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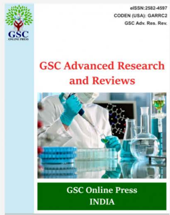

(Research Article)

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Acute toxicity of an effluent and two oil spill dispersants to *Vibrio fischeri*

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Abstract

Contamination of aquatic systems due to different anthropogenic activities including discharge of industrial wastes (effluent) and application of mitigation chemicals (oilspill dispersants) is a major issue in Nigeria as the effect could be detrimental to important organisms of various trophic levels in marine environments. This study aimed at evaluating the acute toxicity of *Vibrio fischeri* to a treated effluent and two dispersants- Corexit 9527 and Finasol OSR. The Omni model 500 Microtox Analyzer for bacterial bioluminescence inhibition, a rapid and ecologically relevant bioassay tool for ecotoxicity assessment during Biological Monitoring Studies was employed. The oil spill dispersants were obtained from licensed oil field chemical stores in Port Harcourt, Rivers State while the treated effluent was sourced from a natural gas production industry that discharges its treated effluent into Bonny Estuary, Rivers State, Nigeria. The experimental procedure involved exposure of reconstituted freeze-dried cells of the marine bioluminescent *Vibrio fischeri* to various dilutions of the toxicants, reference chemical (KCl), including instruments reference chemical for 5minutes and 15 minutes. The percentage reductions in bioluminescence by *Vibrio fischeri* after 5minutes and 15minutes exposure to the toxicants (Treated Effluent, Corexit 9527, and Finasol OSR) was recorded as median effective concentration value (EC_{50}). The 5 minutes-EC_{50} values of Treated Effluent, Corexit 9527, and Finasol OSR for *Vibrio fischeri* were 27.54%, 18.61% and 4.35% respectively while the 15minutes-EC_{50} values of Treated Effluent, Corexit 9527, and Finasol OSR were 13.75%, 15.18% and 4.21% respectively. The test indicated that *Vibrio fischeri* was sensitive to the treated effluent and the two oil spill dispersants. Finasol OSR was more toxic to *Vibrio fischeri* than Corexit 9527. The continuous discharge of effluents (treated and untreated) and the application of oil spill chemicals offshore can lead to inhibition of bioluminescence by marine bacteria an important organism in marine environments.

Keywords: Acute toxicity; *Vibrio fischeri;* Microtox*;* Bioluminescent; Effluent and Dispersants

1. Introduction

Pollution of the oceans is widespread, worsening and in most countries crudely controlled. The pollutants are usually complex mixture of toxic metals, plastics, manufactured chemicals, agricultural wastes, pharmaceutical chemicals and petroleum industrial wastes. It reaches the oceans through rivers, runoff and direct discharges. Effluent as defined by United States Environmental Protection Agency (USEPA) is a liquid (wastewater)- treated or untreated that flows out of industrial outfall. The discharge of this effluent is considered water pollution as it may carry pollutants such as fats/oils/grease (FOG), chemicals, detergents, solids and other wastes based on the possible source – manufacturing industries, mining industries, oil & gas extraction and service industries [1][2].

The most immediate effect of wastewater on the environment is when it contributes toward the contamination and destruction of natural habitats by exposing them to harmful chemicals. It poses hazards that can be toxic, corrosive, reactive and acidic. Toxic compounds in the effluent can disrupt aquatic ecosystem. For instance, the presence of large amount of biodegradable substance will cause the microbes to begin breakdown process thereby utilizing a lot of

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dissolved oxygen of which the dissolved oxygen can become depleted and this becomes life threatening for the higher organisms – fishes.

Another source of marine pollution is oil spill which occurs accidentally or naturally. To rapidly minimize this pollution damage to the aquatic inhabitants, oil spill dispersants are used. Dispersants are the chemical mixture of surface-active substance that helps break up an oil slick into very small droplets (making it more available for microbial degradation) which dilute throughout the water body. These dispersants are usually composed of ionic and non-ionic surfactants in organic solvent, which may cause environmental and health problems. For example, they can induce oxidative stress, mitochondrial dysfunction and apoptosis in mammalian cells [3][4] and it inhibits the growth of oil-degrading bacteria at a dispersant-to-oil ratio of 1:100 and higher [5][4].

In monitoring the biological effects of toxicants, many methods are available. One of such methods is acute toxicity bioassay. Acute toxicity is the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time. In this test, the strength of the toxicant is determined by the responses of living organisms to it. With this method, direct measurement of the toxic properties of the toxicants can be conducted using one or a few species of organisms that are selected due to their relative greater sensitivity, ease of culture, and are considered representatives of the indigenous organisms in the recipient water. *Vibrio fischeri* is one of such selected organisms as outlined by Nigerian Upstream Petroleum Regulatory Commission (NUPRC) [6].

