



## Original article

Bioaccumulation of lead nitrate in tissues and its effects on hematological and biochemical parameters of *Clarias gariepinus*Abdel-Wahab A. Abdel-Warith<sup>a,b,\*</sup>, El-Sayed M.I. Younis<sup>a</sup>, Nasser A. Al-Asghar<sup>a</sup>, Ahmed M. Rady<sup>a</sup>, Hasan Y. Allam<sup>b</sup><sup>a</sup> Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia<sup>b</sup> Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt

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## ABSTRACT

*Clarias gariepinus*, weighing  $119.18 \pm 5.21$  g, were exposed to 0%, 20%, 40%, and 60% of the LC<sub>50</sub> lead nitrate, Pb(NO<sub>3</sub>)<sub>2</sub>, which represents the following treatments 0 (control), 16, 32, and 48 mg/l, respectively, for a period of 10 and 20 days. The results showed that the bioaccumulation of Pb(NO<sub>3</sub>)<sub>2</sub> in gills were significantly increased ( $p < 0.05$ ) after 10 days having  $0.17 \pm 0.07$ ,  $5.05 \pm 1.04$ ,  $6.01 \pm 0.82$ , and  $9.61 \pm 1.76$  mg/100 g wet weight, respectively, for the treatments. However, after 20 days these values increased ( $0.17 \pm 0.07$ ,  $4.34 \pm 1.27$ ,  $10.83 \pm 0.97$ , and  $19.18 \pm 2.40$  mg/100 g) for 0%, 20%, 40%, and 60% of LC<sub>50</sub> Pb(NO<sub>3</sub>)<sub>2</sub> respectively. There was an increase with each increasing concentration level of Pb(NO<sub>3</sub>)<sub>2</sub> as compared with that of the control group. The accumulation of Pb(NO<sub>3</sub>)<sub>2</sub> in the liver, showed a significant increase ( $p < 0.05$ ) with the increasing period of exposed and Pb concentration with LC<sub>50</sub> values ranging between  $3.32 \pm 0.91$  and  $4.42 \pm 0.78$  after 10 days as compared with that of the control group  $0.08 \pm 0.02$  mg/100 g wet weight. Although white muscles and skin displayed lower values of bioaccumulation than gills and liver after 10 days, after 20 days the results were slightly more increased in the white muscles than in the skin. However, the observed pattern of increase was the same compared to that of the control group. Therefore, hematological parameters, such as red blood cells (RBCs), hemoglobin (Hb), and hematocrit (Hct) showed significant ( $p < 0.05$ ) concentration-dependent decreases in fish exposed to Pb(NO<sub>3</sub>)<sub>2</sub> during both periods. However, the values of white blood cells (WBCs) showed a significant reduction when levels of Pb concentration increased. Hepatic enzyme activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) displayed a significant increase with increasing concentrations and exposure time.

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## 1. Introduction

Heavy metals are produced from different sources, including natural and anthropogenic sources (Bauvais et al., 2015). Heavy metal contamination of the aquatic environment results from direct atmospheric deposition or geological weathering or through the evacuation of agricultural, municipal, residential, and manufac-

tural waste products, as well as via agricultural wastewater treatment (Maier et al., 2014; Dhanakumar et al., 2015; Garcia et al., 2015). Fish exist at the end of the aquatic food chain and can accumulate heavy metals and pass them through the food chain to humans causing chronic or acute diseases (Al-Yousuf et al., 2000). Research in the field and laboratory has revealed that the accumulation of pollutants in tissue is fundamentally dependent on water levels of metals and time of exposure. Fish have the capability to consume and accumulate heavy metals directly from the environment or indirectly from other aquatic organisms, such as smaller fish, crustaceans, zooplankton, and phytoplankton (Polat et al., 2015). Pollutants accumulate in fish in their organs, such as the liver, and the effects become obvious when the levels in such tissues attain a high concentration (Omar et al., 2014). Therefore, fish can absorb heavy metals either via ingestion of polluted food through the alimentary duct or through the gills and skin (Drevnick et al., 2006; Sfakianakis et al., 2015). After the uptake

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of metals by fish, they are transferred via the blood stream to the tissues and organs where they form precipitations (Adeyemo et al., 2010; Fazio et al., 2014).

Lead (Pb) is a persistent heavy metal that has been described as a priority hazardous substance (Sfakianakis et al., 2015). Bioaccumulation of Pb in aquatic organisms occurs through the from water and diet. Accumulation of Pb occurs in various fish organs, such as the liver, spleen, and kidneys, as well as the digestive system and gills (Jezierska and Witeska, 2006). Lead bioaccumulation in various fish species has been evaluated in numerous studies (Adeyemo, 2007; Ikeogu et al., 2016; Khalesi et al., 2017) and has been shown to lead to disorders in fish. The characteristic symptoms of lead poisoning include changes in the blood parameters with acute damage to red and white blood cells (WBCs) that cause harmful effects in the nervous system (El-Badawi, 2005). Several metals, such as lead and cadmium, are classified as toxic because they are highly injurious even at minimum levels and when taken up for long periods (Guardiola et al., 2013). Biochemical and physiological descriptions of fish and other aquatic organisms under pollution stress serve as important biomarkers in aquatic ecosystem control (Shalaby et al., 2005; Al-Asgah et al., 2015).

