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RESEARCH PAPER

THE HISTOLOGICAL ALTERATIONS IN GILLS AND LIVEROF *TILAPIA GUINEENSIS* EXPOSED TO COMMON DRILLING FLUIDSIN THE DELTA OF RIVER NIGER

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ABSTRACT

This study investigated the histological changes on gill and liver of fry, fingerling and post-fingerlings of *Tilapia guineensis* exposed to sublethal concentrations of drilling fluid for 12 weeks. The concentrations used were 0.32%, 0.63%, 1.25% and 2.5% vol/vol (Note 1% = 1000ml/L). The results revealed that little or no lesions were observed in the gills and liver with the exception of those exposed to the highest concentration. The gill filaments of the fish exposed to the highest concentration of the toxicants (2.5%) had mild lesion which was characterized by epithelial lifting and hyperplasia of the gill lamellae, while the liver exhibited mild vacuolations, presence of macrophages and slight sloughing of the cell. Although toxic responses had been identified in the tissues of fish exposed to acute concentrations of drilling fluids, the subtle changes identified in the tissues of the exposed fish divulge regenerative response in terms of functions and histology. This implies that drilling fluids released even at low concentrations are capable of inducing changes in the tissues of fishes endemic in the delta.

Keywords: **Drilling, Niger Delta, medial lethal concentration (LC50), median lethal time (LT50)**

INTRODUCTION

Contamination of waters by oil-related operations, which is the most obvious industrial activity in the Niger Delta, is a chronic problem that has drawn considerable attention in the past few years. The enormous growth of the oil and gas industry and its steady advancement into the deeper waters have raised concern about the impact of these activities on the environment, fisheries and other legitimate uses of the water (Sikoki and Zabbey, 2006).

Drilling fluids which are suspension of solid in liquid emulsion and/or dissolved materials with chemical additives are employed during drilling activities to remove drill cuttings. Drilling fluids are of three different types based on the principal components (air, water and oil). However, the oil based fluids which is the non-aqueous based drilling fluid is further divided into groups according to the increasing level of aromatic hydrocarbon concentrations. They include the conventional oil based fluids, low toxicity oil based fluids and the synthetic based fluids (SBF) (Neff, 2010). Drilling fluids contain toxic salts and pollutants which when discharged untreated into the environment generally affect the biota (Vincent-Akpu *et al*., 2010). The solid phase (SP)of drilling fluids have been found to be more toxic to aquatic organisms when compared to their suspended particulate phase (SPP) (Sil*et al*., 2012)

Fish are relatively sensitive to changes in their environment including an increase in pollution. Environmental pollution in aquatic ecosystems is usually at a low level but chronic in nature. Studies have shown that chronic toxicity testing is more relevant to environmental management than acute toxicity results as the aquatic organisms will be exposed in the longer term to the pollution (Melton *et al*., 2000). When exposure time of fish fry to drilling muds increased from 2–4 days to 10–30 days, the concentration of drilling muds that cause lethal effects in fish fry decreased by 2–3 orders of magnitude (Patin, 1999). While acute toxicity testing suggests that SBF are practically non-toxic, the chronic toxicity of this drilling fluid remains relatively unknown. The study by Gagnon and Bakhtyar (2013) on pink snapper (*Pagrusauratus*), exposed to three different mud system showed that SBF has the potential to affect fish health as evidenced in increased EROD activities, accumulation of biliary metabolites and high stress protein level.

Early toxic effects of pollution may be evident on cellular/tissue level before significant changes can be identified in fish behaviour or physiology (Vincent-Akpu and Chindah, 2009). Therefore histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs (Wester *et al.*, 2003). Using histological studies, one can gain insight into the mode and site of action as well as the fish's specific response regarding the time period it is exposed to the drilling fluid.

Regulatory monitoring of Drilling fluids to use in exploration and drilling activities in Nigerian waters is subjected to acute toxicity testing which usually exclude the sublethal effects of these fluids (DPR, 2002).There is need to consider if chronic exposure to drilling muds has the potential to cause histological changes. Therefore, the present study was undertaken to investigate the potential histological changes in gills and liver of three different life stages of *T. guineensis* associated with chronic exposure to Drilling fluid.

