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Acute Toxicity of Xylene on the African Catfish *Clarias gariepinus*

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ABSTRACT: Acute toxicity of xylene on an African catfish (*Clarias gariepinus*) was carried out. A total of 210 catfish *C. gariepinus* juveniles with mean length 15.20±2.3cm, and mean weight of 10.23±2.6g were obtained from the University of Port Harcourt Demonstration Farm. The test was determined for 96hour median lethal concentration using concentrations of 250ml/l, 200ml/l, 150ml/l, 100ml/l, 50ml/l, 25ml/l and 0.0ml/l (control) which gave a LC_{50} value of 63.965ml/l with upper and lower confidence limits at 106.53 ml/l and 37.82ml/l respectively. The median lethal time LT_{50} recorded was 55.7 hours. There was a strong correlation between (%) mortality in Probits and the Log_{10} Dose (R^2=0.9772). There was statistical significance (P>0.05) in the number of mortality observed in the six concentrations from 24 hours to 96 hours of exposure and high percentage mortalities were recorded as the concentration of the toxicant increased. No mortalities were recorded in the control. The cumulative mortality recorded after exposure of *C. gariepinus* to xylene was time dependent. Based on this the high percentage mortalities of the fish species it is therefore recommended that the use of this chemical be minimized and proper contingency plans be carried out before discharging this toxicants into the aquatic environment. Waste from this chemical and spill incidences should be detoxified to a less toxic level before disposing into the aquatic environment. The results obtained may provide valuable information for formulation of environmental policies and serve as a model for bio-monitoring of the aquatic environment.

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Pollution from industrial waste is a common occurrence in the Niger Delta and in nations whose economies are largely dependent on the oil industry. This is the case with Nigeria where exploration and exploitation remained the most important source of revenue generation for the past decades. These activities have been beneficial in many ways but have also resulted in serious detrimental effects on the environment especially the aquatic environment (Uche *et al.,* 2015). Oil exploration and exploitation at the onshore and offshore are carried out at the Niger Delta areas, producing more than 90% of the crude oil in Nigeria and thus hosting most of the terminals of oil activities (Wegwu and Omoedu, 2010). Xylene is one of the top 30 chemicals produced in the United States in terms of volume. Chemical industries produce xylene from petroleum (Condie *et al.,* 2015). It is used, to a lesser extent, as a material in the chemical, plastics, and synthetic fiber industries and as an ingredient in the coating of fabrics and papers (Fuente*, et al.,* 2013). Xylene is released in large quantities to the environment during oil exploration and exploitation such as well stimulation and cleaning in order to remove 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 environment via storage and its flow backs into the waste pit and eventually to the aquatic environment (Osuji, 2002; Kharaka and Dorsey, 2005). These dangerous chemicals have changed the nature of water that influence the fish and other aquatic organisms in the wild (Shanka, *et al*., 2013). When spillage occurs, only a small fraction dissolves and becomes bioavailable to fish and biota (Di Toro *et al*., 2001). Upon dissolution, they rapidly diffuse fish membranes into the blood circulation which convey them to tissue cells where they metabolize to more toxic components that act on macromolecules of exposed fish. The worry that contamination might impact the wellbeing and genetic composition of fish and shellfish stocks has increased in recent years (Suchirita, 2011). These pollutants can affect different stages of the aquatic food chain which may even lead to genotoxicity and can eventually lead to the distortions of the whole ecosystem and cause extinction of fish species (Sikoki *et al.,* 2013). The results obtained may provide valuable information for formulation of environmental policies and serve as a

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model for bio-monitoring of the aquatic environment. The objective of this paper is therefore to investigate the toxicological effect of Xylene on the African Catfish (*Clarias gariepinus*).

MATERIALS AND METHODS

Collection and Acclimation of Experimental Fish: A total of 210 healthy fingerlings of *Clarias gariepinus* with mean length 15.20±2.3cm, and mean weight of 10.23±2.60g were obtained from the University of Port Harcourt Demonstration farm, Choba, Rivers State, Nigeria and were transported in plastic containers to the Department of Fisheries Laboratory in the University of Port Harcourt. The fish were acclimated to laboratory conditions in a 150 litres capacity glass aquarium tank for 14 days at a room temperature of 28 ± 2^0C and were fed with commercial fish-feed twice daily. They were acclimated in glass tanks with an aerator to continuously oxygenate the water. The water in each glass tanks was replaced with tap water from the laboratory every 48 hours.

