Behavioural Toxicity of A Combined Oilfield Chemicals On African Catfish (*Clarias gariepinus*) (Burchell 1822)

ABSTRACT
The toxicity of the combination of xylene and diesel at a 50:50 ratio on the African catfish (*Clarias gariepinus*) was investigated using static bioassays for 96hrs. No statistical significance was observed in the other groups and the control group. Physicochemical parameters after exposing *C. gariepinus* to xylene: diesel for 24 hours was no significant difference (P>0.05) among the different concentration gradients and there was also an increased variation along the concentration gradient observed after 96hours. The behavioral responses of the test fish were observed at 24-96 hours of exposure. Normal behavioral responses were observed in the control. Fish exposed to 25ml/l to 50 mg/l showed normal behavior from 24 to 48 hours but afterward, the fish that were alert stopped swimming and remained static for a while in response to the sudden changes in the surrounding environment. Generally, fish exposed to higher concentrations such as 100ml/l to 250ml/l of the test chemicals showed progressive hyperventilation and abnormal behavior such as gulping of air, an erratic swimming movement, very fast swimming, jumping, and displaying vigorous jerky movement suffocation, and loss of reflex. A faster operculum and tail beat movement was also observed with Spiraling. The behavioral responses increased significantly (P<0.05) with an increase in concentration per time as compared to the control group of fish. The noticeable behavioural difference was recorded for the different concentrations. An eco-friendly chemical that is within the acceptable limits recommended by WHO should be used in well stimulation and cleaning for sustainable biodiversity and a healthy aquatic environment.
Keywords: Behavioural, Responses, *Clarias gariepinus*, Xylene and Diesel

1. INTRODUCTION

The oil and gas exploration and production practices in Nigeria are at their central wellspring of income and power [1,2]. These ventures have been advantageous from numerous points of view but have also led to adverse effects on the environment, especially on the aquatic body [2]. This is particularly a result of the contemporary exploration technology used in our offshore oil exploration which is done in-situ in various water bodies [3,4]. The petroleum industry is a significant component of world energy, which involves various operations such as drilling, and exploration, of crude oil and/or natural gas [5]. Some of the activities also involve reservoir stimulation which is a specialized area in the petroleum industry, carried out for financial benefits [6].

A mixture of diesel and xylene, as an aromatic solvent, has been a common remedy for surface pipelines, wellbore tubular, and especially near well bore cleaning operations, because other means of cleanup, such as heat, dispersants, pigging, scrapping, etc. cannot remove the organic materials like asphaltene or paraffin completely from the near-wellbore area [7]. These toxic chemicals are released into the aquatic body during the well stimulation and cleaning as organic deposits like asphaltene are removed [8].

Usually, wellbore soaking is done by a mixture of diesel and xylene to remove the organic plugs in the petroleum production system thereby imposing a life threat to field staff and the environment through storage and flow back into the waste pit [6]. The introduction of these chemicals into the aquatic environment has resulted in modifications of the physicochemical parameters of water that have caused fish to kill and also affected other aquatic organisms in the wild [9].

One of the major advantages of using data on behavioural response in a toxicological study is that they are more sensitive indicators of potential impacts on the survival of an organism in the field than are measures of deadliness [61]. Most indications from available data for fish suggest that behavioural effects of organic contaminants often occur more at a lower concentration to a higher order of magnitude lower than those found to cause death, [9]. As a result, it is believed that the use of information on behavioural toxicity in ecological risk assessments could be beneficial to the evaluation process, allowing for a considerable and more ecologically significant and preventive risk and exposure conditions.

