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HAEMATOLOGICAL ASSESSMENT AND PISCICIDAL EFFECT OF SODIUM HYPOCHLORITE ON JUVENILE *HETEROBRANCHUS BIDORSALIS* (GEOFFROY ST. HILIARE, 1809)

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ABSTRACT: *Toxicity bioassays are often used in aquatic ecotoxicology and the main objective of such test is to determine the lethal concentration of toxicants for aquatic organisms. Hematological indices are used to determine the health status of fish. In this study juvenile Heterobranchus bidorsalis of 32.9- 46.4 cm in length and 251.9-298.7g in weight was exposed to different concentrations of Sodium hypochlorite (0.0, 0.5, 1.0, 1.5, 2.0 ml) for 96hrs to assess its toxicity and investigate the effect of the toxicant on the hematological parameters of the fish. Mortality and pattern of response of fish in all the exposure concentrations increased with duration of exposure. The hematological results showed a decrease in Packed Cell Volume (23.00±2.83- 16.50 ± 2.12%), Hemoglobin (7.65± 0.92 – 5.05± 0.07g/mm^3) and RBC (2.55 ±0.35- 1.35±0.42) while there was an increase in white blood cell (6.00 ± 2.83± 8.55 ± 7.78 (10^4/ mm^3)). There was no significant difference (P<0.05) in the value of Mean cell hemoglobin concentration, Mean cell volume and Mean corpuscular Hemoglobin concentration. This can be use as an index of toxicity in water to determine the health of an aquatic organism.*

KEYWORDS: heamatology, piscidal, sodium hypochlorite, *heterobrachus bidorsalis*

INTRODUCTION

The recent past decades were a period when many nations and people were conscious of the economic, and recreational roles of fish and the health benefit. Consequently, fish Demand has been on the increase, growing at variance with its supplies but symmetrically with human population industrial and economic growth rates.Fisheries occupies an important position in the agricultural sector of the Nigerian Economy, partly because she is naturally endowed with vast network of rivers, flood, plains, Natural, man -made lakes, creeks, swamps and lagoons. Presently, the total fish requirement of Nigerian is estimated at about 1.5 million tonnes / year (FDF, 1998). Aquatic toxicology is the study of the effects of environmental contaminants on aquatic organisms, such as the effect of pesticides on the health of fish or other aquatic organisms. The capability of a substance to harm fish and other aquatic organisms is determined by its; toxicity, exposure time, dose rate, and persistence in the environment. Almost every activity leaves behind some kind of

waste in the environment. Some wastes contain chemicals that are hazardous to people and the environment. Once these hazardous chemicals are present in the environment, either aquatic or terrestrial, aquatic organism or people can become exposed to them. The effect is much in the aquatic because fish live all their life in the water hence the toxic substance accumulates and cause future problem for the fish. Some chemicals are of potential health concern because of their toxicity and their ability to accumulate in fish tissue. Harmful chemicals also enter the environment through natural processes, industrial and agricultural use, mining, spills, and improper disposal. Fish can take in these chemicals from what they eat or the water in which they live.

Chlorine is a chemical substance used to reduce levels of coliform bacteria and some other pathogens in drinking water thus it can also be used to reduce the levels and numbers of pathogens in water used for fish production (Potts and Jolly., 1998). Chlorine acts as a powerful oxidizing agent and it is toxic to fish at concentrations of less than 0.05 mg/L. Residual chlorine in municipal water supplies is normally between 0.5 and 2.0 mg/L (Parker, 2002). Fish exposed to chlorine lose color and develop non-specific signs of respiratory difficulty as a result of damage to their gill epithelium (Stoskopf, 1993).

Heterobranchus specie is one the common specie of fishes that are most widely cultured fish in Nigeria because of their fast growth rate, efficient use of natural aquatic feeds, omnivorous food habits, resistance to diseases and handling, ease of reproduction in captivity and tolerance to wide range of environmental conditions.

