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Effect of sub-lethal lead exposure at different salinities on osmoregulation and hematological changes in tilapia, *Oreochromis niloticus*

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Abstract. The objectives of this study were to evaluate the effects of sub-lethal lead concentrations on serum osmolality, Na^+ and Cl^- levels, and hematological parameters in Nile tilapia, *Oreochromis niloticus* (L.) at different salinity levels. The serum osmolalities (SO) were not significantly different at any of the salinity levels in the control fish, while in Pb-exposed fish the SO increased with increasing salinity. The concentrations of serum Na^+ and Cl^- in both the control and Pb-exposed fish increased with increasing salinity. The levels of red blood cells (RBC), hemoglobin (Hb), and hematocrit (Ht) in the control fish were not significantly different at any of the salinity levels. Meanwhile, the levels of RBC, Hb, and Ht in Pb-exposed fish increased with increasing salinity levels. The levels of RBC (at 0 and 5 ppt) and Ht (at 0, 5 and 10 ppt) in Pb-exposed fish were lower than in the control fish. The levels of Hb in Pb-exposed fish were lower than in the control fish at all salinity levels. The levels of WBC in the control fish increased with increasing salinity, while its levels in the Pb-exposed fish decreased with increasing salinity. The levels of WBC in the Pb-exposed fish were higher than in the control fish at 0 and 5 ppt.

Keywords: fish, Pb, blood, erythrocytes, leukocytes, ion

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Introduction

Heavy metals are present naturally in the environment; however, as a consequence of industrial, agricultural, and anthropogenic activities levels of them are increasing rapidly. Heavy metals at high concentrations can cause hazardous effects to many aquatic organisms by changing genetic, metabolic, physiological, biochemical, and behavioral parameters (Scott and Sloman 2004, Atli and Canli 2007, Ramesh et al. 2009). Lead (Pb) is a toxic metal that is still a potential problem in aquatic systems because it comes from ore processing, smelting, refining operations, coal burning, cement manufacturing, agricultural runoff, industrial and domestic waste water effluents, and its in gasoline, batteries and paints (Scott and Sloman 2004, Aldoghachi et al. 2015).

The toxic effects of heavy metals depend on a wide range of environmental factors, among which salinity is one of the most important (Erickson et al. 2008). The toxicity of metal decreases as the salinity of the media increases. This inverse relationship is usually explained by increasing free metal ion concentrations with increasing salinity. Free metal ions are the most bioavailable form of metals to aquatic organism (Rainbow 1995). Salinity affects metal

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bioavailability and uptake and its subsequent toxicity by competing with metal ions binding to biological molecules (Erickson et al. 2008, Loro et al. 2012).

Because of their tolerance of a wide range of salinity levels (from 0 to 32 ppt), various tilapia strains can be cultured efficiently in freshwater, brackish water, and seawater (Suresh and Lin 1992, Avella et al. 1993). However, Baroiller et al. (2000) reported that Nile tilapia, *Oreochromis niloticus* (L.), do not tolerate salinity above 20 ppt and might not be suitable for culture in full-strength seawater (37 to 40 ppt). Under natural and culture conditions, tilapia often encounter both water salinity changes and elevated levels of heavy metals, which renders the interaction between salinity acclimation and toxicant response important (Adeyemi et al. 2012). As a consequence of salinity fluctuations, tilapia typically possess highly developed mechanisms for ion regulation. *O. niloticus* is a hyper- and hypo-osmoregulator, thus, at low salinities it actively maintains its blood hyper-osmotic to the external environment, while at high salinities the blood is maintained in a hypo-osmotic state (Fontainhas-Fernandes et al. 2003). As a result, osmo- and ion-regulatory processes occur in order to maintain the homeostasis of the organism (Roast et al. 2001).

The gill is the primary site of osmotic and ionic regulation in fish, but unfortunately it serves as the primary target for the toxic action of various metals (Evans et al. 2005). Metals affect the osmoregulation of various fishes, for example, copper impaired branchial ion-regulation of freshwater rainbow trout by inhibiting both the active transport of Na^+ and Cl^- and increasing the ionic permeability of the gills, which resulted in a net loss of these ions (Lauren and McDonald 1985, 1986). Spry and Wood (1985) report that zinc increased Na^+ and Cl^- effluxes, and Verbost et al. (1987) demonstrated that cadmium increased Ca^{2+} efflux. Lock et al. (1981) demonstrated increased osmotic permeability to water of the gills after exposure to mercury. A neotropical fish, *Prochilodus lineatus*, exposed to lead showed a decrease in Na^+ plasma concentration (Martinez et al. 2004). These passive movements occurred due to increased branchial permeability (Wendelaar Bonga and Lock 1992).

Changes in both water salinity and heavy metal level can also trigger alteration in blood composition and immune mechanisms. Witeska (2005) demonstrated that the red blood cell system of fish reacted to heavy metal intoxication with anemia; however, in some cases, particularly after brief exposure, red blood parameters (hematocrit, red blood cell, mean corpuscular volume, hemoglobin) can increase. Heavy metal intoxication also reduces the white blood cell count, particularly that of lymphocytes (Witeska 2005). This is usually accompanied by the impairment of their activities (Viale and Calamari 1984, Khangarot and Tripathi 1991, Viola et al. 1996). The aim of present study was to evaluate the effects of sub-lethal lead exposure on serum osmolality, ion levels, and the hematological parameters of tilapia, *O. niloticus,* at different salinity levels.

Materials and methods

Fish acclimation

O. niloticus (East Java strain, local name: Jatimbulan) (approximately 13.1 ± 0.4 cm total length; 25.4 ± 0.5 g body weight) were collected from a commercial farm in Pasuruan, East Java, Indonesia. They were transported to the laboratory and acclimated for adaptation for two weeks to different salinities: 0, 5, 10, 15, and 20 ppt with a 5 ppt daily increase in order to avoid osmotic shock. They were fed twice a day with commercial pellets *at libitum.* During acclimation, high mortality was observed at 20 ppt; hence, the fish were acclimated at salinities 0, 5, 10, and 15 ppt for an additional 14 days, respectively. Salinity was measured using a hand held salinity refractometer (Atago, Japan). Seawater was obtained from the coast adjacent to the university, and freshwater was obtained from municipal tap water. Before being used for acclimation and experimentation, the tap water was aerated overnight to accelerate dechlorination (Putranto et al. 2014).

