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Acute toxicity of detergent on juveniles of African catfish (*Clarias gariepinus*)

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Abstract
The acute toxicity of commercial detergent, (Good mama) containing linear alkyl benzene sulphonate (LABS) was determined using juveniles of African catfish, *Clarias gariepinus* in 96 hours bioassay. After acclimatization, the fish were exposed to concentrations of 0mg/l, 20mg/l, 30mg/l, 40mg/l, 50mg/l and 60mg/l respectively. The mean lethal concentration, LC_{50} values for the detergent was 37.65mg/l. With increase in concentration of the toxicant, respiratory disturbances, loss of balance, erratic swimming before death, were observed in the exposed fish. This varied greatly with difference in concentration of the toxicant and these showed that mortality increases with increase in concentration. The differences observed in the mortalities of *C. gariepinus* juveniles were significant (p<0.05), an indication that mortality could be a function of concentration and time of exposure. Therefore, indiscriminate discharge of detergent into aquatic ecosystem should be discouraged.

Keywords: Toxicity, LC_{50}, good mama detergent, *Clarias gariepinus* and water body

Introduction

Human and ecological disorder experienced in industrial settlements as a result of improper disposal of chemicals such as detergent calls for careful surveillance on the state of the environment.

Environmental contaminants such as heavy metals and pesticides are the most important toxic compounds of aquatic habitats. Consequently, fish larvae and fingerlings are the most vulnerable life stages of fish which could be severely affected by pesticides and detergents as non-target organisms as well as by heavy metal pollution. Histopathological alterations of the fish gill, kidney and liver could affect the survival rate, biological activities, osmoregulation, reproduction, buoyancy etc., which could finally lead to failures in stock recruitment and population changes.

Pollution of water resources has become a serious problem in Nigeria and other developing nations in recent times largely due to the rapid increase in human population and the proliferation of industries which have resulted in the discharge of large amounts of effluents and wastes into aquatic environments where they degrade the normal flora and fauna of the ecosystem (Adewoye *et al.*, 2005) [5].

Presently, the Federal Government of Nigeria is emphasizing the need for adequate environmental protection in all technological and socio-economic development projects by strictly directing industrial operators to sustainably manage the disposal of chemicals such as detergents in the natural environment (DPR 2002) [15]. Indiscriminate discharge of such compounds that contain mixtures of heavy metals such as herbicide, pesticides, detergent etc., their careless handling, accidental spillage or discharge of untreated effluents into natural waterways have harmful effects on the fish population and other forms of aquatic life and may contribute to long term effects in the environment (Olojo *et al.*, 2005 [31] and Ayoola a and b, 2008 [9&10]). All detergents can destroy the external mucus layers that protect the fish from bacteria and parasites and they can as well cause severe damage to the gills. Most fish will die when exposed to detergent concentrations of about 15 parts per million (ppm) and concentrations as low as 5ppm can kill fish eggs.

Common detergents used in Nigeria include Omo produced by Unilever Nigeria Plc, Ariel produced by Procter & Gamble Nigeria Limited, others include Good Mama, Viva plus, Bonus, Waw, Klin, Sunlight etc; and they are composed of surface-active agents or surfactants, bleach, builders, foam stabilizers, perfumes, enzymes, optical brighteners etc.

Surfactants are the components mainly responsible for the cleaning action of detergents. In commercial detergents, the surfactant component is between 10 and 20%. The other components include bleach, filler, foam stabilizer, builders, perfume, soil-suspending agents, enzymes, dyes, optical brighteners and other materials designed to enhance the cleaning action of the surfactant (Okpokwasili and Nwabuzor, 1988) [30]. Detergent surfactants are complex organic chemicals where hydrophilic and hydrophobic groups are joined together in the same molecules (Huang *et al.,* 2000) [22]. Detergents are widely used in both industrial and domestic premises to wash equipment, installations, heavy duty machines, vehicles and oil soiled materials. Detergent is a persistent environmental contaminant probably due to its use in the formulation of cleaning agents, pesticides and for dispersing oil spills at seas; so the production, use and exposure of detergents is unavoidable. Xenobiotic compounds usually concentrate in the tissues of aquatic biotas and are known to produce cumulative deleterious effects (Abbas, 1998 [1]; Abbas and Mahmood, 2003, 2004 [3,2]).

