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Toxicological and Histopathological Responses of African Clariid Mud Catfish, *Clarias gariepinus* (Buchell, 1822) Fingerlings Expose to Detergents (Zip and Omo)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IE and OI designed the study, wrote the protocol, and the first draft of the manuscript. Authors AB and JA managed the analyses of the study and performed the statistical analysis. Author NC managed the literature review/searches. All authors read and approved the final manuscript.

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ABSTRACT

The toxicological and histopathological responses of detergents on *Clarias gariepinus* fingerlings was studied. Twenty-five (25) fingerlings were used for each aquarium exposed to four (4) different concentrations of the two detergents (Zip and Omo) and the control group. The fingerlings were exposed to 0.000, 400, 450, 500 and 550 ppm of the two detergents and the experiment was done in duplicate. A total of 400 fingerlings of *Clarias gariepinus* for each detergent were used throughout the study. The mean fingerlings weight used for the study was 1.7 ± 0.2 g. The mortality data trend of fingerlings exposed to different concentrations of the detergents increased with increasing concentration and duration of exposure. The 96 hours LC_{50} value of Zip and Omo on *C. gariepinus*

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fingerlings were 5.45 and 5.60 respectively. The low LC_{50} value for the fingerlings exposed to the detergents denoted a high toxicity of the detergents. Zip detergent was slightly more toxic to the test organism. Due to high toxicity of the detergents, careless discharge of effluents of detergents should be prevented, and the Government should sensitize its citizen on the lethal and sub-lethal effects of detergent to the aquatic eco-system. Also, the discharge of detergent effluents into our environment should be discouraged, in order to maintain a healthy aquatic environment.

Keywords: Toxicological; histopathological; detergents; Clarias gariepinus; fingerlings.

1. INTRODUCTION

Human and ecological disorder experienced in industrial settlements as a result of improper disposal of chemicals such as detergent effluents has called for careful surveillance on the state of the environment [1]. Detergents are sodium salts of long chain alkyl benzene sulphuric acids or sodium salts of long chain alkyl hydrogen sulphate and differs from soap in the chemical structure in that while detergents have long chain alkyl hydrogen sulphate, soaps on the other hand have long chain carboxylic acids [2]. Surfactants are the components mainly responsible for the cleaning action of detergents [3]. Detergent surfactants are complex organic chemicals with joint hydrophilic and hydrophobic groups in the same molecules. There are various types of surfactants used in detergents formulations; the linear alkyl benzene sulfonate (LAS) - ionic surfactants is the most widely used [4]. Detergents are common household and industrial products and their role as polluting agents cannot be ignored, the validity of a detergent as toxicant depends on three factors: response of the test animal, the substances mode of action and toxicity of the substance in relation to its chemical and physical structure. Fish exposed to detergents show reluctance in food consumption, possibly because they could not identify the palatable nature of food quickly [5]. The cheapness of detergent production from petrochemical sources with its ability to foam when used in acid or hard water, gives it an advantage over soaps [6]. Detergents are extensively applied in industrial and domestic cleaning including laundry, dish water detergents and most often, its likely effects on non-target organisms in the environment is usually ignored. A detergent consists of two parts: The hydrophilic part which readily dissolves in water e.g. SO_3, OH or NH_4 and hydrophobic part which is insoluble in water but soluble in oil [6]. The study is aimed at assessing the toxicological and histopathological responses of detergents on *Clarias gariepinus* fingerlings.

2. MATERIALS AND METHODS

2.1 Collection of the Study Fish

A total of 500 *C. gariepinus* fingerlings with a mean weight of 1.7 ± 0.2 g were purchased from the University of Calabar fish farm. Samples were carefully collected and transferred to a plastic container and transported to the postgraduate research laboratory, Department of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria.

