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Effects of Lead on ATPases in Tissues of Freshwater Fish (*Oreochromis niloticus*) in Differing Calcium Levels

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Abstract

The aim of the present study was to investigate the response of ATPases in tissues of freshwater fish *Oreochromis niloticus* following exposure to Pb^{2+} in differing Ca^{2+} levels. Fish were exposed to Pb^{2+} in water containing 15 (conductivity of 161.0±10.7 µS/cm), 30 (conductivity of 242.0±9.50 µS/cm), and 60 (conductivity of 395.2±40.2 µS/cm) mg Ca^{2+}/L. Experiments were conducted using two Pb^{2+} exposure protocols, naming acute (48 h, 20 µM Pb^{2+}) and chronic (14 d, 10 µM Pb^{2+}). Following the exposures, Na^+/K^+-ATPase and Mg^{2+}-ATPase activities in the gill and intestine, and Ca^{2+}-ATPase activity in the muscle were measured. In acute exposure, Ca^{2+} exposures alone caused significant increases in ATPase activities. Combined exposures of Ca^{2+} and Pb^{2+} caused significant increases in ATPase activities at the lowest Ca^{2+} concentration, but almost all ATPase activities significantly decreased at the highest Ca^{2+} concentration. In chronic exposures, Ca^{2+} exposures alone caused a few significant alterations in ATPase activities, clear trend being a decrease in Na^+/K^+-ATPase activity. In combined exposures, there were only significant increases in ATPase activities at the lowest Ca^{2+} concentration. Gill Na^+/K^+-ATPase was found to be the most sensitive enzyme compared to ATPases in the other tissues. Data emphasized that Ca^{2+} concentration of water is an important parameter in assessment of acute or chronic metal toxicity, especially when ATPases are concerned and should be taken into account in evaluations of biomarkers in metal contaminated waters.

Keywords: Calcium, fish, *Oreochromis niloticus*, ATPase

Kurşunun Farklı Kalsiyum Düzeylerinde Tatlısu Balığı (*Oreochromis niloticus*) Dokularında ATPaz'lar Üzerine Etkileri

Özet

Bu çalışmada, farklı Ca^{+2} derişimlerinde Pb^{+2} etkisine kalan tatlısu balığı *Oreochromis niloticus*'un dokularında ATPaz'ların verdiği tepkilerin ölçülmesi amaçlanmıştır. Balıklar 15 (iletkenlik: 161,0±10,7 µS/cm), 30 (iletkenlik: 242,0±9,50 µS/cm) ve 60 (iletkenlik: 395,2±40,2 µS/cm) mg Ca^{+2}/L içeren ortamlarda Pb^{+2} etkisine bırakıldı. Deneyler akut (48 saat, 20 µM Pb^{+2}) ve kronik (14gün, 10 µM Pb^{+2}) olmak üzere iki farklı protokol ile sürdürüldü. Deneyler sonunda solungaç ve bağırsak dokusunda Na^+/K^+-ATPaz ve Mg^{+2}-ATPaz ile kas dokusunda Ca^{+2}-ATPaz aktiviteleri ölçülmüştür. Akut süreçte, sadece Ca^{+2} etkisinde kalan balıklarda ATPaz aktivitelerinde artış görülmüştür. Ca^{+2} ve Pb^{+2}'nin birlikteki etkisine kalanlarda düşük Ca^{+2} derişimlerinde ATPaz aktivitelerinde artış görülürken, yüksek Ca^{+2} derişimlerinde azalış görülmüştür. Kronik süreçte, sadece sadece Ca^{+2} etkisinde kalan balıklarda ATPaz aktivitelerinde hem artış hem azalış görülmüştür. Ca^{+2} ve Pb^{+2}'nin birlikteki etkisine kalanlarda sadece düşük Ca^{+2} derişimlerinde ATPaz aktivitelerinde artış görülmüştür. Diğer dokulardaki ATPaz'larla kıyaslandığında, solungaç Na^+/K^+-ATPaz'ın daha hassas olduğu bulunmuştur. Veriler, suyun Ca^{+2} derişiminin akut ve kronik metal toksisitesinin belirlenmesinde özellikle ATPaz'lar düşünüldüğünde önemli bir parametre olduğunu ve bu nedenle metal kirliliği olan sularda biyomarkırların değerlendirilmesinde dikkate alınması gerekmektiğini vurgulamıştır.

Anahtar Kelimeler: Kalsiyum, balık, *Oreochromis niloticus*, ATPaz aktivitesi.

Introduction

Aquatic organisms are generally exposed to chronic metal contamination, though they may also suffer acute exposures in areas where industrial effluents are discharged. Metal exposure can lead to several toxic effects, such as disruption of ion homeostasis, neurotoxicological abnormalities,

alterations in biochemical and physiological mechanisms, and can ultimately cause mortality of aquatic organisms (Heath, 1995). The water hardness can be defined as the sum of the concentration of divalent cations, particularly Ca^{2+} and Mg^{2+}. Free ion (basically Ca^{2+}) levels in fresh waters are generally higher in hard waters comparing to soft waters which also lead to an increase in water conductivity. When the conductivity of water increases, metal bioavailability and consequent metal toxicity decreases as a result of chelation processes. Thus, one can say that metals are more toxic to fish when they are exposed in soft water compared to hard water (Grosell *et al.*, 2002; Monserrat *et al.*, 2007; Saglam *et al.*, 2013; 2014).

