www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 12: 861-869 (2012) DOI: 10.4194/1303-2712-v1241_14

Investigations on the ATPase Activities and Cadmium Uptake in Freshwater Fish *Oreochromis niloticus* Following Exposures to Cadmium in Increased Salinity

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Received 05 March 2012
Accepted 21 October 2012

Abstract

Anthropogenic activities can increase the salinity of freshwaters. Thus, freshwater fish *Oreochromis niloticus* were exposed to Cd (1.0 µg/mL) in increased salinities (0, 2, 4 and 8 ppt) for 0, 1, 3, 7 and 14 days. Following single and combine exposures to salinity and Cd, Na^+/K^+-ATPase, Mg^{2+}-ATPase, Ca^{2+}-ATPase activities were measured in the gill, kidney and intestine. In general, salinity alone exposures increased Na^+/K^+-ATPase activity in the gill and intestine while there were fewer alterations in the kidney. Mg^{2+}-ATPase activity significantly altered in the intestine and kidney. Ca^{2+}-ATPase in the tissues significantly decreased, the kidney showing the most decrease. Salinity+Cd combine exposures decreased Na^+/K^+-ATPase activity in 2 ppt medium in the gill while the activity increased at 8 ppt medium. Na^+/K^+-ATPase activity in the intestine decreased in relation to salinity increase, though there was no significant decrease in the kidney. Mg^{2+}-ATPase and Ca^{2+}-ATPase activities showed a declining trend with the increase in salinity. Cd accumulation in the tissues decreased as the salinity of medium increased, though accumulation in the gill increased regardless of salinity increase at the longest exposure period. Results emphasized necessity of measuring fish ATPase activities in evaluation of data from environmental monitoring, as they are highly sensitive to the changes in the aquatic medium.

Keywords: ATPase, tilapia, metal, osmoregulation, gill, kidney, intestine

Tatlısu Balığı *Oreochromis niloticus*'un Tuzluluğu Artmış Ortamda Kadmiyum Etkisine Kalmasını Takiben Kadmiyum Alınımının ve ATPaz Aktivitelerinin Araştırılması

Özet

İnsan kaynaklı aktiviteler sonucu tatlısuların tuzluluğunu artabilir. Bu nedenle, tatlısu balığı *Oreochromis niloticus* artmış tuzluluklarda (0, 2, 4 ve 8 ppt) 0, 1, 3, 7 ve 14 gün sure ile Cd (1.0 µg/mL) etkisine bırakıldı. Tuzluluk ve Cd'nin ayrı ayrı ve birlikte etkilerine maruz kalan balıkların solungaç, böbrek ve bağırsak dokularında Na^+/K^+-ATPaz, Mg^{2+}-ATPaz, Ca^{2+}-ATPaz aktiviteleri ölçüldü. Genellikle, sadece tuzluluk etkisinde kalan balıkların solungaç ve bağırsaklarında Na^+/K^+-ATPaz aktivitesi artarken böbrekte daha az değişim görülmüştür. Mg^{2+}-ATPaz aktivitesi de bağırsak ve böbrek dokularında önemli oranda değişim göstermiştir. Ca^{2+}-ATPaz aktivitesinin bütün dokularda azalmakla birlikte böbrekte en fazla azaldığı görülmüştür. Tuzluluk+Cd kombinasyonuna maruz kalan balıkların solungaç Na^+/K^+-ATPaz aktivitesi 2 ppt de azalırken, 8 ppt de artmıştır. Bağırsak Na^+/K^+-ATPaz aktivitesi tuzluluk artışına bağlı olarak azalış gösterirken, böbrek Na^+/K^+-ATPaz aktivitesinde önemli bir azalma görülmemiştir. Mg^{2+}-ATPaz ve Ca^{2+}-ATPaz aktiviteleri tuzluluk artışıyla birlikte azalma eğiliminde göstermiştir. Dokularda Cd birikimi tuzluluk artışına bağlı olarak azalmakla birlikte, en uzun sürede tuzluluğa bağlı olmaksızın solungaç Cd düzeyi artmıştır. Balık ATPaz enzimleri sucul ortamdaki değişimlere oldukça hassas olduğundan, sonuçlar çevresel araştırmalarda ATPaz aktivitelilerinin ölçülmesinin gerekliliğini vurgulamıştır.

Anahtar Kelimeler: ATPaz, tilapia, ozmoregülasyon, solungaç, böbrek, bağırsak.

