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Effect Of Anadrill Containing Bentonite And Clay On Electrolytes And Metabolites In The Liver And Kidney Of *Heterobranchus longifilis*

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ABSTRACT

Water Based Drilling Fluids assist in the oil drilling process, commonly used within the Niger Delta of Nigeria, but not without adverse impacts on the aquatic food chain. A randomized renewal bioassay was conducted using 35 juveniles of *Heterobranchus longifilis* in this study. Fish were acclimated for 21 days, and divided into control and treatment groups. Treatment groups were exposed to 167 mg/L, 333 mg/L, 400 mg/L and 500 mg/L of toxicant following a trial test for 30 days, while maintaining a negative control. At the end of the exposure period, fish were killed and liver and kidney collected, crushed and homogenized with 0.5 ml of normal saline, perchloric acid and deionized water. The solutions were spun in a centrifuge for 15 minutes at 3000 rpm before taken to the chemical laboratory, FMC, Yenagoa for the analysis of electrolytes and metabolites. Electrolytes such as Na^+, and Cl^- in the Liver appreciated significantly ($p<0.05$), while K^+ declined, whereas Mg^{2+} was not significant ($p>0.05$). In the kidney, Na^+ appreciated significantly ($p<0.05$), Cl^- declined, while K^+ and Mg^{2+} values were not significant ($p>0.05$). The values of Total Protein, Albumin and Urea in the Liver and Kidney were not significant ($p>0.05$). Though, Creatinine values significantly ($p<0.05$) declined in the Kidney, it was not significant ($p>0.05$) in the liver. The sublethal test results confirm the toxicity of WBDF on metabolic and electrolyte parameters of juveniles of exposed fish thus; WBDF should be used cautiously under strict regulation with continuous environmental monitoring.

Keywords: Sublethal, Bentonite, Clay, Drilling Fluid.

INTRODUCTION

ANADrill is a common Water Based Drilling Fluid (WBDF) used for the exploration and drilling of oil wells. Bentonite and Clay, additives of the above drilling fluid assist in the process of oil well drilling. In the last few decades in Nigeria, most 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 volume of drilling waste (OGP, 2003). Drilling fluids are any fluids which are circulated through a well in order to remove cuttings from a wellbore (Kpoey, 2017). Petroleum drilling is the primordial step in the success of oilfield exploration

(Mohamed *et al.,* 2019). This success is based, on the one hand, on the important details derived from geological drill formations and, on the other hand, on the good drill-in reservoir conditions (Kpoey, 2017). Thus, paramount drilling objectives are to reach the target safely in the shortest possible time and at the lowest possible cost, with required additional sampling and evaluation constraints dictated by particular application (Khodja *et al.,* 2010; Talalay *et al.,* 2014).

Drilling of oil wells occurs by using drilling fluids to remove rock fragments as the drilling progresses (Cardoso *et al.,* 2010). Traditionally, drilling muds are classified according to the base used to prepare them, which are air, water or oil (Lucas *et al.,* 2009). Most drilling operations in the world use oil-based fluids due to their low toxicity. Nevertheless, these have some disadvantages that can be overcome by the use of water-based fluids (Cardoso *et al.,* 2010). Drilling fluids which are circulated through a well in order to remove cuttings from a wellbore intends to improve fluid rheological properties and filtration capability, allowing penetration into heterogeneous geological formations under the best conditions (Talalay *et al.,* 2014; Chemwotei, 2011).

Currently in the Niger Delta and beyond, food security has become a complex issue, cutting across virtually all aspects of agricultural practice, including crop production, livestock breeding and fish farming or aquaculture (Vincent-Akpu and Sikoki, 2013), which is the main economic stay of the Niger Delta people. Thus, fish and fishery products have been generally regarded as high risk commodities due to possible bioaccumulation and contamination by drilling fluids and its wastes as a result of increasing and unguarded oil exploration activities in the area. These activities have led to reduction in aquaculture in the Niger Delta area, incessant fish kills and death as well as environmental pollution almost on daily basis. Today, the commonly reared African catfish species; (*Heterobranchus)* is going extinct due to the impact of drilling operations and pollution within the region.

Aim of the Study

This study is aimed at evaluating the sublethal toxicity of ANADrill, a water based drilling fluid containing Bentonite and Clay on juveniles of African Catfish (*Heterobranchus longifilis*).

