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Cadmium-Induced Toxicity on Larvae of the Common Asian Toad *Duttaphrynus Melanostictus* (Schneider 1799): Evidence from Empirical Trials

R. A. A. R. Ranatunge · M. R. Wijesinghe · W. D. Ratnasooriya · H. A. S. G. Dharmarathne · R. D. Wijesekera

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Abstract This paper investigates the toxicity of cadmium (Cd) on young stages of the common Asian toad *Duttaphrynus melanostictus* (Schneider 1799). Signs of acute toxicity were evident in tadpoles repeatedly exposed to five concentrations ranging from 0.002 to 2 mg $L^{-1}$of Cd which included environmentally relevant levels. Mortality at concentrations of 0.02 mg L^{-1} and above was enhanced from 2 % at 0.02 mg L^{-1} to 100 % at 1 mg L^{-1}, in a dose-dependent manner. Significant growth impairment was evident at 0.20 mg L^{-1} with the larvae being markedly smaller. Delayed metamorphosis and retarded swimming were also observed. Therefore levels of Cd recorded in some freshwater bodies in Sri Lanka (e.g. 0.2 mg L^{-1}) may be detrimental to the young stages of *D. melanostictus.*

Keywords Cadmium · *Duttaphrynus* · Tadpoles · Toxicity

There is a wealth of evidence to show that heavy metal contaminants are hazardous to aquatic organisms. In Sri Lanka, cadmium (Cd) has become one of the most commonly observed pollutants of freshwater ecosystems with recorded concentrations in water being in the range of 0.002–0.2 mg L^{-1} (e.g. Manage and Wijesinghe 2009; Ranasinghe 2009; Weeraratna and Ariyananda 2009; Bandara et al. 2011). Apart from natural sources, anthropogenic activities such as the production of nickel–cadmium batteries, electroplating, phosphate mining and the use of phosphorus-fertilizers result in the addition of Cd into water bodies. Cd has been responsible for causing direct mortality in fish, amphibians and other aquatic fauna (e.g. Irwin et al. 2003). Additionally Cd causes retardations in growth and development, abnormalities, and disruptions in physiological and biochemical processes (Lugowska 2007; Zhang et al. 2007; Sharma and Patino 2009). In this paper we investigate the impact of Cd on survival, growth, development and swimming activity of young stages of the common Asian toad, *D. melanostictus* (Schneider 1799). Studies of this nature are particularly relevant for a developing country such as Sri Lanka which is also a global amphibian hotspot facing problems of aquatic pollution.

Materials and Methods

Newly hatched tadpoles corresponding to Gosner stages 24–26 were collected from three home-garden ponds from areas which were widely separated and located in Colombo City, Sri Lanka, to ensure that the clutches were from different individuals and from unpolluted sources. The five test concentrations of Cd, between 0.002 and 2 mg L^{-1}, were selected based on both field levels recorded in Sri Lanka, and on previous exposure trials conducted elsewhere (e.g. Formicki et al. 2008). Cadmium chloride ($CdCl_2.2.5H_2O$ = 228.34; BDH laboratory reagents, Poole, England; purity 99%) was used to prepare the stock solution.

R. A. A. R. Ranatunge · M. R. Wijesinghe (✉) · W. D. Ratnasooriya
Department of Zoology, University of Colombo, Cumaratunga Munidasa Mawatha, Colombo 03, Sri Lanka
e-mail: mayuri@zoology.cmb.ac.lk

H. A. S. G. Dharmarathne
Department of Statistics, University of Colombo, Colombo, Sri Lanka

R. D. Wijesekera
Department of Chemistry, University of Colombo, Colombo, Sri Lanka

The 10-day exposure trials were conducted in glass tanks of 22 × 15 × 15 cm containing 2 L of aged tap water (Sumanadasa et al. 2008). Appropriate volumes of the stock solution were added to each tank to obtain the relevant test concentration and mixed well to ensure homogeneity. Heavy metal concentrations were measured in the tanks to ensure that measured concentrations did not deviate significantly from the nominal concentrations. Each treatment and control (without the heavy metal) was maintained in triplicate. Subsequently, 18 tadpoles (six tadpoles from three clutches) were randomly assigned to each tank. Standard body length and the developmental stage were determined prior to exposure. The larvae were fed daily with fish food pellets (San Aquarium, Colombo) with the quantity added to each tank being determined by preliminary observations. Initially, 30 ± 1 mg was added to each tank. This amount was gradually increased to 58 ± 0.9 mg as tadpoles grew, with allowances being made for mortality in each tank on a given day. The tanks were kept at room temperature (27.4 ± 0.04°C) and exposed to the natural photoperiod of 12 h light: 12 h darkness. Water and heavy metal were renewed every other day during the 10-day exposure. Measured Cd concentrations (GBC 932 AB PLUS Atomic Absorption Spectrometer, Australia) were not significantly different from the nominal concentrations. Measured concentrations of 0.002 ± 0.011 mg L^{-1}, 0.019 ± 0.001 mg L^{-1}, 0.182 ± 0.003 mg L^{-1}, 0.939 ± 0.015 mg L^{-1} and 1.879 ± 0.030 mg L^{-1} were obtained for the nominal concentrations of 0.002 mg L^{-1}, 0.02 mg L^{-1}, 0.2 mg L^{-1}, 1.0 mg L^{-1} and 2.0 mg L^{-1}, respectively. Trials were conducted according to OECD guidelines (2008) for exposure trials.

