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## BIOCHEMICAL CHANGES IN THE TOAD *Bufo maculatus* TREATED WITH SUB LETHAL CONCENTRATIONS OF CADMIUM.

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### Abstract

This study evaluates the toxicity of cadmium and its impact on biochemical constituents, like superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and Thiobarbituric acid reactive substances (TBARS) in the liver of *Bufo maculatus* exposed to sub lethal concentrations of the heavy metal for 28 days. Toads exposed to 0.25, 0.50, 1.00 and 2.00 mg/l Cd showed an elevation ( $p < 0.05$ ) in the specific activity of SOD and CAT relative to controls. The increase could be due to enhanced production of these antioxidants to counteract oxidative stress and lipid peroxidation induced by cadmium. Glutathione (GSH) level showed a decline as the concentration of heavy metal increased at  $p < 0.05$  level of significance. Thiobarbituric acid reactive substances (TBARS) increased as concentration of cadmium increased. The increased level of TBARS in the liver of cadmium exposed frogs is an indication of increased membrane lipid peroxidation which could lead to cell damage and may prevent the liver from carrying out its metabolic activities.

**Key words:** Cadmium, *Bufo maculatus*, Superoxide dismutase, catalase, glutathione, thiobarbituric acid reactive substances.

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## Introduction

Globally considerable attention has focused on the possibility that chemical contaminants may be responsible for declining amphibian populations. Heavy metals are ubiquitous pollutants in the aquatic environment which are released from anthropogenic activities. The flow of heavy metals in ecological systems increases through major sources such as mining, smelting, industrial and agricultural use. Heavy metals are one of the most widespread groups of contaminants because of their slow elimination from environmental compartments (Rogival *et al* 2007, Sparling *et al* 2000).

Cadmium is a heavy metal with no known biological function. When cadmium is absorbed, it can be found in different amphibian tissues but primary accumulation is reported in the liver, kidneys and gonads (Flament *et al* 2003, El-Demerdash *et al* 2004). Cadmium has been reported to cause oxidative stress through several mechanisms; the fenton reaction, depletion of cellular glutathione, alteration of mitochondrial electron transfer chain or the inhibition of antioxidant enzymes (Hensen *et al* 2007). Biochemical parameters are the best indicators of stress situations caused by heavy metals. Accumulating reports show that biochemical parameters in the liver of amphibians could change when exposed to heavy metals and these parameters are extremely sensitive to heavy metals (Cicik and Engin 2005, Sobha 2007).

The toxicity of cadmium is attributed to its ability to generate reactive oxygen species that may act as signalling molecules in the induction of gene expression and apoptosis (Sobha 2007).

Once absorbed, cadmium binds with cysteine residues of the low molecular weight protein metallothionein and accumulates in the liver, kidneys and reproductive organs. Cadmium has the potential to induce oxidative stress in the plasma, liver and brain of living organisms resulting in increased lipid peroxidation (El-Demerdash *et al* 2004). The consequence of oxidative stress induced is loss of cell integrity, enzyme function and genomic stability (Halliwell 1999).

Majority of studies with cadmium and amphibians have focused on effects of exposure on larval life history stages (James and Little 2003, Flament *et al* 2003). Very few experiments have addressed adult health.

Akani and Luiselli (2002) in a study of amphibian faunal diversity and conservation status in the Niger Delta Basin, southern Nigeria reported declining amphibian populations due to chemical contaminants, habitat destruction and exploitation. Obviously, no cause can be unequivocally implicated as the cause of the declines but the synergic effects of several environmental pollutants, heavy metals inclusive, cannot be disregarded. *Bufo*

*maculatus* is one of the toads native to the Niger Delta Region of Nigeria and other West African countries. This study is aimed at evaluating the effect of cadmium on the biochemical parameters of the flat backed toad *Bufo maculatus* bearing in mind that the development of meaningful policies and regulatory framework for the protection of the aquatic environment can only be achieved on the availability of reliable and adequate ecotoxicological data addressing the health of target organisms.

