Quantal Response and Histopathological Effects of Sub-lethal Concentrations of a Selected Oilfield Chemical on African Catfish (*Clarias gariepinus*)

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Abstract

The behavioural and histological effects of sublethal concentrations (0.0 ml/L, 12.8 ml/L, 25.59 ml/L, 38.39 ml/L, 51.19 ml/L, and 63.99 ml/L) of Xylene were evaluated in African Catfish (Clarias gariepinus) after 28 days of exposure. Physico-chemical parameters such as temperature, conductivity, hydrogen ion concentration (pH), total hardness, total dissolved solids, dissolved oxygen, total alkalinity, ammonia, and nitrate levels in the experiment were monitored using the standard method. Significant variations were observed in the different units except for the controlled unit. Behavioural changes were observed closely during the sublethal toxicity test using standard procedures. The bioassay experiments were repeated three times and the renewable test method was used. concentrations showed histopathological alterations in the gills and liver. Severely deformations were observed at 12.80ml/l, 38.39ml/l), 51.19ml/l, and 63.99ml/l. No form of abnormalities was observed in the fish gill and liver in the controlled unit. Progressive hyperventilation, faster operculum and tail beat movement, erratic movement, gulping of air, and spiralling. jumping, display of vigorous jerky movement suffocation, and loss of reflex were observed in C. gariepinus exposed to higher sublethal concentrations of Xylene. There was a significant dose-dependent variation in parameters in the experiment. In conclusion, xylene caused an alteration in the histopathological parameters and the behaviour of C. garienpinus. Therefore, we recommend the need for realistic regulatory measures and proper monitoring and sensitization on use to stakeholders.

Keywords: *Catfish; Histopathological; Behavioural Changes, Xylene; Aquatic organisms.*

1. INTRODUCTION

Exploration and production of oil and gas are associated with the introduction of a significant amount of chemicals and the application of solvents in stimulation activities, and these affect the aquatic ecosystem (Davies *et al.*, 2019). Chemical industries produce xylene from petroleum, which is used as a material in the chemical, plastics, and synthetic fibre industries and as an ingredient in the coating of fabrics and papers (Condie *et al.*, 2015; Fuente, *et al.*, 2013). Xylene is released in large quantities to the environment during oil exploration and exploitation such as well stimulation, cleaning and removal of organic deposits like asphaltene. Normally, wellbore soaking by a mixture of diesel and xylene is performed to remove the organic plugs in the petroleum production system. However, these chemicals impose detrimental impacts and continuous threats to field personnel and the environment via storage and its flow back into the waste pit and eventually to the aquatic environment (Kharaka and Dorsey, 2005).

These chemicals have changed the nature of water which influences fish and other aquatic organisms in the wild (Nimet *et al.*, 2020). When spillage occurs during exploration, just a fraction dissolves and becomes bioavailable to fish and biota (Kuppusamy *et al.*, 2020). When completely dissolved, they rapidly diffuse into the fish membranes, then into the blood circulatory system which conveys them to tissue cells where they metabolize into more toxic components that act on macromolecules of exposed fish (Davies *et al.*, 2019). Worried that contamination might impact the well-being and genetic composition of fish and shellfish stocks has increased in recent years (Landrigan *et al.*, 2020; Brusseau *et al.*, 2019).

The aquatic body, in most cases, is the primary recipient of these wastes and other discharges from oil and gas exploration (Lodungi *et al.*, 2016) Seiyaboh *et al.*, 2017). The discharged wastes from these activities can affect different stages of the food chain, leading to genotoxicity and distortions, which may also lead to the extinction of the biota (Okeke *et al.*, 2022; Chow *et al.*, 2021). However, sub-lethal levels of the most toxic substances have proven to be devastating to fish population density (Santos *et al.*, 2019; Madeira *et al.*, 2020). Fish are often considered the most appropriate subjects among other bio-indicators of the aquatic ecosystem (Keke *et al.*, 2020). They are susceptible to modifications in the aquatic ecosystem, especially when exposed directly and indirectly to chemicals from oil production (Rand *et al.*, 2020). Thus, fish are useful biomarkers for bio-monitoring environmental perturbations (Osman *et al.*, 2018; Manzoor *et al.*, 2021).

This paper focuses on the assessment of the sub-lethal toxicity at different concentrations of xylene on the African catfish (*Clarias gariepinus*) to simulate the likely effect of its exposure to the natural aquatic environment. This will enable us to assess its behavioural responses and haematological indices and thus recommend necessary precautionary measures to be taken towards managing the likelihood of dangerous effects it might impact on the aquatic life.

2. MATERIALS AND METHOD

2.1 Fish collection and acclimation

One hundred and twenty (180) healthy *Clarias gariepinus* juveniles with a mean length of 16.40±0.14cm and a mean weight of 11.34±5.1g was collected from the University of Port Harcourt demonstration and research farm and transported in plastic containers to the laboratory at the University of Port Harcourt. The fish were acclimated in a 150-litre capacity glass aquarium tank with an aerator to continuously oxygenate the water to laboratory conditions for 14 days at a room temperature of 28 ± 51^0C and were fed twice daily with a commercial fish feed which 45% crude protein content at 6% body weight. The water in each of the experimental tanks was replenished with fresh water from the laboratory tap every 48 hours as suggested by Davies *et al.* (2022). This juvenile stage of the test organism was chosen because of its high sensitivity to environmental stress (Davies *et al.*, 2019).