2.2 Acclimation of Fingerlings

Fish specimen were transferred into a laboratory aquarium (80 x 30 x 30 cm^3), each containing 80 litres of water and allowed to acclimate under laboratory conditions of 30.02 ± 0.09 ^{0}C and a pH of 8.0 for two weeks. During this period the fingerlings were fed once daily with commercial feed pellets, Copen, at 5% of their body weight. The unconsumed feeds and faeces were removed regularly from the holding tank and the water in the tank was changed every 24 hours as recommended by [7].

2.3 Purchase of Detergents and Preparation of the Stock Solutions

The Zip and Omo detergents used for the study were purchased from Watt market. A stock solution was prepared by dissolving 1g of Zip and Omo detergents in 1liter of water and then stored in separate 1litre beaker and corked. Serial dilutions of 400, 450, 500 and 550 ppm concentrations for the Zip and Omo detergents were made from the respective stock solutions and utilized as exposure concentrations.

2.4 Range Finding Tests

Range finding tests was carried-out before the commencement of the experiment in order to determine the appropriate concentration range of the soaps that had effects on *Clarias gariepinus* fingerlings. A wide range of concentrations like; x_1, x_2 and x_3 mg/l was tested; including one which killed all organisms within 96 hours and another concentration which did not kill the organisms within 24 hours.

2.5 Toxicity Tests

The fingerlings were exposed to 0.000 (control), 400, 450, 500 and 550 ppm concentrations of the zip and Omo detergents in duplicate. Twenty-five (25) fingerlings of *C. gariepinus* were stocked per group in 25 x 15.5 x 15.5 cm^3 glass aquaria and a total of 500 *Clarias gariepinus* fingerlings and 8 aquaria were used in total for the study (for each detergent).

2.6 Histology of the Gills

After the exposure of the fingerlings for 96 hours, the dead fingerlings were picked and preserved in a 10% formalin. Prior to the histological sectioning, the gills were extracted and impregnated in paraffin wax for easy sectioning with the microtome [8]. The samples were then blocked on wooden blocks to aid microtomy and sectioning was performed with a rotary knife at 5µm and stained with haematoxylin and eosin. Photomicrography of sections was mounted on glass slides and pictures taken with digital motic image capture, Laser microtomy model.

2.7 Probit Analysis

The mortality-concentrations data was subjected to probit transformation, regression analysis and LC_{50} computed using Predictive Analytical Software (PASW) version 20.

3. RESULTS

3.1 Toxic Effect of Detergents

After the exposure of *C. gariepinus* fingerlings to the different concentrations of Zip and Omo detergents, the fingerlings exhibited different behavioural and stress responses such as; erratic swimming, gasping for breath, and piping or frequent surfacing were observed and increased with increase in concentration of the detergents. Over time, the fingerlings became weaker and mortality was recorded for the fingerlings that could not withstand the higher concentrations of the toxicant. The trend mortality of *Clarias gariepinus* fingerlings exposed to detergents increased with increase in the concentration of the toxicants. Mortality data were transformed into probits that were plotted against the concentration. The percentage mortality – concentration relationship was determined by linear regression analysis, and this indicated that mortality rate was concentration dependent (Fig. 1a and 1b). The 96 hours LC_{50} (the concentration that will kill 50% of the fingerlings) for Zip and Omo detergents were 5.50± 6.99 ppm (Fig. 1a) and 5.60 ± 6.85ppm (Fig. 1b) (Table 5), having a lower and upper interval of the of 5.236 to 6.099 for Zip and 2.511 to 9.364 for Omo detergent (Table 5). The regression equations for the probit transformation of *Clarias gariepinus* fingerlings exposed to different concentrations of Zip and Omo detergent were y = 7.4899x - 1.8879 and y = 6.549x – 0.856 respectively (Table 2).