Specific Objectives

The objectives of this study are to determine the sublethal toxicity of Water Based Drilling Fluid (ANADrill), containing bentonite and clay on:

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

(ii) Electrolytes; Sodium (Na^+), Potassium (K^+), Chloride (Cl^-) and Magnesium (Mg^{2+}) ions in the Liver and Kidney 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) (mean weight 220.23±34.12 SD) during the renewal sublethal bioassay test in this study. Experimental fish were obtained from Ikuligan Farm at Agudama Epie, within Yenagoa metropolis of Bayelsa State, Nigeria and transported in 50 L plastic cans covered with fishing nets to the wet laboratory of the Department of Biological Sciences, Faculty of Science, Bayelsa Medical University, Bayelsa State, Nigeria for the experiment. ANADrill, a Water Based Drilling Fluid was collected from Schlumberger Nig. Ltd, directly from a drilling site located close to Intel, Aba Road, Portharcourt, River State, Nigeria and ensured that the fluid was prepared according to prescribed standard for use by the company before collection.

At the wet laboratory, fish were separated into ten (10) plastic aquaria with 30 L of water and acclimated for 21 days. A range finder (trial test) was conducted after the acclimation period where four (4) fish were randomly picked and exposed to four arbitral concentrations of toxicant which lasted for two weeks (14 days). This is to enable the researcher obtain the definitive concentrations needed for the definitive test. Following the trial test as described by Inyang (2008), four definitive concentrations were obtained from the stock solution viz; 167 mg/L, 333 mg/L, 400 mg/L and 500 mg/L for the definitive test which lasted for 30 days, while maintaining a negative control. Throughout the experimental period, fish were fed with compounded fish feed at 11:00 hour, water and chemical was renewed with the plastic aquaria cleaned with foam at 24 hours interval. Feeding was discontinued 24 hours before the termination day while maintaining equal light and dark condition under normal temperature. At the end of the exposure period, fish were killed and liver and kidney collected, crushed and homogenized with 0.5 ml of perchloric acid and deionized water. The solutions were spun in a centrifuge for 15 minutes at 3000 rpm before taken to the chemical laboratory, FMC, Yenagoa for the analysis of electrolytes and metabolites.

Statistical Analysis

The data gotten from the 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.

RESULTS

Table 1: Relative activities of electrolytes in the Liver of *Heterobranchus longifilis* exposed to ANADrill for 30 days (mean ±SD).

Conc. of ANADrill (mg/L)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Mg^{2+} (mmol/L)
0.00	10.55 ± 7.0^c	9.75 ± 2.3^c	8.75 ± 6.0^c	0.80 ± 0.1^a
167	47.65 ± 41.3^a	17.05 ± 6.9^a	40.53 ± 36.4^a	0.67 ± 0.5^a
333	2.78 ± 2.3^d	14.43 ± 5.0^b	8.53 ± 4.4^c	0.76 ± 0.2^a
400	$3.55 \pm 2.2b^{cd}$	10.45 ± 3.0^{cb}	7.23 ± 5.3^d	0.69 ± 0.4^a
500	14.83 ± 7.0^b	7.90 ± 5.2^d	11.28 ± 1.3^b	0.50 ± 0.4^a

All data are expressed as mean ± standard deviation using the software programme Statistical Package for Social Sciences version 20. Different superscript indicate a significant variation ($p<0.05$) a^*, b*, c*, d*.

Table 2: Relative activities of metabolites in the Liver of *Heterobranchus longifilis* exposed to ANADrill for 30 days (mean ±SD).

Conc. of ANADrill (mg/L)	T.P (g/L)	ALB (g/L)	UREA (mmol/L)	CREAT (μmol/L)
0.00	2.50 ± 0.5^a	1.50 ± 0.6^a	0.13 ± 0.1^a	45.25 ± 12.4^b
167	2.25 ± 1.0^a	1.25 ± 9.5^a	0.35 ± 0.4^a	54.25 ± 0.1^a
333	2.75 ± 0.5^a	1.25 ± 0.5^a	0.90 ± 0.1^a	23.50 ± 11.2^d
400	2.97 ± 0.2^a	1.32 ± 0.3^a	0.93 ± 0.2^a	32.41 ± 9.5^c
500	2.75 ± 1.0^a	1.50 ± 0.6^a	0.15 ± 0.9^a	45.5 ± 42.3^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*, c*, d*.

Table 3: Relative activities of electrolytes in the Kidney of *Heterobranchus longifilis* exposed to ANADrill for 30 days (mean ±SD).

