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Effect of Bentonite and Clay Based Drilling Fluid on Haematological Parameters and Metabolites in the Muscle of *Heterobranchus longifilis*

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ABSTRACT
Oil drilling activities in recent years have been made successful due to the use of drilling fluids such as Water Based Drilling Fluid (ANADrill) containing Bentonite and Clay. But their waste and discharges has caused debilitating environmental impacts resulting in fish kills and pollution. A total of 35 juveniles of *Heterobranchus longifilis* were exposed to varying concentrations of ANADrill, water based drilling fluid in this study. Experimental fish were divided into one control and four treatment groups with four replicates each. Treatment groups were orally administered with 167, 333, 400 and 500 mg/L of toxicant following a completely randomized renewal bioassay for 30 days. Thereafter, fish were killed and blood samples collected using a 5 ml syringe and needle into EDTA bottles for blood cells analysis. A portion of fish muscle were collected, crushed, homogenized with 0.5 ml of perchloric acid and centrifuged for 15 minutes at 3000 rpm. Samples were poured into plain bottles for chemical assay at Federal Medical Centre, Yenagoa. Metabolites such as Total protein, Albumin and Urea values in the muscle were not significant ($p>0.05$) compared to the control, but Creatinine values appreciated. The values of haematological parameters such as RBC, WBC, Platelet and Hb all declined compared to the control in this study. The results thus confirm the toxicity of WBDF (ANADrill) on measured blood cells and metabolites in the muscle of exposed fish.
Keywords: ANADrill, Concentration, Haematology, Metabolites, *Heterobranchus*, Bentonite, Clay, Metabolites.

INTRODUCTION
Drilling fluids are a complex system that contains a fluid phase, solid phase and a chemical phase (Sahar & Abouzar, 2009). Other than the fluid and solid phases, different types of chemicals and polymers are also used in modern technology to design a drilling mud in the chemical phase to develop the key properties of the mud that meet the functional requirements such as mud rheology, density, mud activity and fluid loss control (Sahar & Abouzar, 2009). Thus, drilling success is based on the type of drilling mud and important details derived from geological drill formations, on one hand, and the good drill-in reservoir conditions in the other hand (Kpoey, 2017). Thus, paramount drilling objectives are to reach the target safely in the shortest possible time and at the lowest possible cost (Khodja *et al.*, 2010; Talalay *et*

al., 2014). But, generally, xenobiotic components of drilling fluids have shown to have environmental impacts on aquatic organisms and thus, the necessity for a routine sublethal bioassay test.

Sublethal ecotoxicity testing is generally used to predict the toxicity of xenobiotics, such as Water Based Drilling Fluids in the environment. In most cases, toxicity testing with fish species is aimed at assessing the possible risk to similar species or other live forms in various ecosystems including aquatic ecosystem, determination of possible water quality criteria for regulatory purposes and to establish correlation with acute testing of other species. African catfish is one of the highly priced fishes in Africa and serves as a ready source of protein and other vital nutrients needed by man in nutrition. But these species are often contaminated by xenobiotic chemicals through bioaccumulation and magnification. Therefore, routine analysis of haematological and biochemical parameters in fishes exposed to Xenobiotics like Water Based Drilling Fluid in ecotoxicological bioassay is expected to enable toxicologists determine the physiological performance of fish exposed to Xenobiotics in the environment as well as establish contamination of aquatic food chain. Such studies also provide a guide for the diagnosis of many diseases in organisms and in evaluating responses to therapy in both animals and humans alike (Solomon & Okomoda, 2012).

Problem Statement

Nigeria, in the recent decades, specifically in the Niger Delta ecosystem, Oil and Gas (petroleum) exploration and production operations have been on the rise simply due to the current economic stay of Nigeria whose source of income is solely from Oil. Certainly, these operations by the Oil and Gas companies have exacerbated the destructive influence of human activities on the aquatic ecosystem within the area, thus resulting in chronic stress conditions with negative impact on aquatic lives (Oil, Gas and Petrochemical, 2003).

