Acute Toxicity of *Tilapia guineensis* Fingerlings Exposed to Treated Produced Water from the Niger Delta Region of Nigeria

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Abstract: This study evaluated the acute toxicity of *Tilapia guineensis* fingerlings exposed to treated produced water from the Niger Delta region of Nigeria. The produced water was obtained from an Oil and Gas facility within Mbo LGA, Akwa Ibom State, along the Calabar estuary in the Niger Delta Region of Nigeria while the fishes were sourced from African Regional Aquaculture Centre (ARAC), Buguma, in Rivers State, Nigeria. The fishes were allowed to acclimatize in an aquarium for 10 days. Range finding tests of the produced water toxicity was conducted. Based on the preliminary results, main test was defined at 3.125, 6.25, 12.5, 25, 50 and 100%. Reference experimental group was also established using potassium chloride at concentrations of 0.016%, 0.031%, 0.063%, 0.125% and 0.250%. The LC\(_{50}\) was calculated from the mortality value following standard procedure. Mortality rates increased significantly (p<0.05) as the concentration of the produced water and reference chemical (potassium chloride) increased. The LC\(_{50}\) values at 24 hours, 48 hours, 72 hours and 96 hours obtained were 50.33%, 24.70%, 17.90% and 13.68% respectively for produced water, and 0.16%, 0.08%, 0.06% and 0.05% respectively for the reference chemical. The LC\(_{50}\) values showed that the treated produced water is toxic to *Tilapia guineensis* fingerlings. Hence, there is need to properly treat produced water before it is discharged into surface water systems, in order to forestall potential toxicity associated with it.

Keywords: Aquatic environment, Produced water, *Tilapia guineensis*, Toxicity, Water quality

1. **INTRODUCTION**

During production of crude oil, water is generated, especially from the hydrocarbon deposits. This is described as “produced water” and is also called “oil field brine or formation water”. Produced water is basically natural water from the hydrocarbon reservoir and injected water during improved oil recovery. Hence, produced water is a combination of numerous chemical constituents including hydrocarbons, heavy metals, and radioactive substances, dissolved and suspended solids, amongst others. Some of its components are harmful to the receiving environment (mostly aquatic ecosystem).

In Nigeria, the Department of Petroleum Resources (DPR), a Regulatory body of the Oil and Gas Industry in Nigeria has stipulated guidelines and standards for the management and discharge of Produced water and has set limits within which waste water generated from the activities of the petroleum industry in Nigeria must meet. This is prior to its discharge into the aquatic ecosystem (brackish and saline water). In an endeavour to operate within these stipulated regulatory limits, most oil companies treat their wastewater before they are discharged into the environment. Nevertheless, studies have revealed that some of the treated produced water do not meet the DPR limits with respect to some of the parameters, before being discharged into the surrounding (Isehunwa and Onovae, 2011).

Similarly, some of these facilities discharge their produced water in “No Discharge Zones” of less than 200ft of water depth and 12 nautical miles distance from shore as stipulated by the Nigerian Guideline (DPR EGASPIN, 2018). The masses of these possibly harmful compounds and metals could be elevated in treated produced water, presenting some worries about chronic ecological harm which could negatively impact on water quality (both surface and groundwater) and sediment, devastating resident flora and fauna within impacted vicinity thus, directly and indirectly affecting the economic and public health of the oil companies’ host communities.
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For aquatic ecosystems, produced water could alter the features of the receiving medium and have resultant impacts on aquatic life (for example planktons - phytoplankton and zooplankton, micro and macro benthic organisms, microbial community, macrophytes and fishes, including shell and fin fish groups) in water.

Even though the micro pollutant constituents of produced water vary from well to well, some of the previously tested parameters include: total and dissolved organic carbon, petroleum hydrocarbons, phenols, trace/heavy metals, radioisotopes, production chemicals, nutrient content, etc. Of utmost environmental concern is the occurrence of saturated and aromatic petroleum hydrocarbons. Overall, aromatic hydrocarbons depict relatively greater water solubility than saturated hydrocarbons of similar molecular mass (Neff et al., 2011; Fakness et al., 2004). Produced water releases only contribute minute levels of organic components, thereby reflecting its limited influence in the depletion of dissolved oxygen in bottom waters (Bierman et al., 2007; Neff et al., 2011). However, a study by Inyang et al., (2018) reported that toluene (a produced water component) causes a modification in some blood cells and enzymes of adult *Clarias gariepinus*.

