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Effect of Heavy Metals on Ca^{2+} Concentration in Muscle Tissue of Grass Carp and Silver Carp

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Abstract: The effect of heavy metals on Ca^{2+}-ATPase from the sarcoplasmic reticulum in fish muscles has been studied, but there is not any evidence about interaction between heavy metals and Ca^{2+} in muscle tissue. The concentration of heavy metals (Zn, Cu, Pb) in two economically important fish species (*Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*) collected from some ponds in Gilan, Iran were determined using flame atomic absorption spectrometry after digestion methods. Trace metals content in fish samples were 0.88-4.50 μg g^{-1} for copper, 1.83-5.92 μg g^{-1} for lead, 5.63-12.63 μg g^{-1} for zinc and 72.31-173.43 μg g^{-1} for calcium. There is no interaction between heavy metals and calcium concentration in muscles.

Key words: Heavy metals, Ca^{2+} in muscle, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*

INTRODUCTION

The concern on the effects of anthropogenic pollution of freshwater ecosystems is growing. Heavy metals from natural and anthropogenic sources are continually released into aquatic ecosystems and they are a serious threat because of their toxicity, long persistence, bioaccumulation and biomagnifications in the food chain. Fish samples are considered as one of the most indicative factors, in freshwater systems, for the estimation of trace metals pollution potential (Papagiannis *et al*., 2004).

Because of complicated kinetics of metals (absorption, distribution, metabolism, elimination, deposition and excretion) and their mutual interactions, it is almost impossible to predict metal effects on tissue concentrations based on their concentrations in the water. Also, the biological availability of an element and its ultimate effect on an organism may depend on a chemical form in which the element is encountered (Kosanovic *et al*., 2007).

In muscle contraction, Ca^{2+} is released from sarcoplasmic reticulum into muscle cells via Ca^{2+}-release channel, Ca^{2+}-ATPase then pumps back the released Ca^{2+} into the SR to cause relaxation. This pump runs as long as ATP and Ca^{2+} are present in the cytoplasm (Toyoshima, 2008). The Ca^{2+}-ATPase of the Sarcoplasmic Reticulum (SR) is an intrinsic membrane enzyme playing an important role in the muscle contraction of skeletal muscle (Godiksen and Jessen, 2002). Contraction is regulated by Ca^{2+}-sensitive molecular switches on the myosin or actin filaments, depending on the muscle and species. At low Ca^{2+}

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levels, actin-myosin interaction is inhibited and actin-activated myosin ATPase is low. At high Ca^{2+} concentrations, the inhibition of actin–myosin interaction is removed and myosin ATPase is activated by actin (Zhao and Craig, 2003). Voltage-gated Ca^{2+} channels represent a route of entry into the cell for a wide variety of divalent and trivalent metals (Zalpus and Koropatnick, 2000). A number of metals have been examined and found to have blocking effects and are likely to permeate Ca^{2+} channels, including Zn (Winegar *et al*., 1991).

Omnivorous, sediment-dwelling, fish species such as carp may therefore, accumulate heavy metals more readily than pelagic species as a result of exposure to the generally higher metal content of sediment compared with the water column (Alam *et al*., 2002). Grass carp (*C. idella*) has received much attention all around the world, primarily due to its potential use for biological control of aquatic vegetation (Vigh *et al.,* 1996). Cd, Cu and Zn contents in edible muscles of pelagic fish species were lower than for benthic fish species. Similarly, the Cd, Cu and Zn contents in muscles of *Hypophthalmichthys molitrix* (pelagic fish) were lower than those in *Cyprinus carpio* (benthic fishes). The Pb contents in muscles showed no correlation with fish species (Chi *et al*., 2007).

In present investigation, the levels of heavy metals and calcium in fish muscles collected from some ponds in Gilan province, Iran, were determined by flame atomic absorption spectrometry after digestion. Then mean concentrations were compared.

MATERIALS AND METHODS

The fish samples (*Ctenopharyngodon idella, Hypophthalmichthys molitrix)* were collected from three different ponds in Gilan province, Iran in 2002. The collected samples were transferred to the laboratory and washed with distilled water, stored in plastic bags at -20°C until dissection.

Samples (50 g) were weighed and then dried at 105°C for 24 h. Approximately 0.5 g of dried samples were digested in 4 mL nitric acid and after 24 h added 1 mL perchloric acid, followed by heating at a temperature of 60°C for 1 h, then temperature was raised to 120 for 1 h. Samples were then allowed to cool and final solutions were made up to 10 mL with double distilled water (Mason and Barak, 1990). In order to determine Ca^{2+} in muscle, samples put in muffle furnace set at a temperature of 105°C for 10 h, then ashes were weighted, heated at 505°C until the ash was free from all visible traces of carbon. The ash is treated with 20.0 cm^3 (1-1) HCl. The solution is transferred quantitatively to a 50 cm^3 volumetric flask. A total of 6.0 cm^3 of 25000 ppm lanthanum is added and the resulting solution diluted to the mark with water (Udoh, 2000).

