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RESEARCH ARTICLE

Metal concentrations in tissues of common carp, *Cyprinus carpio*, and silver carp, *Hypophthalmichthys molitrix* from the Zarivar Wetland in Western Iran

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Abstract. The aim of this paper was to monitor the concentrations of mercury (Hg), cadmium (Cd), lead (Pb), copper (Cu), and nickel (Ni) in the gills, scales, and muscles of two fish species, common carp, *Cyprinus carpio* L., and silver carp, *Hypophthalmichthys molitrix* (Val.), from the Zarivar Wetland in Western Iran, and to identify any relationships between the species. The metal concentrations in the tissues of common carp and silver carp decreased in the following sequence: Cu > Pb > Hg > Ni > Cd. The results indicated no significant differences between the males and females of either fish species ($P > 0.05$). The results also showed that there were significant correlations ($P < 0.05$) between fish total length and weight and concentrations of Cd, Pb, and Cu in the different muscle tissues examined.

Keywords: metal contamination, Hg, Cd, size, weight

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Introduction

Fish is an important source of protein, and with its omega-3 polyunsaturated fatty acids, which reduce cholesterol levels, it contributes to human heart, brain, joint, and immune system health (Anderson and Wiener 1995, Daviglus et al. 2002, Patterson 2002). Fish have been used as one of the most indicative organisms for assessing metal pollution in freshwater and marine systems (Erdogrul and Ates 2006). Fish accumulate considerable amounts of metals in their tissues, especially in the muscles, and it is a major dietary source of these metals for humans (Dural et al. 2006). Metal contamination of freshwater ecosystems is a concern worldwide, because metals are permanent pollutants, and, when they exceed certain concentrations, most of them have toxic effects on living organisms (Ghrefat and Yusuf 2006). Nickel is available abundantly in the Earth's crust, but once released into the environment, it readily forms complexes with many ligands, making it more mobile than most heavy metals (Palaniappan and Karthikeyan 2009). Copper is an essential element that is carefully regulated by physiological mechanisms in many organisms (Erdogrul and Ates 2006). Cadmium and lead are the most significant of all the metals since they are both very toxic and very common. Mercury contamination causes pathological changes in fish, and the consequential inhibition of metabolic processes, hematological changes, declines in fertility and survival, and protein denaturation in the gills (Bhomre et al. 1996, Kaoud 2011). The only significant source of methylmercury exposure for humans is fish consumption (Rice et al. 2000).

Metal pollution in aquatic ecosystems is a concern because of bioaccumulation, biomagnification, and human exposure to these pollutants (Mansouri et al. 2012a). Essential metals, such as Cu and Ni, play important roles in biological systems, while non-essential metals, such as Hg, Cd, and Pb, have no known role in biological systems (Karadede-Akin and Ünlü 2007). Because of its high toxicity at low concentrations, Hg has long been recognized as an environmental pollutant (Khangarot 2003, Ebrahimpour et al. 2010), and in both inorganic and organic forms it ishighly lethal and poses significant threats to aquatic biota (Skubal and Meshkov 2002). It is also a neurological toxicant to humans and can cause mental retardation, blindness, cerebral palsy, and other birth defects (Chevrier et al. 2009). In freshwater ecosystems, the main food sources of wild carp are insects, crustaceans, crayfish, and benthic worms found in sediments. Therefore, this species plausibly accumulate and store metals more rapidly than pelagic species. This is because of connection to the generally higher metal content of the sediments compared to that in the water column (Phillips 1980). Common carp and silver carp are local food sources in the present study area. Therefore, the aim of this study was to determine the concentrations of Hg, Cd, Pb, Cu, and Ni in common carp and silver carp to examine species- and gender-related variations in trace metal accumulation, to identify any relationships between the species, and to determine the significance among mercury concentrations in gills, scales, and muscles.

Materials and Methods

The Zarivar Wetland is located in the Zagros Mountains in Western Iran at an altitude of 1,278 m above mean sea level. It extends from 35°31'30" to 35°37'06" N and from 46°03'52" to 46°56 10'47" E. The main water sources are rainfall (average of 800 mm per year in 2003-2007) and springs on the wetland floor. The water surface area of this wetland varies seasonally from 1,300 to 2,300 ha. The total area and water depth are 720 ha and 4-5 meters, respectively. Based on calculations, the volume of the Zarivar Wetland fluctuates varies from about 22 to 47 million cubic meters of water. The numbers of tourists coming to see the unique plant and animal diversity of the wetland has been increasing steadily in recent years. Furthermore, wastewater discharge from adjacent populated areas, mainly Marivan City, chemical fertilizers and pesticides from farmlands, and improper solid waste management have all increased over the past few years.

