Asian Journal of Biological and Life Sciences | Original Research

Acute toxicity of cadmium and lead to adult toad *Bufo maculatus*

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Submitted : 26.07.2012 Accepted : 17.09.2012 Published : 31.12.2012

Abstract

The toxicity of cadmium and lead to *Bufo maculatus* were assessed using acute toxicity assays in the laboratory. Mortality and behavioral changes were used as measurement end points for acute toxicity. The Organization for Economic Co-operation and Development (OECD) # 203 recommended semi static renewal bioassay with slight modification was used to determine 96hrs LC_{50}. *Bufo maculatus* was exposed to cadmium and lead concentration of 2, 4, 8, 16 and 32mg/l. Toads assumed a semi-erect posture in the highest concentration (32mg/l) lead concentration. Percentage mortality increased with increase in concentration and exposure duration. Mean percentage mortality for *B. maculatus* exposed cadmium and lead were significantly ($p<0.05$) different from control groups. Estimated LC_{50} concentrations were 9.97 and 16.03mg/l for cadmium and lead respectively. This indicated that *B.moculatus* was more sensitive to cadmium than lead. The study suggests that the release of cadmium and lead into the environment could possibly affect the well-being of amphibians and result in further decline of these sensitive organisms that contribute significantly to the food web. There is therefore the need to protect amphibians from habitat alteration due to metal pollution with a view to sustaining the rich biodiversity in the Nigerian Niger Delta ecological zone.

INTRODUCTION

Industrial waste discharge and input of phosphate fertilizers to Agricultural fields can result in an increased accumulation of heavy metals [1]. Heavy metals are usually detected in measurable levels in industrial effluents because metallic compounds are common constituents of several raw materials. Such materials which serve as feed stocks, catalysts and lubricants are employed in industrial production processes [2]. Industrial waste discharge and input of phosphate fertilizers to Agricultural fields can result in an increased accumulation of heavy metals [1, 3].Adult amphibians can acquire heavy metals through their skin or orally by consumption and respiration. Larvae may also absorb them through their skin.

Cadmium is used in the production of television picture tube phosphorus, nickel cadmium batteries, motor oils, curing agents for rubber, fungicides, phosphate fertilizers, stearate stabilizers for plastics (polyvinyl chloride) and shields for nuclear reactors. Cadmium is used primarily for electroplating other metals or alloys to protect them against corrosion and in the manufacture of low melting point alloys or solders. Anthropogenic activities such as mining, production and consumption of cadmium and non-ferrous metals have accelerated the rate of mobilization and distribution of cadmium from non-bioavailable geological matrices into biologically accessible situations far in excess of natural cycling process [4]. These have predisposed animal and human populations to both subtle and direct exposure pathways with an attendant increase in cadmium related pathologies [5]. The toxic effects of cadmium on organisms include nephrotoxicity, carcinoegenicity, teratogenicity and endocrine disruption [6].

Lead is used in the production of batteries, alloys, cables, pigments and anti-knock agents in fuels. Over 450,000 tons of lead emissions from industrial activities are released into the environment annually [7]. In amphibians lead exposure has resulted in a range of effects including decreased erythrocytes and leucocytes; neutrophils and monocytes; sloughing of the skin; excessive bile secretion; hypertrophy of liver, spleen and stomach; decreased muscle tone and loss of normal semi-erect posture; salivation, excitement and muscular twitching; and delayed metamorphosis [8, 9]. Sparling *et al*., [9] reported that 58.6% of the literature on ecotoxicology and contaminants dealt with fishes, 24.9% dealt with birds, 13.0% dealt with mammals and only 3.5% dealt with amphibians and reptiles combined. This infers that amphibians are relatively understudied in ecotoxicological research.

