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Bioaccumulation of Heavy Metals in Freshwater Fish Species, Anzali, Iran

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Abstract The main objectives of study were to monitor the metals concentrations, in freshwater fish species, *Carassius gibelio* and *Esox lucius;* and to identify any relationships between species and bioaccumulation of metals. The highest concentration of metals (cadmium, 1.96; copper, 24.2; zinc, 49.6; lead, 5.4; chromium, 4.4) between the fish species and tissues was in the liver of *Esox lucius,* while the lowest (cadmium,0.21; copper,7.2; zinc,19.4; lead,0.9; chromium, 0.6 μg/g) found in the muscle of *Carassius gibelio.* Results showed that the metal concentrations were in fishes in descending order of zinc > copper > lead > chromium > cadmium, similarly in the tissue liver > kidney > gill ~ intestine > muscle.

Keywords *Carassius gibelio* · Concentration · *Esox lucius* · Tissues · Trophic level

Wetlands are an important facet in the improve of the quality of storm water runoff. Wetlands are increasingly being used in place of urban catchments for this purpose. Anzali Wetland, a coastal lagoon, is an invaluable wetland located in the north of Iran or south west of the Caspian Sea (Fig. 1). It covers an area of about 200 km^2 situated between 37°28′ N and 49°25′ E. This region has a high natural potential for agricultural and industrial developments as a result of relatively high rainfall and rich water resources. Anzali Wetland is one of the most important water bodies in northern Iran. This important wetland was designated as a Ramsar Site on 23 June 1975 (Khaleghizadeh and Behrouzi-Rad 2004), It is a good example of a natural wetland that supports an extreme diverse of wetland flora and fauna. Every year, this precious water body hosts more than 150 species of migratory birds. Anzali wetland supports over 1% wintering birds of the regional Middle East. However, in recent years eutrophication coupled with the high burden of industrial effluents and domestic sewage threaten this ecosystem on the verge of complete extinction (Charkhabi and Sakizadeh 2006).

In aquatic ecosystems, heavy metals have received considerable attention due to their toxicity and accumulation in biota (Dural et al. 2006). Heavy metals from natural and anthropogenic sources are continually released into aquatic ecosystems, and they are a serious threat because of their toxicity, bioaccumulation, long persistence, and biomagnification in the food chain (Erdoĝrul and Ates 2006). Heavy metals, including both essential and non-essential elements, have a particular significance in ecotoxicology, since they are highly persistent and all have the potential to be toxic to living organisms (Storelli et al. 2005). Heavy metal discharges to aquatic environment are of great concern all over the world.

Fish is considered as one of the main protein sources of food for human (Klavins et al. 2009). Water pollution from toxic metals can have sever negative impacts on fish. Pollution might resulted from many sources, e.g., accidental spillage of chemical wastes, atmospheric precipitation contaminated with air-borne pollutants, discharge of industrial or sewerage effluents (Klavins et al. 2009), agricultural drainage, domestic wastewater and gasoline

