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Comparison of the Sensitivity of *Crassostrea gigas* and *Vibrio fischeri* (Microtox) for Toxicity Assessment of Produced Water

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Authors' contributions

This work was carried out in collaboration between both authors. Author LOO designed the study and wrote the protocol. Author CAA performed the statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The toxicity of produced water (untreated and treated) obtained from exploration and production activities of the coast of Nigeria to the oyster (*Crassostrea gigas*) and a bacterium *Vibrio fischeri* was assessed. The sensitivity of the toxicity test procedures for both test organisms was assessed.
Study Design: Mortality [Lethal concentration (LC_{50})] for *Crassostrea gigas* and Inhibition of bioluminescence (Microtox) by *Vibrio fischeri* [Effective concentration (EC_{50})] were the toxicity indices employed for the organisms.
Place and Duration of Study: Biological monitoring department of Halden Laboratories, Port Harcourt, Nigeria, January, 2016.
Methodology: Lethal concentrations (LC_{50}) of *C. gigas* in produced water generated after 96 hr exposure time were compared with percent reductions in light output by *V. fischeri* after 15 min exposure time in the microtox assay.
Results: The 15 min EC_{50} values of the untreated and treated produced water samples for *V.*

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fischeri was 1.0% and 23.27% respectively, while 96 hr LC_{50} values of untreated and treated produced water for *C. gigas* were greater than 1000 parts per thousand (> 1000 ppt). Microtox test indicated the produced water samples were very toxic after 15 min exposure time, while after 96 hrs exposure time *C. gigas* was yet to produce a toxic response as > 1000 ppt LC_{50} indicates no toxicity. The microtox EC_{50} values were one order of magnitude lower compared to those of *C. gigas* indicating greater sensitivity of microtox assay.

Conclusion: Findings from this study therefore support the incorporation and application of microtox test system as a rapid and sensitive bioassay tool in the biological monitoring protocol and ecotoxicological evaluation tests in Nigeria's petroleum industry.

Keywords: Acute toxicity; Crassostrea gigas; oyster-larvae; Vibrio fischeri; microtox; produced water; sensitivity; Nigeria.

1. INTRODUCTION

Biological monitoring includes examining how living organisms indigenous to any habitat (aquatic or terrestrial system), respond to effluents or wastewater discharges into the receiving or recipient environment. The procedure assesses the toxic effects of the discharges on the inhabitants and concomitantly determines the health status of the aquatic system [1]. The environmental guidelines and standards for petroleum industries in Nigeria (EGASPIN) [2] specifies test species and protocols for conducting biological monitoring for environmental compliance monitoring processes. Amongst these tests is the acute toxicity test which uses lethality and sub-lethality (inhibition of a physiological function e.g. bioluminescence) as endpoint. These endpoints are expressed as median lethal and effective concentrations (LC_{50} and EC_{50} respectively) [1]. According to protocol, species representing various trophic levels in an aquatic food chain should be used due to their potential for varying sensitivities to different toxic compounds that may produce a specific response in one species and absent in another species. For instance, the fresh water/brackish water fish (*Tilapia guineensis*), and the mangrove oyster *Crassostrea gigas*, and others, are standard species recommended for biological monitoring as part of environmental compliance monitoring for effluents such as produced water, oily water by EGASPIN [2,3].

Factors that may be responsible for the selection of test species and protocols may include; Environment (fresh water, brackish or marine water), ease of culture, availability (distribution) within a region or sensitivity of species to a toxicant in a habitat over another species. However, standardized acute toxicity tests with fish and invertebrates can be expensive and time- consuming, therefore the application of a simple, sensitive and rapid toxicity testing tool will be a welcomed approach, as this will aid detection of samples/sites with high toxicity [4]. There are successful biological monitoring or ecotoxicology studies that employed bacteria and other biochemical *in vitro* systems [2].

The Microtox test system is based on measuring changes in the light output of a marine luminescent bacterium *Vibrio fischeri* following exposure to single chemicals or complex environmental samples. The degree of change in light output relative to a control is directly proportional to the level of toxicity present in test samples [3]. Bioluminescence is an aerobic oxidation process and the enzyme involved in the production of luminescence is luciferase. The enzyme catalyzes the oxidation of its substrate luciferin and is mediated by reduced coenzyme flavin mononucleotides. The interactions of toxicants with the bioluminescence bacteria cause the inhibition of luminescence production [5].

There is a comprehensive bank of comparative results generated for the Microtox test and several aquatic organisms by many researchers which include but are not limited to studies by [6-8].