Table 1. Probit transformation/analysis of mortality data of *Clarias gariepinus* exposed to different concentrations of Zip and Omo detergent

Zip detergent								
Conc (ppm)	**Log Conc (x)**	**n**	**r**	**p**	**M_R**	**Y**	**R_P**	***P***
0.00	0.00	25	0	0	0	0.0	0.00	0.00
400	5.991	25	1	0.04	4.0	2.19	-1.19	0.088
450	6.109	25	8	0.32	32.0	5.85	2.15	0.234
500	6.215	25	11	0.44	44.0	10.89	0.10	0.436
550	6.310	25	15	0.60	60.0	15.9	-0.91	0.636
Omo detergent								
Conc (ppm)	**Log Conc (x)**	**n**	**r**	**p**	**M_R**	**Y**	**R_P**	***P***
0.00	0.00	25	0	0	0	0.0	0.00	0.00
400	5.991	25	7	0.28	28.0	6.83	0.175	0.27
450	6.109	25	9	0.36	36.0	9.58	-0.58	0.38
500	6.215	25	12	0.48	48.0	12.59	-0.59	0.50
550	6.310	25	16	0.64	64.0	15.47	0.53	0.62

n = Number of fish fingerling tested at each concentration, r = Number of fish fingerling responding, p = Response rate, r/n, M_R = Mortality rate, Y = Expected probit from visual regression line, R_P = Residual probit, P = Probability

Table 2. Results of regression analysis of Log Concentration – probit relationship of *Clarias gariepinus* fingerlings exposed to concentration of Zip and Omo detergent

Conc. (Log unit)	Response rate, p	Equation	Co-efficient of determination, r^2	Significant level, α
Zip detergent				
0.00	0.00			
400	5.991			
450	6.109	Y = 7.489x – 1.887	0.81	0.05 (S)
500	6.215			
550	6.310			
Omo detergent				
Conc. (Log unit)	**Response rate, p**	**Equation**	**Co-efficient of determination, r^2**	**Significant level, α**
0.00	0.00			
400	5.991			
450	6.109	Y = 6.549x – 0.856	0.71	0.05 (S)
500	6.215			
550	6.310			

Table 3. Chi-square tests of *Clarias gariepinus* fingerlings exposed to different concentrations of Zip and Omo detergent

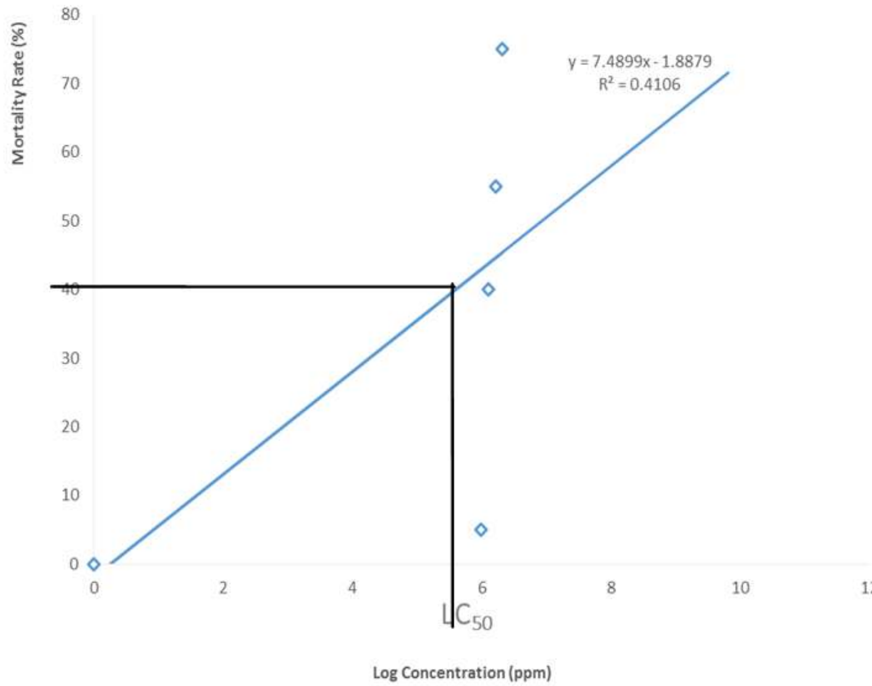
Zip Detergent	Chi square	df[a]	Sig.
PROBIT Pearson Goodness-of-FitTest	1.882	1	0.170[a]
Omo Detergent	**Chi square**	**df[a]**	**Sig.**
PROBIT Pearson Goodness-of-FitTest	0.168	1	0.682[a]

