

## Evaluation of Crude Oil Degradation Under a no-control and Dispersant-Control Settings, Based on Biological and Physical Techniques

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**ABSTRACT:** The degradation of crude oil under a no-control and dispersant-control setting was evaluated using loss of biological activity test, microbial population growth and measurements of optical transmittance of test media. Comparison of the toxicity of crude oil under the no-control setting to crude oil-dispersant mixture revealed that the mixture was about 2 times more toxic than the crude oil under the no response strategy. Furthermore, the results of the toxicity testing experiments also showed that under the no-control setting, there was a loss in the toxicity of the crude oil from 6.03mL/L - 9.43 mL/L compared to crude oil-dispersant mixture where the toxicity of the crude oil-dispersant mixture was found to remain fairly constant with  $LC_{50}$  value of the mixture ranging from 4.0 mg/L to 4.38 mg/L over the 28 days experimental period. On the basis of the regression coefficient factor ( $R^2$ ), loss of biological activity during the no-control setting was found to be about 3 times more than under the dispersant-control setting. The result of the measurement of optical transmittance of crude oil depicting rate of emulsification under the no-control and dispersant-control settings revealed that level of light transmittance under no-control setting ranged from 0% to 84%, while under the dispersant-control setting light transmittance ranged from 0% to 72% over the 28 days of observation. The derived regression factor ( $R^2$ ) however revealed that under the dispersant-control setting, the rate of emulsification and degradation of crude oil was faster ( $R^2 = 0.96$ ) than under the no-control setting ( $R^2 = 0.77$ ). The result of the microbial growth assays also revealed that under the dispersant-control setting, the numbers of microbial colony forming units was about 7 folds higher than the number of colony forming units observed under the no-control setting. The usefulness of the methods for assessing crude oil degradation and its implications for choosing dispersants and making decision on whether or not to deploy dispersants for oil spill control are discussed.

**Key words:** Crude oil, Dispersant, Toxicity, Spill Control, Emulsification

### INTRODUCTION

Almost all activities involved in the exploration and exploitation of crude oil results in the discharge of crude oil into the environment. Recently, the volume of crude oil being spilled into the environment has increased significantly, especially now that oil seems to have taken the center stage as the major source of energy to mankind. Oil spills in Nigeria occur due to a number of causes, they include corrosion of pipelines and tankers (accounts for 50% of all Spills) Sabotage (28%), and oil production operations (21%) (OGP, 1989). The

largest contributor to the spill total is corrosion of pipes and tanks and the eventual rupturing or leaking of production infrastructures. The Nigerian National Petroleum Corporation put the quantity of oil jettisoned into the environment yearly at 2,300 cubic meters with an average of 300 individual spills annually. Among the largest individual spills include the blow out of Texaco offshore station which in 1980 dumped an estimated 400,000 barrels of crude into the Gulf of Guinea and Shell's Forcados Terminal tank failure which resulted in a spillage estimated at 580,000 barrels (Ifeadi and Nwankwo, 1987).

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The spillage of crude oil into the environment demands the development of various control strategies such as containment and recovery using booms, skimmers or pumps, sinkers, burning or use of dispersants (Westermeyer, 1991). The type of control strategies deployed will always be depicted by the volume of the spilled, the sensitivity of the receiving environment, the topography of the area etc. Dispersants are usually deployed when there is a need to urgently eliminate the floating mass of oil shorelines (NRC, 1989; Duke *et al.*, 2000). The immediate effect of dispersants is to break up the oil slicks and move the oil in form of droplets from the surface of the water to the column of the water body thereby increasing the oil-water interface. A good dispersant should be highly effective and less toxic on its own against organisms or in joint action with crude oil when used to control oil spillage. However, it is important to note that most of the dispersants that have been initially introduced into the market were found either to be very toxic on their own or enhance the toxicity of the spilled oil on the receiving habitat or exposed organism when deployed to control oil spills (Horst *et al.*, 2000; Otitolaju, 2005). Recent information on joint-action toxicity of mixtures of compounds has shown that the types of interactions exhibited by components of mixtures are largely dependent on the proportion of their occurrence in the mixture (Otitolaju, 2002, 2005). Therefore, it is not impossible that those dispersants that in the past were found to have enhanced the toxic effect of crude oil to exposed organisms could actually have caused a reduction in the toxic effect of crude oil or the mixture at other dispersal ratios. Indeed, it is a fact that the recommended mixture or dispersing ratio of previously introduced dispersants took into consideration mainly the optimum dispersal ratio that could achieve the greatest emulsifying capabilities with minimal consideration for the type of joint action that may be exhibited by the mixtures at the proposed mixing to ratio (Kingham, 1981; Mitchell and Holdway, 2000). It is therefore important to carry out extensive toxicity evaluation of dispersants and crude oil in various mixing ratios before permits are given to allow the use of such chemicals in managing oil spills in aquatic environments.

