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Full Length Research Paper

Haematological studies of *Oreochromis niloticus* exposed to diesel and drilling fluid in Lagos, Nigeria

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Haematological changes in *Oreochromis niloticus* after exposure to diesel (23.4 mg/L) and drilling fluid (492 mg/L) for 28 days was investigated. The blood parameters of O. *niloticus* revealed a significant decrease in haemoglobin only in fish exposed to drilling fluid and mean cellular volume (MCV), in both diesel and drilling fluid. Blood parameters of the fish such as mean cellular haemoglobin (MCH) and mean cellular haemoglobin concentration (MCHC) increased significantly in both treatments. Significant increase in thrombocytes was also observed although only in fishes exposed to diesel. Diesel and drilling fluid evoked significant changes in the haematological parameters of *O. niloticus*, a fish that has great potentials in the culture fishery of Nigeria.

Key words: Haematology, *Oreochromis niloticus,* diesel, drilling fluid, Nigeria,

INTRODUCTION

Intense activity in oil and gas industrial sector has inevitably increased human destructive influence on the aquatic environment resulting in chronic stress conditions that have negative impact on aquatic life (Mason, 1991). Stress response is characterized by physiological changes and the effect of pollutants on fish is assessed by acute and chronic toxicity tests (Health, 1991). The stress caused by environmental pollution, changes the structure of red and white blood cells (Larsson et al., 1984). Haematological techniques are the most common method to determine the sub-lethal effects of the pollutants (Larsson et al., 1985).

Haematological studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture and greater awareness of the pollution of natural freshwater resources in the tropics. Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Iwama et al., 1976; Chekrabarty and Banerjee, 1988). The count of red blood cells is quite a stable index and the fish body tries to maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation.

In recent years, haematological variables were used more when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances as a result of the close association between the circulatory system and the external environment (Chech et al., 1996; Wendelaar, 1997). As an indicator of pollution, blood parameters are used in order to diagnose and describe the general health condition of fish species following different stress conditions like exposure to pollutants, diseases, metals, hypoxia, e.t.c. (Blaxhall, 1972; Duthie and Tort, 1985). Besides, this type of index reflects certain ecological changes in the environment (Roche and Boge, 1996).

Studies have shown that when the water quality is affected by toxicants, any physiological change will be reflected in the values of one or more of the haematological parameters (Van, 1986). Blood cell responses are important indicators of changes in the internal and/or external environment of animals. In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels. Their changes depend on fish species, age the cycle of the sexual maturity of spawners and diseases (Golovina, 1996; Luskova, 1997). Like in warm blooded animals, changes in the blood parameters of fish, which occur because of injuries of the latter organs or tissues, can be used to determine and confirm the dysfunction of the organs or tissues. However in the fish, these parameters are more related to the response of the whole organism, that is, to the effect on fish survival, reproduction and growth. It

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should be noted that although the mechanisms of fish physiology and biochemical reaction to xenobiotics has not been investigated enough, it is obvious that species differences of these mechanisms exist.

Fish live in very intimate contact with their environment, and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components (Wilson and Taylor, 1993). Thus, water quality is one of the major factors, responsible for individual variations in fish haematology.

Oreochromis niloticus (Nile tilapia) the most widely cultured species of tilapia in Africa is a good fish for warm water aquaculture. They are easily spawned; used as wide variety of natural foods (plankton, aquatic macrophytes, detritus, planktonic and benthic invertebrates and decomposed organic matter) as well as artificial feeds. They tolerate poor water quality, and grow rapidly in warm temperatures. These attributes, along with relatively low input costs, have made it the most widely cultured freshwater fish in tropical and subtropical countries.

This present study was undertaken to establish effect of sub-lethal concentration of diesel and drilling fluid on the haematological parameters of *O. niloticus*, a fresh water fish very important in the capture and culture fishery of Nigeria.

MATERIALS AND METHODS

Fish samples

Live specimens of *O. niloticus* were obtained from a local fishpond in Lagos State, Nigeria and transported in aerated containers to the laboratory. They were acclimatized to laboratory conditions for a minimum period of 2 weeks during which they were fed with artificial (commercial) feed and ground shrimps obtained locally to avoid starvation. The size of the fish varied from 8.00 to 10.00 cm in length and 25.00 to 30.00 g in weight. All sexes were used without discrimination. The natural photoperiod (12 h daylight and 12 h of darkness) was maintained during the acclimatization and experiment periods. The fish were fed before and during the period of the experiment, which lasted for 28 days.