Preparation of a stock solution for Xylene: A working stock solution was prepared form xylene following the method of Lenntech (2008). 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.

Acute Toxicity Test: A Preliminary test was first carried out to establish a range of lethal concentration using a standard range finding method as recommended by Manual of Methods in Aquatic Environmental Research (Reish & Oshida, 1986) during which mortality rate was estimated (USEPA, 2008), dead fish were removed immediately after death to avoid pollution. A definitive test was then carried out in a static exposure period which lasted for 96hours and feeding of the experimental fish was suspended 24 hours before commencement. Six test concentrations: 250ml/l, 200ml/l, 150ml/l, 100ml/l, 50ml/l, 25ml/l and 0.0ml/l (control), each with 10 juveniles were randomly selected and replicated thrice to give a total of 21 samples. Each treatment group of fish was exposed for 96 hours during which mortality rate was estimated at 24, 48, 72 and 96 hour periods and dead fishes were removed immediately to avoid pollution. The aim was to determine concentration-response curves for fish mortality, the $LC5_0$'s, LT_{50}'s and the 95 percent confidence intervals for test organism at 24hr, 48hr, 72hr, and 96hr in a static system. The 96hour LC_{50} was estimated using the arithmetic method of Sprague (1973) by graphic plot of probit mortality versus log concentration.

Mortality Responses: The acute test was conducted after 96hours. The basic criteria that was used to show mortality was total lack of movement. Thus, the test organisms were confirmed dead after been touched or pricked with a forceps and they remained motionless or immobile. The acute exposure was conducted at static bioassay while the upper and lower confidence limits were calculated using the method of UNEP (1989).

Determination of 96 hours LC_{50} and LT_{50}: The 96 hours lethal median concentration (LC_{50}) was obtained from a probit analysis using the arithmetic method of deriving probit mortality from percentage mortality and plotting the former with logarithm of concentrations (Sprague, 1973). The LC_{50} was extrapolated from probit 5 to log concentration. The antilog value gave the LC_{50} in ml/l. The median survival time or median lethal time (LT_{50}) of concentrations was estimated from plot of cumulative mortality versus time by extrapolation from 50% mortality. Percentage mortality probit values were taken from Finneys Table (Finney, 1971).

Statistical Method: Percentage mortality and probit analysis (Finney, 1971) was used to analy the number of mortality recorded and the percentage mortality. This was also used to determine 96hr LC_{50} and LT_{50}. 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 (1955) Multiple Range Test was used to separate differences among means. Differences were considered significant at ($P < 0.05$).

RESULTS AND DISCUSSION

Definitive test for different concentrations of Xylene for 24-96hours: The Mortality of *Clarias gariepinus* exposed to different concentrations of xylene for 24-96hours during the definitive test is presented in Table 1. There was statistical significance ($P>0.05$) in the number of mortalities observed in the six concentrations from 24 hours to 96 hours of exposure and the fishes exposed to the different concentrations of the toxicant showed high mortalities as the concentration of the toxicant increased (Figure 4). Unlike the control, no mortalities were recorded. The values of the percentage mortality at the end of the 96hours assay also increased as the concentrations of the chemical from 24hours at 96hours and this is presented in figure 1 and 2.

Table 1: Mortality of *C. gariepinus* exposed to different concentrations of xylene for 24-96hours during definitive test

Conc. (ml/l)	Log Conc. (ml/l)	Mortality in 3 replicates				% Mortality	% Survival
		24hrs	48hrs	72hrs	96hrs		
0	0	0.0±0.0^{c}	0.0±0.0^{d}	0.0±0.0^{d}	0.0±0.0^{c}	0	100
25	1.40	0.0±0.0^{c}	1.0±0.3^{c}	2.0±0.3^{c}	3.0±0.3^{b}	30	70
50	1.70	0.0±0.0^{c}	2.0±0.6^{c}	2.0±0.3^{c}	4.0±0.3^{b}	40	60
100	2.00	2.0±0.3^{b}	3.0±0.3^{a}	4.0±0.3^{b}	6.0±0.8^{a}	60	40
150	2.18	2.0±0.3^{ab}	3.0±0.3^{ab}	4.0±0.6^{b}	7.0±0.6^{a}	70	30
200	2.30	3.0±0.6^{a}	3.0±0.6^{ab}	4.0±0.3^{b}	8.0±0.7^{a}	80	20
250	2.40	3.0±0.6^{a}	4.0±0.3^{a}	7.0±0.3^{a}	10.0±0.6^{a}	100	00

