Journal of Applied Biology & Biotechnology Vol. 4 (05), pp. 026-032, Sep-Oct, 2016
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2016.40504

Enzymatic responses of *Clarias gariepinus* (Burchell, 1822) exposed to sub-lethal concentrations of an oilfield wastewater

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ARTICLE INFO

Article history:
Received on: 16/04/2016
Revised on: 22/05/2016
Accepted on: 14/06/2016
Available online: 23/10/2016

Key words:
Enzymes, oilfield wastewater, *Clarias gariepinus*, toxicity, aquatic environment.

ABSTRACT

Twenty eight adult *Clarias gariepinus* (mean weight 205 ± 12.89g SD; Mean length; 31.13 ± 3.82cm SD) were exposed to oilfield wastewater, oww (0. 10, 20, 30, 40, 50 and 60%) in quadruplicate for 28 days and the organs (plasma, gill, liver, kidney) and muscle were assayed for enzymes: aspartate transaminase (AST), alanine transaminase (ALT), acid phosphatase (ACP) alkaline phosphates (ALP) and lactate dehydrogenase (LDH). Changes in enzyme response were not concentration-dependent ($P < 0.05$), except ALP in kidney which showed a significant ($p>0.05$) increase with increased concentration of the oilfield wastewater when compared to the control. Other enzymes activities fluctuated around the control with LDH activity showing highest (2812.50±375.00 IU/L) response in all the organs. Lowest activity was recorded for ALT (4.00±0.00 IU/L) in the plasma. The changes observed can be used as an indicator of stress in *C. gariepinus* exposed to elevated levels of an oilfield wastewater. These changes can lead to death of fish and economic loss. The need therefore, for proper treatment of oilfield wastewater prior to discharge into the aquatic environment so as to reduce ecotoxicological problems and public health hazards is advocated.

1. INTRODUCTION

Oilfield wastewater (oww) is an effluent produced alongside with oil and gas during drilling [1]. It contains toxic inorganic and organic constituents such as polyaromatic hydrocarbons, metals such as lead, nickel, chromium and zinc that are potentially hazardous and cause major damages to aquatic and agricultural resources when discharged into the environment [2-6]. Toxicity bioassays involving brent produced water with oyster larvae, water flea (*Daphnia magna*), and shrimp (*Salmo gairdneri*) indicated that it was only toxic at dilutions of less than 20-fold, thereby implying that the toxicity of produced water could be reduced by the dilution effect of sea water in offshore situations [7]. However, the disposal of such effluents on land during onshore operations could pose a threat to fish in fish ponds as well as in open water located around such areas. This is because fish live in very intimate contact with their environment, and are therefore very susceptible to physical and chemical changes which may be reflected in their tissues and blood components [8-9]. An increase of enzymes activity in the extracellular fluid or organs is a sensitive indicator of even minor cellular damage, since the levels of these enzymes within the cell may exceed those in the extracellular fluid by more than three orders of magnitude [10].

The measurement of enzyme activities in the serum have frequently been used as a diagnostic tool in human medicine [11-12]. Enzyme activities have also been most extensively used to predict pesticide toxicity [11, 13]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are liver specific enzymes and provide a more sensitive measure of hepatotoxicity and histopathological changes that can be assessed within a short time [14]. Gabriel *et al.* [15] noted that increases or decreases in the values of ALT and AST indicate tissue damage in liver, kidney, muscle and gill. Alterations in alkaline phosphatase, ALP [16, 17], lactate dehydrogenase, LDH [8, 18-20] and ALP activities in tissues, organs and serum have been reported in fish exposed to toxicants in varying concentrations and have been used as biomarker for tissue damage in fish and a good diagnostic tool in toxicological studies.