There are various types of surfactants used in detergents formulations; the linear alkylbenzene sulfonate (LAS)-ionic surfactants is the most widely used (Ogundiran *et al.,* 2009) [28]. It was introduced as biodegradable alternatives to the non-biodegradable branched-chained alkylbenzene sulfonates. LAS have been reported by WHO (1996) [39], to have a high solid adsorption coefficient, which is attributed to the physicochemical properties of the surfactants. The LAS molecules adsorb to the suspended solid in water bodies and hence end up in sediments along the water course or sludge in treatmente plants (Cavalli *et al.,* 2000) [13]. The recommended LAS that was claimed by some researchers to biodegrade perfectly (Gledhill, 1974 [20]; WHO, 1996 [39] and McAvoy *et al.,* 1997 [25]) have also been reported to poorly degrade in rivers, lakes, ponds and even in soils and this may be toxic to aquatic faunas and floras and can also induce severe damage to vital organs and even haematological, hormonal and enzyme disturbances (Lightowlers, 2004 [24]; Ogundiran *et al.,* 2007 [29] and Ogundiran *et al.,* 2009 [28]). It has also been discovered that detergent surfactant increases microbial populations especially those that are able to use the surfactant as their basic source of carbon or phosphate or both, some of these microorganisms stand as ectoparasites or endoparasites that cause histological degradation in fish species (Eniola and Olayemi, 2002 [16], and Adewoye and Lateef, 2004 [6]).

Toxicity is the degree to which a chemical substance or a particular mixture of substances (toxin or poison) can damage organisms, either humans or animals. Toxic chemicals can cause tissues damage and histopathological degradations as the fish show haematological responses to toxicants. Generally, such degradation of histological origin occurs in the gills, livers, heart, kidney and epidermis of animals. Van Dyk *et al.* (2005) [38] reported sublethal levels of metal mixtures of cadmium and zinc which have influence on the histological responses in exposed specimens with the most histological characteristics identified to be hyalinization of hepatocyte, increased vacuolation associated with lipids accumulation, congestion of blood vessels and cellular swelling. The liver of fish can be considered a target organ to pollutants, therefore alterations in its structure can be significant in the evaluation of fish health (Myers *et al.,* 1998) [26], and exhibit the effects of a variety of environmental pollutants (Hinton *et al.,* 1992) [21]. The extent to which metals in effluents can either increase or decrease histopathological changes, depends on the following: additive effects of the reacting metals in such an effluent, metal concentration, fish species, physiological status of the fish, length of exposure and other factors (Paris-Palacious *et al.,* 2000) [34]. Contamination of aquatic ecosystems by detergent has been reported in aquatic organisms such as fishes (Adham *et al.,* 2002 [7]; Adewoye and Fawole, 2002 [4]; Adewoye *et al.,* 2005 [5]; Ogundiran *et al.,* 2007 [29] and Ogundiran *et al.,* 2009 [28]). These pollutants build up in the food chain and are responsible for the adverse effects and death in aquatic organisms (Farkas *et al.,* 2002) [18]. Acute toxicity test describes the adverse effects of a substance that result either from a single exposure in a short period of time (usually less than 24hours). In acute exposures, organism comes in contact with doses of the toxicant. Acute toxicity tests are generally conducted on organism during the early stage of the organism's life cycle, and are considered 'partial life cycle tests'. Acute toxicity test is not valid if mortality in the control sample is greater than 10%. Results are reported in LC_{50}, or concentration that will affect 50% of the sample size (EPA, 2001) [17]. While chronic toxicity tests on the other hand, are long-term tests (conducted for either weeks, months or years), relative to the test organism's life span (>10% of organism's life span), and generally use sub-lethal endpoints. In chronic exposures, organisms tend to have contact with low, continuous doses of the toxicant. Chronic exposures may induce effects as acute exposures, but can also result in effect that develops slowly. Chronic tests are not considered valid if mortality in the control sample is greater than 20%.

Therefore, the application of environmental toxicology studies on non-mammalian vertebrates is rapidly expanding for the evaluation of the effects of noxious compounds (Ayoola a and b, 2008) [9&10]. Fishes are widely used to evaluate the health of aquatic ecosystems and the physiological changes serve as biomarkers of environmental pollution (Kock *et al* 1996) [23]. *Clarias gariepinus* is most widely used fish species because it is hardy and able to tolerate both well and poorly oxygenated waters. It is a prominent culture species because of its fast growth rate and resistance to diseases and stress factors like over-stocking and poor water quality (Olojo *et al*., 2005) [31]. *Clarias gariepinus* is distributed mainly in fresh waters of Africa hence the name African catfish, although it is also seen in Asia. It is named 'catfish' because they possess prominent barbels which resemble cat's whiskers. *Clarias gariepinus* has a slender body, flat bony head and broad terminal mouth with four barbels. The pectoral fins have spines and its dendritic organ is an accessory breathing organ which is a modification of the gill arches (Ahmed *et al*., 2008) [11]. *Clarias gariepinus* is widely cultivated in Nigeria water bodies, hence used as biological indicators of ecotoxicological studies. Thus, the aim of this study is to investigate the acute toxicity of the detergent (good mama) on the African catfish (*Clarias gariepinus*). This study was conducted to assess the potential toxic effects of 'good mama' on fishes and other aquatic life, by determining the LC_{50}, as well as the manifestation, overturning and survival times of the fingerlings when exposed to various concentrations of the detergent.

