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Acute Toxicity of Aqueous Methanol on Juvenile Guinean Tilapia (*Tilapia guineensis*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was conducted to evaluate the acute toxicity of Juvenile *Tilapia guineensis* exposed to aqueous methanol (Analytical grade). The fishes were obtained from the Nigeria Institute for Oceanography and Marine Research (NIOMR), Buguma, Rivers State, Nigeria. The fishes were acclimated to an aquarium for 14 days. A range-finding test of the toxicity of aqueous analytical methanol was conducted. Based on the preliminary results, a definitive test was conducted at 0ml/l as control (0ml/l), 2.5 ml/l, 5.0ml/l, 10.0ml/l, 15.0ml/l, 20.0ml/l and 25.0ml/l respectively. From the data, the concentration-response curves for fish mortality, the LC_{50}s, and the 95 percent confidence intervals for test organisms at 24hr, 48hr, 72hr, and 96hr in a static system were derived following the standard procedure. The mortality rates increased significantly ($p<0.05$) with an increase in the concentration of the test chemical. The LC_{50} values at 24, 48, 72 and 96 hours recorded were 30.361 ml/l, 16.585 ml/l, 7.369 ml/l, and 3.750 ml/l respectively for the aqueous analytical methanol. The LC_{50} values showed that the test chemical is toxic to the juvenile *T. guineensis*. Therefore, proper handling and discharge of this chemical into the aquatic environment should be minimized to avoid possible toxic effects on the aquatic life therein.

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1. INTRODUCTION

Pollution and contamination from modern waste especially industrial waste is a typical event in the Niger Delta whose economies are generally subject to the oil refining and production business. This is the situation found in Nigeria where exploration and exploitation are the main wellsprings of income for many years [1]. These exercises have been advantageous in numerous ways however, they have additionally brought about greater inconveniencing impacts, particularly on the aquatic environment [2]. The oil and gas exploration and exploitation are carried out both offshore and onshore mostly in the Niger Delta regions, delivering over 90% of the unrefined petroleum in Nigeria and in this way facilitating the majority of the terminals of oil exercises [3].

Nigeria has regulatory bodies such as 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 with stipulated guidelines and safety standards for the management and discharge of waste products in the water body and has set limits within which wastewater is generated and managed from the activities of the petroleum industries in Nigeria [4]. This is before its discharge into the aquatic ecosystem whether brackish or saline water. In an endeavor to operate within these stipulated regulatory limits, most oil companies treat their wastewater before they are discharged into the environment. Nevertheless, studies have discovered that some forms of waste do not meet these limits about some of the guidelines, before being discharged into the surrounding [5].

Methanol is a chemical very useful in different industries as a raw material for many products, including pesticides, soap, solvents, and removers [6]. Due to the large use of this compound, it can be found in the effluent of industries, being described as an environmental contaminant that affects the aquatic biota [7]. Studies have shown that methanol exposure can cause damage to the gastrulation stage of an aquatic organism and methanol is also recognized as a neurotoxin capable of producing visual impairment or blindness, affecting the optic nerve and retina [8]. Some toxic chemical has the potential to change the characteristics of the receiving medium, affecting aquatic life such as planktons; phytoplankton, zooplankton, micro, and macrobenthic faunas, microbial community, macrophytes, and fishes, including shell and finfish groups) in water [9].

Different wastes and other emissions from various oil and gas exploration activities end up in the aquatic environment [10]. The released pollutants from these operations have been shown to have toxic effects, causing hematological and histological abnormalities, death as well as biota extinction [11]. The aquatic body has been the primary recipient of numerous anthropogenic and natural pollutants and harmful compounds, which are the primary drivers of aquatic biota population declines across the world [12]. Sub-lethal doses of most hazardous substances, on the other hand, are disastrous for fish population, composition, and density [13].

Upon dissolution, these compounds can quickly diffuse through fish membranes into the bloodstream, where they are transported to tissue cells and metabolized into more harmful components that act on exposed fish macromolecules [14]. Concerns about pollution affecting the health and genetic makeup of fin and shellfish supplies have grown in recent years [15]. 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 [16]. The findings might be useful in the creation of environmental policy and as a model for aquatic bio-monitoring.