Transition metals such as Fe, Cu, Co and Mn are essential for the metabolism, but may become toxic at high concentrations. Metals such as Cd, Hg, Pb, Sn, and As (known as non-essential metals) are toxic to living organisms at very low concentrations (Rainbow, 1985; Niyogi *et al.*, 2006; Atli and Canli, 2011a; Defo *et al.*, 2014). Pb^{2+} is a non-essential metal for life and known as a common environmental toxicant. Pb^{2+} is released to the environment from various sources such as mining, smelting, coal burning, cement manufacturing, and gasoline (WHO, 1995). Studies showed that Pb^{2+} was an antagonist for Ca^{2+} in fish (Richards and Playle, 1998; Rogers *et al.*, 2003, 2005). Chronic exposure of fish to Pb^{2+} caused the disruption in Ca^{2+} homeostasis and neurological abnormalities (Davies *et al.*, 1976; Mager *et al.*, 2008).

ATPases, as membrane-bound enzymes, are involved in regulation of cellular volume, osmotic pressure, and membrane permeability in fish (Heath, 1995; Marshall and Grosell, 2005). Na^+/K^+-ATPase which are located at the basolateral membrane of the gill epithelium of fish plays a direct role in active transport of Na^+ and K^+ between cell's interior and extracellular fluid (Saravanan *et al.*, 2011; Li *et al.*, 2011). Mg^{2+}-ATPase represents an integral membrane protein and hydrolyses extracellular nucleotides and plays an important role in oxidative phosphorylation and ionic transport, and is responsible for transepithelial regulation of Mg^{2+} ions (Nagy, 1986; Parvez *et al.*, 2006). Ca^{2+}-ATPase is also a significant ATPase which is localized in sarcoplasmic reticulum tubules and removes Ca^{2+} ions from the cytosol both to lumen of the sarcoplasmic reticulum and from the cell to maintain low Ca^{2+} levels in the cell (Watson and Beamish, 1981; Saxena *et al.*, 2000). Considerable alterations of ATPase activities in fish have been reported after metal exposures, osmoregulatory dysfunction being the predominant changes (Grosell *et al.*, 2003; Atli and Canli, 2007, 2011a, 2011b). Studies from our laboratory also showed that Pb^{2+} inhibited ATPase activity in the gill of tilapia (*Oreochromis niloticus*) in relation to Pb^{2+} exposure concentrations (Atli and Canli, 2007). This adverse effect may be associated with the inhibition of the ATPase in osmoregulatory tissues (McGeer and Wood, 2000; Atli and Canli, 2007, 2011b). Pb^{2+} can compete with Ca^{2+} for its molecular targets, namely for the Ca^{2+}-selective channels and therefore, affect fish physiology by changing Ca^{2+} balance (Ay *et al.*, 1999; Heath, 1995; Atli and Canli, 2007).

Fish is one of major sources of food all over the world due to its nutritional values (Yılmaz *et al.*, 2010). The healths of organisms are widely used as early diagnostic tools to estimate the impact of metal toxicity in aquatic systems (Lam and Gray, 2003). For instance, fish have been widely used as experimental models to evaluate the health of aquatic ecosystems. The Nile tilapia is recognized as a good biological model fish as they are easily reproduced and do not have feeding problems in culture conditions. Thus, they are a good source of protein and popular among artisanal and commercial fisheries (Almeida *et al.*, 2002). Thus, the aim of this study was to determine the effects of individual and combined exposure to Ca^{2+} and Pb^{2+} using two exposure protocols (acute, 48 h, 20 µM Pb^{2+} and chronic, 14 d, 10 µM Pb^{2+}) on ATPases in gill, intestine and muscle tissue of *O. niloticus*, and to evaluate whether the enzymes could be used as sensitive biomarkers in water with differing Ca^{2+} levels.

Materials and Methods

Experimental Protocol

Freshwater fish *O. niloticus* have been cultured in Cukurova University (Turkey) for more than 25 years. Fish were taken from the culture pools and transferred to the laboratory where they were acclimatized in experimental aquaria for two weeks before the experiments. Experimental room was air conditioned (20±1ºC) and illuminated for 12 h with fluorescent lamps (daylight 65/80W). The experiments were carried out in glass aquariums sized 40x40x100 cm that contained 120 L contaminated test solution or only test water (dechlorinated) for controls.

