



(RESEARCH ARTICLE)



Evaluation of the ameliorative roles of vitamins A, C and E on reduced glutathione in *Clarias gariepinus* (Burchell, 1822) fingerlings exposed to lead nitrate

Samuel PO ^{1,*}, Arimoro FO ¹, Ayanwale AV ¹ and Mohammad HL ²

¹ Fisheries and Hydrobiology Unit, Department of Animal Biology, Federal University of Technology, Minna, Niger State, Nigeria.

² Biochemistry Department, Federal University of Technology, Minna, Niger State, Nigeria.

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Abstract

The ever-increasing anthropogenic activities all over the world that usually led to release of plethora of pollutants such as lead calls for concern. In the present study the effects of lead nitrate on the production of antioxidants such as reduced glutathione (GSH) in *C. gariepinus* and how such effects can be ameliorated through administration of vitamins were investigated. *C. gariepinus* fingerlings (whose initial weight ranged from 3-11g) were exposed to sub-lethal concentrations of Pb (00, 26mg/L, 44mg/L, 61mg/L and 79mg/L) with replicate in each case. 26mg/L each of the vitamins were administered across all bud. Fresh concentrations of both toxicant and vitamins were administered every 72 hours 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. 3 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 GSH productions levels in each case. The data generated were subjected to one way analysis of variance and considered significant at $P \leq 0.05$. From the results: In the Pb only group, the mean values of the GSH produced in the liver of the control samples were significantly higher than other treatments. The highest mean values of $82.04 \pm 0.13 \mu\text{g/ml}$, $30.84 \pm 0.10 \mu\text{g/ml}$ and $31.30 \pm 0.10 \mu\text{g/ml}$ were obtained in the liver, kidney and gills of the fish, respectively. In fish samples exposed to PbVA group, the highest mean values of $23.57 \pm 0.10 \mu\text{g/ml}$, $58.74 \pm 0.07 \mu\text{g/ml}$ and $52.72 \pm 0.07 \mu\text{g/ml}$ were obtained in the liver, kidney and gills, respectively. In *C. gariepinus* exposed to PbVC group, the highest mean values obtained in the liver, kidneys and gills were $25.79 \pm 0.07 \mu\text{g/ml}$, $28.40 \pm 0.13 \mu\text{g/ml}$ and $37.55 \pm 0.03 \mu\text{g/ml}$, respectively. In PbVE group, the highest mean value of $57.21 \pm 0.03 \mu\text{g/ml}$, $83.51 \pm 0.07 \mu\text{g/ml}$ and $63.29 \pm 0.07 \mu\text{g/ml}$ were obtained in the liver, kidneys and gills, respectively. The liver of the samples exposed to Pb only group displayed higher level of response to the toxicant with the highest GSH produced in the lowest concentration in comparison to other fish organs. In the PbVA group the response was more in the kidney in the highest concentration. There were general low levels of production in all organs of the fish in the PbVC group. The kidneys of the PbVE group exhibited the highest level of GSH production in comparison to other organs. The kidneys and liver of *C. gariepinus* in this research were fully engaged in mitigating the effects of the toxicant in the presence of the vitamins. Administration of higher concentrations of the vitamins could enhance better understanding of the ameliorative roles of the vitamins against the deleterious effects of the toxicant.

Keywords: *Clarias gariepinus*; GSH production levels; Fish organs; Ameliorative roles; Pb treatment groups

* Corresponding author: Samuel PO; E-mail: ajakopatrck@yahoo.com

Fisheries and Hydrobiology Unit, Department of Animal Biology, Federal University of Technology, Minna, Niger State, Nigeria.

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1. Introduction

Fish is a rich source of animal protein throughout the world. African catfish, *C. gariepinus* is an important commercial fish due to its high growth rate, high consumer acceptability, and ability to withstand poor water quality, and oxygen depletion [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 [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 [3]. The common species found are *C. gariepinus*, *C. anguillaris*, *C. buthupogon* and *C. lazera*.

Heavy metals induce significant damage to the physiologic and biochemical processes of the fish and subsequently to fish consumers [4]. Among all the heavy metals, Cd, arsenic, mercury and lead pose highest degree of toxicity and that is of great concern to both plants and human health [5]. Fish are particularly vulnerable and heavily exposed to pollutants due to feeding and living in aquatic ecosystems, because they cannot avoid pollutant harmful effects [6]. Heavy metals enter fish by direct absorption from water through their gills and skin, or by ingestion of contaminated food [7].

Antioxidants that facilitate or confer protective capacity on organisms could be either enzymatic or non-enzymatic. Antioxidant enzymes are crucial in their effort to decrease oxidative stress produced by exposure to toxicants [8]. It has also been reported that antioxidants may ameliorate, protect and remove the oxidative damage to a target organ or molecule [9]. Non-enzymatic 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. For instance, increase in reduced glutathione (GSH) level in fish tissues was attributed to presence of defence system to protect the fish from the oxidative stress or could appear as an antioxidant adaptation to metal exposure [10]. Also, Hermenean *et al.* [11] observed that the liver has a higher capacity and adaptability to counteract ROS compared to kidney. Glutathione has been found in all mammalian cells, and has been demonstrated that GSH is responsible for protection against ROS and RNS, detoxification of endogenous and exogenous toxins of electrophilic nature [12]. In line with this, Saliu and Bawa-Allah [13] reported that the levels of GST-GSH, SOD, CAT and MDA were all reduced in fishes (*Clarias gariepinus*) exposed to $Pb(NO_3)_2$ in comparison with the control indicating the effect of the pollutant on the fish while the reverse was the case in those fish exposed to $ZnCl_2$ with increase in antioxidants except MDA. Sharma and Ansari [14] also demonstrated that GSH level in both the tissues (brain and muscles) were decreased for all exposure periods. Sisein *et al.* [15] attributed the significantly lower GSH value in the liver of *C. gariepinus* from Gbarantoru swamp in comparison with Niger Delta University Agricultural farm (control) partly to the increased accumulation of heavy metals which led to more utilization of GSH to detoxify metals and ROS. In the same vein, Ayoola *et al.* [16] recorded significant differences in GSH, MDA, SOD and total protein in the gills of *Hemichromis fasciatus* and *Chrysichthys nigrodigitatus* collected from polluted Lagos lagoon.

The main biological function of vitamin E is its direct influence on cellular responses to oxidative stress through modulation of signal transduction pathway [17]. Vitamins E and C supplementation can induce protective effects on certain conditions after free radical-mediated cellular damage or disruption [18]. Vitamin E (α -tocopherol) is a fat soluble antioxidant that inhibits the production of reactive oxygen species formed when fat undergoes oxidation. This research therefore, attempted to determine the effects of Pb toxicant on the production of GSH in the exposed samples and how such effects can be ameliorated by the presence of vitamin supplements.

