



Physicochemical Characteristics and Toxicity Studies of Crude Oil, Dispersant and Crude Oil-Dispersant Test Media to Marine Organisms

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Abstract

In this study, the physicochemical characteristics of crude oil, dispersant (Ecobest®) and crude oil-dispersant test systems, and their toxicities on representative marine organisms were assessed. The test media included mechanically dispersed crude oil-in-water (MDO) and its water accommodated fraction (WAF), chemically dispersed crude oil-in-water (CDO) and its water accommodated fraction (CEWAF), the Dispersant (D), and a reference toxicant, sodium dodecyl sulphate (SDS). These test media were used to carry out toxicity studies on *Tilapia guineensis*, *Palaeomontes africanus*, and bacteria – heterotrophic bacteria and hydrocarbon utilizing bacteria. Physicochemical characteristics of the test media were done using standard methods. The static with renewal bioassay option was employed for toxicity tests involving *Tilapia guineensis* and *Palaeomontes africanus*, while the static without renewal option was used for microbial bioassays. Marine organisms were exposed to the following concentrations: 100%, 50%, 25%, 12.5%, 6.25% and 0% of MDO, CDO, WAF, CEWAF and D, respectively. The 96 h LC₅₀ and toxicity factors were determined. Results for physicochemical characteristics of the test media showed that the pH and dissolved oxygen levels were sufficient for sustaining aquatic habitation. Pb metal was present in high amounts in D, but relatively low in CDO and CEWAF. Toxicity data showed that Ecobest® was non-toxic to the test organisms relative to SDS. The 96h LC₅₀ of MDO, CDO, D, WAF, CEWAF and SDS were 89.8, 225.6, 1891.8, 683.9, 528.1 and 1.37 for *T. guineensis*, 275.5, 137.7, 3800.9, 76.1, 168.3 and 9.99 for *P. africanus*; 1658.5, 944.1, 17221.9, 228641.0, 1036319.3 and 3.84 for heterotrophic bacteria, and 250.6, 9544.1, 77.2, 141.4, 12780.8 and 3.6 for hydrocarbon utilizing bacteria. SDS exhibited the greatest toxicity, but the dispersant reduced its toxicity by several folds. However, with increased levels of some heavy metals and polycyclic aromatic hydrocarbons in the test media water, there may be likelihood for bioaccumulation to occur in the tissues of marine organisms.

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1. Introduction

Marine pollution is defined as “the introduction by man, directly or indirectly, of substances or energy into the marine

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environment (including estuaries) resulting in such deleterious effects as harm to living resources, hazardsto human health, hindrance to marine activities including fishing, impairment of quality for use of seawater and reduction of amenities” [1]. Oil spills are inevitable during oil exploration activities, and they are a significant threat to the marine environment. Since oil is of a lower density than water, spilled oil floats on surface water. This can cause harm to aquatic organisms, primarily by limiting the amount of dissolved oxygen required for respiration. Oil spill response techniques have been developed for remediating the ecological consequences of spilled oil in the marine environment [2]. Mechanical methods (skimming) and chemical methods (dispersants and sinking agents) are common methods employed for such purposes. With several shortcomings associated with several cleanup methods, the use of dispersants have been seen as a useful alternative [3].

Dispersants are made up of different chemical compositions, and are available in the market. They help to break up crude oil into oil-in-water emulsions, which allows for easy degradation by hydrocarbon utilizing bacteria indigenous to the environment [4]. They also help in diluting crude oil rapidly in the water column, thereby preventing a drift of the oil slick into ecologically relevant shorelines. The use of dispersants is still controversial because, although they help in dispersing the spilled oil quickly, they do not necessarily remove the oil [5]. Dispersants could also induce high levels of toxic metals and polycyclic aromatic hydrocarbons in the water column, thereby creating harmful conditions for marine organisms.

Many of the earlier used dispersant formulations (prior to 1970) were inherently toxic to aquatic organisms and possessed bioaccumulation potentials (these were historically referred to as first and second generation dispersants, respectively). For instance, the aquatic toxicity of Corexit dispersants to a range of marine organisms in the United States has been reported [6]. The toxicity of these early dispersants led to the development of third-generation dispersants with the belief that they were nontoxic and biodegradable, as against their historical counterparts [7, 8]. The acute toxicity values of some of these third generation dispersants have been reported to range between 190 – 500 mg L⁻¹, as against the earlier formulations, which had acute toxicity values between 20 – 50 mg L⁻¹ [9]. However, the use of these third generation dispersants still needs to be monitored so that they do not cause harm to the environment. The toxicological effects resulting from dispersant use is not a straightforward task, as it requires at least five components: the dispersant, the oil being dispersed, the nature of the exposure, the age and species of the test organism(s). For instance, Finasol OSR52®[®], a third generation dispersant was reported to be toxic to sea urchin embryos, and toxicity was enhanced after the dispersant was added [10]. The same dispersant was also determined to be very toxic to juvenile sea bass, while Finasol OSR51®[®] possessed toxic characteristics to sea urchin embryos [8]. However, Nalco-D4106 was reported to significantly reduce the toxicity of crude oil to *T. guineensis* and *D. trispinosa* [10, 11].

Ecobest®[®] dispersant is a third generation dispersant that is about to be introduced into the Nigeria Oil industry for cleanup

purposes. It is therefore necessary that its toxicity to organisms in the marine environment is evaluated. Presently, there are no studies on the toxicities of this dispersant to tropical marine organisms. Similarly, most studies do not take into account the properties of the habitat water used in their toxicity studies. The aim of this study was to evaluate the physicochemical characteristics of crude oil, dispersant and crude oil-dispersant test media using Ecobest®[®] dispersant, and to study the toxicity of its interaction with crude oil on marine organisms using laboratory-scale experimental approach.

2. Research Methodology

2.1. 2.1 Source of Crude Oil, Dispersant and Habitat Water

Samples of Bonny light crude oil were obtained from an on-shore operational facility situated in the Niger Delta. Ecobest®[®] oil dispersant was obtained from the National Oil Spill Detection and Response Agency (NOSDRA), while habitat water was brackish water collected at intervals from off the shores of OnnePort.

2.2. Collection of Test Organisms

Test organisms recommended by the Department of Petroleum Resources (DPR) of Nigeria were obtained from the African Regional Aquaculture Centre/National Institute of Oceanography and Marine Research (ARAC/NIOMR) in Buguma, Rivers State, Nigeria. They include fish – *Tilapia guineensis* (tertiary consumer) and crustacean – *Palaemonetes africanus* (secondary consumer). Bacteria (primary consumer) was however isolated from brackish water. These test organisms represented the three trophic levels in the food chain within a tropical marine ecosystem.

The fish (body weight ranging between 4.80 g and 6.20 g) were caught with nets and transported to the laboratory in transparent polyethylene bags (air bags) containing habitat water and enough space for air. On reaching the laboratory, organisms were introduced into tanks (1 m × 3 m) containing habitat water for acclimatization. Crustaceans were collected during the dry season (January) when the salinity of the river was high for easy acclimatization. They were transported to the laboratory in the early hours of the morning in transparent polyethylene bags (air bags) containing habitat water and enough space for air. Heterotrophic bacteria (HB) and hydrocarbon utilizing bacteria (HUB) were isolated from brackish water using the spread plate technique on Nutrient Agar [37]. Incubation of cultures was 24 to 48 h for HB and 5-7 days for HUB, all at 24 ± 2°C.