To study the effects of Ca^{+2} on Pb^{+2} toxicity, relatively soft drinking water was used for the experiments, purchasing dirinking water (approx. 1 tonnes) from Pinar Company (Turkey). This water was used as normal control and called as Ca0. Water with added Ca^{+2} were called Ca1 and Ca2. Final Ca^{2+} concentrations of Ca0, Ca1 and Ca2 were 15, 30 and 60 mg Ca^{2+}/L, respectively. A total of 6 fish were used for each exposure group in the experiments. Chemical quality of all control waters and size of fish used in the experiments were given in Table 1. Fish were individually exposed to 20 µM of Pb^{2+} ($Pb(NO_3)_2$) for acute exposure (48 h) and 10 µM of Pb^{2+} for chronic exposure (14 d) at all Ca^{2+} concentrations. During the experiments, exposure waters were renewed every two days just after feeding to reduce contamination with food remains and to

minimize metal loss. At the end of each experimental period, fish were killed by transection of the spinal cord to the decision of Ethic Committee of Cukurova University, and gill, intestine and muscle tissues were dissected. Tissues were stored at -80°C (Revco Ultima II, Newsbreak, UK) until the analyses. Tissues were homogenizedin ice-cold buffer containing 20 mM Tris–HCl, 0.25 M sucrose, and 1 mM ethylene diamine tetraacetic acid (pH 7.7) with a ratio of 1:10 at 9.500 rpm for 2 to 3 min. Homogenates were centrifuged at 13.000x g (4ºC) for 20 min. The supernatants were collected for determination of total protein levels and ATPase activity.

ATPase Activity Assay

The final assay concentrations to measure tissue Na^+/K^+-ATPase and Mg^{2+}-ATPase activity were 40 mM Tris–HCl, 120 mM NaCl, 20 mM KCl, 3 mM MgCl2, 7.7 pH, and 1 mM ouabain. In addition, incubation media (pH 7.7) containing 40 mM Tris–HCl, 4 mM $MgCl_2$, 1 mM $CaCl_2$ and 1 mM EGTA was used for Ca^{2+}-ATPase activity. For measuring ATPase activity, 50 µl of enzyme suspension (~100 µg protein) was added to 850 µl of incubation media and preincubated for 5 min at 37°C. The reaction was started by the addition of 100 µl Na_2ATP (3 mM) and incubated for 30 min. The reaction was stopped by adding 500 µl of ice-cold distilled water. Inorganic phosphate was measured as described by Atkinson *et al.* (1973). Appropriate blanks were included with each assay to correct for non-enzymatic hydrolysis of ATP. KH_2PO_4 (25–250 µM) was used as inorganic phosphate standard and spectrophotometric analysis was carried out at 390 nm using Schimadzu UV-1800 series spectrophotometer. Specific Na^+/K^+-ATPase activity was calculated from the inorganic phosphate liberated from ATP using the differences between the presence (Mg^{2+}-ATPase activity) and absence (Total-ATPase activity) of the ouabain. Ca^{2+}-ATPase activity was measured as the absorbance differences between the presence and absence of $CaCl_2$. All assays were carried out in triplicate. Total protein levels were determined according to Lowry *et al.* (1951) and bovine serum albumin was used as a standard.

Statistical Analysis

Statistical analysis of data (mean ± standard error) was carried out using a statistical program (SPSS 15, IBM Corporation, USA). One-way ANOVA was used to do individual comparisons of variables among controls and treatments. Each parameter from each Pb^{2+} exposure was analyzed separately with its own control. All statistical analyses were done separately for acute and chronic exposures. Significant differences ($P<0.05$) were evaluated by Duncan's test to determine which individual groups were significantly different from appropriate control.

Results

There was no fish mortality in any of the exposure condition during the experiments. Table 2 summarizes statistical results and fluctuations in enzyme activities in controls and Pb^{2+} exposed fish.

Effects of Ca^{2+} on the ATPase Activity

In acute exposures, there were increases in ATPase activities of Ca1 and Ca2 compared to Ca0 (Figure 1-5), the highest increase (120 %) being in gill Na^+/K^+-ATPase activity of Ca1 (Figure 1). In chronic exposures, there were fluctuations in ATPase activity in Ca1 and Ca2 compared to Ca0 (Figure 6-10). The lowest (37%) enzyme activity was measured in gill Na^+/K^+-ATPase in Ca2 (Figure 6), while the highest increase (345%) in enzyme activity was detected in muscle Ca^{2+}-ATPase (Figure 10). Comparisons of acute and chronic controls also showed some significant differences in enzyme activities (Table 3). Generally, there were decreases in enzyme activities of Ca1 and Ca2 at chronic duration compared to acute duration, highest decrease (72%) being in the intestine Mg^{2+}-ATPase in Ca2 (Figure 7).

Effects of Pb^{2+} Exposures in Differing Ca^{2+} Concentrations

In acute exposures, there were alterations in ATPase activities depending on Pb^{2+} or Ca^{2+} concentrations of media and tissue types (Figure 1-5). The lowest value (52%) was detected in gill Na^+/K^+-ATPase activity of fish exposed to Pb^{2+} in Ca2 (Figure 1), while the highest increase (89%) was measured in the intestine Na^+/K^+-ATPase activity in fish exposed to Pb^{2+} in Ca0 (Figure 3). In chronic exposures, Pb^{2+} caused increases in Na^+/K^+-ATPase

Table 1. A summary of the conditions of different exposure media and fish used in the experiments