Table 4. Covariance's and correlation of *Clarias gariepinus* fingerlings exposed to different concentrations of Zip and Omo detergent

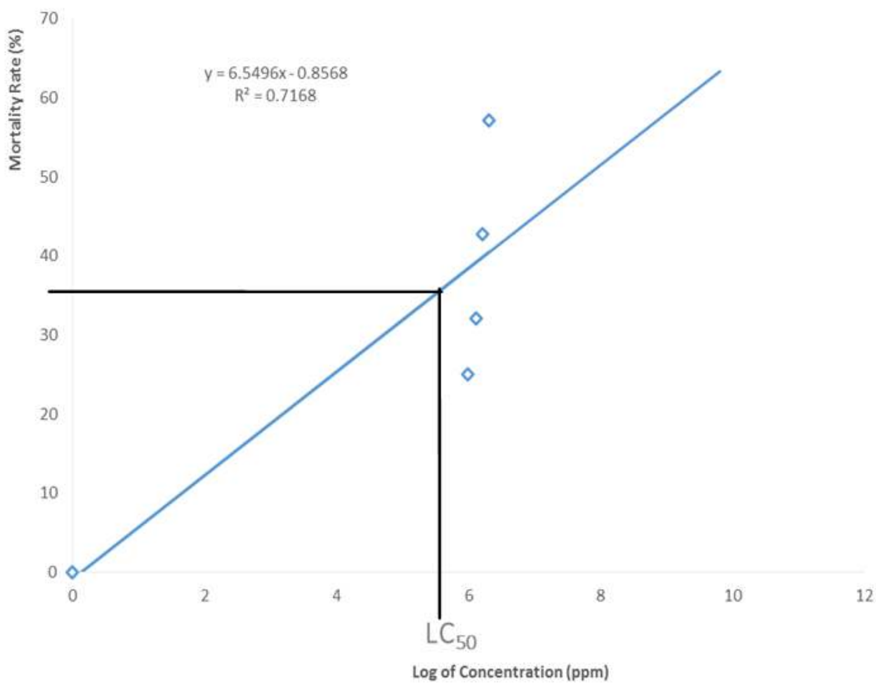
Probit	Concentration	Natural response
Zip detergent		
Concentration	3.184	0.693
Natural Response	0.189	0.023
Omo detergent		
Probit	**Concentration**	**Natural response**
Concentration	9.177	0.890
Natural Response	1.070	0.160

Table 5. Lc_{50} With 95% confidence limits of *Clarias gariepinus* fingerlings exposed to different concentrations of Zip and Omo detergent

LC_{50} WITH ± 95%CL	Confidence limits	
Zip detergent		
	Lower	Upper
5.45 ± 6.99 PPM	5.236	6.099
Omo detergent		
LC_{50} WITH ± 95%CL	**Confidence limits**	
	Lower	Upper
5.60 ± 6.85 PPM	2.511	9.364



(1a)



(1b)

Fig. 1a, b. Probit graph of African Mud Catfish (*Clarias gariepinus*) fingerlings exposed to different concentrations of Zip (2a) and Omo detergent (2b)

3.2 Histopathological Changes

Histopathology examination showed that the gill lamellae of fish in the control group were well distributed without any observed fusion or erosion (Plate 1a and Plate 2a). In the gills of *C. gariepinus* exposed to Omo detergent, fusion of the gill lamellae was observed in 400 ppm (Plate 2b) and hyperplasic gill lamellae cells was observed in 500/550 ppm (Plate 2d). In the gills of *C. gariepinus* exposed to Zip detergent, fusion of the gill lamellae was observed in 400 ppm (Plate 1b), lamellae fusion was observed in 450 ppm, and erosion of lamellae cells/hyperplasia was observed in 500/550 ppm (Plate 1d).

