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Impact of Lead and Cadmium Chronic Exposure on Some Physiological Parameters of the Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

The current work was designed to assess the effects of waterborne lead and cadmium on biochemical and haematological parameters for the Nile tilapia, *Oreochromis niloticus*, after 60 days of exposure to chronic sub-lethal concentrations. A total of 300 fish individuals of *O. niloticus* were randomly divided into five treated groups. The first group was the control one. The second (T_1) and third groups (T_2) were exposed to lead (Pb) at concentrations of 0.72 and 3.6mg/ l, respectively. The fourth (T_3) and fifth (T_4) groups were exposed to cadmium (Cd) at concentrations of 0.62 and 3.1mg/ l, respectively. The results of the current study revealed that tilapia fish exposed to lead and cadmium exhibited several behavioral changes, such as anorexia, rapid unbalanced movement, irregular swimming, an increase in operculum movement and slack movement during the experimental duration.The values of aspartate amino transferase (AST), alanine amino transferase (ALT), serum creatinine, blood urea and serum total cholesterol showed a highly significant increase in a time-dependent manner in the Pb and Cd exposed groups compared to the control group. While serum albumin was significantly decreased in the Pb and Cd exposure experimental groups. Serum total protein and globulin concentrations showed a non-significant difference among different experimental groups at the end of the experiment period. On the other hand, RBCs count, Hb% and Hematocrit levels decreased gradually during exposure time in fish exposed to both low and high concentrations of Pb and Cd. In addition, the WBCs count was significantly decreased in fish exposed to Pb and Cd concentrations, compared to fish in the control group. The present study indicates that waterborne lead and cadmium exposure have a severe negative impact on *Oreochromis niloticus* health, and these impacts are greater in cadmium than in lead.

INTRODUCTION

Heavy metals are extremely hazardous compounds released into the aquatic environment, threatening aquatic animals and human health. These chemicals are non-biodegradable and build up in the environment (**Mahmoud & Abd El Rahman, 2017; Mohanta *et al*., 2020**). Specifically, cadmium, copper, lead, and zinc are considered the most hazardous and toxic elements that enter the food chain and biologically accumulate in aquatic organisms, causing wide detrimental effects on their health (**Huang *et al*., 2020; Khattab *et al*., 2021**; **Suchana *et al*., 2021**). Heavy metal toxicity in fish is multifaceted, manifesting as physiological disturbances and a risky increase in fish mortality (**Ali *et al*., 2020**; **Sarkar *et al*., 2022**).

Cadmium (Cd) is released in considerable amounts through industrial effluents into the aquatic environment. These excess amounts, in addition to the naturally occurring levels can build up toxic levels causing damage to the biotic elements of the aquatic ecosystem **(Friberg, 2017**; **Ahmad *et al.*, 2018)**. Biomagnifications and greater half-life periods of Cd exacerbate its harmful effects. Cd interferes with many protein and carbohydrate metabolisms by inhibiting the enzymes involved in these processes (**Fazio *et al.*, 2014; Khalesi *et al*. 2017**). Moreover, lead is toxic to most forms of life including aquatic organisms **(Afshan *et al*., 2014**; **Shovon *et al*., 2017)**, and aquatic organisms have less capability to regulate lead **(Ali *et al*., 2019)**. However, fish can detoxify Pb by producing lead metallo-protein and storing it in various organs such as liver, gills, kidney, and muscles **(Kim & Kang, 2017a)**.

Generally, the previous studies that have checked the effects of chronic exposure to sub-lethal levels of Pb and Cd manifested some physiological changes in fish, such as hard respiration, reduction in growth, disruption in whole-body or plasma ion regulation, changes in hematology, enzyme activity and other blood parameters that reveal the stress response in fish **(Çiftçi *et al*., 2015; El-Sayed, 2015; Dos Santos *et al*., 2016; Khalesi *et al*., 2017; Kumar *et al*., 2017; Abdel-Warith *et al*., 2020)**.

Egypt's freshwater resources are limited; according to the Egyptian legislation, the aquaculture sector is not allowed to use either irrigation or the Nile water; only the water from agricultural drainages is permitted (**Shaalan *et al.*, 2017; El-Rawy *et al*., 2019**). Human activities have amplified the levels of heavy metal ions in natural water systems. Furthermore, industrial wastes such as paints, pesticides, textiles, fertilizers, leather, pharmaceuticals, and domestic effluents, as well as agricultural runoff, all supply loads of metals to the water ecosystem (**Al Naggar *et al*., 2018; Vajargah, 2021**).

