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High lead accumulation in *Clarias gariepinus* (Arila) inhibited catalase activity

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Abstract
Heavy metals are generally referred to as those metals which possess a specific density or more than 5 g/cm^3 and adversely affect the life cycle of living organisms. The study aimed to determine the lead accumulation in *Clarias gariepinus* and effects on the Catalase activity. The result revealed that the higher the concentration of lead introduced, the higher lead accumulated in the range of 0.020 ±0.0021 mg/Kg – 0.616 ± 0.0057 mg/Kg. The result showed the highest catalase activity of 6.000 µmol/min was found in 2.00 mg/kg concentration, followed by 3.00 mg/kg which had catalase activity of 4.900 µmol/min, 4.00 mg/kg which had catalase activity of 3.200 µmol/min while the least catalase activity was revealed on 1.00 mg/kg with a value of 0.494 µmol/min. Finally, the result revealed that highest catalase activity of 6.000 µmol/min was found in 0.593±0.0014 mg/Kg lead accumulation, followed by 0.538±0.0134 mg/Kg lead accumulation which produced a catalase activity of 4.900 µmol/min, 0.616 ±0.0057 mg/Kg lead accumulation had catalase activity of 3.200 µmol/min while the least catalase activity was revealed on 0.252±0.0417 mg/Kg lead accumulation concentration with a value of 0.980 µmol/min. sub-lethal concentration of lead has effect on catalase activity of *Clarias gariepinus* fingerlings does not cause mortality. Prolonged exposure however can lead to undesirable effects like shorter life span.

Keywords: heavy metals, *clarias gariepinus*, catalase, catalase activity

Introduction
Metals are substances with high electrical conductivity, malleability, and luster, which voluntarily lose their electrons to form cautions (Dimari, 2011) [12]. Metals are found naturally in the earth's crust and their compositions vary among different localities, resulting in spatial variations of surrounding concentrations (Malik, 2014). The metal distribution in the atmosphere is monitored by the properties of the given metal and by various environmental factors (Khlifi and Hamza-chaffai, 2010) [23]. Heavy metals are generally referred to as those metals which possess a specific density or more than 5g/cm^3 and adversely affects the life cycle of fish living organisms (Jarup, 2013) [19]. These metals are quintessential to maintain various biochemical and physiological function in living organisms when in very low concentrations however they becomes noxious when they exceed certain threshold concentrations (Khlifi *et al.*, 2010) [24]. Heavy metals have many adverse health effects and last for a long period of time heavy metal exposure continues and is increasing in many parts of the world (Ashy, 2011) [4]. They are also among the majour environmental pollutants and their toxicity is a problem of increasing importance for ecological evolutionary, nutritional and environmental reasons (Dimari, Abdulkarim, Akan and Garba, 2014) [13]. The most commonly found heavy metals in water include arsenic, cadmium/chromium, copper, lead, nickel and zinc, all of which cause risk for aquatic life and human health and the environment (Lambert *et al.*, 2013) [25]. Heavy metals enter the surroundings by natural means and through human activities. Various sources of heavy metals include soil erosion, natural weathering of the earth's crust, mining, industrial effluents, urban runoff, sewage discharge, insect or disease control agents applied to crops and many others (Morais *et al.*, 2012). Although these metals have crucial biological functions in plants and animals, sometimes their chemical coordination and oxidation-reduction properties have given them an additional benefit so that they can escape control mechanisms such as homeostasis, transport, compartmentalization and binding to required cell constituents (Fathi, Mohammed and Mazlan, 2011) [15]. These metals bind with protein sites which are not made for them by displacing original metals form their natural binding sites causing malfunctioning of cells and ultimately toxicity (Fathi *et al.*, 2011) [15]. Metals are non-biodegradable and are considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals (Rauf *et al.*, 2013) [38]. Heavy metals such a copper (Cu) and Zinc (Zn) are essential for fish metabolism while some others such as lead (Pb) and cadmium (Cd) have known no role in biological systems (Moraes *et al.*, 2012).

