JOURNAL OF FOOD TECHNOLOGY

MEDWELL PUBLICATIONS SINCE 2000

Behavioural Responses of *Clarias gariepinus* (Burchell 1822) Fingerlings Exposed to Varied Concentrations of Xylene and Diesel

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Keywords: Behavioural, responses, *Clarias gariepinus*, fingerlings, xylene and diesel

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Page No.: 13-20
Volume: 20, Issue 2, 2022
ISSN: 1684-8462
Journal of Food Technology

Abstract: The toxicity of the combination of xylene and diesel at 50:50 ratio on the African catfish (*Clarias gariepinus*) was investigated using static bioassays for 96 h. No statistical significance was observed from the othergroups and the control group. Physicochemical parameters after exposing *C. gariepinus* to xylene: diesel for 24 h was no significant difference ($p>0.05$) among the different concentration gradients and there was also an increase variation along concentration gradient observed after 96 h. The behavioral responses of the test fish were observed at 24-96 h of exposure. Normal behavioral responses were observed in the control. Fish exposed to 25-50 mg L^{-1} showed normal behavior from 24-48 h but afterwards, 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 100-250 mL L^{-1} 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 a vigorous jerky movement suffocation and loss of reflex. A faster opercula and tail beat movement was also observed with Spiraling. The behavioral responses increased significantly ($p<0.05$) with increase in concentration per time as compared to the control group of fish. Noticeable behavioural difference was recorded for the different concentration. An eco-friendlier chemical known LC_{50} that is within the acceptable level recommended by WHO in well simulation and cleaning for a sustainable biodiversity and healthy aquatic environment.

INTRODUCTION

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

A mixture of diesel and xylene, as an aromatic solvent, has been a common remedy for surface pipelines, wellbore tubular and especially near wellbore cleaning operations, because other means of clean up, such as heat, dispersants, pigging, scrapping, etc. cannot remove the organic materials like asphaltene or paraffin completely from near wellbore area[5]. These toxic chemicals are released into the aquatic body during the well stimulation and cleaning as organic deposits like asphaltene are removed[6].

Usually, wellbore soaking is done by a mixture of diesel and xylene to remove the organic plugs in the petroleum production system thereby imposing life threat to field staff and the environment through storage and flow backs into the waste pit. The introduction of these chemicals into the aquatic environment have resulted to modifications of the physicochemical parameters of water that have caused fish kill and also affected other aquatic organisms in the wild[7].

MATERIALS AND METHODS

Fish collection and acclimation: One hundred and twenty 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±2.3 cm and mean weight of 10.23±2.60 g; acclimated in a glass tank with an aerator to continuously oxygenate the water to laboratory conditions at a room temperature of 28±2°C in a 150 L 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 tanks was replaced with tap water from the laboratory every 48 h.

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.

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.*[8], Arbel *et al.*[9]. The test chemical was prepared, using the equation: $V_1C_1 = V_2C_2$, where; V_1C_1 = Stock solution attributes and V_2C_2 = New stock solution attributes while the Water-Soluble Faction (WSF) of the Diesel used was prepared using the standard method by Anderson *et al*.[10] and Orlu and Ogbalu[11]. The Combination of both chemicals at 50:50 Ratio was prepared using the method described by Davies *et al.*[5].

Behavioural response: Feeding was suspended 24 h before the static exposure period that lasted for 96 h. Six test concentrations of 0.0 mL L^{-1} (control) 25, 50, 100, 150, 200 and 250 mL L^{-1} were prepared, each test concentration was held in plastic aquarium tank of 15 L 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 96 h during which the behavioural changes of the fish samples were assessed by closely observing the movement of the fishes to report the follows parameters.

On exposure of fish to varied concentration of toxicant between the 24th h and 96th h, respiratory movement (operculum beat) tail fin beat frequency, loss of reflex, hyperventilation, erratic swimming suffocation or spiraling were carefully observed and mortality recorded[12].