These types of evaluations are necessary because when oil is spilled; there is an initial evapo-

ration of lighter, fractions. For example, in the Exxon Valdez or Amoco Cadiz incident, approximately 30% of the spilled oil evaporated. There is further spreading of the oil which makes it thinner as a result of wave action (Wells *et al.*, 1985; Moles *et al.*, 2002). Further biodegradation processes will continue to take place over time. However, control strategies often introduced by man, especially the use of chemical dispersants often cause the formation of water-in-oil emulsions (Sterling *et al.*, 2004). These emulsions are usually more adherent and maybe more harmful than un-emulsified oil in case of a synergistic interaction between the crude oil and the dispersant, therefore causing more harm than if the spilled oil was left uncontrolled (Spies *et al.*, 1986; Duke *et al.*, 2000). It is therefore necessary to evaluate the importance of utilizing the dispersants at the selected dispersal ratio against the possible scenario of a no-control strategy whereby the spilled oil is allowed to degrade over time without introduction of dispersants.

On the basis of the above, the objectives of this study are to estimate the degradation of crude oil under a no-control and a dispersant (OSD - 9460) controlled scenario using physical techniques based on light transmittance, microbial population growth assays and toxicity testing techniques based on evaluating the loss of biological activities of the crude oil: dispersant mixture (dispersal ratio 9:1) against fingerlings of *Clarias gariepinus*.

## MATERIALS & METHODS

Fingerlings of *Clarias gariepinus* (Chordata, Osteichthyes, Siluriformes, and Clariidae) also known as the African catfish used in the bioassays of this experiment. Fingerlings of similar sizes ranging in length from 4 – 6cm and body weight range of 4-10g were purchased from a fish farm in Lagos, Nigeria and transported in an oxygen bag to the laboratory. The fingerlings were kept in a plastic tank (34 x 27 x 48.5) cm which was half filled with de-chlorinated water. The de-chlorinated water was obtained by aerating tap water in a plastic tank with an aerator (Cosmo Aquarium, air pump 11,000) for 24 hours. This was done to allow the rapid evaporation of chlorine gas in the water. During acclimatization, the fingerlings were fed with Coppens fish feed. They were fed twice

daily (morning and evening) and the water was changed once every three days to prevent the accumulation of waste metabolite and food particles. The fingerlings were maintained in the holding tank for a minimum of 5 days to allow them acclimatize to the laboratory conditions (Temperature:  $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ; Salinity:  $0\text{‰}$ ; pH:  $7.2 \pm 0.1$ ) before using them in the bioassays. Fingerlings of similar sizes ( $5.0\text{cm} \pm 1\text{cm}$ ) were selected from the stock tank. They were caught with the aid of a hand net and introduced into bioassay containers containing the media. The quantal response (mortality) was assessed every 24 hours over a period of 4 days. Death of fingerlings was assumed when they showed no response to mechanical stimulation (prodding with a rod). Dead fish were removed at each observation.

Forcados light crude oil used in this experiment was obtained from Shell Petroleum Development Company (SPDC) production platform in Forcados, Port Harcourt, Nigeria. Some of the physico-chemical characteristics of the Forcados light brand of crude oil include: Sulphur, content 0.2%, API gravity 60 / 60F; rapid vapor pressure 2.5psi and pour point 25. The crude oil was stored in a bottle which was tightly covered and kept at  $4^{\circ}\text{C}$  in the refrigerator. OSD 9460 is a hydrocarbon mitigation agent yet to be recommended for oil spill clean-up in Nigeria. It was obtained from a representative of the marketers. It was stored in a plastic bottle and kept in the laboratory at room temperature. A pre-determined volume of the test chemical was introduced into a beaker and made up with water achieve the desired concentration of the test medium. The mixture was mixed properly with a stirrer before the test animals were introduced on the required / pre determined day / time.