Mortality was monitored daily while growth measurements were taken every other day. The one-way ANOVA and Tukey's tests were used to determine the influence of pesticide concentration on mortality and the Pearson's Correlation test was used to examine the nature of the dose-dependent response (Sumanadasa et al. 2008). The developmental stage of the surviving tadpoles was also recorded at the end of the trial. The swimming activity of tadpoles was assessed every other day according to a method described in Sumanadasa et al. (2008) and Wijesinghe et al. (2011). Each tank was placed over a white paper on which a cross had been drawn, and the number of times that tadpoles crossed the lines was counted for 5 min between 0800 and 1200 hours. The split plot design repeated-measures ANOVA and Tukey's tests were used to examine effects of Cd on growth and activity (Ortiz et al. 2004; Wijesinghe et al. 2011).

Results and Discussion

The present study reveals that the current field levels of Cd in some water bodies of Sri Lanka (0.2 mg L^{-1}) are toxic to the young stages of the common Asian toad, *Duttaphrynus melanostictus*. Exposure to Cd at levels of 0.2 mg L^{-1} and above significantly enhanced levels of mortality compared to the control (one-way ANOVA and Tukey tests: $F_{5,12} = 89.74$, $p < 0.05$; Fig. 1). These results are in agreement with the observations of James et al. (2005) where Cd caused marked reductions in survival in the Southern Leopard Frog (*Rana sphenocephala).* It is noteworthy that tadpoles exposed to 1.0 mg L^{-1} and above suffered 100 % mortality while no mortality occurred in the controls indicating high survival rates in uncontaminated water. Cd-induced mortality was also dose-dependent (Pearson correlation $r = 0.92$, $p < 0.001$) which is consistent with results of Fridman et al. (2004) observed in amphibians.

Cadmium also induced growth impairment in *D. melanostictus*. Tadpoles exposed to 0.20 mg L^{-1} were 20 % smaller (4.90 ± 0.08 cm) than those of the control (6.44 ± 0.09 cm) (Table 1) (repeated-measures ANOVA: $F_{12,\ 804} = 19.63$, $p < 0.001$). These results are consistent with those of other studies. Sharma and Patino (2009) documented that Cd exposure caused growth retardation in tadpoles of *Xenopus laevis*. Growth impairment could be attributed to several factors. As evident in the present study, the swimming activity of the tadpoles exposed to Cd (at 0.02 mg L^{-1} and above) was markedly lower (Fig. 2). This may have affected feeding, since tadpoles generally feed while actively swimming close to the water surface. When food consumption and assimilation is reduced, less energy is available for growth. Furthermore, organisms exposed to environmental stressors utilize a fair proportion of energy for maintenance and for the physiological processes that are triggered by these stress factors. For instance, it has been reported that the synthesis of metallothioneins occurs within a few hours after exposure to Cd (Garcia et al. 1999).

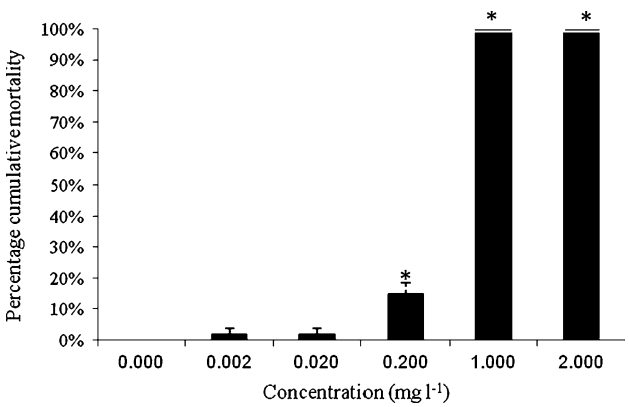

Fig. 1 Mortality in *D. melanostictus* larvae due to Cd exposure. Asterisk indicates significant differences from control