MATERIALS AND METHODS

The drilling fluid was procured from Baker Hughes Nigeria Limited. The physical and chemical characteristics of the drilling fluids were determined in Baker Hughes Nigeria Limited and Institute of Pollution Studies Rivers State University of Science and Technology Port Harcourt laboratories using standard methods (APHA, 1998).

Fry (0.64cm –0.65cm/0.52g – 0.55g), fingerlings (5.51cm–5.57cm/5.52g – 5.56g) and Post fingerlings (7.21 - 7.25cm / >10.5g) of *T. guineensis* grouped according to Odiete (2003) were collected from the African Regional Aquaculture centre (ARAC), Buguma, Rivers State Nigeria. The fish were acclimated for 28 days in holding tanks [120 x 120 x 120cm]. The holding tanks were aerated, cleaned and the water renewed regularly. During the acclimation period, the fish were gradually subjected to the dilution water until they could survive in the uncontaminated dilution water without showing signs of stress. Fish were fed (NIOMR feed - 35% protein) at a maintenance ratio of 3% body weight/day during the acclimation and experimental periods. The basic procedures followed according to the method of standard handling and water renewals (EPA, 2002;Reish and Oshida, 1986). Mortality during the holding period was less than one percent of the whole population.

The LC_{50} was calculated based on the probit analysis from Vincent-Akpu *et al*., (2010), through which five sublethal concentrations 2.5 %, 1.25 %, 0.63 %, 0.32 % and control were obtained in a volume to volume ratio (Vincent-Akpu and Chindah, 2009)(Note 1% = 1000ml/L).

Fifteen 25 liter capacity tanks were used for the fry and fifteen 50 liter capacity tanks for the fingerlings. Ten 500 liter capacity concrete tanks were constructed for the post fingerlings. Healthy ten fish were randomly assigned to the aquaria and screened with a mosquito net to prevent fish escape. Exposure lasted for 12 weeks, during which freshly prepared test solutions were made weekly as the water is changed and tanks cleaned (EPA,2002;Chindah*et al*., 2004). Water parameters (Temperature, pH, DO and alkalinity) of the test solution were monitored weekly throughout the duration of the experiment (APHA, 1998). At the end of the 12 weeks, four fish from each concentration were sacrificed by a sharp blow to the head, and the liver and gills excised from the fish. Care was taken not to squeeze any of the tissues and fixed immediately in 10% neutral formalin in Nalgene container for 36hr to avoid post–mortem changes. The tissues were processed using established methods (Wester *et al.,* 2003). Each slide was reviewed microscopically without any knowledge of its individual treatment and a histological report prepared. Photomicrographs were taken to illustrate some of the tissue pathology recorded.

RESULTS

Composition of drilling fluid: The drilling fluid has a synthetic based fluid (SBF) system called synteq. The base fluid is an olefin isomer base fluid formed by isomerization of linear alpha olefins in the presence of heat and a suitable catalyst (Neff *et al.,* 2000). The physical and chemical composition of drilling fluid is presented in Table 1.

The formulation is 26.2% water and 73.8% base fluid with $CaCO_3$ as weighting agents and other additives. The total hydrocarbon content is 41.00mg/g. The drilling fluid is slightly acidic (6.79) and contains Lead (2.16ppm), Manganese (2.05ppm), Barium (0.004ppm), Chromium (0.096ppm) and Zinc (5.82ppm).