These exploration activities are basically made possible through extensive drilling activities using either water based or non-aqueous drilling fluids, thus generating large volumes of drilling wastes within the region (OGP, 2003). In the Niger Delta today, most drilling muds and their toxic wastes are left to the environment without adequate treatment and they eventually find their way into aquatic environment, and causing severe pollution impact on aquatic lives, including fish. Sometimes, these toxic wastes are left in temporary holding-pits during onshore drilling operations and even dumped directly into surrounding waters such as swamps, rivers and oceans during off-shore drilling (Ifeadi, Nwankwo, Ekaluo & Orubima, 1985). These unprofessional practices by the Oil and Gas companies operating within the Niger Delta has caused debilitating impacts on the ecosystem of the region, thereby destroying the ecosystem and fish farming in the area. This has led to reduction in aquaculture in the Niger Delta area on daily basis.

Today, the commonly cultured African catfish species; (*Heterobranchus)* in the Niger Delta is increasingly endangered by the negative impact of drilling operations and pollution within the region. These have often resulted in fish kills arising from the pollution of both aquatic and terrestrial environment in the Niger Delta area. Available literatures have also shown that drilling fluids and their wastes have caused a lot of effects to aquatic ecosystem such as fish mortality and smothering effects, resulting in oxygen depletion and increase in particle suspension due to oil drilling operations in the aquatic ecosystem (Saasen *et al.,* 2001; Wills, 2000).

Recent studies have shown that some of the water based mud (WBM) additives that were acceptable from an environmental point of view decades ago are now no longer acceptable for current and future drilling operations in environmentally sensitive areas (Sahar & Abouzar, 2009; Amanullah, 2007). The recent clamour by governments all over the world to improve on environmental laws to curb the menace of air pollution, clean water, hazardous waste disposal, as well as the control of occupational health and safety hazards have charted a new course for the petroleum industry to begin to re-evaluate all aspects of drilling and production. These changes have greatly affected drilling fluid additive choices. Thus, drilling fluid additives must not only perform and meet minimum specifications, but must also meet global environmental standards (Neff *et al*.,, 2000), which often leads to oxygen depletion as well as increased particles suspension in water mainly due to the release of Water Based Drilling Muds and their additives during oil drilling operations (Saasen *et al.,* 2001; Wills, 2000). Other debilitating effects include potential damage to aquatic invertebrate population and alteration in feeding and spawning grounds (Saasen *et al.,* 2001).

Water Based Drilling Fluids, their additives and wastes have also been reported in literature to have some negative impacts on man, some of which include skin irritation, contact dermatitis, coughing as well as nausea (Ezemonye *et al.,* 2008). But these effects are mainly dependent on the type, dosage and exposure duration of the chemical (Ezemonye *et al.,* 2008). Drilling muds and their additives get easily released into aquatic ecosystems in the Niger Delta area through accidental discharge, poor waste management and handling, as well as poor management of drill cuttings during the drilling process (Harayama *et al.,* 1999) and this has proven to be harmful to marine flora and fauna (Mojtahid *et al.,* 2006). The growth of flora and fauna are often impacted negatively, thus causing contaminations in the environment (Silva *et al.,* 2009; Paula *et al.,* 2009; Khangarot & Das, 2009).

Discharges of Oil Based Drilling muds have been reported to affect 750 m^2 of area from a disposal site in Congo and West Africa, while the formation of intertidal mudflats was impacted due to oil spillages (Mojtahid *et al.,* 2006; Morvan, 2004). These discharges have been reported to have both short and long-term adverse impacts on aquatic organisms and wildlife present near the shoreline (McCay *et al.,* 2004). All of these attendant effects is believed to have been caused by poor handling and management of drilling mud, its wastes, improper control of hazards due to lack of government guidelines and enforcement, as well as lack of strict adherence to safety standards by oil exploration and production companies during drilling operations.

In all of these challenges, the effect of toxic concentrations of WBDF containing Bentonite and Clay on the haematological and biochemical indices of *Heterobranchus longifilis* have not been effectively reported in the Niger Delta area, and there is also little or no studies been carried out to assess the environmental impact of these fluids on the ecosystem at the early stage of drilling in the Niger Delta. But the researcher believes that these impacts can be mitigated by carrying out effective bioassay testing to evaluate and study the toxicity of drilling fluids and their wastes to aquatic lives (biota), which is the core of this research, and in the end, proffer solutions on how to manage this environmental menace bedeviling the Niger Delta area of Nigeria.