Vibrio fischeri is a Gram-negative, rod-shaped heterotrophic bacterium found in marine environments. This bacterium can be found free living and in symbiosis with various marine animals such as fishes and squids and produces bioluminescence controlled by a small set of genes known as lux gene [7]. Bioluminescence is the production and emission of light by living organism through chemical reactions in their bodies. This chemical reaction requires two chemicals – luciferin (the light emitting compound) and luciferase (oxidative enzyme). Luciferin reacts with oxygen in the presence of luciferase to release energy in form of light.

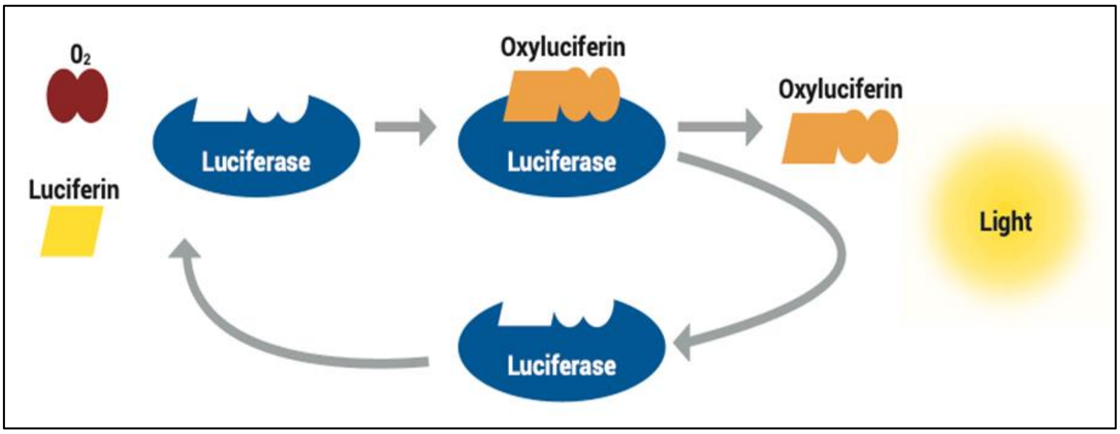

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Figure 1 Reaction of luciferin that leads to production of light

The bioluminescence of *Vibrio fischeri* is caused by transcription of the lux operon which is induced through population-dependent quorum sensing [8] that is, the population of the bioluminescence bacteria has to reach an optimal level to activate the lux operon and stimulate light production.

The microtox bioassay based on the measurement of the natural luminescence emitted by the marine bacterium *Vibrio fischeri* can be used since it is a standardized method, fast, simple and allowing the analysis of a large number of samples [9]. In this study, the luminous bacteria - *Vibrio fischeri* was considered as biological representative to conduct a comparative study on the acute toxicity of treated effluent and oil spill dispersants.

2. Materials and methods

2.1. Collection of test toxicants

The treated effluent was collected into pre-cleaned amber bottle from a natural gas production industry in Bonny, Rivers State while the dispersants were obtained from licensed chemical stores within Port Harcourt, Rivers State.

2.2. Test Organism employed for the studies

The resuscitated freeze-dried preparations of the heterotrophic marine bacterium - *Vibrio fischeri* were obtained from the manufacturer-Omni model 500 Analyzer (Modern Water London).

2.3. Acute toxicity test for bioluminescence marine bacterium

The Microtox-bioluminescence inhibition test method [10] was employed for the definitive acute toxicity test. Using the Omni model 500 Analyzer instrument, the median effective concentration (EC_{50}) of the toxicants – treated effluent, Corexit 9527 and Finasol OSR were estimated.

Freeze-dried cells of the *Vibrio fischeri* were exposed to different dilutions of the toxicants as well as the reference chemical (potassium chloride) for 5minutes and 15minutes. Inhibition of bioluminescence of the bacterium by the luminometer was measured and the EC_{50} for 5minutes and 15minutes were determined using the concentration response curve generated by the analyser (Omni Model 500) software. Toxicity result of a sample was considered valid if the 95% confidence level was not >30% of the EC_{50}.

3. Results and discussion

3.1. Microtox Toxicity Test

Upon exposure to a substance containing toxic materials, changes in the bacteria's light output are measured. The response of bioluminescence inhibition of *Vibrio fischeri* to the test toxicants (Treated Effluent, Corexit 9527 and Finasol OSR) at different exposure time are represented in figure 2. Between the 0mins and 5mins exposure time, the bioluminescence inhibition in all test toxicants was increased at steep slopes and at a gentle slope to 15mins. This pattern which is a typical response of bacteria when exposed to organic contaminants conforms with findings of Choi et al., [11] when *Vibrio fischeri* was exposed to surface sediments from wastewater treatment plant effluent outfall area.

