



Acute toxicity of Cadmium (Cd^{2+}) to the developmental stages of freshwater fish, *Clarias gariepinus* (Burchell, 1822)

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Abstract

Cadmium toxicity on the aquatic environment has negative impact to fresh water organisms including fish such as *C. gariepinus*. However, the study evaluated the impact of short-term exposure for 96 hours of the developmental stages of *C. gariepinus* to waterborne cadmium (Cd^{2+}). The different stages of development of the fish were gotten through artificial reproduction of *C. gariepinus* carried out in the Fishery/Hydrobiology Laboratory, Department of Biology, Ahmadu Bello University, Zaria, Nigeria. For the acute bioassay, 420 embryos of 12 hours post fertilization (hpf) and 180 larvae of 24 hours post hatching (hph) were randomly exposed in one-liter capacity plastic tanks containing 500 ml of Cd^{2+} concentrations of control (0.00), 1.80, 2.00, 2.20, 2.40, 2.60 and 3.00 mg/L and (0.00, 0.80, 1.00, 1.20, 1.40 and 1.60 mg/L) respectively, for the 96-h. However, acute effects of Cd^{2+} to *C. gariepinus* reduced the percentage hatching from 88.89% in the control group to 52.22% in the group exposed to 3.0 mg/L Cd^{2+} , caused malformation of embryos, and LC_{50} values of 2.55 and 1.33 for embryos and larvae respectively. Therefore, the major sources of cadmium (Cd^{2+}) should be minimized from entering into the freshwater aquatic environment.

Keywords: Cadmium (Cd^{2+}), acute toxicity, *C. gariepinus*, developmental stages and embryos

Introduction

Cadmium (Cd), a well-known heavy metal which is extremely toxic has a specific gravity 8.65 times greater than water. It is a common aquatic environmental contaminant, that is associated with a wide variety of human activities and products like pigments, ceramics, plastics, glasses, vehicle tyres and other synthetics (Sen and Mandal, 2019) [24]. Freshwater lotic resources are contaminated with numerous pollutants, which has become a matter of great concern lately (Tabrez *et al.*, 2021) [27]. Among the pollutants, heavy metals are the main culprits Khan *et al.* (2020), resulting from intensive agricultural operations and industrial activities, because of their ubiquitous presence, non-biodegradability, and persistency (Chauhan *et al.*, 2019) [7]. These heavy metals constantly challenge the ecological balance of the recipient water body, diversity of aquatic fauna, quality of seafood, and health of fish consumers (Abiona *et al.*, 2019) [1]. The toxic action of metals is particularly pronounced in the early stages of fish development, (Vinodhini and Narayanan, 2008; Aldavood *et al.*, 2020) [28, 3] and adversely affects various metabolic processes in developing fish (embryos in particular), resulting in developmental retardation, morphological and functional deformities, or death of the most sensitive individuals (Sfakianakis *et al.*, 2015; De Silva *et al.*, 2021) [25, 9]. On the other hand, fish deformities have devastating effects on fish populations since they affect their survival, growth rate, welfare and external morphology (Boglione *et al.*, 2013) [5]. Some of the most common deformities can be located in the vertebral column. However, African catfish has been the most popular choice as test organism because it is cheap and a rich source of animal protein, hardy, found in all freshwater sources. Fishes are considered to be most significant biomonitors in aquatic systems for the estimation of metal pollution level. As reported

by Authman (2008) [4], they offer several specific advantages in describing the natural characteristics of aquatic systems and in assessing changes to habitats (Lamas *et al.*, 2007) [16].

Materials and Methods

Collection and Maintenance of *Clarias gariepinus* Broodstock

Sexually mature male and ripe female breeders (broodstock) of *C. gariepinus* weighing 1.2kg to 1.8kg, with a standard length of 50-57cm were obtained from a fish farm in Ungwan Boro, Kaduna, Kaduna State, Nigeria. The Spawners were selected according to the criteria described by De Graaf and Janssen (1996) [8]. The fish were then transported in oxygenated polythene bags to the Fisheries Laboratory, Department of Biology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. They were fed on a commercial pellet diet (3% of body weight per day) and acclimatized for three weeks in 800 L rectangular tanks containing dechlorinated tap water (conductivity 2000 $\mu\text{S}/\text{cm}$; $\text{pH} \approx 7.5$; Oxygen 90-95% saturation; temperature 25 °C; photoperiod 12:12 Light: Dark).

Artificial Reproduction of *Clarias gariepinus*

Selection, identification, hormone administration, collection of gametes, fertilization and incubation of eggs from the brood stock.

