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Haematological and behavioral response of African catfish (*Clarias gariepinus*) (Burchell, 1822) exposed to sub-lethal concentration of xylene

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Abstract

The study was carried out to examine the haematological and behavioral response of African catfish (*Clarias gariepinus*) exposed to sub-lethal concentrations (0.0 ml/L (as control), 12.8 ml/ L, 25.59 ml/L, 38.39 ml/L, 51.19 ml/L, and 63.99 ml/L) of xylene using a renewable assay for 28 days. A total of one hundred and twenty (120) healthy *Clarias gariepinus* with a mean length of 15.20±2.3 cm and mean weight of 10.23±2.60 g was used for the experiment. Some physicochemical parameters such as temperature, conductivity, hydrogen ion concentration (pH), total hardness and total dissolved solids, dissolved oxygen, total alkalinity, ammonia and nitrate were monitored using standard procedures. These parameters were significantly different (P<0.05) across the concentration gradients with time. behavioral changes observed in *C. gariepinus* exposed to the different concentrations of xylene compared to the control includes increased erratic swimming, hyperactivity, decreased equilibrium status, increased jerky movement and decreased fin movement. There were significant dissimilarities (P<0.05) observed in haemoglobin concentration, packed cell volume, red blood cell counts, white blood cell and blood platelets of the *Clarias gariepinus* species on exposure to the toxicant from the control except for mean corpuscular volume and haemoglobin concentration PCV (24.5±0.33 to 20.8±0.06); HB (6.9±0.58 to 8.2±1.03); RBC (3.5±0.08 to 3.9±0.16); WBE (6.9±0.05 to 10.1±0.04); Platelet (168±1.45 to 214±0.05); MCHC (30.5±0.07 to 32.5±0.01); MCH (20.5±0.01 to 20.4±0.01) and MCV (59±0.07 to 62±0.02) respectively. In conclusion, xylene caused negative changes in the haematological indices and the behavioural pattern of C. *garienpinus*. Hence the need to focus efforts on ensuring a decrease in the discharge of xylene to water bodies.

Keywords: Xylene; *Clarias gariepinus*; Haematological Indices; Behavioural Response

1. Introduction

It is commonly documented that most oil exploring industries utilize various types of oilfield chemicals for well drilling operations, production enhancement, well completion and optimum recovery [1]. Xylene is among the top 30 chemicals originally produced in the United States [2]. They are produced from chemical industries, some naturally occur in petroleum and coal tar and are also created in tiny quantities during forest fires [3, 4]. Xylene is a useful substance used by oil and gas industries in dissolving other substances [5]. It is commonly used as a cleaning agent with other chemicals during stimulation and cleaning to remove organic deposits like asphaltene in most petroleum materials [6]. It is also used as a substance in some plastics and synthetic fibre industries and as an additive in coating [7].

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Xylene is used in mixed form in gasoline and other commercial products like paints, so people are generally exposed to it and not just to single xylene isomers [8,7]. [9] measured the mean concentration of xylene in three groundwater monitoring wells at a Shell Oil service station in California and the concentration was 2.8ppm in the water at oil spill site in northern Virginia. [10] also reported xylene emissions that occurred in the atmosphere, they observed that it had limited potential health effects because its concentration was lower than the corresponding criteria.

It's been documented that xylene exposure in humans even in acute amounts can vary the severity of symptoms expressed in humans including but not limited to as simple as Nausea, vomiting, dizziness, sleepiness, chest pain to as extreme as, convulsions, life-threatening pulmonary oedema and even death. [11]. The discharge of xylene occurs mainly in the aquatic settings and spills on drylands from its use and transportation and sometimes when it is introduced into the soil and water where it eventually vaporizes into the atmosphere [12]. Xylene may also percolate into groundwater, where it perseveres for several months [13].

In the food chain, Fish is an essential source of protein and it is heavily consumed daily with a consumption rate of about 120 g/person/day [14]. An assessment of volatile organic compounds like xylene in fishes has been reported to be a contributor to death and since the blood is an essential component for animals and humans which carries nutrients and oxygen all over the body, it's necessary to check for the effect on haematological indices of the fish [15,16].

