Essential metal (Cu, Zn) exposures alter the activity of ATPases in gill, kidney and muscle of tilapia *Oreochromis niloticus*

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Abstract An acute (96 h—0.1, 0.5, 1.0, 1.5 µg/ml) and chronic (up to 30 days—0.05 µg/ml) protocols of Cu and Zn were applied to freshwater fish *Oreochromis niloticus* to investigate these essential metal effects on the activities of gill, kidney and muscle Na^+/K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase. In vitro effects of both metals (20 min—0.1, 0.5, 1.0, 1.5 µg/ml) were also measured to be able to compare both exposure routes. Data showed that ATPase activities, in general, decreased following all the exposure conditions, though there were some increases especially in Mg^{2+}-ATPase activity. Among the enzymes, Na^+/K^+-ATPase and Ca^{2+}-ATPase appeared to be more sensitive than Mg^{2+}-ATPase to the metals. The data also indicated that effects of Cu on ATPase activity in the tissues of *O. niloticus* were stronger than the effects of Zn, possibly due to higher toxic effects of Cu. In vivo and in vitro exposures of metals showed similar trends with a few exceptions, especially in the gill. Variability of ATPase activity is determined by tissue type, metal species, concentration and duration. This work showed that even essential metals can alter significantly activities of ATPases in fish and thus suggests using them as a sensitive biomarker in metal contaminated waters.

Keywords Acute · ATPase · Chronic · Copper · In vitro · In vivo · *Oreochromis niloticus* · Zinc

Introduction

Aquatic organisms are generally exposed to chronic metal contamination in the field though they may also suffer acute exposures in areas where industrial effluents are discharged. Chronic and acute stresses present different ecological challenges. Organisms are likely to endure specific molecular, biochemical, physiological or morphological responses under each of these conditions. Copper (Cu) and Zinc (Zn), essential metals for fish metabolism and function of a variety of proteins and enzymes, can exert adverse toxicological effects when present in excess amounts (Heath 1987; Schlenk and Benson 2001).

ATPases are the membrane-bound enzymes responsible for the transport of ions through biological membranes and thus regulate, e.g., cellular volume, osmotic pressure, and membrane permeability (Sancho et al. 2003). The key mechanism of metal toxicity has been reported to be an osmoregulatory impairment associated with ATPase inhibition in the osmoregulatory tissues such as gill and kidney (McGeer and Wood 1998). It has been suggested that the assessment of ATPase activity may therefore be used as an early warning signal of pollutant-induced damage to the osmoregulatory and acid-based regulatory system in gills (Stagg et al. 1992). Considerable alterations including both inhibition and stimulation of ATPase activities in fish were shown in literature (Watson and Benson 1987; Ay et al. 1999, Grosell et al. 2003; Atli and Canli 2007).

Cu is a cofactor for a number of enzymes including cytochrome C oxidase and is thus essential for respiration in all eukaryote cells. The redox nature of copper is also what makes this element a potent toxicant. Aquatic organisms can take up copper directly from the water and elevated ambient copper concentrations can lead to excess copper accumulation in several tissues (Lauren and

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McDonald 1987; Kamunde et al. 2002). Oxidative stress is generally accepted as one of the major effects of excessive cellular Cu concentrations but other effects have been reported in osmoregulatory organs of aquatic organisms as well (Sanchez et al. 2005; Atli and Canli 2010). Like Cu, Zn is both a micronutrient involved as a cofactor in several enzymes such as oxidoreductase and transferases, and toxicant which is taken up by an active saturable transport system (Schlenk and Benson 2001). It was suggested that Zn inhibited both Ca^{2+}-ATPase activity through competition with Ca for the Ca-selective channel and Na^+/K^+-ATPase, followed by ion distruption (Watson and Beamish 1980; Heath 1987).

The Nile tilapia *Oreochromis niloticus* is recommended as a bioindicator species for monitoring both in the field and laboratory as it is well known to resist environmental stress (Almeida et al. 2002). Data from literature have showed that fish ATPases are sensitive to metal exposures, though there are conflicting results about the inhibitory and stimulatory effects of metals. The aim of this study was to determine the effects of Cu and Zn exposures on ATPases from the gill, kidney and muscle of *O. niloticus*, and to evaluate whether they could be used as a sensitive biomarker in metal exposures.

