Pakistan J. Zool., vol. 37(2), pp. 101-105, 2005.

Haematological Assessment of Freshwater Catfishes, *Clarias gariepinus* (Burch) and "Heteroclarias" (Hybrid) Exposed to Sublethal Concentrations of Zinc

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Abstract.- Freshwater catfishes: *Clarias gariepinus* and "Heteroclarias" (Hybrid) were subjected to sublethal concentrations (2.5 and 4.0 mg^{-1}, respectively) of zinc for six weeks. The effects of these treatments on some blood parameters were analyzed. Decreased haematocrit, haemoglobin, MCH and MCV and increased blood cell count and white blood cell count were observed in *Clarias gariepinus* exposed to zinc. The "Heteroclarias" showed increases in MCV, MCH and MCHC following zinc treatment. The red blood cell count and white blood cell count of this species increased during the first week of exposure but declined in the preceding weeks of the experiment. Decrease in haematocrit levels of both species following zinc exposure is attributed to haemo-dilution. The observed increase in red blood cell count of *Clarias* species is due to cell shrinkage caused by osmotic alterations of blood by the action of the metal. The decrease in the red blood cell count of the species is attributed to swelling of the erythrocytes possibly as a result of sampling procedures. The most susceptive haematological parameters of these freshwater catfishes following zinc contamination are haematocrit, haemoglobin, red blood cell count and white blood cell count in the systems.

Key words: Toxicology of zinc, haematology, *Clarias gariepinus*, "Heteroclarias" (Hybrid).

INTRODUCTION

Zinc is ubiquitous and an essential trace element in living organisms, being involved in nucleic acid synthesis and occurring in many enzymes. It occurs widely in nature as sulphide, carbonate and hydrated silacate ores, frequently accompanied by other metals, mainly iron and cadmium. It is used for galvanizing in brass and other alloys and some of its compounds, including the oxide, chloride, chromate and sulphide are widely used in other industries (Alabaster and Lloyd, 1982). Consequently, it can be an important pollutant from both mining and other industrial processes.

An effect of sublethal concentration of zinc on fish has been reviewed by several authors. Zinc is taken up by fish directly from the water, especially by the mucus and gills (Skidmore, 1965). Considerable amounts are also found in the eye, kidney, bone and the gastro-intestinal tract, with lower amounts in liver, spleen, and muscle (Alabaster and Lloyd, 1982). High concentrations have also been found in the gonads. The same author found that initial high rate of uptake was followed by a decline after 12 hours. Reports available has shown that at high concentration, zinc has adverse effects in fish such as affecting the development, growth and survival of fish (Skidmore, 1965; Tuurala and Soivio, 1982). Zinc accumulates in fish gills (Crespo *et al*., 1979) and induces a depressive effect on tissue respiration leading to death by hypoxia (Burton *et al*., 1972). Zinc pollution also induces changes in ventilatory and heart physiology (Hughes and Tort, 1985). Sublethal levels of zinc have also been reported to adversely affect hatchability, survival, haematological parameters of fish (Cardeihac *et al*., 1986; Flos *et al*., 1987). Several studies have been done on fish haematology, mostly on marine temperate teleosts (Mishra and Srivastava, 1980; Waiwood, 1980; Koyama *et al*., 1982). However, not much work has been done on haematological parameters of tropical freshwater catfishes.

The analyses are focused on haematological parameters of fish. This is because of their relationship with energetic respiration and defence mechanisms. Changes in blood parameters are often

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0030-9923/2005/0002-0101 $ 4.00/0

quick responses to environmental or physiological changes and can provide an integrated measure of the physiological status of the fish. Haematological analyses also provide quick screening methods for assessing the health of fish and can be used to determine the incipient lethal concentration of a toxicant (McLeavy, 1973). In this study, fingerlings of *Clarias gariepinus* and "Heteroclarias" were subjected to subacute concentrations of 2.5 and 4.0 mgZn/L^{-1}, respectively.

MATERIALS AND METHODS

Fingerlings of *Clarias gariepinus* and "Heteroclarias" (Hybrid) were obtained from Jayess Fish Farm, Ltd., Port Harcourt Rivers State, Nigeria. The *Clarias gariepinus* averaging 14.25±0.41 cm (total length) and 18.28±1.10 gm body weight and the "Heteroclarias" (Hybrid) of total length 10.24±1.38 cm and body weight 17.83±1.63 gm were used for this study. The fishes were held in the laboratory in large water baths of 160 l capacity at 24.5-26.5°C and acclimated for two weeks prior to the experiment. The fishes were fed Pfizer pelted diet during acclimation and tests. A daily photo-period of 16:8 h light:dark was maintained during acclimation and tests.

The African freshwater catfishes were chosen for this study because they are ubiquitous in Africa (Fagnenro, 1982), and constitute one of the main fish families of economic value as food fish. In Nigeria, they are caught all year round in most freshwater and swamps with a variety of gears (Oti, 1999).

