ASSESSMENT OF SOME TOXIC EFFECTS OF LEAD NITRATE ON *HETEROBRANCHUS LONGIFILIS* (VALENCIENNES, 1840) FINGERLINGS

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Abstract
Some toxic effects of lead nitrate $(PbNO_3)_2$ on *Heterobranchus longifilis* under laboratory conditions for periods of 42 days were investigated. 300 fingerlings were obtained and acclimatized for 14 days. The acute toxicity test was conducted for 96 hours to determine the LC_{50}. Superoxide Dismutase (SOD) and Lipid Peroxidase (LPO) production levels were determined in the gills, liver and kidneys of the fish exposed to the sub-lethal concentration of $(PbNO_3)_2$. Four treatments; 27, 41, 54 and 68 mg/L with replicate in each case were taken as 10, 15, 20, 25% of the LC_{50}, respectively. The samples were collected and homogenized in phosphate buffer on 14th, 28th, and 42nd day of exposure. From the results, the LC_{50} was 272.62 mg/L. The LPO level in gills and SOD activities in liver of *H. longifilis* exposed to highest concentration (68 mg/L) of $(PbNO_3)_2$ was significantly ($P<0.05$) lower when compared with the control on day 42. There were increased production levels of LPO in liver, gill and kidney on day 28 in all treatments. The gill had the highest production level of LPO (1.1622±0.42) at 54 mg/L on day 28. Liver SOD activities in fish exposed to the lowest concentrations (27 mg/L) of $Pb(NO_3)_2$ was significantly different ($p<0.05$) on day 14 when compared with control and other treatments. The kidney showed the highest activity of SOD at 41 mg/L on day 28. Fish mortality increased with increased concentration of $Pb(NO_3)_2$ during acute toxicity test. The toxic effects of $Pb(NO_3)_2$ on *H. longifilis* manifested in physical and behavioural changes in both acute and sub-lethal exposures of the fish to the toxicant; as well as varying production levels of the antioxidants (SOD and LPO) and can therefore, be used for early detection of the effects of pollutants in aquatic environment.

Keywords: Lead nitrate, Super oxide dismutase, Lipid peroxidase, LC_{50}, *Heterobranchus longifilis,* Reactive oxygen species, Oxidative stress.

Introduction

Pollution of the environment of aquatic organisms has over the years impacted negatively on their population and survival. Due to feeding and living in the aquatic environments fish are particularly vulnerable and heavily exposed to pollution because they cannot escape from the detrimental effects of pollutants (Mahboob *et al.*, 2013; Saleh, 2014). It is also known that fish, in comparison with invertebrates, are more sensitive to many toxicants and are a convenient test subject for indication of ecosystem health (Zaki *et al.*, 2014). Heavy metals can be taken up into fish either from ingestion of contaminated food via the alimentary tract or through the gills and skin (Kentouri *et al.*, 2015). Heavy metals in fish are usually transported through blood stream to the organs and tissues where they are accumulated (Adeyemo *et al.*, 2010; Fazio *et al.*, 2014).

Defensive mechanisms to counteract the impacts of reactive oxygen species (ROS) are found in many mammalian species including aquatic animals such as fish. Superoxide dismutase (SOD) represents a group of enzymes that use as cofactor copper and zinc, or manganese, iron, or nickel ions. ROS have biological effects on lipids that lead to the formation of lipid peroxides; which can decompose to yield alkanes, ketones and aldehydes (Zielinski and Portner, 2000).

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Oxidative damage is counteracted by antioxidant defence systems and repair mechanisms. The antioxidant defence

systems comprise of a number of enzymes which act as scavengers of the highly reactive intermediates produced in cells during hydrocarbon metabolism to maintain cell homeostasis (Otitoloju and Olagoke, 2011). Notable antioxidant enzymes include superoxide dismutase (SOD), which converts superoxides (O_{2-}) generated in peroxisomes and mitochondria to hydrogen peroxide; catalase (CAT) which removes the hydrogen peroxide by converting it to water and oxygen; glutathione S-transferase (GST); glutathione peroxidase and glutathione reductase_ all of which are involved in the removal of hydrogen peroxide from the system .

Lipid peroxidation involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds in unsaturated lipids and the eventual destruction of membrane lipids, with the production of a variety of breakdown products, including alcohols, ketones, alkanes, aldehydes and ethers (Dianzani & Barrera, 2008). Lipid peroxidation is known to be a causative factor that contributes to the patho-physiology of inflammation (Guéraud *et al.,* 2010). Lipid peroxidation can alter vital membrane protein structure and function, and if it proceeds unchecked could lead to cell death.

Heterobranchus longifilis is a large species of air breathing catfish found widely in rivers and other freshwater habitats of Sub-Saharan Africa, as well as the Nile (*Azeroual et al., 2010*). *H. longifilis* are active at night feeding on any available food, including invertebrates and insects when small, then fish and other small vertebrates when large. It scavenges off large carcasses and offal from riverside villages. It can live for 12 or more years (Froese *et al.*, 2010).

The toxic action of metals is particularly pronounced in the early stages of fish development (Vinodhini and Narayanan, 2008) and adversely affects various metabolic processes in developing fish resulting in developmental retardation, morphological and functional deformities, or death of the most sensitive individuals (Kentouri *et al.,* 2015). Lead (Pb) is a persistent heavy metal which has been characterized as hazardous substance (Kentouri *et al.,* 2015). Although Pb is a naturally occurring substance, its environmental concentrations are significantly increased by anthropogenic sources which include base metal mining, battery manufacturing, Pb-based paints and leaded gasoline (Mager, 2011; Monteiro *et al.,* 2011).