Materials and methods

Experimental protocol

Freshwater fish *O. niloticus* have been cultured in Cukurova University (Turkey) for more than 25 years. Fish (2 years old) were taken from the culture pools and transferred to the laboratory where they were acclimatized in experimental aquaria for 1 month before the experiments. The experimental room was air conditioned ($20 \pm 1°C$) and illuminated for 12 h with fluorescent lamps (daylight 65/80 W). The experiments were carried out in glass aquariums sized $40 \times 40 \times 100$ cm that contained 130 l metal contaminated dechlorinated tap water or only dechlorinated tap water for controls. During the experiments, pH and oxygen levels were estimated as 8.32 ± 0.08 and 5.96 ± 0.44 mg O_2/l, respectively (Orion 5 Star multimeter). Total hardness (with EDTA titration method) and alkalinity (acidimetry method) were measured as 340 ± 29 mg $CaCO_3$/l, 248.6 ± 13.1 mg $CaCO_3$/l, respectively ($n = 20$). Ion levels in aquariums were also measured using a flame atomic absorption spectrophotometer (AAS, Perkin Elmer AS 3100) and estimated as: Na^+: 0.73 ± 0.12 µg/ml, K^+: 0.42 ± 0.07 µg/ml, Mg^{2+}: 0.44 ± 0.02 µg/ml, Ca^{2+}: 13.15 ± 0.82 µg/ml, Cl^-: 28.30 ± 1.20 µg/ml. The detection limits of Na^+, K^+, Mg^{2+} and Ca^{2+} were 0.002, 0.015, 0.0001 and 0.002 µg/ml, respectively.

Fish were exposed to 0, 0.1, 0.5, 1.0 and 1.5 µg/ml concentrations of Cu ($CuCl_2 \cdot 2H_2O$) and Zn ($ZnCl_2$) for 96 h for acute experiments and 0.05 µg/ml concentration of these metals for 0, 5, 10, 20 and 30 days for chronic experiments. A total of six fish were used for each experimental period of acute and chronic metal exposures. As there were no significant differences ($P > 0.05$) among controls in different exposure periods, all control data were pooled. Mean length (15.7 ± 1.21 cm) and weight (61.5 ± 12.8 g) of fish did not differ significantly ($P > 0.05$) among different exposure treatments and controls. The aquaria of controls and metal exposed groups were cleaned every 2 days after 1 h feeding (Pinar Sazan, Izmir, Turkey) to clean food remains and also to minimize metal loss in the exposure medium (Atli 2010). Metal concentrations in exposure medium were controlled using Atomic Absorption Spectrophotometer (Perkin Elmer 3300). Metal levels in the tap water were below the detection limits.

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 gill, kidney and muscle tissues were dissected. Tissues were stored at $-80°C$ until the ATPase 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 9,500 rpm for 2–3 min. Homogenates were centrifuged at $13,000 \times g$ ($+4°C$) for 20 min. The supernatants were collected for determination of total protein levels and ATPase activity.

ATPase activity assay

The final assay concentrations to measure tissue Na^+/K^+-ATPase and Mg^{2+}-ATPase activity were 40 mM Tris–HCl, 120 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 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 ($\sim$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 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 oua-bain. 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 and ATPase activities were calculated as µmol Pi/mg p/h using the standard equation of $y = 0.0012\times$. Total protein levels were determined according to Lowry et al. (1951) and bovine serum albumin was used as a standard.

An in vitro experiment was also designed to test the direct effects of metal ions to ATPases. For this protocol, supernatants from control group containing ATPases were exposed to metals for 20 min and incubation medium was adjusted to the level of acute exposure concentrations (0.1, 0.5, 1.0 and 1.5 µg/ml) of metals. This was followed by the measurement of ATPase activities in metal exposed supernatants.

Statistical analysis

Statistical analysis of data was carried out using SPSS statistical package programs. One-way ANOVA was used to compare variables among controls and treatments. Significant differences ($P < 0.05$) were reanalyzed by Duncan tests to determine which individual groups were significantly different from the control. Data were given as mean ± SE.

Results

In acute series; gill Na^+/K^+-ATPase activity was significantly decreased by Cu and Zn exposures and the lowest enzyme activity (0.86 ± 0.15 µmol Pi mg p/h) was determined after the highest Cu exposure concentration (Fig. 1a). Despite the decrease of gill Mg^{2+}-ATPase activity after all Zn exposures, enzyme activity was not affected by Cu exposure concentrations ($P > 0.05$). The lowest Mg^{2+}-ATPase activity (1.45 ± 0.10 µmol Pi mg p/h) was measured following 0.1 µg Zn/ml exposure (Fig. 1a). All concentrations of both Cu and Zn decreased the gill Ca^{2+}-ATPase activity significantly and 1.0 µg Cu/ml lead to the highest inhibition (0.05 ± 0.03 µmol Pi mg p/h) (Fig. 2a). Kidney Na^+/K^+-ATPase activity also decreased after all metal exposures beside an increase (5.70 ± 0.62 µmol Pi mg p/h) after 1.0 µg Cu/ml. On the other hand, the lowest value was measured as 0.29 ± 0.02 µmol Pi mg p/h after 0.5 µg Cu/ml (Fig. 1b). Mg^{2+}-ATPase activity was increased after Cu exposure and the highest enzyme activity (5.58 ± 0.46 µmol Pi mg p/h) was observed after 0.5 µg Cu/ml exposure in the kidney (Fig. 1b). Similarly with gill tissue Ca^{2+}-ATPase activity was decreased by all metal concentrations in the kidney and the 0.5 µg Cu/ml caused the highest inhibition

(0.06 ± 0.02 µmol Pi mg p/h) (Fig. 2b). In addition muscle Ca^{2+}-ATPase activity also decreased by Cu and Zn exposures whereas 1.0 µg Zn/ml lead to the lowest enzyme activity (0.15 ± 0.07 µmol Pi mg p/h) in this tissue (Fig. 2c).