Introduction

Cadmium, a nonessential heavy metal widespread in the environment as a result of anthropogenic activities, can penetrate into aquatic organisms and exerts adverse effects on aquatic species, even human health. Cd toxicity can cause hematological effects, impaired Ca homeostasis, histological and morphological alterations (Monserrat *et al*., 2007; Ezemonye *et al*., 2011) in ion regulating tissues such as the kidney, gills and intestine and also can lead imbalance of ion concentration in

extracellular fluid and alteration of the osmoregulatory capacity of fish (Heath, 1987; Besirovic *et al*., 2010).

Salinity affects the speciation and bioavailability of trace metals, influencing their uptake by aquatic organisms. Thus, it directly controls the amount of incoming metal that potentially altering the ATPase enzymes in osmoregulatory system (Bianchini *et al*., 2002; Monserrat *et al*., 2007). ATPases, membrane-bound enzymes, regulate the cellular volume, osmotic pressure, and membrane permeability due to the transport of ions through biological membranes (Sancho *et al*., 2003; Li *et al*., 2011). Mg^{2+}-ATPase (EC 3.6.3.2) plays an important role in oxidative phosphorylation and ionic transport and is responsible for transepithelial regulation of Mg^{2+} ions (Parvez *et al*., 2006). Ca^{2+}-ATPase (EC 3.6.3.8) is also a significant ATPase, functioning to remove the Ca^{2+} ions from cytoplasm to protect the low Ca^{2+} levels (Watson and Beamish, 1981). The assessment of ATPase activity may therefore be used as an early warning signal of metal-induced damage to the osmoregulatory and acid-based regulatory system in osmoregulatory organs such as gills, kidney and intestine (Stagg *et al*., 1992; Grosell *et al*., 2002; Monteiro *et al*., 2005; Atli and Canli, 2007). Osmoregulation is the ability to actively maintain osmotic concentrations in extracellular fluids, in spite of the osmolarity (salinity) of the surrounding environment. It is a fundamental physiological adaptation of animals living in estuarine environments and salinity increased freshwaters (Heath, 1987; Monserrat *et al*., 2007). Na^+/K^+-ATPase (EC 3.6.3.9) is present in high concentrations in salt transporting tissues like intestine and gills, where it maintains ionic and electrical gradients necessary for transepithelial salt movements (Lionetto *et al*., 2000).

Biomarkers have gained importance for metal toxicity monitoring programs because it can be beneficial in evaluating the physiological condition of aquatic animals before toxic effects occur. Salinity has a profound effect on a fish's osmoregulatory and ionoregulatory physiology and interferes with the metal uptake by changing their availability (Loretz, 1995; Marshall and Grosell, 2005; Blanchard and Grosell, 2006). Thus, the aim of this study is to investigate alterations in response ATPases following salinity and Cd exposures in freshwater fish (*O. niloticus*) and to evaluate their potentials to use as a sensitive biomarker in environmental studies.

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 one month before the experiments. Experimental room was air conditioned (20±1°C) and illuminated for 12 h with fluorescent lamps (daylight 65/80 W). The experiments were carried out in glass aquariums sized 40 × 40 × 100 cm that contained 120 L contaminated test solution or only test water (dechlorinated) for controls. Total hardness and alkalinity of tap water used in the experiments were 307±20.4 mg $CaCO_3$/L, 142±11.8 mg $CaCO_3$/L, respectively.

Experimental design was based on three types of groups: 1. Control groups, 2. Salinity alone exposed groups, 3. Salinity+Cd combination exposed groups. According to this, 1. group was treated with only tap water, 2. group was treated with only 2, 4 and 8 ppt NaCl (Sigma) and 3. group was treated with both salinity and 1 µg Cd/ml ($CdCl_2.H_2O$). Six fish were used for each group and exposure duration (0, 1, 3, 7 and 14 days). As there were no significant differences ($P>0.05$) among controls in different exposure periods, all control (1. group) data were pooled. Mean length (13.2±1.17 cm) and weight (32.2±6.98 g) of fish did not differ significantly ($P>0.05$) among different exposure treatments and controls. The aquaria of each group were cleaned every two days after 1 h feeding period to reduce contamination with food remains and also to minimize metal loss in exposure medium. The exposure media were controlled daily by a multimeter (Thermoscientific Orion 5-Star). Salinity and conductivity of test solutions were; 0.3 ppt-0.60 ±0.02 mS/cm, 2.2 ppt-4.32±0.13 mS/cm, 4.2 ppt-7.84±0.22 mS/cm and 8.3 ppt-14.8±0.68 mS/cm for control, 2 ppt, 4 ppt and 8 ppt, respectively. During the experiments, pH and oxygen were also measured and estimated as; 8.20±0.19 and 5.80±0.97 mg O_2/L, respectively.