Heavy metals distort the integrity of the physiological and biochemical mechanisms in fish that are not only an important ecosystem component, but also used as a food source (Ibrahim *et al.,* 2013). Lead pollution has become a major environmental problem in many developing and industrializing countries due to both occupational and environmental exposure (Yucebilgic *et al.*, 2003). It has many undesirable effects, including neurological (Sharma *et al.*, 2011), behavioural (Adeniyi *et al.*, 2008), immunological (Rosenberg *et al.*, 2007), renal (Rastogi, 2008) and hepatic problems (Patra *et al.,* 2001). This is why this research sought to determine some toxic effects of lead nitrate on the fingerlings of *H. longifilis.*

Materials and Methods

Fish fingerlings collection and acclimatization

Three hundred fingerlings of *H. longifilis* with an average weight of 4-6g and length of 6-8cm were obtained from National Institute for Freshwater Fisheries Research (NIFFR), New-Bussa, Niger State and transported to the laboratory of the Department of Animal Biology, Federal University of Technology, Minna, Niger State. They were acclimatized for 14 days in two 100 litres aquaria, during which they were fed vital fish feed twice daily, morning

and evening 9.00 hours and 16.00 hours of the day, respectively.

Acute Toxicity Test

Experimental set-up included 20L glass aquaria containing 10 fishes each. The test consisted of five groups of ten fishes each exposed to one of the following nominal $Pb(NO_3)_2$ concentrations: 0 (control), 240, 260, 280 and 300 mg/L. In each tank, the dead specimens were removed as soon as possible. The 96 h LC_{50} value was determined using Probit analysis (Finney, 1971).

Chronic Toxicity Test

In order to assess long-term effects of lead nitrate, the fishes were exposed to five sub-lethal concentrations corresponding to 0% (control), 10%, 15%, 20% and 25% of the LC_{50}; these gave 0 mg/L, 27mg/L, 41mg/L, 54 mg/L, 68 mg/L, respectively. Each treatment had two replicates containing 15 fishes each in 20L glass aquaria. The water was changed and fresh lead nitrate added at every 72 hours according to OECD (2000) standards. Five fishes were sacrificed each from all treatments on every 14th day for the period of 42 days. The liver, gills and kidneys were excised, homogenized in sodium phosphate buffer solution and stored in sample test tubes for analysis.

Preparation of sodium phosphate buffer

The 0.2 mole of sodium phosphate buffer solution was prepared from the mixture of sodium dihydrogen orthophosphate with 0.1 mole and disodium hydrogen orthophosphate with 0.1 mole. The pH was adjusted to 8.0.

Determination of Lipid peroxidase

Lipid peroxidation as evidenced by the formation of Thiobarbituric acid reactive substances (TBARS) was measured by the modified method of Niehaus and Samuelson (1968). Two 0.15ml of tissue homogenate, 0.25M sucrose solution was treated with 2ml of (1:1:1 ratio) TBA-TCA-HCL reagent (0.37%TBA, 0.25N HCL and 15% TCA) and was placed in water bath for 1hr at 90°C. The mixture was cooled and centrifuged at 3000rpm for 5mins. The absorbance of the pink supernatant was measured against a reference blank using spectrophotometer at 535nm. The MDA activity was calculated as follows: Molar extinction of MDA = I.56 x 10^5 $cm^{-1}m^{-1}$ MDA concentration = Absorbance/ I.56 x 105 cm-1m-1 MDA activity (µmol/mg of protein) = MDA concentration/ mg of protein.

Determination of Superoxide Dismutase Activity

Superoxide dismutase (SOD) was determined by the method described by Winterbourn *et al.* (1999). The ability of superoxide dismutase (SOD) to inhibit the auto-oxidation of epinephrine at pH 10.2 formed the basis for a simple assay of dismutase.

An aliquot of 0.1ml of the diluted tissue supernatant was added to 1.25ml of 0.05M phosphate buffer (pH 7.8) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.15ml of freshly prepared 0.3M adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 1.25ml of the phosphate buffer, 0.15ml of substrate (adrenaline) and 0.1ml of distilled water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds as follow:

$$\text{Increase in absorbance per minute} = \frac{A3 - A0}{2.5}$$

Where A_o = absorbance after 30 seconds
A_3 = absorbance after 150 second

Data Analyses

For each parameter analyzed, differences among groups exposed to the same lead concentration for different periods, including the control group, were tested for significance by one way Analysis of Variance (ANOVA) at P≤ 0.05 level of significance. The various production levels of the antioxidants of interest were indicated graphically with error bars.

Results and Discussions

Physical and behavioural changes in *H. longifilis* exposed to lethal and sub-lethal concentrations of lead nitrate

The observed physical changes during lethal concentration exposure of *H. longifilis* include erratic swimming behaviour in first two hours; loss of balance with tendency to jump out of the trough, de-pigmentation after 6 hours; increased opercula movement, loss of appetite after 24 hours. There were no obvious changes in fish behaviour in the lower concentrations (240-260mg/L). The 96 hours acute toxicity of $Pb(NO_3)_2$ on *H. longifilis* indicated increased fish mortality with increased concentration of $Pb(NO_3)_2$. The highest mortality (100%) was recorded in the treatment with the highest concentration (300 mg/L), followed by 80% in 280 mg/L while 240 mg/L and 260 mg/L concentration recorded no mortality throughout the study periods. The 96hr-LC_{50} was 272.63 mg/L (Fig. 3.1). However, fish in the control treatment did not exhibit any abnormal behaviour. Similarly, regression of probit kill of *H.longifilis* exposed to $Pb(NO_3)_2$ indicates a strong relationship between the probit mortality and concentration of lead having an R_2 value of 0.8699 (Fig. 3.2).

The sub-lethal exposure of *H. longifilis* to $Pb(NO_3)_2$ at later stages (day 14-28) showed signs of weakness, slow swimming and loss of appetite. The physical changes ranged from the paling of the skin, excess production of mucous to swelling of the gill region.

