Toxicity of Zinc and Copper on *Heterobranchus bidorsalis* Geoffroy Saint-Hilaire, 1809 Juveniles

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Abstract

Zinc and Copper released into the environment, through mining and waste combustion in Nigeria, are major threats to fish production. In this study, the sub-acute toxicity of Zinc and Copper to a commercially important fish species was investigated. Three hundred and fifty *Heterobranchus bidorsalis* (mean weight: 13.5±1.0g, total length: 15.0±0.5cm) were exposed to four different concentrations of copper and zinc for 96 hours in a static renewal bioassay to assess lethal concentration (LC_{50}). The toxicity on fish was assessed using hematological and histopathological indices. The Median Lethal Concentration (LC_{50}) was determined using probit method. The LC_{50} for copper and zinc were 15.03mg/L and 324mg/L, respectively. Death of fish occurred after 12 hours in the highest concentration of 2000mg/L for zinc treatment and 24 hours in the highest concentration of 80mg/L for copper treatment. The mean percentage mortalities for zinc concentrations of 0.00, 200, 400, 900, 2000 mg/L were 0, 10%, 63%, 100% and 100%, respectively. While mean mortalities for copper concentrations of 0.0,10, 20, 40, 80 mg/L were 0, 10%, 77%, 100% and 100%, respectively. Exposure to copper resulted in frenzied swimming, mucus covering the body and death with their mouths wide open. Similar observations were seen for fish exposed to zinc except that after the death of the fish, there was a release of blood around the gill area. Significant decreases were observed for red blood cells, park cell volume, haemoglobin, and white blood cells, while increases were observed for mean cell volume and mean cell haemoglobin after 96 hours of exposure. Lesions observed include vacuolation, necrosis, cellular infiltration, irregular lamellae, severe sub mucosal congestion, degeneration of cells, haemorrhage and spongiosis in the gills, liver and brain of exposed fish. This study revealed that high doses of zinc and copper are harmful to *Heterobranchus bidorsalis* juveniles.

Keywords: Heavy metals, Catfish, Lethal concentration, Haematology, Liver

Introduction

Sustainable development of aquaculture is hinged on the availability of a friendly environment to the fish species. Thus, the environment should be free of contaminants such as highly toxic chemicals and heavy metals. When these contaminants cause physical or chemical alterations in aquatic environment, they can be rapidly detected through measurable changes in fish (1). This is because fish are inseparably associated with the aquatic environment. Hence, the introduction of substances or energy directly or indirectly by man into the aquatic environment could result in harm or damages

to living resources (2). Fish cannot easily flee from the damaging effects of pollutants, and are thus vulnerable (3) and their sensitivity make them a favourable test subject for evaluating the health of an ecosystem. Thus, finding the relationship between toxicant concentration and its effect on aquatic animals is very essential in managing aquatic life (4).

A collection of anthropogenic and natural activities cause the accumulation of heavy metals. These heavy metal pollutants in aquatic environments are from breakdown of geologic materials, agricultural discharges, domestic, municipal and industrial waste products and atmospheric deposition.

Nevertheless, heavy metals such as zinc and copper are essential in the optimal performance of animals, as they form co-factors for many of the enzymes needed in metabolic activities. Although, excess amounts of these metals may be detrimental to both animals and humans. This is because at higher concentrations, they accumulate in tissues of fish thereby leading to fish poisoning (5). According to Garcia et al. (6), the presence of excess amounts of these metals influence fish reproduction, compromise immunocompetence and induce pathological changes.

Considering the location of fish in the aquatic food chain, they may accumulate metals in their tissue and pass them to man through consumption, and this can result in chronic or acute diseases (7). According to previous studies, concentration of heavy metals in water, exposure period, temperature, oxygen, alkalinity, hydrogen ion concentration, and dissolved organic carbon influence the accumulation of heavy metals in the tissue of fish and its toxicity (8, 9). The increased pollution of water bodies and sediments with metalloids and heavy metals poses a serious threat to humans because of the bioaccumulation and the bio magnification of these metals in the food chain.