The fish for the study were caught in the Zarivar Wetland using commercial fishing nets in February 2012, and then transported to the laboratory. A total of 20 individuals of two species were analyzed for mercury concentrations in the gills, scales, and muscle tissues. The study material comprised common carp (males = 5 and females = 5) and silver carp (males = 3 and females = 7). The total length and weight of the 20 fish were measured and were 411.5 (± 34.3) cm and 753.5 (± 18.1) g for common carp, and 353.5 (± 16.1) cm and 764.8 (± 19.8) g for silver carp, respectively. In the laboratory, personnel wearing clean plastic gloves immediately dissected the fish with stainless steel dissection instruments. Skinless muscle samples were excised from below the dorsal fin using the method by UNEP (1984). Approximately 0.2 g dry weight (d.w.) of each sample (gill, scale, muscle) was dissected and placed in 150 ml Erlenmeyer flasks. Ten ml of nitric acid (65%) was added to each sample, and they were left overnight to digest slowly. Thereafter, 5 ml perchloric acid (70%) was added to each sample. Digestion was performed on a hot plate (sand bath) at 90°C, for about 1 h. After cooling, the solution was transferred quantitatively to 50 ml polyethylene bottles and made up to 25 ml with deionized water (Anderson and Meyers 2000). Then the solution was filtered using 0.45 μm nitrocellulose membrane filters.

Determinations of Hg in the tissues were performed in a Perkin Elmer 3030, and Cd, Pb, Cu, and Ni concentrations were measured in a GFA-EX7i graphite furnace atomic absorption spectrometer. Recovery varied between 98.9 and 100%. The detection limits for each metal were: Cd (0.05), Pb (0.8), Cu (0.55), Ni (0.7), Hg (0.2).

Data analyses were performed using the statistical package SPSS (version 16; SPSS, Chicago, IL). The significance of differences among the means in gill, scales, and muscle tissues of the two fish species were evaluated with one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test. Differences between metal concentrations in the tissues of males and females were tested using Student's *t*-test. Pearson's correlation (r) was used to analyze the correlations. The concentration of metals in the tissues was expressed in micrograms per gram of dry weight. Values are means ± standard deviation (SD), and the level of significance was set at $P \leq 0.05$.

Results and discussion

Variations in metal concentrations in the gills, scales, and muscles of common carp and silver carp are presented in Tables 1, 2, and 3, respectively. The mean concentrations of Hg, Cd and Pb were 1.1, 0.1, and 1.6 μg g^{-1} for gills; 0.8, 0.1, and 1.1 μg g^{-1} for scales; and 1.1, 0.1, and 1.4 μg g^{-1} for muscles of common carp, respectively. For silver carp, the mean concentrations of Hg, Cd and Pb were 1.3, 0.2, and 1.6 μg g^{-1} for gills; 1.1, 0.1, and 1.2 μg g^{-1} for scales; and 0.8, 0.1, and 1.5 μg g^{-1} for muscles, respectively. The mean concentrations of Cu and Ni were 2.4 and 0.4 μg g^{-1} for gills; 1.7 and 0.2 μg g^{-1} for scales; and 2.1 and 0.3 μg g^{-1} for muscles of common carp, respectively. For silver carp, the mean concentrations of Cu and Ni were 2.1 and 0.4 μg g^{-1} for gills; 1.9 and 0.4 μg g^{-1} for scales; and 0.1 and 1.5 μg g^{-1} for muscles, respectively. The results of this study showed that the gills accumulated the highest levels of metals in both species. Metal concentrations in the tissues of common carp and silver carp decreased in the following sequence: Cu > Pb > Hg > Ni > Cd. The results also indicated that there were no significant differences between males and females of either fish species (*t*-test, $P > 0.05$). Pb, Cu, and Ni concentrations differed significantly among the common carp tissues (one-way ANOVA, $P < 0.05$), while there were significant differences in Hg and Ni concentrations in silver carp (one-way ANOVA, $P < 0.05$; Table 4).