Freshwater and diluted seawaters were filtered through gravel, sand, and sponge filters. Throughout the acclimation and experimentation tests, water temperature was measured using glass mercury thermometers (°C), pH was measured with a pH meter (Hanna Model HI 981502, China), and dissolved oxygen (DO) was measured with a DO meter (Lutron DO 5510, Taiwan). The values of water temperature, pH, and dissolved oxygen were 28-30°C, 7.55-8.05, and 7.1-7.6 mg L^{-1}, respectively, with a natural photoperiod.

LC_{50} assessment

The median lethal concentration (LC_{50}) of Pb in tilapia was applied only to fish acclimated in freshwater (0 ppt). The LC_{50} value was then used as the basis for establishing sub-lethal Pb concentrations for the next experiment. A stock solution (1000 mg Pb L^{-1}) was prepared from 1.5985 g of $Pb(NO_3)_2$ (Merck, Germany) in 1000 ml of deionized water. Selected experimental concentrations were made by adding adequate volumes of the stock solution to freshwater. A toxicity range-finding test was conducted prior to initiating a static, acute, definitive toxicity test. The definitive toxicity test was conducted in 63 L plastic tanks containing 50 L of test solution. Each tank contained ten fish, and the media was aerated continuously with an air stone, but it was not renewed. The fish were starved for 24 h before the toxicity test. The nominal test Pb concentrations used for acute toxicity estimations were 0, 50, 100, 200, 400, and 800 mg L^{-1}. Three replicates were performed for all treatments and for control groups. Each day, dead fish were counted and removed from the tanks. The fish were not fed during the experiment. Death was confirmed when the animals were immobile, lacked opercular movement, and showed no response when touched with a glass rod. The data from the experiment were used to estimate the median lethal concentration (96 h LC_{50}) using the trimmed Spearman-Karber method. The 96 h LC_{50} and 95% confidence intervals of Pb to *O. niloticus* were 200 (170.84-234.14) mg L^{-1}. The sub-lethal toxicity of this experiment was 100 mg Pb L^{-1} (≈50% of LC_{50}) (Table 1).

Table 1
Percentage mortality (%) of *O. niloticus* exposed to Pb in freshwater (0 ppt salinity) for 96 h and its medium lethal concentration (LC_{50} with 95% confidence limits) calculated with the trimmed Spearman-Karber method

Lead concentration (mg Pb L^{-1})	Percentage mortality (%)	LC_{50} with 95% confidence limits (mg Pb L^{-1})
0	0	200 (170.84 - 234.14)
50	0	
100	13.3	
200	40	
400	96.7	
800	100	

Sub-lethal effect on serum osmolality, ions, and hematological parameters

Sub-lethal tests were conducted using the static renewal method, with 80% test solutions renewed every 48 h. The fish were exposed for 7 d to sub-lethal concentrations of Pb: 0 (control) and 100 mg L^{-1}, at salinities of 0, 5, 10, and 15 ppt. There were triplicates for each test media, with a total of five fish per replicate. Test media were aerated continuously. During the tests, the fish were fed with commercial fish food twice a day at libitum. Uneaten food and debris were removed daily.

At the end of the exposure period, five fish were chosen at random and removed from each treatment for determinations of serum osmolality, serum ions, and hematological parameters. Prior to blood sampling, the fish were anesthetized with 200 mg L^{-1} clove solution (Mohseni et al. 2008). Blood from each fish was obtained by puncturing the heart using a sterile plastic syringe. Then, blood samples were introduced immediately to tubes containing ethylenediamine tetraacetic acid (EDTA) as an anti-coagulating agent for the assessment of hematological parameters, and to non-EDTA tubes for the assessment of serum osmolalities and serum ions.

Blood samples from non-EDTA tubes were centrifuged at 4500 × g for 10 min to separate blood serum and blood cells (at ambient temperature). The serum was then measured for osmolality and Na^+ and Cl^- concentrations. Serum osmolality was measured using an automated freezing point depression osmometer (Fiske® 210 Micro-Sample Osmometer, USA). The osmolality of serum samples is expressed as mOsm kg^{-1}. The medium from each treatment was also sampled and its osmolality was determined using the same osmometer. Serum ions Na^+ and Cl^- were measured with the potentiometic (ion-selective electrode) method using an automated electrolyte analyzer (Jokoh EX-D, Japan). Blood samples from EDTA tubes were aspirated directly with an automated hematology analyzer (Sysmex XT-2000i, Japan) to assess hematological parameters (the red blood cell (RBC) count, hematocrit (Ht), hemoglobin (Hb) concentration, and white blood cell (WBC) count)). The Sysmex XT-2000i uses the electric resistance detecting method (impedance technology) with hydrodynamic focusing to measure RBC counts and Ht. Fluorescence flow cytometry is used to measure WBC. Hb is measured photocolorimetrically using sodium lauryl sulfate-Hb, a cyanide-free method. The reagents required for operating the Sysmex XT-2000i were obtained from Sysmex Corporation.

Statistical analysis

All data were expressed as mean ± standard deviation, and their normality and homogeneity were verified before using them for statistical analysis. To determine the exposure effects of Pb, salinity and its impact on osmolalities, ion levels, and hematological parameters were analyzed using two-way ANOVA, respectively. When significant differences were detected ($P \leq 0.05$), Duncan's multiple range test was employed for multiple comparisons to determine which treatment had a significant effect on osmolalities, ion levels, and hematological parameters at a significance level of 0.05.

