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Effects of Lead Nitrate on catalase production levels in post juvenile *Clarias gariepinus* (Burchell, 1822)

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The effects of Lead Nitrate on catalase (CAT) production levels in post juvenile *Clarias gariepinus* were investigated. A total of 250 samples of the fish were acclimatized for two week. Six samples of the fish were exposed to the sub-lethal concentrations of 00, 28, 43 and 57 mg/L with replicate in each case for 28 days. Two samples of the fish were randomly selected from the treatments and control for the bioassay of catalase on the 14th and 28th day respectively. The fish organs (kidney, liver and gill) were excised from the fish and homogenized in sodium phosphate buffer (0.2 M, pH 8.0). The data generated were subjected to one-way analysis of variance followed by Duncan Multiple Range test where significant. The results showed that 96 h LC_{50} of *C. gariepinus* exposed to lethal concentrations of Lead Nitrate was 284.189 mg/L. The CAT production levels were significantly higher in the kidney of the fish exposed in 28 mg/L on day 14; 43 and 57 mg/L on day 28, respectively. The CAT production levels were significantly higher in the gill of the fish exposed in 57 mg/L on day 14; 43 and 57 mg/L on day 28 respectively. The CAT production levels were significantly higher in the liver of the fish exposed in 28 and 57 mg/L on day 14 respectively. The highest production mean values of 149.55±43.65 and 152.80±40.40 U/mgprotein were obtained in the kidney of the fish exposed in 57 and 43 mg/L, respectively. Therefore, the kidney of the fish exhibited a better control of the toxicant and as such, catalase production level in this organ should be used in assessing the level of physiological changes in the fish.

Key words: Catalase, Lead Nitrate, oxidative stress, *Clarias gariepinus*, LC_{50}.

INTRODUCTION

Fish is a rich source of animal protein throughout the world. Due to its nutritional value (Tingman et al., 2010), the demand for fish food has been on the increase with increasing human population (FAO, 2010, 2012). Fish culture which is an important source of protein and employment for many people (Gabriel et al., 2007) has been used to bridge the gap between demand and supply of fish from capture fisheries. Clarias gariepinus is a member of the Clarridae family. They occur naturally in South East Asia and in Africa and are sometimes called African catfish or mudfish. C. gariepinus is well appreciated in many African countries (De Graaf et al.,

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1996). Catfishes are opportunistic feeder, feeding on virtually everything that comes their way.

The contamination of freshwaters with a wide range of pollutants has become a matter of concern over the last few decades (Ohe et al., 2004). Heavy metal adulteration may have disturbing effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi et al., 2007). The presence of heavy metals is a potential threat to the life and safety of many aquatic organisms by changing their genetic, physiological, biochemical and behavioural parameters (Scott and Sloman, 2004). Lead (Pb) is one of the heavy metals that has contaminated water bodies on a global scale with adverse effects on human, environment health and aquatic life especially fishes (Markus and McBratney, 2001). Several reports have indicated that Pb can cause neurological, hematological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes all of them related to the dose and time of exposure to Pb (Mirhashemi et al., 2010). Lead as a metal has physical and chemical properties that make it extremely useful in industries especially in lead battery production, coloured inks and paint preparation. Lead thus constitutes an important constituent of wastes discharged from industries to which aquatic animals especially fishes are exposed. Lead and other trace metals have high affinity for animal tissues where they are concentrated to varying levels (Martinez et al., 2004). Metals like Lead which are toxic are known to present greater hazard when they are both persistent and bioaccumulative (De Forest et al., 2007). The quantity of metal accumulated has been reported to be directly related to the concentration to which the organisms are exposed and the period of exposure (Otitoloju and Don-Pedro, 2001). Metals are also preferentially accumulated by different organs of the body (Vinodhini and Narayanan, 2008; Rauf et al., 2009). The concentration of metals in the aquatic environment and the tissue of the fish (Bu-Olayan and Thomas, 2008) often pose serious health problems to fish consumers especially man. Lead is known to be accumulated in different organs of fish including the bone, gills, kidneys, liver and scales (Javid et al., 2007). Fish accumulate toxic chemicals such as Lead nitrate directly from water and diet, and contaminant residues may ultimately reach concentrations hundreds or thousands of times above those measured in the water, sediments and food (Labonne et al., 2001; Goodwin et al., 2003; Osman et al., 2007).

