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Original Research Paper

ACUTE TOXICITY OF LINEAR ALKYLBENZENE SULPHONATE (LAS) DETERGENT ON FINGERLINGS OF THE AFRICAN CATFISH, HETEROCLARIAS (A HYBRID OF *HETEROBRANCHUS BIDORSALIS* AND *CLARIAS GARIEPINUS*)

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Abstract

Background and aim: Fish is one of the most important aquatic organisms affected by detergent pollution. The acute toxicity of Linear Alkylbenzene Sulphonate (LAS) detergent on the African catfish is sparse in literature. We therefore conducted a series of static short-term bioassays to assess the acute toxicity of LAS detergent on the fingerlings of Heteroclarias, a hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*

Method: This study was undertaken in the laboratory to determine the 24, 48, 72 and 96h medium concentration (LC_{50}) of the acute toxicity of the detergent [Linear Alkylbenzene Sulphonate (LAS)] using the static bioassay test and the freshwater African catfish fingerlings: Heteroclarias (A hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*), as experimental animal, over an exposure period of 96 hours.

Results: During the exposure period, the test fish exhibited several behavioural changes before death such as restlessness, rapid swimming, loss of balance, respiratory distress and haemorrhaging of gill filaments amongst others. Opercular ventilation rate as well as visual examination of dead fish indicated lethal effects of the detergents on the fish. The 96-h LC_{50} was computed using probit analysis.The overall mean value of 45.71 mgL^{-1} was obtained

Conclusion: The result shows that the toxicity increases with increase in exposure periods. The present findings indicate that the LAS detergent have mortality effects on Heteroclarias fingerlings and may adversely affect other aquatic organisms. The discharge of detergents or afterwash water into the aquatic environment should be discouraged.

Keywords: Acute toxicity; African catfish; Bioassay; detergents; Heteroclarias; LC_{50}; Lethal concentration;

Introduction

Fish is generally considered very sensitive to all kind of environmental changes resulting from physical chemical, biological or a combination to man's anthropogenic activities as identified by Odiete (1999) and Asrar-Sheriff *et al.* (2012). Fish is one of the most important non-target aquatic organisms affected by detergent pollution. Toxicities of various detergents against fish have been evaluated in a number of studies (Abel 1974; Verma 1980; Okwuosa and Omoregie 1995, Saxena *et al*. 2005; Arimoro and Agbon 2006; Varsha *et al.* 2011; Arivizhivendhan *et al*. 2014; Perieria 2014; Chandanshive, 2013, 2015).

Detergents form a major freshwater pollutant that has attracted special attention worldwide. They are widely used in industries and domestic premises to wash clothes, utensils and vehicles. The "after wash" of the detergents are either drained into the aquatic environment such as ponds, rivers, streams etc. or they find their way into the environment by natural seepage (Ogundele *et al*., 2005). According to Jawahar Ali *et al.* (2015) detergents are the parts of large group of chemical compounds, collectively referred to as surface-active agents or surfactants because they act upon the surface. Okwuosa and Osuala (1993) reported that considerable amount of detergent have been found in Nigeria freshwater where they generally affect several aquatic organisms via commercial washing. A common practice in water bodies in Nigeria and this could lead to a buildup of detergent level in our natural waters.

Eutrophication as a result of excessive phosphate in detergent has also been traced to the effect of detergents (Chandanshive 2015). Therefore the real benefit of using homemade laundry detergent as a cleansing agent in our homes is consequently rendering our environment, fauna and flora in danger of accumulation and bioaccumulation of these toxins or toxicants of detergent. This may also disrupt the natural food chain and food web in the aquatic ecosystem.

Heteroclarias is a hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*. It is commonly cultured in most artificial ponds in Nigeria for its high commercial value. By implication it is consumed by a significant proportion of the Nigeria population. This fish also possesses the ability to grow and thrive well in adverse pond condition. It can tolerate low DO (dissolved oxygen) level due to possession of accessing air breathing organ and above all it is an omnivorous feeder. The acute toxicity of LAS detergent with the trade name 'ZIP' on Heteroclarias is sparse in the literature. We therefore conducted a series of static short-term bioassays to assess the acute toxicity of this detergent on the fingerlings of Heteroclarias.