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Other researchers have also reported changes in the enzyme kinetics of the organs and blood of fish exposed to toxicants [21-24]. In some of these studies, exposure to the toxicants caused either a significant increase or decrease or variable changes in the enzyme activities. Atamanalp *et al*. [25] found a significant decrease in the activities of ALP, LDH, AST and creatinine kinase (CK) and lactate in blood plasma. The activities of acid phosphatase (ACP) remained unchanged while ALP and LDH were depleted in the brain, kidney and liver when the fish, *Labeo robita* was exposed to 0.139 ppm of cypermethrin for 45 days [26]. Gabriel *et al*. [20] also recorded a decline in the activities of enzymes in all the organs (gill, kidney and liver) with ALP mostly affected when *Clarias gariepinus* was exposed to varying concentrations of cypermethrin. Basically, toxicants inhibit the activity or synthesis of enzymes [27], resulting in decreased activities in the organs [12].

In Nigeria, toxicity studies carried out on the effect of effluents from various sources on *C. gariepinus* include cassava processing effluent [28], liquid petroleum effluent [29], oil formation water [30], textile mill wastewater effluent [31], oil dispersant [32], detergent effluent [33, 34], shea butter effluent [35] and abattoir and saw-mill effluent [36]. Oilfield wastewater is being discharged either into aquatic environments or pits on a regular basis from oil wells in oil-producing areas in the Niger Delta, Nigeria. Despite the potential toxicity of oilfield wastewater, there is a dearth of information on the toxicity of oilfield wastewater on *C. gariepinus*. This study therefore, evaluates the enzymatic responses of *C. gariepinus* exposed to sub-lethal concentrations of an oilfield wastewater.

2. MATERIAL AND METHODS

2.1 Collection 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. 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 by car in 50l aquarium containing borehole water. The mouth of the trough was covered with a net and transported to the Department of Applied and Environmental Biology laboratory for analysis. On arrival, the fish was acclimated individually (a fish/tank) in rectangular aquaria for two weeks. The top of the aquaria were covered with perforated lid 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 at 800 and 1700 hrs, respectively.

The test toxicant (oilfield wastewater) was collected from Ebocha Oil Centre (run by AGIP oil Company) in Ogba/Egbema Local Government Area of Rivers State with 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*l* plastic jerry cans 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 stored in a fridge or freezer.

2.2 Trial test

Preliminary investigation was conducted to determine the range of concentrations of oilfield wastewater that exhibited sub-lethal effect on the fish. Five concentrations (10%, 30%, 50%, 70% and 100%) of the oilfield wastewater were prepared by serial dilution from each effluent sample on a volume to volume, v/v ratio [37]:

$$\text{Volume percent} = \frac{volume\ of\ effluent}{V_E + V_{DW}}\ x\ 100$$

Where, V_E = Volume of effluent

V_{DW} = Volume of dilution water

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

One fish was put into aquarium holding 20*l* of test solution with aquarium net. The test solutions were 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 also 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 [38].

2.3 Definitive test

For definitive test 10, 20, 30, 40, 50, and 60%v/v of oilfield wastewater were prepared including a control. Each test concentration had four replicates. A fish was then introduced into 15*l* of the oww in each of the aquaria. The exposure lasted for a period of 28 days and fish was fed as in the acclimation period. The test solution was renewed weekly after washing the aquarium and replaced with new stock.

2.4 Physicochemical Characteristics of Oilfield Wastewater

The following physicochemical characteristics of the various oilfield wastewater samples collected at various times were analyzed [39]. They include temperature, pH, salinity, turbidity, electrical conductivity, total dissolved solids (TDS), total suspended solids (TSS), chloride, alkalinity, total hydrocarbon content and heavy metal.

2.5 Enzyme Analysis

At the end of 28 days, fish per tank was killed with a blow on the head and tissues samples from the plasma, gill, liver, and kidney and muscle were obtained, ground in a mortar, mixed with 5ml of deionized water and centrifuged at the rate of 3000 rpm for 10 minutes. The supernatant was decanted analysed for AST, ALT and ACP calorimetrically following the method of [40]; ALP was determined using the method of [41], while LDH was estimated according to the method of [42]. The data obtained from the tests were subjected to a one way ANOVA using

Statistical Package for the Social Sciences (SPSS) version 17.0 and differences among means were separated by Student Neuman Kuel's test at 95% probability.

