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# Effects of Lead and Cadmium Accumulation on Survival and Growth of the Nile Tilapia (*Oreochromis niloticus*)

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**ABSTRACT**

The current paper was designed to understand the quantitative uptake pattern of lead (Pb) and cadmium (Cd) using the Nile tilapia *Oreochromis niloticus* and their effects on their survival and growth performance, under laboratory conditions. A total of 300 fish individuals of Nile tilapia were randomly divided into five treated groups (control group; 2 groups exposed to low concentration "2% $LC_{50}$" and 2 groups exposed to high concentration "10% $LC_{50}$" of both Pb and Cd). Both control and treated groups were noticed for 60 days. The results of the current study revealed that the mortality rate was higher in fish exposed to Cd than in those exposed to Pb, while no mortality was recorded in the control group. All growth parameters (WG, DWG and RGR) of *O. niloticus* were retarded due to the exposure to either Pb or Cd at different concentration levels compared to the control group. However, the hepatosomatic index (HSI) values were always higher in fishes exposed to Pb at different concentration levels than the fish in control group and these values were sharp in Cd exposures experimental groups during the first 30 days indicating hepatic hyperplasia or hypertrophy. Furthermore, the Pb and Cd accumulations in target organs were gradually increased with an increase in Pb and Cd concentrations and exposure time. After 60 days of exposure, the accumulation values of Pb in fish muscles were 2.64 and 8.67 µg/ g wet weight for $T_1$ and $T_2$, respectively; however, values of Cd in fish muscles were 2.23 and 3.87 µg/ g wet weight for $T_3$ and $T_4$, respectively. Results revealed that the bioconcentration factor (BCF) of Pb and Cd at low concentrations' exposure was greater than that recorded at high concentrations' exposure, especially on the days after the starting point until the saturation point (Saturated point recorded after day 45 in both Pb and Cd exposure).

## INTRODUCTION

Heavy metals are naturally occurring elements that may be found in the aquatic environment; some are essential for life, such as Mn, Fe, Co, Cu, and Zn, while others such as Pb, Hg, and Cd are highly toxic even at low concentrations (**Yildiz *et al*., 2010**). Most of them have the ability to bioaccumulate in fish tissues and biomagnificate through the food chain **(Babatunde *et al*., 2012)**.

Heavy metals reach to aquatic environment through industrial drainage of paints, pesticides, textiles, fertilizers, leather, pharmaceuticals, in addition to agricultural runoff and domestic effluents (**Al Naggar *et al*., 2018; Vajargah, 2021**). Heavy metals make their way into organisms via food, respiratory pathways or through the skin **(AL-Taee *et.al*., 2020)**. Toxicity of heavy metals is associated with interference with metabolic processes through interaction with sulphur-containing biochemical in living beings, such as enzymes and

proteins, which eventually lead to disturbances of metabolism, growth, reproduction, endocrine system and the immune system **(Khaled, 2004; Rubino, 2015)**.

Metals bio-accumulate in fish tissues like liver and muscles, with a variety of pathological responses (**Lalhruaitluanga *et al*., 2010; El-Agri *et al*., 2021; El-Khayat *et al*., 2022)**. Heavy metal toxicity can be mediated by causing oxidative stress through the generation of reactive oxygen and nitrogen species. This toxicity involves neurotoxicity, genotoxicity, hepatotoxicity and nephrotoxicity **(Jakimska *et al*., 2014; Sharma *et al*., 2014)**. Heavy metals accumulation not only affects fish health in aquatic environment, but it also extends through the food chain to the next trophic level **(Chen *et al*., 2019)**. It has major impacts on human health because it promotes different diseases such as cancer and neurological disease **(Cicero *et al*., 2017)**.

Cadmium and lead are among the most toxic heavy metals in aquaculture **(Taslima *et al*., 2022)**. The toxicity of these elements is due to their ability to cause oxidative stress and damage to living tissues in animals and humans **(Pizzino *et al*., 2017)**. The toxic effects of cadmium and lead on fish are manifested by many changes in the physiological and chemical processes of the body system **(Sarkar *et al*., 2021; Suchana *et al*., 2021)**. Accumulation of these metals in different fish organs may cause structural damage, functional disorders (**Emere & Dibal, 2013; Zaki *et al*., 2014; Li & Xie, 2018)**, oxidative stress (**Chang *et al*., 2021)**, immunosuppression and reduce disease resistance (**Mitra *et al*., 2022; Elgendy *et al*., 2022)**. It can also reduce growth and fish survival **(Abdel-Tawwab & Wafeek, 2014; Sharaf *et al*., 2021)**.

Water quality is the most critical issue affecting fish health and performance either in natural fisheries or aquaculture production systems **(FAO, 2014; Elgendy *et al*., 2022**). In addition, aquaculture makes a critical contribution to the world's welfare and prosperity **(FAO, 2020)**.

Freshwater resources are limited in Egypt; therefore, the aquaculture sector is only permitted to use water from agricultural drainages rather than irrigation from the Nile water according to the Egyptian law **(Shaalan *et al*., 2017; El-Rawy *et al*., 2019)**. When partially treated or untreated domestic and industrial wastewater is discharged into agricultural drains, the water quality deteriorates **(Authman *et al*., 2013)**. This leads to the declination of fish production, increasing the risk of diseases and threatening fish health. It also reduces the opportunities for fish exports **(Mur, 2014; FAO, 2020)**. The Nile tilapia, *Oreochromis niloticus*, is the most common fish species in the Nile River. It is one of the most widely cultured, cheapest, and readily available fish to Egyptians. (**Zhou *et al*., 1998**). It may survive in poor environmental conditions and tolerate low oxygen and high ammonia levels **(Ibrahim, 2020)**. It is easily spawned and feeds on natural foods as well as artificial feeds. It grows rapidly, reaching the market size in 6 months time **(FAO, 2022)**.

