

# Changes in the Hematological Characteristics of *Oreochromis niloticus* Exposed to Sublethal Levels of Cadmium

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## ABSTRACT

The 96 h LC<sub>50</sub> of cadmium chloride to young *Oreochromis niloticus* (TL= 60–115 mm, 4.0–17.7 g) was 53 ppm (approximately 29 mg Cd/L). The toxicity of cadmium chloride to *O. niloticus* was affected by the concentration of the metal and pH. Acute exposure of fish to 37 mg/L and 26.5 mg/l cadmium chloride at pH 7.1 to 7.8 and 5.0 to 5.8 induced changes in RBC count, Ht and Hb concentration, and leucocyte count. Leucopenia, and a tendency toward neutrophilia were observed in tested fish.

## INTRODUCTION

Hematological parameters have been useful in assessing the health of fish and in monitoring the stress responses including those due to exposure to sublethal strengths of toxicants (McLeay and Gordon 1977). The blood parameters are easily measurable (Tort and Torres 1988) and the changes in blood parameters often indicate rapid responses to changes in the environment and physiology and provide an integrated measure of the physiological status of the fish. Furthermore, the blood parameters are easily measurable (Tort and Torres 1988).

Among other toxicants, heavy metals have been shown to exert a wide range of effects on fish. These effects include disturbances in osmoregulation (Lewis and Lewis in Tort and Torres 1988), respiration (Majewski and Giles 1981) and tissue damage (Hawkins et al. 1980, Versteeg and Giesy 1986).

No studies have been reported on the hematology of cadmium-exposed *Oreochromis niloticus*. The present work reports on the effects of sublethal cadmium exposure on the Nile tilapia, a common aquacultured fish in the Philippines. The study focused on the hematological parameters because of their relationships with respiration (hemoglobin and related values) and defense mechanisms (leucocyte values).

**Key words:** *Oreochromis niloticus*, blood values, cadmium chloride, hematology, hematocrit, RBC count, leucocrit, hemoglobin concentration, WBC count

## REVIEW OF LITERATURE

Studies on the toxicity of cadmium to some tropical fish have been reported. Banerjee et al. (1978) recorded 50% mortality in *Tilapia mossambica* (12–14 cm long) after 90 hours of exposure to 200 ppm cadmium. Chung (1983) reported that the 96 h LC<sub>50</sub> of cadmium in *Tilapia mossambica* (SL=27 mm) was 80 mg/L. The 24 h LC<sub>50</sub> in *Tilapia nilotica* fry estimated by Andaya and Gotopeng (1982) was 1.6 mg Cd/L.

Cadmium-induced hematological effects on fish have been reported. In carp (*Cyprinus carpio*), treated with 10 mg cadmium chloride/L, Schmidt and Picos (1980) observed that erythrocyte count and hemoglobin content increased while leucocyte number decreased after 3 days, but all indices decreased after 15 days exposure. Beena and Viswaranjan (1987), however, reported that exposure of *Cyprinus carpio* to acute sublethal concentration of cadmium nitrate (24 ppm, 90 h) resulted in significant decrease in erythrocyte count and hematocrit and hemoglobin content while leucocyte and thrombocyte counts did not change significantly. Gardner and Yevich (in Murad and Houston 1988) observed that exposure of the mummichog, *Fundulus heteroclitus*, to 50% of 48 h LC<sub>50</sub> of cadmium for 48 h induced marked progressive decreases in differential lymphocyte and thrombocyte counts with major increases in the overall granulocyte differential count. In *Colisa fasciatus* held for 90 h at 80% of their 96 h LC<sub>50</sub>, Srivastava and Mishra (in Murad and Houston 1988) noted increased total leucocyte, thrombocyte, and lymphocyte counts. In dogfish, *Scyliorhinus canicula*, subjected to sublethal

cadmium exposure, Tort and Torres (1988) recorded decreases of hematocrit and leucocrit and increases of hemoglobin concentration and erythrocyte count after 24 h; after 96 h, the hemoglobin concentration was significantly lower than control values. Hematocrit and erythrocyte declined to control levels while leucocrit was even lower.