Aim of the Study

The aim of this study is to investigate the Bentonite and Clay based drilling fluids effects on haematological parameters and metabolites in the muscle of *Heterobranchus longifilis*.

Specific Objectives

The research objectives are to determine the sublethal effects of bentonite and clay based drilling fluid on:

(i) Haematological parameters such as RBC, WBC, Platelets and Haemoglobin of juveniles of *Heterobranchus longifilis*.

(ii) Metabolites; Total protein (TP), Albumin, Urea and Creatinine in the muscle of juveniles of *Heterobranchus longifilis*.

MATERIALS AND METHOD

A completely randomized experimental design was applied with a total of thirty-five (35) healthy juveniles of *Heterobranchus longifilis* (African catfish) during the renewal sublethal bioassay test in this study. Exposed fish were obtained from Ikuligan Farm at Agudama Epie, Yenagoa, Bayelsa State, Nigeria and transported to the Department of Biology, Bayelsa Medical University, Bayelsa State, Nigeria for the experiment. ANADrill, a Water Based Drilling Fluid containing bentonite and clay was collected from Schlumberger Nig. Ltd, directly from a drilling site located close to Intel, Aba Road, Port Harcourt, Rivers State, Nigeria and ensured that the fluid was prepared by the drilling company and ready for use before collection.

Experimental fish in the laboratory was separated into ten (10) plastic aquaria with 30 L of water and acclimated for 21 days. A trial test was conducted after the acclimation period during which four (4) fish were randomly selected and exposed to four arbitral concentrations of toxicant for fourteen days, to enable the researcher obtain the definitive concentrations for the sublethal test. The method described by

Inyang (2008) was strictly followed. Following the trial test, four definitive concentrations were eventually prepared from the stock solution and presented in milligram per litre viz; 167 mg/L, 333 mg/L, 400 mg/L and 500 mg/L for the definitive test which lasted for 30 days. During the period of the sublethal test, experimental fish were fed with compounded fish feed at 11:00 hour, water while toxicant was renewed at 24 hours interval. Feeding was discontinued 24 hours to day of termination and equal light and dark condition under normal temperature was maintained throughout the experimental period.

At the end of the exposure period, fish were killed and a portion of muscle was collected, crushed and homogenized with 0.5 ml of perchloric acid. The solution was spun in a centrifuge for 15 minutes at 3000 rpm and the supernatant poured into labelled sample bottles before taken to the chemical laboratory, FMC, Yenagoa for the analysis of metabolites. For haematological analysis, blood samples were collected using a 5 ml syringe and hypothermic needle. The needle was placed towards the lower end of the abdomen. The blood was then poured immediately into EDTA bottles and covered to prevent blood clot and lysing of blood cells. These blood samples were then sent to the haematological unit of Federal Medical Centre, Yenagoa for blood cell analysis.

Statistical Analysis

The data gotten from this study was subjected to statistical analysis such as One-way Analysis of Variance (ANOVA) to determine significant differences between the measured parameters. Means and Standard Deviations were also calculated from the data derived from the analysis of the various experimental groups. A Post Hoc Test (Turkey HSD test) was also conducted to separate means between groups and determine their interrelatedness. All statistical analysis were done using the statistical package for social science SPSS version 20.8.

RESULTS

Table I: Activities of haematological indices of *Heterobranchus longifilis* exposed to bentonite and clay based drilling fluid for 30 days (mean ±SD).

Concentration Of WBDF (mg/L)	Red Blood Cell (RBC) g/L (10^{12})	White Blood Cell (WBC) g/L (10^9)	Platelet g/L (10^9)	Haemoglobin (Hb) g/dL
0.00	$2.25±0.01^a$	$203.00±2.45^{bc}$	$53.00±0.08^a$	$9.40±0.03^a$
167	$2.15±0.01^a$	$205.65±2.31^b$	$21.50±0.16^d$	$9.20±0.04^a$
333	$2.30±0.02^a$	$211.30±4.10^a$	$31.50±1.41^c$	$9.30±0.03^a$
400	$2.49±0.05^a$	$201.34±2.09^c$	$33.20±1.33^b$	$8.92±0.02^b$
500	$1.60±0.10^b$	$179.35±3.15^d$	$31.00±2.00^c$	$8.80±0.01^b$

All data are expressed as Mean ± Standard Deviation using the software programme Statistical Package for Social Sciences version 20. Different superscript indicated a significant variation ($p<0.05$) a*, b*, bc*, c*, d*.

