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Contamination of Metals in Tissues of *Ctenopharyngodon idella* and *Perca fluviatilis*, from Anzali Wetland, Iran

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Abstract The objective of the present study was to investigate the levels of metals (Cd, Cu, Zn, Pb and Cr) in muscle, gill, liver, kidney and intestine of two freshwater fish, *Ctenopharyngodon idella* and *Perca fluviatilis*, in Anzali Wetland, Iran. The concentrations were different between the fish species as well as among the tissues of fish. Results showed that the metal concentrations in both fish species were in descending order of Zn > Cu > Pb > Cr > Cd. Results also showed that the Cd, Cu, Zn, and Pb concentrations in the muscle of both fish from Anzali Wetland are below levels of concern for human consumption.

Keywords Carnivore · Concentration · Fish species · Freshwater fish · Herbivore

Anzali Wetland (Anzali lagoon) is located in Northern Iran (Fig. 1). It covers an area of about 200 km^2 situated between 37°28′ N and 49°25′ E. It is located at the south-western coast of the Caspian Sea, close to the city of Bandar-e-Anzali, and colonized by a pretty diverse wetland flora and fauna. The water system is comprised of large, eutrophic freshwater basins, shallow impoundments, marshes and seasonally flooded grasslands. The average length of this complex is about 30 km and its average width is about 3 km. The depth of Anzali Wetland is also subject to changes. The average depth is about 3 m. This wetland has a passage to the Sea with the width of 426 m.

Contamination of aquatic ecosystems (e.g. lakes, rivers, streams, etc.) with metals has been receiving increased worldwide attention due to their harmful effects on human health and other organisms in the environment (Palaniappan and Karthikeyan 2009). Metals are considered to be the most important form of aquatic pollution because of their accumulation by aquatic organisms metals (Ashraf et al. 2006). The contamination of freshwaters with a wide range of pollutants has become a matter of great concern over the last few decades, not only because of the threat to public water supplies, but also with of the damage caused to the aquatic life (Canli et al. 1998). Metals from natural to anthropogenic sources are continually released into aquatic ecosystems, and they are a serious threat because of their toxicity, bioaccumulation, long persistence, and bio-magnification in the food chain (Erdoĝrul and Ates 2006). Once metals are accumulated by an aquatic organism they can be transferred through the upper classes of the food-chain (Canli et al. 1998). Human activities such as metal-related industries have greatly increased the input of trace metals into aquatic systems, where these metals are accumulated by aquatic organisms and may be further transferred up to the top trophic levels (Xu and Wang 2002). Aquatic organisms are widely used to biologically monitor variation in environmental levels of anthropogenic pollutants (Farkas et al. 2003).

There has been a growing interest in assessing the levels of trace metals in food. The ingestion of food is an obvious means of exposure to metals, not only because many metals are natural components of foodstuffs but also because of environmental contamination and contamination during food

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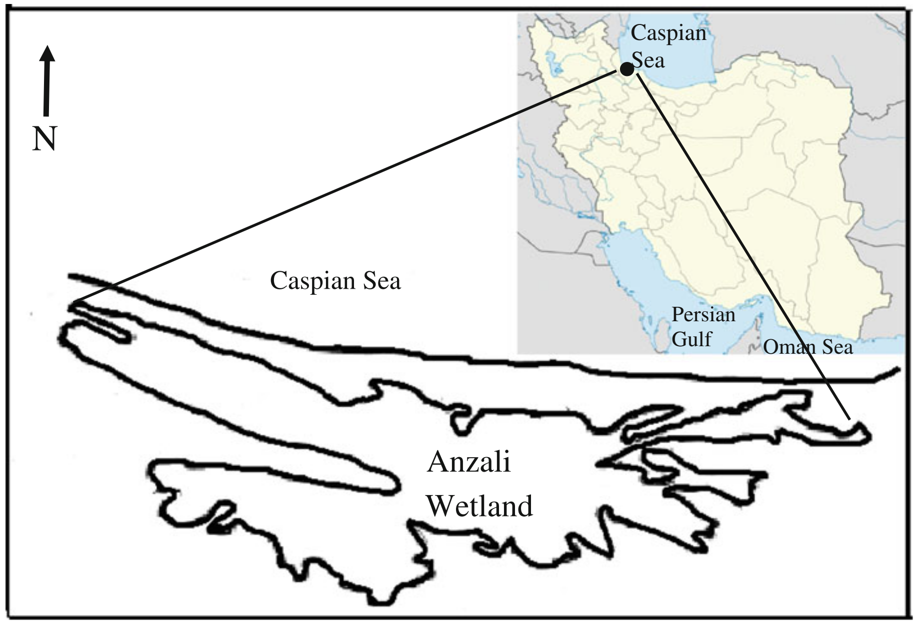