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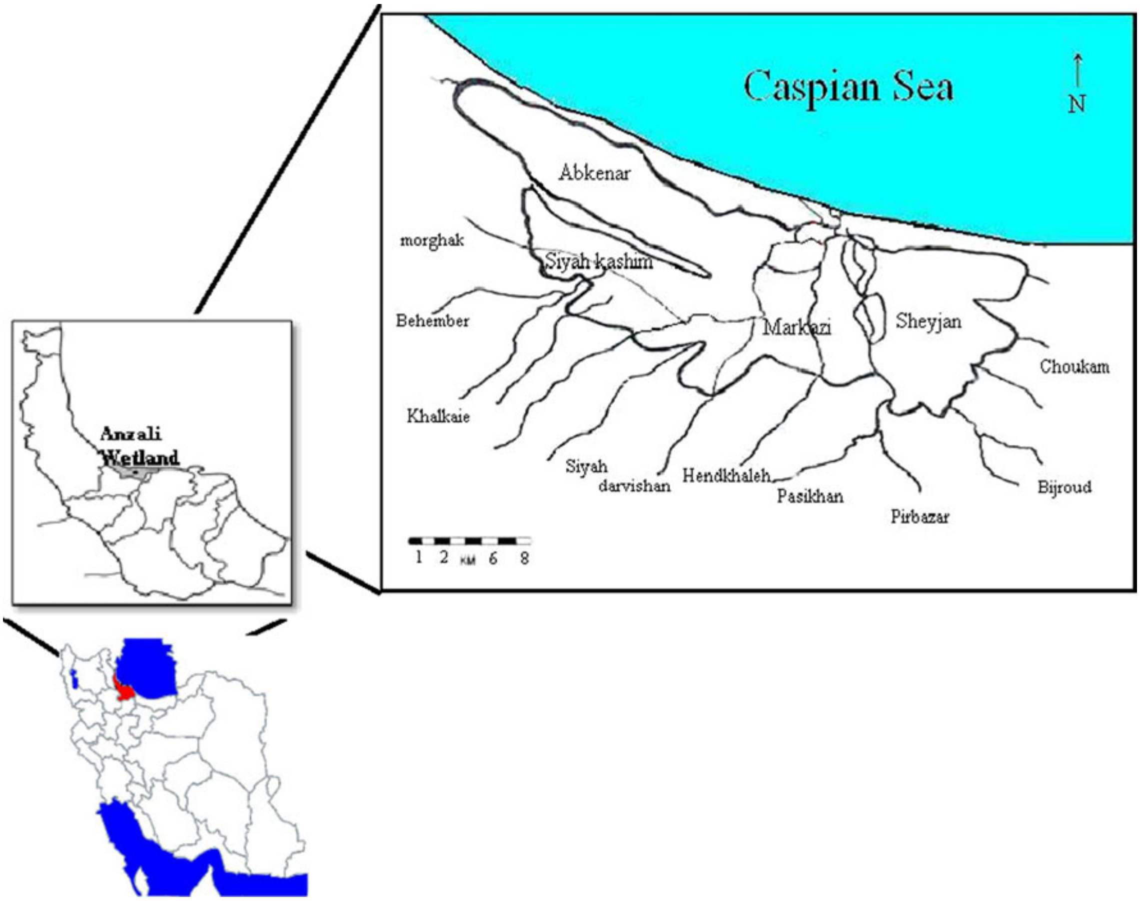

Fig. 1 Map of Anzali Wetland, Iran

from fishery boots. Also fish pollution with heavy metals was reported as a result of contamination of water with fertilizers containing heavy metals (Dural et al. 2006). Contaminants in fish pose risks to human consumers (e.g., recreational and subsistence anglers) and to piscivorous wildlife (Stahl et al. 2009). The aims of this study are: (a) to monitor the concentrations of metals, including cadmium (Cd), copper (Cu), zinc (Zn), lead (Pb) and chromium (Cr), in the tissues, including muscle, gills, liver, kidney and intestine, in two freshwater fish species, *Carassius gibelio* and *Esox lucius*, (b) to determine the tissues' tendency for accumulating these metals, and (c) to identify relationships between fish species and metals bioaccumulation.

Materials and Methods

Fish samples were caught by fishermen's nets in August 2009, and then transported to the laboratory. Total size and weight of fishes were measured (Table 1).

The fish species were selected from different families with different trophic and ecological characteristics. They include the both zooplankton feeders (e.g. *Carassius gibelio*) and piscivorous or fish predators (e.g. *Esox lucius*). *C. gibelio* is the most favorite food for of *E. lucius*. The organs and tissues used in this study were muscle, gill, liver, kidney and intestine. The fish samples were divided into two groups according to their total length. For *C. gibelio,* Group I included samples with a total length below than 220 mm (mean, 200 ± 6.6 mm), while Group II had the larger than 220 mm (mean, 262 ± 35.5 mm) fish. For *E. lucius,* Group I included samples smaller than 320 mm (mean, 296.5 ± 18.3 mm), and Group II had fish larger than 320 mm (mean, 355 ± 18.6 mm) (Table 1). The tissues samples were digested in nitric acid (HNO_3) and perchloric acid ($HClO_4$) mixture (Nussey et al. 2000). One gram of dried muscle, gill and intestine tissues, 0.5 g of liver tissue and 0.25 g of kidney tissue was then accurately weighed into 100 mL Erlenmeyer flasks, 10 mL nitric acid (65%) was added to each sample, and the samples were left overnight to be slowly digested (Ip et al. 2005); 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 cleared and near to dry. After cooling, the solution was quantitatively transferred to 100 mL polyethylene bottles and made