Table1: Physical and chemical characteristic of drilling fluid

Composition/Characteristics	Values
Colour	White
Water	26.2%
Base Fluid	73.8%
Organophilic lignite (Carbongel 11)	12ppd
Organophilic clay (Omniplex)	2.16ppd
Lime	3ppd
$CaCl_2$	32.8ppd
$CaCO_3$	139.3ppd
Synthetic Polyamide (Omnimul)	8.62ppd
pH	6.76
Total Solid	587mg g^{-1}
Total organic carbon	1.65mg g^{-1}
Chloride	0.625mg g^{-1}
Nitrate	1.599mg g^{-1}
Sulphate	-
Phosphate	-
Total hydrocarbon	41.00mg g^{-1}
Lead	2.16ppm
Manganese	2.05ppm
Zinc	5.82ppm
Cadmium	-
Chromium	0.096ppm
Barium	0.004ppm

Physiochemical Parameters: The mean values of the physiochemical parameters ranged from 27.5 – 28.3^{0}C (Temperature); 4.21- 4.96 Mg/L (DO); 21.20 - 23.62Mg/L (Alkalinity) and 6.96 – 7.33(pH).There were no significant differences ($p < 0.05$) in the physiochemical parameters of the drilling fluid treated tanks for each life stages and at different concentrations.

Histopathology: The gills of untreated *T. guineensis* (Fig. 1A) revealed normal parallel arranged gill filaments consisting of primary lamellae and its arrays of delicate secondary lamellae with epithelial cells all intact (Fig. 1A). In the gills of the fry, fingerling and post fingerling exposed to 1.25%, 0.63%, 0.32% of Drilling fluid for 12weeks, no noticeable change was observed and cannot be differentiated from the control.

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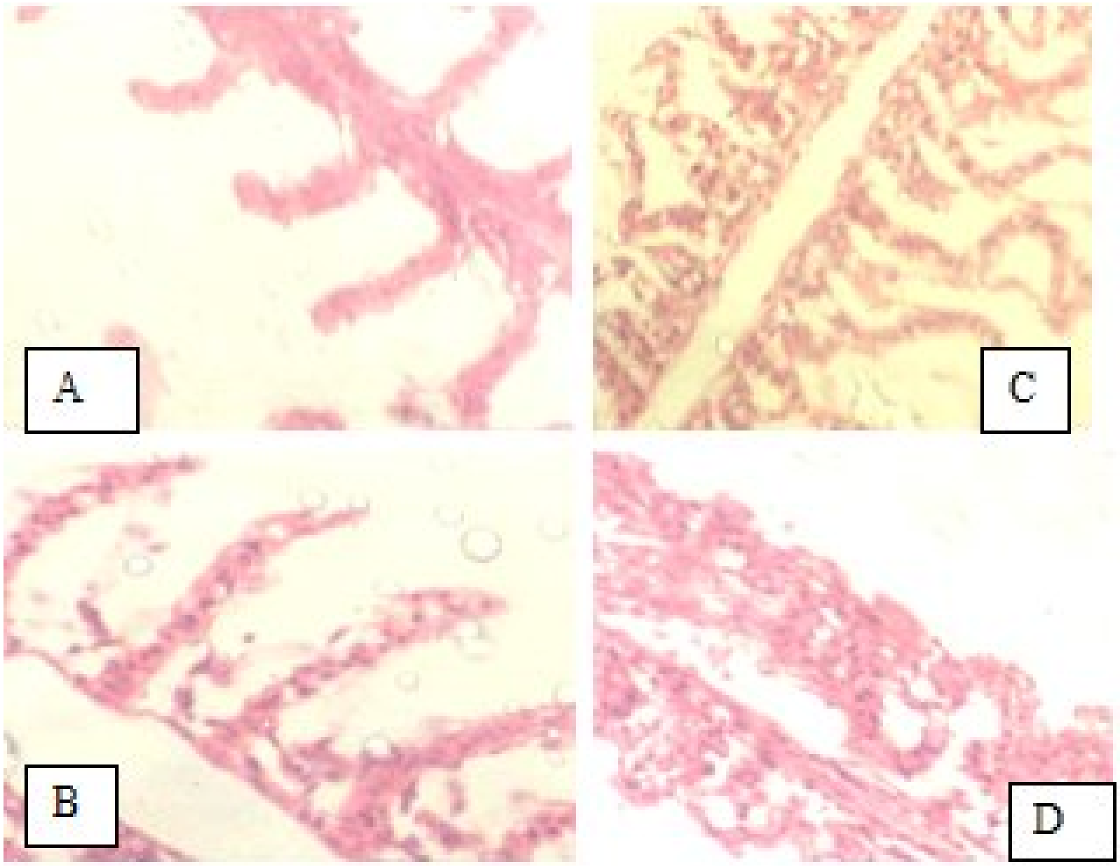

