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Hepatic bioaccumulation of cadmium in the crowned bullfrog, *Hoplobatrachus occipitalis* and flat backed toad, *Bufo maculatus*

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Abstract: The hepatic bioaccumulation of cadmium in *Hoplobatrachus occipitalis* and *Bufo maculatus* were assessed for 14 and 28 days respectively. The amphibians were exposed to sub lethal cadmium concentrations of 0.25, 0.50, 1.00 and 2.00 mg/l. Bioaccumulation in *H. occipitalis* and *B. maculatus* increased significantly ($p<0.05$) in both periods of exposures. There was no significant difference in bioaccumulation between the 14 and 28 days exposures. There was also no significant difference in the bioaccumulation of cadmium between two amphibian species. The study suggested that the release of cadmium into the environment could possibly affect the well-being of amphibians as well as resulting in further decline of these very sensitive organisms that contribute significantly to the food web. There is therefore the need to protect amphibians from habitat alteration due to cadmium pollution with a view to sustaining the rich biodiversity in the Nigerian Niger Delta ecological zone.

Key Words: amphibian, bioaccumulation, cadmium, toxic

Introduction

Aquatic systems may be contaminated with heavy metals released due to industrial and agricultural activities. Heavy metals cannot be destroyed through biological degradation (El-Demerdash *et al*., 2004). When exposed to higher concentrations, organs of aquatic animals may accumulate heavy metals (Ezemonye & Enuneku, 2005, 2011). Cadmium is a biotoxic environmental pollutant which accumulates in body organs such as the lungs, liver, kidneys, bones, reproductive organs and the immune system (Egwurugwu *et al*., 2007, Sofyan *et al*., 2007). 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

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low melting point alloys or solders (Enuneku, 2010). The metal is released into the air, land and water by human activities (Linder 1985, WHO report 1992) and very steep increases in contamination by this metal has been documented (Cabrera *et al*., 1998). 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 (Suru, 2008). These have predisposed animal and human populations to both subtle and direct exposure pathways with an attendant increase in cadmium related pathologies (Satarug & Moore, 2004).

Bioaccumulation of chemicals is an important factor in the assessment of environmental hazard. It has been accepted as a trigger factor for decisions of administrative relevance (Heng *et al*., 2004, Beek, 1991). Bioaccumulation is the building up of a chemical to a toxic level in an organism. It is the net accumulation of a substance by an organism as a result of uptake directly from all environmental sources and from all routes of exposure (ASTM 1998). Primarily, it is the movement of a chemical into the organism from the food or water that is ingested or absorbed. Bioaccumulative contaminants are rapidly absorbed out of water-borne ambient environments and concentrated in the tissues of living aquatic organisms at concentrations that can range from thousands to millions of times greater than levels in the ambient environment (Ogeleka 2007). These absorbed levels might be high enough to cause dysfunction in the organisms and potential harmful effects to humans. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down or excreted (Britton, 1998). Bioaccumulation becomes an environmental problem where chemicals accumulated are toxic, leading to an elevated amount in the organism's body.

The frog, *Hoplobatrachus occipitalis* and toad, *Bufo maculatus* are endemic to West Africa (Roedel 2000). They are typical representatives of amphibians of the Niger Delta ecological zone (Enuneku 2010). They were chosen for this study because they are highly prolific (Roedel, 2000) and easy to handle and maintain under laboratory conditions. *H. occipitalis* is economically important as it is consumed by man. Frogs and toads have been reported to be sensitive to cadmium toxicity (James and Little, 2003, Resenberg *et al*., 2007). Ecologically, they occupy important positions in the food chain as they serve as food for both aquatic and terrestrial predators. Furthermore, these amphibians have been relatively understudied in ecotoxicological research.

The aim of this study was to assess the hepatic bioaccumulation of cadmium in the bullfrog *H. occipitalis* and flat backed toad, *B. maculatus***.**

Materials and Methods

Hoplobatrachus occipitalis were collected from unpolluted spawning ponds (Ezemonye & Tongo, 2010) in Oghara Community in the Niger Delta ecological zone of Nigeria. They were collected using hand nets to prevent injury to animals during capture since they are active animals. Toads (*Bufo maculatus*) were also collected from the same community using handnets in the night. Acclimation to laboratory conditions was done for two weeks prior to experiments (Goulet & Hontella 2003) in plastic tanks measuring 49cm in length × 29cm in width × 24cm in height with dechlorinated tap water (2 litres at a slant). The frogs 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 26-27 °C.

