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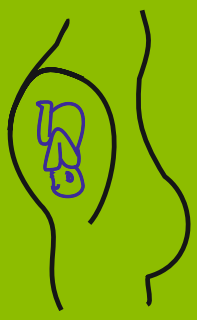

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Original Article

Evaluation of the Ameliorative Roles of Vitamins A, C, and E on Alanine Aminotransferase Production in *Clarias gariepinus* (Burchell, 1822) Fingerlings Exposed to Lead Nitrate

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ABSTRACT

Background: Pollutants from industrial and commercial usage of chemicals all over the world that usually lead to release of myriads of toxic pollutants such as lead call for concern. **Aim and Objective:** The effects of lead nitrate on the production of antioxidants such as Alanine aminotransferase (ALT) in *Clarias gariepinus* and how such effects can be ameliorated through administration of vitamins were investigated. **Materials and Methods:** *C. gariepinus* fingerlings (whose initial weight ranged from 3 to 11 g) were exposed to sublethal concentrations of Pb (00, 26 mg/L, 44 mg/L, 61 mg/L, and 79 mg/L) with replicate in each case. 26 mg/L of the vitamins was administered across all bud. Fresh concentrations of both toxicant and vitamins were administered every 72 h for a period of 12 weeks every time the water medium was changed. The various treatments group include Pb (Pb only), PbVA (Pb + vitamin A), PbVC ((Pb + vitamin C), and PbVE (Pb + vitamin E) with T1-T4 and replicates in each case. Three samples of the fish were randomly selected and sacrificed from each aquarium tank every 2 weeks of the exposure period. The gills, kidneys, and liver were excised from these specimens and homogenized in sodium phosphate buffer. These were then assayed for ALT production levels in each case. The data generated were subjected to one-way analysis of variance and considered significant at $P \leq 0.05$. **Results:** In samples exposed to Pb only group, the ALT production levels indicated that the highest ALT produced in the liver, kidney, and gills was 87.20 ± 0.15 nM/mg, 65.76 ± 0.20 nM/mg, and 69.92 ± 0.05 nM/mg, respectively. Samples exposed to PbVA indicated that the highest ALT produced in the liver, kidney, and gills was 77.12 ± 0.20 nM/mg, 84.75 ± 0.10 nM/mg, and 70.43 ± 0.24 nM/mg, respectively. **Conclusions and Recommendation:** In samples exposed to PbVC, the highest ALT produced in the liver, kidney, and gills was 86.53 ± 0.05 nM/mg, 63.48 ± 0.15 nM/mg, and 66.53 ± 0.15 nM/mg, respectively. In samples exposed to PbVE, the highest ALT produced in the liver, kidney, and gills was 73.82 ± 0.15 nM/mg, 78.05 ± 0.15 nM/mg, and 73.31 ± 0.05 nM/mg, respectively. The samples

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of the fish exposed to sublethal concentrations of the toxicant in the various treatments displayed varying levels of production of the enzyme with higher production levels mostly at higher concentrations of the toxicant. In the Pb only and PbVC groups, the liver of the samples produced the highest ALT, while the kidneys did same in the PbVA and PbVE groups. The high levels of production of the enzyme, especially in higher concentrations suggest physiological imbalances due to the presence of the toxicant.

Keywords: *Alanine aminotransferase, ameliorative roles, Clarias gariepinus, fish organs and Pb treatment groups, vitamin supplements*

Introduction

Fish is a rich source of animal protein throughout the world. Fish and fisheries resources all over the world find its usage in cultural and economic benefits either individually or at community level. African catfish, *Clarias gariepinus* is an important commercial fish due to its high growth rate, high consumer acceptability, and the ability to withstand poor water quality and oxygen depletion (Adewolu *et al*., 2008; Karami *et al*., 2010).[1,2] The African cat fish, *C. gariepinus,* is a tropical hardy species belonging to the Phylum Chordata, class Actinopterygii and family Clariidae. *Clarias* species is a widely distributed fish in Asia and Africa. In these areas, the fish is extremely popular on account of its tasty flesh, its unparalleled hardness, its rapid growth, and its somewhat acceptable market price (FAO, 2003).[3] In Nigeria, *Clarias* species is an indigenous fish occurring in freshwater throughout the country. It is suspected that, apart from tilapia, *Clarias* is the most abundant cultivated fish species in Nigeria (FAO, 2003).[3] The common species found are *C. gariepinus*, *Clarias anguillaris*, *Clarias buthupogon,* and *Clarias lazera*.

The presence of pollutants in the environment of an aquatic organism such as fish can lead to the production of reactive oxygen species (ROS) and consequently, oxidative stress. Heavy metals are known to elicit oxidative stress in organisms when the threshold is exceeded. Heavy metals are also known to promote oxidative damage by increasing the cellular concentration of ROS in fish, consequently, a response of antioxidative defences (Monteiro *et al*., 2010).[4] Heavy metals could be essential or nonessential. Heavy metals such as Fe, Cu, Zn, Ni, Co, Cr, and Mn are vital to human only at lower concentrations, but they become more toxic when they are taken up more than the bio-recommended limits (Shilpi *et al*., 2015).[5] It is also known that even essential metals may be toxic on the biological activities of organisms above certain concentrations (Merciai *et al*., 2014).[6] Fish are particularly vulnerable and heavily exposed to pollutants due to feeding and living in aquatic ecosystems, because they cannot avoid pollutant harmful effects (Ahmed *et al*., 2020).[7] Heavy metals induce significant damage to the physiologic and biochemical processes of the fish and subsequently to fish consumers (Mehana *et al*., 2020).[8]