The Nile tilapia, *O. niloticus*, is a highly commercial fish species in Egypt (**GAFRD, 2020)**. It has many competitive farming attributes. High marketability, tolerance to unfavourable environmental conditions and acceptance of artificial feeds make it the best choice for farming in Egypt **(Kaleem & Bio Singou Sabi, 2021)**. Egypt is ranked first in Africa and the 11th in the world for the production of tilapia aquaculture **(FAO, 2020)**. Although they have a high tolerance to pollution, higher levels of heavy metals including Cd and Pb can devastate tilapia health status, causing detrimental outcomes and great losses **(Abbas *et al*., 2019a; Dutta *et al*., 2022)**.

Lead and cadmium are among the most dangerous metals in the Egyptian aquatic environment. These heavy metals devastate the health of numerous cohabiting aquatic organisms and accumulate in fish, posing serious health risks to fish and subsequently to humans. Therefore, the present study aimed to investigate the effect of bioaccumulation of waterborne lead and cadmium on the physiological parameters of the Nile tilapia under laboratory conditions for long-term chronic exposure.

MATERIALS AND METHODS

1. Experimental design

A total of 300 individuals of tilapia fish (*Oreochromis niloticus*) with an average weight of 26- 28g were obtained from a private commercial fish farm in Sharkia Governorate and were brought to the aquarium laboratory in the animal house of the Faculty of Science, Al-Azhar University. Fish were distributed and allowed to acclimatize in dechlorinated tap water for 2 weeks in 50L aquaria, with constant aeration at 27 ± 2°C, pH ranging between

7.0–7.5 and dissolved oxygen ranging between 6.2–6.9mg/ L. Fish were fed once daily a commercial diet containing 30% raw protein and free of any additives.

Based on our previous findings, the 96-h LC_{50} values of lead and cadmium exposure in the Nile tilapia, *O. niloticus*, were recorded as 36.0 and 31.0ppm, respectively**.** Tilapia fish were randomly distributed into five groups, with each group containing 60 fish (20 × three replicates). The first group was the control one (C). The second (T_1) and third (T_2) groups were exposed to 2% (low concentration: 0.72 mg/l) and 10% (High Concentration: 3.6 mg/l) of LC_{50} from lead. While, the fourth (T_3) and fifth (T_4) groups were exposed to 2% (low concentration: 0.62 mg/l) and 10% (high concentration: 3.1 mg/l) of LC_{50} from cadmium.

Table 1. The experimental trial groups

Group no.	Description	Heavy metal concentration
C	Control	No treatment
T_1	Pb LC	Lead 0.72 mg/l (2% 0f LC_{50})
T_2	Pb HC	Lead 3.6 mg/l (10% 0f LC_{50})
T_3	Cd LC	Cadmium 0.62 mg/l (2% 0f LC_{50})
T_4	Cd HC	Cadmium 3.1 mg/l (10% 0f LC_{50})

2. Blood sampling

After 15, 30, 45, and 60 days of the experiment, blood samples were taken from three fish from each aquarium. Fish were fasted for 24 hours prior to sampling and anaesthetized with MS222 (50 mg L-1) before blood samples were drawn from the caudal vein using a sterile syringe containing heparin solution as an anticoagulant. The blood sample was divided into two parts. The first part was placed in glass tubes containing EDTA for haematological analysis. The second part was left in glass tubes without anticoagulant to clot. After centrifugation at 1500g for 15min, serum was collected and frozen at −20°C for biochemical analysis.

3. Biochemical analysis

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST), blood urea, creatinine, total cholesterol, total protein as well as serum albumin (gm/dl) were estimated as previously described (**Wu, 2006**) using commercial kits (Spectrum Diagnostics, Egypt). Then, serum globulin concentration was calculated by subtracting albumin from total protein concentration. The endpoint of the biochemical reactions was detected according to the kit instructions using a spectrophotometer (AGILENT CARY 100/300 Series UV-Vis, UNITED STATES).

4. Haematological analysis

The red blood cells (RBCs) and white blood cells (WBCs) counts were estimated using an improved Neubauer haemocytometer (**Natt & Herrick, 1952**). Haematocrit (Hct%) was determined using heparinized capillary tubes, centrifuged in a microhaematocrit centrifuge. Haemoglobin concentration (Hb) was recorded using a spectrophotometer.

