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Full Length Research Paper

Effects of zinc exposure on the accumulation, haematology and immunology of Mozambique tilapia, *Oreochromis mossambicus*

Ekrem Şanver Çelik[1]*, Hasan Kaya[1], Sevdan Yilmaz[2], Mehmet Akbulut[1] and Arınç Tulgar[1]

[1]Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Department of Basic Sciences, TR-17100 Çanakkale, Turkey.
[2]Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Department of Aquaculture, TR-17100 Çanakkale, Turkey.

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Changes in the haematological and innate immune parameters and accumulation in the liver, gill and muscle tissues were investigated in Mozambique tilapia (*Oreochromis mossambicus,* L.1758), which were semi-statically exposed to several zinc concentrations *in vivo*. The fish were exposed to low (1 mg L^{-1}), medium (2.5 mg L^{-1}) and high (5 mg L^{-1}) concentrations of zinc for 14 days. In this study, significant changes were seen in the haematological and innate immune parameters of the fish exposed to zinc in comparison to those of the control group ($p<0.05$) at day 14. In all groups exposed to zinc, a decrease in the erythrocyte count (RBC) and lymphocyte percentage and an increase in hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) values and neutrophile percentage occurred ($P<0.05$). A decrease in white blood cell (WBC) count and an increase in mean corpuscular hemoglobin concentration (MCHC) values occurred with medium and high concentrations ($P<0.05$). As per hematocrit (Hct) values, a decrease with high concentrations and an increase with low and medium concentrations were found ($P<0.05$). In all groups exposed to zinc, a decrease in phagocytic activity was found, and an increase in lysozyme and myeloperoxidase activities were observed with medium and low concentrations ($P<0.05$). A decrease was found in nitroblue tetrazolium (NBT) activity with medium and high concentrations; in the lysozyme and myeloperoxidase activities was found with high concentrations ($P<0.05$). In this study, the highest zinc accumulation rate was found in the liver tissue, and the lowest rate was found in the muscle tissue. Accumulation of zinc metal in the tissues was found to increase directly proportional with the ambient concentration and exposure duration ($P<0.05$). In conclusion, it was found that exposure of *O. mossambicus* to Zn concentrations affected haematological and innate parameters adversely. Therefore, these parameters can be used to predict the effect of metals such as zinc on fish populations.

Key word: Zinc, haematology, immunology, accumulation, *Oreochromis mossambicus.*

INTRODUCTION

Zinc is a necessary trace element that contributes to the structure of more than 300 proteins which play a role in the growth, reproduction, development and immune system catalysts in fish (Watanabe et al., 1997). In high concentrations, zinc may have toxic effects in fish and may be fatal (Malik and Sasty, 1998). Similar to other metals, zinc toxicity may change according to environmental conditions such as temperature, dissolved oxygen, pH, water hardness, and other organic and inorganic ligands (Hellawell, 1986).

Zinc may be transported to aquatic ecosystems as a result of both natural (weathering and erosion) and

*Corresponding author. E- mail: sanver_celik@comu.edu.tr.

Abbreviations: MCV, Mean corpuscular volume; **Hb,** hemoglobin; **WBC,** white blood cell; **MCH,** mean corpuscular hemoglobin; **MCHC,** mean corpuscular hemoglobin concentration; **Hct,** hematocrit; **NBT,** nitroblue tetrazolium.

Table 1. Physico-chemical parameters measured in the experiment.

Parameter	Value	Method
Temperature (°C)	25.5±0.4	YSI 556 MPS probe
Dissolved oxygen (mg L^{-1})	6.4±0.15	YSI 556 MPS probe
pH	7.18±0.03	HANNA C 200 (HI 83200) photometer
Total ammonia (mg L^{-1})	0.148±0.02	Thermo aquamate VIS - spectrophotometer
Water hardness (mg $CaCO_3$ L^{-1})	127±5.0	Thermo aquamate VIS - spectrophotometer
Calcium (mmol L^{-1})	0.830±0.006	Varian Liberty Sequential ICP-OES
Magnesium (mmol L^{-1})	0.500±0.001	Varian Liberty Sequential ICP-OES
Sodium (mmol L^{-1})	0.30±0.005	Varian Liberty Sequential ICP-OES
Potassium (mmol L^{-1})	0.050±0.001	Varian Liberty Sequential ICP-OES

All values are expressed as mean ± standard error.

antropogenic (industrial and agricultural) activities (Shuilleabhain et al., 2004). Zinc is taken by the fish through the gills and intestines and transported to other organs (such as liver and kidneys) via blood and accumulated there to be used in the organism or might be excreted (Firat and Kargın, 2010).