Results

LC_{50} assessment

No fish died in the control treatments. Mortality increased with increasing Pb concentration. Mortalities of 100% were observed for fish exposed to 800 mg Pb L^{-1}. The 96 h LC_{50} with 95% confidence limits was 200 (170.84-234.14) mg Pb L^{-1} (Table 1).

Serum osmolality

No fish mortality was noted during the experiments. There was no significant Pb effect ($P = 0.179$) or interactive effect between salinity and Pb ($P = 0.460$) on the SO of *O. niloticus*. However, the effect of salinity on SO ($P = 0.003$) (Table 2) was significant. Duncan's test revealed that the SO values of *O. niloticus* at salinity levels of 0, 5, 10, and 15 ppt without Pb-exposure were not significantly different ($P > 0.05$). In Pb-exposed fish, the highest value of SO was noted in fish exposed to 100 mg Pb L^{-1} at a salinity level of 15 ppt, and the lowest SO was observed at 0 ppt. The levels of SO of Pb-exposed fish at 0 and 5 ppt were not significantly different ($P > 0.05$). There were no significant differences between the SO of control and Pb-exposed fish at the same salinity ($P > 0.05$) (Figure 1).

Serum ions concentration

This experiment showed the significant effects of salinity, Pb, and the interaction between salinity and Pb on serum Na^+ ($P = 0.000$) and Cl^- levels in fish ($P = 0.000$) (Table 2) respectively. The serum Na^+ and Cl^- concentration in *O. niloticus* at different salinity levels both with and without Pb-exposure are presented in Figures 2 and 3, respectively. The concentrations of serum Na^+ and Cl^- in fish at salinity levels 0 and 5 ppt without Pb were not significantly different ($P > 0.05$); however, these levels were significantly lower than those at salinity levels of 10 and 15 ppt ($P \leq 0.05$), respectively. Serum Na^+ and Cl^- concentrations in fish at salinity levels

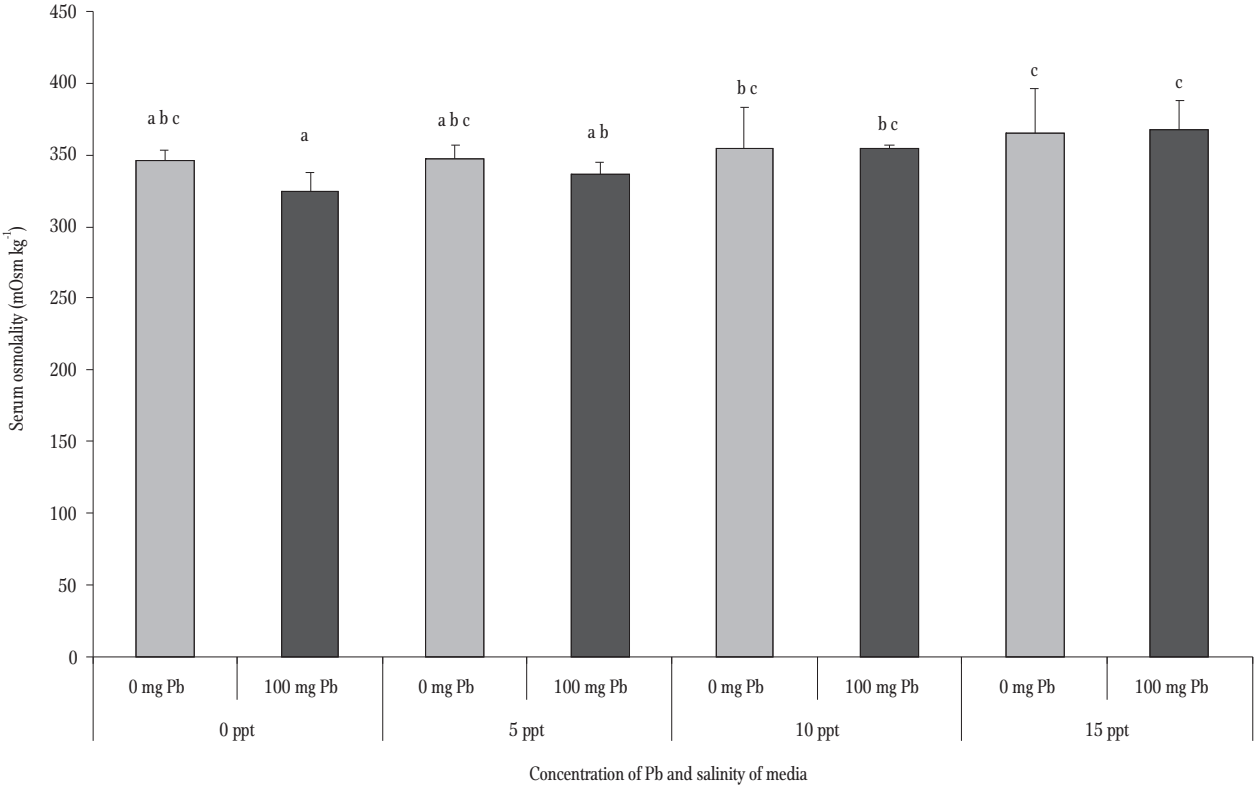

Figure 1. Serum osmolality of *O. niloticus* exposed to 0 and 100 mg Pb L^{-1} under different salinities for 7 d. Groups with different letters indicate significant differences ($P<0.05$, a<b<c). Data are means of five determinations.