2. MATERIALS AND METHODS

2.1. Fish Collection and Acclimation

One hundred and twenty (120) healthy fingerlings of *Clarias gariepinus* were collected from the University of Port Harcourt Demonstration and research farm, Choba campus, and transported in plastic containers to the Department of Fisheries wet laboratory in the University of Port Harcourt. The fish had a mean length of 15.20±2.3cm and mean weight of 10.23±2.60g; acclimated in a glass tank with an aerator to continuously oxygenate the water to laboratory conditions at a room temperature of 28 ± 2^0C in a 150 litres capacity glass aquarium tank for 14 days and were fed commercial fish-feed (45% crude protein) at 6% body weight, twice daily. The water in each glass tank was replaced with tap water from the laboratory every 48 hours.

2.2. Test Chemical

The Diesel used in the study was obtained from the Nigerian National Petroleum Corporation (NNPC) Filling station in Port Harcourt while Xylene (liquid)was bought from a chemical laboratory in Choba, Port Harcourt, Rivers State, and stored under ambient conditions in the laboratory.

2.3. Preparation of a working stock solution for Xylene and Diesel

A working stock solution was prepared from Xylene following the method of Ormerod*et al.*; Arbel, *et al.*, [10,11]. The test chemical was prepared, using the equation: $V_1C_1 = V_2C_2$, where; V_1C_1 = Stock solution attributesandV_2C_2 = New stock solution attributes while the water-soluble fraction (WSF) of the Diesel used was prepared using the standard method by Anderson *et al*. and Orlu and Ogbalu, [12, 13]. The Combination of both chemicals at a 50:50 ratio was prepared using the method described by Davies *et al.*, [7].

2.4. Behavioural Response

Feeding was suspended 24 hours before the static exposure period that lasted for 96 hours. Six test concentrations of 0.0ml/l(control), 25ml/l, 50ml/l, 100ml/l, 150ml/l, 200ml/l and 250ml/l were prepared, each test concentration was held in plastic aquarium tank of 15 litres and filled to 10 mark. Ten fish were randomly selected and put in each of the test concentrations. Each treatment was in replicates. Each treatment group of fish was exposed for 96hours during which the behavioural changes of the fish samples were assessed by closely observing the movement of the fishes on exposure to the varied concentration of toxicant from the 24th and 96th hours to report the following parameters; respiratory movement (operculum beat), tail fin beat frequency, loss of reflex, hyperventilation, erratic swimming suffocation or spiraling were carefully observed and mortality recorded. These were carried out using the method described by Gabriel and Obomanu [14].

2.5. Physico-chemical parameters of the experimental Water.

During the 24th and 96th hours experimental period hydrogen ion concentration (pH), temperature (^{o}C), conductivity (µS/cm), total dissolved solids (ppm), and total hardness (mg/l) were measured using an in-situ handheld multimeter (EZDO Multimeter Model CTS-406) while dissolved oxygen (DO) was measured with an in-situ Milwaukee Multimeter (Model MW600). Total alkalinity (mg/l), ammonia (NH_3-N) (ppm), and nitrate (NO_3-N) (ppm) were monitored using standard procedures as described by APHA, [15].

2.6. Statistical Method

The results were subjected to a one-way Analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS Version 23) to determine significant differences between various treatments and control. The Duncan [16] Multiple Range Test was used to separate differences among means. Differences were considered significant at ($P < 0.05$).

3. RESULTS

3.1. Physico-chemical parameters of the experimental water for 24 hours.

The mean values for physicochemical parameters after exposing *C. gariepinus*to xylene: diesel for 24 hours is represented in Table 1. The highest temperature (27.8±0.05°C) of the experimental water was observed in the test concentration of 250ml/l while the least (26.9±0.03°C) was recorded at the control (0ml/l). There was no significant ($P>0.05$) difference observed across the concentration gradients of the test chemical after 24 hours of exposure. The highest pH (6.7±0.03) was recorded at control