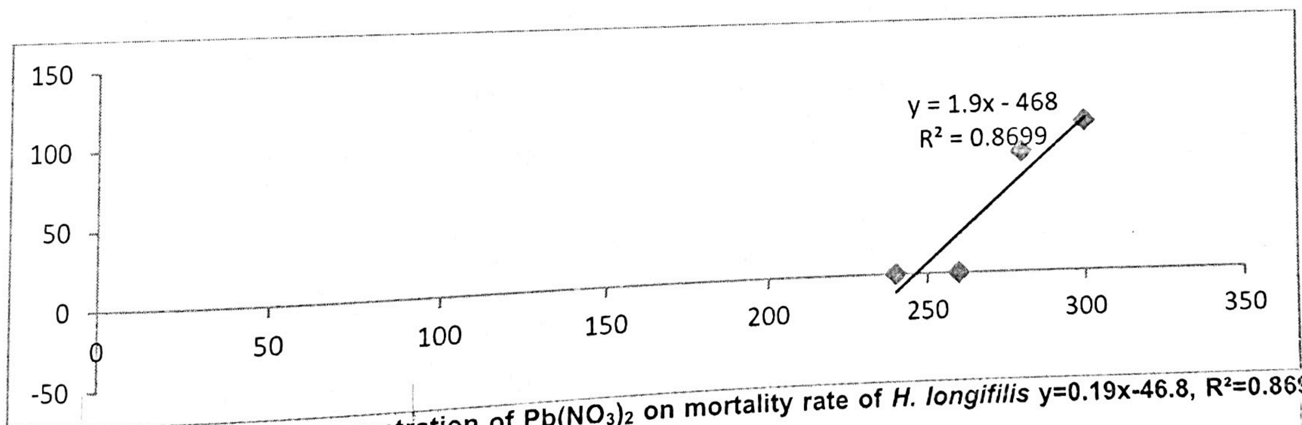

Fig. 3.1: Effects of lethal concentration of $Pb(NO_3)_2$ on mortality rate of *H. longifilis* y=0.19x-46.8, R^2=0.8699, LC_{50}=272.63, LC_{90}=293.68.

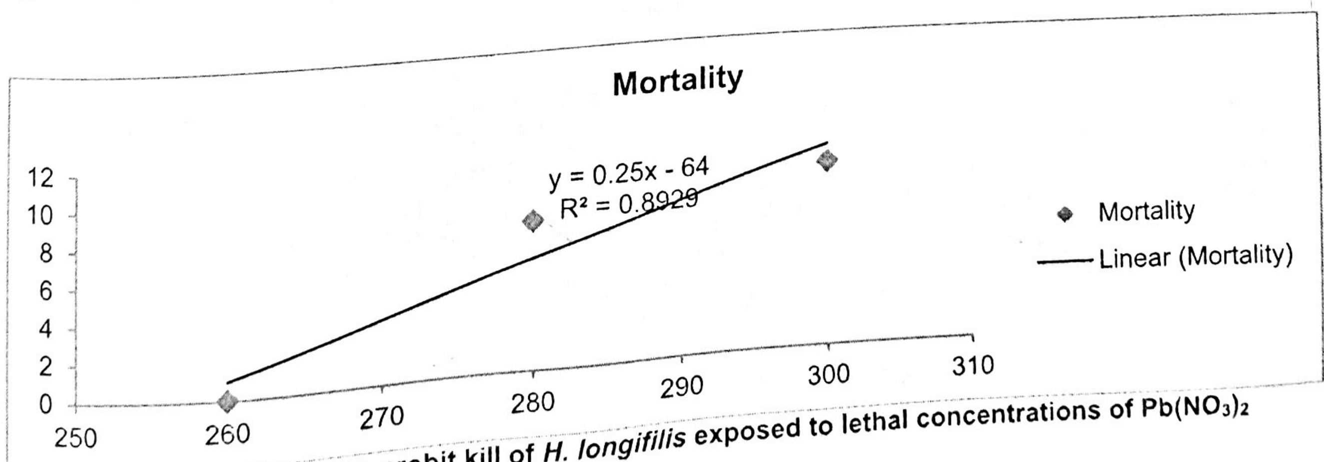

Fig. 3.2: Regression line of the probit kill of *H. longifilis* exposed to lethal concentrations of $Pb(NO_3)_2$

Lipid peroxidase production levels in organs of *H. longifilis* exposed to sub-lethal concentration of $Pb(NO_3)_2$

Liver Lipid Peroxidase

Increased $Pb(NO_3)_2$ concentration resulted in decreased LPO production levels in the liver of *H.longifilis* (Fig. 3.3). LPO levels in liver of test organisms exposed to the highest concentration (68 mg/L) of $Pb(NO3)_2$ were lower than the control on 28th and 42nd day (Table 3.2; Fig. 3.3).

Kidney Lipid Peroxidase

There was an increased LPO production levels in the kidney of the fish exposed to T1 with a value of 0.4836±0.3990µmol/mg protein on day 28 when compared with other treatments and control in all the periods of exposure in which there were no significant differences (Table. 3.3, Fig. 3.4).

Gills Lipid peroxidase

LPO production levels in the gill of the fish increased from day 14 to day 42 in T1. However, in T2-T4 there were initial increases in LPO production levels from day 14 to day 28 and then, decreased on day 42 in all cases (Table. 3.4, Fig.3.4).

Table 3.2: LPO production levels in the liver of *H. longifilis* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ (µmol/mg protein).

Treatments	DAY 14	DAY 28	DAY 42
T_1	0.0936±0.0156^a	0.2262±0.1014^a	0.2340±0.312^a
T_2	0.1170±0.023^a	0.4134±0.1950^a	0.3042±0.1794^a
T_3	0.0858±0.0546^a	0.3198±0.0234^a	0.0468±0.156^a
T_4	0.1560±0.0936^a	0.1248±0.0156^a	0.0468±0.1560^a
Control	0.17940±0.00^a	0.2808±0.1716^a	0.2106±0.1482^a

Control= 0 mg/L; T_1= (27 mg/L); T_2= (41 mg/L); T_3= (54 mg/L); T_4 = (68 mg/L).

