Original Article

Acute Toxicity of a Heavy Metal Cadmium to an Anuran, the Indian Skipper Frog *Rana cyanophlyctis*

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ABSTRACT

Background: There has been increasing awareness throughout the world regarding the remarkable decrease in amphibian population. For such amphibian population decline several causes have been given. Cadmium, a heavy metal is released both from natural sources (leaching of cadmium rich soils) and anthropogenic activities to the aquatic and terrestrial environments. This study evaluated the toxicity of heavy metal cadmium to Indian skipper frog *Rana cyanophlyctis*.

Methods: For the determination of LC_{50} values for cadmium, four-day static renewal acute toxicity test was used. Five replicates each containing ten frogs were subjected to each concentration of cadmium chloride (15, 20, 25, 30, 35, 40, 45 and 50 mg/L). At different exposure periods (24, 48, 72 and 96 h), the mortality of the frog was subjected to Probit analysis with the POLO-PC software (LeOra Software) to calculate the LC_{50} and 95% confidence level.

Results: The LC_{50} values of cadmium chloride for the frog *R. cyanophlyctis* at 24, 48, 72, and 96 h are 32.586, 29.994, 27.219 and 23.048 mg/L, respectively. The results have been discussed with the toxicity reported for other aquatic vertebrate --fish.

Conclusion: Cadmium caused mortality to the frog and this could be one of the reasons for population decline of frogs which inhabit water contaminated with heavy metals.

Keywords: Amphibia, Cadmium, Heavy Metal, LC_{50}, Toxicity.

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INTRODUCTON

The heavy metals are discharged into water resources through industries such as steel and mining, textile dyes, paints and varnishes, fertilizers, feed additives and ceramics [1]. “The global dispersion of heavy metals and their extensive use in modern society pose an enormous challenge to organisms including humans” [2]. In the aquatic and terrestrial environments the release of heavy metal cadmium is through leaching of cadmium rich soils, mining, smelting, electroplating, manufacturing of batteries etc. [3]. “Atmospheric deposition of airborne cadmium, and the application of cadmium-containing fertilizers and sewage sludge on farm land may lead to contamination of soils and increased cadmium uptake by crops and vegetables consumed by human beings” [4]. Cadmium has been considered more toxic (toxic at levels one tenth) that of lead, mercury, aluminium or nickel [5] and ranked the 7th toxicant in the Priority List of Hazardous Substances of the Agency for Toxic Substances and Disease Registry [6, 7]. This element has a long-biological half-life in humans and it gets accumulated in vital organs – especially in liver and kidney throughout their lives [8, 9].

There has been increasing awareness throughout the world regarding the remarkable decrease in amphibian population [10, 11]. A comprehensive global assessment of amphibian species has been performed by The International Union for Conservation of Nature (IUCN)[12]. Almost 32.5% were listed as vulnerable, endangered, or critically endangered. 7.4% species were listed as Critically Endangered, and about 43% were experiencing some form of population decline [13]. For such amphibian population decline several causes have been given which are habitat loss [12]; UV-B radiation [14,15]; infectious disease [16, 17]; contaminants [18-20]; non-native predators [21]; a combination

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of factors [22, 23] and pesticide's effect [19, 20, 24]. There is a wide variation in tolerance levels among amphibians even between closely related species [25]. Conclusions drawn from studies on only a few species cannot reveal the entire effects of any harmful chemicals to amphibians in general [26].

Hence, in this study we have assessed the LC_{50} value of heavy metal cadmium on an anuran species *Rana cyanophlyctis*. This species has been chosen for the experiments as this frog is highly aquatic and remains permanently resident in different types of habitats with pooled water.

MATERIALS AND METHODS

For experiments laboratory bred *R. cyanophlyctis* (both sexes, body wt. 14.74±0.55 g) were selected. Mean weights of the frogs used in the experiment showed no significant difference. Frogs were kept in all glass aquaria containing dechlorinated tap water and acclimatized to the laboratory conditions (under natural photoperiod 11.58-12.38 and temperature 27.2±1.4 °C) for at least two weeks. The physicochemical characteristics of the tap water were pH 7.20 ± 60.1; dissolved oxygen 7.95±0.25 mg/L and hardness as $CaCO_3$ 167.06 ± 5.61 mg/L. Frogs were fed daily with live insects, 2-3 times per day. Water was renewed daily after cleaning the fecal matter. Feeding was stopped 24 h before and during the experimental period to avoid the excretory substances to influence the toxicity test solutions.

All the experimental protocols were approved by the Ethical Committee of Department of Zoology, DDU Gorakhpur University.

