INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 23, No. 1, p. 334-340, 2023

RESEARCH PAPER OPEN ACCESS

Detergents and degreasers toxicity on *Escherichia coli* in marine water from Akwa Ibom, Nigeria

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Key words: Detergents, Degreasers, *Escherichia coli*, Toxicity, Marine habitat

http://dx.doi.org/10.12692/ijb/23.1.334-340 Article published on July 20, 2023

Abstract

Uncontrolled detergent and degreaser release into water bodies and cultivated soil usually results in poor life productivity in various habitats causing significant ecological and economic problems. The research assessed the toxicity of detergents - Biological toilet cleanser (BTC) and Teepol; degreasers - Rigwash and Aquabreak on *Escherichia coli* in marine water. Percentage log survival was used to assess acute toxicity at various concentrations - 10ppm, 100ppm, 1000ppm, 10,000ppm, and 100,000ppm. The result of the toxicity test revealed a decrease in percentage log survival while the percentage mortality increased as the concentration of the chemicals and contact time increased. The decrease in per cent log survival of the test isolate suggests that the functions of the test isolate in the aquatic habitat were impaired. This also reduced the ability of the affected aquatic habitat to maintain the lives of the aquatic organisms. Inhibiting the growth of this bacterium disrupts the equilibrium of biogeochemical cycles in which it was involved. The study also examined the relationship between the chemicals and the susceptibility of the test isolate at different concentrations. The result of this study at a 95% confidence level using ANOVA, revealed a relationship between the susceptibility of the test isolate and the toxicant concentrations but the degree of susceptibility varied. The findings on the median lethal concentration LC_{50} of the chemical showed that Teepol was more toxic than BTC (164.53ppm > 95.41ppm) and Rigwash more toxic than Aquabreak (1358.45ppm >239.56ppm) because the lower the toxicity, the greater the LC_{50}.

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Introduction

Toxicity is the inherent ability of a substance to cause harm (Uffort and Odokuma, 2018). Acute toxicity is the ability of a substance to cause harmful effects in an organism after a short-term exposure while chronic toxicity involves the ability of a substance to cause harmful effects over extended period after long-term exposure (Uc-Peraza and Delgado-Blas, 2012).

Detergents are chemical compounds with both polar and nonpolar characteristics. They are most abundant in polar and non-polar media at phase transitions. Surfactant and phosphate detergents are the two types of detergents with various properties. Surfactant-based detergents are particularly toxic, while phosphate-based detergents are caustic. Detergents, particularly biodegradable detergents if present, in significant concentrations can be hazardous to all types of aquatic life (Lenntech, 2008). Phosphorus-containing detergents can induce eutrophication, releasing toxic chemicals and lowering the streams' oxygen levels. As the algae decompose, the available oxygen decreases (Markina and Aizdaicher, 2007).

Surfactants and detergents are commonly utilized in both households and offices (Azizullah, 2011). According to (Info Mine Research Group, 2012), global detergent production will be around 10 million tons annually, while global surfactant production will be around 15 million tons annually (Van Bogaert, *et al*., 2007 Henkel, *et al.,* 2012; Henkel, *et al.,* 2014). Chlorine concentrations as low as 10ppm can kill certain microorganisms in as little as four hours, according to the previous study (Obire and Nrior, 2014). These compounds pose problems for aquatic species by reducing the water surface tension.

Detergents are commonly used in Nigeria (Smith, 1983). However, discharging waste into the environment, through the use of household detergents and industrial detergents, may have an impact on the microbiota of the receiving environment (Okpokwasili and Odokuma, 1997). Detergents disrupt the ecosystem by damaging gills, lowering water surface tension, causing eutrophication releasing poisonous substances, reduction of oxygen in streams, and lowering aquatic organisms' reproductive abilities. The toxicity of this chemical is mostly based on the sodium silicate solution and the surfactant employed in their compositions (Holding, 2005).

Degreasers are synthetic compounds that remove filth, and oil from automobile motor parts. They come in two varieties: oil-based and water-based. Oil-based degreasers are typically hazardous and combustible. Contamination occurs when little quantity of contaminants enter shallow or deep water. (Nrior and Odokuma, 2015; Schlemmer *et al.,* 2002).