Zinc exhibits accumulation in the tissues and organs of freshwater fish (Cicik, 2003; Murugan et al., 2008) and may cause disorder in osmoregulation (Nussey et al., 2002), cardiac respiratory rhythm (Hughes and Adeney, 1977), changes in the blood gases and acid-alkaline status (Spry and Wood, 1984), tissue hypoxia (Tort et al., 1982), or histopathological organ damage (Abdel-Warith et al., 2011). Zinc may have negative effects in blood serum (Buthelezi et al., 2000; Fırat and Kargın, 2010a, b), haematological and immune parameters of the fish (Witeska and Kosciuk, 2003; Witeska, 2005; Kori-Siakpere and Ubogu, 2008; Ololade and Ogini, 2009) and thus may cause diseases and death. Therefore, it is important to assess the effect of metals on the haematological parameters and immune system (Saxena et al., 2009).

With a high reproductive and growth performance, *Oreochromis mossambicus*, is widely cultured around the world. Moreover, since this species has a high tolerance against stress and diseases, it is often used as an indicator in ecotoxicological studies (Verdegem et al., 1997; Liao and Ling, 2003). There are a few studies available that report the effects of varied Zn concentrations on haematological parameters in *O. mossambicus* (Sampath et al., 1998; Buthelezi et al., 2000; Nussey et al., 2002). However, to our knowlegde, no studies have investigated the accumulation of zinc in tissues and the subsequent effect on the innate immune parameters of *O. mossambicus.*

In this study, the accumulation in tissues (liver, gill and muscle) and the change in the haematological and innate immune parameters were investigated in *O. mossambicus* exposed to sublethal concentrations of zinc.

MATERIALS AND METHODS

Experimental fish

A total of 156 *O. mossambicus* with an average weight of 20.4±1.80 g (mean ± SEM) were obtained from Canakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology.

Experimental design

Each fish was adapted to ambient conditions in 12 aquaria, with 45 x 28 x 80 cm dimensions and containing 80 L rested tap water, for two months. Each aquaria was provided with sponge filters connected via airline to a Resun GF-120 air pump. During the acclimatization, water was exchanged daily at a rate of ~10% of the total volume. The fish were fed with fish food during the adaptation period in the laboratory conditions (protein ratio of 35%, and 10% fat). The experiment was designed in triplicate and 13 fish were placed in each experimental aquarium (12 aquaria, with 45x28x80 cm dimensions). Fish were fed twice a day with 2% of their body weight and feeding was stopped 24 h before starting the experiments. During the experiment, the fish were exposed for 14 days to 1 mg L (low: L), 2.5 mg L^{-1} (medium: M), 5 mg L^{-1} (high: H) concentrations of $ZnSO_4.7H_2O$, and controls were exposed to only rested tap water. These sublethal concentrations were adopted from the literature (Cicik, 2003; Atlı and Canlı, 2007).

During the experiment, fish were fed twice a day based on the 2% of their body weight. The experiment was designed semi-statically, and the water was replaced every day; 75% in the morning and 25% in the evening. After each water replacement, $ZnSO_4.7H_2O$ solution in the same ratio was added to the aquaria together with the water (Smith et al., 2007). The physico-chemical parameters of the rested tap water used in the experiment is given in Table 1. Sampling was done three times during the experiment: on the first day before any chemical application, and on the 7th and 14th day. Haematological and innate immune parameters, and zinc accumulation were analyzed in the fish. Fish experiments were performed in accordance with the guidelines for fish research from the animal ethic committees at Çanakkale Onsekiz Mart University, Çanakkale, Turkey.

Preparation of the $ZnSO_4.7H_2O$ solution and application

Sigma-Aldrich (USA) $ZnSO_4.7H_2O$ heavy metal salt was used in the experiment. In order to prepare the needed concentrations, main

stock solution was prepared in ultra distilled water and appropriate dilutions were made from it and thus the concentrations for the experiments were obtained. Trisodium citrate dihydrate ($C_6H_5Na_3O_7.2H_2O$) solution was used in the experiment in order to enable $ZnSO_4.7H_2O$ solution to be dispersed in the aquariums homogenously and to avoid the precipitation to the floor of the aquarium. Trisodium citrate dihydrate was also added to equal concentrate in the control groups.

Blood sampling

In the experiment, 12 fish on the first day (before any chemical application), 6 fish from each aquarium on the 7th and 14th day were used for blood analyses. Anaesthesia with MS222 was used to kill the fish (Smith et al., 2007). They were well wiped and cleaned in order to avoid mucus mixing into the blood, and then, blood was taken from the fish through the caudal vein by a 5 ml plastic syringe, without harming the fish (Val et al., 1998). Then, a sample of blood was transferred to EDTA tubes, BD Microtainer®, UK for haematological analysis. Plastic biochemistry tubes (Kima-vacutest®, Italy) were used for biochemical analysis. Blood serum was isolated by centrifugation (4000 x *g*, 10 min) and it was stored below -20°C.