BTEX are found mostly in produced water that is untreated. Its extreme volatility explains its rapid loss when produced water is treated (Terrens and Tait, 1996; Aidar et al., 1999; Neff et al., 2011). However, the toxic chemical content of some produced waters, whether in low or high concentration, portends significant toxicity effects on sensitive aquatic organisms. This effect becomes pronounced, especially in shallow, enclosed coastal environments (Neff, 2002).

Due to the potential effects of produced water on aquatic organisms, it becomes necessary to test the effects of produced water discharges on some livelihood dependent organisms in the food chain found in the common recipient water source (saline water) which form the essential part of average delicacies of man.

Bioassays are useful in providing the extent of comparative toxicity potential of effluents or identifying active constituents that result in biological impacts (Krause et al., 1992). Several organisms have been extensively used to test the effect of different toxicants on the environment (including continental and aquatic organisms). Environmental scientists and toxicologists often use fish to test the effects of wastewater and other chemical substances on aquatic organisms (Inyang et al., 2018; Ajuzieogu et al., 2018). On water, fishes have been used to test the effect of toxicants i.e pesticides and other chemical substances (Aghoghovwia et al., 2019 a,b; Aghoghovwia and Izah, 2018 a,b; Inyang et al., 2017, 2016a,b; Ogamba et al., 2015 a, b; Akan et al., 2013; Ahmad, 2012; Okomoda, and Ataguba, 2011; Adedeji, 2010, 2009; Adamu and Francis, 2008).

This present study is aimed at assessing acute toxicity of *Tilapia guineensis* fingerlings exposed to treated produced water from the Niger Delta region of Nigeria

2. **MATERIALS AND METHODS**

2.1. **Source of Test Organisms and Habitat Water**

*Tilapia guineensis* used in this study were procured from African Regional Aquaculture centre ARAC, Buguma, Rivers State, Nigeria. The test organisms were conveyed to the laboratory in oxygen bags with their habitat water. The mean length and body weight of the test organisms employed for the acute toxicity test were 4.0 ± 0.5cm and 215 ± 5mg. Habitat water was also sourced from ARAC and collected into sterilized plastic containers.

2.2. **Source of Produced Water**

The toxicant used was treated produced water. Samples were collected in 25 litres Plastic container, from an Oil and Gas facility offshore Akwa Ibom State, along the Calabar estuary in the Niger Delta Region of Nigeria and transported to the laboratory.

2.3. **Acclimatization of Test Organisms**

Acclimatization was performed for the test organisms in two stages to reduce mortality during acclimatization period in the test laboratory. Prior to shipment of test organisms to the laboratory, acclimatization was conducted for three (3) days within the ARAC facility before onward delivery to the laboratory where the bioassay was carried out. In the laboratory, the fishes were allowed to acclimatize for 10 days.
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The test organisms were allowed to acclimatize in large circular plastic aquaria of 75 litres capacity at 24 +/- 2°C with the habitat water during which they were fed with commercial feed of 5% body weight daily. The aquaria containing the test organism and habitat water were continuously aerated using aquarium air pumps to enable them to get used to the laboratory temperature. Weak, injured and dead organisms were removed during the acclimatization period. The acclimatization water was changed with fresh habitat water every 72 hours to lessen the effect of waste products of metabolism in the water by the test organisms. Rate of mortality during acclimatization was used as an index to the healthy condition of the organisms.

2.4. Range Finding Test

Prior to commencement of the definitive test protocols, 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 definitive toxicity test. Five (05) different concentrations of produced water and Five (05) different concentrations of the reference chemical - Potassium chloride from manufacturer stock of 74.56 weights were prepared for this test. They were conducted for 48 hours and the outcome provided test concentrations for the definitive test.