The initial mean weight of frogs and toads were measured. Since metabolic activity changes with size and affects the parameters to be measured (Canli & Furness, 1993), individuals of similar weights and snout-vent-lengths were used.

Cadmium as $CdCl_2.H_20$ was used for the sub lethal tests. Stock solution of the toxicant was prepared by dissolving toxicant in distilled water to a final volume of 1.0 L. Each treatment solution was prepared after a range-finding test by serial dilution (Ezemonye & Enuneku 2006). Four sub lethal concentrations (0.25, 0.50, 1.00 and 2.00 mg/l) of cadmium were dosed to frogs and toads. For each amphibian species, there were three replicate tanks per treatment and three individuals per tank including controls. The amphibians were fed with termites.

On the 14^{th} and 28^{th} day one frog/toad was sacrificed for the determination of the extent of bioaccumulation in the liver. Digestion of samples was according to FAO/SIDA (1983). To each sample of liver or kidney (0.1g), 10ml of 10% perchloric acid: conc. HNO_3 (3:2 v/v) was added and heat was applied (60 °C) until a clear solution was formed. The volumes were made up to 50ml using distilled water. The samples were then stored in plastic bottles before analysis with atomic absorption spectrophotometer to determine the amount of heavy metal bioaccumulated. The heavy metal concentrations were determined with an atomic absorption spectrophotometer (SOLAR 969 UNICAM SERIES) with air acetylene flame according to APHA (1998). The source of radiation was a hollow cathode lamp which contained a cathode constructed of the same material as that being analysed. After analysis using the AAS, the actual concentration of heavy metal in the tissue was determined as (Olaifa & Olaifa, 2003):

Actual concentration of heavy metal = PPMR × Dilution factor

Where PPMR = AAS reading

$$\textbf{Dilution factor} = \frac{\text{volume of digest used}}{\text{Weight of sample digested}}$$

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

The initial mean weight of frogs was 55.23±0.53 g. The initial mean weight of toads was 27.14±0.34 g. There was no significant difference ($p>0.05$) between the mean weights of frogs and toads used in the experiments.

Bioaccumulation of cadmium in the liver of *H. occipitalis* increased with increase in concentration of the heavy metal relative to the control group. The bioaccumulation of cadmium for the 14 days exposure was not significantly different from the 28 days exposure (Fig.1). Bioaccumulation of cadmium in the liver and kidney of *B. maculatus* increased as the concentration of heavy metal increased. Results indicated that there was no significant difference in the bioaccumulation of cadmium between the 14 days exposure and the 28 days exposure (Fig.2). Figures 3 and 4 compared the hepatic bioaccumulation of cadmium between *H occipitalis and Bufo maculatus*. There was no significant difference in the hepatic bio-accumulation of cadmium and between *H. occipitalis* and *B. maculatus*.

In this study the Bioconcentration factor (BCF) of cadmium in *Hoplobatrachus occipitalis* varied between 0.91and 1.40 in the liver in 14 days exposure. BCF varied between 0.26 to 0.99 in the liver for the 28 days exposure. BCF of cadmium in *Bufo maculatus* varied from 0.28-1.36 in the liver for the 14 days exposure; whereas BCF ranged between 0.06-1.76 in the liver for the 28 days exposure.

Discussion

Although lethal concentration (LC_{50}) is a commonly used measure of environmental toxicity, it does not give any information about long-terms effects of sub lethal concentration in the aquatic environment (Ogeleka, 2007). The main concern of toxicology is the bio-accumulation of substances at levels that are detrimental to the well-being of organisms. This has become a critical consideration in the regulation of chemicals.The liver accumulates relatively higher amounts of heavy metals (Vinidhini & Narayanan 2007). This agrees with the finding of Vinodhini and Narayanan (2009) who reported that cadmium strongly accumulated in liver and kidney of the common carp *Cyprinus carpio* after 32 days exposure.