2.3. Acclimatization of Test Organisms

2.3.1. *Tilapia guineensis*

Due to the difference in salinity between the habitat water and sea water, the test organisms were gradually exposed to increasing levels of salinity. They were first acclimatized in 100% habitat water for 72 h. The acclimatization water was then changed every 72 h with 50:50 of habitat and sea water, followed by 30:70 of habitat and sea water and lastly, 100% sea water for 14 days [13].

2.3.2. *Palaemonetes africanus*

The test organisms were introduced directly into the brackish water, since its habitat was within the salinity range of the brackish water, and they were acclimatized in the laboratory for 14 days at ambient temperature.

2.4. Preparation of Test Media

The test media was made up of five different exposure mixtures. These were the mechanically dispersed crude oil, chemically dispersed crude oil, dispersant, the water accommodated fractions of the mechanically dispersed and chemically dispersed crude oil, respectively. There was also a control (habitat water) set up without crude oil or dispersant, as well as a reference toxicant, sodium dodecyl sulphate (SDS), which is a historically used toxic dispersant.

2.5. Preparation of Test Media

2.5.1. Crude Oil Preparation

Crude oil used for the study was bubbled using aerator for 24h at 25°C to mimic external weathering conditions in a tropical environment to reduce oil volume by 10 percent [13].

2.5.2. Mechanically Dispersed Crude Oil

Mechanically dispersed crude oil (MDO) was prepared using a loading rate of 1g L⁻¹ made up with habitat water [15]. 10 g of crude oil was diluted with 10 L of habitat water in a 15 L pyrex bottle, and agitated with a magnetic stirrer for 30 minutes at medium energy to mimic field conditions. Five different concentrations were prepared (i.e. 100%, 50%, 25%, 12.5% and 6.25%, respectively).

2.5.3. Chemically Dispersed Crude Oil

Chemically dispersed crude oil (CDO) (containing 10 g of crude oil and 1g of dispersant) was obtained by adding each components respectively into 10 L of habitat water in a 15 L pyrex bottle and agitated for 30 minutes using a magnetic stirrer [14]. Five different concentrations were also prepared.

2.5.4. Dispersant

Each gram of dispersant used was mixed with 1 L of water in a glass beaker using a magnetic stirrer for 30 minutes [2]. Five different concentrations were also prepared.

2.5.5. Water Accommodated Fractions

The water accommodated fractions were prepared according to [17]. The water accommodated fraction obtained from mechanically dispersed crude oil (i.e. WAF) was prepared by adding 1 part of crude oil to 9 parts of habitat water in a 15 L pyrex bottle with gentle stirring using a magnetic stirrer. Mixing was done at ambient temperature for 20 h. After mixing, the oil and water phases were allowed to separate for 6 h. The water phase was then collected and immediately used for analysis. Five different concentrations were also prepared.

The same procedure described above was used for preparing the chemically enhanced water accommodated fraction (CE-WAF) from CDO.

2.6. Physicochemical Analysis of Test Media

Unstable parameters such as the pH, electrical conductivity (EC), temperature and total dissolved solids (TDS) were measured *in-situ*. The pH was measured using a hand-held digital pH metre (pHep® Hanna, USA). The EC and TDS were measured using a TDS/conductivity metre. Total solids (TS) and total suspended solids (TSS) were carried out gravimetrically. Dissolved oxygen (DO), biochemical oxygen demand (BOD₅), chemical oxygen demand (COD), nitrate (NO₃⁻), and phosphate were determined using standard methods [18].

2.7. Heavy Metals Analysis

Water samples from the different test media were digested using nitric acid according to [19]. Lead, chromium, vanadium, cadmium, arsenic, mercury, nickel, iron and zinc were quantified using atomic absorption spectrometer (Perkin Elmer 3110). The equipment was calibrated using analytical standards of the respective metals. These standards (1000 mgL⁻¹) were diluted serially to get the working concentrations and subsequently, a calibration graph.

2.8. Total Petroleum Hydrocarbons

Total petroleum hydrocarbon (TPH), was determined according to ASTM D 7066, D3921. 100 mL of water sample was measured using a graduated cylinder into a separating funnel. 1ml of H₂SO₄ was added, followed by 20 mL of tetrachloroethylene at 10 mL each. This was sealed, shaken vigorously for about 1-2minutes with periodic venting to release the inbuilt pressure, and allowed to stand for 10 mins for separation into organic and inorganic layers. The organic layer (i.e. the lower layer) was collected in a beaker. A filter paper was placed in a filter glass funnel and, approximately, 1 teaspoon of silica gel was added, and the solvent layer was drained through it. A cuvette was filled with the solvent layer and placed on an InfraCal 2 analyzer. "RUN" was selected, and the result was displayed in ppm

$$\text{Result}(mg/l) = \frac{C \times V1}{V2}$$

where *C* = concentration obtained from the instrument, *V1* = Volume of solvent used for extraction, and *V2* = Volume of sample used for extraction.

2.9. Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) was extracted using the US EPA Method 3510 liquid-liquid extraction. 100 mL of water sample was collected into a separatory funnel. A known volume of n-hexane and methylene chloride (3:1) was added, and the sample was spiked with ortho-Terphenyl. This was sealed and shaken vigorously for 1 to 2 minutes with periodic venting to release excess pressure, and the organic layer was allowed to separate from the water phase for a minimum of 5 minutes. The extract was filtered through a glass funnel with glass wool and anhydrous sodium sulphate. The volume of the sample extracted was recorded and the extract was transferred to a Teflon-lined screw-cap vial ready for PAH analysis.

A method blank was similarly carried out. The extracts were quantified using gas chromatograph with a flame ionization detector (GC-FID) Model 6890 (Agilent instruments, USA).

2.10. Microbiological Analyses

Microbiological characteristics of the sea water and crude oil used for the acute toxicity test was done by adopting methods from [37] and [38].

2.11. Acute Toxicity Tests

Acute toxicity tests were conducted in two steps: first, a preliminary range finding test was conducted to evaluate the lowest concentration that would result in 100% mortality (lethal concentration) or 100% inhibition of bioluminescence (effective concentration) and the highest concentration that will cause 0% mortality or 0% inhibition of bioluminescence. Second, the actual toxicity test, which involved exposure of the test organisms to varying concentrations of the toxicants, employing the least concentration that would cause mortality or inhibit bioluminescence, which was obtained from the preliminary range finding test as the highest concentration.

Two options were adopted for acute toxicity test. These were the static with renewal option and the static without renewal option. This test was conducted with reference to [20] and [21] Guidelines in Part III E, Section 4.3.2 of the 'Environmental Guidelines and Standards for Petroleum Industry in Nigeria' 2018.