At the end of each experimental period, fish were killed by transaction of spinal cord according to the decision of Ethic Committee of Çukurova University and the gill, kidney and intestine were dissected out with clean equipments. Tissues were stored at -80°C until the analyses. Tissues were homogenized in ice-cold buffer containing 20 mM Tris-HCl, 0.25 M Sucrose, and 1 mM EDTA (pH 7.7) with a ratio of 1/10 at 9500 rpm for 2-3 min. Homogenates were centrifuged at 13,000 g (+4°C) for 20 min. The supernatants were collected for determination of total protein levels and ATPase activity.

ATPase Activity Assay

The final assay concentrations and conditions were optimized in our previous research (Atli and Canli, 2008). The final assay concentrations to measure tissue Na^+/K^+-ATPase and Mg^{2+}-ATPase activity were 40 mM Tris-HCl, 120 mM for NaCl, 20 mM for KCl, 3 mM for $MgCl_2$, 7.7 for pH, and 1 mM for ouabain. In addition, incubation media (pH 7.7) containing 40 mM Tris, 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 a Pi standard and spectrophotometric analysis was carried out at 390 nm using a Cecil 5000 series spectrometer. 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 used as a standard.

Cd Analysis

To measure Cd levels, gill and intestine tissue (kidney Cd levels were not measured) were first dried in an oven at 60°C until they reached a constant weight and then they were transferred into glass flasks for digestion process. A perchloric acid and nitric acid (Merck) mixture (1:2) was added to the digestion flasks and the tissue-acid mixtures were put on a hot plate set to 120°C. After complete digestion, the digests were cooled and diluted with distilled water appropriately in the range of standards that were prepared from stock standard solution of the metal (Merck). Metal concentrations in the gills were measured using an AAS (Kalay and Canli, 2000). Accuracy of the AAS and validity of measurements were tested with a reference material (TORT 1 lobster hepatopancreas, National Research Council, Canada). Mean values and standard deviations of the reference material were 10% of the ranges. Cadmium levels in the tap water were below the detection limit (0.001 µg/ml).

Statistical Analysis

Statistical analysis of data was carried out using SPSS statistical package program. As there was no significant differences ($P>0.05$) among control data (1. group) from different exposure periods, all control data were pooled. However, data from the salinity controls (2. group) differed among different exposure periods, so they were treated individually. Thus, each parameter was analyzed separately by One-way Anova to evaluate the changes in each salinity control (2. group) and combine exposures (3. group). Significant differences ($P<0.05$) were reanalyzed by Duncan tests to determine which individual group was significantly different from controls and represented in figures.

Results

Activities of ATPases

In the gill; Na^+/K^+-ATPase activity generally increased following salinity alone exposures and the highest increase (144 %) was detected in fish exposed 4 ppt salinity for 14 days compared to control group (Figure 1a). However, Mg^{2+}-ATPase activity did not differ significantly from the control group ($P>0.05$) (Figure 1b). Ca^{2+}-ATPase activity decreased significantly (80%) following 7 days exposure to 8 ppt salinity (Figure 1c).

Salinity+Cd combination caused both decreases and increases in gill Na^+/K^+-ATPase activity. Highest (140%) and lowest (29%) enzyme activity were measured after 7 days exposure to 4 ppt salinity+Cd combination and 3 days exposure to 8 ppt salinity+Cd combination, respectively (Figure 1a). On the other hand, Mg^{2+}-ATPase and Ca^{2+}-ATPase activities decreased after the exposure to salinity+Cd combinations. The lowest activities were 56% for Mg^{2+}-ATPase after 1 day exposure to 2 ppt salinity+Cd (Figure 1b) and 67 % for Ca^{2+}-ATPase (Figure 1c) after 3 days exposure to 2 ppt salinity+Cd combine exposures.

In the kidney; the activities of Na^+/K^+-ATPase and Mg^{2+}-ATPase increased significantly in salinity alone groups. The highest activity increase was 169% after 7 days exposure to 2 ppt salinity alone and 537% 8 ppt salinity alone exposures (Figure 2a, b). Total inhibition of Ca^{2+}-ATPase activity was observed after the exposure to salinity alone (Figure 2c).

