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RESPONSE OF *TILAPIA GUINEENSIS* AND *CLIBANARIUS AFRICANUS* TO ACUTE TOXICITY OF TWO OIL SPILL DISPERSANTS

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ABSTRACT: *Activities associated with crude oil, natural gas or condensate fields could result in several accidents that can lead to massive oil spills. To curb the deleterious effect of the hydrocarbon contents of the crude oil, the chemical clean-up method can be utilized in far and deep offshore environments in Nigeria. Using the dispersants weakens the interfacial tension between oil and water and in the process, bitty droplets of the oil are hatched and entrained within the water column. Acute toxicity testing becomes vital as the dispersants have the potential of causing deleterious effects on resident organisms within the recipient environment. This study aimed to determine the acute toxicity of two oil spill dispersants and the response of the euryhaline-Tilapia guineensis (fish) and benthic-Clibanarius africanus (hermit crab) when exposed to the dispersants. The fish and hermit crab which are standard test organisms for acute toxicity testing in Nigeria as specified by Nigerian Upstream Petroleum Regulatory Commission (NUPRC) were sourced from Nigerian Institute for Oceanography and Marine Research (NIOMR) at Buguma, Rivers State while the dispersants - Corexit 9527 and Finasol OSR were obtained from different licensed oil field chemical stores in Port Harcourt, Rivers State, Nigeria. For the test organisms to adjust to a new environment, they were first acclimatized before conducting the range finding test. Based on the results obtained from the range finding test, the definitive test concentration was established at 0.2, 0.4, 0.6, 0.8, 1.0ppt and 2, 4, 6, 8,10 ppt for Tilapia guineensis and Clibanarius africanus respectively. Reference experimental group was also instituted using Potassium Chloride at concentrations of 20, 40, 60, 80 and 100 ppt. The median lethal concentration (LC_{50}) was calculated from the mortality value using Probit software. Mortality rates increased with increase in concentration of the toxicants and exposure time. The 96 hour-LC50 values for Corexit 9527 were 4669mg/l and 181 mg/l for Clibanarius africanus and Tilapia guineensis respectively while that of Finasol OSR were 4268 mg/l and 188 mg/l for Clibanarius africanus and Tilapia guineensis respectively. The study revealed that Tilapia guineensis was more sensitive as the two dispersants were less toxic to hermit crab and more toxic to the fish. Hence, adequate enforcement of regulations on the use of these chemicals should be adhered to in the water column of marine environments and more attention be inclined to biologically derived chemicals (biosurfactants).*

KEYWORDS: Acute toxicity, Dispersants, Tilapia guineensis, Clibanarius africanus.

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INTRODUCTION

Activities associated with crude oil, natural gas or condensate fields such as exploration, exploration drilling, development drilling, production and transportation could result in several accidents that can lead to massive or catastrophic oil spills. To curb the deleterious effect of the hydrocarbon contents of the crude oil, major steps are involved. The steps include containment and recovery through mechanical cleanup (use of booms, skimmers and sorbent materials), chemical cleanup (use of dispersants, gelling agents and/or sinking agents), and natural degradation (Agarwal, 2021).

The third-generation dispersants which contain two or more blends of surfactants in which the concentration stretches between 25% and 65% with glycol and light petroleum distillate solvent weakens the interfacial tension between oil and water (International Petroleum Industry Environmental Conservation Association – International Association of Oil & Gas Producers (IPIECA-IOGP 2015)). This enhances the inevitable evolution of dispersion. That is, bitty droplets of the oil are hatched in larger numbers and entrained within the water column (Uffort & Odokuma, 2018). Although the surfactants used in the formulation of oil spill dispersants are pivotal as they enhance dispersion of slick, they are considered the more noxious constituent. It can cause the alteration of pH of the water thereby resulting in modification of the concentration of dissolved phosphate, nitrate and organic material used by primary producers leading to effects on aquatic organisms' survival and performance (El-Hack et al., 2022). Thus, concerns still persist about the potential toxicity of oil spill dispersants to water column organisms.

