Artículo de investigación

Toxicity of Zinc at sublethal exposure to rainbow trout (*Oncorhynchus mykiss*)

Toxicidad de zinc por exposición subletal en trucha arco iris (Oncorhynchus mykiss)

Toxicidade por exposição ao zinco em trutas arco-íris (Oncorhynchus mykiss)

Fereshteh Nasri [2] ✉; M. Saeed Heydarnejad [1*] ✉; Amin Nematollahi [3] ✉

Filiación:

*Correspondig author: M. Saeed Heydarnejad. Shahrekord University, PO B 115, Shahrekord 88186, Iran.
E-mail: msh@utas.edu.au

Comparte

Abstract

Zinc (Zn), can alter different physiological processes in fish such as growth and serum parameters. The objective of this study was to investigate the toxicity of Zn on sublethal exposure to rainbow trout (*Oncorhnychus mykiss*). Fish exposed to higher levels of Zn grew slower than fish exposed to lower levels of Zn. Weight gain and SGR (Specific Growth Rate) decreased linearly as the Zn level in the water increased. The CF (Condition factor) of fish grown in water with a high concentration of Zn also decreased significantly compared to the low concentration of Zn, whereas the Food Conversion Ratio (FCR) increased with the concentration of Zn in water. Both AST (Aspartate Transaminase) and ALT (Alanine Transaminase) activities were significantly altered by Zn and showed a linear pattern and decreased after 30 days, while ALP (Alkaline Phosphatase) levels showed a remarkable increase from a mean level of control. This study suggests that biochemical parameters of growth and serum could be used as important and sensitive biomarkers to monitor heavy metal toxicity on fish health status.

Keywords: *Rainbow trout, heavy metals, Zinc, growth.*

Resumen

TZinc (Zn), puede alterar diferentes procesos fisiológicos en peces, tales como, el crecimiento y los parámetros séricos. El objetivo de este estudio fue investigar la toxicidad de Zn en la exposición subletal de la trucha arco iris (*Oncorhnychus mykiss*). Los peces expuestos a niveles más altos de Zn crecieron más lentamente que los peces expuestos a niveles más bajos de Zn. El aumento de peso y la SGR (tasa de crecimiento específica) disminuyeron linealmente a medida que aumentó el nivel de Zn en el agua. El CF (factor de condición) de los peces cultivados en agua con una alta concentración de Zn, también disminuyó significativamente en comparación con la baja concentración de Zn, mientras que el índice de conversión de alimentos (FCR) aumentó con la concentración de Zn en el agua. Las actividades AST (Aspartate Transaminase) y ALT (Alanine Transaminase) fueron significativamente alteradas por el Zn y mostraron un patrón lineal y disminuyeron después de 30 días, mientras que los niveles de ALP

1. Shahrekord University, PO B 115, Shahrekord 88186, Iran (Supervisor).

2. Zoology dept. Faculty of Science, Shahrekord University, Shahrekord, Iran. E-mail: fereshtehnasri@ymail.com (Student).

3. Veterinary Dept., Shahrekord University, Shahrekord, Iran. E-mail: anematolahi@yahoo.com (Co-supervisor).

(Fosfatasa Alcalina) mostraron un aumento notable desde un nivel medio de control. Este estudio sugiere que los parámetros bioquímicos de crecimiento y suero podrían usarse como biomarcadores importantes y sensibles para controlar la toxicidad de metales pesados en el estado de salud de los peces.

Palabras clave: *Trucha arcoiris, metales pesados, Zinc, crecimiento.*

Resumo

O TZinc (Zn) pode alterar diferentes processos fisiológicos em peixes, como parâmetros de crescimento e soro. O objetivo deste estudo foi investigar a toxicidade do Zn na exposição subletal à truta arco-íris (*Oncorhnychus mykiss*). Os peixes expostos a níveis mais altos de Zn cresceram mais lentamente do que os peixes expostos a níveis mais baixos de Zn. O ganho de peso e a SGR (Taxa de Crescimento Específico) diminuíram linearmente à medida que o nível de Zn na água aumentou. O CF (fator de condição) de peixes cultivados em água com alta concentração de Zn também diminuiu significativamente em comparação com a baixa concentração de Zn, enquanto a tax on de conversão alimentar (FCR) aumentou com a concentração de Zn na água. As atividades de AST (Aspartate Transaminase) e ALT (Alanine Transaminase) foram significativamente alteradas pelo Zn e mostraram um padrão linear e diminuíram após 30 dias, enquanto os níveis de ALP (Fosfatase Alcalina) mostraram um aumento notável em relação ao nível médio de controle. Este estudo sugere que parâmetros bioquímicos de crescimento e soro podem ser usados como biomarcadores importantes e sensíveis para monitorar a toxicidade de metais pesados no estado de saúde dos peixes.