Fish can be used as an indicator of fish water contamination by heavy metals because they occupy different tropical level in an aquatic ecosystem. High exposure concentration of heavy metals is known to be toxic for aquatic organism but the low metal concentration also cause toxicity when they are introduced into the environment by a wide spectrum of natural and anthropogenic source (Macdonald and Christopher, 2011). Heavy metals are non- biodegradable and once they enter the aquatic environment their bio-accumulation may occur in fish tissue by means of metabolic activities and bioabsorption process (Ahmed and Bibi, 2010) [1]. It is vital to note that the growing rate of anthropogenic waste input into the natural environmental system leads to bioassumulation of heavy metals in biota and their levels of economically important fin fish and shed fishes have become a matter of great concern. Hence, it is important to establish the

levels of heavy metal tolerance by these organisms in other to maintain the food quality environment in this present study. The aim of the study was determine the Lead accumulation in *Clarias gariepinus* and assess the catalase activity.

Materials

Fish samples

Fish (*Clarias gariepinus*) were obtained from Applied Biology and Biotechnology Laboratory, Enugu State University of Science and Technology.

Water Samples

Deionized water samples were obtained from Industrial Chemistry Laboratory Enugu State University of Science and Technology.

Methods

Collection of Fish Samples

The fish (*Clarias gariepinus)* were collected from Applied Biology and Biotechnology fish farm Enugu State University of Science and Technology, Enugu State.

Principle of atomic absorption spectrophotometer (AAS)

In analytical chemistry, atomic absorption is a technique used to determine the concentration of specific metal element in a sample. The techniques can be used to analyze the concentration of over 70 different metals in solution. The technique makes use of absorption spectroscopy to assess the concentration of an analyte, it therefore relies heavy on Beer – Lambert's Law in the process the electrons of the atoms in the atomizer are promoted to a higher level/orbital for a short time by absorbing a set of quantity of energy (light of a given wavelength). This amount of energy (or wavelength) is specific for a particular electron transition in a particular element and in general each wavelength corresponds to one element (heavy metal); this gives the technique its elemental relatively. As the quantity of energy (power) put into the flame is known and the quantity meaning on the other side can be measured.

Determination of heavy metal concentration in the sample

The digest of the samples were analyzed for the presence of heavy metals using atomic absorption spectrophotometer spectra AA model number 240FS under the appropriate wavelength and detection limit for each heavy metal. Heavy metals analysis was conducted using according to the method of American Public Health Association (APHA, 2015). The process of sample analysis involves the following placing the diluted extracts on the bench. The atomic absorption spectrophotometer machines was switched on and set to the required wavelength which is determined by the heavy metal being assayed. The appropriate lamp which is determined by heavy metal was placed in the appropriate place in the machine. The machine was then set to take the absorbance which is displayed on the screen of the front of the machine as well as the concentration aspiration tube.

Assay of catalase activity

A known quantity, 0.5 g of the flesh samples were ground with 3ml of potassium phosphate buffer, centrifuged at 2000g for 10 minutes and the supernatant were used for the assay. A 20 % homogenate was prepared in phosphate buffer. The homogenate was centrifuge and the supernatant was used for the enzyme assay or catalase.

Statistical analysis

The observed catalase value as means $\pm$ SD were statistically analysed with one way ANOVA. Dunnett's test was employed for multiple comparisons against control. $P < 0.05$ was considered significant.

Results

Concentration of lead introduced and the accumulation after seven days exposure

The result shows the concentration of lead introduced and the concentration of lead accumulated in the muscle of the fish (*Clarias gariepinus*) Table 1 shows the highest concentration of lead accumulated value of 0.616 ± 0.0057 followed by 0.593 ± 0.0014 and the least concentration of lead accumulated value of 0.252 ± 0.0417.

Table 1: Content of lead introduced and accumulated after seven days

Conc. of Lead Introduced (mg/ Kg)	Conc. of Lead Accumulated (M $\pm$ SD)
1.00	0.252 ± 0.0417
2.00	0.593 ± 0.0014
3.00	0.538 ± 0.0134
4.00	0.616 ± 0.0057
Control	0.020 ± 0.0021

Concentration introduced and the corresponding catalase activity

The highest catalase activity 6.000 μ mol/min was observed in 2.00 mg/kg concentration of lead introduced followed by 4.900 μ mol/min catalase activity in 3.00 mg/kg and least concentration of lead introduced 1.00 mg/kg had the least catalase activity of 0.980 μmol/min.