Determination of physicochemical variables of test water: At 24th and 96th h experimental period hydrogen ion concentration (pH) temperature (°C) conductivity (μS L^{-1} cm), total dissolved solids (ppm) and total hardness (mg L^{-1}) were measured using an in-situ hand held multimeter (EZDO Multimeter Model CTS-406) while Dissolved Oxygen (DO) was measured with an in-situ Milwaukee Multimeter (Model MW600). Total alkalinity (mg L^{-1}) ammonia (NH_3-N) (ppm) nitrate (NO_3-N) (ppm) was monitored using standard procedures as described by APHA[13].

Statistical method: The results were subjected to one way Analysis of Variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS Version 23) to determine significant difference between various treatments and control. The Duncan[14] Multiple Range Test was used to separate differences among means. Differences were considered significant at ($p<0.05$).

RESULTS AND DISCUSSION

Water quality parameters after exposing *C. gariepinus* to xylene: diesel for 24 h: The mean values for physicochemical parameters after exposing *C. gariepinus* to xylene: diesel for 24 h is represented in Table 1. The highest temperature (27.8±0.05°C) of the experimental water was observed in the test concentration of 250 mL L^{-1} while the least (26.9±0.03°C) was recorded at the control (0 mL L^{-1}). There was no significant

($p>0.05$) difference observed across the concentration gradients of the test chemical after 24 h of exposure. The highest pH (6.7±0.03) was recorded at control (0 mL L^{-1}) while the least (6.2±0.01) was in the 200 mL L^{-1} and 250 mL L^{-1}. There was an observed significant difference ($p<0.05$) along the concentration gradient. Maximum value of conductivity (0.16±0.00 µS cm L^{-1}) was recorded at control, (0 mL L^{-1}) while the minimum value (0.10±0.01 µS cm L^{-1}) was at 150, 200 and 250 mL L^{-1}. There was a statistically significant ($p<0.05$) among the different concentrations. The alkalinity value was highest (38.3±0.01 mg L^{-1}) in the control (0 mL L^{-1}) and least (35.4±0.03 mg L^{-1}) in the 200 and 250 mL L^{-1} concentration. There alkalinity value decreased significantly ($p>0.05$) along concentration gradient. Dissolved oxygen was maximum (4.7±0.01 mg L^{-1}) in the control (0 mL L^{-1}) and minimum (4.4±0.01 mg L^{-1}) in 200 and 250 mL L^{-1} concentrations. A slight significant difference ($p<0.05$) was observed along the concentration gradient. The total dissolved solid value recorded, was highest (159.3±0.3 ppm) at 250 mL L^{-1} concentration and the least (107.3±0.1 ppm) at control (0 mL L^{-1}). The TDS increased significantly ($p>0.05$) along the concentration gradient. The maximum value of ammonia was recorded (0.70±0.03 ppm) in 250 mL L^{-1} concentration while the minimum value was recorded (0.3±0.01 ppm) in the control (0 ml L^{-1}). There was a significant increase ($p>0.05$) along the concentration gradient. Nitrate was highest (0.68±0.05 ppm) in 250 mL L^{-1} concentrations and least (0.3±0.00 ppm) in the control (0 mL L^{-1}). There was significant difference ($p>0.05$) along the different concentrations. The maximum value for the experimental water hardness was (119±0.03 mg L^{-1}) in 250 mL L^{-1} concentrations while the minimum was (63±0.01 mg L^{-1}) in the control (0 mL L^{-1}). The values increased significantly ($p>0.05$) along the concentration gradient.