2.2 Test Chemical

Xylene was purchased from an oilfield chemical laboratory in Rivers State, Nigeria, and was stored in an ambient laboratory condition. A working stock solution was prepared from Xylene following a standard method and a test chemical was prepared, using a volumetric and analytical method as reported by (Davies *et al.*, 2019).

2.3 Selection of Test Organism for the Assay

Ten healthy and active juveniles of uniform size were selected randomly from the acclimation tanks using a hand-held scoop net and transferred carefully into different treatment units for 28 days to test for the sub-lethal effect of the Xylene (Sil *et al.*, 2010). The experiment was carried out in triplicates including the control. The test was performed using a renewal method to maintain toxicant strength and level of dissolved oxygen, minimizing changes due to metabolism by the fish during this experiment (Davies *et al.*, 2022). Feeding was suspended 24 hours before the renewable exposure period that lasted for 28days. Five test concentrations of 0.0ml/l(control), 12.8ml/l, 25.59ml/l, 38.39ml/l, 51.19ml/l, and 63.99ml/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 replicated.

2.4 Determination of the Water Quality of the Test Water.

After 28 days of exposure, the temperature (^{o}C), conductivity (µS/cm), hydrogen ion concentration (pH), total hardness (mg/l), and total dissolved solids (ppm) were measured using a handheld multi-meter (EZDO Multi-meter Model CTS-406) while the dissolved oxygen (DO) was measured with a Milwaukee Multi-meter (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 (1988) the water quality parameters.

2.5 Behavioural Responses

Each treatment group of fish was exposed for 28days during which the behavioural changes of the fish samples were assessed by closely monitoring the movement of the fish to report the carefully observed responses such as the respiratory movement (operculum beat), tail fin beat frequency, loss of reflex, hyperventilation, erratic swimming suffocation or spiralling and

recorded. The changes were observed every week from 7, 14, 21, and 28days of exposure. These were carried out using the method described by Davies and Uedeme-Naa (2022).

2.6 Determination of the Histological effect on *Clarias gariepinus*

At 28-day post-exposure, two active juveniles from each test concentration were euthanized for dissection of the gills and livers of each fish to be harvested and prepared for histological sections. The organs were fixed in Bouin's fluid and subsequently dehydrated through a graded series of ethanol (Oladokun *et al.,* 2020). Following this, they were embedded in paraffin and then manually sectioned with a microtome at 4-5 µm. The sections were dewaxed and stained with hematoxylin and eosin (H&E) and examined using a digital light microscope (Leica® DM500) (Sogbanmu *et al.,* 2019).

2.7 Statistical Method

A one-way Analysis of variance (ANOVA) was used in analysing the results with a Statistical tool software (Package for the Social Sciences; SPSS Version 23) to determine significant differences between various treatments and control. Duncan (1955) Multiple Range Test was used to separate differences between means. Differences were considered significant at ($P < 0.05$).

2.8 Ethical Approval

Ethical approval was gotten from the Office of Research and Development (Research Ethics Committee) at the University of Port Harcourt after due deliberation and consideration this research was approved.

3.0 RESULTS AND DISCUSSIONS

3.1 RESULTS

3.1.1 Physico-chemical parameters of the experimental water after 28 days of exposure.

During the experimental period physicochemical parameters, the experiment water temperature value ranged from 26.4 to 28.6°C, the highest pH value ranged from 5.5 to 6.6, conductivity was 0.18 to 0.31µS/cm, alkalinity value ranged from 34.3 to 36.7±0.02 ml/l, Dissolved Oxygen ranged from 3.2 to 4.6mg/l, Total Dissolved Solid value varied from 163.2 to 101.4ppm, ammonia varied from 0.98 to 0.52ppm, nitrate ranged from 0.38 to 0.64ppm, and total hardness varied from 67 to 105mg/l 3respectively. The values were statistically significant ($P<0.05$) across the concentration gradients.

Table 1: Mean water quality parameters after exposure to Xylene for 28 days.