Many field studies have been conducted to monitor the concentrations of non-essential heavy metals (lead and cadmium) in aquatic environment and their accumulation quantities in different aquatic organisms **(Abdel-Baki *et al*., 2011; Ahmed *et al*., 2012; Aly, 2016)**. On the other hand, some studies have been conducted to understand the early accumulation behavior of heavy metals inside aquatic animals' tissues and their effects on their heath under laboratory conditions for a short-term period **(Atli & Canli, 2011; Al-Asgah *et al*., 2015; Ayegbusi *et al*., 2018)**, however, rare and inadequate studies dealt with the accumulation of Pb and Cd under a long- term accumulation for more than one month **(Zulfahmi *et al*., 2021)**.

Thus, the present study aimed to understand the quantitative uptake pattern of lead and cadmium using a common edible and wide distribution fish, the Nile tilapia *Oreochromis niloticus*. Consequently, a laboratory experiment was conducted in which the Nile tilapia fish were exposed to two sub-lethal concentrations of heavy metals (Pb and Cd), representing 2%

and 10% of the $LC_{50}$ of the target metals for a long exposure period (60 days). In addition, the effect of these concentrations on the survival and growth performance of the fish species under study was determined to report how these toxic metals affect the production of aquaculture fishes, represented by the Nile tilapia.

## MATERIALS AND METHODS

### 1. $LC_{50}$ experiments and estimation of exposure concentrations

Two 96-h $LC_{50}$ trials for both cadmium (Cd) and Lead (Pb) heavy metals were conducted in the fish aquaria lab, in the Animal House at the Faculty of Science, Al-Azhar University. All fish individuals from the Nile tilapia *Orechromis niloticus* were acclimatized to the lab conditions without any treatment for a period of 15 days. A total of 70 fish individuals were used for each $LC_{50}$ experiment trial and distributed in seven aquaria (50-liter), with 10 fish per each (weight range: 20– 25g). Each of the seven fish groups was exposed for 96h to the same concentration sequence (0, 10, 20, 30, 40, 50, and 60 mg/l) of both Pb (Lead chloride) and Cd (Cadmium acetate) compounds. All aquaria, for Cd and Pb $LC_{50}$, operated with good aeration, and all fish were in different experimental groups with the same exposure's start time. Dead fish were removed when movement ceased, and times of mortality were recorded. $LC_{50}$ values were determined according to **Behreues and Karbeur (1953)**.

### 2. Experimental design for lead and cadmium accumulation

Concentrations of long time exposure experiment for the accumulation rate purposes were estimated later to be 2% and 10% of $LC_{50}$ for both metals (lead & cadium) respectively.

A total of 300 fish individuals of the Nile tilapia (*O. niloticus*) with an average weight of 26±3 g were fetched to the laboratory and acclimated to conditions in aerated and de-chlorinated tap water in 50L aquaria for 1 week at 26+2°C under a natural photoperiod. Fishes were randomly divided into five groups, with 60 individuals in each (20 individuals in an aquarium, three replicates per group). The first group was the control one. The second and third groups were exposed to 2% and 10% of the $LC_{50}$ of lead (Pb) (0.72 and 3.6 mg/l), respectively. The fourth and fifth groups were exposed to 2% and 10% of the $LC_{50}$ of cadmium (Cd) (0.62 and 3.1 mg/l), respectively (Table 1).

**Table 1.** The experimental groups for low (LC) and high (HC) concentrations of lead and cadmium accumulation

| Group no. | Description | $LC_{50}$ value (mg/l) | Heavy metal concentration | |
|---|---|---|---|---|
| | | | $LC_{50}$ | mg/l |
| **C** | Control | ---- | 0% | No treatment |
| **$T_1$** | Pb LC | 36.0 | 2% | 0.72 |
| **$T_2$** | Pb HC | | 10% | 3.6 |
| **$T_3$** | Cd LC | 31.0 | 2% | 0.62 |
| **$T_4$** | Cd HC | | 10% | 3.1 |

Fish samples were daily fed during the experiment with artificial food. Three fish samples were taken every 15 days of exposure for 60 days to determine the growth performance and metal accumulation in the target organs.

### 3. Survival rate experiments

Additionally, 150 fish were separately used for a survivorship experiment to detect fish survival rates in different experimental groups. The fish were divided into the previously

recorded five experimental groups (C, $T_1$, $T_2$, $T_3$ and $T_4$) to monitor the survival rate for 60 days. The dead fish were immediately removed from the test aquarium and recorded to monitor the survival rate.