Palavras-chave: *Truta arco-íris, metais pesados, Zinco, crescimento.*

Introduction

An interesting subject of research is the essential heavy metals, also referred to as essential trace elements because they are required at low levels for the function of different biological processes, but at increased levels they are toxic to the organism (Zheng *et al.* 2016). For both terrestrial and aquatic animals, Zn is an essential trace element. It is an integral part of many metalloenzymes (Gropper *et al.* 2005). A lack of Zn resulted in decreased growth, cataracts, skin lesions and dwarfism (Park and Shimizu 1989; Read *et al.* 2014; Zheng *et al.* 2015). Zn deficiency (1 mg Zn kg-1 in food) has been shown, for example, to lead to growth retardation in rainbow trout (Ogino and Yang 1978) and Nile tilapia (Do Carmo Sa *et al.* 2007). In order to maintain health and high growth performance, the diets offered to aquatic animals must therefore contain the optimum Zn level (Fountoulaki *et al.* 2010). As reported, rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*), blue tilapia (*Oreochromis aureus*) and red drum (*Sciaenops ocellatus*) requirements are within the range of 15-30 mg/kg diet (Ogino and Yang 1978; Fountoulaki *et al.* 2010; Read *et al.* 2014).

A number of studies on the accumulation of zinc in aquatic organisms were conducted both under laboratory conditions and in the field (Golovina 1996; Shaw *et al.* 2006; Fountoulaki *et al.* 2010). Zn absorbed by fish is transported through the bloodstream to various tissues where it causes tissue damage, changes in blood parameters and endocrine and metabolic activity (Tuncosoya *et al.* 2016). In fact, fish were used to a large extent in the assessment of aquatic system quality. These organisms are often at the top of the aquatic food chain and can concentrate large quantities of metals

from the waters around them (Rajkowska and Protasowicki 2011). The accumulation of metals in aquatic systems suggests that fish can serve as a useful indicator of contaminating metals in aquatic systems as they respond to changes in the aquatic environment with great sensitivity (Vinodhini and Narayanan 2008).

Sub-lethal exposure to certain heavy metals can cause these metals to accumulate in fish (Hollis *et al.* 1999; McGeer *et al.* 2000). Some organs are often investigated for metal accumulations, such as the liver, intestines, gills, and kidneys. Biochemical parameters are used as an index for detecting physiological changes in several species of fish (Adhikari *et al.* 2004; Suvetha *et al.* 2010), because they are sensitive to potential adverse effects of accumulations of metals. Before hazardous effects occur in fish, the activities of different enzymes are considered as sensitive biochemical indicators and are important parameters for testing water for toxicants (Gul *et al.* 2004). Serum enzymes like ALP, ALT, and AST are considered a useful biomarker for determining levels of pollution during sublethal (chronic) exposure (Basaglia 2000; Ozman *et al.* 2006; Younis *et al.* 2012).

Fish growth depends on water's physiochemical parameters and usually decreases in contaminated waters (Weatherley and Cill 1987; Folmar 1993). Physiological or behavioral stress causes decreased growth during toxicant exposure (Alabater and Loyd 1980). Sublethal Zn exposure in various fish species leads to decreased appetite and poor growth (Alabater and Loyd 1980; Weatherley and Cill 1987; Folmar 1993; Galvez *et al.* 1998; Rowe 2003). The main objective of this study was to determine the influence of sublethal (100 and 250µg / L) Zn on growth and serum biochemical parameters including enzymes such as ALP, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), glucose, triglyceride, cholesterol and TP in rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Experimental fish and design
Rainbow trout was collected from a local hatchery (17.5±0.3 g; 11.4±0.2 cm) and then transferred to the laboratory. Fish were acclimatized for two weeks before experiments to laboratory conditions. The physicochemical characteristics of experimental water were shown in table 1. At random times, the fish were fed twice a day with commercial dry pellets. The feed consisted of 40.69±0.3% crude protein, 53.7±0.18% crude lipid, 25.59±0.11% ash and 11.13±0.5% humidity. Fish were fed for 30 days at a feeding rate of 2% of body weight a day.