Artificial reproduction of *C. gariepinus* was carried out in the Fisheries Laboratory, Department of Biology, Ahmadu Bello University, Zaria, Nigeria. For this experiment, two males and female fishes each were selected based on the external morphological features as described by Marimuthu *et al.* (2013) [17]. However, the mature male was identified by a slightly pointed genital papilla, and females by a swollen abdomen and a reddish swollen vent. In addition, the maturity of the ripe

female was confirmed by a slight pressing of the ventral side of the fish for oozing of eggs. Both the female and male fish were artificially induced by intra-muscular injection with ovulin at 0.5 mL per kilogram of fish body weight. Hormone injected to male and female *C. gariepinus* were then released separately into rectangular cement tanks (500 L) containing dechlorinated tap water. After 12 h of hormone administration, the females were stripped by applying slight pressure on the abdomen. This led to running out of the eggs which were collected into circular plastic trays. The male was sacrificed to expose the testes which were removed and squeezed to let out the milt for fertilizing the eggs. Dry fertilization was then carried out by mixing the milt and the eggs in a plastic bowl using feather. After 2 min of gentle stirring, the fertilized eggs were washed several times with freshwater to remove excess milt. The fertilized eggs were immediately placed in experimental units for embryo toxicity assay. A portion of fertilized eggs were released into the glass aquarium to obtain hatchlings for larval and subsequently raised fingerlings and juveniles for the different stages of bioassay exposure.

Preparation of Metal Test Solutions

Analytical grade Cadmium chloride ($\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$) was obtained from Kaduna central Market (Merck Company, Darmstadt, Germany, Glaxo India Limited, Bombay, India (No. 17584) and used without further purification. Cadmium chloride solutions were prepared with distilled water to the desired concentrations and introduced into glass tanks. The concentration of cadmium chloride was expressed in terms of Cd^{2+} ion in mg/L. Dechlorinated tap water which is stored in a large overhead tank for about ten days was used for conducting the toxicity experiments. A stock solution of 1000mg/L (1g/L) of the cadmium was prepared by adding 2.0 g of cadmium chloride to 1litre of dechlorinated tap water (Reish and Oshida, 1987) [22]. The stock solution was used for preparing different strengths of the test solutions by diluting measured volumes with dechlorinated tap water. The dechlorinated tap water used had the same physical and chemical properties as the one used in acclimatizing the fish. The control solutions were made up of only dechlorinated tap water. The amount of cadmium chloride which contained 1.0 g of cadmium was determined from the molecular and atomic weights.

Experimental Design

Healthy fertilized embryos (12 hours post fertilization) were randomly exposed to 21 plastic containers measuring 1 liter each, containing 500 ml of dechlorinated water and blank control respectively. Twenty (20) embryos (12 hours post fertilization) were placed in each plastic container (totaling four hundred and twenty (420) embryos). This gives 21 experimental set up, for each of the containers containing Cd and the control for the acute toxicity test i.e. seven (7) treatments, including control in three (3) replication. Ten (10) larvae of (24 hours post hatching) were randomly exposed to 15 plastic containers each measuring 1-liter and each container containing 500 mL cadmium concentrations and 3 controls with dechlorinated tap water (blank control) respectively. This gave 18 experimental set ups, for each of the tanks containing heavy metal (Cd) and the control, to give a total of one hundred and eighty (180) fish used for the acute toxicity test i.e. six (6) treatments, including control in three (3) replications for each of the two separate experiments for the larvae.

Acute Toxicity Bioassay

Acute-toxicity test of *Clarias gariepinus* stages of development were conducted according to the OECD Draft Proposal-Fish Embryo Toxicity (FET) Test (OECD, 2013) and a previously proposed method by Fraysse *et al.* (2006). The nominal concentrations of cadmium were 1.8, 2.0, 2.2, 2.4, 2.6 and 3.0 mg/L, selected based on pre-experiment data. Dechlorinated tap water was used to prepare all test solutions, and it served as blank control (0 mg/L). To evaluate the embryonic toxicity, four hundred and twenty (420) normally developed embryos (approximately 12 hpf) were randomly selected and transferred into respective test concentrations. Each experimental treatment and control were conducted in three replicates. The experiments were conducted using one-liter capacity plastic containers containing 500 ml of the toxicant. Treatments were allotted at random in the experimental units. The number of dead individuals and the state of embryonic development were examined daily. Acute toxicity measurement in larvae and fries were conducted following the method of Mu *et al.* (2013). One hundred and eighty (180) larvae of 24 hph were randomly exposed to 1L plastic container containing 500 ml of dechlorinated tap water (blank control), and 0.80, 1.00, 1.20, 1.40 and 1.60 mg/L concentration of cadmium, respectively.

Mortality

Survival rates were calculated by counting the living larvae to the total embryos. Dead larvae were judged via the appearance of blood circulation, heartbeat and body colour changes under the microscope (for larvae) (OECD, 1998). Dead embryos were counted and discarded daily. The heartbeat rates of larvae from each group at corresponding time points were observed under a microscope and recorded (beats/minute) by using a timer. Survival and mortality were recorded from 1-12, 24, 48, 72 and 96 hours. Fishes were considered dead when the opercular movement ceased and there was no response to gentle probing. Dead fish were removed immediately to avoid dissolved oxygen depletion.