Despite the widely use of chlorine, a major constituent of sodium hypochlorite for water treatment and the hypo itself to remove strong stains from cloth, little work has been done on its piscidal effect on some freshwater fish like *Heterobranchus bidorsalis* when it finds its way into body of waters through flooding and drainages after washing. Hence, this study is aimed at determining the piscidal and haematological effects in *Heterobranchus bidorsalis* juveniles exposed to sodium hypochlorite, so as to ascertain their level of tolerance and their suitability as bio-indicator in freshwater ecosystems.

MATERIALS AND METHODS

The experiment was carried out in the fisheries laboratory of JABU using Plastic tank of 75 cm x 40 cm x 40 cm; 50L capacity. Each tank was filled with 30 litres of unchlorinated water. Apparently healthy juvenile catfish *H. bidorsalis* (32.9-46.4 cm length and 251.9-298.7 g weight) were collected at a private fish farm Lagos. The fishes were acclimated to the laboratory condition for two weeks. The fish were fed with coppens fish feed during the acclimation period. Feeding was discontinued 24hours before the commencement of the experiment to minimize the production of waste in the test container. The Sodium Hypochlorite was introduced with 5ml syringe.

Toxicity Test

Range finding test: Preliminary 24h range finding test was conducted to determine the toxic range of sodium hypochlorite to juveniles of catfish *H. bidorsalis*, following static bioassay procedure. The fish were batch weighed and distributed into a set of 12 rectangular glass tanks (75x45x45

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cm) each filled with 30L unchlorinated water. Sodium hypochlorite was introduced at different concentration of 1, 2, 3, 4, 5, to make Six treatments. Each of the test solutions was introduced directly into the plastic tanks in a single dose, representing six replicates treatments per fish. The behavior and mortality of the test fish in each tank were monitored and recorded
every 15 min. for the first one hour, every 1h for the next four hours, and every four hours for the rest of the 24 h period.

Definitive test: Based on the results from the range finding (Lethal toxicity) test described above, 96h definitive tests was carried out, following static bioassay procedures described by Parish (1985) . Batches of seven juveniles *H. bidorsalis* were batch weighed and distributed into a set of 12 rectangular glass tanks (75x45x45 cm) each filled with 30L of Unchlorinated water. Five test solutions of sodium hypochlorite 0.0, 0.5, 1, 1.5, 2.0ml as earlier determined from the range finding test was introduced in a single dose directly into the glass tanks. The test fishes were not fed throughout the 96 h test. The behavioral pattern and mortality of the test in each tank was monitored and recorded every 15min for the first one hour , every hour for the next four hour, once every four hours for the next 24h and once every 24h for the rest 96h. Dead fish was removed immediately with scoop net to avoid contamination due to rotting.

Haematological analysis: Blood (1-3ml $fish^{-1}$) was collected from the fish after 96h of exposure. Collection of blood was done through the vertebral caudal blood vessel with the help of disposable hypodermic syringe and needle. Blood sample was emptied into 10ml sample bottle treated with anticoagulant, Ethylene Diamine Tetracetic Acid (EDTA). Haematological analysis of fish followed the method described by Svobodova *et al*. (1991). Blood cell count (erythrocytes and leucocytes) was carried out in an improved Neubaeurhaemocytometer using a modified Yokoyama diluting fluid. The basic eryhrocyte indices, Mean Cell Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) were computed from hemoglobin values and erythrocyte count.

Water quality analysis: Water quality monitoring was done prior, during, and after the experiment. pH was determined using a digital pH meter (Mettle Toledo, 320). Dissolved Oxygen (DO) was measured using a digital dissolved oxygen meter (Jenway 9071). While, Temperature was measured using a mercury glass thermometer and the conductivity using conductometre.

Statistical analysis: All results were collated and analyzed using computerized probit and logit analysis (Lichtfield and Wilcoxon, 1949). The median lethal concentration, at selected period of exposure and an associated 95% confidence interval for each replicate toxicity test, was subjected to logit and probit analysis (Finney, 1971) MINITAB (release 14.) and hematological and proximate statistical analysis using SAS.

Calculation of lethal concentrations and statistics: The mortality rates observed during the stipulated exposure periods were recorded and used for calculation of $96hLC_{50}$ (Finney 1971; USEPA 2000) method along with slope values.

