Lethal Effects Of Aqueous Methanol On Juvenile Tropical Freshwater Fish (Oreochromis niloticus)

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Abstract*: In this study, juvenile tropical freshwater fish (Oreochromis niloticus) were exposed to different lethal concentrations (2.5 ml/L, 5.0ml/L, 10.0ml/L, 15.0ml/L, 20.0ml/L and 25.0ml/L respectively) of aqueous methanol and (0ml/L) which is a tank without the toxicant to serve as the control. The experiment was conducted using a static nonrenewable bioassay method. The fish were obtained from the African Regional Aquaculture Centre (ARAC), Aluu, Rivers State, Nigeria. The fishes were acclimated to an aquarium for 14 days. In order to determine the definitive test concentration, a range-finding test was conducted. The mortality, LC_{50s} value, and the 95 per cent confidence intervals for test organisms were derived using standard procedures at 24hr, 48hr, 72hr, and 96hr respectively. There was a statistically ($P<0.05$) increase in the mortality rates as the concentration of the test chemical increased. The LC_{50} values at 24, 48, 72 and 96 hours recorded were 30.064ml/L, 26.562ml/L, 11.534ml/L, and 6.347ml/L respectively for the Chemical. The LC_{50} values showed that the chemical is toxic to this tropical freshwater fish. Hence, it is recommended that there should be a regulatory measure in the discharge of this chemical into the aquatic environment, to avert potential toxic effects that may result in the death of non-targeted aquatic organisms which is an edible meal for humans which in turn may affect human health.

***Keywords*: Lethal Toxicity, Oreochromis niloticus, Methanol, Water quality**

I. INTRODUCTION

The aquatic body is the utmost recipient of many anthropogenic and natural inputs of contaminants and toxic substances which are the main causes of the decrease in the population of aquatic biota all over the world (Idowu et al., 2020). However, sub-lethal levels of most toxic substances have proven to be devastating to fish population, composition, and density (Adedeji et al., 2009). There is great worldwide concern about the effect of human activities on the aquatic environment which is an essential component of human life and existence (Lee et al., 2011).

Toxic pollutants and contaminants generated from waste products in most industries are distinctive events in the Niger Delta whose economic activities generate such wastes from oil refining and production business. This is the condition initiated in Nigeria where exploitation and exploration are the focal sources of income for several years (Davies *et al.*, 2019a). These activities have been of great benefit. However, they have additioncreatedbiggerdisrupting impacts, mostly on the aquatic environment (Uche *et al.*, 2015).

The petroleum and gas industry plays a significant role and acts as a component of world energy, which involves various operational activities such as drilling, and exploration of crude oil and natural gas (John *et al.*, 2012). Some of the activities also involve reservoir stimulation using different chemicals which is a specialized area in the petroleum and gas industry conducted to boost the ultimate financial recovery (Firozjaii*et al.*, 2020). However, these chemicals impose life threats to the aquatic organisms in the environment and field staff as well through storage and flow back into the waste pit and the rivers creeks (Davies *et al.*, 2019).

These dangerous chemicals have resulted in modifications in the physicochemical parameters of water that have affected fish and other aquatic organisms in the wild (Suchirita, 2011). Although most companies operate within the stipulated regulatory limits given by the National Environmental Standards and Regulations Enforcement Agency (NESREA), National Oil Spill Detection and Response Agency (NOSDRA), the Federal Ministry of Environment (FME), and the Directorate of Petroleum Resources (DPR) which are the regulatory bodies for these Oil and Gas Industries and their environment in Nigeria, and most oil companies treat their wastewater before they are discharged into the environment. Nevertheless, previous studies have reported that there are some types of waste disposed to the aquatic environment that do not meet these stipulated regulatory standards before being discharged into the environment (Opete*et al.*, 2019; Isehunwa and Onovae, 2011).