**4. Evaluation of growth performance**

Total weight and standard length of each fish specimen in control and all different exposure groups were taken at the start of the experiment and at each 15-day interval to assume growth performance. Weight gains (WG), daily weight gains (DWG) and the relative growth rates (RGR) were calculated according to **Tukmechi *et al.* (2011)**, using the following equations:

**Weight gain (WG) = Final weight − Initial weight**

**Daily weight gain (ADG) = Weight gain⁄Days of feeding**

**Relative growth rate (RGR %) = Weight gain / Initial weight *100**

## 5. Hepatosomatic index (HIS)

The fish sample was wet weighed and dissected, then its liver tissue was excised and wet weighed. Hepatosomatic index (HIS) was calculated according the following equation:

**Hepatosomatic index (HSI) = (liver weight⁄total weight) * 100**

**6. Detection of lead and cadmium in fish tissues**

Fish samples were dissected and the gills, liver, and muscle tissues were excised and freeze-dried. Tissues were cleaned, rinsed in double deionized water, and blotted on filter paper. Then, one gram of tissues taken from the liver, gills, and muscles was placed in a clean screw-capped tube and digested according to the method of **Finerty *et al.* (1990)**. Concentrated nitric and perchloric acid (AR grade) in a 5:5 ratio was used in Teflon beakers on a hot plate at 50°C for about 5 hours until organic matter decomposition took place. The digested solutions were cooled to room temperature, filtered and diluted to a final volume of 50ml with deionized distilled water. The obtained solutions were then analyzed by using inductively-coupled plasma mass spectrometry (ICP-MS) (model ELAN 9000, Perkin Elmer ICP-MS, USA) to measure Pb and Cd concentrations in the Central Laboratory, National Research Centre.

**7. Bioaccumulation Factor (BAF)**

According to EPA guidelines, the bioaccumulation factor (BAF) is defined as the ratio of metal concentration in the organism to that in the surrounding water. BAF was calculated according to **Kalfakakour and Akrida-Demertzi (2000)** as follows:

**BAF = M tissue/M water**

Where; M tissue: is the metal concentration in fish tissue µg/g, and

M water: metal concentration in water mg/L.

**8. Statistical analysis**

Data were analyzed using Statistical Package for the Social Science (SPSS) software (Version 22) (IBM Corp., Armonk, NY). Differences in metal concentration and time exposure were determined using 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

### 1. $LC_{50}$ values of lead and cadmium

The 96-h $LC_{50}$ values of lead and cadmium exposure in the Nile tilapia (*O. niloticus*) were calculated as 36.0 and 31.0 ppm, respectively. Such results were used to calculate the experimental concentrations of lead and cadmium as a percentage of their $LC_{50}$ to obtain low and high concentrations used for each compound's experiments, as shown in Table (1).

### 2. Survival rate

The results of survival rates of *O. niloticus* under five different experimental treatments in a 60-day experimental period are shown in Fig. (1). The exhibited data showed that there was no mortality for fish specimens in the control group. In general, the mortality rate was higher in fish exposed to Cd than in those exposed to Pb.

When fish were exposed chronically to lead, either at a low exposure concentration ($T_1$: 0.72 mg/l) or at a high one ($T_2$: 3.6 mg/l), the survival percentages were 96.6 and 93.33 %, respectively, at the end of the experiment. Moreover, the first death was recorded on day 55 in $T_1$ and day 40 in $T_2$.

On the other hand, the first deaths in the cadmium experiments, either at low exposure concentration ($T_3$: 0.62 mg/l) or at a high one ($T_4$: 3.1 mg/l) were recorded on day 10 after exposure and the survival percentages were gradually decreased, reaching 53.3% in $T_3$ and 46.7% in $T_4$ at the end of the experiment.