Table 3.3: LPO production levels in the kidney of *H. longifilis* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ (µmol/mg protein).

Treatments	DAY 14	DAY 28	DAY 42
T_1	0.0390±0.0234^a	0.4836±0.3990^a	0.1950±0.054^a
T_2	0.1482±0.0390^a	0.1404±0.000^a	0.1872±0.171^a
T_3	0.0858±0.0078^a	0.2574±0.070^a	0.0936±0.078^a
T_4	0.0624±0.0156^a	0.0546±0.0234^a	0.0234±0.0078^a
Control	0.2028±0.109^a	0.1014±0.0078^a	0.1638±0.117^a

Control= 0 mg/L; T_1= (27 mg/L); T_2= (41 mg/L); T_3= (54 mg/L); T_4 = (68 mg/L)

Table 3.4: LPO production levels in the Gill of *H. longifilis* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ (µmol/mg protein).

Treatments	DAY 14	DAY 28	DAY 42
T_1	0.0702±0.54^a	0.2340±0.15^a	0.3666±0.148^b
T_2	0.1326±0.23^b	0.3120±0.24^a	0.2106±0.234^b
T_3	0.1248±0.31^b	1.1622±0.42^b	0.0546±0.007^a
T_4	0.0780±0.46^a	0.2886±0.195^a	0.0780±0.015^a
Control	0.3276±0.31^c	0.3198±0.148^a	0.2574±0.117^b

Mean values with the same superscript across the rows and within the columns are not significantly different ($P > 0.05$) from each other. Control= 0 mg/L; T_1= (27 mg/L); T_2= (41 mg/L); T_3= (54 mg/L); T_4 = (68 mg/L).

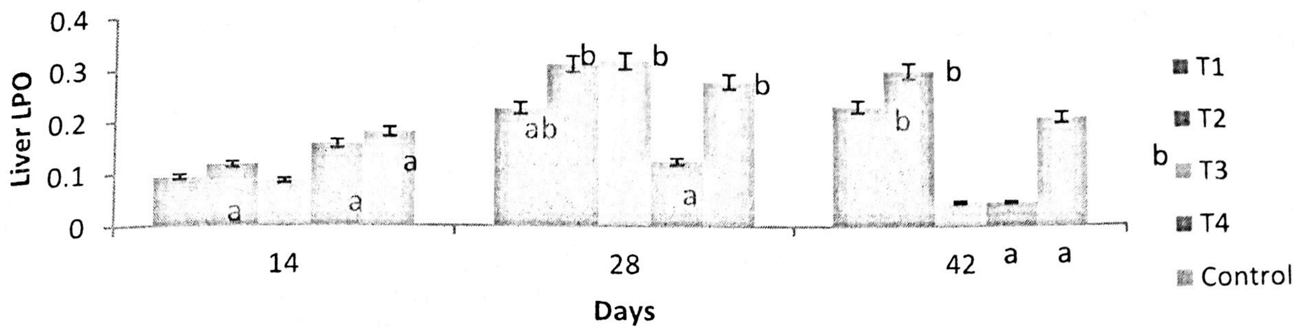

Fig. 3.3: LPO production levels in the Liver of *H. longifilis* exposed to sub-lethal concentrations of $Pb(NO_3)_2$.

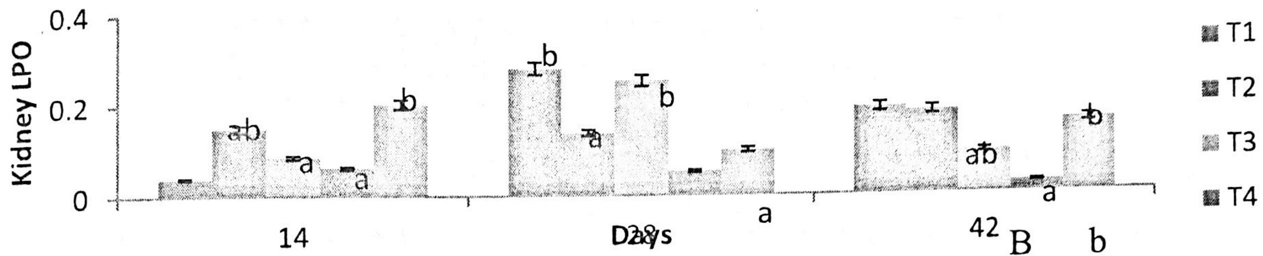

Fig. 3.4: LPO production levels in the Kidney of *H. longifilis* exposed to sub-lethal concentrations of $Pb(NO_3)_2$.

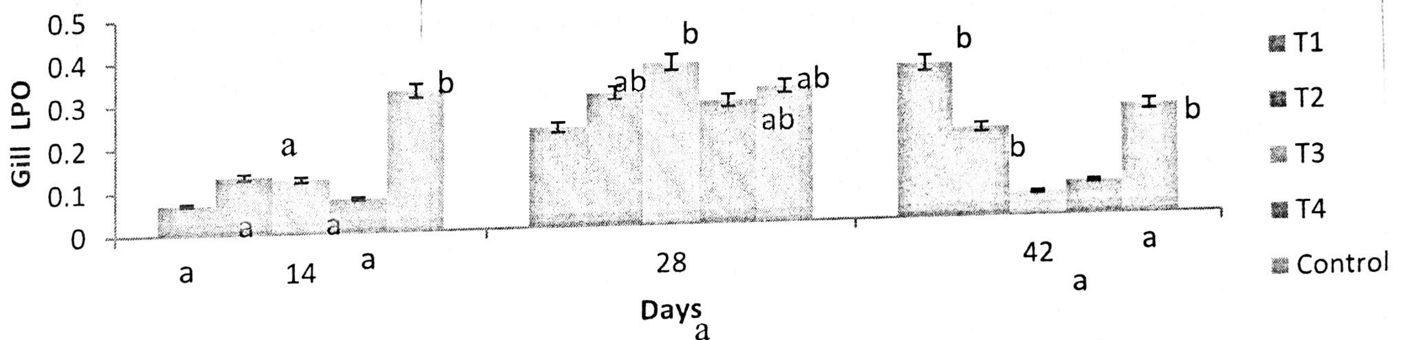

Fig. 3.5: LPO production levels in the gill of *H. longifilis* exposed to sub-lethal concentrations of $Pb(NO_3)_2$.

