



## Toxicological Effects of Different Concentrations of Hypo Bleach on the Mortality of African Clariid Mud Catfish (*Clarias gariepinus*) Fingerlings

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### Authors' contributions

This work was carried out in collaboration between all authors. Author IEA designed the study, author JAP performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author AAB managed the analyses of the study and author NES managed the literature searches. All authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/AJOB/2017/36001

#### Editor(s):

- (1) P. Dhasarathan, Department of Biotechnology, Prathyusha Engineering College, Anna University, India.  
(2) Tulay Askin Celik, Department of Biology, University of Adnan Menderes, Turkey.

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(3) Fatih Polat, Gaziosmanpaşa University, Almus Vocational School, Turkey.  
Complete Peer review History: <http://prh.sdiarticle3.com/review-history/22211>

Original Research Article

Received 7<sup>th</sup> August 2017  
Accepted 27<sup>th</sup> October 2017  
Published 8<sup>th</sup> December 2017

### ABSTRACT

**Aim:** The study was aimed at evaluating the mortality and LC<sub>50</sub> of *Clarias gariepinus* exposed to different concentrations of hypo bleach.

**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%

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observed in the highest concentration. The 96 hours LC<sub>50</sub> 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<sup>2</sup>) of 0.93 at P<0.05. The low LC<sub>50</sub> value for the fingerlings exposed to the toxicant showed its high toxicity.

**Conclusion:** The bleach was highly toxic as shown by its very low LC<sub>50</sub> 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 behavioral changes was observed in the fingerlings when exposed to the toxicant. As a result of the observed toxicity of hypo bleach to fingerlings of *Clarias gariepinus*, more research should be carried out on the hematological and physiological alterations of fingerlings when exposed to bleach.

**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 slime, algae, odour, 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. Bleach is toxic to biological organisms causing mortality, physiological, haemathological alterations. In

human, it causes abdominal pain, burning sensation, cough, Diarrhoea, sore throat and vomiting. *Clarias gariepinus* fingerlings was used for this study because it is the most common fingerlings in the country and has been used by several scholars for toxicity testing. The study is aimed at evaluating the mortality and LC<sub>50</sub> of *Clarias gariepinus* exposed to different concentrations of Hypo bleach.

## 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, transferred to a transparent rectangular plastic container of 60 x 30 x 60 cm<sup>3</sup> 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, which were measured using measuring board and weighing balance to the nearest centimeters (cm) and grams (g) respectively.

In the laboratory, the fingerlings were transferred into a laboratory aquarium (80 x 30 x 30 cm<sup>3</sup>), each containing 80 litres of water and allowed to acclimate with the laboratory conditions of 30.02

$\pm 0.09$  °C and a pH of 8.0 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. The composition of the hypo bleach used was sodium chlorate, caustic soda, di-mineralized water and soda ash. 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 on the test fingerlings.

### 2.4 Preparation of Stock Solution

The hypo bleach commercial solution had a 3.5% weight per volume. 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 using a measuring cylinder, through which serial dilutions was made to 1.20, 1.25, 1.75 and 1.90 mg/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. Also, behavior responses to stress such as erratic swimming, gasping for air, frequent surfacing and loss of balance of the fingerlings were also monitored. 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<sub>50</sub> 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. RESULTS

### 3.1 Toxicological Effect of Bleach

The summary of the mortality data of *Clarias gariepinus* fingerlings exposed to different concentrations of hypo bleach for 24, 48, 72 and 96 hours is shown in Table 1. The mortality of the fingerlings increased with increase in the duration of the exposure to the toxicant. In the control group, no mortality was recorded, but for the 1.20 mg/l exposed group mortality trend of 0, 0, 1 and 1 were recorded after 24, 48, 72 and 96 hour exposure respectively. For the 1.25 mg/l group, 0, 1, 2, 2 and 2 were recorded after 24, 48, 72 and 96 hour exposure respectively. The group exposed to 1.75 mg/l of bleach recorded 2, 3, 7 and 8 mortality after 24, 48, 72 and 96 hour exposure respectively. In the 1.90 group, an increasing mortality trend of 3, 5, 9 and 12 was recorded after 24, 48, 72 and 96 hour exposure period respectively (Table 1).