Table 2
Two-way ANOVA of serum osmolality, ions, and hematological parameters of *O. niloticus* after 7 d of exposure to Pb and salinity treatments (n = 5)

Dependent variable	Source	F	P
Serum osmolality	Salinity	5.897	0.003
	Pb	1.889	0.179
	Salinity × Pb	0.883	0.460
Serum Na+	Salinity	58.751	0.000
	Pb	24.295	0.000
	Salinity × Pb	9.095	0.000
Serum Cl^-	Salinity	58.121	0.000
	Pb	32.597	0.000
	Salinity × Pb	12.366	0.000
Red blood cell count	Salinity	12.088	0.000
	Pb	55.286	0.000
	Salinity × Pb	8.170	0.000
Hemoglobin	Salinity	20.110	0.000
	Pb	188.938	0.000
	Salinity × Pb	14.110	0.000
Hematocrit	Salinity	49.515	0.000
	Pb	208.991	0.000
	Salinity × Pb	38.465	0.000
White blood cell count	Salinity	2.878	0.177
	Pb	1.903	0.051
	Salinity × Pb	60.869	0.000

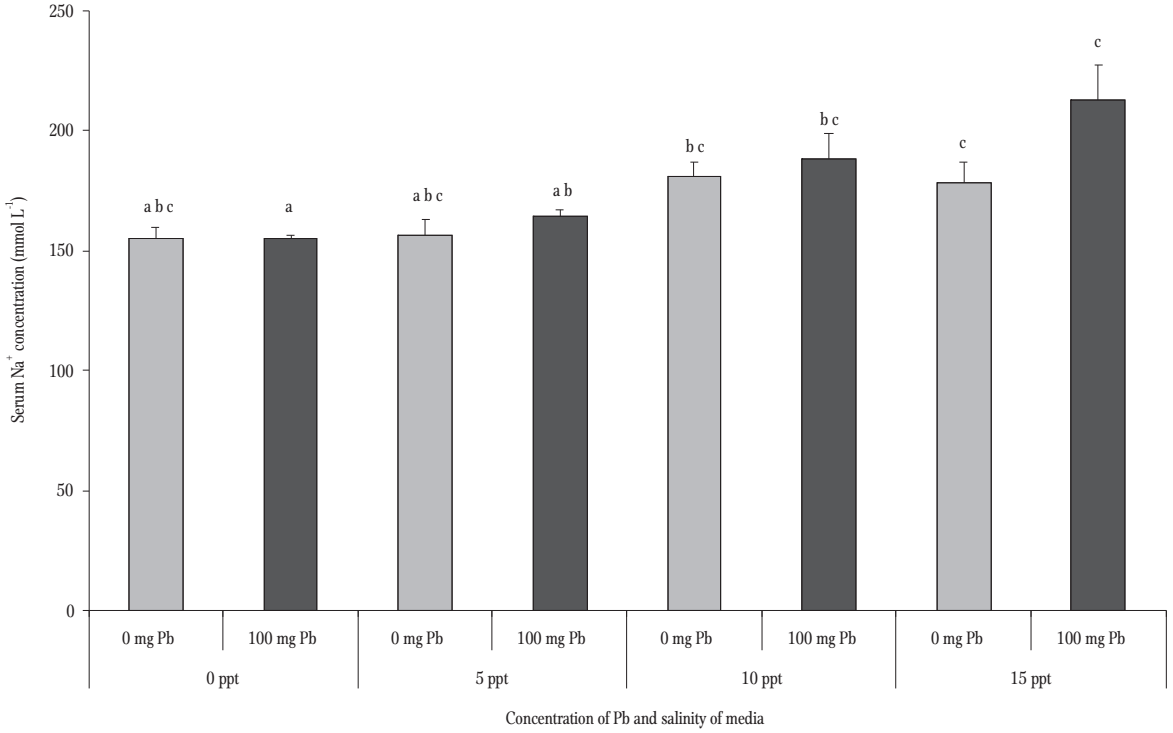

Figure 2. Serum Na^+ concentrations of *O. niloticus* after exposure to Pb (0, 100 mg Pb L^{-1}) at different salinities for 7 d. Groups with different letters indicate significant differences ($P < 0.05$, a<b<c). Data are means of five determinations.

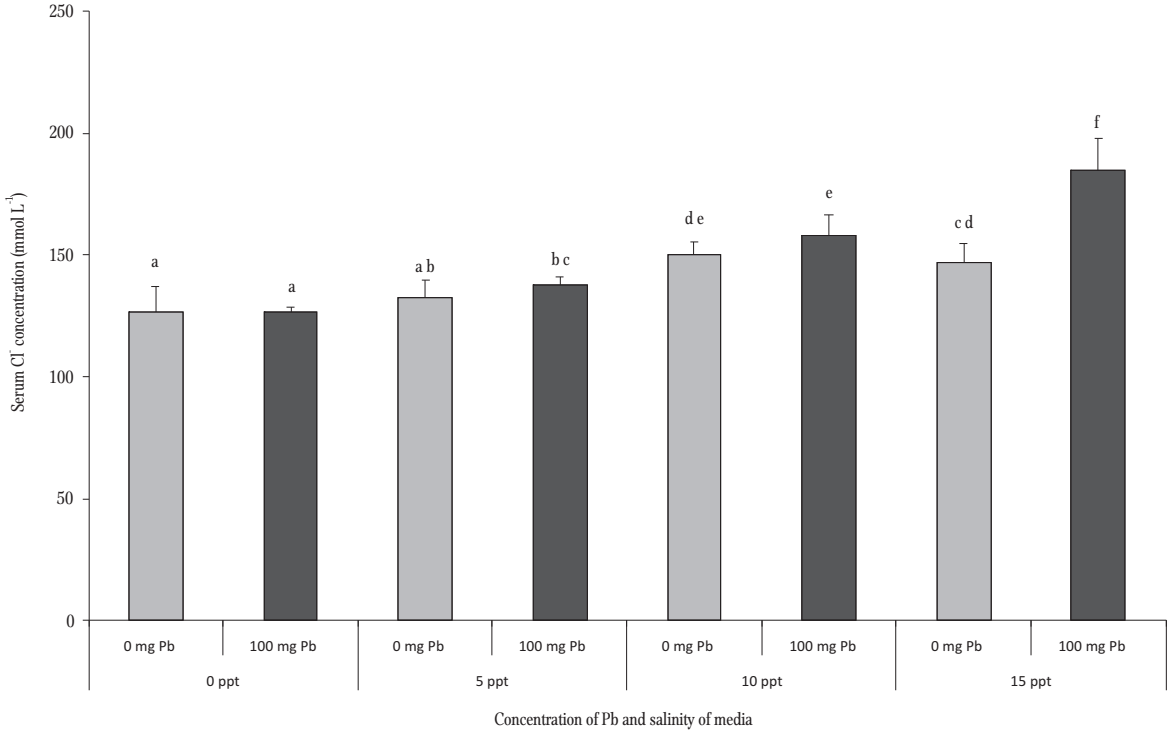

Figure 3. Serum Cl^- concentrations of *O. niloticus* after exposure to Pb (0, 100 mg Pb L^{-1}) at different salinities 7 d. Groups with different letters indicate significant differences ($P < 0.05$, a<b<c<d<e<f). Data are means of five determinations.