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

Table 2: The LC_{50} of acute toxicity test after exposing *C. gariepinus* to Xylene

Time (hrs.)	LC_{50}	Lower 95%	Upper 95%	Regression Equation
24	887.54	357.03	2206.34	y= 0.9366x + 2.239: R^2 = 0.7719
48	537.91	219.87	1315.98	y= 0.8943x + 2.5573: R^2 = 0.9161
72	230.33	107.08	495.42	y= 0.9957x + 2.6483: R^2 = 0.8313
96	66.97	37.82	106.53	y= 1.5032x + 2.29: R^2 = 0.9772

Median lethal concentration (LC_{50}) and median lethal time (LT_{50}): The cumulative mortality recorded after exposure of *C. gariepinus* to xylene was time dependent and increased as the time went by (Figure 3). The Median lethal concentration LC_{50} was 63.965 after 96 hours exposure of the test fish samples (Figure 2) while the median lethal time LT_{50} recorded was 55.7 hours as represented in figure 3. The linear and the regression equation as well as the lower 95% and upper 95% values for xylene at the different times were 37.82% and 106.53% (Table 2).

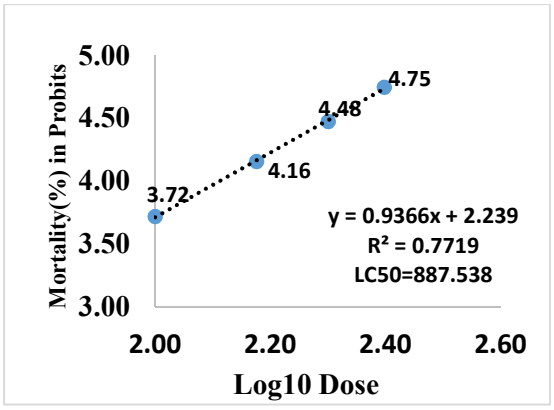

Fig 1: Probit mortality versus log concentration for Xylene at 24 hours exposure

Mean Mortality Rate of Clarias gariepinus Juveniles Exposed to Acute Concentrations of Xylene for 96 Hours: The application of xylene in well cleaning can increase the amount of chemical compound in the environment (Kochhann et *al.,* 2013). The exposed fish samples showed signs of toxic interference which includes air gulping, uncoordinated movements and an attempt to escape, hemorrhag of gills and quiescence before death agrees with earlier reports of Wade *et al.* (2002), Okwuosa and Omoregie (1995) and Awoyinka *et al.* (2010), when they exposed fish to various contaminants. The mortality rate recorded for the fish samples exposed to acute concentrations of the chemical (Xylene) at 250, 200, 150, 100, 50, 25 and control/0.0) ml/l obtained from a range finding test gave a 24 hours LC_{50} value of 887.538ml/l.

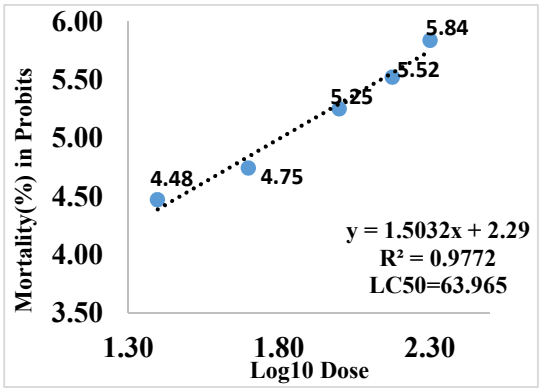

Figure 2: Probit mortality versus log concentration for Xylene at 96 hours exposure

