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Haematological studies of clarias gariepinus and oreochromis niloticus exposed to cadmium chloride

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Abstract
Discharge of heavy metals into aquatic environment from various sources above tolerable limits, cause harmful effects in aquatic organisms. The presence of these pollutants in water bodies coupled with their tendency to accumulate in organisms ultimately produce toxic reactions in aquatic biota, especially fish. The present study was carried out to evaluate sub-lethal effects of cadmium chloride on the juveniles of *Clarias gariepinus* and *Oreochromis niloticus*. Haematological changes were observed; the Hb, RBC and HCT were significantly low as a result of the effects of the pollutant (cadmium chloride) in water. It was observed that in the highest concentration the RBC declined more than the remaining treatments with lower concentration. Exposed fish showed a significant increase in WBC count compared to the control. Hence, the indiscriminate disposal of substances containing heavy metals should be discouraged to prevent possible entering into the aquatic environments.

Keywords: Pollution, fish, aquatic, heavy metals

1. Introduction
One of the serious problems in the world is environmental pollution which affects the health of aquatic ecosystems and physiological changes of aquatic animals. The biochemical and physiological parameters of these animals especially fishes are tools and biomarkers for evaluating pollution impacts [1]**.** Cadmium is in its elemental form a soft, silver-white metal. It is not usually present in the environment as a pure metal, but is most often present as complex oxides, sulphides, and carbonates in zinc, lead, and copper ores. Cadmium does not have any recognizable taste or odour. Cadmium sulphate and cadmium chloride are quite soluble in water, whereas elemental cadmium, cadmium oxide and cadmium sulphide are almost insoluble.
Fish have the ability to accumulate heavy metal in their tissues by absorption along the gill surface and gut tract wall to higher levels than the toxic concentration in their environment [2]. Fish are more sensitive to metals such as cadmium and zinc that inhibit ion-regulation because of the energy demands required to maintain a constant condition/homeostasis in lower hardness water [3]. To accurately test the impacts of cadmium and other metals on fish, it is important that the role of hardness and acclimation be evaluated [3].
Haematology provides the real picture of the toxic effects of heavy metals on the vital functions of living organism. The extent of damages induced on fish in relation to concentration of toxicants is utilized in assessing the toxicity of the pollutants [4]. The use of heavy metals has an influence on non-target organisms such as *Clarias gariepinus* and *Oreochromis niloticus* and this may accumulate in their tissues and can be carried to consumers along the food chain causing harmful effects to both fish and man. Therefore this research is aimed at assessing the effects of Cadmium Chloride on the haematological parameters of juveniles of *Clarias gariepinus* and *Oreochromis niloticus.*

2. Materials and methods
2.1 Experimental Fish
Juveniles of *Oreochromis niloticus* of average length 11.22±1.07cm and body weight of 15.63±1.23g, and *Clarias gariepinus* of average length 19.89±0.61cm and weight of 34.04±1.21g were both obtained from Aqua Haven fish farm; behind cattle market North Bank Makurdi, Benue State. The fish were acclimatized for 14 days in the fish hatchery Department of Fisheries and Aquaculture, University of Agriculture, Makurdi.

The fish were fed twice daily at 0800 and 1600 hours at 5% of their body weight. Prior to and during exposure period fish were starved for 24hrs so as to reduce faeces and ammonia in the experimental containers that could act as contaminants to the experiment.

2.2 Experimental Design

A complete randomized design was used for the experiment. A total of one hundred and eighty (180) juveniles each of *Clarias gariepinus* and *Oreochromis niloticus* were randomly distributed into the plastic containers at a stocking rate of ten (10) fish. The eighteen (18) tanks were assigned to 6 treatments with control inclusive. Sub-lethal concentrations were obtained from the LC_{50} 39.69mg/L and 41.03mg/L for *C. gariepinus* and *O. niloticus* respectively using the method according to Abubakar and Abdulsalami [5] which are 0.51, 1.02, 1.54, 2.05 and 2.58mg/l for *C. gariepinus* and 0.67, 1.33, 2.01, 2.68 and 3.35 for *O. niloticus* respectively. Exposures to various sub-lethal concentrations were done in triplicates following methods by Ayoola, [6]. The exposure period lasted for 8 weeks during which each plastic container was well aerated. Physico-chemical parameters of test solutions were determined following the methods of APHA [7] and measured with Hanna II GRO meter. The fish were fed with commercial floating feed (coppens) at 5% of their body weight.