Twenty-four fish of the *Clarias gariepinus* and "Heteroclarias" were exposed to 2.5 mgZn/L and 4.0 mgZn/1 (analytical grade $ZnSO_4.7H_2O$), respectively, in water bath (160 l capacity). There were two replicates and a control in each treatment. The toxicant and test water were renewed at three days intervals to maintain the toxicant strength and the level of dissolved oxygen as well as minimizing the level of ammonia during the experiment. The water quality was monitored using (APHA, 1981).

Four fish were randomly sampled from each group on weekly basis. The caudal artery was punctured from the caudal peduncle and blood samples collected by use of heparinized microcapillary and sampling tubes. Blood samples were taken at least 40 seconds after collecting the fish from the water bath. Haematocrit (Ht) was analyzed using capillary tubes filled with blood and centrifuged at 11,000 r/min for six minutes. The mean values of haematocrit (%) were measured with a microcapillary reader. Haemoglobin (Hb) levels were obtained by means of Boehringer-Mannheim commercial kits, based on colorimetric determinations. Red blood cell (RBC) count was performed with a Neubauer count chambers diluting the blood (200 times) in Toisson's solution. Similarly, white blood cell count (WBC) was performed with microscope Neubauer count chamber diluting the blood (200 times) in Turk's solution.

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the following formulae:

$$\text{MCV } (\mu m^3) = \frac{\text{Ht (\%) X 10}}{\text{RBCC (Cells mm}^{-3})}$$

$$\text{MCH } (\text{ßgcell}^{-1}) = \frac{\text{Hb (g 100 ml}^{-1}) \text{ x 100}}{\text{RBCC (Cells mm}^{-3})}$$

$$\text{MCHC (g 100 ml}^{-1}) = \frac{\text{Hb (g 100 ml}^{-1}) \text{ x 100}}{\text{RBCC (Cells mm}^{-3})}$$

Results were recorded as Mean±S.E. Statistical analysis was done by one way analysis of variance. In all cases the 5% probability level was regarded as the lowest limit of significance.

RESULTS

Table I gives the mean values and standard errors (S.E.) of all blood parameters for each group of fish. No mortality was recorded in any group. The blood indexes in each treatment varied significantly, although they did not follow coincident patterns.

Exposure of *C. gariepinus* to zinc for six weeks (Table I) produced decreases in haematocrit (58.9% to 27.3%), haemoglobin (10.2 g 100 ml^{-1} to 8.2 g 100 ml^{-1}), MCH (284 pg $cell^{-1}$ to 103 pg $cell^{-1}$),

Table I.- The effect of zinc on haematological parameters in *Clarias gariepinus* and "Heteroclarias" (Hybrid.)

Parameters	Control	Zn – treatment						Significance
		Wk 2	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	
Clarias gariepinus								
Haematocrit (%)	58.9±0.9	58.0±0.5	55.0±1.4	51.7±2.4	35.5±0.3	35.0±1.5	27.3±1.2	
Haemoglobin (g 100ml^{-1})	10.2±0.2	6.5±0.1	4.5±0.1	8.4	7.4	7.6±0.2	8.2	
RBC (x 10^3 Cell mm^{-3})	49.4	452	1182	618	598	808	280	<0.001 1,2
WBC (x10^3 Cell mm^{-3})	49.4	975	834	91.5	19.5	19.5	36.9	<0.001 1,2
MCV (µm^{-1})	1765±26	403±4	473±4	845±60	446±41	446±6	985±57	<0.001 1,2
MCH (pg cell^{-1})	284±11	54±2	49±2	145±5	136±5	103±3	306±37	<0.035[2]
MCHC (g 100 ml^{-1})	182.2±0.2	11.3±0.7	9.2±0.7	16.5±0.7	20.5±0.1	20.3±1.4	27.6±0.8	<0.05[1]
Heteroclarias (Hybrid)								
Haematocrit (%)	17.8±0.1	14.3±0.5	10.3±0.8	8.3±0.8	6.7±0.7	10.3±1.2	9±0.8	<0.05[2]
Haemoglobin (g 100ml^{-1})	5.7±0.1	6.9	7.8±0.1	4.5	4.5±0.1	4.5±0.3	4.9±0.3	
RBC (x 10^3 Cell mm^{-3})	861	1004	484	162	130	146	170	<0.05[2]
WBC (x10^3 Cell mm^{-3})	10	10	10	10	10	10	10	
MCV (µm^{-1})	213±21	158±54	226±60	557±44	528±55	701±62	536±54	<0.034[2]
MCH (pg cell^{-1})	77±1	79±2	172±6	286±37	356±59	324±53	295±43	<0.05[1,2]
MCHC (g 100 ml^{-1})	32±0.6	48.8±3.6	74.5±2	50.4±2.7	67.4±2.4	43.4±1.9	54.3±1.2	<0.05[1,1]

[1]Significance difference between Zn – treated group.
[2]Significance differences between control and Zn – treated groups.