Haematological parameters

Red blood cells (RBC, $10^6 mm^{-3}$) were counted with a Thoma haemocytometer using Dacie's diluting fluid. Hematocrit ratio (Hct, %) was determined using a capillary hematocrit tube. Hemoglobin (Hb, g/dl) concentration was determined by spectrophotometry (540 nm) using the cyanomethaemoglobin method (Blaxhall and Daisley, 1973). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using the following equations (Lewis et al., 2006): MCV (μm^3) = Hct (%) x 10/RBC($10^6 \mu L^{-1}$), MCH (pg)= (Hb (g dL^{-1}) x 10)/RBC($10^6 \mu L^{-1}$), MCHC (%) = (Hb (g dL^{-1}) x10)/Hct (%).

White blood cells (WBC, $10^3 mm^{-3}$) were counted indirectly according to McKnight (1966). Differential leukocytes [lymphocyte (LYM), neutrophil (NEU), and monocyte (MON)] were examined with May-Grunwald-Giemsa-stained peripheral blood smears. Each slide was examined under oil-immersion at 100x magification. For each slides, 100 leukocytes were identified as lymphocytes, neutrophils or monocytes.

Innate immune parameters

Phagocytic activity was estimated by using the method modified by Siwicki and Anderson (1993). It was calculated as: PA= (Number of phagocytic cells/Number of total cells) x 100. The respiratory burst of the neutrophils and monocytes was quantified by the reduction of nitroblue tetrazolium (NBT) to formazan as a measure of the production of oxygen radicals (Siwicki and Anderson, 1993). Plasma lysozyme was assessed using the turbidometric assay (Ellis, 1990). Total myeloperoxidase content in coagulated blood serum was measured according to the study of Quade and Roth (1997).

Tissue zinc analysis

Tissues (liver, gill and muscle) for trace metal analysis were oven dried to a constant weight, digested in 5 ml of concentrated nitric acid, then diluted to 20 ml with deionised water and analysed by ICP-OES for Zn according to the study of Smith et al. (2007).

Percentage tissue moisture content was calculated from wet and dry tissue weights.

Statistical analysis

Each value was expressed as mean ± standard error of mean (SEM) for each parameter measured. The data of total metal concentrations in the tissues, haematological and innate immune parameters in the metal exposure groups were compared to the control group with Student's t-test using SPSS 17.0 packaged software (Logan, 2010). Statistical significance was established at $P<0.05$.

RESULTS

No fish, exposed to zinc concentrations, died during the experiment. The changes in the haematological parameters in the study are given in Table 2.

According to Table 2, RBC count in L and H groups and WBC count in H group at the end of day 7 were found to be significantly lower than those in the control group ($P<0.05$). At the end of the same day, WBC count and MCH values of L and M groups, Hb and MCHC values of M and H groups, Hct ratio of L and M groups, and MCV values of L and H groups were found to be significantly higher than those in the control group ($P<0.05$). At the end of day 14, Hb, MCV and MCH values of L, M and H groups, MCHC values of M and H groups, WBC count of L group, Hct ratio of L and M groups were found to be significantly higher than those in the control group ($P<0.05$). At the end of the experiment, RBC count of L, M and H groups, WBC count of M and H groups and Hct ratio of H group were found to be significantly lower than those in the control group ($P<0.05$).

The changes in white blood cell types in fish exposed to varied Zn concentrations are given in Table 3. According to Table 3, LYM percentages of L, M and H groups at the days 7 and 14, showed significant decrease when compared to the control group ($P<0.05$), while NEU percentages showed significant increase when compared to the control group ($P<0.05$). MON percentages of H groups at day 14 showed significant increase when compared to the control group ($P<0.05$).

The changes of innate immune parameters in *O. mossambicus* exposed to varied Zn concentrations during the experiment are given in Figures 1, 2, 3 and 4. The increase in the phagocytic activity in L and M groups on the 7th day of the experiment was found to be significantly higher compared to those in the control group ($P>0.05$), while the decrease in the phagocytic activity of the same groups compared to those in the control group was found to be significant (at the end of the experiment) ($P>0.05$). The phagocytic activity determined in H group fish at days 7 and 14 decreased significantly in comparison to those in the control group ($P<0.05$) (Figure 1).

The increase in NBT activity in L and M groups on the

Table 2. Effects of different concentrations of zinc on haematological variables.

