INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 22, No. 4, p. 69-75, 2023
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Toxicity assessment of dispersit SPC 1000 on *Escherichia coli* and *Pseudomonas* spp in Fresh and marine water from Akwa Ibom, Nigeria

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Article published on April 18, 2023

Key words: Dispersit SPC- 1000, *Escherichia coli*, *Pseudomonas* spp., Freshwater, Marine water, Toxicity

Abstract

Unsustainable techniques, human activities, and laws used in the exploration and extraction of petroleum resources have wreaked havoc on the environment of the Niger Delta Region. This research assessed the toxicity of oil spill dispersant- Dispersit SPC 1000 on *Escherichia coli* and *Pseudomonas* spp. in water habitats. The bacteria were isolated following standard procedures by the spread plate technique. Percentage log survival was used as the toxicity index. The result of the findings showed that the survival rate decreased with increased concentration of Dispersit SPC 1000 and as the exposure periods increased while the mortality rate increased. The study also investigated the susceptibility of the test organisms to the toxicant concentrations and the result revealed a significant difference between the toxicant concentration and the susceptibility of the test isolates though the degree of toxicity differed in the isolates studied. It was observed that Dispersit SPC 1000 exerted a greater toxic effect on *Pseudomonas* spp. than on *E. coli*. The result of the 24th-hour acute toxicity of the toxicant at various concentrations showed that Dispersit SPC 1000 was more toxic to *Escherichia coli* (386.93) than *Pseudomonas* spp (459.72) in Freshwater and more toxic to *Pseudomonas* spp (15.96) than *Escherichia coli* (1293.96) in Marine water. This was evident in the lower LC_{50} for *Escherichia coli* in freshwater and *Pseudomonas* spp. in marine water.

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Introduction

One of the most important environmental challenges nowadays is hydrocarbon contamination produced by petrochemical sector activities. Accidental discharges of petroleum products are a major environmental problem (Nilanjana and Preethy, 2011).

Dispersants are composite combinations of surfactants in a hydrocarbon solvent that lower the interfacial tension between oil and water, breaking the oil into minute droplets and improving the surface-to-volume ratio and biodegradability of the oil (Prince, 2015). They do not reduce the amount of oil that enters the ecosystem, but they do help to mitigate the spill's effects. While dispersants make the oil spill less visible, they also harm marine life beneath the ocean's surface.

Oil spill dispersants are used to move spilled oil from the surface of the sea/down the seabed, forming minute dews. The water flow associated with any wave motion, whether breaking or non-breaking, holds the tiny oil dewdrops in the water column a few meters above. Tides and other water movements will disperse the dewdrop over a large zone and into a large volume of water over time. The change of gloss of floating oil into extremely minute oil dewdrops results in a considerable increase in oil/water interfacial area.

Because dispersants have a high oxygen demand, their use on spills in contaminated coastal bays or inland waterways with restricted circulation may diminish dissolved oxygen resources, causing harm to the biological community in these places (Hamdan and Fulmer 2011). They are most effective immediately after a spill when the lightest components of the oil have not evaporated. The deployment of dispersants near the shore is predicted to increase the exposure of the aquatic organism to petroleum (Milinkovitch *et al*., 2011).

The use of dispersants poses the risk of exposing marine organisms to larger levels of scattered oil (and soluble components from the dispersed oil) than if none were employed. The extent of injury that marine organisms exposed to dispersed oil will suffer depends on the conditions of contact (concentration of dispersed oil, period of exposure, and the rate of spreading and thinning), in addition to the intrinsic reaction of the specific microbe to the oil dispersed.

Many compounds or waste products discharged into water bodies due to petroleum exploration activities have the potential to be toxic and persist in the ecosystem. There could be short-term or long-term negative consequences (Rhodes and Hendricks 1990; Okpokwasili and Odokuma 1996). Oil, metals, and other contaminants can accumulate in aquatic organisms fed by humans, causing both acute (short-term) and chronic (long-term) health problems. The harmful effects of chemicals on different microbial species depends on different factors which include not only the fresh water species but also the form in which the pollutant occur, therefore to measure the toxicity there, some toxicity tests have to be carried out (Richard *et al*., 2011). The effects of chemicals on aquatic species could be observed in the loss of species, because of the complexity of pollution, the effects of uptake by aquatic species are also dependent on the pollutant characteristics. When two or more toxicants are present in an effluent, they may exert a combined effect which can be additive, synergistic or antagonistic (Nrior and Odokuma, 2017). The research study investigated the toxic effect of Dispersit **SPC 1000** on *Escherichia coli* and *Pseudomonas* spp.