Water quality parameters after exposing *C. gariepinus*to xylene: diesel for 96 h: The mean physicochemical parameters of the experimental water after exposing *C. gariepinus*to xylene: diesel for 96 h is presented in Table 2. The maximum value for temperature (28.5±0.06°C) was observed in 250 mL L^{-1} concentration and the minimum value (26.8±0.06°C) was recorded at the control (0 mL L^{-1}). 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 (0 mL L^{-1}) and the least value (6.2±0.03) was recorded at 250 mL L^{-1}. There was no statistically significant difference ($p>0.05$) among the different concentration gradients. The maximum value for conductivity (0.17±0.00 µS cm L^{-1}) was recorded at control 0 mL L^{-1} while the minimum value (0.09±0.00 µS cm L^{-1}) was recorded at the 250 mL L^{-1}. There was no significant difference ($p>0.05$) among the different concentrations. The total alkalinity value was highest (37.9±0.11 mg L^{-1}) in the control (0 mL L^{-1}) and least (34.0±0.01 mg L^{-1}) in 250 mL L^{-1} concentration. There total alkalinity values decreased significantly ($p>0.05$) along the concentration gradient. Dissolved Oxygen was maximum (4.8±0.01 mg L^{-1}) in the control (0 mL L^{-1}) and minimum (4.5±0.01 mg L^{-1}) in 50, 100, 150, 200 and 250 mL L^{-1} concentrations. No statistical significance ($p>0.05$) was observed along the concentration gradient. The total dissolved solid value was highest (161.7±0.3 ppm) at 250 mL L^{-1} concentration and the least (110.3±3.1 ppm) at control (0 mL L^{-1}). The TDS increased significantly ($p>0.05$) along the concentration gradient. The maximum value of ammonia was recorded (0.70±0.03 ppm) in 250 mL L^{-1} concentrations while the minimum value was recorded (0.3±0.01 ppm) in the control (0 mL L^{-1}). There was a significant increase ($p>0.05$) along the concentration gradient. Nitrate was highest (0.80±0.05 ppm) in 250 mL L^{-1} concentration and least (0.3±0.00 ppm) in the control (0 mL L^{-1}). There were no significant differences ($p>0.05$) along the different concentrations. The total hardness of the experimental water was highest (121±0.03 mg L^{-1}) in 250 mL L^{-1} concentration and the minimum was (61±0.01mg L^{-1}) in the control (0 mL L^{-1}). The values increased significantly ($p>0.05$) along the concentration gradient.

Behavioural response: The behavioral responses of the test fish were observed at 24-96 h of exposure (Table 3 and 4). Normal behavioral responses were observed in the control fish group. Fish exposed to 25-50 mg L^{-1} showed normal behavior from 24-48 h but afterwards, 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 100-250 mL L^{-1} 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 a vigorous jerky movement suffocation and loss of reflex. A faster opercula and tail beat movement was also observed with Spiraling. The behavioral responses increased significantly ($p<0.05$) with increase in concentration per time as compared to the control group of fish.

Opercular Beat Frequency (OBF) and Tail fin Beat Frequency (tbf) of *C. gariepinus* exposed to acute concentrations of xylene: diesel (50:50%) for 96 h: The values of the opercular beat frequency were more responsive to the xylene: diesel at 50:50% ratio than tail beat (Table 5 and 6). However, the responses were directly dependent on the concentration of the toxicant for the OBF and TBF.

Opercular Beat Frequency (OBF) after 96 h: Result of Opercular Beat Frequency (Table 5) for *C. gariepinus* exposed to the toxicant for 96 h showed a statistically significant difference ($p<0.05$) in the opercular beat frequency count among the treatment means and control. The control (0 mL L^{-1}) 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 250 mL L^{-1} 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 h and a decrease from 72 to 96 h before death. The OBF rate decreased significantly ($p<0.05$) with increase in time for the exposure groups unlike those in the control group.

Tail fin beat frequency (TBF) after 96 h: 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±0.6 and 47.0±0.6 beats per minute for the fish exposed to control 25, 50, 100, 150, 200 and 250 mL L^{-1} of xylene: diesel respectively for 96 h. These values showed significant difference ($p<0.05$) between the exposed group and the control after 96 h. The TBF of *C. gariepinus* decreased with time (24 h>48 h>72 h>96 h) and increased as the concentration increased (0>25>50>100>150>200>250 mL L^{-1}). There was also an observed increased tail beat frequency per minute from 24-48 h and a decrease with time of exposure from 72-96 h and before death. There was significant difference ($p<0.05$) in the tail beat frequency in the different concentrations and the control.

