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Effects of Salinity on the Accumulation of Copper in Liver, Gill and Muscle Tissues of *Tilapia nilotica*

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Abstract: In this study, effects of 5‰, 10‰, 15‰ and 20‰ NaCl concentrations on the accumulation of copper in liver, gill and muscle tissues of *Tilapia nilotica* were studied over experimental periods of 1, 7, 15 and 30 days at concentrations of 0.1, 1.0 and 10.0 ppm copper in the medium.

The highest levels of copper were found in the liver and the lowest values in the muscle tissue in a given NaCl and metal concentration. Accumulation of copper in the liver tissue, however, increased with increasing copper concentrations in the medium in all salt concentrations.

The increase of the NaCl concentration in the medium, significantly reduced the accumulation of copper in the liver, gill and muscle tissues of *Tilopia nilotica*, at all copper concentrations.

Key Words: Salinity, Heavy Metal, Accumulation, Fish.

Tilapia nilotica'da Karaciğer, Solungaç ve Kas Dokularındaki Bakır Birikimine Tuzluluğun Etkileri

Özet: Bu çalışmada ‰5, ‰10, ‰15 ve ‰20 NaCl konsantrasyonlarının *Tilopia nilotica*'nın karaciğer, solungaç ve kas dokularındaki bakır birikimine 1, 7, 15 ve 30 günlük sürelerdeki ve 0.1, 1.0 ve 10.0 ppm Cu ortam derişimlerindeki etkisinin belirlenmesi araştırılmıştır.

Belirli bir metal ve NaCl konsantrasyonunda en fazla bakır düzeyi karaciğerde, en düşük bakır düzeyi ise kas dokusunda bulunmuştur. Karaciğer dokusundaki bakır akümulasyonu tüm tuz konsantrasyonlarında ortamdaki bakır konsantrasyonunun artışı ile birlikte artma göstermiştir.

Tilapia nilotica'da çalışılan tüm bakır konsantrasyonlarında ortamdaki NaCl konsantrasyonunun artışı karaciğer, solungaç ve kas dokularındaki bakır birikimini önemli derecede azaltmıştır.

Anahtar Sözcükler: Tuzluluk, Ağır Metal, Birikim, Balık.

Introduction

In recent years, different factors such as industrial development and increases in pesticide usage and mining have led to an increase in the levels of heavy metals, including copper, in aquatic environments (1, 2).

The accumulation of heavy metals in the tissues of fishes, which comprise the most significant group of water-dwelling organisms, may cause various physiological defects and mortality (3-5).

The accumulation of heavy metals in the tissues of aquatic organisms is affected by physical and chemical factors such as temperature (6), water hardness, pH (7) and salinity (8). Additionally, the salinity affects various vital factors such as osmoregulation (11), nitrogen excretion and oxygen consumption (10), hatching (11), embryonic development and distribution of aquatic organisms (12).

This study was carried out to determine the effects of salinity on the accumulation of copper in the muscle, gill and liver tissues of *Tilapia nilotica*, an euryhaline species, exposed to various sublethal copper concentrations for different numbers of days.

Materials and Methods

Tilapia nilotica specimens were brought into the laboratory from pools and acclimatized to laboratory conditions for three months in 4 stock aquaria of 40x100x40 cm. Experiments were run in a constant temperature room at 25±1°C under a 8 h day/16 h night cycle, and the aquaria were aerated during the experimental period. Four series of experiments were conducted for 1,7, 15 and 30 days at environmental salinities of 5‰, 10‰, 15‰ and 20‰ NaCl .

Four glass aquaria (20x60x25 cm) divided into 3 equal volumes were used for each salt concentration. Five litres of solution with each determined salt concentration, containing 0.1, 1.0 and 10.0 ppm copper were added to three of the aquariums. Five litres of salt solution having the same salinities (5, 10, 15 and 20‰) but containing no copper was added to the 4th aquarium for the control group.

In each series, experiments were run three times with one fish in each division. The levels of salinity of the experimental solutions were measured daily by salinimeter (Yellow Spring Instruments) and the solutions were changed every three days.

Some physical and chemical parameters of the experimental environment are given below:

Temperature : 25±1°C

Light Period : 8 hours Light/16 hours darkness

Total Hardness : 233±1.14 ppm $CaCo_3$

Dissolved Oxygen : 7.3±0.25 mg/l.

Copper-Sodium Citrate ($C_6H_5O_7CuNa$) was used to prevent the precipitation of copper (13). A solution of copper-sodium citrate was prepared by mixing copper 2 chloride ($CuCl_2$;H_2O) and tri-sodium citrate ($C_6H_5O_7Na_3$; $5H_2O$) solitions and kept as 1M (6350 ppm Cu) stock solution. Copper solutions of 0.1, 1.0 and 10.0 ppm were prepared by dilution of this stock solution.