A mixture of crude oil and dispersant in pre determined ratio of 9:1 (prescribed by manufacturers for oil spill control in aquatic ecosystems) was prepared. At each pre-determined concentration of mixture required, the proportion of each constituent compound was computed and measured out. Crude oil was put first before the dispersant was put into the bioassay container. Three batches of the test media (for both no-control and controlled tests) were prepared and each batch was utilized at prescribed time interval.

Eight (8) fingerlings of *C. gariepinus* of similar sizes were exposed / introduced into bioassay containers containing the test media at various concentrations which were left over various durations. Each concentration was duplicated, meaning that a total of sixteen (16) fingerlings were exposed per treatment including and untreated control at the various durations.

Fingerlings were exposed to the following concentrations left for 0 days, 14 days and 28 days: 0.5, 2.5, 3.5, 5, 6.5, 8, 10, 12 mL/L and untreated control. Mortality for the various experimental periods was assessed once every 24 hours over 4 days for the various durations. Fingerlings of similar sizes were exposed to various concentrations of the mixtures at ratios 9:1 prepared over various experimental periods in a similar procedure as described in 2.3.1 above. The concentrations are as follows:

Crude oil / OSD – 3, 3.5, 4, 5, 8, 10 mL/L and untreated controls.

Mortality for the various experimental periods was assessed once every 24 hours over 4 days. Degradation of crude oil under a no-control setting or under dispersant controlled setting during the observation period was measured by determination of the optical transmittance of the media with the aid of a colorimeter set at wavelength of 540nm. These were carried out at pre-determined time interval by putting a sample of the media into corvettes' and the optical transmittance read off. Microbial population growth in medium containing crude oil alone (no-control setting) and in media containing crude oil and dispersant; OSD-9460 at dispersal ratio (9:1) (controlled setting) were determined by culturing  $1\mu\text{m}$  of the medium on agar media. The number of distinct colonies formed was observed after incubation for 5 days.

Toxicological data involving quantal response (mortality) for both single and joint studies were analyzed by probit analysis including the equation for probit lines (Finney, 1971). T- test analysis was used to compare the equality of means for the mean number of colony forming units for the microbial load. The t-test was used to show the significant difference between the mean numbers of colony forming units for crude oil when acting singly and when in joint action with dispersant OSD-9460. Regression coefficient ( $R^2$ ) factor was

determined based on the rate of biological activity, which is obtained from the data of toxicity plotted against time.

**RESULTS & DISCUSSION**

The analysis of dose-response (mortality) data for crude oil acting singly based on 96-hLC<sub>50</sub> values for days 0, 14 and 28 were 6.03mL/L, 7.29mL/L and 9.43mL/L respectively (Table 1). This shows that the toxicity of crude oil under the no-control setting reduced over the period of observation. For example, between day 0 and day 28, there was approximately a two-fold loss in the biological activity of the crude oil under the no-control setting (LBA = 1.56) (Table 1). Regression coefficient analysis depicting the rate of loss of biological activity also gave a positive value of 0.98 (Fig. 1). Therefore implying that in instances where no control measures were deployed following an oil spill incident, there will still be a marked decrease in the toxicity of the crude oil over time. The observed decrease in the toxicity of the crude oil under the no-control setting can be attributable to the weathering of the spilled oil by processes such