This study was conducted to assay the bioaccumulation of Pb ( $\text{NO}_3$ )<sub>2</sub> in some tissues, as well as the hematological and biochemical changes in *C. gariepinus* exposed to sublethal concentrations of Pb, which is the most common form of the pollutant in waterways of many countries worldwide.

## 2. Materials and methods

### 2.1. Experimental fish

The samples of *C. gariepinus* were brought from fish hatcheries belonging to King Abdulaziz City for Science and Technology, Mozahmiya, Saudi Arabia, and fish were transported to the lab in special tanks supplied by aeration units then acclimation for 2 weeks to the laboratory conditions before the experiment.

### 2.2. Experimental design

One hundred and sixty fish (average weight  $119.18 \pm 5.21$  g), were divided into four groups. Unexposed group, was treated as a control the remaining three groups were exposed to 20%, 40%, and 60% of the LC<sub>50</sub> for Pb( $\text{NO}_3$ )<sub>2</sub>, which represents 16, 32, and 48 mg/l respectively, for 10 and 20 days. Glass aquaria with a capacity of 100 l ( $100 \times 60 \times 50$  cm) were used, with 20 fish for each aquaria as a duplicate group. The Pb( $\text{NO}_3$ )<sub>2</sub> concentrations were based on the 96 h LC<sub>50</sub> of Pb( $\text{NO}_3$ )<sub>2</sub> for *C. gariepinus* that was previously estimated to be 80.6 mg/l by Okareh and Akande (2015). Dissolved oxygen was added with diffused air, fish were fed two times a day using commercial diet from Maram Feed Factory containing approximately 32% crude protein at the rate of 2% of the biomass.

### 2.3. Hematological and biochemical analyses

At the termination of the experimental period, fish were fasted for 1 day prior to being anesthetized with buffered MS222 (50 mg/l). Then, blood samples were taken from five fish of each group from the caudal vein of the anesthetized fish with a sterile syringe using heparin as an anti-coagulant. Part of the blood sample was centrifuged at 800g for 10 min to separate the plasma of the blood using a Hettich – Zentrifugen (Mikro 22R), and the non-hemolyzed plasma was maintained at  $-20$  °C for subsequent biochemical analyses. The other portion of the blood sample was used to estimate the complete blood count (CBC) using Mindray BC-2800Vet

(Auto Hematology Analyzer) to count the red blood cells (RBCs) and WBCs using the methods of Dacie and Lewis (2006) and Stoskopf (1993), respectively, and determine the hemoglobin (Hb) content using the method described by Van Kampen and Zijlstra (1961). The hematocrit (Hct) value was calculated based on the formulas described by Britton (1963). Furthermore, mean cell hemoglobin (MCH), MCH concentration (MCHC), and mean cell volume (MCV) were determined.

The hepatic enzyme levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed using a SEMI-Automatic Analyzer BTS-350 according to the procedure described by Reitmen and Frankel (1957).

### 2.4. Tissue analysis

Fish were dissected and the gills, liver, and white muscle tissues were excised and freeze-dried, tissues were cleaned, rinsed in double deionized water, and blotted on filter paper. Tissues were dried at 80 °C to reach a constant weight, weighed (to approximately 0.3 g dry), and digested. The digested solutions were diluted by double deionized water and subjected to an atomic absorption spectrophotometer (Varian – Spectra, 220 FS) to measure the lead level concentration in fish tissues according the method described by Allen (1989).

### 2.5. Statistical analysis

A one-way analysis of variance using the Minitab software program and Tukey's least significance difference test were used to compare the variations among the treatments. Differences were considered statistically significant at  $P < 0.05$ .

## 3. Results

### 3.1. Bioaccumulation of Pb( $\text{NO}_3$ )<sub>2</sub> in gills

Accumulation of Pb( $\text{NO}_3$ )<sub>2</sub> was higher in gills than liver, white muscles, and skin in *C. gariepinus*. The concentration of Pb in *C. gariepinus* exposed to 20%, 40%, and 60% of LC<sub>50</sub> Pb( $\text{NO}_3$ )<sub>2</sub> with significant differences ( $p < 0.05$ ), which showed an increase in the accumulation of the metal in gills with increasing of Pb( $\text{NO}_3$ )<sub>2</sub> concentration after 10 days with  $0.17 \pm 0.07$ ,  $5.05 \pm 1.04$ ,  $6.01 \pm 0.82$ , and  $9.61 \pm 1.76$  mg/100 g wet weight for the control, 20%, 40%, and 60% of LC<sub>50</sub> Pb( $\text{NO}_3$ )<sub>2</sub>, respectively. However, the values were much higher in the gills ( $4.34 \pm 1.27$ ,  $10.83 \pm 0.97$ , and  $19.18 \pm 2.40$  mg/100 g) for 20%, 40%, and 60% after a 20 day exposure period that increased with increasing concentration levels of Pb( $\text{NO}_3$ )<sub>2</sub> when compared to that of the control group ( $0.16$  mg/100 g) (Table 1).