Data Analysis

Probit analysis was used to determine the LC_{50} value for the heavy metal using minitab 15 statistical packages. The data collected from embryos hatching rate and larvae, were subjected to one way Analysis of Variance (ANOVA) using IBM SPSS Statics Version 20.0 for Windows 8, statistical analysis software and Duncan's Multiple Range Test (DMRT) was used to test for differences between different levels of treatment and to separate means respectively, where applicable (Duncan, 1955) [10]. Test of significance was at 5% level.

Results

The physicochemical parameters of the test water measured daily during acute exposure of embryos and larvae to cadmium (Cd^{2+}) concentrations are presented in Table 1 and 2. The mean physicochemical parameters of water during acute exposure of embryos to cadmium (Cd^{2+}) concentrations were, Temperature (T) ($^{\circ}\text{C}$) was $24.94 \pm 0.06^{\circ}\text{C}$, Hydrogen ion concentration (pH) was 7.80 ± 0.07 , Electrical Conductivity (EC) ($\mu\text{S}/\text{cm}$) was $479.95 \pm 1.07 \mu\text{S}/\text{cm}$, Total Dissolved Solids (TDS) (mg/L) was $238.29 \pm 0.80 \text{ mg}/\text{L}$ and Dissolved Oxygen (DO) (mg/L) was $3.54 \pm 0.10 \text{ mg}/\text{L}$ (Table 1). While the mean physicochemical parameters of water during acute exposure of larvae to cadmium (Cd^{2+}) concentrations were T ($^{\circ}\text{C}$) was $23.82 \pm 0.09^{\circ}\text{C}$, pH was 8.02 ± 0.41 , EC was $271.33 \pm 2.71 \mu\text{S}/\text{cm}$, TDS was $135.72 \pm 1.34 \text{ mg}/\text{L}$ and DO was $4.14 \pm 0.06 \text{ mg}/\text{L}$ (Table 2).

Table 1: Physico-chemical parameters of test water monitored during the acute exposure of embryos of *Clarias gariepinus* to cadmium (Cd^{2+})

Parameters	Range	Mean \pm S. E	(FEPA, 1991) [11]
Temperature (T) ($^{\circ}\text{C}$)	24.00 – 25.20	24.94 \pm 0.06	26.00
Hydrogen ion Concentration (pH)	7.30 – 8.30	7.80 \pm 0.07	6.0-8.5
Electrical Conductivity (EC) ($\mu\text{S}/\text{cm}$)	469 – 489	479.95 \pm 1.07	2000
Total Dissolved Solids (TDS) (mg/L)	232 – 243	238.29 \pm 0.80	<600
Dissolved Oxygen (DO) (mg/L)	2.90 – 4.10	3.54 \pm 0.10	5.0 – 7.0

Table 2: Physico-chemical parameters of test water monitored during the acute exposure of larvae of *Clarias gariepinus* to cadmium (Cd^{2+})

Parameters	Range	Mean \pm S. E	(FEPA, 1991) [11]
Temperature (T) ($^{\circ}\text{C}$)	23.00 – 24.20	23.82 \pm 0.09	15 - 35
Hydrogen ion Concentration (pH)	7.80 – 8.30	8.02 \pm 0.41	6.0 – 9.0
Electrical Conductivity (EC) ($\mu\text{S}/\text{cm}$)	254 – 288	271.33 \pm 2.71	2000
Total Dissolved Solids (TDS) (mg/L)	127 – 144	135.72 \pm 1.34	<600
Dissolved Oxygen (DO) (mg/L)	3.70 – 4.60	4.14 \pm 0.06	5.0 – 7.0

Behavioural and Morphological Responses of *C. gariepinus* Embryos Exposed to Acute Concentrations of Cadmium (Cd^{2+})

Hatching success

Exposure of eggs to Cadmium resulted in decrease in hatching success. The hatching was highest in control group and lowest in treated group having the highest concentration of Cadmium. The values of hatching success for different Cadmium concentrations i.e. Control, 1.8 mg/L, 2.0 mg/L, 2.2 mg/L, 2.4 mg/L, 2.6mg/L, 2.8mg/L and 3.0 mg/L were determined in percentages (%) as 88.89 \pm 2.2, 79.78 \pm 2.9, 76.67 \pm 1.9, 67.78 \pm 1.1, 65.55 \pm 4.0, 63.33 \pm 1.9, 55.56 \pm 1.1 and 52.22 \pm 2.9 respectively (Fig. 1). The difference in percentage hatching success was significant ($p < 0.05$) at different concentrations. Although the difference in hatching success in the Control and Cadmium

concentration of 1.8 mg/L was insignificant ($p > 0.05$) with, Cd^{2+} concentration of 2.0 mg/L onwards survival of eggs and their hatching decreased significantly. Only 52.22 \pm 2.9% of the embryos hatched out in treatment having the highest concentration 3.0 mg/L in set value of the metal.