2. Material and methods

2.1. Samples/materials collection and Acclimatization

A total number of seven hundred and fifty (750) fingerlings of *C. gariepinus* were purchased from a commercial fish farmer and transported in 50L 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 (3mm) 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 (500g unit each) were purchased from commercial chemical stores. The toxicant Pb (2 units of 500g) analar grades were purchased from commercial chemical stores and stored in a cool dry condition throughout the period of the experiment. These toxicant and vitamins were administered according to the sub-lethal concentrations of the treatments during the chronic phase of the exposure.

2.2. 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 twelve (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 ($\text{Pb}(\text{NO}_3)_2$), the fishes were exposed to five sub-lethal treatments of lead nitrate concentrations; 26mg/L as T1, 44mg/L as T2, 61mg/L as T3 and 79mg/L as T4, respectively. Each treatment was in two replicates containing 20 fish in 20L 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 hours according to Organization for Economic Co-operation and Development [19] standards. Three fish samples were picked at random and sacrificed from each trough on every 14th day for the twelve weeks 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 GSH.

2.3. 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.

2.4. Reduced Glutathione Bioassay

The GSH (reduced glutathione) produced in each organ of the fish from each treatment and replicate were determined from their homogenates in the Laboratory of STEP B, Federal University of Technology, Bosso Campus, Minna, Niger State. The following reagents were used for the analysis: 0.2M phosphate buffer (8.40 g of NaH_2PO_4 and 9.94 g of Na_2HPO_4 was dissolved in distilled water and made up to 1000ml mark in a volumetric flask. The buffer was adjusted to pH8.0); 10% Trichloroacetic acid (10 g of TCA was dissolved in distilled water and made up to 100ml in the volumetric flask); and Ellman' reagent (19.8 mg of 5,5'-Dithiobis Nitro Benzoic acid (DTNB) in 100 ml of 0.1 % sodium nitrate).

To 150 μL of the tissue homogenate (in phosphate-saline pH 7.4), 1.5 ml of 10 % TCA was added, and centrifuge at 1500 g for 5 min. 1.0 ml of the supernatant was treated with 0.5ml of Ellman's reagent and 3.0 ml of phosphate buffer (2.0 m pH 8.0). The absorbance was read at 412 nm. Estimation of Reduced Glutathione was determined by the method of Ellman [20] as described by Rajagopalan *et al.* [21]. The amount of glutathione was calculated using a GSH standard curve and expressed as micron grams of GSH formed/mg protein in each case.

2.5. Data Analyses

The antioxidants levels in samples exposed to sub-lethal concentrations of the toxicant as well as the treatments supplemented with vitamins were analysed 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).

3. Results

3.1. GSH production levels in Liver, Kidneys and gills of *C. gariepinus* exposed to sub-lethal concentrations of $\text{Pb}(\text{NO}_3)_2$ toxicant and the respective supplemented treatments with Vitamins A, C and E for a period of twelve weeks and sampled fortnightly

From the statistical analyses of the results of the samples of *C. gariepinus* exposed to sub-lethal concentrations of $\text{Pb}(\text{NO}_3)_2$, the mean values of the glutathione (GSH) produced in the liver of the control samples were significantly higher than other treatments, with T3 significantly higher than other treatments in the first two weeks of exposure to the toxicant. However, T4 mean values in the 4th week of exposure were significantly higher than the other treatments including the control. Likewise, T2 and T1 mean values were significantly higher in the 6th and 8th week of exposure, respectively than in other treatments. On the 10th week, T3 mean values were significantly higher than other treatments. At the end of the 12th week, T1 mean values were significantly higher than in other treatments with the control following suit. The highest mean value of $82.04 \pm 0.13 \mu\text{g/ml}$ in the liver of the fish was also obtained in T1. (Table 1).

Meanwhile, in the kidneys of the samples exposed to the sub-lethal concentration of the toxicant, T4 and T1 mean values were significantly higher than other treatments including the control in the first 2 weeks of exposure. These however, became significantly lower in the 4th week of exposure in comparison with the control mean values which were significantly higher than other treatments. The control mean values in the 6th week were also significantly higher than other treatments. The T3 mean values (of $30.84 \pm 0.10 \mu\text{g/ml}$) in the kidney of the fish exposed to the sub-lethal concentration of the toxicant was the highest, which were also significantly higher than the other treatments at the end of 8th week. The GSH mean values in the 10th week of exposure in the treatments were significantly lower than the control. In the 12th week however, the T1 mean values were significantly higher than other treatments including the control. (Table 2).

Furthermore, in the gills of the samples exposed to sub-lethal concentration of the toxicant, T3 mean values were significantly higher than other treatments including the control in the first 2 weeks of exposure. On the other hand, there were general low production levels at the end of the 4th week of exposure. T2 and T1 mean values were significantly higher than other treatments in the 6th and 8th weeks of exposure, respectively. Similarly, T4 and T1 mean values were significantly higher than other treatments in the 10th and 12th weeks of exposure, respectively. The highest GSH production level (mean value) in the gill of the fish exposed to the sub-lethal concentration of the toxicant was $31.30 \pm 0.10 \mu\text{g/ml}$ in T2 at the 6th week of exposure. (Table 3).

Table 1 GSH production levels in the Liver of *C. gariepinus* exposed to sub-lethal concentrations of $\text{Pb}(\text{NO}_3)_2$ for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	19.76 ± 0.13^m	7.26 ± 0.13^h	13.51 ± 0.07^j	19.03 ± 0.10^k	9.42 ± 0.07^c	57.83 ± 0.13^n
T1	7.32 ± 0.10^c	8.11 ± 0.10^i	6.70 ± 0.13^c	24.88 ± 0.07^m	9.59 ± 0.10^d	82.04 ± 0.13^o
T2	9.88 ± 0.07^e	7.66 ± 1.34^i	17.49 ± 0.26^l	17.55 ± 0.03^j	19.59 ± 0.10^k	12.60 ± 0.07^e
T3	13.51 ± 0.07^j	5.90 ± 0.46^f	11.45 ± 0.31^f	11.75 ± 0.10^f	46.13 ± 0.13^n	15.22 ± 0.07^h
T4	9.48 ± 0.16^d	11.35 ± 0.13^m	5.37 ± 0.29^b	8.74 ± 0.00^d	27.09 ± 0.03^l	10.16 ± 0.10^c

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$.
GSH unit for each mean value is $\mu\text{g/ml}$.