Concentrations of Hg in the muscles of common carp and silver carp from Zarivar Wetland were compared with those reported for fishes from other areas. Hg concentrations in the muscles of common carp (1.1 μg g^{-1}) and sliver carp (0.8 μg g^{-1}) from the present study were higher than those in torpedo scad, *Megalaspis cordyla* (L.) (0.24 μg g^{-1}), bigeye scad, *Selar crumenophthalmus* (Bloch) (0.13 μg g^{-1}), redtail scad, *Decapterus kurroides* Bleeker (0.11 μg g^{-1}), javelin grunter, *Pomadasys kaakan* (Cuvier) (0.09 μg g^{-1}), and black pomfret, *Parastromateus niger* (Bloch) (0.05 μg g^{-1}) from marine fish in Southeast Asia (Agusa et al. 2007); short-bodied mackerel, *Rastrelliger brachysoma* (Bleeker) (0.14 μg g^{-1}), black pomfret (0.03 μg g^{-1}), and narrow-barred Spanish mackerel, *Scomberomorus commerson* (Lacepède) (0.01 μg g^{-1}) from Malaysia (Hajeb et al. 2009); and Caspian roach, *Rutilus caspicus* (Yakovlev) (0.20 μg g^{-1}) and Caspian sprat, *Clupeonella cultriventris* (Nordmann) (0.05 μg g^{-1}) from the Caspian Sea (Anan et al. 2005). In the present study, Hg concentrations in the muscles of the two fish species examined were lower than those in queen fish, *Scomberoides lysan* (Forsskål) (1.8 μg g^{-1}), goat fish, *Upeneus sulphureus* Cuvier (1.9 μg g^{-1}), and catfish (1.8 μg g^{-1}) from the state of Penang, Malaysia (Sivalingam and Sani 1980). The concentrations of heavy metals were expressed as dry weight; a wet weight (w.w.)-dry (w.d.) weight conversion factor of 0.2 can be used to convert the concentrations (Pourang et al. 2005). The results indicated that the concentrations of Cu (0.42 and 0.38 μg g^{-1} w.w.), Cd (0.02 and 0.02 μg g^{-1} w.w.), Pb (0.28 and 0.30 μg g^{-1} w.w.) and Hg (0.22 and 0.16 μg g^{-1} w.w.) in the fish from the Zarivar Wetland were lower than the guidelines for food summarized by Jones and

Table 1
Mean (±SD) metal concentrations (μg g^{-1} d.w.) in the gills of common carp (*C. carpio*) and silver carp (*H. molitrix*) from the Zarivar Wetland in western Iran

			Heavy metals				
Species	Sex	N	Hg	Cd	Pb	Cu	Ni
Common carp	Male	5	1.0±0.4	0.1±0.1	1.7±0.4	2.6±0.4	0.4±0.2
	Female	5	1.3±0.3	0.1±0.1	1.5±0.6	2.2±0.4	0.3±0.2
	Min-Max		0.2-1.7	0.02-0.3	0.9-2.6	1.8-3.0	0.1-0.7
	Overall mean		1.1±0.4	0.1±0.1	1.6±0.5	2.4±0.4	0.4±0.2
	P-value		NS	NS	NS	NS	NS
Silver carp	Male	3	1.2±0.8	0.2±0.1	1.7±0.3	2.2±0.5	0.3±0.2
	Female	7	1.3±0.5	0.2±0.1	1.6±0.3	2.1±0.5	0.5±0.1
	Min-Max		0.5-2.2	0.05-0.3	0.9-2.1	1.4-2.9	0.1-0.7
	Overall mean		1.3±0.6	0.2±0.1	1.6±0.4	2.1±0.5	0.4±0.2
	P-value		NS	NS	NS	NS	NS

P value for Student's *t*-test to compare between males and females; NS not significant

Table 2
Mean (±SD) metal concentrations (μg g^{-1} d.w.) in the scales of common carp (*C. carpio*) and silver carp (*H. molitrix*) from the Zarivar Wetland in western Iran