RESULTS AND DISCUSSION

Water quality for 24-96 h: The reported value corresponded with the temperature values recorded in other studies which generally varied between 25-35°C[15]. 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 (30°C) by WHO.[16] and National guideline and Standards for water quality (20°C-33°C) in Nigeria for aquatic life, industrial and agricultural uses (FME). Meanwhile[17] reported a lower value of temperature (25.3°C) 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.[16]. Although the values indicated slight acidity as the concentration increased, this agreed with what was reported in similar studies[18,19]. Low pH is linked to increased solubility and toxicity of chemicals[20]. It is also a generally acceptable fact that concentration of to xicants influences the elevation and or reduction of test water in an experimental set up[21,22] coupled with other activities of the fish which might have also affected the physicochemical parameters while trying to survive [23.24]. The mean values of conductivity obtained were lower than the values reported by Aremu[19]. The values were not in agreement with the conductivity range of 160-1600 μS cm L^{-1} of the guideline range as stipulated by WHO.[16]. The mean alkalinity values 34-38 mg L^{-1} were within the permissible levels of 150 mg L^{-1} [18]. The decrease of the values with the concentration of the toxicantscould be due to the carbonates and bicarbonates present in the water which could cause water deformation[25]. 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[26].

Total dissolved solids increased with increased concentration of toxicant. This could be linked to the differences in organic matter that remained in the different experimental water at different concentrations. The total dissolved solid value ranged between 110-161 ppm which fell within the acceptable limit by WHO.[16] and that of National Institute of Standard Technology (NIST). Although the values differ from that reported by Aremu[19] who recorded a value of 1048.67 mg L L^{-1}. Water with high total dissolved solids is undesirable or harmful for both human and aquatic life[27].

The value of ammonia recorded was high compared to the acceptable standard (0.05 ppm) of Department of Environment for experimental water. However, it was within the acceptable range for EPA which was below 2.0 mg L^{-1}. The increase could be attributed to the fact that excreta from fish, decomposed by bacteria can produce ammonia and other ammonium compounds through conversion of nitrogen during ammonification. It is a necessary nutrient source but high amounts of ammonia in water can be toxic to fish and other aquatic lives[28]. These values were lower than that of <45.0)[18]. This implied that the experimental water analyzed contained low levels of oxidized organic matter which appeared in the form of soluble anions such as nitrates. Although, nitrate levels as low as 0.50 ppm could result in significant growth of algae[29]. 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 304.6 ppm reported by Dowden and Bennett[30] for a similar toxic exposure. The mean values fell within the[18] specification limits (500 ppm) for drinking water. The observation is also in

Table 1: Mean water quality parameters after exposing *C. gariepinus* to xylene: diesel for 24 h

	Concentrations (mL L^{-1})						
Parameters	0	25	50	100	150	200	250
Temperature (°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 L^{-1})	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^{-1})	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^{-1})	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}
otal Hardness (mg L^{-1})	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}

Table 2: Mean water quality parameters after exposing *C. gariepinus*to xylene: diesel for 96 h

	Concentrations (mL L^{-1})						
Parameters	0	25	50	100	150	200	250
Temperature (°C)	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 L^{-1})	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±0.00a
Total alkalinity (mg L^{-1})	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^{-1})	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±0.00a	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^{-1})	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 same superscript across the rows are not significantly different, Means with different superscript across the rows are significantly different, {TDS (ppm) = Total dissolved solid}

Table 3: Behavioural response of *C. gariepinus*tosub-lethal concentrations of xylene: diesel after 24 h

	0	25	50	100	150	200	250
Behavioural response	(mL L^{-1})						
Hyperventilation	-	-	-	+	+	++	+++
Erratic swimming	-	-	-	+	++	++	+++
Spiraling	-	-	-	+	++	+++	+++
Loss of reflex	-	-	+	++	++	+++	+++
Suffocation	-	-	+	++	+++	+++	+++

-: None, +: Mild, ++: Moderate, +++: Strong, ++++: Stronger

Table 4: Behavioural response of *C.gariepinus*tosub-lethal concentrations of xylene: diesel after 96 h