## Materials and Methods

Adults of *B. maculatus* were collected from an unpolluted forest in Oghara Community in the Niger Delta ecological zone of Nigeria. Acclimation to laboratory conditions was done for two weeks prior to experiments (Goulet and Hontella 2003) in plastic tanks measuring 49cm in length x 29cm in width x 24cm in height with dechlorinated tap water (2 litres at a slant). The toads were fed *ad libitum* daily with termites. They experienced a natural photoperiod of approximately 10: 14, light/dark period at a laboratory temperature range of 27-28°C. The mean values for the test water quality were as follows; temperature 26±1°C; pH 5.7±0.4; dissolved oxygen 4.7±0.7 ppm and hardness 36±1.24 ppm.

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 (Canli and Furness 1993), individuals of similar weights were used.

Cadmium as CdCl<sub>2</sub>.H<sub>2</sub>O was used for the sub lethal tests. Stock solutions of the toxicant (CdCl<sub>2</sub>) were prepared by dissolving the toxicant in distilled water to a final volume of 1.0 L. Each treatment solution was prepared after a range-finding test by diluting the stock solution with water to achieve the appropriate exposure concentrations (Ezemonye and Enuneku 2006). Four sub lethal concentrations (0.25, 0.50, 1.00 and 2.00 mg/l) cadmium were dosed to toads for 28 days. There were three replicate tanks per treatment and five individuals per tank including controls. The amphibians were fed with termites. On the 28<sup>th</sup> day toads from each tank were sacrificed for the determination of hepatic superoxide dismutase, catalase, glutathione and thiobarbituric acid reactive substances. Each toad was decapitated. The liver was quickly excised and placed on ice until required for homogenization.

The levels of total superoxide dismutase activity in liver homogenate was determined by the method of Misra and Fridovich (1972). Catalase activity was determined according to the method of Sinha (1971). The levels of reduced glutathione in liver homogenates were determined by the method of Jollow *et al* (1974). The

reduced form of glutathione (GSH) in most instances is the bulk of cellular non-protein sulphhydryl groups. This method is based upon the development of relatively 5thiobenzoic acid, resulting from the reaction of Ellman's reagent with reduced glutathione possesses a molar absorption at 412nm. The absorbance at 412nm is proportional to the reduced glutathione content.

A breakdown product of lipid peroxidation, thiobarbituric acid reactive substances (TBARS) was determined by the method of Buege and Aust (1978). Malondialdehyde formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction. Malondialdehyde (MDA) has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535nm.

#### STATISTICAL ANALYSIS

stable yellow colour when Ellman's reagent (5', 5'-dithioibis-2-nitrobenzoic acid) is added to sulphhydryl compounds. The chromophoric product, 2- nitro-

Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multi sample Range post hoc test using SPSS 15 software (SPSS Inc. Chicago). Statistical significance was considered at  $p < 0.05$  level of significance.

#### Results and Discussion

Changes in biochemical parameters in *B. maculatus* exposed to cadmium are presented in figs 1.1-1.4. There was a significant increase ( $p < 0.05$ ) in the specific activity of SOD and CAT in the liver of *B. maculatus* exposed to cadmium relative to controls. The increase was concentration dependent. In the highest cadmium concentration, the levels of SOD and CAT increased by 84.7% and 77.8% respectively, relative to control groups.

