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Comparative toxicity of effluent to *Tilapia guineensis* and *Clibanarius africanus*

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Abstract

The continuous discharge of effluent into the adjoining environment is a major source of aquatic pollution which can trigger critical problems in the aquatic environment including terminating life forms directly or indirectly. This study aimed to comparatively investigate the toxicity of effluent to *Tilapia guineensis* (fish) and *Clibanarius africanus* (hermit crab). Treated effluent sample was sourced from a petroleum producing industry that discharges its effluent into Bonny estuary, Bonny while the test organisms were obtained from National Institute for Oceanography and Marine Research (NIOMR), Buguma both in Rivers State, Nigeria. The range of pH (7.24 – 7.59), salinity (17.46 – 27.31psu), temperature (22.00 – 23.42°C), dissolved oxygen (2.93 – 3.79mg/l) total dissolved solid (1479 – 19980 mg/l) and electrical conductivity (2957 – 29840uscm^{-1}) of the test medium for the different concentrations of effluent were within the limits that permits survival of aquatic organisms. *Clibanarius africanus* displayed more tolerance than *Tilapia guineensis*. The median lethal concentration (LC_{50}) of the treated effluent at 96hours for both *Tilapia guineensis* and *Clibanarius africanus* were very high (409,824mg/l and 299,008mg/l respectively) which implies that the treated effluent was not toxic to both organisms hence would not cause fatal effect on the test organisms. Thus, effluent treatment compliance and management is essential for protection of sensitive aquatic areas.

Keywords: Toxicity; Effluent; *Tilapia guineensis*; *Clibanarius africanus*

1. Introduction

Pollution of the oceans is widespread, worsening and in most countries crudely controlled. The pollutants are usually complex mixture of toxic metals, plastics, manufactured chemicals, agricultural wastes, pharmaceutical chemicals and petroleum industrial wastes. It reaches the oceans through rivers, runoff and direct discharges. Effluent as defined by United States Environmental Protection Agency (USEPA) is a liquid (wastewater)- treated or untreated that flows out of industrial outfall. The discharge of this effluent is considered water pollution as it may carry pollutants such as fats/oils/grease (FOG), chemicals, detergents, solids and other wastes based on the possible source – manufacturing industries, mining industries, oil & gas extraction and service industries [1][2].

The treated effluent from most industries is in most cases discharged into adjoining environment. The inability to effectively and efficiently manage vast amount of waste generated by various anthropogenic activities particularly in developing countries including Nigeria has created critical problems in aquatic environment (alters the physical, chemical and biological nature of receiving water body). Improper treatment of effluent released into water bodies has been creating toxic effects on all type of life forms directly or indirectly [3].

The most immediate effect of wastewater on the environment is when it contributes toward the contamination and destruction of natural habitats by exposing them to harmful chemicals. It poses hazards that can be toxic, corrosive, reactive and acidic. Toxic compounds in the effluent can disrupt aquatic ecosystem. For instance, the presence of large amount of biodegradable substance will cause the microbes to begin breakdown process thereby utilizing a lot of

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dissolved oxygen of which the dissolved oxygen can become depleted and this becomes life threatening for the higher organisms.

In monitoring the biological effects of toxicants, many methods are available. One of such methods is acute toxicity bioassay. Acute toxicity is the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time. In this test, the strength of the toxicant is determined by the responses of living organisms to it. With this method, direct measurement of the toxic properties of the toxicants can be conducted using one or a few species of organisms that are selected due to their relative greater sensitivity, ease of culture, and are considered representatives of the indigenous organisms in the recipient water. *Tilapia guineensis* and *Clibanarius africanus* are such selected organisms as outlined by Nigerian Upstream Petroleum Regulatory Commission (NUPRC) [4].

Clibanarius africanus (hermit crabs) decapods crustaceans of the super family Paguroidea, live at a range of depths from shallow coral reefs and shorelines to deep bottoms. This benthos is a useful bio-indicator as they are visible to the naked eye and easy to monitor because they can be sampled quantitatively and also respond to man-made disturbances. Their sedentary nature makes it possible for them to readily imbibe and accumulate any xenobiotic compounds and other stressors released into the water body [5].