Liver SOD

SOD production levels decreased from day 14 to day 42 in T1 while it increased from day 14 to day 42 in T3. The production levels increased from day 14 to 28 then decreased on day 42 in T2, T4 and control, respectively (Table 3.5, Fig. 3.6).

Gills SOD

SOD activities in gills of *H. longifilis* decreased from day 14 to day 28 and then, increased slightly on day 42 in T1 and T2. However, SOD activities increased from day 14 to day 42 in T3 and T4 (Table 3.6, Fig. 3.7).

Kidney SOD activities

SOD activities in the kidney of the fish decreased from day 14 to day 28 and then increased slightly on day 42 in T1, T2 and T3, respectively. However, SOD activities increased from day 14 to day 42 in T4 and control (Table.3.7, Fig. 3.8).

Table 3.5: SOD production levels in the liver of *H. longifilis* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ (µg/mg protein)

Treatments	DAY 14	DAY 28	DAY 42
T_1	0.0170±0.0002[b]	0.0008±0.0000[a]	0.0080±0.0004[ab]
T_2	0.0052±0.000[a]	0.0108±0.0020[b]	0.0094±0.0002[ab]
T_3	0.0052±0.000[a]	0.0070±0.0034[ab]	0.0130±0.0018[b]
T_4	0.0096±0.0012[ab]	0.0044±0.0032[ab]	0.0042±0.0022[a]
Control	0.0076±0.0016[a]	0.0108±0.0016[b]	0.0096±0.0040[ab]

Mean values with the same superscript across the rows and within the columns are not significantly different ($P > 0.05$) from each other. Control= 0 mg/L; T_1= (27 mg/L); T_2= (41 mg/L); T_3= (54 mg/L); T_4 = (68 mg/L).

Table 3.6: SOD production levels in the gill of *H. longifilis* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ (µg/mg protein).

Treatments	DAY 14	DAY 28	DAY 42
T_1	0.0198±007[b]	0.0013±0.0001[a]	0.0068±0.0028[ab]
T_2	0.0098±0.002[ab]	0.0044±0.0028[a]	0.0050±0.0006[a]
T_3	0.0030±0.002[a]	0.0034±0.001[a]	0.0064±0.0012[ab]
T_4	0.0024±0.0016[a]	0.0042±0.0014[a]	0.0138±0.0038[b]
Control	0.0014±0.001[a]	0.0014±0.0006[a]	0.0070±0.0014[ab]

Mean values with the same superscript across the rows and within the columns are not significantly different ($P > 0.05$) from each other. Control= 0 mg/L; T_1= (27 mg/L); T_2= (41 mg/L); T_3= (54 mg/L); T_4 = (68 mg/L).

Table 3.7: SOD production levels in the Kidney of *H. longifilis* exposed to sub-lethal concentrations of $Pb(NO_3)_2$ (µg/mg protein)

Treatments	DAY 14	DAY 28	DAY 42
T_1	0.0126±0.0006[b]	0.0042±0.0018[a]	0.0090±0.0046[a]
T_2	0.011±0.0018[ab]	0.0304±0.025[b]	0.0054±0.0026[a]
T_3	0.0044±0.0004[a]	0.0032±0.020[a]	0.0040±0.0008[a]
T_4	0.0016±0.0008[a]	0.0044±0.004[a]	0.0070±0.0002[a]
Control	0.0112±0.0068[b]	0.0122±0.0094[b]	0.0140±0.0064[b]

Mean values with the same superscript across the rows and within the columns are not significantly different ($P > 0.05$) from each other. Control= 0 mg/L; T_1= (27 mg/L); T_2= (41 mg/L); T_3= (54 mg/L); T_4 = (68 mg/L).

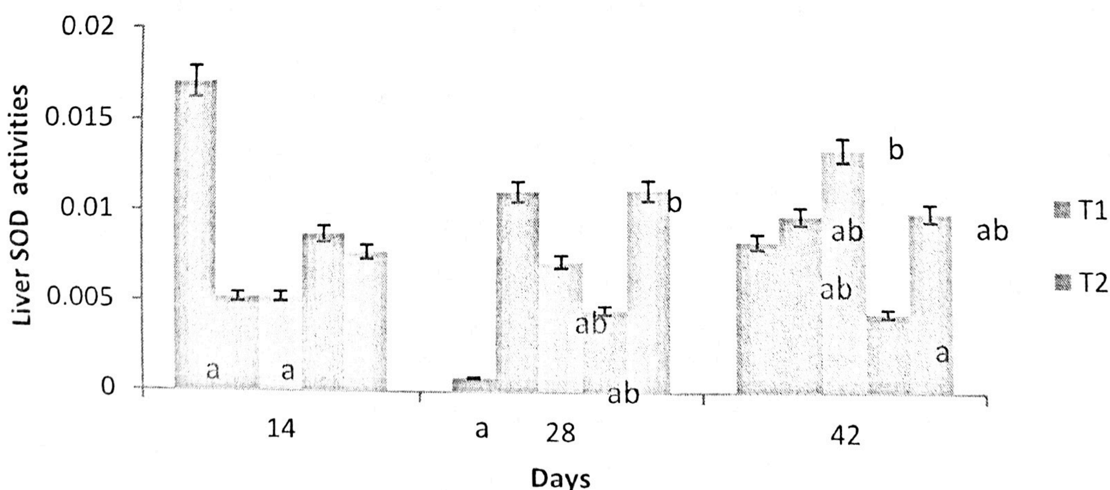

Fig. 3.6: SOD production levels in the liver of *H. longifilis* exposed to sub-lethal concentrations of $Pb(NO_3)_2$