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RESULTS

Lethal concentrations: The respective LC_{50} of sodium hypochlorite at various stages of administration to juvenile *H. bidorsalis* are presented on Fig. 1. The estimated LC_{50} lie within the 95% confidence limits and are given along with the slope and F-values. LC_{50} are found to decrease with increasing exposure periods. The LC_{50} represent the concentration at which 50% of the fish population will be killed when exposed to sodium hypochlorite. From the graph below, the LC_{50} is 1.3437ml of sodium hypochlorite. Therefore, the chemical is very toxic to the juvenile of *Heterobranchus bidorsalis.*

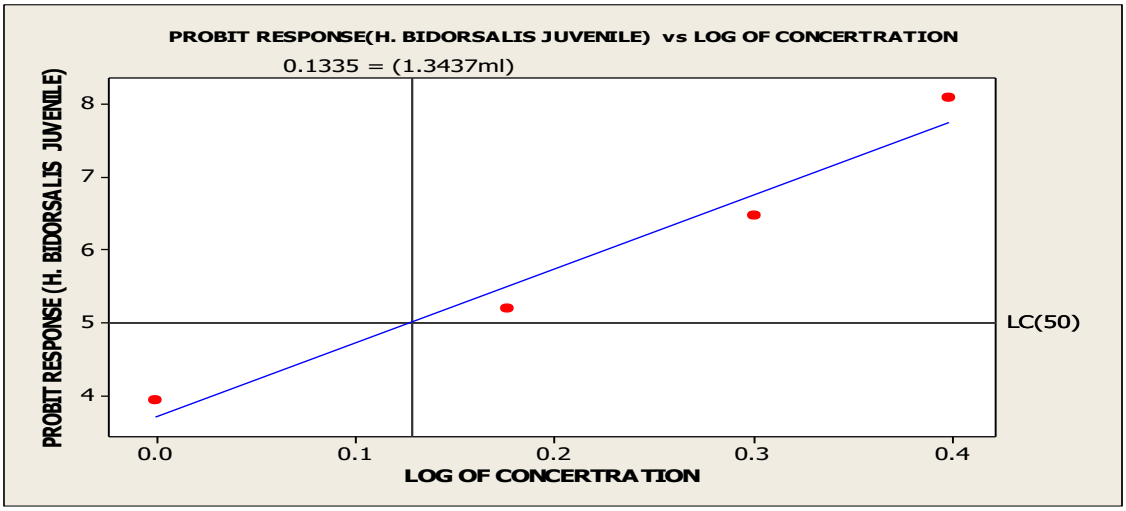

Fig 1: Log of concentration of sodium hypochlorite and its probit value for juvenile *H. bidorsalis*

Behavioral changes: The fish show some behavioural changes at the introduction of the toxicant, such as erratic swimming, air gulping, barbel deformation, molting, and excessive mucus secretion. None of the control fish showed any of these behavioral changes. This is presented in Table 1 The dying ones in the experimental aquaria exhibited vertical positioning with head above the water surface. Finally they lost balance, settled at the bottom of the aquaria and died. No mortality was observed in the control groups.

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Table 1: Behavioural changes observed in the *H. bidorsalis* exposed to Sodium hypochlorite

Behavior/ Exposure Time	24hrs					48hrs				
Concentrations	0.0	0.5	1.0	1.5	2.0	0.0	0.5	1.0	1.5	2.0
Loss of reflex	-	-	-	-	+	-	-	+	-	+
Air gulping	-	+	+	+	+	-	+	+	+	+
Erratic swimming	-	-	+	+	+	-	-	+	+	+
Barbel deformation	-	-	+	+	+	-	-	+	+	+
Excessive mucus	- +	+	+			- +	- +	+		
Molting	-	+	+	-	-	-	+	+	-	+

Keys: + reaction,
- No reaction

Haematological studies: There was significant reduction ($p<0.05$) in the value of blood parameters of *H. bidorsalis* after exposure to sodium hypochlorite for 96h (Table 2). Pack cell volume reduces from 23.00±2.83 % to 16.50±2.12%, heamoglobin reduces from 7.65±0.92g/mm^3 to 5.05 ±0.07 g mm^{3,} Red blood cell reduces from 2.55 ±0.35(10^6 mm^3) to 1.35 ±0.42(10^{6}mm^3) in the highest concentration White blood increased from 6.00±2.83 to8.55^a±7.78 . No significant difference was noticed in the Mean cell haemoglobin concentration ($p<0.05$), Mean cell volume and mean cell haemoglobin.

