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Alterations in ion levels of freshwater fish *Oreochromis niloticus* following acute and chronic exposures to five heavy metals

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Abstract: Freshwater fish, *Oreochromis niloticus*, were individually exposed to 0, 0.1, 0.5, 1.0, and 1.5 µg/mL of Cu^{2+}, Cd^{2+}, Cr^{6+}, Ag^{+}, and Zn^{2+} for 96 h (acute) and 0.05 µg/mL concentrations of the same metals for 0, 5, 10, 20, and 30 days (chronic). Following each period, metal accumulation and ion levels (Na^{+}, K^{+}, Ca^{+2}, and Mg^{+2}) were measured in the gills, kidneys, and muscles.

Except for Ag^{+}, none of the metals killed the fish within 30 days. Silver killed all the fish within 16 days. With the exceptions of Ag^{+} and Cr^{6+}, as their levels were below detection limits, metal accumulation occurred in the tissues following both acute and chronic exposures. Ion levels in the tissues were altered by metal exposure, the general tendency being a decrease in Na^{+} and K^{+} levels and an increase in Mg^{2+} and Ca^{2+} levels. Acute exposure to heavy metals seemed to be more effective in altering ion levels of the tissues than chronic exposure. Na^{+} was the most affected ion while Mg^{2+} was the least affected. Results of this study emphasize that ion levels in the tissues of *O. niloticus* can be altered by heavy metals, both in acute and chronic exposures. This suggests that heavy metals should be monitored carefully in ecotoxicological studies in the field due to their importance in fish physiology.

Key words: Metal, *Oreochromis niloticus*, ATPase, ion

Akut ve kronik olarak beş ağır metal etkisinde kalan tatlısu balığı *Oreochromis niloticus*'da iyon düzeylerindeki değişimler

Özet: Tatlısu balığı *Oreochromis niloticus* 0, 0,1, 0,5, 1,0, 1,5 µg/mL Cu^{+2}, Cd^{+2}, Cr^{+6}, Ag^{+} ve Zn^{+2}'nin 96 saat süreyle (akut) ve 0.05 µg/mL derişimindeki aynı metallerin 0, 5, 10, 20, 30 günlük (kronik) etkisine ayrı ayrı bırakılmıştır. Deney süreleri sonunda solungaç, böbrek ve kas dokularında metal birikimi ve iyon düzeyleri (Na^{+}, K^{+}, Ca^{+2}, Mg^{+2}) ölçülmüştür.

Gümüş hariç hiç bir metal 30 günlük sürede letal etki göstermemiştir. Ag^{+} etkisinde kalan balıkların tamamı 16. gün sonunda ölmüştür. Metaller genellikle akut ve kronik etki sonunda dokularda birikim gösterirken, Ag^{+} ve Cr^{+6} birikimleri saptama sınırlarının altında gözlenmiştir. Metaller doku iyon düzeylerini değiştirmiştir. Metal etkisi sonunda genel eğilim Na^{+} ve K^{+} düzeyinde bir azalma, Mg^{+2} ve Ca^{+2} düzeylerinde ise bir artış yönünde olmuştur. Akut etki sonrasında iyonların kronik etkiye göre daha fazla etkilendiği görülmektedir. Na^{+} en çok Mg^{+2} ise en az etkilenen iyon olmuştur. Sonuçlar hem akut hem de kronik metal etkisi sonucu *O. niloticus*'un dokularında iyon düzeylerinin değişebileceğini gösterirken, balık fizyolojisindeki önemi nedeniyle arazide yapılan ekotoksikolojik araştırmalarda dikkate alınması gerektiğini vurgulamıştır.

Anahtar sözcükler: Metal, *Oreochromis niloticus*, ATPaz, iyon

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Introduction

Metal contamination in the aquatic environment has become one of the most critical environmental issues of recent years. Heavy metals such as Cd^{2+}, Zn^{2+}, Hg^{2+}, Cr^{6+}, and Cu^{2+} reach aquatic systems as a consequence of industrial, agricultural, and anthropogenic activities. As a result, aquatic organisms are exposed to a significant amount of these pollutants (Heath, 1987). The bioaccumulation of trace metals in aquatic organisms occurs when metal uptake rates exceed the depuration rates (Heath, 1987; Tagliari et al., 2004). The metal uptake rate is determined by factors such as concentration, species, pH, ions, and temperature (Heath, 1987; Roesijadi and Robinson, 1994). Heavy metals such as Cu^{2+}, Zn^{2+}, and Fe^{2+} are essential microelements for fish metabolism, although some of these, including Hg^{2+}, Cd^{2+}, and Pb^{2+}, are known to be nonessential for all living creatures. However, both essential and nonessential metals can become toxic for fish when they are exposed to excessive levels (Heath, 1987). Therefore, studies on metal uptake and toxicity have received much attention from those interested in assessing the quality of the environment, both for fish and humans (Davies, 1978; Verbost et al., 1989; Grosell et al., 2002). The initial effects of heavy metal pollution may be evident only at the cellular or tissue levels before significant changes can be identified in fish behavior or external appearance. During waterborne metal exposure, active ion uptake and ionic homeostasis are damaged, primarily in the gills (Pelgrom et al., 1995). It is important to determine the contamination levels of aquatic systems before toxic effects occur.