Parameters	**Concentrations (ml/l)**					
	0	**12.80**	**25.59**	**38.39**	**51.19**	**63.99**
Tempt (^oC)	26.4±0.02^a	26.9±0.15^a	27.2±0.03^a	27.4±0.6 ^{a}	28.1±0.03^a	28.6±0.01^a
pH	6.6±0.01^a	6.6±0.01^a	6.2±0.03^a	6.1±0.06^a	5.8±0.03^b	5.5±0.03 ^{b}
Conductivity (µS/cm)	0.18±0.01^a	0.18±0.01^a	0.21±0.00^{ab}	0.26±0.01^b	0.28±0.01^{bc}	0.31±0.01^c
Total Alkalinity (mg/l)	36.7±0.02^a	35.7±0.03^a	33.8±0.03^{ab}	37.2±0.02^a	36.5±0.01^a	34.3±0.02^a

DO (mg/l)	4.6 ± 0.02^{a}	4.3 ± 0.01^{a}	4.0 ± 0.02^{a}	3.8 ± 0.09^{ab}	3.6 ± 0.02^{b}	3.2 ± 0.02^{b}
TDS (ppm)	101 ± 0.03^{c}	113.7 ± 0.01^{bc}	127.4 ± 0.04^{b}	153.2 ± 0.13^{ab}	158.9 ± 0.31^{a}	163.2 ± 0.20^{a}
Ammonia (NH_3-N) (ppm)	0.52 ± 0.02^{c}	0.56 ± 0.02^{c}	0.63 ± 0.03^{b}	0.88 ± 0.02^{ab}	0.95 ± 0.01^{a}	0.98 ± 0.02^{a}
Nitrate (NO_3-N) (ppm)	0.38 ± 0.01^{a}	0.41 ± 0.02^{a}	0.45 ± 0.02^{a}	0.49 ± 0.02^{a}	0.56 ± 0.01^{a}	0.64 ± 0.01^{a}
Total Hardness (mg/l)	67 ± 0.01^{c}	79 ± 0.02^{bc}	86 ± 0.03^{b}	91 ± 0.01^{ab}	98 ± 0.02^{ab}	105 ± 0.03^{a}

Means with the same superscript across the rows are not significantly different at ($P < 0.05$)
Means with different superscripts across the rows are significantly different at ($P < 0.05$)
{DO =Dissolved Oxygen (mg/l), TDS (ppm) = Total Dissolved solid}

3.1.2 Behavioural Response

The behavioural responses of the test fish were observed from 7 days to 28 days (Tables 3 and 6). Normal behaviour was observed in the controlled group. Fish exposed to 12.80 ml/l to 63.99 ml/l
showed normal behaviour from 7 days to 28 Days 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 51.19(ml/l) to 63.99ml/l of the chemicals showed progressive hyperventilation and abnormal behaviour such as very fast swimming, an erratic swimming movement, gulping of air, jumping, and displaying vigorous jerky movement suffocation and loss of reflex. A faster operculum and tail beat movement was also observed with spiralling. The behavioural responses increased significantly ($P<0.05$) with an increase in concentration per time as compared to the control group of fish.

Table 2: Behavioural response of *C. gariepinus* after 7 Days of exposure to Xylene.

Behavioural response	**0(ml/l)**	**12.80(ml/l)**	**25.59(ml/l)**	**38.39(ml/l)**	**51.19(ml/l)**	**63.99(ml/l)**
Hyperventilation	-	+	-	+	+	+
Erratic Swimming	-	-	-	+	+	+
Spiralling	-	-	-	+	+	+
Hyporeflexia	-	-	+	+	+	+
Suffocation	-	-	+	+	+	+

Key: + = present, - = Not present

Table 3: Behavioural response of *C. gariepinus* after 14 Days of exposure to Xylene.

Behavioural response	**0(ml/l)**	**12.80(ml/l)**	**25.59(ml/l)**	**38.39(ml/l)**	**51.19(ml/l)**	**63.99(ml/l)**

Hyperventilation	-	-	+	+	+	+
Erratic Swimming	-	-	+	+	+	+
Spiralling	-	-	+	+	+	+
Hyporeflexia	-	-	+	+	+	+
Suffocation	-	-	+	+	+	+

Key: + = present, - = Not present

Table 4: Behavioural response of *C. gariepinus*to after 21 Days of exposure to Xylene.

Behavioural response	**0(ml/l)**	**12.80(ml/l)**	**25.59(ml/l)**	**38.39(ml/l)**	**51.19(ml/l)**	**63.99(ml/l)**
Hyperventilation	-	+	+	+	+	+
Erratic swimming	-	+	+	+	+	+
Spiralling	-	+	+	+	+	+
Hyporeflexia	-	+	+	+	+	+
Suffocation	-	+	+	+	+	+

Key: + = present, - = Not present

Table 5: Behavioural response of *C. gariepinus*to after 28 Days of exposure to Xylene.

Behavioural response	**0(ml/l)**	**12.80(ml/l)**	**25.59(ml/l)**	**38.39(ml/l)**	**51.19(ml/l)**	**63.99(ml/l)**
Hyperventilation	-	+	+	+	++	++
Erratic swimming	-	+	+	+	++	++
Spiralling	-	+	+	+	++	++
Hyporeflexia	-	+	+	+	++	++
Suffocation	-	+	+	+	++	++

Key: + = present, - = Not present

3.1.3 Opercular Beat Frequency (OBF) of *C. gariepinus* exposed to Xylene for 28 days

The result in table 2 shows that the opercular beat frequency of the fishes in the control unit was 53.0± 0.3 beats per minute after the 28 days and 54.2±.06 to 58.6±0.3 beats per minute were observed in the group exposed to 12.80ml/l while 68.0±0.6 to 79.0±0.6 beats was recorded in the 63.99ml/l group which was the highest concentration. There was a significant increase ($P<0.05$) in the OBF recorded from the 7^{th} day to 28days and the same increase was observed across different concentration gradients. There was a significant difference ($P>0.05$) in the concentrations and control.

