Lethal Effects of Xyleneand Diesel on African Catfish (*Clariasgariepinus*)

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Abstract:*A total of 210 African catfish (Clariasgariepinus) juveniles with a mean weight of 10.23±2.6g and a mean length of 15.20±2.3cm, and were obtained from the University of Port Harcourt Demonstration Farm. A lethal effect of the mixture of xylene and diesel (at 50:50 ratio) was carried out to determine its effect on the African Catfish (C. gariepinus). Each experimental tanks containing 10 samples of the test fish were subjected to different concentration of the test chemicals. The definite 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 139.76ml/l with upper and lower confidence limits at 195.24ml/l and 85.11ml/l respectively. There was a strong correlation between (%) mortality in Probits and the Log$_{10}$ Dose (R²=0.9738). The results obtained may provide valuable information that will guide formulation of environmental policies and serve as a model for bio-monitoring of Nigerian aquatic system, especially the oil impacted water bodies.*
Key Words: *Acute toxicity, Clariasgariepinus, and Xylene:Diesel*

I. Introduction

The exploration and production of Oil and Gas in the Niger Delta is associated with the introduction of a wide range of chemicals and the application of chemical solvents in stimulation jobs are widely performed in the oil industry (Fernandes and Mazon, 2013). In Nigeria, the oil industry operations are bothonshore and offshore and all the oil terminals and most refineries in the country are located in theNiger Delta region and hence more than 90% oil related activities take place in this region and this has led to the adverse impacts on the aquatic environment (Albers, 2015). A mixture of diesel and xylene, as an aromatic solvent, has been a common remedy for surface pipelines, wellbore tubulars and especially near well bore cleaning operations, because other means of cleanup, such as heat, dispersants, pigging, scrapping, etc. cannot remove the organicmaterials like asphaltene or paraffin completely from near wellbore area. Chemicals are known to cause changes in various components of the environment and the organisms exposed to them (Uche *et al.,* 2015). These activities have impacted negativelyon the aquatic fauna in the Niger Delta environment and constitute a threat to the biodiversityof the aquatic life (Ogundiran*et al.,* 2010). Fish might have some mechanism to resist oil pollution inthe open rivers and creeks because of their abilityto move out of water for some time, but not all can resist oil pollution in a restricted or confirmed environment (Fayeofori, 2012). This paper focus on assess thelethal toxicityat different concentrationgradients of the combined effect of xylene &diesel fuel on the African cat fish (*Clariasgariepinus*)with a view to simulating the likelyeffectof petroleum and aromatic hydrocarbons in the natural aquatic environment.This will enable us assess its biodiversitystatus and thus take necessary precautionary measures towards managing the likelihood of the being endangered or taken into extinction.

II. Materials And Methods

FishCollection and Acclimation

A total of one hundred and twenty (120) healthy fingerlings of *C.gariepinus* were collected from theUniversity of Port Harcourt Demonstration farm, Choba campusand were transported in plastic containers to the Department of Fisheries Laboratory in the University of Port Harcourt.The fishhad a mean length 15.20±2.3cm, and mean weight of 10.23±2.60g.They were acclimated in a glass tanks with an aerator to continuously oxygenate the waterto laboratory conditions with a room temperature of 28±2^{0}Cin a 150 litres capacity glass aquarium tank for 14 days and were fed with commercial fish-feed twice daily.They were acclimated. The water in each glass tanks was replaced with tap water from the laboratory every 48 hours.

Preparation of and a working stock solution for Xylene&Diesel

A working stock solution was prepared form xylene following the method of Bell (1987); King *et al.,* (2015). The test chemical was prepared, using the equation: $V_1C_1 = V_2C_2$, Where; V_1C_1 = Stock solution attributesandV_2C_2 = New stock solution attributes while the water-soluble faction (WSF) of the Diesel used was prepared using the method suggested by previous studies (Anderson *et al*. 1974 and Orlu&Ogbalu, 2013).

Acute Toxicity Test

Each of the experimental tanks contained 10 fish samples and a preliminary test was done to establisha 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, 2002). 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 of 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 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.

LC_{50} Determination

To determine concentration-response curves for fish mortality, 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 ($P < 0.05$).