Tilapia guineensis inhabits creeks, lagoons and other coastal waters of West Africa. It is considered a relatively stenothermal species with a temperature ranged of 14°C to 33^{o}C. Also, it grows and reproduces in salinities of 0-35ppt [6].

To track the response of the organisms when exposed to toxicants, an index/clue is set. These clues include: inability of movement and mortality. This study aimed to comparatively assess the toxicity of effluent to *Tilapia guineensis* and *Clibanarius africanus*

2. Materials and Methods

2.1. Source of test toxicants

The reference chemical-potassium chloride was obtained from a licensed chemical store in Choba, Rivers State.

The treated effluent was collected just before discharge into the recipient water at the point of discharge from a petroleum producing industry that discharges its effluent into Bonny Estuary, Bonny, Rivers State. The samples were collected into pre-cleaned amber bottles and transported to the laboratory in ice-packed coolers.

2.2. Source of test organisms

Juveniles of *Tilapia guineensis* (weight range: 3.3g to 25.6g; length range: 6.9 to 12.1cm) were obtained from National Institute for Oceanography and Marine Research (NIOMR) at Buguma, Rivers State, Nigeria and transported in airbags to the laboratory.

Active *Clibanarius africanus* were obtained from NIOMR and were transported to the laboratory in mesh holding unit. The habitat mud of the hermit crabs was also collected from same site and placed in holding tanks as substrate.

The method of Luke and Odokuma [7] was employed for acclimatization of the fishes and the hermit crab.

2.3. Physicochemical analysis

The standard analytical procedures adopted for the analysis of heavy metals, nutrients and hydrocarbon followed APHA and ASTM protocols. The unstable parameters were measured *in-situ*.

2.3.1. Acute toxicity testing

Employing the static non-renewal method, the acute toxicity test was carried out in line with Nigerian Upstream Petroleum Regulatory Commission (NUPRC) guidelines detailed in part IIIE, section 4.3.2 of *Environmental Guidelines and Standards for Petroleum Industry in Nigeria* and United States Environmental Protection Agency (USEPA) [8]. Ten active juvenile fishes and ten active hermit crabs of similar sizes were exposed in duplicates into aerated glass tanks of various dilutions/concentrations (logarithmic concentrations) of the whole effluent or reference chemical. The range finding test was conducted first to reduce the number of dilutions of the toxicants used in the definitive toxicity test.

This range finding was done with the aim of determining the lowest concentration of the effluent/chemical that could kill 100% of the test organisms within 24hours and the highest concentration that would have no effect (mortality) on the test organisms. The concentrations used for the definitive test are displayed in table 1. The 0ppt concentration tanks were without toxicants (recipient water-seawater) and served as control tanks. Mortality which was used as an index for scoring toxicity was assessed for 96hours at intervals of 24hours. The fishes (juvenile *Tilapia guineensis*) and hermit crabs (*Clibanarius africanus*) were considered dead when they failed to show any sign of movement or response to gentle prodding with sharp object.

Table 1 Concentrations of toxicants used for the bioassay

Test organism	Treated Effluent	Potassium chloride
Tilapia guineensis	0, 200, 400, 600, 800 and 1000ppt.	0, 20, 40, 60, 80 and 100ppt
Clibanarius africanus	0, 200, 400, 600, 800 and 1000ppt.	0, 20, 40, 60, 80 and 100ppt

2.4. Statistical analysis

The mortality rate of the test organisms was calculated by multiplying the quotient of number of dead test organisms and total number of test organism exposed to the test toxicants by 100. This is depicted below:

$$\%\text{Mortality} = \frac{Ci - Cl}{Ci} \times 100$$

Where C_i = initial count of organism and C_l is the count of organism alive after exposure. Toxicological data involving mortality rate were then analyzed using Probit analysis. The toxicity indices derived were median lethal concentration (LC_{50}), No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC). The toxicity factors were computed by dividing the median lethal concentration of the toxicant by the median lethal concentration of the reference chemical.