Material and methods

Water sample collection

Sterile containers were used to collect marine and freshwater from the Ibaka Deep Sea in Oron L.G.A and Nwaniba River in Ifiayong Uruan L.G.A respectively, both in Akwa Ibom State, Nigeria. Before being collected, each container was rinsed with the water sample. This was immediately taken to the laboratory for analysis. Oil spill dispersant- Dispersit SPC 1000 was purchased from a Marine Chemical shop at East-West Road, Port Harcourt.

Isolation and characterization of bacterial isolates

An aliquot (0.1mL) of the water samples was transferred into Nutrient agar plates in duplicates. This was uniformly spread with a sterile spreader and incubated at 37°C and 42°C for 24 hours in an inverted position for *Pseudomonas* spp and *Escherichia coli* respectively. The test isolates were also grown on eosin methylene blue and cetrimide agar as a confirmatory test for *E coli* and *Pseudomonas* spp respectively. Identification of the isolates was based on their cultural characteristics, microscopic examination, carbohydrate fermentation, and other biochemical tests (Kanu and Okereke, 2004).

Toxicity test

The procedure outlined in (APHA, 2005) was followed to prepare different concentrations (10ppm, 100ppm, 1000ppm, 10,000ppm, and 100,000ppm) of the effluent. A loopful of each test organism was put into 10mL of sterile cetrimide and eosin methylene broth for *Pseudomonas* spp and *E coli* respectively and cultured at 28 ± 2°C for two days. This was kept at 4°C in the refrigerator. One (1mL) aliquot was added into the fresh sterile broth (10mL) and cultured for 24 hours (to ensure that actively developing organisms are used for toxicity test). A standard bacterial inoculum was prepared by serially diluting the organism ten folds. Using the spread plate technique an aliquot (0.1ml) of each dilution was inoculated onto cetrimide and eosin methylene agar plates for *Pseudomonas* spp and *E coli* respectively. The plates were incubated for 48 hours. After the incubation periods, the plates were examined for discrete colonies. The dilution that gave between 200 and 300 colonies was noted and used as reference dilution to obtain the standard inoculum for the toxicity bioassay (Nrior and Odokuma, 2017).

The test procedure for the isolates

Five milliliters (5mL) of each of the test organisms were added to 45mL of each toxicant concentration. A control experiment which does not contain any toxicant was also set up. These were plated out immediately after inoculation on same media for each isolate. This is known as zero-hour count plating. These were incubated at room temperature (28±2°C). An aliquot (0.1ml) of each concentration of the toxicants and control were then plated out after 8 hours, 16 hours and 24 hours. The plates were then counted and average colonies were taken, then Colony Forming Unit per milliliter (CFU/ml) was calculated (Nrior and Odokuma 2017; Obire and Nrior, 2014).

The percentage log survival of the isolates in the toxicants.

The percentage log survival of the bacterial isolate in the toxicant was calculated using the Williamson and Johnson formula (Williamson and Johnson 1981; Nrior and Odokuma 2017), obtained by multiplying the log of count in each toxicant concentration by the log of count in the zero-toxicant concentration.

% Log survival= Log C × 100/Log c

Where Log C= log of count in each toxicant concentration

Log c= log of the count in the zero-toxicant concentration

Statistical analysis and LC_{50}

The findings of the toxicity screening were subjected to statistical analysis using Analysis of Variance (ANOVA) at a 0.05 confidence level to determine the significant difference between the susceptibility of the test organisms to the toxicant concentrations in the different habitats. The lethal concentration was also determined using regression probit. The Statistical Package for Social Sciences (SPSS) version 26 was used for all statistical analyses.

Result

When *Escherichia coli* were exposed to different concentrations of Dispersit SPC 1000 at 0, 8, 16 and 24 hour exposure time in freshwater (Fig 1), a gradual decrease in the percentage log survival and an increased mortality rate was observed. The result of the finding showed OSD to have caused a stimulatory effect during the 16 h exposure time and 10 ppm, 100 ppm and 1000 ppm concentration. There was also increase in viable counts during the 8 hr exposure period at 10000 and 100000 ppm concentrations.

No death of cells was observed in the control flask within the periods of exposure. The result of the percentage log survival of *Pseudomonas* spp in freshwater (Fig. 2), revealed a similar trend. The percentage log survival of the bacterium cell decreased with increased exposure time and concentration while growth was stimulated during 16 hr exposure time for 100 ppm and 1000 ppm concentrations while increase in mortality rate was observed at other exposure times and concentrations. The findings of the acute toxicity of the toxicant on the test isolates in marine water are shown in (Fig. 3 and 4). It was revealed that for *Escherichia coli* (Fig. 3), the toxicant caused a stimulatory effect at 1000 ppm concentration during the 16 hour exposure time,100 ppm during the 24 hour exposure time and 10000 ppm during the 8 hr exposure time respectively while an inhibitory effect was observed at other concentrations and exposure time. Dispersit SPC 1000 caused both stimulatory and inhibitory effects on *Pseudomonas* spp in marine water (Fig 4).