Bioassays can be used to determine the degree of effluents' comparative toxicity potential or to discover active ingredients that cause biological effects [17]. Different organisms have been employed extensively to assess the environmental effects of various toxicants including continental and aquatic organisms [18]. Toxicologists and environmental scientists mostly use fish to measure the impact of wastewater and other chemicals on aquatic creatures [19,20]. Fish have been used in the water to assess the effects of toxicants such as pesticides and other chemical compounds [21]. The study aims to assess the acute toxicity of Analytical

Methanol on Guinean tilapia (*Tilapia guineensis*) Juvenile.

2. MATERIALS AND METHODS

2.1 Source of Test Organisms

Guinean tilapia (*Tilapia guineensis*) was used as the test organism. A total of 1,200 healthy juveniles of *T. guineensis* with a mean length of 15.20±0.2cm, and a mean weight of 10.34±0.3g was obtained from the Nigeria Institute for Oceanography and Marine Research (NIOMR), Buguma, Rivers State, Nigeria and transported in plastic containers to the Laboratory. This developmental stage (juvenile) of the test organism was chosen because of its high sensitivity to environmental stress [22].

2.2 Test Chemical

The test chemical analytical grade of methanol (CH_3OH) with a molecular weight of 32.04mol^{-1} and a density of 0.792g/cm^3) was collected in a 2.5little container from a chemical laboratory in Choba, Port Harcourt, and was stored under ambient conditions before usage in the laboratory. The chemical was available in liquid form and was treated directly in the test medium.

2.3 Acclimation of the test Organism

The fish were acclimated to laboratory conditions in a 150 liters capacity glass aquarium tank for 14 days at a room temperature of 27±0.3^{0}C to reduce mortality during the acclimatization period in the test laboratory and were fed with commercial fish feed twice daily with a 2 mm imported Coppens feed 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 [1]. During acclimation, the tank was aerated continuously. The water in each glass tank was replaced with tap water from the laboratory every 48 hours as suggested by [23]. 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 commencement of the definitive test procedures, a preliminary range-finding test was conducted using the toxicants in logarithmic concentrations to determine the most appropriate range of concentrations for exposure of the test organisms during the definitive toxicity test as recommended by [24]. Six (6) different concentrations of the analytical grade of methanol were prepared for this test and each tank was in triplicate with ten (10) juveniles per tank and was exposed for 24 to 96hours during which mortality rate was estimated [25] and the dead fish were discarded immediately to avoid pollution while the outcome provides the test concentrations for the definitive test.

2.5 Definitive Toxicity Test

The Toxicity assessments followed a standard procedure and guidelines [26]. Feeding was suspended 24 hours before and during the static assay and each test concentration (control (0 ml/l), 2.5 ml/l, 5.0ml/l, 10.0ml/l, 15.0ml/l, 20.0ml/l, and 25.0ml/l was held in an aquarium tank of 15 liters and filled to 10 mark. Ten fish were randomly selected and put in each of the test concentrations. Each treatment was in triplicates. Each treatment group of fish was exposed for 96hours during which mortality was determined at 24, 48, 72, and 96-hour periods, and dead fishes were removed immediately to avoid pollution. From the data, the concentration-response curves for fish mortality, the LC_{50}, and the 95 percent confidence intervals for test organism at 24, 48, 72, and 96-hour in a static system was derived. A static nonrenewal bioassay option was employed for this study.

2.6 *In-situ* Analysis of the Physico-chemical Parameters

The various concentrations of the Physico-chemical Parameters analyzed were Dissolved Oxygen (DO), Temperature, Hydrogen Ion Concentration (pH), Conductivity, and Total Dissolved Solids (TDS) using portable meters following American Public Health Association [27] procedures.

2.7 Determination of Mortality

The test organisms were proved dead when they do not respond to repetitive prodding. The mortality rate of the test organisms was calculated with the formula:

$$Mortality\ rate = \frac{Number\ of\ deadtest\ organisms}{Total\ number\ of\ test\ organism\ exposed\ to\ the\ treated\ produced\ water} x100$$

2.8 LC_{50} and Toxicity Factor Determination

Mortality was employed as an indicator of toxicity. Dead organisms were removed and counted for the following periods (0, 24, 48, 72, and 96h). The results at varying time intervals were subjected to a probit analysis.

