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RESPONSE OF MARINE MICROALGAE AND COPEPOD TO TOXICITY OF AN EFFLUENT

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ABSTRACT: *Discharges of treated effluents from producing industries is a continuous source of pollutants to the marine ecosystems. Typically, effluents comprise of one or more pollutants such as hydrocarbons, heavy metal rinses and detergents of which after treatment, the pollutants may be at a level that could affect the marine ecosystem. This study aimed to determine the acute toxicity of the treated effluent and the response of Microalgae (Skeletonema costatum) and Copepod (Acartia tonsa) when exposed to the treated effluent in a marine system. The microalgae and copepod which are standard test organisms for acute toxicity testing as selected by Nigerian Upstream Petroleum Regulatory Commission were sourced from the Nigerian Institute for Oceanography and Marine Research, Buguma in Rivers State, acclimatized to laboratory conditions and utilized in whole effluent acute toxicity test of the effluent. Utilizing different concentrations of the treated effluent, growth inhibition test (for microalgae) and mortality test (for copepod) were performed using static without renewal option at 22^{o}C under continuous white light for 72 hours. Thereafter, median inhibition/lethal concentration (IC_{50}/LC_{50}) was calculated. The treated effluent was more toxic to Acartia tonsa (72 hours - LC_{50} 473.19 mg/l) when compared to Skeletonema costatum which had 72 hours - IC_{50} > 100,000 mg/l. The study revealed that the treated effluent displayed moderate toxicity to the copepod and was non-toxic to the microalgae. Hence, more efforts should be put in place by the regulatory agencies to ensure that operators adhere strictly to effective guidelines of waste water treatment to avoid extinction of sensitive species.*

KEYWORDS: Treated Effluent, Microalgae, Copepod, Toxicity, Toxicity Index.

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INTRODUCTION

Industries are different in terms of processes and raw materials; thus, they discharge wastewater of different compositions which require treatment. This wastewater is termed effluent. The treatment systems for the effluents produced by the petroleum industries involves removal of coarse suspended and floating matters, oil, grease, organic solids through biological processes, colloidal particles and refractory organics (Sathya et al., 2022). This aims at providing compatible water for the secondary treatment which involves use of microbes that metabolize the organic materials present in the water and generate inorganic by-products, after which microbes are eliminated via sedimentation method (Sathya et al., 2022). The treated effluent from most industries is in most cases discharged into the adjoining environment. The inability to effectively and efficiently manage vast amounts of waste generated by various anthropogenic activities particularly in developing countries including Nigeria has created critical problems in the aquatic environment (alters the physical, chemical and biological nature of the receiving water body). Improper treatment of effluents released into water bodies has been creating toxic effects on all types of life forms, directly or indirectly (Ahmed et al., 2021).

The marine ecosystem houses over 300,000 known species and over a million unknown species. These species are bound together through the food chain and each occupy a distinctive position on the feeding level (Egerton, 2007).

Marine copepods (*Acartia tonsa*) are free swimming, planktonic crustacean that range from 0.5 mm to 1.5 mm in length. Their body is translucent and bilaterally symmetrical. These copepods can be differentiated from closely related species by their long first antenna and branched (biramous) second antennae (Gonzalez, 2013). *Acartia tonsa* represents the marine zooplankton. It is common and eats algae plankton, and is eaten itself by larger organisms—bigger crustaceans and fish larvae. They play an important role in the mixing and cycling of nutrients and energy in the marine ecosystem forming a trophodynamic link connecting primary producers and secondary consumers in the oceanic food web (Shore et al., 2021). They are also important regulators of the marine nitrogen cycle, excreting both inorganic and organic nitrogen (Gonzalez, 2013). *Acartia tonsa* has been recommended for standard toxicity tests due to its wide distribution, short life cycle and high reproductive potential (Medina, 2004; EGASPIN, 2018).