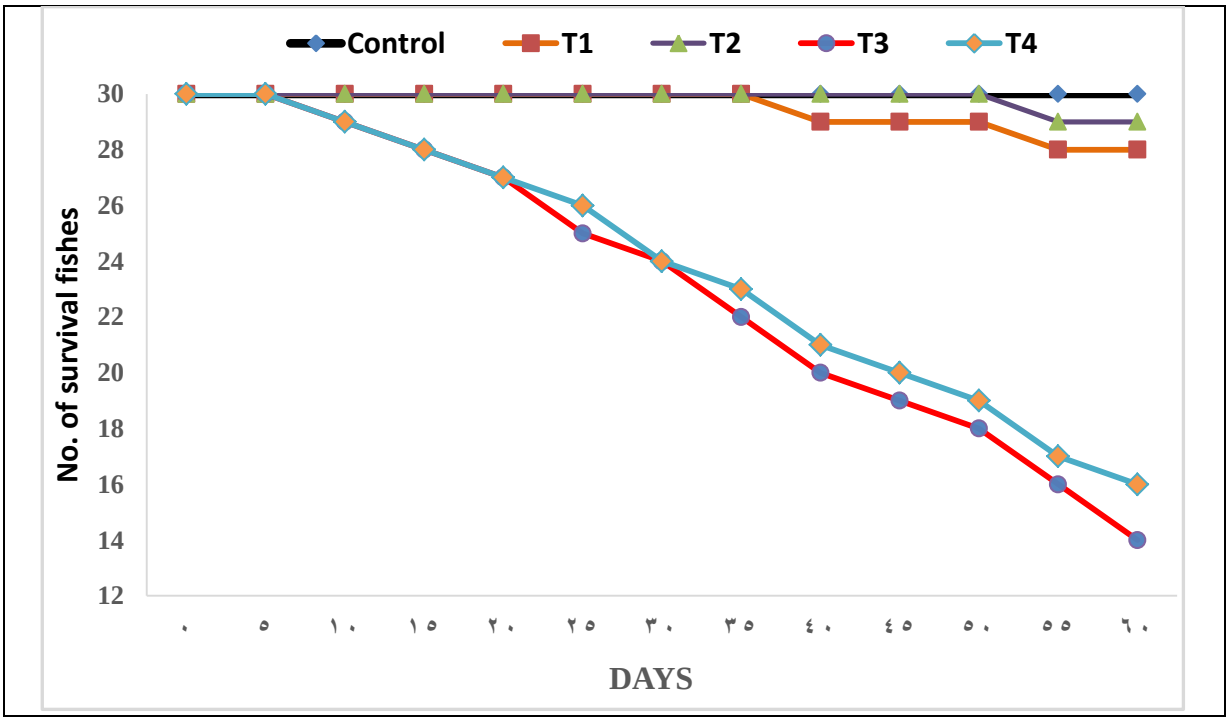


**Fig. 1.** Correlation between different Pb and Cd concentrations and survival rate of *O. niloticus*

### 3. Growth performance

At the beginning of the experiment; in all different experimental trials, the average total weight of the fish was 27.0 ± 0.81 g and gradually increased in the control group until it reached 72.4 ±1.85 g at the end of the experiment. In the same way, the standard length in the control group ranged from 8.8 ±0.97cm on day 0 to 12.2 ±0.28cm on day 60 (Table 2).

Statistically, there were significant differences ($P > 0.05$) between the total weight and standard length in the control group and all in the different trial experiments exposed to low and high concentrations of Pb and Cd. In general, total weight and standard length in all Pb and Cd exposure groups were lower than those of fish in the control group.

Statistical analysis indicates that the total weight and standard length of *O. niloticus* were significantly affected by metal concentrations and time exposure factors in Pb and Cd trial experiments. Contrary to what was expected, it was found that $T_2$ group was greater in total weight and standard length (46.60 ± 4.52g and 11.0±0.97cm, respectively) than $T_1$ group (44.8 ± 2.91g and 10.5 ± 0.7cm, respectively) on day 45. Then, it was reversed where the increase was greater in total weight and the standard length of $T_1$ group (52.7 ± 3.85g and 11.7 ± 0.5cm, respectively) than $T_2$ group (46.6 ± 4.52g and 11.4 ± 0.6cm, respectively) on day 60 (Table 2).

In the same way, the average increase in total weights of fish in the $T_4$ group was greater (46.7 ± 1.48g), compared to those recorded in $T_3$ group (41.9 ± 1.49g) at the end of the experiment (day 60). On the contrary, the increase in the standard lengths of fish in the $T_3$ group (11.0 ± 0.9 cm) was greater than those registered in the $T_4$ group (9.9 ± 0.3 cm) at the end of the experiment (Table 2).

Compared to the control group, all growth parameters (WG, DWG and RGR) of *O. niloticus* were retarded due to the exposure either to Pb or Cd at different concentration levels. The highest WG, DWG and RGR% were found in the control group, whereas the lowest DWG was observed in $T_1$ and $T_3$ groups. On the other hand, the lowest weight gain and RGR% values were recorded in $T_2$ and $T_3$ groups (**Table 2)**.

**Table (2): Growth performance of *O. niloticus* exposed to different concentrations of Pb and Cd among successive intervals (15 days).**

| Parameter | Days | Control | Pb | | Cd | |
|---|---|---|---|---|---|---|
| | | | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| **Weight (g)** | 0 | $28.2^{a}$±3.71 | $27.03^{b}$±2.15 | $27.1^{b}$±1.90 | $25.97^{c}$±0.75 | $26.7^{c}$±1.53 |
| | 15 | $46.3^{a}$±2.83 | $35.4^{b}$±1.48 | $37.75^{b}$±2.75 | $34.0^{c}$±4.93 | $32.8^{c}$±2.5 |
| | 30 | $52.4^{a}$±6.29 | $41.2^{b}$±2.91 | $43.0^{c}$±7.07 | $36.9^{d}$±4.7 | $38.0^{d}$±2.43 |
| | 45 | $60.6^{a}$±1.86 | $44.8^{b}$±6.28 | $46.20^{b}$±5.93 | $38.7^{c}$±1.86 | $40.8^{d}$±1.68 |
| | 60 | $72.4^{a}$±1.85 | $52.7^{b}$±3.85 | $46.60^{c}$±4.52 | $41.9^{d}$±1.49 | $46.7^{c}$±1.48 |
| **Length (cm)** | 0 | $8.8^{a}$± 0.97 | $7.8^{b}$± 0.15 | $7.7^{b}$± 0.65 | $7.9^{a}$± 0.35 | $7.3^{b}$± 0.50 |
| | 15 | $10.0^{a}$± 0.42 | $8.5^{b}$± 1.16 | $9.5^{c}$± 0.53 | $8.9^{b}$± 1.36 | $7.6^{d}$± 0.46 |
| | 30 | $10.9^{a}$± 0.54 | $10.0^{b}$± 1.11 | $10.2^{b}$± 1.72 | $10.6^{a}$± 0.36 | $7.9^{d}$± 0.81 |
| | 45 | $11.5^{a}$± 0.44 | $10.5^{b}$± 0.7 | $11.0^{c}$± 0.97 | $10.6^{b}$± 0.53 | $9.2^{d}$± 0.26 |
| | 60 | $12.2^{a}$± 0.28 | $11.7^{b}$± 0.52 | $11.4^{b}$± 0.56 | $11.0^{c}$± 0.85 | $9.9^{d}$± 0.25 |
| **Weight gain WG (g)** | | 41.4±3.55 | 25.7±1.87 | 19.20± 2.69 | 15.9± 0.81 | 19.9± 0.94 |
| **Daily Weight Gain DWG (g/day)** | | 0.69± 0.06 | 0.29± 0.2 | 0.32± 0.04 | 0.27± 0.01 | 0.31± 0.04 |
| **Relative growth rate "RGR" (%)** | | 134.06±19.91 | 95.15±4.2 | 69.99±6.59 | 61.34±1.99 | 74.80±6.56 |