2.11.1. Acute Toxicity Test for *T. guineensis*

The static renewal option was employed for *T. guineensis*. The habitat water was renewed every 48 h, while the organisms were fed with commercial feed of 5% body weight every 24 h. The acute toxicity test involved exposure of test organisms to five different concentrations (i.e. 100%, 50%, 25%, 12.5% and 6.25%) of MDO, CDO, D, WAF, and CEWAF.

The method is described as follows: 10 healthy acclimatized fish were introduced into an aerated laboratory glass aquarium (18" x 10" x 18") into each unit of the different concentrations. Uniformed concentrations were used for all crude oil systems to allow for comparability of results following CROSERF standards [22]. The setup was done in triplicate at room temperature (22 – 27°C). Mortality and impaired movement were the indices for scoring toxicity. Dead organisms were removed and counted at 0, 24, 48, 72 and 96 hours. Probit analysis [23] was used to analyze the number of mortalities recorded. This was carried out in order to establish the LC₅₀ (median lethal concentration) of the reference toxicant [13].

2.11.2. Acute Toxicity Test for *P. africanus*

The static renewal option was also employed for *P. africanus*: 10 healthy acclimatized organisms were introduced into 1000 mL glass jar of each unit of different concentrations of toxicants. Control was SDS for dispersant, and habitat water for crude oil. The set-up was done at room temperature (22–27°C). Mortality was end point for toxicity. It was scored by organisms' inability to swim and immediately organisms settled

at the bottom of the jar. These organisms were counted at 0, 24, 48, 72 and 96 h. Probit analysis [23] was used to analyze the number of mortality recorded. This was carried out in order to establish the LC₅₀ (median lethal concentration). The toxicity factor was also calculated with respect to the LC₅₀ of the reference toxicant.

2.11.3. Acute Toxicity Test for Bacteria

For bacteria, the static nonrenewal method was employed. 100%, 50%, 25%, 12.5% and 6.25% of MDO, CDO, D, WAF and CEWAF were introduced into 1 L glass beakers. The habitat water without toxicant was used as control. The experiment was set up in triplicate at 22 – 25°C. Aerobic plate count in nutrient agar treated with antifungal antibiotics was used to evaluate toxicity and was done at 0, 24, 48 and 96 hours. Probit analysis was used to analyze the number of mortality recorded.

2.11.4. Toxicity Factor

The toxicity factor (TF) for dispersant and synergistic or joint action factor (JAF) for crude oil-plus-dispersant was determined using the formula described by Odiete (1999):

$$TF = \frac{96 \text{ h LC}_{50} \text{ value of dispersant}}{96 \text{ h LC}_{50} \text{ value of SDS}}$$

$$JAF = \frac{96 \text{ h LC}_{50} \text{ of dispersant} - \text{plus} - \text{crude} - \text{oil}}{96 \text{ h LC}_{50} \text{ value of crude oil}}$$

2.12. Statistical Analysis

All analyses were performed in triplicates. Data were expressed as mean±SD. Significant differences among parameters in the different test media were analysed using Analysis of Variance (ANOVA) using SPSS 22.0 version. Data obtained from acute toxicity tests were also subjected to statistical analysis by Probit method.

3. Results and Discussions

3.1. Physicochemical Characteristics of the Crude Oil Test Media

The physicochemical characteristics of MDO, CDO, CEWAF, WAF, D and habitat water are presented in Tables 1- 6, respectively. The pH of all test media ranged from near-neutral to slightly alkaline. A gradual reduction in pH was observed with decreasing concentration of toxicant in CEWAF and WAF, while in MDO and CDO, the reduction was observed at toxicant concentration of 50%. A pH range of 7.9 - 8.0 has been previously reported in a similar study [16, 24]. A pH range of 6.09 – 8.45 is reportedly ideal for supporting aquatic life including fish [25]; while, waters with a pH value less than 6.0 may result in stunted, reduced or even absent fish population [26]. The pH of the studied test media will therefore support aquatic habitation.

The temperature of the test media fluctuated between 29.0°C and 31.3°C. The temperature of surface waters usually range between 0 – 30°C, but can get to as high as 40°C, depending on the season and the prevailing environmental condition. Temperature affects physical, chemical and biological

processes in water bodies and, therefore, influences the concentration of many variables.

The mean concentration of TDS in the test media ranged as follows: 4365 – 4530 mg L⁻¹ in MDO; 4390 – 4435 mg L⁻¹ in CDO; 3915 – 4450 mg L⁻¹ in CEWAF, 3900 – 3910 mg L⁻¹ in WAF and from 4365 – 4410 mg L⁻¹ in D. There was no significant difference ($p < 0.05$) in TDS levels across the different test media. In terms of TDS, the water in all test media, including the sea water, can still be classified as fresh water, since their concentrations were less than 5,000 mg L⁻¹ [27].

The DO levels ranged from 8.07 – 11.1 mg L⁻¹ in MDO; 9.83 – 10.5 mg L⁻¹ in CDO; 10.3 – 10.9 mg L⁻¹ in CEWAF; 10.1 – 10.2 mg L⁻¹ in WAF; and from 10.5 – 13.1 mg L⁻¹ in D. A decreasing trend with decreasing concentration of toxicant in MDO and D was observed. Comparatively, CEWAF had slightly higher DO levels than WAF; while same trend was observed with MDO and CDO, but at toxicant concentration below 100%. The oxygen level in natural waters usually varies with salinity, temperature, turbulence, the photosynthetic activity of algae and plants, and atmospheric pressure [28]. In freshwaters, DO at sea level normally ranges from 15 mg L⁻¹ at 0°C to 8 mg L⁻¹ at 25°C, while concentrations in unpolluted waters are usually close to, but less than, 10 mg L⁻¹. The DO levels in all test media indicated that the all media water were effectively aerated. The minimum acceptable DO levels that can maintain fish population in aquatic environment is reported to range between 4 and 5 mg L⁻¹, while fish mortality occurs when DO levels are less than 3 mg L⁻¹ [29, 30]. The range of DO concentration that can support fisheries and aquatic lives is 5 – 9 mg L⁻¹ in the EU, 5 – 9.5 mg L⁻¹ in Canada, and 4 – 6 mg L⁻¹ in Russia [28]. Therefore, the toxicant levels were not sufficient to cause any drastic reduction in oxygen levels and hence, could support fish growth and survival.

Biochemical oxygen demand (BOD₅) levels in the river water were relatively high, ranging from 6.23 – 8.25 mg L⁻¹ in MDO; 4.02 – 9.17 mg L⁻¹ in CDO; 4.03 – 9.41 mg L⁻¹ in CEWAF; 4.01 – 9.45 mg L⁻¹ in WAF; and from 4.02 – 6.95 mg L⁻¹ in D. High values were observed in the chemically enhanced media (i.e. CDO and CEWAF, respectively). Additionally, an increase in BOD₅ levels was obtained with increasing concentration of toxicants in the different test media. With respect to BOD₅ levels and aquatic pollution status of waters, BOD concentrations < 1.0 mg L⁻¹ have been classified as being unpolluted; BOD ≥ 2 ≤ 9 mg L⁻¹ has been classified as being moderately polluted, while BOD > 10 mg L⁻¹ has been classified as being heavily polluted [31, 32, 33]. Similarly, the maximum acceptable limits set by [21] and [34] are 10 mg L⁻¹ and 5.0 mg L⁻¹, respectively. Introduction of toxicants significantly altered the BOD levels in all test media at toxicant concentrations greater than 250 mg L⁻¹.