The water column allows sunlight to reach aquatic plants and algae, it allows oxygen and other essential dissolved nutrients to be delivered to aquatic plants and animals. The water column also provides transport for fish eggs and larvae from spawning grounds to nursery and foraging areas. Thus, the water column health is important for pelagic species. The water column is made up of five zones- epipelagic (the sunlight zone which is the upper layer of the water body that allows penetration of sunlight to a depth of 200 meters), mesopelagic (the twilight zone- a layer of water that lies 200 to 1000 meters below the ocean surface just beyond the reach of the sunlight), bathypelagic (midnight zone- a perpetual darkness that extends to about 4000 metres which reaches the ocean floor), abyssopelagic (the seafloor and water column from 3000 to 6500 metres depth where sunlight does not penetrate) and trenches (also known as the hadal zone extending from 6000 to 11000 metres). Epipelagic is a home to phytoplankton and these primary producers become food sources for other organisms (Foulds, 2019).

The crustacean (Arthropoda, Malacostraca, Decapoda, Diogenidae) including *Clibanarius africanus* (hermit crabs) live at a range of depths from shallow coral reefs and shorelines to deep bottoms. The sedentary nature of the benthos makes it possible for them to readily imbibe and accumulate any xenobiotic compounds and other stressors released into the water body (Onyema, 2019). Also, being opportunistic eaters (small fish, invertebrates such as worms, plankton and any floating food particles they can find in water that surrounds them), they help to clean up the sea bottom by harvesting decomposing plant and animal matter. They feed on debris that settle on the bottom of the water and in turn serve as food for a wide range of fishes, in essence, they form part of the aquatic food chain (Idowu & Ugwumba, 2005; Onyema, 2019). They also accelerate the breakdown of decaying organic matter into simpler inorganic forms such as phosphates and nitrates.

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The euryhaline species, *Tilapia guineensis*, inhabit coastal water and are herbivores or detritivores and also feed on zooplankton. Thus, they occupy an intermediate position between primary producers and piscivores. This generates their two main ecological roles: circulation of nutrient metabolites on which primary production depends and supporting the piscivores in the ecosystem including man (Lowe-McConnell, 2000).

Since the oil spill dispersants have the potential to have deleterious effects on resident organisms, acute toxicity (a property of a substance that has toxic effects on an organism when the organism is exposed to a lethal dose) testing becomes vital. The aim of this study is to determine the acute toxicity of two oil spill dispersants- Corexit 9527 and Finasol OSR and the responses of *Tilapia guineensis* and *Clibanarius africanus* when exposed to these dispersants.

MATERIALS AND METHODS

i. Source of oil spill dispersants and reference chemical

Potassium Chloride (RC), Corexit 9527 (D1) and Finasol OSR (D2) were obtained from different licensed oil field chemical stores in Port Harcourt, Rivers State, Nigeria.

ii. Source of test organisms

Juveniles of *Tilapia guineensis* (weight range: 3.3g to 25.6g; length range: 6.9 to 12.1cm) were obtained from National Institute for Oceanography and Marine Research (NIOMR) at Buguma, Rivers State, Nigeria and transported in airbags to the laboratory. Active *Clibanarius africanus* was obtained from NIOMR and was transported to the laboratory in a mesh holding unit. The habitat mud of the hermit crabs was also collected from the same site and placed in holding tanks as substrate.

iii. Acclimatization of test organisms

In order for the organisms to adjust to the laboratory environment, they were subjected to an acclimatization process. To acclimatize the fishes in dilution water (recipient water-seawater) which was used for the toxicity test, they were exposed to different levels of the seawater gradually. The salinity of the seawater was 23 ppt – 26ppt. Due to the differences in salinities of seawater (26 ppt) and brackish water (19 ppt) which was the salinity of the organism's habitat water in Buguma, it was vital to gradually expose the test organisms to increasing concentrations of seawater (salinity).