Fig. 1 Map of Anzali wetland

processing/packaging (Ashraf et al. 2006). Fish samples are considered as one of the most indicative factors, in freshwater systems, for the estimation of trace metals pollution potential (Erdoĝrul and Ates 2006). Fish accumulates substantial amounts of metals in their tissues especially muscles and thus, represent a major dietary source of these metals for humans (Dural et al. 2006). Knowledge of metal concentrations in fish is important both with respect to nature management and human consumption of fish (Ebrahimpour and Mushrifah 2010). Metals are taken up through different organs of the fish because of the affinity between them. In this process, many of these metals are concentrated at different levels in different organs of the body (Erdoĝrul and Ates 2006). Fish in freshwater environments near urban and industrial locations are often subjected to pollutants due to run-off and in most cases deliberate discharges. Knowledge of the distribution of metals in tissues is useful in identifying particular organs that are sensitive and selective to metal accumulation (Gbem et al. 2001). The aims of the present study was to investigate the levels of metals (Cd, Cu, Zn, Pb and Cr) in muscle, gill, liver, kidney and intestine of two freshwater fish, *Ctenopharyngodon idella* and *Perea fluviatilis*, in Anzali Wetland, which are frequently consumed in Gilan province, Iran.

Materials and Methods

Fish were caught by fishermen's nets in August 2009, and then transported to the laboratory. The selected fish species belong to different families and have different trophic ecological characteristics, i.e. they include herbivore (e.g. *C. idella*,) and carnivore (e.g. *Perca fluviatilis*,). Total size and weight of fish were 40.5 (± 2.4) cm and 718.0 (± 67.3) g for *C. idella,* and, 20.7 (± 1.5) cm and 138.2 (± 38.9) g for *P. fluviatilis,* respectively. Fish parts that were analyzed in this study were muscle, gill, liver, kidney and intestine. Kidney samples of *P. fluviatilis* were not measured as kidney was inconsiderable. The tissue samples were digested in a nitric acid (HNO_3) and perchloric acid ($HClO_4$) mixture (Ebrahimpour and Mushrifah 2010). One gram of dried muscle, gill and intestine samples, 0.5 g of liver samples and 0.25 g of kidney samples was weighted into a 100 mL Erlenmeyer flasks, 10 mL nitric acid (65 %) was added to each sample, and the samples were left overnight to be slowly digested (Ebrahimpour and Mushrifah 2010). Thereafter, 5 mL perchloric acid (70 %) was added to each sample. Digestion was performed on a hot plate (sand bath) at 150°C, for about 6 h or until solutions was clear and near to dried. After cooling, the solution was quantitatively transferred to 100 mL polyethylene bottles and made up to 50 mL with distilled water. Then the solution was filtered using 0.45 µm nitrocellulose membrane filter. The metal analysis of tissue samples (Cd, Cu, Zn, Pb and Cr) were carried out using Atomic Absorption Spectrometer (Shimadzu AA 680). The detection limits for each metal were: Cd (0.013), Cu (0.015), Zn (0.01), Pb (0.07), and Cr (0.05). The concentrations of metals in fish tissues presented in 1 g g^{-1} dry weight (dw). The concentrations of metals in fish were expressed as µg/g dw of muscle, gill, liver, kidney and intestine.

The Anderson–Darling′s test was employed to test normal variable distribution. The Levene′s test was employed to test homogeneity of variance, and analysis of variance (ANOVA) was used to determine the differences among the organs (muscle, gill, kidney, liver and intestine) and fish species. The *t* test was used to evaluate differences in the tissues (muscle, gill, kidney, liver and intestine) of *C. idella* and *P. fluviatilis* species in trophic level. Data analysis was performed using the statistical package Minitab (version 14) and the significance was set at 0.05.