Among all the heavy metals, Cd, arsenic, mercury, and lead pose highest degree of toxicity and that is of great concern to plants and human health (Athar *et al*., 2018).[9] Antioxidant enzymes are crucial in their effort to decrease oxidative stress produced by exposure to toxicants (Saglam *et al*., 2014).[10] It has also been reported that antioxidant may ameliorate, protect, and remove the oxidative damage to a target organ or molecule (El-Shenawy and Al-Ghamdi, 2014).[11]

Vitamins A, C, and E are known to play ameliorative roles in the attenuation of the effects of pollutants on organisms. Fishes survive oxidative stress by mobilizing enzymatic as well as nonenzymatic antioxidant defences (Ahmad *et al*., 2008; Van Der Oost *et al*., 2003).[12,13] Furthermore, Vitamins C and E supplementations have been reported to play a positive role in detoxification of mercury toxicity, especially at lower concentrations (Thakur and Kanshere, 2014).[14] It can also reduce Pb and Cu levels in serum and tissues of liver and kidney as well as reduce alanine amino transferase (ALT), Aspartate aminotransferase (AST), urea and creatinine levels in Pb and Cu-intoxicated male rats (Osfor *et al*., 2010).[15] Vitamin C is known to play a crucial role in the immunological and antioxidant properties of vertebrates capable of maintaining the integrity, fluidity of membranes, and capable of controlling the oxidizing reactions of fatty acids, thus keeping cellular respiration and avoiding cell death (Abdel-Warith *et al*., 2011).[16] Nonenzymatic antioxidants such as vitamins C and E can also act to overcome oxidative stress, being a part of the total antioxidant system. They prevent the increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues. The main biological function of vitamin E is its direct influence on cellular responses to oxidative stress through modulation of signal transduction pathway (Pratt *et al*., 2010).[17] Vitamins E and C supplementation can induce protective effects on certain conditions after free radical-mediated cellular

damage or disruption (Yolanda and Maria, 2012).[18] Vitamin E (α-tocopherol) is a fat-soluble antioxidant that inhibits the production of ROS formed when fat undergoes oxidation.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) belong to the plasma nonfunctional enzymes which are normally localized within the cells of liver, heart, gills, kidneys, muscle, and other organs. These enzymes are liberated into the blood in pathological situations and therefore are of clinical importance. AST and ALT are highly conservative indicators in liver and are commonly located in hepatic cytoplasm and would release into the circulation when hepatocytes necrotize (Arenas *et al*., 2017).[19] The presence of pollutant can trigger the utilization or increased production of AST and ALT. Activities of the hepatic enzymes lactate dehydrogenase, alanine aminotransferase (ALAT), and aspartate aminotransferase (ASAT) were found to be significantly elevated, particularly in summer (Yancheva *et al*., 2014).[20] The ameliorative role of vitamins was evident when Vitamin E and metallothionein treatments protected against Cd-induced damage of liver in grass carp by decreasing AST and ALT content, repairing organelles, and maintained the antioxidant system by elevating catalase, superoxide dismutase, and GSH-Px activity and regulating related mRNA transcript expression (Feng *et al*., 2018).[21] Furthermore, increased activities of AST, ALT, and ALP in Indian major carps exposed to nitrite toxicity have been recorded (Das *et al*., 2004).[22] This research, therefore, addresses the effects of Pb toxicant on AST and ALT production levels and how such effects can be attenuated to certain extent by administration of vitamin supplements.

Materials and Methods

Samples/materials collection and acclimatization

A total number of seven hundred and fifty fingerlings of *C. gariepinus* were purchased from a commercial fish farmer and transported in 50 L containers filled with water to the Old Farm Research Unit of the Department of Water, Aquaculture and Fisheries Technology, Bosso Campus, Federal University of Technology, Minna, Nigeria. The fishes were placed in fish ponds with water for acclimatization. The fishes were fed to satiation twice daily (morning and evening) with Blue Crown feed (3 mm) for 14 days (2 weeks) for the acclimatization. The holding water was changed every 3 days during the period.

The Vitamins A, C, and E granules or pellets (500 g in each case) were purchased from commercial chemical stores. The toxicant, Pb (2 units of 500 g) analar grades were purchased from commercial chemical stores and stored in a cool dry condition throughout the period of the experiment. These toxicants were administered according to the sublethal concentrations of the treatments during the chronic phase of the exposure.

Experimental set-up

Five treatments including control with two replicates in each treatment were set-up for the Pb, Vitamin A, C and E; and the sub-lethal exposures were run for a period of 12 weeks. Minimum concentration of the toxicant treatments serves the same basis for the concentration of the vitamins in each treatment group and applied across all the buds. In order to assess long term effects of lead nitrate $Pb(NO_3)_2$, the fishes were exposed to five sublethal treatments of lead nitrate concentrations corresponding to 0% (control), 15%, 25%, 35%, and 45% of the previously determined 96 h LC_{50} (174.72 mg/L) which translated into 26 mg/L as T1, 44 mg/L as T2, 61 mg/L as T3, and 79 mg/L as T4, respectively. Each treatment was in two replicates containing 20 fish in 20 L plastic aquarium for the Pb, Vitamins A, C, and E supplemented exposures. The water was changed and fresh toxicant and the vitamins with the same set of concentrations were added at every 72 h according to Organization for Economic Co-operation and Development (OECD, 2007) standards.[23] Three fish samples were picked at random and sacrificed from each trough on every 14th day for the 12-week exposure period. The liver, gills, and kidney were excised, homogenized in sodium phosphate buffer solution using ceramic mortar and pestle; and stored in sample tubes, then refrigerated until needed for analyses of ALT.