In chronic series; all metal exposures decreased the Na^+/K^+-ATPase activity and the lowest enzyme activity was detected as 0.12 ± 0.0 µmol Pi mg p/h after 20 day Zn exposure in the gill (Fig. 3a). Nevertheless, gill Mg^{2+}-ATPase activity was less affected by Cu and Zn exposures than the Na^+/K^+-ATPase activity. Beside the decrease of the enzyme activity, the highest activity (24.3 ± 1.26 µmol Pi mg p/h) was observed after the 30 day Cu exposure and the lowest enzyme activity was determined after 10 day Zn exposure as 13.7 ± 0.74 µmol Pi/mg p/h (Fig. 3a). A general decrease was also recorded in gill Ca^{2+}-ATPase activity whereas the lowest Zn exposure lead an increase in its activity (5.07 ± 0.25 µmol Pi mg p/h). On the other hand, 10 day Cu exposure caused the maximal decrease of Ca^{2+}-ATPase activity in this tissue (0.94 ± 0.15 µmol Pi mg p/h) (Fig. 4a). Kidney Na^+/K^+-ATPase activity decreased during Cu and Zn exposures and the highest inhibition was measured as 1.0 ± 0.16 µmol Pi mg p/h after 20 day Cu exposure (Fig. 3b). However, kidney Mg^{2+}-ATPase activity was increased by Cu and Zn exposures and the highest enzyme activity was determined as 17.7 ± 1.17 µmol Pi/mg p/h (Fig. 3b). Ca^{2+}-ATPase activity decreased after all metal exposures and the enzyme activity was totally inhibited after the lowest Cu exposure (Fig. 4b). Similarly, muscle Ca^{2+}-ATPase activity was also decreased after these metal exposures and 20 day Cu exposure caused the lowest value in enzyme activity (0.77 ± 0.09 µmol Pi mg p/h) (Fig. 4c).

In vitro metal effects indicated that Na^+/K^+-ATPase (Fig. 5a, b) and Ca^{2+}-ATPase (Fig. 5c) activity was decreased by all metal exposures and the particularly 30 day Cu exposure caused a total inhibition of this enzyme in all tissues. Nevertheless, Mg^{2+}-ATPase activity showed increases and the highest enzyme activity was measured as 15.5 ± 0.12 µmol Pi mg p/h in the gill (Fig. 5a) and 9.07 ± 0.32 µmol Pi mg p/h in the kidney (Fig. 5b) after 1.5 µg Zn/ml and 1.5 µg Cu/ml exposures, respectively.

Discussion

Response of ATPases to acute and chronic metal exposures varied according to the exposure types, concentrations, durations and also tissue types. Data showed that ATPase activities generally decreased in all tissues and all exposures, despite a few increases as summarized in Table 1. The decrease of the enzyme activity could be related to the

Fig. 1 Effects of acute metal exposures on ATPase activities in the gill (**a**) and kidney (**b**) tissues of *O. niloticus*. Data are expressed as mean ($n = 6$) ± SD. * Significant ($P < 0.05$) differences resulted from the Duncan tests between control and individual treatments
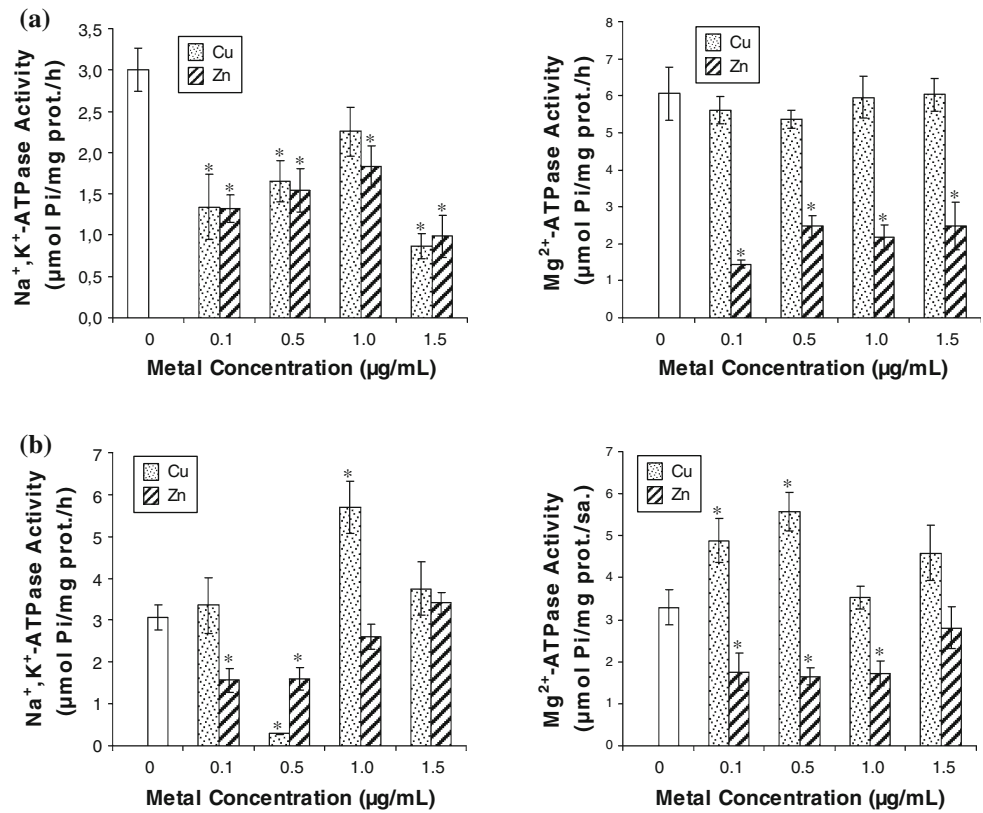