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 2. The mortality of the fingerlings exposed to the hypo bleach was concentration dependent, with the fingerlings mortality increasing with increase in bleach concentration (Table 2).

**Table 1. Mortality data of *Clarias gariepinus* fingerlings exposed to different concentrations of hypo bleach for 24, 48, 72 and 96 Hours**

Bleach concentration (mg/l)	24 Hours	48 Hours	72 Hours	96 hours
0	-	-	-	-
1.20	-	-	1	1
1.25	-	1	2	2
1.75	2	3	7	8
1.90	3	5	9	12

**Table 2. 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

In the control (0.00 mg/l) group, no mortality (100% survivors) was recorded after the 96 hour bioassay. In the 1.20 mg/l concentration of the toxicant, 6.66% mortality (93.33% survivors) were recorded. In the 1.25 mg/l concentration, 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 1.90 mg/l concentration, 96.6% mortality (3.33% survival) was recorded (Table 2).

### 3.2 96 Hours LC<sub>50</sub>

The summary of the probit transformation mortality data for *Clarias gariepinus* exposed to different bleach concentration is shown in Table 3. The fingerlings of *Clarias gariepinus* showed signs of stress and erratic behavior like; skin bleaching, erratic swimming, rapid opercular movement, jumping out, clustering, inactive movement, loss of balance, incessant gulping for air, slime secretion, lying on one side and foam release around the mouth was observed when exposed to 1.25, 1.75 and 1.90 mg/l of bleach, due to respiratory impairment.

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

analysis performed to transformed mortality data showed that mortality depended strongly on concentration (Table 4).

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 was found to be highly significant ( $P < 0.05$ ). The 96 hours LC<sub>50</sub> (the concentration that will kill 50% of the fish) with 95% confidence limit of  $0.19 \pm 1.73$  mg/l was observed (Fig. 1) (Table 5), having a lower and upper interval of 9.647 to 16.411 (Table 5). 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 4).

## 4. DISCUSSION

The damage caused by pollution to the environment cannot be over-emphasized, and has been a hindrance to fish production in Nigeria, and needs to be controlled. Animals well-being lies within its tolerable zone only below the threshold of the toxicant, but above the threshold zone its resistance to toxicant is bridged [8]. Toxicants cause mortality of aquatic organisms and deterioration of the aquatic ecosystem [9,10].

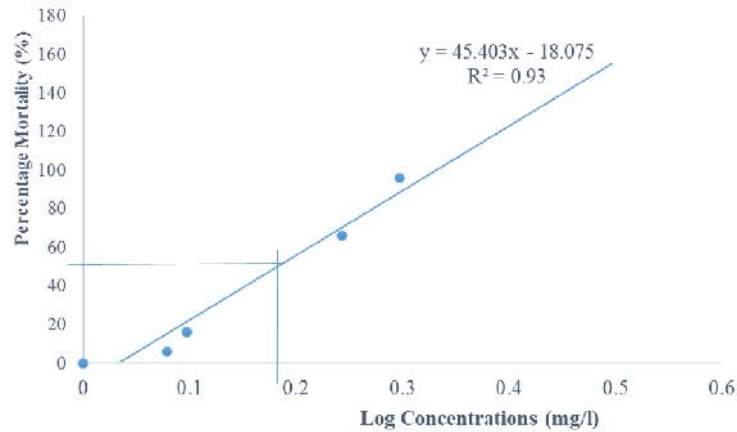
**Table 3. 96 Hours probit transformation of mortality data of *Clarias gariepinus* fingerlings exposed to different concentrations of bleach**

Conc (mg/l)	Log Conc (x)	N	R	P	M <sub>R</sub>	Y	R <sub>p</sub>	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<sub>R</sub> = Mortality rate, Y = Expected probit from visual regression line, R<sub>p</sub> = Residual probit, P = Probability