3. Results and Discussion

Table 2 Treated Effluent and Dilution water (seawater)

Parameters	Treated Effluent	Dilution water
pH	7.49	7.24
EC ($uscm^{-1}$)	655	39650
TDS (mg/l)	328	19830
DO (mg/l)	5.08	3.22
Turbidity	4.8	6.2
Temperature (°C)	27.21	27.38
Salinity (psu)	19.46	25.23
SO_4^{2-} (mg/l)	3.72	48.96
NO_3^- (mg/l)	0.33	0.28
NO_2^- (mg/l)	0.89	
NH_3 (mg/l)	2.20	0.04
PO_4^{3-} (mg/l)	4.41	0.02
TSS (mg/l)	1.23	0.09
TPH (mg/l)	0.25	0.001
THC (mg/l)	0.3	<0.1

PAH (mg/l)	0.005	<0.01
BTEX (mg/l)	<0.001	<0.01
Chloride (mg/l)	346.1	25369.2
Total hardness(mg/l)	100.2	420
Ca^{2+} (mg/l)	28.06	946.9
Mg^{2+}(mg/l)	15.19	129.8
K^{+}(mg/l)	12.36	348.2
Cu^{2+}(mg/l)	0.11	0.05
Zn^{2+}(mg/l)	0.42	0.34
Mn^{2+}(mg/l)	0.10	0.25
Cr^{3+}(mg/l)	0.04	0.15
Pb^{2+}(mg/l)	0.03	0.09
Fe^{2+}(mg/l)	0.39	0.39
Co^{2+}(mg/l)	0.03	0.19

The quality of any water sample is dependent on its physiochemical properties. The physiochemical properties of the treated effluent and the sea water which was used as the dilution water are displayed in Table 2. The values recorded for pH, temperature, salinity and total dissolved solid were within the Environmental Guidelines and Standards for the Petroleum Industry (EGASPIN) [4] limits and conforms with findings of Ntongha & Omokaro [9]. The value recorded for the conductivity of the treated effluent was significantly lower in comparison to the value recorded for the dilution water. The lower the conductivity, the purer the water sample. The dissolved oxygen values were higher than 3.0mg/L as stipulated by NUPRC thus, monitoring is required during treatment and discharge of effluent to ensure safe levels for the recipient environment as high dissolved oxygen can lead to dead zones in the biological floc.

The effect of time and concentration on the physicochemical characteristics of treated effluent test medium containing the test organisms- *Tilapia guineensis* and *Clibanarius africanus* are graphically represented in figures 1 to 12.

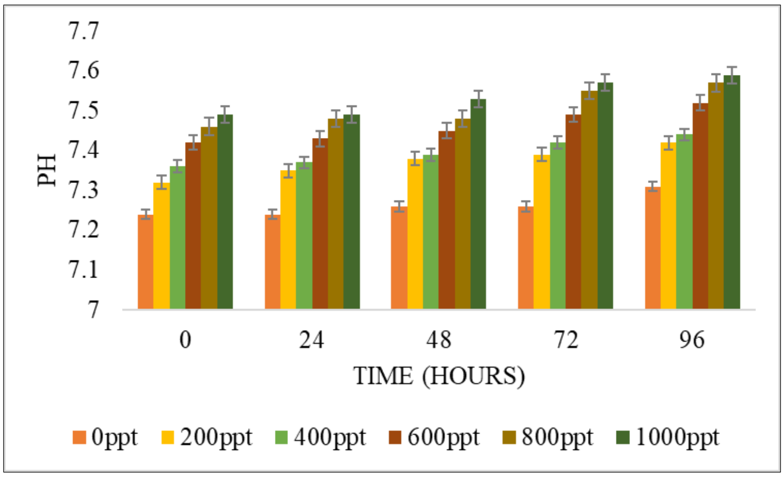

Figure 1 pH at varying concentration of treated effluent test medium for *Tilapia guineensis*

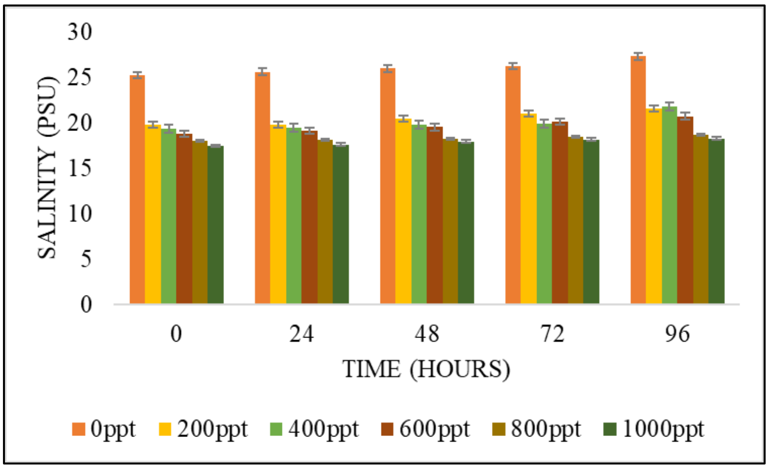

Figure 2 Salinity at varying concentration of treated effluent test medium for *Tilapia guineensis*

