



# International Journal of Fisheries and Aquatic Studies

E-ISSN: 2347-5129

P-ISSN: 2394-0506

(ICV-Poland) Impact Value: 5.62

(GIF) Impact Factor: 0.549

IJFAS 2022; 10(1): 23-28

© 2022 IJFAS

[www.fisheriesjournal.com](http://www.fisheriesjournal.com)

Received: 07-11-2021

Accepted: 09-12-2021

**Jacob Lucky Tabat**

Department of Biology, Ahmadu  
Bello University, Zaria, Kaduna,  
Nigeria

**Adakole JA**

Department of Biology, Ahmadu  
Bello University, Zaria, Kaduna,  
Nigeria

**Gadzama IMK**

Department of Biology, Ahmadu  
Bello University, Zaria, Kaduna,  
Nigeria

**Yerima R**

Department of Biological  
Sciences, Gombe State  
University, Gombe, Nigeria

## Sublethal toxicity of cadmium chloride ( $\text{CdCl}_2$ ) on the growth parameters of *Xenopus laevis* tadpoles (Daudin, 1802)

Jacob Lucky Tabat, Adakole JA, Gadzama IMK and Yerima R

DOI: <https://doi.org/10.22271/fish.2022.v10.i1a.2624>

### Abstract

Intensive agricultural operations and industrial activities release enormous amount of heavy metals, such as cadmium into aquatic ecosystem. The degree of its toxicity indeed pose an environmental problem. The objective of the present work is to study the impact of sub lethal toxicity of waterborne Cadmium chloride for 8 weeks on the growth parameters of *Xenopus laevis* tadpoles. The research was carried out in the Fisheries Laboratory, Department of Biology, Ahmadu Bello University (ABU), Zaria, Nigeria. The tadpoles stage of development was gotten through artificial reproduction of adult *Xenopus laevis* collected from Ahmadu Bello University Reservoir. Some water chemistry such as temperature, pH, electrical conductivity, total dissolved solids and dissolved oxygen were monitored during the study. The tadpoles (Gosner stage 51) were exposed to Cadmium Chloride concentrations of 0.50, 1.00 and 1.50 mg/L derived from already established 96 h  $\text{LC}_{50}$ , (8.86 mg/L  $\text{CdCl}_2$ ) in triplicates. The tadpoles were measured for growth parameters and the number that have completed metamorphosis were counted after an interval of two weeks for a period of eight weeks. Physicochemical parameters of water were within the normal range. The results of growth parameters of *Xenopus laevis* tadpoles showed that, the values of stages of development, Snout Vent Length (SVL), Hind Limb Length (HLL), Tail Length (TaL), Total Length (ToL), Live Weight Gain (LWG) and the percentage of tadpoles that completed metamorphosis of the exposed group of tadpoles decreased significantly ( $p < 0.05$ ) compare with the controls. However, the decrease was concentration-dependent. The present basic information of the growth parameters would serve as a useful tool for further ecological assessment and monitoring of these aquatic organisms, which is considered to be an important food source for human beings and eco-toxicological tool in monitoring aquatic ecosystems in some parts of Nigerian.

**Keywords:** Cadmium, growth, metamorphosis, tadpoles, *Xenopus laevis*

### 1. Introduction

Amphibians are an integral part of an aquatic environment contributing 34% to the species composition. Their early developmental stages (from eggs to tadpoles) are important sources of foods for a wide range of aquatic biota [1], hence they constitute an important link in the energy transfer along the food chain. They can therefore be considered as bio-monitors of aquatic environmental health both as species food components. The activities of humans leading to the accumulation of heavy metals in the ecological habitats is causing an unprecedented decline in the global population of aquatic organisms at an alarming rate [2]. Among all aquatic organisms, amphibians have shown a high vulnerability to exposure to heavy metals [3]. Amphibians possess permeable skins, free of epidermal out growths, and shell less (non-amniotic) eggs, thus being directly exposed to external pollutants present in their habitats [4]. However, growth performance is a factor environmental toxicity in amphibians, and even a small concentration of heavy metals has a negative effect, triggering physiological changes such as growth, metabolism and reducing health and survival rates [5]. Freshwater lotic resources are contaminated with numerous pollutants, which has become a matter of great concern lately [6]. Among the pollutants, heavy metals are the main culprits [7], resulting from intensive agricultural operations and industrial activities, because of their ubiquitous presence, non- biodegradability, and persistency [8]. These heavy metals constantly challenge the ecological balance of the recipient water body, diversity of aquatic fauna [9]. Among the heavy metals, Cadmium (Cd) poisoning has been globally reported causing detrimental health issues with severe toxic effects on certain physiological systems [10].