Table 2: Haematology Means Separated by DUNCAN New Multiple Range Test (DNMRT) p<0.05

Conc./ parameters	T_1(0.0ml)	T_2(0.5ml)	T_3(1.0ml)	T_4(1.5ml)	T_5(2.0ml)
PCV(%)	23.00^a±2.83	21.00^{ba}±2.12	22.50^a±0.71	20.50^{ba}±2.12	16.50^b±2.12
Hb(g/mm^3)	7.65^a±0.92	7.20^a±0.71	7.50^a±0.14	6.80^{ba}±0.71	5.05^b±0.07
WBC(10^4/mm^3)	6.00^a±2.83	6.10^a±9.90	6.70^a±9.90	6.80^a±2.83	8.55^a±7.78
RBC(10^6/mm^3)	2.55^a±0.35	2.35^a±0.21	2.48^a±0.11	2.28^a±0.25	1.35^b±0.42
MCH(pg)	30.05^a±0.49	30.60^a±0.28	30.30^a±0.71	29.85^a±0.21	30.80^a±0.14
MCHC(T/T)	33.25^{ba}±0.07	33.45^{ba}±0.07	33.30^{ba}±0.42	33.15^b±0.07	33.75^a±0.35
MCV(μ)	90.10^a±1.27	90.55^a±0.49	90.85^a±1.06	90.05^a±0.49	90.45^a±0.64
N	60.00^a±2.83	64.50^a±4.95	65.00^a±1.41	64.50^a±6.36	70.50^a±0.71
L	39.00^a±1.41	34.50^{ba}±6.36	34.00^{ba}±0.00	35.00^{ba}±7.07	22.50^b±3.54
M	2.00^{ba}±0.00	1.50^{ba}±0.00	1.50^{ba}±0.00	1.00^b±0.00	3.00^a±1.41

Note: Means with the same column followed by the same letter are not significantly different from each other.

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The LC_{50} decrease with increase in the concentration of the toxicant and also the mortality increases with increase in the concentration of sodium hypochlorite, therefore the death of the fish could be as a result of the presence of high concentration of chlorine in the toxicant used. These values and the trend of the result for juvenile *H. bidorsalis* catfish compared well with those from literature on other toxicants such as Chlorpyrifos , Nickel, insecticide(ethofenprox) to some fish (Ali-Gul,2000; Ololade and Oginni, 2010 and Muniyan and Veeraraghavan,1999).

The result of the $96hLC_{50}$ of this study is also similar to what was obtained by some researchers such as Oladimeji and Ofem, 1989; Ayotunde and Ofem, 2005, 2008; Ayotunde *et al*., 2010 from different toxicant on Clarias fish. Acute toxicity (100% death) occurred at 2.5ml/30L at 48h exposure period. According to Cagauan *et al*. (2004), concentration causing 100% mortality forms the basis of calculating the piscicidal activity of the toxicant.

The obtained $96LC_{50}$ is also comparable to those of diazinon (Adedeji *et al*., 2008), phenol (Cowgill and Milazzo, 1991) and tetrachloromethane (LeBlanc, 1980) but at lower concentrations than those of Benzene (Canton and Adema, 1978), Methanol (Tong *et al*., 1996) and Acetonitrile (Guilhermino *et al*., 2000). The $96hLC_{50}$ value of 1.344ml obtained from this work is higher than 0.04mg/l that was obtained by Olufayo and Akinpelumi (2012) for *Heterobranchus bidorsalis* when exposed to *M. oleifera* extract and that of Hybrid catfish (Heteroclarias) on exposure to textile effluent (Nwanna et al,2000) . Therefore the mortality rates observed in the present study suggests a clear relationship between dose, mortality and exposure period. The concentration of the toxicant is directly proportional to the mortality rate. On the other hand, as the duration of exposure increases the lethal concentration decreases.