2.5. Toxicity Assessment/Definitive Test

The Toxicity assessments followed standard procedure and the National Guideline (EGASPIN, 2018). A static renewal bioassay option was employed for this study. The test condition was a photoperiod of 12 hours of light and 12 hours of darkness.

Varying concentrations (3.125, 6.25, 12.5, 25, 50 and 100%) of Produced water were prepared in Rectangular 10cm x 10cm x 30cm 3 litres capacity glass aquaria based on preliminary range finding test result, using the same procedure as the range finding test but for a period of 96 hours. Potassium chloride served as the reference chemical while the habitat water was used as dilution water. Ten healthy fingerling test organisms from the acclimatization tanks were introduced into each unit in triplicates. The control consisted of a unit of the same set up without the toxicant, also in triplicate. The set up was allowed at a laboratory temperature of 24 +/- 2°C. The organisms were not fed during the test period.

2.6. In-Situ Analysis of the Effluents

The various concentrations of the produced water made during the toxicity test were analyzed for pH, Temperature, Dissolved Oxygen, Conductivity, Salinity and Total Dissolved Solid using portable meters following American Public Health Association (APHA, 2002) procedures.

2.7. Mortality Determination

The test organisms were proved dead when they did not respond to repeated prodding. Mortality rate of the test organisms was calculated with the formula:

\[
\text{Mortality rate} = \frac{\text{Number of dead test organisms}}{\text{Total number of test organism exposed to the treated produced water}} \times 100
\]

2.8. *Lc*<sub>50</sub> and Toxicity Factor Determination

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

The percentage mortality was transformed to probit using Finney’s table. The regression analysis was carried out for probit values against logarithm of the concentration using Microsoft excel. The resultant x value and intercept value were substituted in the equation Y= b + ax in which variable x and b (intercept) were obtained from the regression analysis. The LC<sub>50</sub> was thereafter calculated.

The Toxicity factors were computed by dividing the LC<sub>50</sub> of the toxicant by the LC<sub>50</sub> of the reference chemical.
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3. **Statistical Analysis**

Statistical analysis was carried out with 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 was used to determine the source of the variation. The charts were plotted using graph prism and Microsoft excel.

4. **Results and Discussion**

The effect of time and concentration on the physico-chemical characteristics of produced water test medium containing *Tilapia guineensis* are presented in Tables 1 and 2 respectively, while the p-values of time, concentration; and interaction of time and concentration are presented in Table 3.

The dissolved oxygen level ranged from 5.95 to 6.50 mg/L for time variables (Table 1) and 5.04 to 6.46 mg/L for concentration variables (Table 2). Statistically, there was significant variation (p < 0.05) in the dissolved oxygen level of time, concentration, as well as interaction of time and concentration variables (Table 3). The dissolved oxygen content of the test medium ranged between 5.00 and 6.50 mg/L (Figure 1) which is within the limit that supports the survival of most marine organisms.

The temperature of the produced water test medium ranged from 22.80 to 23.95°C for time variable (Table 1). There were significant variations (p < 0.05) across the time intervals. Waller Duncan multiple test showed that there were no significant variations at 24 hours, 48 hours and 72 hours time intervals. Based on the concentration variables, temperature range d from 23.12 to 23.79°C, being significantly different (p < 0.05) (Table 2). Concentrations at 0.00% and 6.25% were the sources of the observed significant variations (p < 0.05). Based on time and concentration interactions, there were no significant differences (Table 3). The temperature of the produced water test medium was between 22.00 and 24.00°C (Figure 2) which is within the limit that allows the survival of most marine organisms.

