



## Haematological Studies on Freshwater Fish *Clarias gariepinus* (L.) Exposed to Cadmium Chloride

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### Abstract

Heavy metals are significant contaminants in aquatic ecosystems because of their persistence and ability to accumulate in aquatic biota. The effect of Cadmium Chloride on the hematological characteristics of the freshwater fish *Clarias gariepinus* is examined in this study. A local fish farm provided the healthy fish, which weighed about  $121 \pm 0.7$ g and measured  $17.25 \pm 1.70$ cm. They were brought to the laboratory in oxygenated bags. Over 21 days, the African catfish were subjected to 20%, 40%, & 60% of the LC50 for CdCl<sub>2</sub>, or 16, 32, and 48 mg/l, respectively. According to the data collected, Red Blood Cells (RBCs) count, Hemoglobin (Hb) concentration, Hematocrit (Hct), Mean Corpuscular volume (MCV) as well as Mean Corpuscular Hemoglobin (MCH), all significantly decreased as exposure concentration increased. However, compared to the control group, the mean corpuscular hemoglobin concentration (MCHC) rose in the treated groups. The study concludes that *Clarias gariepinus* may have unfavourable hematological effects from exposure to cadmium chloride, even at low concentrations.

**Keywords:** *Clarias gariepinus*, Haematology, Cadmium Chloride, Toxicity, Heavy Metal

### Introduction

Heavy metal pollution is one of the numerous causes of fish's low nutritional value. These inorganic elements are poisonous at higher concentrations, even though they are necessary for growth in tiny amounts. Furthermore, since they are persistent, it is difficult to remove them from the environment using biological degrading techniques (Ali et al., 2018). Al-Asgah et al. (2015) state that heavy metals come from various natural and artificial sources. They can impact on human health and affect the environment and are defined as metals having an atomic number of 11 or higher and a minimum density of 5 g/cm<sup>3</sup> (Tchounwou et al., 2012). Biological systems find it difficult to remove these metals from the environment since they are persistent. Heavy metal emissions from untreated industrial waste, some agricultural techniques, and daily activities notably affect freshwater ecosystems. The negative impacts that toxic heavy metal pollution has on aquatic life and human populations make it a serious problem (Elarabany & Bahnasawy, 2019; Onadje & Akalusi, 2023). Heavy metals originate from several sources, including mining, soil erosion, wastewater, urban runoff, industrial waste, agricultural pesticides, and the Earth's crust breaking down naturally (Sudharshan & Sunitha, 2023). The high pollution levels brought on by industrial growth and a sharp increase in petroleum-fueled transportation are the leading causes of heavy metals in emerging countries' fast-expanding cities (Das et al., 2023; Lemessa et al., 2023). According to Lee et al. (2020), fish can absorb heavy metals through their digestive tracts or gills, allowing them to enter their bloodstream and build up in various organs.

Therefore, fish tend to acquire significant levels of metals from their aquatic environment, and the rate at which they accumulate these metals depends on how well they absorb and remove them (Ahmed et al., 2016). The appealing flavour, durability, ease of farming, and quick growth of the African catfish make it a perfect species for cultivation in tropical Africa and Asia (Qu et al., 2014). Due to its widespread cultivation and ability to live in all freshwater bodies, *Clarias gariepinus* is currently commonly employed as a model organism in fish research (Naglaa et al., 2019). One of the most common freshwater fish in Nigeria is the African catfish *Clarias gariepinus*,

which has high nutritional value and is an affordable source of protein. As a result, it makes an excellent animal model for examining the effects of different environmental contaminants. This species can adapt to various food sources and is resilient to environmental stress. It can also be found day or night (Onadje & Akalusi, 2023).