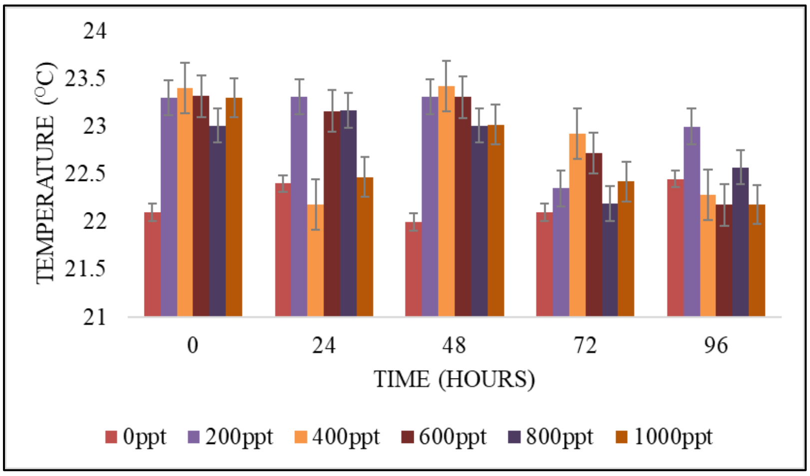

Figure 3 Temperature at varying concentration of treated effluent test medium for *Tilapia guineensis*

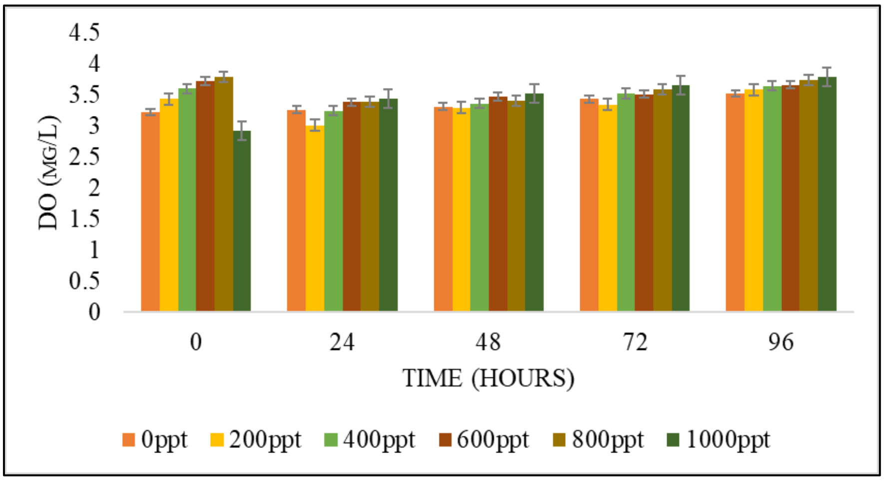

Figure 4 DO at varying concentration of treated effluent test medium for *Tilapia guineensis*