Results and Discussion

Concentrations of Cd, Cu, Zn, Pb and Cr are shown in Table 1. The highest mean concentration of Cd in two fish species and fish tissues was observed in the intestine of *C. idella* at 1.08 µg/g, while the lowest mean concentration of Cd was found in the muscle of *P. fluviatilis* at 0.19 µg/g. The highest mean concentrations of Cu and Pb were found in the liver and kidney of *C. idella* with values of 25.60 and 3.03 µg/g, respectively, while the lowest mean concentration were found in the muscle with values of 6.66 and 0.88 µg/g, respectively. The highest and lowest mean concentrations of Zn were found in the liver and gill of *C. idella* at 31.20 and 20.53 µg/g, respectively. The highest mean concentration of Cr was observed in the liver of *P. fluviatilis* at 4.20 µg/g, while the lowest mean concentration of it was found in the muscle of C. *idella* at 0.48 µg/g. There were significant differences among the organs (muscle, gill, liver, kidney and intestine) and fish species for Cd (Table 2).

In the present study, the levels of metals (Cd, Cu, Zn, Pb and Cr) were investigated in muscle, gill, liver, kidney and intestine of two freshwater fish, *C. idella* and *P. fluviatilis,* which are frequently consumed in Gilan province, Iran. These metals are all regarded as potential hazards that can endanger for humans. The concentrations were different between the fish species as well as among the tissues of fish. Metal concentrations in the tissue of freshwater fish varies considerably among different studies, possibly due to differences in metal concentrations and chemical characteristics of water from which fish were sampled, ecological needs, metabolism and feeding patterns of fishes and also the season in which studies were carried out (Canli et al. 1998; Erdoĝrul and Ates 2006). The predominant pathways for metal uptake appear to be highly variable over the range of metals, contaminant levels, specific ecological situation, and fish species studied. A number of studies show that the concentrations of the non-essential metals (Cd and Pb) in the aquatic organisms depend mainly on their environmental levels (Kargin 1998). Thus, the metal concentrations in fish tissue may appear to be the result of a complex interaction of many factors (Alam et al. 2002). The differences in metal concentrations of the tissues might be as a result of their capacity to induce metal-binding proteins such as metallothioneins. It is well known that one of the most important factors that play a significant role in metal accumulation in marine animals is the metabolic activity (Canli and Atli 2003). Knowledge of metal concentrations in fish is important both with respect to nature management and human consumption of fish.

In the present study, the highest Cd and Pb concentrations were observed in the intestine of *C. idella*, and, Cu and Zn concentrations in the liver of *C. idella,* while the highest concentrations of Cr was observed in the liver of *P. fluviatilis*. The lowest Cd, Cu, Pb and Cr concentrations were observed in the muscles of *C. idella* and *P. fluviatilis*, while the lowest Zn concentrations were observed in the gill of *C. idella* and intestine of *P. fluviatilis.* The value of Cd was about fivefold higher in the intestine and liver than in the muscle of *C. idella* and *P. fluviatilis*. The value of Cr was more than sevenfold higher in the liver than in the muscle of *P. fluviatilis,* while, the value of it was more than threefold higher in the liver than in the muscle of *C. idella.* Results showed that the metal concentrations in both fish species were in descending order of Zn > Cu > Pb > Cr > Cd. The pattern of Cd accumulation in the tissue samples of *C idella* were in descending order of intestine ≥ liver > kidney ≥ gill > muscle, because no significant differences were found between mean metal concentrations of the intestine and liver and between kidney and gill, while in the tissue samples of *P. fluviatilis* were in descending order of intestine ≥ liver > gill ≥ muscle, because no significant differences were found between mean metal concentrations of the intestine and liver and between gill and muscle. Copper accumulation in the tissues of *C idella* were in descending order of liver ≥ intestine > kidney > gill ≥ muscle, while in the tissues of *P. fluviatilis* were in descending order of liver > intestine ≥ gill > muscle. Zinc accumulation in the tissues of *C idella* were in descending order of liver > muscle ≥ kidney ≥ intestine > gill while in the tissues of *P. fluviatilis* were in descending order of liver ≥ gill ≥ muscle > intestine. Lead accumulation in the tissues of *C idella* were in descending order of kidney ≥ intestine ≥ liver > gill ≥ muscle while in the tissues of *P. fluviatilis* were in descending order of intestine > liver > gill ≥ muscle. Chromium concentrations in the tissue samples of *C idella* were in descending order of kidney ≥ intestine ≥ liver > gill ≥ muscle, while in the tissue samples of *P. fluviatilis* were in descending order of liver > intestine > gill ≥ muscle. In general, similar results were reported from a number of fish species that the muscle is not an active tissue in accumulating metals (Tekin-Özan and Kir 2008; Alam et al. 2002; Kargin 1998). Liver and intestine tissues were found to accumulate high concentrations of most metals, with kidney tissue accumulating highest Cr concentrations. Metals mainly accumulate in metabolically active tissues (Kargin 1998). Target organ, such as liver has tendency to accumulate metals in high values, as shown in many species of fish in different areas; (Tekin-Özan and Kir 2008). Liver tissues are believed to be the main site of trace metal