### 3.2. Bioaccumulation of Pb( $\text{NO}_3$ )<sub>2</sub> in liver

Table 1 also illustrates that the bioaccumulation of Pb( $\text{NO}_3$ )<sub>2</sub> in liver tissues after 10 days was  $0.076 \pm 0.02$ ,  $3.32 \pm 0.91$ ,  $3.37 \pm 0.52$ , and  $4.42 \pm 0.78$  mg/100 g wet weight for the control, 20%, 40%, and 60% of LC<sub>50</sub> Pb( $\text{NO}_3$ )<sub>2</sub>, respectively, with significant differences ( $p < 0.05$ ) for the exposed fish and that of the control treatment. However, after 20 days high accumulations showed the same pattern, with significant increases when the concentration of Pb( $\text{NO}_3$ )<sub>2</sub> increased (Table 1), with  $0.123 \pm 0.08$ ,  $3.06 \pm 0.31$ ,  $3.94 \pm 0.38$ , and  $5.54 \pm 2.63$  mg/100 g wet weight for the control, 20%, 40%, and 60% of LC<sub>50</sub> Pb( $\text{NO}_3$ )<sub>2</sub>, respectively, with significant differences ( $p < 0.05$ ) between the exposed fish and that of the control group.

**Table 1**  
Determination of lead in the different tissues of *C. gariepinus* (mg/100 g wet weight) exposed to sublethal concentration for 10 and 20 days.

	After 10-day exposure				After 20-day exposure			
	Control	20% LC <sub>50</sub> (16 mg/l)	40% LC <sub>50</sub> (32 mg/l)	60% LC <sub>50</sub> (48 mg/l)	Control	20% LC <sub>50</sub> (16 mg/l)	40% LC <sub>50</sub> (32 mg/l)	60% LC <sub>50</sub> (48 mg/l)
Gills	0.17 ± 0.07 <sup>c</sup>	5.05 ± 1.04 <sup>b</sup>	6.01 ± 0.82 <sup>b</sup>	9.60 ± 1.76 <sup>a</sup>	0.16 ± 0.05 <sup>d</sup>	4.34 ± 1.27 <sup>c</sup>	10.83 ± 0.97 <sup>b</sup>	19.18 ± 5.40 <sup>a</sup>
Liver	0.08 ± 0.02 <sup>c</sup>	3.32 ± 0.91 <sup>b</sup>	3.38 ± 0.50 <sup>ab</sup>	4.42 ± 0.78 <sup>a</sup>	0.12 ± 0.08 <sup>c</sup>	3.06 ± 0.31 <sup>b</sup>	3.94 ± 0.38 <sup>ab</sup>	5.54 ± 2.63 <sup>a</sup>
White Muscles	0.23 ± 0.13 <sup>b</sup>	0.35 ± 0.38 <sup>ab</sup>	0.39 ± 0.37 <sup>ab</sup>	0.96 ± 0.78 <sup>a</sup>	0.19 ± 0.17 <sup>c</sup>	0.34 ± 0.31 <sup>bc</sup>	0.74 ± 0.17 <sup>b</sup>	1.27 ± 0.47 <sup>a</sup>
Skin	0.09 ± 0.05 <sup>c</sup>	0.38 ± 0.20 <sup>bc</sup>	0.55 ± 0.24 <sup>b</sup>	1.14 ± 0.51 <sup>a</sup>	0.07 ± 0.05 <sup>c</sup>	0.18 ± 0.07 <sup>bc</sup>	0.21 ± 0.06 <sup>ab</sup>	0.32 ± 0.16 <sup>a</sup>

Values in the same row with the same superscript are not significantly different ( $p > 0.05$ ).

### 3.3. Bioaccumulation of $Pb(NO_3)_2$ in white muscles

The results obtained after 10 days were  $0.35 \pm 0.38$ ,  $0.39 \pm 0.38$ , and  $0.96 \pm 0.78$  mg/100 g wet weight for fish exposed to 20%, 40%, and 60% of LC<sub>50</sub>  $Pb(NO_3)_2$ , respectively, with significant increases ( $p < 0.05$ ) in response to increased  $Pb(NO_3)_2$  concentrations between the test and control groups ( $0.23 \pm 0.13$ ). A similar trend was observed for fish exposed for 20 days, and the results obtained were  $0.34 \pm 0.31$ ,  $0.74 \pm 0.17$ , and  $1.27 \pm 0.47$  mg/100 g for fish exposed to 20%, 40%, and 60% of LC<sub>50</sub>  $Pb(NO_3)_2$ , respectively (Table 1).