Employing the method of Luke and Odokuma (2021), the organisms were first acclimatized for 72 hours in 100% habitat water, for another 72 hours, they were acclimatized in 50%/50% (v/v) of habitat and sea water. In the next 72 hours, the organisms were acclimatized again in 30%/70% (v/v) habitat and sea water. Acclimatization in 100% sea water was then conducted for 14 days with renewal of the sea water every 3 days to decrease the impact of introduction of waste-products of metabolism in water by test organisms. The dead and weak organisms were removed during the acclimatization period.

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The method of Luke and Odokuma (2021) was employed for acclimatization of the hermit crabs. The organisms were first acclimatized for 72 hours in 100% habitat water, for another 72 hours, they were acclimatized in 50%/50% (v/v) of habitat and sea water. In the next 72 hours, the organisms were acclimatized again in 30%/70% (v/v) habitat and sea water. Acclimatization in 100% sea water was then conducted for 14 days. Each concentration (v/v) at the different levels was freshly prepared and renewed on a daily basis to decrease the impact of introduction of waste-products of metabolism in water by test organisms. The dead and weak organisms were removed during the acclimatization period.

iv. Acute toxicity test

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.

Ten active juvenile fishes and ten active hermit crabs of similar sizes were exposed in duplicates into aerated glass tanks (12.06cm × 11.43cm × 11.43cm for *Clibanarius africanus* and 36.83cm × 17.78cm × 17.78cm for *Tilapia guineensis*) of the different concentrations (derived after preliminary range finding test) of the test toxicants as outlined below:

Table 1: Concentrations of Toxicants Used for the Bioassay

Test organism	Corexit 9527 (D1)	Finasol OSR (D2)	Potassium chloride (RC)
Tilapia guineensis	0, 0.2, 0.4, 0.6, 0.8, 1.0ppt	0, 0.2, 0.4, 0.6, 0.8, 1.0ppt	0, 20, 40, 60, 80, 100 ppt
Clibanarius africanus	0, 2, 4, 6, 8, 10 ppt	0, 2, 4, 6, 8, 10 ppt	0, 20, 40, 60, 80, 100 ppt

The five different concentrations for each toxicant including the reference chemical - KCl as seen in table 1 - were used for the acute toxicity set up. The 0 ppt was 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. The fishes and hermit crabs were considered dead when they failed to show any sign of movement or response to gentle prodding with sharp objects.

v. Statistical analysis

The mortality rate of the test organisms was calculated by multiplying the quotient of the number of dead test organisms and the total number of test organisms exposed to the test toxicants by 100. Toxicological data involving mortality rate were then analysed by probit analysis. The toxicity indices derived were median lethal concentration (LC_{50}), No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC). The toxicity factors were computed by dividing the median lethal concentration of the toxicant by the median lethal concentration of the reference chemical.

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RESULTS

Concentration Response with Time of *Clibanarius africanus* to Test Toxicants

The lethal response of *Clibanarius africanus* to different concentrations of the different test toxicants, including reference toxicants at different exposure times was examined and recorded. The results as reported graphically are represented in figures 1 to 3.

i. Concentration response with time of *Clibanarius africanus* to Corexit 9527 (D1)

Clibanarius africanus was exposed to different concentrations of D1 (0, 2, 4, 6, 8 and 10 ppt) for an exposure time of 96 hours. The 0ppt had no death (0% mortality) throughout the 96 hours. The least concentration (2ppt) had 0% mortality at 96 hours. The concentration of 6 ppt recorded 60% mortality at 48 hours while the highest concentration-10 ppt killed all the organisms at 96 hours but had 20 % mortality at 12 hours.

ii. Concentration response with time of *Clibanarius africanus* to Finasol OSR (D2)

The result of the response of *Clibanarius africanus* to 0ppt, 2ppt, 4ppt, 6ppt, 8ppt and 10 ppt concentrations of D2 at 0 to 96 hours are represented in figure 2. The crabs were less sensitive to concentrations of 2 ppt and 4 ppt. At 10 ppt (48 hours to 96 hours), the organisms all died (100%). The concentration of 2 ppt recorded 5% mortality at 96 hours.

iii. Concentration response with time of *Clibanarius africanus* to KCl (RC)

Different concentrations (0, 20, 40, 60, 80 and 100 ppt) of the RC were prepared and used as toxicants exposing *C. africanus* to each for 96 hours. The number of organisms alive were counted at a 24-hour interval. The percentage mortality against time is represented in figure 3. 100 ppt concentration recorded the highest mortality rate of 100% for exposure time: 12 hours, 24hours, 48hours, 72 hours and 96 hours.