Teratogenic effects caused by cadmium

A series of morphological abnormalities were induced by Cadmium during embryonic development, including pericardial edema, yolk sac edema, growth retardation, pigmentation defect, and spine deformation. The most pronounced malformations caused by Cadmium were pericardial edema and yolk sac edema which appeared visible in all the concentrations (Fig. 2).

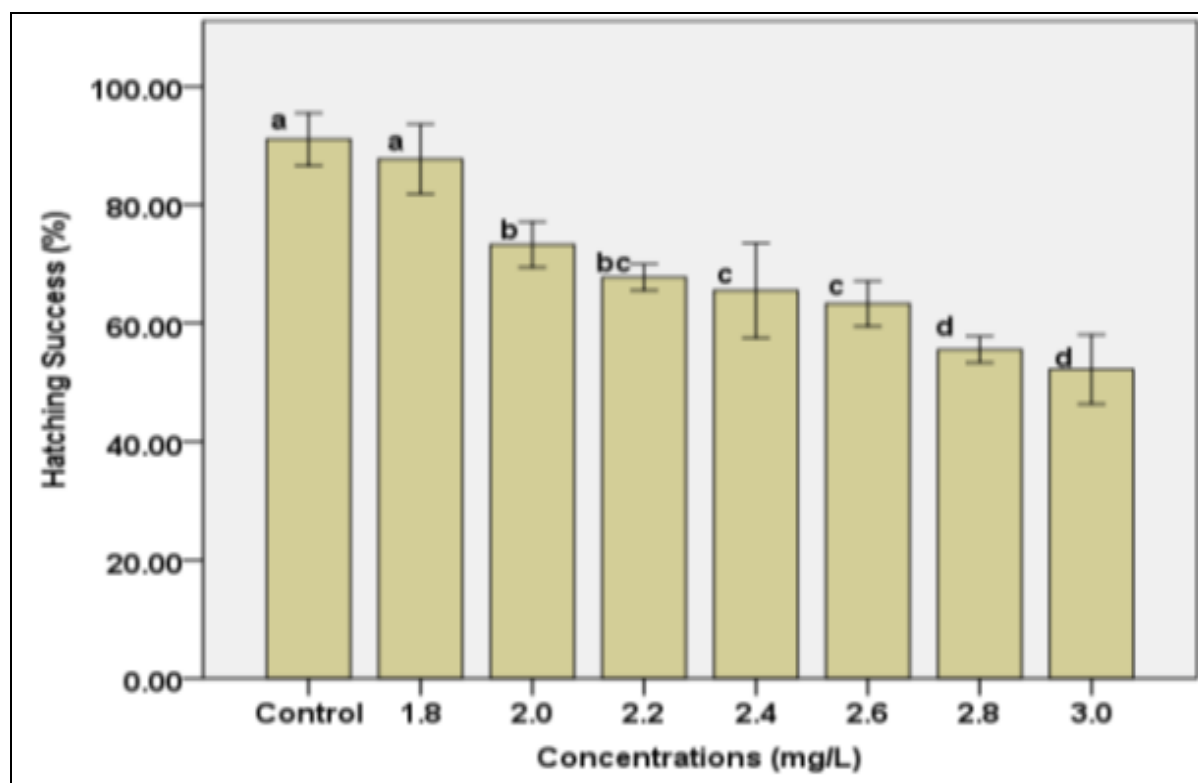


Fig 1: Effects of Cadmium (Cd^{2+}) on *Clarias gariepinus* embryos' hatching rate at 96 hpf (n=30 per concentration). Means with different alphabets are significantly different ($p < 0.05$). Error bars indicate standard error mean (SEM)