2. Material 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 and transported in plastic containers to the Department of fisheries wet laboratory at 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 ^{0}C 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

Xylene was bought from a chemical laboratory in Port Harcourt, Rivers State and stored under ambient conditions in the laboratory. A working stock solution was prepared from Xylene following the method of [17,18]. 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.

2.3. Test Organism (African Catfish (*Clarias gariepinus*)

The fish (*C. gariepinus*) were acclimated to laboratory conditions in a 150 litres capacity glass aquarium tank for 14 days at a room temperature of 28±2^{0}C and were fed with commercial fish feed twice daily (morning and evening). During acclimation, the tank was aerated continuously (Plate 3.3). The water in each glass tank was replaced with tap water from the laboratory every 48 hours as suggested by [19] .

2.4. Selection of test organism for sub-lethal assay

Ten active and healthy fingerlings relatively of uniform size were picked randomly using a hand-held scoop net from the acclimation tanks and transferred carefully into the different treatment units for 28 days to test for the sub-lethal effect of the Xylene [20]. The treatments were in triplicates as well as the control. The test was performed using a renewal method and the exposure medium was renewed every week to maintain toxicant strength, level of dissolved oxygen, and minimize changes due to metabolism by the fish during this experiment.

2.5. Behavioural Response

Feeding was suspended 24 hours before the renewable exposure period that lasted for 28 days. Five test concentrations of 0.0 ml/l (control), 12.8 ml/l, 25.59 ml/l, 38.39 ml/l, 51.19 ml/l, and 63.99 ml/l were prepared, each test concentration was held in plastic aquarium tank of 15 litres and filled to 10 marks. 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 28days during which the behavioural changes of the fish samples were assessed by closely observing the movement of the fishes to report the following parameters; respiratory movement (operculum beat), tail fin beat frequency, loss of reflex, hyperventilation, erratic swimming suffocation or spiralling were carefully observed and recorded. The changes were observed every week from 7,14, and 21 to 28 days of the exposure period. These were carried out using the method described by [21].

2.6. Determination of physicochemical variables of test water.

After 28 days experimental period, the temperature (°C), conductivity (µS/cm), hydrogen ion concentration (pH), total hardness (mg/l) and total dissolved solids (ppm) were measured using a hand-held multimeter (EZDO Multimeter 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 [22].

2.7. Determination of haematological indices

Blood samples were collected using an insulin syringe and needle rinsed with EDTA in a microhematocrit tube sealed at one end with plasticine following the standard procedures by 23 Erhunmwunse and Ainerua (2013). The various Haematological indices such as Packed cell volume (PCV), Haemoglobin (HB), Red Blood Cells (RBC), White Blood Cell (WBC), Platelet (PLA), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Neutrophils (N), Leucocyte (L), Eosinophils (E), Monocytes (M) and Basophils (B) were determined using the method described by 24 Wedemeyer, and Yasutake (1977).

2.8. 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 [25] Multiple Range Test was used to separate differences among means. Differences were considered significant at ($P < 0.05$).

3. Results

3.1. Physicochemical parameters of the experimental water

Table 1 Mean water quality parameters after exposing *C. gariepinus* to Xylene after 28days