Fig. 2 Effects of acute metal exposures on Ca^{2+}-ATPase activities in the gill (**a**), kidney (**b**) and muscle (**c**) of *O. niloticus*, see details in Fig. 1
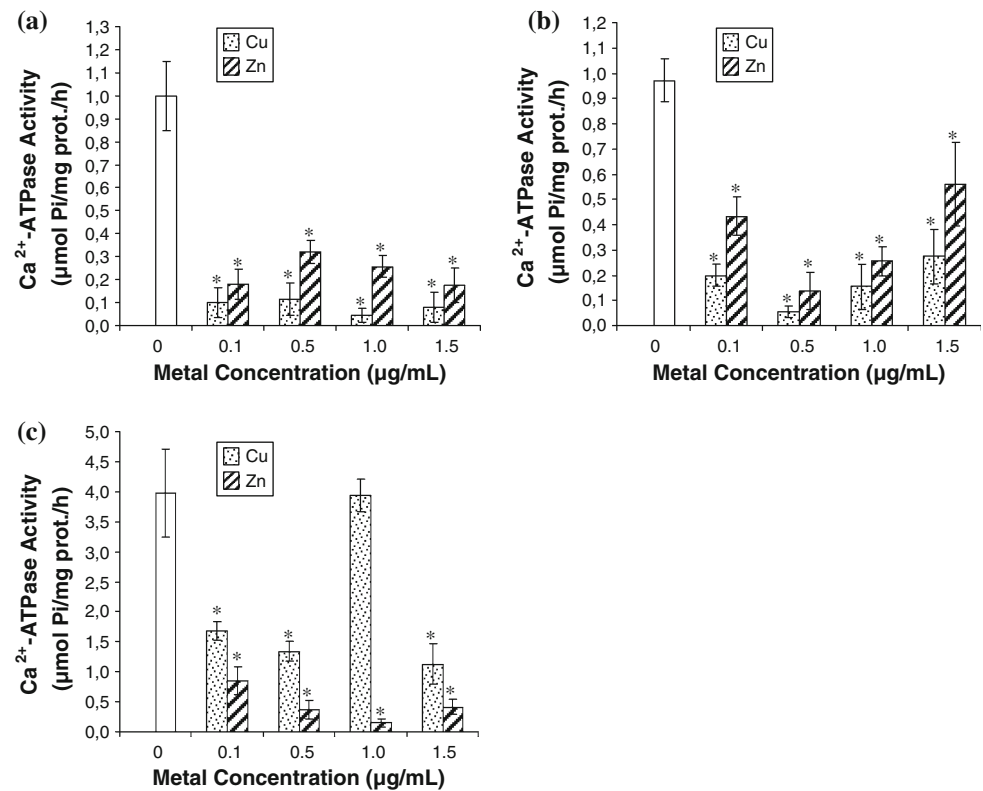
Fig. 3 Effects of chronic metal exposures on ATPase activities in the gill (**a**) and kidney (**b**) tissues of *O. niloticus*, see details in Fig. 1
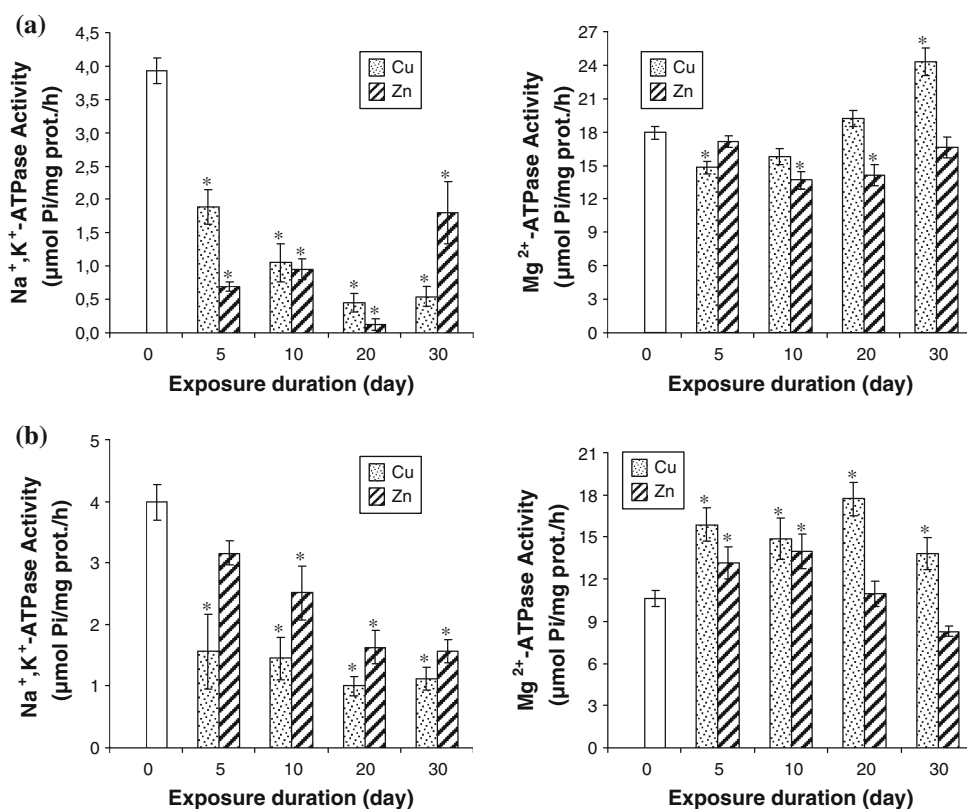