Parameters	Ca0	Ca1	Ca2	P value
pH	7.61±0.25	7.46±0.23	7.34±0.22	NS
Oxygen (mg O_2/ml)	7.03±1.20	7.01±0.76	7.23±0.71	NS
Conductivity (µS/cm)	161.0±10.7	242.0±9.50	395.2±40.2	< 0.05
Hardness (mg $CaCO_3$/L)	91.1±30.3	105.5±12.3	193.3±28.4	< 0.05
Alkalinity (mg $CaCO_3$/L)	95.5±14.0	94.5±12.0	98.0±8.90	NS
Temperature (°C)	17.6±1.43	17.5±1.43	17.6±1.40	NS
Fish Length (cm)	10.3±1.30	10.2±1.20	10.6±1.10	NS

Statistical comparisons were also given in the table

Table 2. A summary of statistical analyses on Na^+/K^+-ATPase and Mg^{2+}-ATPase activity in the gill and intestine and Ca^{2+}-ATPase activity in the muscle of *O. niloticus* exposed individually to different controls and controls+Pb combinations in acute and chronic durations

Exposure Conditions		Gill		Intestine		Muscle
		Na/K-ATPase	Mg-ATPase	Na/K-ATPase	Mg-ATPase	Ca-ATPase
Acute	Ca0	-	-	-	-	-
	Ca1	↑*	-	↑*	↑*	↑*
	Ca2	-	-	↑*	↑*	↑*
	Ca0+Pb	-	↑#	↑#	↑#	-
	Ca1+Pb	↓#	↑#	↑#	-	↓#
	Ca2+Pb	↓#	-	↓#	↓#	↓#
Chronic	Ca0	-	-	-	-	-
	Ca1	↓*	-	-	↑*	-
	Ca2	↓*	-	-	-	↑*
	Ca0+Pb	↑#	↑#	-	-	-
	Ca1+Pb	↑#	-	↓#	↓#	-
	Ca2+Pb	↑#	↑#	↓#	↓#	↓#

↑= increase; ↓= decrease; - = not significant

* indicates significant (P<0.05) difference among controls (Ca0, +Ca1 and +Ca2), # indicates significant difference among individual control and its own Pb^{2+} exposure

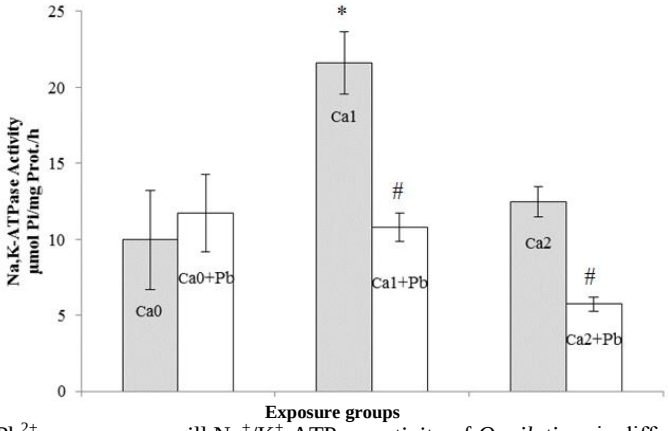

Figure 1. Effects of acute Pb^{2+} exposures on gill Na^+/K^+-ATPase activity of *O. niloticus* in differing calcium concentrations. "*" indicates the significant (P<0.05) differences among different calcium controls (Ca0, Ca1 and Ca2 = 15, 30 and 60 mg Ca^{2+}/L respectively), "#" indicates the significant differences between individual Pb^{2+} exposure and its own control.

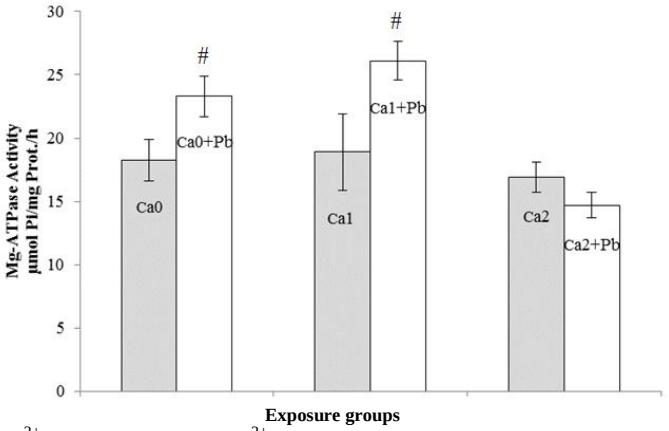

Figure 2. Effects of acute Pb^{2+} exposures on gill Mg^{2+}-ATPase activity of *O. niloticus* in differing calcium concentrations. See details in Figure 1.

and Mg^{2+}-ATPase activities in relation to increases in Ca^{2+} levels of exposure media. Oppositely, there were decreases in enzyme activities in the muscle and intestine (Table 2). The highest increase (349%) was detected in gill Na^+/K^+-ATPase activity of fish exposed to Pb^{2+} in Ca0 (Figure 6), while the lowest (69%) enzyme activity was measured in muscle Ca^{2+}-ATPase activity of fish exposed to Pb^{2+} in Ca2