The African catfish *Heterobranchus bidorsalis* is a common and commercially important fish species in Nigerian waters. They are largely raised in ponds and also live freely in Nigerian freshwater. However, the increase in anthropogenic activities and pollution around inland waters, as necessitated the need to assess the toxicity level and effects of heavy metals especially copper and zinc on *Heterobranchus bidorsalis.*

Materials and Methods

Experimental Fish

The study was carried out in the Aquatic Pollution and Toxicology Laboratory, Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria. *Heterobranchus bidorsalis* fingerlings were procured from a standard farm located in Ibadan and raised to juveniles (13.5±1.00g) at the University of Ibadan Fish Farm. They were acclimatized to laboratory conditions for two weeks in a large bath-tub containing 150 litres of de-chlorinated water. Water was changed daily to get rid of any unconsumed feed and feacal wastes from fish. Fish were fed 40% crude protein diet during this period. After acclimatization, healthy individuals were carefully selected and randomly distributed into units.

Preparation of Toxicants

Copper sulphate ($CuSO_4.5H_2O$) and Zinc sulphate ($ZnSO_4.7H_2O$) were used to prepare the test media. A range finding test with a spacing factor of 10 was carried out using 9 fish per treatment (10) to establish the

concentrations of zinc and copper to be used in the definite test. The ranges of concentration values used in the experiment were determined from the 100% mortality obtained from the trials. Concentrations of copper were 0.1, 1, 10, 100mg/L, while zinc were 1, 10, 100, 1000mg/L.

For the definitive test, accuracy was achieved by closer spacing of the test concentrations while precision was determined by using more fish per concentration. A spacing factor of 2.2 was used as prescribed by Reish and Oshida (11). The final concentration used for the definitive test for copper treatment was 10, 20, 40 and 80mg/L while zinc treatment had 200, 400, 900 and 2000 mg/L for 96 hours.

Two hundred and seventy juveniles used for the definitive test were distributed at random into 27 plastic aquaria at 10 fish per aquarium (25 litres). The aquaria were covered with mesh nets firmly held with twine to prevent the fish from jumping out. Fish were exposed to 12 hours of light and 12 hours of darkness. The behavioural changes in fish were noted and mortalities recorded at 3 hour intervals for 96 hours. Each treatment was replicated three times.

The water quality parameters before and after the introduction of the toxicants and at the end of the experiment were recorded. The pH was measured using a digital pH meter (Model Photoic 20) while a YSI Model 57 Combined Digital Probe was used to measure dissolved oxygen and temperature.

Hematological and Histopathological Analysis

Tuberculin syringes with 24 gauge needles were used to collect blood samples from two fish randomly caught per treatment. Fish were bled serially with needles inserted into the caudal vein of each fish (10). Blood indices were determined and calculated after exposure to the heavy metals for 96 hours. Erythrocytic parameters were determined as described by Jain (12). Neubauer haemocytometer was used to determine white blood cells (13). For Parked Cell Volume (PCV) estimation, fresh blood collected in glass capillary tubes were centrifuged in a microhaematocrit at 3000 rpm for 10 minutes. Haemoglobin concentrations were measured using Drabkin's solution and Colorimetric readings (14).

At the end of the 96-hour exposure, nine fish taken from each treatment were sacrificed and livers, kidneys, brains and gills removed. Organs extracted were fixed in bouins solution for tissue preparation and the tissues were examined for histopathological changes. The techniques and procedures in this study conformed to guidelines for the ethical conduct and reporting of research done with animals (15, 16). All adopted methods and protocols were carefully reviewed and authors adhered to ethical standards

Statistical analysis

The data obtained were subjected to statistical analysis using a One Way Analysis of Variance at 5 % probability level. Least Significant Difference was used as a follow-up test. The lethal concentration dose (LC_{50}) was determined based on the mortality data recorded using probit methods. After the acute toxicity bioassay, the regression coefficient between the log concentration and probit kill of the toxicant was determined.

Results

The Range Finding Test for Zinc and Copper Concentration

Results of the range finding test and percentage mortality for each concentration are presented in Table 1. Fish survived at concentrations up to 100mg/L and 1mg/L of zinc and copper respectively, with 100% mortality recorded at higher doses of these toxicants.

Definitive test

The definitive test revealed no mortality in the control group. For groups treated with zinc sulphate, mortality was low at 200 mg/L but higher when the concentrations increased (Table 2). In addition, high rate of mortality was observed in groups treated with copper sulphate ≥20 mg/L within 24 hours to 75 hours of exposure.

The onset of mortality was at 81 hours (1 mg/L), 45 hours (10 mg/L), 27 hours (100 mg/L) and 24 hour (1000 mg/L) for zinc sulphate treatments. While mortality commenced at 84 hours (0.1 mg/L), 36 hours (1.0 mg/L), 18 hours (10 mg/L) and 12 hours (100 mg/L) for copper sulphate treatments.