			Heavy metals				
Species	Sex	N	Hg	Cd	Pb	Cu	Ni
Common carp	Male	5	0.8±0.4	0.1±0.1	1.1±0.3	1.9±0.2	0.2±0.1
	Female	5	0.7±0.3	0.1±0.1	1.1±0.4	1.6±0.2	0.1±0.1
	Min-Max		0.2-1.3	0.01±0.3	0.6-1.7	1.4-2.2	0.04-0.4
	Overall mean		0.8±0.3	0.1±0.1	1.1±0.3	1.7±0.2	0.2±0.3
	P-value		NS	NS	NS	NS	NS
Silver carp	Male	3	1.1±0.1	0.1±0.1	1.1±0.5	1.6±0.8	0.2±0.1
	Female	7	0.9±0.2	0.1±0.1	1.2±0.3	1.8±0.4	0.2±0.1
	Min-Max		0.6-1.4	0.02-0.2	0.6-1.7	1.1-2.6	0.03-0.4
	Overall mean		1.1±0.2	0.1±0.1	1.2±0.3	1.8±0.5	0.2±0.1
	P-value		NS	NS	NS	NS	NS

P value for Student's *t*-test to compare between males and females; NS not significant

Table 3
Mean (±SD) metal concentrations (μg g^{-1} d.w.) in the muscles of common carp (*C. carpio*) and silver carp (*H. molitrix*) the Zarivar Wetland in western Iran

			Heavy metals				
Species	Sex	N	Hg	Cd	Pb	Cu	Ni
Common carp	Male	5	1.1±0.5	0.1±0.09	1.5±0.3	2.3±0.4	0.3±0.1
	Female	5	1.1±0.4	0.1±0.08	1.4±0.3	1.9±0.6	0.3±0.1
	Min-Max		0.4-1.9	0.01-0.2	0.8-2.3	1.6-2.8	0.1-0.8
	Overall mean		1.1±0.4	0.1±0.08	1.4±0.4	2.1±0.4	0.3±0.2
	P-value		NS	NS	NS	NS	NS
Silver carp	Male	3	0.4±0.3	0.1±0.07	1.5±0.4	1.8±0.6	0.3±0.1
	Female	7	0.9±0.2	0.1±0.07	1.5±0.3	1.9±0.5	0.4±0.1
	Min-Max		0.3-1.3	0.04-0.2	0.8-1.9	1.2-2.7	0.2-0.6
	Overall mean		0.8±0.3	0.1±0.07	1.5±0.3	1.9±0.5	0.4±0.1
	P-value		NS	NS	NS	NS	NS

P value for Student's *t*-test to compare between males and females; NS not significant

Table 4
Statistical analysis of mercury, cadmium, lead, copper, and nickel concentrations in the gills, scales, and muscles of common carp (*C. carpio*) and silver carp (*H. molitrix*)

	Hg		Cd		Pb		Cu		Ni	
	one way ANOVA		one way ANOVA		one way ANOVA		one way ANOVA		one way ANOVA	
Species	F	P	F	P	F	P	F	P	F	P
Common carp	2.00	NS	0.61	NS	3.66	< 0.05	7.11	< 0.01	3.37	< 0.05
Silver carp	3.61	< 0.05	1.85	NS	2.56	NS	1.04	NS	3.27	< 0.05

NS – not significant

Table 5
Correlation coefficients of size, weight, and contaminant levels in muscles of common carp (*C. carpio*) and silver carp (*H. molitrix*) caught in the Zarivar Wetland

Species		Hg	Cd	Pb	Cu	Ni	Size	Weight
Common carp	Hg	1	0.3	0.5	0.06	0.55	0.3	0.11
	Cd		1	0.97**	0.76*	0.57	0.88**	0.92**
	Pb			1	0.71*	0.75*	0.85**	0.83**
	Cu				1	0.36	0.75*	0.74*
	Ni					1	0.49	0.50
	Size						1	0.82**
	Weight							1
Silver carp	Hg	1	-0.35	-0.29	-0.21	-0.19	-0.28	-0.39
	Cd		1	0.85**	0.68*	0.64*	0.89**	0.70*
	Pb			1	0.73*	0.53	0.93**	0.74*
	Cu				1	0.62	0.85**	0.74*
	Ni					1	0.59	0.59
	Size						1	0.87**
	Weight							1