Methanol is an organic aliphatic hydroxy solvent widely used as a raw material in several industries such as the manufacture of formaldehyde, pesticides, photographic film, plastic, textile, soap, artificial leather, etc., and as a solvent for ink, resins, adhesives, and dyes (Kaviraj et al., 2003). It is a chemical used in many industries as a raw substance for different

productions, which includes soap, removers, pesticides, and solvents(Osorio-González, *et al.* (2020). The frequent use of this chemical has been observed in most industrial effluent which has also been reported as a contaminant found to affect the aquatic organism its environmental (Grassi *et al.,* 2012). Some researchers have reported that exposure to methanol could cause damage to the different stages of an aquatic organism (Manzo and Costa, 2020) and this chemical has also been known for its neurotoxin ability in causing visual damage or blindness by affecting the optic nerve and retina (Boia*et al.,* (2020). Some oilfield chemicals also have the potential to change the features of the receiving medium by affecting aquatic life which includes microbial community, planktons, micro and macrobenthic faunas, macrophytes, finfish, and shellfishes in water (Rico *et al.,* 2006)

The aquatic environment has been reported to be the primary recipient of many anthropogenic and natural pollutants and toxic compounds, which are the primary cause of aquatic biota inhabitants,' deterioration across the globe (Idowu *et al.,* 2020).

The release of different pollutants from most industrial operations in the Niger Delta has shown toxic effects thereby causing histological, haematologicalaberrations, and death of the organism (Lakra and Nagpure, 2008). The sub-lethal concentration of the most harmful substances has also been reported to have a catastrophic effect on fish composition, population, and density (Adedeji *et al.,* 2009). When they dissolve in the aquatic environment, they swiftly diffuse through fish membranes into the bloodstream and then transported to tissue, and then metabolized into more harmful components that act on the macromolecules of the fish (Davies *et al.,* 2019b). These contaminants can have an impact on different stages of the aquatic food chain, causing genotoxicity and finally causing ecological disruption and the extinction of the same fish species (Nilsen*et al.,* 2019). The study aims to assess the acute toxicity of an aqueous analytical Methanol on juvenile Nile tilapia(*Oreochromis niloticus*). These results from this study will be useful in formulating models of environmental policymaking and aquatic bio-monitoring.

II. MATERIALS AND METHODS

2.1. Test Fish (*Oreochromis niloticus*) *and Source*

A total of 1,200 healthy with a mean weight of 10.34±0.3g and a mean length of 15.20±0.2cm were obtained from the African Regional Aquaculture Centre (ARAC), Aluu, Rivers State, Nigeria and were transported to the Laboratory.

2.2 Source of Test Chemical

The toxicant used was an analytical grade of methanol (CH_3OH) collected in a 2.5litre container from a chemical laboratory in Port Harcourt, transported to the laboratory, and stored under ambient conditions. The chemical was available in liquid form and was treated directly in the test medium.

2.3 Acclimation of The Test Organism

Acclimatization was performed for the test organisms in two stages to reduce mortality during the acclimatization period in the test laboratory.

The test fish samples were acclimated in a 150 litres capacity glass aquarium tank for 14 days at a room temperature of 27 ± 0.3^0C to reduce mortality during the acclimatization period in the laboratory condition. The fish were fed twice daily with a 2 mm imported commercial fish feed (Coppens) containing 45% crude protein at the rate of 3% body weight during the period. Feeding was terminated 24 hours before the start of the experiment while uneaten feed and wastes were removed daily with subsequent water replenishment. The water was continuously aerated using aquarium air pumps to maintain an ambient laboratory temperature. The water in each glass tank was replaced with tap water from the laboratory after 48 hours as suggested by Davies *et al.*(2019a). The rate of mortality during acclimation was used as an indicator of the healthy condition of the organisms.

2.4. Range Finding Test

Before the start of the definitive test procedures, a preliminary test was conducted using the toxicants in logarithmic concentrations to determine the most suitable range of concentrations to be used for the exposure of the test organisms during the definitive toxicity test (Reish and Oshida, 1986). Six concentrations of the test chemical were prepared for the test and the tanks were in triplicate with ten (10) juveniles per tank and were exposed for 96hours during which mortality rate was estimated (USEPA 2002) and the dead fish were immediately removed and buried to avoid being contaminated and the final result will be used as the test concentrations for the definitive test.

