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Toxicity of Drilling Fluid Parateq on Microbial Load and Survival of *Oreochromis niloticus* Fingerlings

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ABSTRACT

Pollution arising from petroleum exploration is one of the major challenges facing the inhabitants of Niger Delta region in Nigeria. The toxicity of parateq, a synthetic base mud, on the microbial population of *Oreochromis niloticus* fingerling was evaluated in the laboratory. The fingerlings were exposed to different concentrations (0, 1000, 3000, 5000 and 9000 mg L⁻¹) of drilling fluid. Fish mortality data were recorded at 24, 48, 72 and 96 h and analysed using Finney's Probit Analysis method to calculate LC₅₀ values (concentration of drilling fluid in water that will kill 50% of the fish population in 96 h). The 96 h LC₅₀ was 2210 mg L⁻¹. The mortality increased with increase in concentration while, the lethal times decreased as concentration increased. The bacterial biomass ranged from 1.59×10⁸-6.0×10⁸ CFU mL⁻¹ while fungal biomass ranged from 1.0×10³-2.1×10⁴ CFU mL⁻¹. Results revealed that there was an increase in fungal biomass with increase in mud concentration and a decrease in bacterial biomass with increase in mud concentration.

Key words: Parateq, microbial population, median lethal concentration, median lethal time, TVC, drilling fluid

INTRODUCTION

In West Africa, Nigeria (Niger Delta) a lot of oil and gas exploration and development activities are carried out using drilling fluids. Two types of fluids normally use in drilling operations are water based fluids and non-aqueous drilling fluids (OGP, 2003). After use, these drilling fluids are disposed of into temporary or permanent pits for land-based drilling and into the surrounding water body or swamp-cut for offshore drilling (Ifeadi *et al.*, 1985). Drilling waste in water bodies may kill marine life, smother or suffocate it with plume of suspended particles (Saasen *et al.*, 2001; Wills, 2000). This will result in potential damage to fish and invertebrate population and alteration in feeding and spawning grounds (Neff *et al.*, 2000; Soegianto *et al.*, 2008). Rhodes and Hendricks (1990) discovered that the components of drilling fluids inhibit the growth of microbial communities that are important in some biological cycles present in the ecosystem which may affect the productivity of such system.

In aquatic ecosystems, Fish are located at the top of the food chain and are highly visible resources. They are known to be affected by pollutants due to their being in direct contact with water via their gills and body surface. Food security is a complex issue, fish and fishery products are generally regarded as high risk commodity in respect of pathogen contents, natural toxins and possible contaminants and alterations (Yousuf *et al.*, 2008). The estimation of bacterial numbers

in fish is frequently used to retrospectively assess microbial quality or to assess the presumptive safety of the product (Okonko *et al.*, 2008). When microbial growth and actions are affected by the presence of contaminants in the aquatic system, the fishes are exposed to harmful organisms which are subsequently passed on to man.

Ekpo and Uzegbu (2004) studied the effect of drilling additives (chrome lignosulfonate) on *Pachymelania fusca* and *Tympanotonus fuscatus* and observed an increase in the microbial biomass with increase in concentration of drilling additives. However, little information exists on effect of drilling fluid and its additives on microbial load of fish. This study aims to determine any significant changes in the bacterial and fungal biomass in *O. niloticus* when exposed to parateq a commonly used drilling fluid in the Niger Delta wetlands of Nigeria.

MATERIALS AND METHODS

Test organisms: Fingerlings (8.45 cm) of *Oreochromis niloticus* were obtained from the African Regional Aquaculture Centre (ARAC), Aluu, Rivers State-Nigeria.

In the laboratory, the fish were transferred immediately to the holding tanks [120×120×120 cm]. The holding tanks were aerated, cleaned and the water renewed daily (Reish and Oshida, 1986). Fish were fed twice daily with Nigeria Institution of Oceanography and Marine Research feed (NIOMR) (35% protein).

Preparation of drilling fluid: Drilling fluid was obtained from Bariods Nigeria Ltd. and prepared in advance. The fluid was stored in a 5 L bucket at approximately 5°C. Immediately before the fluid was used, it was mixed in a storage bucket with a mixer at 3450 rpm for ½ h to ensure sample homogeneity.

Acute toxicity of drilling fluid: A complete randomised designed was used and the tests were conducted in the laboratory under room temperature using static non-renewal bioassay (Cal/EPA, 2004). The solutions (0000, 1000, 3000, 5000 and 9000 mg L⁻¹) were thoroughly mixed for 20 min and allowed to settle for 1 h before the introduction of fish (Reish and Oshida, 1986; FAO, 1987). The drilling fluid settled into a thin layer covering the bottom of the test chambers.

One hour after the preparation of the test solutions, ten healthy organisms were randomly selected and placed in the tanks in 3 replicates and covered with a net that was fastened with a rubber band to prevent fish escape. The fish were not fed during the 96 h bioassay. Water quality parameters (temperature, alkalinity, pH and dissolved oxygen) of the test tanks were monitored daily for the replicates using standard methods (APHA, 1998).

Mortality was used as a measure of toxicity in terms of 96 h LC₅₀. The numbers of dead and active fishes were recorded. A fish is presumed dead and removed immediately when there are no opercula or body movement after gentle prodding.

Microbial analysis: At the end of the 96 h, the Total Viable Count (TVC) of heterotrophic bacterial and fungal population on the fish body was determined using nutrient agar (APHA, 1998). The pour plate method was used after a serial dilution of the homogenized fish. The plates were incubated at 37 and 25°C for bacteria and fungi, respectively. Based on their morphological and biochemical characteristics, the different species were identified. The average counts of colony forming unit (CFU mL⁻¹) were calculated and recorded.