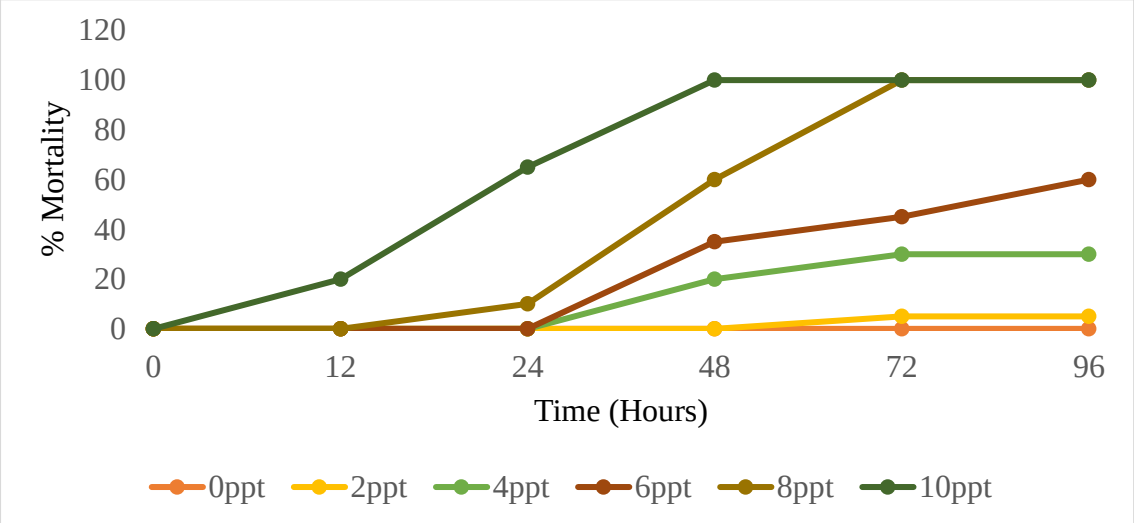

Figure 1: Concentration response with time of *Clibanarius africanus* to D1

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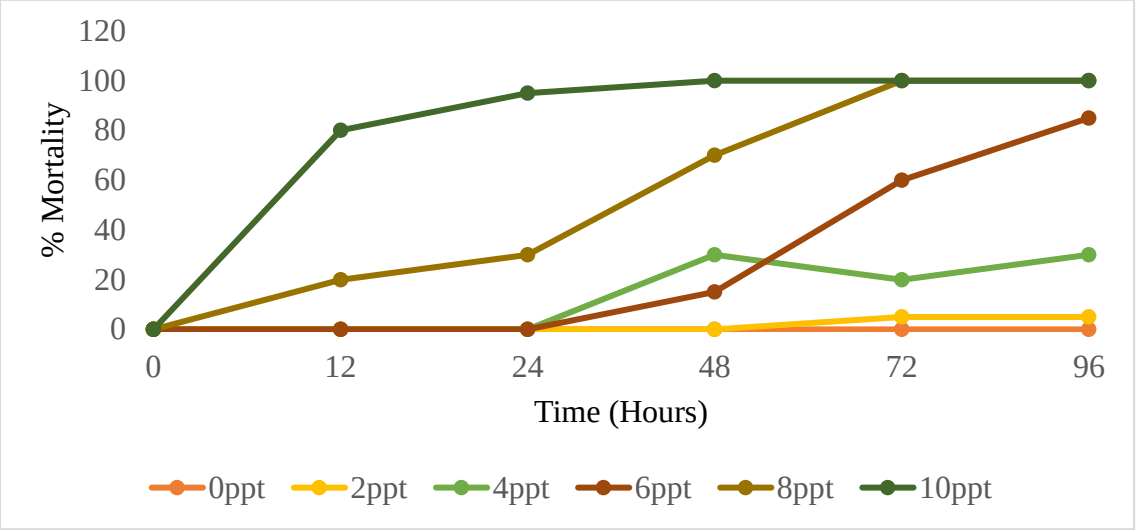