Fig 1: Sections of gills of control and the three life stages of T. guineensis exposed to 2.5% drilling fluid for 12 weeks. A (control) shows normal arrangement, while B, C and D show gills of fry, fingerlings and post fingerling respectively.

At the highest concentration of toxicant (2.5%), the gill filaments of the exposed fish had mild lesion which was characterized by epithelial lifting and hyperplasia of the gill lamellae. The gills of fry, fingerlings and the post fingerling showed epithelial lifting (Figs 1B and C). In addition toepithelial lifting observed in the gills of the post fingerling, hyperplasia was observed (Fig 1D).

The livers of fish from the control tanks were intact and had no gross lesions. The liver cell is characterized by sheets of hepatocytes, which are polyhedral cell and arrayed about the central vein. The cells have distinct central positioned nucleus(s). Most times the sinusoids were empty or collapsed and hepatocytes are often filled with glycogen, giving a vacuolated pale appearance in Haematoxylin and Eosin (H&E) section as seen in Fig.2A. The liver of all the life stages of *T. guineensis* exposed to 1.25%, 0.63%, 0.32% of drilling fluid had little or no lesion and could not be differentiated from those of the control. However, fry, fingerlings and post fingerlings exposed to the highest concentration of the toxicants (2.5%) exhibited mild vacuolations, presence of macrophages as shown in Figs 2B, 2C and 2D. Slight sloughing of the cells were further observed in liver of drilling fluid treated post fingerlings (Fig. 2D).