For chemical oxygen demand (COD), the concentrations ranged from 31.8 – 124.1 mg L⁻¹ in MDO; 20.0 – 62.4 mg L⁻¹ in CDO; 21.5 – 64.3 in CEWAF; 20.0 – 64.2 mg L⁻¹ in WAF; and from 20.0 – 48.3 mg L⁻¹ in D. Water is considered relatively unpolluted when the COD levels is less than or equal to 20 mg L⁻¹. In this study, the sea water could be said to be relatively unpolluted, with a COD concentration of 20.0 mg L⁻¹. In the

different test media and varying toxicant concentrations, as expected, COD levels were all greater those of the sea water, and an increasing concentration was observed with increasing toxicant concentration in all test media. This shows that there is a positive influence on the amount of oxidisable organic matter in water by the added toxicants.

3.2. Heavy Metals Content in Test Media

The concentration of heavy metals at different exposure concentrations in test media are also presented in Tables 1-6 above. Ni had the highest concentration with respect to other metals in all test media. Pb was present in high amounts in D; and its concentration decreased with decreasing concentration of exposure test media.

Trace metals such as Ni, V, Cu, Cd and Pb are naturally found in crude oil, and, water contaminated with crude oil may exhibit higher concentrations of these metals. The concentration of Cu was within the 2 mg L⁻¹ permissible limit for drinking water in all test media and at the different exposure concentrations. Pb is also a normal constituent of crude oil. Its maximum permissible limit of 0.01 mg L⁻¹ in drinking water was exceeded at all exposure concentrations in D. This indicates that the dispersant itself is a toxin of ecotoxicological importance. In MDO and WAF, exposure concentrations < 50% were within the permissible limit for Pb. In CEWAF, only exposure concentration at 100% exceeded the permissible limit, while in CDO, Pb concentration was within the permissible limit in the different exposure concentrations. For CEWAF and CDO, some form of positive synergy between crude oil and dispersant, which limited the bioavailability of Pb in the test media, was observed.

Cd and Ni, like Pb and Cu, are also natural constituents of crude oil. Their permissible limits (0.003 mg L⁻¹ for Cd and 0.1 mg L⁻¹ for Ni) were significantly exceeded in all test media and at the different exposure concentrations. This also is indicative of some degree of toxicity due to Cd and Ni, to aquatic species.

For Cr, its permissible limit of 0.05 mg L⁻¹ was slightly exceeded even in sea water. With increasing toxicant concentrations, this limit was well exceeded in all test media, which implied that there will likely be some level of toxicity to aquatic species. Fe levels were slightly elevated in the different test media compared to control medium. However, Fe concentrations were within the WHO permissible limit of 0.3 mg L⁻¹ in MDO, WAF and D; but were slightly elevated in CDO and CEWAF. Zn, As and V were found in concentrations that were within their respective permissible limits in drinking water, hence, no toxic property is expected from these metals.

3.3. Total Petroleum Hydrocarbons and PAHs in Test Media

The total concentration of TPH in the different test media are shown in Figures 1-3. The TPH concentrations did not vary significantly in MDO and CDO, but there were significant variations in TPH levels in WAF and CEWAF test media. A limit value for TPH has been set at 10 mg L⁻¹ in many water contamination regulations, i.e., the maximum concentration of TPH

Table 1: Physicochemical characteristics of MDO test media

| Parameters | Concentration of exposure medium (%) | | | | |
|----------------|--------------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | 100 | 50 | 25 | 12.5 | 6.25 |
| pH | 6.9±0.01 ^a | 7.00±0.02 ^a | 7.90±0.02 ^a | 6.90±0.02 ^a | 6.90±0.01 ^a |
| Temp. (°C) | 29.9±1.03 ^a | 31.1±0.5 ^a | 31.3±0.1 ^a | 29.1±0.1 ^a | 29.1±0.1 ^a |
| EC (uS/cm) | 9060±3.1 ^a | 8770±2.5 ^a | 8860±2.1 ^a | 8810±3.0 ^a | 8750±2.1 ^a |
| TDS (mg/L) | 4530±2.3 ^a | 4385±3.1 ^a | 4450±3.0 ^a | 4430±2.5 ^a | 4415±1.9 ^a |
| TSS (mg/L) | 1.20±0.5 ^a | 1.00±0.02 ^a | 1.05±0.1 ^a | 1.05±0.01 ^a | 1.05±0.01 ^a |
| Salinity (PSU) | 0.50±0.03 ^a | 0.40±0.01 ^a | 0.40±0.01 ^a | 0.40±0.2 ^a | 0.40±0.2 ^a |
| DO (mg/L) | 11.1±1.1 ^a | 9.80±0.1 ^a | 10.6±0.2 ^a | 10.9±1.1 ^a | 10.8±0.2 ^a |
| BOD (mg/L) | 18.2±1.1 ^a | 13.0±2.1 ^b | 9.40±1.5 ^b | 4.70±1.1 ^c | 4.30±1.05 ^c |
| COD (mg/L) | 124±2.5 ^a | 88.4±2.1 ^b | 64.3±1.8 ^b | 32.2±1.9 ^c | 29.7±1.5 ^c |
| Pb (mg/L) | 0.17±0.01 ^a | 0.06±0.002 ^b | ND | ND | ND |
| Cr (mg/L) | 0.17±0.001 ^a | 0.13±0.001 ^a | 0.13±0.01 ^a | 0.13±0.01 ^a | 0.10±0.001 ^a |
| Cd (mg/L) | 0.13±0.01 ^a | 0.11±0.01 ^a | 0.09±0.001 ^b | 0.08±0.01 ^b | 0.06±0.01 ^b |
| Ni (mg/L) | 0.54±0.02 ^a | 0.75±0.15 ^a | 0.97±0.20 ^b | 1.19±0.25 ^c | 1.20±0.20 ^c |
| Fe (mg/L) | 0.25±0.001 ^a | 0.39±0.01 ^a | 0.35±0.002 ^a | 0.30±0.001 ^a | 0.39±0.02 ^a |
| Cu (mg/L) | 0.06±0.001 ^a | 0.16±0.02 ^b | 0.32±0.02 ^c | 0.45±0.02 ^d | 0.51±0.15 ^d |
| Zn (mg/L) | 0.04±0.001 ^a | 0.05±0.001 ^a | 0.04±0.015 ^a | 0.04±0.002 ^a | 0.03±0.001 ^a |
| V (mg/L) | 0.09±0.001 ^a | 0.09±0.001 ^a | 0.09±0.001 ^a | 0.09±0.01 ^a | 0.09±0.01 ^a |

values are expressed as mean ± standard deviation. Superscripts with same letters on the same row indicate no significant difference, while superscripts with different letters indicate significant difference at $p < 0.05$

