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Assessment of the Toxic Effect of Oily Drill Cuttings on Mangrove Littoral Periwinkle (*Tympanotonus Fuscatus*) of the Lagos Lagoon

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Abstract

The acute toxicity of oily drill cuttings against the littoral mangrove periwinkle (*T. fuscutas*) of the Lagos Lagoon was evaluated in the laboratory bioassay. In this study, the result showed that the acute toxicity of oily drill cuttings based on immobility response of *Tympanotonus fuscatus* increased with time of exposure. The concentration that caused 50% immobility in the organisms at 24hrs, 48hrs, 72hrs and 96hrs were 3808.80ml/L 660.89ml/L, 302.28ml/L and 102.43ml/L respectively. The median lethal concentration of drill cuttings against *T. fuscatcus* decreased as the duration of exposure increased. The analysis of variance (ANONA) showed that there was significant difference ($p < 0.05$) between all the treatments at 24, 48, 72 and 96 hours of exposure. The significance of these result is the need to include bio accumulators such as *T.fuscatus* in monitoring programmes aimed at establishing the environmental level of such pollutant as oily drill cuttings in aquatic ecosystem.

Keywords: Oily drill cuttings; Toxicity; Tympanotonus fuscatus; Periwinkle; Lagos Lagoon.

1. Introduction

The activities of hydrocarbon production generate large amounts of impacts and effects, arising not only from accidental blowouts, but also from permissible discharges of such waste as drill cuttings which impact negatively on aquatic biota. Drill cuttings are particles of crushed rocks produced by the grinding action of drill bit as it penetrates into the earth [1, 2]. Although the drill cuttings are considered toxicologically inert, there is the concern that any adhering fluid additive may be toxic particularly if the cuttings are produced during drilling with oil based mud or synthetic based mud [3]. Drilling muds are used to lubricate and cool the drilling apparatus, transport drill cuttings to the surface and seal off geologic formations [4-6]. The three basic types of drilling mud are Water Based Mud (WBM), Oil Based Mud (OBM) and Synthetic Based Mud [4]. Water based mud are by far the most commonly used mud both onshore and offshore. They are mostly used in shallow wells and shallow portions of deep wells but are not very effective in deeper wells [7]. However, due to some limitation of WBMs, Oil Based Mud (OBMs) have been developed to overcome the problems associated with WBM and are effective for wide range of special situations which include high temperature, hydrate shale, high angle, extended reach well, high density mud and drilling through salt. [7, 8]. Researchers have abundantly shown that drilling mud additives may contain toxic substances such as heavy metals, hydrocarbons, biocides chromates, organic polymers and trace elements that have the tendency to bioaccumulation and interfere with normal biological activities of organisms. Oily drill cuttings are sometimes unintentionally or intentionally released into water bodies and can affect the bottom dwellers especially periwinkles [9]. The toxic effect could be due to the paraformaldehyde biocide and heavy metals included in the different mud formulations/compositions which increases the toxicity to aquatic bottom dwellers. Drill cuttings in water bodies may kill marine lifes, smother or suffocate them with plume of suspended particles [10, 11]. Acute ecotoxicity testing is commonly used to predict the toxicity of oily drill cuttings in the marine environment. Marine species are exposed to a range of test concentration under laboratory conditions to determine the LC50 of the test substance using established protocols for drilling fluids. In carrying out acute toxicity tests, it is particularly important to consider benthic organism which are very venerable to oil spills, [12] and which forage the bottom sediments into most pollutant. Most of these benthic organisms are mollusc which are important animal component of mangrove swamps. *Tympanotonus fuscatus* commonly called periwinkle is found in mangrove swamps and mud flat in low salinity areas of the Lagos lagoon. *T. fuscatus* is a dominant benthic specie in West Africa coast line and also a high source of animal protein. Therefore, this present study is designed to investigate the relative acute toxicity of oily drill cuttings acting singly on macro-invertebrate *Tympanotonus fuscatus.*