Water quality of tilapia culture pond

The fish pond water was light green in color due to presence of algae and pH 7.12, salinity 0.01 ppt , conductivity 31.65 μScm^{-1}, dissolved oxygen 7.5 mg/L, total dissolved solids, 160 mg/L, total suspended solids 0.01 mg/L, biological oxygen demand (BOD) 5.2 mg/L and oil and grease 0.04 mg/L were recorded.

Exposure to sub-lethal concentration

This was conducted by exposing fish species to sub-lethal concentrations extrapolated from results of acute toxicity test. Twenty test organisms, *O. niloticus* were exposed to sub-lethal concentrations (492.0 mg/L and 23.4 mg/L of drilling fluid and diesel, respectively) for a period of 28 days in the laboratory.

Blood collection

Every 7 days, five (5) organisms were harvested from each test tank containing sub-lethal concentration of drilling fluid and diesel; also five organisms were taken from the control tank which contained no test chemical. The fishes were then placed belly upwards and blood samples obtained from the caudal vein with the aid of a heparinised 2 cm^3 disposable plastic syringe and a 21 gauge disposable hypodermic needle. The use of plastic syringe is a necessary precaution with fish blood because contact with glass results in decreased coagulation time (Smith et al., 1952). The site chosen for puncture (about 3 to 4 cm from the genital opening) was wiped dry with tissue paper to avoid contamination with mucus. The needle was inserted at right angle to the vertebral column of the fish and was gently aspirated during penetration. It was then pushed gently down until blood started to enter as the needle punctured a caudal blood vessel. Blood was taken under aspiration until about 1 ml was obtained from each of the five harvested fishes. Thereafter, the needle was withdrawn and the blood pooled together, thoroughly mixed and gently transferred into special tubes containing potassium EDTA as an anticoagulant agent to make 5 ml.

Blood analysis

Haematological parameters were measured at a maximum of 3 h after blood samples were taken. An Automated Sysmex Counter (KX - 21N model) was used to count RBC (Red Blood Cells), HGB (Haemoglobin), HCT (Haematocrit), MCV (Mean Cellular Volume), MCH (Mean Cellular Haemoglobin Concentration) and PLT (Trombocyte). The instrument only aspirated approximately 0.2 µl of each blood sample. In this study leucocytes, WBC (White Blood Cells) counting were ignored since fish erythrocytes have nuclei and because of the concern that they can be counted at the same time with erythrocytes RBC (Red Blood Cells).

The analysis of variance (ANOVA) was used to determine the significance of the blood parameters in *O. niloticus*. Differences were considered significant at $p < 0.05$ (Zar, 1996)

RESULTS AND DISCUSSION

The red blood cell, haemoglobin, haematocrit, platelet count and derived erythrocyte indices (MCV, MCH and MCHC) of *O. niloticus* in control and those exposed to drilling fluid and diesel are as shown in Figures 1 – 7.

In this study, the concentration of haemoglobin in the red blood cells were much lower in the exposed fishes to drilling fluid and diesel than in the control fish, thereby depicting an anaemic condition. This study revealed that haemoglobin decreased in both treatments compared to the control and considerably only in fishes exposed to drilling fluid. The decrease in haemoglobin was significant ($p < 0.05$) only in fish exposed to drilling fluid ($p = 0.001$). Buckley et al. (1976) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and degeneration of the erythrocytes could be ascribed as pathological conditions in fishes exposed to toxicants.

The MCV fluctuated and decreased at the 28 days in fish exposed to both treatments (drilling fluid and diesel) when compared to the control. The decrease in MCV over 28 day period was significant ($p < 0.05$) in treatments, drilling fluid and diesel ($p = 0.00025$ and 0.00115, respectively), while increase in MCH and MCHC recorded by both treatments was significant ($p < 0.05$)