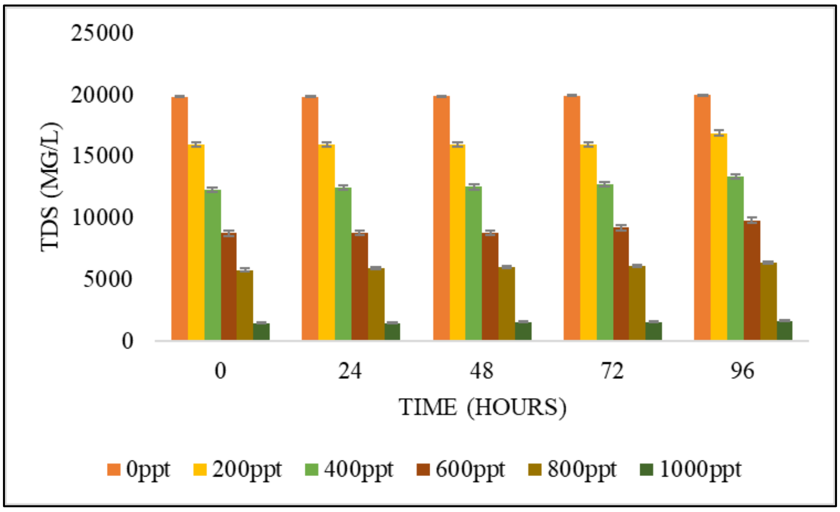

Figure 5 TDS at varying concentration of treated effluent test medium for *Tilapia guineensis*

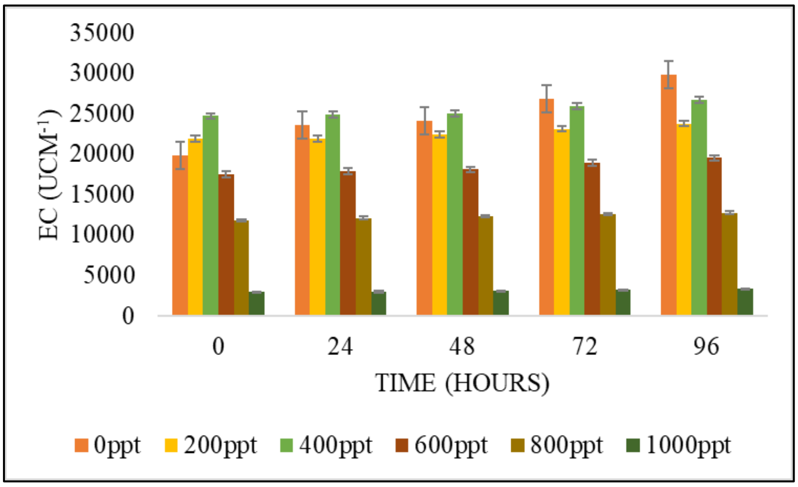

Figure 6 EC at varying concentration of treated effluent test medium for *Tilapia guineensis*

The acute toxicity test medium for the different concentrations and varying exposure time were analyzed. The pH ranged from 7.32 to 7.59 for that of *Tilapia guineensis* and 7.30 to 7.49 for those of *Clibanarius africanus*, which is within the limit that allows the survival of most aquatic organisms.

The values of the salinity were within the limit that permits the survival of most saline aquatic species as the test media of which *T. guineensis* and *C. africanus* were exposed to had a salinity range of 17.46ppt to 21.79ppt

The temperature of the test media was within the range of 21-22.9°C while the values for the dissolved oxygen were slightly above the EGASPIN stipulated limit.

The values for Total Dissolved Solid (TDS) of the test media decreased as the concentration increased but increased with increase in the exposure time. The values range from 1479 to 17990mg/L for *C. africanus* and 1479 to 16900mg/L for the test media of *T. guineensis*.

Apart from been within values previously reported in marine ecosystem, the conductivity level was also within the limit that permits survival of marine organisms. The values ranged from 2957 to 21920µs/cm.

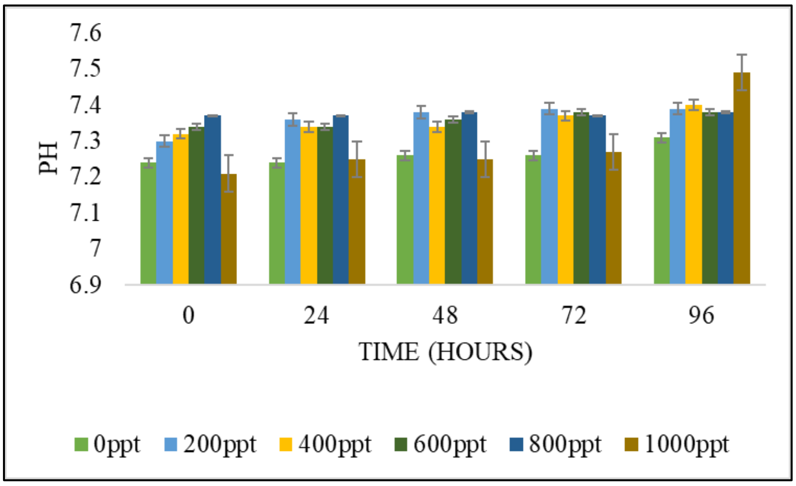

Figure 7 pH at varying concentration of treated effluent test medium for *Clibanarius africanus*