Many oil-based degreasers vanish easily, contributing to brown haze and ozone. Water-based cleansers are often less harmful to humans and the environment. Trichloroethylene is one of the most commonly used degreasers. CAHs (chlorinated aliphatic hydrocarbons) can contaminate water supplies and directly or indirectly harm human health (Mesdaghinia, 2005). Due to inappropriate removal practices, TCE has always been identified in groundwater and is considered a likely cancer-causing substance (Wartenberg *et al*., 2000) having numerous other unfriendly impacts on humans and other organisms (CEPA, 1993; Wartenberg *et al*., 2000).

Although there are works on the toxicity of detergents and degreasers, environmental conditions in the sea and other aquatic ecosystems differ from place to place. Therefore more research is needed on the likely toxicity of industrial detergents and degreasers on various aquatic habitats.

Materials and methods

Collection of Sample

Using sterile plastic containers, marine water samples were collected from Ibaka Deep Sea Oron L.G.A. in Akwa Ibom State, Nigeria. The container was rinsed with the water sample to be collected and transported to the laboratory after collection.

Two detergents - Biological toilet cleanser (BTC) and Teepol and two degreasers-Rigwash and Aquabreak were obtained from Marine Chemical Shop, East-West Road, Port Harcourt, Rivers State, Nigeria.

Source of microorganisms for toxicity testing

- *Escherichia coli* used for the toxicity test were isolated from the marine habitat.

Isolation of test organism

A sterile 1ml pipette was used to transfer one millilitre of homogenously mixed sample into sterile test tubes containing 9ml sterile distilled water as diluent. One (1) millilitre of the sample was transferred to a sterile 9-millilitre diluent and thoroughly mixed. A tenfold dilution was carried out until a dilution of 10^{-5} was produced. An aliquot (0.1ml) of the water sample from dilution 10^{-3} was picked and dropped on different Nutrient agar plates. The inoculum was uniformly dispersed with a sterile spreader and incubated for 24 hours in an inverted position, at 42°C. (Nrior and Odokuma, 2017)

Characterization of the test isolate

The isolate was identified using culture morphology, microscopic examination, carbohydrate fermentation, and other biochemical assays (Cheesbrough, 2006). For bacterial identification, (Bergey's Manual of Systematic Bacteriology, 1984) was consulted. To confirm the isolate, it was also cultured on Eosin methylene blue agar. A pure culture was prepared on an agar slant for further research.

Preparation of test medium

The concentration of the toxicants (10ppm, 100ppm, 1000ppm, 10000ppm and 100000ppm respectively), was prepared following procedures according to (APHA (2005).

Test organism preparation

A loopful of the test organism was placed in 10ml of sterile appropriate broth and cultivated at room temperature (28 ± 2°C) for 48 hours. One millilitre was transferred to ten millilitre of fresh sterile broth and cultured for 24 hours to ensure that actively developing organisms were used for toxicity test.

Standard bacterial inoculum preparation

The organisms were serially diluted ten folds and an aliquot (0.1ml) of each dilution was inoculated onto eosin methylene agar for 48 hours in triplicates.

The plates were checked for distinct colonies after the incubation durations. The dilution that yielded between 200 and 300 colonies was identified and used as the toxicity bioassay reference dilution (Nrior and Odokuma, 2017).

The test procedure for the Escherichia coli

Five millilitre (5ml) of the test organism was added to 45ml of each toxicant concentration. Control experiments without any toxicant were also set up. These were plated on eosin methylene blue immediately to determine the zero-hour count plating and were incubated at ambient temperature (28 ± 2°C). Aliquots (0.1ml) of each toxicant concentration were plated out after 8 hours, 16 hours, and 24 hours. The Colony Forming Unit per millilitre (CFU/ml) was calculated by counting the plates and calculating the average colonies.

The bacterial isolate percentage log survival in the toxicants.

The percentage log survival of the bacterial isolate in the toxicant used for the experiment was calculated using the (Williamson and Johnson, 1981) formula, the log of the count at each toxicant concentration was multiplied by the log of the count at zero toxicant concentration.

% Log survival= Log C× 100/Log c

Where Log C= log of count in each toxicant concentration

Log c = log of 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 a 95 % confidence level to find out the significant difference in susceptibility of the test organisms in the different habitats at various concentrations of the test toxicants. The median lethal concentration was calculated using regression probit analysis by SPSS version 26.

Result and discussion

Result

The result of acute toxicity of BTC to *Escherichia coli* (Fig. 1) showed a gradual decrease in the percentage log survival of the isolate and as the toxicant concentration increased from 10ppm to 100,000ppm, the percentage mortality increased.

At 100ppm, a 1% increase in viable cells was observed whereas in the control there was no death of viable cells. A similar trend of increase in mortality and decrease in percentage log survival was observed for Teepol (Fig. 2).