Figure 2: Concentration response with time of *Clibanarius africanus* to D2

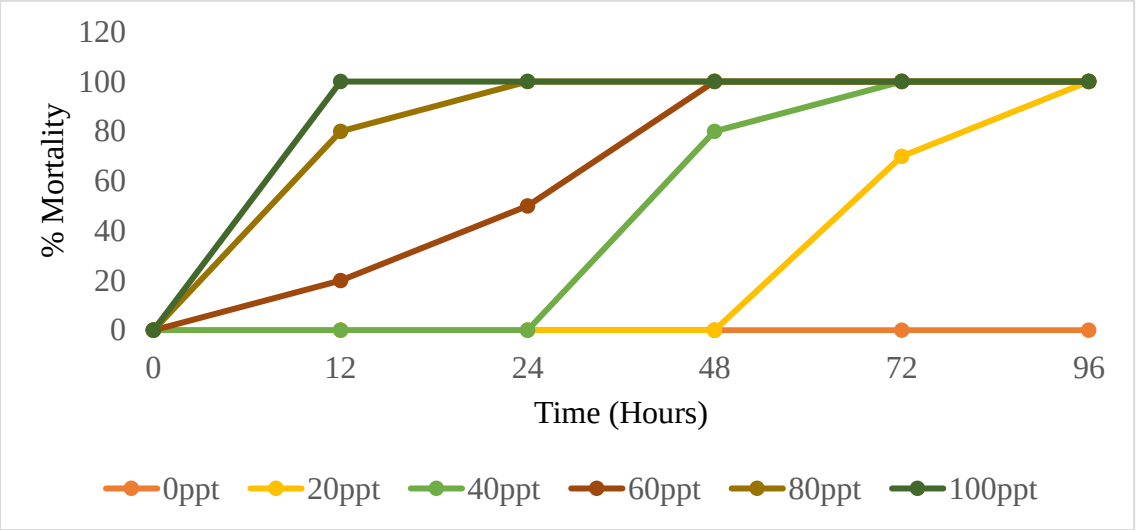

Figure 3: Concentration response with time of *Clibanarius africanus* to RC

Concentration Response with time of *Tilapia guineensis* to Test Toxicants

Using Corexit 9527 and Finasol OSR as test toxicants, KCl as reference chemical, *Tilapia guineensis* was exposed to their different concentrations within 0 to 96 hours exposure time. The response (mortality rate) of the test organism is represented in figures 4 to 6.

i. Concentration response with time of *Tilapia guineensis* to Corexit 9527 (D1)

The response of *Tilapia guineensis* to different concentrations (0, 0.2, 0.4, 0.6, 0.8 and 1.0ppt) at different exposure times is represented in figure 4. The highest concentration used-1.0ppt

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recorded 90% mortality at 12 hours exposure. 0.2ppt recorded 0% mortality at 12 hours, 15% mortality at 24 hours and 65% mortality at 96 hours exposure.

ii. Concentration response with time of *Tilapia guineensis* to Finasol OSR (D2)

Figure 5 displays the percentage mortality of *Tilapia guineensis* to different concentrations of D2 at 0 to 96 hours. To 0.2ppt, *Tilapia guineensis* recorded 5% mortality at 24 hours while to 1.0ppt, the mortality was 65% at 12 hours and 100% at 24 hours exposure time.

iii. Concentration response with time of *Tilapia guineensis* to KCl (RC)

Different concentrations of KCl were observed to express different degrees of mortality on *Tilapia guineensis* with 96 hours exposure time. The result is represented in figure 6. 60ppt, 80 ppt and 100 ppt killed all organisms (100% mortality) at all exposure time. At 20ppt, the mortality rate of *Tilapia guineensis* was 45% when exposed for 24 hours.