Fish are an important link in the aquatic food chain and accumulate considerable amounts of metals from water, diet, or sediment. This makes them a useful indicator for environmental toxicity studies. Reduced plasma osmolarity, Cl^-, and Na^+ concentrations in various fish species exposed to metal in freshwater confirmed that metals act as an osmoregulatory toxicant (Stagg and Shuttleworth, 1982; Morgan et al., 2004; Oner et al., 2008). It is believed that they target the Na^+ and Cl^- transport systems in the gills of freshwater animals. Osmoregulation can be defined as the maintenance of the extracellular osmotic concentrations against the external osmolarity and is an indispensable process for physiological adaptation in aquatic organisms. Therefore, regulation of ions, such as Na^+, K^+, and the divalent cations Mg^{2+} and Ca^{2+}, which are required for stabilization of membrane permeability, play a pivotal role in fish metabolism.

Aquatic organisms are generally exposed to chronic metal contamination in the field, although they may also suffer acute exposures in areas in which 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. In this sense, chronic stress is known to possibly induce an acclimation process, defined as increased tolerance to a concentration of a toxicant, which arises from chronic exposure to a sublethal concentration of that toxicant (McDonald and Wood, 1993). The aim of this study was to determine alteration in tissue ion levels of the freshwater fish *O. niloticus* following acute and chronic exposures to 5 metals.

Materials and methods

Freshwater fish *O. niloticus* has been cultured at Çukurova 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 aquariums for 1 month before the experiments. The 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 measuring 40 ′ 40 ′ 100 cm that contained 130 L of contaminated test solution, or test water only (dechlorinated) for controls. During the experiments, pH and oxygen levels were estimated at 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 (acidimetric method) were measured as 340 ± 29 mg $CaCO_3$/L and 248.6 ± 13.1 mg $CaCO_3$/L, respectively. Ion levels in the aquariums were also measured 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; and Cl^-, 28.3 ± 1.2 µg/mL.

Fish were exposed to 0, 0.1, 0.5, 1.0, and 1.5 µg/mL concentrations of Cu ($CuCl_2.2H_2O$), Zn ($ZnCl_2$),

Cd ($CdCl_2$. H_2O), Cr (K_2CrO_4), and Ag ($AgNO_3$) for 96 h for acute experiments and a concentration of 0.05 μg/mL of these metals for 0, 5, 10, 20, and 30 days for chronic experiments. Trisodium citrate 2-hydrate was used to prevent metal precipitation in the aquariums. A total of 6 fish were used for acute and 8 fish for chronic metal exposures. As there were no significant differences ($P > 0.05$) among the 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 the fish did not differ significantly ($P > 0.05$) among the different exposure treatments and controls. A total of 350 fish were used in this study. The aquariums of the control and metal-exposed groups were cleaned every 2 days, 1 h after the feeding period, to reduce contamination with food remains and also to minimize metal loss in the exposure medium.

At the end of each experimental period, the fish were killed by transaction of the spinal cord. Gills, kidneys, and muscle tissues were dissected out with clean equipment. Tissues of 2 fish of the same size were pooled and were immediately stored at −80 °C (Revco Ultima II) until analysis. In order to measure metal and ion levels, the tissues were first dried in an oven at 60 °C until they reached a constant weight, and then they were transferred into glass flasks for the 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 appropriately diluted with distilled water in the range of standards that were prepared from a stock standard solution of the metals (Merck). Metal and ion concentrations in the tissues were measured using a flame atomic absorption spectrophotometer (Perkin Elmer AS 3100). The detection limits of metals were 0.001, 0.002, 0.002, 0.003, and 0.002 μg/mL for Cd^{2+}, Zn^{2+}, Cu^{2+}, Cr^{6+}, and Ag^{+}, respectively. The detection limits of Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+} were 0.002, 0.015, 0.0001, and 0.002 μg/mL, respectively. 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. Metal levels in the tap water were below the detection limits.

Statistical analysis of data, presented as mean and standard error, was performed using the SPSS statistical package program. One-way ANOVA was applied to compare variables among control and treatments at each exposure period. Post hoc comparisons were done using Duncan's test to determine which individual groups were significantly different from the control when significant differences were found ($P < 0.05$).

Results

In acute experiments, metal accumulation occurred in the tissues when compared to control fish, although Ag^{+} and Cr^{6+} levels in the tissues were not detected, except in the gills (Table 1). The highest metal accumulation rate was in the kidneys of Cd^{2+} exposed fish. In chronic experiments, mortality was observed in Ag^{+}-exposed fish after day 12, and all fish died at day 16. Therefore, there is no data for days 20 or 30 of Ag^{+} exposure. Tissue metal concentrations increased significantly compared to control values, with the exception of Ag^{+} and Cr^{6+} exposures. The highest metal accumulation rate was in the kidneys of Cu^{2+}-exposed fish.