Table 1. Mean (±SD) acclimatization parameters of water quality, cations, anions and background metals.

Parameter	Acclimation water
Temperature (°C)	9 ± 1
pH	7.8 ± 0.2
Dissolved oxygen (mg/L)	7.8-8.3
Total Hardness (mg/L as CaCO$_3$)	104.2
Total alkalinity (mg/L as CaCO$_3$)	78.8
Total dissolved solids (mg/L)	173
Sodium (mg/L)	5.0

Parameter	Acclimation water
Calcium (mg/L)	31.0
Potassium (mg/L)	0.5
Magnesium (mg/L)	6.4
Cl (mg/L)	11.3
NH_3 (mg/L)	0.05
SO_4^{-2} (mg/L)	15.0
PO_4^{-3} (mg/L)	0.05
Copper (µg/L)	0.63
Cadmium (µg/L)	0.57
Zinc (µg/L)	91.37

Experimental exposure

After two weeks of acclimatization, 20 fish were transferred to experimental tanks (160 l) containing dechlorinated, continuously aerated tap water. The fish were exposed to: (i) unexposed group or control: nominally zero Zn [actual measured 'in-tank' value: 91.37 µg/l], (ii) low Zn [100µg/l] and (iii) high Zn [250µg/l] for 1, 15 and 30 days. Zn added as $ZnSo_4.2H_2O$ (Merck, Germany). The experiment was performed based on the three replicates. Every 2 days, the water was changed to minimize metal loss and maintain metal concentration. During the experimental period, the water quality parameters mentioned were evaluated at the collection days. The performance of growth was calculated as follows:

Condition Factor (CF) = 100 weight (g)/length³ (cm).

Specific Growth Rate (as percentage of body weight gain per day) = 100 × [ln final weight (g) - ln initial weight (g)] /time (days).

Feed conversion ratio (FCR) = feed intake/weight gain
Weight Gain Percentage = [final weight (g) - initial weight (g)] / initial weight (g).

Biochemical analysis

Before sampling, fish were not fed for 24 hours. Four specimens of fish from each tank were anesthetized with clove oil (25 mg/l) and blood samples were taken from the caudal vein with a sterile syringe containing an anticoagulant heparin solution (There were sacrificed after taking the blood sample). At the beginning and at the end of the experiment, fish length and weight were measured. Blood samples were stored for up to 4 hours in the refrigerator. Serum was obtained for 10 minutes by blood centrifugation at 4000 g and stored for further biochemical analysis at –48 °C. Biochemical analyzer Hitachi 911 (Japan) was used to determine AL, AST, and ALP activity levels with glucose, triglyceride, cholesterol, and total serum protein concentrations.

Statistical analysis

With the Kolmogorov-Smirnov test, normal data distribution was tested. All values with the standard mean error (SEM) were presented as averages. The one-way variance analysis (ANOVA) was used to test the difference between the control means and

the sampling times in each exposure group and the growth parameters. The post-hoc comparisons, i.e. the multi-range Duncan test, were performed with SPSS14 between treatment means. Significance at $P < 0.05$ was accepted.

Results

Growth

During the experiment, there were no fish deaths. Fish exposed to higher levels of Zn grew slower than fish exposed to lower levels of Zn (Table 2). This shows a clear dose-dependent response to test fish growth. There were significant differences ($p < 0.05$) in the total weight gain and SGR of fish grown in water containing diffe-rent concentrations of zinc compared to the value. Weight gain and SGR decreased linearly as zinc in the water increased. The CF of fish grown in water with high Zn concentration also decreased significantly ($p < 0.05$) compared to the low Zn concen-tration. Fish exposure to different water concentrations of Zn ($p < 0.05$) significantly reduced feed consumption compared to control – a direct correlation with water concentration levels of Zn. The values of the FCR increased with the concentration level of Zn in water, indicating poor use of food in the fish. Results on the growth performance of fish subjected to different sub-lethal (chronic) concentrations of Zn are presented in table 2.