**Corresponding Author:**

**Jacob Lucky Tabat**

Department of Biology, Ahmadu  
Bello University, Zaria, Kaduna,  
Nigeria

Investigation by <sup>[11]</sup> James *et al.* show that cadmium contamination can cause high mortality and delayed metamorphosis in two anurans, *Bufo americanus* and *Rana sphenoccephala*. Chronic exposure of cadmium caused disruption in the activities of adrenal glands in an urodelan *Triturus carnifex* <sup>[12]</sup>. Cadmium (Cd) is one of the PBTs (persistent, bioaccumulative and toxic) chemicals recognized as primary toxicant, found at low concentrations in natural water <sup>[13]</sup>. Anthropogenic activities like lead mining and smelting operations, electroplating plants, fossil fuel consumption, usage of phosphate fertilizers, and manufacture of nickel cadmium batteries are some of the main sources by which the metal is mobilized into the biosphere <sup>[14]</sup>. Cadmium (Cd) has been labelled as an ecological concern because it leaches through water into the soil and finally bioaccumulates in organisms <sup>[15]</sup>. The half-life of cadmium in organisms is 20 years <sup>[16]</sup>.

Xenopus embryos and tadpoles are a popular model system for a wide variety of biological studies <sup>[17]</sup>. This animal is widely used because of its powerful combination of experimental tractability and close evolutionary relationship with humans, at least compared to many model organisms <sup>[17]</sup>. Thus, *Xenopus* is the only vertebrate model system that allows for high-throughput *in vivo* analyses of gene function and high-throughput biochemistry. Additionally, several African clawed frogs were present on the space shuttle Endeavour (which was launched into space on September 12, 1992) so that scientists could test whether reproduction and development could occur normally in zero gravity <sup>[18]</sup>. *Xenopus laevis* is also notable for its use in the first well-documented method of pregnancy testing when it was discovered that the urine from pregnant women induced *X. laevis* oocyte production. Human chorionic gonadotropin (HCG) is a hormone found in substantial quantities in the urine of pregnant women. Today, commercially available HCG is injected into *Xenopus* males and females to induce mating behavior and to breed these frogs in captivity at any time of the year <sup>[19]</sup>. The African Clawed Frog (*X. laevis*) is also seen as a model species for many studies on toxicity, this study therefore, was designed to generate information on the effects of cadmium chloride at acute and sub-lethal exposure concentrations on the development and growth of *X. laevis* which may serve as model for developmental and toxicological studies, since different species of tadpoles are known to respond in a different manner against heavy metals <sup>[20, 21]</sup>, therefore, understanding the impact of cadmium on *X. laevis* tadpoles may throw a light on its survival ability and may partly contribute to identify the prime reason behind their population decline. This decline suggests that these organisms may be sensitive to water quality, which implies that amphibians might have utility as indicator species of aquatic degradation <sup>[22]</sup>. Therefore, there is need to study the effect of chemicals on the tadpole stages of development; since amphibian tadpoles are potentially more informative as biological indicators of aquatic pollution.

## 2. Materials and methods

### 2.1 Collection of Animals

Five individuals (males and females) of sexually matured *Xenopus laevis* were collected from Ahmadu Bello University Zaria Dam using hand net. The specimens were placed in two aerated glass tanks containing 30L of water made of Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX) and then transported to Fishery Laboratory, Department of

Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

### 2.2 Acclimatization of the Experimental Frog

The frogs were each allowed to acclimatized for 14 days in a separate 40-L glass aquaria containing 18-L of Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX) medium (625.0 NaCl, 96.0 NaHCO<sub>3</sub>, 30.0 KCl, 15.0 CaCl<sub>2</sub>, 60.0 CaSO<sub>4</sub>.H<sub>2</sub>O and 70.0 MgSO<sub>4</sub> all in mg/L dissolved de-chlorinated tap water <sup>[23]</sup>. They were then held at 12:12h light: dark regimen and 24 ± 2°C, median pH: 8.2 and fed with grounded beef liver (three days per week).

### 2.3 Adults breeding for eggs production

For each male and female frog, a single priming dose of 500 international units (i.u.) human chorionic gonadotropin was injected into the dorsal lymph sac of five adult female *X. laevis* each in order to induce ovulation. This was followed two days later by a second dose of 750 i.u. to induce egg-laying. The five males each was injected with 300 (i.u) human chorionic gonadotropin on the day of the females' second dose, for gonadotropin stimulation. The five breeding pairs were then transferred to glass breeding tanks measuring 50 cm in length x 24cm in width x 24cm in height containing 30-L of FETAX medium and equipped with false bottoms (Tanks were fitted with plastic grating held about 3.0 mm off the bottom of the tank) to allow the fertilized eggs to fall to the bottom of the tanks and avoid disturbance by frogs in amplexus.