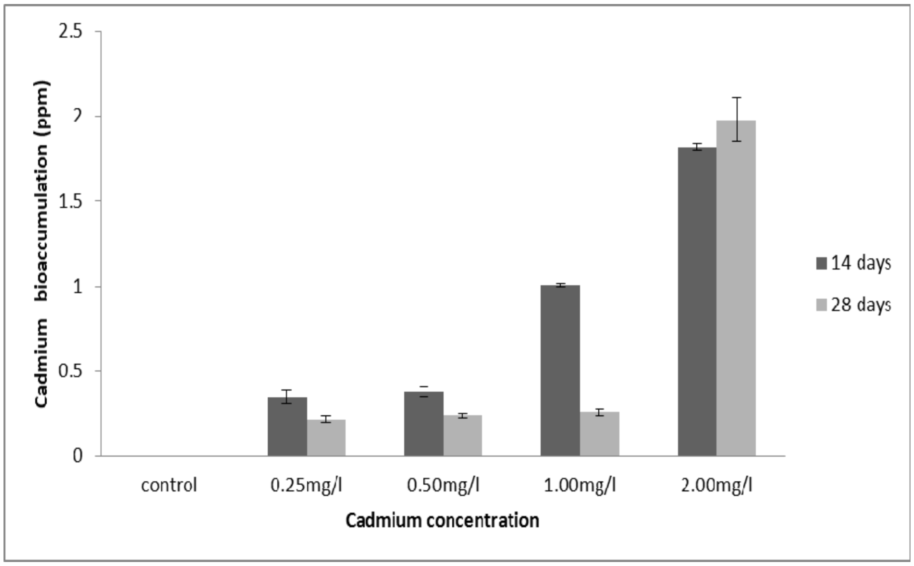

Fig.1: Bioaccumulation of cadmium in the liver of *H. occipitalis* after 14 and 28 days

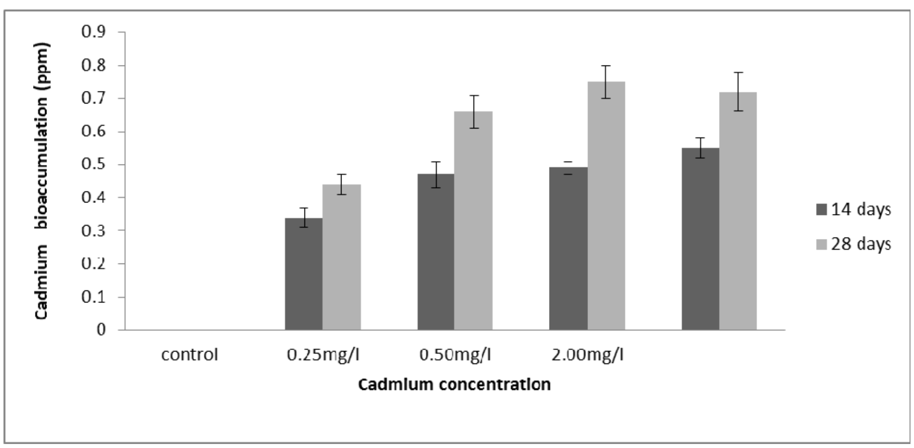

Fig.2: Bioaccumulation of cadmium in the liver of *B. maculatus* after 14 and 28 days

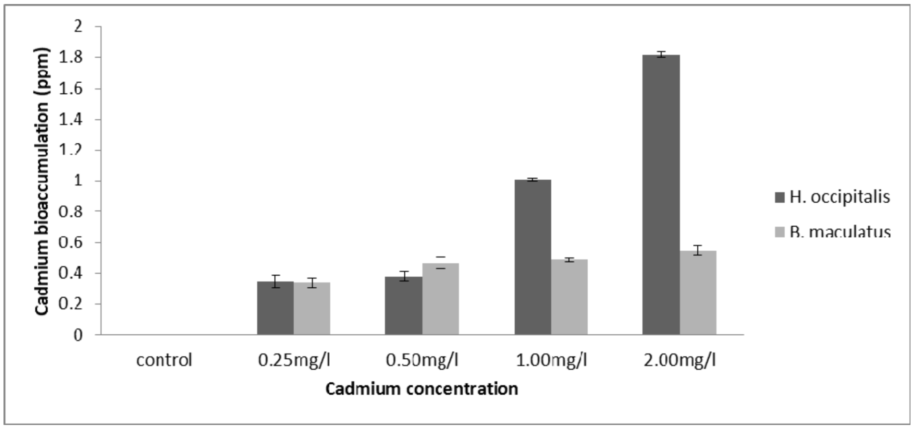

Fig. 3: Differential hepatic bioaccumulation of cadmium in *H. occipitalis* and *B. maculatus* for 14 days