(0ml/l) while the least (6.2±0.01) was in the 200ml/l and 250ml/l. There was an observed significant difference ($P<0.05$) along the concentration gradient. Maximum value of conductivity (0.16±0.00µS/cm) was recorded at control, (0ml/l) while the minimum value (0.10±0.01µS/cm) was at 150ml/l, 200ml/l and 250ml/l. There was a statistically significant ($P<0.05$) among the different concentrations. The alkalinity value was highest (38.3±0.01mg/l) in the control (0ml/l) and least (35.4±0.03mg/l) in the 200ml/l and 250ml/l concentration. The alkalinity value decreased significantly ($P>0.05$) along the concentration gradient. Dissolved Oxygen was maximum (4.7±0.01mg/l) in the control (0ml/l) and minimum (4.4±0.01mg/l) in 200ml/l and 250ml/l concentrations. A slight significant difference ($P>0.05$) was observed along the concentration gradient. The total dissolved solid value recorded, was highest (159.3±0.3ppm) at 250ml/l concentration and the least (107.3±0.1ppm) at control (0ml/l). The TDS increased significantly ($P>0.05$) along the concentration gradient. The maximum value of ammonia was recorded (0.70±0.03ppm) in 250ml/l concentration while the minimum value was recorded (0.3±0.01ppm) in the control (0ml/l). There was a significant increase ($P>0.05$) along the concentration gradient. Nitrate was highest (0.68±0.05ppm) in 250ml/l concentrations and least (0.3±0.00ppm) in the control (0ml/l). There was a significant difference ($P>0.05$) along with the different concentrations. The maximum value for the experimental water hardness was (119±0.03mg/l) in 250ml/l concentrations while the minimum was (63±0.01mg/l) in the control (0ml/l). The values increased significantly ($P>0.05$) along the concentration gradient.

Table 1: Mean water quality parameters after exposing *C. gariepinus*to Xylene: Diesel for 24 hours

Parameters	Concentrations (ml/l)						
	0	**25 ml/l**	**50 ml/l**	**100 ml/l**	**150 ml/l**	**200 ml/l**	**250 ml/l**
Temperature (^{o}C)	26.9±0.03^{a}	27.4±0.06^{a}	27.4±0.03^{a}	27.3±0.06^{a}	27.3±0.03^{a}	27.7±0.06^{a}	27.8±0.05^{a}
pH	6.7±0.03^{a}	6.3±0.00^{a}	6.3±0.03^{a}	6.3±0.03^{a}	6.3±0.03^{a}	6.2±0.01^{a}	6.2±0.01^{a}
Conductivity (µS/cm)	0.16±0.00^{a}	0.15±0.01^{a}	0.13±0.00^{a}	0.13±0.01^{a}	0.10±0.00^{a}	0.10±0.01^{a}	0.10±0.00^{a}
Total Alkalinity (mg/l)	38.3±0.01^{a}	37.6±0.01^{a}	37.6±0.01^{a}	36.6±0.03^{a}	36.8±0.01^{a}	35.4±0.01^{a}	35.4±0.01^{a}
Dissolved Oxygen (mg/l)	4.7±0.01^{a}	4.5±0.01^{a}	4.5±0.00^{a}	4.5±0.00^{a}	4.5±0.00^{a}	4.4±0.01^{a}	4.4±0.01^{a}
TDS (ppm)	107.3±0.1^{da}	111.3±0.3^{a}	122.3±0.9^{a}	134.1±0.6^{a}	149.2±0.6^{a}	156.4±0.9^{a}	159.3±0.3^{a}
Ammonia (NH_3^- N) (ppm)	0.3±0.01^{c}	0.50±0.01^{a}	0.50±0.01^{a}	0.60±0.03^{a}	0.64±0.03^{a}	0.69±0.03^{a}	0.70±0.03^{a}
Nitrate (NO_3^-N) (ppm)	0.3±0.00^{c}	0.50±0.07^{a}	0.52±0.03^{a}	0.54±0.03^{a}	0.60±0.06^{a}	0.64±0.06^{a}	0.68±0.05^{a}
Total Hardness (mg/l)	63±0.01^{b}	91±0.03^{b}	96±0.01^{b}	101±0.01^{a}	108±0.01^{a}	115±0.07^{a}	119±0.03^{a}