### 2.4 Tadpole collection and culture

The egg masses were slowly acclimated with laboratory conditions by maintaining the temperature between 18 and 22 °C, which is ambient temperature variation in the wild for the species. The eggs were then transferred into four (4) separate plastic containers of 20L each. The plastic containers were kept covered with polypropylene mosquito mesh net with sieve opening of 707 µm to avoid any external interferences. Since the containers were meant for nurturing the early (embryological) life phase(s) of the species, we termed it as Neonatal chambers for convenience. Once the tadpoles hatched, they were fed *ad libitum* with coppers in Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX) medium. Containers were monitored daily for feed decline and supplementary feed (same as regular diet) was provided as and when needed. To avert any possibilities of introducing victual bias, existing food particles from all containers were sieved out before providing new installment of feed ration. All the experimental protocols were approved by the Ethical and Scientific Committee of Department of Biology, Ahmadu Bello University, Zaria, Nigeria. Sub-lethal Toxicity.

### 2.5 Preparation of Metal Test Solutions

A stock solution of 1 g/L of Cd was prepared by dissolving CdCl<sub>2</sub> powder into distilled water (CdCl<sub>2</sub>, 99.99% purity; Sigma-Aldrich). Three Cd concentrations of 0.50, 1.00 and 1.50 mg/L for the sub-lethal bioassay derived from a fraction of (1/5, 1/10 and 1/20 of the 96 h LC<sub>50</sub> of 8.86 mg/L) made by using stock solution and control was achieved with dechlorinated tap water respectively.

### 2.6 Experimental Design

A 12-L solution was made for each concentration, and then equally divided into 3 small aquaria. That is, each small

aquarium (50 × 20 × 10 cm) contained a 4-L solution. In total, 3 replicates of 3 Cd concentrations of 0.50, 1.00 and 1.50 mg/L and 3 replicates of control (12 small aquaria) were prepared for the experiment. Eight-week static-renewal test was employed for understanding the impacts associated with a long-term exposure to Cd on the physiology of the tadpoles [24]. Upon reaching Gosner stage 49–51, control and metal-treated concentration groups each consisted of three replicates of 10 randomly allocated tadpoles, mixed from the four investigational stock chambers. The tadpoles were kept undisturbed during the period of toxicity testing and fed *ad libitum* with Coppens. All physicochemical variables were within the ranges described for the acute exposure experiment. The entire experiment was conducted in Observational chambers as those used for the acute exposure experiment, containing five of test metal solutions (treatment groups) and five of dechlorinated water (controls). The total experiment time was for eight weeks, and data was taken bi-weekly at 2, 4, 6 and 8 weeks respectively.

### 2.7 Data collection

Mortality was recorded every day for two weeks. Body mass and length were calculated from eight randomly selected tadpoles per treatment, where the average values (n = 8) were taken. The tadpoles for measuring mass and length were different within each treatment group to avoid causing external stress on particular individuals. Body mass was quantified using a portable electronic balance (Ohaus Scout® Pro) to the nearest 0.01g. A piece of absorbent paper towel (several layers thick) was placed on the plate of the balance, on top of which we placed a piece of fiberglass fly screen to avoid adherence of tadpoles to the paper. For length measurements, tadpoles were dried swiftly on a piece of paper towel, settled on graph paper sheets and measured using Vernier's Calipers (0.1 mm accuracy) to gauge the total length (To/L), Snout Vent Length (SVL) and Tail Length (Ta/L). The investigators randomly selected 20 tadpoles per treatment to record developmental stages according to [25] Gosner (1960). The range(s) of the maximum observed Gosner stage(s) were considered for each test groups at the end of the two-weeks interval (including all the days of monitoring in that week). It was later categorized as ranges; the tadpole hatching (stage 35-36), tadpole feeding (stage 45), Premetamorphosis (stage 51), Prometamorphosis (stage 55-58), Climax with tail (stage 61-63) and Climax without tail (stage 66).

### 2.8 Data analysis

In the chronic toxicity experiment, data were analyzed using repeated measures ANOVAs where the repeated measure is the time period. The ANOVA results were used to compare the effects recorded among the variables through different concentrations at weeks 2, 4, 6 and 8 respectively.