Table 1. Effect of time on the physico-chemical characteristics of produced water test medium with *Tilapia guineensis*

| Hours | DO, mg/l  | Temperature, ºC | pH       | Salinity as Chloride, mg/L | Conductivity, µS/cm | TDS, mg/L |
|-------|-----------|-----------------|----------|----------------------------|---------------------|-----------|
| 24    | 5.95 ± 0.39a | 23.95 ± 0.98b   | 7.37 ± 0.22a | 8,237.38 ± 344.02a          | 24,196.67 ± 807.49a | 14,708.10 ± 280.21a |
| 48    | 6.08 ± 0.44b | 23.54 ± 0.30b   | 7.38 ± 0.24a | 8,241.43 ± 311.21a          | 24,228.37 ± 841.51a | 14,692.38 ± 244.07a |
| 72    | 6.09 ± 0.44b | 23.70 ± 0.25b   | 7.45 ± 0.22a | 8,215.24 ± 279.15a          | 24,229.52 ± 801.06a | 14,733.33 ± 182.22a |
| 96    | 6.50 ± 0.64c | 22.80 ± 0.55a   | 7.37 ± 0.21a | 8,253.81 ± 255.76a          | 24,219.05 ± 775.64a | 14,745.71 ± 292.58a |

The values are arranged as mean ± standard deviation (descriptive statistics) (n=21). The same letters along the column indicate no significant variations at p = 0.05 according to Waller-Duncan test statistics.

Table 2. Effect of concentration on the physico-chemical characteristics of produced water test medium with *Tilapia guineensis*

| Concentration | DO, mg/l  | Temperature, ºC | pH       | Salinity as Chloride, mg/L | Conductivity, µS/cm | TDS, mg/L |
|---------------|-----------|-----------------|----------|----------------------------|---------------------|-----------|
| 0 %           | 6.19 ± 0.14b | 23.79 ± 0.54b   | 7.15 ± 0.06a | 8,278.75 ± 162.70b         | 24,243.33 ± 346.39b | 14,521.67 ± 248.70a |
| 3.125 %       | 6.32 ± 0.19c | 23.58 ± 0.54ab  | 7.32 ± 0.12ab | 8,230.00 ± 208.97b         | 24,541.67 ± 302.89b | 14,605.83 ± 346.37a |
| 6.25 %        | 6.36 ± 0.27d | 23.12 ± 1.17a   | 7.37 ± 0.15bc | 8,335.00 ± 210.95b         | 24,533.33 ± 320.04b | 14,730.00 ± 192.87ab |
| 12.50 %       | 6.46 ± 0.29e | 23.38 ± 0.60ab  | 7.50 ± 0.18bc | 8,332.50 ± 244.06b         | 24,541.67 ± 452.18b | 14,711.67 ± 207.58ab |
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|                | 25.00 % | 50.00 % | 100 %   |
|----------------|---------|---------|---------|
|                | Mean    | Mean    | Mean    |
|                | ± Standard Deviation (n=12) | ± Standard Deviation (n=12) | ± Standard Deviation (n=12) |
| Dissolved oxygen | 6.36 ± 0.36d | 6.37 ± 0.30d | 5.04 ± 0.02a |
| Temperature    | 23.63 ± 0.86ab | 23.32 ± 0.65ab | 23.70 ± 0.34b |
| pH             | 7.47 ± 0.15bc | 7.55 ± 0.21c | 7.40 ± 0.34bc |
| Salinity as chloride | 8,360.00 ± 150.82b | 8,382.50 ± 247.57b | 7,740.00 ± 162.70a |
| Conductivity   | 24,541.67 ± 370.40b | 24,627.50 ± 206.23b | 22,500.00 ± 426.40a |
| Total dissolved solid | 14,774.17 ± 145.63ab | 14,775.00 ± 155.88ab | 14,920.83 ± 234.46b |

The values are arranged as mean ± standard deviation (descriptive statistics) (n=12). The same letters along the column indicate no significant variations at p = 0.05 according to Waller-Duncan test statistics.