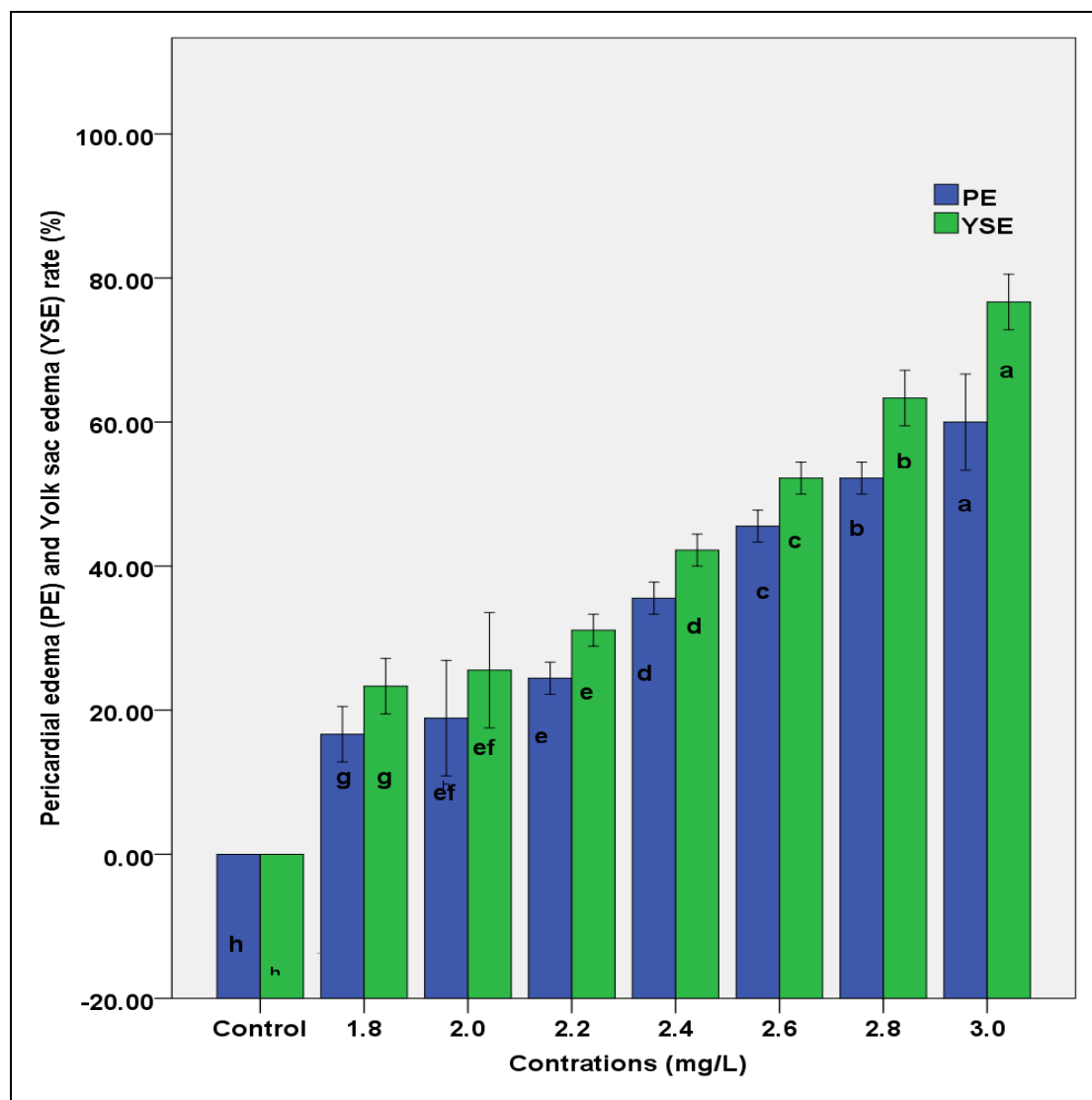


Fig 2: Rate of pericardial edema (PE) and yolk sac edema (YSE) caused by Cadmium (Cd^{2+}) (n = 3 replicates, with 10 embryos per replicate) at 96 hpf, means with different alphabets are significantly different ($p < 0.05$). Error bars indicate standard error mean (SEM)

This shows increase in a concentration dependent pattern from 1.8-3.0 mg/L of Cadmium, with the highest percentage rate of pericardial edema and yolk sac edema at concentration 3.0 mg/L to be 60% and 76.67% respectively, and the lowest pericardial edema and yolk sac oedema were seen at the lowest concentration of 1.8 mg/L to be 16.67% and 23.33% mg/L respectively. However, the percentage of pericardial edema and yolk sac edema in 1.8-3.0 mg/L Cadmium treated groups and the control shows a significant increase ($p \leq 0.05$). The morphological abnormalities were dose dependent. Also, abnormalities increased with increase in concentration of the toxicant.

Morphological aberrations in post-hatching stages

Four major categories of gross morphological abnormalities (irregular head shape, pericardial oedema, yolk sac oedema and notochordal defect) and two minor deformities (short tail defect and growth retardation) were observed. Some of the affected embryos were recorded with combinations of these abnormalities. Embryos and newly hatched embryos of *Clarias gariepinus* after exposure to Cadmium / showing (a) Normal embryo after 20 hpf in the control (b) Coagulated and unhatched embryo exposed to 1.8 mg/L Cadmium at 48hpf (c-h) embryo with irregular head shape, collapsed tail and growth retardation exposed to 2.4 mg/L Cadmium at 48hpf (i) normal hatched embryo 144hpf in the control, (j-l) embryo with

pericardial edema and yolk sac edema exposed to 1.8- 3.0 mg/L at 72 hpf, Notochordal abnormality (body curvature) in the embryos of *Clarias gariepinus* after Cadmium exposure (m) Lordosis (96 hpf exposed to 3.0 mg/L cadmium concentrations, (n) kyphosis (72hpf exposed to 3 mg/L Cadmium), (o-p) scoliosis (96 hpf exposed to 2.8 mg/L Cadmium, (o) Body shortened and (q) V-shaped curvature (Plate I).