**Correlation is significant at a level of 0.01
*Correlation is significant at a level of 0.05

Franklin (2000) (Cu, 20 µg g^{-1} w.w.; Cd, 0.2 µg g^{-1} w.w.; Pb, 2.0 µg g^{-1} w.w.; Hg, 0.3 µg g^{-1} w.w.). These two fish species are consumed abundantly in the region, and based on the concentrations of metals found in the muscle tissues, pose no health risks to the local people. However, the discharge of Marivan municipal wastewater, fertilizers, pesticides, and agricultural wastewater into the wetlands, and increasing pressure from tourists might have serious effects on this water body. High concentrations of metals in wastewater discharge and their possible bioaccumulation and biomagnification might cause increases of metal concentrations in fish tissues in the future.

The gills and scales were observed to have the highest and lowest concentration of metals in common carp and silver carp, respectively. The high concentrations of metals in the gills could have stemmed from the elements complexing with mucus in the gills, which come into direct contact with the surrounding waters (Tekin-Özan and Kir 2007). In other words, the gills are the main sites of metallothionein (MT) production and metal retention (Asagba et al. 2008). Therefore, the main reason for the high concentration of metals in this organ is its capacity to accumulate metals by the induction of the metal-binding protein MT. The severity of damage in gills depends on the concentration of toxicants and the duration of exposure (Koca et al. 2008). Of all the organs, gills receive the most exposure to environmental pollutants (Oliveira-Filho et al. 2010), and are important sites for the entry of heavy metals that provoke lesions and gill damage (Vinodhini and Narayanan 2008). Thus, the concentration of metals in gills might be a reflection of their concentration in the water column (Ikem et al. 2003). In the present study, there was no significant difference in metal accumulation between male and female fish, which might stem from the similar foraging strategies of both sexes of these two species. Keenan and Alikhan (1991) did not observe any differences in cadmium concentrations between males and females of *Cambarus bartoni* (Fabricius) specimens; however, Al-Yousuf et al. (2000) report that metal concentrations were higher in females than in males in a study of heavy metals in the tissues of *Lethrinus lentjan* (Lacepčde).

Pearson's correlation coefficients of size, weight and metal concentrations in common carp and silver carp caught from the Zarivar Wetland are presented in Table 5. Significant positive correlations ($P < 0.001$) were observed between total length and weight in these two fish species. Highly positive correlations for Cd and Pb concentrations were noted with length and weight ($P < 0.01$). Moderately positive correlations were noted between Cu concentrations and length and weight ($P < 0.05$) in common carp. On the other hand, highly positive correlations between Cd and Pb concentrations and length and weight ($P < 0.01$) were observed in common carp, while highly and moderately positive correlations were noted for Cd, Pb, and Cu concentrations with length ($P < 0.01$), and weight ($P < 0.05$) in silver carp. In contrast, negative correlations were found between muscle Hg concentrations and body length and weight in silver carp from the Zarivar Wetland. Conflicting data exist with respect to length-dependent and weight-dependent metal body burdens in fish. Some studies report positive correlations of metal concentrations with length and weight, while others report negative correlations in various fish species. Anan et al. (2005) reported positive correlations between Pb, Se, Co, and Hg concentrations for muscles and body weight in fishes collected from the coastal waters of the Caspian Sea. Agusa et al. (2005) also noted positive correlations between total length and concentrations of Cu, Cd, and Se. Conversely, Honda et al. (1983) reported concentrations of Mn, Cu, Cd, and Pb in muscles decreased with increased body weight in the Antarctic fish *Pagothenia borchgrevinki* (Boulenger) Al-Yousuf et al. (2000) also observed negative correlations between muscular Mn, Cu, and Cd concentrations and total length in the Persian Gulf fish *L. lentjan*. Generally, length-dependent and weight-dependent variations of metal concentrations are affected by several factors such as different ecological needs, metabolic rates, feeding patterns, and growth dilution of metals (Phillips 1980, Langston and Spence 1995, Yilmaz 2003). The present results indicated there was a highly positive correlation between Cd and Pb ($P < 0.001$) in both fish species, and moderately positive correlations between Cd with Cu, and Pb with Cu and Ni ($P < 0.001$) in common carp, and Cd with Cu and Ni, and Pb with Cu ($P < 0.001$) in silver carp. This suggests that metals with highly positive relationships are probably from the same contamination sources (Mansouri et al. 2012b).

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