Table 2: Physicochemical characteristics of CDO test media

| Parameters | Concentration of exposure medium (%) | | | | |
|----------------|--------------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | 100 | 50 | 25 | 12.5 | 6.25 |
| pH | 6.82±0.20 ^a | 6.63±0.02 ^a | 6.86±0.01 ^a | 6.83±0.01 ^a | 6.81±0.02 ^a |
| Temp. (°C) | 29.1±0.25 ^a | 30.5±0.05 ^a | 29.0±0.10 ^a | 31.0±0.25 ^a | 29.0±1.05 ^a |
| EC (uS/cm) | 8720±3.00 ^a | 8710±1.25 ^a | 8830±2.05 ^a | 8820±1.95 ^a | 8810±2.05 ^a |
| TDS (mg/L) | 4410±2.05 ^a | 3925±0.50 ^a | 4415±1.05 ^a | 4415±2.05 ^a | 4410±1.85 ^a |
| TSS (mg/L) | 1.05±0.50 ^a | 1.04±0.01 ^a | 1.06±0.01 ^a | 1.06±0.02 ^a | 1.06±0.01 ^a |
| Salinity (PSU) | 0.43±0.10 ^a | 0.43±0.01 ^a | 0.44±0.01 ^a | 0.44±0.01 ^a | 0.44±0.02 ^a |
| DO (mg/L) | 10.3±0.15 ^a | 10.3±0.15 ^a | 10.1±1.50 ^a | 10.3±1.95 ^a | 10.2±0.50 ^a |
| BOD (mg/L) | 4.05±1.05 ^a | 4.03±1.00 ^a | 4.72±0.05 ^a | 4.05±0.01 ^a | 4.02±0.20 ^a |
| COD (mg/L) | 21.9±0.95 ^a | 21.5±1.00 ^a | 32.1±1.05 ^b | 28.6±1.05 ^b | 22.9±1.05 ^a |
| Pb (mg/L) | ND | ND | ND | ND | ND |
| Cr (mg/L) | 0.11±0.01 ^a | 0.11±0.02 ^a | 0.12±0.001 ^a | 0.12±0.02 ^a | 0.11±0.001 ^a |
| Cd (mg/L) | 0.12±0.02 ^a | 0.16±0.15 ^a | 0.18±0.01 ^a | 0.18±0.04 ^a | 0.16±0.01 ^a |
| Ni (mg/L) | 1.04±0.10 ^a | 2.19±0.02 ^b | 1.80±0.15 ^b | 1.20±0.15 ^a | 1.15±0.30 ^a |
| Fe (mg/L) | 0.09±0.001 ^a | 1.00±0.001 ^b | 0.47±0.20 ^c | 0.47±0.20 ^c | 0.41±0.02 ^c |
| Cu (mg/L) | 0.75±0.01 ^a | 0.30±0.03 ^b | 0.32±0.002 ^b | 0.31±0.10 ^b | 0.31±0.01 ^b |
| Zn (mg/L) | 0.07±0.02 ^a | 0.07±0.02 ^a | 0.05±0.001 ^a | 0.05±0.001 ^a | 0.05±0.001 ^a |
| V (mg/L) | 0.10±0.03 ^a | 0.08±0.001 ^a | 0.24±0.01 ^b | 0.21±0.001 ^b | 0.20±0.01 ^b |

Table 3: Physicochemical characteristics of WAF test media

| Parameters | Concentration of exposure medium (%) | | | | |
|----------------|--------------------------------------|-------------------------|-------------------------|-------------------------|--------------------------|
| | 100 | 50 | 25 | 12.5 | 6.25 |
| pH | 7.38±0.01 ^a | 7.33±0.10 ^a | 7.29±0.25 ^a | 7.02±0.15 ^a | 6.98±0.01 ^a |
| Temp. (°C) | 29.5±1.05 ^a | 29.5±0.50 ^a | 29.3±1.05 ^a | 29.1±1.05 ^a | 29.1±0.50 ^a |
| EC (uS/cm) | 7820±2.00 ^a | 7810±3.05 ^a | 7990±2.95 ^a | 7970±3.50 ^a | 7970±2.50 ^a |
| TDS (mg/L) | 3915±1.95 ^a | 3910±2.95 ^a | 3905±2.50 ^a | 3900±2.80 ^a | 3900±1.85 ^a |
| TSS (mg/L) | 0.55±0.01 ^a | 0.55±0.01 ^a | 0.49±0.10 ^a | 0.42±0.01 ^a | 0.40±0.15 ^a |
| Salinity (PSU) | 0.39±0.01 ^a | 0.39±0.02 ^a | 0.43±0.01 ^a | 0.43±0.01 ^a | 0.43±0.02 ^a |
| DO (mg/L) | 10.2±2.05 ^a | 10.2±0.10 ^a | 10.3±0.05 ^a | 10.2±0.05 ^a | 10.1±0.10 ^a |
| BOD (mg/L) | 9.45±2.05 ^a | 9.13±0.20 ^a | 7.33±0.10 ^a | 4.79±0.10 ^b | 4.01±1.50 ^b |
| COD (mg/L) | 64.3±1.05 ^a | 62.9±0.25 ^a | 46.3±0.55 ^b | 33.5±0.10 ^c | 20.8±0.05 ^d |
| Pb (mg/L) | 0.88±0.01 ^a | 0.06±0.001 ^b | ND | ND | ND |
| Cr (mg/L) | 0.13±0.01 ^a | 0.09±0.001 ^a | 0.07±0.001 ^a | 0.03±0.001 ^b | 0.008±0.001 ^c |
| Cd (mg/L) | 0.24±0.001 ^a | 0.19±0.02 ^a | 0.17±0.001 ^a | 0.12±0.01 ^b | 0.11±0.01 ^b |
| Ni (mg/L) | 0.45±0.01 ^a | 0.75±0.02 ^a | 0.97±0.03 ^b | 1.19±0.40 ^c | 0.54±0.002 ^a |
| Fe (mg/L) | 0.40±0.01 ^a | 0.40±0.001 ^a | 0.40±0.01 ^a | 0.30±0.01 ^a | 0.25±0.02 ^a |
| Cu (mg/L) | 0.50±0.02 ^a | 0.50±0.01 ^a | 0.50±0.01 ^a | 0.40±0.01 ^a | 0.06±0.002 ^a |
| Zn (mg/L) | 0.04±0.001 ^a | 0.04±0.001 ^a | 0.04±0.001 ^a | 0.04±0.001 ^a | 0.04±0.001 ^a |
| V (mg/L) | 0.14±0.001 ^a | 0.09±0.001 ^a | 0.09±0.001 ^a | 0.09±0.001 ^a | 0.09±0.001 ^a |