Yolk sac edema

Yolk sac edema was observed at 120h and 144h-pf (Plate I. 3 j-m) in the groups exposed to 2.0 to 3.0 mg/L of Cadmium. Malformed embryos were characterized by poorly developed mouth and jaws. Also, yolk sac oedema was often associated with notochordal (spinal cord) curvature. Oedematous embryos were usually shorter than the normal ones.

Notochordal defect

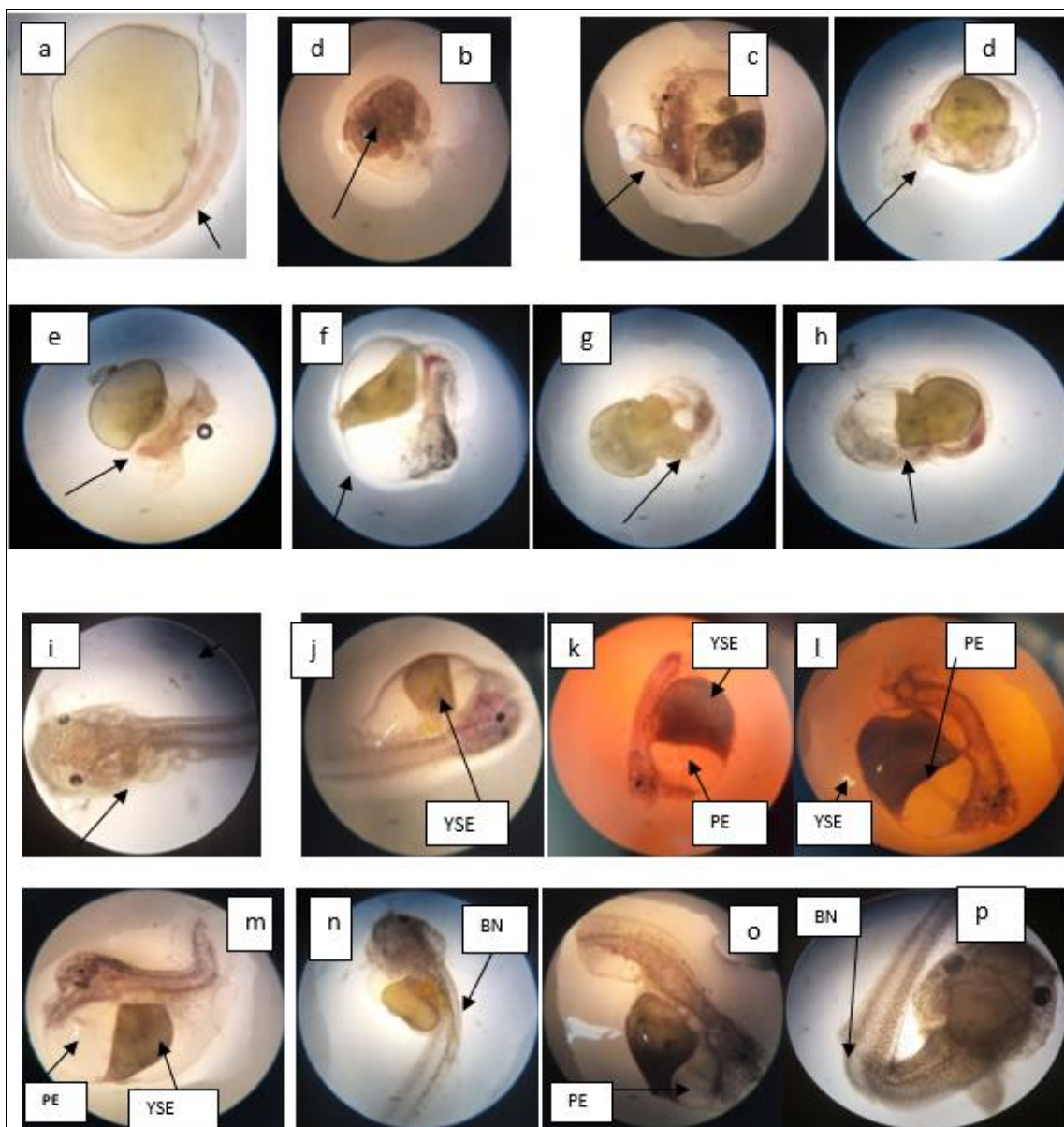
The most frequent observed gross morphological deformation was a bent body or a notochordal (spinal chord) curvature. Different types of spinal cord curvature were recorded (Plate I. 3m-o) (1) lordosis (dorsoventral curvature) (Plate I. 3m), (2) kyphosis (ventrodorsal curvature) (Plate I. 3n), (3) scoliosis (lateral curvature) (Plate I.3o), and (4) V-shaped curvature (Plate I. 3q).