The percentage mortality was transformed 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 were substituted in the equation Y= b + ax in which variables x and b (intercept) were obtained from the regression analysis. The LC50 was thereafter calculated. 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

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

3. RESULTS

3.1 Definitive Tests for *Tilapia guineensis* for 24 to 96 Hours

The number of mortalities recorded in the definitive test increased with an increase in the concentrations of the test chemical from 24 to 96hours of exposure (Figs. 1 to 5 and Table 1). Unlike the control, no mortalities were recorded and no variation was 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 and regression equation of *T. guineensis* exposed to different concentrations of Methanol for 96 hours. The LC_{50} of 3.750 was recorded for *T. guineensis* while the regression equation ($y = 1.5523x + 4.1095$ and $R^2 = 0.9595$) is represented on Table 1 while the plot of log of concentration are represented in Figs. 1 to 4.

3.2 Physiochemical Parameters after 96 hours

The data on the physicochemical parameters are presented in Table 3. There was a slight variation observed in the parameter when compared with the controlled (0ml/l) group.

The observed values of the temperature varied relatively ranging from 26.6^0C to 29.5^0C across all test concentrations with the highest value (29.5±0.61) in the highest concentration of 25.0 ml/l and the least in the controlled unit (26.6±0.06) while the Dissolved Oxygen decreased (DO) values varied from 3.5 to 5.2mg/l with a decrease in the concentration, the highest concentration of DO was observed in the control (5.2±0.01mg/l) and the least value (3.5±0.01mg/l) observed in the highest concentration of 25.0ml/l. The pH values varied from 5.9 to 6.8. the highest value was observed in the controlled unit (6.8±0.03ml/l) while the lowest value (5.9±0.0ml/l) was reported in the concentration unit of 25.0 ml/l indicating slight variation from alkaline to a slightly acidic state.

Table 1. Mean values of the mortality recorded after exposure for 24 to 96hours

Conc. (ml/l)	Mean mortality				% Mortality	% Survival
	24hrs	48hrs	72hrs	96hrs		
0	$0±0.01^a$	$0±0.001^a$	$0±0.00^a$	$0±0.000^a$	0	100
2.5	$0±0.01^d$	$2±0.001^c$	$3±0.33^b$	$4±0.577^a$	40	60
5.0	$1±0.01^d$	$2±0.001^c$	$3±0.58^b$	$5±0.577^a$	60	40
10.0	$2±0.01^d$	$4±0.001^c$	$6±0.33^b$	$7±0.000^a$	70	30
15.0	$2±0.33^d$	$4±0.001^c$	$6±0.33^b$	$8±0.000^a$	80	20
20.0	$4±0.01^d$	$5±0.001^c$	$7±0.33^a$	$9±0.577^a$	90	10
25.0	$6±0.33^c$	$7±0.001^{bc}$	$9±0.33^a$	$10±0.000^a$	100	00

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

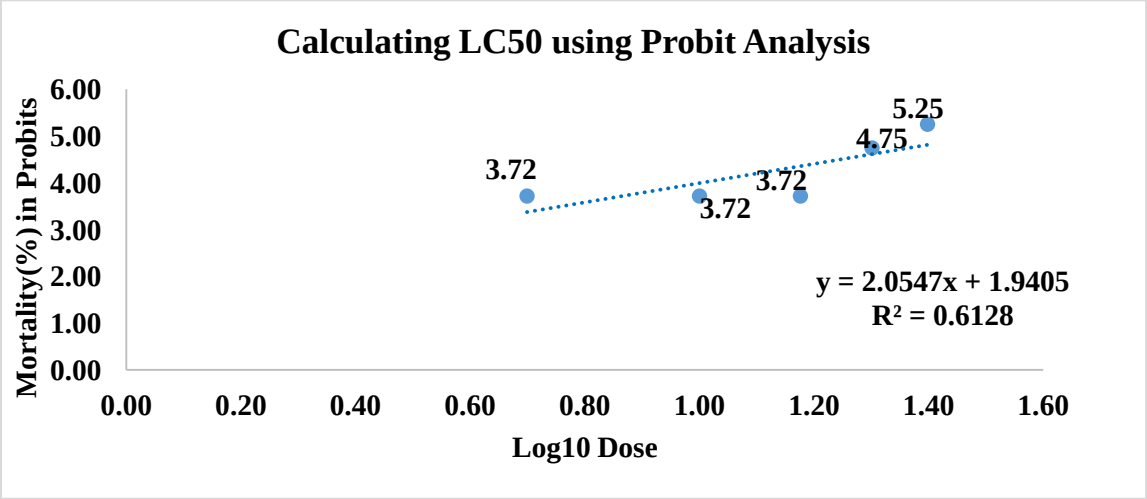

Fig. 1. The plot of log of concentration versus probit at 24Hrs for *Tilapia guineensis* exposed to exposure to Methanol