Materials and Methods

Heteroclarias are readily available and survive well under laboratory conditions. Their fingerlings were used because of the convenience of their size for handling. The fingerlings were transported to the laboratory in open plastic buckets from a fish farm in Sapele, Delta State, Nigeria either in the early morning or late in the evening to avoid possible heat stress. In the laboratory, healthy fingerlings were transferred into large holding tanks containing aerated water, where they were left for at least 14 days to acclimatize to laboratory conditions.The fingerlings were considered fully acclimatized when no deaths were observed for 4 consecutive days.At the end of the acclimatization period, the fingerlings were used for the test.Any deaths recorded during the tests were thus attributed to the effect of the detergent.

All bioassay followed the standard semi-static procedure with the observation of all necessary traditions and precautions (Sprague, 1973, APHA, 1998, EPA. 2000). Appropriate modifications were made where necessary. In essence, the process involved a range determination step during which the experimental fish were exposed to a wide range of concentrations of the detergents “ZIP” relying on preliminary trials data. After establishing the ranges of concentrations to be tested, necessary dilutions from the appropriate stock solution were made for the experimental concentrations for acute tests based on logarithmic bisection of the intervals. In all cases, the dilution water was aerated to saturation *ab initio* and all the test were replicated with the appropriate controls.

The containers used consisted of plastic aquaria 15 litres capacity containing 12 litres of test materials. The upper part of each aquarium was covered with a lid made of fine polyethylene gauze screen of 1 mm mesh size. Each experimental set up and control (each aquarium) was stocked with 20 fish specimen. The acute toxicity experiments lasted 4 days (96hours).

Stock solution of the detergent “ZIP” was prepared from 1g granules in 1 litre of distilled deionized water to form 100% concentration. From this stock solution, various concentrations used in the investigations were prepared based on a logarithmic bisection of intervals. The water quality parameters of the diluting water used in the tests and determined by standard methods were as presented in Table 1.

The fish were not fed twenty-four prior to the commencement of the experiment. The dose that caused a death in 50% of the organisms within the 96 h is expressed as the 96-h LC_{50}. A range-finding test was first carried out to determine the range within which the detergent affected the fish. A fish was considered dead when there was a lack of opercular movement and it was found floating or lying at the bottom without movement. A series of tanks was set up containing a range of toxicant concentrations (0.0, 50, 70, 90, 100, 150 mg/L). Three replicates of the tests were conducted. Mortality was recorded every 24 hours though the aquaria were inspected every three hours for dead fish, which were immediately removed. Behavioural responses of the exposed fish were observed every hour. During the exposure period, Temperature, pH and Dissolved oxygen, Free Carbonioxide and Alkalinity were monitored using mercury in glass thermometer, pH meter and Dissolved Oxygen meter respectively.

Data Analysis

The 96-hour LC_{50} was determined using graphical method and regression equation extrapolated. Results obtained.from the physicochemical parameters of the test tanks were subjected to statistical analysis using the analysis of variance (ANOVA) method to test for significance at 0.05 probability level.

Table 1: Water Quality Parameters.

Parameter	Values
pH	6.64 $\pm$0.34
Temperature	28.8 $\pm$ 1.3^{0}C
Dissolved Oxygen	7.34 $\pm$ 1.04 mgL^{-1}
Free Carbon Dioxide	4.85 $\pm$ 0.08 mgL^{-1}
Alkalinity	34.8$\pm$1.65 mgL^{-1}
Hardness	129.65 $\pm$12.65 mgL^{-1}

Results

The mean values and standard deviation recorded for the various concentrations compared with those of the control aquaria are presented in Table 2. The mean physicochemical parameters of temperature, dissolved oxygen, pH, Free CO_2, and alkalinity of various concentrations did not vary significantly ($P>0.05$) from those of the control. The value of total hardness however showed significant increase ($P<0.05$) with increase in concentration. The 96 hour LC_{50} determined graphically during this investigation was 45.71mg/L as shown in Fig. 1.