Following acid digestion, all samples were analyzed for elements by flame atomic absorption spectrometry (Shimadzu.AA68V). The operating parameters for working elements were set as recommended by the manufacturer (Table 1). Lead, zinc, copper and calcium were determined in air-acetylene flame. A blank solution is also prepared and analyzed. There are several methods for the determination of metallic elements in biological samples. Atomic absorption spectroscopy is arguably faster, more sensitive and reliable but the determination

Table 1: Instrumental analytical conditions of investigated elements conditions for absorption spectrometry

Elements	Acetylene (L min^{-1})	Air (L min^{-1})	Wavelength (nm)	Slit width (nm)
Ca	2	17	422.7	0.5
Pb	2	17	283.3	1.0
Zn	2	17	213.9	0.7
Cu	2	17	324.8	0.7

of calcium poses a problem because of the interference of phosphate ion. This interference is usually overcome by the addition of lanthanum to the sample solution prior to analysis (Fritze *et al*., 1987). All digested samples were analyzed three times for each metal.

All reagents were of analytical reagent grade, unless otherwise stated. Double deionised water (Milli-Q Millipore 18.2 MX cm^{-1} resistivity) was used for all dilutions. HNO_3, H_2O_2 and HCI were of Suprapur quality (E.Merck, Darmstadt, Germany). All the plastic and glassware were cleaned by soaking in dilute HNO_3 (1/9, v/v) and were rinsed with distilled water prior to use. The element standard solutions used for calibration were produced by diluting a stock solution of 1000 mg L^{-1} of the given e lement, supplied by Sigma Chemical Co. (St. Louis, MO). The standard solutions were prepared from Pb $(NO_3)_2$, $CuSo_4$, $ZnCl_2$ and $CaCO_3$.

RESULTS AND DISCUSSION

All metal concentrations were determined on a dry weight basis. The levels of zinc, copper, lead and calcium in muscle tissue of grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*) are given in Table 2.

Statistical analysis of data was carried out using SPSS statistical package program. According to these data, the high metal accumulation levels in the species were found in *C. idella* for Cu, Zn and *H. molitrix* for Pb, respectively. The highest and lowest concentrations of calcium in *H. molitrix* were found to be 173.43 and 83.35 μg g^{-1}, respectively. While mean concentration of calcium in *C. idella* ranged from 72.31 to 109.75 μg g^{-1}. Average copper content of *H. molitrix* samples varied from 0.88 to 2.63 μg g^{-1}. The maximum and minimum copper levels in *C. idella* were 1.13 and 4.50 μg g^{-1}, respectively. Our copper value was higher than those reported earlier (Vigh *et al*., 1996). The highest and lowest levels of lead (5.92 and 1.83 μg g^{-1}) were detected in *H. moltrix*. Our lead value was higher than those reported earlier (Chi *et al*., 2007). In addition, maximum lead level was higher than those reported from Iranian Fisheries Research Organization (Sadeghi, 1993). Mean zinc concentrations ranged from 5.63-12.63 μg g^{-1} measured in *C. idella*. Mea zinc content in samples of *H. molitrix* was between 6.88-12.13 μg g^{-1} which was lower than those reported earlier (Chi *et al*., 2007).

No correlation was reported, however, between metal concentration in the water and fish muscles. Muscle was a poor indicator of low level copper and zinc contamination. That is also true for most other metals, except for mercury which shows higher affinity to the muscles comparing to other metals (Dural *et al*., 2007).

According to standard deviation in Table 3, there is no significant difference between mean calcium concentrations in silver grass carp and Pb, Zn, Cu accumulation in muscle. In addition, mean difference between calcium and heavy metals in muscle tissue of silver carp

Table 2: Maximum and minimum levels of heavy metals (μg/g dw) in *C. idella* and *H. molitrix*

Species	Metals	N	Mean	SD	Minimum	Maximum
Hypophthalmichthys molitrix	Ca	9	110.69	33.02000	83.35	173.43
	Cu	9	1.74	0.54000	0.88	2.63
	Pb	9	3.60	1.26000	1.83	5.92
	Zn	9	9.71	2.17000	6.88	12.13
Ctenopharyngodon idella	Ca	9	89.29	14.40000	72.31	109.75
	Cu	9	2.26	1.34000	1.13	4.50
	Pb	9	3.83	0.78111	2.50	5.13
	Zn	9	8.68	2.62000	5.63	12.63