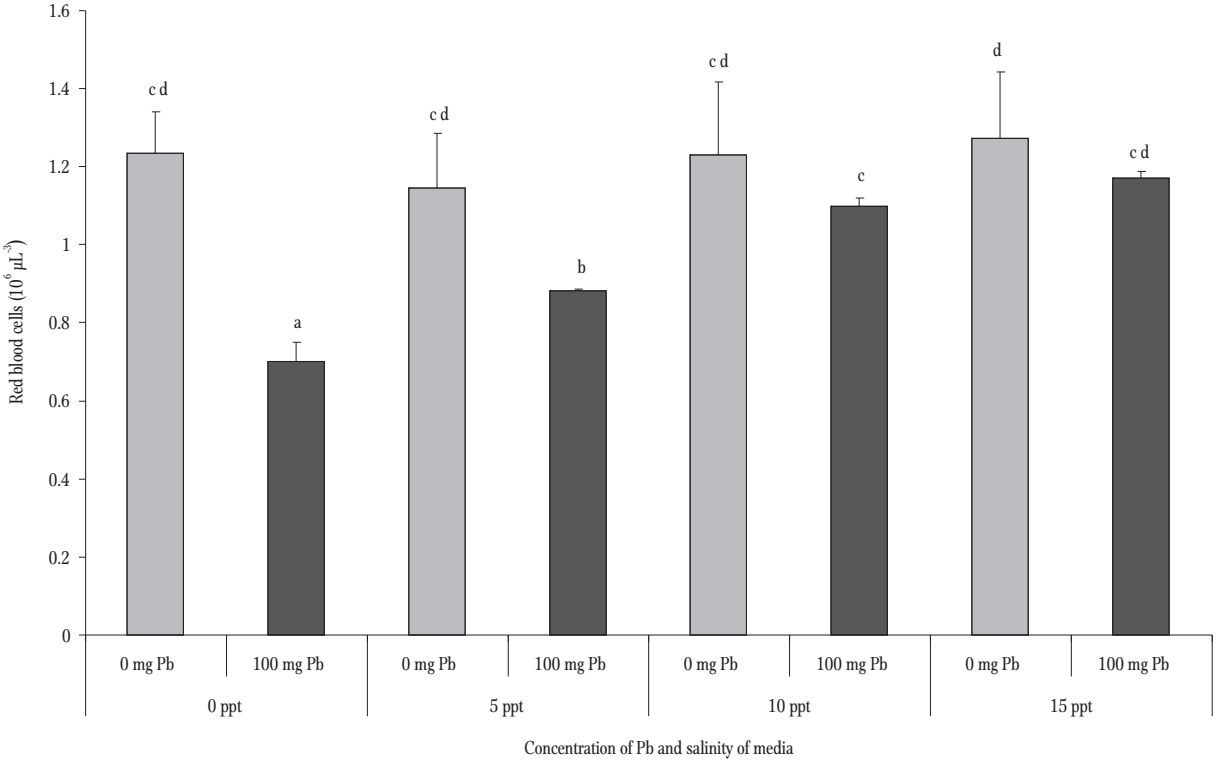

Figure 4. Red blood cells of *O. niloticus* after exposure to Pb (0, 100 mg Pb L^{-1}) at different salinities 7 d. Groups with different letters indicate significant differences ($P < 0.05$, a<b<c<d). Data are means of five determinations.