Haematological parameter	Experimental period (day)		
	0	7	14
RBC (x10^{6}mm^{-3})			
Control		1.73±0.08	1.65±0.08
Low	1.71±0.11	0.90±0.07*	1.03±0.09*
Medium		1.60±0.08	0.90±0.06*
High		0.94±0.08*	1.02±0.08*
WBC (x10^{3}mm^{-3})			
Control		50.29±1.34	49.71±1.08
Low	48.50±1.71	61.43±0.65*	60.57±2.03*
Medium		63.71±0.84*	38.29±0.71*
High		42.00±3.13*	28.00±1.76*
Hct (%)			
Control		27.00±0.62	26.57±0.57
Low	26.50±0.65	30.86±0.91*	32.43±1.13*
Medium		33.00±0.53*	30.86±0.77*
High		26.57±1.07	23.00±0.82*
Hb (g/dl)			
Control		8.34±0.34	8.14±0.28
Low	8.24±0.41	9.43±0.66	9.78±0.46*
Medium		13.27±1.11*	12.94±0.32*
High		10.60±0.57*	10.07±0.48*
MCV (µm^3)			
Control		158.58±8.48	163.55±10.46
Low	155.9±96.43	355.95±31.90*	334.76±45.52*
Medium		209.21±10.95	351.68±22.88*
High		293.30±22.17*	233.43±17.18*
MCH (pg)			
Control		48.51±1.33	50.13±3.63
Low	48.35±1.66	107.83±9.67*	99.51±10.55*
Medium		83.28±6.75*	147.39±9.36*
High		119.15±13.51*	103.83±11.10*
MCHC (%)			
Control		31.08±1.74	30.63±0.83
Low	31.06±1.01	30.82±2.60	30.40±1.85
Medium		40.06±3.03*	42.15±1.74*
High		40.48±3.12*	43.96±2.28*

RBC, Count of erythrocytes; WBC, count of leucocytes; Hb, hemoglobin concentrations; Ht, hematocrit values; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration. Exposure groups are represented as follows control: 0 , low (1 mg L^{-1}), medium (2.5 mg L^{-1}), high (5 mg L^{-1}); asterisks indicate significant differences between control and exposure groups (P<0.05).

7th day of the experiment and the decrease in NBT activity in L group on 14th day were found to be non-significantly (P>0.05) compared to those in the control group. The decrease in NBT activity in H group at the end of the first week and in M and H groups at day 14, were found to be significant compared to those in the control

Table 3. Effects of different concentrations of zinc on white blood cell types.

Parameter	Experimental period (Day)		
	0	7	14
Lymphocyte (%)			
Control	83.50±2.90	82.57±2.68	79.57±2.18
Low		64.14±0.91*	64.29±1.52*
Medium		66.86±1.18*	60.43±0.65*
High		62.86±0.74*	58.71±0.97*
Neutrophil (%)			
Control	16.00±2.74	18.14±2.34	19.71±2.09
Low		34.86±0.83*	33.71±1.46*
Medium		32.00±1.40*	37.57±0.75*
High		34.57±0.92*	37.86±1.10*
Monocyte (%)			
Control	1.00±0.00	1.25±0.19	1.25±0.19
Low		1.75±0.36	2.00±0.44
Medium		1.60±0.34	2.33±0.31
High		2.57±0.57	3.43±0.57*

Exposure groups are represented as follows control: 0 , low (1 mg L^{-1}), medium (2.5 mg L^{-1}), high (5 mg L^{-1}); *Significant differences between control and exposure groups (P<0.05).