Cadmium (Cd) has garnered significant attention among heavy metals due to its toxicity and widespread presence in industrial applications. Due to its corrosion resistance, cadmium is a very poisonous heavy metal that is not physiologically necessary. However, it is widely employed in the manufacturing of Ni-Cd batteries, the mining and metal industries, and dentistry (Sobha et al., 2007). A highly toxic heavy metal with a specific gravity of 8.65 times that of water, cadmium (Cd) is usually found in nature in low concentrations, mostly in argillaceous and shale deposits, where it appears as green rocks (Cds) or otavite ( $\text{CdCO}_3$ ). Cadmium is frequently found in sulphide form with zinc, lead, or copper (Cameron, 1992). It has been placed on the European community's 'black list' and classified as a B-class (soft) metal (Mason, 1996). Exposure to cadmium affects the hypothalamic-pituitary-interrenal axis and is closely associated with endocrine disruption (Liu et al., 2017). Hematology is a vital biomarker in many environmental monitoring, research, and evaluation fields, including environmental surveillance, toxicology, safety analysis, and chemical risk assessment (Naz et al., 2020). Toxins such as heavy metals continuously expose blood-forming tissues to their detrimental effects after entering the fish's body (Ullah et al., 2021). Hematological parameters are used as indicators of stress caused by contaminants and environmental changes (Prakash & Verma, 2022). They are essential instruments for diagnosing and monitoring how organisms respond to stresses, and they provide insight into the state of health under such adverse conditions (Parmar & Prakash, 2022; Singh & Prakash, 2022). The initial indications of pathological illnesses brought on by various toxicants are changes in the parameters of blood, a sensitive tissue impacted by the environment. They are, therefore, a great way to keep an eye on the health of fish. Fish health can be evaluated by tracking haematological and biochemical markers (Ullah & Li, 2019). Numerous haematological indicators, such as hemoglobin, hematocrit, white blood cells (WBCs), red blood cells (RBCs), mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration, have been used to detect metal pollution in the aquatic environment (Hassan, 2018). Haematological characteristic indices have effectively monitored status in such adverse conditions (Elarabany et al., 2019; Tabat & colleagues, 2021; Batool et al., 2023; Chandra & Verma, 2022). This study examined how sublethal amounts of calcium chloride affected the haematological traits of African catfish or *Clarias gariepinus*.

## Materials and Methods

### Preparation of Toxicant

One gram of cadmium metal was dissolved in 100 grams of distilled water to create a stock solution of the test metal ( $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ ). The formula below was used to determine the concentration.

$$\frac{\text{Desired concentration of Cd} \times \text{Molecular weight of CdCl}_2 \cdot \text{H}_2\text{O}}{\text{Atomic Weight of Cd}}$$

Reish and Oshida (1987)

### Experimental Fish

In this study, we used the African catfish *Clarias gariepinus*, which weighed around  $121 \pm 0.7\text{g}$  and measured about  $17.25 \pm 1.70\text{cm}$  in length. Live, healthy specimens of this species were acquired from a local fish farm and transported to the laboratory in an oxygenated bag. To give them time to adjust, they were placed in plastic tanks with dimension of  $40 \times 35 \times 70\text{ cm}$  and contained 60 L of dechlorinated tap water that was changed daily for 14 days. The fish were given a commercial basal diet each day. The mortality rate was less than 2 percent during the acclimatization stage.

### Experimental Procedure

A total of 120 fish were separated into four groups. The other three groups were exposed for 21 days to 20 percent, 40 percent, and 60 percent of the  $\text{LC}_{50}$  for  $\text{CdCl}_2$ , or 16, 32, and 48 mg/l, respectively, while the control group was left untreated.

### Haematological Analyses

After determining the fish's weight and length, they were put belly up, and a  $2\text{ cm}^3$  heparinized plastic syringe and a 21 gauge disposable hypodermic needle were used to draw blood samples from the caudal circulation. Because contact with glass shortens the coagulation period, it is essential to use a plastic syringe when handling fish blood

(Smith et al., 1952). To avoid mucus contamination, tissue paper was used to wipe the puncture site, which was about 3–4 cm from the vaginal opening. The needle was positioned perpendicular to the fish's spinal column and gently aspirated during penetration. A caudal blood vessel was perforated by the needle, which was then gently pulled down until blood started to flow in. After gently aspirating the blood until about 1 cm<sup>3</sup> was acquired, the needle was taken out, and the blood was carefully transferred into heparinised plastic containers. After that, the samples were carefully combined thoroughly. Hemoglobin concentration, hemoglobocrit, total white blood cell count, and red blood cells count were measured in a few blood samples. Blood samples were centrifuged. The plasma obtained was then transferred into a 1 cm<sup>3</sup> plastic syringe, put in a universal bottle, refrigerated, and used for the blood analysis. All analyses were performed in duplicates for each sample. The Stoskopf (1993) and Dacie and Lewis (2006) methodologies were used to calculate RBCs and WBCs, respectively. The Packed Cell Volume (PCV) / Haematocrit (Hct) values were calculated using Britton's (1963) formula. Additionally, mean cell haemoglobin (MCH), mean cell volume (MCV) and mean cell concentration (MCHC) were measured using the techniques of (Dacie & Lewis, 1977).