Preparation of sodium phosphate buffer

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

Alanine aminotransferase

Fish tissues' ALT was determined as described by Reitman and Frankel (1957)[24] from all the treatments and replicates. Spectro-photometric method was used for the assay of alanine aminotransferase. The homogenates were prepared in the laboratories as follows: 100 µl (0.1 ml) of the tissue homogenate was added into test tubes with 500 µl (0.5 ml) of reagent 1 (buffer). The mixture was incubated for 30 min at 37°C. Subsequently, 500 µl (0.5 ml) of reagent 2 (2, 4-dinitrophenylhydrazine) was added and kept for 20 min at 25°C. The reaction was terminated with the addition of 5000 µl (5.0 ml) of 0.4 Mol/L NaOH to the mixture. The blank was prepared with 500 µl (0.5 ml)

of reagent1 and 0.1 µl (100 µl) of distilled water. The absorbance was read at 546 nm.

Data analyses

The antioxidants levels in samples exposed to sub-lethal concentrations of the toxicants as well as those treatments supplemented with vitamins were analyzed using one-way analysis of variance, followed by duncan multiple range test to separate the means where significant at $P \leq 0.05$ level of significance using SPSS Statistical Package (version 20.0 for Windows).

Results and Discussions

ALT production levels in Liver, Kidneys, and gills of *Clarias gariepinus* exposed to sub-lethal concentrations of $PbNO_3)_2$ toxicant and the respective supplemented treatments with Vitamins A, C, and E for a period of 12 weeks and sampled fortnightly

From the results of the samples exposed to sublethal concentrations of $Pb(NO_3)_2$, the ALT production levels indicated that T1 and T4 mean values in the 2nd and 4th weeks of exposure, respectively, are significantly higher than other treatments, including the control. Furthermore, the T4 mean values of both 6th and 8th weeks of exposure are significantly higher than other treatments including the control. Similarly, the T3 mean values in the 10th and 12th weeks of exposure, respectively, are significantly higher than other treatments including the control. The highest mean value of ALT produced in the liver of the samples was 87.20 ± 0.15 nM/mg obtained in T4 at the 4th week of exposure [Table 1]. On the other hand, the T1 mean values in the kidneys of the fish are significantly higher than other treatments including the control in both 2nd and 4th weeks of exposure. The control mean values in the 6th, 8th, and 10th weeks of exposure are significantly higher than other treatments. The T3 mean values, however, in the 6th week of exposure are significantly higher than other treatments. The T4 mean values in the 12th week of exposure are significantly higher than other treatments. This T4 mean value (65.76 ± 0.20 nM/mg) in the 12th week of exposure was also the highest ALT produced in the kidney [Table 2]. Furthermore, T1, T3, and T4 mean values produced in the gill in the 2nd, 4th and 6th weeks of exposure, respectively, are significantly higher than other treatments including the control. The T1 and T3 mean values in the 8th and 12th weeks of exposure, respectively are significantly higher than other treatments including the control. The control mean values in the 10th week of exposure are significantly higher than other treatments. The highest ALT mean value produced in the gill was 69.92 ± 0.05 nM/mg obtained in T3 at the end of the 12th week of exposure [Table 3].

From the results of the samples exposed to sublethal concentrations of Pb $(NO_3)_2$, and supplemented with vitamin A, the ALT production levels in the liver indicated that T1 mean values in both 2nd and 4th weeks of exposure, respectively, are significantly higher than other treatments including the control. Furthermore, the T3, T4, and T3 mean values of 8th, 10th and 12th weeks of exposure are significantly higher than other treatments including the control. The highest mean value of ALT produced in the liver of the samples was 77.12 ± 0.20 nM/mg obtained in T1 at the 4th week of exposure [Table 4]. On the other hand, the T1 mean values in the kidneys of the fish are significantly higher than other treatments in 2nd week of exposure. The T2 and T3 mean values in the 4th and 8th weeks of exposure are significantly higher than other treatments. The T1 and T3 mean values in the 10th and 12th weeks of exposure are significantly higher than other treatments. The highest mean value of ALT produced in the kidney was 84.75 ± 0.10 nM/mg in T3 at the end of the 12th week of exposure [Table 5]. Furthermore, T4 and T1 mean values produced in the gill in the 2nd and 4th weeks of exposure, respectively, are significantly higher than other treatments. The T1, T4, and T3 mean values in the 8th, 10th, and 12th weeks of exposure, respectively, are significantly higher than other treatments. The highest ALT mean value produced in the gill was 70.43 ± 0.24 nM/mg obtained in T4 at the end of the 10th week of exposure [Table 6].

Table 1: Alanine amino transferase production levels in the Liver of *Clarias gariepinus* exposed to sub-lethal concentrations of Pb $(NO_3)_2$, for a period of 12 weeks

	1st	2nd	3rd	4th	5th	6th
CR	48.48±0.20^{d}	8.73±0.15^{c}	66.69±0.05^{d}	65.17±0.05^{d}	48.90±0.15^{a}	23.12±0.15^{a}
T1	55.43±0.20^{e}	8.90±0.05^{d}	57.71±0.15^{c}	49.32±0.10^{c}	67.46±0.20^{d}	43.48±0.15^{c}
T2	27.12±0.10^{b}	4.49±0.15^{b}	26.02±0.15^{a}	2.54±0.10^{a}	63.90±0.20^{c}	26.19±0.15^{b}
T3	34.66±0.05^{c}	1.01±0.10^{a}	26.19±0.05^{b}	5.76±0.10^{b}	70.26±0.05^{e}	71.27±0.05^{e}
T4	26.78±0.10^{a}	87.20±0.15^{e}	68.48±0.10^{e}	72.46±0.15^{e}	62.12±0.05^{b}	49.66±0.10^{d}

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$. The ALT mean value unit in each case is nM/mg. ALT: Alanine amino transferase, CR: Control

Table 2: Alanine amino transferase production levels in the Kidney of *Clarias gariepinus* exposed to sub-lethal concentrations of Pb (NO_3)$_2$, for a period of 12 weeks