Statistical analysis

Data were analyzed using statistical Package for the social science (SPSS) software (Version 22) (IBM Corp., Armonk, NY). Differences in hematological and biochemical parameters and time exposure were determined using the analysis of variance (ANOVA). Data were presented as mean and quartiles with a *P*-value that was considered significant at $P \leq 0.05$.

RESULTS

Tilapia fish exposed to lead and cadmium exhibited several behavioral changes. These changes include anorexia, rapid unbalanced movement and irregular swimming, increase in the operculum movement and the slack movement with resting at the bottom of aquaria, as well as difficulties in breathing, which seems much heavier than the normal breathing. Such abnormalities in fish behavior were not observed in the control group.

1. Serum liver enzymes

The changes in biochemical parameters of tilapia fish under five different experimental treatments over a 60-day experimental period are shown in Table (2) and Fig. (1).

Statistical analysis indicates that the values of aspartate amino transferase (AST) and alanine amino transferase (ALT) activities showed a highly significant increase in time-dependent manner (P<0.05) in the Pb and Cd exposed groups, compared to the control group. There was no significant difference in the hepatic enzymes of the control group, which recorded values of 47.0 ±4.4 IU/L at day 0; 46.0 ±4.6 IU/L at day 60 for AST enzyme; and 17.1 ±3.1 IU/L at day 0; values of 20.7±4.5 IU/L was recorded at day 60 for ALT enzyme (Fig. 1).

As illustrated in Fig. (1), the results reveal that the liver enzymes were impacted by the exposure time and among the increasing in the concentrations in both studied metals. In general, the elevation in liver enzymes by the end of the experimental period was recorded in the two cadmium-exposed fish groups more than in the two lead-exposed fish groups. AST was markedly elevated in the T_3 and T_4 exposure groups by 2.3 and 3.1 folds, which recorded values of 137.0±15.1 IU/L and 186.0±7.9 IU/L at day 60, respectively, and 1.2 to 1.4 folds for the T_1 and T_2 exposure groups, which recorded values of 75.7.0±12.7 IU/L and 85.0 ±6.1 IU/L at day 60, respectively.

Table 2. Changes in biochemical parameters of ***O. niloticus*** exposed to different concentrations of lead (Pb) and cadmium (Cd) after 60 days of exposure (Data: mean ± SD**)**

Parameter / Groups	Control	T_1	T_2	T_3	T_4
AST (IU/L)	46.0^{a}±4.6	75.7^{b}±12.7	85.0^{b}±6.1	137.0^{c}±15.1	186.0^{d}±7.9
ALT (IU/L)	20.7^{a}±4.5	34.0^{b}±7.6	57.3^{c}±6.5	70.0^{d}±3.6	85.7^{d}±6.0
Serum Urea (mg/dl)	5.37^{a}±0.64	8.30^{b}±0.46	9.17^{c}±0.25	10.0^{c}±0.80	12.7^{d}±1.35
Serum Creatinine (mg/dl)	0.19^{a}±0.02	0.27^{b}±0.02	0.30^{b}±0.02	0.46^{c}±0.04	0.65^{d}±0.03
Total cholesterol (mg/dl)	96^{a}±7.5	146^{b}±8.1	163^{b}±11.3	191^{c}±10.1	209^{c}±13.6
Serum Albumin (g/dL)	1.20^{a}±0.08	1.02^{a}±0.08	0.97^{b}±0.08	0.81^{c}±0.03	0.71^{c}±0.02
Total protein (g/dL)	4.22^{a}±0.07	3.93^{b}±0.1	4.17^{a}±0.07	4.09^{c}±0.05	4.05^{c}±0.08
Serum Globulin (g/dL)	3.01^{a}±0.05	2.91^{a}±0.18	3.20^{b}±0.11	3.28^{b}±0.09	3.34^{c}±0.10

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT).
Means with the same letter within the same raw are not significantly different (P>0.05).

On the other hand, ALT was significantly elevated in the T_3 and T_4 exposure groups by 3.9 to 4.8 folds, which recorded values of 70.0±3.6 IU/L and 85.7±6.1 IU/L at day 60, respectively and 1.9 to 3.2 folds for T_1 and T_2, which recorded values of 34.0±7.6 IU/L and 57.3±6.5 IU/L at day 60, respectively, at the end of the experiment period (Table, 2).