2.5. Definitive Toxicity Test

Each test concentration in an aquarium tank of 15 litres was filled to the 10 marks. Ten fish were randomly selected and put in each of the test concentrations(0 ml/L, 2.5 ml/L, 5.0ml/L, 10.0ml/L, 15.0ml/L, 20.0ml/L, and 25.0ml/l). Each treatment group of fish in triplicates was exposed for 96hr duration and mortality was determined at 24, 48, 72, and 96hrs periods. The dead fish were removed immediately to avoid contamination. The LC_{50}, concentration-response curves for mortality, and the 95 per cent confidence intervals for test organisms at 24, 48, 72, and 96-hour in a static system were derived. A static nonrenewal bioassay option was employed for this study. The assessments were carried out using a standard procedure and guidelines (DPR, 2018).

2.6. Water Quality Analysis

The water quality was analyzed using portable meters following American Public Health Association (APHA, 2002) procedures. The parameters analyzedwere Dissolved Oxygen (DO), Temperature, Hydrogen Ion Concentration (pH), Conductivity, and Total Dissolved Solids (TDS)

2.7. Determination of Mortality

The test organisms were confirmed dead when they remained immobile after repetitive prodding with forceps. The mortality rate of the test organisms was calculated with the formula:

Mortality rate = *Number of dead test organism* X 100 *Total number of test organism exposed to the treated produced water*

2.8. LC_{50} and Toxicity Factor Determination

Mortality and motionlessness were used as an indicator of toxicity. The dead fishes were removed and counted at different intervals following the 0, 24, 48, 72, and 96hperiods. The results at varying time intervals were subjected to a probit analysis. The percentage mortality was converted to probit using Finney's table. The regression analysis was carried out for probit values against the logarithm of the concentration using Microsoft excel. The resultant x value and intercept value was substituted in the equation Y= b + ax in which variables x and b (intercept) were obtained from the regression analysis. The$LC_{50}$was calculated thereafter. The Toxicity factors were computed by dividing the LC_{50} of the toxicant by the LC_{50} of the reference chemical.

2.9. Statistical Analysis

The statistical analysis was carried out using the SPSS version. Data were expressed as mean ± standard deviation. Two-way ANOVA was carried out to show the significant variation in the treated produced water's Physico-chemical characteristics. Where significant variations (p = 0.05) exist, Duncan'smultiple choice test statistics were used to determine the source of the variation. The charts were plotted using graph prism and Microsoft excel.

III. RESULTS AND DISCUSSION

3.1 Results

3..1.1 Definitive test for Methanol from 24 hours to 96hours.

The number of mortalities recorded in the definitive test increased with an increase in the test chemical concentrations from 24 to 96hours of exposure (Table 1). Unlike the control, no mortalities were recorded and no variations were observed after 96 hours. There was significance (P<0.05) in the number of mortalities recorded among the different concentrations from 24 hours to 96 hours. The Probit curve of mortality of *O. niloticus* exposed to different concentrations of Methanol for 96 hours is shown in Figures 1, 2, 3, and 4. The LC_{50} valueof6.347 was recorded for the *O.niloticus*while the regression equation (y=1.5032x+3.7932 & R^2 = 0.9772) is represented in Table 2.

Table 1:Mean values of the mortality recorded after Orieochromisniloticus juveniles were exposed to Methanol for 24 to 96hours.

Conc. (ml/l)	Mean mortality				% Mortality	% Survival
	24hrs	48hrs	72hrs	96hrs		
0	0±0.000[a]	0±0.000[a]	0±0.000[a]	0±0.000[a]	0	100
2.5	0±0.000[c]	0±0.000[c]	1±0.333[b]	3±0.577[a]	30	70
5.0	0±0.000[d]	1±0.000[c]	2±0.577[b]	4±0.577[a]	40	60
10.0	1±0.000[c]	2±0.000[b]	3±0.333[b]	6±0.000[a]	60	40
15.0	2±0.333[d]	3±0.000[c]	5±0.333[b]	7±0.000[a]	70	30
20.0	2±0.000[b]	3±0.000[b]	7±0.333[a]	8±0.577[a]	80	20
25.0	5±0.333[c]	6±0.000[c]	9±0.333[a]	10±0.000[a]	100	00