Fig. 4 Effects of chronic metal exposures on Ca^{2+}-ATPase activities in the gill (**a**), kidney (**b**) and muscle (**c**) of *O. niloticus*, see details in Fig. 1
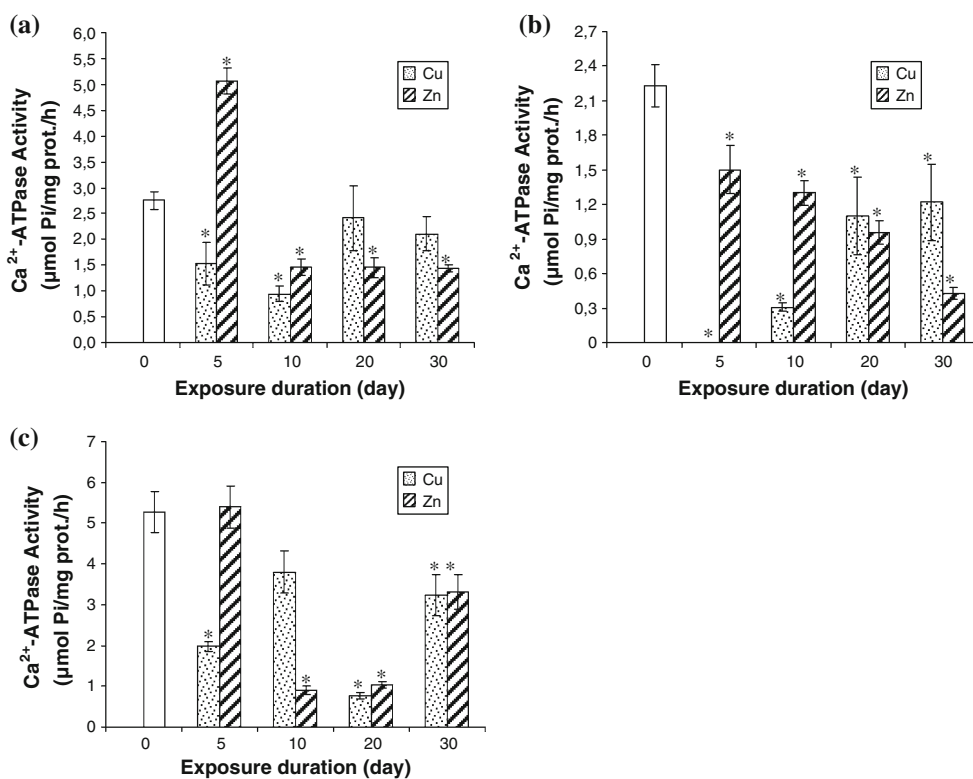
high affinity of metals to -SH groups on the enzyme molecule, membrane rupture or disturbance of the ion homeostasis, while increases in ATPase activity could possibly occur due to an adaptation period dependent upon the continuing metal effect or maintenance of the ion flux. The present data are in accordance with previous studies