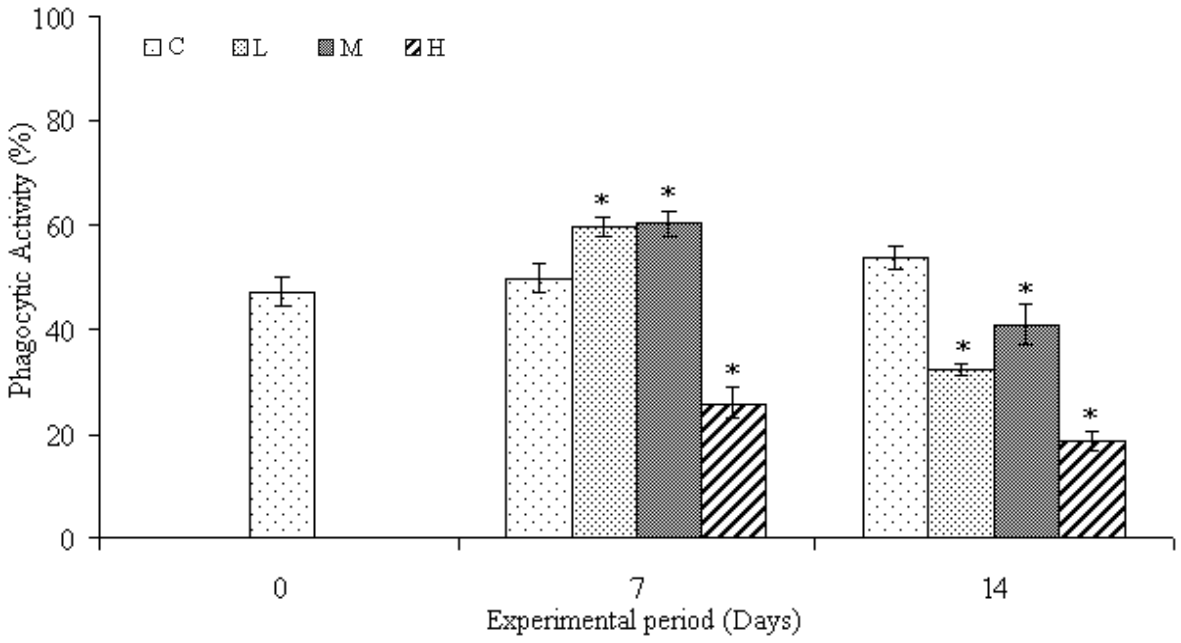

Figure 1. Phagocytic activity in *O. mossambicus* exposed to Zn for 7 and 14 days (C, 0 mg L^{-1}; L, 1 mg L^{-1}; M, 2.5 mg L^{-1}; H, 5 mg L^{-1}). *Significant differences between control and exposure groups (P<0.05). Values are mean ±SEM; *n=6.*

group (P<0.05) (Figure 2).

The increase in the lysozyme activity in L and M groups on the 7th and 14th days was found to be significant compared to those in the control group (P<0.05). A significant decrease of lysozyme activity was found in H group during whole experiment compared to that in the control group (P<0.05) (Figure 3).

MPO activity in the M group on the 7th day and in L

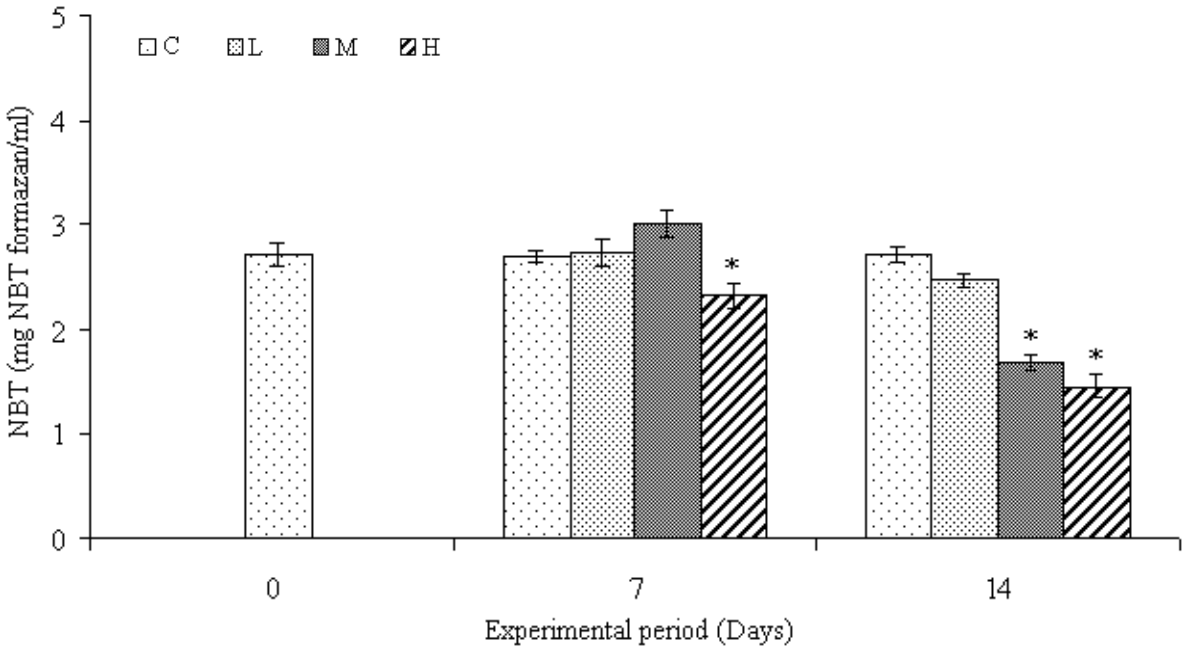

Figure 2. Nitroblue tetrazolium activity in *O. mossambicus* exposed to Zn for 7 and 14 days (C, 0 mg L^{-1}; L, 1 mg L^{-1}; M, 2.5 mg L^{-1}; H, 5 mg L^{-1}). Asterisks indicate significant differences between control and exposure groups (P<0.05). Values are mean ±SEM; *n=6.*