	1st	2nd	3rd	4th	5th	6th
CR	48.48±0.20^d	21.87±0.10^d	68.39±0.15^d	12.54±0.10^e	54.32±0.05^e	16.10±0.10^a
T1	55.43±0.20^e	32.29±0.15^e	51.44±0.15^b	5.00±0.15^b	53.65±0.15^d	32.71±0.10^b
T2	27.12±0.10^b	8.65±0.20^b	0.00±0.00	6.01±0.24^c	49.32±0.20^b	57.20±0.15^d
T3	34.66±0.05^c	5.34±0.15^a	59.75±1.42^c	3.82±0.15^a	20.76±0.15^a	46.95±0.10^c
T4	26.78±0.10^a	12.29±0.15^c	46.44±0.10^a	7.71±0.15^d	52.37±0.10^c	65.76±0.20^e

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$. The ALT mean value unit in each case is nM/mg. ALT: Alanine amino transferase, CR: Control

Table 3: Alanine amino transferase production levels in the Gill of *Clarias gariepinus* exposed to sub-lethal concentrations of Pb (NO_3)$_2$, for a period of 12 weeks

	1st	2nd	3rd	4th	5th	6th
CR	26.61±0.20^c	10.68±0.20^b	7.80±0.10^a	19.32±0.10^c	77.37±0.05^e	24.49±0.05^a
T_1	63.06±0.10^e	2.37±0.10^a	37.88±0.15^b	24.66±0.05^e	53.22±0.10^b	57.71±0.15^d
T_2	11.01±0.10^a	48.56±0.05^d	60.68±0.10^d	22.04±0.10^d	54.24±0.10^c	45.00±0.05^b
T_3	26.70±0.05^b	61.36±0.10^e	60.09±0.05^c	4.07±0.20^a	30.17±0.10^a	69.92±0.05^e
T_4	57.03±0.15^d	17.54±0.05^c	66.87±0.15^e	9.41±0.05^b	55.17±0.15^d	45.76±0.10^c

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$. The ALT mean value unit in each case is nM/mg. ALT: Alanine amino transferase, CR: Control

Table 4: Alanine amino transferase production levels in the Liver of *Clarias gariepinus* exposed to sub-lethal concentrations of Pb (NO_3)$_2$, and supplemented with Vitamin A for a period of 12 weeks

	1st	2nd	3rd	4th	5th	6th
CR	48.48±0.20^c	8.73±0.15^a	66.67±0.05	65.17±0.05^d	48.90±0.15^b	23.14±0.15^a
T1	56.02±0.05^e	77.12±0.20^e	0.00±0.00	22.63±0.15^a	61.87±0.10^c	48.90±0.15^d
T2	41.87±0.20^a	23.14±0.15^c	0.00±0.00	44.07±0.10^b	26.36±0.15^a	27.20±0.05^c
T3	55.51±0.05^d	18.14±0.10^b	0.00±0.00	74.07±0.20^e	0.00±0.00	56.02±0.15^e
T4	48.22±0.05^b	63.56±0.20^d	0.00±0.00	50.34±0.10^c	64.75±0.10^d	24.83±0.15^b

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$. The ALT mean value unit in each case is nM/mg. ALT: Alanine amino transferase, CR: Control

From the results of the samples exposed to sub-lethal concentrations of Pb (NO_3)$_2$, and supplemented with vitamin C, the ALT production levels in the liver indicated that T4 mean values in both 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. Also, the T3, T1 and T2 mean values of 8th, 10th and 12th weeks of exposure are significantly higher than other treatments. The highest ALT mean value produced in the liver was 86.53 ± 0.05 nM/mg obtained in T4 at the end of the 4th week of exposure [Table 7]. On the other hand, the T2 mean values in the kidneys of the fish are significantly higher than other treatments in both 2nd and 4th week of exposure. The T1, T4, and T2 mean values in the 8th, 10th, and 12th weeks of exposure are significantly higher than other treatments. The highest ALT produced in the kidney was 63.48 ± 0.15 nM/mg obtained in T2 at the end of the 12th week of exposure [Table 8]. Furthermore, T1and T2 mean values produced in the gill in the 2nd and 4th weeks of exposure, respectively, are significantly higher than other treatments. The T2 mean values in both 8th and 12th weeks of exposure, respectively, are significantly higher than other treatments. The highest ALT mean value produced in the gill was 66.53 ± 0.15 nM/mg obtained in T2 at the end of the 12th week of exposure [Table 9].

From the results of the samples exposed to sublethal concentrations of Pb(NO_3)$_2$, and supplemented with vitamin E, the ALT production levels in the liver indicated that T2 and T4 mean values in both 2nd and 4th weeks of exposure, respectively, are significantly higher than other treatments. Furthermore, the T4, T1, T4, and T2 mean values of 6th, 8th, 10th, and 12th weeks of exposure, respectively, are significantly higher than other treatments. The highest ALT mean value produced in the liver was 73.82 ± 0.15 nM/mg obtained in T4 at the end of the 10th week of exposure [Table 10]. On the other hand, the T1 and T4 mean values in the kidneys of the fish are significantly higher than other treatments in both 2nd and 4th week of exposure. There were gradual increases in the levels of production from the 2nd to the 6th weeks of exposure in both T2 and T3 samples. The

Table 5: Alanine amino transferase production levels in the Kidney of *Clarias gariepinus* exposed to sub-lethal concentrations of Pb $(NO_3)_2$ and supplemented with Vitamin A for a period of 12 weeks

	1st	2nd	3rd	4th	5th	6th
CR	65.68±0.15^e	21.87±0.10^c	69.39±0.15	12.54±0.10^a	54.32±0.05^a	16.10±0.10^c
T1	34.75±0.20^d	17.88±0.05^b	0.00±0.00	13.05±0.10^b	62.29±0.15^c	46.70±0.05^d
T2	16.78±0.10^b	84.75±0.10^d	0.00±0.00	54.66±0.15^d	0.00±0.00	10.00±0.10^b
T3	27.37±0.15^c	0.00±0.00	0.00±0.00	23.05±0.10^c	0.00±0.00	84.83±0.0.15^e
T4	7.31±0.19^a	16.02±0.05^a	0.00±0.00	60.00±0.10^e	54.41±0.10^b	7.34±0.24^a