### 3.4. Bioaccumulation of $Pb(NO_3)_2$ in skin

A similar trend in relation to fish skin exposed to different levels of  $Pb(NO_3)_2$  for 10 days occurred. The data recorded were  $0.09 \pm 0.01$ ,  $0.38 \pm 0.20$ ,  $0.55 \pm 0.24$ , and  $1.14 \pm 0.51$  mg/100 g wet weight for the control and fish exposed to 20%, 40%, and 60% of LC<sub>50</sub>  $Pb(NO_3)_2$ , respectively, with significant increases ( $p < 0.05$ ) when the concentrations of  $Pb(NO_3)_2$  increased for the treatments and that of the control group. In the same way, results obtained after 20 days showed a high amount of  $Pb(NO_3)_2$  in fish skin exposed for 20 days and the results were  $0.07 \pm 0.05$ ,  $0.18 \pm 0.07$ ,  $0.21 \pm 0.06$  and  $0.32 \pm 0.16$  mg/100 g for the control and exposed fish to 20%, 40%, and 60% of LC<sub>50</sub>  $Pb(NO_3)_2$ , respectively (Table 1).

### 3.5. Hematological and biochemical parameters

#### 3.5.1. After 10 days of exposure

Hematological parameters of *C. gariepinus* exposed to  $Pb(NO_3)_2$  for 10 days showed significant differences ( $p < 0.05$ ) when compared with that of the control group (Table 2). The values decreased when the levels of  $Pb(NO_3)_2$  increased and the RBC counts were  $2.92 \pm 0.18$ ,  $2.71 \pm 0.12$ ,  $2.61 \pm 0.09$ , and

$2.37 \pm 0.23 \times 10^6/mm^3$  for the control and catfish exposed to 20%, 40%, and 60% of the  $Pb(NO_3)_2$  LC<sub>50</sub>, respectively. Additionally, the values of hemoglobin content (Hb) were  $12.82 \pm 0.42$ ,  $12.23 \pm 0.42$ ,  $11.21 \pm 0.82$ , and  $10.35 \pm 0.71$  g/dl for the control and fish exposed to 20%, 40%, and 60% of the LC<sub>50</sub> for  $Pb(NO_3)_2$ , respectively. Furthermore, the hematocrit (Hct) exhibited the same trend with  $42.34\% \pm 2.24\%$ ,  $40.01\% \pm 1.94\%$ ,  $37.15\% \pm 2.34\%$ , and  $33.11\% \pm 2.82\%$  for the control and exposed fish (Table 2). A similar trend was observed for MCHC (g/dl) and MCH (pg), which increased significantly in proportion with the increase in  $Pb(NO_3)_2$  concentration. However, MCV (fl) increased with the increase of  $Pb(NO_3)_2$  levels in the environment after 10 days of exposure. Data recorded were  $131.50 \pm 4.16$ ,  $136.38 \pm 3.30$ ,  $139.54 \pm 2.61$ , and  $148.02 \pm 4.35$  fl for the control and exposure groups. Therefore, results of WBCs ( $\times 10^9/l$ ) were  $189.44 \pm 4.04$ ,  $179.10 \pm 4.19$ , and  $168.34 \pm 6.35$  ( $\times 10^9/l$ ) for fish exposed to 20%, 40%, and 60% of LC<sub>50</sub>  $Pb(NO_3)_2$ , respectively. Compared with the values of the control group ( $201.76 \pm 3.04 \times 10^9/l$ ), those of the treatment groups decreased proportionally with increase in pollutant. Additionally, results for lymphocytes and monocytes followed this trend, and the counts decreased with increase in  $Pb(NO_3)_2$ . Moreover, data obtained for PLT ( $10^9/l$ ) showed significant differences ( $p < 0.05$ ) between the control high volumes  $57.60 \pm 3.59$  and fish exposed to different concentrations of  $Pb(NO_3)_2$ ; the values were  $48.40 \pm 3.94$ ,  $40.40 \pm 4.84$ , and  $29.00 \pm 2.45$  ( $10^9/l$ ) (Table 2).

Data for biochemical parameters, including the hepatic enzymes AST and ALT activities showed significant differences ( $p < 0.05$ ) between unexposed (control) and exposed groups, wherein values increased with each increase in  $Pb(NO_3)_2$  concentration. The data obtained for AST were  $70.40 \pm 6.05$ ,  $117.40 \pm 6.10$ ,  $163.60 \pm 4.61$ , and  $168.80 \pm 4.13$ , and for ALT were  $17.00 \pm 1.82$ ,  $18.2 \pm 2.15$ ,  $19.60 \pm 2.09$ , and  $22.40 \pm 2.32$  IU/l for the control and fish exposed to 20%, 40%, and 60% of LC<sub>50</sub>  $Pb(NO_3)_2$ , respectively, after 10 days (Table 2).