2.3 Procedures for Haematological Studies of *Oreochromis niloticus* and *Clarias gariepinus* Exposed to Sub-Lethal 2.3.1 Concentrations of Cadmium Chloride

At the end of the 8th weeks, blood samples were taken by randomly selecting fish from the various treatments and injecting in a 2mm needle and syringe through the dorsal aorta puncture and placed in ethylene-diamine-tetra-acetic-acid (EDTA) treated bottles to prevent coagulation and analyzed for the following: haemoglobin (Hb), Packed Cell Volume (PCV), Platelets (PLT), Red Blood Cell (RBC), White Blood Cell (WBC) using an automated haemoglobin analyzer (Cobus U 411) model, while Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were determined by calculations.

2.3.2 Mean Corpuscular Volume

This measure was attained by multiplying a volume of blood by the proportion of blood that is cellular (haematocrit), and dividing that product by the number of erythrocytes in that volume.

$$MCV = \frac{\text{Haematocrit (\%)} \times 10}{\text{RBC count (million/mm}^3\text{ blood)}}$$

2.3.3 Mean Corpuscular Haemoglobin

This measure was attained by multiplying a volume of blood by the proportion of haemoglobin, and dividing that product by the number of erythrocytes in that volume.

$$MCH = \frac{\text{Haemoglobin (g/100ml)} \times 10}{\text{RBC number in millions}}$$

2.3.4 Mean Corpuscular Haemoglobin Concentration

This measure was attained by multiplying a volume of blood by the proportion of blood that is cellular (haemoglobin), and dividing that product by the haematocrits in that volume.

$$MCHC = \frac{\text{Haemoglobin (g/100ml)} \times 100}{\text{Haematocrit (\%)}}$$

All the haematological indices were calculated from the equations given by Svobodova. [7]

2.4 Data Analysis.

Data were analyzed using Minitab 14 (Minitab, 2013) for summary statistics; this was carried out to determine if differences exist in water quality parameters for the sub-lethal concentrations. Means in the haematological parameters were analyzed using analysis of variance (ANOVA) at 0.05% level by Genstat version 13, to calculate the significant difference between control and experimental means, where differences exists between the mean, the means were separated using least significant difference (LSD).

3. Results

The physico-chemical parameters of experimental water during sub-lethal toxicity test of cadmium chloride on *Clarias gariepinus* for 56days were shown in table 1. It shows that pH, temperature, TDS, EC & DO varied significantly from the control. The control had the lowest TDS of 174.67 while treatment 5 had the highest TDS of 184.67. The control had the lowest EC of 349.33 while treatment 5 had the highest EC of 369.33. The control had the highest DO of 4.70 while treatment 5 had the lowest DO of 4.61. The pH and temperature show a slight significant difference between the control and the various treatments.

Table 1: Physico-chemical parameters of experimental water during sub-lethal toxicity test of cadmium chloride on *Clarias gariepinus* for 56days

Treatments (mg/L)	pH	Temp (°C)	TDS (ppm)	EC (µS)	DO (mg/L)
0.00	7.56±0.00d	25.65±0.01	174.67±0.33a	349.33±0.67a	4.70±0.00a
0.51	7.55±0.00d	25.65±0.00	175.00±0.58a	350.00±1.15a	4.70±0.00b
1.02	7.54±0.00c	25.66±0.00	176.33±0.33b	352.67±0.67b	4.69±0.00c
1.54	7.54±0.00ab	25.64±0.00	177.00±0.58b	354.00±1.15b	4.69±0.00c
2.05	7.53±0.00ab	25.65±0.01	182.67±0.33c	365.33±0.67c	4.63±0.00de
2.58	7.52±0.00a	25.66±0.01	184.67±0.33d	369.33±0.67d	4.61±0.00e

Mean in the same column with different superscripts differ significantly (P<0.05).

The physico-chemical parameters of experimental water during sub-lethal toxicity test of cadmium chloride on *Oreochromis niloticus* for 56days were shown in table 2. It shows that the pH, temperature, TDS, EC and DO varied significantly ($P<0.05$) from the control. The control had the lowest TDS of 167.00 while treatment 5 had the highest TDS of 177.67. The control had the lowest EC of 334.00 while treatment 5 had the highest EC of 355.33. The control had the highest DO of 4.77 while treatment 5 had the lowest DO of 4.70. There were slight significant difference in pH and temperature between the control and the various treatments.

