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# Hematological Parameters of *Clarias gariepinus* Juveniles and Some Physico-Chemical Parameters of Water Exposed to Linear Alkyl Benzene Sulphonate (LAS)

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## ABSTRACT

This research was done to find out the lethal effects of detergent LAS on *Clarias gariepinus* juveniles. The impact of exposure to linear alkyl benzene sulphonate (klin) on *Clarias gariepinus* juveniles were evaluated using standard methods A total of eighty (80) juveniles of *Clarias gariepinus* were randomly distributed into eight (8) plastic tanks for the test. They were subjected to concentrations of 0.0, 2.5, 3.3 and 4.2 mg/L of LAS for 56 days chronic exposure study following a static renewal protocol. The blood parameters were analyzed in the fish. The parameters like PCV and WBC of *Clarias gariepinus* vary significantly ($P<0.05$). HB, RBC and MCH were decreased while WBC, MCV and MCHC increased. Water quality examination showed increase in pH, DO record was higher in the control but reduces as concentration increases. Temperature did not differ significantly ($P=0.05$). Analyses of variance were used to find out if there was a significant different in the treatment. It was concluded that LAS causes a lot of harmful effects to fish.

**Keywords:** *Clarias gariepinus*, Hematological parameters, Linear alkyl benzene sulphonate, Water

## INTRODUCTION

Water is essential natural resource of living organisms. Man depends on water for various purposes such as fish production, drinking, cooking, irrigation and even disposal of waste products. The aquatic environment plays an important role in the life of all living organism due to its physicochemical and biological properties and formation of food webs and the aquatic ecosystem. The environment is getting polluted due to entry of different effluents into the water bodies through domestic, agrochemicals, fertilizers, pesticides and industrial discharges, etc. These polluting agents are toxic and deteriorate the water quality by changing its physicochemical nature that cause an ecological imbalance leading to stress of different kinds on aquatic organisms [1].

Detergents are organic pollutants that accumulate in freshwater sediment, constituting pollutant mixtures [2]. One of the most common domestic wastes that enter the aquatic ecosystem is detergent, which is a non-biodegradable chemical substance [3].

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. [4].

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 [5].

Fish can serve as bio indicators of environmental pollution (ecotoxicological studies) and therefore can be used for the assessment of the quality of aquatic environment [6].

African catfish (*Clarias gariepinus*) is of great commercial importance and it is the most common freshwater fish widely consumed in Nigeria [7].

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*Clarias gariepinus* is readily recognized by their cylindrical body with scale less skin, flattened bony head, small eyes, elongated spineless dorsal fin and four pairs of barbells around a broad mouth. The upper surface of the head is coarsely granulated in adult fishes but smooth in young fish [8].

## MATERIALS AND METHODS

### Study area

The study was carried out in the General Purpose Laboratory, Department of Fisheries and Aquaculture, University of Agriculture, Makurdi, Benue State, Nigeria.

### Sample collection

Juveniles of *Clarias gariepinus* were obtained from of the University of Agriculture, Makurdi, Benue. The fishes were acclimatized for 14 days in the fish hatchery, Department of Fisheries and Aquaculture, University of Agriculture, Makurdi. The fishes were fed twice daily at 5% of their body weight during acclimatization. Prior to and during exposure period fish were starved.

### Experimental procedure

After the acclimation period, a pilot test was carried out on linear alkyl benzene sulphonate (klin), so as to determine the lethal concentration of LAS. Three concentrations were determined for the test at the end of the trial which were; 2.50 mg/l, 3.30 mg/l and 4.20 mg/l.

### Toxicity test

A total of one hundred and eighty (180) juveniles of *Clarias gariepinus* were randomly distributed into eighteen (18) plastic tanks. The tanks were assigned to 6 treatments (control inclusive) and the treatments were in triplicates. Ten fish were distributed randomly and stocked in each tank. The exposure period lasted for 8 weeks. Fish were fed with commercial floating feed (coppens) at 3% of their body weight.