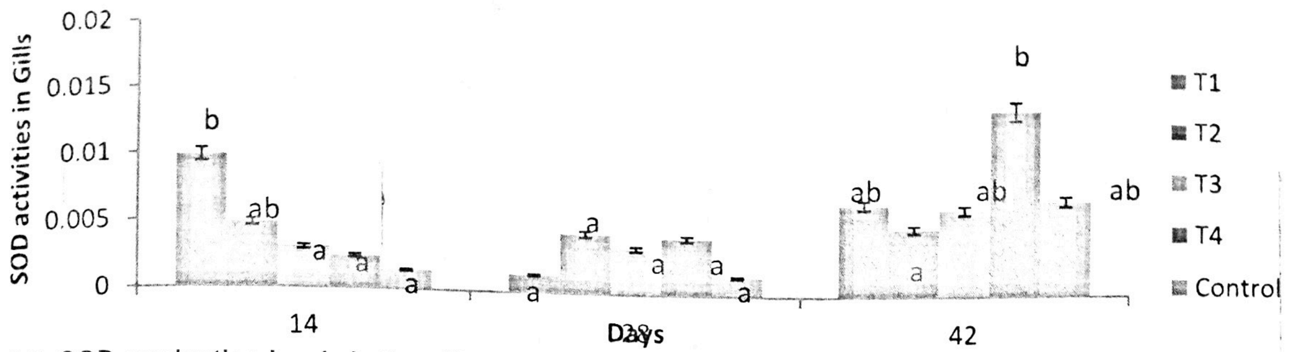

Fig. 3.7: **SOD production levels in the gill of *H. longifilis* exposed to sub-lethal concentrations of $Pb(NO_3)_2$.**

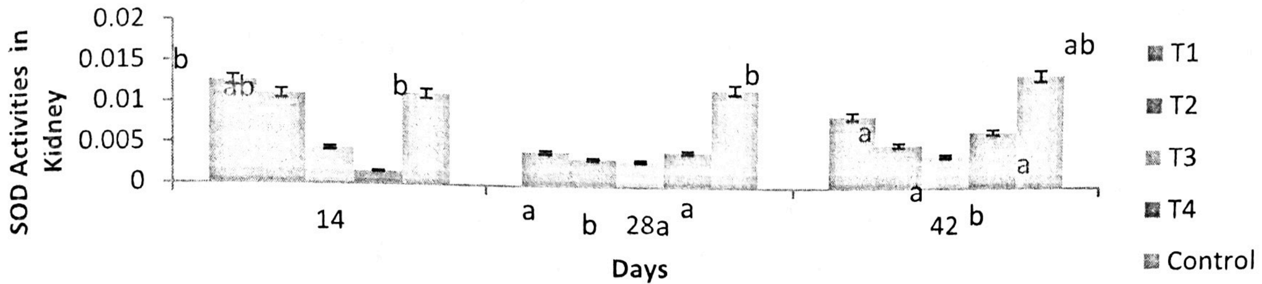

Fig. 3.8: SOD production levels in Kidney of *H. longifilis* exposed to sub-lethal concentrations of $Pb(NO_3)_2$.

Discussions

Acute toxicity provides data on the relative toxicity likely to arise from a single or brief exposure to any substance.aIn the present study, increased fish mortality was recorded with increase in concentration of $Pb(NO_3)_2$ from 280mg/L to 300 mg/L. This is probably because the immunity of the test samples has been subdued as the concentrations of the toxicants increased. This is in conformity with the findings of Samuel *et al.* (2018) on *Clarias gariepinus* but however, obtained a higher 96hours LC_{50} of 284.189mg/L. This observation is also similar to the findings of Zabbey *et al.* (2014) who reported increased mortality in *C. gariepinus* as the concentration of the lead nitrate increases.

The 96- hr LC_{50} in this study was 272.63mg/L. This is higher than findings of Esmail *et al.* (2012) who reported 246.45mg/L against crass carp (*Ctenopharyngodon idella*) exposed to $Pb(NO_3)_2$. Awoyemi *et al.* (2014) reported 55.12 mg/L against *Clarias gariepinus* exposed to $Pb(NO_3)_2$. While Martinez *et al.* (2004) reported values of 95 mg/L against *Prochilodus lineatus* exposed to $Pb(NO_3)_2$. The reason for high acute toxicity of $Pb(NO_3)_2$ in this study in comparison with previous literatures could be attributed to the fish species differences, as the present study used *H. longifilis* as opposed to *C. gariepinus* and *Prochilodus lineatus* used in the studies cited above.

The results of the regression analysis of the exposure concentration of the toxicant to the mortality of the fish indicate a strong relationship or affinity between the graded concentration of lead nitrate and the mortality of *H. longifilis* with a R_2 value of 0.8699. The R_2 value reported in this study is lower than R_2 value of 0.986 reported for *C. gariepinus* exposed to lead nitrate (Bawa-Aallah and Saliu, 2015). On the other hand, lower R_2 value of 0.7501 was reported for *D. innoxia* leaf extract against *Clarias gariepinus* (Ayuba *et al.*, 2012). These variations could be attributed to the difference in the toxicant and fish species at different instances. Also continuous exposure of the fish to lead may result to death of the fish due to damage or injury that comes from the stress that the fish is subjected to (Azua and Akaahan, 2017).