Changes in hematological parameters after subchronic or chronic exposure to sublethal levels of cadmium have also been reported in fishes. According to Johansson-Sjoberck and Larsson (1978), the hematological response of the flounder, *Pleuronectes flesus* L., exposed to sublethal levels of cadmium for 4 to 9 weeks were significant reductions in hematocrit, hemoglobin concentration and RBC count, and increase in the number of lymphocytes. In the rainbow trout, *Salmo gairdneri*, chronically exposed to sublethal concentrations of cadmium, Majewski and Giles (1981) reported significant increases in blood hematocrit and hemoglobin concentration. Murad and Houston (1988) observed that in the goldfish, *Carassius auratus*, exposure to sublethal cadmium concentrations for 21 days resulted in sharp reductions in total white cell numbers with significant decline in lymphocyte and thrombocyte counts, while granular leucocytes increased in both absolute and relative numbers.

## MATERIALS AND METHODS

### *Source and Maintenance of Fish*

Young samples of Nile tilapia, *Oreochromis niloticus* (TL=60–115 mm, 4.0–17.7 g), collected from the fishponds of the Natural Science Research Institute, University of the Philippines, Quezon City, were brought to the laboratory for acclimation. They were held in 15-gal and 30-gal glass aquaria supplied with aerated aged tap water; with each aquarium holding a maximum of 20 and 40 fish, respectively. Testing was done in 15-gal aquaria after at least one week of acclimation.

Fish were fed with a commercial fish food to satiation daily during acclimation. They were not fed 24 hrs before and during the tests. Excess food and waste materials were removed daily. Water was replaced every other day during acclimation and daily during bioassay. However, water was not changed during acute exposure to sublethal levels of cadmium. Water temperature, pH, and dissolved oxygen were measured at least once during acclimation (before water replacement) and twice during tests (1st and 4th day). An ordinary laboratory thermometer and a pH meter

(CG 810 TOA Electronics Ltd., Japan) were used to measure temperature and pH, respectively. The Rideal-Stewart Modification of the Winkler Method was used to determine dissolved oxygen content.

### *Blood Collection and Hematological Techniques*

Fish were caught individually using a hand net and blood samples withdrawn immediately from the caudal circulation with a disposable syringe and hypodermic needle rinsed with 10% EDTA (disodium ethylenediamine tetracetic acid, AR, BDH Chemicals Ltd., England).

RBC counts (millions/ml) were done by standard method with the aid of an improved Neubauer hemocytometer (Boeco, Austria) and with Yokoyama's solution (Katz 1951) as diluting fluid.

Total WBC counts (thousands/ml) were done by the indirect method (McKnight 1966) from panoptically stained blood smears (Lucky 1971) using Jenner's solution (Gatenby and Painter 1937) as fixative. Relative WBC counts (%) were done from stained smears similar to the method of Lucky (1971) for differential leucocyte counts.

Hematocrit values (%) were determined by the microhematocrit method (Miale 1982). Leucocrit values (%) were obtained according to the method described by McLeay and Gordon (1977). Heparinized capillary tubes (75 mm long, 1.1–1.2 mm I.D., Chase Instruments Corp., N.Y.), plastic sealing clay (Seal-Ease, Clay-Adams, N.Y.), a clinical model centrifuge, and a vernier caliper were used.

Hemoglobin concentrations (g%) were determined by the cyanmethemoglobin method (Miale 1982). Drabkin's Reagent and Hemoglobin Standard (Medical Center Trading Corp., Manila) were used. Readings were done at 540 nm on a Spectronic 21 (B & L) spectrophotometer.

### *Bioassay*

Test concentrations were prepared by dissolving the appropriate amounts of  $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$  (BDH Chemicals Ltd., England) in a liter of deionized water and by adding this to aquaria containing aged tap water to make up 50 L. Ten fish were placed in each of the following concentrations: 0 (control), 0.05, 0.1, 0.5, 1, 6, 7, 8, 9, 10, 20, 30, 45, 50, 55, and 60 ppm. Water temperature recorded during the tests was 28°C; pH ranged from 6.2 to 7.8; and dissolved oxygen, 3.2 to 5.9 cc/L.

Three replicates of 10 fish each were done for test concentrations that resulted in mortality in 96 h. Data were pooled to calculate the % survival in each concentration. The 96 h LC50 was determined by the Arithmetic Graphic Method (Reish and Oshida 1987).

Water samples containing 0, 45, 50, and 60 ppm cadmium chloride were submitted to the Analytical Services Laboratory of the Institute of Chemistry, University of the Philippines for analysis of total cadmium content.

