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Sublethal Effects of Cadmium on Some Selected Haematological Parameters of Heteroclarias (A Hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*)

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Abstract: Experiments were conducted to ascertain the sublethal effects of waterborne cadmium at 0.5 and 1.0 mg L^{-1} on a freshwater fish *Heteroclarias* (a hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*) following a 15 days exposure in a renewal static bioassay system. The effects were assessed by examining the haematological profile. Exposure to cadmium caused a dose-dependent decrease in haemoglobin, haematocrit and erythrocyte counts. These changes are obvious indication of anaemia of the normochromic type. The erythrocytic indices of MCHC, MCH and MCV were similarly decreased apart from one case. Differential leucocyte count and erythrocyte sedimentation rate were also affected. Plasma levels of protein were lowered and glucose levels were elevated in the exposed fish when compared to the control. The results of this study highlight the stress to which freshwater fish are exposed through the uncontrolled discharge of heavy metals in the aquatic environment.

Key words: Cadmium, haematology, anaemia, glucose, protein, *Heteroclarias*, Nigeria

Introduction

Cadmium is a non-essential element with no known biological function (Martìnez *et al*., 1999), naturally found at low concentrations in natural waters (Allen, 1994 and Bennet-Chamber *et al*., 1999). Cadmium is a highly toxic metal that has been studied for years (Nordberg, 1978) and its toxicity to freshwater organisms has been well documented (Brown *et al*., 1994). Cadmium pollution sources are diverse, but it is commonly accepted that electroplating plants are mainly responsible (Cuthbert *et al*., 1976).

The effects of pollutants on fish are evaluated by acute and chronic toxicity tests (Sprague, 1973; Rand and Petrocelli, 1985; Van Der Merwe *et al*., 1993; Svobodova *et al*., 1994; Nussey *et al*., 1996). Death is an easily detected deleterious response, where the criterion for death is usually lack of movement, for example no gill movement and a lack of reaction to prodding (Sprague, 1973; Parrish, 1985). In view of the fact that normal physiological processes are affected long before the death of an organism and because death is to an extreme a criterion for determining whether a substance is lethal or not, scientists had to search for physiological and biochemical indicators of health and sublethal toxicants effects (Zachariassen *et al*., 1991).

Blood physiology is currently considered as an essential index to the general health status in a number of fish species (Larsson *et al*., 1985). Changes in haematological variables are now in use when clinical diagnosis of fish physiology is applied to determine the effects of external stressors. It has been

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illustrated that the use of haematological variables as indicators of stress, toxic substances as well as metals can provide information of the physiological response fish make to a changing external enviroument. This is the result of the close association between the circulatory system and the external environment (Casillas and Smith, 1977), because fish live in the closest possible contact with their environment (Blaxhall, 1972).

A review of literature on piscian haematology showed that very little work has been done so far on the alteration of blood constitutions following exposure of fresh water fish to cadmium. Therefore, the present study was undertaken to evaluate some haematological effects resulting from the exposure of freshwater fish *Heteroclarias* to sublethal concentrations of cadmium in the water. *Heteroclarias* was chosen for this study because this hybrid constitutes one of the main fish species of economic importance in the Niger Delta. It is an abundant cultural fish species in Nigeria and very popular with fish farmers and consumers. It has also been observed that *Heteroclarias* grow at a fast rate and in more isolated cases *Heteroclarias* were bigger than *Heterobranchus bidorsalis* of the same age (Fagbenro, 1992).

Materials and Methods

Apparently healthy specimens of *Heteroclarias* were obtained from a local fish farm at Ughelli town, Delta State, Nigeria and were transported in containers to the laboratory. In the laboratory fishes were kept in large plastic bowls containing 60 L of clean tap water and acclimatized for 14 days to the laboratory conditions, during which they were provided with artificial feed and grounded shrimps to avoid possible effects of starvation on any of the haematological parameters of the fish. The size of the fish varied from 17.0-27.0 cm in standard length and 39.0-110.4 g in weight. Fish of both sexes were used without discrimination.

Stock solution of the test metal compound Cadmium acetate $(CH.COO_2)$ Cd. $2H_2O$ was prepared by dissolving 2.37 g of cadmium acetate $[(CH_3\ COO_2)$ Cd. $2H_2O]$ equivalent to 1 g of cadmium metal in a distilled water at concentration of 1000 mg L^{-1}.