Studies revealed that organisms exposed to toxicants usually exhibit changes in opercula rate, erratic and sudden jerky swimming movements and different behavioral activities which is demonstrated to be a sensitive indicator of physiological stress in fish subjected to sub-lethal concentration of pollutants (Maikai *et al*., 2008). The fish in the test medium shows some abnormal signs such as Loss of reflex, Molting, Air gulping, erratic movement, Babel deformation and Excessive mucus secretion. The behavioral responses obtained from the study compared favorably with the observation of Pascual *et al*. (1994) when formalin at different concentrations were used on sea bass (*Lates calcarifer*) fry and *M. oleifera* on *heterobrachus bidorsalis* (Olufayo and Akinpelumi (2012).The exhibited clinical signs and eventual deaths of exposed fish may be due to direct poisoning leading to pathological alterations in their tissues and organs(Gabriel, *et al*, 2007; Mohammed, 2008) or indirectly due to changes in the physicochemical conditions of their immediate external environment(Abalaka and Auta, 2010).

Haematological indices have been reported to indicate secondary responses of an organism to irritants (Rogers *et al*., 2003). The changes in the value of blood parameters of juveniles *H. bidorsalis* after exposure to 96 h in sodium hypochlorite in this experiment is similar to the results obtained from the work of Saleh *et al*. (1998) who studied the effect of inhalation of the pyrethroidinsecticide, tetramethrin, on hematological and biochemical parameters in albino rats. Cruz *et al* (1988) state the importance of haematology in fish disease and assessment of the effects of pollution has been widely accepted. The result of haematological parameters (Table2) of

juveniles *H. bidorsalis* showed significant differences in higher concentrations after 96h of exposure. Exposure of juveniles *H. bidorsalis* to acute concentration of sodium hypochlorite caused a significant ($p<0.05$) decrease in packcell volume (PCV), Haemoglobin (Hb) and erythrocyte(RBC) of the fish. PCV increased from 23.00± 2.83 -16.50± 2.12; Hb (7.65± 0.92-5.05± 0.07) and RBC (2.55± 0.35 -1.35± 0.42). Similar reduction was reported by Adeyemo, 2005, Okomoda *et al* (2010) and Aderolu *et al* (2010), when they exposed fish to pollutants under laboratory conditions. The significant reduction in these parameters could be indication of severe anemia caused by destruction of erythrocytes (Omoniyi *et al* , 2002; Kori- siakpere *et al* 2011) Haemodilution (Adeyemo,2005 and Ayuba,2008) resulting from impaired osmoregulation across the gill epithelium and it could be as a result of the destruction of intestinal cells caused by chlorite, hydroxide and sodium which are the main constituent of the toxicant. Decrease in RBC could also be as a result of haemodilution through haemolysis (Gardner & Yevich,1970), these observation is similar to what was earlier observed by Mason *et al* (1994). WBC increased from 6.00 ± 2.83-8.55± 7.78, this could be as a means of fighting against the presence of the toxicant in the blood since WBC function as an Antigen that fight any unwanted microorganisms or infections in the body. Increase in WBC counts and lymphocytes percentage with the decrease in blood platelets agrees with the findings of Saleh *et al*., 1998 on albino rat. The result of this work is similar to the work of Ferrira *et al* (1981) who reported an increase in haematocrit level of *Cyprinus carpio* anaesthetized with benzocaine hydrochloride. The MCHC, MCH and MCV, show no significant changes $p<0.05$ from the control, this is supported by Okomoda *et al* (2010).

Therefore in conclusion, the toxicity effect of sodium hypochlorite on juvenile catfish *H. bidorsalis* had a positive correlation with exposure. From the toxicity tests sodium hypochlorite concentration value of 1.0 ml – 1.5 ml in the medium can be potentially hazardous to some fish species in freshwater. Therefore, acute toxicity data of the present study provide baseline information needed to develop models of sodium hypochlorite effects on ecological systems. More information is needed to assess their potential impacts on aquatic environment.

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