#### Exposure to Sublethal Levels of Cadmium Chloride

Fish were exposed to 0, 37 (= 70% 96 h LC50), and 26.5 mg/L (= 50% 96 h LC50) cadmium chloride concentrations at near neutral pH (7.1–7.8) and at low pH (5.0–5.8) for 96 h. Acidification was done by adding 2–2.5 ml concentrated sulfuric acid to 50 L of test concentration. Temperature of aquarium water during the tests ranged from 28° C to 30° C; pH, from 5.0 to 7.8; and D.O., from 3.5 to 5.1 cc/L.

#### Statistical Analysis

Means  $\pm$  1 S.E.M. were determined for the hematological values of exposed fish. Comparisons were made between control values and those from treated fish. Significant differences were tested by Student's t test at  $P < 0.05$ .

## RESULTS AND OBSERVATIONS

#### Bioassay

Fish exposed to cadmium chloride concentrations lower than 45 mg/L showed 100% survival after 96 h in the first test run while mortalities were observed in fish exposed to 45–60 mg/L. The survival data resulting from the exposures are shown in Table 1.

The results of analysis for total cadmium in water samples

from 0, 45, 50, and 60 mg/L cadmium chloride concentrations were: <0.10, 22.5, 29.0, and 30.0 mg/L, respectively.

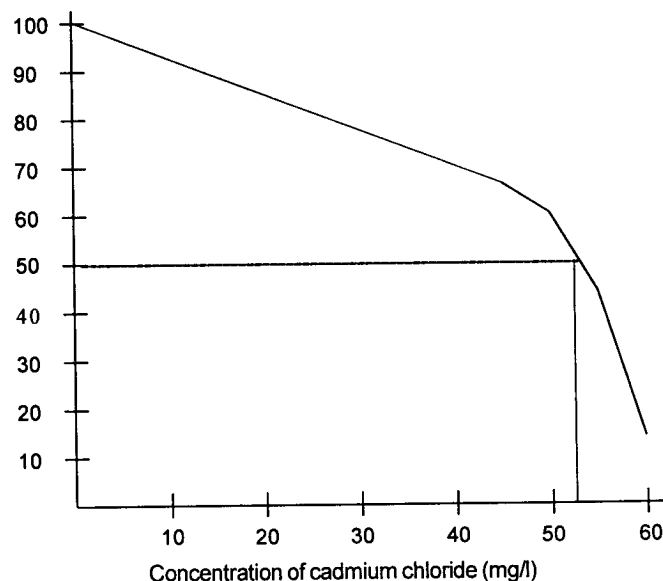


Fig. 1. Survival in *O. niloticus* exposed to cadmium chloride concentrations for 96 h.

The graph formed by plotting concentration against % survival on a graph paper is shown in Fig. 1. The LC50 obtained from the graph is 53 mg/L. Based on the total cadmium analysis data, this contained approximately 29 mg Cd/L.

Blood values of fish exposed to 37 mg/L cadmium chloride concentration at pH 7.1–7.8 are shown in Table 2. As shown in the table, erythrocyte count (RBC) and hematocrit (Ht) and hemoglobin concentrations (Hb) were not significantly different from the control at 24 h, but after 48 h and 72 h exposure, RBC count and Ht markedly decreased. RBC count remained depressed at 96 h, while Ht increased but remained below the control value (not significant). Hb concentrations in treated fish were lower than control at

Table 1. Survival of *Oreochromis niloticus* in different concentrations of  $CdCl_2$  at 96 hours exposure

$CdCl_2$ concentration ppm	No. of exposed fish	No. of dead fish	No. of live fish	% Survival
0	30	0	30	100
45	30	10	20	66.7
50	30	12	18	60
55	30	17	13	43.3
60	30	25	5	16.7

Table 2. Blood values of *Oreochromis niloticus* exposed to 37 ppm CdCl<sub>2</sub> at near neutral pH