*Means with same superscript across the rows are not significantly different at (P < 0.05)

**Means with different superscript across the rows are significantly different at (P < 0.05)

*** {TDS (ppm) = Total Dissolved solid}

3.2. Physico-chemical parameters of the experimental water 96 hours.

The Physico-chemical parameters of the experimental water are presented in Table 2. The maximum value for temperature (28.5±0.06°C) was observed in 250ml/l concentration and the minimum value (26.8±0.06°C) was recorded at the control (0ml/l). There was no significant difference(P>0.05) among the different concentration gradients. The highest pH value (6.5±0.03) was observed in the control (0ml/l) and the least value (6.2±0.03) was recorded at 250 ml/l. There was no statistically significant difference (P>0.05) among the different concentration gradients. The maximum value for conductivity (0.17±0.00µS/cm) was recorded at control 0ml/l while the minimum value (0.09±0.00µS/cm) was recorded at the 250ml/l. There was no significant difference (P>0.05) among the different concentrations. The total alkalinity value was highest (37.9±0.11mg/l) in the control (0ml/l) and least (34.0±0.01mg/l) in 250ml/l concentration. Their total alkalinity values decreased significantly (P>0.05) along the concentration gradient. Dissolved Oxygen was maximum (4.8±0.01mg/l) in the control (0ml/l) and minimum (4.5±0.01mg/l) in 50ml/l, 100ml/l, 150ml/l, 200ml/l and 250ml/l concentrations. No statistical significance (P>0.05) was observed along the concentration gradient.

The Total Dissolved Solid value was highest (161.7±0.3ppm) at 250ml/l concentration and the least (110.3±3.1ppm) at control (0ml/l). The TDS increased significantly (P>0.05) along the concentration gradient. The maximum value of ammonia was recorded (0.70±0.03ppm) in 250ml/l concentrations while the minimum value was recorded (0.3±0.01ppm) in the control (0ml/l). There was a significant increase (P>0.05) along the concentration gradient. Nitrate was highest (0.80±0.05ppm) in 250ml/l concentration and least (0.3±0.00ppm) in the control (0ml/l). There were no significant differences (P>0.05) along with the different concentrations. The total hardness of the experimental water was highest (121±0.03mg/l) in 250ml/l concentration and the minimum was (61±0.01mg/l) in the control (0ml/l). The values increased significantly (P>0.05) along the concentration gradient.

Table 2: Mean water quality parameters after exposing *C. gariepinus* to Xylene: Diesel for 96 hours

Parameters	**Concentrations (ml/l)**						
	0	**25 ml/l**	**50 ml/l**	**100 ml/l**	**150 ml/l**	**200 ml/l**	**250 ml/l**
Temperature (^oC)	26.8±0.06[a]	27.8±0.06[a]	27.3±0.03[a]	27.3±0.06[a]	28.2±0.03[a]	28.1±0.06[a]	28.5±0.06[a]
pH	6.5±0.03[a]	6.4±0.00[a]	6.4±0.03[a]	6.3±0.03[a]	6.3±0.00[a]	6.3±0.03[a]	6.2±0.03[a]
Conductivity (µS/cm)	0.17±0.00[a]	0.17±0.01[a]	0.16±0.00[a]	0.16±0.01[a]	0.16±0.00[a]	0.12±0.01[a]	0.09±0.00[a]
Total Alkalinity (mg/l)	37.9±0.11[a]	36.8±0.03[a]	36.2±0.01[a]	35.6±0.03[a]	35.8±0.01[a]	34.7±0.00[a]	34.0±0.01[a]
Dissolved Oxygen (mg/l)	4.8±0.01[a]	4.6±0.01[a]	4.5±0.00[a]	4.5±0.00[a]	4.5±0.00[a]	4.5±0.00[a]	4.5±0.01[a]
TDS (ppm)	110.3±3.1[b]	121.3±0.3[b]	135.0±0.3[ab]	146.0±0.6[a]	159.3±0.6[a]	160.2±0.3[a]	161.7±0.3[a]
Ammonia (NH_3^-N) (ppm)	0.3±0.01[a]	0.50±0.01[a]	0.50±0.01[a]	0.60±0.03[a]	0.64±0.03[a]	0.69±0.03[a]	0.70±0.03[a]
Nitrate (NO_3^-N) (ppm)	0.3±0.00[b]	0.52±0.07[b]	0.57±0.03[b]	0.62±0.03[ba]	0.66±0.06[b]	0.76±0.06[a]	0.80±0.05[a]
Total Hardness (mg/l)	61±0.01[b]	96±0.03[ba]	101±0.01[a]	105±0.01[a]	111±0.01[a]	118±0.07[a]	121±0.03[a]