For determination of metal accumulation in fishes, they were removed from the aquaria, washed with distilled water and placed on filter paper to remove the excess liquid. Their gill, muscle and liver tissues were dissected separately and dried at 105°C for 48 hours. They were then digested with concentrated nitric and perchloric acid (2:1 v/v) at 120°C for 3 hours and completed to 5 ml with distilled water. After dilution copper concentrations of the tissues were measured at 324.8 nm by using an Instrumentation Laboratories model 751 Atomic Absorbtion Spectrophotometer.

Regression and variance analysis and Student-Newman Keul's Test (S.N.K.) were used for the statistical analysis of the data (15).

Results

At a 10 ppm copper concentration, the rate of mortality was 14% after 30 days. At the end of each experimental period copper levels in the tissues were measured three times for each metal and salt concentration, and their arithmetic means with standard errors are given in Figures 1-3.

In order to determine the effects of exposure time, the determined salt solutions and the different test concentrations on the accumulation of copper in the tissues, the data were analysed by S.N.K. test and the results are given in Table 1.

The statistical test was not applied for gill and muscle tissues. At all salt concentrations tested, the liver accumulation of copper increased according to the experimental time and copper concentration in the medium (Table 1).

At all copper concentrations, a decrease was observed in the liver accumulation of copper with increasing salt concentrations in the medium for each test period (Table:1).

By increasing salt concentration from 5‰ to 20‰, the accumulation of copper in liver decreased 59.72%, 57.77% and 48.48% at 0.1, 1.0 and 10.0 ppm copper concentrations, respectively (Table 1).

Discussion

The highest levels of copper were measured in liver of *Tilapia nilotica*, whereas accumulation was lowest in muscle tissue. It was found that the liver accumulation of copper increased parallel to the medium copper concentration and exposure time at a given salt concentration.

Studies carried out with various fish species have shown that liver tissue is the main site that accumulates and stores copper (16, 17). Fish respond to heavy metal exposure by producing metallothioneins (MTs) particulary in the liver (18). MTs preferentially bind metals and thus project cellular proteins from metal poisoning. An increase was observed in liver MT synthesis in *Oncorhynhus kisutch* and *Pleuronectes platessa* parallel to the increase of metal concentration in the medium (19). It was observed that the MT synthesis under the effects of Cu, Zn and Cd increased for four weeks and then remained at a steady state level in *Salmo gairdneri* (16).

In this study, the lowest accumulation of copper occurred in the muscles and this was followed by gill tissue. The accumulations of heavy metals in fish have shown the copper accumulation capacity of gill (20) and muscle tissues (21) to be lower than that of other metals. The muscle tissue copper accumulation of *Clarias batrachus* was observed to be lower than that of other tissues. (22).

Table: 1 Effects of medium concentration (μg Cu/g dry.wt.) and time on the accumulation of copper in the liver of *Tilapia nilotica* at different NaCl concentrations.

		NaCl Concentrations							
		5‰		10‰		15‰		20‰	
Time (Day)	Cu Content. (ppm)	X±SE	*	X±SE	*	X±SE	*	X±SE	*
	0.0	N.D.		N.D.		N.D.		N.D.	
	0.1	22.16±0.63	ax	16.08±1.43	ay	12.36±0.55	az	9.33±0.04	az
1	1.0	35.14±1.16	bx	26.77±1.15	by	22.03±1.17	by	17.19±0.83	bz
	10.0	54.25±0.87	cx	43.50±0.29	cy	33.69±1.10	cz	25.66±0.67	ct
	0.0	N.D.		N.D.		N.D.		N.D.	
	0.1	61.53±0.82	ax	51.34±1.33	ay	41.48±0.63	az	31.52±0.26	at
7	1.0	85.76±2.40	bx	73.31±1.36	by	65.66±1.03	by	57.63±0.29	bz
	10.0	128.35±1.17	cx	105.71±1.99	cy	85.14±2.37	cz	71.99±1.85	ct
	0.0	N.D.		N.D.		N.D.		N.D.	
	0.1	99.35±2.23	ax	82.12±0.67	ay	60.78±0.21	az	42.80±0.83	at
15	1.0	144.25±0.11	bx	122.73±1.45	by	100.77±2.04	bz	81.39±1.30	bt
	10.0	236.77±1.45	cx	203.55±2.72	cy	147.59±3.82	cz	104.45±0.76	ct
	0.0	N.D.		N.D.		N.D.		N.D.	
	0.1	152.19±3.01	ax	101.66±1.27	ay	78.65±0.65	ay	61.30±2.22	at
30	1.0	211.56±1.61	bx	141.70±0.34	by	115.28±1.67	bz	89.33±2.31	bt
	10.0	294.72±1.84	cx	241.68±1.51	cy	178.23±2.88	cz	151.23±1.49	ct

X±Sx : Mean ± Standard Error

* : SNK; Letters a, b and c show differances among copper; x, y, z and t among NaCl concentrations. Data shown with different letters are statistically significant at the $P<0.01$ level.