**Note: Data are expressed as mean ± standard error. Means with the same letter within the same raw are not significantly different (p > 0.05).**

## 4. Hepatosomatic Index (HSI)

During the entire duration of the experiment, the hepatosomatic index (HSI) is always high in fishes exposed to Pb at different concentration levels, its average reached to (2.03) and (2.37) at day 60 in $T_1$ and $T_2$, respectively, compared with fishes in the control group, its

average was 1.99 at day 60. Such increases in the HSI were sharp in both Cd concentration exposure experiment trial groups during the first 30 days, recording 1.9 and 2.12 in $T_3$ and $T_4$ groups, respectively. Then the HSI values slightly decreased at day 45, being 1.71 and 1.83 in $T_3$ and $T_4$ groups, respectively. It was still higher than the control. However, HSI at day 60 reached 1.73 and 1.87 in $T_3$ and $T_4$, respectively (**Table 3)**.

**Table (3): Hepatosomatic index (HIS) of *O. niloticus* exposed to different concentrations of Pb and Cd among successive intervals (15 days).**

| Days | Control | Pb | | Cd | |
|---|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| 0 | $1.02^{a}$±0.29 | $1.01^{a}$± 0.45 | $1.01^{a}$± 0.45 | $1.01^{a}$± 0.45 | $1.01^{a}$± 0.45 |
| 15 | $1.26^{a}$±0.13 | $1.37^{b}$± 0.14 | $1.47^{c}$± 0.21 | $1.49^{c}$± 0.28 | $1.54^{c}$± 0.22 |
| 30 | $1.60^{a}$±0.32 | $1.86^{b}$± 0.23 | $2.04^{c}$± 0.18 | $1.9^{b}$± 0.30 | $2.12^{c}$± 0.33 |
| 45 | $1.84^{a}$± 0.19 | $1.99^{b}$± 0.09 | $2.17^{c}$± 0.52 | $1.71^{d}$± 0.24 | $1.83^{a}$± 0.31 |
| 60 | $2.01^{a}$±0.22 | $2.03^{a}$± 0.24 | $2.37^{b}$± 0.35 | $1.73^{c}$± 0.38 | $1.78^{c}$± 0.27 |

**Note: Data are expressed as mean ± standard error. Means with the same letter within the same raw are not significantly different (p > 0.05)**

## 5. Metal accumulation in fish tissues

Changes in Pb and Cd concentrations in liver, gills, and muscles of *O. niloticus* fish, among experiment successive intervals (15 days), exposed to different concentrations were presented in **Table (4)**. Statistical analysis indicated that there is a significant increase (p> 0.05) of both Pb and Cd concentration in all target organs over the exposure time.

The uptake of lead by the different organs of tilapia fish was varied. The preferred organ tissues for lead accumulation were the gills, which recorded the highest accumulation values among the exposure times ranging from 3.47 ±0.35 and 7.22 ±0.81 µg/g wet weight (at day 15) to 13.21 ±1.34 and 29.37 ±2.4 µg/g wet weight (at day 60) in $T_1$ and $T_2$, respectively. It followed by the liver, while the lowest concentrations were found in muscle tissue. A significant increase was observed in all target organs with the prolongation of the exposure period and the increase in metal concentration in water. The lead uptake on day 15 and day 60 of $T_1$ was higher in the liver and gills, but in $T_2$ the uptake of metal in the gills and the liver was high for 45 days and then decreased through the last 15 days. While in muscle tissue, Pb gradually accumulates in different concentrations **(Table 4)**.

The preferred organ for accumulation of cadmium was found in the liver as it ranged from 6.02 ±0.44 and 22.16 ±1.52 µg/g wet weight (at day 15) to 37.16 ±4.13 and 70.79 ±5.55 µg/g wet weight (at day 60) in $T_3$ and $T_4$, respectively. It followed by the gills, with much lower concentrations found in muscle tissue compared to other target organs. The mean value of Cd accumulation was ordered as follows: liver > gills > muscles. The Cd accumulation in the liver, gills, and muscles was gradually increased by an increase in Cd concentration and exposure time, irrespectively of the uptake route **(Table 4).**

## 6. Bioconcentration factor (BCF)

Results in **Table (5)** indicated that the bioconcentration factor (BCF) for Pb attained its high values in the gills in the groups ($T_1$ and $T_2$), among the entire exposure period, and the lower values in the muscles. The bioconcentration factor of $T_1$ was greater than that of $T_2$ in all the target organs except in muscles, in which its BCF values of $T_2$ group during the first 30 days were greater than that of $T_1$ group. However, the bioconcentration factor for cadmium attained its high rate in the liver, regardless of the concentration level. Cadmium bioconcentration factor was minimal in the muscles during all exposure periods. By

comparing the two groups of concentrations for all the studied target organs, the BCFs of $T_3$ are always greater than that of $T_4$ **(Table 5)**.