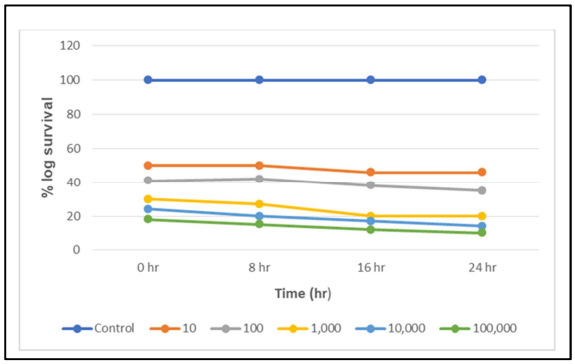

Fig. 1. Percentage log survival of *Escherichia coli* in BTC at different concentrations.

By 24-hour exposure at 100,000ppm, there was 0 percent survival of the test isolate, that is 100 % mortality while the control flask showed no death of viable cells within the 24 hours of exposure to the toxicant. Rigwash caused an inhibitory effect on Esherichia coli at all concentrations and exposure periods. There was increased mortality as the concentration and exposure time increased while the control showed 100% survival of the test isolate.

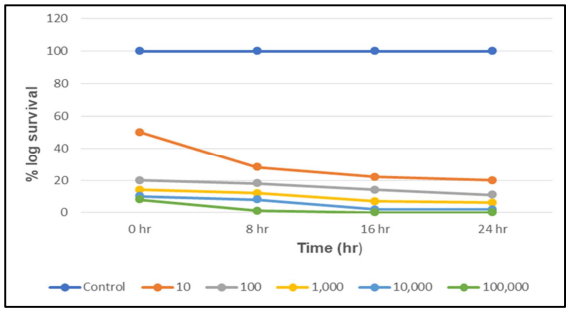

Fig. 2. Percentage log survival of *Escherichia coli* in Teepol at different concentrations.

The result of lethal toxicity of aquabreak to *Escherichia coli* (Fig. 4) showed a decrease in percentage log survival and increased mortality as the concentration of aquabreak increased with an increased exposure period. A stimulatory effect was observed at 10ppm, 100ppm and 1000ppm during the 16- hour exposure time while no death of viable cells was observed in the control flask. The result of the median lethal concentration (LC_{50}) of the four (4) chemicals shown in Figure 5 revealed aquabreak to have the highest LC_{50} which means the lowest toxicity while Teepol had the lowest LC_{50}, which means the highest toxicity, because the lower the LC_{50} the higher the toxicity and vise versa.

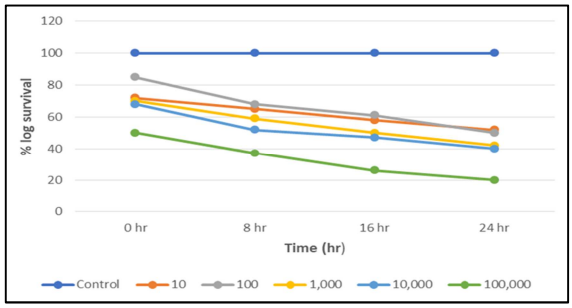

Fig. 3. Percentage log survival of *Escherichia coli* in Rigwash at different concentrations.

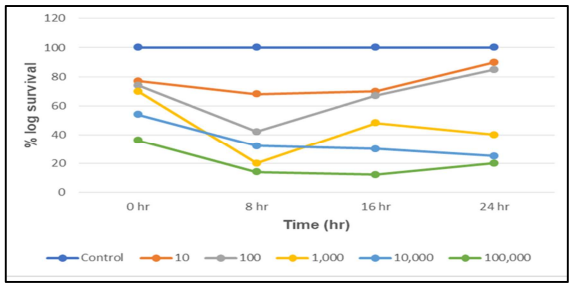

Fig. 4. Percentage log survival of *Escherichia coli* in Aquabreak at different concentrations.

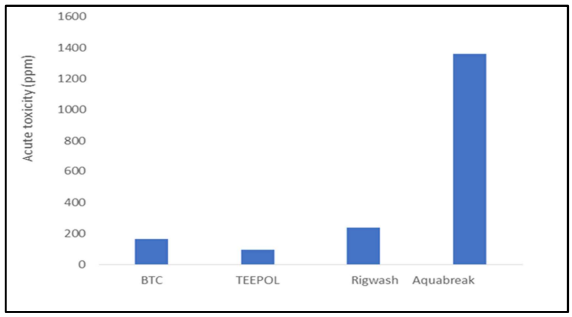

Fig. 5. LC_{50} of the chemicals (ppm).