Table 4: Physicochemical characteristics of CEWAF test media

| Parameters | Concentration of exposure medium (%) | | | | |
|----------------|--------------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | 100 | 50 | 25 | 12.5 | 6.25 |
| pH | 7.98±0.15 ^a | 6.99±0.02 ^a | 6.93±0.15 ^a | 6.82±0.15 ^a | 6.63±0.02 ^a |
| Temp. (°C) | 31.3±0.10 ^a | 29.1±0.05 ^a | 29.1±0.10 ^a | 29.1±1.05 ^a | 30.5±0.05 ^a |
| EC (uS/cm) | 8860±2.05 ^a | 8810±0.30 ^a | 8750±1.55 ^a | 8720±2.55 ^a | 8710±1.65 ^a |
| TDS (mg/L) | 4450±3.15 ^a | 4430±1.05 ^a | 4415±1.50 ^a | 4410±1.50 ^a | 3925±1.05 ^a |
| TSS (mg/L) | 1.05±0.01 ^a | 1.07±0.15 ^a | 1.06±0.10 ^a | 1.05±0.10 ^a | 1.04±0.01 ^a |
| Salinity (PSU) | 0.44±0.01 ^a | 0.44±0.01 ^a | 0.43±0.01 ^a | 0.43±0.01 ^a | 0.43±0.01 ^a |
| DO (mg/L) | 10.6±1.55 ^a | 10.9±1.05 ^a | 10.8±0.20 ^a | 10.3±1.55 ^a | 10.3±0.01 ^a |
| BOD (mg/L) | 9.41±1.05 ^a | 4.70±0.15 ^b | 4.25±1.05 ^b | 4.06±0.25 ^b | 4.03±0.01 ^b |
| COD (mg/L) | 64.3±2.05 ^a | 32.1±1.55 ^b | 29.7±0.20 ^b | 21.9±1.05 ^c | 21.5±0.50 ^c |
| Pb (mg/L) | ND | ND | ND | ND | ND |
| Cr (mg/L) | 0.10±0.01 ^a | 0.10±0.001 ^a | 0.12±0.001 ^a | 0.12±0.01 ^a | 0.10±0.01 ^a |
| Cd (mg/L) | 0.16±0.01 ^a | 0.15±0.01 ^a | 0.18±0.02 ^a | 0.15±0.001 ^a | 0.15±0.001 ^a |
| Ni (mg/L) | 1.40±0.10 ^a | 0.97±0.15 ^b | 0.80±0.02 ^b | 0.80±0.02 ^b | 0.57±0.025 ^c |
| Fe (mg/L) | 0.43±0.20 ^a | 0.20±0.001 ^b | 0.13±0.001 ^c | 0.09±0.001 ^c | 0.08±0.01 ^c |
| Cu (mg/L) | 0.27±0.01 ^a | 0.16±0.01 ^b | 0.13±0.01 ^b | 0.13±0.001 ^b | 0.12±0.01 ^b |
| Zn (mg/L) | 0.04±0.001 ^a | 0.04±0.01 ^a | 0.04±0.01 ^a | 0.04±0.01 ^a | 0.04±0.015 ^a |
| V (mg/L) | 0.23±0.10 ^a | 0.18±0.01 ^a | 0.17±0.02 ^a | 0.17±0.02 ^a | 0.17±0.001 ^a |

Table 5: Physicochemical characteristics of Dispersant test media

| Parameters | Concentration of exposure medium (%) | | | | | |
|----------------|--------------------------------------|------------------------|----|-------------------------|-------------------------|-------------------------|
| | 100 | 50 | 25 | 12.5 | 6.25 | |
| pH | 7.38±0.01 ^a | 7.33±0.10 ^a | | 7.29±0.25 ^a | 7.02±0.15 ^a | 6.98±0.01 ^a |
| Temp. (°C) | 29.5±1.05 ^a | 29.5±0.50 ^a | | 29.3±1.05 ^a | 29.1±1.05 ^a | 29.1±0.50 ^a |
| EC (uS/cm) | 7820±2.00 ^a | 7810±3.05 ^a | | 7990±2.95 ^a | 7970±3.50 ^a | 7970±2.50 ^a |
| TDS (mg/L) | 3915±1.95 ^a | 3910±2.95 ^a | | 3905±2.50 ^a | 3900±2.80 ^a | 3900±1.85 ^a |
| TSS (mg/L) | 0.56±0.01 ^a | 0.55±0.01 ^a | | 0.49±0.10 ^a | 0.42±0.01 ^a | 0.40±0.15 ^a |
| Salinity (PSU) | 0.39±0.01 ^a | 0.39±0.02 ^a | | 0.43±0.01 ^a | 0.43±0.01 ^a | 0.43±0.02 ^a |
| DO (mg/L) | 10.2±2.05 ^a | 10.3±0.10 ^a | | 10.3±0.05 ^a | 10.2±0.05 ^a | 10.1±0.10 ^a |
| BOD (mg/L) | 9.45±2.05 ^a | 9.13±0.20 ^a | | 7.33±0.10 ^a | 4.78±0.10 ^b | 4.01±1.50 ^b |
| COD (mg/L) | 64.3±1.05 ^a | 62.9±0.25 ^a | | 46.3±0.55 ^b | 33.5±0.10 ^b | 20.8±0.05 ^c |
| Pb (mg/L) | 0.85±0.25 ^a | 0.73±0.25 ^a | | 0.54±0.01 ^a | 0.52±0.01 ^a | 0.08±0.01 ^b |
| Cr (mg/L) | 0.13±0.01 ^a | 0.08±0.01 ^a | | 0.07±0.02 ^a | 0.07±0.01 ^a | 0.08±0.015 ^a |
| Cd (mg/L) | 0.20±0.001 ^a | 0.19±0.01 ^a | | 0.16±0.02 ^a | 0.14±0.01 ^a | 0.13±0.01 ^a |
| Ni (mg/L) | 1.04±0.50 ^a | 0.99±0.01 ^a | | 0.88±0.025 ^a | 0.80±0.02 ^a | ND |
| Fe (mg/L) | ND | ND | | ND | ND | ND |
| Cu (mg/L) | 0.45±0.05 ^a | 0.40±0.01 ^a | | 0.36±0.01 ^a | 0.40±0.01 ^a | 0.30±0.01 ^a |
| Zn (mg/L) | 0.05±0.01 ^a | 0.04±0.01 ^a | | 0.04±0.001 ^a | 0.04±0.001 ^a | 0.03±0.001 ^a |
| V (mg/L) | ND | ND | | ND | ND | ND |

Table 6: Physicochemical characteristics of habitat water

| Parameters | Concentration of brackish water (%) |
|----------------|-------------------------------------|
| pH | 7.23±0.01 |
| Temp. (°C) | 31.3±0.50 |
| EC (uS/cm) | 8780±1.05 |
| TDS (mg/L) | 4390±1.00 |
| TSS (mg/L) | 1.04±0.01 |
| Salinity (PSU) | 0.43±0.01 |
| DO (mg/L) | 9.20±0.02 |
| BOD (mg/L) | 4.71±0.50 |
| COD (mg/L) | 20.0±0.50 |
| Pb (mg/L) | ND |
| Cr (mg/L) | 0.08±0.001 |
| Cd (mg/L) | 0.20±0.01 |
| Ni (mg/L) | 0.42±0.02 |
| Fe (mg/L) | ND |
| Cu (mg/L) | 0.12±0.001 |
| Zn (mg/L) | 0.37±0.015 |
| V (mg/L) | 0.16±0.001 |

should be present in waters during the discharge of oilfield waters (Özdemir, 2018). For drinking water, the limit is 0.5 mg L⁻¹ [35] 2008) and 10 mg L⁻¹ [21]. In this study, the TPH level in sea water was 4.43mg L⁻¹, which was within the DPR limit. In the test media, the TPH concentrations ranged from 25.8 – 914.7 mg L⁻¹ in CDO; 19.8 – 239.9mg L⁻¹ in CEWAF; 7.5 – 21.8 mg L⁻¹ in WAF; and from 4.30 – 11.1mg L⁻¹ in D. The levels in D were generally within the limit for TPH in discharged oilfield waters, but well above the drinking water limit. For all crude oil-based media, significant pollution was observed.