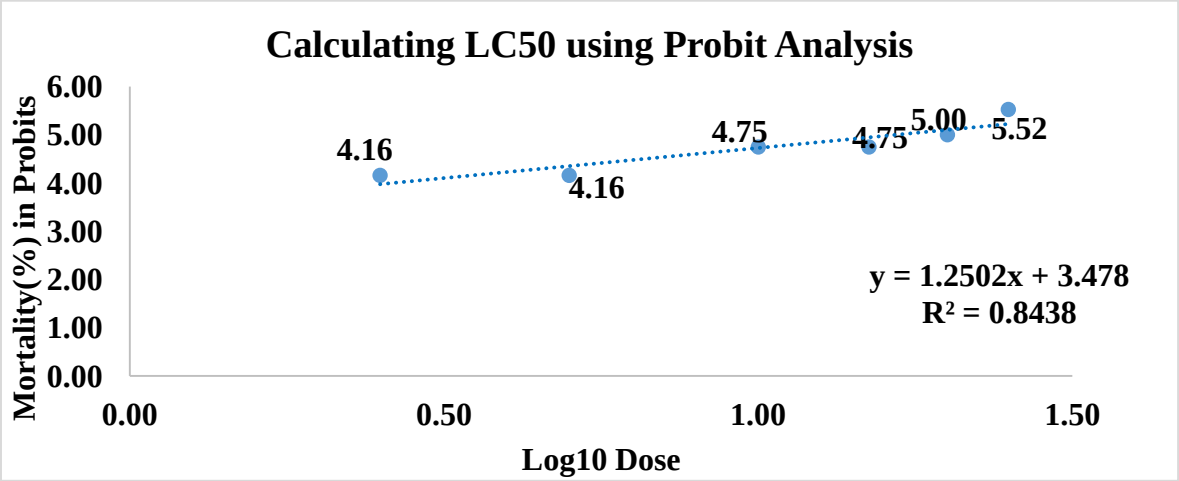

Fig. 2. The plot of log of concentration versus probit at 48Hrs for *Tilapia guineensis* exposed to exposure to methanol

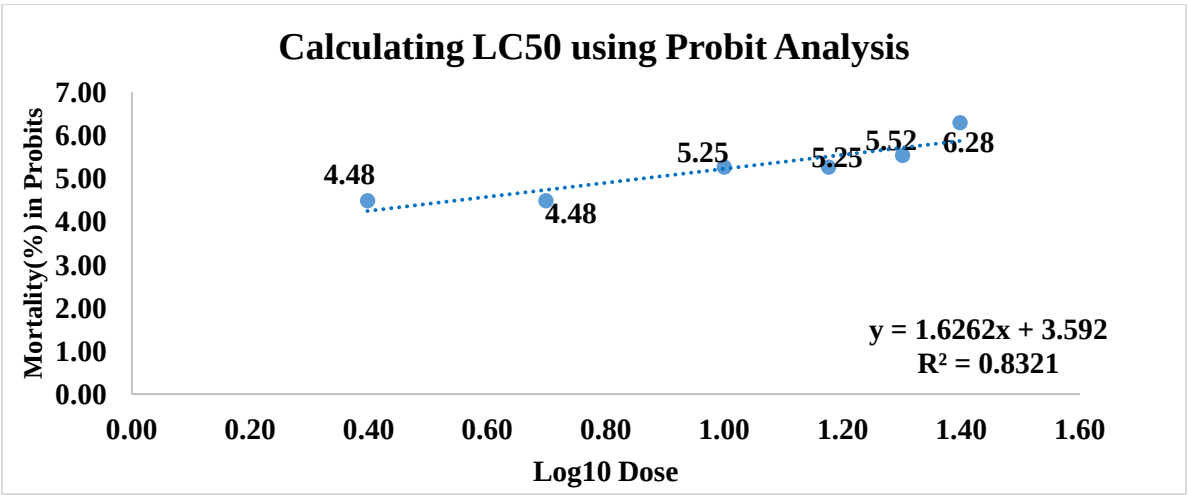

Fig. 3. The plot of log of concentration versus probit at 72Hrs for *Tilapia guineensis* exposed to exposure to Methanol