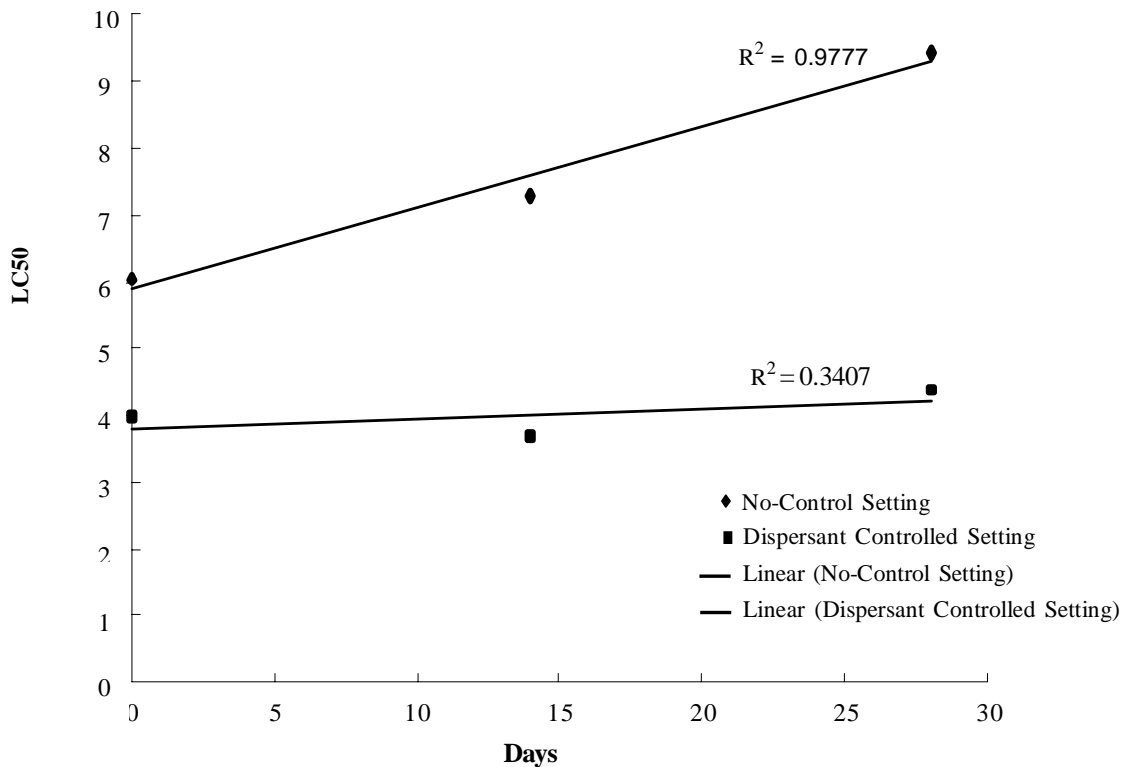
as evaporation, sedimentation, oxidation and biodegradation (Well *et. al.*, 1995). All these processes contribute to the degradation or decrease in the concentration of the spilled oil even when no control measures are deployed. This is in agreement with the work of Reed *et. al.*, (1999), who observed that natural weathering processes including vertical and horizontal mixes even in light wind, brought subsurface hydrocarbon concentrations to background levels within few days following a 10 day blowout scenario releasing 11,000 bbl per day of light crude.

The analysis of dose – response (mortality) data for the loss of biological activity of crude oil by dispersant OSD 9460 at dispersal ratio 9:1 showed that the 96-hLC<sub>50</sub> values for days 0, 14 and 28 were 4.0, 3.67 and 4.38mL/L respectively (Table 2). These results revealed that the toxicity of crude oil-dispersant mixture under the dispersant-control setting did not reduce significantly over the period of observation. For example, between day 0 and day 14, there was an increase in the toxicity of the mixture (LBA = 0.93), though by

**Table 1. Acute toxicity of crude oil, Forcados light over a 28-day period (0, 14 and 28) against fingerlings of *C. gariepinus* under a no-control / dispersant-control setting for crude oil spillage**

Chemical	LC50(C.L)	Slope ±S.E	D.F	Probit line equation	LBA*
<b>No-control Setting</b>					
Day 0	6.034 (8.680-4.280)	3.625 +1.52	1	Y = 2.170 + 3.625	1
Day 14	7.289 (19.381-5.651)	5.330 +3.547	1	Y = 0.402 + 5.330	1.21
Day 28	9.432(13.083-6.815)	3.252 + 2.284	2	Y = 1.831 + 3.525 x	1.56
<b>Dispersant Controlled Setting</b>					
Day 0	3.962 (6.082 – 2.516)	2.611 ± 1.147	3	Y = 3.439 + 2.611 x	1
Day 14	3.671 (5.562 – 2.365)	2.952 ±1.406	2	Y = 3.333 + 2.952 x	0.93
Day 28	4.375 (6.205-3.070)	3.065 + 1.549	2	Y = 3.036 + 3.065 x	1.10