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2. Materials and Methods

2.1. Test Animals

The test animals used for this bioassay were periwinkles, *Tympanotonus fuscatus* (Mollusca, Gastropoda, Mesogastropoda, Potamidae). *Tympanotonus fuscatus* were collected from the mangrove flats of Lagos lagoon. Each of this animal was handpicked into a separate 10 litres plastic bucket containing water from the habitat. The animals were of unknown age but approximately the same range (Length of shell 3.0 ±0.5, diameter of aperture 0.8 – 1.0mm). In the laboratory, sand from the site of collection was placed at the bottom of the holding tanks serving as substrate. 450 test animals were put in different holding tanks (113cm x 54cm x80cm) and half filled with lagoon water. These holding tanks were aerated with a 220v air pump and then changed every 48 hours to prevents acclimatize to laboratory conditions (28 + 2, 72.2%R.H) for 7 days before used for experiment.

2.2. Test Compound

40 litres of drill cuttings used for this study were collected in two 20 litres plastic bucket from the main discharge point at the Shell Development Petroleum Corporation Warri. Chemical characterization of the cutting samples showed a pH value.

2.3. Bioassay Procedures

The bioassays were carried out in glass tanks (22cm x 15cm x 18cm). These tanks are observed to be advantageous when compared to plastic containers as they minimize absorption of toxicants and prevents risk of corrosion and chemical reactions. Some plastic are known to react with some crude oil components [13]. The soil substrate have been observed to increase the sensitivity of benthic organisms including *Tympanotonus.* The substrate used in this study was obtained from the site of collection of test animals and subjected to standardization procedure after Tokolo [14]. The mud and sand substrate were dried on a flat wooden board in the open air during the day and in the laboratory at night for 8 days. Drying was done to standardize moisture content and particles sizes, although it reduced the number of naturally occurring microorganisms. The dry soil was grinded with a stone and sieved with a sieve (0.25mm) so as to obtain uniform particles as substrate. A weighed mass of sieved soil (100g) was poured into each bioassay container. Lagoon water was used as the medium for the entire bioassay test conducted. Pre-determined volumes of prepared oily drill cuttings were measured using measuring cylinder and introduced into the soil substrate at the volume made up to 100ml/l by adding appropriate volumes of lagoon water. There were controls in which test medium was lagoon water with similar substrate to the tested tanks but no toxicant was introduced.

To assess the immobility response for the *T. fuscatus*, they were carefully picked out of the test medium, one at a time using a pair of forceps into a clean petri dish containing lagoon water and observed for 20 minutes. They animals were taken to be immobile if they failed to respond by moving to gentle prodding with a glass.

2.4. Relative Toxicity of Oily Drill Cuttings Against Tympanotonus Fuscatus

Active periwinkles of similar sizes were placed randomly in treated and untreated media in bioassay tanks already holding soil substrate. Twenty periwinkles were introduced per container including untreated control. In all cases a total of twenty periwinkles were introduced per container and usually three replicates per treatment giving a total of sixty animals. The test animal were exposed to various concentrations of test compounds as: drill cuttings against *T. fuscatus* at 50ml/L, 150ml/L, 200ml/L, 250ml/L, 300ml/L and untreated control.

2.5. Statistical Analysis

Toxicity dose response data involving quantal response (Immobility) were analyzed by probit analysis Finney [15] based on a computer programmed by Ge le Pattourel, Imperial College, and London as adopted by Don Pedro [16]. The indices of toxicity measurement derived from this analysis were:

EC_{50} =Median effective concentration that courses 50% (immobility) of exposed organisms.

EC_{95} = Effective concentration that causes 95% (immobility) of exposed organisms.

EC_5 = Effective sub lethal concentration that causes 5% response (immobility) of exposed organisms and their 95% confidence limits (l)

TF =Toxicity factor of relative potency measurement e.g 96-h EC_{50} of another compound tested against same species.

One-way analysis of variance (ANOVA) and comparison of means by student Newman-Keul (SNK)test were used to test for statistical differences in the result toxicity tests.