The LC_{50} determined based on the result from the definitive test was 15.03mg/L for copper sulphate treatment and 323.6mg/L for zinc sulphate treatment. This implied that exposure of *Heterobranchus bidorsalis* to 15.03mg/L of $ZnSO_4.7H_2O$ and 323.6mg/L of $CuSO_4.5H_2O$, would cause a mortality of 50% for the fish (Figure 1 and 2).

Effects of Copper and Zinc on the Behaviour of Fish

Fish in the control groups showed no sign of stress and survived; exhibiting normal behaviour. However, *Heterobranchus bidorsalis* exposed to varying concentrations of the toxicants, showed immediate response immediately after contact with the toxicants. The swimming became faster suggesting stressful conditions in the water and the fish became hyperactive at higher concentrations of the toxicants. Twelve hours after the introduction of copper at high concentrations, there was reduced fish activity and weakness. Fish were covered in thick mucus with foam present on the surface of all treatments except the control, after 24 hours. Further observations revealed irritable behaviour over a period of time. The erratic display increased among the treated fish as the concentration of copper and zinc increased. Treated fish swam frequently to the surface with rapid movement of the mouth and opercula. Fish activities subsequently reduced including swimming and frequent surfacing. Also there was a change in pigmentation from black to pale with mucus covering the body.

Table 1: Range finding test for zinc sulphate and copper sulphate treatment on *Heterobranchus bidorsalis* juveniles (n = 9)

Treatment	Concentration (mg/L)	Total mortality	% Mortality
CTR	0.0	0	0
Zn1	1.0	0	0
Zn2	10.0	0	0
Zn3	100.0	0	0
Zn4	1000.0	9	100
Cu1	0.10	0	0
Cu2	1.00	0	0
Cu3	10.0	2	22
Cu4	100.0	9	100

CTR: control group

Table 2: Rate of mortality of *Heterobranchus bidorsalis* juveniles exposed to varying concentrations of copper sulphate and zinc sulphate

Treatment	Concentration (mg/L)	hours 0	6	12	18	24	30	36	42	48	54	60	66	72	78	84	90	96	Total mortality	% mortality
Control	0																		0	0
ZN1	1														01	01	01		03	10
ZN2	10								02	06	07	04	05						24	80
ZN31	100						05	08	16	01									30	100
ZN41	1000					02	10	13	05										30	100
Cu1	0.1															01	02		03	10
Cu2	1							05	03		04	05	01		01				19	63
Cu3	10				15	12	03												30	100
Cu4	100			11	14	05													30	100

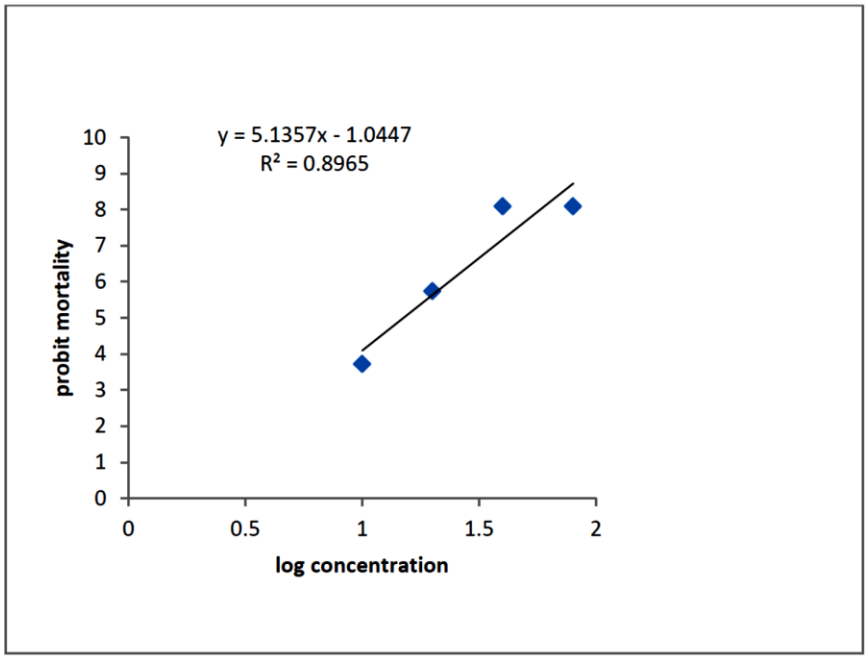


Figure 1: Determination of LC_{50} of *Heterobranchus bidorsalis* juveniles exposed to copper sulphate, using probit method