Table 3. *P*-value of the physico-chemical characteristics of produced water test medium with *Tilapia guineensis*

| Parameters                  | Time | Concentration | Interaction of time and concentration |
|-----------------------------|------|---------------|---------------------------------------|
| Dissolved oxygen            | 0.000| 0.000         | 0.000                                 |
| Temperature                 | 0.000| 0.049         | 0.210                                 |
| pH                          | 0.608| 0.001         | 0.998                                 |
| Salinity as chloride        | 0.963| 0.000         | 0.999                                 |
| Conductivity                | 0.993| 0.012         | 1.000                                 |
| Total dissolved solid       | 0.904| 0.000         | 0.998                                 |

**Figure 1.** Dissolved oxygen at varying concentrations of produced water test medium used for *Tilapia guineensis* toxicity testing

**Figure 2.** Temperature at varying concentrations of produced water test medium used for *Tilapia guineensis* toxicity testing
The pH of the test medium ranged from 7.37 to 7.45 for time variable (Table 1). There were no significant variations (p>0.05) across the time intervals. The pH of concentration variables ranged from 7.15 to 7.55, being significantly different (p<0.05). Post hoc showed that there were significant variations (p < 0.05) between 00.00% and 50.00% (Table 2). In addition, there was no significant interaction (p > 0.05) between the time and concentration variables (Table 3). The pH of the test medium was between 7.00 and 7.60 (Figure 3) which is within the limit that allows the survival of most aquatic organisms.

Salinity as chloride concentration of the test medium ranged from 8,215.24 to 8,253.81 mg/L for time variable (Table 1). There were no significant variations (p>0.05) across the time intervals. The salinity level for the concentration variables ranged from 7,740.00 to 8,382.50 mg/L, being insignificantly different (p>0.05) apart from 100% concentration (Table 2). In addition, there was no significant interaction (p>0.05) between the time and concentration variables (Table 3). The test medium salinity level ranged between 7,700 and 8,400 mg/L (Figure 4) which is within the limit that allows the survival of most aquatic species in a saline environment.

The conductivity level of the produced water test medium ranged from 24,196.67 to 24,229.52 µS/cm for time consideration (Table 1). There were no significant variations (p>0.05) across the diverging time intervals. The conductivity level of the concentration variables ranged from 22,500.00 to 24,627.50 µS/cm, being insignificantly different (p<0.05) apart from the 100% concentration variable (Table 2). In addition, there was no significant interaction (p>0.05) between the time and concentration considerations (Table 3). In general, conductivity level ranged from 22,400.00 to 25,000.00 µS/cm (Figure 5) which is within values previously reported in marine ecosystem.

Figure 3. pH at varying concentrations of produced water test medium used for Tilapia guineensis toxicity testing

Figure 4. Salinity as chloride at varying concentrations of produced water test medium used for Tilapia guineensis toxicity testing
The total dissolved solids content of the test medium ranged from 14,692.38 to 14,745.71 mg/L for time consideration (Table 1). There were no significant variations (p>0.05) across the time intervals. The total dissolved solid level of the concentration variable ranged from 14,521.67 to 14,920.83 mg/L, being significantly different (p<0.05). Waller Duncan multiple comparison showed that there were no significant variations (p>0.05) between 0.00% and 3.125% concentration variables, and between 0.00%, 3.125%, 6.25%, 12.50%, 25.00% and 50% concentration variables. On the other hand, significant variation (p<0.05) existed between 0.00% and 3.125% with 100% concentration variables (Table 2). In addition, there was no significant interaction (p>0.05) between the time and concentration variables (Table 3). In general, total dissolved solids content of the test medium ranged from 14,500.00 to 15,000.00 mg/L (Figure 6). The total dissolved solids content is within levels that are supportive to most aquatic life forms found within marine environments.

Figure 5. Conductivity at varying concentrations of produced water test medium used for Tilapia guineensis toxicity testing

Figure 6. Total dissolved solids at varying concentrations of produced water used for Tilapia guineensis toxicity testing

The effects of varying concentration and time of produced water on Tilapia guineensis toxicity are presented in Tables 4 and 5, while the p-value is presented in Table 6.