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$. The ALT mean value unit in each case is nM/mg. ALT: Alanine amino transferase, CR: Control

Table 6: Alanine amino transferase production levels in the Gill of *Clarias gariepinus* exposed to sub-lethal concentrations of Pb $(NO_3)_2$ and supplemented with Vitamin A for a period of 12 weeks

	1st	2nd	3rd	4th	5th	6th
CR	26.61±0.20^a	10.68±0.20^b	7.80±0.10	19.32±0.10^b	77.37±0.05^e	24.49±0.05^c
T_1	51.44±0.15^d	26.78±0.10^e	0.00±0.00	59.49±0.10^e	56.19±0.05^a	23.82±0.05^b
T_2	33.56±0.10^c	26.01±0.15^d	0.00±0.00	6.19±0.15^a	64.15±0.05^b	10.93±0.15^a
T_3	33.31±0.05^b	0.76±0.05^a	0.00±0.00	56.61±0.10^d	69.92±0.05^c	54.83±0.15^e
T_4	63.05±0.10^e	18.98±0.20^c	0.00±0.00	55.00±0.15^c	70.43±0.24^d	31.87±0.10^d

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$. The ALT mean value unit in each case is nM/mg. ALT: Alanine amino transferase, CR: Control

Table 7: Alanine amino transferase production levels in the Liver of *Clarias gariepinus* exposed to sub-lethal concentrations of Pb $(NO_3)_2$ and supplemented with Vitamin C for a period of 12 weeks

	1st	2nd	3rd	4th	5th	6th
CR	48.48±0.20^e	8.73±0.15^b	66.70±0.05	65.17±0.05^c	48.90±0.15^a	23.14±0.15^a
T1	8.90±0.15^a	0.34±0.20^a	0.00±0.00	21.10±0.05^a	66.36±0.15^d	42.20±0.10^d
T2	21.19±0.10^c	14.32±0.05^c	0.00±0.00	46.27±0.20^b	0.00±0.00	68.39±0.15^e
T3	12.88±0.10^b	23.98±0.15^d	0.00±0.00	65.34±0.05^d	54.41±0.10^b	33.73±0.10^c
T4	41.87±0.10^d	86.53±0.05^e	0.00±0.00	0.00±0.00	59.66±0.10^c	25.93±0.10^b

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$. The ALT mean value unit in each case is nM/mg. ALT: Alanine amino transferase, CR: Control

Table 8: Alanine amino transferase production levels in the Kidney of *Clarias gariepinus* exposed to sub-lethal concentrations of Pb $(NO_3)_2$ and supplemented with Vitamin C for a period of 12 weeks

	1st	2nd	3rd	4th	5th	6th
CR	65.68±0.15^e	21.87±0.10^c	68.39±0.15	12.54±0.10^a	54.32±0.05^a	16.10±0.10^a
T1	17.37±0.15^c	26.10±0.10^d	0.00±0.00	62.12±0.15^d	60.41±0.15^b	23.05±0.0.10^b
T2	18.39±0.15^d	59.58±0.15^e	0.00±0.00	27.37±0.15^b	0.00±0.00	63.48±0.15^d
T3	5.51±0.05^a	16.87±0.05^a	0.00±0.00	48.48±0.10^c	62.63±0.15^c	41.19±0.10^c
T4	16.61±0.20^b	19.41±0.15^b	0.00±0.00	0.00±0.00	63.05±0.10^d	0.00±0.00

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$. The ALT mean value unit in each case is nM/mg. ALT: Alanine amino transferase, CR: Control

T3, T4, T4 and T3 mean values in the 6th, 8th, 10th, and 12th weeks of exposure are significantly higher than other treatments. The highest ALT produced in the kidney was 78.05 ± 0.15 nM/mg obtained in T4 at the end of the 4th week of exposure [Table 11]. Furthermore, T2, T1, and T2 mean values produced in the gill in the 2nd, 4th, and 6th weeks of exposure, respectively, are significantly higher than other treatments. The T3 mean values in both 10th and 12th weeks of exposure, respectively, are significantly higher than other treatments. There were gradual increases in the levels of production of ALT from the 2nd to the 6th weeks of exposure in T4 samples. The highest ALT mean value produced in the gill was 73.31 ± 0.05 nM/mg obtained in T3 at the end of the 10th week of exposure [Table 12].

Table 9: Alanine amino transferase production levels in the Gill of *Clarias gariepinus* exposed to sub-lethal concentrations of Pb $(NO_3)_2$ and supplemented with Vitamin C for a period of 12 weeks

	1st	2nd	3rd	4th	5th	6th
CR	26.61±0.20^{e}	10.68±0.20^{b}	7.80±0.10	19.32±0.10^{c}	77.37±0.05^{b}	24.49±0.05^{a}
T_1	17.23±0.11^{d}	10.40±0.23^{a}	0.00±0.00	20.96±0.11^{d}	57.12±0.17^{a}	39.21±0.06^{c}
T_2	16.02±0.15^{c}	42.04±0.10^{e}	0.00±0.00	35.59±0.10^{e}	0.00±0.00	66.53±0.15^{e}
T_3	9.75±0.05^{a}	26.19±0.05^{c}	0.00±0.00	7.12±0.10^{a}	0.00±0.00	49.83±0.10^{d}
T_4	11.10±0.05^{b}	32.20±0.10^{d}	0.00±0.00	15.76±0.20^{b}	0.00±0.00	36.27±0.10^{b}

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$. The ALT mean value unit in each case is nM/mg. ALT: Alanine amino transferase, CR: Control