*Means with the same superscript across the rows are not significantly different

**Means with different superscripts across the rows are significantly different.

*** {TDS (ppm) = Total Dissolved solid}

3.3. Behavioural Response

The behavioral responses of the test fish were observed at 24-96 h of exposure (Tables 3 and 4). Normal behavioral responses were observed in the control fish group. Fish exposed to 25ml/l to 50 mg/l showed normal behavior from 24 to 48 hours but afterward, the fish that were alert stopped swimming and remained static for a while in response to the sudden changes in the surrounding environment.

Generally, fish exposed to higher concentrations such as 100ml/l to 250ml/l of the test chemicals showed progressive hyperventilation and abnormal behavior such as gulping of air, an erratic swimming movement, very fast swimming, jumping, and displaying vigorous jerky movement suffocation and loss of reflex. A faster operculum and tail beat movement was also observed with Spiraling. The behavioral responses increased significantly ($P<0.05$) with an increase in concentration per time as compared to the control group of fish.

Table 3: Behavioural response of *C. gariepinus* after 24 hours exposure

Behavioural response	**0(ml/l)**	**25(ml/l)**	**50(ml/l)**	**100(ml/l)**	**150(ml/l)**	**200(ml/l)**	**250(ml/l)**
Hyperventilation	-	-	-	+	+	++	+++
Erratic swimming	-	-	-	+	++	++	+++
Spiraling	-	-	-	+	++	+++	+++
Loss of reflex	-	-	+	++	++	+++	+++
Suffocation	-	-	+	++	+++	+++	+++

None-, mild+, moderate ++, strong +++. stronger ++++

Table 4: Behavioural response of *C. gariepinus*to after 96 hours exposure

Behavioural response	**0(ml/l)**	**25(ml/l)**	**50(ml/l)**	**100(ml/l)**	**150(ml/l)**	**200(ml/l)**	**250(ml/l)**
Hyperventilation	-	-	+	++	+++	++++	++++
Erratic swimming	-	-	+	++	+++	++++	++++
Spiraling	-	-	+	++	+++	++++	++++
Loss of reflex	-	-	+	++	+++	++++	++++
Suffocation	-	-	+	++	+++	++++	++++

None-, mild+, moderate ++, strong +++. stronger ++++

3.4. Opercular Beat Frequency (OBF) and Tail fin beat frequency (TBF) of *C. gariepinus*

The values of the opercular beat frequency were more responsive to the Xylene: Diesel at a 50%:50% ratio than tail beat (Tables 5 and 6). However, the responses were directly dependent on the concentration of the toxicant for the OBF and TBF**.**

3.4.1. Opercular Beat Frequency (OBF) for 96 hours of exposure

The result of Opercular Beat Frequency (Table 5) for *C. gariepinus* exposed to the toxicant for 96hours showed a statistically significant difference ($P<0.05$) in the opercular beat frequency count among the treatment means and control. The control (0ml/l) recorded the least opercula beat frequency (51.0±0.3 beats per minute) and the maximum opercula beat frequency value (79.6±0.9 beats per minute) was observed at 250ml/l exposed concentration of the toxicant. The fish in the different treatment groups showed a progressive stressed movement with time before death. They displayed an initial erratic movement; rapid opercula beat and increased mucus secretion on their skins and gills. There was also an increase in the opercula beat frequency from 24 to 48 hours and a decrease from 72 to 96 hou before death. The OBF rate decreased significantly (P<0.05) with an increase in time for the exposure groups unlike those in the control group.