Table 1 Mean body lengths of *D. melanostictus* larvae exposed to Cd

Day	Body length (mean ± SE) (mm)					
	Concentration (mg L^{-1})					
	0.0	0.002	0.02	0.20	1.00	2.00
1	4.56 ± 0.05 (n = 54)	4.39 ± 0.07 (n = 54)	4.40 ± 0.07 (n = 54)	4.43 ± 0.07 (n = 54)	4.40 ± 0.06 (n = 54)	4.52 ± 0.06 (n = 54)
3	5.24 ± 0.06 (n = 54)	5.07 ± 0.06 (n = 54)	4.98 ± 0.07 (n = 54)	4.53 ±0.06 (n = 51)	4.28 ± 0.13 (n = 13)	–
5	5.62 ± 0.07 (n = 54)	5.71 ± 0.06 (n = 53)	5.53* ± 0.07 (n = 54)	4.69* ± 0.09 (n = 47)	4.26* ± 0.05 (n = 5)	–
7	5.99 ± 0.08 (n = 54)	6.06 ± 0.06 (n = 53)	6.21 ± 0.07 (n = 54)	4.81 ± 0.08 (n = 45)	–	–
9	6.44 ± 0.09 (n = 54)	6.33 ± 0.08 (n = 53)	6.38 ± 0.08 (n = 53)	4.90* ± 0.08 (n = 45)	–	–

Means for each concentration were calculated using three replicates

– Indicates where 100 % mortality occurred

* Indicates where the mean body length significantly differs from the control

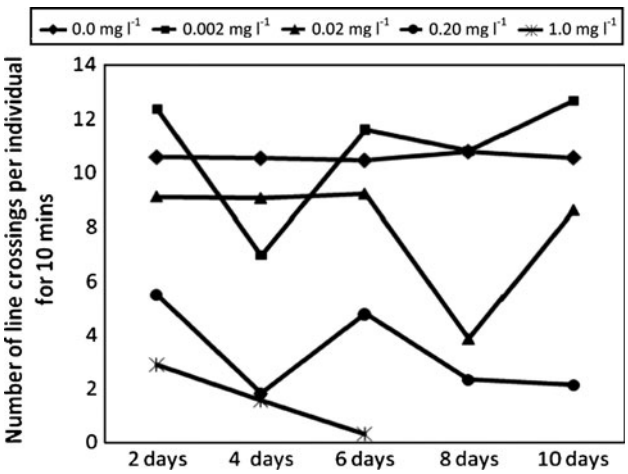

Fig. 2 Variation in swimming activity of *D. melanostictus* larvae over a 10 day exposure to Cd

The present study revealed that Cd had a negative impact on larval development. Over 80 % of tadpoles not exposed to Cd completed metamorphosis by the 40th day. In contrast, rates of metamorphosis were lower in those exposed to Cd; at 0.002 mg L^{-1} only 55 % of the tadpoles metamorphosed and at 0.2 mg L^{-1} the surviving tadpoles only progressed to the hind limb stage (Gosner stage 31–40). These results indicate developmental delays induced by Cd. In other studies, Zhang et al. (2007) observed that tadpoles of *Bufo raddei* suffered developmental delays due to Cd exposure. Similarly *Rana luteiventris* tadpoles exposed to metal mixtures of Cd, Pb and Zn required a relatively longer time to complete metamorphosis than those not exposed (Lefcort et al. 1999). Contradictory results have been reported; Gross et al. (2009) found that stress accelerates development in tadpoles and state that this may be beneficial for the tadpoles to overcome unfavorable environmental conditions.

Swimming activity was reduced significantly in tadpoles exposed to Cd at 0.02 mg L^{-1} and above (Repeated measures ANOVA, $F_{12,\ 32} = 15.83$, $p < 0.05$; Fig. 2). Lefcort et al. (1999) reported such abnormal swimming in tadpoles of *R. luteiventris* exposed to heavy metals. Sumanadasa et al. (2008) observed that diazinon also impaired swimming activity in tadpoles of the same species of toad as was used in the present study. Reduced swimming in tadpoles exposed to Cd could be attributed to several factors; Cd accumulation has been shown to occur in muscles, probably resulting in muscle damage (Vinodhini and Narayanan 2008). Cd-induced behavioral changes have also been noted in fish (Garcia et al. 1999).

In conclusion, the results of the present study are alarming because Cd at levels of 0.2 mg L^{-1} and above are acutely toxic to the early-life stages of *D. melanostictus*. These findings are particularly significant since the harmful impacts were observed at environmentally relevant concentrations recorded from some of the freshwater bodies of Sri Lanka.

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