Table 1 Statistics result of fish biometry with standard division

Factor	*C. gibelio*		*E. lucius*	
	Big size (SD)	Small size (SD)	Big size (SD)	Small size (SD)
Weight (g)	314.2 ± 150.3	134.1 ± 11.6	290 ± 33.8	176 ± 30.7
Standard length (mm)	212.3 ± 3.3	164 ± 7.1	309.5 ± 1.5	271.5 ± 29.1
Fork length (mm)	240.5 ± 34.3	186.3 ± 6	533 ± 81	283.5 ± 17.7
Total length (mm)	262.9 ± 35.5	200.8 ± 6.6	355 ± 18.6	296.5 ± 18.3
Age	<3	<3	<3	<3

SD Standard division

up to 50 mL with distilled water. Then the solution was filtered using 0.45 µm nitrocellulose membrane filter. The determination of Cd, Cu, Zn, Pb and Cr in the fish tissues was carried out using a flame atomic absorption spectrometer, model (Thermo, Solar AA Report). The detection limits for each metal were: Cd (0.013), Cu (0.015), Zn (0.01), Pb (0.07), Cr (0.05). The concentrations of metals in fish tissues presented in µg g^{-1} dry weight (dw).

The Anderson–Darling's and Levene's test were used to test the normal distribution of variable and the homogeneity of variance. One way ANOVA test was used to evaluate differences among the organs and fish species. The *t* test was used to evaluate differences in the tissues of *C. gibelio* and *E. lucius* species in trophic level. The significance was set at 0.05 and data analyses were carried out using Minitab (Release 14).

Result and Discussion

Among the species and the organ tissues *E. lucius* liver has the highest mean concentration of Cd, Cu, Zn and Cr (1.96, 24.2, 49.6, 4.4 µg g^{-1}, respectively)., while the lowest mean concentration of Cd was found in the muscle (0.21 µg/g) of *C. gibelio* and *E. lucius* (Table 2). *C. gibelio* muscle tissues have mean concentration of Cu and Zn at 7.2 and 19.4 µg g^{-1} (see Tables 3, 4). For Cr, this is *E. lucius* muscle that have the lowest amount (0.6 µg/g, See Table 6). A high mean concentration of Cd was observed in intestine of *E. lucius* at 1.27 µg g^{-1}. There were significant differences among the organs and fish species for Cd (Table 7). High mean concentrations of Cu, Zn and Cr were also observed in the kidney of *E. lucius* at 20.8, 41.6, 4.0 µg g^{-1}, respectively. There were significant differences among the organs and fish species for Cu, Zn and Cr (Table 7). However, the highest mean concentration of Pb in for the both fish species and organ tissues was observed in the liver and kidney of *E. lucius* (5.4 µg g^{-1}). On the contrary, the lowest mean concentration of Pb was found in the muscles of *E. lucius* at 0.9 µg/g (Table 5). Also, a high mean concentration of Pb was observed in the kidney of *C. gibelio* at 3.9 µg g^{-1}. There were significant differences among the organs and fish species for Pb (Table 7).