### 3. Results

The physicochemical parameters of the test water measured daily during sublethal toxicity bioassay with Cadmium chloride concentrations are presented in Table 1. The Temperature (T) (°C) range was between 24.80 to 25.70 °C, Hydrogen ion concentration (pH) was between 7.52 to 8.50, Electrical Conductivity (EC) (µS/cm) was between 141 to 235 µS/cm, Total Dissolved Solids (TDS) (mg/L) was between 70 to 117 mg/L and Dissolved Oxygen (DO) (mg/L) was between 4.00 to 5.20 mg/L (Table 1).

### 3.1 Chronic toxicity on the growth of *Xenopus laevis* tadpoles exposed to CdCl<sub>2</sub>

The results of growth parameters of *Xenopus laevis* tadpoles exposed to sub-lethal concentration of Cadmium chloride after 8 weeks of exposure is presented in (Table 2). The values of Gosner stages of development (GSD), Snout Vent Length (SVL), Hind Limb Length (HLL), Tail Length (TaL), Total length (ToL) and Live Weight Gain (LWG) of the tadpoles exposed to the control was significantly higher than the exposed group at week 2, 4, 6 and 8 respectively. However, Live Weight gain of tadpoles exposed at 0.5 mg/L was higher compared with the control during the entire period of the exposure, but the difference was not significant. This clearly shows that; the control group gave the best growth rate in all the growth parameters measured from week 2 to 8. However, Tail Length and Total length were observed to increase to a peak from week 2 to week 4, and started declining from week 4 to 8. Among the exposed group of tadpoles, the tanks with the lowest concentration of Cadmium chloride 0.50 mg/L were significantly higher than the exposed group at the highest concentrations of Cadmium chloride 1.00mg/L and 1.50 mg/L at week 2, 4, 6 and 8 respectively.

**Table 1:** Physico-chemical Parameters of Diluting Water Monitored during the Chronic Toxicity Test for Cadmium Chloride on *Xenopus laevis* for 8 Weeks

Parameters	Range	Mean± S.E
Temperature(T) (°C)	24.80 – 25.70	25.23±0.08
Hydrogen ion Concentration (pH)	7.52 – 8.50	8.01±0.05
Electrical Conductivity (EC) (µS/cm)	141– 235	187.42±10.41
Total Dissolved Solids (TDS) (mg/L)	70 –117	93.33±5.22
Dissolved Oxygen (DO) (mg/L)	4.00 – 5.20	4.46±0.12

However, the different variables of length were seen to be following the trends of the above morphometric characters where the test samples inhabiting 1.50 mg/L showed lesser values as compared to the other treatment groups. Tail length at week 8 drastically reduced in most of the tadpoles in the control compare with the exposed group of tadpoles. While total length was also observed to decline since the growth of the tadpoles was approaching a climax. The growth rate of tadpoles exposed to Cadmium chloride concentrations, was clearly concentrations dependent. Also, growth parameters reduce with increase in concentration of toxicant (Table 2).

### 3.2 Percentages of stage 51 *X. laevis* onset and completion of metamorphosis exposed to sub-lethal concentrations of CdCl<sub>2</sub> after 8 weeks

Percentages of Stage 51 *X. laevis* Onset and Completion of Metamorphosis exposed to sub-lethal Concentrations of CdCl<sub>2</sub> after 8 weeks is presented in Table 3. Tadpoles exposed to sub-lethal concentrations of cadmium chloride (CdCl<sub>2</sub>) after 8 weeks shows that, at the onset of metamorphic the mean values and their respective percentages were 2.67 (26.7%), 2.00 (20%), 1.33 (13.3%) and 1.00 (10.0%) in the control (0.00). 0.50. 1.00 and 1.50 mg/L respectively (Table 3). They were observed to show no significant difference between the control and the exposed group of tadpoles to cadmium chloride (CdCl<sub>2</sub>). However, at the completion of metamorphosis, tadpoles in the control with the mean 6.00 (60.00%) were significantly different compare with the exposed group at 1.00 and 1.50 mg/L Cd. While among the tadpoles exposed to sub-lethal concentrations of cadmium chloride (CdCl<sub>2</sub>) with the mean 3.33 (33.3%) in 0.50 mg/L,



2.67 (26.7%) in 1.00 mg/L and 2.00 (20.00%) in 1.50 mg/L were not significantly different. However, the Gosner stage(s) of 20 randomly chosen tadpoles from the treatment groups were compared to check the advancement of the metamorphic process.

The range of Gosner stages observations (per monitoring in two weeks interval week) from each treatment group revealed that the tadpoles at 1.00 mg/L and 1.50 mg/L Cd displayed

delayed metamorphosis as compared with the tadpoles in the controls and 0.50 mg/L Cd respectively (Table 3). At the end of 8th week, maximum tadpoles at 1.00 mg/L and 1.50 mg/L Cd were in late larval stage while the controls and tadpoles at 0.5 mg/L Cd reached the prometamorph stage. The rate of advancement of the Gosner stage was also very slow for the tadpoles at 1.00 mg/L and 1.50 mg/L Cd as compared with the controls and 0.50 mg/L Cd (Table 3).