Mortality rates

Mortality of embryos

The results of the acute toxicity bioassay showing mean mortality of *C. gariepinus* are presented in Table 3. Mortality of fish was observed in all the treatment groups except the control. Mortality was first observed at twelve hours in the 2.4, 2.6, 2.8 and 3.0 mg/L concentrations. The highest mortality of 49 embryos were recorded in the highest concentration (3.0mg/L)

of cadmium while the least mortality of 14 embryos was observed in the 2.0 mg/L treatment. Similarly, the total mortality, percentage mortalities, probit kill values, regression equation and the LC₅₀ in mg/L of *C. gariepinus* exposed to acute nominal concentrations of cadmium are presented in Table 3



YSE= yolk sac edema, PE= pericardial edema, BN= bent notochord, HPF= Hours post fertilization).

Plate I: Morphological aberrations in post-hatching stages of *C. gariepinus* exposed to acute of cadmium (Cd²⁺) after 96 hours (a) Normal embryo after 20 hpf in the control.

Table 3: Mortality rates, percentage mortality and probit kill values, regression equation and LC₅₀ of *C. gariepinus* embryos`exposed to acute concentrations of cadmium

Conc. (mg/L)	Log of conc.	Total no. of fish exposed	Mortality	% Mortality	Probit value	Regression equation (R ² -value)	LC ₅₀ (mg/L)
Control	0	60	0	0	0	Y=11.838x + 0.2004	2.55
2.0	0.3	60	14	23.33	4.26		
2.2	0.34	60	17	28.33	4.42		
2.4	0.38	60	21	35	4.61		
2.6	0.41	60	31	51.67	5.05		
2.8	0.45	60	32	53.33	5.08		
3.0	0.48	60	49	81.67	5.92		

The percentage mortality was highest in the 3.0 mg/L treatment and lowest in the 2.0 mg/L treatment, while no mortality occurred in the control group. Mean mortality was observed to

be concentration-dependent. The 96-hour median lethal concentration (LC₅₀) of cadmium for *C. gariepinus* embryos-

larvae using the probit method was found to be 2.55 mg/L (Table 3).

Mortality of larvae

The results of the acute toxicity bioassay showing mean mortality of *C. gariepinus* are presented in Table 4. Mortality of fish was observed in all the treatment groups except the control. Mortality was first observed at twelve hours in the 0.8, 1.0, 1.2, 1.4 and 1.6 mg/L concentrations. The highest mortality of 26 larvae was recorded in the highest concentration cadmium (1.6 mg/L) while the least mortality of 8 larvae was observed in the

0.8 mg/L treatment. Similarly, the total mortality, percentage mortalities, probit kill values, regression equation and the LC₅₀ in mg/L of *C. gariepinus* exposed to acute nominal concentrations of cadmium are presented in Table 4. The percentage mortality of larvae was dose dependent from the highest concentration of Cd²⁺ (1.6 mg/L) to the lowest Cd²⁺ concentration (0.8 mg/L), while no mortality occurred in the control group. The 96-hour median lethal concentration (LC₅₀) of cadmium for *C. gariepinus* larvae using the probit method was found to be 1.33 mg/L.

Table 4: Mortality rates, percentage mortality and probit kill values, regression equation and LC₅₀ of *C. gariepinus* larvae exposed to acute concentrations of cadmium

Conc. (mg/L)	Log of conc.	Total no. of fish exposed	Mortality	% Mortality	Probit value	Regression equation (R ² -value)	LC ₅₀ (mg/L)
Control	0	30	0	0	0	Y=10.082x + 3.7588	1.33
0.8	-0.097	30	8	26.67	4.39		
1.0	0	30	12	40	4.76		
1.2	0.079	30	15	50	5.00		
1.4	0.146	30	21	70	5.62		
1.6	0.204	30	26	86.67	6.13		

Discussion

Fishes play important roles in the aquatic food web; they respond to low levels of toxicants and can bioaccumulate contaminants. These characteristics make them useful as sentinel organisms in toxicological studies (Pandey *et al.*, 2018) [21]. The physicochemical parameters of the test water (containing cadmium) measured during the bioassay were within acceptable values for the growth of *C. gariepinus* (FEPA, 1991) [11]. Ajiboye *et al.* (2015) [2] stated that the optimum pH level for growth of fish should be within the range of 6.5 and 9.0, and the values recorded from this study were within that range. This implied that the changes in fish behaviour and mortality observed during the bioassay may not be due to poor water quality but may be due to the cadmium. In the present work, exposure to cadmium reduced the hatching success and the hatching rate from 88% in the control group to 52% in the group exposed to 3.0 mg/L Cadmium. The hatching success was also dose-dependent. Hatching success in the control group (88%) was higher than in the exposed group. This may be that, during the normal hatching process of fish embryos, the chorion was digested by the hatching enzyme, which is a proteolytic enzyme secreted for the successive disintegration of the egg shell from hatching gland cells of the embryo, and with the help of which the twisting embryos tear up the chorions and become free pro-larvae. The hatching process involves the interaction of biochemical (enzymatic), biophysical (mechanical) and osmotic mechanism (Cao *et al.*, 2009; Zhao *et al.*, 2018) [6, 31]. However, the dose-dependent hatching retardation of fish embryos observed in the present study might be due to disturbance of the hatching enzyme and hypoxia induced by Cadmium. For example, hatching success of common carp under optimum conditions is usually above 70%, while the hatchability of metal-exposed embryos is much lower, because many newly hatched larvae died just after hatching (Jeziarska *et al.*, 2009a; De Silva *et al.*, 2021) [14, 9]. Similar results of reduced hatching rate in cadmium exposure have been reported by Zhang *et al.* (2012) in soldatove's catfish (*Silurus soldatovi*). Also, Aldavood *et al.* (2020) [3] reported that cadmium markedly reduced zebra fish (*Danio rerio*) embryos hatching rates at 48 hpf. Acute toxicity data has been used to derive water quality guidelines for regulatory measures (Santos and Martinez, 2012) [23]. The present study shows that the LC₅₀ for Cadmium at 96- h was 1.33 and 2.55 for larvae and embryos of *C. gariepinus* respectively. As shown in the present study,