Discussion

Individuals and organizations have been contaminating the aquatic environment with various types of chemicals, thus, the role of microorganisms in the food chain and as agents of substance degradation in aquatic environments has been hampered (Wenedo and Nrior). According to studies, cell walls, cytoplasmic membranes, enzyme-mediated activities, and genetic machinery are all targets of toxicant activity in the bacterial system (Kobeticova, 2012).

The toxicity of detergents (BTC and Teepol) and degreasers (Rigwash and Aquabreak) used in the oil exploration and discovery industries on *Escherichia coli* was examined in this study. Bacteria are usually chosen for toxicity test because of their exceptional role in trophic interaction in both water and terrestrial environments (Jonas 1989). When compared to a variety of eukaryotic 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 toxicity of BTC and Teepol to *Escherichia coli* in marine water revealed that Teepol had a significant effect on the test organism, as seen by the decrease in percentage log survival at various concentrations and contact times. BTC had an inhibiting impact on *Escherichia coli* at various concentrations. The findings of the Teepol toxicity test revealed that the toxicant killed *Escherichia coli* completely during 16- and 24-hour exposure periods at a concentration of 100,000ppm. This result is in agreement with (Wenedo and Nrior, 2016) findings, which revealed that increased concentrations of domestic detergents killed the test fungus (*Mucor racemosus*), as well as inhibited and altered the degradative process in the aquatic habitat. They concluded from the study that detergents had a depressing effect on aquatic fungus and that using detergents to wash domestic and industrial goods in surface waters could generate ecotoxicological issues. Domestic detergents may be toxic to microorganisms because they hinder the respiratory mechanism in the organism (Stanier *et al.,* 1982). This happens within the organism's cell membrane. Toxic compounds in the aquatic environment may interact with respiratory enzymes located in cell membranes, causing the process to be disrupted (Obire and Nrior, 2014).

The detergents were also found to have a stimulating impact on the test organism. (Obire and Nrior, 2014) observed that domestic detergent proved stimulatory to *Pseudomonas* sp. and *Mucor* sp., as shown by 100% log survival of these organisms, observed also when the detergent concentrations were increased. Researchers previously revealed that the bacteria mentioned above can digest (biodegrade) crude oil and refined products for metabolism (Atlas and Bartha, 1972; Foght and Westlake, 1987)

Factors such as differences in genotype (Patrick *et al*., 1991), previous or prolonged exposure to the detergent effluent (Nrior and Odokuma, 2015), structural changes (Zelibor *et al*., 1987), and relative use of the detergent effluent for metabolism, could all play a role in the way bacteria respond to different detergents metabolism (Atlas and Bartha, 1972; Foght and Westlake, 1987).

Rigwash and Aquabreak were also tested for toxicity on *Escherichia coli.* As the concentration and exposure period increased from 10ppm to 100,000ppm and 0 hours to 24 hours, respectively, the percentage log survival decreased. The death of microbial cells (increased mortality), shows that the petroleum product concentration has a direct relationship with the microbial populations and so, using concentrations that are more than specification would cause harm to the microbial populations. This observation is in concurdance 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.

Odokuma and Akponah (2008) showed that the percentage survival of *Nitrobacter*, *Nitrosomonas,* and *Escherichia coli* reduced with increasing drilling fluid concentration and exposure period, especially at high concentrations (10.0, 100, and 1000mg/l). At lower concentrations of 0.01, 0.1, and 1.0mg/l, several fluids improved bacterial growth. As the content and exposure length of the four fluids tested increased, *Nitrosomonas* nitrite accumulation, *Nitrobacte*r nitrite consumption, and *E. coli* carbon IV oxide evolution all decreased. *E. coli* is a facultative anaerobe that is involved in denitrification in the nitrogen cycle (reduction of nitrates to nitrites) process. This could have an impact on the efficiency of agriculture in the Niger Delta, as they disrupt the nitrogen cycle involving both nitrifying and aerobic bacteria.

Conclusion

Industrial chemicals could be lethal to aquatic microbial organisms; however, the level of toxicity varies depending on the toxicant and the water habitat. From the study it was observed that rigwash was more toxic to the test isolate than aquabreak while BTC was less toxic than Teepol. It is therefore advisable that the use of the detergent, BTC and the degreaser, aquabreak be encouraged during oil exploration activities.

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