	Control	24 hours	48 hours	72 hours	96 hours
No. fish examined	28	17	15	15	17
Fish length (mm)	84 ± 2	87 ± 2	92 ± 2	95 ± 3	90 ± 2
Fish weight (g)	9.9 ± 0.6	11.7 ± 0.5	12.3 ± 0.6	14.6 ± 0.7	11.5 ± 0.5
RBC (x 10 <sup>6</sup> mm <sup>-3</sup> )**	2.2 ± 0.1	2.2 ± 0.1	1.9 ± 0.1*	1.7 ± 0.1*	1.8 ± 0.1*
Ht (%)	30.3 ± 1.5	27.1 ± 1.0	23.4 ± 1.9 *	22.6 ± 2.0 *	28.8 ± 2.1
Hb (g%)	5.3 ± 0.3	5.3 ± 0.2	4.9 ± 0.2	4.9 ± 0.3	4.8 ± 0.2
Lt (%)	3.6 ± 0.4	2.4 ± 0.3*	3.1 ± 0.4	2.9 ± 0.2*	4.0 ± 0.4
WBC (x 10 <sup>3</sup> mm <sup>-3</sup> )	19.5 ± 1.0	10.2 ± 0.9*	9.1 ± 0.7*	14.3 ± 1.1*	15.6 ± 1.1*
Lymphocytes					
(x 10 <sup>3</sup> mm <sup>-3</sup> )	10.4 ± 0.6	6.1 ± 0.7*	4.6 ± 0.4*	4.3 ± 0.6*	4.9 ± 0.4*
( % )	55.2 ± 2.8	59.6 ± 3.8	50.1 ± 2.9	31.1 ± 4.6*	32.4 ± 2.6*
Neutrophils					
(x 10 <sup>3</sup> mm <sup>-3</sup> )	9.0 ± 0.9	4.0 ± 0.5*	4.5 ± 0.5 *	10.0 ± 1.2	10.7 ± 0.9
( % )	44.5 ± 2.7	40.2 ± 3.7	49.9 ± 2.9	68.7 ± 4.6 *	67.6 ± 2.6 *

\* significantly different from control at P < 0.05

\*\* (x10<sup>6</sup> per cubic millimeter)

48 h through 96 h, but the differences were not significant. Leucocrit (Lt), total leucocyte count (WBC), and absolute lymphocyte count in treated fish significantly decreased from control levels except for the Lt at 48 h and 96 h which were not significantly different. Lymphocyte percentage at 24 h was higher than the control (not significant) but decreased after 48 h (not significant) through 72 h (significant) and 96 h (significant). Absolute neutrophil counts were reduced at 24 h and 48 h (significant) but increased at 72 h through 96 h (not significant). Percent neutrophils decreased at 24 h (not significant) but increased at 48 h (not significant) through 72 h and 96 h (both significant).

All control fish survived while 10.9% mortality occurred among treated fish.

### 37 mg/L Cadmium Chloride at pH 5.0–5.8

The blood values of fish exposed to 37 mg/L cadmium chloride at pH 5.0–5.8 are shown in Table 3. The RBC count and related values (Ht and Hb concentration) tended to increase in treated fish, the differences from the control being significant for RBC count at 72 h and 96 h, and for Ht at 48 h and 72 h. Leucocrit decreased at 24 h and 48 h (not significant) but increased at 72 h (significant) and 96 h (not significant). Total WBC count increased (not

significant) at 24 h and 48 h due to increased neutrophil counts, but decreased sharply at 72 h and 96 h. Absolute and relative lymphocyte counts in treated fish were all significantly reduced from the control levels. Absolute neutrophil count at 24 h was markedly increased but declined progressively at 48 h through 96 h to levels not significantly different from control. Percent neutrophils in treated fish were consistently higher (significant) than control value.

Mortality in treated fish was 7.5 %.

### 26.5 mg/L Cadmium Chloride at pH 7.1–7.8 (Table 4)

RBC count, Ht, and Hb concentrations did not differ significantly from the control except for the increased Ht at 24 h and 48 h.

Leucocrit decreased significantly at 24 h but recovered after 48 h. Total WBC count increased slightly at 24 h (not significant), then decreased at 48 h (not significant) and at 72 h (significant), and increased to a level below the control at 96 h (not significant). Absolute and relative lymphocyte counts progressively decreased in treated fish, differences from the control being significant at 48 h through 96 h. Absolute neutrophil counts in treated fish were all higher than the control but differences were not significant.