Table 5: Mean Opercular Beat Frequency (OBF) of *C. gariepinus* exposed from 24 to 96 hours

Acute concentration (ml/l)	OBF (beat/min)			
	24hrs	**48hrs**	**72hrs**	**96hrs**
0	51.7±0.3^{g}	51.7±0.3^{g}	52.3±0.3^{f}	51.6±0.3^{g}
25	55.7±0.3^{f}	57.3±0.3^{f}	51.7±0.9^{f}	47.3±0.9^{f}
50	63.3±0.3^{e}	65.0±0.6^{e}	59.7±1.2^{e}	55.7±1.5^{e}
100	68.3±0.3^{d}	70.7±0.3^{d}	67.0±0.6^{d}	63.7±0.9^{d}
150	76.3±0.9^{c}	79.3±0.9^{c}	74.7±0.7^{c}	70.0±1.5^{c}
200	82.0±0.6^{b}	83.3±0.3^{b}	77.7±0.9^{b}	74.3±0.9^{b}
250	88.7±0.3^{a}	91.0±0.6^{a}	85.0±0.6^{a}	79.3±0.9^{a}

-Means with the same superscript down the column are not significantly different
-Means with different superscripts down the column are significantly different.
-Duncan′s Multiple Range Test at a 5% level of significance (P<0.05).

3.4.2. Tail fin beat frequency (TBF) for 96hours of exposure

The result in Table 6 shows the values for the tail beat frequency of *C. gariepinus* as 32.0±0.6, 31.0±0.6, 35.0±0.3, 37.3±0.9, 43.3±0.9, 45.0±0.6and 47.0±0.6beats per minute for the fish exposed to control 25ml/l, 50ml/l, 100ml/l, 150ml/l, 200ml/l and 250ml/l of Xylene: Diesel respectively for 96hours. These values showed a significant difference (P<0.05) between the exposed group and the control after 96 hours. The TBF of *C. gariepinus* decreased with time (24hrs >48hrs >72hrs >96hrs) and increased as the concentration increased (0>25>50>100>150>200 >250ml/l). There was also an observed increased tail beat frequency per minute from 24hours to 48hours and a decrease with time of exposure from 72 to 96 hours and before death. There was a significant difference (P<0.05) in the tail beat frequency in the different concentrations and the control.

Table 6: Mean Tail Beat Frequency (TBF) of *C. gariepinus* exposed from 24 to 96hours

Acute concentration (ml/l)	TBF (beat/min)			
	24hrs	**48hrs**	**72hrs**	**96hrs**
0	32.6±0.3^{f}	34.0±0.5^{f}	34.3±0.3^{f}	32.0±0.6^{e}
25	36.7±0.3^{e}	35.7±0.9^{e}	33.7±0.3^{f}	31.0±0.6^{d}
50	40.7±0.3^{d}	43.5±0.3^{d}	38.6±0.9^{e}	35.6±0.3^{bc}
100	42.0±0.5^{c}	48.0±0.6^{c}	41.0±0.6^{d}	37.3±0.9^{c}
150	48.3±0.9^{b}	54.6±0.3^{b}	43.0±0.6^{c}	43.3±0.9 ^{b}
200	51.6±0.3^{a}	62.7±0.3^{a}	54.0±0.6$^{\mathbf{b}}$	45.0±0.6^{b}
250	53.0±0.6^{a}	61.6±0.3^{a}	51.3±0.9^{a}	47.0±0.6^{a}

-Means with the same superscript down the column are not significantly different
-Means with different superscripts down the column are significantly different.
-Duncan′s Multiple Range Test at a 5% level of significance (P<0.05).