Parameters	Concentrations (ml/l)					
	0	**12.80**	**25.59**	**38.39**	**51.19**	**63.99**
Tempt. (°C)	26.5±0.05^{a}	26.8±0.06^{a}	26.7±0.03^{a}	26.6±0.06^{a}	26.6±0.03^{a}	26.7±0.06^{a}
pH	6.5±0.01^{a}	6.4±0.01^{a}	6.3±0.01^{a}	6.4±0.01^{a}	6.4±0.01^{a}	6.3±0.01^{a}
Conductivity (µS/cm)	0.16±0.01^{a}	0.16±0.01^{a}	0.17±0.01^{a}	0.16±0.01^{a}	0.15±0.01^{a}	0.13±0.01^{a}
Total Alkalinity (mg/l)	34.3±0.03^{a}	34.5±0.03^{a}	35.2±0.03^{a}	35.8±0.03^{a}	36.2±0.03^{a}	36.5±0.03^{a}
Dissolved Oxygen (mg/l)	4.9±0.01^{a}	4.3±0.01^{a}	4.1±0.01^{a}	3.6±0.01^{a}	3.3±0.01^{a}	3.2±0.01^{a}
TDS (ppm)	95.2±0.03^{b}	102.1±0.03^{b}	105.2±0.03^{ab}	109.1±0.03^{b}	112.3±0.03^{a}	118.2±0.03^{a}
Ammonia (NH_3^-N) (ppm)	0.3±0.01^{b}	0.41±0.01^{a}	0.44±0.01^{a}	0.52±0.01^{a}	0.53±0.01^{a}	0.58±0.01^{a}
Nitrate (NO_3^-N) (ppm)	0.3±0.05^{a}	0.42±0.05^{a}	0.43±0.05^{a}	0.48±0.06^{a}	0.51±0.05^{a}	0.59±0.06^{a}
Total Hardness (mg/l)	56±0.03^{c}	78±0.03^{b}	94±0.01^{ab}	99±0.03^{ab}	109±0.01^{a}	114±0.05^{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}

The water temperature ranged from 26.5 °C to 26.7 °C for 28 days. There was a slight variation in the values of temperature across the concentrations. No significant difference (P<0.05) was observed along the concentration gradients and the control. pH value ranged from 6.3 to 6.5 and was not concentration-dependent. No significant difference (P>0.05) was observed along the concentration gradient and the control. The maximum value for conductivity (0.17±0.01 µS/cm) was recorded at 25.59 ml/l while the minimum value (0.13±0.01 µS/cm) was at 63.99 ml/l. No significant difference (P>0.05) was observed in the values recorded for the concentrations and the control. The alkalinity recorded had a range of 34.3 to 36.5 mg/l. Alkalinity increased significantly (P>0.05) along the concentration gradient (12.80> 25.59> 38.39> 51.19> 63.99 ml/l). The dissolved oxygen was maximum (4.9±0.01 mg/l) in the control (0ml/l) and the least (3.2±0.01 mg/l) in 63.99 ml/l. There was no statistical significance (P>0.05) observed along the concentration gradients. The total dissolved solid value recorded was highest (118.2±0.03 ppm) at 63.99 ml/l concentration and the least (95.2±0.03 ppm) at the control (0 ml/l). The TDS increased significantly (P>0.05) with increase in the concentration (0>12.80>25.59>38.39>51.19>63.99 ml/l). Ammonia recorded the maximum value (0.58±0.01 ppm) in 63.99 ml/l concentration and the minimum (0.3±0.01ppm) in the control. Nitrate was highest (0.68±0.05 ppm) in 63.99 ml/l and least (0.3±0.05 ppm) in the control. There was no statistical significance (P>0.05) between the different concentrations and the control. The water hardness was highest (114±0.05 mg/l) in 63.99 ml/l concentration and least (56±0.03 mg/l) in the control. There was a significant difference (P<0.05) across the concentration gradients (Table 1).

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

Analysis of OBF of the fishes in the control recorded mean values of 53.0± 0.3 beats per minute for the 28-day periods and 54.2±.06 to 58.6±0.3 beats per minute in the group of fish exposed to 12.80 ml/l and 68.0±0.6 to 79.0±0.6 beats in the group 63.99 ml/l with maximum concentration. There was a significant increase (P<0.05) of the OBF from the 7th day to 28 days and the same increase was observed across different concentration gradients. There was a significant difference (P>0.05) in the concentrations and the Control (Table 2).

Table 2 Mean 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 superscript down the column are significantly different; Duncan's Multiple Range Test at a 5 % level of significance (P<0.05).

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

Table 3 Mean 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 superscript down the column are significantly different; Duncan's Multiple Range Test at a 5 % level of significance (P<0.05).

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 3). 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.