Fig. 5 Effects of metal in vitro on Na$^+$,K$^+$-ATPase and Mg^{2+}-ATPase activities in the gill (**a**) and kidney (**b**) and Ca^{2+}-ATPase activity in the muscle (**c**) of *O. niloticus*, see details in Fig. 1
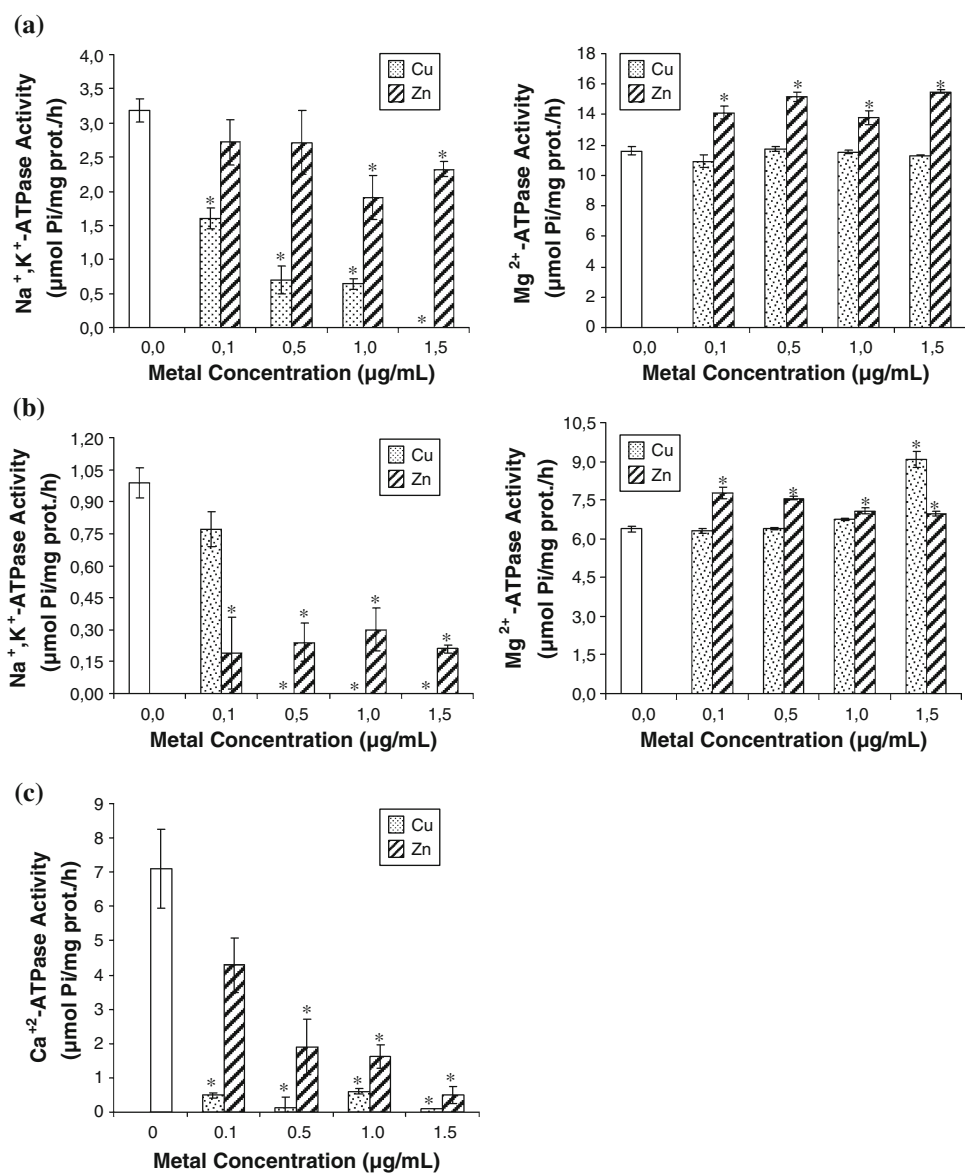
(Larsson et al. 1985; Canli and Stagg 1996; Ay et al. 1999; Grosell et al. 2003; Atli and Canli 2007).