4. DISCUSSION

4.1. Water quality for 24 to 96 hours

The reported value corresponded with the temperature values recorded in other studies which generally varied between 25 ºC to 35 ºC (Alabaster and Lloyd, [17]). The values were not significantly different (P<0.05) from the control group of fish and the other concentrations. The values reported in this work were within the recommended values (30ºC) by WHO [18] and National Guideline and Standards for water quality (20ºC-33ºC) in Nigeria for aquatic life, and industrial and agricultural uses (FME, [19]). Meanwhile Manilla and Frank [20] reported a lower value of temperature (25.3ºC) in their investigation and this could be related to the different climatic conditions at that particular geographical location and period.

The pH values did not agree with the range (6.5-8.9) recommended by WHO [18]. Although the values indicated slight acidity as the concentration increased, this agreed with what was reported in similar studies (Edimeh *et al.,* Aremu *et al.,* [21,22]). Low pH is linked to increased solubility and toxicity of chemicals (Calvalho *et al.,* [23]). It is also a generally accepted fact that the concentration of toxicants influences the elevation and or reduction of test water in an experimental setup (Heijerick *et al.;* Ayotunde *et al.,* [24,25]) coupled with other activities of the fish which might have also affected the physicochemical parameters while trying to survive (Adewoye, Ogundiran*et al*., [26.27]). The mean values of conductivity obtained were lower than the values reported by Aremu *et al.* [22]). The values were not in agreement with the conductivity range of 160-1600 µS/cm of the guideline range as stipulated by SASO; GCCS [28,29] and WHO [18]. The mean alkalinity values (34 to 38mg/l) were within the permissible levels of WHO [18] (150mg/l).

There were significant slight reductions in the DO with enhanced toxicant concentration. The decrease was significant throughout the concentration gradient. The observed reduction in the DO of water may suggest that some fractions of xylene which became bio-available were sufficient to deplete the oxygen level in the water (Di Toro *et al.*, [31]).

The total dissolved solids increased with the increased concentration of toxicant. This could be linked to the differences in organic matter that remained in the different experimental water at different concentrations (Singh and Chandel, [32]). The total dissolved solid value ranged between 110 to 161ppm which fell within the acceptable limit by WHO [18] and that of the National Institute of Standard Technology (NIST, [33]). Although the values differ from that reported by Aremu*et al.* [22] who recorded a value of 1048.67 mg/L. Water with high total dissolved solids is undesirable or harmful for both human and aquatic life (Saksena and Kaushik, [34]).

The value of ammonia recorded was high compared to the acceptable standard (0.05ppm) of the Department of Environment (DOE, [35]) for experimental water. However, it was within the acceptable range for EPA [36] which was below 2.0 mg/l. The increase could be attributed to the fact that excreta from fish, decomposed by bacteria can produce ammonia and other ammonium compounds through the conversion of nitrogen during ammonification (ATSDR; Sylvia *et al.*, [37,38]). It is a necessary nutrient source, but high amounts of ammonia in water can be toxic to fish and other aquatic lives (Uddin *et al.,* [39]). These values were lower than that of WHO [18] (<45.0). This implied that the experimental water analysed contained low levels of oxidized organic matter which appeared in the form of soluble anions such as nitrates. Although, nitrate levels as low as 0.50ppm could result in significant growth of algae (Dowden and Bennett, [40]). However, excess levels of *nitrates* in water could create conditions that make it difficult for *aquatic* organisms to survive. The mean value was by far lower as compared to 304.6ppm reported by Perveen*et al.* [41] for similar toxic exposure. The mean values fell within the WHO [18] specification limits (500ppm) for drinking water. The observation is also in agreement with the findings of other workers in similar studies (Aremu *et al.,* Edimeh, [22,42]). Some reported studies found very low values for water hardness parameter: Kusti (Sudan) which ranged from 55.0-59.0 mg/l (Ibrahim *et al.,* [43]), in Kontagora (Nigeria) with 56.0 mg/l for dry and 49.0 mg/l for rainy seasons (Ibrahim, [44]) and in Bhopal (India), Choudhory *et al.,* [45].