For the determination of LC_{50} values for cadmium, four-day static renewal acute toxicity test [27] was used. Five replicates each containing ten frogs (kept in glass aquarium containing 30 L of the test solution) were subjected to each concentration of cadmium chloride (15, 20, 25, 30, 35, 40, 45 and 50 mg/L). Cadmium chloride was firstly dissolved in distilled water and then desired volume of the solution was mixed in tap water to obtain the above mentioned toxicant concentrations. For each toxicant, a control group with five replicates (each containing 10 frogs) kept in 30 L tap water was also run. The solutions of all the aquaria (control and experimental) were renewed daily. Precautions were taken to remove the dead frog immediately because dead animals deplete dissolved oxygen which greatly affects toxicity data. At different exposure periods (24, 48, 72 and 96 h), the mortality of the frog was subjected to Probit analysis with the POLO-PC software (LeOra Software) to calculate the LC_{50} and 95% confidence level.

RESULTS

The per cent mortality of *R. cyanophlyctis* after exposure to various concentrations of cadmium chloride for 24, 48, 72 and 96 h is shown in Figures 1-4. The LC_{50} values (Table 1) for cadmium chloride at 24, 48, 72 and 96 h are 32.586, 29.994, 27.219 and 23.048 mg/L, respectively. Table 1 also depicts the slope functions and upper and lower confidence limits for *R. cyanophlyctis.*

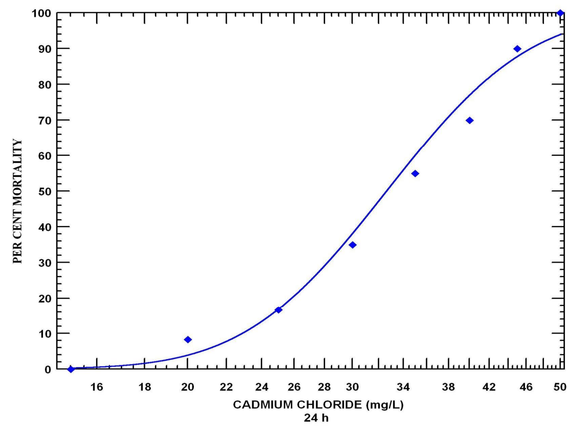

Figure1. Per cent mortality of the frog *Rana cyanophlyctis* after 24 h exposure to different concentrations of cadmium chloride.

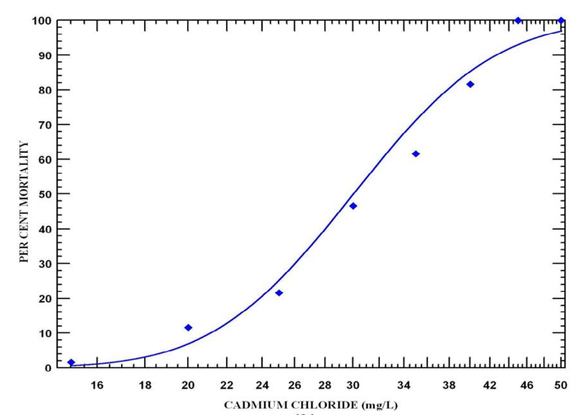

Figure 2. Per cent mortality of the frog *Rana cyanophlyctis* after 48 h exposure to different concentrations of cadmium chloride.

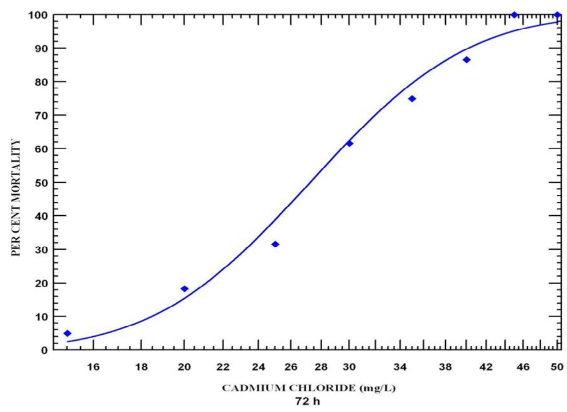

Figure 3. Per cent mortality of the frog *Rana cyanophlyctis* after 72 h exposure to different concentrations of cadmium chloride.

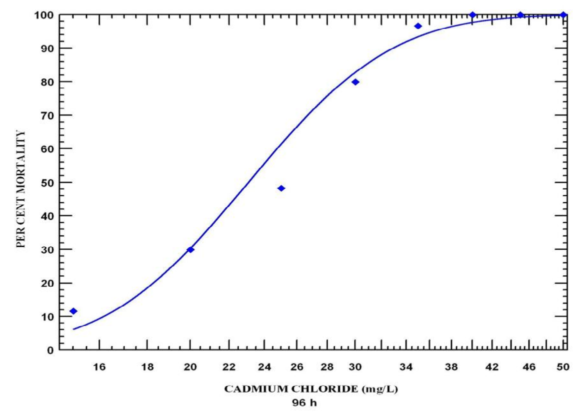

Figure 4. Per cent mortality of the frog *Rana cyanophlyctis* after 96 h exposure to different concentrations of cadmium chloride.