RESULTS AND DISCUSSION

The various physicochemical parameters (temperature- 28.15 ± 0.65 ; pH- 6.61 ± 0.31 ; alkalinity- 23.38 ± 0.019 ; dissolved oxygen- 3.83 ± 0.28) did not vary significantly ($p > 0.05$) from those of the control except a slight decrease in the Dissolved Oxygen (DO) which Ifeadi *et al.* (1985) attributed to the effect of oil based fluid.

No mortality was observed in the control, however, in the other treatments, mortality increased with increase in drilling fluid concentration over time. At 24 h, no death was recorded at the lowest concentration of parateq. However, as the time of exposure increased, more deaths were recorded. At the end of 96 h, the result showed 26, 33, 73 and 80% mortality recorded in 1000, 3000, 5000 and 9000 mg L^{-1} , respectively (Fig. 1). The LT_{50} also decrease with increase in concentration.

The results indicated that as the concentration increased, more deaths were recorded and as the time increased the LC_{50} reduced (Table 1). The toxicity of Drilling fluids has been attributed to their hydrocarbon content (Neff *et al.*, 2000; Tagatz *et al.*, 1985). Low hydrocarbon content in the drilling fluid can be responsible for the low toxicity and also Synthetic based fluid does not readily disperse in water (Wills, 2000). Parrish and Ward (1988) recorded LC_{50} range of 0.45-39% using laboratory prepared drilling fluid on mysid and posited that the increased toxicity was due to the presences of hydrocarbon. The 96 h LC_{50} value of the static bioassay with parateq was 2210 mg L^{-1} ($1\% = 1000 \text{ mg L}^{-1}$), this LC_{50} was relatively low. This is probably because the test was not renewed daily during the 96 h bioassay period (Neff *et al.*, 1981).

Table 2 shows the heterotrophic fungal and bacterial counts of *O. niloticus* exposed to parateq. The total fungal counts ranged from 1.0×10^3 - 2.1×10^4 CFU mL^{-1} where the highest counts were found in fish exposed to 9000 mg L^{-1} . The fish in control tanks and in concentration 1000 mg L^{-1} had total viable count of 1.0×10^3 . While in the other concentrations, the cells increased from the lowest to the highest concentration. The bacterial count decreased with increase in parateq concentration. The fish in control had bacterial counts of 1.59×10^8 CFU mL^{-1} while those in highest concentration (9000 mg L^{-1}) had a bacterial count of 6.0×10^6 CFU mL^{-1} . The total counts for all the

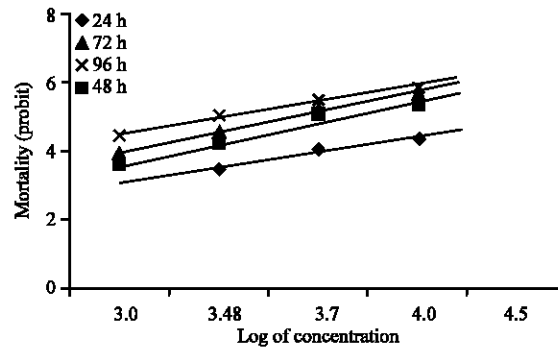


Fig. 1: Mortality in probit of *O. niloticus* exposed to different concentration of parateq drilling fluid 96 h

Table 1: Median lethal concentrations (LC_{50}) of parateq on *O. niloticus* in 96 h

Time (h)	LC_{50} (mg L^{-1})
48	3310
72	2710
96	2210

Table 2: Viable count of colonies from the fungal and bacterial isolates

Concentrations (mg L ⁻¹)	Fungal counts (CFU mL ⁻¹)	Bacterial counts (CFU mL ⁻¹)
0000	1.00×10 ³ ±141.4	1.59×10 ⁸ ±79.2
1000	1.50×10 ³ ±282.8	1.55×10 ⁸ ±8.50
3000	1.00×10 ³ ±282.8	1.12×10 ⁸ ±15.6
5000	1.70×10 ³ ±282.8	2.70×10 ⁷ ±8.5
9000	2.10×10 ³ ±424.3	6.00×10 ⁶ ±1.4

samples examined in this study were generally high exceeding the FAO/WHO Standard limits of 1.0×10^2 CFU mL⁻¹ that can be present in fish (ICMSF, 1986). This indicated that there is presence of harmful microorganisms on the fish body. Similar trend of bacterial growth was reported by Ekpo and Uzegbu (2004) where the coliform counts ranged between 5.4×10^2 and 8.5×10^5 for shrimp and 5×10^2 - 4.4×10^4 cells for prawn when exposed to drilling mud additives. They further stated that when the total count reaches 10^6 CFU per gram or milliliter, the product is assumed to be, or nearing, spoilage. As noted by earlier workers (Smith *et al.*, 1982), micro organisms are usually the normal flora on the body of the fish. Their growth and multiplication are either encouraged or inhibited by toxicants. In this study, the growth of the bacterial population was inhibited while the growth of the fungal population was encouraged. Nweke and Okpokwasili (2003) observed that 1% drilling fluid waste elicited differential toxic effects on four bacterial isolates from the mangrove swamps in the Niger Delta. This they attributed to the natural ability of some bacteria to biodegrade the base oil. When bacterial growth and action are hindered by the presence of drilling fluid, fishes are exposed to harmful materials.

CONCLUSION

Despite the importance and sensitive nature of the Niger Delta, the area is replete with oil exploration and exploitation activities which involve the use of drilling fluids. Most of the cuttings produced contain considerable proportion of drilling fluids which are dumped at the well site or deposited into the water. Drilling fluids elicited differential effects on the microbial population on the fish body. This has been attributed to the natural ability of some bacteria to biodegrade the base fluid. Thus micro organisms can increase or decrease in number depending on the effect of base fluid on the species.

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