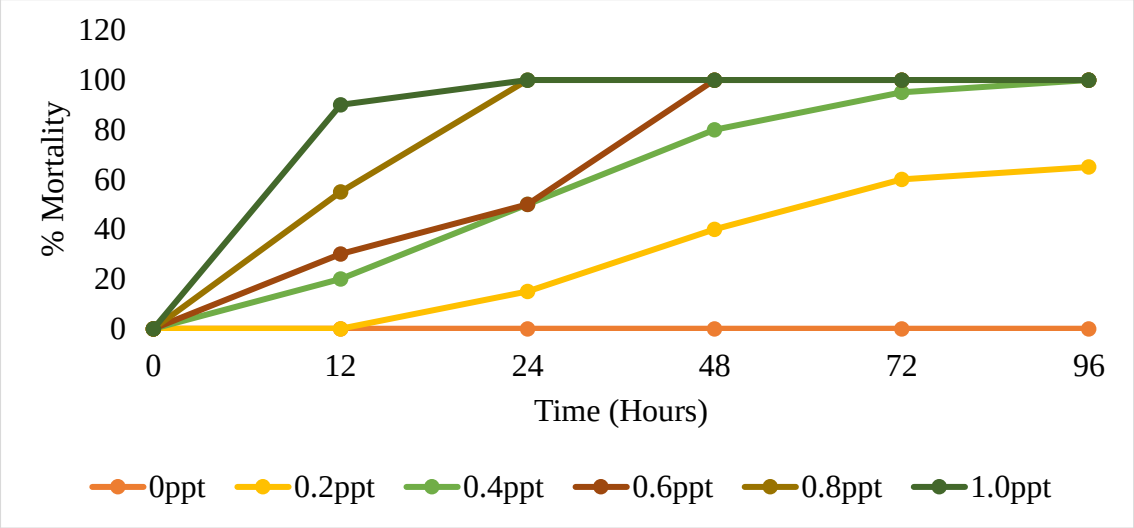

Figure 4.18: Concentration response with time of *Tilapia guineensis* to D1

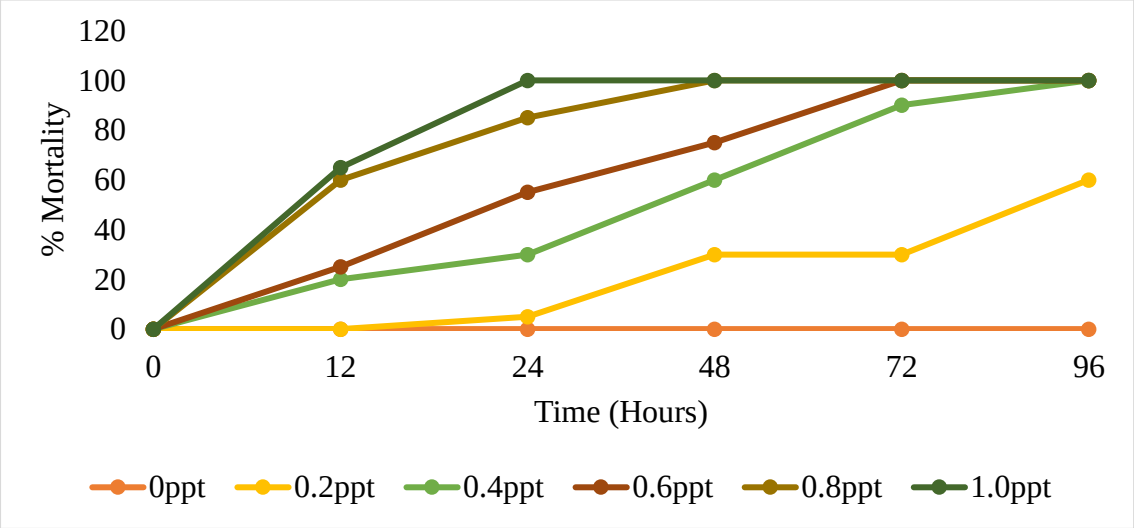

Figure 4.19: Concentration response with time of *Tilapia guineensis* to D2

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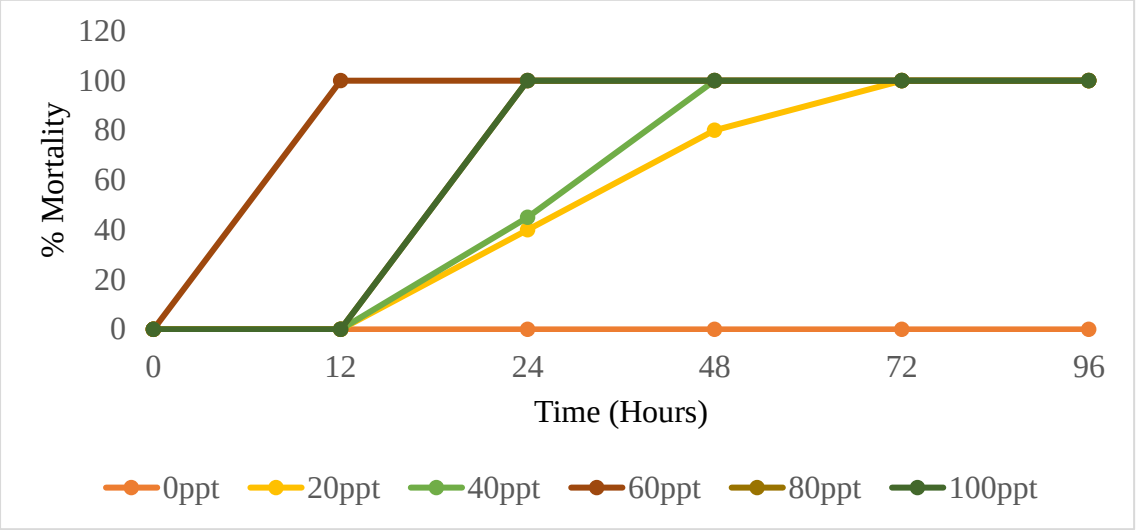

Figure 4.20: Concentration response with time of *Tilapia guineensis* to RC

Acute toxicity test

The toxicity indices including lethal concentration that can kill 50% of the organisms, Lowest Observed Effect Concentration, No Observed Effect Concentration, Acute Toxicity Unit and Chronic Toxicity Unit (LC_{50}, LOEC, NOEC, TU_a and TU_c respectively) of the test oil spill dispersants and reference chemical on the benthos- *Clibanarius africanus* and the aquatic vertebrate- *Tilapia guineensis* were determined and are presented in tables 2 to 4. The toxicity factor is presented in table 5.

Table 2: Acute Toxicity Indices of Corexit 9527

Test organism	Exposure time	NOEC (mg/l)	LOEC (mg/l)	LC_{50} (mg/l)	TU_a	TU_c
Clibanarius africanus	96 hours	2857	3381	4669	0.02142	0.03500
Tilapia guineensis	96 hours	131	146	181	0.55249	0.76336

Table 3: Acute Toxicity Indices of Finasol OSR

Test organism	Exposure time	NOEC (mg/l)	LOEC (mg/l)	LC_{50} (mg/l)	TU_a	TU_c
Clibanarius africanus	96 hours	2773	3215	4268	0.0234	0.03606
Tilapia guineensis	96 hours	136	152	188	0.53191	0.7353

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Table 4: Acute Toxicity Indices of KCl

Test organism	Exposure time	NOEC (mg/l)	LOEC (mg/l)	LC_{50} (mg/l)	TU_a	TU_c
Clibanarius africanus	96 hours	2480	2189	1724	0.058	0.040
Tilapia guineensis	96 hours	2121	1876	1482	0.067	0.047