Table 2. Rainbow trout (Oncorhynchus mykiss) growth performance in control, low exposure (100 μg / L) and high exposure (250 μg / L) zinc over the 30-day experimental period.

Parameters/groups	Control	Low Zn	High Zn
MWi	17.63 ± 0.38[a]	17.15 ± 0.48[a]	17.53 ± 0.50[a]
MWf	47.05 ± 1.75[a]	33.4 ± 3.46[b]	32.7 ± 1.55[b]
MBLi	11.63 ± 0.09[a]	11.5 ± 0.05[a]	11.63 ± 0.13[a]
MBLf	16.07 ± 0.18[a]	14.8 ± 0.51[b]	14.26 ± 0.23[b]
Cfi	1.12 ± 0.01[a]	1.12 ± 0.16[a]	1.10 ± 0.01[a]
CFf	1.13 ± 0.02[a]	1.02 ± 0.04[b]	1.12 ± 0.05[a]
SGR	3.27 ± 0.67[a]	2.20 ± 0.32[b]	2.07 ± 0.07[b]
BWG	166.12 ± 13.23[a]	94.66 ± 8.75[b]	83.44 ± 7.02[c]
FCR	0.67 ± 0.17[b]	0.91 ± 0.17[a]	0.95 ± 0.17[a]

MWi. initial mean weight (g); MWf. final mean weight (g); MBLi. initial mean body length (cm); MBLf. final mean body length (cm); CFi: initial condition factor; CFf: final condition factor; SGR: specific growth rate; BWG: body weight gain; FCR: feedconversion ratio. Significant differences between values in columns are indicated with letters ($p < 0.05$); values in columns with the same letters are not significantly different.

Serum enzymes and biochemical parameters

Zn markedly changed the activity levels of AST and ALT activity and displayed a linear pattern and decreased compared to the control after 30 days (Figure 1). For fish exposed to 100 μg / l Zn (lower concentrations). this reduction was more noticeable in both cases. By contrast. the level of ALP showed a remarkable increase from the mean level of control when the fish were exposed to both sublethal concentrations of zinc. However, no significant differences were found in days 15 and 30 between the two groups (lower and higher concentrations of Zn).
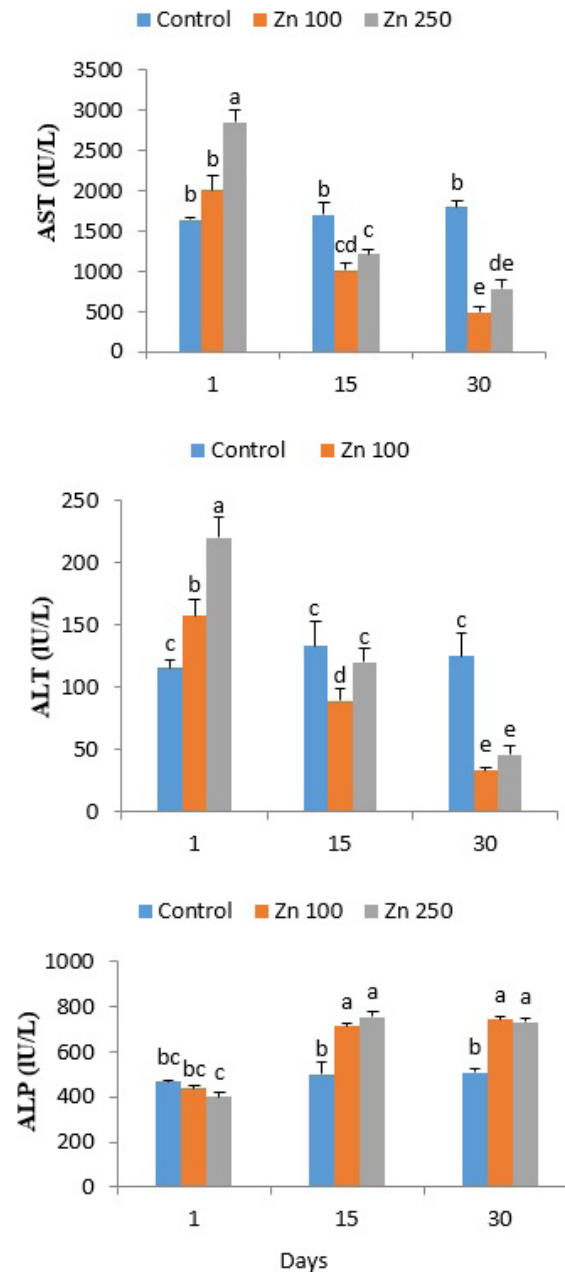
Figure 1. Effects of various concentrations of sublethal zinc on serum enzyme activity. (Upper) Serum aspartate transaminase (AST). (middle) serum alanine transaminase (ALT). (low) serum alkaline phosphatase (ALP) in zinc-exposed *Oncorhynchus mykiss*. Data is expressed as mean error ± standard (SE). Significantly different meanings with different letters (p<0.05)