Table 2 GSH production levels in the Kidney of *C. gariepinus* exposed to sub-lethal concentrations of $\text{Pb}(\text{NO}_3)_2$ for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	9.88 ± 0.07^e	11.70 ± 0.13^n	13.80 ± 0.03^k	16.13 ± 0.13^i	18.80 ± 0.10^j	12.55 ± 0.10^d
T1	13.00 ± 0.03^h	3.23 ± 0.10^b	13.00 ± 0.03^h	6.58 ± 0.07^a	11.75 ± 0.10^e	23.11 ± 0.10^m
T2	5.56 ± 0.13^a	10.94 ± 0.04^l	0.00 ± 0.00	6.81 ± 0.13^b	12.83 ± 0.07^f	16.30 ± 0.10^i
T3	6.18 ± 0.03^b	8.34 ± 0.10^k	12.32 ± 0.10^g	30.84 ± 0.10^n	7.21 ± 0.03^a	14.93 ± 0.10^g
T4	13.34 ± 0.10^i	3.97 ± 0.13^c	10.33 ± 0.07^e	16.13 ± 0.07^i	7.21 ± 0.03^a	6.53 ± 0.10^b

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$.
GSH unit for each mean value is $\mu\text{g/ml}$.

From the statistical analysis of the results of the fish samples exposed to sub-lethal concentrations of $\text{Pb}(\text{NO}_3)_2$ and supplemented with vitamin A, the T3 and T2 mean values of the liver of the fish were significantly higher than other treatments in the 2nd and 4th week of exposure, respectively. However, only T2 mean values were significantly higher than other treatment in the 8th week of exposure. T1 mean values in the 10th and 12th weeks were significantly higher than in other treatments. The highest mean value of $23.57 \pm 0.10 \mu\text{g/ml}$ in the Liver of the fish subjected to sub-lethal concentrations of the toxicant and supplemented with vitamin A was obtained in T1 at the 10th week of exposure. (Table 4).

On the other hand, the kidneys of the fish indicated that T1 mean values were significantly higher than other treatments at the 2nd week of exposure. T2 and T1 mean values were significantly higher than other treatments in the 4th and 8th week of exposure, respectively. However, T4 mean values in the 10th and 12th weeks of exposure were significantly higher than other treatments. The highest mean value of 58.74±0.07µg/ml in the kidney was obtained in T4 at the 12th week of exposure. (Table 5).

Table 3 GSH production levels in the Gill of *C. gariepinus* exposed to sub-lethal concentrations of Pb(NO₃)₂ for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	12.55±0.16 ^g	2.72±0.07 ^a	4.88±0.07 ^a	13.29±0.13 ^g	16.35±0.07 ⁱ	13.68±0.10 ^f
T ₁	12.09±0.16 ^f	7.04±0.07 ^g	19.99±0.07 ^m	21.92±0.13 ^l	13.91±0.03 ^g	23.06±0.07 ^l
T ₂	15.33±0.07 ^l	12.32±0.10 ^o	31.30±0.10 ⁿ	9.42±0.07 ^e	8.29±0.07 ^b	21.92±0.07 ^k
T ₃	24.88±0.13 ⁿ	5.62±0.10 ^e	13.74±0.07 ⁱ	14.71±0.16 ^h	14.88±0.13 ^h	19.48±0.03 ^j
T ₄	15.10±0.13 ^k	5.28±0.10 ^d	9.82±0.03 ^d	6.92±0.07 ^c	29.65±0.13 ^m	5.33±0.07 ^a

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. GSH unit for each mean value is µg/ml.

In addition to the forgoing, the gills of the samples of the fish exposed to the sub-lethal concentrations of the toxicant and supplemented with vitamin A, the T4 mean values were significantly higher than other treatments. There were general low GSH production levels in the gills of the fish at the 4th week of exposure. The T2 and T1 mean values were significantly higher than other treatments at the 8th and 10th weeks of exposure, respectively. T1 mean values were significantly higher than other treatments at the 12th week of exposure. The highest mean value of 52.72±0.07 µg/ml in the gill of the fish samples was obtained in T1 at the end of 12 weeks of exposure. (Table 6).

Table 4 GSH production levels in the Liver of *C. gariepinus* exposed to sub-lethal concentrations of Pb(NO₃)₂ and supplemented with vitamin A for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	19.76±0.13 ^l	7.26±0.13 ^f	13.51±0.07 ^b	19.03±0.10 ^o	9.42±0.07 ^a	57.83±0.13 ^m
T ₁	10.90±0.20 ^g	5.96±0.10 ^d	0.00±0.00	16.47±0.13 ^m	23.57±0.10 ⁿ	17.89±0.03 ^h
T ₂	9.25±0.16 ^e	13.17±0.20 ^m	0.00±0.00	17.43±0.16 ⁿ	17.09±0.10 ^h	13.91±0.10 ^e
T ₃	21.01±0.07 ^m	12.21±0.10 ^l	0.00±0.00	3.97±0.07 ^a	14.22±0.07 ^c	13.00±0.03 ^c
T ₄	9.20±0.13 ^d	5.90±0.07 ^c	0.00±0.00	9.14±0.10 ^e	16.92±0.07 ^g	0.00±0.00

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. GSH unit for each mean value is µg/ml.

Table 5 GSH production levels in the Kidney of *C. gariepinus* exposed to sub-lethal concentrations of Pb(NO₃)₂ and supplemented with vitamin A for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	9.88±0.07 ^f	11.70±0.13 ^k	13.80±0.03 ^c	16.13±0.13 ^l	18.80±0.10 ^l	12.55±0.10 ^b
T ₁	11.87±0.10 ^h	7.15±0.07 ^g	0.00±0.00	13.34±0.10 ^j	15.39±0.10 ^d	17.21±0.03 ^g
T ₂	8.46±0.10 ^b	34.37±0.10 ⁿ	0.00±0.00	11.53±0.10 ^f	12.26±0.13 ^b	14.37±0.03 ^f
T ₃	4.14±0.03 ^a	0.00±0.00	0.00±0.00	9.03±0.10 ^d	17.15±0.07 ⁱ	26.07±0.03 ^j
T ₄	9.03±0.16 ^c	3.80±0.10 ^b	0.00±0.00	4.54±0.07 ^b	18.74±0.07 ^k	58.74±0.07 ⁿ

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. GSH unit for each mean value is µg/ml.

Table 6 GSH production levels in the Gill of *C. gariepinus* exposed to sub-lethal concentrations of Pb(NO₃)₂ and supplemented with vitamin A for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	12.55±0.16 ⁱ	2.72±0.07 ^a	4.88±0.07 ^a	13.29±0.13 ⁱ	16.35±0.07 ^e	13.68±0.10 ^d
T₁	18.51±0.13 ^k	8.12±0.10 ^h	0.00±0.00	7.21±0.03 ^c	25.50±0.10 ^o	52.72±0.07 ^l
T₂	12.83±0.39 ^j	8.91±0.89 ⁱ	0.00±0.00	15.90±0.13 ^k	16.75±0.30 ^f	43.47±0.10 ^k
T₃	21.81±0.20 ⁿ	6.75±0.49 ^e	0.00±0.00	12.66±0.03 ^h	20.16±0.10 ^m	11.64±0.03 ^a
T₄	24.31±0.20 ^o	8.97±0.07 ^j	0.00±0.00	12.15±0.07 ^g	17.21±0.10 ^j	23.40±0.20 ⁱ

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. GSH unit for each mean value is µg/ml.