In acute experiments, Na^{+} levels decreased in all tissues, except in the gills of fish exposed to Zn^{2+}. In chronic experiments, Na^{+} levels also generally decreased in all tissues, except in the gills of Zn^{2+}- and Cr^{6+}-exposed fish (Table 2). In the acute series, gill K^{+} levels decreased after all metal exposures (Table 3), although this decrease was only seen in the kidneys of Cr^{6+}- and Ag^{+}-exposed fish and in the muscles of Cu^{2+}-, Zn^{2+}-, and Cr^{6+}-exposed fish. In the chronic series, Cd^{2+}, Cr^{6+}, and Ag^{+} exposures decreased K^{+} levels, but Zn^{2+} exposure increased gill K^{+} levels. K^{+} levels in the kidneys decreased only in Cd^{2+}-exposed fish, while muscle K^{+} levels did not show any change. In the acute series, Mg^{2+} levels increased in the kidneys of Cu^{2+}-, Cr^{6+}-, and Ag^{+}-exposed fish and in the gills of Cd^{2+}-exposed fish (Table 4). However, Zn^{2+} exposure caused a decrease in the muscle levels. In the chronic series, similar increases in Mg^{2+} levels were observed in the kidneys following Cu^{2+} and Ag^{+} exposures, in the muscle following Ag^{+} exposure, and in the gills following Zn^{2+} exposure. In the acute series, a general increase in Ca^{+} levels was observed in all tissues, except in the gills after Ag^{+} exposure and in the muscle of Ag^{+}- and Cr^{6+}-exposed fish (Table 5).

Table 1. Total metal levels (µg/g dry weight) in the tissues of *O. niloticus* exposed to acute and chronic metal effects (mean ± SE; control group, N = 21; metal-exposed group, N = 3; asterisks indicate significant differences between control and metal exposed groups, P < 0.05).

		ACUTE				CHRONIC		
Metal	Concentration (µg/mL)	Gill	Kidney	Muscle	Exposure duration (days)	Gill	Kidney	Muscle
Cd	0	1.26 ± 0.12	<0.001	<0.001	0	1.26 ± 0.12	<0.001	<0.001
	0.1	14.1 ± 1.08*	22.8 ± 8.9*	3.74 ± 1.69*	5	8.59 ± 3.24*	<0.001	3.57 ± 0.33*
	0.5	6.2 ± 0.15*	45.1 ± 22.5*	2.04 ± 0.36*	10	17.1 ± 2.59*	<0.001	4.96 ± 0.81*
	1	8.56 ± 0.81*	49.7 ± 9.1*	2.33 ± 0.4*	20	20.3 ± 3.11*	2.25 ± 2.25	3.78 ± 0.38*
	1.5	19.9 ± 3.78*	97.3 ± 15.3*	0.66 ± 0.23	30	21.8 ± 5.16*	14.3 ± 2.74*	5.14 ± 0.69*
Cu	0	3.35 ± 0.32	<0.002	<0.002	0	3.35 ± 0.32	<0.002	<0.002
	0.1	4.2 ± 0.01	<0.002	<0.002	5	6.57 ± 0.49*	41.5 ± 9.08*	3.71 ± 0.13*
	0.5	8.88 ± 1.13*	<0.002	<0.002	10	8.45 ± 0.59*	45.8 ± 1.17*	5.16 ± 0.83*
	1	7.68 ± 0.25*	20.6 ± 20.6	1.4 ± 0.7*	20	9.6 ± 0.3*	48.6 ± 9.05*	2.14 ± 1.25*
	1.5	10.3 ± 1.13*	9.4 ± 9.4	<0.002	30	10.2 ± 0.47*	60.9 ± 9.4*	1.98 ± 1.16*
Zn	0	69.2 ± 1.74	157.2 ± 15.5	32.1 ± 0.83	0	69.2 ± 1.74	157.2 ± 15.5	32.1 ± 0.83
	0.1	69.9 ± 5.2	160.6 ± 75.7	41.9 ± 8.57	5	78.1 ± 4.08	251.5 ± 8.16*	35.4 ± 2.05
	0.5	79.6 ± 6.15	146.7 ± 4.84	46.1 ± 9.35*	10	89.1 ± 8.23*	230.9 ± 23.2*	33.5 ± 1.44
	1	84.2 ± 6.76*	193.2 ± 0.46	36.3 ± 4.95	20	77.7 ± 0.94	269.5 ± 25.9*	33.0 ± 1.21
	1.5	91.1 ± 6.28*	270.9 ± 31.7	44.5 ± 3.86	30	83.1 ± 5.51*	261.9 ± 31.0*	32.0 ± 1.40
Cr	0	<0.003	<0.003	<0.003	0	<0.003	<0.003	<0.003
	0.1	2.0 ± 2.0	<0.003	<0.003	5	<0.003	<0.003	<0.003
	0.5	<0.003	<0.003	<0.003	10	<0.003	<0.003	<0.003
	1	4.26 ± 2.14*	<0.003	<0.003	20	<0.003	<0.003	<0.003
	1.5	5.13 ± 2.57*	<0.003	<0.003	30	<0.003	<0.003	<0.003
Ag	0	<0.002	<0.002	<0.002	0	<0.002	<0.002	<0.002
	0.1	2.21 ± 0.35*	<0.002	<0.002	5	<0.002	<0.002	<0.002
	0.5	2.48 ± 0.55*	<0.002	<0.002	10	<0.002	<0.002	<0.002
	1	2.24 ± 0.23*	<0.002	<0.002	16	<0.002	<0.002	<0.002
	1.5	2.84 ± 0.74*	<0.002	<0.002				

Table 2. Total Na+ levels (mg/g dry weight) in the tissues of *O. niloticus* exposed to acute and chronic metal effects (mean ± SE; control group, N = 21; metal-exposed group, N = 3; asterisks indicate significant differences between control and metal exposed groups, P < 0.05).