**Table 4. 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 <sup>2</sup>	Significant level, α
0.00	0.00			
1.20	0.06			
1.25	0.16	Y = 45.403x - 18.075	0.93	0.05 (S)
1.75	0.66			
1.95	0.96			



**Fig. 1. Probit transformation graph of *Clarias gariepinus* fingerlings exposed to different concentrations of bleach**

**Table 5. LC<sub>50</sub> with 95% confidence limits of *Clarias gariepinus* fingerlings exposed to concentrations of bleach**

LC <sub>50</sub> with ± 95%CL	Confidence limits	
	Lower	Upper
0.19 ± 1.73 mg/l	9.647	16.411

Data obtained from this study showed that percentage mortality of the fingerlings was concentration and duration dependent. The percentage mortality increased with corresponding increase in the bleach concentration and exposure duration which corroborated with the findings of [11,12,13,14]. [15] observed that toxicant in the aquatic environment may not necessarily result in the outright mortality of aquatic organisms but can result in several physiological disfunction in the fish, and a similar situation was observed in the present study. Several behavioral changes like; skin bleaching, erratic swimming, rapid opercular movement, jumping out, clustering, inactive movement, loss of balance, incessant gulping for air, slime secretion, lying on one side and foam release around the mouth were observed when the fingerlings were exposed to 1.25, 1.75 and 1.90 mg/l concentration of hypo bleach due to

respiratory impairment, and this corroborated the reports of [16,17,12,13,14]. The mortality and behavioural changes of *Clarias gariepinus* fingerlings exposed to different concentrations of bleach in the present study confirms the fact that toxicants causes mortality of aquatic organisms and deterioration of the aquatic ecosystem [9,10].

The low lethal concentration (LC<sub>50</sub>) value of 0.19 mg/l for hypo bleach on *Clarias gariepinus* fingerlings is an indication of its high toxicity as shown by the mortality of 50% of the fingerlings at such low concentration of the toxicant due to the sodium chlorite, caustic soda, di-mineralized water and soda Ash composition of the hypo bleach. The 96 hours LC<sub>50</sub> value of 0.19 ± 1.73mg/l for the toxicant on *Clarias gariepinus* fingerlings at 95% confidence varied from the findings of other scholars. The 0.19 mg/l LC<sub>50</sub> observed in this study was lower than the 0.634 mg/l reported by [18] where the toxicity of bleach (sodium hypochlorate) was tested against *Acanthalburnus microlepis* over a 96 hours period, the 112.20 mg/l reported by [19] where the toxicity of formalin was tested against *Clarias gariepinus*, the 40.86 mg/l 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 96 hours, 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 respectively were tested against *Clarias gariepinus* fingerlings over a 96hour period. Also the 0.19 mg/l 96 hour LC<sub>50</sub> value of hypo bleach in this study was lower than the 1.39 mg/l value for *Lepomis macrochirus*, 4.8 mg/l for *Pimephales promelas* but higher than the 0.032 mg/l for *Oncorhynchus kisutch*, 0.065 mg/l for *Clupea harengus*, 0.09 mg/l for *Leiostomus xanthurus* reported by [21] over a 96 hour exposure period. These discrepancies in the LC<sub>50</sub> values could be due to the differences in the toxicity of the toxicants, fish species and environmental conditions [22]. It could also be due to the difference in the composition of the different bleaches used compared to that of the present study.

The behavioral 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 [23] 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 [24], 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 LC<sub>50</sub> 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 behavioral changes was observed in the fingerlings when exposed to the toxicant. As a result of the observed toxicity of hypo bleach to fingerlings of *Clarias gariepinus*, more research should be carried out on the hematological and physiological alterations of fingerlings when exposed to bleach.

## ETHICAL CONSIDERATION

The authors ensured that the all the ethical and other basic principles underlying behavior and advancing welfare for the use of animals in

research including handling, relevant laws and regulations were considered before proceeding with the research.

## ACKNOWLEDGEMENTS

Authors acknowledge Prof. Albert Ekanem of the Institute of Oceanography, University of Calabar, for providing the laboratory facilities for carrying out this research work.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:

The peer review history for this paper can be accessed here:  
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