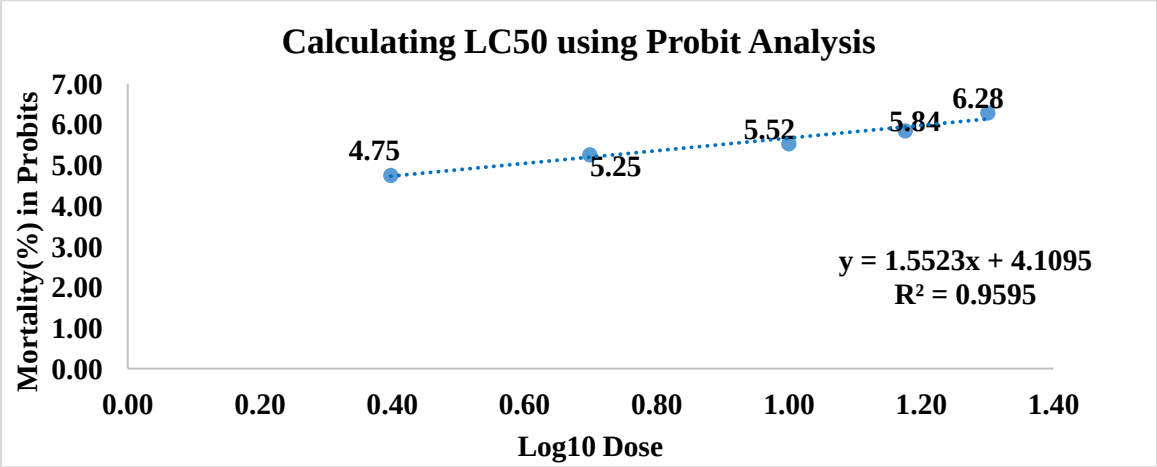

Fig. 4. The plot of log of concentration versus probit at 96Hrs for *Tilapia guineensis* exposed to exposure to Methanol

Table 2. The LC_{50} and the acute toxicity test after exposing *T. guineensis* to methanol

Time (hrs.)	LC_{50}	Lower 95%	Upper 95%	Regression equation
24	30.361	19.620	46.984	y = 2.0547x + 1.9405 R² = 0.6128
48	16.585	9.002	30.553	y = 1.2502x + 3.478 R² = 0.8438
72	7.369	4.563	11.901	y = 1.6262x + 3.592 R² = 0.8321
96	3.750	2.203	6.383	y = 1.5523x + 4.1095 R² = 0.9595

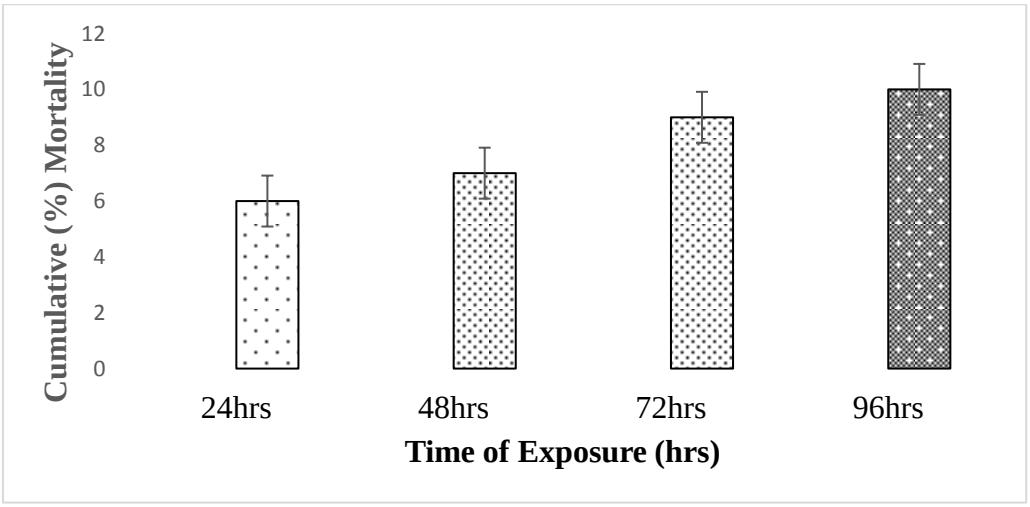

Fig. 5. Mortalities of *T. guineensis* exposed to different concentrations of methanol

The Total Dissolved Solids (TDS) value was highest (337.2±0.02 ml/l) in the test concentration with 25.0ml/l of the test chemical while the least value (180±0.31 ml/l) was observed in the controlled unit. The values range from 180 to 373.2ppm. The electrical conductivity varied from 267 to 453µs/cm. The conductivity increased from the lower concentration (0ml/l) to the higher concentration (25ml/l) of the toxicant. Where the highest value (453±0.01) was observed in the concentration of 25ml/l while the least was recorded in the controlled group (267±0.43).