3. RESULTS

3.1 Physicochemical characteristics of oilfield wastewater

The result of the mean values of the physico-chemical properties of constituted concentrations of the oilfield wastewater is presented in Table 1. Generally, the values increased with increased concentration of the effluent: turbidity (1.5 ± 0.05 – 3.5 ± 0.0NTU), conductivity (183.33 ± 28.87 – 13666.67 ± 288.68 µs/cm), TDS (48.33 ± 2.89 – 7316.67 ± 275.3ppm), chloride (9.33 ± 1.16 – 3726 ± 64.29 ppm), BOD (0.82 ± 0.03 – 1.73±0.02ppm), ammonia (0.0 ± 0.0 – 0.02 ± 0.0ppm), nitrite (0.001 ± 0.0 – 0.01±0.0ppm) 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.

3.2 Responses of *Clarias gariepinus* to oww

Results of activity of enzymes in tissues of *C. gariepinus* exposed to various concentrations of oww are presented in Tables 2 - 6 The activity of AST in the plasma at the control, 7.00±0.00 IU/l and that at the exposure concentrations 20%, 40 - 60% were similar ($p \geq 0.05$) but differed ($p \leq 0.05$) from those at 10%, 25.75±14.86 and 30%, 25.00±2.31 IU/L (Table 2). The toxicant did not cause pronounced change ($p \geq 0.05$) in the activity of ALT in the plasma relative to the control values. Exposure of *C. gariepinus* to the treated wastewater raised the activity of ACP in the plasma at some of the concentrations (20%, 6.80±0.29 IU/L and 50% 8.33±0.62 IU/L) above ($p \leq 0.05$) the other treated groups and in the control, 4.83±0.91 IU/L (Table 2). The response of ALP in the treated group was variable, differing ($p \leq 0.05$) among themselves (Table 2) with that at 30% higher ($p \geq 0.05$) than the control by 5 units. LDH-P activity at 30 - 60% (375.00±68.60 IU/L) was the same, similar to that at 10% (450.00±173.21 IU/L) and control, but differed ($p \leq 0.05$) only from that at 20%, 675.00±86.00 IU/L (Table 2). Oilfield wastewater caused progressive decline ($p \leq 0.05$) in the activities of AST in the gills of exposed fish from 185.00±133.60 IU/L at 10% to 42.5±8.60 IU/L, below that at the control at 30%, 50% and 60%, 46.25±14.36 IU/L (Table 3). The activity of ALT at the control, 30.00±11.55 IU/L was higher ($p \geq 0.05$) than that at the exposed concentrations by 5 - 10 units, but ACP activity at the control differed ($p \leq 0.05$) from that of all the exposure concentrations which were different among themselves (Table 3).

Table 1: Mean ± standard deviation values of Physico-chemistry of constituted concentrations of oilfield wastewater used during the study.