Nonetheless, comparative interspecies records with *C. gigas* and *V. fischeri* (microtox) are limited. Majority of multispecies comparison studies focused on correlating invertebrate or fish LC_{50} with microtox EC_{50} for toxicity assessment of various chemicals and effluents. The aim of this study was to assess the application of microtox (*V. fischeri*) protocol as a sensitive and rapid bioassay tool for toxicity assessment of produced water in comparison with the standard Oyster-larval biological monitoring test species, *C. gigas*.

2. MATERIALS AND METHODS

2.1 Test Samples

Samples of untreated and treated produced water were collected from an offshore operational production facility situated in the Atlantic ocean about 90 km south-west of Warri, Delta State, Nigeria. Samples were treated in the following manner (e.g., filtered, centrifuged, dechlorinated, or pH-adjusted) prior to the initiation of testing with species. All samples were stored at 4°C prior to testing. Sea water used for acclimation and as dilution water was obtained from the Atlantic Ocean, 1000m from the facility.

2.2 Physicochemical Analysis of Test Samples

The pH, temperature, electrical conductivity, dissolved oxygen (DO), biochemical oxygen demand (BOD), salinity, total dissolved solids (TDS), total suspended solids (TSS), nitrates and phosphate of both produced water (PW) samples were analyzed following standard methods by American public health association (APHA) [9].

2.3 Detection of Heavy Metals

Lead, chromium, cadmium, arsenic, mercury, nickel, iron and zinc were detected by flame analysis Method 7000B using the Atomic absorption Spectrophotometer Model AA500 (PG instruments) after sample preparation and digestion according to the method described by [9].

2.4 Gas Chromatography of Oils

Total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAH) and monocyclic aromatic hydrocarbons (MAH) were extracted and quantified using Gas chromatograph equipped with single flame ionization detector (GC-FID) Model 6890 (Agilent instruments USA) according to the method adopted from US Environmental protection agency (USEPA) [1] (USEPA 8015 and 8270c). Benzene, toluene, ethyl benzene and xylene (BTEX) were analysed using the sri8610c purge and trap Gas chromatography according to the method described by [1] (USEPA 5030).

2.5 Enumeration of Total Culturable Heterotrophic Bacteria

Total culturable heterotrophic bacterial (TCHB) counts were determined using spread plate method on plate count agar (PCA) described by [10]. From each sample 1 ml was homogenized in 9 ml of 0.85% normal saline using Heindolph vortexing machine. Serial dilutions (10-fold) of the samples were prepared and dilutions (10^{-4}-10^{-5}) of samples were plated out on agar medium and incubated at 30°C for 24 h. The colony forming units were afterwards enumerated.

2.6 Enumeration of Hydrocarbon Utilizing Bacteria

Hydrocarbon utilizing bacteria (HUB) were enumerated by a method adopted from [11] which involved the dilutions of appropriate sample and plating out on Bushnell-Haas agar (Sigma-Aldrich, USA). Hydrocarbons were supplied through the vapour phase by placing sterile Whatman No.1 filter papers impregnated with 5 ml Bonny light crude oil on the lids of the inverted plates and incubated for 7 days at 30°C.

2.7 Test Organisms

Freeze-dried reagent of the luminescent marine bacterium *V. fischeri* was obtained from the manufacturer (MODERN WATER INC, Delaware, USA) and used for conducting the Microtox tests. Oyster larvae (spats) were obtained from African Regional Aquaculture Center/ Nigerian Institute of Oceanography and Marine Research (ARAC/NIOMR) Buguma, Rivers State and transported the laboratory in steel cages.

2.8 Test Methodology

2.8.1 Oyster-larval survival test (acute toxicity)

Oyster-larval survival test was conducted following method from [12-14]. The static non-renewal option was applied. Six logarithmic concentrations [0.01 parts per thousand (ppt), 0.1ppt, 1.0 ppt, 10 ppt, 100 ppt and 1000 ppt] of untreated produced water, treated produced water and diesel (reference toxicant) were prepared in glass aquaria tanks (30cm × 30cm× 30 cm) with sea water as dilution water, after a preliminary range finding test had been conducted to determine the least concentration of the toxicants that caused 100% mortality of the Oyster larvae (*C. gigas*) and the highest concentration that caused 0% mortality. Ten healthy oyster larvae (*C. gigas*) each from acclimation unit were introduced into glass tanks

bearing the various toxicant concentrations. *C. gigas* was subjected to quality control testing using diesel in reference tests. Each assay on the produced water samples was accompanied by diesel as the positive control (reference toxicant). Control was sea water without toxicant. Each set up was in triplicates and were incubated at laboratory temperature (22-24°C) at 16 hrs light and 8 hrs dark photoperiod. Mortality was end point for toxicity and mortality was scored by a partial or complete opening of the bivalve (two shells) and stench in some cases. Dead spats were isolated and recorded at (0, 24, 48, 72 and 96 hrs). The median lethal concentration (LC_{50}) at 96 hrs was calculated using probit analysis.