Antioxidants are substances capable of decreasing the harmful effects of oxidative stress. Antioxidant defense systems are affected by contact with chemicals and therefore, antioxidant defense systems can act as biological markers for environmental pollution monitoring. Antioxidants are organisms' first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. Catalase is a common enzymatic antioxidant found in nearly all living organisms exposed to oxygen (such as bacteria, plants and animals). It catalyses the decomposition of hydrogen peroxide to water and oxygen (Aebi, 1984). It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). Likewise catalase, has one of the highest turnover number of all enzymes, one catalase molecule can convert approximately 5 million molecules of hydrogen peroxide to water and oxygen each second (Goodsell, 2004).

The heavy metals are of particular concern due to their persistence and un-degradable nature. The metal contamination in aquatic ecosystem is considered to be unsafe not only for fishes but, also for human beings because they consume fishes which are best sources of proteins and essential amino acids. Consumption of such staple food in contaminated forms calls for concern. This is why the effects of toxicants on the immunity and physiology of the fish need to be put in check as soon as possible; since fish like any other vertebrate, are endowed with antioxidants to deal with the effects of xenobiotics.

MATERIALS AND METHODS

Collection and acclimatization of post juvenile *C. gariepinus*

A total of two hundred and fifty (250) samples of juvenile *C. gariepinus* were purchased from fish farm and were transported to the laboratory in a well aerated 25 L plastic bucket with water in it. The fish were distributed in 12 different plastic tanks (Aquaria) for a period of two weeks (14 days) in which they were fed with vital feed (2 mm) morning and evening every day of the acclimatization period.

Lethal concentration (96 h LC_{50}) determination

Experimental set-up for the determination of LC_{50} were as follows: 240, 260, 290, 320, 340 and 00 mg/L as control. The experiment was conducted using 6 fishes per tank. From the Experiment, LC_{50} value was determined using Probit analysis (Finney, 1971).

Chronic exposure (Sub- lethal concentration)

The LC_{50} value gotten from the Probit analysis was used to derive the sub- lethal concentration at 10, 15 and 20% of the lethal concentration and approximated to the nearest decimal place. The sub-lethal treatments had 00, 28, 43 and 57 mg/L of Lead Nitrate with replicate in each case. Six samples of the fish were exposed in each replicate and control.

The experiment was carried out for 28 days and the water in the tanks were changed every 3 days (72 h) and Lead Nitrate was dissolved into the water each time the water was changed aside control setup. The feeding process was carried out every 9.A.M in the morning and 9.P.M in the night.

Sample harvesting and preparation

On the 14^{th} day 2 fish samples were selected at random from each

Table 1. The LC_{50} determination of *Clarias gariepinus* exposed to lead nitrate.

Exposure period (hours)	Concentration (mg/L)	No. of Fish	Mortality (%)
24	350	6	100
48	320	6	66.7
72	290	6	50
96	260	6	0
120	240	6	0

Table 2. Catalase production levels in kidney of *C. gariepinus* exposed to sub-lethal concentration of lead nitrate.

Concentration (mg/L)	Day 14	Day 28
Control	96.35 ± 0.05^{b}	$101.0.2\pm0.00^{b}$
C1	111.35 ± 11.85^{c}	93.80 ± 3.80^{a}
C2	91.85 ± 7.45^{b}	152.80 ± 40.40^{c}
C3	72.80 ± 6.20^{a}	149.55 ± 43.65^{c}

Mean values with the same superscript in the column are not significantly different (P>0.05) from each other. C1-C3 represents sub-lethal concentration of lead nitrate as: 28, 43 and 57 mg/L, respectively.

treatment and replicate for dissection and organ extraction. On the 28th day also 2 fish samples were selected and dissected for organ extraction. The extracted organs (gills, liver and kidney) were homogenized in sodium phosphate buffer using ceramic mortar and pestle. Each homogenate was placed in a test tube containing the buffer and was refrigerated. Buffer solution was prepared from mixture of 8.40g of sodium dihydrogen phosphate (NaH_2PO_4) and 9.94 g of disodium hydrogen phosphate ($NaHPO_4$) and the pH of buffer was adjusted to 8.0.

Estimation of catalase

Catalase production levels in each of the organ were assayed for according to Aebi (1984). Supernatant/Serum (0.1 ml) was added to cuvette containing 1.9 ml of 50 mM phosphate buffer, pH 7.0. Reaction started by the addition of 1.0 ml of freshly prepared 30 mMH_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/mg protein.

