ACUTE TOXICITY OF OILY DRILL CUTTINGS ON MANGROVE LITTORAL PERIWINKLE (*Pachymelania aurita*) OF THE LAGOS LAGOON

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ABSTRACT
Acute toxicity of oily drill cuttings against the littoral mangrove periwinkle (Pachymelania. aurita) of the Lagos Lagoon was evaluated in the laboratory bioassay. 450 specimens of P. aurita were collected from Lagos lagoon and mangrove swamp, put in different holding tanks, half-filled with lagoon water, and aerated. Sand was placed at the bottom of the holding tanks serving as substrate. 20 litres of oily drill cuttings were collected. The bioassays were carried out in glass tanks. The result showed that the acute toxicity of drill mud cuttings based on the immobility response of P. aurita increased with time of exposure. The concentration that caused 50 % immobility in the organisms at 24 hours, 48 hours, 72 hours, and 96 hours was 2057.93 ml/L 394.11 ml/L, 321.85 ml/L, and 122.15 ml/L respectively. The median lethal concentration of drill cuttings and fluids against P. aurita decreased as the duration of exposure increased. There was a significant difference ($p< 0.05$) between all the treatments at 24, 48, 72, and 96 hours of exposure. There is a need to include bio accumulators such as P. aurita in monitoring programmes to establish the environmental level of drill cuttings in Aquatic Ecosystems.
Keywords: Probit response, Drilling muds, immobility, bioaccumulation, acclimatization,

INTRODUCTION

In Nigeria, there is a lot of oil and gas exploration and development activities going on. The practice of dumping drilling mud and cuttings into the aquatic environment by oil and gas operators poses a potential health risk to resident fauna (Ifeadi *et al*., 1985, Shohel *et al.*, 2017). Drill cuttings are particles of crushed rocks produced by the grinding action of the drill bit as it penetrates the earth (Kholood *et al*., 2017). 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 (Neff *et al*., 2000). Drilling muds are essential components of the rotary system and are carefully formulated to achieve specific desired characteristics and function to transport cuttings to the surface, to balance subsurface and formation pressure, prevent blowout and to cool, lubricate and support part of the drill bit and drill pipe. (Neff *et al*., 2000, Okoro 2011). Drilling mud additives may contain toxic substances such as heavy metals, hydrocarbons, biocides chromates, organic polymers, and trace elements that they tend to bioaccumulation and interfere with normal biological activities of organisms (Odokuma and Akponah 2008, Vincent-Akpu *et al.*, 2010). The oil-based drill cuttings may be discharged directly or carried onshore for treatment before disposal into the water body.

In many countries, muds and cuttings are discharged on-site into the ocean. However, in Nigeria, the regulator, Department of Petroleum Resources (DPR) requires that a toxicity test be conducted to ascertain their safety before releasing into the environment. The lethal concentration (LC50) is a standard toxicity test to determine the concentration of substances which will prove lethal to 50 % of a test population of the organisms in a specified duration. *Pachymelania aurita* is regarded as a dominant member of the faunal community of Lagos lagoon with a mean annual production rate that varied from 1.59 g and 0.99 g/0.5 m/ year (Brown 1991). The species is edible and serves as a source of protein to local inhabitants.

The aim of this study, therefore, is to estimate the short term toxicity of oil-based drill cuttings on periwinkle (*P. aurita*) of the Lagos lagoon. These organisms were chosen because they are mostly sessile, relatively immobile, or sedentary and may serve as useful *in situ* sentinel in biomonitoring studies of drill cuttings pollutant in Nigerian coastal waters.

MATERIALS AND METHODS

Test Specimens and Acclimatization

The test specimens used for this bioassay were periwinkle *P.* aurita (Mollusca, Gastropoda, Mesogastropoda, Melanidae). *P. aurita* were collected from the edge of the Lagos lagoon and adjacent mangrove swamp at low tide. Each of these specimens was handpicked into a separate 10 litre-plastic bucket containing water from the habitat. The specimens were of known age but were approximately the same range (Length of shell 3.0 ± 0.5, the diameter of aperture 0.8 – 1.0 mm). The specimens were taken to the laboratory and left in the holding tanks. Sand from the site of the collection was placed at the bottom of the holding tanks serving as substrate. 450 test specimens were put in different holding tanks (113 cm x 54 cm x 80 cm) and half-filled with lagoon water. These holding tanks were aerated with a 220 V air pump and then changed every 48 hours to prevents acclimatization to laboratory conditions (28 + 2, 72.2 %R.H) for 7 days before used for the experiment.