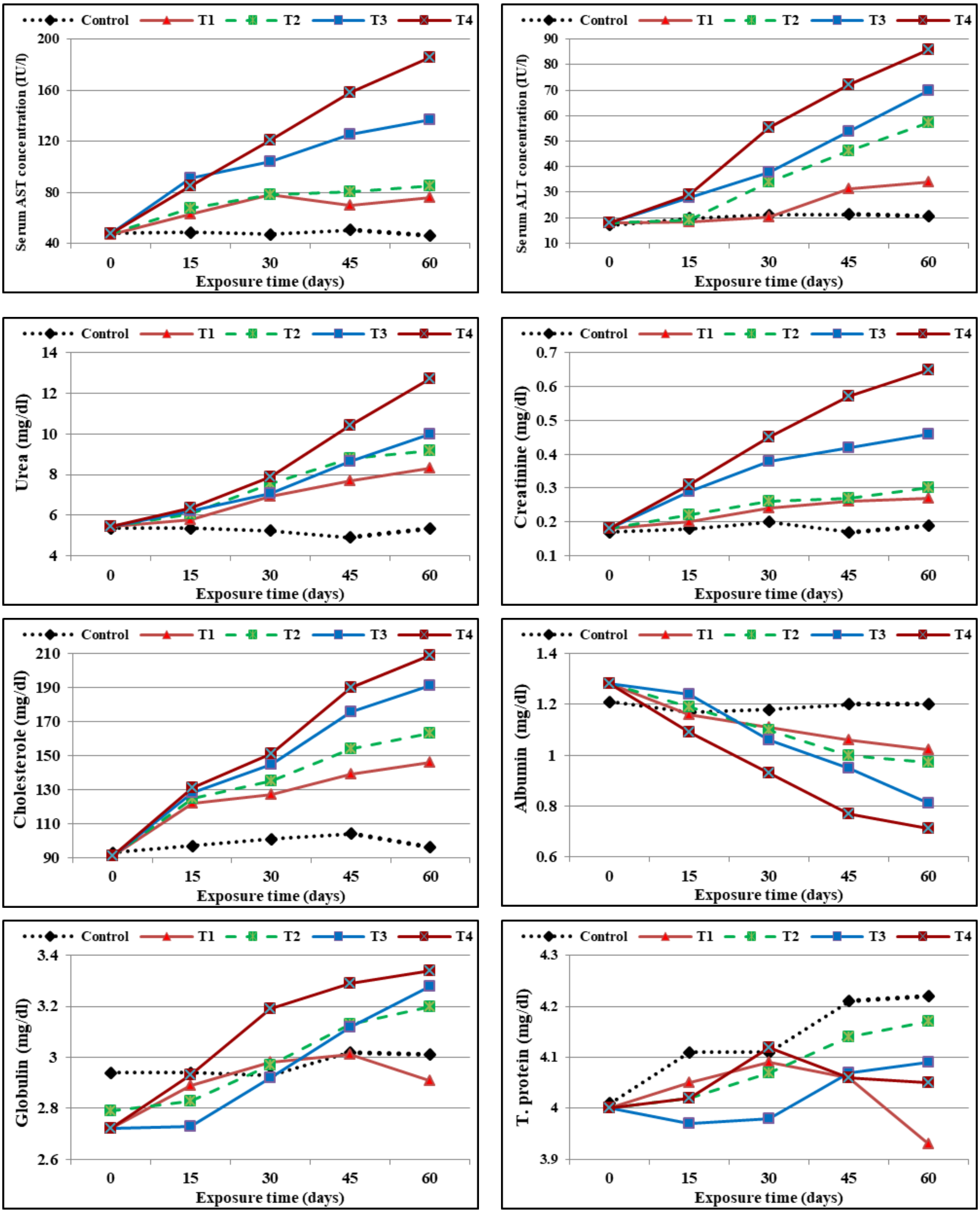

Fig. (1): Effect of different exposure times to lead and cadmium on different serum biochemical parameters of Nile tilapia, *Oreochromis niloticus*