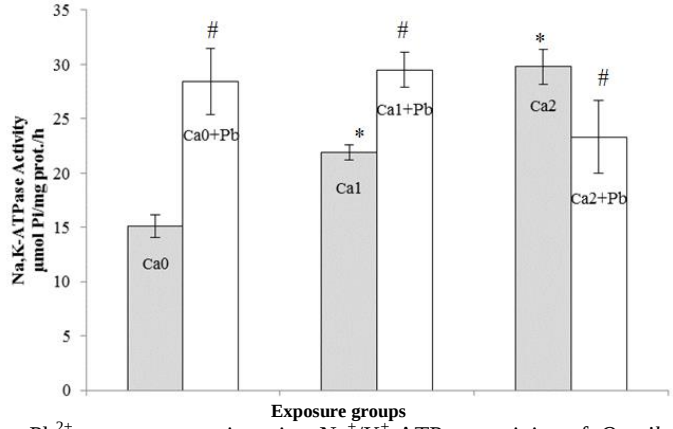

Figure 3. Effects of acute Pb^{2+} exposures on intestine Na^{+}/K^{+}-ATPase activity of *O. niloticus* in differing calcium concentrations. See details in Figure 1.

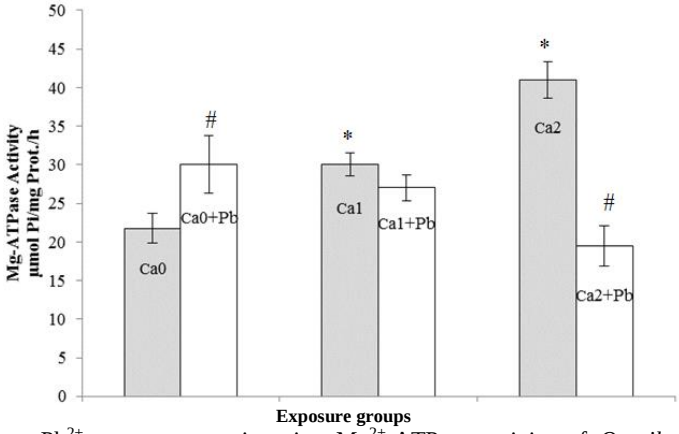

Figure 4. Effects of acute Pb^{2+} exposures on intestine Mg^{2+}-ATPase activity of *O. niloticus* in differing calcium concentrations. See details in Figure 1.

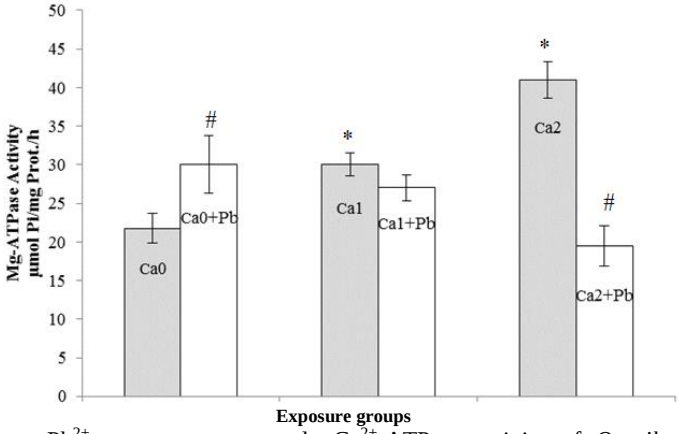

Figure 5. Effects of acute Pb^{2+} exposures on muscle Ca^{2+}-ATPase activity of *O. niloticus* in differing calcium concentrations. See details in Figure 1.

(Figure 10).

Discussion

There was no fish mortality in the present study at all exposure conditions, despite soft hardness value of experimental water. Literature data showed that metals can be more toxic to fish when administered in soft waters (Heath ,1995; Saglam *et al.*, 2013; Dogan *et al.*, 2014). The water hardness, altered largely by Ca^{+2} levels of water, can affect fish physiology, bioavailability, uptake and toxicity of metals in fish.

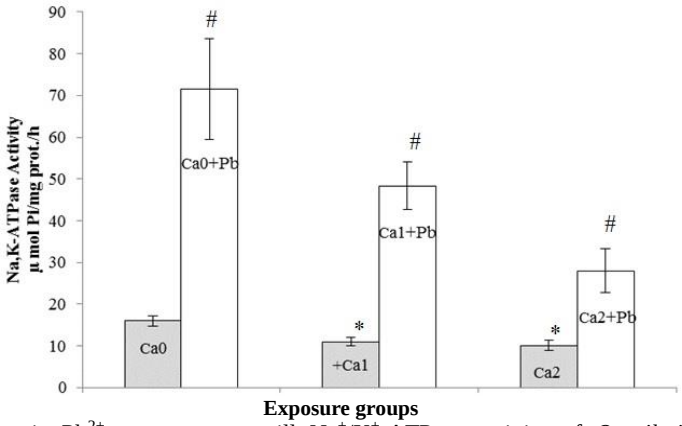

Figure 6. Effects of chronic Pb^{2+} exposures on gill Na^{+}/K^{+}-ATPase activity of *O. niloticus* in differing calcium concentrations. See details in Figure 1.