Toxicity tests in conjunction with appropriate chemical data can establish potential causes [9]. Recent research findings reveal that the monitoring of environmental toxicants using biological organisms is more realistic than physico-chemical measures of concentrations in water, soil or sediment. This has led to the development of lethal (acute) and sub lethal (chronic) toxicity testing using biota and the search for biomarkers of sub lethal toxicity [10].

MATERIALS AND METHODS

Collection and acclimation of test organisms

Adult toads were obtained from an unpolluted forest in Oghara Community, Delta State, Southern Nigeria. The initial mean weight of toads was 27.14±0.34g. There was no significant difference ($p>0.05$) between the mean weights of toads used in the experiments. Since metabolic activity changes with size and affects the parameters to be measured [11], individuals of similar weights were used.

Acclimation to laboratory conditions was done for two weeks prior to experiments in plastic tanks measuring 49cm in length x 29cm in width x 24cm in height with dechlorinated tap water (2 litres at a slant to create a terrestrio-aquatic environment).

Test Chemicals

Cadmium as $CdCl_2.H_20$ and lead as PbO were used for the acute toxicity tests. Cadmium and lead are used in industrial and agricultural activities and can result in heavy metal contamination and uptake by aquatic organisms [12].

Bioassay procedure

Acute toxicity of toad exposed to cadmium and lead was determined according to the Organization of Economic Development and Cooperation (OECD) #203 (1992) method [12]. The semi-static renewal bioassay procedure started with a range finding test. This was used to determine the range of concentrations for the definitive test. The definitive test was carried out with five different concentrations of cadmium and lead (2, 4, 8, 16 and 32mg/l). Cadmium as $CdCl_2.H_2O$ and lead as PbO was used. Stock solutions of the toxicants were prepared by dissolving toxicants in distilled water to a final volume of 1.0 liter. Each treatment solution was prepared after a range-finding test by serial dilution. There were three replicate tanks per treatment and 10 individuals per tank including controls. Test tanks used in bioassay were 36 cuboidal tanks measuring 36cm x 29cm x 24cm with perforated lid and kept at a slant to simulate a terrestrio-aquatic environment.

The test solutions were renewed daily and their physic-chemical constituents were measured throughout the duration of the experiment. They experienced a natural photoperiod of approximately 10: 14, light/dark period at a laboratory temperature range of 27-28.The mean values for the test water quality were as follows; temperature 26±1; pH 5.7±0.4; dissolved oxygen 5.8±0.7 mg/l and hardness 36±1.24 mg/l. The amphibians were not fed during the 96-h acute toxicity test.

Mortality

Mortality assessments for the toads were recorded at 24, 48, 72 and 96 hours for each toxicant. Dead toads were immediately removed from test media. Toads were considered dead when they showed no movements even when probed with a glass rod. There was no mortality observed in the control groups throughout the experiments. Toads in the control groups appeared healthy throughout the test duration.

Statistical analysis

Statistical evaluation for LC_{50} values were carried out using probit analysis (software) [13]. This is the concentration that kills 50% of the total number of test organisms used at a specified period of time. Computation of the confidence intervals of mortality was also obtained from the probit analysis.

RESULTS

The results of acute toxicity of cadmium and lead to *B. maculatus* are presented in tables 1.0 and 2.0. Adults of *B. maculatus* exposed to nominal concentrations (2, 4, 8, 16 and 32mg/l cadmium showed increase in percentage mortality with increase in cadmium concentration. The mean 96-h percentage mortality value for cadmium was 100% (fig 1).The mean LC_{50} value in *B. maculatus* for 24 hours were not determined due to low mortality. However, LC_{50} values for 48, 72 and 96 hours were 17.74, 13.39 and 9.97mg/l respectively

Adults of *B. maculatus* exposed to nominal concentrations (2, 4, 8, 16 and 32mg/l) lead showed an increase in percentage mortality with increase in concentration. The mean 96hr percentage mortality value for lead was 93% (fig. 1). Toads assumed a semi-erect posture in the highest concentration (32mgl/l) lead concentration.