2. Serum biochemical parameters

In both groups exposed to Pb and Cd, the levels of creatinine and urea in the serum increased gradually, compared to the control group in which such increase was markedly detected to begin at day 15 until the end of the experimental period. Statically, there was a significant increase ($P < 0.05$) in serum creatinine and urea levels in the Pb and Cd exposure groups. (Table 2 & Fig. 1). The effect of Pb and Cd exposure on the kidney functions of the Nile tilapia appeared significantly where the serum creatinine and urea levels were strongly elevated over time of exposure, i.e., the creatinine level was 0.18± 0.03 at day 0 and increased to 0.24±0.03 and 0.26 ±0.2 at day 30 and finally recorded a value of 0.27±0.02 and 0.30 ±0.02 mg/dL for T1 and T2 exposure groups, respectively, and increased to 0.38±0.02 and 0.45 ±0.4 at day 30 and finally recorded a value of 0.46±0.04 and 0.65 ±0.03 mg/dL for T3 and T4 exposure groups, respectively. On the other hand, the serum urea level was recorded as 5.43 ± 0.76 at day 0 and elevated to 6.93±0.55 and 7.53 ±0.55 at day 30 and finally recorded 8.30±0.46 and 9.17 ±0.25 mg/dL for the T1 and T2 exposure groups, respectively. While, blood urea levels in the T3 and T4 exposure groups increased to 7.07± 0.02 and 7.87± 0.91mg/ dL at day 30; they eventually reached 10.0 ±0.80 and 12.7± 1.35 mg/ dL at day 60, respectively (Table 2 & Fig. 1).

Total cholesterol level was significantly increased ($P < 0.05$) in the Pb and Cd exposure experimental groups, which recorded 91 ± 11.9mg/ dL at day 0 which gradually increased with increasing exposure time to become 146 ±8.1mg/ dL and 163±11.38mg/ dL at day 60 for T_1 and T_2 exposure groups, respectively, and 191 ±10.1mg/ dL and 209±13.6mg/ dL at day 60 for T_3 and T_4 exposure groups, respectively, On the contrary, no differences were detected in total cholesterol values in the control group, which averaged about 96 ±7.5mg/ dL at the end of the experimental period. (Table 2 & Fig. 1).

3. Serum protein profile

The results showed that serum albumin decreased significantly ($P< 0.05$) in the experimental groups for exposure to Pb and Cd in comparison with the control group, which had semi-constant values during the experiment's duration (1.21±0.08g/ dL at day 0 and 1.20 ± 0.08g/ dL at day 60). Whereas, the serum albumin values decreased with increasing exposure time to become 1.02 ± 0.08g/ dL and 0.97 ± 0.08g/ dL at day 60 for the T1 and T2 exposure groups, respectively; the lowest values were recorded (0.81 ± 0.03 and 0.71 ± 0.02g/ dL) at day 60 in the T3 and T4 exposure groups, respectively (Table 2 & Fig. 1). Statistically, serum total protein and serum globulin have no significant differences between all experimental trial groups. There was a slight elevation in serum total protein concentration in all experimental trail groups, except in the T1 exposure groups, which showed a slight decrease compared to the control group. Specifically, the serum total protein concentrations at day 60 for the T1 and T2 exposure groups were 3.93±0.03g/ dL, 4.17±0.07g/ dL, respectively, and 4.09±0.05g/ dL, 4.05±0.08g/ dL for the T3 and T4 exposure groups, respectively, while the control group recorded 4.22±0.07g/ dL at day 60 (Table 2 & Fig. 1). At the same time, serum globulin was slightly increased in all experimental groups, with values of 2.91 ±0.18g/ dL and 3.20 ±0.11g/ dL at day 60 for T1 and T2 exposure groups, respectively; Values of 3.28 ±0.09g/ dL and 3.34 ±0.10g/ dL were recorded at day 60 for T3 and T4 exposure groups, respectively, and the values of 3.01±0.05g/ dL were registered at day 60 for the control group (Table 2 & Fig. 1).