Salinity+Cd combine exposure caused increases in Na^+/K^+-ATPase activity and the highest activity increase was 538% compared to control group (Figure 2a). Mg^{2+}-ATPase and Ca^{2+}-ATPase showed different trends as their activity fluctuated. Significant increase (183 %) and decrease (66%) were measured in Mg^{2+}-ATPase activity after 4 ppt+Cd combination and 8 ppt+Cd combination at day 14 and 7, respectively (Figure 2b). Total inhibition (100%) of Ca^{2+}-ATPase activity was observed following 7 days exposure to 2 ppt+Cd combination, though it caused a significant increase in its activity at day 14 (Figure 2c).

In the intestine; Na^+/K^+-ATPase activity increased at all salinity alone groups and the highest (422%) enzyme activity was measured after 4 ppt salinity exposure at day 14 (Figure 3a). Mg^{2+}-ATPase activity showed both decreases and increases following salinity alone exposures. 2 ppt salinity exposure caused the highest activity (24%) at day 3 while the lowest activity (58%) was observed at day 7 (Figure 3b). Ca^{2+}-ATPase activity decreased following 2 and 4 ppt salinity exposures and the lowest enzyme activity (80%) was measured after 3

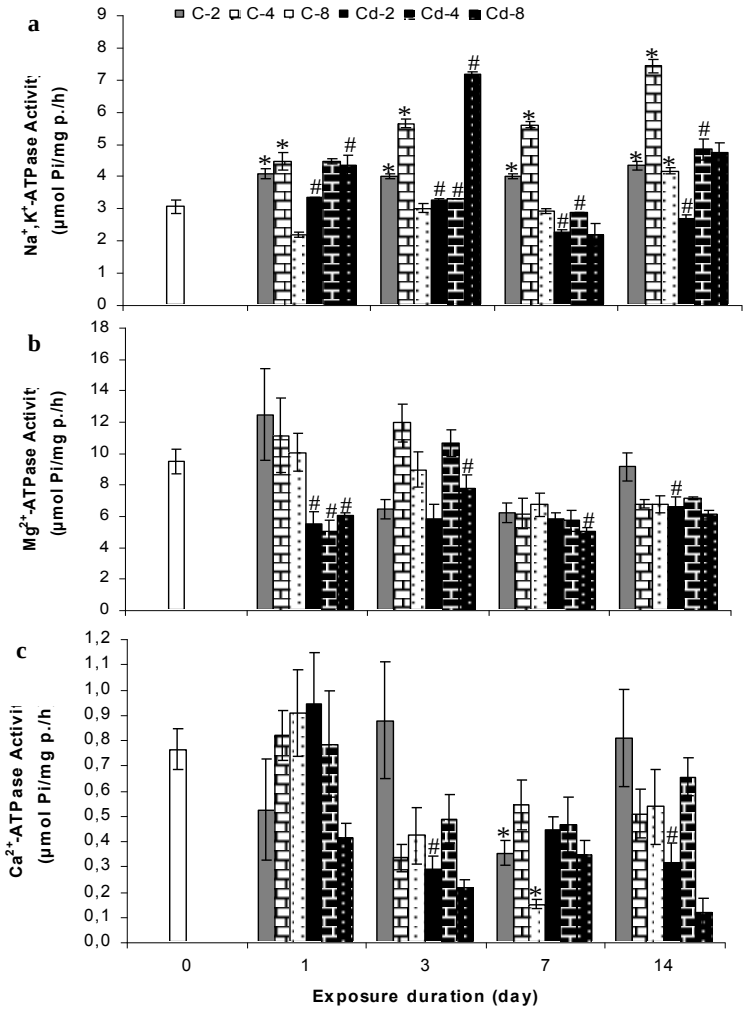

Figure 1. Effects of salinity alone (2, 4 and 8 ppt) and salinity+Cd (1.0 µg Cd/ml) combination exposures on a) Na^+/K^+-ATPase activities, b) Mg^{2+}-ATPase activities and c) Ca^{2+}-ATPase activities in the gill of *O. niloticus*. Data are expressed as mean (n=6)±standard error. * indicate significant (P<0.05) differences resulted from the Duncan tests between control and salinity control treatments and # indicate the differences between salinity control and salinity+Cd combination treatments.

days exposure to 4 ppt salinity (Figure 3c).