Table 3. Mean water quality parameters after exposure for 96 hours

Parameters	Concentrations (ml/l)						
	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 (ºC)	26.6±0.06^{c}	27.2±0.26^{b}	27.4±0.23^{b}	27.8±0.36^{ab}	28.5±0.22^{b}	28.9±0.16^{a}	29.5±0.61^{a}
pH	6.8±0.03^{a}	6.7±0.00^{a}	6.5±0.03^{a}	6.2±0.03^{ab}	6.1±0.02^{b}	6.0±0.03^{b}	5.9±0.01^{b}
Conductivity (µS/cm)	267.0±0.0^{d}	284.1±0.01^{c}	314.0±0.02^{b}	356.1±0.01^{b}	367.1±0.02^{b}	422±0.01^{a}	453±0.01^{a}
Dissolved Oxygen (mg/l)	5.20±0.01^{a}	5.1±0.02^{a}	4.5±0.01^{ab}	4.3±0.01^{ab}	4.1±0.02^{b}	4.1±0.01^{b}	3.5±0.01^{c}
Total Dissolved Solid (ppm)	180.0±3.1^{d}	188.1±0.02^{cd}	192.2±0.03^{c}	188.6±0.06^{c}	198.5±0.06^{c}	272.1±0.01^{b}	373.2±0.02^{a}

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

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

4. DISCUSSION

4.1 Physiochemical Parameters

The rate of change in the physiological reproductive, and life cycle functions is regulated by the temperature of the water, which is a determining factor for aquatic life [28]. The temperature increased progressively from the lowest concentration to the highest with values ranging from 26.6^0C to 29.5^0C. There was a significant difference in the temperature value ($P<0.05$) observed in the parameter when compared with the controlled (0ml/l) group. Increases in water temperatures or broad fluctuations may be caused by metabolic processes, which can cause other physicochemical parameters to speed up, slow down, or halt entirely [29]. Similar results were reported by [30] in the physicochemical properties of the Aleto water body in Eleme, Rivers. [28] also recorded a similar result in selected rivers in Port Harcourt, Niger Delta of Nigeria. The increase in temperatures may be due to a large number of suspended solids from fecal waste from the fish and the time of exposure is believed to have been influenced by the intensity of sunlight at the time of collection of the result [31, 32].

The present investigation indicated that the concentration of Dissolved Oxygen (DO) decreased fluctuated from 3.5 to 5.2mg/l with a decrease in the concentration. Dissolved oxygen (DO) had a marked difference in the exposure media. A remarkable trend was observed in the different exposure media tanks, where the mean Dissolved oxygen (mg/L) level in the control tank (0ml/l) which was 5.2±0.01mg/l drastically dropped to (3.5±0.01mg/l) in the highest concentration of 25.0ml/l. The DO value was lower than the permissible limits of [33] and [34] of (>5mg/l) standard in all for the drinking and aquatic life. The reduction was consistent across all concentrations, with the highest concentration of 25.0mg/l greatest reduction. This suggests that the effluent is primarily an oxygen-limiting toxicant with a clear effect on the fish's health and physiology [35]. According to [33], this water having declined DO level may indicate the presence of pollution because the healthy water value of DO should be within the range of 5-14.6mg/l. Any water body with less than 5 or greater than 14.6 indicates the impairment of the water which is a problem for an aquatic body.

The pH values varied and ranged from 5.9 to 6.8. the highest value was observed in the controlled unit (6.8±0.03ml/l) while the lowest value (5.9±0.0ml/l) was reported in the highest concentration tank indicating a slight variation from alkaline to a slightly acidic state. The pH value was lower than the permissible limits of [33] and [34] of (6.5-8.5). This could be based on the effect of the increased effluent concentrations as a further decrease in the pH of the various tanks led to more slight acidity which will become harmful to the test organism as time goes by. However, the different concentrations in the tanks were not significant at $p < 0.05$ with permissible limits of [33] and [34]. The pH of most natural water, according to [32], ranges from 6.5 to 8.5, which is a divergence from the neutral 7.0 value due to the CO/bicarbonate balance.