2.8.2 Microtox test

Microtox acute toxicity test was conducted with the Model 500 Microtox Analyzer (MODERN WATER INC, Delaware, USA) using protocols for the Basic Test [4,15,16,17]. A standard procedure is detailed in the manufacturer's manual. Each test consisted of a blank and four serial dilutions of produced water samples. The Inhibition test involved exposure of the reconstituted freeze-dried bacteria to test samples. The reconstituted freeze-dried bacteria were distributed to cuvettes containing cooled (15^oC) 2% saline solution (diluent). An initial light output (I_0) from each cuvette was recorded after a 15-min stabilization period. Bioluminescence was measured in a temperature-controlled Luminometer. This was followed by the addition of test solutions (also precooled to 15^oC) to appropriate cuvettes. After the 15-min exposure period, the final light output (I_{15}) was measured relative to a control. The inhibition of the luminescence was correlated with the toxicity of the water samples tested. The test organism was subjected to quality control testing using zinc sulfate in reference tests. Each assay on the PW samples was accompanied by Zinc sulphate as the positive control (reference toxicant). The results of reference toxicant test conducted during the study period fell within the acceptable range for the species and reference material.

2.9 Statistics and Data Analysis

In the case of the oyster larvae, median lethal concentrations (LC_{50}) at 96 hrs were calculated using probit analysis. Low LC_{50} values, indicated greater the toxicity. Also, a > 1000 ppt indicated that the sample was not toxic to the test organism.

Median effective concentrations (EC_{50}) for *V. fischeri* were calculated using the software that accompanied the Microtox system known as MicrotoxOmni software which uses linear regression analysis. A set of developed guidelines (with categories broadly defining the degree of toxicity) by the manufacturer was used for interpreting the results of the Microtox Inhibition tests for toxicity assessment. The results of tests were compared against toxicity categories developed.

The "Nontoxic" category captures EC_{50} values ranging between (> 100%), the "Slightly toxic" category ranging between (EC_{50} of 80-99%), "Moderately toxic" ranging between (EC_{50} of 60-79%), "Toxic" ranging between (EC_{50} of 40-59%), "Very toxic" ranging between (EC_{50} of 20-39%) and "Extremely toxic" ranging between (EC_{50} of 0-19%).

Sensitivity factor which indicates the degree of sensitivity of microtox relative to *C.gigas* was also calculated. Values less than 1 (< 1) indicated that *C. gigas* was more sensitive than microtox (*V. fischeri*). Values greater than 1 (> 1) indicated that microtox (*V. fischeri*) was more sensitive than *C. gigas* [1].

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties of Untreated and Treated Produced Water

The Physicochemical properties of produced water (PW) and sea water samples are shown in Table 2. Untreated PW, treated PW and sea water samples had alkaline pH (8.21, 8.02 and 7.80) respectively) due to the presence of high levels of carbonates. The Total petroleum hydrocarbons (TPH), Total hydrocarbon content (THC) and Oil and Grease levels in the treated produced water were lower than that of untreated produced water. This could be attributed to the treatment process (Hydro cyclone units and Induced gas floatation units) the untreated produced water was subjected to. A higher turbidity was observed for Untreated PW compared to Treated PW because of higher levels of TPH, THC, Oil and Grease, total dissolved solids (TDS) and total suspended solids (TSS) in the untreated PW, while that of sea water was negligible. Monocyclic aromatic hydrocarbons (MAH) and Benzene, Toluene, Ethyl benzene and Xylene (BTEX) were detected at negligible concentrations, while polycyclic

aromatic hydrocarbon (PAH) was not detected in all three samples. Heavy metals analysed were detected also at negligible concentrations. Results from physicochemical analyses reveal that pH, temperature and TSS in treated PW were within recommended discharge limit. Some constituents of the PW samples were above Department of Petroleum Resources' (DPR) recommended offshore discharge limits even though their concentrations were reduced in the treated PW compared to untreated PW. TPH, THC, Oil and Grease, total dissolved solids (TDS) and chlorides exceeded DPR set limits [18] as presented in Table 1. This agrees with the findings of [19-22] who confirmed that produced water sourced from some Nearshore and Offshore production and treatment facilities in the Niger Delta, Nigeria, were yet to meet DPR allowable discharge limits.

