Behavioural and Haematological Changes in Adult *Hoplobatrachus occipitalis* and *Bufo maculatus* Exposed to Sub Lethal Concentrations of Lead

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Abstract

Hoplobatrachus occipitalis and *Bufo maculatus* were exposed to sub lethal concentrations (0.25, 0.50, 1.00 and 2.00mg/l lead oxide) for 28 days. There were 3 replicate tanks per treatment and 5 individuals per tank including control groups. A behavioural change in coloration in skin (dark permanent colour) were observed in the toads at the end of the experimental period. The haematological alterations after the examination of blood indices during the period of exposure showed that total erythrocyte count (TEC), haematocrit (Hct) and haemoglobin (Hb) concentration decreased ($p<0.05$) in both amphibian tetrapods relative to controls. The decrease was concentration-dependent with increase in the concentration of lead. There was elevation in total leucocyte counts (TLC) with increase in the concentration of lead. There was no significant variation in all the haematological parameters studied between both species. The study has shown that the exposure of the frog *Hoplobatrachus occipitalis* to lead could cause alterations in the haematological indices which could induce unfavourable physiological changes in the target organism ultimately leading to death.

Keywords: Frog, Toad, Haematology, Nigeria, Lead, Pollution

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Introduction

Global declines in amphibian populations in the wild have caused concern in the scientific community [1, 2, 3] and provided the impetus for this study. Numerous physical and chemical causes have been postulated[4] and in some instances, interaction of multiple causes have been implicated.[5] In many cases, heavy metals from industrial and agricultural activities have been implicated.[5]

Heavy metals have long been recognised as serious pollutants of the aquatic environment.[7] They are one of the most widespread groups of contaminants because of their slow elimination from environmental compartments.[8, 9]

Lead is an environmentally persistent heavy metal that causes neurological, haematological, gastrointestinal, circulatory, immunological and reproductive pathologies. The propensity for lead to catalyze oxidative reactions and generate reactive oxygen species has been demonstrated in multiple studies.[10, 11] In Nigeria, over 200 children died in Arika Local Government area of Zamfara State in 2010 and 2011 due to environmental lead pollution from illegal mining activities. Lead toxicity continues to be a major health problem. The pathogenesis of lead toxicity is multi-factorial as lead directly interrupts enzyme activation, completely inhibits trace mineral absorption, binds to sulphydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis and lowers the level of available sulphydryl antioxidant reserves in the body.[12] 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, 13] Given the paucity of information on the ecotoxicology of heavy metals to amphibians, the scientific challenge is to develop sensitive methodology to detect the specific role of heavy metals in heavy metal toxicity.

Maheswaran *et al*.[6] have reported that the main components of blood; Haemoglobin, (Hb) total erythrocyte count (TEC), total leucocyte count (TLC) and haematocrit (Hct) have been used as indicators of heavy metal pollution in the aquatic environment.

The frog, *Hoplobatrachus occipitalis* and toad, *Bufo maculatus* are endemic to West Africa. They are typical representatives of amphibians of the Niger Delta ecological zone. They were chosen for this study because they are easy to handle and maintain under laboratory conditions. Secondly these amphibians are relatively understudied with

available ecotoxicological data. *H. occipitalis* is economically important as it is consumed by man. Frogs and toads have been reported to be sensitive to lead.[14]

Materials and Methods

Hoplobatrachus occipitalis were collected from unpolluted spawning ponds[15] 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[16] in the night. Acclimation to laboratory conditions was done for two weeks prior to experiments[17] 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 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^{0}C.The initial mean weight of frogs was 55.23±0.52g. The initial mean weight of toads was 27.14±0.34g. Because metabolic activity changes with size and affects the parameters to be measured, individuals of similar size were used.[18] The frogs and toads were acclimated to laboratory conditions in dechlorinated tap water for 2 weeks prior to the assay in plastic tanks measuring 49cm x 29cm x 24 cm. The mean values for the test water quality were as follows; temperature 22±1^{0}C; pH 5.7±0.4; dissolved oxygen 4.7±0.7 ppm; hardness 36±1.24ppm. The frogs were fed *ad libitum* daily with termites. Frogs and toads maintained a photoperiod of 10 light, 14h dark regime.