Results of PAHs concentration in the different test media are presented in Table 7. Only two concentrations of the different test media (i.e., 100% and 50%) were reported in this study due to the significance of their results relative to the other concentrations. A decreasing concentration of PAHs was observed in all test media with decreasing concentration of test media. When compared with the PAH concentration of the crude oil (464.3 mg L⁻¹), there was a significant drop in PAHs concentration in the crude oil test media. A maximum PAHs concentration of 55.1 mg L⁻¹ was observed in CDO test media concentration of 100%, while the least concentration of 2.04mg L⁻¹ was found in D test media concentration of 50%. All 16 US EPA priority PAHs were detected in WAF test media, with Σ_{16} PAHs concentrations of 21.3mg L⁻¹ and 12.5mg L⁻¹ at toxicant concentrations of 100% and 50%, respectively. The least Σ_{16} PAHs concentrations was detected in D, with a concentration of 2.04mg L⁻¹. A value of 0.2 µg L⁻¹ (0.0002 mg L⁻¹) has been set as the maximum permissible limit for total PAHs in drinking water, while that of benzo[a]pyrene is 0.1 µg L⁻¹ (0.0001 mg L⁻¹) [34]. The results of this study indicated that all test media, including the control, had Σ_{16} PAHs that greatly exceeded the permissible limit by several orders of magnitude. This in-

indicated a strong likelihood for significant bioaccumulation of these contaminants in aquatic organisms. For benzo[a]pyrene, which is regarded as the most carcinogenic of all PAHs, it was detected in all test media except CEWAF and the control.

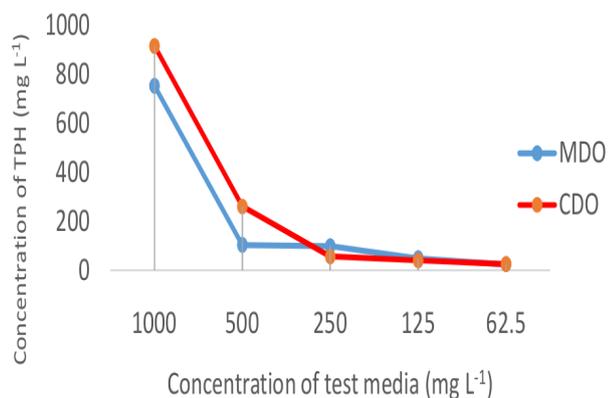


Figure 1: Comparing the concentration of TPH in MDO and CDO

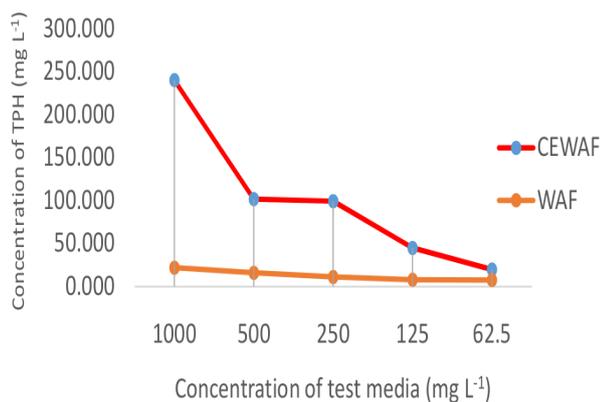


Figure 2: Comparing the concentration of TPH in WAF and CEWAF

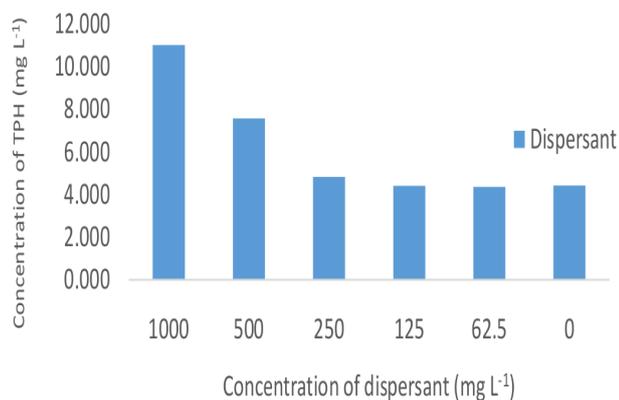


Figure 3: Concentration of TPH in D

3.4. Acute toxicity test result for test organisms

The acute toxicity of the control toxicant and the different test media on the *T. guineensis* is presented as the 96h median

lethal concentration (LC₅₀) as shown in Tables 8, 9 and 10, respectively. The LC₅₀ of the different toxicant test media on *T. guineensis* followed the order: SDS > MDO > CDO > CEWAF > WAF > D. Following the description on 96h LC₅₀, the reference toxicant was moderately toxic, with a value of 1.37. The dispersant solution exhibited the least toxicity, and was non-toxic to *T. guineensis*, having a value greater than 1,000. Several studies have also shown that SDS exhibited the greatest toxicity to marine organisms relative to the dispersant and the dispersant plus crude oil test system [11, 12]. The different exposure media ranged from being non-toxic to being practically non-toxic. The toxicity factors (i.e. the index for assessing toxicity) showed that SDS was 1,380 times more toxic to *T. guineensis* than the dispersant, and 65.7 times more toxic to *T. guineensis* than MDO.

Acute toxicity tests on *P. africanus* showed that the chemically modified test media (i.e., CDO and CEWAF) exhibited slightly greater toxicities to *P. africanus* than MDO and D; but lesser toxicities compared to the reference SDS toxicant (Table 9). The LC₅₀ of the different toxicant test media followed the order: SDS > CDO > CEWAF > WAF > MDO > D. The dispersant also exhibited the least toxicity to *P. africanus*. For the two tested organisms, which are representatives of the secondary and tertiary consumers in the marine food chain, Ecobest® dispersant was shown to be less toxic to both test organisms. However, the LC₅₀ test result suggested that *P. africanus* was more tolerant to SDS relative to *T. guineensis*, as seen in the LC₅₀ values for SDS in both organisms.

The toxicity of the different dispersants on the two types of microbial populations were determined using the 96hLC₅₀ of the toxicants on the test organisms are presented in Table 9. The toxicity of the toxicant test media followed the order: CEWAF < D < CDO < MDO < COA < SDS for heterotrophic bacteria, and CEWAF < CDO < MDO < WAF < D < SDS for hydrocarbon-utilising bacteria. The toxicants generally expressed a lesser toxic effect on the hydrocarbon utilizing bacteria than the total heterotrophic bacteria when compared with their 96 h LC₅₀ values.