		ACUTE				CHRONIC		
Metal	Concentration (µg/mL)	Gill	Kidney	Muscle	Exposure Duration (day)	Gill	Kidney	Muscle
Cd	0	7.01 ± 0.31	14.01 ± 0.65	4.18 ± 0.13	0	7.01 ± 0.31	14.01 ± 0.65	4.18 ± 0.13
	0.1	4.39 ± 0.32*	11.89 ± 0.75	4.20 ± 0.35	5	4.69 ± 0.12*	5.33 ± 0.28*	2.19 ± 0.15*
	0.5	4.93 ± 0.28*	8.66 ± 0.88*	3.80 ± 0.42	10	4.63 ± 0.15*	6.29 ± 0.30*	2.54 ± 0.14*
	1	7.39 ± 0.43	7.99 ± 0.53*	3.10 ± 0.54*	20	5.28 ± 0.30*	5.74 ± 0.12*	2.63 ± 0.20*
	1.5	5.77 ± 0.60	7.57 ± 0.71*	2.91 ± 0.09*	30	5.08 ± 0.23*	5.90 ± 0.39*	2.47 ± 0.30*
Cu	0.1	1.95 ± 0.0*	14.59 ± 1.12	2.69 ± 0.0*	5	4.63 ± 0.11*	8.31 ± 1.01*	2.61 ± 0.17*
	0.5	6.74 ± 2.56	13.78 ± 1.5	1.07 ± 0.77*	10	5.10 ± 0.38*	14.48 ± 0.92	2.51 ± 0.32*
	1	4.68 ± 0.22*	8.22 ± 0.57*	2.83 ± 0.21*	20	6.05 ± 0.30	6.13 ± 0.47*	2.56 ± 0.22*
	1.5	5.44 ± 0.51	8.08 ± 1.36*	2.50 ± 0.10*	30	5.67 ± 0.20	8.71 ± 0.73*	2.87 ± 0.31*
Zn	0.1	5.61 ± 0.59	11.60 ± 0.96	2.62 ± 0.31*	5	6.04 ± 0.19	7.80 ± 0.19*	2.91 ± 0.23*
	0.5	6.90 ± 0.83	4.20 ± 0.62*	2.39 ± 0.09*	10	7.46 ± 0.76	7.24 ± 0.48*	2.86 ± 0.14*
	1	6.48 ± 0.87	4.56 ± 1.42*	2.31 ± 0.19*	20	6.23 ± 0.12	7.96 ± 0.91*	2.86 ± 0.11*
	1.5	6.02 ± 0.64	4.82 ± 0.83*	1.79 ± 0.16*	30	7.16 ± 0.45	18.83 ± 0.96*	3.06 ± 0.04*
Cr	0.1	4.44 ± 0.30*	5.87 ± 0.18*	2.76 ± 0.30*	5	5.46 ± 0.21	6.13 ± 0.45*	2.92 ± 0.09*
	0.5	4.92 ± 0.14*	6.30 ± 0.61*	2.72 ± 0.21*	10	5.56 ± 0.16	6.14 ± 0.29*	2.43 ± 0.18*
	1	5.50 ± 0.61	7.43 ± 1.87*	2.67 ± 0.17*	20	6.54 ± 0.61	7.74 ± 0.58*	2.97 ± 0.39*
	1.5	5.42 ± 0.52	6.04 ± 0.17*	2.27 ± 0.03*	30	5.95 ± 0.51	7.16 ± 0.58*	2.69 ± 0.30*
Ag	0.1	4.40 ± 0.81*	5.15 ± 0.41*	2.11 ± 0.27*	5	4.50 ± 0.12*	8.45 ± 0.41*	2.29 ± 0.11*
	0.5	4.74 ± 0.52*	4.82 ± 0.28*	2.25 ± 0.19*	10	5.81 ± 0.40	8.07 ± 0.22*	2.76 ± 0.15*
	1	3.67 ± 0.34*	4.89 ± 0.73*	2.09 ± 0.23*	16	5.27 ± 0.41*	9.10 ± 1.31*	2.60 ± 0.08*
	1.5	5.55 ± 0.41*	3.86 ± 0.58*	2.28 ± 0.14*				

Table 3. Total K+ levels (mg/g dry weight) in the tissues of *O. niloticus* exposed to acute and chronic metal effects (mean ± SE; control group, N = 21; metal-exposed group, N = 3; asterisks indicate significant differences between control and metal exposed groups, P < 0.05).