Salinity+Cd combine exposure caused significant decreases in Na^+/K^+-ATPase activity and the slowest decrease (88%) was observed following 7 days exposure to 4 ppt salinity+Cd combination (Figure 3a). However, 14 day 4 ppt salinity+Cd combine exposure increased (79%) the Mg^{2+}-ATPase activity while 1 day 8 ppt salinity+Cd combine exposure decreased (43%) its activity (Figure 3b). Ca^{2+}-ATPase activity was totally inhibited (100 %) after 4 ppt salinity+Cd combine exposure at day 1 whereas it significantly increased (85 %) after 14 day exposure (Figure 3c).

A summary table (Table 1) was prepared to see all significant alterations in ATPase activities in salinity alone exposure and combine exposures, indicating significant increases and decreases with up and down arrows, respectively.

Cd Accumulation

Cd levels in the tissues of salinity alone exposed groups were below the detection limits of AAS. There was no Cd accumulation at first day of exposures. Cd accumulation occurred after 3 days combination exposures. Cd accumulation in the gill was negatively correlated with salinity increase up to 7 days, but this correlation was opposite at the longest exposure period (Figure 4a). There were significant Cd accumulations in the intestine and this increase negatively correlated with salinity increase (Figure 4b).

Discussion

No fish mortality occurred following exposure to increased salinities and Cd in the present conditions that accords with our previous studies (Atli and Canli, 2003; Atli and Canli, 2007; Atli and Canli, 2011). Actually, relatively hard nature of the tap water used in the experiments might be one of the reasons for low toxicity as water hardness are known to reduce metal uptake (Heath, 1987; Monserrat *et al*., 2007). Similarly, salinity increase is also known to reduce

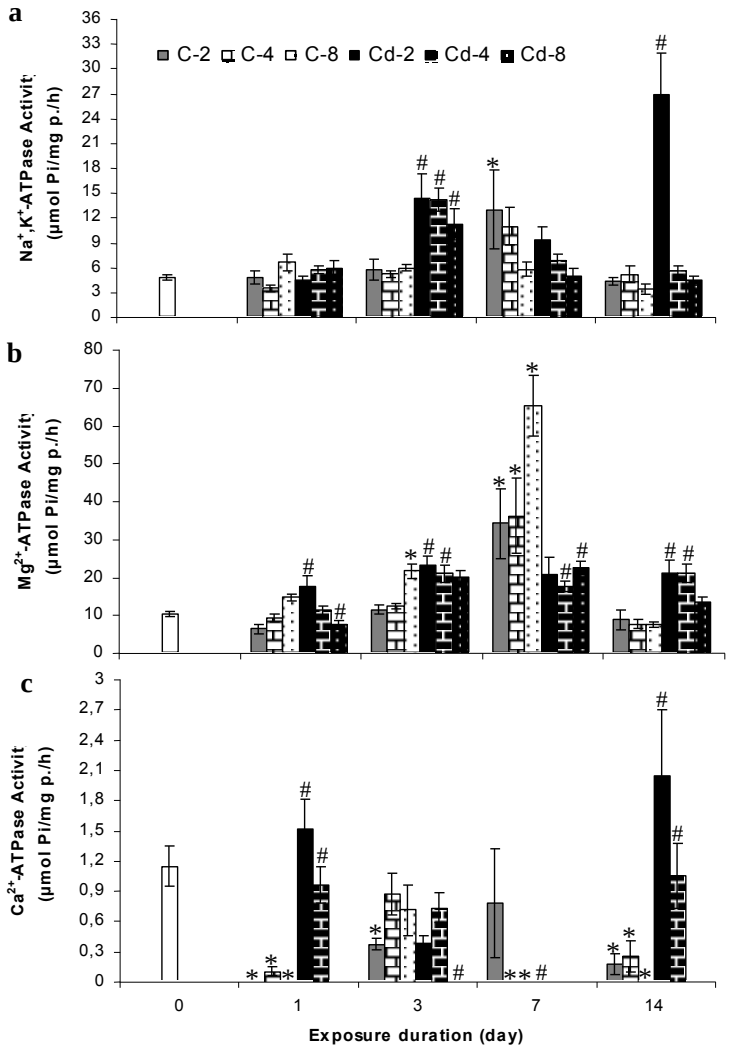

Figure 2. Effects of salinity alone (2, 4 and 8 ppt) and salinity+Cd (1.0 µg Cd/mL) combination exposures on a) Na^+/K^+-ATPase activities, b) Mg^{2+}-ATPase activities, c) Ca^{2+}-ATPase activities in the kidney of *O. niloticus.* See details in Figure 1.