Table 2: Concentration of lead introduced (mg/kg) and catalase activity in μ mol/min

Conc. of Lead Introduced (mg/kg)	Catalase Activity in μ mol/min
1.00	0.980
2.00	6.000
3.00	4.900
4.00	3.200

Concentration of lead accumulated by the fish and the catalase activity

The highest catalase activity 6.000 μ mol/min was observed in 2.00 mg/kg concentration of lead accumulated followed by 4.900 μ mol/min catalase activity in 3.00 mg/kg and least concentration of lead accumulated 1.00 mg/kg had the least catalase activity of 0.980 μ mol/min.

Table 3: Conc. of lead accumulated by the fish (*Clarias gariepinus)* and the catalase activity

Concentration of lead accumulated	Catalase Activity (μmol/min)
0.252 ± 0.0417	0.980
0.593 ± 0.0014	6.000
0.538 ± 0.0134	4.900
0.616 ± 0.0057	3.200

Discussion

Clarias gariepinus is an economic important species of fish to man, some people farm the fish for consumption, while some people farm the fish for commercial purposes. Lead is a heavy metal that is both poisonous and a ubiquitous environmental toxicant. Lead metal causes toxicity in living cells by following ionic mechanism and that of oxidative stress. The lead accumulated by the *Clarias gariepinus* and the effects on catalase was carried out after exposing the fish to sublethal dose of lead for a period of seven days. The statistical analysis observed catalase value as mean ±SD with one way ANOVA and Dunnet's test employed for multiple comparisons against control. $P < 0.05$ were also considered significant. In the analysis as shown in table 1 revealed the concentrations of lead (1.00 mg/kg – 4.00 mg/kg) introduced and concentration of the lead accumulated after 7 days. The result of table was shown that at concentration of 4.00 mg/kg introduced the concentration of lead accumulated was 0.616 ± 0.0057, which is the highest value, it is also shown in 1.00 mg/kg concentration, the concentration of lead accumulated was shown to be 0.252 ± 0.0417 which is the lowest value in the results the concentrations of 2.00 mg/kg and 3.00 mg/kg with respective concentrations of lead accumulated 0.593 ± 0.0014 and 0.538 ± 0.00134. The implication of the result in table 1 was showing higher to *Clarias gariepinus.* The higher the concentration of lead accumulated; catalase activity levels.

The catalase activity levels in the *Clarias spp* exposed to sublethal concentration of lead introduced varied with the concentration introduced and ranged from 0.980 µmol/min – 6.000 µmol/min. The highest catalase activity of 6.000 µmol/min was found in 2.000 mg/kg concentration, followed by 3.000 mg/kg which had catalase activity of 4.900 µmol/min, 4.00 mg/kg which had catalase activity of 3.200 µmol/min while the least catalase activity was revealed on 1.00 mg/kg with a value of 0.980 µmol/min. The catalase activity result suggested an increase in catalase activity in all the fishes assayed but in 1.00mg/kg of lead introduced and the catalase activity of 0.980 µmol/min had the lowest value, this could be due to the fact that lead is an essential heavy metal needs to be in higher quantity to be exert toxic effect.

The findings also revealed the concentration of lead accumulation and Catalase activity. The highest Catalase activity of 6.000 µmol/min was found in 0.593±0.0014 lead accumulation concentration, followed by 0.538±0.0014 lead concentration accumulation had catalase activity of 4.90µmol/min, 0.616±0.0057 lead concentration accumulation had catalase activity of 3.200 µmol/min while the least catalase activity was revealed on 0.252±0.0417 lead accumulation concentration with a value of 0.980 µmol/min. The findings is in correlation with Osman *et al.* (2007) who stated that the highest production mean values of 149.55±43.65 and 152.80±40.40 µ/mg protein were obtained in the flesh of the fish exposed to sub-lethal concentrations of 57 and 43 mg/L, respectively. However it can be suggested that lead could be introduced in higher quantity to induce toxicity.

Conclusions

The study conducted on lead accumulation in *Clarias gariepinus* and effect on catalase activity. Sublethal concentration of lead has effect on catalase activity of *Clarias gariepinus* fingerlings does not cause mortality. Prolonged exposure however and higher concentrations inhibits catalase activity and may lead to other undesirable effects like shorter life span. It induced oxidative stress in the muscle tissue of *Clarias gariepinus,* showing that enzymes can serve as biomarkers for early detection of pollution during bio-monitoring programs. Further investigation should be carried out on other part of fish like kidney and gills of *Clarias gariepinus*.

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