		ACUTE				CHRONIC		
Metal	Concentration (µg/mL)	Gill	Kidney	Muscle	Exposure Duration (day)	Gill	Kidney	Muscle
Cd	0	4.57 ± 0.14	10.93 ± 0.80	8.83 ± 0.55	0	4.57 ± 0.14	10.93 ± 0.80	8.83 ± 0.55
	0.1	3.03 ± 0.21*	10.43 ± 0.70	5.90 ± 1.29	5	3.28 ± 0.64*	6.77 ± 0.20*	8.54 ± 0.01
	0.5	3.25 ± 0.49*	7.84 ± 0.22	6.21 ± 1.25	10	3.64 ± 0.04*	6.98 ± 0.29*	9.07 ± 0.04
	1	4.18 ± 0.11	8.21 ± 0.79	9.93 ± 5.44	20	4.20 ± 0.23	6.56 ± 0.10*	8.93 ± 0.37
	1.5	3.82 ± 0.25*	8.28 ± 0.51	4.85 ± 0.72	30	3.83 ± 0.10	6.50 ± 0.36*	9.60 ± 0.20
Cu	0.1	1.66 ± 0.0*	11.93 ± 0.73	10.96 ± 0.0	5	3.60 ± 0.09*	10.02 ± 0.94	6.09 ± 0.88
	0.5	4.29 ± 2.24	11.74 ± 2.06	5.25 ± 0.57*	10	4.08 ± 0.42	8.73 ± 0.24	9.76 ± 0.14
	1	3.33 ± 0.18*	9.16 ± 0.74	4.07 ± 0.43*	20	4.18 ± 0.16	7.46 ± 0.28	7.93 ± 1.49
	1.5	3.56 ± 0.26*	10.32 ± 1.56	4.96 ± 0.20*	30	3.95 ± 0.16	7.78 ± 0.43	9.64 ± 0.17
Zn	0.1	3.67 ± 0.20*	7.16 ± 0.46	6.69 ± 0.09	5	4.38 ± 0.17	8.42 ± 0.42	10.27 ± 0.24
	0.5	4.0 ± 0.04	7.76 ± 0.65	4.18 ± 2.8*	10	5.69 ± 0.52*	8.61 ± 0.20	10.52 ± 0.01
	1	3.87 ± 0.38	6.99 ± 0.40	2.66 ± 0.54*	20	4.93 ± 0.10	9.32 ± 0.38	9.83 ± 0.17
	1.5	3.71 ± 0.20*	8.19 ± 0.52	5.33 ± 0.93*	30	5.34 ± 0.36	10.12 ± 0.49	9.82 ± 0.32
Cr	0.1	3.16 ± 0.18*	6.10 ± 0.18	6.70 ± 0.0	5	4.06 ± 0.16	8.17 ± 0.61	9.72 ± 0.14
	0.5	3.68 ± 0.03*	7.49 ± 1.04	6.19 ± 1.03	10	4.07 ± 0.03	7.80 ± 0.25	8.57 ± 2.11
	1	3.99 ± 0.23	5.45 ± 1.32*	5.34 ± 1.52	20	4.62 ± 0.34	8.20 ± 0.27	10.2 ± 1.34
	1.5	4.61 ± 0.0	6.23 ± 0.56*	5.09 ± 0.27*	30	4.26 ± 0.32	7.96 ± 0.33	10.2 ± 0.29
Ag	0.1	3.40 ± 0.27*	6.10 ± 0.18*	7.93 ± 1.17	5	3.46 ± 0.06*	7.79 ± 0.36	7.56 ± 1.15
	0.5	3.09 ± 0.21*	6.50 ± 0.45*	9.62 ± 0.38	10	4.19 ± 0.23	7.24 ± 0.15	8.92 ± 0.11
	1	2.76 ± 0.18*	6.38 ± 0.56*	9.25 ± 0.54	16	3.83 ± 0.28*	10.0 ± 1.01	8.65 ± 0.24
	1.5	1.95 ± 1.20*	6.41 ± 0.38*	9.07 ± 0.34				

Table 4. Total Mg^{+2} levels (mg/g dry weight) in the tissues of *O. niloticus* exposed to acute and chronic metal effects (mean ± SE; control group, N = 21; metal exposed group, N = 3; asterisks indicate significant differences between control and metal exposed groups, $P < 0.05$).

	ACUTE				CHRONIC			
Metal	Concentration (µg/mL)	Gill	Kidney	Muscle	Exposure Duration (day)	Gill	Kidney	Muscle
Cd	0	1.13 ± 0.07	1.09 ± 0.05	1.54 ± 0.06	0	1.13 ± 0.07	1.09 ± 0.05	1.54 ± 0.06
	0.1	1.14 ± 0.12	1.12 ± 0.01	1.73 ± 0.21	5	1.02 ± 0.08	0.80 ± 0.03	1.34 ± 0.04
	0.5	1.36 ± 0.05	1.31 ± 0.14	1.79 ± 0.14	10	1.09 ± 0.09	0.89 ± 0.06	1.30 ± 0.06
	1	1.54 ± 0.09*	1.31 ± 0.15	2.11 ± 0.41	20	0.94 ± 0.08	0.89 ± 0.02	1.17 ± 0.06
	1.5	1.63 ± 0.27*	1.47 ± 0.22	1.55 ± 0.07	30	1.13 ± 0.10	0.79 ± 0.06	1.48 ± 0.06
Cu	0.1	0.99 ± 0.01	2.12 ± 0.73*	1.14 ± 1.14	5	0.97 ± 0.06	1.33 ± 0.22	1.38 ± 0.07
	0.5	1.68 ± 0.25	1.25 ± 0.11	1.34 ± 0.06	10	0.99 ± 0.13	2.65 ± 1.19*	1.86 ± 0.32
	1	0.85 ± 0.09	0.90 ± 0.13	1.22 ± 0.11	20	1.12 ± 0.10	0.91 ± 0.08	1.32 ± 0.11
	1.5	0.93 ± 0.26	0.83 ± 0.01	1.16 ± 0.11	30	0.84 ± 0.11	2.39 ± 0.66*	1.53 ± 0.13
Zn	0.1	1.26 ± 0.23	1.33 ± 0.01	1.44 ± 0.15	5	1.32 ± 0.08	1.19 ± 0.10	1.93 ± 0.08
	0.5	1.31 ± 0.07	1.11 ± 0.35	1.39 ± 0.11	10	1.34 ± 0.21	1.09 ± 0.11	1.37 ± 0.11
	1	1.59 ± 0.10	1.46 ± 0.51	0.90 ± 0.12*	20	1.61 ± 0.11*	1.45 ± 0.22	1.88 ± 0.05
	1.5	1.20 ± 0.11	1.22 ± 0.13	0.86 ± 0.05*	30	1.66 ± 0.01*	1.26 ± 0.05	1.97 ± 0.19
Cr	0.1	1.33 ± 0.14	0.94 ± 0.01	1.52 ± 0.13	5	1.16 ± 0.18	0.96 ± 0.17	1.83 ± 0.17
	0.5	1.27 ± 0.08	1.56 ± 0.22*	1.52 ± 0.23	10	1.33 ± 0.19	0.98 ± 0.25	1.34 ± 0.06
	1	1.46 ± 0.04	0.91 ± 0.13	1.53 ± 0.08	20	1.11 ± 0.05	0.96 ± 0.18	1.39 ± 0.12
	1.5	1.54 ± 0.19	0.99 ± 0.01	1.63 ± 0.10	30	1.04 ± 0.12	0.84 ± 0.11	1.34 ± 0.11
Ag	0.1	1.16 ± 0.13	0.91 ± 0.10	1.83 ± 0.14	5	1.29 ± 0.10	1.04 ± 0.15	1.87 ± 0.24
	0.5	1.23 ± 0.06	1.44 ± 0.25*	1.77 ± 0.14	10	1.52 ± 0.24	1.45 ± 0.15*	1.77 ± 0.06
	1	1.18 ± 0.09	1.51 ± 0.23*	1.66 ± 0.12	16	1.52 ± 0.06	2.37 ± 0.22*	2.37 ± 0.17
	1.5	1.49 ± 0.23	1.60 ± 0.18*	1.79 ± 0.21				