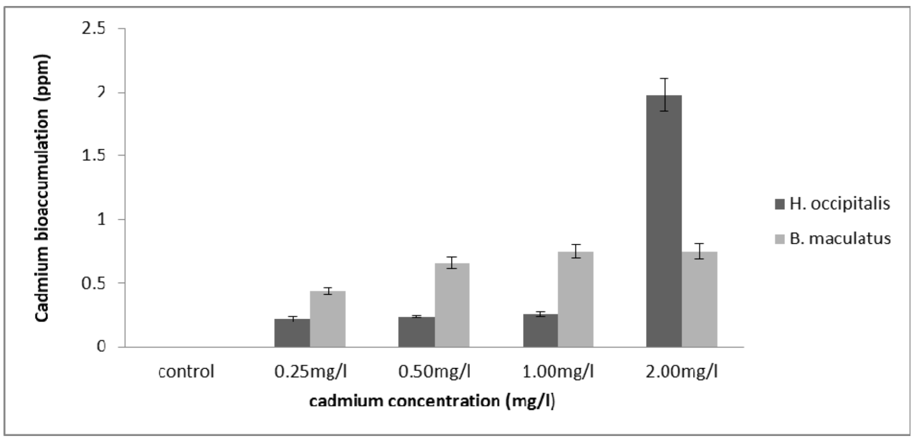

Fig.4: Differential hepatic bioaccumulation of cadmium in *H. occipitalis* and B. maculatus for 28 days

The results of this study indicated that cadmium accumulation gradually increases during the exposure period. The high accumulation in liver may alter the levels of various biochemical parameters (Ezemonye and Enuneku 2011). This may also cause severe liver damage (Mayers & Hendricks, 1984). Results from this study also revealed that there was no significant difference in the bioaccumulation of cadmium in the liver of the experimental animals between the 14 days exposure and the 28 days exposure.

A chemical with BCF greater than 1000 tends to persist in the environment (Ogeleka 2007). This is because at such levels, chemicals persist in the environment and tend to bioaccumulate in the tissues of organisms since the transport medium and catabolic pathways for them may not have evolved. Although values of BCF were less than 1000 in both periods of exposures in the two amphibians, sub lethal effects give a true picture of the effects of chemicals on the tested organisms.

Accumulation of cadmium in the investigated amphibians was significantly higher than the WHO (1992) safe limit (0.005mg/l). Hence, there is serious need for the monitoring of cadmium residues in water, food and the environment, as this will go a long way towards preventing various environmental hazards in the Nigerian ecosystems.

This study showed that the release of cadmium into the environment could threaten amphibian survival. Sub lethal exposure to cadmium resulted in bioaccumulation in the liver of *H. occipitalis* and *B. maculatus*. The results obtained indicated that the heavy metal is toxic and could be lethal as it bioconcentrates along the food chain. The assay used in this study could therefore be employed in the monitoring of amphibian habitats.