The BCF values in each tissue in lead groups ($T_1$ and $T_2$) were relatively close to each other except in the gills. Whereas, in the gills, it ranged from 4.81 to 18.34 after 15 to 60 days of exposure in $T_1$ group; and from 2.01 to 8.16 after 15 to 60 day of exposure in $T_2$ group. However, it ranged in the liver from (1.56 on day 15) to (4.71 on day 60) and from (0.55 on day 15) to (3.12 on day 60) in the $T_1$ and $T_2$ groups, respectively. Also, in muscles, it ranged from (0.42 on day 15) to (3.67 on day 60) and from (0.56 on day 15) to (2.41 on day 60) in $T_1$ and $T_2$ groups, respectively.

By comparing the ratios of cadmium BCF in $T_3$ with the ratios in $T_4$, it was observed that these values were relatively doubled in $T_3$ (low concentration) than the ratios in $T_4$ in all target organs. **(Table 5)**.

**Table (4): Accumulation of Pb and Cd in liver, gills and muscles (µg/g wet weight) in different experimental groups of *O. niloticus* fishes, among successive intervals (15 days)**

| Metal | Days | Liver | | Gills | | Muscles | |
|---|---|---|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $T_1$ | $T_2$ | $T_1$ | $T_2$ |
| Pb | 0 | $0.04^{a}$± 0.02 | $0.04^{a}$± 0.02 | $0.03^{b}$± 0.02 | $0.03^{b}$± 0.02 | $0.01^{c}$± 0.01 | $0.01^{c}$± 0.01 |
| | 15 | $1.12^{a}$± 0.1 | $1.97^{b}$± 0.37 | $3.47^{b}$± 0.35 | $7.22^{c}$± 0.81 | $0.31^{d}$± 0.63 | $2.02^{e}$± 0.47 |
| | 30 | $1.52^{a}$± 0.17 | $4.27^{b}$± 0.42 | $5.26^{c}$± 0.63 | $14.82^{d}$±0.77 | $0.76^{d}$± 0.14 | $5.83^{e}$± 0.83 |
| | 45 | $2.26^{a}$± 0.1 | $10.68^{b}$± 1.79 | $8.06^{c}$± 1.60 | $25.60^{d}$± 2.86 | $1.74^{d}$± 0.22 | $7.38^{e}$± 0.70 |
| | 60 | $3.39^{a}$± 0.38 | $11.23^{b}$± 0.53 | $13.21^{c}$± 1.34 | $29.37^{d}$± 2.4 | $2.64^{d}$± 0.23 | $8.67^{e}$± 1.19 |
| Cd | Days | $T_3$ | $T_4$ | $T_3$ | $T_4$ | $T_3$ | $T_4$ |
| | 0 | $0.05^{a}$± 0.04 | $0.05^{a}$± 0.04 | $0.01^{c}$± 0.01 | $0.01^{c}$± 0.01 | $0.01^{e}$± 0.004 | $0.01^{e}$± 0.004 |
| | 15 | $6.02^{a}$± 0.44 | $22.16^{b}$± 1.52 | $3.50^{c}$± 0.39 | $4.25^{c}$± 1.07 | $0.29^{e}$± 0.12 | $1.14^{f}$± 0.37 |
| | 30 | $15.15^{a}$± 1.94 | $35.90^{b}$± 2.44 | $4.68^{c}$± 0.39 | $12.52^{d}$± 1.16 | $0.83^{e}$± 0.14 | $2.22^{f}$± 0.19 |
| | 45 | $28.04^{a}$± 2.48 | $43.81^{b}$± 2.63 | $7.71^{c}$± 0.56 | $15.70^{d}$± 2.11 | $1.73^{e}$± 0.30 | $3.04^{f}$± 0.30 |
| | 60 | $37.16^{a}$± 4.13 | $70.79^{b}$± 5.55 | $13.48^{c}$± 1.09 | $21.74^{d}$± 2.52 | $2.23^{e}$± 0.15 | $3.87^{f}$± 0.52 |

**Note: Data are expressed as mean ± standard deviation; Means with the same letter within the same raw are not significantly different (*P*> 0.05)**

**Table (5): Bioconcentration factor of Pb and Cd in liver, gills and muscles (µg/g wet weight) in different experimental groups of *O. niloticus* fishes**

| Metal | Days | Liver | | Gills | | Muscles | |
|---|---|---|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $T_1$ | $T_2$ | $T_1$ | $T_2$ |
| Pb | 15 | 1.56±0.14 | 0.55±0.10 | 4.81±0.49 | 2.01±0.22 | 0.42±0.09 | 0.56±0.13 |
| | 30 | 2.11±0.24 | 1.19±0.12 | 7.31±0.87 | 4.12±0.22 | 1.05±0.20 | 1.62±0.23 |
| | 45 | 3.14±0.15 | 2.97±0.50 | 11.20±2.23 | 7.11±0.80 | 2.41±0.31 | 2.05±0.19 |
| | 60 | 4.71±0.53 | 3.12±0.15 | 18.34±1.87 | 8.16±0.67 | 3.67±0.32 | 2.41±0.33 |
| Cd | Days | $T_3$ | $T_4$ | $T_3$ | $T_4$ | $T_3$ | $T_4$ |
| | 15 | 9.71±0.70 | 7.15±0.49 | 5.65±0.62 | 1.34±0.35 | 0.46±0.19 | 0.37±0.12 |
| | 30 | 24.43±3.13 | 11.58±0.79 | 7.55±0.63 | 4.04±0.37 | 1.34±0.23 | 0.72±0.06 |
| | 45 | 45.23±4.0 | 14.13±0.85 | 12.43±0.90 | 5.07±0.68 | 2.79±0.48 | 0.98±0.10 |
| | 60 | 59.93±6.67 | 22.84±1.79 | 21.73±1.76 | 7.01±0.81 | 3.59±0.24 | 1.25±0.17 |