3. Results and Discussion

3.1. Relative Toxicity of Oily Drill Cuttings Against *Tympanotonus Fuscatus*

The results of toxicity based on immobility response measurements of oily drill cuttings against *T. fuscatus* at 24hrs, 48hrs, 72hrs and 96hrs of exposure are shown in tables 1 and 2. From Tables 1, the concentration that will cause 50% immobility in the organisms at 24hrs, 48hrs, 72hrs and 96hrs were 3808.80ml/L, 660.89ml/L, 302.28ml/L and 102.43ml/L respectively. The median lethal concentration of oily drill cuttings against *T. fuscatus* decreased as the duration of the exposure increased (Tables 1). Figure 1 shows the graph of probit response and log dose of drill cuttings against *T. fuscatus* drawn from probit line equation tables. The analysis of variance showed that there was significant difference ($P< 0.05$) between all the treatments (concentrations) at 24, 48, 72 and 96 hours of

exposure. Using the student Newman-Keul's (SNK) test at 5% significant level, the mean immobility response of 50ml/L, 100ml/L, 150ml/L, 200ml/L, 250ml/L and 300ml/L were significantly different from the control at 24hrs of exposure (Table 2). At 96 hours of exposure all concentration were significantly different from control, even though there was no significant difference between 100m/L and 150m/L exposure. There was also no significant difference between 150m/L and 200ml/L immobility response at 96 hours.

However 300ml/L treatment was significantly different from the 250ml/L, 200ml/L, 150ml/L, 100ml/L, 50ml/L and control treatments at 96hrs exposure (Table 2). Table 2 shows the detailed statistical difference between all concentration pairing at 24, 48, 72 and 96 hours of exposure in the SNK test (P = 0.05).The acute toxicity based on the 96hrs value of oily drill cuttings was found to be 102.43ml/L when tested against *T. fustcatus*. The drill cutting was significantly more toxic to *T.* fuscatus when exposed for 96hrs than each other exposure periods (24,48,72) due to no overleap in their $EC_{50.}$ Analysis of variance (ANOVA) showed a significant difference ($p < 0.05$) in the immobility response of T. fuscatus to different treatments of drill cuttings at 24, 48, 72 and 96 hours of exposure. Oily drill cuttings were found to be 37.2 times more toxic on T. fuscatus when actingly singly at 96hrs (102.43ml/L) of exposure than 24hrs (3508.80ml/L).The animals at high concentration were taken to be immobile if they failed to respond by moving to gentle prodding with a glass. This could be attributed to the presence of some dissolved compounds in the oily drill cuttings (e.g chrome salt, surfactant, paraformaldehyde biocide, metals and oil). Hydrocarbon oil, one of the components of oil based mud enter through the gills, and disturb the main functional systems such as respiration, nervous system, blood formation and enzyme [17]. Chukwu and Odunze [18] reported that the relative acute toxicity of spent lubricant oil and detergent (omo) against estuarine benthic macro invertebrate *Cucumis africanus* (*Aurivillus*) and *T. fuscatus* was more toxic to the test organisms at 96hrs of exposure. Don-Pedro [19] has also reported that the different response of organisms to chemical compounds can be attributed to several factors such as the permeability of body membranes, cuticles, sex, age, body size, site of action and behaviour. The ability of molluscs to isolate themselves temporarily from external medium has been emphasized [20, 21]. Both authors observed that gastropods retracted into their shell under the influence of irritants or stress. This study has shown that *T. fuscatus* is susceptible to the harmful effects of oily drill cuttings and the degree of retraction into the shell could probably account for this. These results are indications of poor treatment drill cuttings which is likely to pose higher than acceptable levels of danger to living organisms in the environment. More so, these organisms may be highly susceptible to the constituents of drill cuttings. It has been reported that high biological oxygen demand brings about high microbial activities, which utilizes oxygen for aerobic metabolism. The utilization of dissolved oxygen by microbial activities leads to depletion, thus animals confined to water bodies stands a high risk of dying by asphyxiation.