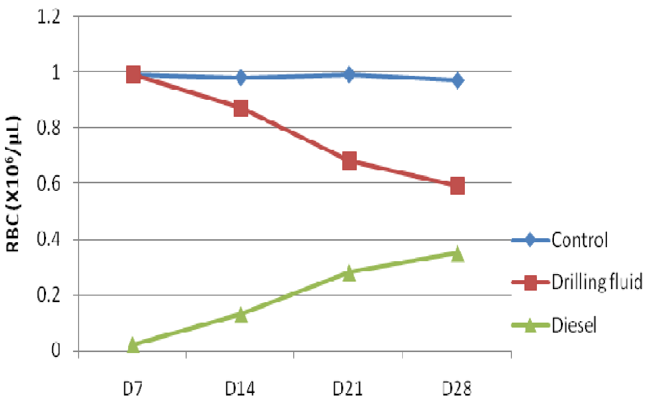

Figure 1. Red blood cells ($x10^6$ µL) of *O. niloticus* in control and those expose to drilling fluid and diesel over a 28-days period under laboratory conditions.

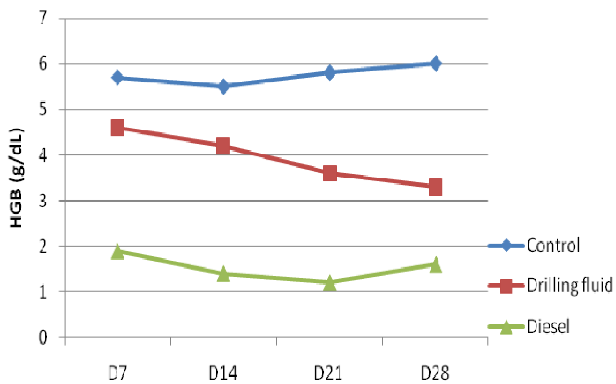

Figure 2. Haemoglobin concentration (g/dl) of *O. niloticus* in control and those expose to drilling fluid and diesel over a 28-days period under laboratory conditions.

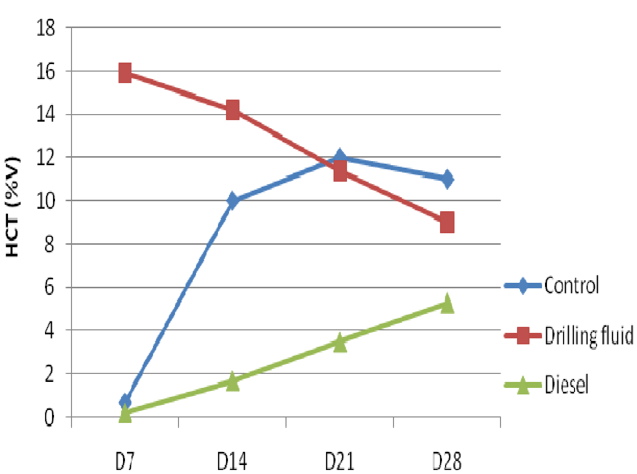

Figure 3. Haematocrit volume (%V) of *O. niloticus* in control and those expose to drilling fluid and diesel over a 28-days period under laboratory conditions.

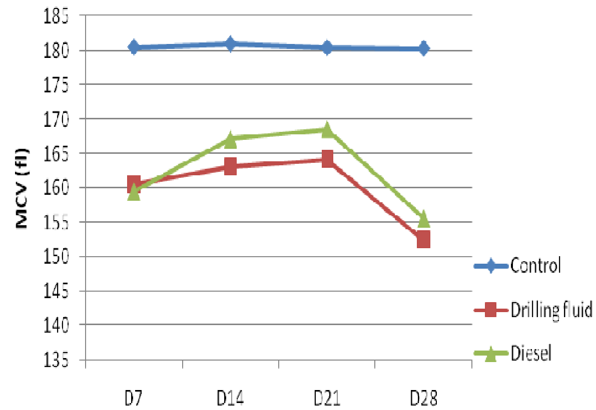

Figure 4. Mean corpuscular volume (fl) of *O. niloticus* in control and those expose to drilling fluid and diesel over a 28-days period under laboratory conditions.

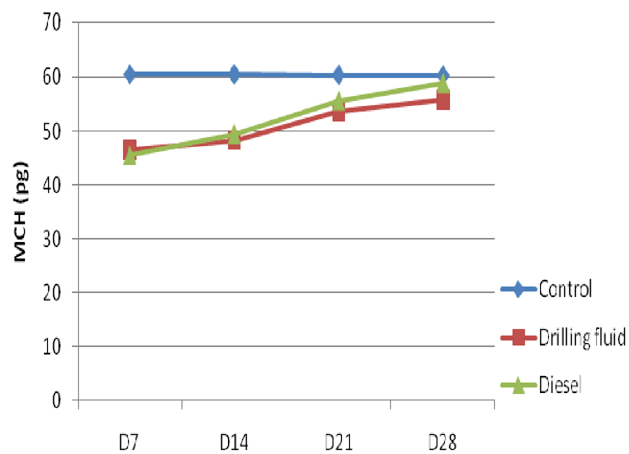

Figure 5. Mean concentration of haemoglobin (pg) of *O. niloticus* in control and those expose to drilling fluid and diesel over a 28-days period under laboratory conditions.