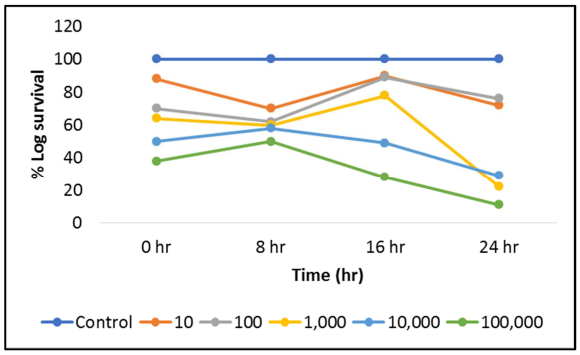

Fig. 1. Percentage log survival of *Escherichia coli* when exposed to Dispersit SPC 1000 in Freshwater.

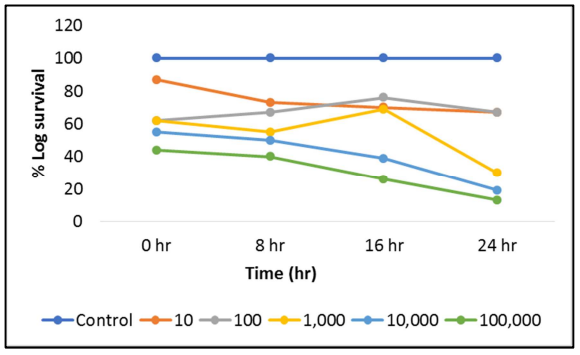

Fig. 2. Percentage log survival of *Pseudomonas* spp when exposed to Dispersit SPC 1000 in Freshwater.

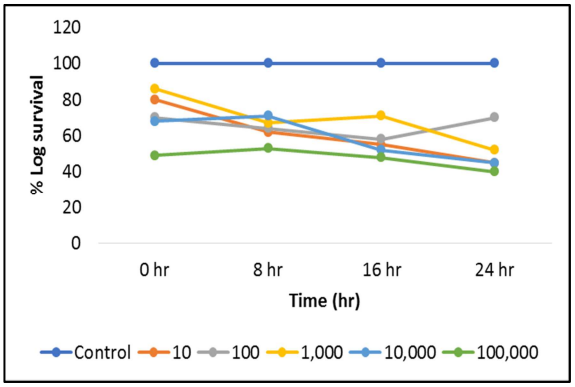

Fig. 3. Percentage log survival of *Escherichia coli* when exposed to Dispersit SPC 1000 in Marine water.

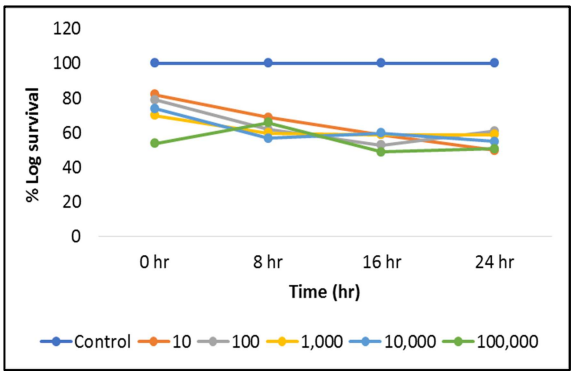

Fig. 4. Percentage log survival of *Pseudomonas* spp when exposed to Dispersit SPC 1000 in Marine water.

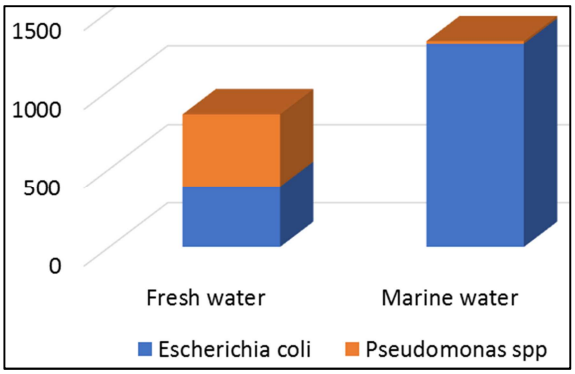

Fig. 5. Lethal toxicity LC_{50} (ppm) of the toxicant on the isolates.

The stimulatory effect was observed at 10000 ppm concentration during 16 hour exposure period and 100000 ppm (during 8 hour exposure time) respectively. The result of the lethal toxicity LC_{50} of the toxicant showed its adverse effect on *Pseudomonas* spp in marine water with less effect on *Escherichia coli* while in freshwater the chemical was more toxic to *Escherichia coli* than *Pseudomonas* spp. (Fig. 5).

