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Hematological changes of *Oreochromis niloticus* (Linne 1757) juveniles exposed to kiln(R)

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Abstract
This research was done to find out the sub lethal effects of detergent kiln® on *Oreochromis niloticus* juveniles due to the contamination of fresh waters with a wide range of pollutants. The Nile Tilapia, *Oreochromis niloticus* was exposed to linear alkyl benzene sulphonate (kiln (R)) for 56 days after obtaining the LC_{50}-96 which was 22.26mg/l. Fractions of 11, 1.5 and 1.9mg/l for *Oreochromis niloticus* representing 1/20, 1/15 and 1/12 respectively of these LC_{50} values were used for the 56day chronic exposure study following a static renewal protocol so as to monitor its effects on the fish's haematology. Blood were collected after 56 days by randomly selecting fish from the various treatments and inserting a 2mm needle and syringe through the dorsal aorta puncture and placed in ethylene-diamine-tetra-acetic-acid (EDTA) treated bottles to prevent coagulation. The hematological parameters estimated include haemoglobin, mean cell haemoglobin, mean cell haemoglobin concentration, packed cell volume, mean cell volume, Red blood cell count sand white and red blood cell counts. Results showed that the PCV, WBC, RBC, MCH, MCHC, and Hb were significantly different and decreases with increasing concentration ofklin.

Keywords: Oreochromisniloticus, Linear Alkyl Benzene Sulphonate, klin(R), Haematology.

1. Introduction
Detergents are organic pollutants that accumulate in freshwater sediment, constituting pollutant mixtures [1]. One of the most common domestic wastes that enter the aquatic ecosystem is detergent, which is a non-biodegradable chemical substance [2].
Generally detergents are xenobiotic components which are usually washed into water bodies and are made up of several compounds of which the active components are the surface-active agents or surfactants [3]. Surfactants are of various types which are used in the formulation of detergents; the LAS (Linear alkyl benzene sulphonate) are the most widely used [4]. At first the most common active substance of detergent used is Alkyl Benzene Sulfonate (ABS) that is a non-biodegradable chemical substance [1]. Linear Alkyl-benzene Sulfonate (LAS) is an anionic surfactant that has replaced the usage of ABS. According to [5], LAS was four times more toxic than ABS, however it is biodegradable. Effluents of LAS were found in the marine, brackish and fresh water ecosystems [6]. Linear Alkyl benzene Sulphonate (LAS) is the most widely used anionic surfactant in household and cleaning products that lower the surface tension of water, enabling soils and stains to loosen and release from fabrics and surfaces. These anionic surfactants are reported to be acutely toxic to aquatic organisms [7]. Contamination of natural water by detergents has become a matter of concern in recent years because of their large scale use in home and industrial applications, such as, washing powders, dye fasteners, formulation of shampoos, industrial and household cleansing agents, toothpaste, tooth powder and dispersing oil spills etc. [8, 9]. LAS is the primary cleaning agent used in many liquid and powder laundry detergents and specialty household cleaners at concentrations up to 25 percent of the total formulation.
Fishes live in very intimate contact with their environment and are therefore very susceptible to physical and chemical changes in it, which may be reflected in their blood components.Recently, haematological parameters have become promising biomarkers in measuring the effects of chemical pollutant in fish. Blood samples can regularly be obtained from test organisms, thus allowing the use of non- destructive approach in effect assessment [10]. Typically, haematological parameters are non-specific in their responses towards chemical

Stressors. Nevertheless, they may provide important information in assessment studies, by providing an indication as to the general physiology and health status of the organism under investigation [11]. Several researcher have investigated the toxicity, uptake and tissues distribution and hematological changes of pollutants in fish [12, 13, and 14] and the use of hematological techniques in fisheries research is growing rapidly, as it is very important in toxicological research which result in monitoring and predicting health conditions of the fish [15].

Pollutants such as herbicides, pesticide and industrial effluent are known to alter the haematological indices of fish [14, 13].

The tilapia family (of which *O. niloticus* is a member) is the second most intensively farmed species in the world.

2. Materials and Methods

2.1. Study area and Collection of Fish Samples

Juveniles of *Oreochromis niloticus* was obtained from of the fish farm, University of Agriculture Makurdi, Benue state using net. The fish were acclimatized for 14 days in the fish hatchery, Department of Fisheries and Aquaculture, University of Agriculture, Makurdi. The fish were fed twice daily at 5% of their body weight during acclimatization. Prior to and during exposure period fish were starved. Linear Alkyl benzene Sulphonate (LAS) detergent with the trade name Kiln in Nigeria was used for this investigation. It was obtained from the modern market in Makurdi, Benue State. After obtaining the 96hr LC_{50}, 3 sub-lethal concentrations were chosen for both fish by taking fractions; one twenty (1/20), one fifteen (1/15) and one twelve (1/12) of the concentrations. (10) *Oreochromis niloticus* were randomly selected and stocked with triplicates. Fish were fed with commercial floating feed (coppens) at 3% of their body weight. The exposure period lasted for 8 weeks.At the end of the 8th week, blood samples of *Oreochromis niloticus* were taken by injecting a 2mm needle and syringe through the dorsal aorta puncture and placed in ethylene-diamine-tetra-acetic-acid (EDTA) treated bottles to prevent coagulation. The blood samples was analysed in Q.A diagnostics medical laboratory, Makurdi, Benue State for the following: Haemoglobin (HB), Packed Cell Volume (PCV), Red Blood Cell (RBC), and White Blood Cell (WBC) while Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC).