the larvae stage of *C. gariepinus* are more sensitive to the toxicant than the embryo. The mortality of the fish was also concentration and time dependent. The possible reasons for the sensitivity of *C. gariepinus* larvae may be that first, unlike embryos, newly hatched larvae lack chorion, which serves as a protective barrier against exposure to waterborne chemicals (De Silva *et al.*, 2021) [9]. Similarly, reported that common carp larvae were more sensitive to Cadmium compared to embryos. The anecdotal degree of mortality reported in the present study was unswerving with the report of Sparling *et al.* (2001) [26], which said that the variance in an organism's biological modification response to change in water chemistry and osmotic conditions, depends on the stages of development. Also, morphological abnormalities such as pericardial oedema, yolk sac oedema, lack of head and tail, incomplete eye pigmentation and hyperactivity within the chorion were observed in the late embryonic stage. No abnormal developed embryos and larvae were observed in the controls. At the end of the tests, morphological abnormalities in 3.0mg/L Cd²⁺ solutions were higher than in others. These abnormalities included; pericardial oedema, yolk sac oedema, growth retardation, pigmentation defect, and spine deformation, pericardial oedema, yolk sac oedema and spinal curvature in the larvae. However, the mortality was dose-dependent. The malformations observed may be as a result of a sudden increase of metal concentration in the egg during osmosis that leads to plasmolysis (shrinkage) in the egg which probably caused stress in the embryos, resulting in premature hatching of underdeveloped larvae or mortality of larvae. The level of swelling affected the entire embryonic development. However, properly swollen eggs allow the embryo to change its position every 5-10 seconds, whereas eggs that do not swell enough are too small. Therefore, embryos have too little space to move, which might have resulted in hatching of abnormal larvae. De Silva *et al.* (2021) [9] reported Growth retardation, Shrinkage of chorion, Scoliosis and Pericardial edema (PE), Lack of pigmentation, tail deformities, Hemorrhages (H) and Yolk – sac edema (YSE) in zebrafish embryo exposed to acute concentrations of Cadmium after 96 hpf. Similarly, also revealed that Copper, Cadmium and Lead reduced swelling in a concentration-dependent way which was different when compared to about 40% (as the increase in egg diameter) in the control groups. Pericardial oedema and yolk sac oedema were most pronounced with 60% and 76.67% occurring in the highest

Cd concentrations of 3.0 mg/L. Pericardial oedema is a non-specific abnormality, as it is reported in response to other inorganic or organic pollutants (Hallare *et al.*, 2005) [13]. However, these two severe defects were lethal and led to the death of the malformed embryos few hours after hatching and were consequently not observed later on. Exposure to Cd could have adverse effects on morphological development of various fish species. For instance, common carp eggs exposed to 0.02mg/L of Cd²⁺ could have up to 47% spinal deformity in the embryos.

Conclusion

Acute exposure of *C. gariepinus* embryos to Cadmium (Cd²⁺) reduced the hatching rate from 88% in the control group to 52% in the group exposed to 3.0 mg/L Cd²⁺. Also, acute exposure of embryos to Cd²⁺ caused morphological abnormalities such as pericardial edema and yolk sac edema with percentages of 16.67% and 23.33% respectively, at the lowest concentration of 1.8mg/L and 60% and 76.67% respectively, at the highest Cd²⁺ concentration of 3.0mg/L after 96 hours. However, the LC₅₀ of cadmium recorded for embryos and larvae were 2.55mg/L and 1.33mg/L respectively, Therefore, intentional and unintentional release of heavy metals into the aquatic environment could threaten fish survival. This study could therefore be used as a tool to assess the effects of cadmium to fish in the course of the monitoring of waters in Nigeria.

Recommendation

It is recommended that treatment of all kinds of wastewaters sewage and Agricultural waste must be conducted before discharge into the aquatic system. Also, enforcement of all articles of laws and legislations regarding the protection of aquatic environments must be taken into considerations.

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