III. Results

Definitive test for different concentrations of Xylene: Diesel (50%:50%) for 24-96hours.

The Mortality rate of *C. gariepinus* exposed to different concentrations of Xylene: Diesel (50%:50%) for 24-96hours during definitive test is presented in table 1. There was significant ($P>0.05$) increase in the number of mortality observed in the six concentrations from 24 to 96 hours of exposure and the fishes exposed to the different concentrations of the toxicant showed high mortalities as the concentration of the chemicals increased (figure 2). No mortalities were recorded in the control.

The values of the percentage mortality at the end of the 96hours exposure also increased as the concentrations of the chemical increased, throughout the experiment. The probit mortality curve versus the log concentration for the definitive test at 96hour exposure is presented in figure 3.

Median lethal concentration (LC_{50}) and median lethal time (LT_{50}).

The cumulative mortality recorded after exposure of *C. gariepinus* to Xylene: Diesel (50%:50%) was also time dependent and increased with increasing time (table 2). The Median lethal concentration LC50 recorded in this study was 316.768as represented in figure 3and the median lethal time LT_{50} was 63.3 hoursas represented in figure 1. The lower 95% and upper 95% values were 85.11 and 195.24 after 96 hours. The linear and the regression equation are shown in table1.

Table 1: The LC50 of acute toxicity test after exposing *C. gariepinus*to Xylene: Diesel at a 50:50 ratio

Time (hrs.)	LC50	Lower 95%	Upper 95%	Regression Equation
24	316.77	217.36	461.64	y= 2.5719x + 1.4316 R^2 = 0.9995
48	401.59	182.47	883.84	y= 1.0073x + 2.3769 R^2 = 0.9613
72	220.31	125.07	388.09	y= 1.3913x + 1.7374 R^2 = 0.9182
96	139.76	85.11	195.24	y= 1.9037x + 0.9844

$R^2 = 0.9738$

Table 2: Mortality rate of *C. gariepinus* exposed to different concentrations of Xylene: Diesel (50%:50%) 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[c]	0.0±0.0[d]	0.0±0.0[d]	0	100
25	1.40	0.0±0.0[c]	0.33±0.3[c]	1.33±0.3[c]	1.67±0.3[c]	10	90
50	1.70	0.33±0.3[c]	0.33±0.3[c]	1.33±0.3[c]	2.0±0.0[c]	20	80
100	2.00	0.67±0.3[b]	2.0±0.57[b]	3.0±0.0[b]	4.3±0.3[b]	40	60
150	2.18	1.67±0.3[a]	2.67±0.3[b]	3.0±0.6[b]	5.06±0.3[b]	50	50
200	2.30	3.0±0.0[a]	3.33±0.3[ab]	4.67±0.3[a]	6.3±0.67[a]	70	30
250	2.40	3.7±0.3[a]	4.0±0.57[a]	5.33±0.7[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.
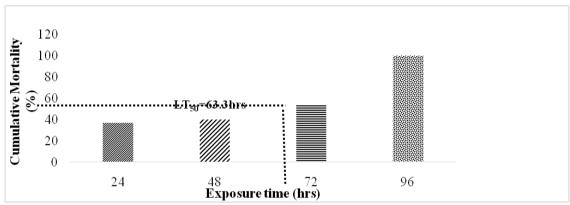

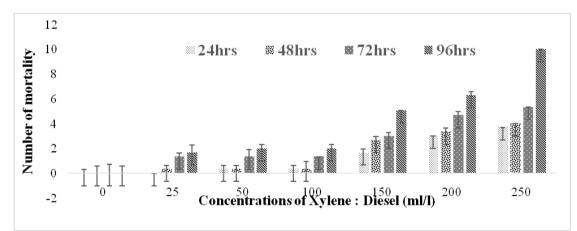**Figure 1**: Cumulative Mortality recorded at different time of exposure of *C. gariepinus* to Xylene: Diesel

Figure 2: Number of mortality of *C. gariepinus* recorded at different time and concentration of Xylene: Diesel
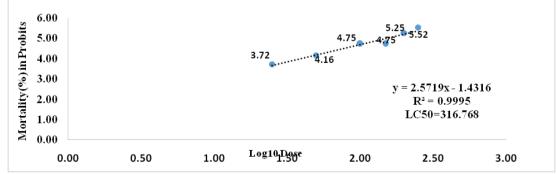