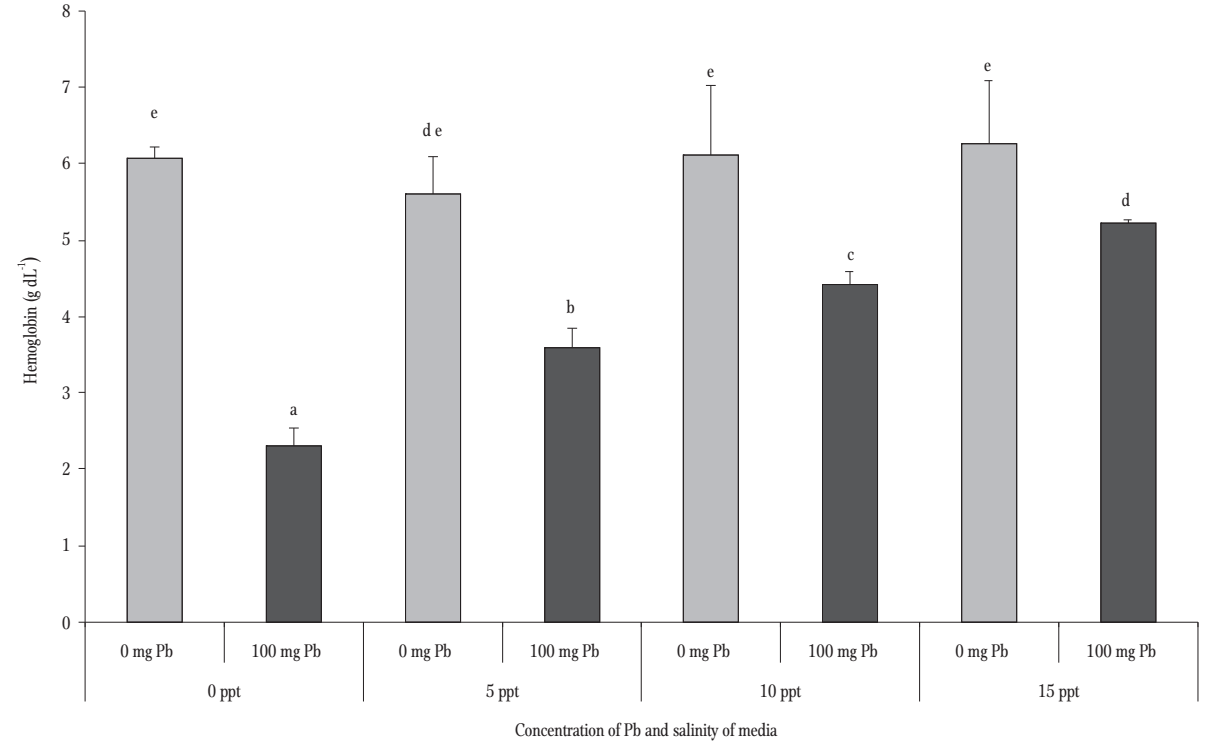

Figure 5. Hemoglobin of *O. niloticus* after exposure to Pb (0, 100 mg Pb L^{-1}) at different salinities 7 d. Groups with different letters indicate significant differences ($P < 0.05$, a<b<c<d<e). Data are means of five determinations.

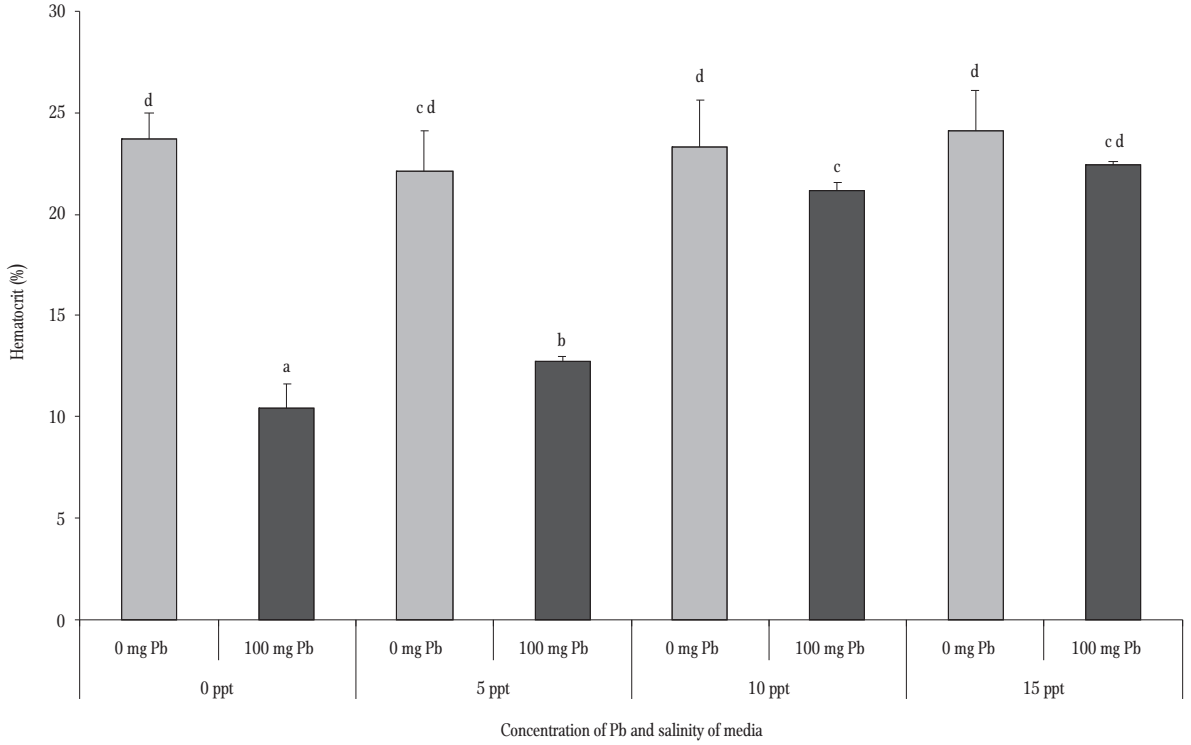

Figure 6. Hematocrit of *O. niloticus* after exposure to Pb (0, 100 mg Pb L^{-1}) at different salinities 7 d. Groups with different letters indicate significant differences ($P < 0.05$, a<b<c<d). Data are means of five determinations.