The mortality rate at 3.125 %, 6.25 %, 12.50 %, 25.00 % and 100 % concentrations were 3.33 %, 12.50 %, 23.33 %, 28.33 %, 55.83 % and 100.00%, respectively. Statistically, there was significant difference (p<0.05) across the various concentrations. Across the time interval, mortality rate at 24 hours, 48 hours, 72 hours and 96 hours was 21.11 %, 33.89 %, 42.78 % and 51.11 % respectively, being significantly different (p<0.05).
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Table 4. The effects of varying concentrations of produced water on *Tilapia guineensis*

| Concentration, % | *Tilapia guineensis* (n=12) |
|------------------|-----------------------------|
| 3.125            | 3.33±4.92a                  |
| 6.25             | 12.50±9.65b                 |
| 12.5             | 23.33±15.57c                |
| 25               | 28.33±20.38d                |
| 50               | 55.83±23.14e                |
| 100              | 100.00±0.00f                |

The values are arranged as mean ± standard deviation (descriptive statistics). The same letters along the column indicate no significant variations at p = 0.05 according to Waller-Duncan test statistics.

Table 5. The effects of varying time of produced water on *Tilapia guineensis*

| Time, Hours | *Tilapia guineensis* (n=18) |
|-------------|-----------------------------|
| 24          | 21.11±37.71a                |
| 48          | 33.89±33.63b                |
| 72          | 42.78±34.61c                |
| 96          | 51.11±32.16d                |

The values are arranged as mean ± standard deviation (descriptive statistics); the same letters along the column indicate no significant variations at p=0.05 according to Waller-Duncan test statistics.

Table 6. *P*-values of *Tilapia guineensis* exposed to produced water

| Test Organism | Concentration | Time | Interaction |
|---------------|---------------|------|-------------|
| *T. guineensis* | 0.000         | 0.000| 0.000       |

Figures 7 and 8 represent the concentration-mortality rate of the various time intervals of *Tilapia guineensis* exposed to produced water and the reference chemical, respectively.

The mortality rate at 3.125 %, 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100 % concentrations were 0.00 %, 0.00 %, 0.00 %, 0.00 %, 26.67% and 100 % respectively (at 24 Hrs), 0.00 %, 10.00%, 23.33%, 26.67%, 43.33% and 100%, respectively (at 48 hours), 3.33%, 16.67%, 30.00%, 33.33%, 73.33% and 100%, respectively (at 72 hours), and 10.00%, 23.33%, 40.00%, 53.33%, 80% and 100%, respectively (at 96 hours) for treated produced water (Figure 7).

**Figure 7.** Concentration-mortality rate of the various time intervals of *Tilapia guineensis* exposed to produced water

For the reference chemical; at 0.016%, 0.031%, 0.063%, 0.125% and 0.250% concentration, the mortality rate were 0.00%, 0.00%, 3.30%, 26.67% and 86.67% respectively (at 24 Hrs), 0.00%, 10.00%, 13.33%, 16.67%, 43.33% and 100.00%, respectively (at 48 hours), 6.67%, 20.00%, 26.67%, 60.00% and 100%, respectively (at 72 hours), and 10.00%, 33.33%, 46.67%, 76.67% and 100.00% respectively (at 96 hours) (Figure 8).
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**Figure 8.** Concentration-mortality rate of the various time intervals of *Tilapia guineensis* exposed to potassium chloride (reference chemical).

The Plot of Probit against Log of Concentration with the Regression equation at different time intervals for *Tilapia guineensis* exposed to produced water and potassium chloride (reference chemical) are shown in Figures 9 to 16.

**Figure 9.** Plot of Log of Conc versus Probit at 24Hrs for *Tilapia guineensis* exposed to Produced water

**Figure 10.** Plot of Log of Conc versus Probit at 48Hrs for *Tilapia guineensis* exposed to Produced water
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**Figure 11. Plot of Log of Conc versus Probit at 72Hrs for *Tilapia guineensis* exposed to Produced water**

**Figure 12. Plot of Log of Conc versus Probit at 96Hrs for *Tilapia guineensis* exposed to Produced water**

**Figure 13. Plot of Log of Conc versus Probit at 24Hrs for *Tilapia guineensis* exposed to Potassium chloride (Reference chemical)**
Figure 14. Plot of Log of Conc versus Probit at 48Hrs for Tilapia guineensis exposed to Potassium chloride (Reference chemical)

Figure 15. Plot of Log of Conc versus Probit at 72Hrs for Tilapia guineensis exposed to Potassium chloride (Reference chemical)

Figure 16. Plot of Log of Conc versus Probit at 96Hrs for Tilapia guineensis exposed to Potassium chloride (Reference chemical)
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The LC$_{50}$ values of *Tilapia guineensis* exposed to produced water and potassium chloride are presented in Table 7.