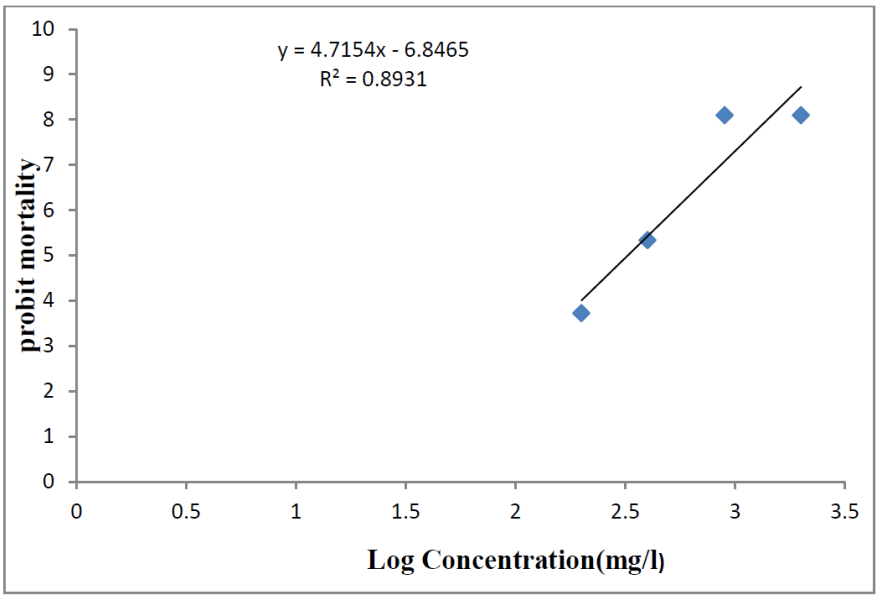


Figure 2: Determination of LC_{50} of *Heterobranchus bidorsalis* juveniles exposed to zinc sulphate, using probit method

Water Quality Parameters of Treatments Exposed to Varying Concentrations of Copper and Zinc

Zinc sulphate reduced the pH of water at concentrations higher than 200mg/L, while water temperature increased slightly (Table 3). Dissolved oxygen decreased with increased zinc concentration, with Zn4 (2.60mg/L) being the least. The pH of water containing various concentrations of copper sulphate ranged from 6.81 (Cu1) to 7.80 (Cu5) (Table 4). The dissolved oxygen of Copper sulphate treatments followed the same trend observed for Zinc sulphate with values ranging from 2.83mg/L (Cu4) to 6.07mg/L (control).

Table 3: Water quality parameters of aquaria exposed to various concentrations of Zinc Sulphate (mean±standard error)

Treatment	pH	Temperature (ºC)	Dissolved Oxygen (mg/L)
CTR	7.02±0.02	24.87±0.06	6.02±0.02
Zn1	6.48±0.01	25.20±0.01	3.50±0.20
Zn2	6.13±0.06	25.97±0.06	3.24±0.02
Zn3	5.93±0.06	26.17±0.12	2.97±0.01
Zn4	5.40±0.01	26.77±0.15	2.60±0.02

Table 4: Water quality parameters of aquaria exposed to various concentrations of Copper Sulphate (mean±standard error)

Treatment	pH	Temperature (ºC)	Dissolved Oxygen (mg/L)
CTR	6.81±0.27	24.37±0.25	5.83±0.42
Cu1	7.17±0.35	24.77±0.05	4.40±0.30
Cu2	7.43±0.03	24.97±0.25	3.50±0.26
Cu3	7.61±0.03	25.63±0.25	2.97±0.25
Cu4	7.80±0.17	26.20±0.20	2.83±0.15

Haematological Changes in *Heterobranchus bidorsalis* Exposed to Varying Concentrations of Zinc sulphate and Copper sulphate

The PCV was significantly reduced in *H. bidorsalis* from 24.67% (control) to 19.67% (Zn4) in fish exposed to the highest concentration of zinc sulphate (Table 5). The haemoglobin level increased from 8.6g/dl (control) to 9.33g/dl (Zn1) and 9.17g/dl (Zn2). There was a sharp reduction in these values for Zn3 and Zn4 (6.33g/dl and 6.77g/dl, respectively). The white blood cell increased significantly from 11.80 x 10^6/µl in the control group to 17.66 x 10^6/µl in Zn4. However, RBC of *H. bidorsalis* decreased significantly from 3.36 x 10^6/µl (control) to 1.67x 10^6 /µl (Zn3). Values for mean cell volume were significantly different in all zinc treated fish and ranged from 81.53fl (in Zn1) to 117.53fl (in Zn3). Mean cell haemoglobin count showed no significant variation across treatments, however mean cell haemoglobin varied from 27.33pg in Zn2 to 39.63 in Zn3.