Table 3. Blood values of *Oreochromis niloticus* exposed to 37 ppm CdCl<sub>2</sub> at low pH

	Control	24 hours	48 hours	72 hours	96 hours
No. fish examined	28	9	8	8	9
Fish length (mm)	84 ± 2	81 ± 2	79 ± 3	79 ± 3	79 ± 2
Fish weight (g)	9.9 ± 0.6	8.1 ± 0.4	7.7 ± 0.9	7.2 ± 0.6	7.2 ± 0.6
RBC (x 10 <sup>6</sup> mm <sup>-3</sup> )	2.2 ± 0.1	2.6 ± 0.2	2.4 ± 0.1	2.8 ± 0.1*	2.5 ± 0.1*
Ht (%)	30.3 ± 1.5	35.4 ± 3.6	40.3 ± 3.4 *	42.9 ± 4.9*	31.9 ± 2.7
Hb (g%)	5.3 ± 0.3	5.2 ± 0.3	5.4 ± 0.6	5.9 ± 0.4	5.5 ± 0.3
Lt (%)	3.6 ± 0.4	3.3 ± 0.7	3.1 ± 0.2	5.6 ± 0.6*	4.0 ± 0.9
WBC (x 10 <sup>3</sup> mm <sup>-3</sup> )	19.5 ± 1.0	21.7 ± 2.9	21.3 ± 2.8	15.1 ± 1.6*	12.4 ± 1.2*
Lymphocytes					
(x 10 <sup>3</sup> mm <sup>-3</sup> )	10.4 ± 0.6	4.8 ± 0.7*	8.1 ± 0.9 *	4.5 ± 0.6*	4.4 ± 0.7*
(%)	55.2 ± 2.8	24.8 ± 3.7*	39.6 ± 3.9 *	27.0 ± 3.4*	33.2 ± 3.8*
Neutrophils					
(x 10 <sup>3</sup> mm <sup>-3</sup> )	9.0 ± 0.9	16.8 ± 3.1*	13.2 ± 2.4	12.7 ± 2.1	7.9 ± 0.8
(%)	44.5 ± 2.7	74.8 ± 3.8*	60.0 ± 3.8*	73.0 ± 3.4*	65.8 ± 3.5*

\* significantly different from control at p<0.05

However, % neutrophils that were consistently higher than the control were significantly different at 48 h through 96 h.

Mortality in fish exposed to cadmium chloride concentrations was 10.7 %.

#### 26.5 mg/L Cadmium Chloride at pH 5.0–5.8 (Table 5)

RBC count and related values in exposed fish were not significantly different from the control except for the lower Hb concentration at 48 h.

Table 4. Blood values of *Oreochromis niloticus* exposed to 26.5 ppm CdCl<sub>2</sub> at near neutral pH

	Control	24 hours	48 hours	72 hours	96 hours
No. fish examined	28	11	10	10	8
Fish length (mm)	84 ± 2	73 ± 1	73 ± 2	72 ± 1	77 ± 2
Fish weight (g)	9.9 ± 0.6	5.6 ± 0.3	6.0 ± 0.2	5.8 ± 0.2	6.4 ± 0.4
RBC (x 10 <sup>6</sup> mm <sup>-3</sup> )	2.2 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0.2
Ht (%)	30.3 ± 1.5	35.7 ± 1.8*	35.1 ± 1.5*	31.8 ± 1.8	33.9 ± 2.3
Hb (g%)	5.3 ± 0.3	4.9 ± 0.3*	5.3 ± 0.2	4.6 ± 0.2	5.3 ± 0.4
Lt (%)	3.6 ± 0.4	2.4 ± 0.4*	3.9 ± 0.8	4.4 ± 0.5	3.4 ± 0.6
WBC (x 10 <sup>3</sup> mm <sup>-3</sup> )	19.5 ± 1.0	20.8 ± 1.7	16.9 ± 1.5	15.5 ± 1.4*	17.4 ± 2.6
Lymphocytes					
(x 10 <sup>3</sup> mm <sup>-3</sup> )	10.4 ± 0.6	9.3 ± 1.8	7.0 ± 0.9*	4.8 ± 1.0*	4.1 ± 0.9 *
(%)	55.2 ± 2.8	44.1 ± 5.8	40.8 ± 3.2*	30.5 ± 4.5*	24.2 ± 3.9 *
Neutrophils					
(x 10 <sup>3</sup> mm <sup>-3</sup> )	9.0 ± 0.9	11.4 ± 1.6	9.9 ± 0.9	10.7 ± 0.2	13.3 ± 2.1
(%)	44.5 ± 2.7	55.7 ± 5.8	59.1 ± 3.2*	69.5 ± 4.5*	75.8 ± 3.9 *