Table-1. Relative Acute Toxicity of Drill Cuttings Against Tympanotonus Fuscatus at 24, 48, 72 And 96 Hours of Exposure

TIME (HRS)	EC_{50} (95% CL)	EC_{95} (95%CL)	EC_5 (95%CL)	SLOPE ± S.D	D.F	Probit Equation	TF
24	3.808.80	414203	35.02	0.81 ± 0.49	4	Y = 2.11 + 0.81X	1
48	660.89 (298.65 – 124.93)	622239 (3456.765 - 1.037436)	7.02(1.181475E -15 -32.85751)	0.83 ± 0.39	4	Y = 2.70 + 0.83X	5.7
72	302.28 (187.89752 -3323.53746)	15843.60 (2062.90612 -76214787029)	5.77 (0.00010 -25.11815)	0.96 ±	4	Y = 2.63 + 0.96X	37.2
96	102.43 (65.80236 - 134.05959)	1010.42 (531.71137 - 5107.57270)	10.38 (1.18526 - 24.17567)	165 ± 0.38	4	Y = 1.67 + 1.65X	37.2

TF = Toxicity Factor = $\frac{EC_{50} \text{ of Test Compound at 24 hours}}{EC_{50} \text{ of Test Compound at other hours (48, 72, 96hrs)}}$

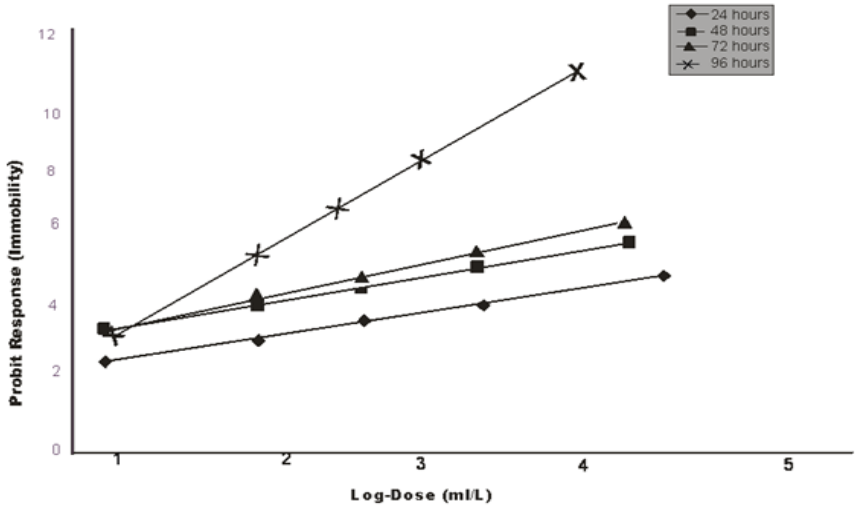
SD = Standard Deviation
DF = Degree of Freedom
CL = Confidence Limit
EC = Effective Concentration

Table-2. Percentage Mean Immobility of T. Fuscatus Exposed to Different Concentrations of Drill Cuttings for 96 Hours

Concentration (Ml/L)	No. of Organisms	% Immobility/Time (Hours)			
		24	48	72	96
CONTROL	30	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
50	30	6.7^{bb}	0.0^{a}	26.7^{b}	36.7^{b}
100	30	10^{bc}	23.3^{b}	30.0^{b}	46.7^{c}
150	30	13.3^{bc}	26.7^{b}	33.3^{bc}	53.3^{cd}
200	30	13.3^{bc}	30.0^{bc}	40.0^{cd}	60.0^{d}
250	30	16.7^{bc}	36.7^{cd}	46.7^{d}	73.3^{e}
300	30	20^{c}	43.3^{d}	56.7^{d}	90.0^{f}

Mean followed by the same superscript letter in a column are not significantly different in the SNK test (P = 0.005).

Figure-1. Probit Response (immobility) log-dose Graph Depicting Relative Taxicity of Drill Cuttings Againts T. Fuscatus



4. Conclusion

The result obtained from this study revealed that the release of oily drill cuttings in aquatic environment constitute a potential threat to environmental-sustainability and human health. This is due to the fact that most of the oily drill cuttings released into the environment sorbs to sediment particles where they cause harm to organisms and overlying waters. Therefore, reliance only on chemical analysis of individual waste waters to identify potential toxic components may sometimes be misleading, and so integrated or complimentary strategies are recommended. Such strategies should involve the inclusion of such bio accumulators like *T. fuscatus* in monitoring programs aimed at deriving realistic water quality criteria and standards for the protection of our aquatic ecosystem in Nigeria.

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