Materials and Methods

Description of Study Area

The study was carried out in the indoor hatchery of the department of fisheries technology, Federal College of Freshwater Fisheries Technology Baga. Federal College of

Freshwater Fisheries Technology Baga is location at Kukawa Local Government Area of Borno State but was relocated to Maiduguri, the state capital because of the insurgency in the Northeastern part of Nigeria.
It is located at latitude 11º 15N and longitude 13º 15E. The state occupied a land area of 70,898 Km^2 (27, 37459 mile) making it the second largest state in Nigeria in terms of land area after Niger state with a population of 4,151,193 (Census 2006) and with high ambient temperature. The sahelial climate is characterized with their distinct seasons: rainy and dry seasons. The rainy season starts from June to October, with mean annual rainfall ranging from 200mm to 500mm; dry season starts from November to April/May which is a period of very low temperature and dry harmattan wind. The dry hot season starts from March to June with temperature ranging from 27ºC to 40ºC making it the driest period with intense heat (CBDA 1981). The state is mainly dominated by the Kanuri, Babur, other minority tribes are Shuwa Arabs ethnic groups, Margi and Gwoza. Borno is a habitable state with many other tribes living for businesses and other proposes.

Specimen collection
300 healthy juveniles of *Clarias gariepinus* of the same parent were obtained from Federal College of Freshwater Fisheries Technology Baga hatchery. The fish were acclimatized for two weeks in plastics bowls filled with de chlorinated water in the hatchery, during which they were fed with dried commercial fish feed (BlueCrown) containing 45% crude protein twice daily at 5% body weight. *Clarias gariepinus* as specie was selected based on availability, adaptability to laboratory conditions, convenience, handling size, In-depth knowledge of species biology and ecology, biological significance and economic values. There was 5% mortality recorded during the period of acclimatization. Also, water was changed frequently to avoid accumulation of faecal matter and uneaten feed.

Experimental detergent
Locally made detergent, "GOOD MAMA" manufactured by Eko Supreme Resource Nigeria Limited which has the following ingredients: Linear Alkyl Benzene sulfonate (LABS), Sodium Tripolyphosphate (STPP), Sodium Carbonate Na_2CO_3, Sodium sulphate Na_2SO_4, and perfume; was tested for its toxicity. Different concentrations of the detergent were weighed using a sensitive weighing balance in the laboratory.

Bioassay experimental setup
The bioassay for the acute toxicity test was carried out in twelve plastic bowls of about 70litres. The fishes were not fed for 24hours before the experiment and during the exposure which lasted for 96hours. Five different concentrations for the range test and a control were used for this investigation and each concentration was replicated. The aqueous solution was introduced at different concentrations (0mg/l, 20mg/l, 30mg/l, 40mg/l, 50mg/l and 60mg/l). Twenty *Clarias gariepinus* juveniles were weighed using a sensitive weighing scale with mean weight, 10.00g and mean length of 12.25cm and distributed randomly, twenty in each plastic bowl containing 20litres of water. The behavior and mortality of the test fish in each plastic bowls was monitored and recorded for every 24hours within the 96hours period.

Measurement of physio-chemical parameters of Water
The physio-chemicals parameters of water determined were Temperature, pH, Dissolved Oxygen (DO), Electrical Conductivity and Total Dissolved Solid.
The dissolved oxygen of each treatment was determined using the DO meter, while pH, Temperature, Electrical Conductivity and Total Dissolved Solid were determined using a multi-parameter water checker.

Data Analysis
The 96hours LC_{50} was determined as a probit analysis using the arithmetic method of percentage mortality data. The lower and upper confidence limits of the LC_{50} were determined as described by UNEP (1989) [37]. The data collected was subjected to statistical analysis of variance (ANOVA) for differences between levels of treatment (P>0.05).