3.2. Haematological Parameters of *C. gariepinus* exposed to Xylene for 28 Days

Results of the haematological parameters are shown in Table 4. From this study, it was observed that parameters such as packed cell volume, haemoglobin, red blood cells, white blood cell, platelet, mean corpuscular haemoglobin concentration, mean corpuscular, mean corpuscular volume of *C. gariepinus* exposed to xylene for 28 Days for at different concentration showed significant difference (p < 0.05). There were significant variations in the parameters recorded for the control (0ml/l). The values deviated in the volume of red blood cells, platelet, white blood cell, mean corpuscular haemoglobin concentration, mean corpuscular volume and mean corpuscular and varied significantly (p < 0.05) as the concentration of the toxicant increased.

Table 4 Mean values of haematological parameters of *C. gariepinus* exposed to Xylene for 28 Days.

TRT	PCV (%)	HB (g/dl)	RBC ($X10^{12}/L$)	WBE ($X10^9/L$)	Platelet ($X10^9/L$)	MCHC (g/l)	MCH (pg)	MCV (fl)
0	24.5±0.33^a	8.2±1.03^a	3.9±0.16^a	6.9±0.05^c	168±1.45^c	32.5±0.01^a	20.5±0.01^a	59±0.05^a
12.80	23.5±0.03^a	7.8±0.32^{ab}	3.8±0.06^b	7.6±0.08^b	181±0.03^b	32.5±0.02^a	20.5±0.01^a	62±0.02^a
25.59	23.7±0.03^a	7.9±1.45^{ab}	3.8±0.12^b	7.7±0.09^b	189±0.02^b	31.7±0.04^{ab}	20.5±0.01^a	61±0.03^a
38.39	22.8±0.05^{ab}	7.6±0.07^{ab}	3.7±1.73^{ab}	8.8±0.07^{ab}	199±0.05^{ab}	31.3±0.03^{ab}	20.5±0.01^a	61±0.04^a
51.19	21.8±0.06^b	7.3±0.70^b	3.6±0.58^c	9.5±0.06^a	206±0.04^a	30.8±0.08^b	20.5±0.01^a	60±0.07^a
63.99	20.8±0.06^b	6.9±0.58^b	3.5±0.08^c	10.1±0.04^a	214±0.05^a	30.5±0.07^b	20.4±0.01^a	59±0.07^a

*TRT= Treatment (mg/l) PCV= Packed cell volume (%) HB= Haemoglobin (g/dl); RBC = Red Blood Cells; WBC =White Blood Cell; PLA= Platelet; MCHC= Mean Corpuscular Haemoglobin Concentration (gdl^-) MCH= Mean Corpuscular Haemoglobin (pg); MCV= Mean Corpuscular Volume (fl).

3.2.1. Packed Cells Volume (PCV)

The mean packed cell volume values of *C. gariepinus* juveniles exposed to the different sub-lethal concentrations of xylene for 28 Days recorded the highest value of 20.8±0.0% in the highest concentration (63.99 ml/l) while the least value (24.5±0.33) was observed in the control. The results show that the packed cells volume values decreased with an increase in the concentration of the chemicals and the differences in mean PCV values obtained across the different concentrations were significantly different between the control and the three highest concentrations (P<0.05) as shown in table 4.

3.2.2. Haemoglobin (Hb) Concentration

The result of the haemoglobin of *C. gariepinus* is presented in table 4. The result shows that the haemoglobin of *C. gariepinus* decreases with an increase in concentration. The controlled unit recorded the highest value (8.2±1.03 g/dL) of haemoglobin while the lease value (6.9±0.58 g/dL) was observed in the highest concentration (63.99 ml/l) of the toxicant. the results in the haemoglobin values of the fish exposed to the xylene were significantly different at p < 0.05.

3.2.3. Red Blood Cell Counts (RBC)

The red blood cell counts of *C. gariepinus* juveniles exposed to sub-lethal concentrations of xylene are presented in table 4. The control recorded the highest value (3.9±0.16 $X10^{12}/L$) of haemoglobin and the least value (3.5±0.08$X10^{12}/L$) was observed in the highest concentration (63.99ml/l) of the chemical. The result shows that the mean red blood cell counts of the fish exposed to the toxicant decreased with an increase in the concentration. This result revealed that there was a statistical significance (P < 0.05) between the concentrations and the control.

