

Toxicological Effects of different Concentrations of Hypo Bleach on the Survival of African Clariid Mud Catfish (*Clarias gariepinus*) Fingerlings

ABSTRACT

AIM: The study was aimed at studying the toxicological effects of hypo bleach on the survival of *Clarias gariepinus* fingerlings.

METHODOLOGY: Thirty (30) fingerlings were used for each aquarium exposed to four (4) different concentrations of the toxicant and the control group. The fingerlings were exposed to 0, 1.20, 1.25, 1.75 and 1.90 mg/l of the bleach and the experiment was done in duplicate. A total of 300 *C. gariepinus* fingerlings were used throughout the study. The fingerlings were of the same age group, ranging from 9.2 to 10.4 cm in length and 5.5 to 6.5g in weight.

RESULTS: The mortality data trend of fingerlings exposed to different concentrations of the toxicant were dependent on the concentration and exposure duration, with the highest mortality of 96.6% observed in the highest concentration. The 96 hours LC₅₀ value with 95% confidence limit of *Clarias gariepinus* fingerlings exposed to hypo bleach was 0.19 ± 1.73 mg/l, and was significant with a determination coefficient (r^2) of 0.93 at $P < 0.05$. The low LC₅₀ value for the fingerlings exposed to the toxicant showed its high toxicity.

CONCLUSION: The bleach was highly toxic as shown by its very low LC₅₀ value and are capable of causing mortality to organisms even at low concentration. The toxicity of the toxicant was concentration and exposure duration dependent. Also, several behavioural changes was observed in the fingerlings when exposed to the toxicant.

Keywords: *Toxicological, Hypo bleach, survival, Clarias gariepinus and fingerlings*

1. INTRODUCTION

Globally, two million tons of sewage, industrial and agricultural waste are discharged into the world's waterways [1], which empties into the ocean and this have ample effects on marine organisms. The aftermath effects of unregulated discharge of waste water into the coastal water ways are; eutrophication, foul smell, change in biodiversity, impairment of buffering capacity, ecosystem degradation and loss of ecosystem services. Hypo is a registered (proprietary) for Multi-pro enterprise limited Nigeria, the makers of Hypo bleach. The bleaching agent present in Hypo bleach is a chemical compound called sodium hypochlorite. Sodium hypochlorite is produced from the reaction of chlorine with sodium hydroxide. It is unstable compound due to its volatile nature [2]. The stability of sodium hypochlorite is most influenced by parameters like; pH, temperature, sunlight and concentration of certain impurities. Sodium hypochlorite decomposes at pH below 11 and is stable at pH between 12 and 13 [2]. When bleach is dissolved in water, it forms hypochlorous acid, which is responsible for its bleaching effect. Sodium hypochlorite is everyday compound due to its wide usage in homes and industries. Bleach is used for cleaning and disinfection of surfaces such as; toilet, floor and counters. It is also used for the control of smile, algae, odour and destruction of organic matter and removal of ammonia. It is also used in the petro-chemical industry to refine petroleum products [3].

According to [4,5], aquatic environment where fish and other aquatic organisms live, is subjected to different types of pollutants which enter water bodies through industrial, domestic and agricultural discharge systems, thereby introducing stress to living creatures. The study is aimed at evaluating the acute toxicity of Hypo bleach on the survival of *Clarias gariepinus*.

2. MATERIALS AND METHODS

2.1 Bioassay method

The bioassay experiment was carried out in the Department of Zoology and Environmental Biology Laboratory, University of Calabar, Nigeria. The experiment was conducted using standard static bioassay method [6] which involved controlled environmental condition as to define the response of the organism to the test toxicant.

2.2 Collection of test organism and acclimation

The *Clarias gariepinus* fingerlings used for the study were purchased from the University of Calabar fish farm and were immediately transported to Department of Zoology and Environmental Biology Laboratory, University of Calabar, Nigeria. All the fingerlings were of the same age group, ranging from 9.2 to 10.4 cm in length and 5.5 to 6.5g in weight, when measured using measuring board and weighing balance respectively.