Table 1 Mean, standard deviation and range of Cd, Cu, Zn, Pb and Cr concentrations (μg/g dw) in the tissues of two fish species

	Ctenopharyngodon idella					*Perca fluviatilis*				
	Muscle	Gill	Kidney	Liver	Intestine	Muscle	Gill	Kidney	Liver	Intestine
Cd										
Mean	0.21	0.35	0.70	1.00	1.08	0.19	0.41	–	1.00	1.02
SD	0.05	0.09	0.35	0.28	0.20	0.01	0.19	–	0.24	0.36
Range	0.15–0.25	0.25–0.50	0.40–1.30	0.80–1.20	0.80–1.35	0.10–0.30	0.25–0.85	–	0.70–1.40	0.70–1.60
Cu										
Mean	6.66	8.78	15.30	25.60	18.32	10.02	11.62	–	21.99	15.18
SD	6.66	1.88	5.40	0.85	1.84	2.20	2.32	–	2.57	3.81
Range	4.45–9.90	6.15–11.05	9.80–23.9	25.0–26.20	16.20–20.55	7.30–13.00	8.95–14.5	–	17.60–25.80	9.90–19.10
Zn										
Mean	27.25	20.53	26.57	31.20	25.49	27.76	28.82	–	29.01	21.54
SD	4.09	3.57	4.92	2.26	4.34	6.90	9.62	–	6.65	2.22
Range	23.25–33.00	14.85–23.65	21.65–34.10	29.60–32.80	20.55–32.00	16.45–37.05	17.25–43.50	–	22.20–41.00	18.70–23.70
Pb										
Mean	0.88	1.56	3.03	2.40	2.69	1.18	1.84	–	2.27	2.34
SD	0.39	0.65	1.01	0.28	0.71	0.80	0.80	–	0.54	0.48
Range	0.55–1.55	0.95–2.50	1.80–4.50	2.20–2.60	1.95–3.80	0.55–2.30	0.95–3.35	–	1.30–3.00	1.90–3.10
Cr										
Mean	0.48	0.76	1.56	1.10	1.46	0.53	0.79	–	4.20	1.14
SD	0.16	0.23	0.87	0.42	0.35	0.28	0.37	–	0.85	0.36
Range	0.25–0.70	0.50–1.10	0.70–2.90	0.80–1.40	0.85–1.70	0.20–0.95	0.45–0.40	–	3.60–4.85	0.60–1.50

Table 2 Statistical analysis of Cd, Cu, Zn, Pb and Cr concentrations in the muscle, gills, liver, kidney and intestine of fish

Fish species	Cd		Cu		Zn		Pb		Cr	
	One-way *F* valu	ANOVA *p* value	One-way *F* value	ANOVA *p* value	One-way *F* value	ANOVA *p* value	One-way *F* value	ANOVA *p* value	One-way *F* value	ANOVA *p* value
C. idella	13.90	<0.001	19.28	<0.001	3.02	<0.05	7.62	<0.001	4.49	<0.01
P. fluviatilis	26.13	<0.001	30.88	<0.001	3.87	<0.05	5.18	<0.01	15.61	<0.001