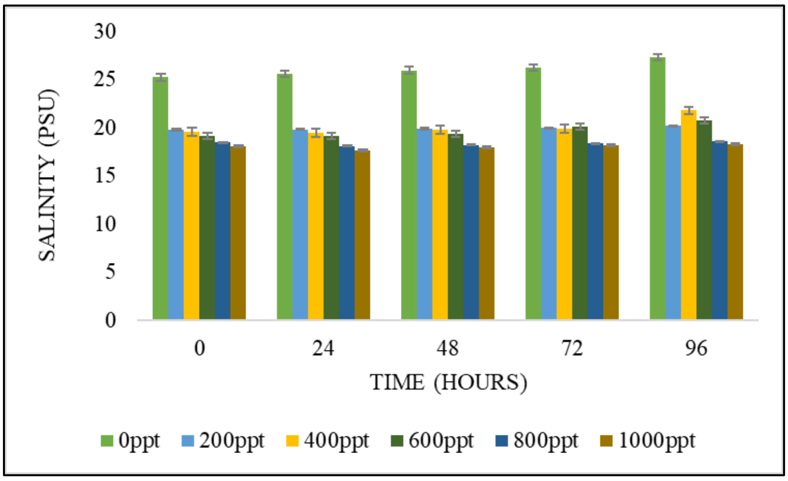

Figure 8 Salinity at varying concentration of treated effluent test medium for *Clibanarius africanus*

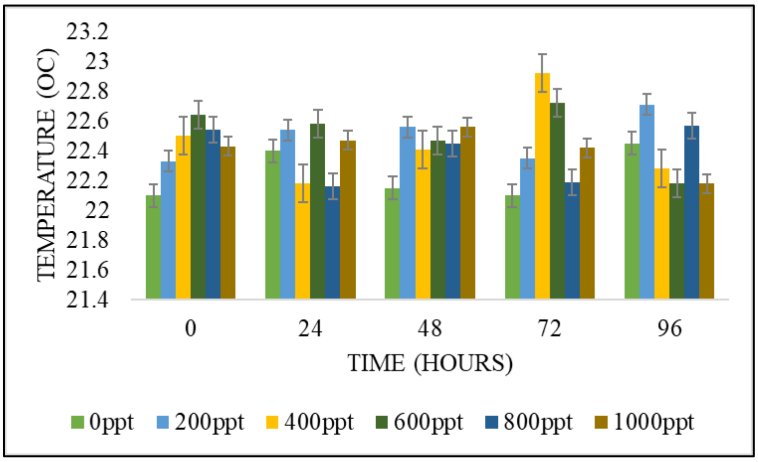

Figure 9 Temperature at varying concentration of treated effluent test medium for *Clibanarius africanus*

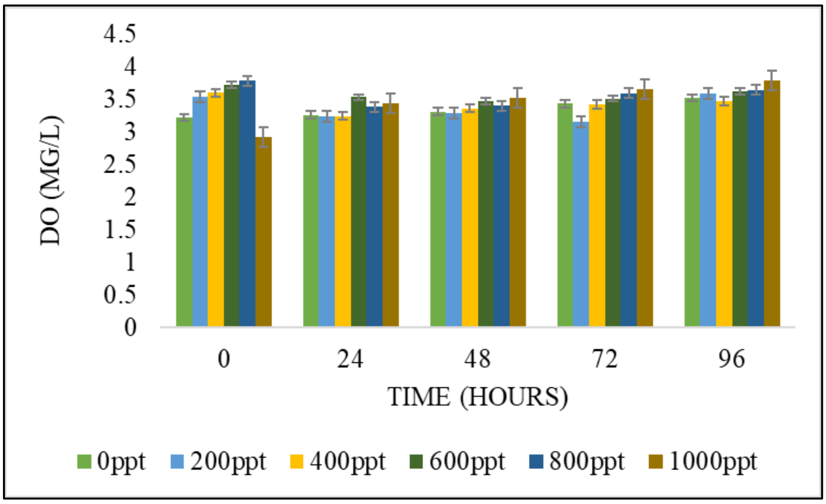

Figure 10 DO at varying concentration of treated effluent test medium for *Clibanarius africanus*