Stock solutions of PbO were prepared by dissolving toxicant in distilled water to a final volume of 1.0 L. Each treatment solution was prepared by diluting the stock solution with water to achieve the appropriate exposure concentrations (3).The toxicant in the test chamber was renewed completely with fresh solution of the same concentration every 24 hour.

Four sub lethal concentrations (0.25, 0.50, 1.00 and 2.00 mg/l) of lead were prepared in addition to control groups and 5 frogs were introduced in each concentration per tank. For this sub lethal exposure 3 replicates were used. These same conditions were also replicated for the toads but the tanks for the toads were kept at a slant to create a terrestrio-aquatic environment. The frogs and toads were fed with termites *ad libitum* as in controls. On the 28th day each frog/toad was decapitated and blood collected by heart puncture by means of a hypodermic syringe and needle. The blood collected from the heart was transferred to ethylenediaminetetraacetic

acid (EDTA) containers for hematological analysis.[19] The treatment and control blood samples were used to estimate the hematological parameters.

Ethylenediaminetetraacetic acid (EDTA) was used as anticoagulant and noncoagulated blood was tested shortly after collection for hemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC) and haematocrit (Hct). Hemoglobin (Hb) concentration was estimated as cyanomethemoglobin according to Ochei and Kolhakter.[20] TEC and TLC were determined according to the classical method using the Neuber hematocytometer. Haematocrit was determined using microhaematocrit tubes and a haematocrit centrifuge (Juan Model A 13). Blood was centrifuged at 1200 x g for 5 minutes and the haematocrit value was obtained.[21]

Statistical Analysis

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). Control groups were compared with treatment groups. Statistical significance was considered at $p<0.05$ level of significance.

Results

The water quality parameters (pH, temperature, dissolved oxygen, hardness and turbidity) monitored during the exposure periods for acute and sub lethal assays were not significantly different between various concentrations of the heavy metal and the control groups and within concentrations ($p > 0.05$) .The parameters were almost uniform all through the study irrespective of the treatment, duration and the organisms. Changes in coloration on the skin (Dark permanent colour) were observed in the toads exposed to lead for 28 days. With the exception of control groups coloration deepened with time in all treatment groups.

Alterations in haematological parameters of *H. occipitalis* and *B. maculatus* exposed to lead are presented in Figure 1-4. Lead inflicted a drastic reduction ($p<0.05$) in the total erythrocyte count (TEC), haemoglobin (Hb) and haematocrit (Hct) in both amphibians after 28 days relative to controls. However total leucocyte counts increased as concentration of lead increased in both amphibians. There was no significant variation ($P > 0.05$) in TEC, Hct, TLC and Hb between *H. occipitalis* and *B. maculatus* exposed to lead (Fig.1-4).

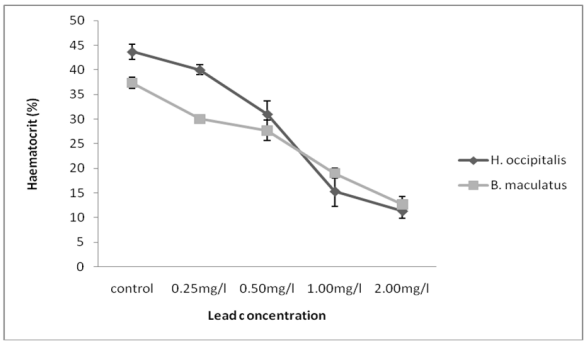


Fig. 1: Variation in haematocrit value in *H. occipitalis and B. maculatus* exposed to lead

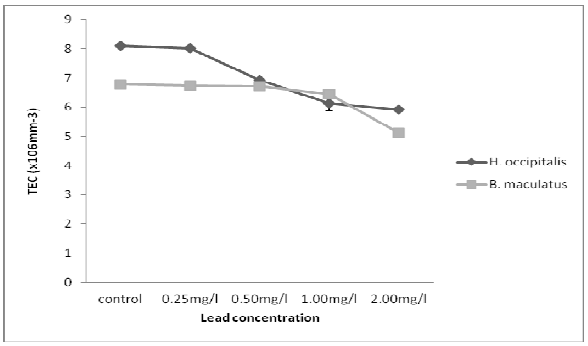


Fig. 2: Variation in total erythrocyte count in *H. occipitalis and B. maculatus* exposed to lead