4.2. Behavioral Responses Of *C. gariepinus* Exposed To The Combined Chemicals

There has been much research works on the extent of damage posed by several industrial activities such as oil spillage etc. [46] (Nwani *et al.,* 2013). The toxicity of chemicals has been reported to vary depending on species, developmental stages [47] (Bridges and Semlitsch, 2000), and testing protocols [48] (Jones *et al.,* 2009). Environmental pollution resulting from industrial effluents and other anthropogenic activities has become a global issue because of the extent of damage caused to the aquatic ecosystems and the disruption in the natural food chain Davies *et al.,* [49].

Behavioural changes in any fish species are very sensitive parameters to measure in an organism's response to stresses associated with aquatic environmental contaminants (Vanzella *et al.,* [50]). From the result, there were remarkable behavioural changes in *C. gariepinus* exposed for 96 hours, and these were increased behavioural changes with increased concentrations while the control group recorded no abnormal changes. The changes observed were hyperactivity, decreased equilibrium status, increased erratic swimming, decreased fin movement, and increased jerky movement.

The behavioral study gives a direct response of the fish to the different concentrations of combined chemicals (Xylene: Diesel). According to Radhaiah *et al.* [51], the behavioral activity of organisms represents the final integrated result of diversified biochemical and physiological processes. Behavioural changes served as ecological and physiological tools used for environmental pollution study processes (Gerhardt, [52]). The observed behavioral alterations in this study on acute concentrations of Xylene: Diesel are consistent with previous reports by Nwani*et al.,* Sharbidre *et al.,*[53,54]. Other chemicals such as cypermethrin (Ansari *et al.*,) [55], Profenofos (Pandey *et al.* [56],), and malathion (Ahmad *et al.,* [57]). The observed behavioral changes could also be attributed to the neurotoxic effect of either of the toxicants (Xylene: Diesel). The inhibition interferes with normal neurotransmission in cholinergic synapses and neuromuscular junctions of the nervous system and may affect the normal functioning of the nerves (Miron*et al*., [58]).

This study has shown that there were noticeable behavioural changes observed at the different concentrations of the chemicals (Xylene: Diesel**)** which were below those concentrations that led to mortality. Toxicity of chemicals to aquatic organisms has also been reported to be affected by dissolved oxygen, size, age, water quality, and formulations of chemicals (Pandey *et al.*, [59]) and death as reported by (Davies *et al*., [9]).

Umejuru [60] reported that an acute toxicity test of the water-soluble fraction of crude oil on juvenile crawfish (*Procambarus clarkii*) resulted in anxiety, upside-down imbalance swimming movements, gathering at the surface for breathing, and hitting the side walls of aquaria.

5. CONCLUSION

In conclusion, the results shows that the tail and operculum beat frequency of the *C. gariepinus*, which measures respiratory rate, was altered with increase in the concentration as compared to the controls. The fish exhibited an erratic swimming, sluggishness, an increase in surface activity, abnormal postures, gradual loss of equilibrium, and the spread of excess mucus all over their body surface. The different concentrations effect of these chemicals has shown a harmful effect on the biochemical and physiological activities of the fish which may have led to the observed changes in the behavioral pattern. Therefore, the use of this mixture of chemicals for remediation of surface pipelines wellbore tubular, and near-wellbore cleaning should be strongly monitored and regulated to avoid chronic aberration-related toxicity on the aquatic organisms.

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