Data analysis

One-way analysis of variance (ANOVA) was used to compare the means of the results obtained from biochemical analysis followed by Duncan Multiple Range test where significant at P≤0.005. Bar charts were used to represent the effects of Lead Nitrate on catalase production levels in *C. gariepinus.*

RESULTS

Lethal dose of fish exposed to Lead Nitrate

The number of fish and the mortality rate in the final phase of the LC_{50} determination is indicated 100% mortality in the treatment exposed to 350 mg/l. there was no mortality in the treatments 240 and 260 mg/l, respectively (Table 1).

Catalase production levels in *C. gariepinus* exposed to sub-lethal concentrations of Lead Nitrate

The highest catalase (CAT) production levels of kidney of *C. gariepinus* exposed to sub-lethal concentration of Lead Nitrate was gotten from treatments C2 and C3; and are significantly higher than the control on day 28. On day 14 however, the CAT production levels obtained from Kidney was significantly lower than control in C2 and C3 but significantly higher in C1 (Table 2 and Figure 1).

The CAT production levels in the Gills of *C. gariepinus* expose to sub-lethal concentration of Lead Nitrate were significantly higher in C3 but significantly lower in C2 on day 14. On day 28 however, the CAT production levels in the gill of the fish exposed in C2 and C3 were significantly higher than control (Table 3 and Figure 2).

CAT production levels in C1 and C3 are significantly higher than the control in the Liver of *C. gariepinus* exposed to sub-lethal concentration on day 14. On day 28, the CAT production levels in all treatments were significantly lower than the control (Table 4 and Figure 3).

DISCUSSION

The toxicity of Lead Nitrate or any chemical toxicant can alter the physiological state of the vertebrate exposed to it thereby impairing the various metabolic activities. A number of defense systems are involved in combating the accumulation of ROS and CAT is a major antioxidant

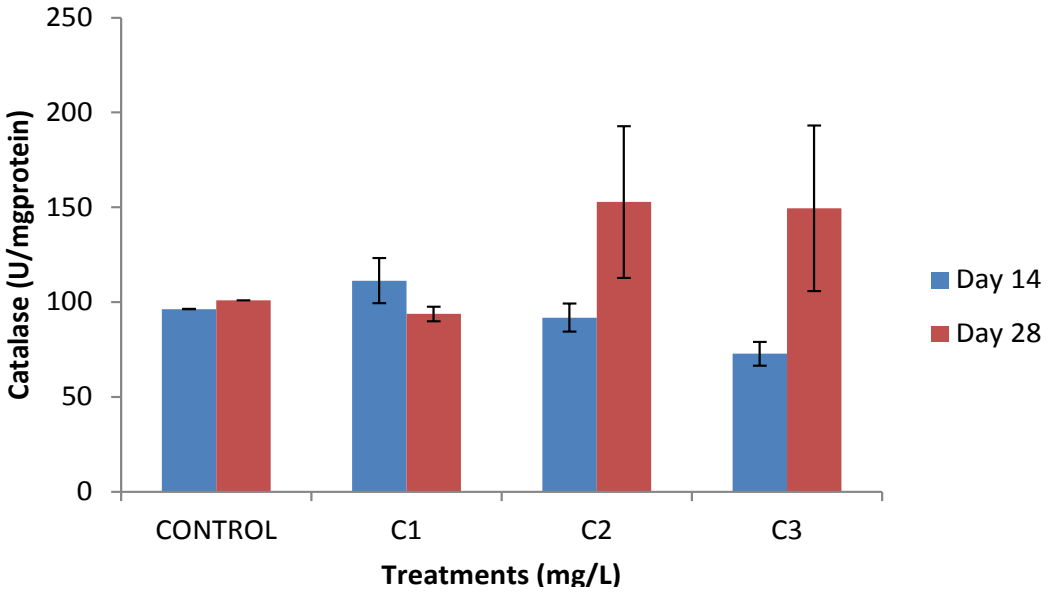

Figure 1. Catalase production levels in the kidney of *C. gariepinus* exposed to sub-lethal concentration of lead nitrate.

Table 3. Catalase production levels in Gill of *C. gariepinus* exposed to sub-lethal concentration of lead nitrate.

Concentration (mg/L)	Day 14	Day 28
Control	97.15±0.050[a]	96.81±0.10[d]
C1	101.70±10.00[a]	91.35±12.55[a]
C2	89.40±2.30[a]	114.80±2.80[b]
C3	112.95±15.35[a]	121.10±18.40[c]

Mean values with the same superscript in the same column are not significantly different P>0.05) from each other.