Table 6: Opercular Beat Frequency (OBF) of *C. gariepinus* Exposed to Xylene for 28 days.

Concentration (ml/l)	OBF (beat/min)			
	7Days	14Days	21Days	28Days
0	53.0±0.3^{d}	53.0±0.3^{e}	53.0±0.3^{e}	53.0±0.3^{e}
12.80	54.2±.06^{d}	57.8±0.3^{d}	58.6±0.3^{d}	58.3±0.6^{d}
25.59	57.3±0.3^{c}	58.0±0.6^{d}	61.0±0.3^{cd}	65.0±0.6^{cd}
38.39	62.0±0.6^{b}	64.0±0.6^{c}	65.6±0.3^{c}	67.0±0.9^{c}
51.19	67.3±0.9^{ab}	69.0±0.9^{ab}	72.0±0.6^{ab}	71.0±0.3^{b}
63.99	68.0±0.6^{a}	71.0±0.6^{a}	75.0±0.3^{a}	79.0±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.

3.1.4 Tail Beat Frequency (TBF) of *C. gariepinus* Exposed to Xylene for 28days

The results for the tail beat frequency (TBF) of *C. gariepinus* exposed to Sub-lethal (SL) test concentrations of xylene for 28 days gave range values of 33.2±0.3 to 34.0±0.6, 34.4±0.3 to 39.0±0.6, 37.5±0.3 to 43.0±0.6, 41.0±0.6 to 46.0±0.6, 48.3±0.3 to 48.4±1.2, 52.5±1.3 to 54.8±0.3 beats per minute for the group of fish exposed to control (0), 12.80, 25.59, 38.39, 51.19, 63.99ml/l of xylene respectively (Table 4.17). The values were significantly different ($P<0.05$) from the control group. A significant increase ($P<0.05$) was also observed in the TBF for the fish exposed to the different concentration gradients (0>12.80> 25.59> 38.39> 51.19> 63.99ml/l) from the 7th to the 28th day.

Table 7: Tail Beat Frequency (TBF) of *C. gariepinus* exposed to Xylene for 28 days.

Concentration (ml/l)	TBF (beat/min)			
	7Days	14Days	21Days	28Days
0	33.2±0.3^{e}	33.8±0.3^{e}	33.6±0.3^{f}	34.0±0.6^{e}
12.80	34.4±0.3^{e}	34.8±0.9^{e}	38.0±1.0^{e}	39.0±0.6^{d}
25.59	37.5±0.3^{d}	38.3±0.3^{d}	41.0±0.6^{d}	43.0±0.6^{cd}
38.39	41.0±0.6^{c}	43.0±0.6^{c}	46.0±0.6^{c}	45.7±0.3^{c}
51.19	48.3±0.3^{b}	49.8±0.3^{b}	46.7±1.2^{b}	48.4±1.2^{b}
63.99	52.5±1.3^{a}	54.7±0.3^{a}	55.2±0.6 ^{a}	54.8±0.3^{a}

Means with the same superscript down the column are not significantly different
Means with different superscripts down the column are significantly different.

3.1.5 Histopathological alterations observed in *C. gariepinus* Exposed to Xylene for 28days

The histological sections of the gills of *Clarias gariepinus* were exposed to sublethal concentrations of Xylene for 28 days. In the Controls (a), the histologic section of the gill filament shows preservation of the cartilaginous support of the primary lamellae, with long primary lamellae and comb-like secondary lamellae projecting from both sides of each primary lamella indicating normal gill (X400). At 12.80ml/l (b) Concentration, the histologic sections of gills show shortening and blunt secondary lamellae but the preservation of the primary lamellae indicates mild lamellar necrosis (X400); At the concentration of 25.59ml/l (c), the histologic sections of tissue show necrosis and destruction of both primary and secondary lamellae indicating severe lamellar necrosis (X400); At the concentration of 38.39ml/l (d), the histologic section of gill filament shows necrosis and destruction of both primary and secondary lamellae

indicating severe lamellar necrosis (X400); At the concentration of 51.19ml/l (e), the histologic section of gill filament shows severe secondary lamella necrosis (SLN), epithelial lifting (EL), mild deformity of the secondary lamella (DSL), At the concentration of 63.99ml/l (f) the histologic section of gill filament also shows severe secondary lamella necrosis (SLN), epithelial lifting (EL), severe deformity of the secondary lamella (DSL).