Test Compound and Bioassay Procedures.

Drill cuttings used for this study were collected in two 20 litre- plastic buckets from the main discharge point at the Shell Development Petroleum Corporation Warri. The cuttings were coated with oil-based mud used during the drilling process with a pH value of 5.9. The bioassays were carried out in glass tanks (22 cm x 15 cm x 18 cm).

Preparation of Substrate and toxicants to test media.

The substrate used in this study was obtained from the site of the collection of the test specimen and subjected to standardization procedure after Tokolo (1988). The mud and sand substrate was 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 particle sizes, although it reduced the number of naturally occurring microorganisms. The dried soil was ground with stone and sieved with a sieve (0.25 mm) to obtain uniform particles as a substrate. A weighed mass of sieved soil (100 g) was poured into each bioassay container. Lagoon water was used as the medium for the entire bioassay test conducted. Pre-determined volumes of prepared drill cuttings were measured using a measuring cylinder and introduced into the soil substrate and the volume made up to 100 ml/l by adding appropriate volumes of lagoon water. These were controls in which test medium was lagoon water with a similar substrate to the tested tanks but no toxicant was introduced.

Relative Toxicity of Drill Cuttings against *Pachymelania aurita.*

Active *P. aurita* of similar sizes were picked up from the holding tank and introduced into the bioassay containers holding untreated and treated medium in the presence of sandy substrate and three replicates per treatment giving a total of sixty specimens. The test specimens were exposed to various concentration of test compounds as follows: Drill cuttings against *P. aurita*: 100 ml/L, 150 ml/L, 200 ml/L, 250 ml/L, 300 ml/L, 350 ml/L)

Statistical Analysis.

Dose-Response Data Analysis.

Toxicity dose-response data involving quantal response (Immobility) were analyzed by probit analysis (Finney, 1989) based on a computer programme by Ge le Pattourel Imperial College, London as adopted by Don-Pedro (1989). The indices of toxicity measurement derived from this analysis were:

EC50 =Median effective concentration that courses 50 % (immobility) of exposed organisms.

EC95 = Effective concentration that causes 95 % (immobility) of exposed organisms.

EC5 = 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 EC50 of another compound tested against the 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 results of toxicity tests.

RESULTS

The results of dose immobility analysis of oily drill cuttings against *P. aurita* at 24 hrs, 48 hrs, 72 hrs and 96 hrs of exposure are shown in Table 1

Table 1: Relative Acute Toxicity of drill cuttings against *Pachymelania aurita* 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
24	2057.9	57400.9	73.7	1.14 ± 0.69
48	384.11(293.19- 1021.5)	2970.33 (1093.5 - 150892.7)	52.29 (5.8–92.6)	1.88 ± 0.55
72	321.85 (248.7 –646.2)	2623.14 (1012.4 -105169.4)	39.49(3.1– 76.5)	1.81 ± 0.55
96	122.15	1149.34	12.98	1.68 ± 0.53

CL = Confidence Limit, EC = Effective Concentration

From Table 1, the concentration that will cause 50 % immobility of the organisms at 24 hrs, 48 hrs, 72 hrs, and 96 hrs were 2057.93 ml/L, 394.11 ml/L, 321.85 ml/L and 122.15 ml/L respectively. The median effective concentration of drill cuttings against *P. aurita* decreased as the duration of the exposure increased (Table 1).

Figure 1 shows the graph of probit response and log dose of drill cuttings against *P. aurita* drawn from the probit line equation. The analysis of variance shows that there was a significant difference ($P < 0.05$) between all the treatments at 24, 48, 72, and 96 hours of exposure.