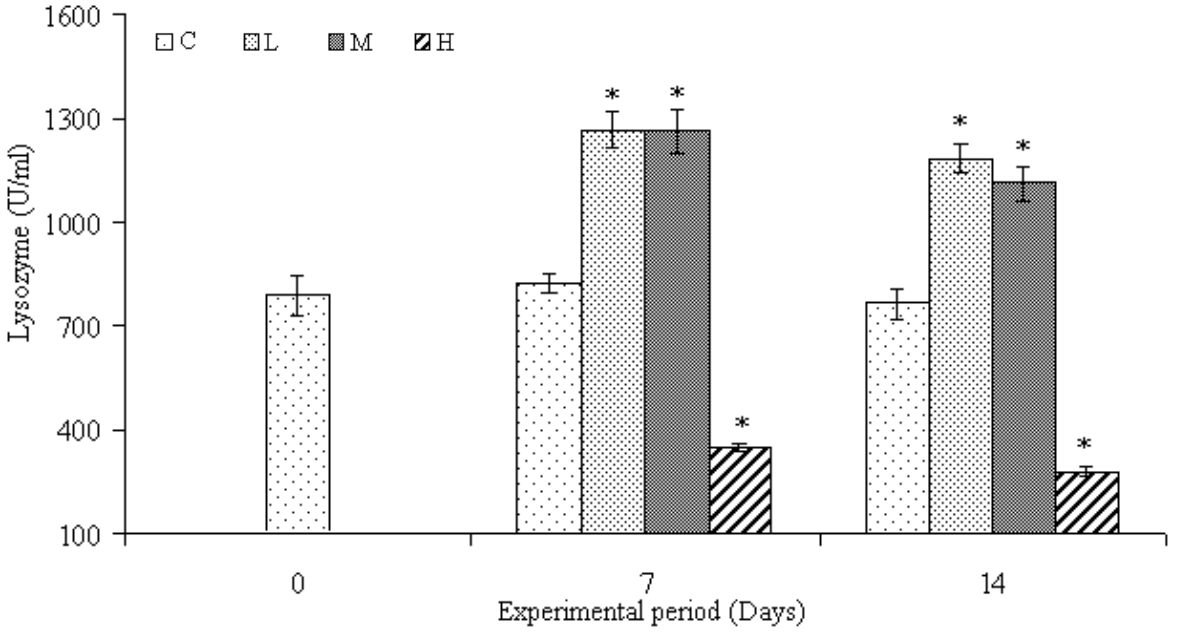

Figure 3. Lysozyme activity in *O. mossambicus* exposed to Zn for 7 and 14 days (C, 0 mg L^{-1}; L, 1 mg L^{-1}; M, 2.5 mg L^{-1}; H, 5 mg L^{-1}). Asterisks indicate significant differences between control and exposure groups (P<0.05). Values are mean ±SEM; n=6.

and M groups on the 14th day increased significantly compared to that in the control group (P<0.05). The decrease in MPO activity in H group at the end of the 7th day was not significant compared to the control group (P>0.05), while the decrease in the end of the experiment was found to be significant (P<0.05) (Figure 4).

The total zinc levels in the tissues of fish are shown in Table 4. A statistically significant increase was found in

Table 4. Total zinc concentrations (Mean±SE µg/g dry weight; *n* = 6) in the tissues of *O. mossambicus.*

Parameter	Experimental period (Day)		
	0	7	14
Gill			
Control		96.81±2.45	96.81±2.45
Low	96.81±2.45	200.78±1.83*	229.03±2.22*
Medium		236.25±1.26*	256.97±1.34*
High		266.19±0.69*	285.26±2.64*
Liver			
Control		84.07±0.98	84.07±0.98
Low	84.07±1.70	92.05±0.84*	157.14±1.56*
Medium		130.18±0.68*	225.53±0.90*
High		187.60±0.92*	366.06±2.62*
Muscle			
Control		46.44±0.47	46.44±0.47
Low	46.44±0.81	64.99±0.42*	72.50±1.77*
Medium		70.81±0.23*	65.42±1.22*
High		83.78±1.47*	132.49±1.52*

Exposure groups are represented as follows control: 0 , low (1 mg L^{-1}), medium (2.5 mg L^{-1}), high (5 mg L^{-1}); asterisks indicate significant differences between control and exposure groups (P<0.05).