Microalgae (*Skeletonema costatum*), a photosynthetic phytoplankton, is an important species that frequently causes microalgal blooms in the sea. The species belongs to the group of diatoms (Bacillariophyceae), cosmopolitan, unicellular, colonial or solitary algae, characterized by the presence of the external siliceous cell wall named frustules (Culcea, 2017). They account for about 20% of global primary productivity and thus play a crucial role in the global cycling of carbon and silicon (Hangxiao et al., 2021). This phytoplankton has been selected due to its importance in aquaculture, affinity towards a wide range spectrum of nutrients and its dominance in various ecosystems (Redzuan & Milow, 2019). To determine the potential of these contaminated matrices to cause deleterious harm to the indwellers, methods such as acute toxicity are employed. Acute toxicity testing involves testing the power of a stimulus which is estimated by how living organisms respond to it (EGASPIN, 2018). These test organisms are selected based on a number of factors which include sensitivity to a wide range of compounds, accessibility ease, representative of a diversity of environments, ease of preservation, being culturable under laboratory environment and being representatives of the different trophic levels (EGASPIN, 2018). The test organisms are usually exposed to a

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sequence of concentrations of the environmental sample, and the inhibition or mortality that occurs over one to four days is recorded. To track the response of the organisms when exposed to toxicants, an index/clue is set. These clues include inability of movement, growth inhibition and mortality. This study aimed to determine the acute toxicity of the treated effluent and the response of Microalgae (*Skeletonema costatum*) and Copepod (*Acartia tonsa*) when exposed to the treated effluents in a marine system.

METHODOLOGY

The acute toxicity testing included sourcing of test organisms, acclimatization of test organisms to laboratory conditions, conducting whole effluent acute toxicity tests (range finding tests and definitive toxicity tests) of the treated effluents and the reference chemical, Potassium chloride (KCl) using Nigerian Upstream Petroleum Regulatory Commission (NUPRC) approved test organisms: *Skeletonema costatum* (microalgae) and *Acartia tonsi* (copepod).

Source of Toxicants (Treated Effluent and Reference Chemical)

The treated effluent sample was collected with sterile amber glass containers from the industrial treatment plant (at the point of discharge) of a liquefied natural gas-producing company in Bonny Island, Nigeria. Potassium chloride which served as the reference chemical was purchased from a licensed chemical store within Port Harcourt, Rivers State.

Source of Test Organisms

Acartia tonsa was obtained from Nigerian Institute for Marine and Oceanography Research (NIOMR), Buguma, Rivers State and transported to the laboratory in a 2 litres container before noon because these crustaceans are sensitive to excessive sunlight. The organisms were transferred into a 500 ml beaker containing seawater. The seawater was renewed every two days to reduce the influence of waste products of metabolism introduced into the water by the test organisms.

Skeletonema costatum was isolated from natural culture systems of Skeletonema within Nigerian Institute for Marine and Oceanography Research (NIOMR), Buguma, Rivers State using selective media (Guilard F/2 media). The phenotypic identification was done using conventional algal keys then the algal population was scaled up.

Physicochemical analysis of Treated Effluent

The physicochemical properties of the treated effluent were determined using standard analytical procedures recommended by American Public Health Association (APHA 2002). Unstable parameters were measured *in situ*.

Acute Toxicity Test

A two-stage approach was employed in the acute toxicity test: preliminary range finding test and definitive test.

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i. Range Finding Tests

This test was conducted to reduce the number of dilutions of the toxicant (Treated Effluent and Reference Chemical) used in the definitive toxicity test. The test organisms were exposed to various dilutions/concentrations (logarithmic concentrations) of the whole effluent or test chemical with the aim of determining the lowest concentration of the effluent/chemical that could kill 100% of the test organisms within 24 hours and the highest concentration that would have no effect (growth inhibition/mortality) on the test organisms.

Broadly spread logarithmic concentrations (0, 0.01, 0.1, 1.0, 10, 100 and 1000 ppt) of the toxicants (Effluent and KCl) were prepared and transferred into 10 ml test tubes containing 5 ml of microalgal suspension for the preliminary range finding test. This was incubated at laboratory temperature (22–24^{o}C) under continuous white light. Cell counting was performed using a haemocytometer after every 24 hours for 3 days.