Table 3: Comparison of heavy metals and calcium in muscle tissue of grass carp

Species/metal	Species/metal	Mean difference	SD
C. idella/Ca	*H. moltrix*/Ca	-21.40667*	0.001
C. idella/Ca	*H. moltrix*/Cu	87.55056*	0.000
C. idella/Ca	*H. moltrix*/Pb	85.68544*	0.000
C. idella/Ca	*H. moltrix*/Zn	79.57833*	0.000
C. idella/Ca	*C. idella*/Cu	87.02278*	0.000
C. idella/Ca	*C. idella*/Pb	85.45111*	0.000
C. idella/Ca	*C. idella*/Zn	80.60611*	0.000
C. idella/Cu	*H. moltrix*/Ca	-108.4294*	0.000
C. idella/Cu	*H. moltrix*/Cu	0.52778	0.931
C. idella/Cu	*H. moltrix*/Pb	-1.33733	0.825
C. idella/Cu	*H. moltrix*/Zn	-7.4444	0.222
C. idella/Cu	*C. idella*/Ca	-87.02278*	0.000
C. idella/Cu	*C. idella*/Pb	-1.057167	0.796
C. idella/Cu	*C. idella*/Zn	-6.41667	0.292
C. idella/Pb	*H. moltrix*/Ca	-106.8577*	0.000
C. idella/Pb	*H. moltrix*/Cu	2.09944	0.729
C. idella/Pb	*H. moltrix*/Pb	0.23433	0.969
C. idella/Pb	*H. moltrix*/Zn	-5.87278	0.335
C. idella/Pb	*C. idella*/Ca	-85.4511*	0.000
C. idella/Pb	*C. idella*/Cu	1.57167	0.796
C. idella/Pb	*C. idella*/Zn	-4.84500	0.425
C. idella/Zn	*H. moltrix*/Ca	-102.0127*	0.000
C. idella/Zn	*H. moltrix*/Cu	6.94444	0.255
C. idella/Zn	*H. moltrix*/Pb	5.07933	0.404
C. idella/Zn	*H. moltrix*/Zn	-1.02778	0.865
C. idella/Zn	*C. idella*/Ca	-80.60611*	0.000
C. idella/Zn	*C. idella*/Cu	6.41667	0.292
C. idella/Zn	*C. idella*/Pb	4.845	0.425

*Significant difference

Table 4: Comparison of heavy metals and calcium in muscle tissue of silver carp

Species/metal	Species/metal	Mean difference	SD
H.moltrix/Ca	*H. moltrix*/Cu	108.95722*	0.000
H. moltrix/Ca	*H. moltrix*/Pb	107.09211*	0.000
H. moltrix/Ca	*H. moltrix*/Zn	100.98500*	0.000
H. moltrix/Ca	*C. idella*/Ca	21.40667*	0.001
H. moltrix/Ca	*C. idella*/Cu	108.42944*	0.000
H. moltrix/Ca	*C. idella*/Pb	106.85778*	0.000
H. moltrix/Ca	*C. idella*/Zn	102.01278*	0.000
H. moltrix/Cu	*H. moltrix*/Ca	-108.9572*	0.000
H. moltrix/Cu	*H. moltrix*/Pb	-1.86511	0.758
H. moltrix/Cu	*H. moltrix*/Zn	-7.97222	0.192
H. moltrix/Cu	*C. idella*/Ca	-87.55056*	0.000
H. moltrix/Cu	*C. idella*/Cu	-0.52778	0.931
H. moltrix/Cu	*C. idella*/Pb	-2.09944	0.729
H. moltrix/Cu	*C. idella*/Zn	-6.94444	0.255
H. moltrix/Pb	*H. moltrix*/Ca	-107.0921*	0.000
H. moltrix/Pb	*H. moltrix*/Cu	1.86511	0.758
H. moltrix/Pb	*H. moltrix*/Zn	-6.10711	0.316
H. moltrix/Pb	*C. idella*/Ca	-85.68544*	0.000
H. moltrix/Pb	*C. idella*/Cu	1.33733	0.825
H. moltrix/Pb	*C. idella*/Pb	-0.23433	0.969
H. moltrix/Pb	*C. idella*/Zn	-5.07933	0.404
H. moltrix/Zn	*H. moltrix*/Ca	-100.9850*	0.000
H. moltrix/Zn	*H. moltrix*/Cu	7.97222	0.192
H. moltrix/Zn	*H. moltrix*/Pb	6.10711	0.316
H. moltrix/Zn	*C. idella*/Ca	-79.57833*	0.000
H. moltrix/Zn	*C. idella*/Cu	7.44444	0.222
H. moltrix/Zn	*C. idella*/Pb	5.87278	0.335
H. moltrix/Zn	*C. idella*/Zn	1.02778	0.865

*Significant difference

were compared, there is no standard deviation (Table 4). The Ca-ATPase activity in the muscle decreased due to heavy metals exposure. 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 (Atli and Canli, 2007). There is not any evidence which demonstrate direct interaction between heavy metals and Ca^{2+} in muscles.

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