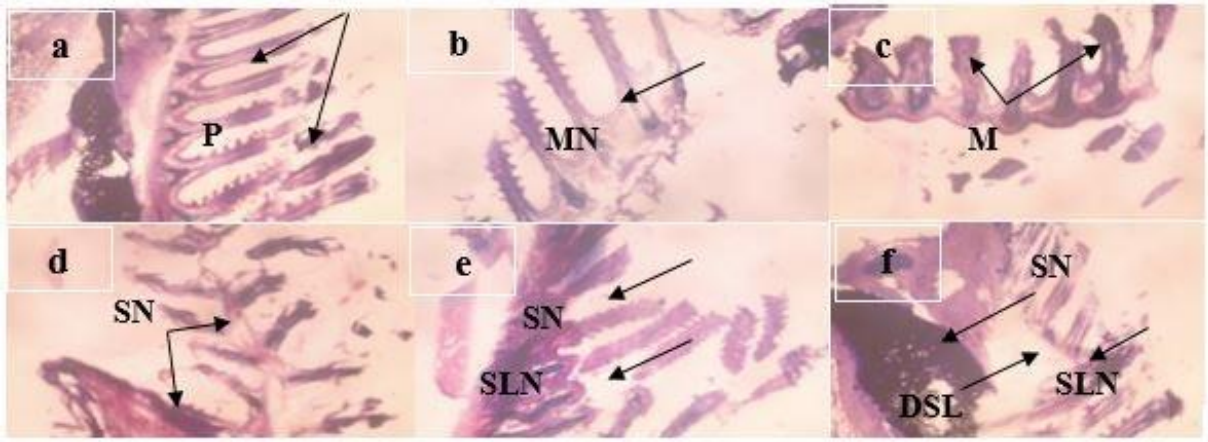

Figure 1: Photomicrography of histological sections showing the gills of *Clarias gariepinus* exposed to sublethal concentrations of Xylene over 28 days at X400 magnification.
Notes: P = Primary gill filaments; S = Secondary lamellae; MN = Mild Necrosis; SN = Severe Necrosis; SLN = secondary lamella necrosis.

Figure 2 Photomicrography of histological sections of the liver of *Clarias gariepinus* exposed to sublethal concentrations of Xylene for 28 days: in Control (group a), the histologic sections of the liver show parallel radially arranged plates of hepatocytes, and no form of abnormalities was observed which is an indicator for a normal liver. At 12.80ml/l (group b), the concentration and vascular congestion were observed. Meanwhile, the group with 25.59ml/l (c) concentration showed cytoplasmic vacuolation and malignancy in the tissue of the liver. Whereas, at the concentration of 38.39ml/l (d) Necrosis and Vascular congestion were seen. At the concentration of 51.19ml/l (e), Pigment, wrinkling of oocyte membrane and cellular degeneration were observed. While the concentration of 63.99ml/l (f) shows pigment, cellular degeneration and inflammatory cells.

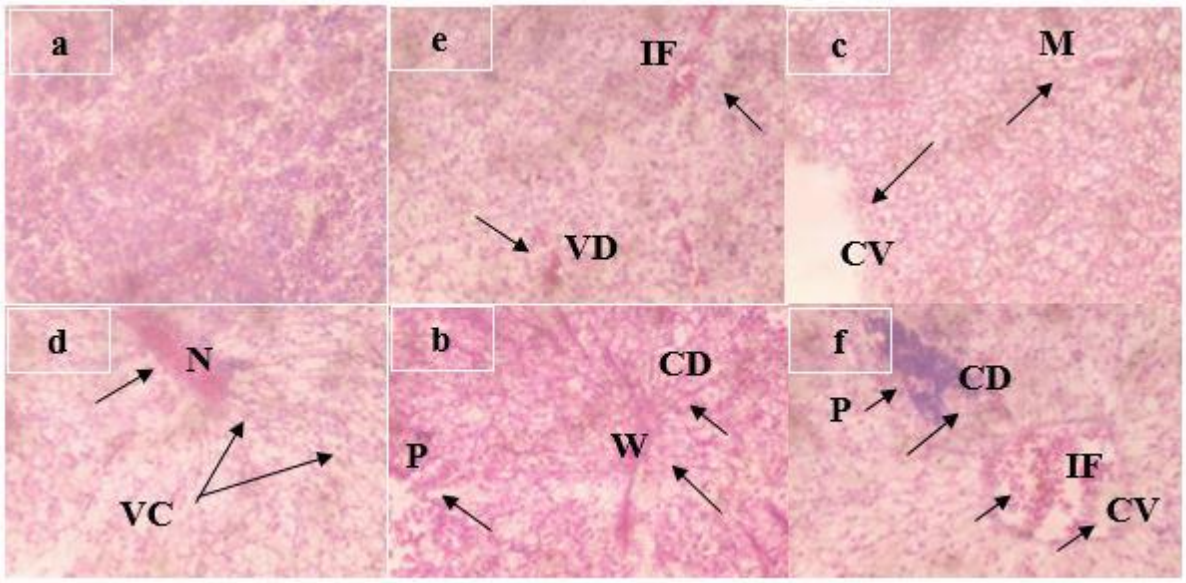

Figure 2 Photomicrography of histological sections showing the Liver of *Clarias gariepinus* exposed to sublethal concentrations of Xylene over 28 days.
Notes: CD = cellular degeneration, **IF** = Inflammatory cells, **VC** = Vascular congestion, **VD** = Vascular degeneration, **W** = Wrinkling of oocyte membrane and **CV** = Cytoplasmic vacuolation, **M**= Malignancy **N=** Necrosis, **P**=Pigment, **CD**= cellular degeneration, Haematoxylin & Eosin stain, x400.