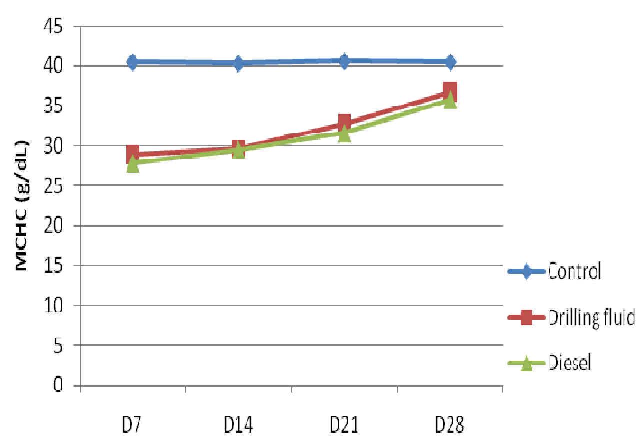

Figure 6. Mean corpuscular haemoglobin concentration (g/dl) of *O. niloticus* in control and those expose to drilling fluid and diesel over a 28-days period under laboratory conditions.

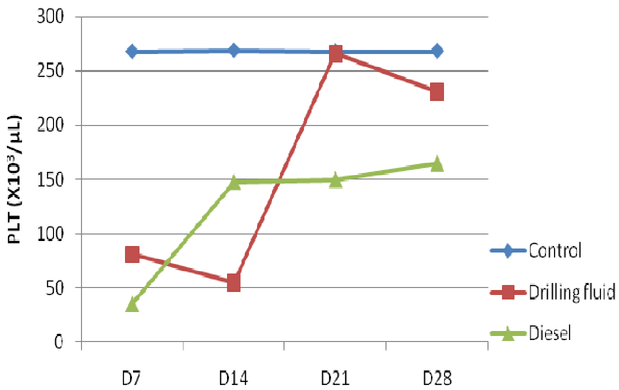

Figure 7. Platelets count (x10^3/µl) of *O. niloticus* in control and those expose to drilling fluid and diesel over a 28-days period under laboratory conditions.

compared to the control (p = 0.0056 and 0.0366) and (p = 0.0031 and 0.0017), respectively. These alterations were attributed to direct or feedback responses of structural damage to red blood cells membranes resulting in haemolysis and impairment in haemoglobin synthesis and stress-related release of red blood cells from the spleen and hypoxia, induced by exposure to toxicant (Shah, 2006).

Thrombocytes are compared to mammal blood platelets and play an important role in the blood clotting which prevents blood loss from hemorrhaging. The thrombocytes increased in fish exposed to both drilling fluid and diesel treatments. The increase in thrombocytes were significant ($p < 0.05$) and only in fishes exposed to diesel (p = 0.0031). Haematology is used as an index of fish health status in number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia, etc (Blaxhall, 1972; Duthie and Tort, 1985). Previous studies have shown that when the water quality is affected by toxicants, physiological changes will be reflected in the values of one or more of the haematological parameters (Van, 1986), the same trend was observed in this study. Water quality can thus be regarded as one of the major factors responsible for individual variations in fish haematology.

CONCLUSION

This study has shown that:

1. Drilling fluid alone significantly lowered the haemoglobin of *O. niloticus*, while both diesel and drilling fluid lowered significantly the mean cellular volume of the fish.
2. Both drilling fluid and diesel significantly increased the mean cellular haemoglobin (MCH) and mean cellular haemoglobin concentration (MCHC) of *O. niloticus*, while diesel alone significantly increased thrombocytes in the fish.

This study has shown that physical and chemical changes induced by diesel and drilling fluid in the environment are reflected in the haematology of the fish. The blood physiology of *O. niloticus* is therefore a useful tool in determining the effects of external stressors and toxic substances in its environment.

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