MCV (1,765 µm^3 to 985 µm^3). The haematocrit values decreased significantly (P<0.05) after six weeks of exposure of zinc. During the first two weeks of exposure, the haemoglobin and MCH values were found to be lower than those of the control. However, these values improved in the preceding weeks towards recovery. There was significant (P<0.001) increase in RBCC during the experimental period. The WBCC increased significantly (P<0.001) during the first three weeks of exposure but declined in the following weeks of the experiment. The MCV values decreased throughout the experimental period. The MCHC values decreased during the first three weeks of experiment but increased in the following weeks.

Exposure of "Heteroclarias" to 4.0 mgZn/1 for six weeks (Table I) showed significant (P<0.05) decrease in haematocrit. The values of haemoglobin obtained from zinc-treated "Heteroclarias" did not differ significantly from those of the control. RBCC and WBCC increased significantly (P<0.05) during the first week of exposure but increased significantly (P<0.05) in the subsequent weeks. There were significant increases (P<0.05) in MCH and MCHC values of "Heteroclarias" during the six weeks exposure to zinc.

DISCUSSION

The results of control values obtained in this study are within the normal range for teleost blood parameter. The *Clarias gariepinus* presented low RBCC. The RBCC of teleost is within the range of 700,000 – 2,000,000 cells mm^3 (Saunders, 1966). Teleost also have bigger corpuscular volumes and high MCH (close to 300 Pg cells^{-1} (Saunders, 1966). The MCH value of control Heteroclarias (77±1 Pg cells^{-1} was found to be very low in comparison with those reported by Saunders (1966) for teleost.

Metal exposure induces changes in blood parameter possibly as result of alterations in blood water content. The fish muscle has been known as the water exchange tissue with blood (Waiwood, 1980). Both haemoconcentration and haemodilution have been described in previous works. Mishra and Srivastava (1979, 1980) observed haemoconcentration after copper exposure and haemodilution following zinc exposure in *Colisa fasciatus*. In the present study, decrease of haematocrit following zinc exposure in *C. gariepinus* and "Heteroclarias" may be an indication of haemodilution. Tort and Torres (1988) also reported decrease of haematocrit following 24h exposure of dogfish, *Scyliorhinus*

canicula to cadmium contamination. They attributed such decrease in haematocrit to haemodilution. However, Waiwood (1980) and Flos *et al*. (1987) observed increase in haematocrit levels in different fish species after zinc treatment. They attributed such increase in haematocrit values to increase in size of the erythrocytes as has been demonstrated for chromonium and zinc treated rainbow trout.

The RBCC of the *Clarias gariepinus* increased significantly ($P<0.00$), whereas that of the "Heteroclarias" (Hybrid) did not. Mishra and Srivastava (1980) observed increase in RBC in *Colisa fasciatus* exposure to copper at 3 ppm for 96h. The RBCC elevation observed in the *Clarias gariepinus* may be due to blood cell reserve combined with cell shrinkage as a result of osmotic alterations of blood by the action of the metal (Tort and Torres, 1988). Mishra and Srivastava (1979) observed decrease in RBCC in *Colisa fasciatus* followed an exposure to 100 ppm of zinc. El-Domiaty (1987) also reported decrease in RBC in *Clarias lazera* exposed to copper. The decrease in RBCC observed in the "Heteroclarias" may be due to the swelling of the red cells. Haemolysis of erythrocytes has also been described in rainbow trout exposed to zinc (Koyama *et al*., 1982). According to Flos *et al*. (1987), the swelling of the erythrocytes may be due to increased protein-carbon-dioxide in the blood. These changes could be the result of hypoxia or stress caused by sampling procedures.

Spleen contraction after stress has been detected in fish (Abrahamsson and Nilsson, 1975). Cells released from the spleen which is an erythropoietic organ would have lower MCV value in this study. Lower MCV values were observed in *C. gariepinus* treated with zinc salt. A similar observation was made for *Cyprinus carpio* after cadmium exposure (Philips and Rainbow, 1994), Mishra and Srivastava (1979) observed that 100 ppm of zinc caused decrease in WBCC in *Colisa casciatus.* Flos *et al*. (1987) reported decrease in WBCC in females of *Scyliorhinus canicula* following zinc exposure. Similar observations were made in this study for both *Clarias* and "Heteroclarias" species. Joshi (1980) suggested that developing gonads through a gradually decreasing space in the abdominal cavity depletes feeding in female fishes. This may be the reason for the low number of leucocytes, because WBCC in fish blood exhibit a direct correlation with the feeding status (Smimova, 1965).

Haematological variables related to oxygen transport (RBCC, Hb, Ht) and calculated indexes (MCV, MCH and MCHC) varied between control and zinc treated groups of both *Clarias* and "Heteroclarias" species. Therefore, it appears that the exposure of these freshwater catfishes to sublethal zinc levels act as a stressor, producing the usual haematological and blood metabolites level response to unspecified stressor. The most susceptible haematological parmeters of these freshwater catfishes appeared to be haematocrit hemoglobin RBCC and WBCC. Zinc causes a stress inducing effect on freshwater catfishes during the course of long-term exposure as indicated by alterations in blood parameters. In such cases the fish becomes more susceptible to infections and may be more vulnerable to predators.

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(Received 20 September 2003, revised 13 August 2004)