Table 5. Total Ca^{+2} levels (mg/g dry weight) in the tissues of *O. niloticus* exposed to acute and chronic metal effects (mean ± SE; control group, N = 21; metal exposed group, N = 3; asterisks indicate significant differences between control and metal exposed groups, $P < 0.05$).

		ACUTE				CHRONIC		
Metal	Concentration (µg/mL)	Gill	Kidney	Muscle	Exposure Duration (day)	Gill	Kidney	Muscle
Cd	0	42.8 ± 1.54	0.52 ± 0.05	0.39 ± 0.04	0	42.8 ± 1.54	0.52 ± 0.05	0.39 ± 0.04
	0.1	43.1 ± 2.21	0.94 ± 0.03	40.9 ± 0.98*	5	45.3 ± 5.0	0.43 ± 0.02	17.2 ± 2.24*
	0.5	46.9 ± 1.15	1.02 ± 0.2	29.2 ± 6.3*	10	42.7 ± 1.84	0.58 ± 0.03	4.51 ± 1.66*
	1	62.6 ± 4.04*	1.94 ± 0.38*	7.81 ± 4.46*	20	46.0 ± 2.99	0.51 ± 0.09	10.1 ± 3.14*
	1.5	55.6 ± 3.86*	1.14 ± 0.47*	7.71 ± 0.72*	30	36.5 ± 2.58	0.49 ± 0.02	0.44 ± 0.08
Cu	0.1	42.8 ± 0.01	2.41 ± 0.49*	0.24 ± 0.01	5	44.0 ± 2.04	0.58 ± 0.17	14.4 ± 1.31*
	0.5	76.0 ± 14.4*	1.28 ± 0.65*	9.53 ± 1.63*	10	38.0 ± 0.96	1.53 ± 0.29*	0.97 ± 0.19
	1	41.2 ± 3.07	0.62 ± 0.17	0.33 ± 0.09	20	44.4 ± 1.98	0.48 ± 0.09	6.96 ± 3.65*
	1.5	58.1 ± 7.05*	1.14 ± 0.19	0.54 ± 0.01	30	34.5 ± 2.14	0.92 ± 0.11*	0.36 ± 0.03
Zn	0.1	50.5 ± 4.94	0.62 ± 0.07	0.29 ± 0.07	5	41.4 ± 0.95	0.47 ± 0.06	0.26 ± 0.01
	0.5	54.5 ± 6.36*	0.48 ± 0.05	0.73 ± 0.50	10	48.0 ± 3.72	0.41 ± 0.02	0.37 ± 0.03
	1	54.3 ± 7.08*	1.38 ± 0.44*	5.54 ± 2.61*	20	48.8 ± 1.20	0.37 ± 0.02	0.28 ± 0.03
	1.5	54.1 ± 4.75*	0.53 ± 0.13	0.88 ± 0.08	30	56.7 ± 3.05*	0.46 ± 0.06	0.26 ± 0.02
Cr	0.1	44.2 ± 2.78	0.95 ± 0.15*	0.39 ± 0.04	5	46.6 ± 4.95	0.76 ± 0.02	0.57 ± 0.12
	0.5	49.1 ± 3.49	1.62 ± 0.09*	0.32 ± 0.04	10	46.6 ± 6.88	1.88 ± 0.38*	0.37 ± 0.08
	1	56.6 ± 3.95*	0.57 ± 0.11	0.48 ± 0.19	20	39.3 ± 1.14	1.71 ± 0.26*	0.32 ± 0.11
	1.5	52.9 ± 4.66	0.63 ± 0.09	0.18 ± 0.07	30	48.1 ± 6.44	0.65 ± 0.05	0.42 ± 0.03
Ag	0.1	42.3 ± 2.97	0.68 ± 0.14	0.70 ± 0.35	5	38.9 ± 1.95	0.63 ± 0.08	0.42 ± 0.03
	0.5	45.3 ± 6.49	1.05 ± 0.29*	0.29 ± 0.01	10	53.8 ± 5.12*	1.70 ± 0.29*	0.51 ± 0.09
	1	39.9 ± 1.35	0.94 ± 0.22	0.27 ± 0.02	16	56.1 ± 6.22*	2.12 ± 0.26*	0.61 ± 0.15
	1.5	54.7 ± 9.74	0.98 ± 0.06*	0.27 ± 0.02				