Accumulation of toxins particularly metals by fish is potentially dangerous since these could be transferred directly upon consumption to humans (Asuquo et al. 2004). The five metals (Cd, Cu, Zn, Pb and Cr) studied in the present investigation are considered as hazardous material and can severely effect animal and human health. It has been reported that contamination of the environment by metals can pose a great hazard to man and other organisms when they are in excess in the biological food chain (Asuquo et al. 2004). Knowledge of heavy metal concentrations in fish is therefore important both with respect to

Table 2 Cadmium concentration (SD) in the tissues of *Carassius gibelio* and *Esox lucius*(µg/g)

Tissue	*Carassius gibelio*				*Esox lucius*			
	Group I (n = 15)		Group II (n = 15)		Group I (n = 21)		Group II (n = 21)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Muscle	0.29 (0.19)	0.05–0.55	0.21 (0.09)	0.1–0.35	0.22 (0.10)	0.15–0.35	0.21 (0.05)	0.10–0.95
Gill	0.40 (0.17)	0.20–0.70	0.45 (0.29)	0.2–0.95	0.42 (0.17)	0.20–0.70	0.44 (0.27)	0.20–0.95
Kidney	0.65 (0.19)	0.40–0.80	0.64 (0.30)	0.20–1.0	0.92 (0.23)	0.60–1.20	1.0 (0.32)	0.60–1.4
Liver	1.05 (0.34)	0.60–1.4	0.90 (0.14)	0.8–1.0	1.96 (0.4)	1.40–2.40	1.96 (0.56)	1.3–2.6
Intestine	0.63 (0.22)	0.40–0.90	0.80 (0.43)	0.30–1.20	1.27 (0.13)	1.15–1.45	1.10 (0.18)	0.95–1.35

SD Standard division

Table 3 Copper concentrations (SD) in the tissues of *Carassius gibelio* and *Esox lucius*(μg/g)

Tissue	*Carassius gibelio*				*Esox lucius*			
	Group I (n = 15)		Group II (n = 15)		Group I (n = 21)		Group II (n = 21)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Muscle	7.4 (1.1)	6.0–9.4	7.2 (1.7)	5.1–9.9	9.1 (3.3)	6.0–15.5	8.8 (3.3)	6.0–15.5
Gill	11.9 (4.1)	8.9–19.9	14.0 (2.8)	10.5–18.8	12.7 (1.6)	9.9–14.4	14.0 (2.8)	10.5–18.8
Kidney	17.1 (3.0)	13.8–21.0	16.9 (2.1)	15.4–20.6	19.8 (3.0)	16.4–23.2	20.8 (4.7)	15.8–28.0
Liver	20.5 (3.8)	15.6–24.6	24.1 (5.4)	20.4–28.0	22.8 (3.2)	19.7–27.6	24.2 (6.1)	18.7–34.1
Intestine	12.2 (2.1)	9.8–14.3	17.2 (1.4)	15.2–18.4	18.5 (3.0)	15.5–22.5	21.9 (2.3)	19.5–24.4

SD Standard division

Table 4 Zinc concentration (SD) in the tissues of *Carassius gibelio* and *Esox lucius*(μg/g)

Tissue	*Carassius gibelio*				*Esox lucius*			
	Group I (n = 15)		Group II (n = 15)		Group I (n = 21)		Group II (n = 21)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Muscle	19.4 (2.8)	14.9–22.8	22.2 (4.1)	16–28.1	28.6 (4.0)	20.9–33.7	21.3 (3.72)	15–25.5
Gill	31.3 (10.0)	18.7–45.0	27.0 (7.3)	18.7–35	33.6 (4.9)	24–37.3	29.2 (6.9)	18.7–35
Kidney	28 (4.6)	24.6–34.6	23.9 (2.5)	21.8–28	41.6 (10.0)	29.8–55.2	36.9 (6.4)	28.6–46.2
Liver	27.4 (3.0)	24.2–31.4	26.8 (1.9)	25.4–28.2	46.5 (10.0)	38.8–54.9	49.6 (12.5)	38.8–68.7
Intestine	25.8 (2.5)	23.1–28.1	23.2 (2.3)	21.1–25.7	28.9 (6.9)	20.5–37.1	27.5 (3.3)	23.5–31.5

SD Standard division

Table 5 Lead concentration in the tissues of *Carassius gibelio* and *Esox lucius*(μg/g)