Physicochemical properties	Concentration of Oilfield Wastewater (%)							
	0	10	20	30	40	50	60	FEPA Limits
Temp.	26.17±0.29	26.07±0.40	26.50±0.50	25.00±0.0	26.83±0.29	27.33±0.29	27.00±0.00	35
pH	7.10±0.10	7.47±0.31	7.67±0.12	7.23±0.25	7.47±0.31	8.00±0.00	8.00±0.00	6.5 -8.5
Salinity (ppm)	0.00±0.00	47.67±2.52	517.33±28.31	1500.0±50	2443.3±309.25	3233.3±251.66	4556.00±51.07	
Turbidity (NTU)	1.50±0.50	5.33±0.58	13.33±4.16	16.0±1.00	23.33±3.06	32.00±2.0	35.00±0.00	
Conductivity (us/cm)	183.33±28.87	3766.67±251.66	1060±121.66	2166.7±288.7	4216.7±225.46	8433.3±404.15	13666.7±288.68	400
TDS(ppm)	48.33±2.89	976.67±25.17	1620.00±158.8	2966.7±152.75	4783.3±256.58	6146.7±128.58	7316.7±275.38	2000 (max.)
TSS (ppm)	0.48±0.03	1.23±0.25	2.33±0.15	2.70±0.17	3.00±0.00	3.27±0.12	3.50±0.0	30
Chloride (ppm)	9.33±1.16	1760±52.92	1926.67±110.2	2316.7±189.29	2726.7±253.25	3472.7±25.33	3726.7±64.29	
DO (ppm)	3.97±0.451	3.40±0.20	3.30±0.10	2.43±0.15	2.33±0.12	2.3±0.00	2.20±0.00	5
BOD (ppm)	0.82±0.03	1.02±0.03	1.18±0.06	1.52±0.06	1.58±0.02	1.64±0.00	1.73±0.02	10
COD (ppm)	0.01±0.00	0.01±0.0	0.02±0.00	0.02±0.00	0.04±0.01	0.05±0.0	0.07±0.01	40
Alk. (ppm)	9.33±1.16	20.00±2.00	23.5±0.50	26.67±3.06	31.33±1.16	32.00±0.00	14.00±0.00	
Ammonia (ppm)	0.00±0.0	0.02±0.015	0.01±0.00	0.01±0.001	0.02±0.002	0.02±0.00	0.02±0.00	0.1
Nitrite (ppm)	0.00±0.0	0.01±0.001	0.01±0.0	0.01±0.001	0.01±0.00	0.01±0.00	0.01±0.00	
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±3.12	
Pb (ppm)	0.00±0.0	0.00±0.00	0.01±0.00	0.03±0.044	0.01±0.00	0.02±0.00	0.03±0.00	0.05
Cd (ppm)	0.00±0.00	0.0±0.0	0.0±0.0	0.00±0.00	0.0±0.0	0.00±0.00	0.00±0.00	<0.1
Zn (ppm)	0.001±0.002	0.002±0.001	0.0±0.0	0.00±0.00	0.01±0.00	0.00±0.0	0.01±0.00	1.0
Co (ppm)	0.0±0.0	0.00±0.00	0.0±0.0	0.00±0.00	0.00±0.00	0.00±0.0	0.00±0.00	1.5
Fe (ppm)	0.001±0.001	0.01±0.004	0.04±0.001	0.07±0.003	0.08±0.0	0.06±0.08	0.21±0.01	<1.0
PAH (ppm)	0.00±0.00	0.01±0.01	0.02±0.01	0.03±0.01	0.03±0.01	0.08±0.02	0.07±0.00	

Table 2: Enzyme activity in the plasma of *C. gariepinus* exposed to different concentrations of an oilfield wastewater for 28 days (mean±SD).

Conc. of wastewater (%)	Enzyme activity (IU/L)				
	AST	ALT	ACP	ALP	LDH-P
0	7.00±0.00^{a}	4.00±0.00^{a}	4.83±0.91^{ab}	60.00±11.55^{c}	525.00±86.60^{ab}
10	25.75±14.86^{b}	7.00±3.83^{a}	5.33±0.17^{b}	21.50±1.73^{a}	450.00±173.21^{a}
20	10.00±3.46^{a}	4.00±0.00^{a}	6.80±0.29^{c}	33.50±7.51^{b}	675.00±86.60^{b}
30	25.00±2.31^{b}	4.00±0.00^{a}	5.10±0.41^{ab}	65.00±2.31^{c}	375.00±86.60^{a}
40	10.00±0.00^{a}	4.00±0.00^{a}	4.08±0.61^{a}	38.25±11.44^{b}	375.00±86.60^{a}
50	13.00±0.00^{a}	6.00±2.31^{a}	8.33±0.62^{d}	11.50±1.73^{a}	375.00±86.60^{a}
60	10.00±3.46^{a}	6.00±4.00^{a}	4.63±0.56^{ab}	12.67±5.17^{a}	375.00±86.60^{a}
Level of sig. p≤0.05	0.001	0.302	0.001	0.001	0.003