The toxicity factor of D to *T. guineensis* and *P. africanus* were 1380 and 380, which implied that SDS was about 1380 and 380 times more toxic to *T. guineensis* and *P. africanus*, respectively. The synergistic action /joint action factor of crude oil-dispersant test systems to the tested organisms implied that the dispersant reduced the toxicity of crude oil. The toxicity of a given crude oil-dispersant mixture varies, and this depends on the proportion of addition of the mixture components [36].

4. Conclusion

Ecobest® dispersant and its product of interaction with crude oil in a marine environment was shown to be non-toxic to representative marine organisms in this study, based on the LC₅₀ values obtained. *P. africanus* exhibited greater tolerance to the effect of crude oil-dispersant in the different test media relative to *T. guineensis*. However, with increased levels of some metals and polycyclic aromatic hydrocarbons present in

Table 7: Concentration of PAHs (mg L⁻¹) in different test media

| | CEWAF | | WAF | | CDO | | MDO | | D | | Brackish water |
|--------|---|--------|-------|-------|------|------|------|-------|--------|--------|----------------|
| PAHs | Concentration (%) of toxicant in test media | | | | | | | | | | |
| | 100 | 50 | 100 | 50 | 100 | 50 | 100 | 50 | 100 | 50 | |
| Nap | 0.08 | 0.0727 | 0.9 | 0.6 | 3.5 | 0.72 | 0.13 | 0.25 | 0.0069 | 0.036 | 0.027 |
| Ace | 0.37 | 0.238 | 3.3 | 2.1 | 14.8 | 2.6 | 1.3 | 1.7 | ND | ND | 0.037 |
| Acp | 0.23 | 0.15 | 1.04 | 0.9 | 6.88 | 1.6 | 2.49 | 1.09 | 0.04 | ND | 0.009 |
| Flu | 0.19 | 0.14 | 2.56 | 1.13 | 9.94 | 1.88 | 2.65 | 0.64 | 0.0042 | 0.035 | 0.031 |
| Phe | 0.61 | 1.36 | 3.98 | 2.44 | ND | 2.35 | ND | 1.33 | 0.064 | 0.024 | 0.017 |
| Ant | 0.72 | ND | 0.61 | 0.23 | 19.6 | 0.25 | 6.96 | 2.38 | 0.007 | 0.019 | ND |
| Flo | ND | 0.25 | 0.59 | 0.35 | ND | 26.8 | 0.27 | ND | 0.01 | 0.0075 | 0.014 |
| Pyr | ND | 0.8 | 1.28 | 0.92 | ND | ND | 12.6 | 5.74 | 0.0005 | 0.016 | 0.0076 |
| Chry | 0.17 | 0.04 | 2.35 | 1.45 | ND | ND | ND | ND | 0.043 | 0.03 | 0.021 |
| BaA | 0.054 | 0.02 | 1.49 | 0.9 | ND | ND | ND | ND | 0.076 | 0.024 | 0.067 |
| BbF | 0.22 | ND | 1.05 | 0.35 | ND | 0.55 | 0.11 | 0.23 | 0.16 | ND | 0.56 |
| BkF | 0.16 | ND | 0.62 | 0.25 | ND | 1.6 | 0.13 | 0.13 | 0.084 | ND | ND |
| BaP | ND | ND | 0.21 | 0.1 | 0.3 | 0.67 | 0.18 | 0.078 | 0.41 | ND | ND |
| Indeno | ND | ND | 0.37 | 0.25 | ND | 0.27 | 4.3 | ND | 1.8 | ND | ND |
| Dah | 4.3 | ND | 0.46 | 0.3 | ND | 0.34 | 0.24 | ND | ND | 1.6 | ND |
| Bghi | 0.048 | 0.33 | 0.38 | 0.2 | ND | 0.29 | 0.18 | 4.08 | 1.13 | 0.017 | 4.08 |
| Total | 7.28 | 3.43 | 21.27 | 12.47 | 55.1 | 39.4 | 31.6 | 17.7 | 3.89 | 2.04 | 4.87 |

Table 8: Acute effect of the different test media on *Tilapia guineensis*

| S/N | Toxicants | 96 h LC ₅₀ (ppt) | Toxicity Factor 1 (MDO) | Toxicity Factor 2 (SDS) | Inference |
|-----|-----------|--------------------------------|-------------------------------|-------------------------------|--|
| 1. | MDO | 89.99 | - | 65.7 | Mechanically dispersed crude oil is 65.7 times less toxic than SDS. |
| 2. | CDO | 225.60 | 2.51 | 164.7 | Chemically dispersed crude oil is at least 2.5 times less toxic than mechanically dispersed crude and 164.7 times less toxic than SDS. |
| 3. | D | 1891.80 | 21.04 | 1380.8 | Dispersant is at least 21.04 times less toxic than mechanically dispersed crude oil and 1380.88 times less toxic the SDS. |
| 4. | WAF | 683.99 | 7.60 | 684 | WAF is 7.6 less toxic than mechanically dispersed crude oil and 684 times less toxic than SDS. |
| 5. | CEWAF | 528.02 | 5.87 | 385.4 | CEWAF is 5.86 times less toxic than mechanically dispersed crude oil and 385.41 times less toxic than SDS. |
| 6. | SDS | 1.37 | 0.015 | - | SDS is 0.015 times more toxic than mechanically dispersed crude oil. |

Table 9: Acute effect of the different test media on *Palaemonetes africanus*

| S/N | Toxicants | 96 h LC ₅₀ (ppt) | Toxicity Factor 1 (MDO) | Toxicity Factor 2 (SDS) | Inference |
|-----|-----------|-----------------------------|-------------------------|-------------------------|--|
| 1. | MDO | 275.5 | - | 27.8 | Mechanically dispersed crude oil is 27.8 less toxic than SDS. |
| 2. | CDO | 137.77 | 0.05 | 13.78 | Chemically dispersed crude oil is 0.05 times more toxic to crustacean than mechanically dispersed crude but 13.78 times less toxic than SDS. |
| 3. | D | 3800.92 | 13.80 | 380.24 | Dispersant is 13.80 times less toxic than mechanically dispersed crude oil and 380.24 times less toxic than SDS. |
| 4. | WAF | 176.13 | 0.64 | 17.62 | WAF is 0.64 times less toxic than mechanically dispersed crude oil. |
| 5. | CEWAF | 168.29 | 0.61 | 16.83 | CEWAF is 0.61 more toxic than mechanically dispersed crude oil but 16.83 times less toxic than SDS. |
| 6. | SDS | 9.99 | - | 0.036 | - |

Table 10: Acute effect of the different test media on bacteria

| Toxicant | HB LC ₅₀ | HUB LC ₅₀ |
|-----------------|---------------------|----------------------|
| CDO | 1,658.5 | 9,544.117 |
| MDO | 944.131 | 250.608 |
| D | 17,221.9 | 77.19 |
| WAF | 228,641.0 | 141.408 |
| CE-WAF | 1,036,319.3 | 12,780.87 |
| Crude oil alone | 185.837 | 2,962.383 |
| SDS | 3.842 | 3.60 |

the habitat water in the test media, there might be a likelihood for bioaccumulation to take place.

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