Table 1. The DPR standard limits for produced water discharge in Nigeria

Parameters	DPR limit
pH	6.5-8.5
Temperature °C	30.0
Oil/Grease content (mg/l)	40
THC (mg/l)	40
Salinity (mg/l)	No limit
Total dissolved solid (mg/l)	5000
Total suspended solids (mg/l)	50
Chemical oxygen demand (mg/l)	125
Biochemical oxygen demand (mg/l)	125
Chloride (mg/l)	2000
Chromium (mg/l)	0.5
Zinc (mg/l)	5.0
Turbidity (mg/l)	15

Source: DPR, 1998

Table 2. Physicochemical and microbiological properties of Produced water and sea water samples

Parameters	Untreated produced water	Treated produced water	Sea water
Ph	8.21	8.02	7.80
Temperature	27.0	27.0	25.0
Electrical conductivity (mS/cm)	22.1	16.0	45.8
TDS (mg/l)	12,870	9,040	22,900
Salinity (mg/l)	16,256	11,896	28,741
Turbidity, NTU	906	113	< 0.10
DO (mg/l)	1.98	2.77	5.68
BOD (mg/l)	22.8	17.0	9.50
Nitrate (mg/l)	11.0	0 .80	0.80
Phosphate (mg/l)	6.40	1.46	0.28
Chlorides (mg/l)	10,562	7,210	17,419
TPH (mg/l)	714	48.2	1.93
BTEX (mg/l)	0.005	<0.0001	< 0.0001
PAH (mg/l)	-	-	-
MAH, mg/l	0.005	<0.0001	< 0.0001
Oil and Grease (mg/l)	852	65.2	6.14
THC (mg/l)	801	58.7	3.80
Lead (mg/l)	<0.05	<0.05	<0.05
Chromium (mg/l)	<0.05	<0.05	<0.05
Cadmium (mg/l)	<0.05	<0.05	<0.05
Arsenic (mg/l)	<0.001	<0.001	<0.001
Mercury (mg/l)	<0.001	<0.001	<0.001
Nickel (mg/l)	<0.05	<0.05	<0.05
Iron (mg/l)	0.28	<0.05	<0.05
Zinc	<0.05	<0.05	<0.05
TCHB (cfu/ml)	4.5×10^5	3.2×10^4	1.05×10^4
HUB (cfu/ml)	4.0×10^5	2.5×10^4	3.2×10^2

3.2 Microbial Population Found in the Produced Water

Microbial activity in the produced water was determined by the enumeration of total culturable heterotrophic bacteria and total hydrocarbon utilizing bacteria as presented in Table 2. The total culturable heterotrophic bacteria (TCHBC) and hydrocarbon utilizing bacteria (HUB) counts were highest in the untreated produced water with mean values of 4.5×10^5 and 3.2×10^4 cfu/ml respectively, while treated PW had mean values of 4.0×10^5 and 2.5×10^4 cfu/ml for TCHBC and HUB respectively. This is consistent with findings from [23,24] who stated that population densities of microorganisms in produced water is usually not very high with total bacterial counts reaching up to 10^5 - 10^6 cfu/ml. They suggested that these low population densities indicate that oil field waters constitute a nutrient limiting environment.

The untreated PW had TCHBC of 4.5×10^5 cfu/ml and 7.1% of it had the capability to degrade hydrocarbons. Treated PW also had TCHBC of 4.0×10^5 and 6.25% of it had the capability to degrade hydrocarbons. These findings reveals that PW samples had an appreciable population of hydrocarbon utilizing bacteria (HUB), suggesting that the components of the PW samples maybe biodegradable. This corroborates the findings of [25,26] who isolated a wide variety of Hydrocarbon utilizers (HUB) from produced water sourced from Escravos tank farm, Lagos, Nigeria and also confirmed that chemical constituents of the produced water is biodegradable.