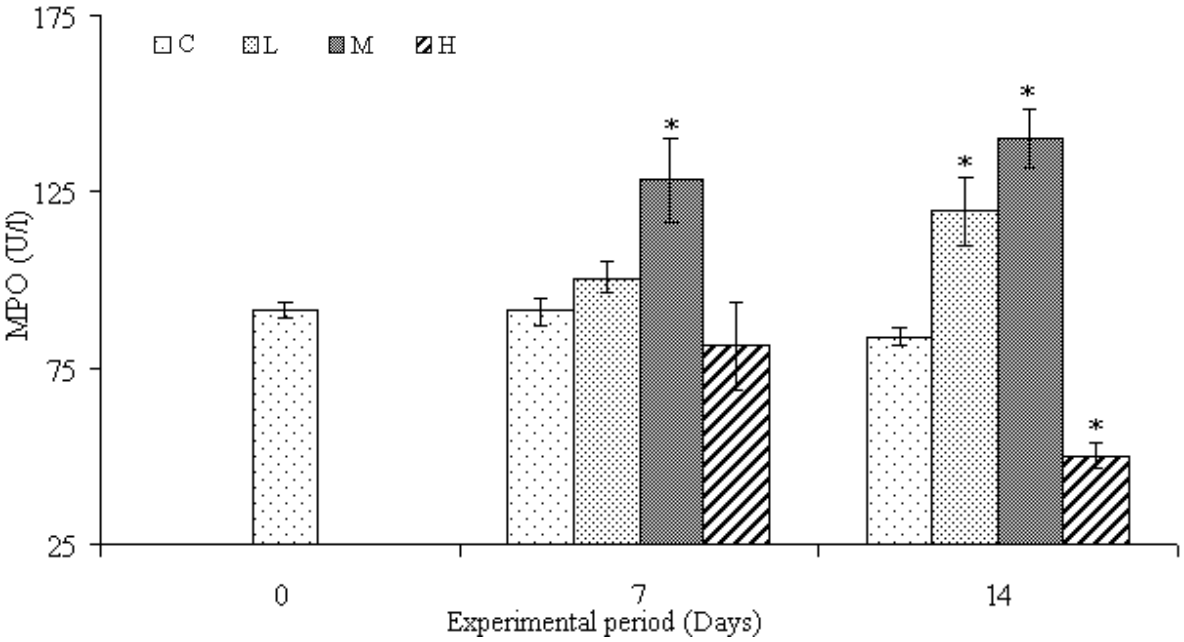

Figure 4. Myeloperoxidase activity in *O. mossambicus* exposed to Zn for 7 and 14 days (C, 0 mg L^{-1}; L, 1 mg L^{-1}; M, 2.5 mg L^{-1}; H, 5 mg L^{-1}). Asterisks indicate significant differences between control and exposure groups (P<0.05). Values are mean ±SEM; *n=6.*

all the tissues in which metal accumulation was investigated in groups exposed to 1, 2.5 and 5 mg L^{-1} zinc concentrations compared to those in the control group (P<0.05). The tissues with the highest accumulation at the end of the experiment were liver (366.06±2.62 µg/g), then gill (285.26±2.64 µg/g) and the finally was the muscle (132.49±1.52 µg/g). It was found that zinc accumulation in liver, gill and muscle tissues tended to increase in a concentration and time-dependent manner (P<0.05).

DISCUSSION

Blood is a pathophysiological reflector of the whole body, and blood parameters are important in the diagnosis of the structural and functional statuses of animals exposed to toxicants (Sampath et al., 1998). Toxicants have important effects which cause several physiological functional disorders in fish (Omoregie et al., 1990). Zinc is an essential element for plants and animals. However, at higher concentrations, it causes structural damage, which may affect the survival, development, and growth of fish (Tuurala and Soivio, 1982).

It was found in the present study that Hct ratio and RBC count in fish decreased significantly, and that Hb, MCV, MCH, and MCHC values increased significantly as a result of zinc application, especially in high doses. Such a situation can be an indicator for haemolytic anemia, as was found in some fish species exposed to paraquat (Salazar-Lugo, 2007). Haemolytic anemia is a genetic and molecular disease and has also previously been seen in fish in anoxic and low pH conditions previously. This disorder causes rupture of the erythrocytes, and an increase of free haemoglobin in blood, and damage in the tissues and organs of the fish, and death, if the condition continues (Hárosi et al., 1998; Pia Koldkjær and Berenbrink, 2007). Therefore, haemolytic anemia is reported to be an important parameter in the evaluation of fish health (Hárosi et al., 1998).

In the present study, erythrocyte counts in L and M groups were found to be significantly lower (except M group, at day 7) and Hct was significantly higher compared to those in the control group following the 7th and 14th days of exposure. In addition, MCV, MCH and MCHC values in the M and H groups were significantly higher compared to those in the control group following 14th days of exposure, and this fact shows that low and medium dose zinc application to the fish may cause macrolytic anemia. Similar results were seen in *O. mossambicus* (Nussey et al., 2002) exposed to zinc and in *Clarias albopunctatus* (Mgbenka et al., 2005) exposed to organophosphorous pesticide (actellic).