Table 10: Alanine amino transferase production levels in the Liver of *Clarias gariepinus* exposed to sub-lethal concentrations of Pb $(NO_3)_2$ and supplemented with Vitamin E for a period of 12 weeks

	1st	2nd	3rd	4th	5th	6th
CR	48.48±0.20^{b}	8.73±0.15^{b}	66.70±0.05^{c}	65.17±0.05^{c}	48.90±0.15^{b}	23.14±0.15^{c}
T1	46.61±0.10^{a}	4.07±0.10^{a}	39.24±0.05^{a}	54.32±0.05^{b}	73.31±0.15^{d}	11.10±0.05^{a}
T2	54.58±0.10^{c}	35.68±0.05^{d}	66.78±0.20^{d}	0.00±0.00	44.15±0.05^{a}	53.39±0.10^{d}
T3	52.04±0.10^{c}	10.43±0.15^{c}	58.22±0.15^{b}	0.00±0.00	64.58±0.10^{c}	0.00±0.00
T4	53.90±0.10^{d}	36.44±0.10^{e}	67.20±0.05^{e}	50.34±0.20^{a}	73.82±0.15^{e}	20.17±0.10^{b}

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$. The ALT mean value unit in each case is nM/mg. ALT: Alanine amino transferase, CR: Control

Table 11: Alanine amino transferase production levels in the Kidney of *Clarias gariepinus* exposed to sub-lethal concentrations of Pb $(NO_3)_2$ and supplemented with Vitamin E for a period of 12 weeks

	1st	2nd	3rd	4th	5th	6th
CR	65.68±0.15^{e}	21.87±0.10^{a}	68.39±0.15^{d}	12.54±0.10^{a}	54.32±0.05^{d}	16.10±0.10^{a}
T1	53.14±0.05^{d}	75.17±0.05^{d}	15.93±0.39^{a}	48.98±0.10^{b}	53.48±0.15^{c}	19.49±0.10^{b}
T2	41.70±0.20^{b}	56.10±0.10^{b}	57.88±0.15^{b}	0.00±0.00	25.85±0.15^{a}	28.48±0.10^{c}
T3	52.37±0.10^{c}	58.31±0.10^{c}	68.39±0.05^{d}	0.00±0.00	47.63±0.10^{b}	57.71±0.15^{e}
T4	15.00±0.15^{a}	78.05±0.15^{e}	66.61±0.10^{c}	68.14±0.10^{e}	59.58±0.15^{e}	42.63±0.05^{d}

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$. The ALT mean value unit in each case is nM/mg. ALT: Alanine amino transferase, CR: Control

Table 12: Alanine amino transferase production levels in the Gill of *Clarias gariepinus* exposed to sub-lethal concentrations of Pb $(NO_3)_2$ and supplemented with Vitamin E for a period of 12 weeks

	1st	2nd	3rd	4th	5th	6th
CR	26.61±0.20^{b}	10.68±0.20^{c}	7.80±0.10^{a}	19.32±0.10^{a}	77.37±0.05^{e}	24.49±0.05^{d}
T_1	56.10±0.10^{d}	63.82±0.05^{e}	14.07±0.20^{b}	24.66±0.15^{b}	69.32±0.10^{c}	2.37±0.10^{a}
T_2	56.36±0.15^{e}	8.65±0.10^{b}	61.27±0.15^{e}	0.00±0.00	32.37±0.10^{a}	13.98±0.15^{b}
T_3	37.37±0.05^{c}	2.46±0.15^{a}	56.78±0.10^{d}	0.00±0.00	73.31±0.05^{d}	69.49±0.10^{e}
T_4	12.54±0.20^{a}	18.90±0.15^{d}	31.19±0.10^{c}	0.00±0.00	46.70±0.05^{b}	16.27±0.10^{c}

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$. The ALT mean value unit in each case is nM/mg. ALT: Alanine amino transferase, CR: Control

Discussions

ALT production levels in Clarias gariepinus exposed to sublethal concentrations of Pb toxicant and the respective supplemented treatments with Vitamins A, C and E

From the results of the samples exposed to sublethal concentrations of Pb $(NO_3)_2$, the ALT production levels indicated that T1 and T4 mean values in the 2nd and 4th weeks of exposure, respectively, are significantly higher than other treatments including the control. The need for upregulation of the defence system was probably elicited from the beginning of the exposure, especially in the lowest concentration; given that, AST and ALT are highly conservative indicators in liver and are commonly located in hepatic cytoplasm and would release into the circulation when hepatocytes necrotize (Arenas *et al*. 2017).[19] Subsequently, the higher concentrations witnessed increased production