### Statistical analysis

The differences between the treatments were evaluated using a one-way analysis of variance (ANOVA) with statistical differences set at  $P < 0.05$  for the data collected using the Graph Pad Prism 10 software.

### Results

After 21 days of exposure to a sublethal dose of CdCl<sub>2</sub>, *Clarias gariepinus*'s haematological parameters differed significantly ( $p < 0.05$ ) from those of the unexposed/control group (Table 1). RBC and WBC levels decreased as toxicant concentrations increased. WBC counts were  $204.81 \pm 3.02$ ,  $192.32 \pm 4.01$ ,  $173.31 \pm 4.37$ , and  $161.10 \pm 3.33$  for the unexposed and test specimens exposed to 20 percent LC<sub>50</sub>, 40 percent LC<sub>50</sub>, and 60 percent LC<sub>50</sub>, respectively, while RBC counts were  $2.91 \pm 0.72$ ,  $2.77 \pm 0.14$ ,  $2.63 \pm 0.07$ , and  $2.31 \pm 0.15$ . Additionally, the unexposed fish and those exposed to 20 percent LC<sub>50</sub>, 40 percent LC<sub>50</sub>, and 60 percent LC<sub>50</sub> had thrombocyte values of  $64.13 \pm 12.1$ ,  $61.57 \pm 9.6$ ,  $57.38 \pm 5.7$ , and  $54.34 \pm 14.3$ , respectively. A similar pattern was also seen in haemoglobin levels, which decreased as the toxicant's concentration increased. The control value was  $64.13 \pm 12.1$ , but at the 60 percent concentration, it fell to  $54.34 \pm 14.3$ . Both MCH (pg) and MCHC (g/dl) exhibited a fast decline in direct proportion to the increase in CdCl<sub>2</sub> concentration. Nevertheless, after 21 days of exposure, MCV (fl) increased in proportion to the growth in ambient CdCl<sub>2</sub> levels. The matching values for the treated and unexposed groups were  $143.58 \pm 3.42$  fl,  $133.21 \pm 2.43$ ,  $137.32 \pm 3.46$ , and  $129.46 \pm 3.17$  fl. Furthermore, the results for the neutrophil and eosinophil counts indicated decreasing values as the levels of CdCl<sub>2</sub> concentration increased.

**Table 1:** Haematological Parameters of *C. gariepinus* exposed to sublethal concentration of CdCl<sub>2</sub> for 21 days (Mean  $\pm$  SE).

Parameters	Treatments			
	Control	20% LC <sub>50</sub>	40% LC <sub>50</sub>	60% LC <sub>50</sub>
Red Blood Cells (RBCs) (count $\times 10^6/\text{mm}^3$ )	$2.91 \pm 0.72$	$2.77 \pm 0.14$	$2.63 \pm 0.07$	$2.31 \pm 0.15$
White Blood Cells WBCs) ( $\times 10^9/\text{l}$ )	$204.81 \pm 3.02$	$192.32 \pm 4.01$	$173.31 \pm 4.37$	$161.10 \pm 3.33$
Thrombocytes ( $10^3/\mu\text{l}$ )	$64.13 \pm 12.1$	$61.57 \pm 9.6$	$57.38 \pm 5.7$	$54.34 \pm 14.3$
Haemoglobin (Hb) (g/dl)	$12.71 \pm 0.24$	$12.22 \pm 0.12$	$11.12 \pm 0.91$	$10.41 \pm 0.17$
Eosinophil (%)	$2.57 \pm 0.27$	$3.15 \pm 1.22$	$3.97 \pm 87$	$4.21 \pm 2.45$
Neutrophils (%)	$7.86 \pm 0.81$	$6.65 \pm 1.12$	$6.32 \pm 1.08$	$6.21 \pm 0.58$
Packed Cell Volume (PCV) / Hematocrit (HCT) (%)	$42.43 \pm 2.14$	$40.01 \pm 1.49$	$37.51 \pm 2.43$	$33.12 \pm 2.28$
Mean Corpuscular Hemoglobin Concentration (MCHC) (g/dl)	$35.48 \pm 2.69$	$31.64 \pm 1.08$	$30.60 \pm 1.74$	$27.07 \pm 3.13$
Mean Corpuscular Hemoglobin (pg)	$53.14 \pm 1.75$	$51.12 \pm 2.98$	$48.02 \pm 2.19$	$44.06 \pm 2.03$
Mean Corpuscular Volume (MCV) (fl)	$129.46 \pm 3.17$	$133.21 \pm 2.43$	$137.32 \pm 3.46$	$143.58 \pm 3.42$