**Table 2:** Growth Parameters of 51 *X. laevis* Tadpoles Exposed to Sublethal Concentrations of (CdCl<sub>2</sub>) for 8 Weeks

WEEKS	Conc.mg/L	GSD	SVL±SE (mm)	TaL±SE (mm)	ToL±SE (mm)	HLL±SE (mm)	WT±SE (g)
0 (Initial)	Control	51	13.78±1.06 <sup>a</sup>	28.93±0.49 <sup>a</sup>	42.71±1.06 <sup>a</sup>	Nil	1.56±0.06 <sup>a</sup>
	0.50		13.99±0.76 <sup>a</sup>	28.53±1.33 <sup>a</sup>	42.52±1.73 <sup>a</sup>	Nil	1.58±0.07 <sup>a</sup>
	1.00		14.10±0.63 <sup>a</sup>	28.67±1.35 <sup>a</sup>	42.77±1.76 <sup>a</sup>	Nil	1.38±0.03 <sup>a</sup>
	1.50		14.20±0.74 <sup>a</sup>	28.07±1.62 <sup>a</sup>	42.27±1.82 <sup>a</sup>	Nil	1.55±0.06 <sup>a</sup>
2	Control	55	21.67±1.31 <sup>a</sup>	38.13±1.34 <sup>a</sup>	59.80±2.50 <sup>a</sup>	4.87±0.47 <sup>a</sup>	2.19±0.12 <sup>a</sup>
	0.50		18.27±1.35 <sup>b</sup>	33.40±1.62 <sup>b</sup>	51.67±2.94 <sup>b</sup>	3.93±0.70 <sup>ab</sup>	2.21±0.14 <sup>a</sup>
	1.00		16.40±1.10 <sup>bc</sup>	30.73±1.49 <sup>bc</sup>	47.17±2.42 <sup>bc</sup>	2.60±0.39 <sup>b</sup>	1.57±0.09 <sup>b</sup>
	1.50		15.67±0.90 <sup>c</sup>	28.40±1.63 <sup>c</sup>	44.07±2.55 <sup>c</sup>	1.87±0.29 <sup>c</sup>	1.56±0.07 <sup>b</sup>
4	Control	58	25.10±0.75 <sup>a</sup>	46.10±1.11 <sup>a</sup>	71.20±1.64 <sup>a</sup>	8.87±0.62 <sup>a</sup>	2.23±0.11 <sup>a</sup>
	0.50		19.33±0.70 <sup>b</sup>	42.67±1.10 <sup>ab</sup>	62.00±1.91 <sup>b</sup>	7.53±0.79 <sup>a</sup>	2.26±0.14 <sup>a</sup>
	1.00		16.67±0.84 <sup>c</sup>	40.20±1.83 <sup>bc</sup>	56.87±2.59 <sup>c</sup>	5.87±0.65 <sup>b</sup>	1.84±0.13 <sup>b</sup>
	1.50		16.27±0.65 <sup>c</sup>	36.10±1.70 <sup>c</sup>	52.37±2.17 <sup>cd</sup>	4.60±0.39 <sup>bc</sup>	1.70±0.08 <sup>b</sup>
6	Control	61-63	27.80±0.57 <sup>a</sup>	48.47±2.18 <sup>a</sup>	76.27±2.50 <sup>a</sup>	9.20±1.94 <sup>a</sup>	2.39±0.11 <sup>a</sup>
	0.50		19.80±0.73 <sup>b</sup>	40.27±1.97 <sup>b</sup>	60.07±2.48 <sup>b</sup>	7.57±2.31 <sup>ab</sup>	2.49±0.10 <sup>a</sup>
	1.00		17.87±0.84 <sup>b</sup>	37.20±1.90 <sup>b</sup>	55.07±2.58 <sup>c</sup>	6.87±1.71 <sup>bc</sup>	1.91±0.10 <sup>b</sup>
	1.50		16.73±0.82 <sup>bc</sup>	36.78±1.20 <sup>b</sup>	53.51±1.79 <sup>c</sup>	5.13±1.36 <sup>c</sup>	1.72±0.90 <sup>b</sup>
8	Control	66	29.83±0.65 <sup>a</sup>	38.99±2.18 <sup>a</sup>	68.8 <sup>a</sup> ±1.06 <sup>a</sup>	9.77±1.52 <sup>a</sup>	2.63±0.28 <sup>a</sup>
	0.50		24.50±0.29 <sup>ab</sup>	30.27±1.97 <sup>b</sup>	54.77±1.73 <sup>bc</sup>	7.60±1.78 <sup>ab</sup>	2.75±0.20 <sup>a</sup>
	1.00		21.20±0.49 <sup>bc</sup>	37.20±1.90 <sup>c</sup>	58.40±1.76 <sup>b</sup>	6.88±1.58 <sup>bc</sup>	1.95±0.19 <sup>b</sup>
	1.50		16.90±0.37 <sup>c</sup>	36.79±1.20 <sup>c</sup>	53.69±1.82 <sup>c</sup>	5.15±1.58 <sup>bc</sup>	1.76±0.10 <sup>c</sup>