In the samples of *C. gariepinus* exposed to sub-lethal concentrations of Pb(NO₃)₂ and supplemented with vitamin C, T₁ mean values in the liver of the fish were significantly higher than other treatments at the end of the second week of exposure. T₂ and T₁ mean values in the 4th and 8th weeks of exposure, respectively were significantly higher than other treatments. While T₂ and T₄ mean values in the 10th and 12th weeks of exposure, respectively were significantly higher than other treatments. The highest mean value of 25.79±0.07µg/ml in the liver of the fish in this case, was obtained in T₄ at the 12th week of exposure. (Table 7).

On the other hand, T₁ and T₄ mean values in the kidneys of the fish in the 2nd and 4th weeks of exposure were significantly higher than other treatments. T₂ mean values at the 8th week of exposure were significantly higher than in other treatments. Also, the T₄ and T₂ mean values were significantly higher than other treatments in the 10th and 12th weeks of exposure. The highest mean value of 28.40±0.13µg/ml was obtained in T₂ at the 12th week of exposure. (Table 8).

Table 7 GSH production levels in the Liver of *C. gariepinus* exposed to sub-lethal concentrations of Pb(NO₃)₂ and supplemented with vitamin C for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	19.76±0.13 ^m	7.26±0.13 ^d	13.51±0.07 ^b	19.03±0.10 ^j	9.42±0.07 ^b	57.83±0.13 ^m
T₁	20.84±0.10 ⁿ	9.37±0.10 ^f	0.00±0.00	22.32±0.10 ^l	5.90±0.07 ^a	18.63±0.13 ⁱ
T₂	14.42±0.07 ^k	18.63±0.13 ^l	0.00±0.00	14.93±0.10 ^h	15.84±0.03 ^j	17.83±0.07 ^h
T₃	14.08±0.07 ^j	9.42±0.07 ^g	0.00±0.00	10.05±0.10 ^e	12.21±0.10 ^e	11.87±0.36 ^d
T₄	13.46±0.10 ⁱ	0.00±0.00	0.00±0.00	0.00±0.00	12.26±0.07 ^f	25.79±0.07 ^k

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. GSH unit for each mean value is µg/ml.

Table 8 GSH production levels in the Kidney of *C. gariepinus* exposed to sub-lethal concentrations of Pb(NO₃)₂ and supplemented with vitamin C for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	9.88±0.07 ^d	11.70±0.13 ^j	13.80±0.03 ^c	16.13±0.13 ⁱ	18.80±0.10 ^l	12.55±0.10 ^e
T₁	11.92±0.20 ^e	9.88±0.52 ^h	0.00±0.00	4.93±0.10 ^a	15.16±0.10 ^j	10.10±0.13 ^c
T₂	8.63±0.07 ^c	11.07±0.10 ⁱ	0.00±0.00	10.33±0.07 ^f	11.13±0.07 ^c	28.40±0.13 ^l
T₃	7.78±0.10 ^b	8.06±0.07 ^e	0.00±0.00	5.56±0.07 ^c	11.98±0.16 ^d	13.34±0.03 ^f
T₄	4.65±0.13 ^a	14.59±0.03 ^k	0.00±0.00	0.00±0.00	23.57±0.10 ^m	0.00±0.00

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. GSH unit for each mean value is µg/ml.

Furthermore, the T4 mean values in the gills of the fish exposed to the sub-lethal concentrations of the toxicant and supplemented with vitamin C were significantly higher than other treatments in the 2nd, 4th, 8th and 10th weeks of exposure, respectively. However, the T1 mean values in the 12th week of exposure were significantly higher than other treatments. The highest mean value of $37.55 \pm 0.03 \mu\text{g/ml}$ in T4 was obtained in the gills of the fish in this case at the 8th week of exposure. (Table 9).

Table 9 GSH production levels in the Gill of *C. gariepinus* exposed to sub-lethal concentrations of Pb(NO₃)₂ and supplemented with vitamin C for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	12.55±0.16 ^f	2.72±0.07 ^b	4.88±0.07 ^a	13.29±0.13 ^g	16.35±0.07 ^k	13.68±0.10 ^g
T₁	15.05±0.10 ^l	7.15±0.13 ^c	0.00±0.00	8.57±0.50 ^d	14.82±0.16 ^h	21.07±0.10 ^j
T₂	13.12±0.10 ^g	7.26±0.13 ^d	0.00±0.00	5.33±0.13 ^b	12.38±0.07 ^g	13.34±0.10 ^f
T₃	13.17±0.07 ⁱ	2.09±0.10 ^a	0.00±0.00	21.47±0.13 ^k	14.82±0.03 ^h	9.20±0.07 ^b
T₄	22.38±0.13 ^o	21.24±0.07 ^m	0.00±0.00	37.55±0.03 ^m	14.82±0.01 ^h	6.92±0.07 ^a

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. GSH unit for each mean value is $\mu\text{g/ml}$.

Table 10 GSH production levels in the Liver of *C. gariepinus* exposed to sub-lethal concentrations of Pb(NO₃)₂ and supplemented with vitamin E for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	19.76±0.13 ^l	7.26±0.13 ^f	13.51±0.07 ⁱ	19.03±0.10 ^h	9.42±0.07 ^a	57.83±0.13 ^m
T₁	15.84±0.10 ^j	5.84±0.16 ^d	12.21±0.23 ^f	10.39±0.10 ^e	12.21±0.10 ^d	7.49±0.07 ^c
T₂	9.25±0.10 ^d	7.66±0.10 ^g	17.83±0.07 ^l	0.00±0.00	22.66±0.03 ^m	3.85±0.07 ^a
T₃	10.67±0.13 ^g	6.47±0.13 ^e	36.75±0.10 ⁿ	0.00±0.00	10.67±0.07 ^c	57.21±0.03 ^l
T₄	8.57±0.03 ^c	6.47±0.13 ^e	10.45±0.07 ^e	9.08±0.13 ^c	13.85±0.07 ^h	39.31±0.13 ^j

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. GSH unit for each mean value is $\mu\text{g/ml}$.