The survival test was conducted according to methods of Odokuma and Ogba (2002) and Ajuzieogu and Odokuma (2018). Concentrations (0, 0.1, 1, 10, 100 and 1000 ppt) of the toxicants (Effluent and KCl) were used. Twenty (20) healthy individuals were introduced into the test tubes of varying concentrations. Active organisms were counted at 0, 24, 48 and 72 hours.

ii. Definitive Tests

Employing the static non-renewal method, this 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.*

The 5 different concentrations for each toxicant (treated effluent and KCl) as seen in Table 1 were used for the definitive acute toxicity set up. The 0 ppt were without toxicants and served as controls. Mortality which was used as an index for scoring toxicity was assessed for 96 hours at intervals of 24 hours.

Table 1: Concentrations of Toxicants Used for the Bioassay

Test organism	Treated Effluent	Potassium chloride (RC)
Skeletonema costatum	0, 200, 400, 600, 800, 1000 ppt	0, 0.1, 1.0, 10, 50, 100 ppt
Acartia tonsa	0, 200, 400, 600, 800, 1000 ppt	0, 2, 4, 6, 8, 10 ppt

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The inability of *Acartia tonsa* to swim shown by darting movements within the dilution water and their settling at the bottom of the test tubes was used as a sign of mortality. Live organisms were counted during the following periods (0, 24, 48 and 72 hours). Probit software was used to determine LC_{50}. Inhibition of cell (*Skeletonema costatum*) division measured by cell count decrease (Inhibition Concentration) was employed as the index for toxicity. Cell counts were measured using a haemocytometer. Probit software was used to determine the toxicity, IC_{50}.

RESULTS AND DISCUSSION

The results for physicochemical analysis of the treated effluent are displayed on Table 2. From the table presented, the results show that Total Petroleum Hydrocarbon (TPH) and Polyaromatic Hydrocarbon (PAH) were detected in the effluent. These are chemical indicators believed to be from anthropogenic sources and are persistent and recalcitrant. The pH value for the treated effluent was 7.49 which is within the *Environmental Guidelines and Standards in the Petroleum Industry in Nigeria* (2018) limit. The salinity was 19.46 ppt. Heavy metals and hydrocarbon concentrations were examined and recorded as well.

The result revealed that the treated effluent had a pH within alkaline range which fell within the acceptable range given by EGASPIN (2018). This range indicates that the effluent will be unlikely to have an adverse impact on the receiving environment. This is in conformity with the work of Osuaha and Nwaichi (2019) and Ntongha and Omokaro (2021). The lower the conductivity, the less saline the effluent/water; thus, the electrical conductivity recorded was significantly low in relation to the stipulated limits by EGASPIN (2018). The dissolved oxygen (DO) value of 5.08 mg/L was higher than 3.0 mg/L as stipulated by EGASPIN (2018). High DO can lead to dead zones in the biological floc. Although Calcium was high, the low values for other heavy metals can be attributed to adequacy in treatment. The presence of hydrocarbons is an indicator that the treated effluent was in connection with petroleum compounds/volatile organic compounds and this could have toxic effects on marine organisms.

The effect of the different concentrations (0 ppt, 200 ppt, 400 ppt, 600 ppt, 800 ppt and 1000 ppt) of treated effluent on *Acartia tonsa* is displayed graphically in Figure 1. The least concentration (200 ppt) had no effect as it recorded a zero percent mortality rate at the different time exposure. 400 ppt at 72 hours killed half (50%) of the copepods. However, the concentration of 1000 ppt recorded the highest mortality rate of 100% at 72 hours exposure.

Figure 2 represents the response of *Acartia tonsa* to different concentrations (0, 2, 4, 6, 8 and 10 ppt) of KCl (reference toxicant). The highest concentration (10 ppt) had 40% mortality at 12 hours exposure and at 72 hours exposure, all organisms died. The concentration of 6 ppt had 70% mortality at 24 hours of exposure. Generally, the percentage mortality increased with increase in exposure time.