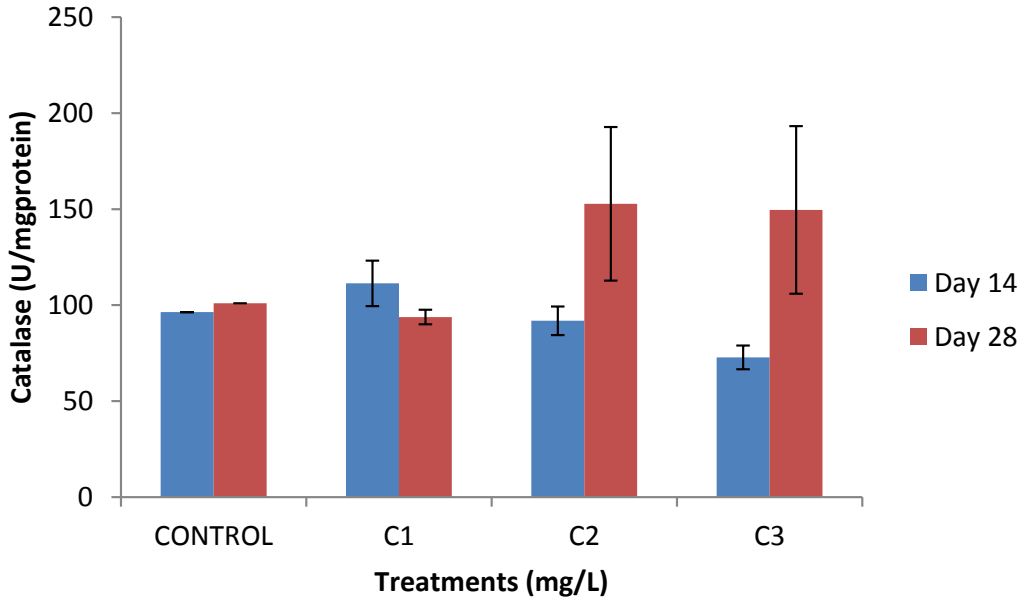

Figure 2. Catalase production level in the gill of *C. gariepinus* exposed to sub-lethal concentration of lead nitrate.

Table 4. Catalase production levels in liver of *C. gariepinus* exposed to sub-lethal concentration of lead nitrate.

Concentration (mg/L)	Day 14	Day 28
Control	115.09±0.10[a]	121.55±0.05[d]
C1	121.95±8.25[c]	112.30±4.50[c]
C2	109.00±8.30[a]	96.70±3.80[b]
C3	118.55±3.15[b]	69.65±1.85[a]

Mean values with the same superscript in the same column are not significantly different (P>0.05) from each other.

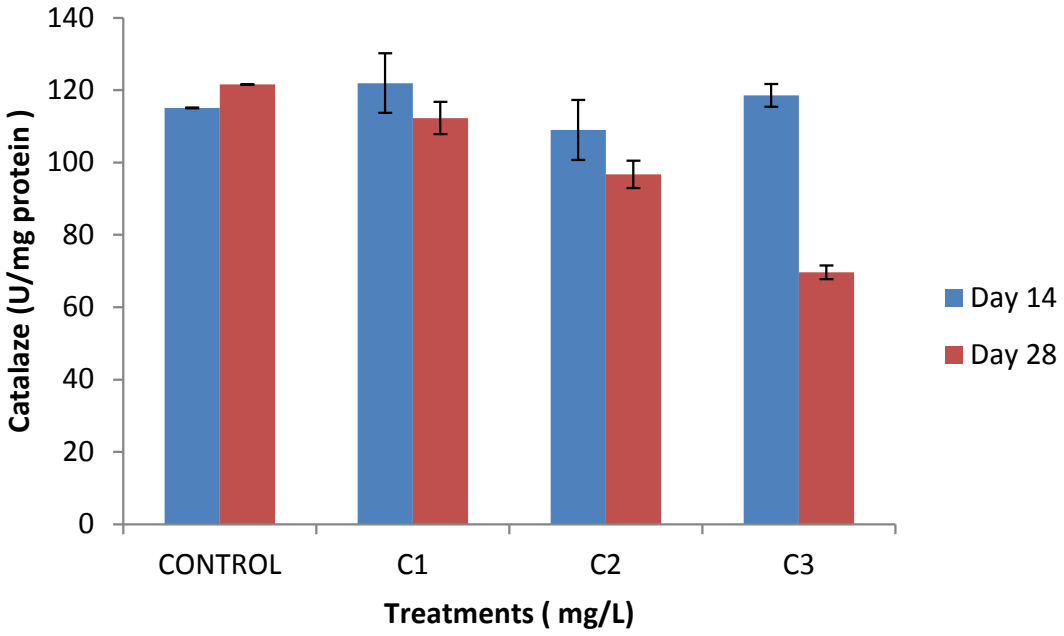

Figure 3. Catalase production level in the liver of *C. gariepinus* exposed to sub-lethal concentration of lead nitrate.

