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Assessment of drilling fluids toxicity on *Escherichia coli* and *Pseudomonas* spp. in marine water from Akwa Ibom State, Nigeria

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Abstract

The contamination of environments with various kinds of petroleum products has been a long - term practice and as such can affect the role of microbes in food chain and as agents of biodegradation of substances in aquatic ecosystems. The acute toxicity of drilling fluids, water -based and oil-based, was assessed on *Escherichia coli* and *Pseudomonas* spp. The test organisms were isolated from marine water by spread plate technique and further confirmed by growth on eosin methylene blue and cetrimide agar for *Escherichia coli* and *Pseudomonas* spp respectively. Percentage log survival was used as index for toxicity assessment. The result of the study revealed a decrease in percent log survival of the test isolates as the concentration of the toxicants and time of exposure increased. A stimulatory effect was observed for *Pseudomonas* spp and *Escherichia coli* in oil - based drilling fluid at 1,000ppm concentration during the 0- and 8-hour exposure periods and 100ppm during 24 - hour exposure period, respectively. The significance of the toxicity of the fluids to the susceptibility of the test isolates was analyzed by ANOVA using SPSS and the result revealed that the isolates were susceptible to the fluids concentrations at varying degrees. The result of the LC_{50} of the drilling fluids revealed water - based drilling fluid to be less toxic than oil - based drilling fluid to both isolates; *Escherichia coli* (168.77ppm, 15. 431ppm) and *Pseudomonas* spp (5776.69ppm, 372.92ppm), respectively. The higher the LC_{50} the lower the toxicity.

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Introduction

The release of numerous hazardous mixtures into water, air, and soil has come from increased economic development and substances used in agricultural activities, resulting in various environmental issues (Jaffrezic-Renault and Dzyadevych, 2008). Human and animal disease can be caused by living organisms being exposed to harmful levels of pollution. Toxic compounds can change the rate at which normal life procedures occur, including, suppression of species development, reproduction and migration. As a result, toxic chemical monitoring and detection are critical for the overall safety and security of humans and all biota on the planet. Toxicity tests entail exposing test organisms (fish, shrimp, bacteria, earthworms) to a medium and monitoring how pollution affects the organisms' survival, development, growth and other characteristics. These can assist to establish whether a contaminant's volume in a habitat is large enough to harm an organism (Atuanya and Tudarado- Aherobo, 2015).

Drilling fluids are mixtures of natural and artificial compounds that are useful in cooling hole and lubricating the drill bit, removing cuttings from the lower part and bringing them to the surface, managing pressure during formation and expanding the drill string and equipment (Burke and Veil, 1995). They are fluids which are circulated through a well in order to remove cuttings from a wellbore (Ahmed and Kalkan, 2019). The composition of a drilling fluid differs with the type of drilling fluid (Nwaiche, 2015). There are many drilling fluids such as the oil-based, the synthetic-based, and the water-based fluid. Muds for drilling in Nigeria are divided into two categories: Muds made of water and oil (mineral/artificial or pseudo-mineral/artificial (EGASPIN, 2002). Drilling off-shore is becoming more used as a means of discovering new oil reserves and this constitutes a new source of petroleum pollution (Readman *et al.*, 1992).

Drilling fluids and cuttings pose a major harm to natural ecosystems biota (Odokuma and Ikpe, 2003). Nevertheless, a good drilling fluid should have a stable rheological property which covers wide pressure and temperature range and should be able to prevent loss of water by forming cakes (Sulaimon, *et al.,* 2017).