C.L = Confidence Limit  
 D.F = Degree of freedom  
 S.E = Standard error



**Fig. 1. Regression analysis of toxicity indices depicting rate of loss of biological activity of test media oil under a no-control and dispersant-control settings (Regression analysis factor (R<sup>2</sup>F) = Ratio of R<sup>2</sup> under the no-control setting / R<sup>2</sup> under the dispersant-control setting = 2.88)**

**Table 2. Comparisons of toxicity of crude oil and crude oil-dispersant OSD-9460 mixture against fingerlings of *C. gariepinus* over a 28-day period of observation**

Chemical	LC50(C.L) mL/L	LC50(C.L) mL/L	T.F
	No-control Setting	Dispersant-control Setting	
Day 0	6.03 (8.680-4.280)	3.96 (6.082 – 2.516)	1.52
Day 14	7.29 (19.381-5.651)	3.67 (5.562 – 2.365)	1.99
Day 28	9.43 (13.083-6.815)	4.38 (6.205-3.070)	2.16

**KEY: C.L = Confidence Limit    D.F = Degree of freedom    S.E = Standard error**

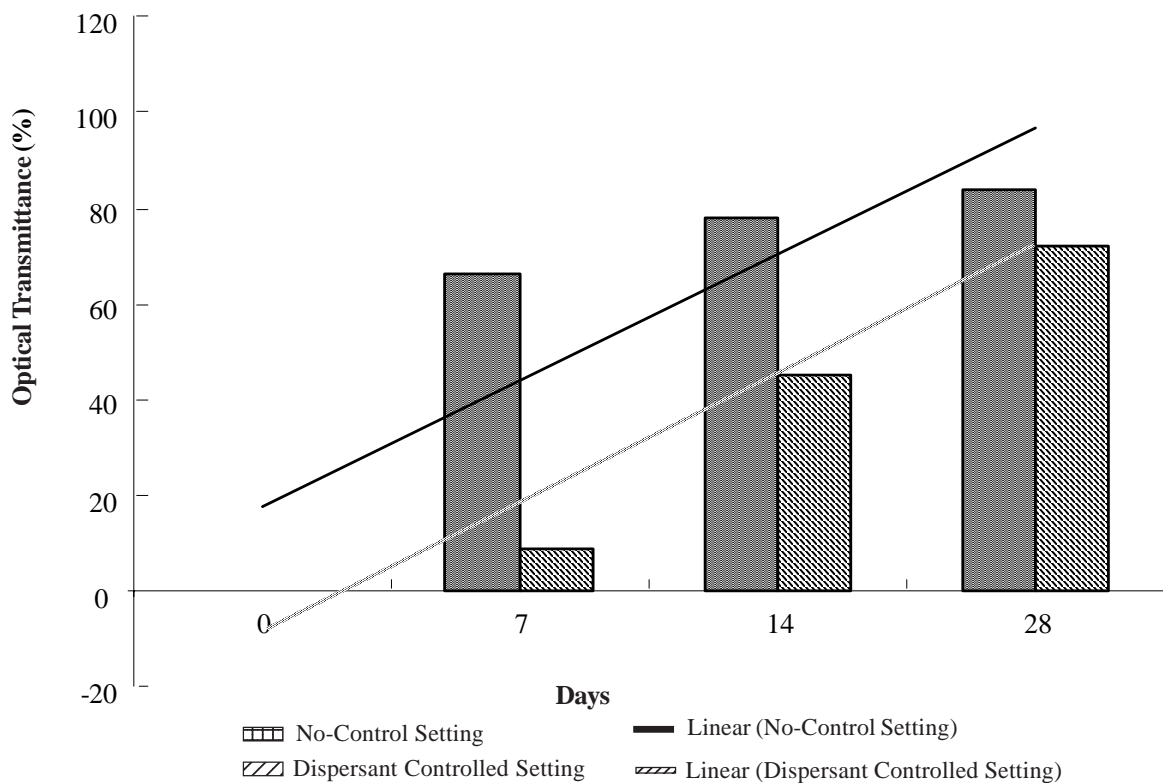
the 28<sup>th</sup> day, there was a slight loss in the biological activity of the mixture (1.14) (Table 1). Regression coefficient analysis depicting the rate of loss of biological activity also gave a weak positive value of 0.34 (Fig. 1). Under the dispersant-control setting, the toxicity of the crude oil and dispersant mixture at the recommended mixing ratio of 9:1 respectively, was also found to be about two-times more toxic than the crude oil acting singly over the 28 days period of observation. This implies that the deployment of this dispersant to emulsify the spilled oil will increase the toxicity level to which organisms inhabiting such ecosys-