Conc. of ANADrill (mg/L)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Mg^{2+} (mmol/L)
0.00	5.88 ± 2.8^d	4.13 ± 1.4^b	10.63 ± 3.9^a	0.53 ± 0.1^a
167	2.25 ± 1.2^e	6.60 ± 1.5^a	10.53 ± 3.3^a	0.73 ± 0.1^a
333	13.85 ± 9.7^c	2.45 ± 2.8^d	7.78 ± 7.3^b	0.49 ± 0.2^a
400	15.45 ± 7.9^b	3.23 ± 1.9^c	6.87 ± 3.2^{bc}	0.42 ± 0.3^a
500	26.75 ± 22.7^a	4.28 ± 2.1^b	5.10 ± 1.8^c	0.32 ± 0.2^a

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*, c*, d*.

Table 4: Relative activities of metabolites in the kidney of *Heterobranchus longifilis* exposed to ANADrill for 30 days (mean ±SD).

Conc. of ANADrill (mg/L)	T.P (g/L)	ALB (g/L)	UREA (mmol/L)	CREAT (μmol/L)
0.00	2.50 ± 1.3^{a}	1.75 ± 1.0^{a}	0.40 ± 0.3^{a}	49.75 ± 7.9^{a}
167	2.5 ± 0.6^{a}	1.25 ± 0.5^{a}	0.15 ± 0.1^{a}	26.75 ± 8.0^{d}
333	2.25 ± 0.5^{a}	1.50 ± 1.0^{a}	0.30 ± 0.2^{a}	44.00 ± 3.6^{b}
400	2.12 ± 0.8^{a}	1.15 ± 0.3^{a}	0.23 ± 0.5^{a}	42.05 ± 5.3^{bc}
500	2.5 ± 1.3^{a}	1.25 ± 0.5^{a}	0.63 ± 0.4^{a}	39.25 ± 9.0^{c}

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*, c*, d*.

DISCUSSIONS

From the results presented above, the value of Na^+ ion in the liver of fish at first treatment (167 mg/L) with WBDF recorded a significant ($p<0.05$) appreciation to 47.65±41.3 mmol/L. But further increase in concentrations of toxicant to 333 mg/L and 400 mg/L resulted in a significant ($p<0.05$) decline of its values to 2.78±2.3 mmol/L and 3.55±2.2 mmol/L compared to the control (10.55±7.0 mmol/L) respectively. This report is in tune with the findings of Inyang *et al.,* (2010). The authors also reported similar results of alterations in the values of liver electrolytes after exposing *Clarias gariepinus* to sublethal concentrations of diazinon for 30 days. At the highest treatment concentration (500 mg/L), there was a significant increase (14.83±7.0 mmol/L) in the value of Na^+ ion in the liver compared to the control (10.55±7.0 mmol/L). The recorded decline in the value of sodium ion at lower concentrations (333 mg/L and 400 mg/L) and sudden appreciation at the highest concentration (500 mg/L) compared to the control is an indication of the toxicity of WBDF to electrolytes in fish. This finding is in consonance with the report of Ogamba *et al.*, (2011) that also reported alterations in the values of Na^+ and K^+ in the liver and muscle of *Clarias gariepinus* exposed to Paraquat. This finding is similar to the report of Patani *et al.*, (2020) that also reported alterations in electrolyte levels in probe rabbits exposed to varying concentrations of Chlorpyrifos.

Potassium (K^+) ion values recorded in the liver of treated fish at highest concentration of toxicant (500 mg/L) fluctuated to 7.90±5.2 mmol/L compared to the control (9.75±2.3 mmol/L). Though there was an initial appreciation in its values viz; 17.05±6.9 mmol/L at 167 mg/L, 14.43±5.0 at 333 mg/L and 10.45±3.0 mmol/L at 400 mg/L compared to the control (9.75±2.3 mmol/L). While that of Chloride (Cl^-) measured in the liver of fish at first intoxication (167 mg/L) with WBDF appreciated to 40.53±36.4 mmo/L, but further increase in the concentration of toxicant (333 mg/L and 400 mg/L) resulted in a significant decline ($p<0.05$) in its values to 8.53±4.4 mmol/L and 7.23±5.3 mmol/L. But the value of Cl^- slightly appreciated to 11.28±1.3 mmol/L at 500 mg/L compared to the control (8.75±6.0 mmol/L). On the other hand, there was no significant ($p>0.05$) difference in the value of Magnesium ion (Mg^{2+}) measured in the liver of fish treated with WBDF and the control. This possibly indicate the fact that WBDF is less toxic to magnesium ions or that it is due to the low concentration of toxicant used in this study. The result so far indicates the toxic effect of WBDF to liver electrolytes (Na^+, K^+ and Cl^-) and consequently its impact on the metabolic function of the liver.