4. Hematological analysis

Results of the statistical analysis showed that there were non-significant differences in RBC count and hematocrit values in the control group during the experimental period, and these values ranged from 1.53×10^6/µl at day 0 to 1.57×10^6/µl at day 60 and from 20.53 % at day 0 to 22.20 % at day 60, respectively. However, a significant increase ($P< 0.05$) was detected in Hb % content in the control groups. The increase was gradual and ranged from 10.3g/ dL at day 0 to 12.2g/ dL at day 60. In contrast, a highly significant decrease ($P<0.05$) was observed in RBC count, Hb%, and hematocrit levels in the Pb and Cd exposure groups compared to the control group. As shown in Table (3) and Fig. (2), RBC count, Hb% and hematocrit levels gradually decreased in fish exposed to low and high concentrations of Pb and Cd starting from 15 days of exposure to the end of the experiment. The decrease in RBC count, Hb%, and hematocrit levels was greater in Cd-exposed fish, which recorded values of 0.92×10^6/µl; 7.30g/ dL and 10.89%, respectively, for T3 and 0.91×10^6/µl; 7.20g/ dL and 10.82%, respectively, for T4 at day 60 than in Pb-exposed fish, which recorded values of 1.23×10^6/µl; 8.9 g/dL and 14.97%, respectively, for T1, and 0.92×10^6/µl, 9.0g/ dL and 15.58%, respectively, for T2 at day 60.

Statistical analysis revealed that there was a non-significant difference in WBCs in the control group, with semi-constant values during the experiment duration. On the other hand, the WBCs count was significantly decreased (*P*<0.05) in fish exposed to low and high concentrations of Pb and Cd, which recorded 109.93±5.6 $\times10^3$/ µl at day 0 that gradually decreased with increasing exposure time to become 89.61 ±2.61$\times10^3$/ µl and 85.69 ±3.72$\times10^3$/ µl at day 60 for T_1 and T_2 exposure groups, respectively, and 56.01±6.66 $\times10^3$/ µl and 42.69 ±4.04 $\times10^3$/ µl at day 60 for T_3 and T_4 exposure groups, respectively, as shown in Table (3) and Fig. (2).

Table 3. Changes in hematological parameters for *O. niloticus* exposed to different concentration of lead (Pb) and cadmium (Cd) after 60 days of exposure (Data: mean ± SD)

Groups / Parameter	Control	T_1	T_2	T_3	T_4
Hb (g/dl)	12.2^a±0.5	8.9^b±0.26	9.0^b±0.25	7.30^c±0.44	7.20^c±0.30
RBCS $\times10^6$ /µl	1.57^a±0.17	1.23^b±0.08	0.92^c±0.18	0.92^c±0.18	0.91^c±0.08
HCT (%)	22.20^a±0.4	14.97^b±0.82	15.58^b±0.69	10.89^c±1.03	10.82^c±0.86
WBCS $\times10^3$ /µl	113.5^a±9.8	89.61^b±2.61	85.69^b±3.72	56.01^c±6.66	42.69^c±4.04

Hemoglobin (Hb); Red blood cells (RBCs); Hematocrit (Hct), and White blood cells (WBC_S).
Means with the same letter within the same raw are not significantly different (*P*>0.05).