Table 8: Histological changes of *C. gariepinus* of liver and gills exposed to Xylene at different concentrations.

TRT (ml/I)	Duration	Organs	Lesion	Malignancy	Necrosis	Inflammation	Pigment	Cellular degeneration	Inclusion bodies
0	**28**	Gill	-	-	-	-	-	-	-
		Liver	-	-	-	-	-	-	-
12.8	**28**	Gill	+	+	+	+	-	-	-
		Liver	+	+	+	+	-	-	-
25.59	**28**	Gill	+	+	+	++	++	-	-
		Liver	+	++	++	++	++	+	-
38.39	**28**	Gill	++	++	++	+++	+++	++	-
		Liver	++	++	++	+++	+++	+++	+
51.19	**28**	Gill	+++	+++	+++	+++	++++	++++	++
		Liver	+++	+++	+++	+++	++++	++++	++
63.99	**28**	Gill	++++	++++	++++	++++	++++	++++	+++
		Liver	++++	++++	++++	++++	++++	++++	+++

Legend: - (absent), + (present), + + (mild), + + + (severe).
***TRT=** treatments.

3.2 DISCUSSION

3.2.1 Physico-chemical Parameters

The results from mean water quality parameters measured in the tanks during the 28 day experimental period showed that the non-targeted aquatic organisms (*C. gariepinus*) used for the experiment may be at risk of adverse changes in the physio-chemical parameters and biological effects from exposure to Xylene from non-point sources. The physicochemical parameters as reported in this study are very important indicators of the state and health of aquatic lives and their environment (Audu *et al.,* 2014, Chindah *et al.,* 2004) In this study, the parameters were observed to be significantly different from that in the control unit. The values of the physicochemical parameters of the water were not within the ranges recommended by (WHO,2000) and Nigeria Industrial Standard Technology ((NIST, 2007). The fact that the water quality parameters recommended for the test fish did not differ statistically from the control is proof that the changes observed in fish were toxicant-induced. although there was a significant difference in the values of the parameters along the concentration gradient, the variation could be due to pollutants, impurities, and toxicants capable of changing the different physicochemical parameters (WHO 2006). Gbem *et al.,* 2001 Idodo-Umeh *et al.,* 2002)

3.2.2 Behavioural Response

The behavioural response of *Clarias gariepinus* was conducted every week for 28days during the sublethal assay and the behavioural responses of the test fish were observed weekly from the 7^{th} to the 28^{th} day of exposure. In the control groups, *C. gariepinus* showed normal behaviour during the test period while some changes in behaviour began after the 7^{th} day. Contrary to the control tank, loss of equilibrium, slowing their motion, and spending at the bottom were observed in all the concentration gradients as observed weekly for the 28days. The fishes were repetitively swimming sideways, with increased operculum movement and gaping their mouth for air while reactions like hanging vertically in the water, lordosis, and motionlessness were observed. The fish exposed to the control showed normal behaviour whereas those in the exposed concentration units were observed to be alert, stopped swimming and remained static in a position after 7 days which may be a response to the sudden changes in the surrounding environment (Fewtrell and McCauley, 2012)

The fish samples exposed to higher concentrations of xylene showed abnormal behavioural responses while trying to avoid the experimental water by moving very fast, displaying erratic and jumping vigorously with jerky movements, which is a confirmation that the fish showed a direct response of the fish to the toxicant (Davies *et al.,* 2022). A faster opercula bite movement, surfacing, hyperexcitation, and gulping of air were also observed this agrees with Inyinbor *et al.* (2018) who stated that apart from natural contamination, man's impact on the aquatic environment poses more serious damage than man has intended. Similar toxic behaviour of an aquatic organism has also been reported by Mahmood *et al.* (2016). According to Laughlin *et al.* (2017), the behavioural activity of organisms represents the final cohesive result of diversified physiological and biochemical alterations. The observed behavioural alterations in this study agree with previous reports by (Pulgar *et al.,* 2019; Xia *et al.,* 2018; Wang *et al.,* 2021; Boyle *et al.,* 2020).

The behavioural changes observed may be attributed to the neurotoxic effect of the chemical by its inhibition (Ogungbemi *et al.,* 2019). This inhibition affects the normal neurotransmission of cholinergic synapses and neuromuscular junctions of the nervous system, thereby affecting the normal functioning of the nerves (Ogungbem *et al.,* 2020). The impaired physiological and

biochemical activities of the organism may have led to the observed changes in behavioural patterns which may cause dose-dependent mortality (Depledge 2020). The study showed that Xylene had a negative effect on the behaviour of the test fish and could be a general adaptation syndrome (Ogungbemi *et al.,* 2020). The restlessness of the fishes could be attributed to the release of the stress hormones to initiate a series of physiological changes as the hormone control system began to compensate (Bryant *et al.,* 2022). Significant changes in opercula beat frequency and tail beat frequency reported in this study propose that the *C. gariepinus* were stressed by the test chemical which may have caused the fish to exhibit avoidance syndrome as earlier reported by Sharma *et al.* (2019) and Bailey *et al.* (2016).