**Note: Data are expressed as mean ± standard deviation.**

## DISCUSSION

In the current study, the 96-hr $LC_{50}$ of lead in *O. niloticus* was 36 ppm (mg/L). This finding was lower than that found by **Asim Ullah *et al*. (2016),** whom reported that the 96-hr $LC_{50}$ value of lead nitrate for *O. niloticus* was 44 mg/l. However, it was higher than that reported by **Arya *et al.* (2018),** whom found that the $LC_{50}$ value of lead acetate at 96-hr was 17.33 mg/L for *O. mossambicus*. On the other hand, the 96-hr $LC_{50}$ of cadmium in *O. niloticus,* in the present work, was 31 ppm (mg/L). Our result was lower than that reported by **Faheem *et al*. (2012)** on the same species exposed to cadmium chloride with a 35.9 mg/l $LC_{50}$, and higher than that found by **Al-Asgah *et al.* (2015)** and **Abdel-Aziz *et al.* (2022),** where they reported that the $LC_{50}$ of cadmium in *O. niloticus* was 16.8 and 28.01 mg/L, respectively. Such $LC_{50}$ variability may be attributed to several factors, including differences in weight, sex, and composition of the toxic substance, as well as the experimental conditions such as water hardness, water temperature, and pH **(Abdullah *et al*., 2007; Atli & Canli, 2011; Abedi *et al*., 2012**; **Kim *et al*., 2020)**.

Regarding fish survivability, early mortalities were noticed in both cadmium exposure groups on day 10 of the experiment. Furthermore, the first mortality recorded in Pb exposure groups was after day 40. The toxic effects of heavy metals on fish are multi-directional and manifested by numerous changes in physiological and chemical processes (**Emere & Dibal, 2013; Javed & Usmani, 2017).** The presence of lead and cadmium at low concentrations in water may lead to bioconcentration and accumulation of these metals in various organs of fish and may cause functional disturbances (**Mahmoud *et al*., 2014; Paul & Small, 2021**). According to **Li & Xie (2018)**, fish exposed to an acute lethal concentration of cadmium are related to the loss of sodium ions, which causes disruption of the cardiovascular system, which leads to fish kill. Long-term metal exposure causes numerous histopathological changes, particularly in fish gills, that may lead to respiratory dysfunction, hypoxia and fish death (**Agatha, 2010; Abdel-Baki *et al*., 2011; Mahamood *et al*., 2021**). **Çiftçi *et al.* (2015a)** recorded that no mortality was observed in *O. niloticus* exposed to 0.2 ppm Pb over 30 days, which could be due to the tested concentrations of these metals not being lethal to this species at the experimental periods tested. The exaggerated mortalities noticed in the present study could be due to the length of exposure time.

The changes in the total weight and standard length at chronic Pb and Cd exhibit negative effects on growth during the experimental period as compared with the control group. This reduction in growth is attributed to the poor utilization of food during the exposure period (**Cai *et al*., 2020; Taslima *et al.*, 2022**). It was observed that treated fish lost portability and food conversion with the passage of time (**Ayegbusi *et al*., 2018; Fazio *et al*., 2022**). In agreement with our results, **Ahmed *et al*. (2012)** studied the growth of grass carp fingerlings exposed to different concentrations of Cd and Pb for 8 weeks. They have observed that the cadmium treatments caused a significant reduction in growth as compared to lead treatments and the control group. Similar observations were noticed by **Vincent *et al.* (2002)**, whom studied the impact of a waterborne sublethal concentration of cadmium on *Catla catla* fish and found that the cadmium exhibited depletion in food utilization parameters in fish and that it was dependent on metal concentration.

Generally, the growth rate in fish is generally retarded as the response to exposure to toxicants because the allocation of energy assigned to growth and reproduction will transfer to tissue repair (**Segner, 2011; Xie *et al*., 2019).** Experimentally, the metal accumulation at high concentration can induce retarded growth in fish development, which has an influence fish size and such findings were reported by many authors **(Al-Asgah *et al*., 2015; Cai *et al*.,**

**2020).** In the current work, both weight gain and specific growth rates of *O. niloticus* declined with Pb and Cd chronic exposure. Similar results were noticed by **Ayegbusi *et al.* (2018)** and they revealed that the exposure of *Clarias gariepinus* to sub-chronic concentrations of Pb significantly affected the survival and growth performance, specific growth rate, and food conversion efficiency. The findings on the effect of exposure to chronic concentrations of Pb and Cd on growth performance may be due to induced oxidative stress (**Yuan *et al.*, 2017; Araujo *et al.*, 2022**). **Erdogan *et al.* (2005)** suggested that the reduction in weight gain could have been due to the decrease in food intake, or to the overall increased degeneration of lipids and proteins as a result of cadmium toxicity. Previous studies have reported that the reduction of fish growth by exposure to lead and cadmium may be due to the consequent increase in metabolic demands (**Naz *et al.*, 2013; Ayyat *et al.*, 2017; Paul & Small, 2021**).