3.3 Oyster-larval Survival Test (Acute Toxicity)

The results from the oyster-larval survival test were subjected to probit analysis, to calculate the median lethal concentration (LC_{50}) at 96 hrs. The results are presented in Table 3. Median lethal concentration (LC_{50}) at 96 hrs for untreated and treated PW samples were greater than 1000 ppt (> 1000 ppt) or > 100% indicating that at this concentration of produced water in the aquatic environment, half of the entire natural population of *C. gigas* larvae will still remain alive. *C. gigas* larvae were not sensitive but tolerant to the PW samples. A study by [27] supports findings from this study. They reported that small size (larvae) oyster showed lower mortality at all test toxicant (Nigerian crude oil) concentrations, hence they are more resistant than the larger oysters to oil pollution. Their observation was that the oyster embryos used in their study were less affected by both the degraded oil fraction and the water soluble fraction (WSF) with adverse effects observed only at 5% (50 ppm) and full strength. Furthermore, they informed that degraded oil is a substrate for the ATP-dependent multi-xenobiotic resistance (MXR) transporter also known as P-glycoprotein, which is known to impart resistance to a wide range of xenobiotic chemicals throughout a number of phyla and oysters from contaminated sites have been shown to have increased MXR activities [28], thus, an inducible mechanism for resistance to organic contaminants. *C. gigas* possess the transporter (MXR).

Subsequently, [29,30] indicated high resistance of oysters to crude oil in their studies. They stated that crude oil is toxic to larvae and eggs of the oyster *Crassostrea angulata* and *Crassostrea gigas* only at very high concentrations which are rarely obtained in the sea. [27] further informed that small oysters show high resistance to crude oil pollution than larger ones and attributed the reason for such response to greater physiological vibrancy of the smaller oysters, which makes them less susceptible to stress in general. The resilience of the oysters in bioassays was attributed to their ability to close their shells permanently against toxicants and depuration of toxic substances [29].

Table 3. Toxicity of produced water samples and reference toxicants to microtox and *C. gigas*

Effluent type	Microtox 15-min EC_{50} (%)	*C. gigas* 96 hrs LC_{50} (%)	Sensitivity factor[a] (LC_{50}/EC_{50})
Untreated produced water	1.00%	> 100	100
Treated produced water	23.27%	> 100	4.297
Zinc sulphate	4.849 mg/l	-	-
Diesel	-	15.6	-

[a] *Degree to which Microtox is more sensitive than C. gigas. Values less than 1 indicate that C. gigas is more sensitive than Microtox. Values greater than 1 indicate Microtox is more sensitive than C. gigas*

A contrasting finding was reported by the study of [31], who evaluated effluent toxicity using embryo-larval developmental stages of the bivalve mollusk (*Mytilus galloprovincialis*) and recoreded great sensitivity of the embryo-larvae to effluents with EC_{50}-48h values ranging from 1.69%-12.5% for industrial and organic effluents.

Diesel was used as the reference toxicant for each assay on the produced water samples in the oyster-larval survival assay with 96hrs LC_{50} of 156 ppt. These levels were consistent throughout this assay indicating the reproducibility of the test.

3.4 Microtox Toxicity Tests

The results of the Microtox assay on the produced water samples (untreated and treated PW) are summarized in Table 3. The toxicity of two (2) samples corresponded to changes in reagent light output in the Microtox test in both samples. Untreated PW was most toxic with a 15 min EC_{50} value of 1.0%, while the treated had a 15min EC_{50} value of 23.27%. This implies that 1% concentration of untreated produced water in the marine environment will cause 50% inhibition of luminescence by *V. fischeri* and 23.27% concentration of treated produced water in the marine environment will cause 50% inhibition of luminescence by *V. fischeri*.

Findings from this study showed that untreated and treated were acutely toxic to *V. fischeri* (Microtox) though at varying degrees when compared against toxicity categories developed by manufacturers for interpreting results. The 15 min EC_{50} values for Microtox (*V. fischeri*) exposed to untreated and treated PW were 1.0% and 23.27% respectively, suggesting that the untreated PW lies under the “Extremely toxic” category (0-19%) and treated lies under the “Very toxic” category (20-39%). The variance in toxicity could be attributed to the general physicochemical characteristics of the PW samples. As presented in Table 2, the untreated produced water had higher concentrations of Oil and Grease (852 mg/l), total hydrocarbon content [THC (801 mg/l)] and total petroleum hydrocarbon [TPH (714 mg/l) than that of treated PW, and these have been reported as major compounds of environmental concern as they contribute to the toxicity of effluents [19,21,22]. Although concentrations of Oil and Grease, TPH and THC in treated PW were reduced compared to the untreated PW, the treated PW was however, “very toxic” to *V. fischeri* and this could be attributed to the fact that concentrations of the compounds listed above were all above the Department of Petroleum Resources allowable offshore discharge limits for such compounds, hence the persistence of toxicity.