tem are exposed. Furthermore, studies on the loss of biological activity revealed that there was very minimal loss in the toxicity of the medium under the dispersant-controlled setting when compared to the loss in toxicity that was about two-fold for crude oil under the no-control setting. This observation that toxicity in the dispersant-controlled medium persisted for longer than under the no-control setting tends to suggest that the toxic action of the dispersant itself is responsible for the sustained toxicity in the medium since a marked reduction in toxicity of crude oil under the no-control was observed. This observation is in agree-

ment with the findings of Powell et al. (1985) and Oyewo (1986) who reported that some dispersants used in Nigeria were highly toxic to some aquatic organisms and that their toxicity were higher than the crude oil that they are often deployed to control.

The use of physical measurement technique based on optical transmittance of the spilled oil under a no-control/dispersant-control setting revealed that there was a gradual degradation of the spilled oil in both settings. The results showed that the level of light transmittance for the test media under the no-control setting ranged from

0% - 84%, while under the dispersant-control setting the light transmittance ranged from 0% - 72% over the 28 days period of observation (Table 3). The determination of the rate of degradation of crude oil based on regression coefficient analysis revealed that the rate of degradation of the spilled oil was faster in the dispersant-control setting ( $R^2 = 0.96$ ) than in the no-control ( $R^2 = 0.77$ ) setting (Fig. 2). This apparently is as a result of the ability of the dispersant to emulsify the spill oil so that actions of natural weathering processes especially microbial action would be enhanced under the dispersant-control setting.



**Fig. 2. Regression analysis of the optical transmittance indexes depicting rate of emulsification of crude oil under a no-control and dispersant-control settings (Regression analysis factor ( $R^2F$ ) = Ratio of  $R^2$  under the no-control setting /  $R^2$  under the dispersant-control setting = 0.8)**

**Table 3. Optical Transmittance of crude oil under a no-control and dispersant-control settings**

Days of Observation	No-control Setting (%)	Dispersant-control Setting (%)
0	0	0
7	66	9
14	78	45
28	84	72

The results of the optical transmittance properties of the spilled oil as a good measure of degradation under a no-control or dispersant control setting was further confirmed by the microbial population growth assays, which showed that the number of colony forming units under the dispersant-control setting was consistently higher than the number detected in samples from the no-control setting. For instance, within seven days of dispersant deployment an average of 160cfu/ml of microbes were detected in samples collected from the dispersant-control units compared to 22.33cfu/ml detected in samples from the no-control setting (Fig. 3). The dispersant-control media therefore had an increase in microbial population growth that was about 7-fold higher than the microbial population obtained under the no-control scenario. Further test of significance base on t-test to compare the mean number of colony forming units under the no-control and dispersant-control settings showed that on day 7, there mean number of microbial units in the dispersant-control media was significantly ( $P < 0.5$ ) higher than the mean number of units detected in the no-control test media. However, for days 14 and 28, there was no significant ( $P > 0.5$ ) difference in the mean number of colony forming units in both the dispersant-