The Total Dissolved Solids (TDS) value range between 180 to 373.2ppm across the test medium. The level of total dissolved solids varied significantly ($P<0.05$) as the test contraptions increased the values were within the recommended range of 500-1000 by [34]. TDS may affect the aesthetic quality of water, interfering with other chemical parameters [36]. [33] recommends that water containing more than 1000 mg L^{-1} of dissolved solids is not be used if other less mineralized supplies are available.

The electrical conductivity of water is a metric for ion concentration. The environment, mobility, and water sources all have an impact on ion concentrations. The bulk of soluble ions in surface water comes from rock mineral dissolution [37]. The conductivity increased from 267 and 453 S/cm, with the maximum value found at 25ml/l concentration. This value is higher than [34] drinking permitted limit of 400 S/cm. As a result of the chemical reaction with experimental water, the test water obtains a large amount of dissolved inorganic compounds in ionised form. This assertion aggresses [33] stated with the conductivity of water depends upon the concentration of ions and its nutrient status and variation in dissolved solid content. The chemical conductivity of water shows that it receives a large number of dissolved inorganic compounds in the ionised form [38]. The limited diluting impact of the higher concentration of the chemical utilised could explain the rise in conductivity seen in the research area [39].

4.2 Mortality

The acute toxicity results for *Tilapia guineensis* Juveniles Exposed to Methanol for 96 hours giving an LC_{50} value of 3.750ml/l with a concentration range from 2.5ml/l to 25ml/l. There was a significant increase in the numbers recorded with an increase in the concentrations of the test chemical from 24 to 96hours of exposure. The number of mortalities in *T. guineensis* increased as the concentration increased. There was an increase. percentage of mortality with an increased concentration. There was no mortality recorded in the control tank from 24 to 96 hours. Meanwhile, there were significant variations in the numbers of mortality across the different test concentrations of 2.5ml/l to 25ml/l after 96 hours. The high number of mortalities could be attributed to the obstruction of the respiratory structures of the test organism which is caused by the increasing concentrations [40]. The high number of mortalities could also be attributed to the assertion that the exposed test fish may have suffered from oxygen reduction brought by the organic compounds in the test chemicals [41]. The values fall within the range of methanol toxicity reported for other species as reported (Reyes- [42]). A comparison of methanol toxicity for other aquatic species as reported by [43] shows that *Nitocra spinipes, Mytulis edulis, and Alburnas alburnas,* which are all brackish/marine had an LC_{50} value of 15,900 mg/L as determined in this study. It's worth noting that they only tested for 24 hours and didn't double-check the methanol content. In our study, *T. guineensis* in the 25ml/l concentration did not survive beyond 72 hours and were dead at 96 hours.

Rodrigues [44] reported that after 96 hours of exposure to SWFs of diesel and gasoline on marine pejerrey *Odontesthes argentinensis,* the median lethal concentration after 96 hours (LC50) was 13.46% and 5.48%, compared to 15% in our current study. [45] investigated a 96 hrs. static acute toxicity test on the juveniles *C. gariepinus* (African catfish) and *C. anguillaris* (mudfish) on exposure to different concentrations of crude oil-polluted water and reported an LC_{50} value of while that of *C. garieinus* was 0.000219% of the highest exposed concentration and 0.0000122 % for *C. anguillaris* (mudfish). The variation in the numbers of mortality observed between *T. guineensis* and *O. niloticus* exposed to the same concentrations of Methanol for 96 hours was significant and could be attributed to the selective toxicity of Methanol to species of cichlid fish from both marine and freshwater aquatic bodies and then 95% confidence intervals at 24 and 48 h of exposure [46].

5. CONCLUSION

In the present study, the LC_{50} values showed that Methanol was toxic to the *Tilapia guineensis* juvenile. The number of mortalities increased with an increase in concentrations. Hence, it is recommended that there is a need for proper handling and discharging of this chemical into the aquatic environment, to manage the potential toxicity associated with its interaction with the aquatic life therein. Therefore, the discharge of methanol in the aquatic environment may result in the death of non-targeted aquatic organisms and edible species which in turn affect human health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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