3.2.4. White Blood Cell (WBC)

The white blood cell counts of *C. gariepinus* juveniles exposed to sub-lethal concentrations of xylene are presented in table 4. The result shows that the white blood cell counts of the test organism exposed to the toxicant increased with an increase in the concentration with the highest value of 10.1±0.04X10^9/L recorded in the highest concentration (63.99 ml/l) while the least value (6.9±0.05 X10^9/L) was observed in the control (0 ml/l). There was an observed statistically different across the different concentrations and the control ($p < 0.05$).

3.2.5. 8.2.5. Mean Corpuscular Haemoglobin (MCH)

The mean corpuscular haemoglobin values of *C. gariepinus* juveniles exposed to the different sub-lethal concentrations of xylene recorded the highest value of 20.5±0.01 pg in the control while the least value (20.4±0.01 pg) was observed in the highest concentration (63.99 ml/l). The results show that the mean corpuscular haemoglobin values decreased with an increase in the concentration of the chemicals and did not differ significant ($P > 0.05$) in mean values obtained across the different concentrations including the control as shown in table 4.

3.2.6. Mean Corpuscular Haemoglobin Concentration (MCHC)

The Mean corpuscular haemoglobin concentration of *C. gariepinus* juveniles exposed to the sub-lethal concentrations of xylene is presented in table 4. The highest value **(**32.5±0.01 g/l) of MCHC was observed in the control while the least value (30.5±0.07 g/l) was observed in the highest concentration (63.99 ml/l). The different concentrations recorded no statistically difference ($P > 0.05$).

3.2.7. Blood Platelets

From the result, the blood platelets showed a declining trend with an increase in the concentrations. The highest platelet value (214±0.05X10^9/L) of the test fish specie was observed in the highest concentration (63.99 ml/l) of the toxicant while the least value (168±1.45X10^9/L) was recorded in the control. The differences in the platelet values across the different concentrations were statistically significant ($P < 0.05$) as shown in table 4.

3.2.8. Mean Corpuscular Volume (MCV)

Table 4 shows the mean corpuscular volume for *C. gariepinus* exposed to sub-lethal concentrations of xylene. The highest value (59±0.05 fL**)** was observed in the control concentration while the least value (58±0.07 fL) was recorded in the highest concentration (63.99 ml/l) of the toxicant. There was a slight increase observed in mean corpuscular volume with an increase in the concentration of xylene. The mean corpuscular volume values of the different concentrations were not significantly different from the control ($P > 0.05$).

4. Discussion

4.1. The Physicochemical Parameters

Ecological parameters such as temperature, pH, dissolved oxygen, electric conductivity and total dissolved solids are vital indicators of the state of health of aquatic lives [26,27]. In this study, the parameters were observed to be significantly different from the control. The values of the physicochemical parameters of the water are within the ranges recommended by [28] and that of Nigeria Industrial Standard Technology [29]. 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. There were slight variations in the temperature values along the concentration gradient and the variation could be due to impurities, pollutants and toxicants capable of elevating the different physicochemical parameters [30,31,32]. The temperature values were within the acceptable range of [33] for water quality management. According to [34], the temperature of natural water bodies can vary between 25 ^{0}C and 30 ^{0}C.

The pH values were not significantly different from the control. Although it was within the acceptable limit (6-9) of [35] at the initial concentration later decreased with increased concentrations. Low pH is linked to increased solubility and toxicity strength of the chemical [36]. It is a generally accepted fact that concentration influences the elevation and reduction of test water during an experiment [37,16] coupled with the activities of the aquatic organisms which might also affect the physicochemical parameters while trying to survive [38,39].

The electrical conductivity (EC) values of all samples were not in agreement with the conductivity range 160-1600 µS/cm of the guideline range for fish as indicated by [40,41,42]. No significant change ($P<0.05$) was evident in conductivity at different concentrations with time. [43] reported that the low conductivity of any sample reflects the level of total dissolved solids in waters and the amount of total dissolved salts.