Table 2: Activities of metabolites in the muscle of *Heterobranchus longifilis* exposed to bentonite and clay based drilling fluid for 30 days (mean ±SD).

Conc. of WBDF (mg/L)	T.P (g/L)	ALB (g/L)	UREA (mmol/L)	CREAT (µmol/L)
0.00	$2.00±0.8^a$	$1.20±0.0^a$	$0.38±0.2^a$	$489.25±177.2^c$
167	$2.00±8.0^a$	$1.75±0.5^a$	$0.20±0.1^a$	$555.00±221.0^b$
333	$2.5±1.3^a$	$1.75±0.5^a$	$0.18±0.1^a$	$401.5±121.4^d$
400	$2.0±1.4^a$	$1.69±0.4^a$	$0.21±0.1^a$	$413.2±145.5^{cd}$
500	$2.75±0.5^a$	$1.75±0.6^a$	$0.30±0.1^a$	$731.00±192.5^a$

All data are expressed as mean ± standard deviation using the software programme Statistical Package for Social Sciences version 20.8. Different superscript indicated a significant variation ($p<0.05$) a*, b*, c*, cd*, d*.

DISCUSSION

Haematological parameters have often been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health of fish and other animals. In Table 1 of this study, there was significant ($p<0.05$) fluctuation in all the values of haematological parameters of fish exposed to varying concentrations of WBDF (ANADrill) containing bentonite and clay in this study. The result reveal thus: Red Blood Cells (RBC) 2.15±0.01 g/L at 167 mg/L to 1.60±0.10 g/L at 500 mg/L compared to the control (2.25±0.01 g/L). White Blood Cell (WBC) also reduced viz; 205.65±2.31 g/L at 167 mg/L to 179.35±3.45 g/L at 500 mg/L compared to the control (203.00±2.45 g/L) respectively. This is in line with the report of Bakhtyar and Gagnon, (2012), who also recorded decline in the values of some haematological parameters after exposing fish to xenobiotics.

In the same vein, Platelet count recorded in this study also declined significantly ($p<0.05$) from 53.00±0.08 g/L (control) to 31.00±2.00 g/L at 500 mg/L compared to the control. Similarly, Haemoglobin count also significantly ($p<0.05$) declined thus; 9.20±0.01 g/L at 167 mg/L, to 8.80±0.01 g/dL at 500 mg/L compared to the control (9.40±0.03 g/dL) respectively. Lowering of haemoglobin count in freshwater fish Channa Punctatus after acute exposure to diazinon has also been reported by Arees, (1978).

The depreciation in the values of various blood parameters recorded in this study were observed to be non-dose dependent and may have interfered with the processes of neural transmission, blockage of ionic channels as well as inducing histopathological activities in fish. According to Begum, (2006), this effect could interfere with proper functioning of enzyme activities, which possibly might lead to hormonal changes or imbalances as well as inhibition effects. This result is similar to that of Omoniyi *et al.,* (2002). The authors reported that similar xenobiotic chemicals like tobacco leaf dust extracts used to determine the weight and haematological changes of *Clarias gariepinus* showed significant reduction in different haematological parameters such as haematocrit, haemoglobin number, leukocyte count, red blood cell (RBC) count, and mean corpuscular haemoglobin concentration etc.

The decline in haematological parameters measured in treated fish in this study may also infer that the primitive stem cells responsible for blood production in fish (*Heterobranchus longifilis*) would have been hampered due to the toxic effect of the toxicant. According to Dixon and Dick (1985), this could result in anaemia (low red blood cells) or leucopenia (low white blood cell) in exposed fish. In line with the results of this study is the report by Gabriel, *et al.,* (2009), who stated that reduction in haematological parameters in exposed organisms possibly indicates xenobiotic toxicity to some receptor enzymes in exposed fish. Hence, these changes may adversely impact upon and alter the normal blood cell physiology of the fish.