In the laboratory, the fingerlings were allowed for two weeks to acclimatize with the laboratory conditions for two weeks, during which they were fed twice daily with 4% of their body weight. The culture water was changed every day in order to maintain adequate environmental conditions. Feeding stopped two days to the commencement of the study.

2.3 Test Toxicant and Range Finding

The test toxicant was purchased from express supermarket along Mount Zion Street. A range finding test was carried-out as described by [7], where the fingerlings were exposed to a wide range of concentrations of the bleach; in order to realize the most appropriate concentrations that has lethal effect of the test fingerlings.

2.4 Preparation of Stock Solution

The stock solution was prepared by dissolving 10ml of the hypo bleach with 990ml of water to have a 1000ml stock solution of the test toxicant, through which serial dilutions was made to 1.20, 1.25, 1.75 and 1.90mg/l of the hypo bleach which the fingerlings were exposed to.

2.5 Experimental Procedure

The bioassay experiment was carried out in duplicate using ten (10) rectangular aquaria. A total of three hundred (300) fingerlings were used for the study, with each aquarium containing 30 fingerlings. Each group of fingerlings was exposed to 1.20, 1.25, 1.75 and 1.90 mg/l and there was also a control group; where no toxicant was introduced. After introduction of the bleach into the culture water, the fingerlings were monitored for mortality after 24, 48, 72 and 96 hours of exposure. The number of death fingerlings were recorded in each cases, and the death fingerlings removed immediately using a scoop net.

2.6 Statistical analysis

The mean of the mortality for the replicate of each toxicant concentration group and the control group was computed. The mortality-concentration data were subjected to probit transformation, regression analysis and LC_{50} values were computed using Predictive analytical software (PASW) version 20. The significance of the slope were tested using chi-square. Graphs were drawn using Microsoft excel version 2013.

3.0 RESULTS

3.1 Toxicological effect of bleach

The summary of the 96hours survival, percentage survival, mortality and percentage mortality of *Clarias gariepinus* exposed to different concentrations of bleach is shown in Table 1. The fingerlings were of the same age group, ranging from 9.2 to 10.4cm in length and 5.5 to 6.5g in weight. In the control (0.00mg/l) concentration of the toxicant, no mortality (100% survivors) was recorded after the 96 hour bioassay. In the 1.20mg/l concentration of the toxicant, 6.66% mortality (93.33% survivors) were recorded. In the 1.25mg/l concentration of the toxicant, 16.66% mortality (83.33% survivors) were recorded after 96 hours. In the 1.75mg/l concentration of the toxicant, 66.66% mortality (33.33% survivors) was recorded, while in the 1.90mg/l concentration, 96.6% mortality (3.33% survival) was recorded (Table 1).

Table 1: 96 Hours. Survival, Percentage Survival, Mortality and Percentage Mortality of *Clarias gariepinus* Fingerlings exposed to different Concentrations of Bleach

Bleach Conc. (ppm)	Survival	% Survival	Mortality	% Mortality
0	30	100	0	0
1.20	28	93.33	2	6.66
1.25	25	83.33	5	16.66
1.75	10	33.33	20	66.66
1.90	1	3.33	29	96.60

3.2 96 hours LC₅₀

The summary of the probit transformation mortality data for *Clarias gariepinus* exposed to different bleach concentration is shown in Table 2. As can be observed, the mortality trend were dependent on the concentrations. The number of survivors decreased with increasing concentration of the toxicant. Similarly, mortality of the fingerlings increased with increasing concentration (Table 1). The fingerlings of *Clarias gariepinus* showed signs of stress and erratic behaviour and gasping for air when exposed to 1.25, 1.75 and 1.90 mg/l of bleach, due to respiratory impairment.

Table 2: 96 Hours Probit Transformation of Mortality Data of *Clarias gariepinus* Fingerlings exposed to different Concentrations of Bleach

Conc (mg/l)	Log Conc (x)	N	R	P	M _R	Y	R _p	P
0	0.00	30	0	0.00	0	0.00	0.00	0.00
1.20	0.079	30	2	0.06	6.00	2.534	-0.534	0.084
1.25	0.097	30	5	0.16	16.00	3.785	1.215	0.126
1.75	0.243	30	20	0.66	66.00	23.284	-3.284	0.776
1.90	0.297	30	29	0.96	96.00	26.688	2.312	0.890

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

Mortality data were transformed into probits that were plotted against the concentration. The percentage mortality – concentration relationship was determined by linear regression analysis (Table 2), and result of the probit transformation revealed that mortality rate increased with increased linearly with increase in concentration of bleach. Regression analysis performed to transformed mortality data showed that mortality depended strongly on concentration (Table 3).