Table 5: Toxicity Factors of Test Organisms Exposed to the Oil Spill Dispersants and Potassium Chloride

Test organisms	D1	D2
Clibanarius africanus	2.70	2.47
Tilapia guineensis	0.12	0.12

DISCUSSION

The hermit crab- *Clibanarius africanus* which is a prominent species among the members of the benthic community in coastal water constitute the key components of aquatic food webs, linking organic matter and nutrient resources with higher trophic levels (Kiljunem et al., 2020; Nkwoji, 2023). At a concentration of 10 ppt of D1, D2, RC at 96 hours exposure time, 100% mortality was recorded. Generally, the percentage mortality of *Clibanarius africanus* when exposed to the different toxicants including the reference chemical displayed a trend of increase in percentage mortality with increase in concentration of toxicant and increase in exposure time. This trend of increased percentage mortality corroborates with findings of King et al. (2012) where exposure of hermit crab to different concentrations of crude oil and petroleum products showed that the toxicants were differentially toxic to the test hermit crab. On the basis of 96 hour-LC50 values, D2 (4268 mg/l) was found to be more toxic than D1 (4669 mg/l). With reference to other toxicity indices, the toxicity of the test toxicants on *Clibanarius africanus* was in order of D2>D1>RC.

The aquatic vertebrate-*Tilapia guineensis* was exposed to different concentrations of the test toxicants. Using mortality as a measure of toxicity, the percentage mortality was seen to increase with increase in concentration and exposure time. This conforms to findings of Awodele et al. (2017) and Kingsley et al. (2021). Their findings revealed that production chemicals (corrosion inhibitor, biocide, oxygen scavenger and defoamer) were toxic to *Tilapia guineensis*. The two oil spill dispersants showed that they were lethal to *Tilapia guineensis* as the 96 hour-LC50 values obtained were within the range of 181 – 188 mg/l. The hermit crab-*Clibanarius africanus* was more tolerant to the test toxicants when compared to *Tilapia guineensis*.

The toxicity factors of *Clibanarius africanus* exposed to D1 and D2 at 96 hours were 2.70 and 2.47 respectively while the toxicity factors for *Tilapia guineensis* exposed to D1 and D2 were the same (0.12). The toxic effect of the reference chemical – Potassium Chloride - could be as a result of denaturation of proteins as a result of increased alkalinity in the toxicity test medium. In comparison of the test organisms, *Tilapia guineensis* was more sensitive to the exposure to

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the two test oil spill dispersants than *Clibanarius africanus*. This could be attributed to their basic structures (tissues) which provide high resistance to penetration as the hermit crabs carry mobile shelters (shells) which cover its soft body while the fishes possess the hydroxyapatite, calcium carbonate protective scales. In the comparison of the toxicity of the oil spill dispersants, FinasolOSR (D2) was more toxic compared to Corexit9527 (D2) which could be dependent on the chemical compositions which could cause harm to tissues and can lead to oxidative pressure. Thus, subjection of the test organisms to the test dispersants could cause modification in hematological indices, biochemical and metabolites that assume the test organism's basic function (Opete et al., 2019; Inyang et al., 2018).

CONCLUSION

This study investigated the response of *Tilapia guineensis* and *Clibanarius africanus* to acute toxicity of two dispersants (Corexit 9527 and Finasol OSR). The results showed that as the concentration of dispersants and reference chemicals increased, the mortality rates increased. The study revealed that Corexit 9527 and Finasol OSR were moderately toxic to *Clibanarius africanus* and more toxic to *Tilapia guineensis*. The euryhaline and the benthos are both important aquatic species in view of their vast contribution to the needs of many nations in terms of nutrition, growth and development. Thus, their exposure to high concentrations of toxicants poses serious health and environmental challenges hence adequate enforcement of regulations on the use of these chemicals should be adhered to. Also, attention should be tilted to the use of biologically derived substances that can reduce the interfacial tension between oil and water as they can be readily available for biodegradation.

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