Table 11 GSH production levels in the Kidney of *C. gariepinus* exposed to sub-lethal concentrations of Pb(NO₃)₂ and supplemented with vitamin E for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	9.88±0.07 ^f	11.70±0.13 ^h	13.80±0.03 ^k	16.13±0.13 ^g	18.80±0.10 ^k	12.55±0.10 ^f
T₁	11.30±0.16 ^h	12.78±0.10 ^j	0.00±0.00	2.89±0.10 ^a	83.51±0.07 ^o	22.89±0.10 ^h
T₂	8.34±0.10 ^b	12.66±0.16 ⁱ	13.63±0.07 ^j	0.00±0.00	63.80±0.10 ⁿ	4.93±0.10 ^b
T₃	7.43±0.10 ^a	39.14±0.10 ⁿ	8.40±0.07 ^b	0.00±0.00	9.48±0.03 ^b	56.41±0.03 ^k
T₄	9.71±0.23 ^e	14.03±0.16 ^l	9.03±0.16 ^c	10.28±0.16 ^d	16.24±0.07 ⁱ	33.40±0.13 ^j

Mean values and standard errors with different alphabets along the column are significantly different from each other at P≤0.05. GSH unit for each mean value is $\mu\text{g/ml}$.

From the results of the samples of fish exposed to sub-lethal concentrations of Pb(NO₃)₂ and Supplemented with vitamin E, the T1 and T2 mean values were significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. The T3 mean values in the 6th week were significantly higher than other treatments. Also, T2 and T3 mean

values in the 10th and 12th weeks, respectively were significantly higher than other treatments. The highest mean value of $57.21 \pm 0.03 \mu\text{g/ml}$ in the liver of the exposed samples of the fish was obtained in T3 at the 12th week of exposure. (Table 10). The T1 and T3 mean values in the kidneys of the samples were significantly higher than in other treatments in the 2nd and 4th weeks of exposure, respectively. Likewise, the T2 and T4 mean values in the 6th and 8th weeks of exposure, respectively were significantly higher than in other treatments. Also, the T1 and T3 mean values in the 10th and 12th weeks of exposure, respectively were significantly higher than other treatments. The highest mean value of $83.51 \pm 0.07 \mu\text{g/ml}$ in the kidneys of the sample was obtained in T1 at the 10th week of exposure. (Table 11). Furthermore, T4 and T1 mean values in the gills were significantly higher than in other treatments in the 2nd and 4th weeks of exposure, respectively. T4 and T3 mean values were also significantly higher than other treatments in the 6th and 10th weeks of exposure, respectively. The T3 mean value of $63.29 \pm 0.07 \mu\text{g/ml}$ obtained at the 12th week was the highest and was significantly higher than other treatments. (Table 12).

Table 12 GSH production levels in the Gill of *C. gariepinus* exposed to sub-lethal concentrations of $\text{Pb}(\text{NO}_3)_2$ and supplemented with vitamin E for a period of 12 weeks

	1 st	2 nd	3 rd	4 th	5 th	6 th
CR	12.55 ± 0.16^i	2.72 ± 0.07^b	4.88 ± 0.07^a	13.29 ± 0.13^f	16.35 ± 0.07^j	13.68 ± 0.10^g
T₁	19.65 ± 0.26^k	29.08 ± 0.13^m	13.12 ± 0.03^h	7.89 ± 0.10^b	13.23 ± 0.16^g	0.00 ± 0.00
T₂	23.40 ± 0.20^m	2.55 ± 0.10^a	9.37 ± 0.03^d	0.00 ± 0.00	12.49 ± 0.13^e	9.71 ± 0.10^d
T₃	25.45 ± 0.26^n	4.76 ± 0.13^c	12.32 ± 0.10^g	0.00 ± 0.00	18.91 ± 0.10^l	63.29 ± 0.07^n
T₄	25.65 ± 0.13^o	13.46 ± 0.10^k	26.30 ± 0.10^m	0.00 ± 0.00	12.66 ± 0.10^f	11.24 ± 0.07^e

Mean values and standard errors with different alphabets along the column are significantly different from each other at $P \leq 0.05$.
GSH unit for each mean value is $\mu\text{g/ml}$.

4. Discussion

4.1. GSH production levels in *C. gariepinus* exposed to sub-lethal concentrations of Pb toxicant and the respective supplemented treatments with Vitamins A, C and E

Antioxidants serve as superior therapy compared to conventionally-used chelating agents which may have attendant side effects in treatment of lead toxicant. Antioxidants possess both chelating and ROS scavenging capacity enabling elimination of lead from intracellular sites and blood stream which are effective while the subject is still exposed [22]. Also, GSH is the most important non-protein thiol in all living cells and has vital role in protection of intracellular body against toxins such as Cu and Zn through the action of GR, GST and GPx [23]. From the analysis of the results of the samples of *C. gariepinus* exposed to sub-lethal concentrations of $\text{Pb}(\text{NO}_3)_2$, the mean values of the glutathione (GSH) produced in the liver of the control samples were significantly higher than other treatments in the first two weeks of exposure to the toxicant. However, T4 mean values in the 4th week of exposure were significantly higher than in the other treatments including the control. This is most likely because at the initial stages of the exposure the samples were adapting to the changing environmental condition but at the 4th week of exposure there was an increase in the level of production in T4 probably to counteract the deleterious effects posed by the presence of the toxicant. As the duration of the exposure increased, the effects were probably being felt such that T2 and T1 mean values became significantly higher in the 6th and 8th week of exposure, respectively than other treatments. This is also probably the reason why at the end of the 12th week, T1 mean values were significantly higher than other treatments; and also had the highest mean value of $82.04 \pm 0.13 \mu\text{g/ml}$ in the liver of the fish. Ibrahim [24] reported that liver enzymes (such as AST and ALT) showed a significant increase in malathion exposed groups; and the activity of SOD, CAT, GSH and GST showed a significant increase ($p < 0.05$) when compared to the control groups of *Oreochromis niloticus* exposed to malathion. Also, pendimethalin has been reported to induce leukocytosis, hyperglobulinemia, hyperglycemia and increased lipid peroxidation and decreased levels of glutathione, SOD, catalase, glutathione reductase in the liver tissues [25]. At lower concentrations of the toxicant, the effects are probably minimal. In line with this, Jhamtani *et al.* [26] reported that hepatic GSH levels in Zebra fish were not altered when exposed to 2ppm of $\text{Pb}(\text{NO}_3)_2$. Also in line with the assertions above, increase in reduced glutathione (GSH) level in fish tissues was attributed to presence of defence system to protect the fish from the oxidative stress or could appear as an antioxidant adaptation to metal exposure [10]. In like manner, Hermenean *et al.* [11] observed that the liver has a higher capacity and adaptability to counteract ROS compared to kidney.