For fish administered Copper sulphate, the PCV values significantly reduced from 31.33% in Cu1 to 23.33% in Cu4 (Table 6). Haemoglobin values ranged from 7.27g/dl in

Cu4 to 10.00g/dl in the control. The white blood cell values ranged from 14.97 $x10^6$/µl (control) to 16.50 $x10^6$/µl (Cu3 and Cu4). There were reductions in the values recorded for RBC of *H. bidorsalis* from 3.35 $x10^6$ /µl (control) to 1.67 $x10^6$ /µl (Cu4). Platelets was significantly higher in the control (184.33 x 10^3 /µl) with values in the copper treated groups ranging from 128.66 x 10^3 /µl in Cu1 to 174.33 x 10^3 /µl in Cu2. Hematocrit ranged from 24.67% in Cu3 to 36.67% in the control. Mean cell volume was significantly higher in Cu4 (139.13 fl) and least in Cu3 (92.58 fl). There were no significant differences in the values of mean cell haemoglobin counts, while mean cell haemoglobin ranged from 28.77pg in Cu3 to 43.51pg in Cu4.

Histopathological Examination of Organs of *Heterobranchus bidorsalis* Juvenile Exposed to Different Concentrations of Copper sulphate and Zinc sulphate

There were no significant changes in the liver, brain and gill tissues of fish in the control group. The gills of fish in this group consisted of well-arranged primary and secondary lamella. However, the fish exposed to different concentrations of Copper sulphate showed varying degrees of changes in the gill structures and the intensity of change increased with increase in concentration (Table 7). The brains of fish exposed to concentrations of zinc above 200 mg/L showed severe spongiosis of the medulla and angulation. This was the same trend observed in fish exposed to copper concentrations above 10 mg/L. The livers of fish exposed to zinc presented a marked disorganized liver structure. At 2000 mg/L of zinc, the intra hepatic blood vessels were congested. Similarly, the hepatocytes were degenerated at 80 mg/L of copper exposure.

Discussion

In fish biology, copper and zinc are classical limiting factors because they are both essential and toxic. In this study, the fish exposed to different concentrations of copper and zinc were observed to be highly irritable and displayed frenzied swimming when approached, and their bodies were covered with mucus as time progressed with eventual death of the fish and their mouths wide open. Irritability and erratic displays increased with increase in heavy metal concentrations in the experimental units. Oxygen depletion in the zinc treated aquaria was evidenced by the increased movement of the fish to the water surface coupled with rapid movement of their mouth and opercula. Overtime, the occurrence of mucus on the skin may be attributed to increased excretion of Cu and Zn in tissues. According to Khalaf *et al*. (17) and Hilmy *et al*. (18), the skin is an important excretory organ in fish, especially when concentration is high within the aquatic ecosystem.

The water physico-chemical parameters revealed a rise in temperature as the toxicant concentration increased. Increased oxidation of metals is reported to be associated with increased temperature (19). This also had a resultant negative effect on the dissolved oxygen concentration which was significantly reduced as toxicant concentration increased. The toxicants stimulation of chemical reactions in the aquatic environment could have produced stressors, thus increasing respiratory activities in the fish and eventual death due to lack of oxygen. Increased biological oxygen demand has been reported when toxicants increasingly find their way into aquatic systems (2, 20).

Table 5: Haematological parameters of *Heterobranchus bidorsalis* juvenile subjected to different concentrations of Zinc Sulphate