Percentage mortality increased significantly ($P<0.05$) with increase in the detergent concentration. Fingerlings exposed to 0.00mg/L and 50mg/L had 0% and 25% mortality respectively: while 42%, 60%, 85% and 100% mortality were observed in aquaria with concentrations 70 mg/L, 90 mg/L, 100mg/L and 150mg/L respectively as shown in Table 3.

The following behavioural responses were exhibited by the test organism fish during the exposure period: restlessness, erratic swimming, frequent attempts at jumping out of the aquaria, loss of balance, rapid opercula movement, and excess mucus, secretion towards the ninety-six hour. The colour of exposed fish became darker, the skins of the dead fish were dry and had lost their mucus lining. Haemorrhaging of the gill filament were also observed on the dead fish. These responses were not observed in the control experiment.

Table 2 Water physico-chemical parameters during the exposure of the catfish, Heteroclarias to lethal concentrations of LAS detergent.

Parameters/ detergent concentration (mgL^{-1})	0.00 (control)	50.0	70.0	90.0	100.0	150.0
Temp (OC)	28.8+1.34	28.6+1.32	28.8+1.42	27.9+1.03	28.2+1.32	28.7+1.27
DO (mgL-1)	7.33+1.04	7.02+1.32	7.42+1.02	7.06+1.05	7.10+1.29	7.28+1.22
pH	6.64+0.34	6.63+0.21	6.58+0.25	6.65+0.36	6.54+0.26	6.40+0.20
Free CO_2 (mgL-1)	4.94+0.03	4.64+0.07	4.82+0.04	4.86+0.03	4.96+0.04	4.72+0.05
Alka (mgL-1)	32.83+0.2	34.17+0.20	34.26+0.20	36.88+0.28	33.5+0.03	32.97+0.32
Total Hardness (mgL-1 CaCO3)	104+6.45	114+8.42	118+6.41	120+8.42	124+10.21	129.65+12.65

Note: Values are mean ± SD, Temp, Temperature; DO, Dissolved oxygen; Free CO_2, free carbon IV oxide; Alka, Alkalinity.

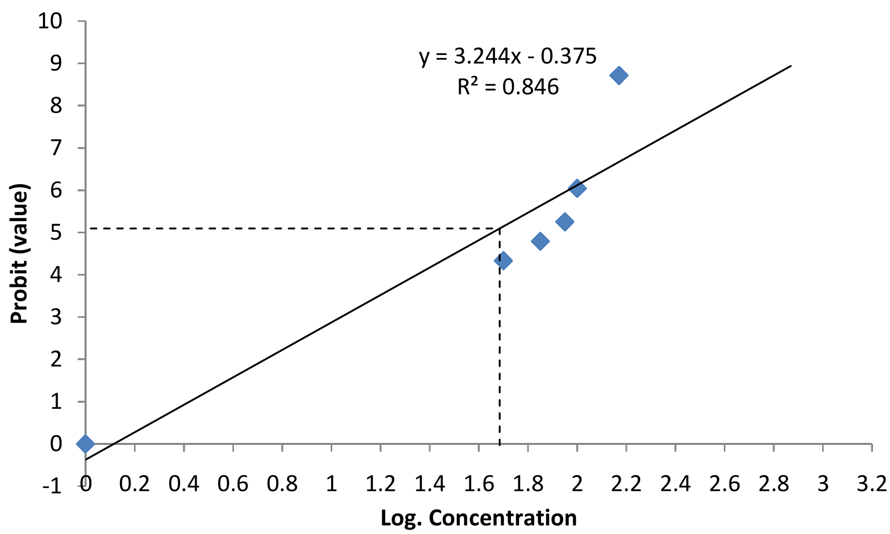

Fig 1. Linear relationship Probit Mortality and Log. Concentration of Heteroclarias exposed to various concentration of Linear Alkylbenzene Sulphonate (LAS) Detergent (ZIP) (Log 1.66, antilog = 45.71mg/L).