Thrombocytes (platelets) are nucleated cells responsible for blood clotting in fish and other animals. Therefore, the declining trend of Platelets in this study may possibly signify the effect of the toxicant on thrombocytes (platelets) formation by the primitive stem cells responsible for the production of blood. This correlates with the report of Adeyemo (2005), who stated that reduced effect of platelets in fish could lead to haemophilia (continuous bleeding) and possible death with time due to reduced clotting factors caused by the toxic effect of xenobiotic chemicals on thrombocytes of fish.

The decrease in the values of WBC as presented in this study is in sharp contrast with widely accepted belief, that WBC fight against foreign bodies, aided by phagocytosis to produce increased amount of antibodies. It is thus expected that its values would have increased as a result of the introduction of toxicant (antigen) or foreign body in exposed fish. The earlier exposure of fish to lower concentrations of toxicant (167 mg/L and 333 mg/L) indicated a slight increase in WBC number, which shows the defensive response of fish to the presence of the toxicant antigen (foreign body). However prolonged exposure of fish to WBDF at higher concentration (500 mg/L) revealed the toxic nature of bentonite and clay containing WBDF on the immune system of fish.

Thus, the initial appreciation in the values of WBC in exposed in this study is a clear indication showing the ability of host immune system to fight against foreign invasion when the need arises. But subsequent decline in WBC of exposed fish clearly shows that WBDF is toxic to WBC. Hence, the exposure of fish to WBDF may have caused a decline in the established immune response against a variety of antigenic substances, thus capable of causing immune depression. This result is similar to the findings of Shah and Altindag, (2005) as well as that of Inyang (2008), who also confirmed at different times in their research of depreciation in blood cells after exposing fish to xenobiotic chemicals.

These alterations in leukocyte values may manifest in the form of leukocytosis (heterophilia and lymphopenia), which are characteristics of leukocytic response in fish exhibiting stress. Red Blood Cell (RBC) is the number of red blood per cubic mm of blood. It is responsible for all transportation and circulation of materials and nutrients in the body, thus, are essential parameters in fish and suitable for evaluating the effects of chemicals (Patani *et.al.*, 2020).

The recorded decline in the values of RBC variables of exposed fish in this study also suggests that there was an osmotic disturbance and alteration in the oxygen carrying capacity of fish exposed to WBDF. This result is also similar to the reports by Banaee *et al.,* (2008) and Inyang (2008), who also recorded decrease in Red Blood Cell (RBC), Haemoglobin (Hb) and Haematocrit values of fish exposed to xenobiotics like Diazinon (an organophosphate pesticide) and related it to the destructive effects of toxicants to cells. Also, the decline in RBC due to increased concentration of WBDF can be attributed to the toxic effect of the toxicant on Haematopoietic Stem Cells (HSC) responsible for blood production. This effect could lead to reduced supply of RBC, either due to less production, increased rate of removal from circulation or rapid destruction due to effect of toxicant.

This may equally affect respiratory potentials of the fishes. The overall decrease in the values of haematological indices in this study may eventually lead to low oxygen consumption in fish (*Heterobranchus longifilis*) due to injury to RBC corpuscles by the toxicant following exposure. This reduction also could possibly result in hypochromic microcytic anaemia. This result is in line with the reports of Adeyemo (2005), Aderolu *et al.*, (2010), and Okomoda *et al*,.(2010). The authors at different times reported the toxic effect of glyphosate based herbicides on haematological parameters of fresh water fishes.

Haemoglobin is the chemical molecule responsible for the red pigmentation of blood. Its function is tremendous as it is responsible for carrying oxygen in the blood. From the results obtained in this study, there was a significant ($p<0.05$) fall in haemoglobin values in the blood of exposed fish. This decline may be as a result of the toxic effect of the toxicant on the synthesis of this molecule or due to low concentrations of toxicant used in this study. The current result possibly shows disruption in the synthetic pathway of the compound (haemoglobin) by affecting the activity of the enzymes involved in the synthesis of Haemoglobin, thus, consequently, reducing the level of Haemoglobin of exposed fish (*Heterobranchus longifilis)*.