The mean value for alkalinity agreed with the range value reported by [44,45] for natural water. The highest value (36.2 mg/l) was reported in the control which reduced as the concentration increased. Generally, the alkalinity from the different concentrations and times was within the permissible limit of [41, 42] though there was statistical significance between the control group and other varying concentrations. There were a slight reduction in the dissolved oxygen (DO) values with an increase in the toxicant. The observed reduction in dissolved oxygen of water may suggest that the bio-availability of xylene may have sufficiently depleted the oxygen level coupled with the rapid consumption of the DO by the test fish which could have depleted the available oxygen [46]. [47] reported that a lower concentration of dissolved oxygen increases toxicity of an aquatic body leading to fish kills and signifying that low dissolved oxygen is a very vital factor to consider.

The total dissolved solid was lowest in the control group (95.2 ppm) and highest (112.3 ppm) at the highest concentration after 96 hours of exposure to xylene. Although it is within the stipulated guidelines for water quality by [28] which is <500 mg/l, it may have acted synergistically with the toxicant to affect the behaviour as well as mortalities recorded in the different concentration gradients. Water with high total dissolved solids is undesirable or harmful for both human and aquatic life and some specific mineral salts that make up the total dissolved solids pose a different health hazard [48]. Ammonia is a necessary nutrient source, but high ammonia in water can lead to fish kill [49]. The mean average value was high compared to the acceptable standard (0.05 ppm) of the Department of Environment in Bangladesh [50] for an aquatic body. The increase in the value of ammonia could be because ammonia is a natural by-product of fish metabolism via metabolizing food into the energy, nutrients, and proteins they use for survival and growth [51].

Unlike other physicochemical parameters, nitrates usually do not affect aquatic organisms directly. However, excess levels of nitrates in water can create difficulty for the survival of aquatic organisms. From the experimental, the nitrate values were between 0.3 and 0.59 ppm for 96 hours. This agrees with [52] and [53] who reported that nitrate toxicity to aquatic invertebrates increases with increased concentrations and exposure times to the toxicant.

The hardness of water reported for aquatic bodies ranged between 1-1000 ppm as $CaCO_3$. It reflected the composite measure of polyvalent cations whereas Calcium and Magnesium are the primary constituents of hardness [54]. The mean value which ranges between 114 ppm was low as compared to the recorded mean value (304.6 ppm) reported by [55] for a similar toxic exposure time. The values of total hardness for this experiment were within the [28] WHO specification limits (500 ppm). This agreed with the findings of other research on similar studies [56,57].

4.2. Behavioral responds

The study showed that the toxicants harmed the behaviour of the test fishes and this could be best described using the general adaptation syndrome [58]. Generally, fish exposed to higher concentrations of the pesticide showed abnormal behaviour and tried to avoid the test water by swimming very fast, jumping and displaying erratic with vigorous jerky movements, faster opercula movement, hyperexcitation, surfacing and gulping of air. The restlessness of the fishes observed in this study 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 [59]. As time went by, there was a success in the componentry process which led to reduced bioenergy cost of the fish and exhaustion which lead to death due to biological tolerant limit, nervous disorders and insufficient gaseous exchange across the gills [59]. The significant ranges in opercula ventilation and tail beat frequency reported in this study suggest that the test fish were stressed by Xylene which could have caused the fish to exhibit avoidance syndrome as earlier reported by [60] and [61].

The exposed fish samples swam faster to escape from the toxic area which increased their tail fin beat frequency and opercula beat frequency to assist them to gain more metabolism oxygen. However, [62] reported that hyperventilation resulted in lowered CO_2 in fish cells and might increase the blood pH thereby constricting blood vessels to the brain. This could lead to nervous dysfunction and toxicity in the fish. Fish eventually became fatigued resulting in a drop in opercula ventilation and tail beat frequency. The combined effects of fatigue and the direct effects of the chemical on the fish could have led to subsequent death at last [63]. [64] noted that opercula hyperventilation had been reported for stressed fish in an unfavourable environment.