Table 2: Physico-chemical parameters of experimental water during sub-lethal toxicity test of cadmium chloride on *Oreochromis niloticus* for 56days

Treatments (mg/L)	pH	Temp (°C)	TDS (ppm)	Cond. (µS)	DO (mg/L)
0.00	7.61±0.01d	25.58±0.00a	167.00±1.00a	334.00±2.00a	4.77±0.01a
0.67	7.61±0.00d	25.57±0.00ab	168.33±0.67a	336.67±1.33a	4.76±0.00c
1.33	7.59±0.01c	25.56±0.00ab	170.67±0.33b	341.33±0.67b	4.74±0.00b
2.01	7.58±0.00bc	25.57±0.01b	173.33±0.33c	346.67±0.67c	4.73±0.00b
2.68	7.57±0.00ab	25.57±0.01b	175.67±0.33d	351.33±0.67d	4.71±0.01a
3.35	7.56±0.01a	25.55±0.01b	177.67±0.33e	355.33±0.67e	4.70±0.01a

Mean in the same column with different superscripts differ significantly (P<0.05).

Table 3 shows the haematological parameters of juveniles of *Clarias gariepinus* exposed to sub-lethal concentration of cadmium chloride. The haematological parameters shows Red blood cell (RBC), Haematocrits (HCT), Haemoglobin (HB), Platelets (PLT), White blood cell (WBC), Mean cell volume (MCV), Mean cell haemoglobin concentration (MCHC), and Mean cell haemoglobin (MCH) varied significantly from the control. The values of blood parameters were concentration dependent. The control had the highest haematocrits of 27.18 while treatment 5 had the lowest haematocrits of 19.83. The control had the highest haemoglobin of 9.26 while treatment 5 had the lowest haemoglobin of 6.06. The control had the highest RBC of 4.05 while treatment 5 had the lowest RBC of 1.95. The control had the highest MCHC of 34.06 while treatment 5 had the lowest MCHC of 30.57. The control had the lowest WBC of 3.85 while treatment 5 had the highest WBC of 11.35. The control had the lowest PLT of 30.50 while treatment 5 had the highest PLT of 83.50. The control had the lowest MCV of 67.11 while treatment 5 had the highest MCV of 101.74. The control had the lowest MCH of 22.86 while treatment 5 had the highest MCH of 31.10.

Table 3: Effects of sub-lethal concentrations of cadmium chloride on haematological parameters of juveniles of *Clarias gariepinus*.

Treatment (mg/L)	0.00	0.51	1.02	1.54	2.05	2.58
Parameters						
HCT (%)	27.18±0.03^{e}	26.33±0.03^{d}	26.39±0.01^{d}	21.34±0.02^{c}	20.52±0.04^{b}	19.83±0.01^{a}
HB (g/dl)	9.26±0.03^{e}	8.62±0.00^{d}	8.65±0.00^{d}	7.14±0.02^{c}	6.46±0.04^{b}	6.06±0.01^{a}
WBC (x10^{9}/L)	3.85±0.05^{a}	5.40±0.00^{b}	5.23±0.03^{b}	7.68±0.03^{c}	9.70±0.10^{d}	11.35±0.05^{e}
RBC(x10^{12}/L)	4.05±0.05^{e}	3.23±0.03^{d}	3.20±0.00^{d}	2.76±0.06^{c}	2.43±0.03^{b}	1.95±0.05^{a}
PLT (x10^{9}/L)	30.50±0.50^{a}	42.50±0.50^{b}	52.00±1.00^{c}	61.00±1.00^{d}	71.00±1.00^{e}	83.50±1.50^{f}
MCH (Pg)	22.86±0.35^{a}	26.73±0.21^{b}	27.03±0.00^{b}	25.91±0.47^{b}	26.64±0.11^{b}	31.10±0.85^{c}
MCV (fL)	67.11±0.77^{a}	81.65±0.54^{ab}	82.47±0.03^{c}	77.47±1.49^{b}	84.63±0.71^{c}	101.74±2.59^{d}
MCHC (%)	34.06±0.13^{e}	32.74±0.04^{c}	32.78±0.01^{c}	33.45±0.05^{d}	31.48±0.13^{b}	30.57±0.06^{a}

Mean in the same row with different superscripts differ significantly.