Table 3. Mortality rate of Heteroclarias exposed to various concentrations of Linear Alkylbenzene Sulphonate (LAS) Detergent for 96 hr.

Con. mgL^{-1}	Log. Conc.	Number of death at 96hrs Replicates			Total death out of 60	% mortality
		1	2	3		
Control 0	0	0	0	0	0	0
50	1.698	5	4	6	15	25.0
70	1.845	9	7	9	25	42.0
90	1.954	12	11	13	36	60.0
100	2.000	18	16	17	51	85.0
150	2.170	21	18	20	60	100.0

Discussion

Results obtained from this investigation indicated that concentrations of LAS detergents led to increased mortality rate of the catfish hybrid “Heteroclarias”. The value of 96-h LC_{50} of 45.71 mg/L with lower and upper confidence limit of 24.56 mgL^{-1} and 98.84 mgL^{-1} respectively reported is not too different from 25.11 mgL^{-1} reported by Okwuosa and Omoregie (1995) for the African tooth carp, *Aphyosemion gairdneri* and also the range of 0.4 to 40.00 mgL^{-1} earlier reported by Abel (1974), for several fish species. It is however important to note that the differences in fish species, age, handling and experimental conditions were perhaps the reason for the disparity or variability in this present investigation from those of earlier workers. However the fish species ‘Heteroclarias’ a hybrid of *Heterobranchus* and *Clarias* species is more hardy and resistant to environmental toxicants.

During the exposure period, the test fish exhibited various behavioural changes before death finally occurred. These include hyperventilation, erratic swimming, loss of balance and respiratory distress. These responses were not observed in the control experiment. These observations are in agreement with earlier reports of Ogundale *et al.* (2005), Oronsaye and Ogbebor (1997), and Omoregie *et al.* (1998). Also, in this investigation mucus accumulation was observed on body surface and gill filament of dead fish. According to Chandanshive (2013), mucus accumulation results from increase in the activity of mucus cells subsequent to exposure to pollutants. This results in an increase in the production of mucus over the body of the fish.

It has been reported that nearly all detergents destroy the external mucus layers that protect the fish from bacteria and parasites and they can cause severe damage to the respiratory apparatus ‘gills’ of aquatic fishes. Most fish will die when detergents concentrations approach 15 parts per million (Asrar-Sheriff *et al.* 2012). These behavioural responses are indications of death due to nervous disorder and insufficient oxygen supply. Hemorrhaging of gill filaments also indicated that detergents could damage the gills of exposed fish. Omoregie and Ufodike (1991) noted that gill damage will greatly reduce proper exchange of respiratory gases between the fish and the surrounding water thereby leading to air gulping by the stressed fish. Abel (1974) had earlier reported that reflexes of air gulping can be associated with detergents toxicity in fish.

The physicochemical parameters of the test solution fluctuated slightly during the bioassay but were not thought to have affected the fish mortality since they were within the adjusted tolerance range (Mackereth, 1963). However, the pH values fluctuated between 6.65-6.40. This indicated the slightly acidic nature of the water. Ogundale et al (2005) affirmed that detergent concentration as little as 0.9 mg/L at a pH of 5.5 is lethal to fish.

Conclusion

The use of detergent in homes and industries cannot be discontinued. However better methods of disposing of the 'after wash' need to be worked out. This investigation showed that the harmful effects of 'Heteroclarias' subjected to LAS detergent will subsequently lead to death. If the present rate at which detergents are introduced into the aquatic environment is not checked, the continuous existence of the aquatic fauna, including biologically important fish species will be in jeopardy.

Author's Contributions:

FOA, EOA, LA and FII designed the study, performed the statistical analysis and wrote the draft of the manuscript while FOA managed the literature searches and probit analysis and edited the final draft. All authors read and approved the final manuscript.

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