**Table 2**  
Hematological and biochemical parameters of *C. gariepinus* exposed to sublethal concentration of  $Pb(NO_3)_2$  for 10 days. (Mean ± SE  $n = 5$ ).

Parameters	Treatments			
	Control	20% LC <sub>50</sub>	40% LC <sub>50</sub>	60% LC <sub>50</sub>
RBC (count $\times 10^6/mm^3$ )	$2.92 \pm 0.18^a$	$2.71 \pm 0.12^{ab}$	$2.61 \pm 0.09^{ab}$	$2.37 \pm 0.23^b$
Hb (g/dl)	$12.82 \pm 0.42^a$	$12.23 \pm 0.42^a$	$11.21 \pm 0.82^{ab}$	$10.35 \pm 0.71^b$
Hct (%)	$42.34 \pm 2.24^a$	$40.01 \pm 1.94^{ab}$	$37.15 \pm 2.34^{ab}$	$33.11 \pm 2.82^b$
MCHC (g/dl)	$35.94 \pm 2.96^a$	$31.46 \pm 1.80^{ab}$	$30.06 \pm 1.47^{ab}$	$27.70 \pm 3.31^b$
MCH (pg)	$52.04 \pm 1.66^b$	$51.12 \pm 3.27^{ab}$	$47.50 \pm 2.09^{ab}$	$44.06 \pm 2.31^a$
MCV (fl)	$131.50 \pm 4.16^b$	$136.38 \pm 3.30^b$	$139.54 \pm 2.61^{ab}$	$148.02 \pm 4.35^a$
WBCs ( $\times 10^9/l$ )	$201.76 \pm 3.04^a$	$189.44 \pm 4.04^{ab}$	$179.10 \pm 4.19^{ab}$	$168.34 \pm 6.35^b$
Lymphocyte	$147.50 \pm 1.70^a$	$142.12 \pm 4.49^a$	$145.21 \pm 6.56^a$	$137.13 \pm 5.41^a$
Monocyte	$11.49 \pm 0.487^a$	$9.71 \pm 0.248^a$	$9.02 \pm 0.400^a$	$8.90 \pm 0.411^a$
PLT ( $10^9/l$ )	$57.60 \pm 3.59^a$	$48.40 \pm 3.94^{ab}$	$40.40 \pm 4.84^b$	$29.00 \pm 2.45^c$
AST (IU/l)	$70.40 \pm 6.05^c$	$117.40 \pm 6.10^b$	$163.60 \pm 4.61^a$	$168.80 \pm 4.13^a$
ALT (IU/l)	$17.00 \pm 1.82^a$	$18.20 \pm 2.15^a$	$19.60 \pm 2.09^a$	$22.40 \pm 2.32^a$

Values in the same row with the same superscript are not significantly different ( $p > 0.05$ ).

### 3.5.2. After 20 days of exposure

Hematological and biochemical parameters of *C. gariepinus* exposed to various levels of  $\text{Pb}(\text{NO}_3)_2$  for 20 days are illustrated in Table 3. There was a significant difference ( $p < 0.05$ ) as RBCs decreased in comparison with that of the control group. The results obtained were  $2.22 \pm 0.24$ ,  $1.89 \pm 0.14$ ,  $1.76 \pm 0.14$ , and  $1.49 \pm 0.07$  ( $\times 10^6/\text{mm}^3$ ) for the control group, and *C. gariepinus* exposed to 20%, 40%, and 60% of the  $\text{LC}_{50}$  for  $\text{Pb}(\text{NO}_3)_2$ , respectively. Similar results for hemoglobin content (Hb) were also seen and values decreased significantly ( $p < 0.05$ ) after fish were exposed for 20 days to  $\text{Pb}(\text{NO}_3)_2$ ; the results were  $11.11 \pm 1.34$ ,  $9.93 \pm 0.65$ ,  $9.03 \pm 0.98$ , and  $8.01 \pm 1.11$  g/dl and data for Hct (%) were  $29.56 \pm 1.96\%$ ,  $26.68 \pm 2.74\%$ ,  $23.81 \pm 3.61\%$ , and  $20.24 \pm 2.82\%$  for the control and fish exposed to 20%, 40%, and 60% of the  $\text{LC}_{50}$  for  $\text{Pb}(\text{NO}_3)_2$ , respectively. Furthermore, a similar trend was observed for data obtained for MCHC (g/dl) and MCH (pg) that decreased with each increase in  $\text{Pb}(\text{NO}_3)_2$  concentration. However, data for MCV (fl) observed that there was a significant difference wherein values decreased when the concentration of  $\text{Pb}(\text{NO}_3)_2$  increased. Detected data were  $180.38 \pm 3.69$ ,  $170.00 \pm 2.40$ ,  $159.16 \pm 2.20$ , and  $140.94 \pm 4.17$  fl for the control and fish exposed to different concentrations of  $\text{Pb}(\text{NO}_3)_2$ . Moreover, results of WBCs ( $\times 10^9/\text{l}$ ) revealed a significant difference between unexposed and exposed groups. Data recorded were  $187.74 \pm 7.46$ ,  $158.92 \pm 6.38$ , and  $136.82 \pm 6.63$  for fish exposed to 20%, 40%, and 60% of the  $\text{LC}_{50}$  of  $\text{Pb}(\text{NO}_3)_2$  when compared to that of unexposed fish (control  $198.54 \pm 7.36 \times 10^9/\text{l}$ ). Similarly, data for lymphocytes and monocytes revealed there was a significant difference between groups wherein values decreased as the concentration of  $\text{Pb}(\text{NO}_3)_2$  increased (Table 3). A similar trend was detected for PLT ( $10^9/\text{l}$ ) where values decreased with each increase in  $\text{Pb}(\text{NO}_3)_2$  level and the data were  $84.24 \pm 2.58$ ,  $71.96 \pm 2.51$ ,  $64.80 \pm 1.89$ , and  $61.16 \pm 4.36$  ( $10^9/\text{l}$ ) for the control and fish exposed to 20%, 40%, and 60% of the  $\text{LC}_{50}$  for  $\text{Pb}(\text{NO}_3)_2$ , respectively (Table 3).