metal uptake (Heath, 1987; Bianchini *et al*., 2002) as occurred in the intestine of fish in the present study. Cd accumulation showed a negative relationship with salinity increase in the intestine. Cd accumulation in the gill also followed the similar trend for exposures up to 7 d, though the opposite was true for 14 d exposure that may be explained as the adsorption of Cd on gill surface for a longer period, uncontrolled Cd influx due to membrane disruption or other changes in fish physiology. It is interesting to note that two epithelia (gill and intestine) are the first interfaces of the organism exposed to the aquatic environment and for this reason a primary target for the action of Cd on fish. Therefore, alterations in their physiological functions could put the survival of the fish at risk. Salinity can alter the bioavailability and speciation and, by consequence, the toxicity of metals depending upon its influence on the metal uptake by fish (Heath, 1987; Bianchini *et al*., 2002) that generally agree with the present study.

Taking into account the significance and potential severity of metal toxicity in aquatic environment, the present study focused mainly on toxicological responses at physiological levels of osmoregulatory mechanisms in freshwater fish. ATPase activities, one of the significant key physiological responses, were analyzed with Cd and ion concentrations due to the importance of salinity as a changing variable in waters. Data indicated stimulation trend for Na^+/K^+-ATPase and inhibitory trend for Ca^{2+}-ATPase after salinity alone exposures. However, this was not true for Mg^{2+}-ATPase as it did not show clear trend towards either way. These responses of ATPases could be related to the changes in ion levels and the adverse effects of Cd exposure as results of Cd binding on enzyme molecules (Canli and Stagg, 1996; McGeer *et al*., 2000; Haque *et al*., 2011). Likewise, variable responses of ATPases following salinity+Cd combine exposures were also in accordance with the alterations in ion levels. The present data showed that Na^+/K^+-ATPase was the most affected ATPase by both salinity and Cd exposures and the gill was the most influenced tissue. This could be due its localization as the first target

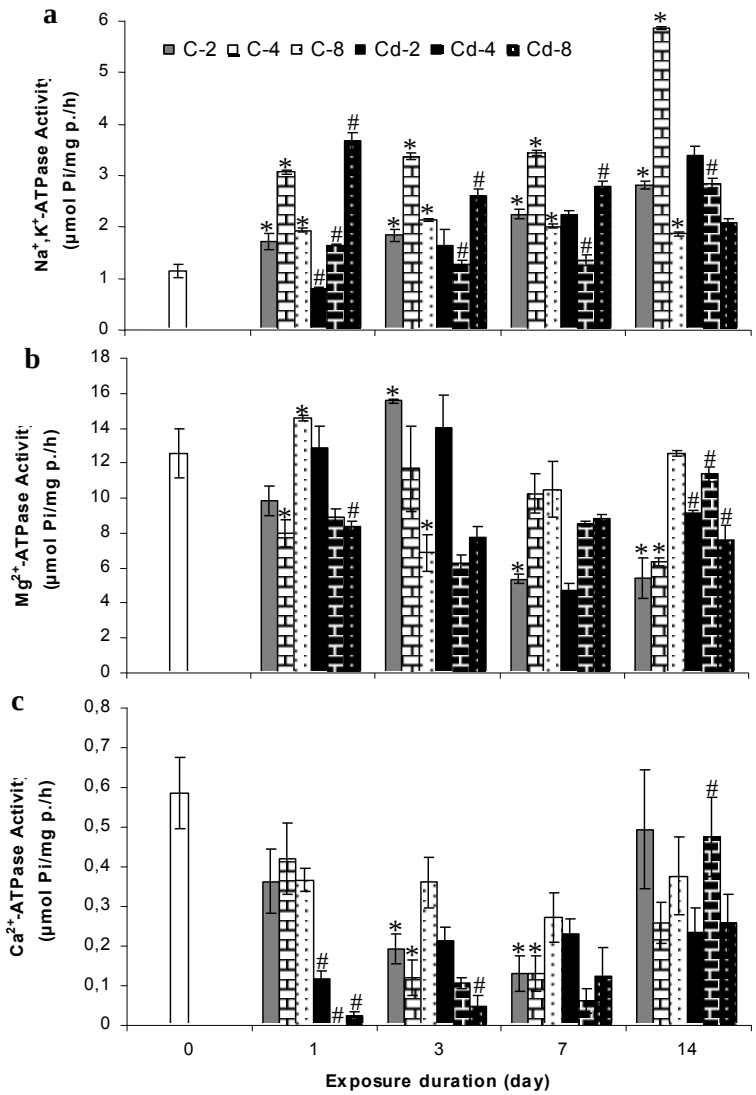

Figure 3. Effects of salinity alone (2, 4 and 8 ppt) and salinity+Cd (1.0 µg Cd/mL) combination exposures on a) Na^+/K^+-ATPase activities, b) Mg^{2+}-ATPase activities, c) Ca^{2+}-ATPase activities in the intestine of *O. niloticus*. See details in Figure 1.