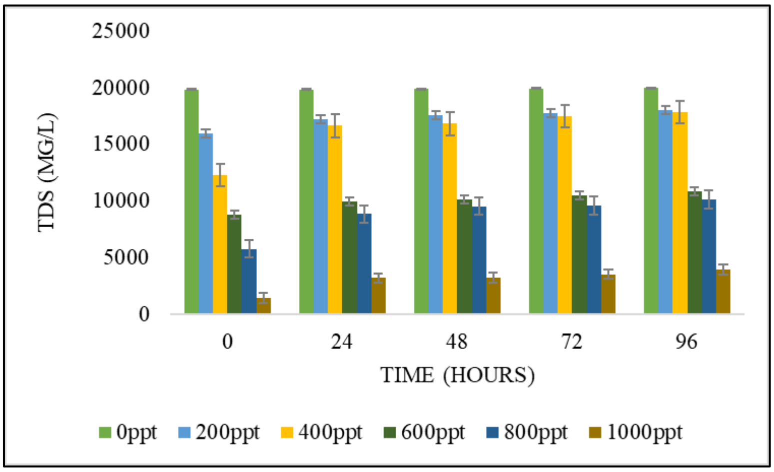

Figure 11 TDS at varying concentration of treated effluent test medium for *Clibanarius africanus*

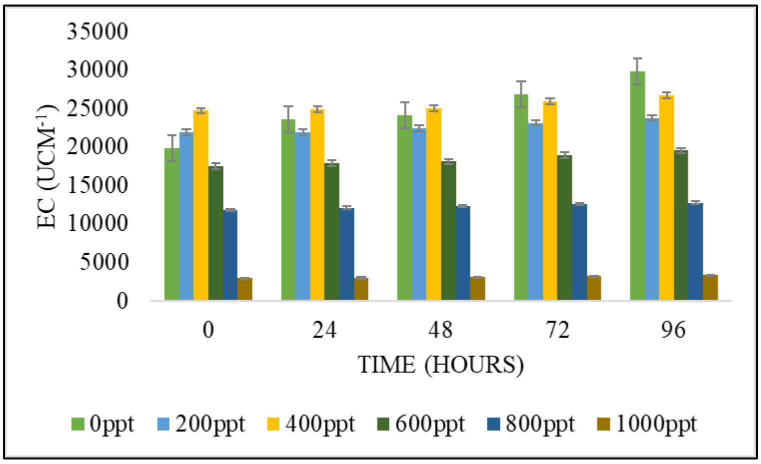

Figure 12 EC at varying concentration of treated effluent test medium for *Clibanarius africanus*

Table 3 Acute toxicity indices of the treated effluent and reference chemical

Test organism	Exposure time	LC_{50} (mg/l) of treated effluent	LC_{50} (mg/l) of KCl
Tilapia guineensis	96hours	409824	1482
Clibanarius africanus	96hours	299008	1724

The results of the toxicity indices for the treated effluent and the reference chemical are presented in table 3. It showed that the 96hourLC_{50} for both *Tilapia gunineensis* and *Clibanarius africanus* were very high. This implies that the treated effluent was not toxic to the test organisms (*Tilapia guineensis* and *Clibanarius africanus*). The reference chemical was more toxic to the test organisms as the 96hourLC_{50} for *T. guineensis* and *C. africanus* were 1482mg/l and 1724mg/l respectively.

Generally, the response of the test organisms- *Clibanarius africanus* and *Tilapia guineensis* indicated that the higher concentration of the treated effluent would be required to cause fatal effect as the percentage mortality increased with increase in toxicant concentration and exposure time. This is in line with findings of Luke & Odokuma [7] [10].

4. Conclusion

This study investigated the acute toxicity of treated effluent when exposed to *Clibanarius africanus* and *Tilapia guineensis* and it revealed that increase in the concentration of the treated effluent and reference chemical increased the mortality rate. The median lethal concentration values showed that the treated effluent still induces varying levels of mortality on *Clibanarius africanus* and *Tilapia guineensis.* Hence, there is need to properly treat before discharging into the aquatic ecosystem.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have declared that no conflict of interest exists.

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