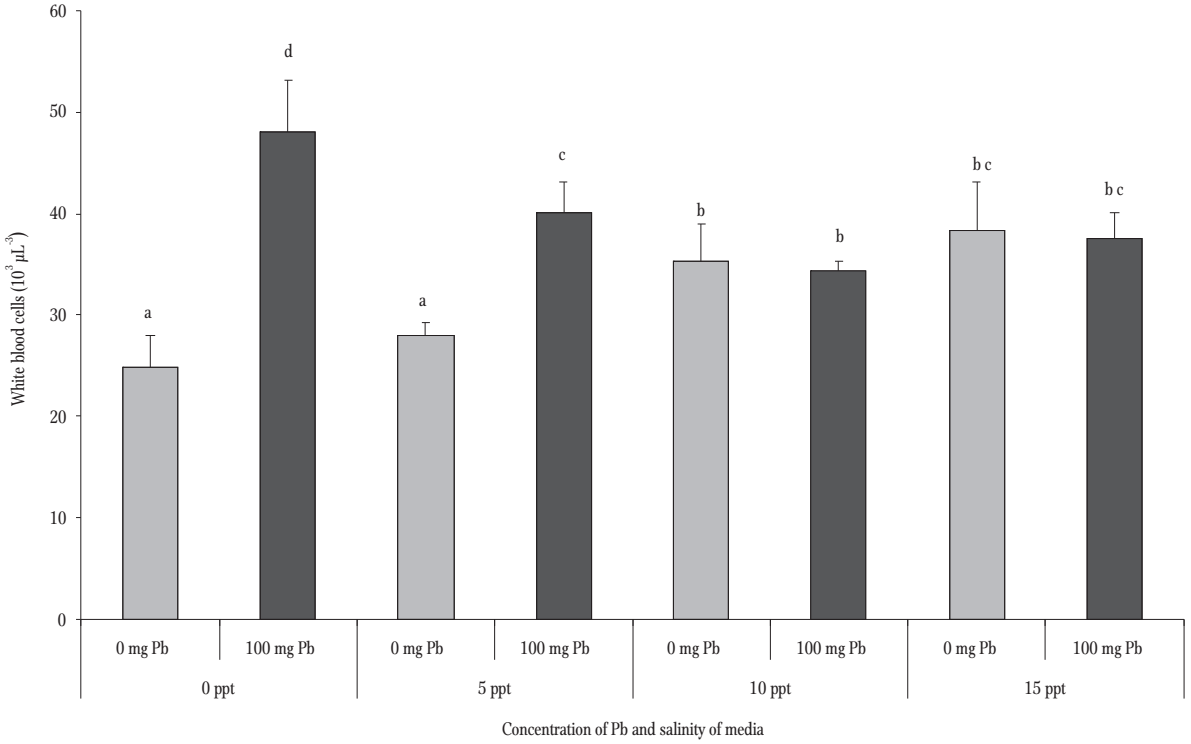

Figure 7. White blood cell counts of *O. niloticus* after exposure to Pb (0, 100 mg Pb L^{-1}) at different salinitiesb 7 d. Groups with different letters indicate significant differences ($P < 0.05$, a<b<c<d). Data are means of five determinations.

10 and 15 ppt without Pb were not significantly different, respectively. The concentrations of serum Na^+ in fish at 0 and 5 ppt under Pb exposure were not significantly different ($P > 0.05$), but it increased at 10 ppt, and reached the highest level at 15 ppt ($P \leq 0.05$). The serum Cl^- concentrations in Pb-exposed fish increased significantly in the following order: 15 ppt>10 ppt>5 ppt>0 ppt. The Na^+ levels in control and Pb-exposed fish were not significantly different at salinity levels 0, 5, and 10 ppt. At salinity level 15 ppt, the level of Na^+ of Pb-exposed fish was higher than that of the controls ($P \leq 0.05$). The levels of Cl^- in control and Pb-exposed fish were not significantly different at salinity levels 0, 5, and 10 ppt respectively ($P > 0.05$). The levels of Cl^- of Pb-exposed fish were significantly higher than those of the controls at salinity level 15 ppt ($P \leq 0.05$).

Hematological parameters

Significant effects of salinity, Pb, and the interactivity between salinity and Pb on RBC counts, Hb, and Ht were observed (Table 2). There were significant interactive effects between salinity and Pb on WBC; however, salinity level and Pb had no significant effect on the WBC of fish (Table 2). The levels of RBC, Hb, and Ht at all salinities in the control fish were not significantly different ($P > 0.05$) (Figures 4, 5, 6). In Pb-exposed fish, the levels of RBC, Hb, and Ht increased with the increasing salinity level of the media ($P \leq 0.05$). At salinities 0 and 5 ppt, the levels of RBC of Pb-exposed fish were significantly lower than those in the control ($P \leq 0.05$). Pb-exposed fish demonstrated lower levels of Ht and Hb when compared to the controls at salinity levels 0, 5, and 10 ppt ($P \leq 0.05$). The levels of WBC of control fish and Pb-exposed fish were not significantly different at salinity levels 10 and 15 ppt ($P > 0.05$); however, their levels were significantly higher than those at 0 and 5 ppt ($P \leq 0.05$) (Figure 7). In Pb-exposed fish, the levels of WBC decreased with increasing media salinity.

Discussion

Under normal conditions, unpolluted waters contain only trace amounts of Pb (0.03-0.05 $\mu g\ L^{-1}$); however, in polluted estuaries Pb concentrations can reach between 10 and 25 mg L^{-1} (Fatoki and Mathabatha 2001, Yilmaz and Sadikoglu 2011). Since the 96 h LC_{50} of *O. niloticus* fingerlings was 1.5 mg L^{-1} (Taweel et al. 2013), the Pb levels found in contaminated waters could be dangerous for the fingerlings (3 cm length, 1.5 g weight; Taweel et al. 2013). Studying the effects of Pb to the osmoregulation and hematological responses of fingerlings is more difficult than it is with adults, because of the difficulty of collecting blood samples from fingerlings. For this reason, adult *O. niloticus* were used in this study.

The 96 h LC_{50} of Pb in adult *O. niloticus* in this study (200 mg L^{-1}) was comparable to the findings of Jiraungkoorskul et al. (2008) at 182.12 mg L^{-1} and of Kosai et al. (2011) at 182.38 mg L^{-1}. In some studies, concentrations of Pb, such as 29, 95, 300 and 820 mg L^{-1}, were estimated as 96 h LC_{50} for *Carassius auratus* (L.), *Prochilodus lineatus* (Val.), *Tinca tinca* (L.), and *Heteropneustes fossilis* (Bloch), respectively (Martinez et al. 2004, Shah 2006, Srivastav et al. 2013, Khan et al. 2014). This great variability could be attributed to species-specific responses to toxic metals (Martinez et al. 2004).