Parameters	Treatment				
	CTR	Zn1	Zn2	Zn3	Zn4
PCV (%)	24.67±0.58[b]	27.67±0.58[a]	28.33±0.58[a]	19.67±0.58[c]	19.67±0.58[c]
Hb (g/dl)	8.60±0.10[b]	9.33±0.06[a]	9.17±0.06[a]	6.63±0.12[c]	6.77±0.06[c]
RBC ($x10^6$/µL)	3.36±0.02[a]	3.39±0.01[a]	2.51±0.01[b]	1.67±0.01[c]	1.72±0.01[c]
WBC($x10^6$/µL)	11.80±0.25[d]	14.97±0.10[c]	17.10±0.05[a]	16.01±0.28[b]	17.66±0.57[a]
PLA (x103/ µL)	184.33±5.77[b]	268.6±4.55[a]	156.33±0.53[d]	148.00±1.00[e]	170.33±0.77[c]
LYM (%)	63.67±0.58[b]	67.33±0.58[a]	65.67±0.58[a]	54.33±0.58[c]	56.33±0.58[c]
HCT (%)	36.67±1.15[a]	23.67±0.58[c]	25.33±0.58[b]	38.67±0.58[a]	25.33±0.58[b]
MON (%)	3.33±0.58[b]	4.00±0.00[a]	2.33±0.58[c]	3.00±0.00[b]	4.00±0.00[a]
EO (%)	5.67±0.58[b]	5.33±0.58[b]	6.67±0.58[a]	2.33±0.58[c]	3.67±0.58[c]
BA (%)	1.00±0.00[a]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	1.00±0.00[a]
MCV (FI)	98.13±1.89[b]	81.53±1.84[c]	84.40±1.65[c]	117.53±3.25[a]	114.13±3.75[a]
MCHC (%)	34.88±1.20[a]	33.73±0.61[a]	32.37±0.61[a]	33.77±1.42[a]	34.43±1.20[a]
MCH (pg)	34.23±0.49[b]	27.50±0.17[c]	27.33±0.12[c]	39.63±0.64[a]	39.27±0.25[a]

Means with same superscript along rows were not significantly different ($p<0.05$)

CTR, Control treatment without toxicant; Zn1, 200 mg/L of toxicant; Zn2, 400 mg/L of toxicant; Zn3, 900 mg/L of toxicant; Zn4, 2000 mg/L of toxicant. PCV= Packed cell volume, Hb = Haemoglobin, RBC= Red blood cell (erythrocyte), WBC= White blood cell count, PLA= Platelets, LYM= Lymphocytes, HCT=Hematocrit, MO, Monocytes, EO= Eosinophils = BA, Basophils MCV= Mean cell volume, MCHC= Mean cell haemoglobin count, MCH= Mean cell haemoglobin

Table 6: Haematological parameters of *Heterobranchus bidorsalis* juvenile subjected to different concentrations of Copper Sulphate ($CuSo_4.5H_2O$)

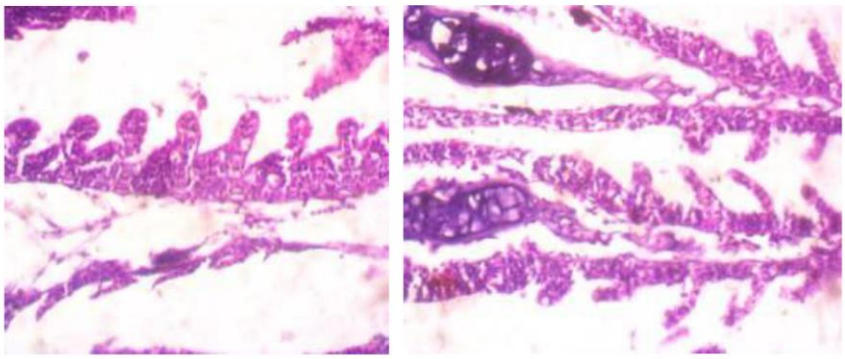
	Treatment				
Parameters	CTR	Cu1	Cu2	Cu3	Cu4
PCV (%)	24.67±0.58^{b}	31.33±1.15^{a}	25.33±0.58^{b}	30.67±0.58^{a}	23.33±0.58^{b}
Hb (g/dl)	8.60±0.10^{b}	10.00±0.20^{a}	8.40±0.20^{b}	9.53±0.15^{a}	7.27±0.12^{c}
RBC ($x10^6$/µL)	2.51±0.02^{b}	3.35±0.10^{a}	2.58±0.10^{b}	3.31±0.01^{a}	1.67±0.20^{c}
WBC ($x10^6$/µL)	14.97±0.25^{b}	16.02±2.51^{a}	16.16±2..87^{a}	16.50±5.00^{a}	16.50±5.00^{a}
PLA ($x10^3$/µL)	184.33±5.77^{a}	128.66±5.77^{d}	174.33±5.35^{b}	136.00±1.00^{c}	144.33±5.77^{c}
LYM (%)	63.67±0.58^{b}	68.67±0.58^{a}	65.67±0.58^{b}	67.00±0.00^{a}	63.67±0.58^{b}
HCT (%)	36.67±1.15^{a}	27.00±1.00^{b}	34.00±1.00^{a}	24.67±0.58^{c}	27.00±1.00^{b}
MON (%)	3.33±0.58^{ab}	2.67±0.58^{b}	4.00±0.00^{a}	3.00±1.00^{ab}	2.33±0.58^{c}
EO (%)	5.67±0.58^{a}	1.00±0.00^{c}	2.00±0.00^{b}	5.33±0.58^{a}	5.67±0.58^{a}
BA (%)	1.00±0.00^{a}	1.00±0.00^{a}	0.00±0.00^{b}	0.00±0.00^{b}	1.00±0.00^{a}
MCV (fI)	98.13±1.89^{b}	93.52±3.20^{b}	98.20±1.91^{b}	92.58±1.69^{b}	139.73±3.81^{a}
MCHC (%)	34.88±1.20^{a}	31.94±1.35^{a}	32.75±1.47^{a}	31.08±1.07^{a}	31.13±0.29^{a}
MCH (pg)	34.23±0.49^{b}	29.84±0.64^{c}	32.54±0.71^{b}	28.77±0.51^{c}	43.51±0.86^{a}