control and no-control test media. The seven-fold increase in microbial load under the dispersant-control setting is attributable to the emulsification of the oil slick floating on the surface of the water thereby increasing the surface area for the micro-organisms to act and multiply accordingly. The results obtained from this study indicate that the deployment of dispersant for oil spill control does have the benefit of bringing about a faster rate of degradation of spilled oil under the experimental conditions. This is in agreement with the work of Reed et al. (1999), Moles et al. 2002 and Lindstorm & Braddock (2002) who showed that deployment of dispersant further reduces potential surface action effects of spilled oil. This benefit can however be overshadowed by the increase in toxicity and persistence of the biological action of the dispersant-crude oil mixture at the mixing ratio of 9:1. Therefore, from an environmental safety point of view, the factors that should govern the choice of dispersants for oil spill control are to include (i) the toxicity of the dispersant in relation to that of crude oil; (ii) the persistence of the dispersant's toxicity in receiving environment and (iii) the type of joint action (synergistic, antagonistic or additive) exhibited by mixture of the dispersant and crude oil at the recommended dispersal ratio.

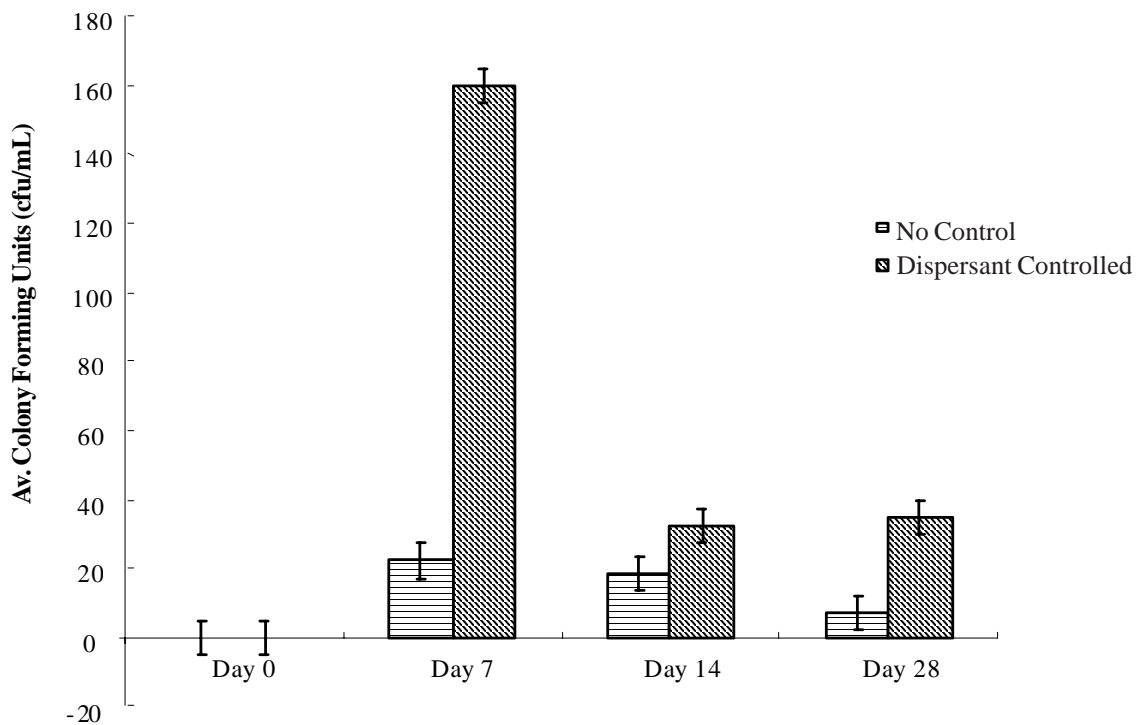


Fig. 3. Mean number of colony forming microbial units detected in test media for the no-control and dispersant-control settings

## CONCLUSION

The toxicity of crude oil under the no-control setting was found to reduce over the period of observation while the toxicity of crude oil-dispersant mixture under the dispersant-control setting persisted for the duration of the bioassays. The deployment of the dispersant for oil spill control was however found to have the benefit of bringing about a faster rate of degradation of spilled oil under the experimental conditions. Therefore, the choice of dispersants for oil spill control must factor in, the toxicity of the dispersant in relation to that of crude oil, the persistence of the dispersant in the receiving ecosystem and the type of joint action (synergistic, antagonistic or additive) exhibited by mixture of the dispersant and crude oil at the recommended dispersal ratio.

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