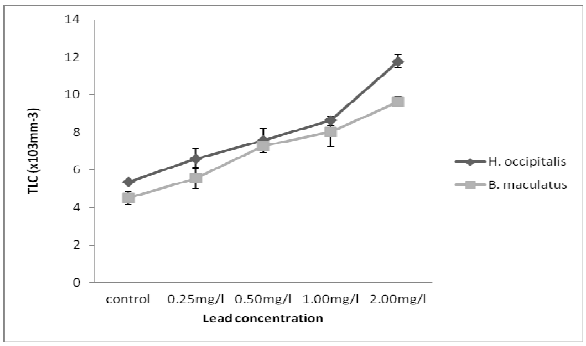


Fig. 3: Variation in total leucocyte count in *H. occipitalis and B. maculatus* exposed to lead

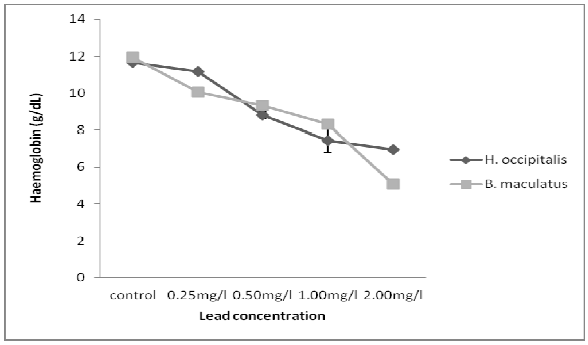


Fig. 4: Variation in haemoglobin content in *H. occipitalis and B. maculatus* exposed to lead

Discussion

Changes in coloration were observed in the toads exposed to lead for 28 days in this study. Minimal mucous secretion was observed in the 1.00 and 2.00mg/l treatment groups. In similar studies, lead exposure to amphibians has resulted in a range of behavioural effects including decreased 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, 13]

Patil *et al*[22] reported that one of the most sensitive targets for lead toxicity is the haematological system. This propensity was also reported by Fufeyin *et al*.[23] *Hoplobatrachus occipitalis* and *Bufo maculatus* exposed to sublethal concentrations of lead for 28 days showed a significant decrease in haematocrit (Hct), total

erythrocyte counts (TEC) and haemoglobin (Hb). In this study lead caused a decline total erythrocyte count in both amphibians studied. Erythrocytes have a high affinity for lead, binding 99% of the lead in the blood-stream. Lead has a destabilizing effect on cellular membranes, and in red blood cells the effect decreases cell membrane fluidity and increases the rate of erythrocyte haemolysis. Haemolysis appears to be the end result of ROS-generated lipid perioxidation in the red blood cell memberane.[24] Lead can also bind directly to phosphatidylcholine in the red blood cell membrane leading to a decrease in phospholipid levels.[25] Hypochromic or normochromic anaemia is the hallmark of lead exposure. It results from ROS generation and subsequent erythrocyte haemolysis. Lead elevates and suppresses blood levels of the antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase.[26, 27, 28]

In this study, total leucocyte counts increased with increase in lead concentration. Leucocytes play a major role in the defence mechanism of animals and consist of granulocytes, monocytes, lymphocytes and thrombocytes. Granulocytes and monocytes function as phagocytes to salvage debris from impaired tissue, and lymphocytes produce antibodies.[29] Blood of all experimental groups showed higher concentration of leucocytes with increased lead concentration than controls. The increase in leucocytes observed in this investigation could be attributed to a stimulation of the immune system in response to tissue damage caused by lead accumulation. Tissue damage or injury could induce stimulation of the immune system manifested by increase in lymphocytes.[30] Increase in leucocytes count due to heavy metal exposure in experimental animals have been reported by various researchers.[31, 32]

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