Findings from this research showed decrease in production level of LPO and SOD with increase in concentration of

$Pb(NO_3)_2$. It been established that SOD catalytically scavenges superoxide radical, toxicity of oxygen and provides defence against oxygen toxicity (Kadar *et al.*, 2005). The low level of SOD in the liver (0.0008±0.0000µg/mg protein) on day 28 in T_2 are in agreement with the findings of Awoyemi *et al.* (2014) who recorded a significant decrease in the activity of SOD in the liver of *C. gariepinus* exposed to sub-lethal concentrations of lead and Zinc after 15 and 30 days as compared to control. The kidney had the highest production of SOD (0.0304±0.025 µg/mg protein) in T_2 on day 28 which is greater than what was obtained in the control (0.0122±0.0094 µg/mg protein). This probably brings to bare the detoxification capacity of the kidney in counteracting the effects of the toxicant at this stage of the exposure. Similarly, the gill recorded the highest production of LPO (1.1622±0.42 µg/mg protein) in T_3 on day 28 when compared to control (0.03198±0.148 µg/mg protein). These were probably because the SOD produced in the kidney and LPO produced in the gill due to presence of toxicant may have been used-up by the fish in counteracting the effects of ROS produced in the solution culminating in the need for increased production of the antioxidants. Bagnyukova *et al.* (2007) reported increased activities of SOD, CAT and GPx as well as increased levels of lipid peroxides and GSSG in Goldfish exposed to arsenic. Likewise, dab exposed to hydrocarbon-contaminated sediments had increased hepatic SOD activity (Livingstone *et al.*, 1993). These were however, field researches without specific toxicant.

Results from this study showed general reduction in production levels of LPO after 42 days in gills, kidney and liver of organisms exposed to sub-lethal concentrations of $Pb(NO_3)_2$ when compared to the control. The reduced activity observed for organism exposed to $Pb(NO_3)_2$ is in agreement with the findings of Olagoke (2008) who studied Lipid peroxidation and antioxidant defence enzymes in *C. gariepinus* as useful biomarkers for monitoring exposure to polycyclic aromatic hydrocarbons. He reported decreased level of LPO and GST in the exposed fishes as compared to the control. Saliu and Bawa-Alla (2012) also reported reduction in LPO as compared to control in *Clarias gariepinus* exposed to sub-lethal concentrations of $Pb(NO_3)_2$.

Conclusions and Recommendations

Conclusions

The toxic effects of $Pb(NO_3)_2$ on *H. longifilis* manifested in physical and behavioural changes in both acute and sub-lethal exposures of the fish to the toxicant; as well as varying production levels of the antioxidants. Fish mortality increased with increase in concentration of $Pb(NO_3)_2$ from 280mg/L to 300mg/L. The LC_{50} obtained for *H. longifilis* was 272.62 mg/L. The gill recorded the highest production levels of LPO. The kidney was more responsive to SOD production than other organs. However, there was general decrease in production levels of LPO and SOD. This indicated their roles in counteracting the oxidative stress generated due to presence of the toxicant in fish environment.

Recommendations

The gills and kidneys of fish from this research was more responsive to LPO and SOD productions; and as such, could be used for early detection of pollution in aquatic environment.

There is paucity of literatures on *H. longifilis* hence; more research should be conducted using *H. longifilis* as a model.

REFERENCES

Adeniyi, T. T., Ajayi, G. O. & Akinloye, O. A. (2008). Effect of Ascorbic acid and *Alliumsativum* on tissue lead in female *Rattus navigicus, Nigerian Journal of Health Biomedical Science*, 7(2), 38-41.

Adeyemo, O. K., Adedeji, O.B. & Offor, C.C. (2010). Blood lead level as biomarker of environmental lead pollution in feral and cultured African catfish *(Clarias gariepinus). Nigerian Veterinary Journal,* 31, 139-147.

Ayuba, V.O, Ofojekwu, P. C & Musa, S. O. (2012). Acute toxicity of *Clarias gariepinus* exposed to *Datura innoxia* leaf extract, *Journal of Medicinal Plants Resources,* 6(12), 2453- 2457.

Awoyemi, O. M., Bawa-Allah, K. A. & Otitoloju, A. A. (2014). Accumulation and Anti-oxidant Enzymes as Biomarkers of Heavy Metal Exposure in *Clarias gariepinus* and *Oreochromis niloticus. Applied Ecology and Enviironmental Sciences,* 2 (5), 114-122.

Azua, E. T. & Akaahan, T. (2017). Toxic stress exhibited by juveniles of *Clarias gariepinus* exposed to different concentration of lead. *Journal of Research in Environmental Science and Toxicology,* 6(1), 008-011.

Azeroual, A., Bills, R., Getahun, A., Hanssens, M., Kazembe, J.; Marshall, B. & Moelants, T. (2010). "Heterobranchus longifilis". IUCN Red List of Threatened Species. *Version 2015.2.* International Union for Conservation of Nature.

Bagnyukova, T. V., Luzhna, L. I., Pogribny, I. P. & Lushchak, V. I. (2007). Oxidative stress and antioxidant defenses in goldfish liver in response to short-term exposure to arsenite. *Environmental and Molecular Mutagenesis*, 48, 658-665.

Barrera, G., Pizzimenti, S., & Dianzani, M. U. (2008). Lipid peroxidation: control of cell proliferation, cell differentiation and cell death. *Molecular Aspects of Medicine*, 29, 1-8.

Bawa-Allah, K. A. & Saliu, J. K. (2015). Acute toxicity and bioaccumulation patterns of lead and Zinc in juveniles of *Clarias gariepinus. IOSR Journal of Environmental Science, Toxicology and Food Technology,* 9(1), 42- 47.

Esmail, G., Hamed, N., Alireza, A., Mohammad, R. Imanpour & Vahid, T. (2012). Effect of lead nitrate on survival rate and chronic exposure on growth performance of grass carp (*Ctenopharyngodon idella*). *Journal of Toxicology and Environmental Health Sciences* 4(10), 171-174.

Fazio, F., Piccione, G., Tribulato, K., Ferrantelli, V., Giangrosso, G., Arfuso, F. & Faggio, C. (2014). Bioaccumulation of heavy metals in blood and tissue of striped mullet in two Italian Lakes. *Journal Aquatic Animal Health*, 26(4), 278-284.

Finney, D. J. (1971). *Probit Analysis*. (3rd ed.). UK: Cambridge University Press. Pp.333.