organ for metals in water and its key role in osmoregulation. Although ATPase activities generally decreased after salinity+Cd combine exposures, there were also increases in the activity especially in the kidney, indicating its detoxification role. On the other hand, Cd exposure provoked Mg^{2+}-ATPase activity which is related to the transport of Mg across the gill epithelium.

Na^+/K^+-ATPase is present in high concentrations in salt transporting tissues like intestine and gills, where it maintains ionic and electrical gradients necessary for transepithelial salt movements (Lionetto *et al*., 2000; Niyogi *et al*., 2008). Euryhaline teleosts are able to osmoregulate across a broad spectrum of salinities. Acclimation to changing environmental salinity requires pre-existing mechanisms to respond to altering conditions such as activation of gill Na^+/K^+-ATPases, crucial for the fish acclimation (Heath, 1987; Wood, 2012). It is known that Cd inhibits enzymes involved in osmoregulatory ion transport particularly ATPases (Watson and Benson, 1987; Pratap and Wendelaar Bonga, 1993; Lionetto *et al*., 2000, De la Torre *et al*., 2007; Atli and Canli 2007). The present data agree with previous studies, as toxic effects of Cd on fish ATPases differ from species to species, and from organ to organ with also duration of exposure (De la Torre *et al*., 2000; Atli and Canli, 2007). In the gills of juvenile *Cyprinus carpio* inhibited Na^+/K^+-ATPase activity did not recover in animals kept in Cd-free media, though stimulated Mg^{2+}-ATPase after Cd exposure return to the normal in Cd-free media (Kramer *et al*., 1986; Carfagna *et al*., 1996; Atli and Canli, 2011). In the shark rectal gland Mg^{2+}-ATPases remained unaffected by in vitro cadmium treatment (Kinne-Saffran *et al*., 1986). Similarly, there was no change in Mg^{2+}-ATPase activities in the gills of bluegill sunfish, fathead minnows and golden shiners after in vivo exposure to heavy metals (Watson and Benson, 1987), supporting the present work. It can be emphasized that one consequence of Cd exposure was an important disturbance in the ion-based

Table 1. A summary of alterations of ATPase activities in the tissues of *O. niloticus*. Significant increases and decreases were shown with up and down arrows respectively

ATPase	Salinity	Day	Salinity Alone Exposure			Combine Exposures		
			Gill	Kidney	Intestine	Gill	Kidney	Intestine
Na^+/K^+-ATPase	2	1	↑	–	↑	↓	–	↓
		3	↑	–	↑	↓	↑	–
		7	↑	↑	↑	↓	–	–
		14	↑	–	↑	↓	↑	–
	4	1	↑	–	↑	–	–	↓
		3	↑	–	↑	↓	↑	↓
		7	↑	–	↑	↓	–	↓
		14	↑	–	↑	↑	–	↓
	8	1	–	–	↑	↑	–	↑
		3	–	–	↑	↑	↑	↑
		7	–	–	↑	–	–	↑
		14	↑	–	↑	–	–	–
Mg^{2+}-ATPase	2	1	–	–	–	↓	↑	–
		3	–	–	↑	–	↑	–
		7	–	↑	↓	–	–	–
		14	–	–	↓	↓	↑	↑
	4	1	–	–	↓	↓	–	–
		3	–	–	–	–	↑	–
		7	–	↑	–	–	↓	–
		14	–	–	↓	–	↑	↑
	8	1	–	–	↑	↓	↓	↓
		3	–	↑	↓	↓	–	–
		7	–	↑	–	↓	↓	–
		14	–	–	–	–	–	↓
Ca^{2+}-ATPase	2	1	–	↓	–	–	↑	↓
		3	–	↓	↓	↓	–	–
		7	↓	–	↓	–	↓	–
		14	–	↓	–	↓	↑	–
	4	1	–	↓	–	–	↑	↓
		3	–	–	↓	–	–	–
		7	–	↓	↓	–	–	–
		14	–	↓	–	–	↑	↑
	8	1	–	↓	–	–	–	↓
		3	–	–	–	–	↓	↓
		7	↓	↓	–	–	–	–
		14	–	↓	–	–	–	–