Electrolytes such as Na^+ ion recorded in the kidney declined to 2.25±1.2 mmol/L on first treatment of fish with toxicant (167 mg/L) compared to the control (5.88±2.8 mmol/L. This decline in the value of Na^+ ion is significant with toxicant effect on electrolytes in exposed fish occasioned by induced stress of toxicant. But as the concentration increased, there was a significant ($p<0.05$) appreciation in Na^+ ion values in the treatment group to 13.85±9.7 mmol/L at 333 mg/L, 15.45±7.9 mmol/L at 400 mg/L and 26.75±22.7 mmol/L at 500 mg/L compared to the control (5.88±2.8 mmol/L) respectively. The value of chloride (Cl^-) ion measured in the kidney of exposed fish also declined from 10.53±3.3 mmol/L at 167 mg/L to 7.78±7.3 mmol/L at 333 mg/L, 6.87±3.2 mmol/L at 400 mg/L and 5.10±1.8 mmol/L at 500 mg/L compared to the control (10.63±3.9 mmol/L). The declining trend of chloride ion recorded across the treatment groups compared to the control appears to be dose dependent. While values of magnesium (Mg^{2+}) and potassium (K^+) recorded in the kidney compared to the control of this study was not significant ($p>0.05$).

Though, an initial appreciation (6.60±1.5 mmol/L) in the value of K^+ ion was recorded in the kidney at first treatment (167 mg/L) of fish with toxicant compared to the control (4.13±1.4 mmol/L), at the second and third treatments of fish with toxicant, a significant ($p<0.05$) decline in K^+ values was recorded in the kidney viz; 2.45±2.8 mmol/L at 333 mg/L and 3.23±1.9 mmol/L at 400 mg/L and later stabilized to 4.28±2.1 mmol/L at the highest concentration (500 mg/L) compared to the control (4.13±1.4 mmol/L) respectively. The appearance of electrolyte stabilization recorded in this study may be as a result of low concentrations of toxicant used in this study or that the toxicant is less toxic to electrolytes such as Magnesium and Potassium ions in the kidney of exposed fish. Whereas, the variance observed in the values of Sodium (Na^+) and Chloride (Cl^-) ions in the kidney as shown in the results of this study revealed the toxic effect of ANADrill (toxicant) to some electrolytes in the kidney, thus could cause dysfunction in osmoregulation and ultra-filtration activities as well as nephrotoxicity in treated fish.

As presented in the tables above, the values of Total Protein, Albumin, Urea and Creatinine in the liver of toxicant treated fish compared to the control are not significantly different ($p>0.05$). This could be an indication that the toxicant (ANADrill) is less toxic to liver metabolites or that it is as a result of the low concentration of toxicant used in this study. The values of Total Protein, Albumin and Urea recorded in the kidney of the treatment group in this study were not also significantly ($p>0.05$) different from the control group, but on the contrary, a sharp decline in the values of Creatinine (CREAT) was recorded in the kidney of toxicant treated fish compared to the control. Related report was also published by Patani et al., (2020) when they studied the xenobiotic effect of 2,4-Dimethylamoine salt (720 g/L) on electrolytes and metabolites in New Zealand Rabbits (*Oryclotagus cuniculus*).

Creatinine values depreciated irregularly viz; 26.75±8.0 µmol/L at 167 mg/L, 44.00±3.6 µmol/L at 333 mg/L, 42.05±5.3 µmol/L at 400 mg/L before declining further to 39.25±9.0 µmol/L at 500 mg/L compared to the control (49.75±7.9 µmol/L) respectively. The depreciation in the values of Creatinine recorded in the Kidney of exposed fish in this study could be attributed to the toxic effect of the toxicant on some kidney metabolites, which can eventually lead to renal dysfunction in exposed fish. This could also be a sign of toxicant effect on renal function of the kidney towards regulating the amount of metabolites as well as low protein metabolism in the kidney of exposed fish.

CONCLUSION

The findings of this research reveal the toxicity of ANADrill (a water based drilling fluid) containing bentonite and clay on metabolite and electrolyte parameters in the liver and kidney of juveniles of African catfish (*Heterobranchus longifilis*). Thus; its use for exploration purposes in the Niger Delta ecosystem should be done with caution and strictly regulated with continuous environmental monitoring.

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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