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

**Means with different superscripts down the column are significantly different.

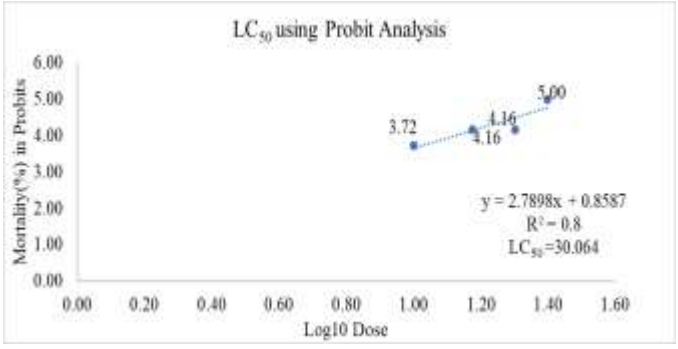

Figure 1: The Plot of Log of Concentration Versus Probit at 24hrs for O. niloticusexposed to exposure to Methanol.

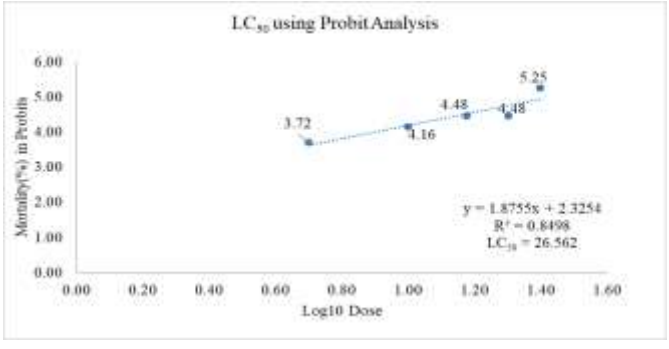

Figure 2: The Plot of Log of Concentration Versus Probit at 42hrs for O. niloticusexposed to exposure to Methanol.

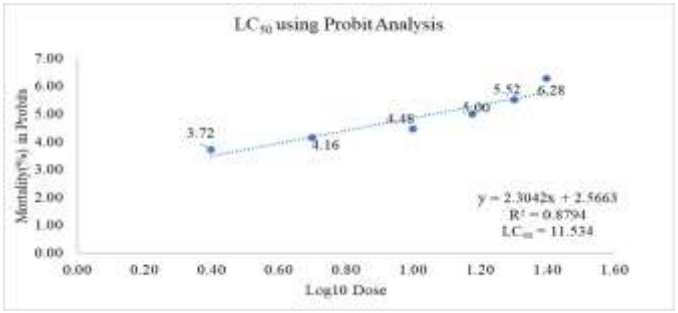

Figure 3: The Plot of Log of Concentration Versus Probit at 72hrs for O. niloticusexposed to exposure to Methanol.

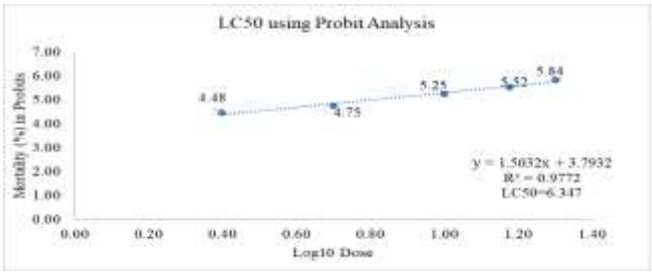

Figure 4: Probit curve of mortality of O. niloticus exposed to different concentrations of Methanol for 96 hours.