A significant increase was seen in the phagocytic and lysozyme activities in the L group, in the phagocytic, lysozyme, and myeloperoxidase activities in the M group, compared to those in the control group on the 7th day of the study. A significant decrease was found in innate immune parameters of H group compared to those in the control group on the 7th and 14th days (except myeloperoxidase activity on the 7th day). It has been reported that zinc can activate natural killer cells (NK), macrophages, antigen-specific cytotoxic, T-lymphocytes and cytokines cells (Chandra and Au, 1980; Bhaskaram, 2002). It has also been found that zinc in high concentrations decreased the natural killer cells (Ferry and Donner, 1984). In addition, zinc has been show to stimulate neutrophiles, monocytes, and macrophages, if used in optimum ratios, and triggered the production of oxygen which plays an important role in killing pathogens (Chandra, 1997; Shankar and Prasad, 1998). However, zinc in high doses inhibits macrophage activation, mobility, phagocytosis, and decreases the oxygen production in humans and animals (Shankar and Prasad, 1998). In many studies, it has been reported that zinc had an immune suppresssive effect on fish (Dunier and Siwicki, 1993; Sanchez-Dardon et al., 1999; Witeska, 2005). The decreases in NBT, phagocytic, lysozyme and myeloperoxidase activities, used in the present study to determine reactive oxygen production also support these results.

In this study, WBC count significantly increased in L and M groups on the 7th day compared to those in the control group, and only in L group on the 14th day. This increase in WBC count may be as a result of the prevention of damage caused by zinc in the gill, kidney, and liver tissues (Buthelezi, 2000; Nussey et al., 2002). This may be explained by a reaction of the defense mechanism of the fish by leucocytosis under pathological conditions and against foreign bodies (John, 2007). WBC count decreased significantly in M and H groups on the 14th day. This decrease presumably resulted from the toxic effect of zinc on WBC or the stress caused on the cell production activity of the spleen (Yamamoto, 1988; Fırat, 2007). In addition, the decrease in WBC count can be associated to the increase in the secretion of corticosteroid and cortisol hormones, since these hormones play an important role in the prevention and healing of inflammation.

Moreover, when the white blood cell types were examined, a significant decrease of lymphocytes were seen in all fish exposed to varied zinc concentrations compared to those in the control group. This effect of zinc can be explained by the suppressive effect of cortisol hormone on lymphocyte production (Rink and Kirchner, 2000; Nussey et al., 2002). In addition, it was found that neutrophil percentage significantly increased in all zinc applied concentrations and monocyte percentage was found higher in high zinc applications compared to those in the control group on the 14th day. This effect may be associated to the damage of metals on the defense cells of the fish (Witeska, 2005). It has been further reported that zinc decreased WBC count, neutrophiles and lymphocytes in carp fish (Witeska, 2005), WBC count in *O. mossambicus* (Buthelezi et al., 2000) and WBC count in *C. gariepinus* (Ololade and Ogini, 2009). On the other hand, it has been found that zinc increased WBC count, lymphocytes, basophyles, and neutrophiles in Mozambique tilapia (Sampath et al., 1998), and WBC count in *Heteroclarias sp* (Kori-Siakpere and Ubogu, 2008). It has also been reported that these effects of zinc may decrease or increase the activation or counts of white blood cells at different concentrations in humans and animals (Schlesinger et al., 1993; Rink and Kirchner, 2000; Ibs and Rink, 2003).

In all groups exposed to varied zinc concentrations (1,

2.5 and 5 mg L^{-1}) during 14 days, a significant increase of zinc accumulation in fish tissues (liver, gill, muscle) was seen compared to those in the control group. The highest zinc accumulation was seen in the liver, and lowest in the muscle tissue. In other studies, similar results have been reported; the highest accumulation was obtained in the liver and the lowest in the muscle tissue in *Channa punctatus* exposed to varied Zinc concentrations (6.62, 13.24 mg L^{-1}) for 45 days (Murugan et al., 2008), and in *Cyprinus carpio* exposed to zinc (5 mg L^{-1}) for 15 days (Cicik, 2003). The difference seen between tissues and organs from the metal accumulation point of view can be explained by the differences in tissue and organ functions (Cicik, 2003).

The muscles are not very active tissues for metal accumulation in fish (Ay et al., 1999). In this study, the lowest zinc accumulation was found in the muscle, the edible part of the fish. The liver is an organ with important functions such as chemical changes in the metals entering into the body, excretion, and detoxification (Çoğun, 2008). Metals taken from the aquatic ecosystem are bound to metal chelating proteins such as metellathionine (MT) and are excreted (Heath, 1987; Canlı et al., 1997; Ay et al., 1999). Liver is the main production place of metal chelating proteins. Therefore, heavy metals are found in this tissue in high concentrations (Thomas et al., 1983; Allen, 1994; Ay et al., 1999).

In conclusion, significant changes were found in haematological and innate immune parameters of *O. mossambicus* exposed to zinc concentrations, compared to those in the control group. It was also found that zinc accumulation increased significantly in liver, gill and muscle tissue according to the concentrations of the ambient, depending on the duration of exposure. Haematological and innate immune parameters can be used in the follow-up of health status of *O. mossambicus* species exposed to Zn concentrations. These parameters can be used to predict the effect of metals such as zinc on fish populations.

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