In the kidneys of the samples reactions of the fish to the presence of the toxicant indicated that T4 and T1 mean values are significantly higher than other treatments including the control in the first 2 weeks of exposure. This is probably an initial surge in the production level of the antioxidant to curtail the effects of the toxicant. The T3 mean values (of $30.84 \pm 0.10 \mu\text{g/ml}$) in the kidney of the fish exposed to the sub-lethal concentration of the toxicant was the highest at the end of the 8th week most likely because the body's defence mechanisms have to be up-regulated to deal with the prevailing conditions. This also probably became evident when the GSH mean values in the 10th week of exposure in the treatments were significantly lower than the control when the production capacity of the body must have dwindled. In line with this, Samuel *et al.* [27] reported how GSH production levels were significantly lower in the kidneys of *C. gariepinus* exposed to sub-lethal concentrations of lead nitrate at 28mg/L and 43mg/L, respectively. Also, Saliu and Bawa-Allah [13] reported reduced values of GST, GSH, SOD, CAT and MDA in comparison with the control when *C. gariepinus* was exposed to sub-lethal concentration of lead nitrate for 28 days.

Furthermore, in the gills of the samples exposed to sub-lethal concentration of the toxicant, there were general low production levels at the end of the 4th week of exposure. This is probably because the principal organs of detoxification such as liver and kidney were engaged much more than the gills that served as entrance to the toxicant. The highest GSH production level in the gill of the fish was $31.30 \pm 0.10 \mu\text{g/ml}$ in T2 at the 6th week of exposure. This is probably because at this period of the exposure there is the need to up-regulate the body's defence mechanisms to counteract the effects of the influx of the toxicant. In line with this, Pb has been reported to have significantly increased G-6-PDH activity and decreased GSH level in the gill, both Pb and Cd significantly increased MDA levels in liver and kidneys while Pb increased its level in gills of the fish and that the combination of Pb and Cd increased MDA level in livers and decreased GSH level in gills [28].

Vitamin A is a fat-soluble vitamin that plays an important role in vision, bone growth, reproduction, cell division, cell differentiation, growth and general maintenance in animals. From the analysis of the results of the fish samples exposed to sub-lethal concentrations of $\text{Pb}(\text{NO}_3)_2$ and supplemented with vitamin A, T2 mean values were significantly higher than other treatment in the 8th week of exposure. T1 mean values in the 10th and 12th weeks were significantly higher than in other treatments. In the lower concentrations (T1 and T2) the GSH production levels were high probably due to the interventions of the presence of vitamin A coupled with the lower concentrations. The highest mean value of $23.57 \pm 0.10 \mu\text{g/ml}$ in the Liver of the fish also obtained in T1 at the 10th week of exposure probably because as the duration of exposure increases there is constant need for the up-regulation of the body's defence mechanisms even at lower concentrations to ensure better physiology and survival. Udo [29] demonstrated how dietary supplementation with vitamin A led to improved growth rate in *C. gariepinus* and recommended that 833-1666 IU/Kg units of vitamin A should be included in the diet for optimum growth and efficient feed utilization. Also, feed consumption and conversion efficiency, protein efficiency ratio, growth, percentage growth, relative growth rate, assimilation and metabolism were greatly improved in feeds supplemented with 400mg/Kg units of vitamin A [30].

Similar scenario played out in the kidneys of the fish such that T1 mean values were significantly higher than other treatments at the 2nd week of exposure. Likewise, T2 and T1 mean values were significantly higher than other treatments in the 4th and 8th week of exposure, respectively. However, T4 mean values in the 10th and 12th weeks of exposure were significantly higher than other treatments. Also, the highest mean value of $58.74 \pm 0.07 \mu\text{g/ml}$ in the kidney was obtained in T4 at the 12th week of exposure. This is probably because as the duration and concentration increased the initial succor provided by the vitamin may have been overwhelmed; hence, the need for sustained increase in the production of the antioxidant to contain the deleterious effects to manageable level. In addition to the foregoing, the gills of the samples of the fish indicated that there were general low GSH production levels in the gills of the fish at the 4th week of exposure. The T2 and T1 mean values were significantly higher than other treatments at the 8th and 10th weeks of exposure, respectively. As the duration of exposure increases, the part of the body in constant touches and has larger surface area with the immediate environment, probably up-regulated its defence mechanism to deal with the effects at that point. This is also probably why T1 mean values were significantly higher than other treatments at the 12th week of exposure and produced the highest mean value of $52.72 \pm 0.07 \mu\text{g/ml}$.

In the samples of *C. gariepinus* exposed to sub-lethal concentrations of $\text{Pb}(\text{NO}_3)_2$ and supplemented with vitamin C, T1 mean values in the liver of the fish were significantly higher than other treatments at the end of the second week of exposure. T2 and T1 mean values in the 4th and 8th weeks of exposure, respectively were significantly higher than other treatments. This is probably because the rate of production of the antioxidant elicited by the presence of the toxicant out-weighs the effects at these stages in the treatments with the lower concentrations. However, as the duration of exposure increases there were probably constant needs for the up-regulation of the defence system as evident when the T2 and T4 mean values in the 10th and 12th weeks of exposure, respectively are significantly higher than other treatments; and thus the highest mean value of $25.79 \pm 0.07 \mu\text{g/ml}$ in the liver of the fish in this case, was obtained in T4 at the 12th week of exposure. This is also probably because, fish accumulate pollutants preferentially in their fatty tissues

like liver and the effects become apparent when concentrations in such tissues attain a threshold level [31]. In addition to this, Vitamins C and E, or in combination (as antioxidants) has been reported to have ameliorated the hepato-renal and testicular toxicity of abamectin, but were not completely protective, especially in liver tissue [32].

On the other hand, T2 mean values at the 8th week of exposure were significantly higher than other treatments. Also, the T4 and T2 mean values were significantly higher than other treatments in the 10th and 12th weeks of exposure. The GSH production level triggered in T2 probably continued through-out the experiment. This up-regulation of the defence system is probably why the highest mean value of $28.40 \pm 0.13 \mu\text{g/ml}$ was obtained in T2 at the 12th week of exposure. In line with this, Shaymaa *et al.* [33] reported how, supplementation of group III with vitamin C ameliorated the toxic effect induced by engine oil through improvement of kidney function parameters, elevation of the antioxidant status of tissue and improvement of the histological images of renal and muscle tissues.

Furthermore, the same scenario played out in the gills as the T4 mean values were significantly higher than other treatments in the 2nd, 4th, 8th and 10th weeks of exposure. The elicited GSH production levels were sustained till the end of the research probably in order to combat the effects of the toxicant and ensure survival. This is also probably why the highest mean value of $37.55 \pm 0.03 \mu\text{g/ml}$ in T4 was obtained in the gills of the fish in this case at the 8th week of exposure for sustained up-regulation of the defence system of the fish at the highest level of concentration and duration; and that the presence of the vitamin may have reduced the burden in lower concentrations. In line with this, Sahiti *et al.* [34] reported how supplementation of vitamins C and E either alone or jointly had significantly decreased ($p < 0.01$; $p < 0.05$) levels of accumulated heavy metals in investigated tissues compared to the control and exposed groups.