However, the glucose concentration showed severe reduction on the first day of the experiment. it increased significantly after 15 days in both Zn exposed groups. especially at 250 μg/L Zn concentration. and increased to 131 ± 8.39 mg/dl compared to 30.67 ± 2.96 mg/dl in the control group. This glucose elevation was temporary and dropped sharply in high Zn exposed group at 30 days to 73.67 ± 6.39 mg/dl but was still higher than control group (Figure 2). With respect to triglycerides. the triglyceride

level decreased significantly to its minimum value after 15 days of exposure to different concentrations of Zn. reaching 301.33 ± 16.95 mg/dl in low exposure and 290.33 ± 31.47 in high exposure Zn groups compared to 486.67 ± 29.36 mg/dl in the control group. However, this significant depression followed by a rapid 30-day elevation so that the serum triglyceride level in both groups (concentrations of 100 and 250 µg/L Zn) returned to the same level in the control group (Figure 2).
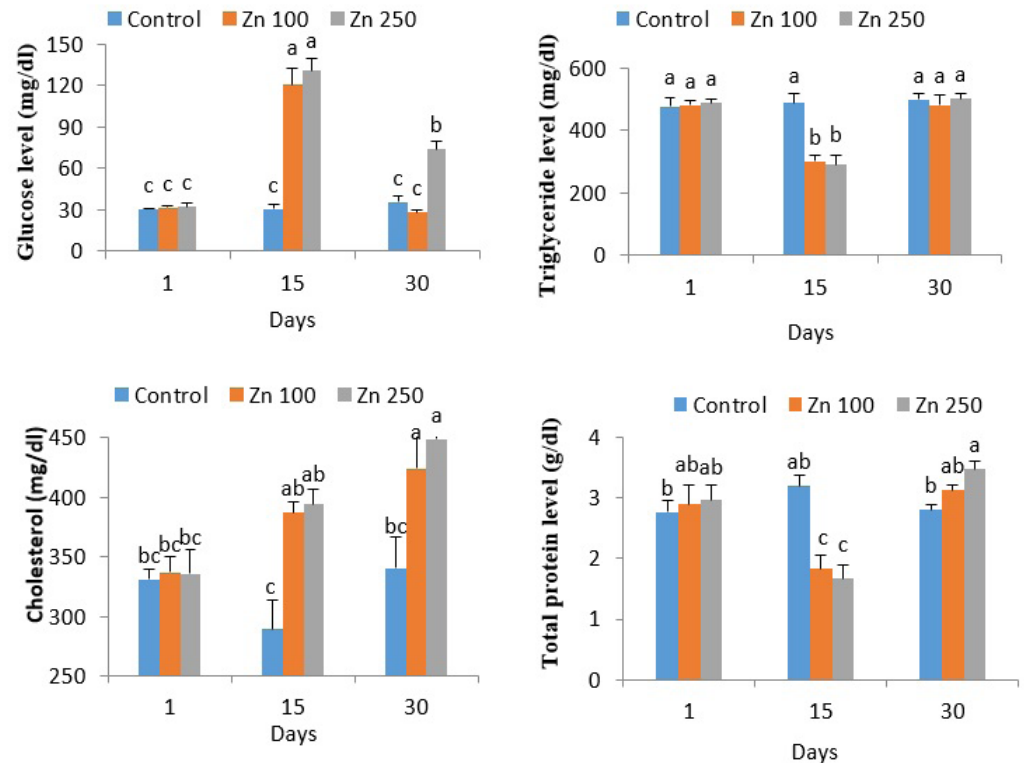

Figure 2. Effects of various concentrations of sublethal zinc on biochemical parameters. Serum glucose level (upper-left). serum triglyceride level (lower-left) serum cholesterol level (lower-right) *Oncorhynchus mykiss* serum TP level exposed to zinc. Data is expressed as mean error ± standard (SE). Significantly different meanings with different letters (p<0.05). Values with the same letters do not differ significantly.