of ALT. This is probably why the T4 mean values of both 6th and 8th weeks of exposure are significantly higher than other treatments including the control and the highest mean value of ALT produced in the liver of the samples (87.20 ± 0.15 nM/mg.) was also obtained in T4 at the 4th week of exposure. In order to counter the effects of the toxicant, the immune system probably had to be improved upon. This up-regulation was also probably necessary in T3 mean values in the 10th and 12th weeks of exposure, respectively + which are significantly higher than other treatments including the control. This could also be due to the fact that, ALT is a cytoplasmic enzyme found in very high concentration in the liver (Arbonnier, 2004);[25] and that AST is less specific than ALT as marker of liver damage, but elevation in the serum levels of the two enzymes is an indicator of tissue damage and altered membrane ability (Satpal and Punnia, 2010).[26] Furthermore, reduction in plasma protein levels may be due to impaired protein synthesis or metabolism (Ramesh *et al*., 2014).[27] On the other hand, the T1 mean values in the kidneys of the fish are significantly higher than other treatments including the control in both 2nd and 4th week of exposure. This is probably because the production of the enzyme was triggered in this treatment by the toxicant but minimally utilized due to the low concentration of the toxicant. The sensitivity of the kidney in producing ALT in response to the effects of the toxicant is also probably minimal. This is probably why the control mean values in the 6th, 8th, and 10th weeks of exposure are significantly higher than other treatments. The T4 mean values in the 12th week of exposure are significantly higher than other treatments. This T4 mean value (65.76 ± 0.20 nM/mg) in the 12th week of exposure was also the highest ALT produced in the kidney. At higher concentration, however, there must probably be an upregulation of the defence system to deal with the effects of the toxicant. In line with this, Al-Balawi *et al*. (2011)[28] reported how exposure of *C. gariepinus* to lead acetate at all concentrations caused reduced growth rate, had significant effects on erythrocyte count, hemoglobin concentration and hematocrit values, increased plasma GOT and GPT, and sperm motility was also hampered after 4 weeks. Furthermore, T1, T3, and T4 mean values produced in the gill in the 2nd, 4th, and 6th weeks of exposure, respectively, are significantly higher than other treatments including the control. In the gill, the first point of call, there was elevation of ALT production level, especially in lower concentration and subsequently in T3 and T4. This is again probably because of the need to regulate the body's mechanisms. The T1 and T3 mean values in the 8th and 12th weeks of exposure, respectively, are significantly higher than other treatments including the control. The highest ALT mean value produced in the gill was 69.92 ± 0.05 nM/mg obtained in T3 at the end of the 12th week of exposure probably due to the same reason given above. Similarly, Kim and Kang (2015)[29] reported a significant increase in the GOT and GPT of Korean rockfish, *Sebastes schegelli* exposed to dietary lead. Similarly, Muralisankar *et al*. (2014)[30] reported that dietary zinc exposure increases the GOT and GPT in the fresh water prawn, *Macrobranchium rosenbergii.*

From the results of the samples exposed to sub-lethal concentrations of Pb $(NO_3)_2$, and supplemented with Vitamin A, the ALT production levels in the liver indicated that T1 mean values in both 2nd and 4th weeks of exposure, respectively, are significantly higher than other treatments. This is probably because there is less utilization of the enzyme unlike in other higher concentrations. This may also be why the highest mean value of ALT produced in the liver of the samples (77.12 ± 0.20 nM/mg) was also obtained in T1 at the 4th week of exposure. However, as the duration of the exposure and the concentrations of the treatments increased there were probably the needs for the up-regulation of the body's immune system to counteract the deleterious effects of the toxicant. In line with this, the T3, T4, and T3 mean values of 8th, 10th and 12th weeks of exposure are significantly higher than other treatments. Okonkwo and Ejike (2012)[31] and Olojo *et al*. (2012)[32] similarly reported that elevations in ALT and AST concentrations in serum of the catfish may be attributed to disruption of hepatic cells as a result of necrosis or altered membrane permeability after exposure to lead. On the other hand, the T1 mean values in the kidneys of the fish are significantly higher than other treatments in 2nd week of exposure. The T2 and T3 mean values in the 4th and 8th weeks of exposure are significantly higher than other treatments. This is probably because as the concentration and duration of exposure increased there is constant need for the up-regulation of the body's defence system as in other cases. The T1 and T3 mean values in the 10th and 12th weeks of exposure are significantly higher than other treatments. The highest mean value of ALT produced in the kidney was 84.75 ± 0.10 nM/mg in T3 at the 12th week of exposure probably due to the same reason stated above. Furthermore, T4 and T1 mean values produced in the gill in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. At the portal of entry, the highest concentration elicited the significant production of the enzyme at early stage of the exposure probably to ensure

survival and avoid being overwhelmed by the effects of the toxicant. As the duration of the exposure increased other concentrations followed suit. This is probably why the T1, T4 and T3 mean values in the 8th, 10th and 12th weeks of exposure, respectively are significantly higher than other treatments. The highest ALT mean value produced in the gill was 70.43 ± 0.24 nM/mg obtained in T4 at the end of the 10th week of exposure. At this stage and concentration the deleterious effects of the toxicant may have elicited the up-regulation of the body's immune system to put it in check.

From the results of the samples exposed to sub-lethal concentrations of Pb $(NO_3)_2$, and supplemented with vitamin C, the ALT production levels in the liver indicated that T4 mean values in both 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. In the highest treatment the effects of the vitamin may not have been able to thoroughly deal with the effects of the toxicant especially at the early stages of the exposure. This is probably why the effects were elicited and sustained in T4 which also produced the highest ALT mean value (86.53 ± 0.05 nM/mg) in the liver at the end of the 4th week of exposure. The production levels of the enzyme in lower concentrations were probably not much at early stage due to the presence of the vitamin. However, at later stages of the exposure there were probably the needs for up-regulation of the immune system, especially in the treatments with lower concentrations. This is probably why the T3, T1, and T2 mean values of 8th, 10th and 12th weeks of exposure are significantly higher than other treatments. Similar findings by Ellakany and Gaafar (2002)[33] reported that in *Oreochromis niloticus*, the ALT activities in liver and muscle were found to increase during the time course of endogenous cortisol elevation induced by ochratoxin intoxication and the results also indicated that the tissue injury in toxicated fish recovered when they were fed dietary ascorbic acid because the AST and ALT activities in fish exposed to the lower or higher dose of ochratoxin + vitamin C became similar to those of control fish. Also, vitamin E and C can reduce Pb and Cu levels in serum and tissues of liver and kidney as well as reduce alanine amino transferase (ALT), aspartate amino transferase (AST), urea and creatinine levels in Pb and Cu intoxicated male rats (Osfor *et al*., 2010).[15] On the other hand, the T2 mean values in the kidneys of the fish are significantly higher than other treatments in both 2nd and 4th weeks of exposure. Perhaps, the ALT elicited at these stages may not have been put to much utilization and hence, its availability and significance coupled with the presence of the vitamin. This is also probably why the highest ALT produced in the kidney (63.48 ± 0.15 nM/mg) was also obtained in T2 at the end of the 12th week of exposure which was also significant at this stage. Similar finding by Ikeogu *et al*., 2020)[34] indicated significant increase in ALT and urea when *C. gariepinus* was exposed to sub-lethal concentrations of glyphosphate but there were decreases in AST, ALT, and urea in the treatments supplemented with vitamin C. Furthermore, T1 and T2 mean values produced in the gill in the 2nd and 4th weeks of exposure, respectively are significantly higher than other treatments. In the lower concentrations and in the presence of vitamin C, there are usually high production levels of the enzyme or antioxidant in question. This is probably because the presence of the vitamin normally mitigates the effects of the toxicant; and as such, leads to underutilization of the already elicited production of the enzyme and or, the antioxidant. This is also probably why the T2 mean values in both 8th and 12th weeks of exposure, respectively, are significantly higher than other treatments and the highest ALT mean value produced in the gill (66.53 ± 0.15 nM/mg) was also obtained in T2 at the end of the 12th week of exposure. This is probably because Vitamins E and C supplementation can induce protective effects on certain conditions after free radical-mediated cellular damage or disruption (Yolanda and Maria, 2012).[18]