The mean LC_{50} value in *B. maculatus* for 24, 48 and 72 hours were not determined since mortality was low. The 96hour LC50 value for lead was 16.03 mg/l.

DISCUSSION

The phenomenon of declining amphibian populations has

Table 1. Mean acute toxicity profile of toad exposure to cadmium at 48.72 & 96-h.

48HRS	LC_{50}	95%confidence limit	Probit line equation	Slope
Tank 1	18.47	8.84-88.29	Y=2.152+2.249Xlog(conc)	2.76
Tank 2	18.18	12.25-29.57	Y=0.533+3.546Xlog(conc)	1.90
Tank 3	16.58	10.76-26.28	Y=0.800+3.444Xlog(conc)	1.94
Mean	17.74			
SD	1.0			
72 HRS				
Tank 1	13.72	3.04-27.64	Y=2.408+2.279Xlog(conc)	2.72
Tank 2	14.37	8.79-21.63	Y=0.989+3.465Xlog(conc)	2.17
Tank 3	12.09	8.47-17.42	Y=1.084+3.618Xlog(conc)	2.50
Mean	13.39			
SD	1.2			
96 HRS				
Tank 1	10.58	7.10-15.52	Y=1.694+3.277Xlog(conc.)	2.03
Tank 2	11.15	7.30-16.95	Y=1.918+2.943Xlog(conc)	2.17
Tank 3	8.17	4.36-12.66	Y=2.726+2.492Xlog(conc)	2.50
Mean	9.97			
SD	1.6			

Table 2. Mean acute toxicity profile for toad exposure to lead at 72 and 96-h.

72 HRS	LC_{50}	95%confidence limit	Probit line equation	Slope
Tank 1				
Tank 2				
Tank 3	30.42	20.37-22.94	Y=0.351+3.135Xlog(conc)	2.07
Mean	30.42			
SD	-			
96 HRS				
Tank 1	17.50	11.10-29.97	Y=1.242+3.023Xlog(conc)	2.72
Tank 2	16.58	10.76-26.28	Y=0.800+3.444Xlog(conc)	2.17
Tank 3	13.10	8.98-24.43	Y=2.097+2.533Xlog(conc)	2.50
Mean	16.03			
SD	1.8			

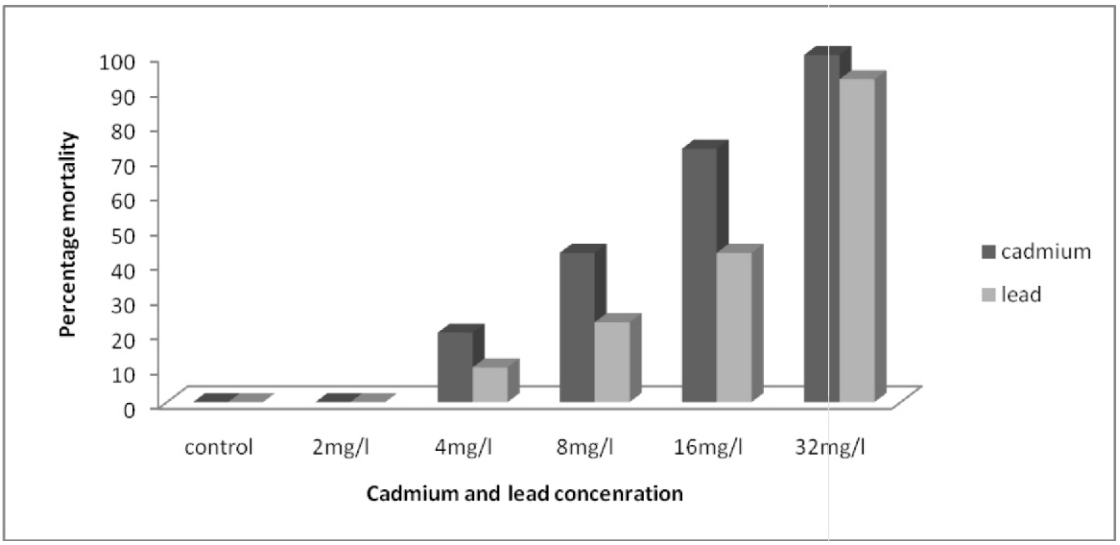

Fig. 1 : Percentage mortality (96-h) in *B. maculatus* exposed to cadmium and lead.