Tissue	*Carassius gibelio*				*Esox lucius*			
	Group I (n = 15)		Group II (n = 15)		Group I (n = 21)		Group II (n = 21)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Muscle	1.3 (0.5)	0.8–2.3	1.1 (0.4)	0.7–0.8	0.9 (0.3)	0.6–1.4	1.2 (0.7)	0.6–2.7
Gill	3.1 (1.6)	1.7–6.0	2.7 (1.1)	1.4–4.7	3.1 (0.8)	2.5–4.5	3.7 (1.0)	2.3–4.9
Kidney	3.9 (0.3)	1.6–2.2	3.9 (0.7)	3.2–5.0	3.7 (0.8)	2.6–4.8	5.4 (0.6)	4.8–6.4
Liver	3.1 (0.6)	2.6–4.0	2.2 (0.3)	1.7–2.7	3.3 (0.7)	2.6–4.0	5.4 (1.1)	4.0–6.7
Intestine	2.3 (0.5)	1.9–3.1	2.8 (0.6)	2.1–3.5	3.0 (0.7)	2.3–3.8	2.4 (0.8)	0.8–3.5

SD Standard division

nature management and human consumption of fish. Chemical and biological factors (Amundsen et al. 1997), ecological needs and metabolic activities (Dural et al. 2006), influence the bioavailability and accumulation of metals in fish.

Moreover, numerous potential interactions between different elements may influence both the assimilation and toxicity of the metals. Thus, the metals concentrations in fish tissue may appear to be the result of a complex interaction of many factors (Amundsen et al. 1997). Results of many studies showed that target organs, such as liver, kidney and gill have tendency to accumulate heavy metals in high values (Karadede et al. 2004; Tekin-Özan and Kır 2006; Tekin-Özan and Kir 2008; Tekin-Özan 2008).

Fish liver tissue is more often recommended as an environmental indicator for water pollution than other fish organs. This can possibly be attributed to the tendency of the liver to accumulate pollutants of various kinds at higher levels from the environment. The literature shows that in many cases, the liver does have 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). In this study,

Table 6 Chromium concentration in the tissues of *Carassius gibelio* and *Esox lucius*(μg/g)

Tissue	*Carassius gibelio*				*Esox lucius*			
	Group I (n = 15)		Group II (n = 15)		Group I (n = 21)		Group II (n = 21)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Muscle	0.7 (0.2)	0.4–1.0	0.7 (0.1)	0.5–0.9	0.7 (0.3)	0.4–1.1	0.6 (0.2)	0.3–0.9
Gill	1.0 (0.4)	0.3–1.5	1.4 (0.6)	0.5–2.2	1.5 (0.4)	0.3–1.9	1.7 (0.3)	1.25–2.0
Kidney	2.5 (0.6)	1.8–3.2	2.6 (0.7)	1.6–3.4	4.0 (1.0)	2.6–5.4	2.1 (0.9)	1.0–2.1
Liver	2.7 (0.9)	1.6–3.8	2.7 (0.9)	1.6–3.8	2.8 (1.3)	0.7–4.9	4.4 (1.5)	2.5–6.3
Intestine	1.1 (0.3)	0.7–1.4	1.1 (0.4)	0.6–1.5	1.7 (0.4)	1.2–2.2	1.4 (0.5)	1.0–2.1

SD Standard division

Table 7 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 ANOVA		One- way ANOVA		One- way ANOVA		One- way ANOVA		One- way ANOVA	
	F value	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value
C. *gibelio*	13.6	<0.001	31.2	<0.001	4.1	<0.05	8.3	<0.001	30.7	<0.001
E. lucius	57.5	<0.001	30.4	<0.001	12.8	<0.001	19.6	<0.001	30.2	<0.001