4. DISCUSSION

Detergent finds its way into the aquatic environment through indiscriminate use, careless detergent effluent discharge, accidental spillage or discharge of detergent effluents. Humans make use of this water for various purposes such as drinking, cooking, bathing, washing [9]. The sensitivity of fishes and other water organism towards detergents depends on type of detergent, its concentration and organism species, chemical structure, water, pH and hardness, oxygen concentration and temperature of water [10]. Fish exposed to detergents show reluctance in food consumption, possibly because they could not identify the palatable nature of food quickly [5]. Synthetic detergents are known to cause damage the fish gills, asphyxiation of the fish, growth retardation of juveniles, loss of appetite and abnormal movements [11]. Detergents, including the biodegradable ones may induce poisonous effects and osmo-regulatory imbalances in aquatic organisms especially if present in concentrations that exceed metabolic demand [9]. The study revealed the alterations in the gills of *C. gariepinus* fingerlings compared to the control when exposed to Zip and Omo detergents, and similar observations was reported by [12] who reported that detergent effluents induce severe damage to vital organs in *C. gariepinus* like the gills, kidney, liver, skin, heart and the brain. Zip and Omo detergents caused mortality of the fingerlings, similar findings was also reported by [13, 14, 15, 16, 17] who all reported that contamination of aquatic environment by detergents have great impacts in aquatic organisms such as fishes, snails, sea turtle, and crustaceans. The toxicity of the

Plate 1a. (control)

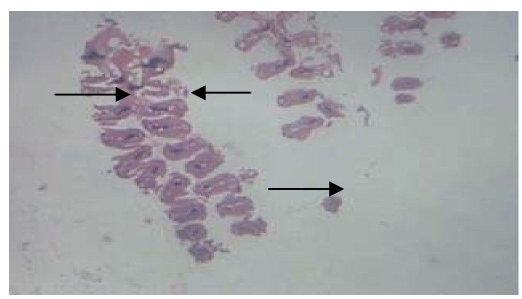


Plate 1b. (400 ppm of Zip detergent)

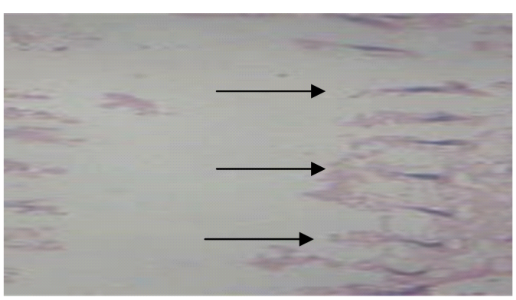


Plate 1c. (450 ppm of Zip detergent)

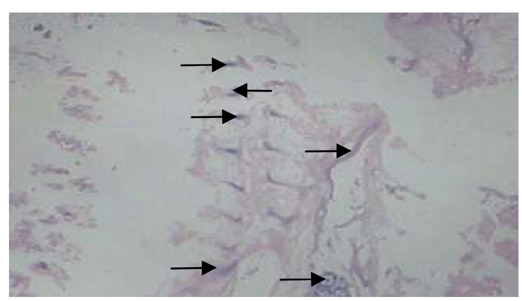


Plate 1d. (500/550 ppm of Zip detergent)