Therefore, results of hepatic enzyme parameters AST and ALT showed a significant differences ( $p < 0.05$ ) between that of the control and exposed fish that were subjected to increasing concentrations of  $\text{Pb}(\text{NO}_3)_2$  after 20 days of exposure. Data obtained for AST (IU/l) were  $189.60 \pm 5.90$ ,  $260.00 \pm 8.09$ ,  $289.60 \pm 9.85$ , and  $330.80 \pm 8.91$  and for ALT were  $21.00 \pm 4.02$ ,  $24.40 \pm 3.67$ ,  $28.60 \pm 3.49$ , and  $33.20 \pm 2.08$  for the control and fish exposed to 20%, 40%, and 60% of the  $\text{LC}_{50}$  for  $\text{Pb}(\text{NO}_3)_2$ , respectively (Table 3).

## 4. Discussion

Results of the present study showed that the highest concentration of  $\text{Pb}(\text{NO}_3)_2$  was observed in gill, liver, skin, and white muscle tissues. These residues increased 57, 58, 13, and 4-fold,

respectively, after 10 days of exposed time, as compared with that of the control group. However, the bioaccumulations in these tissues were found to be more greatly increased to 120, 46, 4 and 7-fold for gill, liver, skin, and white muscle, respectively, after 20 days of exposure. Therefore, similar to our findings, Al-Asgah et al. (2015) reported that compared with other tissues, the bioaccumulation rate of Cd after 10 days of exposure was the lowest in the muscles. In the current study, across all concentrations, the bioaccumulation trends of  $\text{Pb}(\text{NO}_3)_2$  after 10 days followed the order: the gills  $\geq$  liver > white muscles > skin; however, after 20 days the following was the order: gills > liver > skin > white muscles. The finding of this study is in accordance with Brown et al. (2006) who reported that the heavy metals may be uniformly distributed in the body tissues of fish but accumulate differently. Therefore, this is incongruous with our results, suggesting that heavy metals accumulated dependent predominantly upon the time of the exposure and the kind of tissues. Studies on different fish species have demonstrated that the liver is the prime organ for metal bioaccumulation and plays an important role in storage, elimination of toxicity, redistribution, and transformation of heavy metals (Evans et al., 1993).

In addition, muscles are the edible part of the fish; therefore, residues of heavy metals in muscle could be hazardous to consumers. Ozturk et al. (2009) results incongruous with our results, which showed the lowest accumulation of Pb in white muscles. Investigations conducted by other researchers on Pb accumulation in different fish species (Ozturk et al., 2009; Ganbi, 2010; Victor et al., 2012) confirmed the data obtained in this study. This might be related to the increasing growth factor of muscle tissue as growth may dilute the heavy metal concentration (Lohner et al., 2001).

However, different bioaccumulation capabilities of *C. gariepinus* exposed to different concentrations of  $\text{Pb}(\text{NO}_3)_2$  showed different amounts of Pb in various tissues indicated its high bioavailability. Accumulation of heavy metals might cause physiological changes in the structure and function of different tissues (Banaee et al., 2013). Yap et al. (2004) and Łuszczek-Trojnar et al. (2013) reported different rates of accumulation and clearance of Pb in various organ tissues; this may be related to the different mechanisms of heavy metal binding and regulation in these tissues. In this study, the results confirmed that  $\text{Pb}(\text{NO}_3)_2$  had a high possibility to accumulate in various tissues of *C. gariepinus*, particularly in smooth tissues, such that lower accumulation of Pb was found in the muscle tissues, when compared with that of other tissues.