The individual components of the chemical additives in drilling fluids inhibit the growth of some microbial populations that are important in the biogeochemical cycles of the affected environment, which may thereby affect agricultural productivity of that ecosystem (Rhodes and Hendricks, 1990; Okpokwasili and Odokuma, 1996). Drilling fluids have been found to prevent the breakdown of NO_2 to NO_3, the second step in the nitrogen cycle nitrification process (Odokuma and Okpokwasili 2003a). Also, certain Gram-positive bacteria and fungi have been shown to use drilling fluids and cuttings (Nnubia and Okpokwasili, 1993).). They also noticed that these fluids slowed the growth of marine microorganisms to varied degrees and attributed this finding in part to the fact that oil-based fluids are more hazardous. It is therefore critical to investigate the response of these organisms to these drilling fluids (effects on nitrification, aerobic respiration, and mortality) in order to develop less toxic and more readily biodegradable fluids, particularly if the ones in use are toxic, and so fail requirements by the regulatory body. The research study, therefore, investigates the toxicity of water-based and oil-based drilling fluids on the test isolates.

Materials and methods

Collection of the sample

Using sterile containers, marine water samples were collected from the Ibaka Deep Sea, Oron L.G.A. in Akwa Ibom State. Before being collected, the containers were rinsed with the water to be collected. This was immediately transported to the laboratory for bacteria isolation and toxicity testing. Drilling fluids, water-based and oil-based were obtained from the Marine Chemical shop, East-West Road, Port Harcourt, Nigeria.

Isolation of bacteria

Aliquot (0.1ml) portions of the water samples were 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 isolates were characterized and identified based on their culture morphology, microscopic examination, carbohydrate fermentation, and other biochemical tests (Kanu and Okereke, 2004).

Toxicity test

Various concentrations (10ppm, 100ppm, 1000ppm, 10000ppm, and 100000ppm respectively), of the toxicants, were prepared (APHA, 2005).

A loopful of each test organism was put into 10ml sterile cetrimide and eosin methylene blue broth for *Pseudomonas* spp and *Escherichia coli* respectively. These were incubated at room temperature (28 ± 2 0 C) for 2 days and later, were kept at 4°C in the refrigerator for preservation. One milliliter (1ml) was put into fresh sterile broth (10ml) and incubated for 24 hours to ensure actively developing cells were used for the toxicity test (Uffort and Odokuma, 2018).

The inoculum was serially diluted ten folds and an aliquot (0.1ml) of each dilution was inoculated onto eosin methylene blue and cetrimide agar for *E. coli* and *Pseudomonas spp* respectively, using the spread plate technique. The plates were incubated for 48 hrs and were checked for distinct colonies after the incubation period. 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). Five milliliters (5ml) of each test organism was inoculated into 45mls of each toxicant concentration and plated out on the appropriate media immediately. This was to determine the Zero-hour plate count. Aliquots (0.1ml) of each toxicant concentration were plated out in triplicates at 8 hours, 16 hours, and 24 hours. These were incubated at room temperature (28 ± 2° C). The colonies were then counted and the average colony was calculated (Nrior and Odokuma, 2017). Controls that contained the inoculum with no toxicant were set up.

The percentage log survival of the bacterial isolates in the toxicants.

The percentage log survival of the bacterial isolate in each toxicant concentration was calculated by dividing the count in the zero-toxicant concentration by the log of count in each toxicant concentration multiplied by 100 (Williamson and Johnson, 1981; Odokuma and Nrior, 2015).

% 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

The results of the toxicity screening were statistically analyzed using SPSS version 26 by analysis of variance (ANOVA) at 95 % confidence level to find out the significant difference in susceptibility of the test organisms in different habitats at various concentrations of the test toxicants. The median lethal concentration was calculated using regression probit analysis by SPSS version 26.

Result

The result of the percentage log survival of *Pseudomonas* spp at various concentrations (Fig. 1) revealed a decrease in viable counts at all concentrations and exposure periods except at 24 hour exposure period and 10ppm concentration, when an increase in viable count was observed. The decrease suggests the ability of the chemical to cause death of the isolate at those concentrations. The result of the toxicity of water - based drilling fluid to *Pseudomonas* spp revealed that water - based drilling fluid was toxic to the isolate with increased toxicity as the concentration increased from 10ppm to 100000ppm and from 0 hour to 24 hour exposure times. By 24-hour exposure time at 100000ppm, the isolate log percentage survival decreased to 9 %. The control flask showed 100 % survival of the isolate. The effect of the different concentrations of drilling fluids on *E coli* was also assessed (Fig. 3, Fig. 4). The findings revealed a decrease in percentage log survival as concentration and exposure time increases