Means with the same superscript along columns are not significantly different ( $p \geq 0.05$ ) (Means values± SE), n=3

**Table 3:** Mean±SE and Percentages of Stage 51 *X. laevis* Onset and Completion of Metamorphosis at Various Concentrations of CdCl<sub>2</sub> after 8 Weeks of Sub lethal Exposure

Metamorphosis	Concentration mg/L	Metamorphosis Mean±SE	% completion
Onset	Control	2.67 ±0.67 <sup>a</sup>	26.70
	0.50	2.00 ±0.58 <sup>a</sup>	20.00
	1.00	1.33 ±0.33 <sup>a</sup>	13.30
	1.50	1.00 ±0.58 <sup>a</sup>	10.00
Completion	Control	6.00±1.15 <sup>a</sup>	60.00
	0.50	3.33±1.33 <sup>ab</sup>	33.33
	1.00	2.67±0.33 <sup>b</sup>	26.70
	1.50	2.00±0.58 <sup>b</sup>	20.00

Means with the same superscript along columns are not significantly different ( $p \geq 0.05$ ) (Means values± SE), n=3

#### 4. Discussion

Tadpoles exposed at 1.5 mg/L suffered from reduced growth and showed delayed metamorphosis than those from the rest of the treatments. This sub-lethal effect of Cd can be explained through understanding the biochemical interventions of this heavy metal in the physiological aspects of metamorphosis. In a study by [26] Sharma and Patiño, it was observed that chronic exposure of *Xenopus laevis* tadpoles to Cd caused reduction in the activities of thyroid gland- the endocrine gland with imperative role in the amphibian metamorphosis [27]. *Bufo raddei* tadpoles suffered delays in metamorphosis when exposed to Cd for a long time while the metal was detected to impede the activities of two enzymes, Superoxide dismutase and ATPase [28]. It was observed that low concentrations of Cd like 0.50mg/L for *Xenopus laevis* tadpoles don't impact the metamorphic progression- similar observations were recorded by [10] Ojha *et al.*, where they

found low concentrations of Cd like 0.3mg/L for *Polypedates maculatus* (Anura, Rhacophoridae) tadpoles relatively accelerate the time to complete metamorphosis as compared to the tadpoles in higher concentrations of Cd. We speculated that moderate stress induced by Cd accelerates development in tadpoles and this may be beneficial for the tadpoles to avoid adverse environmental conditions. Meanwhile, the present study showed that high-dose Cd caused tadpole size inhibition. At metamorphic climax (Gosner stage 61-66), significant reduction in body size—such as total length, hind-limb length, and body mass—was observed with exposure to high-dose Cd (1.00 mg/L and 1.50 mg/L). This inhibitory effect was also found in other studies. In tadpoles of *X. laevis*, reduction of body size was present after exposure to Cd [26], and Cd exposure caused growth impairment in *D. melanostictus* [29]. Cadmium accumulation may cause damage of organs that favor normal growth, such as the thyroid gland, which leads to smaller sized tadpoles [30]. Moreover, Cd exposure may affect tadpole feeding activities, and lack of energy resulted in growth impairment. During the feeding period, we found that with the increase of the concentration of heavy metals, the amount of food surplus increased normally. In addition, in our study, at the completion of metamorphosis (Gosner stage 66), average total length and hind-limb length were significantly decreased only in the 1.50/L of Cd group, which indicated that the accumulation of high-dose Cd may have obvious inhibition effects at this stage.