However, in the chronic series, Ca^{2+} levels in the gills increased following Zn^{2+} and Ag^{+} exposure; in the kidneys following Cu^{2+}, Cr^{6+}, and Ag^{+} exposure; and in the muscle after Cd^{2+} and Cu^{2+} exposure.

Discussion

There was no fish mortality in acute (96 h) or chronic (30 days) exposures to Cu^{2+}, Cd^{2+}, Zn^{2+}, and Cr^{6+}. However, the same concentration of Ag^{+} killed all fish within 16 days, starting from day 12. It is well known that Ag^{+} is highly toxic to fish, and the principal toxic effect of Ag^{+} in fish is ionoregulatory disturbances (Morgan et al., 1997). Generally, Ag^{+} is among the most toxic heavy metals, together with Hg^{2+}, As^{2+}, and Cu^{2+} and followed by Cd^{2+}, Pb^{2+}, and Zn^{2+} (Heath, 1987). In a previous study, we also observed mortality after 6 days of exposure of O. niloticus to 1 mg Cu^{2+}/L (Atlı and Canlı, 2003). In addition to metal species, factors such as fish species, sex, and age and the temperature, salinity, hardness, and pH of the water play significant roles in metal toxicity (Heath, 1987).

In the acute series, metal accumulation generally occurred in the gills, although only the Cd^{2+} level in the kidneys and the Cd^{2+}, Cu^{2+}, and Zn^{2+} levels in the muscle increased. Ag^{+} and Cr^{6+} accumulations were below the detection limits. Although there were variations in ion levels in relation to metal species, exposure concentrations, and tissues, results can be summarized as follows: Ca^{2+} and Mg^{2+} levels increased while Na^{+} and K^{+} levels decreased in the tissues of metal-exposed fish. The gills, kidneys, and intestines are important tissues due to their pivotal role in osmoregulation. Plasma Na^{+} and Cl^{-} are key factors in the maintenance of osmotic pressure. In addition, K^{+}, Mg^{2+}, and Ca^{2+} are significant in osmotic and ionic regulation in the intra- and extracellular fluids of vital mechanisms (Simkiss and Taylor, 1989). The fish gill is a key tissue constituting large surfaces and facilitating gas exchange, acid-base regulation, ionic transport, and the excretion of nitrogenous wastes. The kidney is a significant tissue, associated with a higher rate of metal accumulation and metal-binding protein synthesis. Muscle is also a key tissue, important as a food source in the food chain. Gills, the primary target of metals and the first site of accumulation, as well as kidneys, a metal-selective feature tissue, have gained importance in metal studies (Heath, 1987; Canli and Atli, 2003).

Ions move across the cell by diffusion, or via specific cytosolic carriers, prior to export from the cell. Thus, the overall process involves adsorption, import, intracellular trafficking, and export. Some electrolytes may move between the cells by diffusion. The driving force for transport is often an energy-requiring pump (primary transport) located on the basolateral membrane (Handy et al., 2002). Ionic disturbances associated with Cd^{+2} and Zn^{+2} exposure have been shown to result from changes in Ca^{2+} influx kinetics, or competition with Ca^{2+} for apical uptake channels (Hogstrand and Wood, 1996; Verbost et al., 1989). In the literature, there is wide variety of data concerning the effect of metals on ion levels, both supporting and contradicting the present data. This probably results from the different experimental conditions, species, and tissues targeted in different studies. Garcia-Santos et al. (2006) showed that there was no variation in plasma Na^{+} concentration, although plasma Ca^{2+} levels in *O. niloticus* declined in a dose-dependent manner following acute (96 h) Cd^{2+} exposure. However, tissue Na^{+} levels of *O. niloticus* decreased, while Ca^{2+} levels increased in the acute series of this study. Pelgrom et al. (1995) found an increase in whole tissue Ca^{2+} levels in *O. mossambicus* following individual and combined Cd^{2+} and Cu^{2+} exposures, in results similar to the present study's. Since freshwater fish take up most ions that are necessary for homeostasis from the water via their gills, alteration of ion levels may occur as a result of impaired active ion uptake by the chloride cells of the gill. Studies have shown that Cd^{2+} blocks Ca^{2+} uptake because it competes with Ca^{2+} for the high affinity Ca^{2+} binding sites (Torreblanca et al., 1989). Variation of tissue ion levels in fish exposed to metals is dependent upon the fish and metal species, concentration of metals, and the physicochemical properties of the water (Morgan et al., 1997; Rogers et al., 2003). Several studies indicated that Cu^{2+} causes a reduction in Na^{+}/K^{+}-ATPase activity, thereby decreasing tissue Na^{+} and K^{+} levels (Li et al., 1998; Handy et al., 2002). This is also in line with our data, which show a decline in Na^{+} and K^{+} levels in the gills and kidneys. Morgan et al. (2004) indicated that Cd^{2+}, Zn^{2+}, and Hg^{2+} block skeleton and heart Na^{+} channels as a result of binding to sulfhydryl groups.