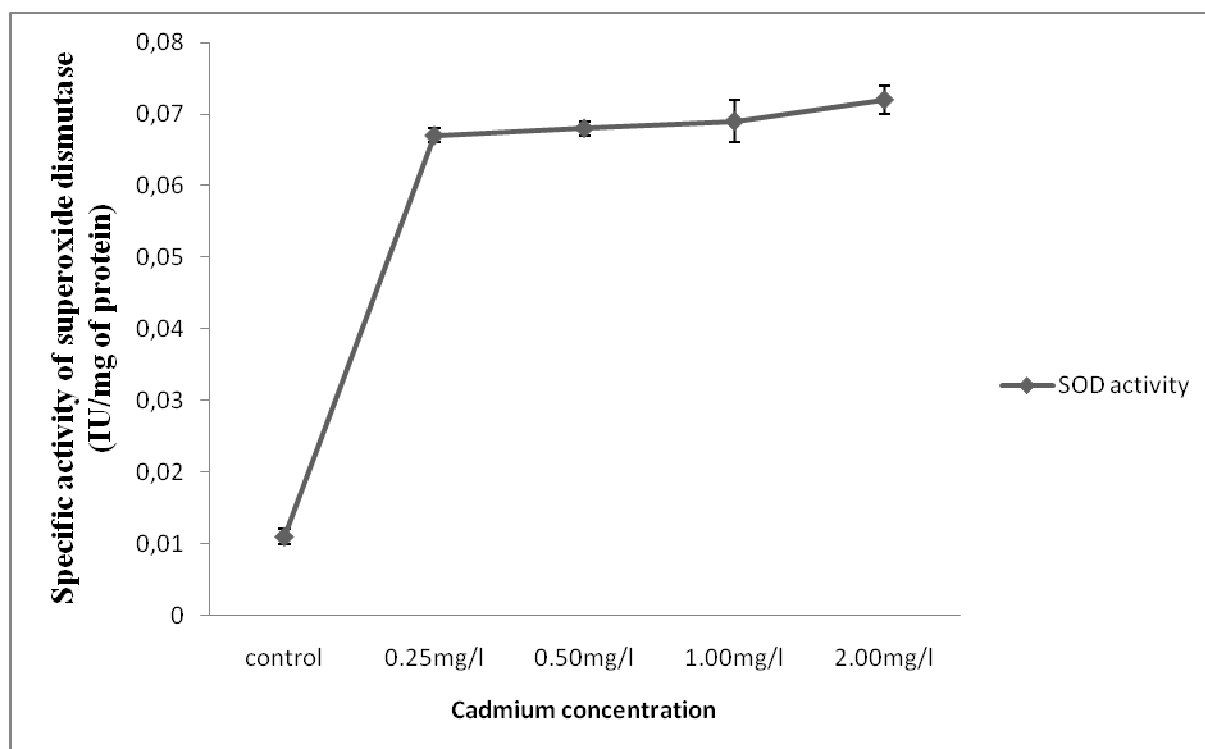


Fig. 1.1 Specific activity of SOD in *B. maculatus* exposed to cadmium.