Test fish (*C. gariepinus*) exposed to higher concentrations of the toxicant swam faster to escape it. This may have increased their tail fin beat frequency and opercula beat frequency to gain more metabolic oxygen (Cheema *et al.*, 2018). Camarillo *et al.* (2020) however reported that higher performance and ventilation in extremophile fish caused by toxic exposure could have led to nervous dysfunction and toxicity in the test fish in this study. *C. gariepinus* in this study ultimately became exhausted, thereby resulting in the drop of the opercula and tail beat frequency. These effects of exhaustion and the direct effects of the toxicant on the fish may have resulted in subsequent death observed in the end (Bailey *et al.*, 2016). Bryant *et al.* (2022) noted that opercula hyperventilation had been reported for stressed fish in an unfavourable environment.

3.2.3 Histopathological Changes in The Liver

The liver is where metabolism takes place (Stoyanova *et al.*, 2020). The liver normally develops lesions and other histological changes as a result of toxicant exposure and build-up. It is crucial to understand how contaminants are transformed biochemically during the toxification process (Wang *et al.*, 2020). Additionally, contaminants may have an impact on the organ's strong metabolic ability (Authman *et al.,* 2015). The liver of the fish (*C. gariepinus*) was exposed to sub-lethal concentrations of Xylene for 28 days compared to the control showed varying degrees of alteration and degeneration in the present study. These alterations may have been induced by the sub-lethal concentration effect of the toxicant in the liver which may have led to hepatocytes such as necrosis, vacuolization, and nuclear condensation as reported by Wolf and Wheeler, (2018). From the results, the fish could not regenerate new liver cells and this could be attributed to the toxic alteration that has led to necrosis and vascular degeneration (Wolf and Wheeler, 2018). The degeneration of hepatocytes may be due to the direct effects of the varying concentrations of toxic pollutants on hepatocytes as found in Xylene toxicity (Maurya *et al.,* 2019).

The liver fish also had a vacuolated cell showing evidence of fatty degeneration. Necrosis of certain portions of liver tissue may probably result from the excessive work required by fish to get rid of the toxicant from its body during process of detoxification and a similar report observed by Stoyanova *et al.* (2020). The degenerative effect observed in the liver may have led to the inability of the fish to regenerate new liver cells thereby leading to necrosis (Mohammod *et al.,* 2015) because the liver has an important detoxicate role in endogenous waste products as well as externally derived such toxicity (Li *et al.,* 2016).

Histopathological effects of xylene on the liver of *C. gariepinus* showed some form of a lesion, malignancy necrosis, cellular degeneration, pigment, and inflammation in the liver after 28 days

of exposure. This also agrees with Renu *et al.,* 2021) who reported impairment and physiological dysfunction observed as a molecular mechanism of heavy metals-induced hepatotoxicity. At higher concentrations, there was fatty degenerative necrosis and severe oedema. This agrees with Mohammad *et al.* (2015) who reported a similar alteration in the liver of silver barbs after chronic exposure to quinalphos where liver showed disorganized hepatic cords, coagulative necrosis of liver hepatocytes, and severe oedema. The degeneration and necrosis of liver hepatocytes and the hepatic tissue may be due to the severe effect of the increase in the concentrations of the toxicant (Eriegha *et al.,* 2019; Saha *et al.,* 2021). Sani *et al.* (2020) reported that some fish exposed to toxicants cause some forms of abnormalities such as irregularly shaped hepatocytes, cytoplasmic vacuolation, and nuclei positioned laterally revealing evidence of cytoplasmic, nuclear vacuolation, nuclear degeneration, and focal necrosis in the fish livers. These changes, which have been described as being more severe, are typically connected to the fish's exposure to higher concentrations of toxins (Audu *et al.,* 2020; Flores-Lopes *et al.,* 2020).

The contaminant effect of xylene also affected the proper function of the liver and its optimal metabolic capacity (Vijayakumar *et al.,* 2017).

Histological alterations observed in this study agree with (Singh et *al.,* 2019) who noticed different toxicological changes in the liver of fish after exposure to different toxicants. The congestion of the central vein in the fish liver was also reported by (Sula *et al.,* 2020; Singh *et al.,* 2019). The most frequently encountered alterations in the liver of fish exposed to sub-lethal concentrations of xylene in this study are those of vascular degeneration and necrosis. The necrosis of the liver tissues and vascular degeneration in this study may be attributed to the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver (Adewumi *et al.,* 2018; Shah and Parveen, 2022). Generally, fish hepatic alterations can be classified into different levels and units according to their relative significance, most especially as indicators of toxicants (Tashla *et al.,* 2018).