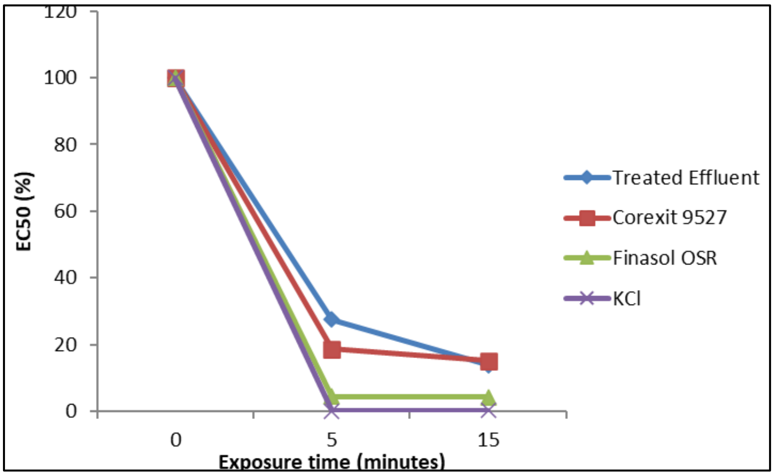

Figure 2 The response of bioluminescence inhibition to the test toxicants at 0, 5 and 15minutes exposure time

Table 1 Acute toxicity indices

Toxicant	Microtox 15-min EC_{50} (%)	95% Confidence range
Treated effluent	13.78	13.01 – 14.59
Corexit 9527	15.18	10.52 – 25.17
Finasol OSR	4.21	2.514 – 6.841
Potassium chloride	0.306	0.287 – 0.328
Zinc sulphate	0.00048	0.00029 – 0.00081

The test toxicants toxicity value (15mins-EC_{50}) with the 95% confidence range are given in table 1. Finasol OSR was more toxic with a 15mins-EC_{50} value of 4.21%. Treated effluent recorded a 15mins-EC_{50} value of 13.78%. In this study, the result showed that the test toxicants- treated effluent, Corexit 9527 and Finasol OSR were acutely toxic to *Vibrio fischeri* (Microtox) when compared against toxicity categories developed by manufacturers

for interpreting results. Potassium chloride and Zinc sulphate were used as reference toxicants and the 15mins EC_{50} values were 0.306% and 0.00048% respectively. All the test toxicants were within the "extremely toxic" category (0 – 19%) [12]. This finding collaborates with findings of Ajuzieogu et al. [13]; Ngwoke et al. [14] and Hawrylik et al. [15]. Ajuzieogu et al. [13] employed the Microtox test procedure to assess produced water discharges. Their findings indicated that the treated and untreated produced water samples were very toxic and extremely toxic respectively after 15minutes exposure time as the 15min-EC_{50} values of the treated and untreated produced water for *Vibrio fischeri* was 23.27% and 1.0% respectively. To evaluate toxicity level of produced water effluent on indigenous organisms in Delta State, Nigeria, Ngwoke et al. [14] carried out acute toxicity tests using Microtox® Model 500 and the result of the acute toxicity tests on *Vibrio fischeri* revealed that the average inhibitive concentration (IC_{50}) for treated produced water at 5minutes and 15minutes was 22.20% and 31.17% and the no effect concentration (NOEC) and low effect concentration (LOEC) at 5 minutes and 15minutes was 5.63% and 5.63% respectively. Also, to assess the toxicity of sewage sludge from the municipal sewage treatment plant in Bialystok, Hawrylik et al. [15], used Microtox Model 500 kit for the toxicity analyses. The EC_{50} index (concentration of toxic substances resulting in a 50% reduction in the intensity of luminescence of the bacterial strains used) was calculated and the results obtained showed high and very high ecotoxicity of the raw sewage and sewage sludge from the primary settling tank.

The process of light emission is somehow a reflection of the bacterial metabolic and enzymatic intensity [16] thus, the toxicity of the test treated effluent and the dispersants can be attributed to their physicochemical properties and chemical complexity which can cause disruption of the metabolism.

4. Conclusion

The study has revealed that treated effluent and the two oil spill dispersants- Corexit 9527 and Finasol OSR were extremely toxic to the bioluminescence bacteria- *Vibrio fischeri* in the order of Finasol OSR> treated effluent > Corexit 9527. Since bacterial bioluminescence is tied directly to cellular respiration, any inhibition of cellular metabolism due to toxicity results in a decrease in the light emission of the affected cells. The continuous discharge of effluents (treated and untreated) and the application of oil spill chemicals offshore can lead to inhibition of bioluminescence by marine bacteria an important organism in marine environments. Therefore, emphasizing the need for adequate monitoring and enforcement of guidelines and set limits by regulatory agencies.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have declared that no conflict of interest exists.

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