Figure 3: Probit mortality versus log concentration for Xylene: Diesel at 24 hour exposure
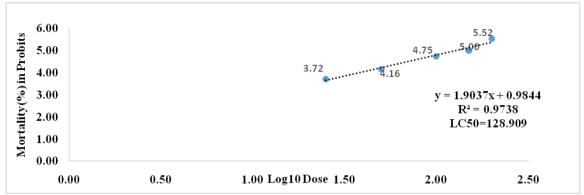
Figure 4: Probit mortality versus log concentration for Xylene: Diesel at 96 hours exposure

IV. Discussion

The result from the 96 hours exposure shows that the concentration ranged of 25ml/l to 250ml/l of the test chemical recorded mortalities that ranged between 10 to 100%. Although *C. gariepinus*are known to tolerate harsh environmental conditions (Jamabo, *et al.,* 2013), the high mortality rate could be an indication of the clogging of these respiratory structures caused by the combined effect of xylene and diesel. It is possible that the fish had suffered from oxygen stress induced by the organic compounds in the test chemicals (Dede and Kaglo 2001). Similar, it could be attributed to oxygen stress imparted by the water soluble fractions of the petroleum hydrocarbon on aquatic life (Igloh*et al.,* 2001). Also the rate of mortality in this study indicated agrees with the earlier work by Fayeofori (2012) that the extent of depletion of oxygen in the water is often a function of the concentration of the chemical pollutant in it. The result from the present study indicated increased death rate with an increase in the concentration of the test chemicals and the percentage mortalities were concentration dependent. The higher the concentration, the higher the percentage mortalities. Similar report was presented by Ogundiran*et al.,* (2010) when investigating toxicological impacts of detergent effluent in fingerlings of African catfish *Clariasgariepinus*, Calta*et al.,* (2004). The lowest concentration (25ml/l) recorded 0, 1, 1, and 2 deaths respectively within the 96 hours with 10% mortality rate while the highest concentration (250ml/l) recorded 3, 4, 4, 5 and 10 deaths respectively within 96hours with 100% mortality rate.

The median lethal concentrations for the 24 and 96 hours (LC50) for xylene and diesel were 128.91 and 316.77ml/l, respectively (figure 4.48 and 4.9). The upper and lower confidence limits calculated were 85.11 and 195.24ml/l for 96h LC_{50} value. The 96hrs lethal median time LT_{50} (survival time) was determined using cumulative frequency versus time (figure 4.6) to be 63.3 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 0.633% for the combined test chemicals (Xylene: Diesel) for *Clariasgariepinus* with mean weight of 10.23±2.60g, and mean length 15.20±2.3cm. The results from this study differ from those reported bySchwaiger*et al.(*2010) 1.069 ml/l for Nile Tilapia *Oreochromisniloticus* 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 *Clariasgariepinus* exposed to petroleum products (King, *et al.*, 2012). There was a significant differences in LC50 values (P<0.05) as time of exposure increased from 24 to 96hours. In aquatic organisms, sensitivity to pollutant is related to the physiological and biological activity. Xylene: Diesel showed significant dose dependent increase in mortality from 24 to 96 hours.

The safe concentration of 0.633% Xylene: Diesel for *Clariasgariepinus* observed in this study also different from safe concentration of 0.356% reported for *Sarotherodonniloticus*and0.288% *Oreochromisninoticus*exposed to aged liquid petroleum Ojuola and Onuoha (2017) and different from safe values of 7.1%, 1.3% and 0.53% estimated for larvae of marine pejerry fish *Odontesthesargentinensis* exposed respectively to crude oil, diesel and gasoline (Rodrigues,*et al.*, 2010).it could be concluded from the result of this findings that a very lowconcentrations of the test chemical mixture of petroleum hydrocarbon could be lethal to *C. gariepinus*and this is could affecting thebiodiversity of the species and other aquatic life. More efforts should be made by the oil companies operatingin the Niger Delta region of Nigeria to minimizethe pollution of the aquatic environment with this aromatic solventto ensure a balance in the biodiversity of this fish species and to prevent themfrom becoming endangered or extinct.

Acknowledgement

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