Means with the same superscript in the column are not significantly different at $p \geq 0.05$

Table 3: Enzyme activity in the gills of *C. gariepinus* exposed to different concentrations of an oilfield wastewater for 28 days (mean±SD).

Conc. of oww (%)	Enzyme activity (IU/L)				
	AST	ALT	ACP	ALP	LDH-P
0	50.00±17.32^{a}	30.00±11.55^{a}	33.00±2.61^{c}	236.25±17.02^{d}	1125.00±433.01^{a}
10	185.00±133.60^{a}	20.00±0.00^{a}	22.75±2.90^{b}	137.50±16.58^{c}	1125.00±433.01^{a}
20	156.25±106.57^{a}	20.00±0.00^{a}	18.00±2.89^{ab}	78.75±25.29^{b}	1500.00±0.00^{a}
30	42.50±8.66^{a}	20.00±0.00^{a}	18.00±8.66^{ab}	57.50±8.66^{ab}	750.00±0.00^{a}
40	50.00±0.00^{a}	25.00±10.00^{a}	10.50±0.00^{a}	67.50±20.21^{ab}	750.00±0.00^{a}
50	42.50±8.66^{a}	25.00±10.00^{a}	15.50±0.00^{ab}	50.00±0.00^{ab}	1125.00±433.01^{a}
60	46.25±14.36^{a}	20.00±0.00^{a}	23.00±2.89^{b}	41.75±9.53^{a}	1125.00±433.01^{a}
Level of sig,P≤0.05	0.014	0.3	0.001	0.001	0.055

Means with the same superscript in the same column are not significantly different at p≥0.05

Table 4: Enzyme activity in the liver of *C. gariepinus* exposed to different concentrations of an oilfield wastewater for 28 days (mean±SD).

Conc. of oww (%)	Enzyme activity (IU/L)				
	AST	ALT	ACP	ALP	LDH-P
0	72.50±19.37^{ab}	20.00±0.00^{a}	23.00±2.89^{ab}	775.00±28.87^{d}	1500.00±0.00^{b}
10	61.25±22.50^{ab}	20.00±0.00^{a}	30.50±11.55^{b}	92.50±8.67^{ab}	1500.0±0.00^{b}
20	103.75±36.60^{b}	30.00±11.55^{a}	30.50±5.77^{b}	250.00±70.71^{c}	1125.0±433.01^{ab}
30	35.00±0.00^{a}	20.00±0.00^{a}	20.50±5.77^{ab}	33.50±0.00^{a}	2625.0±433.01^{c}
40	35.00±0.00^{a}	20.00±0.00^{a}	13.00±2.89^{a}	33.50±0.00^{a}	1312.5±375.00^{b}
50	97.50±20.21^{ab}	30.00±11.55^{a}	25.50±0.00^{ab}	67.13±30.68^{a}	750.00±0.00^{a}
60	97.50±55.45^{ab}	20.00±0.00^{a}	23.00±8.66^{ab}	130.00±21.21^{b}	1500.0±0.00^{b}
Level of sig,P≤0.05	0.006	0.055	0.014	0.00	0.00

Means with the same superscript in the same column are not significantly different at p≥0.05

Table 5: Enzyme activity in the kidney of *C. gariepinus* exposed to different concentrations of an oilfield wastewater for 28 days (mean±SD).