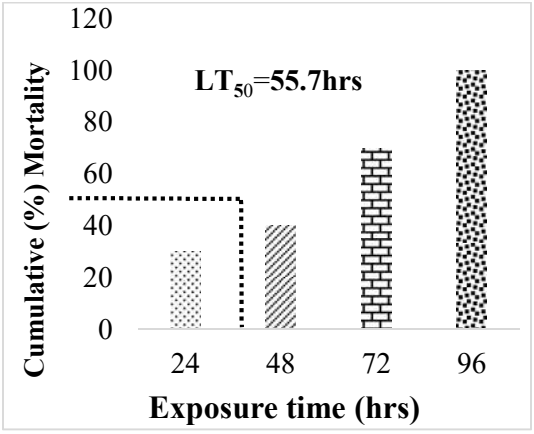

Fig 3: Cumulative Mortality recorded at different time of exposure of *C. gariepinus* to Xylene

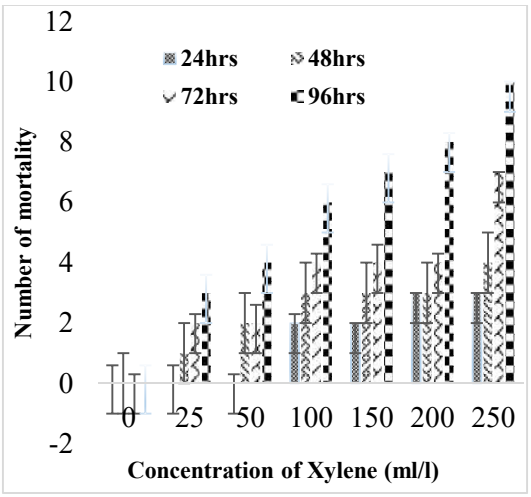

Fig 4: The rate of mortality of *C. gariepinus* recorded after exposure to Xylene at different time and concentration

Xylene showed significant dose dependent increase in mortality rate from 24-96 hours. The Median Lethal Concentration for 96 hrs (LC_{50}) determined graphically from the probit versus log concentration plot gave 63.96ml/l (Figure 2), with upper and lower confidence limits calculated to be of 106.537 and 37.818ml/l for 96h LC_{50} value. The 96hrs lethal median time LT_{50} (survival time) was determined using cumulative frequency versus time (figure 3) to be 55.7 hours where greater than 50% mortality was observed. The safe concentration was determined by multiplying LC_{50} with a factor of 0.01 (Koesoemadinate, 1980) which gave a value of 0.639% Xylene for *Clarias gariepinus* (with mean weight of 10.23±2.60g, and mean length 15.20±2.3cm). The value of 96 hour LC_{50} for xylene reported in this study differed from those of whole crude oil exposed to fingerlings of *Clarias gariepinus* which gave values of 125.89mg/l as reported by Ndimele *et al.,* 2010) and 9.355ml/l (Michael *et al*., 2012). Udofia (2010) reported 1.069 ml/l for Nile Tilapia *Oreochromis niloticus* exposed to Qua Iboe Light crude oil and 2.449 ml/l for petrol; 7.839ml/l for diesel and 8.095 for kerosene reported for fingerlings of *Clarias gariepinus* exposed to petroleum products (Michael *et al*., 2012). Similarly, the dose dependent progressive decrease in median lethal time LT_{50} due to increased toxicity, reported in this study agrees with the report of Ojuola and Onuoha (1987).

The safe concentration of 0.639% xylene for *Clarias gariepinus* reported in this study is different from safe the concentration of 0.288% reported for *Oreochromis ninoticus* and 0.356% for *Sarotherodon niloticus* exposed to aged liquid petroleum (Ojuola and Onuoha, 1987) and different from the safe values of 7.1%, 1.3% and 0.53% estimated for larvae of marine pejerry fish *Odontesthes argentinensis* exposed respectively to crude oil, diesel and gasoline (Ricardo *et al*., 2010). Differences in the safe values reported may be attributed to differences in size, species, and age, method of estimation and variation of derivatives in the different chemicals. Safe values for fish provides information for estimating human benchmark for a 15kg child (USEPA, 2008).

Conclusion: The results from the experiment shows that xylene is toxic to aquatic organism at a very low concentration. Therefore it could be concluded that the level of xylene in the aquatic environment should not exceed the 10% of its 96 hours LC_{50}. Based on the high mortalities rate of the fish species, it is therefore recommended that the use of this chemical be minimized and proper contingency plans be carried out before discharging into the aquatic environment. Waste from this chemical and spill incidences should be detoxified to a less toxic level before disposal.

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