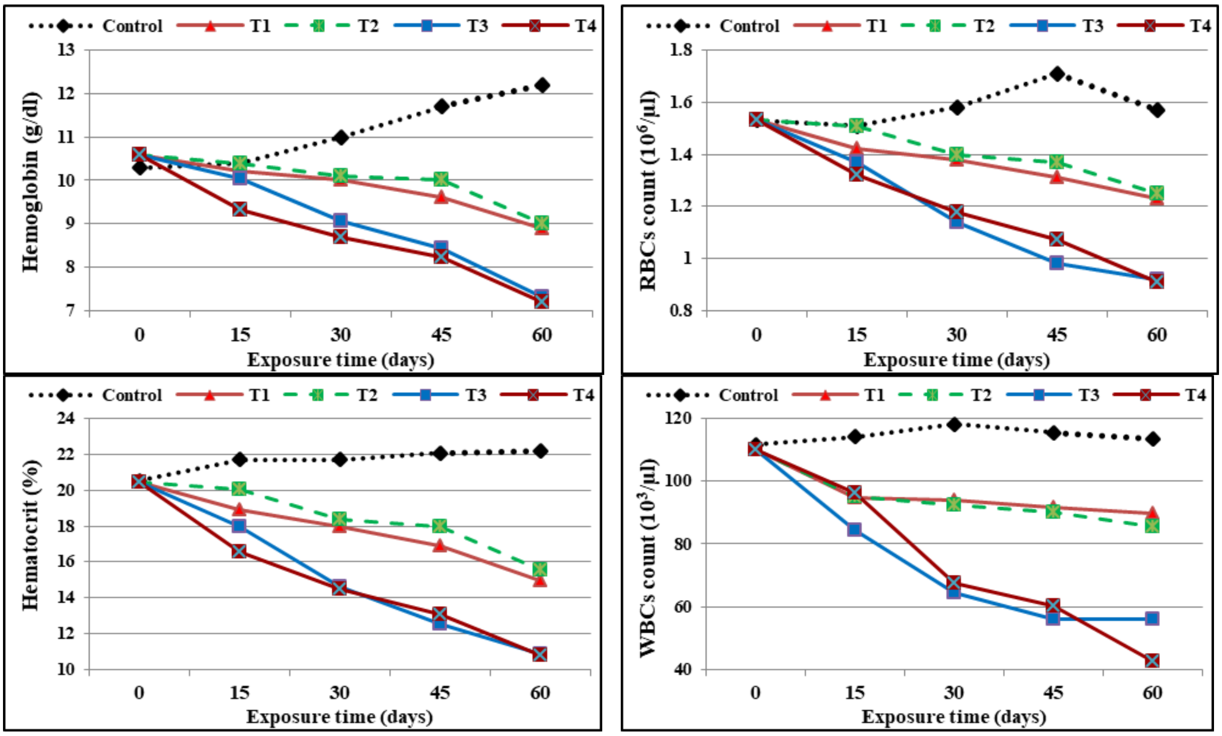

Fig. (2): Effect of different exposure times to lead and cobalt on different hematological parameters of Nile tilapia, *Oreochromis niloticus*

DISCUSSION

The expansion of fish farming is the best choice for solving food security problems in Egypt (**Elgendy *et al*., 2022**). Lead and cadmium are among the most prevalent contaminants in the water used in fish farming in Egypt. They are toxic to aquatic animals at low concentrations and have negative effects on their physiological processes, causing oxidative stress (**Chang *et al*., 2021**), immunosuppression and reduced disease resistance (**Mitra *et al*., 2022**; **Elgendy *et al*., 2022**). In addition to the previous effects, these elements reduce fish survival (**Sharaf *et al*., 2021**). The present findings showed that exposure to waterborne Pb and Cd caused numerous abnormal clinical manifestations in the Nile tilapia, such as anorexia, rapid unbalanced movement, irregular swimming, and slack movement, which may be due to neurological impairment in the central nervous system. The increase in operculum movement and heavy breathing, observed during this study, may be relevant to respiratory distress caused by toxic heavy metal concentrations; similar signs were recorded by other studies **(Singh & Saxena, 2020; Abalaka *et al*., 2022)**. The behavioral changes increased with increasing heavy metal concentrations in concordance with **Hesni *et al*. (2011**).

ALT and AST enzymes are indicators of liver function and integrity (**Heydarnejad *et al*., 2013; Abdel-Moneim *et al*., 2016**). They play a critical role in carbohydrate and amino acid metabolism (**Garai *et al*., 2021; Paulusma *et al*., 2022**). In the present study, serum AST and ALT were elevated in the Pb and Cd exposure groups, compared to the control fish. This elevation was in tandem with increasing exposure time and metal concentration. Our findings suggest that these toxic metals caused liver damage, resulting in the leakage of these enzymes into the blood (**Thirumavalavan, 2010**). These enzymes are typically elevated during acute hepatotoxicity or hepatocellular injury (**Al-Asgah *et al*., 2015; Mohiseni *et al*., 2016**). Similarly, a significant increase was reported in plasma (AST) and (ALT) in the Nile tilapia fish exposed to chronic Cd toxicity for 25 days. Additionally, **El-Khayat *et al.* (2022)** recorded an increase in ALT& AST activities in acute and chronic exposures to Pb in *O. niloticus.* Toxic metal concentrations may also cause necrosis of the liver (due to the toxicant), resulting in the loss of liver enzymes into the blood (**Authman *et al*., 2013**).

Results showed a gradual decrease in serum albumin level in the Pb and Cd exposure groups, compared to the control group. The same result was obtained by **Al-Asgah *et al*. (2015)**, who observed a significant decrease in serum albumin in the Nile tilapia exposed to varying levels of cadmium chloride. Identically, **El-Khayat *et al.* (2022)** reported a decrease in serum albumin as an acute and chronic outcome of *O. niloticus* exposure to Pb. The decreased albumin levels (hypoalbuninemia) reflect hepatic and renal damage (**Zuluaga Rodriguez *et al*., 2015; Hayati *et al*., 2017**).