	0	25	50	100	150	200	250
Behavioural response	(mL L^{-1})						
Hyperventilation	-	-	+	++	+++	++++	++++
Erratic swimming	-	-	+	++	+++	++++	++++
Spiraling	-	-	+	++	+++	++++	++++
Loss of reflex	-	-	+	++	+++	++++	++++
Suffocation	-	-	+	++	+++	++++	++++

-: None, +: Mild, ++: Moderate, +++: Strong, ++++: Stronger

agreement with the findings of other workers in similar studies[19]. Reported studies found very low values for water hardness parameter: Kusti(Sudan) which ranged from 55.0-59.0 mg L^{-1} in Kontagora (Nigeria) with 56.0 mg L^{-1} for dry and 49.0 mg L^{-1} for rainy seasons[31] and in Bhopal[32].

Behavioral responds: There have been many research works on the extent of damage posed by several industrial activities such as oil spillage etc[33]. Toxicity of chemicals has been reported to vary depending on species, developmental stages and test ing protocols[34,35]. Environmental pollution result in g from industrial effluents and other anthropogenic activities has become aglobal issue because of the extent of damage caused to the aquatic ecosystems and the disruption in the natural food chain[36].

Behavioural changes in any fish species are very sensitive parameters to measure in an organism's response to stresses associated with aquatic environmental contaminants[37]. From the result, there were remarkable behavioural changes in *C. gariepinus* exposed for 96 h and these were increased behavioral change with increased concentrations while the control group recorded

Table 5: Mean Opercular Beat Frequency (OBF) of *C.gariepinus* exposed to lethal concentrations of xylene: diesel (1:1) from 24-96 h

Acute concentration (mL L^{-1})	OBF (beat/min) 24 h	48 h	72 h	96 h
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 same superscript down the column are not significantly different, Means with different superscript down the column are significantly different, Duncan's Multiple Range Test at 5% level of significance (p<0.05)

Table 6: Mean Tail Beat Frequency (TBF) of *C.gariepinus* exposed to lethal concentrations of xylene: diesel (50%:50%) from 24 to 96h

Acute concentration (mL L^{-1})	TBF (beat/min) 24 h	48 h	72 h	96 h
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^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 same superscript down the column are not significantly different, Means with different superscript down the column are significantly different, Duncan's multiple range test at 5% level of significance (p<0.05)

no abnormal changes. The changes observed were hyperactivity, decreased equilibrium status, increased erratic swimming, decreased fin movement and an increased jerky movement.

The behavioral studygives direct response of the fish to the different concentrations of combined chemical (xylene: diesel). According to Vanzella *et al.*[38] and Umejuru[47] the behavioral activity of organisms represents the final integrated result of a diversified biochemical and physiological processes. Behavioural changes served as ecological and physiological tools used for environmental pollution study processes[39]. The observed behavioral alterations in the this study on acute concentrations of xylene: diesel are consistent with previous reports by Nwani[40]. Other chemicals such as cypermethrin[41] Profenofos[42] and malathion[43]. 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 may affect the normal functioning of the nerves[44].

This study have 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[45] and death as reported by Davies[7].

Acute toxicity test of 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[46].

CONCLUSION

From the current study it is concluded that the commercial formulation of the mixture of xylene and diesel as an aromatic solvent used as a common remedy for surface pipelines, wellbore tubular and especially near wellbore cleaning operations is very toxic to fish and other related organisms of the aquatic environment. The different concentrations of combined effect of xylene and diesel have the potential to impair physiological and biochemical activities of the aquatic organism which may have led to observed changes observed in behavioral pattern of *Clarias gariepinus* and consequently lead to dose dependent mortality. The use of the mixture of xylene and diesel as an aromatic solvent at the riverside and coast during rededication of surface pipelines, wellbore tubular and near wellbore cleaning operations should be strongly monitored and regulated to avoid chronic aberration related hazards such mutation or mortality of the aquatic organisms in that area.

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