Froese, R. & Paul,D. (2010). Heterobranchus longifilis" in FishBase.*"Animal.discovery.com".*

Guéraud. F., Atalay, M., Bresgen, N., Cipak, A. & Eckl, P. M. (2010). Chemistry and biochemistry of lipid peroxidation products. *Free Radic Res*, 44, 1098-1124.

Ibrahim, S.A., Authman, M. M. N., Gaber, H. S. & El-Kasheif, M. A. (2013). Bioaccumulation of heavy metals and their histopathological impact on muscles of *Clarias gariepinus* from El-Rahawy drain, Egypt. *International Journal of Environment Sciences*, 4, 51-73.

Kadar, E., Costa, V. & Santos, R. S. (2005). Distribution of micro essential (Fe, Cu, Zn) and toxic(Hg) metals in tissues of two nutritionally distinct hydrothermal shrimps. *Science of Total Environment*, 65-70.

Kentouri, M., Sfakianakis, D. G., Renieri, E. & Tsatsakis, A. M. (2015) Effect of heavy metals on fish larvae deformities: A review. *Environmental Resources*, 137, 246-255.

Livingstone, D. R., Lemaire, P., Matthews, A., Peters, L., Bucke, D. and Law, R. J. (1993). Pro-oxidant, antioxidant and 7-ethoxyresorufin *O*-deethylase (EROD) activity responses in liver of Dab (*Limanda limanda*) exposed to sediment contaminated with hydrocarbons and other chemicals. *Marine Pollution Bulletin*, 26, 602-606.

Mager, E. M. (2011). Lead. Academic Press, New York, USA. Academic Press, New York, USA, Elsevier Inc.

Mahboob, S., Al-Balawi, H.F.A., Al-Misned, F., Al-Quraishy, S. & Ahmad, Z. (2014). Tissue metal distribution and risk assessment for important fish species from Saudi Arabia. *Bulletin of Environmental Contamination Toxicology,* 92, 61-66.

Martinez, C. B. R., Nagae, M. Y., Zaia, C. T. B. & Zaia, D. A. M. (2004). Acute Morphological And Physiological Effects Of Lead In The Neotropical FISH *Prochilodus lineatus. Brazilian Journal of Biology,* 64(4), 797-807.

Monteiro, V., Cavalcante, D. G., Viléla, M. B. F. A., Sofia, S. H. & Martinez, C. B. R. (2011). In vivo and in vitro exposures for the evaluation of the genotoxic effects of lead on the Neotropical freshwater fish *Prochilodus lineatus. Journal of Aquatic Toxicology*, 104, 291-298.

Olagoke, O. (2008). Lipid Peroxidation and Antioxidant Defense Enzymes in *Clarias gariepinus* as Useful Biomarkers for Monitoring Exposure to Polycyclic Aromatic Hydrocarbons. (M.Sc Thesis). University of Lagos, Lagos, Nigeria, 70pp.

Otitoloju, A. & Olagoke, O. (2011). Lipid peroxidation and antioxidant defense enzymes in *Clarias gariepinus* as useful biomarkers for monitoring exposure to polycyclic aromatic hydrocarbons. *Environment Monitoring Assessment*, 182, 205-213.

Patra, R. C., Swarup, D. & Dwidedi, S. K. (2001). Antioxidant effects of α-tocopherol, ascorbic acid and L-methionine on lead-induced oxidative stress of the liver, kidney and brain in rats. *Toxicol.*, 162(2), 81-88.

Rosenberg, C. E., Fink, N. E. & Salibian, A. (2007). Humoral immune alterations caused by lead. Studies on an adults lead model. *Acta. Toxicology Argentina,* 15(1), 16- 23.

Saliu, J. K. & Bawa-Allah, K. A. (2012). Toxicological Effects of Lead and Zinc on the Antioxidant Enzyme Activities of Post Juvenile *Clarias gariepinus.* Resources *and Environment*, 2(1): 21-26 DOI: 10.5923/j.re.20120201.03.

Samuel, P. O., Okoye, L. C., Abiola, P. O., Arimoro, F. O. and Auta, Y. I. (2018). Acute toxicity assessment of lead nitrate on *Clarias gariepinus* (Burchell, 1822). *International Journal of Fisheries and Aquatic Studies*; 6(2), 459-462.

Sharma. S., Pracheta, V. & Sharma, S. H. (2011). Therapeutic potential of hydromethanolic root extract of *Withania somnifera* on neurological parameters in Swiss albino mice subjected to lead nitrate. *International Journal of Current Pharmaceutical Research*, 3(2), 52-56.

Saleh, Y. S. & Marie, M. A. S. (2014). Assessment of metal contamination in water, sediment, and tissues of *Arius thalassinus* fish from the Red Sea coast of Yemen and the potential human risk assessment. *Environmental Science and Pollution*

Research, DOI 10.1007/s11356-014-3780-0.

Vinodhini, R. & Narayanan, M. (2008). Bioaccumulations of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). *International Journal of Environmental Science Technology,* 5:179-182.

Winterbourn, C. C. and Buss, I. H. (1999). Protein Carbonyl Measurement by Enzyme-Linked Immunosorbent Assay. *Methods in Enzymology*, 300, 106-111.

Yucebilgic, G., Bilgin, R. & Tamer, L. (2003). Effects of lead on Na+- K+ ATPase and Ca2+ ATPase activities and lipid peroxidation in blood of workers. *International Journal of Toxicology.* 22, 95-97.

Zaki, M. S., Authman, M. M. N., Hammam, A. M. M. & Shalaby, S. I. (2014). Aquatic environmental pollution in the Egyptian countryside and its effect on fish production (Review). *Life Sciences Journal,* 11, 1024-1029.

Zielinski, S. & Portner, H. O. (2000). Oxidative stress and antioxidative defense in cephalopods: a function of metabolic rate or age? *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology,* 125(2), 147-60.