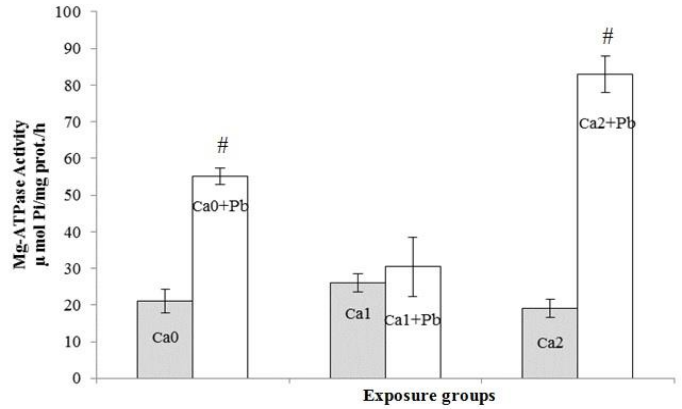

Figure 7. Effects of chronic Pb^{2+} exposures on gill Mg^{2+}-ATPase activity of *O. niloticus* in differing calcium concentrations. See details in Figure 1.

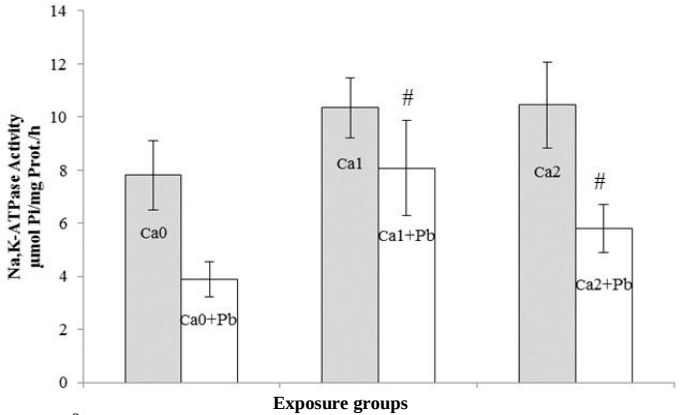

Figure 8. Effects of chronic Pb^{2+} exposures on intestine Na^{+}/K^{+}-ATPase activity of *O. niloticus* in differing calcium concentrations. See details in Figure 1.

Metal bioavailability generally decreases when the conductivity of water increases, indicating the negative relationship between metal toxicity and the conductivity (Verbost *et al.*, 1989; Hollis *et al.*, 2000; Ebrahimpour *et al.*, 2010; Dogan *et al.*, 2014). Thus, the present study was carried out to understand better the response of the osmoregulation system of *O. niloticus* after Pb^{+2} exposure in increased Ca^{+2} concentrations.

The present data showed that significant alterations in ATPase activities occurred following exposure to Ca^{2+} and/or Pb^{2+}, depending on tissue types and exposure protocols. In acute exposure, Ca^{2+} exposures alone (positive controls) caused significant

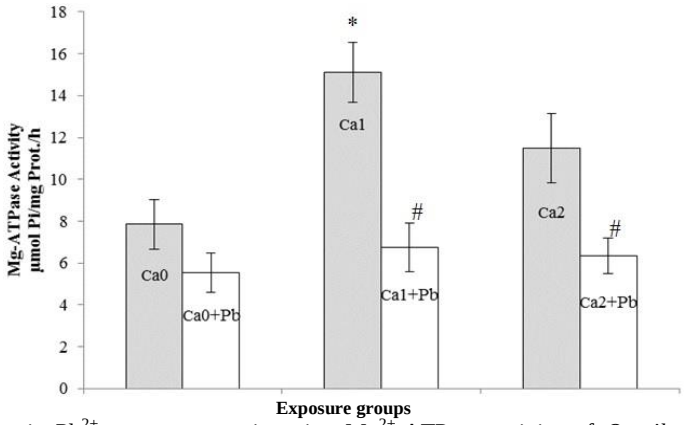

Figure 9. Effects of chronic Pb^{2+} exposures on intestine Mg^{2+}-ATPase activity of *O. niloticus* in differing calcium concentrations. See details in Figure 1.

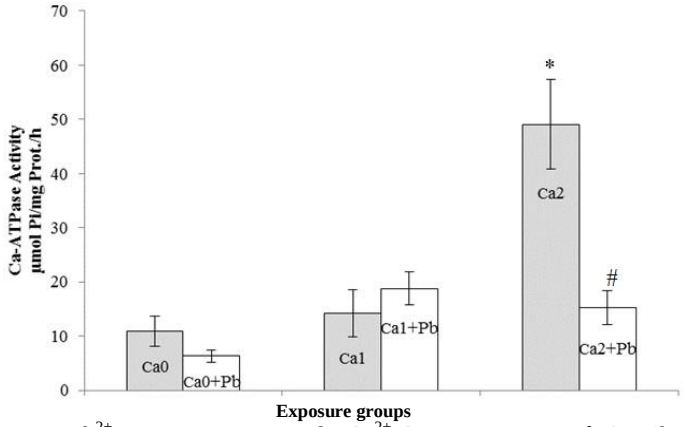

Figure 10. Effects of chronic Pb^{2+} exposures on muscle Ca^{2+}-ATPase activity of *O. niloticus* in differing calcium concentrations. See details in Figure 1.