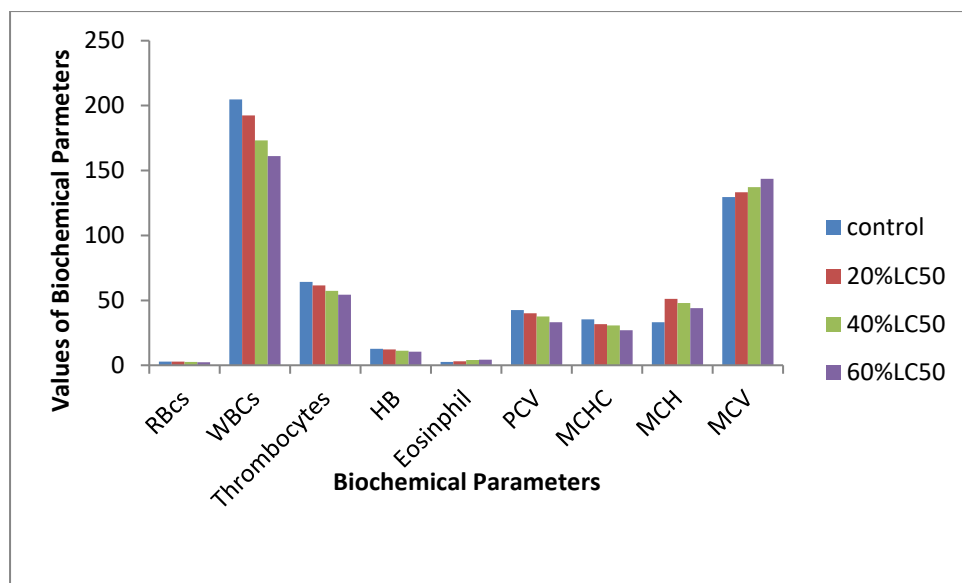


Figure 1: Haematological Parameters of *C. gariepinus* exposed to sublethal concentration of  $\text{CdCl}_2$  for 21 days (Mean  $\pm$  SE).

### Discussion

In recent years, haematological parameters have played a more significant role in defining the sublethal doses of pollutants (Hassan, 2018; Elarabany et al., 2019; Batool et al., 2024). The results of this investigation indicate that the concentration and duration of exposure to cadmium ( $\text{Cd}^{2+}$ ) had a substantial effect on the haematological variables of the fish exposed to this metal. Statistically significant ( $P < 0.05$ ) decreases in several of the values of the evaluated haematological parameters are common in fish exposed to sublethal amounts of toxicants and therapies.

### RBC COUNTS, HB and HT

Fish exposed to Cadmium have their RBC counts, Hb, and Ht displayed in Table 1. The RBCs are the most common type of cells in fish and are primarily responsible for delivering oxygen to all tissues. They can function as a "red blood cell immune system," similar to white blood cells, which helps the immune system (Shen et al., 2018). They can also limit angiogenesis and RBC formation, which can help fish with anaemia (Abdel-Tawwab et al., 2017). Stress-induced anaemia is a crucial indicator for evaluating environmental stress, as fish under stressful circumstances tend to have decreased RBCs, Ht levels, and Hb concentrations (Parrino et al., 2018). Ht is a straightforward measurement of the proportion of RBCs in blood volume and is crucial for assessing fish's ability to carry oxygen in their blood (Witeska et al., 2023). Anaemia, hemodilution, and fish fin bleeding brought on by different metal exposures or environmental stresses are all strongly associated with a decrease in Ht (Batool et al., 2024). Hb, an iron-containing oxygen transport metalloprotein found in red blood cells, serves as an oxygen transporter, an antioxidant, and a controller of iron metabolism (Shen et al., 2018). Research conducted by Abdel-Tawwab and Wafeek (2017) revealed a substantial decrease in RBCs, Ht, and Hb in the significant commercial fish *Labeo rohita* after exposure to varying Cadmium Chloride concentrations. Likewise, Elarabany et al., in 2019, observed a significant reduction in RBCs, Ht, and Hb in *Clarias gariepinus* when exposed to 10% and 30% of the LC50 level of Cadmium, suggesting that cadmium had an unfavourable impact on the metabolic and hematopoietic functions in fish. In 2020, Naz et al. reported a significant decrease in RBCs, Ht, and Hb in *Catla catla*, a major carp species, after exposure to 1.35 and 1.8 mg/L of Cd. Moreover, Samuel et al. (2021) found a significant decrease in RBCs and Hb in *Clarias gariepinus* after exposure to 12 mg/L of Cd, indicating that the iron content in the blood was reduced, consequently reducing the oxygen transport capacity of the blood and impacting the production of erythrocytes. Consequently, the reduction in blood parameter values suggests anaemia in *C. gariepinus* due to cadmium exposure in this study.