**Table 7. LC$_{50}$ values (%) of *Tilapia guineensis* exposed to produced water and potassium chloride**

| Time, Hours | Toxicant | *Tilapia guineensis* |
|-------------|----------|----------------------|
| 24          | PW       | 50.33                |
|             | KCl      | 0.16                 |
| 48          | PW       | 24.70                |
|             | KCl      | 0.08                 |
| 72          | PW       | 17.90                |
|             | KCl      | 0.06                 |
| 96          | PW       | 13.68                |
|             | KCl      | 0.05                 |

PW = Produced water; KCl = Potassium chloride

At 24 hours, 48 hours, 72 hours and 96 hours the LC$_{50}$ values were 50.33%, 24.70%, 17.90% and 13.68% respectively for produced water, and 0.16%, 0.08%, 0.06% and 0.05% respectively for the reference chemical.

Basically, as the test duration increases, the LC$_{50}$ value decreases. This trend has been narrated in acute toxicity of fishes exposed to pesticides (Aghoghovwia et al., 2019 a; Aghoghovwia & Izah 2018 a,b; Ojesanmi et al., 2017), cassava mill effluents (Seiyaboh and Izah, 2018), palm oil mill effluents (Aghoghovwia et al., 2019b).

The toxicity factor of *Tilapia guineensis* exposed to produced water and potassium chloride are presented in Table 8. At 24 hours, 48 hours, 72 hours and 96 hours, the toxicity factors were 314.56, 308.75, 298.33 and 273.60 respectively.

**Table 8. Toxicity factors of *Tilapia guineensis* exposed to produced water and potassium chloride**

| Time, Hours | *Tilapia guineensis* |
|-------------|----------------------|
| 24          | 314.56               |
|             | 308.75               |
| 72          | 298.33               |
| 96          | 273.60               |

The mortality rate and LC$_{50}$ values indicate that the acute toxicity was prolonged as the percentage concentration of the produced water increased. At the point when test organisms were subjected to produced water, depending on the chemical qualities of the produced water, it could cause a modification in some hematological indices, biochemicals, electrolytes and metabolites that assume basic functions in test organisms (Inyang et al., 2017, 2016 a, b; Banaee et al., 2013). Authors have reported that toxicants could harm tissue and lead to oxidative pressure in exposed organisms (Izah and Richard, 2019; Banaee et al., 2013). The lower LC$_{50}$ values of the reference chemical however suggest that it is more potent compared to the treated produced water. The mortality level induced by the produced water suggests that it could affect fishes especially when their concentration is high.

5. **Conclusion**

This study investigated the acute toxicity of *Tilapia guineensis* fingerlings exposed to treated produced water from the Niger Delta region of Nigeria. The fishes were obtained from African Regional Aquaculture centre (ARAC), Buguma, Rivers State, Nigeria, while the treated produced water was obtained from an Oil and gas facility, Offshore Akwa Ibom state along the Calabar Estuary, within the Niger Delta region of Nigeria. The study found that as the produced water and reference chemical concentration increased the mortality rates increased. The LC$_{50}$ values showed that treated produced water in the Niger Delta still induce varying levels of mortality on *Tilapia guineensis*. Hence, there is need to properly treat the produced water before discharging it into the aquatic ecosystem.

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**Citation:** O. S. E. Opete, et.al. “Acute Toxicity of Tilapia Guineensis Fingerlings Exposed to Treated Produced Water from the Niger Delta Region of Nigeria”. International Journal of Research Studies in Biosciences (IJRSB), 7(12), pp. 8-21. DOI: http://dx.doi.org/10.20431/2349-4050.0712002

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