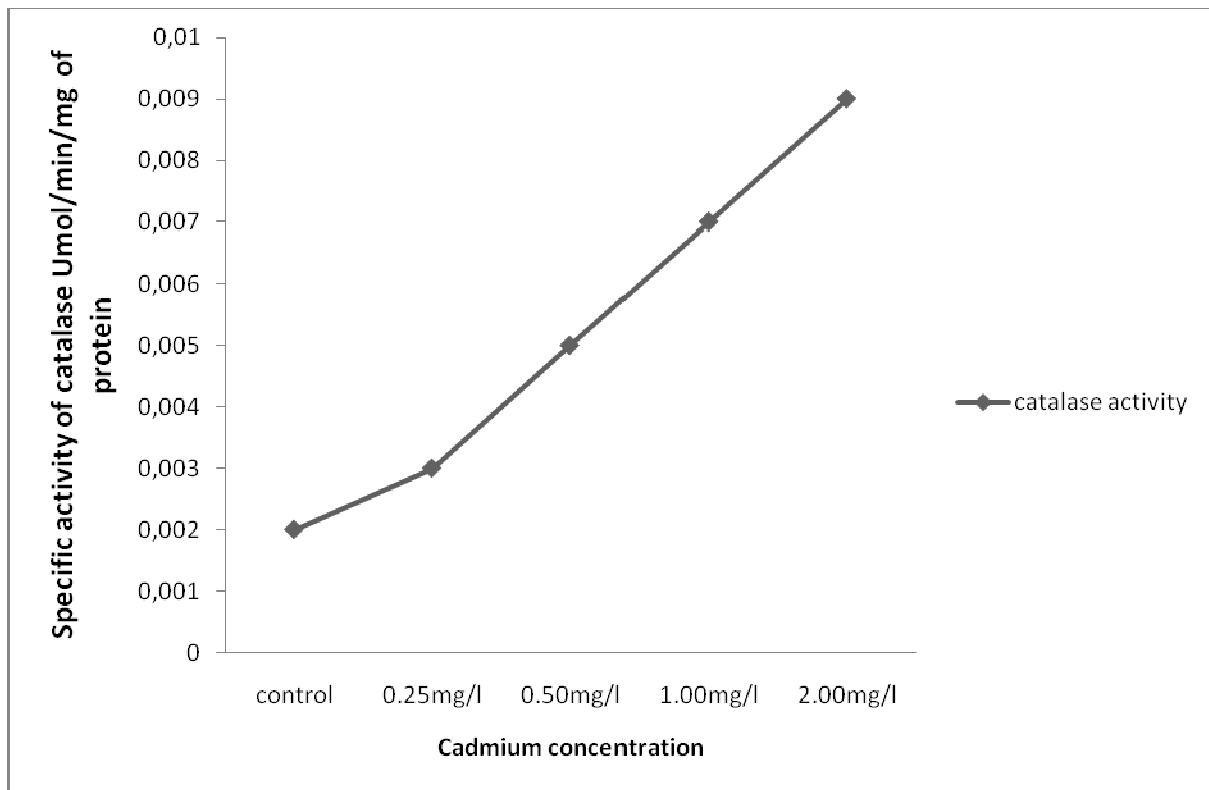


Fig.1.2 Specific activity of catalase in *B. maculatus* exposed to cadmium.

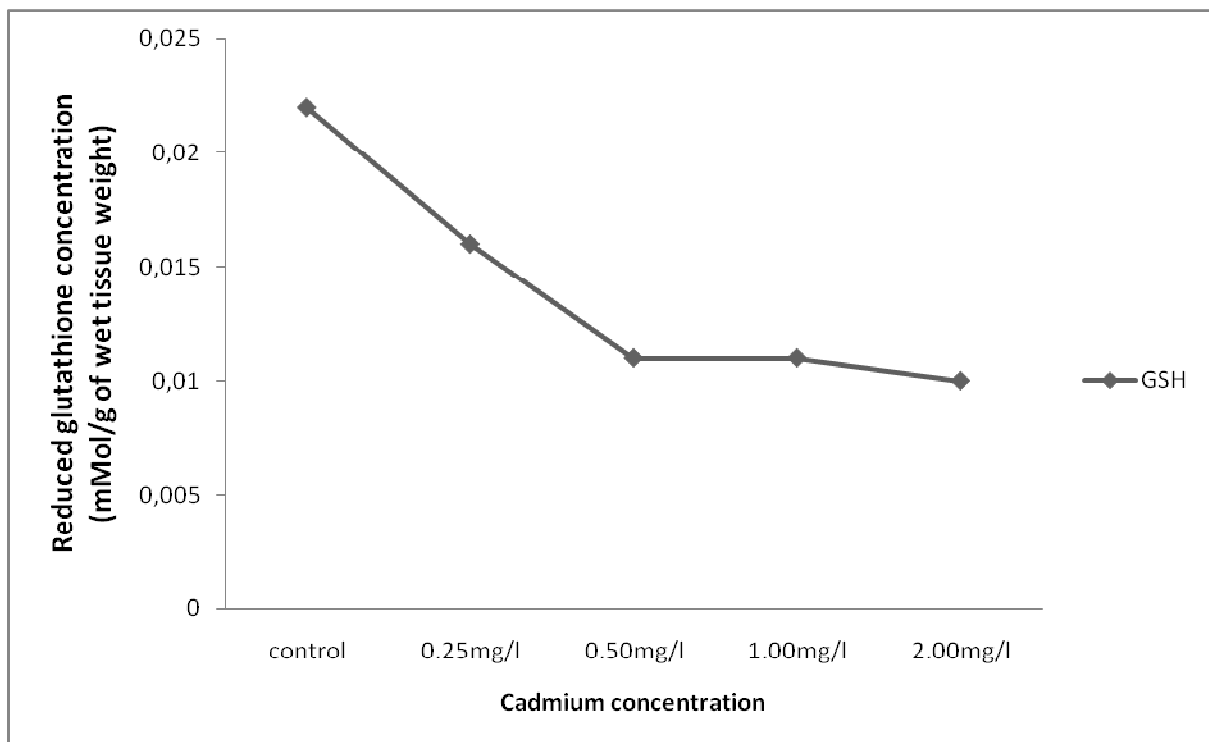
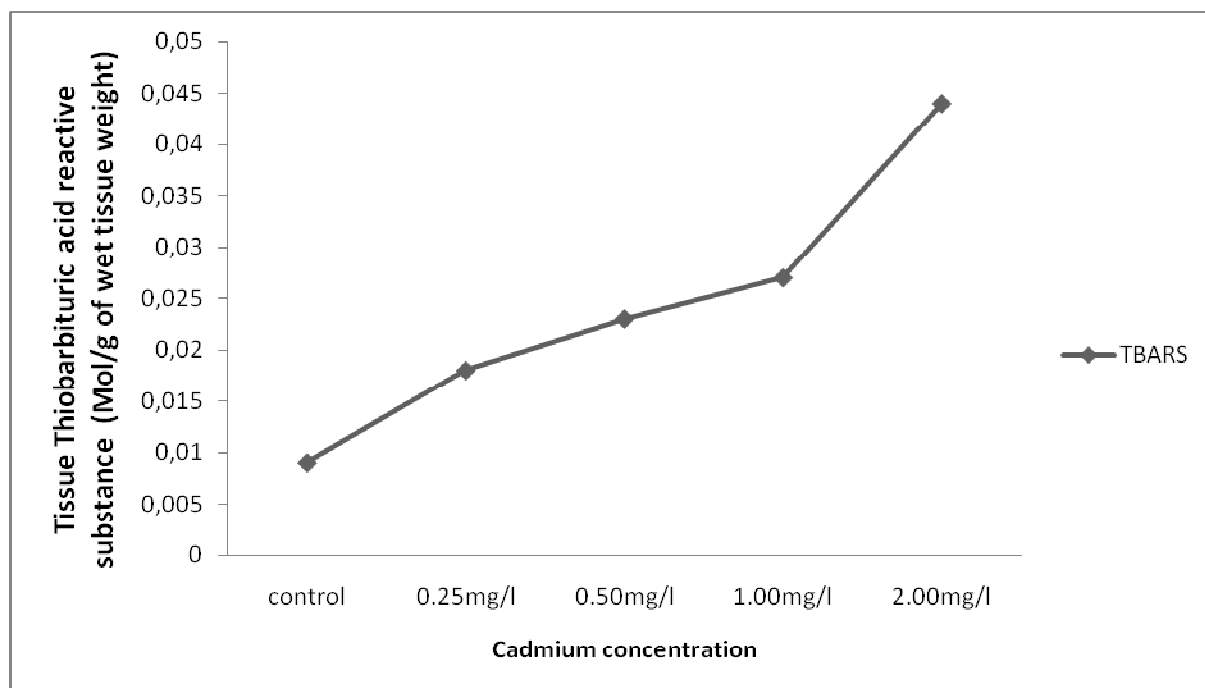