At the end of the $8^{th}$ week, blood samples of *Clarias gariepinus* were taken by randomly selecting fish from the various treatments and injecting a 2 mm needle and syringe through the dorsal aorta puncture and placed in Ethylene-Diamine-Tetra-Acetic-Acid (EDTA) treated bottles to prevent coagulation [9]. The blood samples of *Clarias gariepinus* were analysed at Laboratory College of veterinary medicine, University of agriculture Makurdi, Benue State for the following: Hemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC) and White Blood Cell (WBC) while Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) using methods as described [10].

### Water quality determination of test solution

The following parameters were determined during the experiment:

**Temperature:** Dissolved oxygen meter model HI98123 which have ability to read both dissolved oxygen and temperature was used. The probe was immersed in the water samples and allowed to stand for 2 min for equilibrium in each case. The readings were taken and recorded.

**Dissolved oxygen:** Dissolved oxygen meter model HI98123 was used to determine the dissolved oxygen in the water samples. The probe was dipped in the samples of water and after waiting for about 2 min for the reading to stabilized, the result was displayed on the LCD and the records were taken.

**pH (hydrogen ion concentration):** The hydrogen ion concentration was measured using the Hanna multi parameter water tester model H198129. The probe was immersed in each of the water samples. It was stirred gently and readings was taken and recorded after it has stabilized.

**Total dissolved solids:** TDS measurements were also done using Hanna HI 98129 multi parameters water checker. The meter was set to measure the TDS by use of the MODE keypad. The probe was immersed in each of the water samples. It was gently stirred and then reading was taken when stabilized.

**Electrical conductivity:** This was done with the use of Hanna multi parameters water checker meter.

## DATA ANALYSIS

The data obtained from these experiments were analysed using the minitab $17^{th}$ edition and one way Analysis of Variance (ANOVA).

## RESULTS AND DISCUSSION

Hematological parameters of juveniles of *Clarias gariepinus* exposed to sub lethal concentrations of LAS are shown in **Table 1**. Hematological indices such as Packed Cell Volume (PCV) and White Blood Cell differed significantly ($P<0.01$) but hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentrations were not significantly different across the treatment. Packed Cell Volume (PCV) differed significantly ($p<0.05$) with values ranging from 35.50 ± 1.00% in 0.0 mg/l to 27.00 ± 2.50 in 4.20 mg/l. White Blood Cell (WBC) also differed significantly ranging from 11.85 ± 0.85 in 4.20 mg/l to 4.15 ± 0.65 in the control (0.0 mg/l).

**Table 1.** Mean hematological parameters of *Clarias gariepinus* juvenile's exposed to concentrations of linear alkyl benzene sulphonate (LAS).

| Parameters | Concentration (mg/L) | | | | P-Value |
|---|---|---|---|---|---|
| | 0.00 | 2.50 | 3.30 | 4.20 | |
| PCV (%) | 35.50 ± 1.00$^{a}$ | 33.00 ± 1.00$^{b}$ | 33.50 ± 2.50$^{b}$ | 27.00 ± 2.50$^{c}$ | <0.01 |
| RBC ($10^9$/l) | 5.15 ± 1.35 | 4.65 ± 1.65 | 4.70 ± 1.10 | 5.10 ± 1.35 | 0.99$^{ns}$ |
| WBC ($10^{12}$/l) | 4.15 ± 0.65$^{c}$ | 5.85 ± 0.75$^{c}$ | 7.00 ± 0.40$^{b}$ | 11.85 ± 0.85$^{a}$ | <0.01 |
| Hb (g/dl) | 11.85 ± 0.30 | 11.00 ± 0.30 | 11.15 ± 0.85 | 9.00 ± 0.85 | 0.45$^{ns}$ |
| MCV (fl) | 75.30 ± 0.31 | 82.00 ± 0.31 | 50.60 ± 2.80 | 81.80 ± 2.46 | 0.72$^{ns}$ |
| MCH (pg) | 25.00 ± 1.04 | 27.30 ± 1.04 | 25.55 ± 7.73 | 18.55 ± 8.40 | 0.86$^{ns}$ |
| MCHC (g/dl) | 33.20 ± 0.10 | 33.30 ± 0.10 | 33.25 ± 0.05 | 33.30 ± 0.20 | 0.90$^{ns}$ |

*Means of the same row with different superscript are statistically significant (p<0.05), ns: not significant*
*KEY: PVC: Packed Cell Volume; RBC: Red Blood Cell; WBC: White Blood Cell; Hb: Hemoglobin; MCV: Mean Corpuscular Volume; CH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration*