In general, the aquatic environment is affected by pollutants; any physiological alterations will be inverted in the amounts of most measurements of hematological parameters. Thus, aquatic ecology is an important factor accountable for the individual

**Table 3**

Hematological and biochemical parameters of *C. gariepinus* exposed to sublethal concentration of  $\text{Pb}(\text{NO}_3)_2$  for 20 days. (Mean  $\pm$  SE  $n = 5$ ).

Parameters	Treatments			
	Control	20% $\text{LC}_{50}$	40% $\text{LC}_{50}$	60% $\text{LC}_{50}$
RBC (count $\times 10^6/\text{mm}^3$ )	$2.22 \pm 0.24^a$	$1.89 \pm 0.14^{ab}$	$1.76 \pm 0.14^{ab}$	$1.49 \pm 0.07^b$
Hb (g/dl)	$11.11 \pm 1.34^a$	$9.93 \pm 0.65^a$	$9.03 \pm 0.98^a$	$8.01 \pm 1.11^b$
Hct (%)	$29.56 \pm 1.96^a$	$26.68 \pm 2.74^{ab}$	$23.81 \pm 3.61^{ab}$	$20.24 \pm 2.82^b$
MCHC (g/dl)	$37.96 \pm 2.06^a$	$35.96 \pm 1.65^a$	$39.68 \pm 3.11^a$	$36.44 \pm 2.88^a$
MCH (pg)	$71.84 \pm 5.48^a$	$67.08 \pm 6.87^a$	$66.22 \pm 6.60^a$	$62.84 \pm 4.92^a$
MCV (fl)	$180.38 \pm 3.69^a$	$170.00 \pm 2.40^b$	$159.16 \pm 2.20^c$	$140.94 \pm 4.17^d$
WBCs ( $\times 10^9/\text{l}$ )	$198.54 \pm 7.36^a$	$187.74 \pm 7.46^a$	$158.92 \pm 6.38^b$	$136.82 \pm 6.63^b$
Lymphocyte	$141.04 \pm 7.17^a$	$139.78 \pm 9.08^a$	$127.28 \pm 9.21^{ab}$	$114.92 \pm 6.58^b$
Monocyte	$10.14 \pm 0.84^a$	$9.21 \pm 0.69^{ab}$	$7.98 \pm 0.48^b$	$7.40 \pm 0.72^b$
PLT ( $10^9/\text{l}$ )	$84.24 \pm 2.58^a$	$71.96 \pm 2.51^b$	$64.80 \pm 1.89^{bc}$	$61.16 \pm 4.36^c$
AST (IU/l)	$189.60 \pm 5.90^d$	$260.00 \pm 8.09^c$	$289.60 \pm 9.85^b$	$330.80 \pm 8.91^a$
ALT (IU/l)	$21.00 \pm 4.02^b$	$24.40 \pm 3.67^{ab}$	$28.60 \pm 3.49^{ab}$	$33.20 \pm 2.08^a$

Values in the same row with the same superscript are not significantly different ( $p > 0.05$ ).



differences in fish hematological parameters because they are susceptible to slight fluctuations that might occur in their internal environment (Fernandes and Mazon, 2003).

In this study, *C. gariepinus* exposed to Pb showed a significant reduction in their RBCs, Hb, and Hct in both periods, 10 and 20 days when compared to that of the control group. However, after 20 days of exposure hematological parameters were highly altered. This is in agreement with data obtained by Ikeogu et al. (2016) who found that RBC counts, Hb, and Ht of *C. gariepinus* decreased following exposure to 20 and 35 mg/l of Pb(NO<sub>3</sub>)<sub>2</sub>. In addition, these findings are in agreement with those of Mahmoud et al. (2013) who reported that *C. gariepinus* exposed to 7 mg/l of lead exhibited a significant decrease in their RBCs, Hb, and Hct. These changes were caused by direct responses that were structurally harmful to RBC membranes resulting in hemolysis and deterioration in hemoglobin structures, stress-related release of RBCs from the spleen and hypoxia, which were stimulated when fish were exposed to lead (Shah, 2006). Therefore, induced reduction in hematological parameters in this study might be also attributed to disequilibrium of the osmotic pressure inside and outside the blood cells (Heath, 1995). Moreover, Tawari-Fufeyin et al. (2008) and Borane and Zambare (2006) reported reduced RBC counts, Hb and Ht in *C. gariepinus* exposed to lead and cadmium. This reduction in the amounts of the RBC, Hb, and Ht might be caused by hemolysis resulting in hemodilution, a mechanism for diluting the level of the toxicants in the blood stream (Smith et al., 1999).