Three groups of ten fish were subjected to serial dilutions of the stock solution of cadmium of 0 (control), 0.1 and 0.5 mg L^{-1} in three large plastic bowls of 60 L capacity.

The test was performed by the semistatic (renewal) method in which the exposure medium was exchanged every 24 h to maintain toxicant strength and level of dissolved oxygen as well as minimizing the level of ammonia excretion during this experiment (Kori-Siakpere, 1996). The water quality parameters of the diluting water used in the tests and determined by standard methods were as presented in Table 1. The exposure period lasted 15 days.

Blood samples were taken from the control and experimental fish at the end of the 15 days exposure period. The blood samples were taken by puncturing the caudal vessels, using EDTA (ethylene diamine tetraacetate) as anticoagulant. Haematocrit were determined by using

Table 1: Water quality parameters

Parameter	Values
pH	6.58±0.32
Temperature(°C)	28.3±1.3
Dissolved oxygen (mg L^{-1})	7.32±1.04
Free carbon dioxide (mg L^{-1})	4.95±0.08
Alkalinity (mg L^{-1})	32.8±1.75
Hardness (mg L^{-1})	128.56±12.65

microhaematocrit heparinized capillary tubes and a microhaematocrit centrifuge (12,000 for 3 min). Haemoglobin concentrations were determined with the aid of a commercial kit by the cyanhaemoglobin method, using wavelength of 540 nm. RBCC and WBCC were made under light microscope with an improved Neubauer haemocytometer using the appropriate diluting fluids. Differential leucocyte counts were made from Leishman/Giemsa stained blood smears. Erythrocyte sedimentation rate was determined with microhaematocrit tubes filled with blood and allowed to stand for 60 min. The derived blood indices of Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated using standard formulae. Total plasma protein and plasma glucose concentration were determined using commercial kits (Randox Laboratories Ltd, United Kingdom).

The mean values of the various haematological parameters for the control and experimental fish were analysed for statistical significance using the student's t-test. The calculations of statistical significance by the student's t-test at 0.01 and 0.05 levels were aided using SPSS.7.5 for windows 98 and Microsoft Excel 2000.

Results and Discussion

The main haematological responses in this study include slightly non-significant decrease in red blood cell counts, haematocrit and haemoglobin concentration (Table 2). The white blood cell counts were also decreased with change in the composition as seen from the differential white blood cell counts. While a decrease in the total plasma protein was recorded; plasma glucose levels were increased in all the concentration of cadmium to which the experimental fish were exposed. The erythrocytic indices of MCHC, MCH and MCV were similarly decreased in most of the exposure media.

Increased levels of toxic metals in the aquatic environment as a result of point (e.g., industrial) or diffuse source (e.g., mining) can have both lethal and sublethal effects on organisms including fish. Sublethal concentrations of toxicants in the aquatic system do not necessarily result in outright mortality of aquatic organisms but could have significant effects, which can result in several physiological disfunctions in fish (Omoregie *et al*., 1990).

Table 2: Changes in haematological parameters[#] as a result of exposure of *Heteroclarias* to the various sublethal concentrations of cadmium in the water

	Exposure media		
Parameters	Control (0 mg Cd L^{-1})	0.5 mg Cd L^{-1}	1.0 mg Cd L^{-1}
Haematocrit (%)	38.4±0.94	37.6±0.93	28.4±0.68*
Haemoglobin (g dL^{-1})	15.2±0.77	12.6±0.070	10.0±0.28**
RBCC (10^6 mm^{-3})	1.63±0.13	1.39±0.06*	1.30±0.08*
WBCC ($10^3$$mm^{-3}$)	8.56±0.07	8.20±0.09	7.20±0.13**
MCHC (%)	40.38±3.19	33.75±3.92	33.34±5.33
MCH(ρg)	97.28±12.63	90.66±11.27	82.0±10.52
MCV (μg)	271.14±22.12	240.38±14.39	207.05±15.23*
ESR (mm h^{-1})	28.8±1.07	27.8±1.28	25.8±1.16*
Plasma protein (g dL^{-1})	3.11±0.31	2.99±0.27**	2.64±0.69**
Plasma glucose(mg dL^{-1})	258.54±4.84	274.55±6.62	296.86±7.42
Lymphocytes	29.52±1.33	22.72±0.03	33.32±0.02*
Basophils	10.95±0.54	0	0
Neutrophils	15.29±0.24	25.82±0.08	0
Eosinophils	15.27±0.32	37.04±0.12	50.00±7.01
Thrombocytes	27.09±0.03	15.15±0.34	16.69±0.01

[#]The values are expressed as the mean±SE and for each parameter, mean values were calculated from 5fishes from each exposure and control; each result was duplicated so that the mean±SE given here were calculated from 10 observations. * = significance at 0.05 level , ** = significance at 0.01 level

Changes in haematological values occur in relation to physiological stress, disease and toxic environmental conditions (Blaxhall, 1972; Barnhart, 1969; Swift and Lloyd, 1974; Soivio and Oikari, 1976; Wedemeyer and Yasutake, 1977).