Table 2: The LC_{50} and the Acute Toxicity Test After exposing O.niloticus to Methanol

Time (hrs.)	LC_{50}	Lower 95%	Upper 95%	Regression Equation
24	30.064	21.141	42.752	y = 2.7898x + 0.8587 R^2 = 0.8
48	26.562	16.873	41.814	y = 1.8755x + 2.3254 R^2 = 0.8498
72	11.534	8.106	16.411	y = 2.3042x + 2.5663 R^2 = 0.8794
96	6.347	3.782	10.653	y = 1.5032x + 3.7932 R^2 = 0.9772

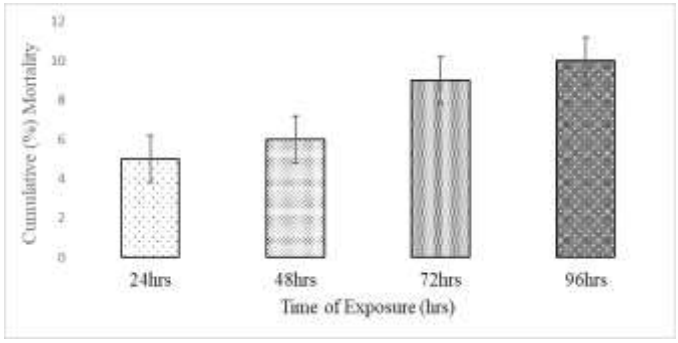

Figure 5: Mortalities of O. niloticus exposed to different concentrations of Methanol

3.1.2 Physicochemical parameters of the experimental water after 96 hours

The data on the physicochemical parameters are presented in Table 3. The parameter varied slightly when compared with the control sample (0ml/L). The temperature was observed to have remained relatively constant ranging from 26.7^0C to 28.5^0C across all test concentrations, while the Dissolved Oxygen decreased (DO) from 5.2 to 4.1mg/l as the concentration increased, the highest concentration of DO was observed in the control (5.2mg/L). The pH values varied from (6.8 to 5.9) in the control to the highest concentration (25.0ml/L) indicating that the water changed from being slightly alkaline to slightly acidic. Total Dissolved Solids were highest in the test concentration of 25.0ml/L while the least was observed in the control ranging from 180.0 to 363.7ppm. The electrical conductivity varied from 267.0 to 452.9µs/cm. The conductivity increased from the lower concentration (0ml/L) higher concentration (25ml/L) of the toxicant. There was statistical ($P<0.05$) across all the parameters along the concentration gradients.

Table 3:Mean water quality parameters after exposing *O. niloticus*to Methanol for 96 hours.

Parameters	Concentrations (ml/L)							WHO (2011)
	0	2.5 ml/L	5.0 ml/L	10.0 ml/L	15.0 ml/L	20.0 ml/L	25.0 ml/L	
Temperature (^{o}C)	26.6 ±0.06^b	26.7 ±0.06^b	26.7 ±0.03^b	27.3 ±0.06^{ab}	27.8 ±0.03^{ab}	28.4 ±0.06^a	28.5 ±0.06^a	<25
pH	6.8 ±0.03^a	6.7 ±0.00^a	6.7 ±0.03^a	6.2 ±0.03^b	6.1 ±0.00^b	6.1 ±0.03^b	5.9 ±0.03^b	6.5-8.5
Conductivity (µS/cm)	267.0 ±0.0^b	298.1 ±0.01^b	346.0 ±0.00^{ab}	367.1 ±0.01^{ab}	379.1±0.00^{ab}	442.2 ±0.01^a	452.9 ±0.00^a	400
Dissolved Oxygen (mg/L)	5.20 ±0.01^a	5.0 ±0.01^a	4.7 ±0.00^{ab}	4.6 ±0.00^{ab}	4.3±0.00^b	4.3 ±0.00^b	4.1 ±0.01^c	>5
Total Dissolved Solid (ppm)	180.0 ±3.1^d	182.3 ±0.3^d	185.0 ±0.3^d	186.0 ±0.6^{dc}	195.3±0.6^c	260.2 ±0.3^b	363.7 ±0.3^a	250

*Means with different superscripts across the rows are significantly different.

*Means with the same superscript across the rows are not significantly different.