Conc. of oww (%)	Enzyme activity (IU/L)				
	AST	ALT	ACP	ALP	LDH-P
0	35.00±0.00^{a}	20.00±0.00^{a}	28.00±2.89^{a}	275.00±28.89^{a}	2625.00±433.01^{c}
10	53.75±14.36^{ab}	20.00±0.00^{a}	28.00±2.89^{a}	517.50±37.53^{c}	1500.00±0.00^{b}
20	130.00±25.17^{c}	20.00±0.00^{a}	20.50±0.00^{a}	778.75±132.75^{d}	750.00±0.00^{a}
30	42.50±8.66^{ab}	20.00±0.00^{a}	20.50±11.55^{a}	392.50±49.08^{b}	1125.00±433.01^{ab}
40	50.00±17.32^{ab}	25.00±10.00^{a}	30.50±11.55^{a}	375.00±78.42^{b}	1500.00±0.00^{b}
50	72.50±8.66^{b}	25.00±10.00^{a}	28.00±2.88^{a}	942.50±8.66^{e}	2625.00±433.01^{c}
60	50.00±12.24^{ab}	25.00±10.00^{a}	28.00±2.88^{a}	775.00±28.86^{d}	2812.50±375.00^{c}
Level of sig,P≤0.05	0.00	0.677	0.226	0.00	0.00

Means with the same superscript in the same column are not significantly different at p≥0.05.

The activity of ALP in the gills of control (236.25±17.02 IU/L) fish exceeded twice (p≤0.05) than at all the exposure concentrations. Also the activity at 10%, 137.50±16.58 IU/L was higher (p≥0.05) than those at the other exposure concentrations which did differ from one another. The LDH-P in the gill responded in a similar manner: 1125±433.01 IU/L at 0, 10, 50 and 60% oilfield wastewater with the highest values 1500.00±0.00 IU/L at 20%. However, at 20% and 30% the activity diminished (p≤0.05) to 750.00±0.00 IU/L (Table 3). AST activity in liver responded similarly to oilfield wastewater exposed at 30 and 40% (35.00±0.00 IU/L) and 50% and 60% (97.50±20.21 IU/L), respectively (Table 4). The activity at the control, 72.50±17.37IU/L did not differ (p≥0.05) from those at the other concentrations except 30 and 40%. (Table 4). ALT and ACP activities in the respective controls and test concentrations were not different, p≤0.05 (Table 4). Exposure to oilfield wastewater resulted in 3 - 2.3 times decline (p≤0.05) in the activity of ALP in the gills of exposed fish. The activity level at the control was 775.00±28.87 IU/L which differed (p≤0.05) from those in the test concentrations. The responses among the treated group were variable, p≤0.05 (Table 4). The oilfield wastewater caused highest elevation in the AST of the kidney at 20%, 130.00±25.27 IU/L which differed (p≤0.05) from that at the other concentrations and the control, 35.00±0.00 IU/L (Table 5).

In the kidney, it did not cause pronounced changes (p≥0.05) in the ALT and ACP of the treated groups (Table 5). ALP activity in the kidney was variable among treated groups (p≤0.05) and raised (p≤0.05) above the control value, 275.00±28.89 IU/L (Table 5). LDH-P activity at the control (2625.00±433.01 IU/L), 50% (2625.00±433.02 IU/L) and 60% (2812.50±375.00 IU/L) were similar (p≥0.05) but higher than those at the other concentrations (Table 5).The activity of AST in muscle at the control, 187.50±39.48 IU/L was not different from some other concentrations, 20%, 157.50±25.98 IU/L; 40%, 167.50±14.43 IU/L and 50%, 150.00±63.51 IU/L. However, they differed (p≤0.05) from 10% (115.00±23.09 IU/L), 30% (72.50±19.37 IU/L) and 60% (115.00±23.09 IU/L) which did not differ from one another (Table 6). The highest ALT response (p≥0.05) to oilfield wastewater occurred at 20%. The activity of ACP in the muscle under oilfield wastewater stress was low for both the control and treated groups ranging from 5.50±0.00 IU/L - 8.00±2.89 IU/L (Table 6). ALP responded in a variable manner among the treated groups with those at 20% (142.50±8.66), 30% (325.00±28.87 IU/L) and 40% (267.50±20.21 IU/L) differing among themselves and from 50, 60 and 0%. The highest activity of LDH-P, 1500.00±0.00 IU/L in the muscle was recorded at 50% followed (p≤0.05) by the control, 1125±433.01 IU/L (p≤0.05) and the others, 750.00±0.00 IU/L (Table 6).