The formula used in determining LC_{50}

$$LC_{50}=Dm-\Sigma\frac{(z x d)}{n}$$

Where:
Dm = the largest dose which killed all animals
Z = Mean of dead fish between 2 successive groups.
d = the constant factor between 2 successive dose.
n = Number of animals in each group.
∑ = the sum of (z x d).

Results
The results of the mean mortality rates of the *C.gariepinus* juvenile exposed to detergent (Good mama) are presented in Table 1. This shows the logarithmic probability curves of the mean mortality rates. It was observed during the exposure that the juveniles placed in media devoid of the toxicant survived the 96hours period. The 96hours LC_{50} of *C.gariepinus* juveniles exposed to various concentrations of the toxicant (Good mama detergent) was 37.65mg/l with upper and lower confident limits of 45.24mg/l and 31.33mg/l respectively. The regression equation of the relationship was calculated to be Probit = 3.828log of Conc-1.032 on X and Y square value (r2) of 0.993. The regression equation (r2) valued indicates that mortality rate of the experimental fish and concentration of the toxicant are positively correlated. This shows that the mortality rate of the fish increased with increase in the concentration of the toxicant (Detergent).

Table 1: Mortality of *Clarias gariepinus* juveniles exposed to different concentration of Good mama detergent (linear alkyl Benzene sulphanate)

Concentration (Mg/L)	No of Fish	Total Mortality	% Mortality	Log. Of Conc	Probit Value
0	20	0	0	0	0
20	20	2	10	1.3005	3.95
30	20	6	30	1.4760	4.60
40	20	12	60	1.6023	5.14
50	20	14	70	1.6992	5.40
60	20	16	80	1.7782	5.84

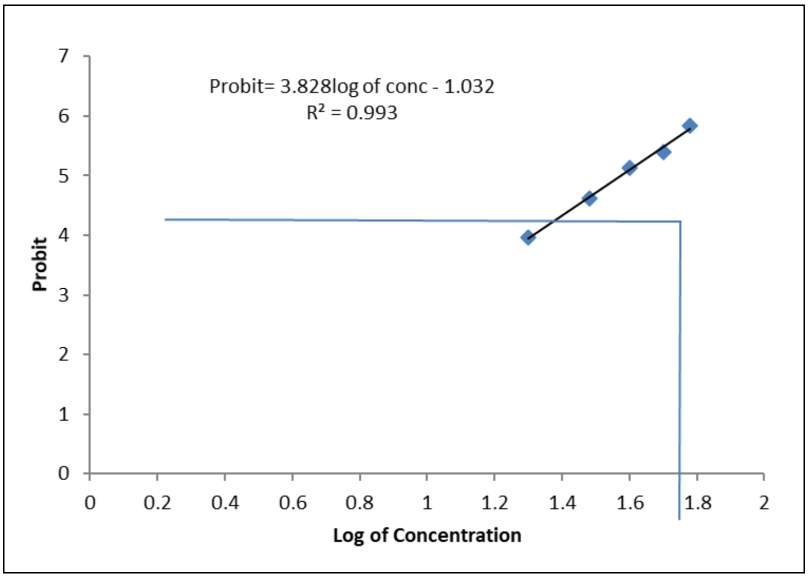

Fig 1: Linear relationship between mean probit mortality and log concentration of *Clarias gariepinus* juveniles exposed to acute concentrations of Good mama detergent (linear alkyl benzene sulphanate) for 96 hours.

Results of the physico chemical parameters of the test water are presented in Table 2. The water quality parameters in the various treatments and replicates fluctuate slightly**.** The dissolved oxygen (DO) values decreases slightly with increase in the concentration of the toxicant. Total dissolved solid (TDS) and Electrical conductivity (EC) values increase slightly with higher increase in the concentration of the toxicant compare to the control. The temperature and pH values did not show much variation. Generally, the water quality parameters determined did not show significant differences (P<0.05) between the various concentrations of the toxicant (Detergent) and the control.

Table 2: The water quality parameters of experimental units during the acute toxicity test of Good mama detergent (linear alkyl benzene sulphanate) on *Clarias gariepinus* juveniles.