Means with same superscript along rows show no significant difference ($p<0.05$)

Keys: CTR, Control treatment without toxicant; Cu1, 10 mg/L of toxicant; Cu2, 20 mg/L of toxicant; Cu3, 40 mg/L of toxicant; Cu4, 80 mg/L of toxicant. PCV= Packed cell volume, Hb = Haemoglobin, RBC= Red blood cell (erythrocyte), WBC= White blood cell count, MCV= Mean cell volume, MCHC= Mean cell haemoglobin count, MCH= Mean cell haemogblobin, PLA= Platelets, LYM= Lymphocytes, HCT=Hematocrit, MO, Monocytes, EO= Eosinophils = BA, Basophils, MCV=Mean cell volume, MCHC= Mean cell haemoglobin count, MCH= Mean cell haemoglobin

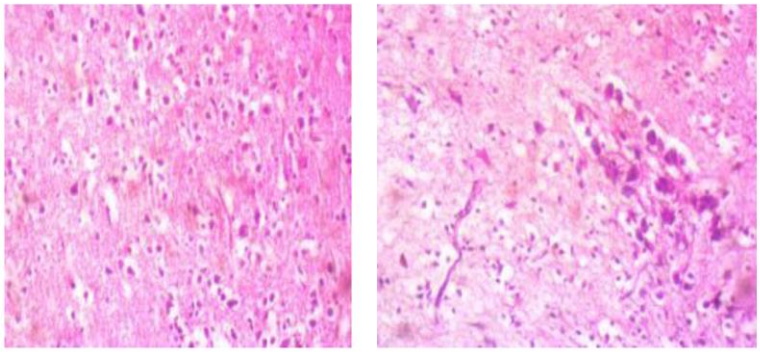
Table 7: Results of histopathology of gills, brain and liver of *Heterobranchus bidorsalis* subjected to varying concentrations of copper sulphate or zinc sulphate

Treatment	Gill	Brain	Liver
CTR	No lesion	No lesion	No lesion
Zn1	degenerative changes in epithelial cells of secondary gill filaments	Mild spongiosis	cells were degenerated; the normal architecture of liver was markedly disorganized
Zn2	moderate necrosis in inter lamellar epithelial cells	severe spongiosis of the medulla and angulation	Hypertrophy of hepatocytes which had pycnotic nuclei
Zn3	moderate necrosis in inter lamellar epithelial cells	severe spongiosis of the medulla and angulation	dilated sinusoids with congestion were noticed
Zn4	twisting of gill filament tips and cells in the primary axis infiltrated.	severe spongiosis of the medulla and angulation	dilated sinusoids and intra hepatic blood vessels with congestion were noticed
Cu1	showed irregular lamellae and mild degeneration of epithelial cells	mild spongiosis of the cortex	mild vacuolation of the hepatocytes
Cu2	necrosis and further degeneration of epithelial cells	severe spongiosis of the medulla	diffused glycogen vacuolation
Cu3	haemorrhage, fusion and distortion of secondary lamellae	severe spongiosis of the medulla and angulation	diffused glycogen vacuolation
Cu4	moderate atrophy of the secondary lamella, severe sub mucosal congestion and necrosis	ischaemic necrosis of neurons	congestion of central venule, atrophy and degeneration of hepatocytes



A **B**

Plate 1: Histology of *Heterobranchus bidorsalis* gills in the control (A) showing a well arranged lamellae and gill epithelium and fish exposed to 10mg/L of copper (B) showing irregular lamellae and mild degeneration of epithelial cells.