Previous work from the present laboratory have showed that both essential and non-essential metals accumulate in tissues of *O. niloticus* in relation to exposure concentration and period (Eroglu et al. 2005; Atli and Canli 2003; Atli and Canli 2008; Oner et al. 2009). Metal concentrations used in the present study are relevant for polluted waters. Cu is a common aquatic contaminant varying from 1 μg/ml in unpolluted sites to between 32 and 505 μg/ml within polluted waterways. Natural background levels of Zn are usually <0.1–50 μg/ml in freshwater and in anthropogenically contaminated places, Zn levels of up to 4 μg/ml in water (WHO 2001). Recently, we also studied the effects of water hardness on metal uptake in tissues of *O. niloticus* and found that metal uptake is significantly higher in soft

water compared to hard water (unpublished data). There are also data in the literature indicating water hardness affect metal uptake by fish, namely metal uptake is higher in soft water (Pratap and Wendelaar Bonga 1993; Hollis et al. 2000; Ebrahimpour et al. 2010). The tap water used in the present study is considerably hard (340 ± 29 mg CaCO$_3$/l), thus the effects observed in the present study might be much higher if softer water was used in the exposure medium.

Studies have shown that Cu can enter the cells through Na-sensitive pathways and can cause toxicity by inhibition Na$^+$/K$^+$-ATPase in gill tissue with consequential whole-body ionoregulatory disturbance (Morgan et al. 1997; Handy et al. 2002; Grosell et al. 2004). Although there were inhibitions in ATPase activity following Cu exposure, Mg^{2+}-ATPase activity especially in the kidney, was

Table 1 A summary of ATPase activities in the tissues of *O. niloticus* following Cu and Zn exposures. Significant increase, decrease and statistically not significant ATPase activity were indicated by symbols as "↑", "↓" and "−", respectively

Metal	µg/ml (acute)	In vivo							In vitro				
		Na$^+$, K$^+$–ATPase		Mg^{2+}-ATPase		Ca^{2+}-ATPase			Na$^+$, K$^+$-ATPase		Mg^{2+}-ATPase		Ca^{2+}-ATPase
		Gill	Kidney	Gill	Kidney	Gill	Kidney	Muscle	Gill	Kidney	Gill	Kidney	Muscle
Cu^{2+}	0.1	↓	−	−	↑	↓	↓	↓	↓	−	−	−	↓
	0.5	↓	↓	−	↑	↓	↓	↓	↓	↓	−	−	↓
	1.0	−	↑	−	−	↓	↓	−	↓	↓	−	−	↓
	1.5	↓	−	−	−	↓	↓	↓	↓	↓	−	↑	↓
Zn^{2+}	0.1	↓	↓	↓	↓	↓	↓	↓	−	↓	↑	↑	−
	0.5	↓	↓	↓	↓	↓	↓	↓	−	↓	↑	↑	↓
	1.0	↓	−	↓	↓	↓	↓	↓	↓	↓	↑	↑	↓
	1.5	↓	−	↓	−	↓	↓	↓	↓	↓	↑	↑	↓
Metal	Day (chronic)	Na$^+$, K$^+$-ATPase		Mg^{2+}-ATPase		Ca^{2+}-ATPase							
		Gill	Kidney	Gill	Kidney	Gill	Kidney	Muscle					
Cu^{2+}	5	↓	↓	↓	↑	↓	↓	↓					
	10	↓	↓	−	↑	↓	↓	−					
	20	↓	↓	−	↑	−	↓	↓					
	30	↓	↓	↑	↑	−	↓	↓					
Zn^{2+}	5	↓	−	−	↑	↑	↓	−					
	10	↓	↓	↓	↑	↓	↓	↓					
	20	↓	↓	↓	−	↓	↓	↓					
	30	↓	↓	−	−	↓	↓	↓					

stimulated. This could be an indication of the detoxifying role of the kidney. In such cases, it seems likely that homeostatic control mechanisms could be activated during metal exposures (McGeer et al. 2000; Grosell et al. 2004). Osmoregulatory disturbances induced by metals were associated with an increased epithelial permeability and inhibition of active ion uptake, subsequently to reduction of Na$^+$/K$^+$-ATPase activity and a decrease in the number of active chloride cells (Lauren and McDonald 1987; Monserrat et al. 2007; Atli and Canli 2007; Eyckmans et al. 2011). Differences in the response of ATPases among different exposure protocols are also common in the literature. For instance, although acute laboratory exposure to Cu decreased gill Na$^+$/K$^+$-ATPase activity in the carp and rainbow trout (Lauren and McDonald 1987; De Boeck et al. 2001), chronic exposures led to either a return to normal activity of the gill Na$^+$/K$^+$-ATPase (Lauren and McDonald 1987) or an increase (McGeer et al. 2000). Nevertheless, both Cu and Zn decreased ATPase activity even at shorter exposure periods in the present study. The differences among different studies might be due to the differences in species, water quality and exposure conditions.

Gill tissue represents a thin and extensive surface (up to 90% of the total body surface) in intimate contact with water. Due to the constant contact with the external environment, gills are the first target of waterborne pollutants and the main place for waterborne metal uptake (Heath 1987). Although fish gill play a quantitatively larger role for ionic regulation, kidney is the primary organ for elimination of water. Kidney is particularly important for freshwater species due to the efficient ion reabsorption mechanisms to minimize the loss of ion in this tissue. Therefore, the decrease of Na$^+$/K$^+$-ATPase activity in the gill and increase in Mg^{2+}-ATPase activity in the kidney may be attributed to the specialized structure of these tissues based on their different role in fish.