Haemoglobin is the oxygen-carrying component in the blood of fish and its concentration can be used as a good indicator of anaemia (Blaxhall and Daisley, 1973). The decreased haemoglobin in the experimental fish exposed to cadmium could thus be an indication that anaemic condition occurred in the fish during exposure. Decreased haemoglobin following metal exposure usually results in haemodilution. This haemodilution has been regarded as a mechanism that reduces the concentration of the toxicant/pollutant in the circulatory system (Smit *et al*., 1979).

Haematocrit is used to determine the ratio of plasma to corpuscles in the blood as well as the oxygen-carrying capacity of the blood (Larsson *et al*., 1985). The significant decrease in the Haematocrit in this study could be attributed to gill damage and/or impaired osmoregulation causing anaemia and haemodilution.

Blood cells of teleost are produced from haemopoietic tissues of the kidney and spleen (Heath, 1982). The red blood cells have the important function of haemoglobin transport which carries oxygen to all tissues in the body (Hibiya, 1982). The decreased red blood cell number following exposure to cadmium could be as a result of haemolysis or destruction of the red blood cells. The cause of the reduction of circulating erythrocytes of stressed fish has been attributed to aggregation of red blood cells in damaged gills (Sing and Sing, 1982). Decreases in the red blood cells could also be as a result of internal bleeding caused by damaged kidney.

Erythropaenia could also be as a result of damaged gills and impaired osmoregulation during sublethal cadmium exposure, which cause haemodilution leading to a decrease in the number of red blood cells through haemolysis (Wedemeyer and Yasutake, 1977). Though histopathology of the gills was not investigated in this study, cadmium has been implicated as responsible for various gill lesions (Gardner and Yevich, 1970).

In fish, the white blood cells respond to various stressors including infections and chemical irritants (Christensen *et al*., 1978). Thus increasing or decreasing numbers of white blood cells are a normal reaction to a chemical such as cadmium as in the present study, demonstrating the effect of the immune system under toxic conditions. The decreased number of white blood cells (leucopaenia) may be the result of bioconcentration of the test metal in the kidney and liver (Agrawal and Srivastava, 1980). Decreased number of white blood cells may also be related to an increased level of corticosteroid hormones. The secretion of these hormones is a non-specific response to any environmental stressor (Iwama *et al*., 1976; Ellis, 1981).

Cortisol plays an important role to prevent the development of inflammation and causing resolution thereof. The cortisol decreases the migration of white blood cells into the inflamed area and also suppresses the immune system by causing a decrease in lymphocyte production (Guyton, 1991). This reaction is also indicated in this study with the observed decrease in the lymphocytes of the exposed fish. In the same way, changes in the various types of white blood cells could be attributable to the stimulation of the immune system of the fish in a bid to eliminate the effects of the cadmium.

The total plasma protein levels in fish is generally accepted as an indicator of the nutritional status.

In many fish species, the blood/plasma glucose level has the tendency to increase due to experimental stress. Both the forced muscular activity during capture and the removal of the fish from the water are known to elevate the circulating levels of glucose (Mackay and Beatty, 1968; Wedemeyer, 1972). Hyperglycaemic response in this study is an indication of a disrupted carbohydrate metabolism possibly due to enhanced breakdown of liver glycogen and mediated perhaps by adenocortical hormones and reduced insulin secretory activity. Similar hyperglycaemic response has been reported in the flounder, *Pleuronectes fleusus* following exposure to sublethal concentrations of cadmium (Larsson, 1975).

On the whole, the results of this study highlight the stress to which freshwater fish are exposed through the uncontrolled discharge of heavy metals in the aquatic environment. Results obtained from exposure of fish to the various toxicants, despite specific shortcomings such as experimental error, water hardness, water-pH and capture stress enable us to predict the fate of fish populations exposed to these conditions.

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