The HSI value informs us about the health of the fish along with the quality of the aquatic eco-system and it mostly used as a bioindicator of toxic exposure (**Çiftçi *et al.*, 2015b; Łuczyńska *et al.*, 2018**). Our results showed that the HSI value was higher in fish exposed to Pb at different concentration levels compared with fish in the control group. Such increases in the HSI were sharp in both Cd concentration exposure experiment trial groups during the first 30 days, then the value slightly decreased until the end of the experiment. Previous studies indicate that metal exposure may result in either increased HSI due to hepatic hyperplasia or hypertrophy (**Morado *et al.*, 2017; Aissioui *et al.*, 2022**). According to **Hansson *et al.* (2017)** the decreased HSI is due to loss of hepatocytes, probably caused by hepatic apoptosis, atrophy, or necrosis.

The heavy metals accumulation in aquatic animals has taken place through several mechanisms, including the direct uptake from water by gills and skin, and other ways through the digestive tract via food **(Isani *et al.*, 2009; Annabi *et al.*, 2013**; **Sauliutė & Svecevičius, 2015**). The concentration of heavy metals in the gills reflects their level in the fish's surrounding environment, whereas the concentration in the liver and kidneys represents metal storage (**Rao & Padmaja, 2000**). Thus, more than any other fish organ, fish gills and liver are recommended as ecological indicator devices for water contamination (**Obasohan *et al.*, 2008; Yilmaz, 2009**). In the present study, *O. niloticus* has exposed to waterborne Pb and Cd, resulting in accumulation in their tissues and redistribution among tissues. The Pb accumulation was distributed as follows: gills > liver > muscles. A similar distribution was observed in field studies for the Pb concentrations in the same species' tissues **(Abdel-Baki *et al.*, 2011; Akan *et al.*, 2012** and **Aly, 2016).** Higher Pb concentrations in the gills could be due to the metal combined with the mucus, that is practically impossible to completely remove from the gill lamellae before being prepared for analysis (**Eneji *et al.*, 2011**). Furthermore, the direct inflow of polluted water (through the gills chamber during inhalant breathing current) makes the gills the first target for pollutants in water and could also have a significant influence on the total metal accumulation of the gill (**Çiftçi *et al.*, 2015a**). Target organs such as gills and the liver are metabolically active parts that can accumulate heavy metals at higher levels (**Shovon *et al.*, 2017; El-Khayat *et al.*, 2022**). On the other hand, the present data showed that the distribution of accumulated Cd in *O. niloticus* organs was mainly higher in the liver, followed by the gills, and muscle. These results are in full agreement with the findings obtained by **Badr *et al.* (2014); Al-Asgah *et al.* (2015)** and **Radwan *et al.* (2022**). They found a similar distribution pattern for Cd in the same species' tissues. The liver is one of the most important organs in eco-toxicological investigations due to its main accumulation tissue for metal exposure in fish, and it functions in amino acid and energy metabolism, blood composition, and toxic detoxification (**Authman *et al.*, 2015**). The high fat content of the liver of tilapia may explain the higher concentration of heavy metals

compared to the muscle tissues (**Zaki *et al*., 2014**; **Ferreira *et al*., 2019**), unlike muscle tissues, which have lower metal accumulation than other organs because of their low-fat content (**Azab *et al*., 2016**).

The Bioconcentration factor (BCF) is the ratio of a waterborne heavy metals concentration in an organism to the concentration in water and usually determined in laboratory studies (**USEPA, 2016).** The present results revealed that the BCFs of low Cd concentration exposure are always greater than that of high concentration exposure. The same is the case with lead, except for muscles where the opposite has happened after 30 exposure days. According to **McGeer *et al.* (2003)** whom collected the metal BCF data for different aquatic organisms and generally noticed a contrary relationship between BCF and exposure concentration, they mentioned that the BCFs are highest at low metal concentrations (low potential for toxicity) and lowest at higher exposure concentrations where toxicity is more likely. This idea is based on the fact that organisms will actively uptake essential metals at low concentrations to serve metabolic needs and that non-essential metals are frequently regulated to varying degrees because the mechanisms for regulating essential metals are not metal-specific (**DeForest *et al*., 2007**).

## CONCLUSION

The toxicity of both Lead and cadmium affects the survivorship and growth performance of *O niloticus* and these impacts are sharp in Cadmium than in Lead. The level of concentration and the time of exposure significantly affect survivability and growth negatively. On the contrary, there were increases in the HSI values that were higher group in fish exposed to Pb with high and low concentrations than those in the control group and this value was sharp in Cd exposures trials during the first 30 days indicating hepatic hyperplasia or hypertrophy.

The current study showed that the gills and liver are among the metabolically active parts in which heavy metals can accumulate at high levels, while lead prefers to accumulate in the gills, and cadmium prefers to accumulate in the liver, and each replaces the other in the arrangement of organs as the second preferred organ in the other metal. The present results showed that the BCF was higher in the case of exposure to the lower concentration than exposure to the higher concentration, especially in the days after the starting point until the saturation point, which will be shorter in the compounds with higher toxicity, as in the case of cadmium.

## 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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