However, GOT and GTP were altered, GSH was significantly reduced and haemoglobin and haematocrit were substantially decreased in juvenile olive flounders (*Paralichthys olivaceus*) exposed to zinc for a period of 2 weeks [35].

From the results of the samples of fish exposed to sub-lethal concentrations of $\text{Pb}(\text{NO}_3)_2$ and supplemented with vitamin E, the T1 and T2 mean values were significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. There may be probably low utilization of this antioxidant in the lower concentrations than in higher concentrations.

The T2 and T3 mean values in the 10th and 12th weeks, respectively were significantly higher than other treatments. The highest mean value of $57.21 \pm 0.03 \mu\text{g/ml}$ in the liver of the exposed samples of the fish was obtained in T3 at the 12th week of exposure probably because the initial utilization may have been overtaken by constant sustained production of the antioxidant. In line with this, Kadry *et al.* [36] posited that oxidative damage in the liver tissues was evident in the increased levels of lipid peroxidation (LPO) and reduced glutathione content (GSH) in the samples of *C. gariepinus* exposed to chronic toxicity of Atrazine. The T1 and T3 mean values in the kidneys of the samples were significantly higher than other treatments in the 2nd and 4th weeks of exposure, respectively. Likewise, the T2 and T4 mean values in the 6th and 8th weeks of exposure, respectively were significantly higher than other treatments. This is probably because the level of utilization varies with the concentration and duration of exposure to the toxicant.

Also, the T1 and T3 mean values in the 10th and 12th weeks of exposure, respectively were significantly higher than other treatments. The highest mean value of $83.51 \pm 0.07 \mu\text{g/ml}$ in the kidneys of the sample was obtained in T1 at the 10th week of exposure. This could also be as a result of low utilization of the available antioxidant as well as the constant need for the up-regulation of the defence system to deal with the challenges posed by the toxicant. Furthermore, T4 and T3 mean values in the gills of the fish were also significantly higher than other treatments in the 6th and 10th weeks of exposure, respectively. The T3 mean value of $63.29 \pm 0.07 \mu\text{g/ml}$ obtained at the 12th week was the highest and was significantly higher than other treatments.

Also, the need for constant up-regulation of the defence system may have come to play. In line with this, Azeez and Braimah [37] reported how the depleted endogenous antioxidants such as GPx, GST and GSH were restored in fish fed vitamin E-supplemented feeds when *C. gariepinus* were exposed to potassium dichromate. In like manner, Azeez and Braimah [38] reported how the depleted endogenous antioxidants such as GPx, GST and GSH were restored in fish fed vitamin E-supplemented feeds when *C. gariepinus* were exposed to copper sulphate.

5. Conclusion

The liver of the samples exposed to Pb only group displayed higher level of response to the toxicant with the highest GSH produced in the lowest concentration in comparison to other fish organs. The GSH highest mean value produced in the liver was $82.04 \pm 0.13 \mu\text{g/ml}$.

In the PbVA group the response was more in the kidney in the highest concentration. The kidneys of the samples produced $58.74 \pm 0.07 \mu\text{g/ml}$ as the highest GSH mean value.

There were general low levels of production in all organs of the fish in the PbVC group. The highest GSH value was produced in the gill with $37.55 \pm 0.03 \mu\text{g/ml}$.

The kidneys of the PbVE group exhibited the highest level of GSH production in comparison to other organs. There were also improved production levels in the gills and liver of the fish. The highest GSH mean value in the kidney was $83.51 \pm 0.07 \mu\text{g/ml}$.

The kidneys and liver of *C. gariepinus* in this research were fully engaged in mitigating the effects of the toxicant in the presence of the vitamins. Higher concentrations of the vitamins should be administered to ensure higher manifestation of the effects of the vitamin in ameliorating the effects of the toxicant.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is conflict of interest whatsoever.

References

- [1] Adewolu MA, Adeniji CA, Adejobi AB. Feed utilization, growth and survival of *Clarias gariepinus* (Burchell, 1822) fingerlings cultured under different photoperiods. *Aquaculture*. 2008; 283: 64-67.
- [2] Karami A, Christianus A, Ishak Z, Courtenay SC, Sayed MA, Noor AM, Noor SH. Effect of triploidization on juvenile African catfish (*Clarias gariepinus*). *Aquaculture International*. 2010; 18: 851-858.
- [3] FAO. Food Security: concepts and measurement. Rome: Food and Agriculture Organization of the United Nations. In FAO (Ed.), *Trade Reforms and Food Security*. 2003; 25-34.
- [4] Mehana EE, Khafaga AF, Elblehi SS, AbdEl-Hack ME, Naiel MAE, Bin-Jumah M, SarahI Othman SI, Allam AA. Biomonitoring of Heavy Metal Pollution Using *Acanthocephalans* Parasite in Ecosystem: An Updated Overview. *Animals*. 2020; 10: 811, 1-15.
- [5] Athar T, Waris AA, Nisar M. A review on toxicity and environmental implications of heavy metals. *Emergent Life Sciences Research*. 2018; 4(2): 31-37.
- [6] Ahmed NF, Sadek KM, Soliman MK, Khalil RH, Khafaga AF, Ajarem JS, Maodaa SN, Allam AA. Moringa Oleifera Leaf Extract Repairs the Oxidative Misbalance following Sub-Chronic Exposure to Sodium Fluoride in Nile Tilapia *Oreochromis niloticus*. *Animals*. 2020; 10: 626.
- [7] Ayyat MS, Ayyat AM, Naiel MA, Al-Sagheer AA. Reversal effects of some safe dietary supplements on lead contaminated diet induced impaired growth and associated parameters in Nile tilapia. *Aquaculture*. 2020; 515: 734580.
- [8] Saglam D, Atli G, Dogan Z, Baysoy E, Gurler C, Eroglu A, Canli M. Response of the antioxidant system of freshwater fish (*Oreochromis niloticus*) exposed to metals (Cd, Cu) in different hardness. *Turkish Journal of Aquatic Science*. 2014; 14: 43–52.
- [9] El-Shenawy NS, Al-Ghamdi OA. Phenthoate induced oxidative stress in fresh isolated mice hepatocytes. Alleviation by ascorbic acid. *Toxicology, Environment and Health Science*. 2014; 6(2): 67–80.
- [10] Marzouk M, Abdelkader EM, El-zayat M, Aboushady A. Assessing environmental impact indicators in road construction projects in developing countries. *Sustainability*. 2017; 9(5): 843.