The Pb(NO<sub>3</sub>)<sub>2</sub> induced alterations towards increases or reductions in MCHC, MCH, and MCV levels were detected in the current study. These were incongruous with data obtained by Adeyemo (2007) who found that *C. gariepinus* exposed to Pb showed a significant increase in MCV values. However, data in the current study were in accordance with finding by Oluah and Omerebele (2010) who reported reductions in MCH and MCHC of *C. gariepinus* exposed to lead. In contrast, Shah (2006) reported that there was a significant increase in MCHC, MCH, and MCV in tenth (*Tinca tinca*) after exposure to lead that might have been based on different species of fish. Moreover, Ololade and Oginni (2009) found similar results for *C. gariepinus* exposed to Zinc. These chemicals induced changes in MCV, MCH, and MCHC that were caused by a direct or feedback response to structural injury to RBC membranes resulting from hemolysis and impaired hemoglobin structure or stress-related release of RBCs from the spleen and hypoxia (Marei et al., 1998).

Toxicity of chronic Pb showed characteristic symptoms that include changes in the blood measurements with acute harm to red blood and white blood cells and disadvantages to the nervous system (El-Badawi, 2005). Lead depletes and reduces main antioxidants in the cell, particularly thiol-containing antioxidants and enzymes, and can cause considerable increases in the production of reactive oxygen species (ROS), followed by a situation known as oxidative stress that causes different dysfunction in lipids, proteins, and DNA (Ercal et al., 2001). Shah and Altındağ (2005) found that significant increases in immunological measurements after exposure to Pb, and believed that Pb could weaken the immune response, resulting in an increased potential to cause the infections.

Leucocytes or WBCs are cells of the immune system that play a key role in both non-specific and specific immune responses in protecting the body against foreign substances. A review of the literature showed two opposite lines of responses of leucocytes to different heavy metals. Previous investigations reported an increase in WBC values in fish in response to heavy metal toxicity (Raina and Sachar, 2014; Sharma and Langer, 2014). The results in this study showed that a decline in WBC counts of the tested

groups for each period exhibited significant reductions also, with an increase in the concentrations of Pb(NO<sub>3</sub>)<sub>2</sub> as the pollutant when compared with the unexposed group (control) (Tables 2 and 3). This might be attributed to the release of epinephrine during stress that causes decreases in WBC counts that occur during the weakening of the immune system. These results were in agreement with data obtained by Adeyemo (2007) who reported that adult *C. gariepinus* exposed to various concentrations (25, 50, 100, and 200 mg/l) of lead nitrate for 96 h observed a decline in leucocyte counts of the experimental groups when compared with that of the control group. In addition, increases in WBC counts can be caused by prompting an immune system in response to tissue harm caused by heavy metals. Moraes (2007) reported that the primary method to estimate the immune response in channel catfish (*Ictalurus punctatus* Raf.) was to explore changes in the WBC count.

In this study, enzyme activities observed were significantly increased ( $P < 0.05$ ) with each increase in Pb(NO<sub>3</sub>)<sub>2</sub> and period and AST and ALT amounts increased in the tested groups compared with that of the control (Tables 2 and 3). These might be attributed to the significant alterations in the enzyme activities in blood plasma that indicate tissue deterioration caused by stress (Svoboda, 2001). Therefore, the rising of AST and ALT levels in fish detected enzymes released from the liver into the blood stream (Yang and Chen, 2003; Perez-Rostro et al., 2004). Therefore, Mahmoud et al. (2013) found that *C. gariepinus* exposed to Pb exhibited increased AST and ALT levels that could be promoted by similar data as that of Olojo et al. (2012), who stated there was an increase in AST and ALT values for *C. gariepinus* after exposure to lead. It has been shown that an alteration in the enzyme activities in the plasma directly caused major pathologic alterations in the permeable membrane of the cell or liver cell shredding. However, plasma of AST and ALT increased and might be attributed to hepatocellular injury or cellular degeneration caused by Cd, probability in the liver, heart, or muscle (Yamawaki et al., 1986).

## 5. Conclusion

This study was conducted to evaluate that exposure to lead nitrate at sublethal concentrations for a time exposure 20 days by Pb(NO<sub>3</sub>)<sub>2</sub>, which may lead to alterations in the bioaccumulation in gills, liver, white muscle, and skin tissues. However, muscles are considered an important tissue, which represents an edible part of fish that may influence human health. Therefore, contamination by Pb(NO<sub>3</sub>)<sub>2</sub> can produce significant changes in the physiology of *C. gariepinus* as demonstrated by alterations in the hematological and biochemical parameters. Additionally, profound effects on blood parameters may have caused a disruption of the inner physiology. It is obvious from the above that water contamination with Pb(NO<sub>3</sub>)<sub>2</sub> has adverse effects on fish health, which is reflected in economic status, as well as human health.

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## Declaration of Competing Interest

All of this work (conception; acquisition, analysis, data interpretation; drafting of the manuscript; critical review of the manuscript and statistical analysis) were done by the authors. Also, the authors have declared that no competing interests exist.

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