Table 6: Enzyme activity in the muscle of *C. gariepinus* exposed to different concentrations of an oilfield wastewater for 28 days (mean±SD).

Conc. of oww (%)	Enzyme activity (IU/L)				
	AST	ALT	ACP	ALP	LDH-P
0	187.50±39.48^{c}	20.00±0.00^{a}	5.50±0.00^{a}	49.63±12.87^{a}	1125.00±433.01^{b}
10	115.00±23.09^{ab}	20.00±0.00^{a}	8.00±2.89^{a}	103.75±45.16^{b}	750.00±0.00^{a}
20	157.50±25.98^{c}	40.00±23.09^{a}	8.00±2.89^{a}	142.50±8.66^{c}	750.00±0.00^{a}
30	72.50±19.37^{a}	20.00±0.00^{a}	5.50±0.00^{a}	325.00±28.87^{e}	750.00±0.00^{a}
40	167.50±14.43^{c}	30.00±11.55^{a}	5.50±0.00^{a}	267.50±20.21^{d}	750.00±0.00^{a}
50	150.00±63.51^{c}	30.00±11.55^{a}	5.50±0.00^{a}	41.75±9.53^{a}	1500.00±0.00^{c}
60	115.00±23.09^{ab}	30.00±11.55^{a}	5.50±0.00^{a}	42.50±8.66^{a}	750.00±0.00^{c}
Level of sig,P≤0.05	0.001	0.677	0.226	0.001	0.001

∗Means with the same superscript in the same 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 [43, 44]. The characteristics of the oilfield wastewater used in this study have been evaluated by Akani and Gabriel [45]; the values agree with that of Wemedo *et al*. [12], but differ from that of Obire and Amusan [2]. Akani and Gabriel reported that all the physico-chemical properties except temperature, DO, BOD and THC fell above acceptable limits of the Federal Environmental Protection Agency [46] of 35 ^{O}C, 5.0 ppm, 50.0 ppm and 48.0 ppm respectively [45]. The low levels of DO indicate that the environment is stressed [47]. *C. gariepinus* has been shown to have a wide tolerance for temperature ranges, low BOD, DO and salinity [48]. However, it was reported that the main cause of mortality in aquarium fish was the inadequate maintenance of the water environment [49]. In this study, all the monitored parameters were within the acceptable limits of FEPA except for TDS, salinity, conductivity, and alkalinity which had values above FEPA limits while, BOD and DO, had values below FEPA limits. Total dissolved solids (TDS) are a measure of inorganic salts, organic matter and other dissolved materials in matter [50]. Infact, *C. gariepinus* has been successfully cultured in this range of salinity, 47.67 ±2.52 - 4556.0 ±51.07ppm [48]. This ability may have accounted for the minimal changes in the AST, ALT, and ACP in the tissues of the experimental fish.

Enzymes assays such as ALP, AST, ALT, ACP and LDH are parts of standard laboratory tests to detect abnormalities in animals [23, 51, 52]. Changes in the activity of these enzymes resulting from toxicant or contaminant effects in various organs of fish have been reported [53-55]. Such alterations in fish are aimed at maintaining equilibrium in the presence of these toxicants which are known to disrupt physiological and biochemical processes [56].

In this study, activities of these enzymes increased, or decreased as the concentration of oilfield wastewater increased in all organs tested, but they were not dose dependent.