Red blood cell were not significantly (P=0.99) different among the treatments but ranged from 5.15 ± 1.35 in 0.0 mg/l to 4.65 ± 1.65 in 2.50 mg/l. Hemoglobin value ranged from 11.85 ± 0.30 in 0.0 mg/l to 9.00 ± 0.85 in 4.20 mg/l. Mean corpuscular hemoglobin concentration ranged from 33.30 ± 0.10 in 2.50 and 4.20 mg/l to 33.20 ± 0.10 in 0.0 mg/l, mean corpuscular hemoglobin ranged from 27.30 ± 1.04 in 2.5 mg/l to 18.55 ± 8.40 in 4.20 mg/l and mean corpuscular volume ranges from 82.00 ± 0.31 in 2.5 mg/l to 50.60 ± 2.80 in 3.3 mg/l.

**Physico-chemical parameters of the water used during the experiment**

**Table 2** shows the physico-chemical parameters obtained during the 8 weeks sub lethal test *Clarias gariepinus* treated with LAS. There were significant difference (P<0.05) of the tested water among different concentrations with the exception of temperature. Dissolved oxygen decreased with increase in concentrations from 6.75 ± 0.05 mg/l in control to 4.45 ± 0.25 mg/l in 4.20 mg/l, Total dissolved solid increased with increase in concentrations also from 339.50 ± 10.50 from control to 969.00 ± 18.00 mg/l in 4.20 mg/l and so was electrical conductivity from 696.50 ± 24.50 in control to 1943.00 ± 36.00 in 4.20 mg/l and pH from 7.15 ± 0.15 in control to 8.10 ± 0.14 in 4.20 mg/l.

**Table 2.** Mean physico-chemical parameters of the water during exposure of *Clarias gariepinus* juveniles to concentrations of linear alkyl benzene sulphonate (LAS).

| Parameters | Concentrations (mg/L) | | | | P-Value |
|---|---|---|---|---|---|
| | 0.00 | 2.50 | 3.30 | 4.20 | |
| Dissolved Oxygen (mg/l) | 6.75 ± 0.05$^{a}$ | 6.55 ± 0.05$^{a}$ | 5.10 ± 0.20$^{b}$ | 4.45 ± 0.25$^{c}$ | <0.01 |
| Total Dissolved Solids (mg/l) | 339.50 ± 10.50$^{d}$ | 623.50 ± 0.30$^{c}$ | 877.00 ± 29.00$^{b}$ | 969.00 ± 18.00$^{a}$ | <0.01 |
| Electrical conductivity (us/cm) | 696.50 ± 24.50$^{c}$ | 1303.00 ± 26.00$^{b}$ | 1825.50 ± 94.50$^{a}$ | 1943.00 ± 36.00$^{a}$ | <0.01 |
| pH | 7.15 ± 0.15$^{c}$ | 7.85 ± 0.15$^{b}$ | 8.55 ± 0.05$^{a}$ | 8.10 ± 0.14$^{ab}$ | <0.01 |
| Temperature (°C) | 29.90 ± 0.20 | 28.80 ± 0.30 | 29.35 ± 0.05 | 28.90 ± 0.10 | 0.07$^{ns}$ |

*Means of the same row with different superscript vary significantly (p<0.05); ns: not significant*

## DISCUSSION

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. Some researchers [11-15] stated that hematology may be useful tool in monitoring stress levels of aquatic pollution on fish. Hematological parameters are closely related to the response of the animal and to the environment, an indication that environment where the fish lives exert some influence on the hematological characteristics [16]. Hematology 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 [17]. 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 hematological data [18]. Hence, comparison of reference interval should be done with caution in respect to variation in environmental condition.