from 10ppm to 100000ppm and 0 hour to 24 hour respectively. From the result, oil-based drilling fluid was observed to cause a stimulatory effect on *Escherichia coli* at 100ppm during 24 - hour exposure times. Water- based drilling fluid effect on *Escherichia coli* (Fig. 4) revealed a similar trend. Water- based drilling fluid caused an increase in mortality rate for all the periods of exposure and concentrations while an appreciable increase in the percentage survival of the isolates was observed at 100ppm during 24-hour exposure period. The result of the LC_{50} (Fig. 5) showed water-based drilling fluid to be less harmful to the two isolates than oil - based drilling fluid. This could be attributed to its composition as noted by Okoro (2011). "When the function of composition and toxicity in the degradability of drilling muds was investigated, it was discovered that oil-based muds were relatively toxic to ecosystems and have the most negative impact on the local environment, particularly diesel." The oil - based and water - based drilling fluids were revealed to exhibit more acute toxicity on *Escherichia coli* than *Pseudomonas* spp.

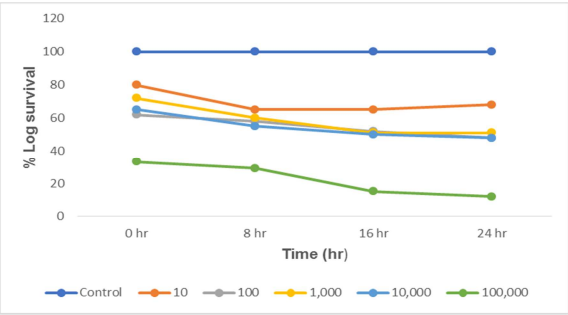

Fig. 1. Percentage log survival of *Pseudomonas* spp in oil- based drilling fluid at different concentrations.

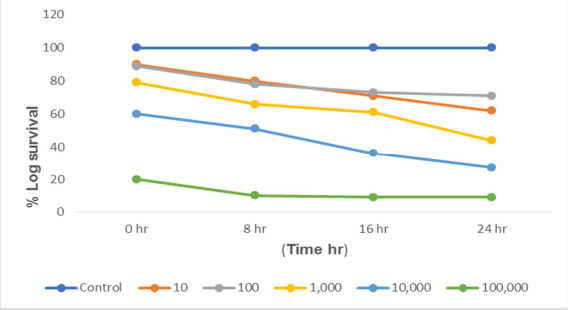

Fig. 2. Percent log survival of *Pseudomonas* spp in water-based drilling fluid at different concentrations.

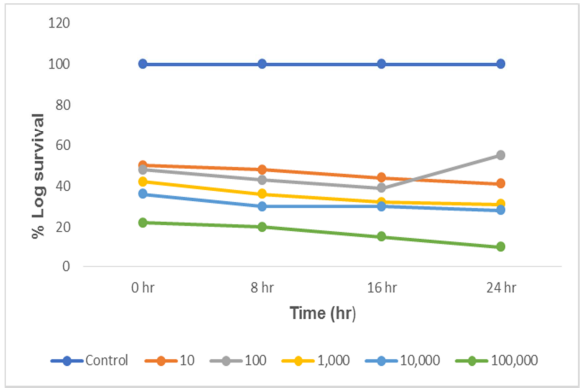

Fig. 3. Percentage log survival of *Escherichia coli* in oil - based drilling fluid at different concentrations.