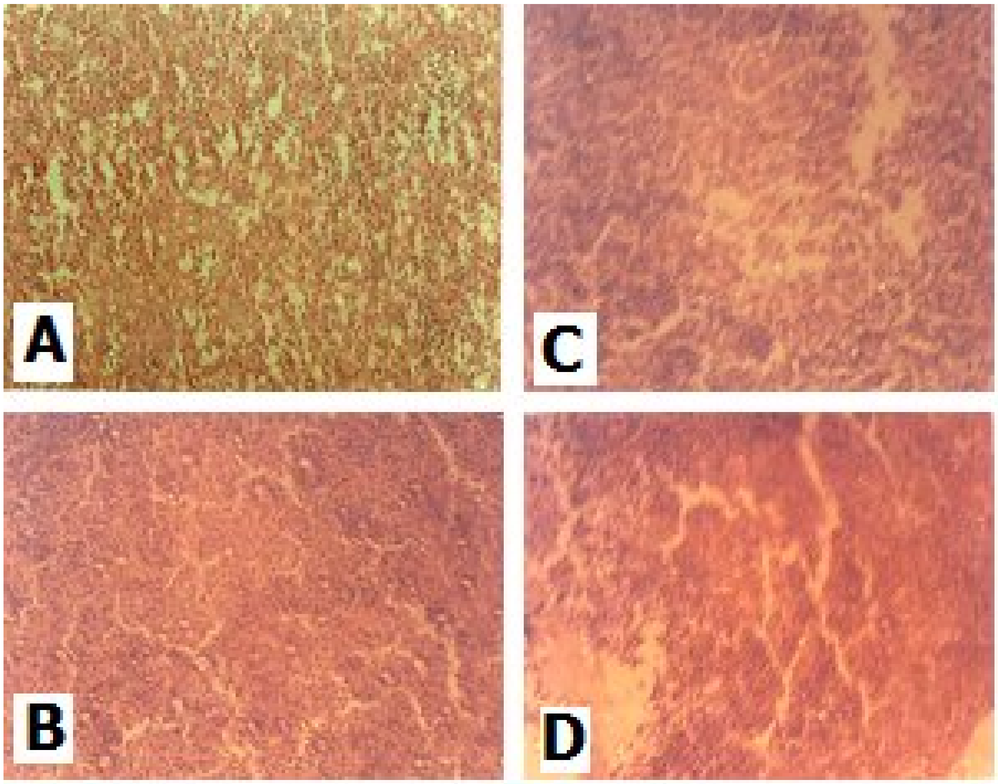

Fig 2: Photomicrographs from liver of fry, fingerling and post fingerling exposed to 2.5% of Drilling fluid (X100). A is Control), while B, C and D represents those of Fry, Fingerling and post fingerling exposed to 2.5% drilling fluid respectively.

DISCUSSION

Most of the physicochemical parameters of the drilling fluid were within the range allowed by DPR (2002). However, the hydrocarbon content (41.00 mg/l in Drilling fluid) was higher compared to 20mg/l that is acceptable for discharge in near shore waters. The toxicity of drilling fluid has been attributed to the presence of hydrocarbons (Neff et al., 2000). Also lead and chromium were higher than the concentration permitted to be discharged.

The limited variations in the water quality observed between control and treated tanks suggested that the drilling fluid did not adversely alter the water quality integrity. The water quality readings indicated that all the physicochemical parameters measured remained at sufficient levels that will sustain biological life.

Gill exposed to the drilling fluid showed widespread damage. These include epithelial lifting and lamella disruption or fusion, symptoms consistent with gill irritants. Studies of the morphological changes induced by drilling fluid in fish gill tissues have shown two types of responses: defense and compensatory response, both responses help to bar the entry of toxicants and prevent them from reaching the blood stream and to a lesser extent, prevent damage caused by direct effect of the toxicant. However, because the gills are the main site of gas exchange and other important function such as ionic and osmotic regulation and acid-based equilibrium, histopathological changes in gill structure involve respiratory disturbance and electrolytic imbalance (Soegianto, *et al*., 2008, Fernandes and Mazon, 2003).

The liver is an organ that is highly susceptible to damage, by virtue of its detoxifying role and active metabolic functions. It is possible, therefore that localized lesions are normally present. The liver of drilling fluid treated fish also showed histological alterations, characterized by the presence of macrophage, mild vacuolations and sloughing of liver cells. The histopathology changes of liver in this study were similar to changes described in various papers in young and adult fish (Nwakanma and Hart, 2013, Wolf and Wolfe, 2005). Constitutively present in the exposed liver of the fish used in this study is the macrophages. Though this is a generalized lesion of liver toxicity, the presence of macrophage in liver is most likely due to the effect of the drilling fluids. Macrophages aggregates serve as repositories for products of cell membrane and erythrocyte breakdown and function in iron storage, sequestration and detoxification of endogenous and exogenous substances amongst others. Proliferation of macrophage aggregates has been associated with several conditions including starvation, tissue catabolism, toxicant-induced hemolytic anemia, heat stress and others (Wolf and Wolfe, 2005, Wester *et al.*, 2003).

The histopathological effects of chronic exposure to pollutants are more difficult to evaluate because these processes develop gradually and prolonged exposure to a sublethal concentration of toxicant increases the organism's tolerance to the toxicant and establishing new physiological state (Saliu and Salami (2013).Sublethal exposure to the drilling fluid resulted in noticeable mild alterations in contrast to changes in acute exposure as observed inVincent-Akpu *et al* (2010).

CONCLUSION

Subtle changes seen in the liver exposed to sublethal levels of the toxicant, divulge a regenerative response in the fish. This implied that although toxic response had been identified in the tissues of fish exposed over the short-term period (Nwakanma and Hart, 2013, Vincent-Akpu *et al*., 2010), the fish were able to adapt to the low level of exposure to drilling fluid in terms of liver function and histology. However the long term effects of the new induced adaptive changes in the tissues of the fishes endemic in the delta is not evident.

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