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Table 2: Treated Effluent and Recipient Water

Parameters	Treated Effluent
Ph	7.49
EC (uscm^{-1})	655
TDS (mg/l)	328
DO (mg/l)	2.9
Turbidity	4.8
Temperature(°C)	27.21
Salinity (psu)	19.46
SO_4^{2-} (mg/l)	3.72
NO_3^- (mg/l)	0.33
NO_2^- (mg/l)	0.89
NH_3 (mg/l)	2.20
PO_4^{3-} (mg/l)	4.41
TSS (mg/l)	1.23
TPH (mg/l)	0.25
THC (mg/l)	0.3
PAH (mg/l)	0.005
BTEX (mg/l)	<0.001
Chloride (mg/l)	346.1
Total hardness (mg/l)	100.2
Ca^{2+} (mg/l)	28.06
Mg^{2+} (mg/l)	15.19
K^+ (mg/l)	12.36
Cu^{2+} (mg/l)	0.11
Zn^{2+} (mg/l)	0.42
Mn^{2+} (mg/l)	0.10
Cr^{3+} (mg/l)	0.04
Pb^{2+} (mg/l)	0.03
Fe^{2+} (mg/l)	0.39
Co^{2+} (mg/l)	0.03

Source: *Laboratory Analysis*

The response of the microalgae to varying concentrations of treated effluent is graphically represented in Figure 3. The control (0 ppt) tubes had no inhibition of growth as they recorded negative percentages, which means the algal growth was supported (increased). Similar response was recorded in concentration of 200 ppt at 24 hours and 48 hours exposure time. The 1000 ppt recorded the highest percentage (52.7%) of growth inhibition at 72 hours exposure time.

The reference chemical (KCl) had a high growth inhibition on the microalgae. The lowest concentration (0.1 ppt) recorded an inhibition percentage of 21.6 at 72 hours while the highest concentration (100 ppt) recorded growth inhibition of 70.2% and 98.9% at 24 hours and 72 hours exposure time respectively. The concentration response is graphically represented in Figure 4.

The results of the toxicity indices for the treated effluent and the Reference chemical (KCl) are presented in Table 3–4. The treated effluent was toxic to *Acartia tonsa* (72h LC_{50}, 473.19

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mg/L) when compared to the other test organism, *Skeletonema costatum* (72h IC_{50}, >1000 mg/L). The reference chemical (KCl) was more toxic to all the test organisms than the treated effluent.

The trend of mortality recorded when *Acartia tonsa* was exposed to the toxicants (treated effluent and KCl) was an increase in percentage mortality with increasing concentration and exposure time. This corroborates with the findings of Picone et al. (2021). This trend was also seen in the algal growth inhibition as the microalgae (*Skeletonema costatum*) was more sensitive (less growth) when exposed to higher concentration of the toxicants.

Skeletonema costatum was more tolerant to the toxicants when compared with the copepods, *Acartia tonsa*. Generally, the reference chemical (KCl) had a higher toxic effect on the test organisms than the treated effluent.