4.3. Haematological parameters

Haematological indices are often associated with health status and are of diagnostic importance in the routine clinical evaluation of the state of health of fish exposed to toxicants [65]. The responses in fish to stresses are non-specific and may enable the fish to cope with the condition in maintaining their homeostatic state [66]. But when the stress becomes severe and persistent, the response may become malignant and threaten the fish's health and wellbeing [26]. The changes observed in fish PCV can be attributed to the swimming frequency resulting from the increased concentration level of the toxicant. Despite the increased erratic movement observed in the *Clarias garienpinus* which should have accounted for a rise in PCV and RBC, there was rather a steady significant decline in the RBC, PCV and haemoglobin due to the increasing doses of Xylene. This agrees with [67] who reported a low level of PCV and Haemoglobin in an aquatic organism with sedentary behaviour in some lentic water bodies and a higher value in active aquatic lives with spleen contraction which also resulted in a distorted shape and size of RBC as well [68.69] and [70].

There were significant variations were observed in haemoglobin concentration, packed cell volume, red blood cell counts, white blood cell and blood platelets of the *Clarias gariepinus* species on exposure to the various concentrations of xylene during the study. Although no significant differences were observed in the mean corpuscular haemoglobin concentration, mean corpuscular volume. Similar results were reported after exposure of fish to various toxicants [71]. This toxicant interaction could interfere with the osmoregulation processes which may lead to a decrease in plasma osmolarity which is also associated with a rise in blood volume and tissue water content. The increased influx of water in the gills due to changes in gill permeability of water may lead to the failure of electrolyte regulatory mechanisms as well [72]. This could probably be an explanation for the observed variations in the haematological indices of blood cells which may also cause anaemia and leucopenia as reported by [73].

The changes in the values of the haematological indices of the test fish are similar to that reported by [65]. The haemoglobin concentration, packed cell volume, and red blood cell counts are good indicators of the oxygen transportation capacity of fish which makes it possible to establish a relationship between the oxygen concentration availability in the aquatic habitat and the health status of the fish [26]. On the other hand, the white blood cells protect against infectious agents caused by microbial organisms and toxic factors [74]. In the *Clarias gariepinus* species, the significant reduction of haemoglobin concentration, packed cells volume, red blood cell counts, white blood cell counts, and platelet counts could indicate severe anaemia caused by the destruction of erythrocytes or haemo-dilution from impaired osmoregulation across the gill epithelium as dissolved oxygen levels declined significantly [75].

Packed cell volume is used to determine the ratio of plasma to corpuscles in the blood as well as the oxygen-carrying capacity of the blood [76]. [77] attributed damage and impaired osmoregulation of gills cause anaemia and hemodilution, as well as a significant decrease in packed cell volume.

Haemoglobin concentration decline of the exposed *C. gariepinus* may be caused by reduced production of haemoglobin as well as reduced oxygen-carrying capacity. This may be caused by disruption of the haemoglobin synthesis pathway by the extract [65]. The haemoglobin concentration falls lower than the range reported for catfish [78]. In some cases, this decline may be the result of an increased rate of red blood cell breakdown and/or reduced rate of red blood cell formation due to the plant extract [79].

The haemoglobin concentration and packed cell volume correlate directly with erythrocyte counts, possibly due to the synergistic linking of blood cells. [26]. In stressed conditions, the white blood cells and platelets likely increase to provide protection against disease and improve the fish's health [80]. When fish exposed to toxicants have low haemoglobin levels, they are at risk of Hypoxia as well as erythrocyte degeneration [55]. The changes observed in the values of haematological indices in these studies were concentration-dependent and may be attributed to the concentrations and the duration of exposure to the toxicant.

5. Conclusion

In conclusion, xylene caused negative changes in the haematological parameters of *C. garienpinus* by decreasing the red blood cell and packed cell volume, MCV while the white blood cell increased while trying to fight the changes in the organism's system. Even at a very low concentration, it elicited these abnormal changes in the metabolic processes of *C. gariepinius* showing the level of toxicity of the chemical on organisms. Water physicochemical parameters were also affected by the toxicant leading to stress factors that were reflected in behaviours distress like increased respiratory rate, gasping for air, restlessness, and loss of balance of the test fish. Hence the need to concentrate efforts on ensuring activities that increase the discharge of xylene to water bodies be checked its potential to alter the food chain in the coming years, hindering the target of the Sustainable development goals (SDG) and health complications in man.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this document.

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