Ca^{2+}-ATPase was significantly reduced during all the acute and chronic, Cu and Zn exposures. This may result from the breakdown of the active transport mechanism depending upon the altered membrane permeability and also due to the disturbed Ca homeostasis. Significant inhibition of Ca^{2+}-ATPase in the gill of rainbow trout after in vitro Zn exposure (Hogstrand et al. 1996) also supported the present data. It was indicated that a decrease in Ca^{2+}-ATPase activity was shown after metal exposures possibly resulted from an inhibition of Ca transport (Wong and Wong 2000; Rogers and Wood 2004). In our previous, we demonstrated that the significant inhibition of muscle Ca^{2+}-ATPase activity in *O. niloticus* after Cu, Cd, Zn and Pb exposures (Atli and Canli 2007) was also in accordance with this data. Inhibition of Ca^{2+}-ATPase activity could be

related to the blocked active transport system by metals and followed by disturbed osmoregulatory mechanisms.

Mg^{2+}-ATPase enzyme is found in association with Na^+/ K^+-ATPase in fish, related to the transport of Mg across the gill epithelium, and is also essential for the integrity of the cellular membrane and for the stabilization of branchial permeability (Reddy et al. 1991). Significant increases in Mg^{2+}-ATPase activity were observed especially in the kidney particularly after chronic metal exposures. Recovery in enzyme activity may possibly occur by increasing the number of enzyme molecules and/or increasing the turnover rates of the enzyme present in order to compensate for the activity of lost enzymes. De la Torre et al. (2000) found that branchial ATPase activities in *Cyprinus carpio* were impaired in a dissimilar way after chronic Cd exposure: Na^+/K^+-ATPase was inhibited by approximately 30%, while Mg^{2+}-ATPase was significantly activated by 70%. These results relate to our present study. Lower sensitivity or insensitivity of Mg^{2+}-ATPase to metals comparing to Na^+/K^+-ATPase are also recorded in literature (Watson and Benson 1987; Lemaire-Gony and Mayer-Gostan 1994; Lionetto et al. 2000).

In vivo and in vitro exposures of Cu and Zn showed similar alterations in ATPase activities as they generally decreased the enzyme activities. However, there were differences between in vivo and in vitro Zn exposures on kidney Mg^{2+}-ATPase activity and no significant alteration was detected in gill Mg^{2+}-ATPase activity in vitro Cu exposures. Lionetto et al. (2000) also showed that Mg^{2+}-ATPase activity did not alter significantly by in vitro Cd exposure either in the gills or in the intestine. However, Bansal et al. (1985) demonstrated inhibition of Ca^{2+}-ATPase activity after in vitro metal exposures with an exception of increase after lower concentrations of some metals, indicating metals displaced Ca^{2+} ions from protein carriers involved in facilitated diffusion. Data obtained from in vitro studies could be beneficial in understanding the toxic effects that metals elicit on fish in vivo. However, in vivo studies could be more appropriate to determine the effects of metals for ecotoxiological research as they represent better the challenges that fish face in the aquatic environment. In addition, in vitro data exhibit only direct effect of metals without considering internal factors due to its isolated structure whereas in vivo data represents the whole biochemical and physiological processes in organisms.

Anthropogenic activities have led to a widespread release of metals in aquatic systems and aquatic organisms develop several species-specific defense mechanisms to cope with pollution. In fact, the impairment of the activity of enzymes acting as key physiological roles could cause dysfunction of fish physiology. Responses of biomarkers are restricted to lower levels of biological complexity, such as molecular, cellular and tissue levels. Thus, they precede

alterations at population or communities levels and, consequently may be considered as ''early warning'' signals in environmental quality assessment (De la Torre et al. 2007). The measured biomarkers were selected considering their physiological significance as indicators of basic aspects of homeostatic mechanisms in aquatic organisms. Thus, ATPase activity was considered as an index of the ion-oregulatory capacity status.

Conclusion

The present results showed that both Cu and Zn are able to alter ATPase activities in the tissues of *O. niloticus* regardless of their application routes, most alterations being a decrease in the activity. The data also indicated that effects of Cu on ATPase activity in the tissues of *O. niloticus* were more than Zn, possibly due to higher toxicity of Cu. It seems that both branchial and renal osmoregulatory function is an important target side of toxic impact for waterborne metals. Nevertheless, the increases in ATPase activities seen here suggest that fish metabolisms try to cope with the toxic effects of metals up to a threshold level by increasing possibly turnover rates of the enzyme. The present findings warrant future studies to explore ATPases as possible candidate for biomarkers of exposure in ecotoxicology.

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