Hematological parameters of *Clarias gariepinus* such as PCV, HB, RBC and MCH showed reduction with increase in concentration while WBC, MCV and MCHC increased with increased LAS concentrations. This situation is similar to that reported by some researchers [19-21] on *T. guinensis*. Low PCV value obtained in this study may be attributed to reduction in red blood cell caused by osmotic changes [22]. This finding is in agreement with the findings of others who investigated hematological changes in mudfish *Clarias gariepinus* exposed to sub lethal concentrations of copper and lead [23,24]. The observed decrease in the PCV and Hb of *C. gariepinus* juvenile exposed to LAS could be indicative of hemodilution due to erythrocyte sequestration. Some workers have attributed changes in such blood parameters to erythrocyte swelling [25] or hemolysis [26]. Some have reported that the RBC count decreased significantly in the fresh water fish, *Labeo rohita* on exposure to herbicide glyphosate [27,28]. While others reported decreased hemoglobin content in the fresh water fish, *Tilapia mossambica* on exposure to arsenic [29,30]. Certain researchers made similar observation in *Cirrhinus mrigala* exposed to detergent tide [31,32]. All these observations are in conformity with the findings of the present study. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs [33].

Although the increasing or decreasing number of the White Blood Cell (WBC) of *Clarias gariepinus* is a normal reaction on the exposure of fish to toxicants [34], this study showed increase with increased concentration of LAS to *Clarias gariepinus*. This is in agreement with the work of certain scientists who reported increased WBC with increase in concentration of tobacco leaf dust. This may be attributed to increase in leucocytes synthesis as a defense mechanism against the destruction of erythrocytes [35].

MCH and MCHC is an indicator of red blood cell swelling. In the findings, MCH of *Clarias gariepinus* reduced with increase in concentrations of LAS where as MCHC and MCV increased with increase in concentration of LAS in *Clarias gariepinus*. This is in consonant to the findings of which reported a decrease in MCH and increase in MCV [36]. The increase in MCV is contrary to the findings of some of the researchers who reported lower MCV values in *Clarias gariepinus* treated with Zinc salt [37].

**Effects on water quality parameters**

The temperature reading for acute and sub lethal effect of LAS on *Clarias gariepinus* fell within 23-30°C in the study which is in accordance with the range (20-30°C) suggested by the Federal Environment Protection Agency for optimum physiological state of fish. Dissolved oxygen reduced with increase in concentrations of with control having the highest dissolved oxygen in both acute and sub lethal test and lowest value was recorded in the highest concentration for acute and sub lethal test. The heighten activities of the fish due to poison can also remove oxygen from the water body had earlier reported that indiscriminate deposition of effluent into an aquatic system might decrease the dissolved oxygen concentration, which stand to impair respiration leading to asphyxiation (which is an indication of unconsciousness or death produced by failure of the blood to become properly oxygenated in the lungs) and may ultimately result into organ architectural degradation such as liver dysfunction. Decrease in dissolved oxygen in this study can be attributed to the presence of the chemical (LAS) in the water. TDS, EC and pH increased with increase in concentrations. The water quality parameters of the study were significantly different from the control. However, they were within acceptable tolerance range and also could not have had any negative effect on the tested fish. The water quality parameters showed significant difference ($p<0.05$) with exception of the Temperature both in the acute and sub lethal of *Clarias gariepinus* on LAS.

A parameter like temperature can influence metabolic rate in the bodies of organisms affect density of ambient water of the organism and even food availability [15]. In the present experiment, temperatures did not vary both acute and sub lethal test of *Oreochromis niloticus* but was between 26 and 28°C which are within tolerance limits for the survival of fish [16]. Some recommended a pH range of 6.5-9. The pH of both acute and sub lethal test of *Oreochromis niloticus* were also within the tolerance limit of fish survival, it could not have affected the fish, though slight changes in pH could grossly affect ammonium nitrogen toxicity [38]. It was affirmed that detergent concentration as little as 0.9 mg/l at a pH of 5.5 is lethal to fish. Significant variations in oxygen concentrations were observed, though within tolerance limit, reduced levels as observed with increased LAS concentration could have led to stress.

## CONCLUSION

1. In conclusion, linear alkyl benzene sulphonate was found to be toxic to juveniles of *Clarias gariepinus*.

2. Histopathological studies further revealed that acute concentrations of LAS damaged the gills and liver of treated fishes and led to their deaths.
3. Sub lethal concentrations of LAS adversely affected the growth performances of *C. gariepinus* and *Oreochromis niloticus*.
4. The present study revealed that sub lethal concentrations of LAS in *C. gariepinus* and *Oreochromis niloticus* created hematological disturbances.

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