On the first day of the experiment. serum cholesterol did not show significant differences in both the exposed Zn groups and the control group. but significantly increased on day 15 to 387 ± 9.61 mg/dl in low exposure and 289.33 ± 24.26 mg/dl in high exposure Zn groups compared to 301.33 ± 16.95 mg/dl in the control group. After 30 days. serum cholesterol showed 24.58% and 31.93% increases in low and high concentrations of Zn. respectively. but this increase showed no significant difference in both exposed Zn groups. This finding suggests that the dose-dependent response is not the effect of zinc on cholesterol level. Total serum protein did not show a regular pattern so that after a sharp 15-day reduction it increased rapidly to 3.22 ± 0.18 g/dl (low dose) and 3.46 ± 11 g/dl (high dose) compared to 2.7±0.23 g/dl in the control group after 30 days. (Figure 2).

Discussion

Growth

In the present study. the effects of zinc exposure to rainbow trout were assessed using fish growth along with biochemical parameters. Numerous field studies have shown that the rate of growth in fish exposed to metal mixtures by enzymatic capacity changes is lower (Lan *et al.* 1995; Zikic *et al.* 2001) and changes in the food base in contaminated waters in some situations (Lévesque *et al.* 2003). In this study. exposure to Zn reduced growth parameters such as condition fact (at low doses) or SGR and total weight gain. resulting in lower food consumption. Reduced growth due to Zn exposure may be due to metabolic demands related to metal detoxification. Such higher metabolic requirements divert resources from normal growth processes (Health 1995). In yellow catfish *Pelteobagrus fulvidraco* exposed to Zn. Zheng *et al.* (2016) also found reduced growth performance. They then suggested energy depletion accompanied by decreased lipid content for the growth and condition factor observed after Zn treatment. Furthermore. qualitative observations of fish feeding behavior indicated in our study that fish in exposed Zn groups did not actively feed and a severe reduction in the level of feeding activity was observed in the highest Zn exposure.

Serum enzymes and biochemical parameters

Stress conditions in fish caused by heavy metals increase energy requirements mainly from carbohydrates such as glucose and non-carbohydrate sources such as proteins and lipids through gluconeogenic enzymes such as AST and ALT (Tuncosoya *et al.* 2016). In general, nearly all biochemical parameters follow a similar pattern so that early elevation or depression in chronic exposures to heavy metals followed by a return to baseline values (Detholff *et al.* 1999). This pattern suggests acclimation over a period of time to the toxicant. In the current study. on the first day of exposure. sera AST and ALT trout activities increased and in each case their values were found to be higher in 250 µg/l Zn compared to 100 µg/l Zn. Similarly, the activities of sera AST and ALT increased to 5.0 ppm higher than the concentration of 1.0 ppm Zn in *C. gariepine* (Tuncosoya *et al.* 2016). Nevertheless. this study's level of both AST and ALT decreased linearly over a period of 30 days and the higher concentration caused a more significant effect on fish. Zn. on the other hand, increased the activities of sera AST and ALT in *O. niloticus* in short and long periods of exposure (Younis *et al.* 2012). The contrast between our studies and others on Zn may be due to species. stage of life history. sex. age of fish and temperature. hardness and water pH (Health 1995).

ALP is a bloodstream enzyme that helps break down proteins in the body. It acts as transphosphorylase at alkaline pH and plays an important role in the mineralization of the aquatic animal skeleton and in the activities of membrane transport (Bernet *et al.* 2001; Grosell *et al.* 2004). The present study showed a linear pattern of increasing ALP over time with Zn exposure which after 30 days resulted in recognizable physiological and functional changes. Similarly, after 14 d of 5. 10 and 20 M Zn exposure. ALP activity in *O. niloticus* serum increased (Oner *et al.* 2008). By contrast. in fish (*Salmo trutta*) living in naturally contaminated water. ALP activity decreased (Oner *et al.* 2008). The decrease in ALP activity may result from disruption of the membrane transmission system. although the increase in activity may be associated with tissue damage (Bernet *et al.* 2001).