Treatments (mg/L)	pH	Temp (°C)	TDS (ppm)	Cond. (µS)	DO (mg/L)
0.0	8.20± 0.01^{f}	24.34± 0.05	355.0±1.00^{f}	712.0±2.00^{f}	4.24± 0.01^{a}
20	8.62± 0.01^{e}	24.41± 0.00	364.5± 0.50^{e}	729.0±1.00^{e}	4.20± 0.00^{b}
30	8.71± 0.01^{d}	24.34 ± 0.05	368.5±0.50^{d}	738.5±0.50^{d}	4.10± 0.00^{bc}
40	8.75± 0.01^{c}	24.31± 0.00	375.0±0.50^{c}	745.0±0.00^{c}	4.09± 0.01^{c}
50	8.83± 0.01^{b}	24.36± 0.05	374.0±0.00^{b}	755.5±0.50^{b}	4.07± 0.01^{d}
60	8.86± 0.00^{a}	24.34± 0.15	380.5±0.50^{a}	763.0±1.00^{a}	4.05± 0.01^{e}
P-value	0.000	0.951	0.000	0.000	0.000

Mean in the same column with different superscripts differ significantly (P<0.05).

Discussion.

The results of *C.gariepinus* juveniles exposed to Detergent show that *C. gariepinus* juveniles of mean weight 10g and 12.20cm mean length were generally sensitive to the detergent aqueous solution. At a concentration of 60mg/l, there was 80% mortality. Mean mortalities of 10, 30, 60, 70 and 80% were recorded in the treatments containing 20, 30, 40, 50, and 60mg/l of the toxicant. No mortality was recorded in the control (0 mg/l). From the results, the 96hours LC_{50} value for *C. gariepinus* juveniles was calculated to be **37.65mg/l.** there was a significant positive correlation between LC_{50} values and the exposure time. Hence LC_{50} of the toxicant increased in concentration per time.

Physical changes observed in *C. gariepinus* juveniles exposed to acute toxicity of detergent include stress, restlessness, loss of balance, gasping for breath, erratic swimming before death; this is in line with the findings of Tawari-Fufeyin *et al.* (2008) [36]; Ololade and Oginni (2010) [33]; Okomoda *et al.* (2010) [32] who made similar observations when they exposed *C. gariepinus* to different toxicants. The stressful and erratic behaviours of the fish in this investigation may signal respiratory impairment and this may be as a result of the effect of the detergent on the gills. This is consistent with the opinion of Ogundiran *et al.* (2009) [28]. Behavioral response of the fish to most toxicants and differences in reaction times, are due to the effects of chemicals, their concentrations, species, size and specific environmental conditions (Bobmanuel *et al.* 2006) [12]. Results obtained from this research revealed that the 96hours LC_{50} for the African catfish (*C. gariepinus)* juveniles exposed to detergent (Good mama) was 37.65mg/l. The result obtained from this research is greater than the 96hours LC_{50} earlier reported for *C. gariepinus* by Ogundiran *et al.* (2009) [28] which was 0.018mg/l for detergent effluents, and Nkpondion *et al.* (2016) [27] who also recorded LC_{50} values' for 96hours acute bioassay test to be 0.11mg/l for toxicity effects of detergent on enzymatic and protein activities of African catfish *C.gariepinus*. while the result is similar to that of Christopher *et al.* (2013) [14] who have reported the median lethal concentration LC_{50} 96hours for *C.gariepinus* (female) and *H. longifilis* (male) fingerlings exposed to Omo and Ariel detergents values range between 33.03-35.19ppm and 37.43-39.79ppm for Omo and Ariel respectively. However, the 96hours LC_{50} value of 37.65mg/l obtained from this study is different from most of the reported literatures which could be attributed to the type of detergent, their concentrations, age and size of the fish, environmental factors, water parameters and selective action of toxicants.

The behavioral alterations that occurred before death in this study may be as a result of nervous impairment due to blockage of nervous transmission among the nervous system and various affected sites, failing organs and retarded physiological processes in the fish body functions as alluded to by Shah and Altindag (2004) [35]. This may have resulted from enzyme dysfunction and paralysis of respiratory muscle or depression of respiratory center and disturbances in energy pathways leading to depletion of energy (Gabriel *et al*., 2010) [19]. Since aquatic organisms are in continuous contact with polluted medium, breathing and feeding is impaired due to the damages to respiratory and other organs of the body.

Conclusion

The 96 hours LC_{50} for the African catfish (*C.gariepinus*) juveniles exposed to detergent (Good mama) has been determined to be 37.65mg/l with upper and lower confident limit of 45.24mg/l and 31.33mg/l respectively.

Hence, it is clear from this result that Good mama detergent is very toxic against the freshwater fish, *C.gariepinus.* It can be concluded that the presence of detergent in aquatic environment causes damages to the tissue and organs, which might make all the living entities in polluted environment vulnerable to disease, and eventually lead to death.

General monitoring of water quality on a regular basis should be made compulsory and as a result, any abnormal changes in the physiology of the aquatic organisms can easily be detected and appropriate action taken before the outbreak of epidemics and eventual death.

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