detoxification within fish. This can possibly be attributed to the tendency of the liver to accumulate pollutants of various kinds at higher levels from the environment (Licata et al. 2005). The literature shows that in many cases the liver also has an important role in contaminant storage, redistribution, detoxification or transformation and acts as an active site of pathological effects induced by contaminants (Licata et al. 2005). The liver tissue is highly active in the uptake and storage of metals. Fish respond to heavy metal exposure by producing metallothionein, particularly in liver (Kargin 1998). The gill is a tissue which was active and passive exchanges occur between the animal and aquatic environment. First high levels of metals accumulate in the gill tissues by absorption and adsorption (Kargin 1998). The metal concentration in muscle tissue is important for the edible parts of the fish (Erdoĝrul and Ates 2006).

The studied fish species in the Anzali Wetland system are being exploited for human consumption. In the present study, metal concentrations in muscle tissues of both fish species were analyzed because these concentrations provide information on the potential risk to the fish themselves or to other organisms that consume them (especially humans). For this purpose, the concentrations of metals have been expressed mostly on a wet weight basis. A wet weight-dry weight conversion factor of 0.2 can be assumed (Pourang et al. 2005). According to the FAO (1983) maximum permissible concentrations are for Cd: 0.5 μg/g (in this study 0.04 μg/g for both fish species), Pb: 0.5 μg/g (in this study 0.18 μg/g for *C. idella* and 0.24 μg/g for *P. fluviatilis*) Cu: 30 μg/g (in this study 1.33 μg/g for *C. idella* and 2.04 μg/g for *P. fluviatilis*) and Zn: 30 μg/g (in this study 5.45 μg/g for *C. idella* and

5.55 μg/g for *P. fluviatilis*). The Cd, Cu, Zn, and Pb concentrations in the muscle of both fish from Anzali Wetland are below levels of concern for human consumption as defined by the (FAO 1983). Chromium is not usually an analytical target within routine surveillance of pollutants in fish, and there is absence of contemporary information available for comparison purposes (Storelli et al. 2005).

There has been a growing interest in assessing the levels of trace metals in food. The ingestion of food is an obvious means of exposure to metals, not only because many metals are natural components of foodstuffs but also because of environmental contamination and contamination during food processing (Ashraf et al. 2006). The hypothesis of higher metal concentration in the higher trophic level was met for the examined fish species. So, levels of metals (Cd, Cu, Zn, Pb and Cr) in tissues (muscle, gill, kidney, liver and intestine) of *C. idella* (herbivore) and *P. fluviatilis* (carnivore) was investigate to evaluate whether metal concentrations varied in two different trophic level. With a few exceptions, results of *t* test showed that there were no significant difference between the carnivores fish species (*P. fluviatilis)* and herbivores (*C. idella)* ($p < 0.05$). *C. idella* (herbivores) showed higher Cu concentrations than *P. fluviatilis* (carnivores) in the liver and intestine, while, Cu concentrations were higher in the muscle and gill of *P. fluviatilis* than in the muscle and gill of *C. idella.* Also, Cr concentration was higher in liver of *P. fluviatilis* than in the liver of *C. idella.* The *t* value and *p* value of the data were as follows: Concentration of Cu in the muscle tissue, $t = 12.55$ and $p < 0.001$; in the gill, $t = 2.42$ and $p < 0.05$; in the liver, $t = 3.16$ and $p < 0.05$; in the intestine, $t = 8.26$ and $p < 0.001$. Concentration of Cr in the liver tissue, $t = 3.30$ and $p < 0.05$. Trophic-level differences in metal levels have been reported for a number of contaminants (Burger et al. 2002). It seems that although food is often the most important sources of metals in marine species, it could not always follow that predators at higher trophic levels contain the highest metal concentrations. In general, carnivorous species have higher levels than herbivorous species but herbivores sometimes have higher levels than carnivores (Burger et al. 2002). Fish accumulates substantial amounts of metals in their tissues especially muscles and thus, represent a major dietary source of these metals for humans. The studies on the transfer of metals through the food chain can provide useful information for the development of surveillance programs aimed at ensuring the safety of the food supply and minimizing human exposure metals (Ashraf et al. 2006). In the present study, carnivore and herbivore fish were accumulated the same metal concentrations in their tissues, so, metal concentrations were not varied in two different trophic levels.

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