Fig. 1.3 Reduced GSH concentration in *B. maculatus* exposed to cadmium.



**Fig. 1.4** Changes in hepatic TBARS in *B. maculatus* exposed to cadmium.

Reduced glutathione concentration in *B. maculatus* exposed to cadmium decreased ( $p < 0.05$ ) with increase in concentration of cadmium. When compared with control groups, the decline was by 54.5% in the highest cadmium concentration exposed. TBARS which is an index of lipid peroxidation increased with increase in the concentration of cadmium in the liver of toads ( $p < 0.05$ ). In the 2.00mg/l cadmium, TBARS was elevated by 79.5% compared with control groups.

The present study has demonstrated the ability of cadmium to induce oxidative stress in toad liver as evidenced by increased lipid peroxidation (TBARS) after 28 days of cadmium treatment. This finding is consistent with several reports demonstrating that cadmium induces oxidative stress in tissues by increasing lipid peroxidation and by altering antioxidant status in several tissues (El-Demerdash *et al* 2004, Sarker *et al* 1997, Manca *et al* 1991). Cytosolic cadmium indirectly generates reactive oxygen species capable of inflicting peroxidative damage on biologic membrane lipids and a variety of transport proteins (Stohs and Bagchi 1995). Superoxide dismutase and catalase are important components of the antioxidant defence system and they help to counteract oxidative stress. The significant increase in the hepatic activities of these enzymes could be due to increased production to adequately quench oxidative stress as evident by marked increase in thiobarbituric acid reactive substances. Gupta *et al* 1991 reported that cells could increase the production of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase in order to circumvent oxidative stress.

Cellular GSH is very sensitive acting as the first line of defense in cadmium toxicity (Singhal *et al* 1987). The

observed decline in liver GSH of toads may be due to the effect of cadmium on GSH because of its high affinity to this molecule where a sulphhydryl, an amino and two carboxylic acid groups as well as two peptide linkages represent reactive sites for metals. Reaction of metals with glutathione may lead to either the formation of complexes or the oxidation of glutathione (Regoli and Principato 1995).

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