N.D.: Not Detectable.

Table: 2 Effects of medium concentration (μg Cu/g dry.wt.) and time on the accumulation of copper in the gills of *Tilapia nilotica* at different NaCl concentrations.

		NaCl Concentrations			
		5‰	10‰	15‰	20‰
Time (Day)	Cu Content. (ppm)	X±SE	X±SE	X±SE	X±SE
	0.0	N.D.	N.D.	N.D.	N.D.
	0.1	N.D.	N.D.	N.D.	N.D.
1	1.0	N.D.	N.D.	N.D.	N.D.
	10.0	N.D.	N.D.	N.D.	N.D.
	0.0	N.D.	N.D.	N.D.	N.D.
	0.1	N.D.	N.D.	N.D.	N.D.
7	1.0	7.88±0.28	N.D.	N.D.	N.D.
	10.0	14.0±0.96	8.21±0.28	5.08±0.31	N.D.
	0.0	N.D.	N.D.	N.D.	N.D.
	0.1	4.91±0.21	N.D.	N.D.	N.D.
15	1.0	11.21±0.10	6.88±0.35	N.D.	N.D.
	10.0	24.68±0.95	12.36±0.55	7.70±0.29	N.D.
	0.0	N.D.	N.D.	N.D.	N.D.
	0.1	8.31±0.14	N.D.	N.D.	N.D.
30	1.0	17.19±0.80	10.36±0.20	5.81±0.01	N.D.
	10.0	30.12±0.45	18.52±0.32	11.96±0.98	N.D.

N.D.: Not Detectable.

In *Mytulis edulis*, an increase was observed in the copper uptake at low salinities, and a decrease of salinity from 35‰ to 15‰ caused an increase in the metal uptake (23). Cadmium-binding protein levels in *Palaemonetes pugio* increased with increases in cadmium exposure concentration and in temperature, and with decreases in salinity (24). The increase of salt concentration in the medium reduces the mortality caused by cadmium and chromium in *Callinectes sapidus* (25).

Accumulation of copper in liver, gill and muscle tissues of *Tilapia nilotica* decreased with increasing salt concentrations in the medium and with a particular copper solution.

The toxicity of heavy metals to fish is reported to be a function of the free metal ion concentration, which is controlled by the chloride content of the water (26, 27). As the chloride ion concentration increases, so the concentration of free metal ion decreases relative to the total metal concentration, due to its complication with chloride ions. On the other hand, it is possible that increased toxicity at reduced salinities may be linked to osmoregulatory impairment (28). When the negative potential difference of the inner body wall increases with decreased salinity, ion transport into organisms consequently increases.

Table: 3 Effects of medium concentration (μg Cu/g dry.wt.) and time on the accumulation of copper of *Tilapia nilotica* in the muscle at different NaCl concentrations.

		NaCl Concentrations			
		5‰	10‰	15‰	20‰
Time (Day)	Cu Content. (ppm)	X±SE	X±SE	X±SE	X±SE
	0.0	N.D.	N.D.	N.D.	N.D.
	0.1	N.D.	N.D.	N.D.	N.D.
1	1.0	N.D.	N.D.	N.D.	N.D.
	10.0	N.D.	N.D.	N.D.	N.D.
	0.0	N.D.	N.D.	N.D.	N.D.
	0.1	N.D.	N.D.	N.D.	N.D.
7	1.0	5.08±0.31	N.D.	N.D.	N.D.
	10.0	10.18±0.51	N.D.	N.D.	N.D.
	0.0	N.D.	N.D.	N.D.	N.D.
	0.1	N.D.	N.D.	N.D.	N.D.
15	1.0	9.33±0.04	N.D.	N.D.	N.D.
	10.0	14.49±0.50	6.11±0.16	N.D.	N.D.
	0.0	N.D.	N.D.	N.D.	N.D.
	0.1	6.44±0.14	N.D.	N.D.	N.D.
30	1.0	13.12±0.47	N.D.	N.D.	N.D.
	10.0	17.51±0.36	9.88±0.22	N.D.	N.D.

N.D.: Not Detectable.

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