The study also revealed that the whole-tadpole body burdens of Cd were positively correlated with the increase in Cd concentrations [31]. It was found that the average total body length and related morphometric parameters of length were significantly decreased ( $p < 0.05$ ) in the treatment groups of

1.00 mg/L and 1.50 mg/L Cd. This observation follows the findings of <sup>[32]</sup> Sun *et al.*, wherein they reported that chronic exposure to 0.70 mg/L of Cd caused significant reduction in average body length and hind limb length of *Bufo gargarizans* tadpoles. Their investigations indicated that a high dosage of Cd negatively affected the ossification in bones of the tadpoles as the metal disrupted the functioning of thyroid hormone responsible for regulating endochondral ossification and bone development. <sup>[26]</sup> Sharma and Patiño, also observed reductions in snout vent length, hind limb length and total length of *Xenopus laevis* tadpoles during long term exposure to 0.86 mg/l Cd. In the same study, it was seen that the tadpoles reaching metamorphic climax in Cd-exposed media were remarkably lesser than the controls <sup>[26]</sup>. <sup>[32]</sup> Sun *et al.*, observed the body mass of *B. gargarizans* tadpoles exposed to 0.1 and 0.5 mg/l Cd was significantly less than that of the control group. This is conforming with the present findings where the tadpoles at 1.00 mg/L and 1.50 mg/L Cd showed significant reduction in the body mass at all the weeks of chronic exposure. In a study on *Xenopus laevis* tadpoles, it was found that increasing concentration of Cd is associated with decrease in body length <sup>[33]</sup>. Higher mortality was observed in all the studies on Cd toxicity and tadpoles where the increased Cd contamination caused higher deaths <sup>[34]</sup> - it was observed in a similar line with the given study where tadpoles at 1.50 mg/L Cd experienced maximum deaths as compared to the other treatment groups.

## 5. Conclusion

Exposure to cadmium chloride at sub lethal doses of 0.5mg/l 1.0mg/l and 1.5mg/l affected growth parameters of *X. laevis* tadpoles in delaying metamorphosis, reducing snout-vent length, hind limb length, tail length total length and weight. The present study suggest that Cadmium Chloride affects the survival of *X. laevis* Gosner stage 51 tadpoles. Therefore, the results can be used as an eco-toxicological tool in the monitoring of aquatic ecosystems in the Northern Nigerian ecological zone.

## 6. 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.

## 7. References

- Daniel Hocking J, Kimberly Babbitt J. Amphibian Contributions to Ecosystem Services. *Herpetological Conservation and Biology*. 2014;9(1):1-17.
- Ali H, Khan E, Ilahi I. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry*. 2019, 1-14.
- Enuneku AA, Ezemonye LI. Acute toxicity of cadmium and lead to adult toad *Bufo maculatus*. *Asian Journal of Biological and Life Sciences*. 2012;1(3):238-241.
- Rourke DP. Amphibians used in research and teaching. *ILAR Journal*. 2007;48(3):183-187.
- Hussain SM, Javed M, Asghar S, Hussain M, Abdullah S, Raza SA, *et al.* Studies on growth performance of metals mixture stressed *Cirrhina mrigala* in earthen ponds. Pakistan. *Journal of Agricultural Science*. 2010;47(3):263-270.
- Tabrez S, Zughabi TA, Javed M. Bioaccumulation of heavy metals and their toxicity assessment in *Mystus species*. *Saudi Journal of Biological Sciences*. 2021;28(2):1459-1464.
- Khan MS, Javed M, Rehman MT, Urooj M, Ahmad MI. Heavy metal pollution and risk assessment by the battery of toxicity tests. *Scientific Reports*. 2020;10(1):1-10.
- Chauhan A, Islam A, Javed H, Kumar S. Facile fabrication of Amberlite XAD-16 with dipicolylamine for remediation of industrial wastewater containing lead and copper: Isotherm, kinetics, thermodynamics and selectivity studies. *Microchemical Journal*. 2019;146:606-613.
- Abiona OO, Anifowose AJ, Awojide SH, Adebisi OC, Adesina BT, Ipinmoroti MO. Histopathological biomarking changes in the internal organs of Tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) exposed to heavy metals contamination from Dandaru pond, Ibadan, Nigeria. *Journal of Taibah University for Science*. 2019;13(1):903-911.
- Ojha S, Roy A, Mohapatra AK. Environmentally relevant concentrations of Cadmium impair morpho-physiological development and metamorphosis in *Polypedates maculatus* (Anura, Rhacophoridae) tadpoles. *Environmental Chemistry and Ecotoxicology*. 2021;3:133-141.
- James SM, Little EE, Semlitsch RD. Metamorphosis of two amphibian species after chronic cadmium exposure in outdoor aquatic mesocosms. *Environmental Toxicology and Chemistry: An International Journal*. 2005;24(8):1994-2001.
- Gay F, Laforgia V, Caputo I, Esposito C, Lepretti M, Capaldo A. Chronic exposure to cadmium disrupts the adrenal gland activity of the newt *Triturus carnifex* (Amphibia, Urodela). *Biomedical Research international*. 2013;13:1-6.
- Viarengo A. Biochemical effects of trace metals. *Marine Pollution Bulletin*. 1985;16(4):153-158.
- McCarthy J, Shugart L. Biomarkers of environmental contamination, Lewis Publication. Boca Raton. USA. 1990.
- Idrees N, Tabassum B, Abdallah EF, Hashem A, Sarah R, Hashim M. Groundwater contamination with cadmium concentrations in some West UP Regions, India. *Saudi Journal of Biological Sciences*. 2018;25(7):1365-1368.
- Altındağ A, Yiğit S. Assessment of heavy metal concentrations in the food web of lake Beyşehir, Turkey. *Chemosphere*. 2005;60(4):552-556.
- Wallingford JB, Liu KJ, Zheng Y. *Xenopus*. *Current Biology*. 2010;20(6):R263-R264.
- Morris BL. Understanding and targeting the C-terminal Binding Protein (CtBP) substrate-binding domain for cancer therapeutic development. Virginia Commonwealth University. 2016.
- Green WW, Mirza RS, Wood CM, Pyle GG. Copper binding dynamics and olfactory impairment in fathead minnows (*Pimephales promelas*). *Environmental science and technology*. 2010;44(4):1431-1437.
- Rohr JR, Kerby JL, Sih A. Community ecology as a framework for predicting contaminant effects. *Trends Ecological Evolution*. 2006;21:606-613.
- David M, Kartheek RM. Malathion acute toxicity in tadpoles of *Duttaphrynus melanostictus*, morphological and behavioural study. *The Journal of Basic and Applied*