References

- ✓ American Public Health Association (APHA). America Water Works Association, Water Pollution Control Federation (1998) Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association., New York.
- ✓ Beek, B. (1991) Bioaccumulation in aquatic systems. In Bioaccumulation in Aquatic Systems: Contributions to the Assessment, R. Nagel and R. Loskin (Eds), Weinheim, Germany.
- ✓ Britton, L.N. (1998) Surfactants and the environment. JSD 1: 109–117.
- ✓ Cabrera, C., Ortega, E., Lorenzo, M. and Lopez, M. (1998) Cadmium contamination of vegetable crops, farmlands, and irrigation waters. Rev. Environ. Contam. Toxicol. 154: 55–81.
- ✓ Canli, M. and Furness, R.W. (1993) Toxicity of heavy metals dissolved in sea water and influences of sex and size on metal accumulation and tissue distribution in the Norway lobster *Nephrops norvegicus.* Mar. Environ. Res. 36: 217-236.
- ✓ Egwurugwu, J.N., Ufearo, C.S., Abanobi., O.C., Nwokocha, C.R., Duribe, J.O., Adeleye, G.S., Ebunlomo, A.O., Adetola, A.O. and Onwufuji, O. (2007) Effect of Ginger (*Zingiber officinale*) on cadmium toxicity. Afr. J. Biotechol. 6 (18): 2078-2082.
- ✓ El-Demerdash, F.M., Yousef, M.I., Kedwany, F.S., Baghdadi, H.H. (2004) Cadmium-induced changes in lipid peroxidation, blood haematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and β-carotene. Food. Chem. Toxicol. 42: 1563-1571.
- ✓ Enuneku, A.A. (2010) Toxicological and Biochemical alterations in adult Amphibians (*Hoplobatrachus occipitalis* and *Bufo maculatus* exposed to Cadmium and Lead. Ph.D thesis, University of Benin, Nigeria.
- ✓ Ezemonye, L. and Enuneku, A. (2005) Evaluation of acute toxicity of cadmium and lead to amphibian tadpoles (toad: *Bufo maculatus* and frog: *Ptychadena bibroni*). J Aquat. Sci. 20 (1): 33-38.
- ✓ Ezemonye, L. and Enuneku, A. (2006) Stage-dependent acute toxicity of exposure of *Bufo maculatus* and *Ptychadena bibroni* tadpoles to cadmium (Cd^{2+}). J. Appl. Sci. Technol. 11(1-2): 78-82.
- ✓ Ezemonye, L. and Enuneku. A. (2011) Biochemical changes in the toad, *Bufo maculatus* treated with sub lethal concentrations of cadmium. World J. Biol. Res. 4(1): 15-20.
- ✓ Ezemonye, L. and Tongo, I. (2010) Acute Toxic Effects of Endosulfan and Diazinon pesticides on adult amphibians (*Bufo regularis*). J. Environ. Chem. Ecotoxicol. 2 (5): 73-78.
- ✓ FAO/SIDA (1983) Manual of methods in aquatic environmental research part 9. Analysis of metals and

organochlorines in fish. FAO fisheries / Technical paper 212.

- Goulet, N.B. and Hontella, A. (2003) Toxicity of Cadmium, Endosulfan and Atrazine in Adrenal Steriodogenic cells of two amphibian species; *Xenopus laevis* and *Rana catesbeiana*. Environ. Toxicol. Chem. 22(22): 2106-2113.
- Heng. L.Y., Mokhtar, M.B. and Rusin, S. (2004) The bioaccumulation of Trace Essential Metals by the freshwater Snail *Turritella sp*. Found in the Rivers of Borneo East Malaysia. J. Biol. Sci. 4(4): 441–444.
- James, S.M and Little, E.E. (2003) The effects of cadmium exposure on the American toad, *Bufo americanus* tadpoles. Environ. Toxicol. Chem. 22: 377-380.
- Linder, M.C. (1985) Nutritional Biochemistry and metabolism with clinical application. Elsevier, Amsterdam.
- Mayers, T.R. and Hendricks, J.D. (1984) Histopathology. In G.M Rand, S.R. Petrocelli, Eds. Fundamental of aquatic toxicology, Washington DC. Hemisphere.
- Ogeleka, D.F. (2007) Studies on the toxicity of industrial detergent and corrosion inhibitor on biological organisms. A Ph.D Thesis. University of Benin.
- Roedel, M.O. (2000) Herpetofauna of West Africa. Vol 1. Amphibians of West African Savana. Edition Chimaira, Frankfurt.
- Rosenberg, C.E., Fink, N.E. and Salibian, A. (2007) Humoral immune alterations caused by lead: Studies on an adult toad model. Act. Toxicol. Arg. 1851-3743.
- Satarug. S. and Moore, M.R. (2004) Adverse health effects of chronic exposure to low–level cadmium in foodstuffs and cigarette smoke. Environ. Health. perspect. 112: 1099-1103.
- Sofyan, A., Rosita, G., Price, D. and Birge, W. (2007) Cadmium uptake by *Ceriodaphnia dubia* from different exposures: Relevance to body burden and toxicity. Environ. Toxicol. Chem. 26(3: 470-477.
- Suru, S.M. (2008) Onion and garlic extracts lessen cadmium-induced nephrotoxicity in rats. Biometals. 21: 623-633.
- Vinodhini, R. and Narayanan, M. (2007) Bioaccumulation of heavy metals in organs of freshwater fish *Cyprinus carpio* (Common carp). Int. J. Environ. Sci. Tech.5 (2): 179-182.
- Vinodhini ,R. and Narayanan, M. (2009) Heavy metal induced histopathological alterations in selected organs of *Cyprinus carpio* L (Common carp). Int. J. Environ. Res. 3(1): 95-100.
- WHO, (1992) Environment Health Criteria, 134, Cadmium, World Health Organization, GenevaWHO report.