Plate 1a-d. Micrograph of the gills tissues of Clarias gariepinus showing histopathological changes at different concentrations (0.00 – 500/550 ppm) of Zip detergent. X100

detergents on the test fingerlings were concentration dependent, and this corroborated with the report of [18]. In the present study, it was observed that the 96h LC_{50} value were 5.45 and 5.60 ppm for Zip and Omo detergent respectively and similar values were reported by [19,15]. The 96h LC_{50} value of Zip detergent was slightly lower than that of Omo, and this denotes that the Zip detergent is slightly more toxic than Omo detergent. This slight variation in the toxicity of the detergents could be due to the differences in physical and chemical compositions of the detergent surfactants. This discrepancy in toxicity could also be due to the difference in level of toxicity of the toxicant, physical and chemical compositions of the toxicant, solubility of detergent and the reaction of the exposed organism [19,9,1,20]. When the fingerlings were exposed to the detergents, they exhibited different behavioural changes like; incessant jumping and gasping for breath, erratic swimming, piping or frequent surface to bottom movement, restlessness, loss of skin coloration, sudden change of direction during movement, resting at the bottom, loss of equilibrium and gradual onset of inactivity. Similar observations were reported by [21,22,23,1,19,15,9]. This behavioural abnormalities could be due to nervous and respiratory impairment as a result of blockage of nervous transmission between the nervous systems and various effectors sites, enzyme dysfunctions that may induce paralysis of respiratory muscles and/or depression of respiratory center and disturbances in energy or metabolic pathways which results in depletion of energy [19]. The gills of the control group did not show any recognizable changes and consisted of primary filament and secondary lamellae which agrees with findings of [17] for *C. gariepinus* exposed to lethal and sub-lethal concentration of soap and detergents. The histopathological studies of the sectioned gills of *C. gariepinus* during this study showed marked histological alterations after exposure to the various detergents, which were; erosion of secondary gill lamellae, fusion of gill lamellae and hyperplasic gill lamellae cells and this corroborated with the report of [24,25,26] who also reported such anomalies in the gill structure for fingerlings of fish. These abnormalities could be due to the increase in the activities of the test organisms exposed to the changing environment, diffusion distance from surrounding water to capillaries and at the same time and increase in the amount of tissue (blood corpuscles) in the blood spaces of secondary lamellae [17].

Plate 2a. (control)

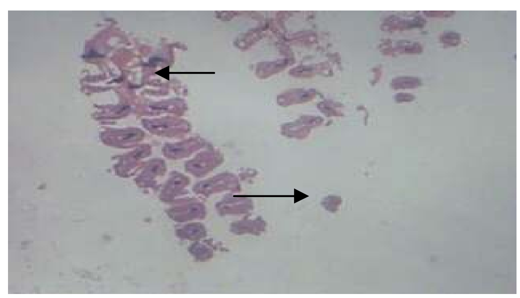


Plate 2b. (400 ppm of Omo detergent)

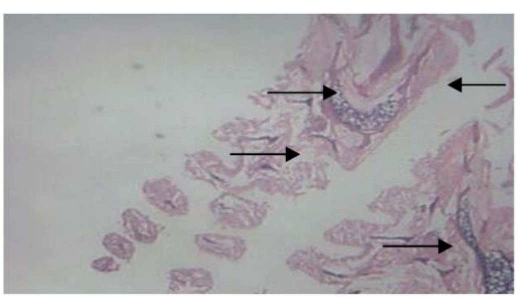


Plate 2c. (450 ppm of Omo detergent

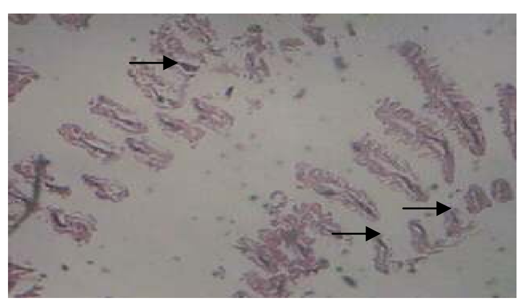


Plate 2d. (500/550 ppm of Omo detergent)

Plate 2a- d. Micrograph of the gills tissues of *Clarias gariepinus* showing histopathological changes at different concentrations (0.00 – 500/550 ppm) of Omo detergent. X100

5. CONCLUSIONS

The study revealed that both detergents were toxic to *Clarias gariepinus* fingerlings, with its toxicity effect increasing with increase in the detergents concentrations. It was also observed that the Zip detergent was slightly more toxic.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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