- [11] Hermenean A, Damache G, Albu P, Ardelean A, Ardelean G, Ardelean DP, Dinischiotu A. Histopathological alterations and oxidative stress in liver and kidney of *Leuciscus cephalus* following exposure to heavy metals in the Tur River, North Western Romania. *Ecotoxicology and Environmental Safety*. 2015; 119: 198-205.
- [12] Luschak VI. Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology*. 2011; 1: 13-30.
- [13] Saliu JK, Bawa-Allah KA. Toxicological effects of lead and zinc on the antioxidant enzyme activities of post juvenile *Clarias gariepinus*. *Resources and Environment*. 2012; 2(1): 21-26.
- [14] Sharma DK, Ansari BA. Effects of deltamethrin on CAT, LPO and GSH in tissues of zebra fish, *Danio rerio*. *Research Journal of Environmental Toxicology*. 2013; 7(1): 38-46.
- [15] Sissein EA, Diepreye E, Tonkiri, A, Asara AA. Nonenzymatic antioxidant levels as pollution biomarkers in liver of *Clarias gariepinus* harvested from Gbaratoru Swamp: a polluted site in Bayelsa State. *Journal of Chemical, Biological and Physical Sciences*. 2014; 4(3): 2325-2332.
- [16] Ayoola SO, Idowu AA, Babalola OE, Ademoye MK. Heavy metals accumulation and Enzymatic Biomarker in Commercially Important Fish Species (*Hemichromis fasciatus* and *Chrysichthys nigrodigitatus*) at the Landing Site, Bariga, Lagos Lagoon. *World Applied Sciences Journal*. 2014; 29(2): 298-306.
- [17] Pratt TC, Cullen FT, Sellers CS, Thomas WL, Madensen TD, Daigle LE, Fearn NE, Gau J. The empirical status of social learning theory: a meta-analysis. *Justice Quarterly*. 2010; 27(6): 765-802.
- [18] Yolanda M, Maria LI. Use of antioxidants for the treatment of cognitive and behavioural disorders in individuals with fragile X syndrome. 2012; WO2012080554.
- [19] Organization for Economic Cooperation and Development. Maximum Acceptable Contaminants: guidance safety level. Fresh water fish (RPWS 1991). 2007; 12-28.
- [20] Ellman GL. Tissue sulfhydryl groups. *Archive Biochemistry and Biophysics*. 1959; 82: 70-77.
- [21] Rajagopalan R, Kode A, Penumatha SV, Kallikat NR, Venugopal PM. Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. *Journal of Pharmacy and Pharmaceutical Science*. 2004; 83: 2747-2752.
- [22] Lamidi IY, Akefe IO. Mitigative effects of antioxidants in lead toxicity. *Clinical Pharmacology and Toxicology*. 2017; 1(1): 3.
- [23] Saddick S, Afifi M, Zinada AO. Effects of zinc nano-particles on oxidative stress-related genes and antioxidant enzymes activity in the brain of *Oreochromis niloticus* and *Tilapia zilli*. *Saudi Journal of Biological Sciences*. 2017; 24: 1672-1678.
- [24] Ibrahim AT. Biochemical and histopathological response of *Oreochromis niloticus* to malathion hepatotoxicity. *Journal of Royal Science*. 2019; 1(1): 10-15.
- [25] Zaahkook SAM, Abdel-Raheid HG, Ghanem MH, Al-sharkawy SM. Physiological and oxidative stress biomarkers in the freshwater catfish (*Clarias gariepinus*) exposed to pendimethalin-based herbicide and recovery with EDTA. *International Journal of Advanced Research*. 2016; 4(10): 243-264.
- [26] Jhamtani RC, Shukla S, Dahiya MS, Agarwal R. Evaluation of acute effects of lead at sub-lethal concentrations in zebra fish. *Research Journal of Environmental Toxicology*. 2017; 11: 97-103.
- [27] Samuel PO, Abiola OP, Arimoro FO, Ayanwale AV, Mohammed AZ, Auta YI. Effects of lead nitrate on glutathione levels in *Clarias gariepinus* (Burchell, 1822) post juvenile. *Journal of Aquatic Sciences*. 2017; 32(2): 223-229.
- [28] Elarabany N, Bahnasawy M. Comparative and interactive biochemical effects of sub-lethal concentrations of cadmium and lead on some tissues of the African catfish (*Clarias gariepinus*). *Toxicological Research*. 2019; 35(3): 249-255.
- [29] Udo IU. Effects of dietary vitamin A level on growth, feed utilization and survival of juvenile North African catfish (*Clarias gariepinus*). *Livestock Research for Rural Development*. 2017; 29(2).
- [30] Jeyaraj N, Rajan MR, Santhanam P. Dietary requirement of vitamin A and biochemical composition of common carp, *Cyprinus carpio* var *communis*. *Journal of Fisheries and Aquatic Science*. 2012; 7: 65-71.
- [31] Omar WA, Saleh YS, Marie MAS. Integrating multiple fish biomarkers and risk assessment as indicators of metal pollution along the Red Sea coast of Hodeida, Yemen Republic. *Ecotoxicology and Environmental Safety*. 2014; 110: 221-231.

- [32] Magdy BW, Mohamed FE, A Seleem AA, Sarhan-Rana S. Ameliorative effects of antioxidants (vitamins C and E) against abamectin toxicity in liver, kidney and testis of male albino rats. *The Journal of Basic and Applied Zoology*. 2016; 77: 69–82.
- [33] Shaymaa HM, Ebtihal MME, Rehab AG, Mona KG. Molecular, biochemical and histopathological studies on the ameliorative effects of vitamin C on the renal and muscle tissues of Nile tilapia fish (*Oreochromis niloticus*) affected by the usage of engine oil. *Aquaculture Research*. 2019; 50(11): 3357-3368.
- [34] Sahiti H, Bislimi K, Rexhepi A, Dalo E. Metal Accumulation and Effect of Vitamin C and E in Accumulated Heavy Metals in Different Tissues in Common Carp (*Cyprinus carpio*) Treated with Heavy Metals. *Polish Journal of Environmental Studies*. 2020; 29(1): 799-805.
- [35] Kim J, Kang J. Toxic effects on hematological parameters and oxidative stress in juvenile olive flounder, *Paralichthys olivaceus* exposed to water borne zinc. *Aquaculture Reports*. 2019; 15: 100225.
- [36] Kadry S, Marzouk MS, Amer AM, Hana MI. Vitamin E as an antioxidant in female African catfish (*Clarias gariepinus*) exposed to chronic toxicity of Atrazine. *Egyptian Journal of Aquatic Biology and Fisheries*. 2012; 16(2): (1110-6131), 83-98.
- [37] Azeez OI, Braimah SF. Mitigating effect of vitamin-E on copper sulphate-induced toxicity in African catfish (*Clarias gariepinus*). *European Journal of Medical and Health Sciences*. 2020a; 2(4).
- [38] Azeez OI, Braimah SF. Protective effects of vitamin E on potassium dichromate-induced haemotoxicity and oxidative stress in African catfish (*Clarias gariepinus*). *Asian Journal of Environment & Ecology*. 2020b; 13(2): 18-31.