In clinical examinations, reduction of Haemoglobin is of severe significance, knowing that the deficiency of Haemoglobin in the blood could possibly lead to low concentration of oxygen in the blood which is the primary cause of anaemia, suffocation and possible cardiac arrest in fish including animals. Similar findings to this result is the report of Gaafar *et al.*, (2010) who reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and electrolytes degeneration in erythrocytes.

Metabolites are major catabolic products of muscle activity and purine metabolism (Patani, *et al.*, 2020). The results as shown in Table 2 of this study reveals that the values of Total Protein (TP), Albumin (ALB) and Urea (UREA) in the muscles of exposed fish in the treatment groups compared to the control were not significant ($p>0.05$), except for creatinine values that significantly appreciated at the highest concentration compared to the control. This point to the fact that, the toxicant is less toxic to metabolites in the muscle or that it could be as a result of the low concentration of toxicant used in this study. But Creatinine values appreciated significantly ($p<0.05$) in the muscle of treated fish in this study viz; 555.00±221.0 µmol/L at 167 mg/L, 401.5±121.4 µmol/L at 333 mg/L, 413.2±145.5 µmol/L at 400 mg/L and 731.00±192.5 µmol/L at 500 mg/L compared to the control (489.25±177.2 µmol/L) respectively. Similar to this result was reported by Patani *et al.*, (2020), the authors reported slight appreciation in the value of some metabolites like cortisol in the brain of Rabbits exposed to sublethal concentrations of Chlopyrifos.

The recorded appreciation in the values of Creatinine in the muscle of exposed fish in this study could be attributed to the potential toxic effect of the toxicant on some metabolic indices in exposed fish. Creatinine is a waste product of muscle activity in fish and other animals, filtered by the kidney from the blood and excreted through the urine of fish. The high value of Creatinine, a product of Creatine phosphate in muscle is also an indication of increased muscle mass. Thus, the recorded appreciation of Creatinine in the muscle of exposed fish possibly, indicate increased activity of muscle mass due to increased protein metabolism in exposed fish induced by the toxicant.

CONCLUSION

The results of this investigation confirm the sublethal toxicity of the Water Based Drilling Fluid (ANADrill), containing Bentonite and Clay on haematological parameters and some metabolites in the muscle of exposed juveniles of African catfish (*Heterobranchus longifilis*). The use of Bentonite and Clay based drilling fluids for oil drilling and exploration should be done with caution and strictly regulated with continuous environmental monitoring. Also, Water Based Drilling Fluid (ANADrill) containing Bentonite and Clay should be used in a safe manner around rivers, oceans, lakes, swamps and even flood prone environments to avoid contamination and pollution of the aquatic food chain in the Niger Delta area of Nigeria.

To curb the menace of Water Based Drilling Fluids (ANADrill) on fish, other non-target aquatic organisms and the environment, it necessary that further studies be carried out on the toxicity of drilling fluids to the environment and constant industry and organization awareness campaigns be done in the Nigerian Oil and Gas industry on the toxic effects of drilling fluids, their waste as well as safety measures to be adopted with regards to handling, storage, treatment and disposal of drilling wastes. It is also imperative that proper waste management methods be employed to prevent excessive runoff of these fluids and wastes into water bodies like ponds, lakes and rivers. This is expedient because of the tendencies of bioaccumulation, resulting in fish contamination. Fish killed around exploration and drilling sites should not be used as source of food almost immediately, but should be allowed to stay in fresh water for a period of time before eaten. This will allow decontamination of accumulated poisons in exposed fish and prevent human food poisoning.

Ethical Issues

The authors are all aware of ethical issues as stipulated by the laws of the land and thus completely complied with best practices while carrying out this research. As regard authorship, everything was done to avoid competing interests, compliance with policies on research ethics as well as dual submission research findings in other journals. Authors therefore strictly adhered to the conditions of publication and attest that this research is authentic and have not been published elsewhere in any form.

Competing Interest

Authors declare that there is no conflict of interest that would jeopardize the authenticity and originality of this scientific manuscript.

Authors Contribution

All authors of this research made equal imputes both for data collection, data analyses and manuscript writing.

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