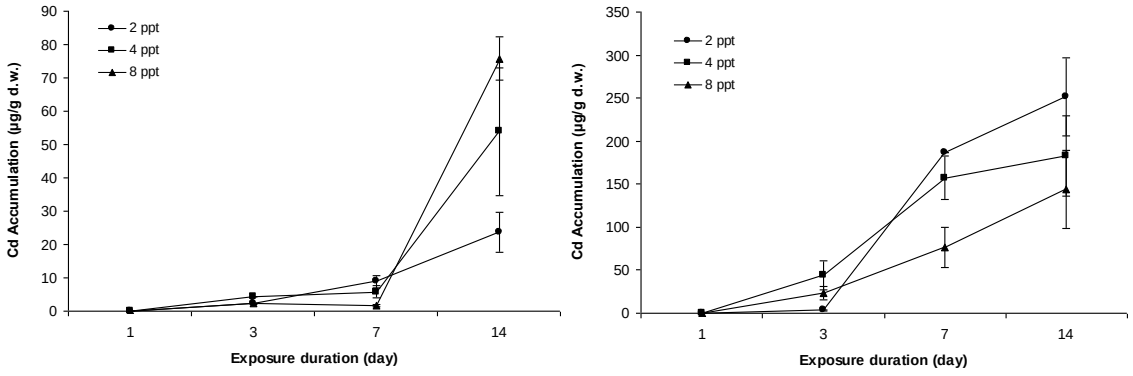

Figure 4. Effects of salinity+Cd combination exposures on Cd accumulation a) in the gill and b) in the intestine of *O. niloticus*. See details in Figure 1.

osmoregulation of fish. Ca^{2+}-ATPase activity decreased generally following salinity alone exposures though several increases were observed after salinity and metal combine exposures. Decrease of this enzyme may result from the breakdown of the active transport mechanism and also due to the disturbed Ca^{2+} homeostasis (Verbost *et al*., 1988). Altered Ca^{2+} levels due to the metal exposures (Atli and Canli, 2011) supported the decline of Ca^{2+}-ATPase activity in this research. Verbost *et al*. (1987) indicated the inhibition of Ca influx due to the Ca transport inhibition after the critical Cd concentration

occurred in the gills of rainbow trout *Salmo gairdneri*. Thaker *et al*. (1996) indicated that inhibition of Ca^{2+}-ATPase activity in the gill, kidney and intestine of *Periophthalmus dipes* exposed to Cr^{6+} was related to the blocked active transport system by Cr^{6+} and thus decreased enzyme activity was observed due to the affected osmoregulatory mechanism. Wong and Wong (2000) showed that the Ca^{2+}-ATPase activity in the gill of *Oreochromis mossambicus* decreased in relation to Cd^{2+} exposures. They concluded that the Cd^{2+} accumulation in gill tissue led to an inhibition of Ca^{2+}-transport by blocking the activity of Ca^{2+}-ATPase. In addition increase in the enzyme activity could be linked with the salinity which prevents the metal bioavailability.

The heavy metal can alter the activity of enzymes either by binding to their functional groups such as sulphidryl, carboxyl and imidazol or by displacing the metal associated with the enzyme (Viarengo, 1985). ATPase enzymes are –SH rich enzyme which play a pivotal role in teleost intestine and gill physiological functions such as salt- and osmoregulation and acid–base balance. Moreover, salt absorption in the intestine and excretion of the excess salt in the gills, which represent the main osmoregulatory processes in teleosts require a fully functional ATPase enzymes. Variable responses might contribute to the diversity observed in the inhibitory mechanism.

In conclusion, data indicated the variability of ATPase responses in the osmoregulatory tissues, due to the type of stress factors, tissues and exposure durations providing a valuable data for biomonitoring the Cd toxicity on fish metabolism, especially in freshwater with increased salinities. Data also emphasized that salinity as an environmental factor should be taken into account during ecotoxicological investigations. Nevertheless, results suggested the necessities of further data are required to estimate the mechanisms of other physico-chemical properties of water on osmoregulation systems of fish. In light of the above, it could be suggested that metal-induced inhibitory effects on osmoregulatory enzymes would be useful as exposure/effect biomarkers in fish, including those living in estuarine areas (Monserrat *et al*., 2007).

Acknowledgement

This study supported by The Scientific and Technical Research Council of Turkey (TUBITAK) with the project number of 108T269 and Research Grant (FEF2008YL15) to Berna Kulaç from Çukurova University.

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