They showed a significant Ag^+ accumulation in the gills of *O. mykiss* with reductions in Na^+ uptake and Na^+/K^+-ATPase activity following 24 h of exposure to 1.92 μg Ag^+/L. Ionoregulatory dysfunctions, related to loss of Na^+ and Cl^-, can cause fish mortality. This could be a result of Na^+/K^+-ATPase inhibition in the gills of fish exposed to Ag^+, which involves fish metabolism via Na^+ channels by mimicking Na^+ (Heath, 1987; Grosell et al., 2000; Morgan et al., 2004). The similarity between the toxic effects of Ag^+ and Cu^{2+} is evident, as they both reduced tissue Na^+ and K^+ levels and increased Ca^{2+} and Mg^{2+} levels in this study.

In the chronic experiments, significant accumulation of Cd^{2+}, Cu^{2+}, and Zn^{2+} in the tissues occurred, although only Cd^{2+} and Cu^{2+} accumulation was detected in the muscle. Following chronic metal exposures, tissue ion levels behaved differently, as Na^+ and K^+ levels decreased and Mg^{2+} and Ca^{2+} levels increased. Chronic exposure of fish to waterborne Cu^{2+}, Cd^{2+}, or Zn^{2+} was shown to cause a variety of physiological and behavioral changes, including loss of appetite, reduced growth, ion loss, decreased aerobic scope, and mortality (Hogstrand and Wood, 1996). The variation in ion levels at the beginning of the experiments returned to control levels at the end of some exposure durations, and this may be explained by compensation mechanisms related to the damage-repair model (McDonald and Wood, 1993). Li et al. (1998) found an alteration in gill chloride cell structure with a decrease in plasma Na^+ levels in a duration-dependent manner and an increase in Cu^{2+} levels in the gills of *O. mossambicus* after 28 days of exposure to 3.2 μM Cu^{2+}. Differences in metal accumulation and distribution among the species and variations in cell defense systems, such as antioxidant mechanisms, cause significant differences in metal sensitivity between species (Heath, 1987; Basha and Rani, 2003; Atlı et al., 2006). On the other hand, different responses are recorded following metal exposures depending upon the differences between metal sensitivities with variable metal binding characteristics to the high-affinity sites (Grosell et al., 2002; Garcia-Santos et al., 2006). For instance, *O. mykiss* exhibited variable responses to different concentrations of Cu^{2+}, Cd^{2+}, and Zn^{2+}, such as dysfunction of whole body Na^+ and Ca^{2+} homeostasis (McGeer et al., 2000).

Trends in metal accumulation in the tissues of *O. niloticus* were dose-dependent in the acute series and duration-dependent in the chronic series. Tissue concentrations of metals generally increased sharply in the early phase but slowed down in the later stage. This could be explained by the rapid saturation of the metal-binding capacity of most of the ligands (i.e. metallothioneins and mucus) in the tissues (Wong and Wong, 2000). The highest accumulation rate in the acute series in this study was in the kidneys of Cd^{2+}-exposed fish, although the highest accumulation rate overall was in the gills of Cd^{2+}-exposed fish in the chronic series. Atlı and Canlı (2003) also recorded that the rate of Cd^{2+} accumulation was highest in the livers of *O. niloticus* when compared to the accumulation rates of other metals (Cu^{2+}, Zn^{2+}, Pb^{2+}, and Fe^{2+}). Hollis et al. (2000) showed that there were positive correlations in Cd^{2+} accumulation and exposure duration in the gill, liver, and kidney of *O. mykiss*. However, a low accumulation rate of Ag^+ in the tissues of the fish was evident. Bury (2005) found only a 2-fold increase in Ag^+ levels in the gills of *O. mykiss* in acute exposure, although there was no significant change in chronic exposure, indicating that the low accumulation levels of Ag^+ in the liver was possibly due to excretion. Following exposure to Ag^+, the gills rapidly accumulate the metal, reaching a peak within a few hours. This is then followed by a decrease in branchial Ag^+ concentrations, due to a reduction in apical Ag^+ entry (Wood et al., 2002; Morgan et al., 2004). This was also observed in the present study as Ag^+ levels increased following acute exposure in the gills, although Ag^+ levels in the tissues were below the detection limit in the chronic series.

In summary, acute and chronic exposures to 5 heavy metals (Cu^{2+}, Cd^{2+}, Cr^{6+}, Ag^+, and Zn^{2+}) affected mostly Na^+ levels, followed by Ca^{2+}, K^+, and Mg^{2+}, in the tissues of *O. niloticus*. Generally, Na^+ and K^+ levels decreased while Ca^{2+} and Mg^{2+} levels increased. None of the metals killed the fish within 30 days, except Ag^+. Metal accumulation occurred in the tissues following both acute and chronic exposures. However, accumulation levels of Ag^+ and Cr^{6+} were below the detection limits. The trends in metal accumulation were dependent upon exposure concentrations in the acute series, while they were dependent upon exposure duration in the chronic series. Although the kidney exhibited

the highest metal accumulation rate, the gill was the most affected tissue. This study highlights the need for further studies to better investigate the effects of acute and chronic metal exposures on enzymatic systems, particularly ATPases, which play a pivotal role in maintaining ion homeostasis in fish.

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