### MCV, MCH, and MCHC

MCV, MCH, and MCHC in fish exposed to Cd are demonstrated in Table 1. MCV and MCH are important indicators for evaluating the diagnosis of anaemia in most vertebrates, including fish. The MCV, MCH, and MCHC in fish exposed to Cd are displayed in Table 1. MCV and MCH are important indicators for diagnosing anaemia in most vertebrates, including fish. MCHC is a helpful biomarker for evaluating haemoglobin production reductions and RBC enlargement. Regarding toxicant-induced changes in erythropoiesis and oxygen transport capacity, these metrics offer crucial information about fish health (Witeska et al., 2023). The investigation results showed that as concentrations increased, MCHC and MCH decreased. While not in line with Elarabany et al. (2019), who reported a significant increase in MCHC, MCH, and MCV in *C. gariepinus* exposed to LC50 levels of cadmium at 10 and 30 percent, this result supports earlier findings by Batool et al. (2024). According to Deen et al. (2009), *Oreochromis niloticus* treated with 10 mg/L Cd showed a considerable rise in MCV but a decrease in MCH and MCHC, suggesting that the fish's haematopoietic function and metabolic activity were compromised. According to Ibrahim et al., (2021) haemolysis of RBCs and a decrease in iron in Hb caused MCHC to dramatically decrease in *Oreochromis niloticus* after 0.36 mg/L Cd, while MCV and MCH significantly increased after 0.12 and 0.36 mg/L Cd.

### WHITE BLOOD CELLS (WBCS) / LEUCOCYTES

WBCs, called leucocytes, are immune system cells necessary for specific and non-specific immune responses that protect the body against foreign pathogens. According to a literature review, leucocytes reacted in two different ways to different heavy metals. Per previous research, WBC levels increased in fish subjected to heavy metal toxicity (Batool et al., 2024; Elarabany et al., 2019; Hassan (2018). As the quantities of the pollutant CdCl<sub>2</sub> rose, the study's results showed that the WBC counts of the tested groups declined significantly during each period compared to the unexposed group (control) (Table 1). This may help clarify why stress leads to the release of adrenaline, which reduces white blood cell counts and weakens the immune system (Abdel-Warith et al., 2020).

### Conclusion

This study aimed to evaluate the effects of exposure to sublethal amounts of cadmium chloride over 21 days. The present study illustrated how *Clarias gariepinus* fish exhibited altered haematological parameters due to exposure to cadmium. Changes in these parameters might be utilised as biomarkers or sensitive indicators to evaluate the toxicity of metals in aquatic environments. The results of this study also aid in determining a lower concentration of these metals in the aquatic environment and elucidate the toxicological endpoint of aquatic contaminants, both of which are necessary to protect aquatic life. Moreover, it is evident from the above that water contaminated with CdCl<sub>2</sub> has a detrimental effect on human and fish health, reflected in economic status.

### Recommendations

Following the findings of this study, the following are thus recommended:

1. Water bodies should be regularly monitored for heavy metal pollutants, particularly cadmium, to avoid toxic accumulation.
2. Strict control over the amount of cadmium that mining, agriculture, and industry release into aquatic habitats.
3. Fish health parameters should be encouraged as **early warning signals** of water pollution.
4. Water from possible contaminated areas should not be used for aquaculture
5. The relevant stakeholders (communities, industries and fish farmers) should be educated on the dangers of heavy metal pollution
6. Government should encourage cleaner production methods and enforce environmental protection legislation.
7. Further researches should be carried out to examine the synergistic impact of cadmium and other heavy metals on fish health.

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