increases in ATPase activities. Similarly, Ca^{2+} and Pb^{2+} combined exposures caused significant increases in ATPase activities at the lowest Ca^{2+} concentration, though ATPase activities decreased at the highest Ca^{2+} concentration. In chronic exposures, Ca^{2+} exposures alone caused fewer alterations in ATPase activities compared to the acute exposures, clear trend being a decrease in Na^{+}/K^{+}-ATPase activity. Ca^{2+} and Pb^{2+} combination exposures caused only significant increases in ATPase activities at the lowest Ca^{2+} concentration. Oppositely, there were decreasing trend in ATPase activities at higher Ca^{2+} concentrations. Comparing the tissues, the intestine showed most significant alterations, followed by the gill and muscle. Data also indicated that the gill Na^{+}/K^{+}-ATPase activity was most sensitive enzyme compared to the other ATPases. The present data agreed with previous studies on the effects of $Pb2^{+}$ (Jain *et al.*, 1997; Ay *et al.*, 1999; Shrivastava *et al.*, 2001; Grosell *et al.*, 2003; Atli and Canli, 2007). A decrease in enzyme activity could be related to high affinity of metals to –SH groups on the enzyme molecule, membrane rupture, osmoregulation dysfunctions, compete with the single or divalent metal ions or disturbance of the ion homeostasis. Increases in enzyme activity might be explained by possible compensation and repair mechanisms. Although it seems that metals inhibit Na^{+}/K^{+}-ATPase *in vitro*, inhibition of this enzyme by metals *in vivo* can be compensated by homeostatic regulation and its activity may return to normal. This recovery may possibly occur by increasing the number of enzyme molecule and/or increasing the turnover rates of enzymes present in order to compensate the activity of lost enzymes (Atli and Canli, 2007, 2011a, 2013).

Na^{+}/K^{+}-ATPase is a membrane bound enzyme located mostly in osmoregulatory tissues such as gill and intestine where it maintains ionic and electrical gradients necessary for transepithelial salt movements (Lionetto *et al.*, 2000; Grosell *et al.*, 2003; Monserrat *et al.*, 2007). The movement of Mg^{2+} over the apical membrane is passive, down an electrochemical gradient. The cytosolic Mg^{2+} concentration is kept well below its equilibrium concentration. The extrusion over the basolateral plasma membrane is mediated by an ATP-consuming enzyme (Flik *et al.*, 1993). Ca^{2+}-ATPase localised in sarcoplasmic reticulum tubules serves on the transport of Ca^{2+} from the cytoplasm to maintain low calcium concentrations in cells (Watson and Beamish, 1981; Saxena *et al.*,

2000). Studies showed that metals inhibit ATPase activity in tissues of aquatic animals, affecting some vital metabolisms. For example, mitochondrial Mg^{2+}-ATPase is the most important associated with energy metabolism and inhibition of Mg^{2+}-ATPase distrupts oxidative phosphorylation. However, it has lower sensitivity to metals compared to other ATPases (Lemaire-Gony and Mayer-Gostan, 1994; Lionetto *et al.*, 2000; Atli and Canli, 2013). Pb^{2+} inhibited Ca^{2+}-ATPase in fish muscle and as a result, changes in calcium levels were observed (Rogers *et al.*, 2003). Studies also showed that Ca^{2+}-ATPase activity decreased in several tissues of fish after metal exposures, possibly inhibiting Ca^{2+} transport (Wong and Wong, 2000; Rogers and Wood, 2004; Atli and Canli, 2011a). Na^{+}/K^{+}-ATPase is the most important ATPase in osmoregulation of fish, yet it is the most sensitive one to metal exposures. Metal exposures, in general, can change ATPase activity either by binding to their functional groups or by displacing the metal associated with the enzyme (Viarengo, 1985; Heath, 1995; Atli and Canli, 2011a, 2013).

Lead is a non-essential trace metal with no biological function and can be toxic to aquatic animals when given in access amounts. Previous studies have shown that Pb^{2+} causes disruption of Na^{+}, Cl^{-} and Ca^{2+} regulation and disruption in hemoglobin synthesis (Hodson *et al.*, 1978; Rogers *et al.*, 2003, 2005; Rogers and Wood, 2004). Pb^{2+} interacts with other elements synergistically or antagonistically. There are evidences on the antagonism between Pb^{2+} and Ca^{2+}, by which this metal directly competes with Ca^{2+} for uptake at calcium binding sites and can enter the cells through similar transport pathways (Verbost *et al.*, 1987, 1989; Spry and Wood, 1985; Rainbow and Blackmore, 2001). Similarly, waterborne Ca^{2+} has a marked protective effect against waterborne Cd^{2+} toxicity to brook trout (Carrol *et al.*, 1979), tilapia (Pratap *et al.*, 1989; Pratap and Wendelaar Bonga, 1993) and rainbow trout (Hollis *et al.*, 2000; Hansen *et al.*, 2002; Baldisserotto *et al.*, 2005). Our previous study showed that there were exposure dependent decreases in the branchial Na^{+}/K^{+}-ATPase activity in the tissues of *Tilapia zillii* exposed to Cu^{2+} and Pb^{2+} (Ay *et al.*, 1999). Similarly, MacDonald *et al.* (2002) showed that effect of Pb^{2+} with competitive inhibition of apical Ca^{2+} channel in fish gills disrupt Ca^{2+} homeostasis. In another study, we also demonstrated that activities of ATPases in tissues of *O. niloticus* altered significantly after exposure to metals (Cd^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+}) for 14 days with an inhibition trend of Na^{+}/K^{+}-ATPase activity in the gill and Ca^{2+}-ATPase activity in the muscle (Atli and Canli, 2007). ATPase activities in the gill, kidney and muscle of *O. niloticus* exposed to Cd^{2+}, Cr^{6+} and Ag^{+} for 96 h decreased, in general (Atli and Canli, 2013). They concluded that decreased ATPase activities were due to the direct effects of metals, and fluctuations in Mg^{2+}-ATPase activity could be associated to its location in the cell.