The present study used 100 mg L^{-1} Pb as the sub-lethal concentration. Although this level was higher than that found in the polluted environment, the physiological and hematological impact of sub-lethal Pb (100 mg L^{-1}) on adult fish could be experienced by fingerlings when exposed to sub-lethal Pb levels in their habitat. In media without Pb, increasing salinities did not affect the SOs of *O. niloticus*, while, in contrast, the SOs of Pb-exposed fish increased with increasing salinity levels. There were no significant differences between the SOs of the control fish and the Pb-exposed fish at any of the salinity levels. Na^+ and Cl^- increased with increasing salinities in both the control and Pb-exposed fish. As the major ions in the body fluid, Na^+ and Cl^- (Gilles and Delpire 1997) determined blood osmolality. In Pb-exposed fish, the SO, Na^+, and Cl^- levels in the blood serum that increased with increasing salinity levels should be caused by the osmotically-induced removal of water from the fish and the uptake of ions

from the hyperosmotic environment into the fish (Hwang et al. 1989, Ghahremanzadeh et al. 2014). Whereas increases of Na^+ and Cl^- but the stable osmolality in the control fish exposed to higher salinities suggested that it is a temporary state of ion imbalance as has been reported in other fish species (Wilson and Laurent 2002, Laiz-Carrion et al. 2005). Increases of Na^+ and Cl^- in fish following exposure to higher salinities might indicate that ion uptake mechanisms were not yet down-regulated, resulting in greater net uptake under conditions of greater NaCl availability (Kolbadinezhad et al. 2012). The higher levels of Na^+ and Cl^- in Pb-exposed fish than in the control fish at 15 ppt could be caused by greater uptake capacity with the proliferation of chloride cells, a decrease in ion efflux rates because of mucus secretion during metal exposure (Wood et al. 1988, Wood 2001), and/or a fluid shift from plasma to tissue that can occur during metal-induced stress (Wood et al. 1988, Pane et al. 2003). Chowdhury et al. (2004) report that the greater levels of plasma protein in fish provide indirect evidence of fluid loss from plasma in fish exposed to metals. Increased Na^+ levels and serum osmolality are also reported in the ray-finned fish *P. lineatus* after exposure to water-soluble fractions of gasoline (Simonato et al. 2013). These increases were accompanied by an increase in the quantity of chloride cells in the lamellae and of N^+/K^+-ATPase activity. They suggest that these results reflect the stimulation of the pathway to Na^+ uptake, as demonstrated by the activation of Na^+/K^+-ATPase activity, which resulted in an increase in Na^+ concentration and plasma osmolality. Other possible reasons for increased ATPase activities could be related to a period of adaptation and/or an increased number of enzyme molecules or the turnover rates of the enzyme to maintain ion flux during metal toxicity (Atli et al. 2016).

The direct effects of metals on blood parameters are usually associated with increased erythrocyte disintegration or to damage to the hemopoietic system (Svobodova et al 1994, Heath 1995). In the present study, lower RBC counts and Hb and Ht levels were noted in Pb-exposed fish as compared to the controls at low salinity (0 and 5 ppt). Allen (1993) observed a decrease in RBC counts and Hb and Ht in *Oreochromis aureus* (Steindachner) treated with 19 mg L^{-1} of Pb. Olanike et al. (2008) reported a decrease in RBC counts and Hb and Ht in *Clarias gariepinus* (Burchell) exposed to 25-200 mg L^{-1} of Pb. The significant decrease in the number of RBC suggested that Pb may inhibit RBC production through erythrocyte destruction (McLeay 1973). Al-Rudainy (2015) points out that the reduction in Hb content in fish could also be due to the inhibitory effect of Pb on the enzyme system responsible for Hb synthesis. The decrease in RBC counts coupled with the decrease in Hb and Ht is an indication that *O. niloticus* experienced anaemic conditions or hemodilution. Changes in these hematological parameters can be interpreted as a compensatory response that improves the oxygen carrying capacity to maintain gas transfer, but it also indicates a change in the water blood barrier for gas exchange in gill lamellae (Jee et al. 2005, Al-Rudainy et al. 2015). In this condition, the ability of fish to provide sufficient oxygen to the tissues is considerably restricted and will result in decreased physical activity (Wepener et al. 1992a, 1992b, Nussey et al. 1995). At higher salinities, although the RBC counts and Ht levels in Cd-exposed fish were not significantly different from those in the control fish, the Hb levels of Pb-exposed fish were lower than those in the control fish. Decreases in Hb might have resulted from the release of immature red blood cells with lower Hb content from hematopoietic tissues. Immature cells are released to compensate for the loss of RBC (Benna and Viswaranjan 1987).

A significant increase in WBC counts was noted in Pb-exposed fish in comparison to control fish at lower salinity levels (0 and 5 ppt). Santos and Hall (1990) reported WBC increases in *Anguilla anguilla* (L.) subjected to Pb exposure, and they suggest that Pb-induced tissue damage might have activated an immune response in the fish. Ghazaly (1991) observed an increase in Pb levels in blood, liver, and kidneys, which indicates that not only circulating blood leukocytes could be affected by Pb but that the peripheral organs could also be affected. The significant increase in WBC counts could also stem from an increase in antibody production that helps the

survival and recovery of fish exposed to heavy metals (Joshi and Deep 2002).

Conclusions

In conclusion, our study shows that Pb did not change the osmolalities and caused minimum alteration in ionic regulation in tilapia, *O. niloticus*. Pronounced alteration occurred in the levels of the hematological parameters. At lower salinities (0 and 5 ppt), Pb-exposed fish demonstrated lower levels of RBC and Ht and higher levels of WBC in comparison to the control fish. These hematological changes could be a compensatory and adaptive response of the fish to cope with the toxic effects of Pb since osmoregulation in the fish did not change. At higher salinities, the unchanged levels of RBC (at 10 and 15 ppt), Ht (at 15 ppt), and WBC (at 10 and 15 ppt) in Pb-exposed fish could indicate that salinity plays a protective role in *O. niloticus* against Pb intoxication.

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Author contributions. M.H.A., A.Z., B.I., A.S. conceived and designed the research; M.H.A., A.Z., T.W.C.P., and A.S. performed the research; M.H.A., A.Z., and A.S. analyzed the data and wrote the paper.

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