A similar study by [20], reported EC_{50} values for 17 produced water samples analysed from 14 different North sea oil platforms ranging from 3.74 – 37.34% with majority (14) having EC_{50} values between 3 and 10%.

[32] also recorded a 15min EC_{50} value for Brent Delta production water to be between 6.2 and 4.3% for the same platform. Somerville et al., 1987, recorded values of between 5 and 6% for the same platform.

[33], in their study on produced water toxicity using luminescent marine bacteria (*V. fischeri*), observed that with normal alkanes, there appeared to be a trend of increasing carbon number. This supports findings from this study as we observed greater toxicity (EC_{50} = 1.0%) with untreated produced water, which contained higher amounts of C14 (n-tetradecane) compared to treated produced water which had very reduced amounts of same compound (C14), resulting in an increased or high EC_{50} value (EC_{50} = 23.27%).

[33], emphasized that indeed, aliphatic hydrocarbons contribute to produced water toxicity as against the general assumption that aromatic hydrocarbons contribute significantly to produced water toxicity [34]. They added that, the toxicity contribution of the aliphatics appeared to be related to their chemical structure, molecular weight and their octanol-water partition coefficient (K_{ow}).

[35] studied on toxicity (as EC_{50}) of methyl, ethyl, n-propyl, n-butyl and benzyl paraben to different bioassays including microtox (*V. fischeri*), and reported that microtox was the most sensitive test (EC_{50} ranging from 0.02 to 2.9 mg/L) compared to others [*Daphinia magna* (EC_{50} ranging from 1 to 15 mg/L), *Photobacterium leiognathi* (EC_{50} ranging from 0.4 to 8.5 mg/L), and *Tetrahymena thermophile* (EC_{50} ranging from 0.5 to 11 mg/L)].

This study reports different sensitivities for two (2) different species to produced water. We suggest that the difference in sensitivity could be related to the chemical structure of the components of the produced water and their

mechanism of action on the different marine species. Studies by [33], underpins this finding. [33], measured EC_{50} of pentane and hexane using *V. fischeri,* and compared results with findings of [36] who carried out same study though using a marine invertebrate (*Daphnia magna*). While [37] recorded lower pentane and hexane EC_{50} values for *Daphnia magna*, [33] recorded higher EC_{50} values for *V. fischeri.*

[33], further informed that the mechanism of alkanes is thought to be non-polar narcosis. Since the endpoint for *Daphnia* test is immobilization, and *V. fischeri* is inhibition of luminescence, it goes further to show that the different sensitivities may reflect different modes of action in both test species.

Zinc sulphate was used as the reference toxicant in the microtox assay with a 15 min EC_{50} of 3.75 mg/L. These levels were within the ranges suggested in the manufacturer's operations manual (15- min EC_{50}: 3 - 10 mg/L), indicating consistency and reproducibility of this assay.

It was observed in this study that the sensitivity factor of microtox relative to *C.gigas* was greater than one (> 1) indicating that microtox (*V. fischeri*) was more sensitive than *C. gigas*. This finding is consistent with that of [3,38,32], who established the sensitivity of microtox over other marine invertebrates (*Daphnia* species) when tested on various effluents and wastewaters from industrial production facilities.

4. CONCLUSION

This study compared the sensitivities of two (2) species (*C. gigas* and *V. fischeri*) to untreated and treated produced water. It was observed that microtox (*V. fischeri*) was the most sensitive to untreated and treated produced water samples with EC50 values of 1.0 and 23.27% respectively. These differences in sensitivity could be attributed to the chemical nature of the components of the produced water samples and their mechanism of action on the different marine species.

Also, this study revealed that the treated produced water analysed in this study was yet to meet the DPR permissible discharge limit as some of its constituents (Oil and Grease, TDS, THC, TPH and chlorides) were above set standard limits for discharge into the marine environment, as shown in comparison of Table 1 and Table 2. These findings, emphasizes the need for adequate monitoring and enforcement of disposal guidelines and set limits by regulatory agencies. It further establishes that microtox test is more sensitive to the test samples (produced water) and can be employed in addition to toxicity assessment protocols involving *C. gigas* during biological monitoring of produced water, since the microtox test requires relatively short exposure times, involves little or no test organism maintenance and does not require a fully equipped laboratory facility. It is worthy to note that this study realizes the importance of multiple species toxicity evaluation, since natural systems are a habitat for a wide range of organisms.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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