The present study demonstrated that Ca^{2+} exposure alone altered ATPase activity especially in acute period, though alterations were leveled by fish metabolism at the chronic period. Craig *et al.* (2007) also recorded that gill Na^{+}/K^{+}-ATPase activity in zebra fish *Danio rerio* significantly increased up to the third day, but by the seventh day it was approached to the level following adaptation to soft water for 7 days. Schoenmakers (1992) indicated that intestinal Ca^{2+} absorption played a significant role in the overall Ca^{2+} status in freshwater fish. Mozambique tilapia (*O. mosembicus*) has been shown to become hypocalcemic when fed cadmium-containing food and could not compensate the decraese of intestinal Ca^{2+} uptake (Pratap *et al.*, 1989). This may be the result of dietary Cd^{2+} entering the blood and inhibiting branchial Ca^{2+} uptake, as was shown by Baldisserotto *et al.* (2005) in rainbow trout (*Oncorhynchus mykiss*). Oppositely, the authors also showed that an increase in Ca^{2+} levels reduced both intestinal and branchial Cd^{2+} uptake. Similar results were also observed by Franklin *et al.* (2005), as high dietary Ca^{2+} reduced the toxic effect of waterborne Cd^{2+} exposure in rainbow trout. Alves and Wood (2006) also showed that elevated dietary Ca^{2+} reduced Pb^{2+} uptake at the intestine, indicating protective role of Ca^{2+} against bioaccumulation of Pb^{2+}. They indicated that this protective effect might result from direct Ca^{2+} versus Pb^{2+} competition for uptake mechanisms at the gastrointestinal system. The protective effects of elevated Ca^{2+} may become important in new environmental regulations and approaches to Pb^{2+} toxicity (Paquin *et al.*, 2002; Alves and Wood, 2006). Nevertheless, it should be noticed that the different responses could be attributed to the different routes of the metal uptake after water borne or dietary exposure to metals (Baldisserotto *et al.*, 2005; Alves and Wood, 2006).

The present results showed that both Ca^{2+} and Pb^{2+} alone and in combination were able to alter ATPase activities in tissues of *O. niloticus*, as supported by previous studies (Ay *et al.*, 1999; Morgan *et al.*, 2004; Silvestre *et al.*, 2005; Atli and Canli, 2007, 2011b; Baysoy *et al.*, 2013; Saglam *et al.*, 2013). Rogers *et al.* (2003) indicated that inhibitory effects of Pb^{2+} on gill Na^{+}/K^{+}-ATPase might be connected with the disturbance of the ion homeostasis rather than membrane rupture, respiratory failure or acid-base disturbance. On the other hand, increases in ATPase activities could be related to developing resistance against to Pb^{2+}, changes in water ion levels or compensation mechanisms (Flik *et al.*, 1993). Following Pb^{2+} exposures, Mg^{2+}-ATPase and Na^{+}/K^{+}-ATPase activities in the intestine mostly decreased in relation to increased Ca^{2+} concentrations in the present study. This could be related to Pb^{2+} toxicity or compensation with divalent ion Mg^{2+} (Monserrat *et al.*, 2007). Inhibition of muscle Ca^{2+}-ATPase could be related to

distruption of Ca^{2+} homeostasis, because Pb^{2+} is known to show high sensitivity to Ca^{2+} channels. Alves and Wood (2006) indicated that waterborne Pb^{2+} caused the disruption of Na^{+}, Cl^{-} and Ca^{2+} regulation, because divalent metals such as Pb^{2+} are known as a Ca^{2+} antagonist, directly competing with Ca^{2+} for uptake at Ca^{2+} binding sites. Another interesting finding of the present study was high resistance of tilapia to Pb^{2+} exposures even at low Ca^{2+} levels in both acute and chronic durations when compared to other fresh water fish species such as rainbow trout (Roger *et al.* 2003).

In conclusion, the present study demonstrated that individual and combined exposure to Ca^{2+} and Pb^{2+} altered the ATPase activities in tissues of *O. niloticus*. Exposure of fish to Ca^{2+} alone increased ATPase activity in acute period, though ATPase activities leveled at chronic exposure. Pb^{2+} exposures of fish mostly decreased the ATPase activities, especially in the intestine, indicating the ineffective protection of Ca^{2+} presence in the medium. Data from the present study indicated that physicochemical characteristics of water and the metabolic activity of a specific organism are very important factor to measure the effects of metals in water, especially on ATPases and emphasized water ion levels should be measured in the evaluation data from the field.

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