2.3 Data Analysis

The data obtained from these experiments were analysed using the mini tab 17th edition and one way analysis of variance (Anova).

3. Results

3.1 Haematological Analysis of *Oreochromis niloticus* juveniles exposed to Sub lethal Concentrations of kiln®.

Haematological parameters of juveniles of *Oreochromis niloticus* exposed to sub lethal concentration of kiln® revealed a significant difference in all of the parameters across the concentrations as shown in table 10. Packed cell volume (PCV) differed significantly ($p<0.05$) with values ranging from 25.50±0.50% in 0.0mg/l to 21.65±0.65% in 1.9mg/l. White Blood Cell (WBC) varied significantly from 5.38±0.01 in 0.0mg/l to 3.57±0.07 in 1.9mg/l. Red Blood Cell (RBC) differed from 3.53±0.02 in 0.0mg/l to 2.73±0.06 in 1.9mg/l.

Mean Corpuscular Volume (MCV) differed significantly ($p<0.05$) with values ranging from 83.76±0.43 in 1.1mg/l to 71.90±0.50 in 1.9mg/l. Mean Corpuscular Haemoglobin (MCH) also differed with the highest value recorded in 1.5mg/l as 26.73±0.16 and least value rcorded in 1.9mg/l as 21.91±0.21. Mean Corpuscular Haemoglobin Concentrtion (MCHC) showed significant difference ranging from 30.34±0.06 in 1.1mg/l to 33.77±0.02 in 0.0mg/l. Haemoglobin ALS differed from 6.31±0.21 in 1.9mg/l to 8.68±0.12 in 0.0mg/l significantly.

Table 1: Mean Haematological parameters of *Oreochromis niloticus* juvenile's exposure to Sub lethal Concentrations of kiln for 8weeks.

Parameters	Concentration (Mg/L)				P-Value
	0.00 (Control)	1.10	1.50	1.90	
PCV (%)	25.50±0.50a	24.00±0.00ab	23.75±0.25b	21.65±0.65c	<0.01
RBC(109/l)	3.53±0.02a	2.81±0.06b	2.82±0.06b	2.73±0.06b	<0.01
WBC(109/l)	5.38±0.01a	5.20±0.10a	5.28±0.08a	3.57±0.07b	<0.01
Hb(g/dl)	8.68±0.12a	7.35±0.05b	7.66±0.13b	6.31±0.21c	<0.01
MCV(fl)	74.24±0.14b	83.76±0.43a	83.26±0.26b	71.90±0.50c	<0.01
MCH(pg)	25.10±0.10b	25.36±0.06b	26.73±0.16a	21.91±0.21c	<0.01
MCHC(g/dl)	33.77±0.02a	30.34±0.06b	33.01±0.51a	30.85±0.35b	<0.01

Means of the same row with different superscript vary significantly ($p<0.05$)

4. Discussion

Haematological parameters of juveniles of *Oreochromis niloticus* exposed to sub lethal concentration of kiln revealed a significant difference in all of the parameters across the concentrations as shown in table 1.Blood is recognized as a potential index of fish response to water quality, and can be used to ascertain the effects of pollutants in the environment. (16) Stated that haematology may be useful tool in monitoring stress levels of aquatic pollution on fish. Haematological parameters are closely related to the response of the animal and to the environment, an indication that the environment where the fish lives exert some influence on the haematological characteristics [17]. Haematology and clinical chemistry analysis, although not used regularly, can provide substantial diagnostic information once reference values are established. Unfortunately, reference values are not used on a routine basis in fish and the number of studies in which reference intervals have been determined for fish is limited [18]. However, it is well known that blood sampling, laboratory techniques, seasoned variation, size, genetic properties, sex, population density, lack of food supply, environmental stress and transportation could affect haematological data [19]. Hence, comparison of reference interval should be done with caution in respect to variation in environmental condition.

All haematological indices of *Oreochromis niloticus* varied significantly across concentrations. Packed cell volume (PCV), White blood cell (WBC), Red blood cell (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Heamoglobin Concentration (MCHC), and Haemoglobin decreased as the concentrations increased. Similar, reduction of blood parameters were reported studied by various authors. [20] Have noted that the RBC count decreased significantly in

the fresh water fish, *Labeorohita*on exposure to herbicide glyphosate. [21] Observed the decreased haematocrit value (PCV) in the fish, *Aspidopariamorar*on exposure to lindane. Similar reduction of haematological indices had been reported by [22, 23, 24, 25, 26, 27, and 28] in their respective studies on fish. Moreover, the reduction in these blood metabolites is an indication of anaemia caused by exposure to the toxicant. This anaemic response of fish might be attributed to the destruction of erythrocyte or inhibition of erythrocyte production [29] or haemodilution [30]. All these observations confirm the findings of the present study. [31] Also reported that erythrocyte count, MCV, MCH and MCHC decreased in *Heteropneutessp*. Treated with insecticides with con-comitant decline in oxygen transport.

5. Conclusion

The present study revealed that sub lethal concentrations of klinon *Oreochromis niloticus* created haematological disturbances. The differences in the haematological parameters may be as a result of different levels of the concentrations of kiln administered.

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