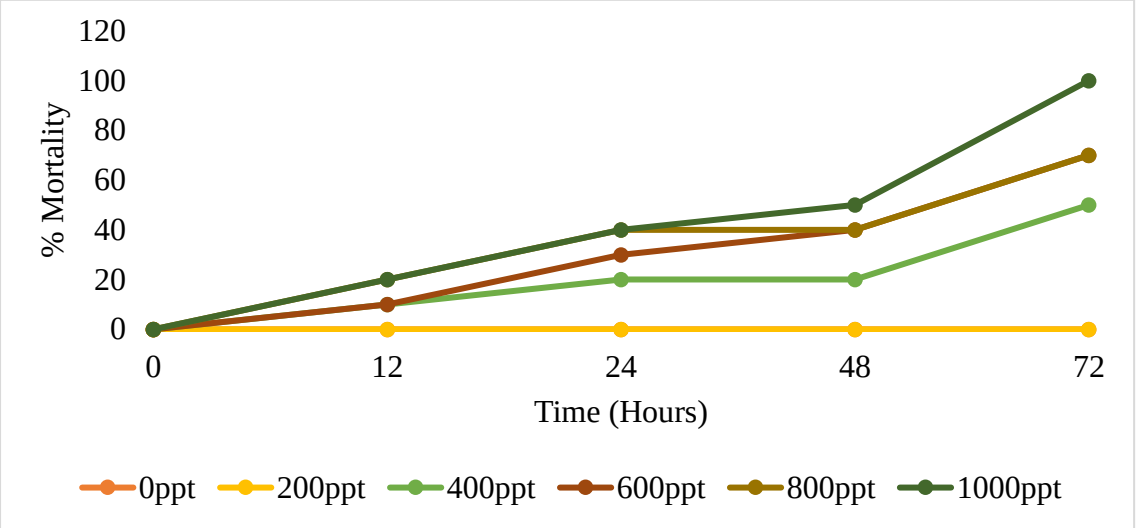

Figure 1: Concentration response with time of *Acartia tonsa* to Treated Effluent