Table 3: Results of regression analysis of 96 Hours Log Concentration–Probit relationship of *Clarias gariepinus* fingerlings exposed to different concentrations of bleach

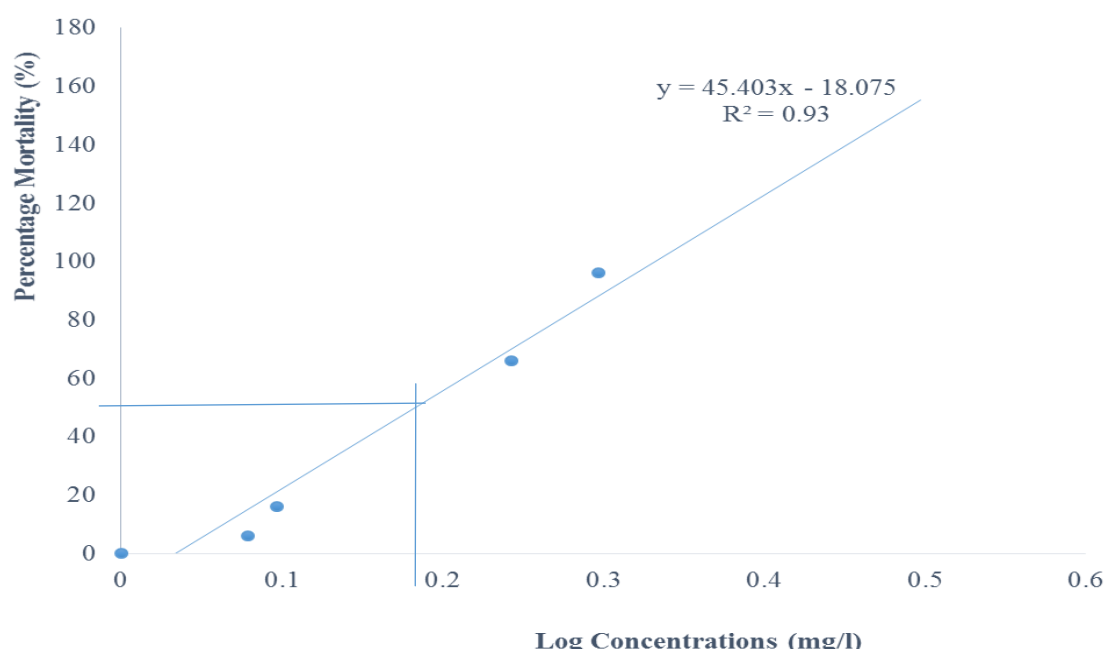
Conc. (mg/l)	Response rate, p	Equation	Co-efficient of determination, r^2	Significant level, α
0.00	0.00	$Y = 45.403x - 18.075$	0.93	0.05 (S)
1.20	0.06			
1.25	0.16			
1.75	0.66			
1.95	0.96			

Table 4: LC₅₀ with 95% confidence limits of *Clarias gariepinus* fingerlings exposed to concentrations of bleach

LC ₅₀ with $\pm$ 95%CL	Confidence limits	
	Lower	Upper
$0.19 \pm 1.73\text{mg/l}$	9.647	16.411

A correlation coefficient and a coefficient of determination (r^2) of 0.93 was obtained for *Clarias gariepinus* fingerlings, indicating a strong, positive linear relationship between concentration of bleach and the fingerlings mortality. The r^2 value of 0.93, for the slope of regression line, which was tested for significance and found to be highly significant ($P < 0.05$). The 96 hours LC₅₀ (the concentration that will kill 50% of the fish) with 95% confidence limit of $0.19 \pm 1.73\text{mg/l}$ was observed (Figure 1) (Table 4), having a lower and upper interval of 9.647 to 16.411 (Table 4). The regression equation for the probit transformation of *Clarias gariepinus* fingerlings exposed to different concentration of the bleach was $y = 45.403x + 18.075$ (Table 3).