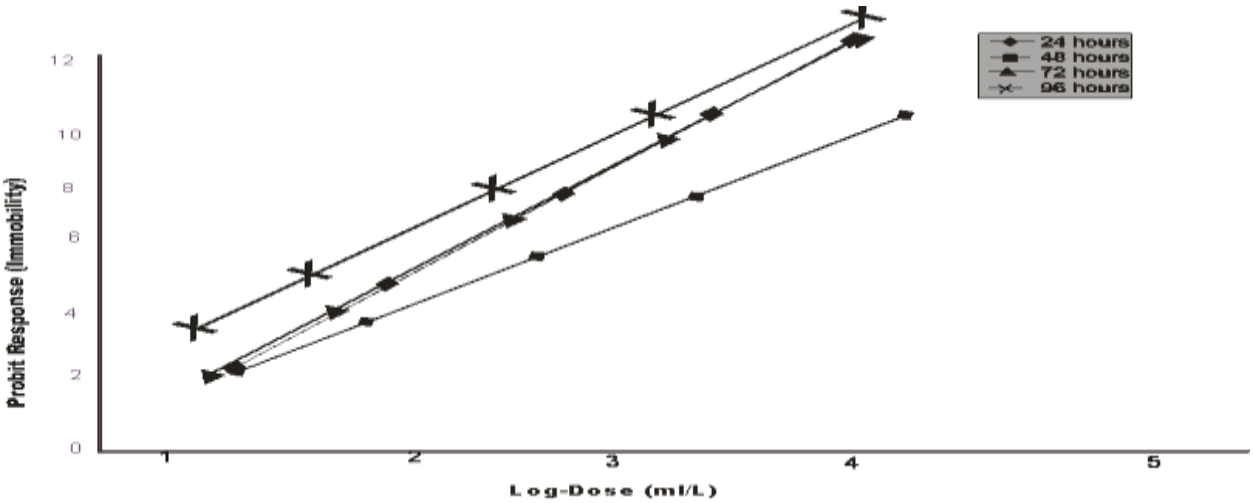

Fig 1: Probit Response (Immobility) Log-dose Graph Depicting Relative Toxicity of Drill Cuttings against *P. aurita*

Table 2: Percentage Mean Immobility of *P. aurita* 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^{ab}	13.3^{b}	20.0^{b}	46.7^{b}
100	30	10^{bc}	23.3^{c}	26.7^{bc}	56.7^{c}
150	30	13.3^{bc}	26.7^{c}	33.3^{cd}	60.0^{c}
200	30	13.3^{bc}	33.3^{c}	40.0^{de}	66.7^{cd}
250	30	16.7^{bc}	43.3^{d}	46.7^{e}	73.3^{d}
300	30	20^{d}	46.7^{d}	56.7^{f}	83.3^{e}
350	30	20^{d}	46.7^{d}	56.7^{f}	83.3^{e}

Mean followed by the same superscript letter in a column are not significantly different in the SNK test (P = 0.05).

Based on the SNK test at P =0.05, no significant difference was observed in the mean immobility response of 100 ml/L, 150 ml/L, 200 ml/L, and 300 ml/L at 24 hours exposure (Table 2). There was also no significant difference between 250 ml/L and 300 ml/L at 96 hours of exposure.

However, 350 ml/L was significantly different from all the concentration. The summary of the statistical difference between all concentrations pairing at 24, 48, 72, and 96 hours of exposure using the SNK is shown in Table 2.

DISCUSSION

The observed high variability in percentage immobility among treatment (drill cuttings) that progressed from low immobility with no mobility in the control tank for the 96 h duration of the test, indicates that the test conditions were appropriate and thus immobility recorded in the test solution could have been induced from the effect of oily drill cuttings. The rate of immobility of *P. aurita* was attributed to the concentration of the oil in the drill cuttings, the period of exposure, the ability of the shellfish to bio-accumulate, and the nature of the oil (Ewa-oboho and Otogo 2009). Don-Pedro (1996) has also reported that the different responses of the organisms to the chemical compound 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 the external medium has been emphasized (Crapp 1971, Dick 1976). Both authors observed that gastropods retracted into their shells under the influence of irritants or stress. This study has shown that *P. aurita* 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 of drill cuttings which is likely to pose higher than acceptable levels of danger to living organisms in the environment. Moreso, these organisms may be highly susceptible to the constituents of drill cuttings.

CONCLUSION

The result obtained in this study suggests that the estuarine benthic macroinvertebrate, *P. aurita* which plays key roles in the environment may serve as useful *in situ* sentinel for biomonitoring studies of drill cuttings pollutant in Nigerian coastal waters.

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