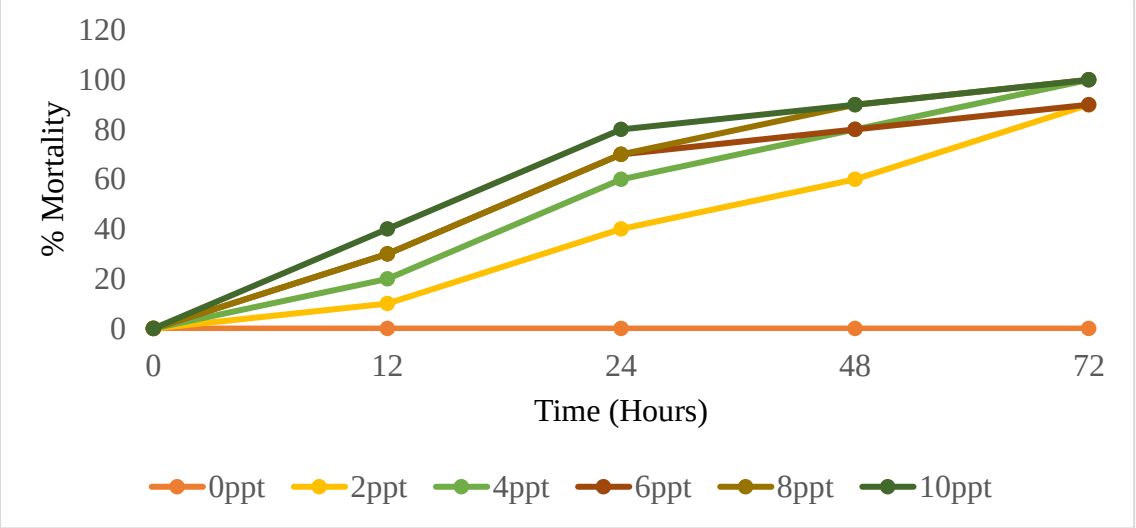

Figure 2: Concentration response with time of *Acartia tonsa* to KCl

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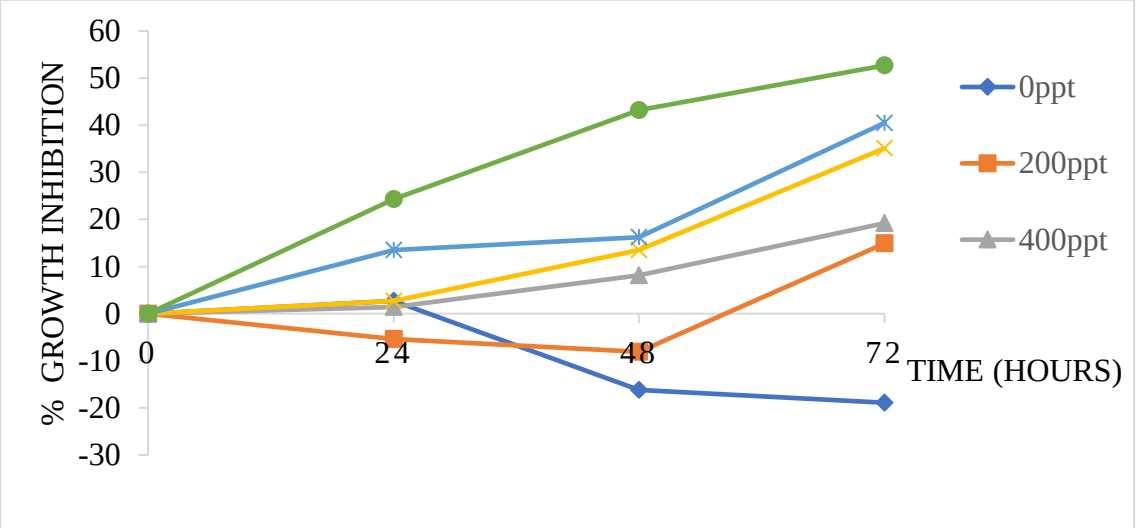

Figure 3: Concentration response with time of Skeletonema to Treated Effluent

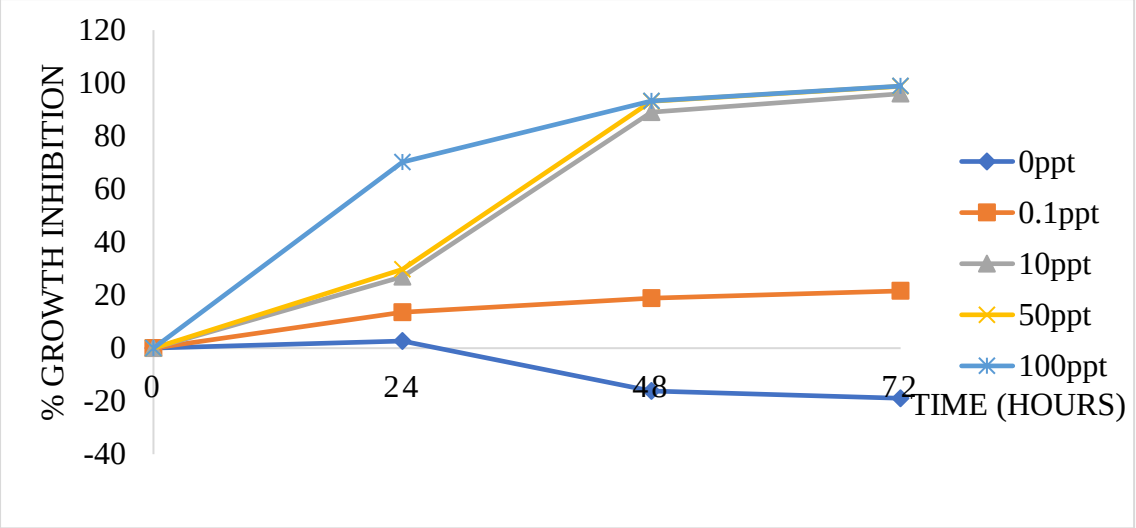

Figure 4: Concentration response with time of Skeletonema to KCl

Table 3: Acute Toxicity Indices of Treated Effluent

Test organism	Endpoint	Duration (hours)	IC_{50} (mg/l)	LC_{50} (mg/l)
Acartia tonsa	Growth inhibition	72	-	473.19
Skeletonema costatum	Mortality	72	>1000	-

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Table 4: Acute Toxicity Indices of KCl

Test organism	Endpoint	Duration (hours)	IC_{50} (mg/l)	LC_{50} (mg/l)
Acartia tonsa	Growth inhibition	72	-	1.027
Skeletonema costatum	Mortality	72	30	-

CONCLUSION

The study has revealed that the treated effluent was not toxic to the microalgae (*Skeletonema costatum*) but displayed moderate toxicity to the copepod (*Acartia tonsa*). Hence, more efforts should be put in place by the regulatory agencies to ensure that operators adhere strictly to effective guidelines of waste water treatment to avoid extinction of sensitive species.

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