defense component which catalyzes the decomposition of H_2O_2 to water (Livingstone, 2001). The results from this research indicated that the catalase production levels in the kidney of *C. gariepinus* exposed to sub-lethal concentration of Lead Nitrate in treatments C2 and C3 were significantly higher than what was obtained in the control at day 28. Likewise, the values obtained in treatment C1 at day 14 were significantly higher than the control. This is probably because as the concentration of the metal increases in the body of the fish there is corresponding need for increase in catalase production to counteract the effects of oxidative stress created due to the presence of the toxicant. Qi et al. (2015) attributed the significantly greater CAT production level relative to control in the most polluted site in Tai Lake (China) to oxidative damage occurring at this site. Likewise, Sumit et al. (2014) attributed the high CAT level (386.6±10.64 µmol/mg protein) in the kidney of the fish to effective antioxidant system in this tissue where there is higher metal bioaccumulation and related to metal binding protein synthesis.

Catalase production levels in the liver of the fish exposed in treatment C1 and C3 were significantly higher than control at day 14. Liver is the site of multiple oxidative reactions and maximal free radical generation (Avci et al., 2005). This is probably why there was increase in the catalase production levels to act in opposition to the effects of oxidative stress due to the presence of the pollutant. This finding is in line with previous investigation made by Basha and Rani (2003) who reported that CAT activity increased in the liver of *Oreochromis niloticus* due to an effective antioxidant defense system acting against oxidative stress caused by metal exposures. They demonstrated that there were simultaneous increases in the levels of CAT activity in the liver following cadmium exposure of the fish. On day 28 however, the catalase production levels in the liver of the fish were significantly lower than that of control in C1 to C3. This could be as a result of long term exposure of the fish to the toxicant. The initial response of the fish that

necessitated the surge in CAT production levels may have died down probably because the fish has adapted to the situation. In line with this, Basha and Rani (2003) indicated that there was a possible shift toward a detoxification mechanism under long term exposure of *O. niloticus* to cadmium.

The CAT production levels in the gill of the fish exposed in treatment C2 and C3 were significantly higher than the control. Gill is the first organ in contact with the toxicant in terms of respiration. Closely associated with the gills are iron oxides involved in the carriage of respiratory gases. The presence of iron oxide in the gills is known to enhance Lead disposition and Lead is known to strongly adsorb onto iron oxide and can be bound to it externally (Hares et al., 1991). Catalase, a primary antioxidant defense component protects fish from oxidative stress by converting the hydrogen peroxide to oxygen and water (Atli and Canli, 2007). This is probably why there were increased CAT production levels especially in the highest concentration. The larger surface area of the gills in contact with the medium then could probably account for the higher production levels of the antioxidant.

CONCLUSIONS

The catalase production levels in the various organs (kidney, gill and liver) of *C. gariepinus* exposed to sub-lethal concentration of Lead Nitrate varied from concentration to concentration. The CAT production levels were significantly higher in the kidney of the fish exposed in sub-lethal concentration of 28 on day 14; 43 and 57 mg/L on day 28 respectively relative to control. The CAT production levels were significantly higher in the gill of the fish exposed in sub-lethal concentration of 57 mg/L on day 14; 43 and 57 mg/L on day 28 respectively relative to control. The CAT production levels were significantly higher in the liver of the fish exposed in sub-lethal concentrations of 28 and 57 mg/L on day 14 respectively relative to the control. The highest production mean values of 149.55±43.65 and 152.80±40.40 U/mg protein were obtained in the kidney of the fish exposed to sub-lethal concentrations of 57 and 43 mg/L, respectively. Therefore, the kidney of the fish exhibited a better control of the toxicant.

RECOMMENDATIONS

The kidney of *C. gariepinus* exhibited a better control of the oxidative stress and therefore, can be used as an indicator organ in the evaluation of catalase production levels as biomarkers of oxidative stress due to Lead Nitrate. More research that would indicate catalase production levels at the 7^{th}, 21^{st} and 42^{nd} days should be carried-out for better explanation of the enzyme activities at these stages in the exposure of the fish to the toxicant.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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