\* significantly different from control at p<0.05

Table 5. Blood values of *Oreochromis niloticus* exposed to 26.5 ppm CdCl<sub>2</sub> at low pH

	Control	24 hours	48 hours	72 hours	96 hours
No. fish examined	28	9	10	10	10
Fish length (mm)	84 ± 2	72 ± 1	69 ± 2	80 ± 3	79 ± 3
Fish weight (g)	9.9 ± 0.6	5.8 ± 0.3	5.1 ± 0.3	7.0 ± 0.6	7.1 ± 0.9
RBC (x 10 <sup>6</sup> mm <sup>-3</sup> )	2.2 ± 0.1	2.0 ± 0.2	2.2 ± 0.2	2.4 ± 0.1	2.1 ± 0.5
Ht (%)	30.3 ± 1.5	26.4 ± 2.2	29.3 ± 1.9	33.0 ± 1.5	30.3 ± 1.4
Hb (g%)	5.3 ± 0.3	4.5 ± 0.4	4.3 ± 0.3*	5.8 ± 0.4	5.6 ± 0.3
Lt (%)	3.6 ± 0.4	5.1 ± 0.9	3.0 ± 0.5	2.4 ± 0.2*	2.3 ± 0.3*
WBC (x 10 <sup>3</sup> mm <sup>-3</sup> )	19.5 ± 1.0	12.6 ± 1.9*	13.9 ± 2.6*	16.0 ± 2.6*	16.8 ± 1.3
Lymphocytes					
(x 10 <sup>3</sup> mm <sup>-3</sup> )	10.4 ± 0.6	3.8 ± 0.8*	3.9 ± 1.1*	2.8 ± 0.5*	3.9 ± 0.6*
(%)	55.2 ± 2.8	30.4 ± 4.0*	27.7 ± 4.1*	22.1 ± 3.4*	24.9 ± 4.4*
Neutrophils					
(x 10 <sup>3</sup> mm <sup>-3</sup> )	9.0 ± 0.9	8.7 ± 1.4	8.0 ± 1.2	12.5 ± 2.1	12.9 ± 1.6*
(%)	44.5 ± 2.7	69.1 ± 4.1*	72.3 ± 4.1*	77.9 ± 3.4*	75.1 ± 4.4*

\* significantly different from control at p<0.05

Leucocrit increased (not significant) at 24 h, then decreased progressively, the difference from the control becoming significant after 72 h. Total WBC count, and absolute and relative lymphocyte count were reduced in treated fish, the differences being significant except for the total WBC count at 96 h. Absolute neutrophil count decreased (not significant) at 24 h and 48 h, but increased at 72 h (not significant) and 96 h (significant). Percent neutrophils were consistently and markedly increased in treated fish.

All treated fish survived the test period.

## DISCUSSION

The 96 h LC<sub>50</sub> of cadmium in *Oreochromis niloticus* determined in the present study (53 mg/L cadmium chloride or approximately 29 mg Cd/L) was lower than the values obtained by either Banerjee et al. (1978, 200 mg/L cadmium) or Chung (1983, 80 mg Cd/L) in *Tilapia mossambica*. This may be due to interspecific differences in sensitivity to cadmium toxicity. The data suggest that *Oreochromis niloticus* is less resistant to cadmium toxicity than the carpine species, *Tilapia mossambica*. The LC<sub>50</sub> value obtained in the present study is higher than the 1.6 mg/L 24 h LC<sub>50</sub> reported by Andaya and Gotopeng (1982) for *O. niloticus* fry. The higher value obtained in the present study could be due to the older and bigger fish used. Banerjee

et al. (1978) who tested bigger fish also obtained higher LC<sub>50</sub> value than Chung (1983) who used smaller-sized fish.

The results of the bioassay as well as the exposure of fish to two sublethal levels of cadmium chloride indicate that the toxicity of the metal is dependent on the concentration, the higher concentration being more toxic. Thus, at the same pH range, lower % mortality was observed in fish exposed to the lower concentration of cadmium chloride. At near neutral pH (7.1–7.8) mortalities observed in exposed fish were 10.9 % in 37 mg/L, and 10.7% in 26.5 mg/L. At lower pH (5.0–5.8), mortalities were 7.5% and 0% in fish exposed to 37 mg/L and 26.5 mg/L, respectively.