Discussion

The toxicity of the oil spill dispersant (OSD) Dispersit SPC 1000 on two bacterial isolates- *Escherichia coli* and *Pseudomonas* spp was studied. Though, according to previous study, the harmful effect of complex combinations like surfactants cannot be attributable to just one or a few molecules (Nrior and Odokuma 2015a). The result of the study showed how the organisms responded to Dispersit SPC -1000. It was observed that, there is a significant difference between the susceptibility of the test organisms and the toxicant concentrations.

According to some studies, the cell walls, cytoplasmic membranes, enzyme-mediated activities, and genetic machinery are all targets of toxicant activity in the bacterial system (Kobeticova *et al*., 2012). Because these organisms are Gram-negative rods, their cell walls share a similar shape (Krieg and Holt 1994). It is possible that the difference in how these bacterial isolates react to petroleum compounds in various aquatic settings (freshwater and marine water) is due to their general makeup (Patrick *et al*., 1991).

Acute toxicity was used as the index for measuring the toxicity of DSPC 1000 (Dispersit SPC 1000) to the test isolates. An increase was observed in percentage mortality while the percentage log survival decreased with increasing concentration and exposure periods or contact time. This finding agreed with the works of (Nrior and Odokuma 2017; Obire and Nrior 2014; Luke and Odokuma 2017; Uffort and Odokuma 2018). Death of microbial cells (increased mortality), shows that the petroleum product concentration has a direct relationship with the microbial populations and so, using concentrations above required would cause a detrimental effect on the microbial populations. The observation is in agreement with the works of Ozoude *et al.* (2018), while monitoring the bio-utilization of a petroleum hydrocarbon (*Paraquat dichloride*) by some soil bacteria. They observed death of bacterial cells at increased concentrations of paraquat dichloride.

The result of the findings also revealed that the oil spill dispersant caused both stimulatory and inhibitory effects on *Pseudomonas* spp in marine water. There was increase in viable cells at 100000 ppm concentration during 8 hour exposure period and 10000 ppm during 16- hour exposure time while for *E. coli,* the stimulatory response was observed at 100 ppm,1000 ppm and 100000 ppm during the 24 hr, 16 hr and 8 hr exposure times in marine water sample. The toxicant showed decreased percentage survival rate as exposure time and toxicant concentration increased. Similar findings were made by Okpokwasili and Odokuma (1997) who found that when *Nitrobacter* was exposed to three oil spill dispersants and five household detergents, the percentage log survival decreased with increasing contact time and concentration.

The toxicity of dispersants to organisms, according to Odokuma and Okpokwasili (2003a) might have occurred from the dispersant's influence on any of the organism's target locations. According to Odokuma and Akponah (2008), a toxicant site of action is determined by its type, concentration, and contact time. The result of the 24-hour LC_{50} (concentration of toxicant sample capable of killing 50 % of the test organism in 24 hours) of OSD- Dispersit SPC 1000 on *Escherichia coli* and *Pseudomonas* spp were shown to be 386.93 ppm, 459.72 ppm respectively in fresh water and 1293.96 ppm, 15.96 ppm respectively, in marine water making *E. coli* more susceptible in fresh water and *Pseudomonas* spp more susceptible in marine water, to the toxicant effect. Generally, OSD - Dispersit SPC 1000 exerted a greater toxic effect on *Pseudomonas* spp than *E. coli*, considering the values of the LC_{50} in both aquatic habitats.

Conclusion

The toxicity of Dispersit SPC -1000 caused cell mortality that led to the reduction in viable counts of the test organisms. This could be attributed to metabolic process of the test isolates within the exposure periods. The toxicity effect of Dispersit SPC 1000 to the test isolates can interfere with their natural role or activity such as their denitrification and degradative capabilities which can cause an imbalance in or upset the biogeochemical cycles where these organisms play key roles.

Also, certain concentrations of the toxicants can bioaccumulate and could via food chain (microbes play a role in food chain) affect humans and pose serious safety/health issues. In this way, the life of the marine creatures and community health would be threatened. Therefore, use of Dispersit SPC 1000 in offshore activities in Nigeria, should be done with caution to avoid adverse effects on aquatic microbes. The findings of this study revealed that Dispersit SPC 1000 was more toxic to *Escherichia coli* than *Pseudomonas* spp in Freshwater and more toxic to *Pseudomonas* spp than *Escherichia coli* in Marine water.

Recommendations

Regulatory authorities should enforce compliance by industries, to the regulatory rules on the use of chemicals. They should also strengthen their surveillance team to closely monitor the effluents from the industries to ensure compliance before final discharge.

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