KEY; HCT=Haematocrit, HB=Haemoglobin, WBC=White blood cell counts, RBC=Red blood cell count, PLT= Platelets, MCH= Mean corpuscular haemoglobin, MCV= Mean corpuscular volume, MCHC=Mean corpuscular haemoglobin concentration.

Table 4 shows the haematological parameters of juveniles of *Oreochromis niloticus* exposed to sub-lethal concentration of cadmium chloride. It shows that Haematocrit, Haemoglobin, WBC, RBC, Platelets, MCH, MCV and MCHC varied significantly from the control. The control had the highest haematocrit of 19.11 while treatment 5 had the lowest haematocrit of 15.64. The control had the highest haemoglobin of 6.66 while treatment 5 had the lowest haemoglobin of 5.13. The control had the highest RBC of 2.70 while treatment 5 had the lowest RBC of 1.22. The control had the highest MCHC of 34.83 while treatment 5 had the lowest MCHC of 32.78. The control had the lowest WBC of 2.53 while treatment 5 had the highest WBC of 7.66. The control had the lowest PLT of 15.29 while treatment 5 had the highest PLT of 26.52. The control had the lowest MCH of 24.66 while treatment 5 had the highest MCH of 42.18. The control had the lowest MCV of 70.78 while treatment 5 had the highest MCV of 128.68.

Table 4: Effects of sub-lethal concentrations of cadmium chloride on haematological parameters of juveniles of *Oreochromis niloticus*.

Treatment (mg/L) Parameters	0.00	0.67	1.33	2.01	2.68	3.35
HCT (%)	19.11±0.01^{d}	19.06±0.01^{d}	18.70±0.30^{d}	17.93±0.03^{c}	17.24±0.01^{b}	15.64±0.04^{a}
HB (g/dL)	6.66±0.03^{e}	6.14±0.02^{d}	6.12±0.01^{d}	5.91±0.02^{c}	5.42±0.02^{b}	5.13±0.03^{a}
WBC(x10^{9}/L)	2.53±0.03^{a}	2.63±0.02^{b}	3.36±0.04^{c}	4.14±0.02^{d}	6.69±0.02^{e}	7.66±0.01^{f}
RBC(x10^{12}/L)	2.70±0.02^{f}	2.51±0.01^{e}	2.24±0.04^{d}	1.98±0.03^{c}	1.64±0.04^{b}	1.22±0.01^{a}
PLT(x10^{9}/L)	15.29±0.04^{a}	16.64±0.04^{b}	18.21±0.01^{c}	20.31±0.01^{d}	23.18±0.03^{e}	26.52±0.02^{f}
MCH (Pg)	24.66±0.28^{a}	24.49±0.01^{a}	27.37±0.40^{b}	29.83±0.53^{c}	33.14±0.80^{d}	42.18±0.03^{e}
MCV (fL)	70.78±0.49^{a}	76.09±0.19^{a}	83.71±2.65^{b}	90.55±1.50^{c}	105.49±2.20^{d}	128.68±0.25^{e}
MCHC (%)	34.83±0.15^{c}	32.19±0.10^{ab}	32.71±0.55^{b}	32.95±0.04^{b}	31.41±0.10^{a}	32.78±0.09^{b}

Mean in the same row with different superscripts differ significantly.

KEY; HCT=Haematocrit, HB=Haemoglobin, WBC=White blood cell counts, RBC=Red blood cell count, PLT= Platelets, MCH= Mean corpuscular haemoglobin, MCV= Mean corpuscular volume, MCHC=Mean corpuscular haemoglobin concentration.

4. Discussion

Haematological indices have different sensitivities to various environmental factors, in fish for example, changes in these parameters and their peculiarities depend upon the concentration of heavy metals and the duration of exposure. Blood indices are more frequently utilized when clinical diagnoses of fish physiology are used to ascertain sub-lethal and chronic exposure of contaminant [9]. The decline in RBC count in the current study might have resulted from inhibition of RBC manufacturer by cadmium chloride. Likewise Li *et al.* [10] reported a reduction of total content of RBC in the blood of rainbow trout exposed to verapamil (VPR). Sharma, [11] and Abedi Z [12] saw reductions in haemoglobin of *Clarias gariepinus, Anabas scandens* and common carp treated with mercury chloride and chromium respectively; this is also seen in the present study for both species.