Measurable levels of blood glucose are generally considered a good indicator of heavy metal stress in fish (Chowdhury *et al*. 2004; Bedii and Kenan 2005) and changes in blood glucose levels can be attributed to renal failure. liver damage and lack of nutrition (Pratap and WenderlaarBonga 1990). After the first 15 days. this study showed a dose-dependent glucose level increase pattern. In 15-d Zn-exposed fish (131±8.39 mg/L). the highest increase in glucose concentration was in comparison with the control value (30.67± 2.96 mg/L; Figure 2). Similarly, in *Oncorhynchus mykiss*. which was exposed to zinc for 7 days. McLeay (1977) reported that sera glucose levels increased. Furthermore. with the exception of 24 hours. sera glucose levels of *C.gariepinus* exposed to 1.0 and 5.0 ppm Zn increased with exposure time. This may result from stimulation of glycogenolysis in the liver and muscle depending on the demand for energy under the zinc effect (Ln and Vosyliene 1999). However, the level of glucose produced in the current study for the next 30 days. The higher level of glucose established during the first days of exposure may result from glycogenolysis (the release of glucose into the blood from energy resources stored in the muscles and liver as glycogen). initiated by hormones (cortisol and catecholamines) when the organism was in unfavorable condition. The reduced glucose level at the end of exposure probably reflected the exhaustion of the organism's energy reserves and the impairment of the fish's capacity to restore them and the acclimatized condition (Welker *et al*. 2016). However, Welker *et al*., (2016) found no significant differences in rainbow trout between the level of glucose and Zn exposure.

In general, the level of serum cholesterol appears to increase due to stress caused by metal exposure. The concentration of triglycerides is important for the evaluation of lipid metabolism and may result in higher levels due to liver and kidney failure causing cholesterol release into the bloodstream (Oner *et al*. 2008). When metabolized. triglycerides play an important role as an energy source and can be used to describe the fish's nutritional status. However, while the present study showed a reduction in serum triglyceride levels in 15 days and returned to control levels after 30 days. there is no consistency in the literature of serum triglyceride concentrations with Zn exposed fish. For example, when Zn was exposed to *O. niloticus*. no changes were observed in fish serum triglyceride concentrations. In addition, Lévesque *et al*. (2003) showed that *Perca flavescens* triglyceride levels exposed to Zn sublethal levels varied by seasons. These variations in concentrations of serum triglycerides may be due to differences in exposure concentration. lipid metabolism. and impairment of glycogen storage in different species of fish.

In this study. concentrations of cholesterol in the serum of Zn-exposed fish were generally increased in 30-d Zn-exposed fish compared to the control value and the highest increase. Because lipid homeostasis is one of the main functions of the liver. any change in the concentration of serum cholesterol may be a clear indication of liver and/or kidney dysfunction. Other studies supporting the present data show that concentrations of serum cholesterol in Zn-exposed fish are increasing (McLeay 1977; Canli 1995; Oner 2008). In *Clarias gariepinus* exposed to Zinc. therefore. Tuncsoya *et al.,* (2016) also showed that cholesterol levels increased. This increase in cholesterol concentrations is due. in particular, to the membranes. to the dangerous effects of Zinc on the cell membrane. Higher cholesterol levels are therefore good indicators of fish environmental stress.

The level of TP has been frequently reported as an indicator of fish health and may be due to liver damage. reduced absorption and protein alternations (Oner *et al*. 2008). Although in 15 days of the present study there was a reduction and non-significant

of TP. the value of TP increased again and reached the control fish value at day 30. Similarly, in Zn-exposed *Oncorhynchus mykiss*. a marked increase was observed in blood serum TP. However, in *O. niloticus* after Zn exposure. Oner *et al*. (2008) found no significant change in TP concentration. Thus. it appears that in different fish species no consistent pattern of TP in relation to zinc has been reported. This was associated by the authors with differences in concentration of exposure and species.

Conclusions

This research suggests that growth performance and changes in selected biochemical parameters used in this study. as bioindicators in aquatic ecosystems. play an important role in monitoring the toxicity of heavy metals to fish health status.

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