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160

161 **Fig 1: Probit transformation graph of *Clarias gariepinus* fingerlings exposed to different**
 162 **Concentrations of bleach**
 163

164 4. DISCUSSION

165 The damage caused by pollution to the environment is irreversible, and has been a hindrance to fish
 166 production in Nigeria, and needs to be controlled. Animals lies within its tolerable zone only below the
 167 threshold, but above the threshold zone is the resistance zone [8]. Toxicants cause mortality of aquatic
 168 organisms and deterioration of the aquatic ecosystem [9,10].

169
 170 Data obtained from this study showed that percentage mortality of the fingerlings was concentration
 171 dependent, increasing with the corresponding increase in the bleach concentration and exposure duration
 172 and this corroborated with the findings of [11,12,13,14]. [15] observed that toxicant in the aquatic
 173 environment may not necessarily result in the outright mortality of aquatic organisms but can result in
 174 several physiological dysfunction in the fish, and a similar situation was observed in the present study.
 175 Several behavioural changes like; skin bleaching, erratic swimming, rapid opecular movement, jumping
 176 out, clustering, inactive movement, loss of balance, incessant gulping for air, slime secretion, lying on one
 177 side and foam release around the mouth were observed when the fingerlings were exposed to the
 178 toxicants due to respiratory impairment, and this corroborated the reports of [16,17,12,13,14]. The
 179 mortality and behavioural changes of *Clarias gariepinus* fingerlings exposed to different concentrations of
 180 bleach in the present study confirms the fact that toxicants causes mortality of aquatic organisms and
 181 deterioration of the aquatic ecosystem [9,10].

182
 183 The low lethal concentration (LC₅₀) value of 0.19 mg/l for bleach on *Clarias gariepinus* fingerlings is an
 184 indication of the high toxicity of bleach showing that 50% of the fingerlings will be killed by such low
 185 concentration of the toxicant. The 96 hours LC₅₀ value of 0.19 ± 1.73mg/l for the toxicant on *Clarias*
 186 *gariepinus* fingerlings at 95% confidence varied from the findings of other scholars. The 0.19 mg/l LC₅₀
 187 observed in this study was lower than the 0.634 mg/l reported by [18] where the toxicity of bleach (sodium
 188 hypochlorate) was tested against *Acanthalburnas microlepis* over a 96 hours period, the 112.20 mg/l
 189 reported by [19] where the toxicity of formalin was tested against *Clarias gariepinus*, the 40.86 mg/l
 190 reported by [20] where *Clarias gariepinus* were exposed to copper sulphate over a 96 hours period of

exposure, the 1.80 ppm reported by [12] where *Clarias gariepinus* fingerlings was tested against cypermethrin over 96hours, the 1.70 ppm reported by [13] where *Raphia hookeri* extracts was tested against *Clarias gariepinus* fingerlings for 96 hours, and the 5.45, 5.60 ppm reported by [14] where ZIP and OMO detergents were tested against *Clarias gariepinus* fingerlings over a 96hour period. These discrepancies in the LC₅₀ values could be due to the differences in the toxicity of the toxicants, fish species and environmental conditions [21].

The behavioural and mortality effect of the toxicant in the study was minimal in the lower concentrations, but became more rapid and pronounced in the 1.75 and 1.90 mg/l concentrations, this was similar to the findings of [22] who reported reduced mortality in exposed fish and linked it to its ability to tolerate low concentration of the toxicant as well as that of [23], who reported that death or physical damages in fingerlings occurs when the concentration of toxic substances is higher than what the homeostasis of the fish can control.

5. CONCLUSION

The study revealed that bleach is highly toxic as shown by its very low LC₅₀ value and are capable of causing mortality to organisms even at low concentration. The toxicity of the toxicant was concentration and exposure duration dependent. Also, several behavioural changes was observed in the fingerlings when exposed to the toxicant.

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