From the results of the samples exposed to sublethal concentrations of $Pb(NO_3)_2$, and supplemented with vitamin E, the ALT production levels in the liver indicated that T2 and T4 mean values in both 2nd and 4th weeks of exposure, respectively, are significantly higher than other treatments. This is probably because there were responses to overcome first, the elicitation threshold that was reached in T2 and at early stage of the exposure; and second, the urgent need to up-regulate the production of the enzyme to counteract the onslaught of the toxicant in the highest concentration in the 4th week. Furthermore, this is probably why the T4 mean values in both 6th and 10th weeks of exposure, respectively, are significantly higher than other treatments and the highest ALT mean value produced in the liver (73.82 ± 0.15 nM/mg) was also obtained in T4 at the end of the 10th week of exposure. Apart from the elicitation of the enzyme production in T2 in the 2nd week of exposure, the effects of the toxicant only became evident in lower concentrations at later stages of the exposure as T1 and T2 mean values of 8th and 12th weeks of exposure, respectively, are significantly higher than other treatments probably courtesy of the presence of the vitamin. In line with this, Mahmoud *et al*. (2012)[35] reported that supplementation of selenium and vitamin E decreases the toxic effects of mercury, significantly increased the mean values of Na+, urea, creatinine, AST, ALT, and ALP in comparison to control values; since,

vitamins C and E are natural nonenzymatic antioxidants that are able to scavenge free radicals and decrease lipid peroxidation (Zhai *et al*., 2015).[36] On the other hand, the T1 and T4 mean values in the kidneys of the fish are significantly higher than other treatments in both 2nd and 4th week of exposure. The same reason and explanation given above may also be tenable here. There were gradual increases in the levels of production from the 2nd to the 6th weeks of exposure in both T2 and T3 samples. This trend may have been occasioned by the increasing need for the up-regulation of the body's defence systems to counter the effects of the toxicant as the duration of exposure increased. The T3, T4, T4 and T3 mean values in the 6th, 8th, 10th and 12th weeks of exposure are significantly higher than other treatments; and highest ALT produced in the kidney (78.05 ± 0.15 nM/mg) was also obtained in T4 at the end of the 4th week of exposure probably buttressing the fact that the responses are concentration and duration dependent banking on the sensitivity of the kidney in detecting the effects. Similar report was given by Azeez and Braimah (2020)[37] when they showed how plasma ALT, and AST and ALP activities were increased when *C. gariepinus* was exposed to varying concentrations of copper sulphate. Also, TL, AST and ALT in *Channa punctata* exposed to lead acephate were significantly increased (Satish *et al*., 2018).[38] Furthermore, T2, T1 and T2 mean values produced in the gill in the 2nd, 4th and 6th weeks of exposure, respectively, are significantly higher than other treatments. In this scenario, the ALT production levels in the gills indicate the elicitation and sustenance in lower concentration at the early stages of the exposure. At later stages however, the T3 mean values in both 10th and 12th weeks of exposure, respectively, are significantly higher than other treatments; and the gradual increases in the levels of production of ALT from the 2nd to the 6th weeks of exposure in T4 samples probably express the need for constant up-regulation of the immune system to deal with the changing environment. This is also probably why the highest ALT mean value produced in the gill (73.31 ± 0.05 nM/mg) was also obtained in T3 at the end of the 10th week of exposure. Similarly, Mahmoud *et al*. (2013)[39] found that *C. gariepinus* exposed to Pb exhibited increased AST and ALT levels, which is also in line with Olojo *et al*. (2012)[32] who stated that there was an increase in AST and ALT values for *C. gariepinus* after exposure to lead.

Conclusions and Recommendations

The samples of the fish exposed to sublethal concentrations of the toxicant displayed varying levels of production of the enzyme with higher production levels mostly at higher concentrations of the toxicant. In the Pb only and PbVC groups the liver of the samples produced the highest ALT, while the kidneys did same in the PbVA and PbVE groups. The high levels of production of the enzyme, especially in higher concentrations suggest physiological imbalances due to the presence of the toxicant. Similarly, the low levels of production of Alanine amino transferase in the treatments with lower concentrations of the toxicant suggest the ameliorative capacity of the vitamins.

The highest ALT mean values produced in each treatment groups are: Pb only (87.20 ± 0.15 nM/mg), PbVA (84.75 ± 0.10 nM/mg), PbVC (86.53 ± 0.05 nM/mg) and PbVE (78.05 ± 0.15 nM/mg). The outcome of this research buttresses the relevance of liver and kidneys in the detoxification of xenobiotics in the environment of living organisms.

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Conflicts of interest

There are no conflicts of interest.

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