A **B**

Plate 2: Histology of *Heterobranchus bidorsalis* brain in the control (A) showing no significant lesion and fish exposed to 10mg/L of copper (B) revealing mild spongiosis of the cortex in brain

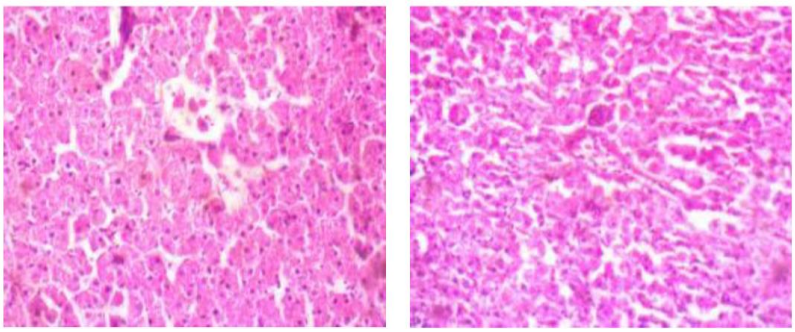


Plate 3: Histology of *Heterobranchus bidorsalis* liver in the control (A) with no lesion and fish exposed to 10mg/L of copper (B) showing mild vacuolation of the hepatocytes

The 96 hour – LC_{50} of Copper sulphate and Zinc sulphate for *H. bidorsalis* obtained in the study using probit method were 15.03 mg/L and 324 mg/L, respectively. The LC_{50} for copper was lower than 21.48 mg/L reported for *Clarias gariepinus* by Ezeonyejiaku *et al*. (21) using the same toxicant. Edeh (22) reported a 96 hour LC_{50} for *Clarias gariepinus* as 59.38 mg/L and 65.15mg/L for copper and zinc, respectively. The lower value of LC_{50} observed in this study may be as a result of the effect of factors such as solubility and physico-chemical characters of test solution on heavy metal toxicity, as affirmed by Ezeonyejiaku *et al*. (21).

Histological changes in fish organs are useful biomarkers to indicate environmental pollution and health status of aquatic ecosystem and fish (23). Fish respond to pollutants from agricultural, sewage and industrial sources with changes in their gills, liver, kidneys and gonads (24, 25). In this study, the histological changes observed in the organs are usually associated with hepatocytes response to toxicants (26), and not specific to metals. The cumulative effect of copper and zinc at high concentration results in the degeneration and necrosis of hepatocytes in the liver (27). Similarly, oxygen deficiency resulting from gill degeneration or vacuolar dilation have been reported to cause cellular degeneration in the liver with intravascular haemolysis observed in the blood vessels (28). It must be noted that the respiratory system provides the most extensive interface of a fish with water and is frequently the first system to be affected by dissolved pollutants (29). Histopathological alterations in gills are linked with specific classes of toxicants. Gills of *Labeo rohita* exposed to tannery effluent revealed fusion and clumping of primary lamellar epithelium (30). Usually, degenerative changes in lamellae and edema were observed in gills of fish exposed to heavy metals (31).

Fish organs adapt to high concentration of toxicants by manifesting a number of histological alteration (32). This assertion may be responsible for the histopathological alterations observed in the gills including degeneration of the epithelial cells of secondary gill filaments, necrosis in inter lamellar epithelial cells and the twisting of the tips of gill filament study.

The haematological indices further highlight the pattern of response to the two heavy metals. The red blood cells and haemoglobin reduced with increased concentration of toxicants in the rearing waters. This suggests the destruction of red blood cells and haemoglobin, leading to anaemia. The size and state of the red blood cells can be inferred from the values of MCV. Increase in values may be attributed to red blood cells' swelling as a result of hypoxic conditions or osmotic stress. According to Larsson *et al*. (33), this may be related to macrocytic anaemia in fish exposed to heavy metal pollution. Higher MCV, MCH and MCHC values were also reported when *Oreochromis mossambicus* was exposed to Zinc (34) and when *Clarias albo-punctatus* was exposed to organo-phosphorous pesticide (35).

Conclusion

This study revealed that high doses of zinc and copper cause severe damages to the gill, brain and liver cells of *Heterobranchus bidorsalis* juveniles. The abundance of this and other species in water-bodies in areas with high anthropogenic activity is hence threatened. Measures must therefore be taken to protect the aquatic ecosystem against heavy metal contamination.

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