3.2.4 Histopathological Changes in The Gills

The gill of *Clarias gariepinus* is made up of the primary lamellae which are arranged in a double row and protrusive on the lateral sides of which are a series of alternately arranged secondary lamellae (Mazumder, 2022). Among the gills are a cartilaginous supporting rod and blood vessels with touches of sinusoidal blood space. This study shows no histopathological changes in the controlled group without the toxicant. The gill is the primary organ of first contact with the pollutant. The gills exposed to the different gradients of Xylene were observed to be swollen in comparison to the control fish because of the effect of the toxicant which is a sign of hyperplasia of the gill epithelial cells (Ullah *et al.,* 2019; Abdel-Tawwab *et al.,* 2019). The histology results also show a pathological change in the gills of the fish after exposure to sub-lethal concentrations of xylene for 28 days. These changes observed after exposure of the gills to the chemical include fragmentation, loss of coracobrachialis bones, loss of secondary lamellae, and elongated primary lamellae.

The histopathological changes in the gills, of *C. gariepinus* observed in this study showed severe damage to the gills which is an indication of a toxic effect of the different sublethal concentrations of xylene, which may have led to an impairment in the gaseous exchange efficiency of the gills Oedematous of the lamella and hyperplasia (Okey *et al.,* 2021). This study

agrees with the work by Mishra *et al.* (2008) who also observed similar alterations in the gill and stated that this can provide a quick means of detection of the effects of toxicants. The pathological changes observed in the gills could have resulted in the inability of the gills to function properly (Olojo *et al.*, 2005). The sublethal exposure also showed marked degenerative changes in the gill's structures and tissues. The gill of the fish epithelial hyperplasia, lamellar fusion, epithelial lifting, necrosis, desquamation, aneurism, and curling of secondary lamellae were observed with excessive mucous secretion as the concentration increased. A similar toxic response was observed in other fish species exposed to other toxicants (Olojo *et al.*, 2005; Figueiredo-Fernandes *et al.*, 2007).

The fish gill is a critical organ for the respiratory, therefore, the osmoregulatory functions and injuries observed in gill tissues reported in this study may reduce oxygen consumption and disrupt the osmoregulatory functions of the fish (Abdelkhalek *et al.*, 2015; Mishra and Mohanty, 2008). Xylene is highly soluble in water and therefore can easily penetrate the biological membranes thereby causing cellular degeneration by inducing oxidative stress (Asadi *et al.*, 2017); Engwa, (2018). Therefore, the histopathological alterations in the gill tissues of *Clarias gariepinus* were diverse and the degeneration observed was comparatively more obvious in gills at the higher concentrations. This could be attributed to the fact that gills exposed to direct contact with toxic chemicals will eventually cause alteration (Braz-Mota *et al.*, 2018); Amin and Hashem, 2012). The observed abnormal behaviour and altered histopathology of vital organs demonstrate the severity and adverse effects of xylene. Therefore, the sublethal exposure may have caused severe physiological and anatomical alterations which eventually will lead to the death of fish (Ullah *et al.*, 2018; Hinton *et al.*, 2018). As the concentrations of the chemical increased, the fusion of secondary lamellae, oedema, necrosis, and desquamation of lamellar epithelium was observed.

The observed fusion of secondary lamellae, which may be due proliferation of mucous and epithelial cells, may be considered a defence mechanism for chemical exposure rather than a direct effect of the toxicants (Doherty *et al.*, 2011). The observed proliferated mucous cells were for continuous secretion of mucous, which is a mechanism that helps to protect and clean up these respiratory surfaces in facilitating the removal of trapped toxicants from them (Ojogu *et al.*, 2017). The defensive role of increased mucous secretion shrinks due to the rapid depletion of mucous cells with substantial loss of mucous after prolonged exposure, resulting in the erosion of the exposed fish's skin's superficial cells (Zulkipli *et al.*, 2021),

4.0 CONCLUSION

The toxic effects of Xylene on the behaviour and histopathology of gills and liver of *C. gariepinus* were investigated and the study shows that non-target organisms in the aquatic ecosystems like *C. gariepinus* may be at risk of toxic effects of the environmentally related sub-lethal effect of Xylene from point and/or non-point sources. Significant alterations were observed in the gills and liver. The implication is that the toxicity of Xylene is high, thus causing more damage to the aquatic environment. The study has predicted that the introduction of this chemical into water bodies could be a threat to life and their existence. Therefore, the release of Xylene in large quantities into the aquatic environment during oil exploration and exploitation should be discouraged particularly in areas close to an aquatic environment.

4.1 RECOMMENDATIONS

The study has established those behavioural responses and the histopathological effects can be effectively used as biomarkers for toxic exposure to this oilfield chemical in aquatic environment. There is therefore a need for actual and realistic regulatory measures and proper monitoring in the use of this oilfield chemical in order to reduce the hazardous effects on the aquatic environment.

COMPETING INTERESTS

The Authors declare no conflicts of interest exist and have no relevant financial or non-financial interests to disclose.

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