The WBC increased in both species of the present study, this simply reflects the protective response of defense mechanism to counter cadmium mediated stress; This is aimed at stimulating the immune system of the fish to overcome stress [13]. WBC are involved in the adjustment of immunological work in many organisms and the saw increased in WBC count in cadmium chloride treated fish shows a generalized immune reply and a defensive response to cadmium chloride [14]. Rousing effect of the toxicant on immune system and liberate of lymphocytes from lymphomyeloid tissue as a protection mechanism may also redound to expansion in WBC count in fishes [15]. The present study also shows increase in WBC count. The increased number of WBC also may be the result of bio concentration of the tested metal in the kidney and liver. Other authors reported leucocytes concentration show greater and quite different pattern of change with the effect of mercuric chloride when compared with erythrocyte level of control fishes. Allen, [16] observed increased WBC count in *Auraus oreochromis* after mercury exposure. The increase in number of WBC observed in the present study may also be attributed to the stimulation of immune system in response to tissue damage caused by cadmium chloride.

In the values obtained for the haematological indices, *Clarias gariepinus* and *Oreochromis niloticus* showed a decrease in values for haematocrit (Hct), Haemoglobin (Hb), Red blood cell (RBC), and mean corpuscular haemoglobin concentration (MCHC) whereas white blood cell (WBC), Platelete (PLT), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) increased with increase in concentration of the toxicant solution used. There were significant changes in MCV and MCH especially at higher concentration. However, slight fluctuations were recorded in the MCV and MCH when compared with the control. A similar observation was made for *Cyprinus carpio* after cadmium exposure [17].

The significant change ($P<0.05$) in the MCH of both experimental fish (*C. gariepinus* & *O. niloticus*) when compared with the control may be due to reduction in cellular blood iron, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis [18]. Annune *et al.* [19] found that there were increased in MCH and MCHC observed when *Oreochromis niloticus* were exposed to zinc. But different with the findings of Dawson, [20] who reported that, MCV was reduced in striped bass exposed to mercury chloride. The expansion of MCV studied in individual of H. malabaricus exposed to lead chloride may be illustrated by the existence of a larger amount of older or larger RBC as delineated by Hardig and Hogland [21]. In the later phases the high percentage of more red blood cells in the circulation might be the cause for MCV decrease in low concentration. The significant expansion of MCHC value might result from sphaerocytosis as mentioned by Sobecka, [22].

The mean corpuscular haemoglobin concentration (MCHC) is a good indicator of red blood cells swelling [23]. The MCHC, is not influenced by the blood volume neither by the number of cells in the blood, but can be interpreted incorrectly only when new cell with a different haemoglobin concentration are released into the blood circulation [24]. This study is in agreement with Bala *et al*. [4] who reported that prolong reduction in the haemoglobin content is deleterious to oxygen transport and degeneration of the erythrocyte which could be due to pathological condition in fish exposed to toxicant.

5. Conclusion

Pollution by heavy metals has become a serious environmental and public health threats due to their bio-accumulative and non-biodegrable properties; their presence in water produced deleterious effects in a wide variety of fish and other aquatic organisms. From the results obtained in the present study, it can be concluded that cadmium chloride is toxic to *Clarias gariepinus* and *Oreochromis niloticus* as well. This study also shows that exposure of *Clarias gariepinus* and *Oreochromis niloticus* juveniles to sub-lethal concentration of cadmium chloride for 56days must have affected their normal functions. Haematological changes observed in *Clarias gariepinus* and *Oreochromis niloticus* juveniles exposed to cadmium chloride shows that there were significant differences ($P<0.05$) between *Clarias gariepinus* and *Oreochromis niloticus* juveniles of the control and those exposed to the various treatments in all the haematological parameters measured.

6. Recommendation

Due to the serious sub-lethal effects of cadmium chloride on the tested fish, it is imperative that industries are educated on the toxic effects of indiscriminate disposal of waste containing toxic substances on land and water. In this way aquatic organisms could be protected from these kinds of toxic chemicals. Similarly the discharge of cadmium chloride substances (car battery,) directly into fields, ditches, ponds, lake, streams, river and agricultural land in Nigeria and other developing countries is strongly discouraged.

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