Above results also indicate that pH influences the toxicity of cadmium to *O. niloticus*, being more toxic at pH 7.1–7.8 than at pH 5.0–5.8. Similar results were obtained in brown trout exposed to zinc (Everall et al. 1989) in which toxicity was noted to be positively correlated with decreasing acidity over the pH range 5–7, the metal being most toxic at pH 8–9. The interaction of pH with cadmium toxicity may be similar to that of zinc, being complex and apparently dependent on the physical and chemical transformations of the metal (Everall et al. 1989). Kaviraj and Konar (1982) also reported that the acute toxicity of cadmium to *Tilapia mossambica* was influenced by pH.

The significant reductions in RBC count, Ht, and Hb

concentration observed in *O. niloticus* exposed to 37 mg/L cadmium chloride concentration at pH 7.1–7.8 over a period of 96 h were also reported in *Cyprinus carpio* exposed to sublethal concentration of cadmium nitrate for 90 h (Beena and Viswaranjan 1987). Significant reductions in the three parameters were similarly observed in flounders chronically exposed to cadmium (Johansson-Sjobeck and Larsson 1978). In carp exposed to acute sublethal levels of mercuric chloride, reductions in the 3 RBC-related parameters were observed (Beena and Viswaranjan 1987), and reduced RBC count was reported in the same fish species exposed to acute sublethal levels of copper (Dick and Dixon 1985).

The reduced RBC values observed in treated fish may be due to hemolysis (Johansson-Sjobeck and Larsson 1978) or to hemodilution. Metal exposure induces changes in hematological values generally because of changes in blood water content (Tort and Torres 1988). The reduction in RBC-related values observed in the present study suggests stressor-induced hemodilution. In order to optimize gas exchange during stressful conditions, the catecholamines released act to increase blood flow to, and permeability of, gill tissue. Exchange of water ions also increases, resulting in water uptake in freshwater (hemodilution) and water loss in salt water (hemoconcentration) (Dick and Dixon 1985).

Results of exposure of *O. niloticus* to 37 mg/L cadmium chloride concentration at a low pH range (pH 5.0–5.8) over a period of 96 h (increases in RBC counts, Ht, and Hb concentrations over a period of 72 h with a trend to return to control levels at 96 h) are similar to those reported in the dogfish in which increases in RBC count and hemoglobin concentration after 24 h exposure to sublethal concentration of cadmium with a decline to control levels at 96 h were also observed (Tort and Torres 1988). Likewise, increased blood hematocrit and hemoglobin content were noted in carp chronically exposed to sublethal concentration of cadmium (Majewski and Giles 1981), and elevated RBC count and Hb amount after 3 days exposure (Schmidt and Picos 1980). The elevated RBC values could be a consequence of blood cell reserve release (Tort and Torres 1980).

The effects of exposure of *O. niloticus* to 26.5 mg/L cadmium chloride at near neutral pH on the RBC values were not significant except for the initial increase in hematocrit which declined to control levels after 72 h. The increase in Ht could be a result of erythrocyte swelling due to osmotic alterations of the blood by the metal.

The results of exposure to 26.5 mg/L cadmium chloride at pH 5.0–5.8 on the RBC parameters were all insignificant except for the reduced Hb concentration at 48 h. The insignificant trend was reduction over a period of 48 h and recovery after 72 h. Reduction may be due to hemolysis (Johansson-Sjobeck and Larsson 1978) or hemodilution (Tort and Torres 1988).

The general trend toward leucopenia and neutrophilia with consistent lymphopenia observed in cadmium-treated fish studied indicate stressful effects of the sublethal cadmium chloride concentrations tested. Leucopenia is a general consequence of stress (Ellis in Tort and Torres 1988) and metal exposure (Mishra and Srivastava in Tort and Torres 1988). Decreased leucocyte count was also reported in carp exposed to acute sublethal concentration of cadmium nitrate (Beena and Viswaranjan 1987) and cadmium chloride (Schmidt and Picos 1980). Likewise, sharp reductions in white cell and lymphocyte counts, and increase in absolute and relative granular leucocyte counts were observed in cadmium-exposed goldfish (Murad and Houston 1988). Similar decreases in differential lymphocyte count and increases in granulocyte differential count in *Fundulus heteroclitus* after acute exposure to sublethal levels of cadmium were reported (Gardner and Yevich in Murad and Houston 1988).

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