Table 1. LC_{50} value, slope function and confidence limits for short-term exposure of cadmium chloride at different intervals for the frog *R. cyanophlyctis.*

Exposure Periods	Effective dose (mg/L)	Limits(mg/L)* LCL	UCL	Slope Function	't' ratio	Hetero-geneity
	LC_{10}=22.858	19.731	25.163	8.322		
24 h	LC_{50}=32.586	30.415	34.800	±	12.652	1.687
	LC_{90}=46.454	42.502	53.089	0.658		
	LC_{10}=21.121	17.646	23.598	8.413		
48 h	LC_{50}=29.994	27.555	32.407	±	13.101	2.307
	LC_{90}=42.594	38.644	49.553	0.642		
	LC_{10}=18.448	15.782	20.504	7.586		
72 h	LC_{50}=27.219	25.225	29.148	±	13.258	1.532
	LC_{90}=40.161	36.823	45.371	0.572		
	LC_{10}=16.153	13.485	18.115	8.301		
96 h	LC_{50}=23.048	21.085	24.895	±	12.571	1.863
	LC_{90}=32.886	30.010	37.587	0.660		

*The upper and lower confidence limits for LC_{50} values calculated at 0.05 levels.

DISCUSSION

In the present study the LC_{50} values for cadmium chloride for adult *R. cyanophylctis* were 32.58, 29.99, 27.21 and 23.04 at 24, 48, 72 and 96 h, respectively. The percentage mortality of the frogs increased as the concentration of cadmium increased. This increase was also time dependent. This is consistent with the reports of Ezemonye and Enuneku [28] who have also noticed increased mortality of tadpoles of *Bufo maculatus* and *Ptychudena bibroni* with increased lead concentration.

"The mean LC50 value in *Bufo. maculates* for 24 h were not determined due to low mortality. However, LC50 values for 48, 72 and 96 hours were 17.74, 13.39 and 9.97mg/l respectively" [29]. Woodal et al. [30] have reported 90 h LC_{50} of cadmium for *Xenopus laevis* larvae between 80 and 100 mg/L. 48 h LC_{50} value for *Xenopus laevis* larvae has been found as 11.7 mg/L [31] and 7.36 mg/L [32]. 96 h LC_{50} value for cadmium for *Bufo arenarum* larvae has been reported as 2.19- 6.77 mg/L [33]. Sparling and Lowe [18] have reported LC_{50} value of cadmium for *Rana clamitans* as 1.9 mg/L. Grillitch and Chovanec [34] recorded LC_{50} value of cadmium as 0.45 mg/L for larvae of *Rana ridibunda*. For *Rana ridibunda* tadpoles the 96 h LC_{50} has been reported as 71.8 mg/L [35]. 96 h LC_{50} value for cadmium for larvae of *Rana ridibunda* has been reported as 51.2 mg/L [11].

The toxicity of cadmium for teleosts has been studied extensively. The 96 h LC_{50} value of cadmium for various teleosts has been reported by several investigators as – 46.8 mg/L for *Carassius auratus* [36], 0.63 (in soft water) and 73.5 mg/L (in hard water) for *Pimephalus promelas* [37], 3.15 mg/L for *Notemigonus crysoleucas* [38], 6.72 mg/L for *Barilius vagra* [39], 89.5 mg/L for *Labeo rohita* [40] and 360 mg/L for *Heteropneustes fossilis* [41].

The toxicity of cadmium to various amphibians has been studied mostly in tadpole/larval stages. There exists studies on LC_{50} value of cadmium for amphibians which reported the values as 50.0 mg/kg (for 48 h in males of toad) [42] and as 9.90 mg/L (for 96 h for *Bufo maculates*) [29]. In the present study the 96 h LC_{50} for cadmium for adult *R. cyanophlyctis* has been found as 23.04 mg/L. This indicates that *R. cyanophlctis* is more resistant/tolerant to cadmium as compared to *Bufo maculatus*. "Tolerance is an important mechanism by which an organism reacts to an adverse environment. Mechanisms that might be responsible for tolerance include decreased uptake, metal speciation, increased excretion and redistribution of metals to less sensitive target sites [29].

The results of the present and previous studies indicate that the toxicity of cadmium in amphibians is species dependent. The toxicity of cadmium correlates negatively with water hardness in aquatic organisms [43]. The toxicities of a particular toxicant to different species are influenced by factors such as temperature, pH, hardness and dissolve oxygen content of test water and physiological condition of the test animal [35].

CONCLUSION

The present study clearly indicates that cadmium caused mortality to the frog and this could be one of the reasons for population decline of frogs which inhabit water contaminated with heavy metals.

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