The present results revealed a slight elevation in serum total protein concentration in all experimental groups, except in the T_1 group, which showed a slight decrease compared to the control group. Furthermore, serum globulin increased slightly in all experimental groups. Previous studies exhibited no consistent pattern of response in total protein (TP) assessments; protein levels changed by heavy metal exposures (**Oner *et al*., 2008; Heydarnejad *et al*., 2013**). The increase in serum total protein (hyper-proteinemia) and globulin levels detected after Pb and Cd exposure indicate the disruption of physiological and immune response mechanisms in fish (**Sayed & Authman, 2018**). These high protein levels may explain an attempt to detoxify the contaminants and overcome the stress responses (**Authman *et al*., 2013**).

The results of the present study showed an elevation of blood urea levels with a significant increase in serum creatinine in the Pb and Cd exposure groups, compared to the control group. The current finding coincides with those of **El-Khadragy *et al*. (2017)** and **El-Khayat *et al*. (2022)** who reported a significant increase in creatinine and urea levels in *Oreochromis niloticus* exposed to Pb and Cd. Urea and creatinine levels increase in in metal-intoxicated exposed fish may be attributed to glomerular insufficiency, production of reactive oxygen species, and kidney dysfunction **(Javed *et al*., 2017; Germoush *et al*., 2021)**.

In the current work, the significantly increase in the serum total cholesterol levels was detected in all treated groups, compared to the control group. As a response to environmental stress in fish, the cholesterol level increased because organisms required excess energy reserves to minimise the stress effects. Moreover, liver and kidney failure led to the release of cholesterol into the blood stream (**Mohiseni *et al*., 2016**; **Roohi *et al.*, 2017**). **Osman *et al*. (2018)** recorded similar results regarding the increase in cholesterol levels in the Nile tilapia and African catfish collected from polluted sites in the Nile River, compared to those collected from non-polluted sites.

Pb and Cd waterborne exposure resulted in a decrease in RBC count, Hb%, and hematocrit levels. **El-Khadragy *et al*. (2017**) postulated similar results; they noticed a significant decrease in RBCs count, Hb concentration, and HCT values in the Nile tilapia exposed to sub-lethal concentrations of cadmium and lead for 2 weeks. Also, **Osman *et al*. (2018)** recorded a remarkable decrease in erythrocyte count, Hb and HCT values in Nile tilapia and African catfish collected from polluted sites in the Nile River compared to those collected from non-polluted sites. The decline in RBCs, HCT, and Hb may be explained as a compensatory response to oxidative stress outcomes, including RBC destruction and a decrease in RBC synthesis **(George *et al*., 2017; Khalil *et al.*, 2017)**.

Different types of cadmium-induced anaemia were reported, including iron deficiency anaemia caused by cadmium competing with iron in the intestinal mucosal iron transport system, hypoplastic anaemia caused by Cd inhibiting the growth of erythrocyte progenitor cells or bone marrow cells, and hemolytic anaemia caused by red blood cell sequestration in the spleen (**Enuneku & Ezemonye, 2013**). On the other hand, the Pb-induced anaemia may be a result of the inhibition of the red cell delta aminolevulinate hydrogenase enzyme (**Sharma *et al*., 2014**).

In the present study, fish exposure to different concentrations of Pb and Cd showed a significant decrease in WBCs count. Similarly, **Osman *et al*. (2018)** observed a reduction in the WBCs count in the Nile tilapia and African catfish collected from contaminated sites in the Nile River, compared to unpolluted sites. White blood cells play an important role in the defence mechanism (**Abbas *et al*., 2019b**). The suppression in WBCs count highlights a reduction in the immune response mechanisms after exposure to these toxic substances (**Khalil *et al*., 2017**).

CONCLUSION

According to our findings, the toxic effects of lead and cadmium accumulation on *O. niloticus* had a negative impact on the physiological processes of its organs. This negative effect is exacerbated by the increase in the concentration of the poison and the periods of exposure, which led to a disruption in physiological processes, affecting the health of the fish and reducing their chances of survival.

Ethical approval

The study was ethically carried in compliance with the National Research Centre Animal Care Committee and following regulations for the care of animals in research under number **(12711122021)**

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