- Zoology. 2015;72:1-7.
22. Guzy JC, McCoy ED, Deyle AC, Gonzalez SM, Halstead N, Mushinsky HR. Urbanization interferes with the use of amphibians as indicators of ecological integrity of wetlands. *Journal of Applied Ecology*. 2012;49(4):941-952.
  23. ASTM Association of Test and Material. Standard guide for conducting the Frog Embryo Teratogenesis Assay *Xenopus* (FETAX), Philadelphia. 1998, 16.
  24. Environmental Protection Agency. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (5th Edition) (1200 Pennsylvania Avenue, Washington DC, USA). 2002.
  25. Gosner KL. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetological*. 1960;16(3):183-190.
  26. Sharma B, Patino R. Exposure of *Xenopus laevis* tadpoles to cadmium reveals concentration-dependent bimodal effects on growth and monotonic effects on development and thyroid gland activity. *Toxicological Sciences*. 2008;105(1):51-58.
  27. Uhlenhuth E. Relation between thyroid gland, metamorphosis, and growth. *The Journal of general physiology*. 1919;1(4):473-482.
  28. Zhang Y, Huang D, Zhao D, Long J, Song G, Li AN. Long-term toxicity effects of cadmium and lead on *Bufo raddei* tadpoles. *Bulletin of Environmental Contamination and Toxicology*. 2007;79(2):178-183.
  29. Ranatunge RAAR, Wijesinghe MR, Ratnasooriya WD, Dharmarathne HASG, Wijesekera RD. Cadmium-Induced Toxicity on Larvae of the Common Asian Toad *Duttaphrynus Melanostictus* (Schneider 1799): Evidence from Empirical Trials. *Bulletin of Environmental Contamination and Toxicology*. 2012;89(1):143-146.
  30. Wang C, Liang G, Chai L, Wang H. Effects of copper on growth, metamorphosis and endocrine disruption of *Bufo gargarizans* larvae. *Aquatic Toxicology*. 2016;170:24-30.
  31. Gross JA, Chen TH, Karasov WH. Lethal and sublethal effects of chronic cadmium exposure on northern leopard frog (*Rana pipiens*) tadpoles. *Environmental Toxicology and Chemistry: An International Journal*. 2007;26(6):1192-1197.
  32. Sun N, Wang H, Ju Z, Zhao H. Effects of chronic cadmium exposure on metamorphosis, skeletal development, and thyroid endocrine disruption in Chinese toad *Bufo gargarizans* tadpoles. *Environmental Toxicology and Chemistry*. 2018;37(1):213-223.
  33. Haywood LK, Alexander GJ, Byrne MJ, Cukrowska E. *Xenopus laevis* embryos and tadpoles as models for testing for pollution by zinc, copper, lead and cadmium. *African Zoology*. 2004;39(2):163-174.
  34. Hu YC, Tang Y, Chen ZQ, Chen JY, Ding GH. Evaluation of the sensitivity of *Microhyla fissipes* tadpoles to aqueous cadmium. *Ecotoxicology*. 2019;28(10):1150-1159.