been a problem of global dimension. Chemical contaminants have been hypothesized to be largely responsible for these declines. The indiscriminate discharge of heavy metals into the aquatic environment where amphibians live and breed will ultimately expose them to the toxic effects of such chemicals and in the case of heavy metals, may not only cause behavioral, developmental, physiological, reproductive and histopathological alterations but also mortality [15, 16]. The purpose of toxicity tests with animal species is to help in the assessment of possible risk to similar species in natural environments, as an aid in determination of possible water quality criteria for regulatory purposes and for use in correlation with toxicity tests of other species. Environmental toxicity test is an important tool used to enhance the understanding of the chemical interactions between biota and environment.

In this study, there was no mortality observed in the control groups throughout the experiments. This indicated that the mortality recorded in various treatments may be as a result of the heavy metal toxicity (cadmium and lead) studied in the exposed toads. Adults of *Bufo maculatus* exposed to nominal cadmium concentrations showed a concentration dependent increase in percentage mortality. The increase in percentage mortality was also time dependent. This finding is in agreement with the reports of Ezemonye and Enuneku [17] that exposed *Bufo maculatus* and *Ptychadena bibroni* tadpoles to increasing cadmium concentration and time.

In this study, percentage mortality of the test organisms increased as the concentration of lead increased. The increase was also time dependent. This is consistent with the finding of Ezemonye and Enuneku [2] where percentage mortality of tadpoles (*Bufo maculatus* and *Ptychudena bibroni*) increased with increased lead concentration**.** Toad assuming a semi-erect posture in the highest concentration (32mg/l) lead concentration was a behavioural observation probably as a result of toxic stress.

The 96-h median lethal concentration for cadmium was 9.97mg/l. Ferrari *et al.,* [18] reported 96h LC_{50} value of cadmium on *Bufo arenarum* larvae as 2.19-6.77mg/l. Sparling and Lowe [19] reported the LC_{50} value of cadmium on *R. calamitans* as 1.9mg/l. Grillitch and Chovanec [20] found the LC_{50} value of cadmium as 0.45mg/L in their study on *R. ridibunda* larvae. A higher 96-h LC_{50} value of 51.2mg/l of cadmium on *R. ridibunda* larvae was reported by Selvi *et al*., [21].

The 96hrs median lethal concentration for lead was 16.03 mg/l for *Bufo maculatus*. This indicates that *B. maculatus* has the potential to tolerate lead than cadmium. Tolerance to heavy metal toxicity is dependent on species type. In a similar study, *Bufo maculatus* tadpoles were found to have a higher tolerance level for lead than cadmium [2]. 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.

The adoption of limitation standards and guidelines obtained from data from other countries may not be effective since their environmental conditions and species assemblage are quite different. It is therefore imperative that safe limit/standard for protection of amphibians and other aquatic fauna in the Nigerian environments should be developed using data obtained from heavy metal ecotoxicological studies of Nigerian species.

CONCLUSION

The mortality of toads exposed to cadmium and lead in this study and lack of mortality in the control groups showed that the heavy metals may have caused the death of the toads. The study showed that the release of heavy metals into aquatic ecosystems where amphibians live and breed may threaten amphibian survival and further exacerbate the problem of declining amphibian populations.

The results from this study can be used as an ecotoxicological tool in the monitoring of aquatic ecosystems in the Nigerian Niger Delta ecological zone.

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