Increased activities of AST, ALT and ALP in Indian major carps exposed to nitrite toxicity have been recorded [10]. They suggested that the increase of transferases is as a result of diversion of alpha-amino acids in the tricarboxylic acid (TCA) cycle as keto-acids to augment energy production. In addition, the enzymatic activities in all the organs were inhibited at different concentrations of oww with ACP activity being mostly affected. The lowered activity of AST, ALT and ACP in this study showed that inactive transamination and oxidative deamination occurred. Cellular toxicity of organophosphates have been attributed to an increase in AST, ALT and ALP in the liver of albino rats exposed to monocrotophos, methyl-parathion acid and dimethoate given orally for 90days [57]. In this study AST activity was elevated in the muscles probably to enable the fish cope with the energy demand during stress condition. Similar findings, suggest that this energy demand could be satisfied through amino acid. ALP activity and decreased in all the tissues tested when compared to the control except kidney and muscles [20]. Similar observation was made in the muscle of *Heteropneustes fossilis* exposed to 0.2mg/l of cadmium for a period of 15-60 days [58] According to them the decrease in the various organs probably resulted from a decline in the rate of synthesis caused by lowered metabolic demand. ALT and ACP activity in all the tissues tested did not show any appreciable difference but fluctuated around the control value in this study probably because the oilfield wastewater did not affect these organs or disturb the integrity of the cell membranes [59, 60]. Other researchers however, recorded contrary findings as ALT and ACP activities increased when black jaw Tilapia (*Sarotherodon melanotheron)* was exposed to industrial effluents and suggested that the liver tissues of the exposed fish have been impaired [12]. This view was supported by Gabriel and George (2005) when *C. gariepinus* was exposed to glyphosate in the laboratory [61]. ALT activity reflects a change in endoplasmic reticulum mass; it is also known to occur in the cell membrane and may be involved in metabolic activities [62]. This increase may denote an increase in metabolic transport [63] which may eventually result in a shift in biosynthesis and the energy metabolism pathway of the exposed organism [64]. The decrease in ACP activity in the tissues reflects a possible decrease in biosynthetic activities and anaerobic capacity of *C. gariepinus* [65], while an elevation in ACP, suggests an increase in lysosomal mobilization and cell necrosis due to effluent toxicity [66].

Generally, LHD activity increased in all the tissues tested but the highest activity was recorded in kidney at 60% concentration of the toxicant when compared with the control. This may be due to a decrease in the glycolytic process which lowered metabolic rate in the presence of the toxicant. It may also be due to a shift towards anaerobic respiration [67], possibly due

to the hypoxic environment as exhibited by the oilfield wastewater in this study. Others reported similar observations in *C. gariepinus* exposed to cypermethrin and pesticides which caused a drop in LDH in the tissues [20, 68]. Alteration in enzymes activities of the exposed fish is one of the major biomarkers indicating the level of changes consequent upon exposure to xenobiotics in fish that can be recognized and associated with established health impaired processes [69]. The changes observed in this study can therefore be used as a marker in *C. gariepinus* exposed to oilfield waste-water.

5. CONCLUSIONS AND RECOMMENDATION

This study revealed that exposure of *C. gariepinus* to sublethal levels of oilfield wastewater produced significant changes in the physiology as manifested by changes in the enzyme activities and disruptive changes in the organ of the fish essential for functional metabolism. Persistent exposure through pollution by the effluent in aquatic and terrestrial environments can lead to mortality of *C. gariepinus* and economic loss. Proper treatment of oilfield wastewater prior to disposal is hereby advocated to prevent ecotoxicological problems and associated health hazards.

Financial support and sponsorship: Nil.

Conflict of Interests: There are no conflicts of interest.

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How to cite this article:

Akani NP, Gabriel UU. Enzymatic responses of *Clarias gariepinus* (Burchell, 1822) exposed to sub-lethal concentrations of an oilfield wastewater. J App Biol Biotech. 2016; 4 (05): 026-032. doi: 10.7324/JABB.2016.40504