatic and haematological consequences. Comparative Biochemistry and Physiology c-Toxicology and Pharmacology, 134, 109-121, 2003.

[37] S. Tiwari and A. Singh. Metabolic changes in the Snake head fish Channa punctatus, due to lattices of Euphorbia royleana, Asian Fisheries Science, 16, 147–155, 2003.

[38] P. Reeta, S. Bhargava and D. K. Saraf. Toxic effects of some biocides on total serum protein in Heteropneustes fossilis. Indian Journal of Environmental Toxicologyy, 3, 5–6, 1993.

[39] S. Ravichandran, K. Midhunashanthi and N. Indira. Impact of phenol on protein metabolism in the freshwater fish Oreochromis mossambicus. Journal of Ecotoxicology and Environmental Monitoring, 4, 33–37. 1994.

[40] S. P. Bradbury, D. M. Symonic, J. R Coats and G. J. Atchison. Toxicology of fenvalerate and its constituent's isomers to the fathead minnow (Pimephales promelas) and blue gill minnow (Lepomis macrochirus). Bulletin of Environment, Contamination and Toxicology, 38, 727-735, 1987.

[41] P. Y. Ram, S. Digvijay, S. K. Singh and A. Singh. Metabolic changes in fresh water fish Channa punctatus due to stem-bark extract of Crton tiglium. Pakistan Journal of Biological Sciences, 6, 1223–1228, 2003.

[42] L. H. Jee, F. Marsroor and J. Ch. Kang. Response of cypermethrin- induced stress in haematological parameters of Korean rockfish, Sebastes schlegeli (Hilgendrof). Aquaculture Research, 36, 898-905, 2005.

[43] O. O. Fafioye, S. O. Fagade and A. A. Adebisi. Toxicity of Raphia vinifera P. beauv fruit extracts on biochemical composition of Nile tilapia (Oreochromis niloticus, Trewavas). Biokemistri, 17 (2), 137-142, 2005.

[44] R. K. Murray, D. K. Sranner, P. A. Mayes and V. W. Rodwell.. Biochemia Harpera (Harpers Biochemistry) 1995, PZWL, Warszawa.

[45] M. T. Yakubu, L. S. Bilbis, M. Lawal and M. A. Akanji. Evaluation of selected parameters of rats' liver and kidney function following repeated administration of yohimbie. Biokemistri, 15 (2), 50–56, 2003.

[46] K. Khleifat, J. Shakhanbeh and K. Tarawneh. The chronic effects of Teuerium pollum on some blood parameters and Histopathology of the liver and kidney in the rat. Turkish Journal of Biology, 26, 65–71, 2002.

[47] U. M. Mahmoud, I. A. Mekkawy and A. A. Ibrahim. Biochemical response of the African catfish, Clarias gariepinus (Burchell, 1822) to sublethal concentrations of mercury chloride with supplementation of selenium and vitamin E. Toxicology and Environmental Health Sciences, 4(4), 218–234. 2013.

[48] O. Kori-Siakpere. Acute toxicity of Potassium permanganate to fingerlings of the African catfish, Clarias gariepinus (Burchell1822). African Journal of Biotechnology, 7(14), 2514–2520, 2008.