3.2 Discussion

3.2.1 Physicochemical Parameters.

The parameter varied significantly across the test medium during toxicity testing. All the values except Conductivity and Total Dissolved Solid were below the WHO recommended limit for freshwater bodies. The quality of water in aquatic systems is a predisposing factor to the biological living of aquatic organisms inhabiting in it (Idowu *et al.*, 2020). The variation could be due to the interaction of the fish with the toxicant at an increasing concentration, thereby distorting the oxygen consumption level in the water. Any distortion of the natural

state of water will lead to the agitation of the specie which can lead to an imbalance in the biological sanctity of the water body thereby resulting in a direct effect on the species that is in a close relationship with it (Davies *et al.,* 2019). Holden (1973) in his earlier work reported that when toxicants are introduced into an aquatic system, it might decrease dissolved oxygen concentration, which may lead to asphyxiation. Since most fish breathe in the water they live in, changes in the chemical properties may be reflected in the animal's respiratory activity, particularly if the environmental factors affect respiratory gas exchanges (Bellan*et al.,* 1981).

3.2.1Mortality of Oreochromis niloticus Juveniles

In this study, the acute toxicity level based on the 96 hours' LC_{50}value of Methanol with a concentration range from 2.5ml/l to 25ml/l was found to be 6.347ml/l when tested against the fingerlings of *Oreochromis niloticus*. The percentage of mortality increased as the concentrations increased. No mortality was observed in the control from 24 to 96 hours. There were significant variations in the numbers of mortality across the different test concentrations from 24 to 96 hours. The high mortality rate could be due to the clogging of these respiratory structures caused by the increasing concentrations or similar alteration from oxygen stress induced by the organic compounds in the test chemicals (Dede and Kaglo 2001). Similarly, it could be attributed to oxygen stress imparted by Methanol on the aquatic body (Iglo*het al.*, 2001). The rate of mortality in this study also agrees with the earlier work by Davies *et al.,*(2019) who reported that the extent of depletion of oxygen in the water is often a function of the concentration of the toxicant. The higher increased death rate with an increase in the concentration of the test chemicals and the percentage of mortalities was concentration-dependent (Fafioye, 2007). Ogundiran*et al.*, (2010) reported similar toxicological impacts of detergent effluent in fingerlings of *Clarias. Gariepinus.*

The median lethal concentrations for the 24 and 96 hours (LC_{50})from the study of methanol was 6.347ml/l and the safe concentration was determined by multiplying the LC_{50} with a factor of 0.01 as recommended by Ezike, (2017) which gave a value of 0.0634% for the methanol. This result differs from that reported by Schwaiger*et al.* (2010) which is 1.069ml/l for *Oreochromis niloticus*exposed to Qua Iboe Light crude oil and 2.449 ml/l for petrol. This is significantly different from King *et al.* (2012) who reported for fingerlings of *Clariasgariepinus* exposed to petroleum products (7.839ml/lfordieseland8.095forkerosene).
Inaquaticorganisms, sensitivity to pollutantsisrelated to physiological and biological activity. Ojuolaand Onuoha(2017) also reported different safe concentrations of0.356% for *Sarotherodonniloticus* and 0.288% for *Oreochromis niloticus* exposedtoaged liquid petroleum. Meanwhile, Rodrigues*etal.* (2010) reported differentsafe values of 0.53%,1.3%and7.1% estimated for larvae of *Odontes the sargentinensis* exposed respectively tocrudeoil, diesel, and gasoline.

IV. CONCLUSION

In conclusion, the physicochemical parameter of the experimental water varied significantly during toxicity. All the values except Conductivity and Total Dissolved Solid were below the WHO standard. The percentage of mortality increased as the concentrations increased. No mortality was observed in the control from 24 to 96 hours. There were significant variations in the numbers of mortalityacrossthe different test concentrations during the assay. The high mortality rate was attributed to the change in the water chemistry which caused reduced dissolved oxygen stress leading to the clogging of the respiratory structuresinduced by the organic compounds in the test chemicalsresulting in the death of *Oreochromis niloticus*.It is therefore necessary for the proper handling of the discharge of methanol into the aquatic environment during explorations in the oilfield.

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