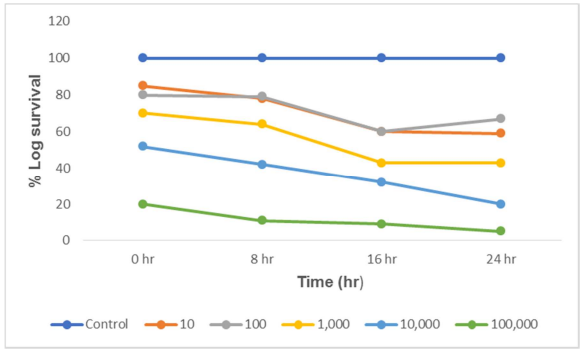

Fig. 4. Percentage log survival of *Escherichia coli* in Water - based drilling fluid at different concentrations.

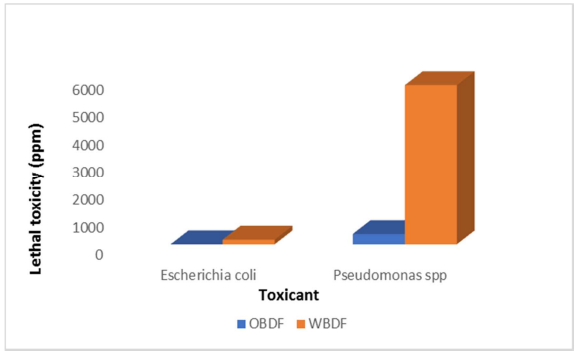

Fig. 5. Median lethal concentration of the toxicant (ppm).

Discussion

The research study investigated the toxic effect of the drilling fluids, water - based and oil - based on the two test isolates; *Escherichia coli* and *Pseudomonas* spp. Bacteria were chosen because of their exceptional role to trophic interaction in both water and terrestrial environments (Jonas, 1989). When compared to a variety of eucaryotic organisms, bacteria are very easy to standardize for toxicity

studies (Bauda and Block, 1985). They combine dissolved and particulate organic carbon to create an enhanced source of particulate organic carbon (De la Cruz, 1978). The result of the study revealed oil-based drilling fluid to be more poisonous to the two isolates than the water - based drilling fluid though the level and extent of toxicity differs, this is observed in the low LC_{50} of oil - based drilling fluid (the lower the LC_{50}, the more toxic the chemical). According to Odokuma and Okpokwasili (1992); Nrior and Odokuma 2017). The low LC_{50} of oil - based drilling fluid could be attributed to their chemical composition. The toxicity result of oil -based drilling fluid on test organisms is in agreement with the works of Nrior and Odokuma, (2017).

The percentage log survival decreased as concentration and time of incubation increased while at lower concentration of 100ppm, a stimulatory effect was observed at different exposure periods. The result is in agreement with the findings of Odokuma and Akponah (2008). In their work, the percentage survival of *Nitrobacter*, *Nitrosomonas*, and *Escherichia coli* was noted to decrease as the drilling fluid concentration and exposure length of the three bacteria increased, especially at high concentrations (10.0, 100, and 1000mg/l). Some fluids enhanced bacterial growth at lower concentrations (0.01, 0.1, and 1.0mg/l). *E. coli* is a facultative anaerobe that is involved in the nitrogen cycle denitrification process (reduction of nitrates to nitrites) process. This process could be inhibited in *E coli* which will indirectly affect agro-soils. *Pseudomonas* spp is capable of degrading variety of compounds, this capability can be impaired in the organism and that can give rise to the accumulation of wastes and environmental pollution. The finding also agrees with the previous reports that with these fluids, marine bacteria growth is reduced to varying degrees. This was attributed to the higher toxicity of oil-based fluids due to their compositions (Nnubia and Okpokwasili, 1993).

Conclusion

Drilling fluids cause acute and chronic toxic effects in microorganisms. The acute toxicity of drilling fluids, water- based and oil- based to *E coli* and *Pseudomonas* spp was investigated. Bacteria are preferred over macro-organisms in toxicity testing because they grow faster and multiply into millions in a short period of time, making the process of obtaining bacteria for biological monitoring easy, accurate, quick, and less costly. From the research results, it was revealed that oil - based drilling fluid was more toxic to the test organisms than the water - based drilling fluid.

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