the highest concentrations of Cd, Cu, Zn, Pb and Cr were observed in the liver of *E. lucius*, followed by kidney, while the lowest metal concentrations of these metals were observed in the muscle. For *E. lucius*, the cadmium concentration in the liver was 9 times higher than the muscle tissues. For *C. gibelio* it is 5 times bigger than in the muscle. Similar ratios have been observed for Cr concentrations in liver and muscle tissues of E. *lucius* (7-folds) and *C. gibelio* (2-fold). Comparable results were reported from a number of fish species that the muscle is not an active tissue in accumulating metals (Amundsen et al. 1997; Alam et al. 2002; Karadede et al. 2004, Tekin-Özan and Kir 2008). The low concentrations of Cd, Cu, Zn, Pb and Cr in the muscle of fish species may reflect the low levels of binding proteins in the muscle (Terra et al. 2008).The metal concentrations were lower in the gill compared to the liver, kidney and intestine. The concentration of Cd in *E. lucius* and *C. gibelio* liver was more than 4 and 3-folds higher than in the gills. The Cr value was more than 4 times higher in the liver than in the gill for *E. luciu* and *C. gibelio.* The metal concentrations in the gill reflect the concentrations of metals in the waters where the fish species live, whereas the concentrations in liver represent storage of metals (Tekin-Özan and Kir 2008). Also, the metal concentrations (Cd, Cu, Zn, Pb and Cr) were from 1.5 to 6-fold higher in the kidney than in the muscle. The value of Cd was more than 5-fold higher in the kidney than in the muscle of *E. lucius.* On the other hand, the value of Cr was about 6-fold higher in the kidney than in the muscle of *E. lucius.* A relatively high concentration of metals was also accumulated in the intestine tissue. The value of Cd was more than 5.5-fold higher in the intestine than in the muscle of *E. lucius,* while the value of Pb was about 3.5 -fold higher in the intestine than in the muscle of *E. lucius.* Results of this study showed that the metal concentrations uptake in both fish species were in descending order of Zn > Cu > Pb > Cr > Cd. Also, the pattern of metal concentrations accumulated in the tissue samples of both species were in descending order of liver > kidney > gill ~ intestine > muscle. There was no significant difference between mean metal concentrations of the gill and intestine in both species. The major findings of this study were that the metal concentrations in the muscle, gills, liver, kidney and intestine samples in both species from Anzali Wetland were relatively higher than those in other regions, and in general displayed significant variation from tissue to tissue. In the present study, significant differences were recorded in the different tissues of the two fish species for Cd, Cu, Zn, Pb and Cr.

The hypothesis that higher metal concentrations are accumulated in larger fish size (length) has been shown in this study. There was no significant difference ($p > 0.05$) between mean metals concentrations and size in both fish species with a few exceptions. There were significant differences between Pb concentration and fish size in the kidney ($p < 0.01$), and between Cu concentration and fish size in the *C. gibelio* intestine ($p < 0.05$). Results also showed that there were significant differences between Zn concentration and fish size in the muscle ($p < 0.05$), between Pb concentration and fish size in the kidney

($p < 0.01$), between Cr concentration and fish size in the kidney ($p < 0.05$), and, between Pb concentration and fish size in the liver ($p < 0.01$) for *E. lucius.*

In the present study Cd, Cu, Zn, Pb and Cr concentrations in the tissues of two fish species *Carassius gibelio* and *Esox lucius* from Anzali Wetland was measured to indicate if higher concentration of these metals occurred in higher trophic level. 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 (Moriarty 1999). With some exceptions, results of t test showed that there was a significant difference between the zooplankton feeder (*C. gibelio)* and piscivorous (*E. lucius)* ($p < 0.05$). *E. lucius* (piscivorous) showed the highest accumulation of Cd, Cu, Zn, Pb and Cr than *C. gibelio* (zooplankton feeder), where the organs of accumulation was found in the muscle, gill, kidney, liver and intestine. The t value and p value of the data are as follows: Concentration of Cd and Cr in the gill tissue, $t = 2.5$ and $p < 0.05$ for Cd; $t = 2.1$ and $p < 0.05$ for Cr. Concentrations of Cd, Cu, Zn and Pb in the kidney tissue, $t = 2.7$ and $p < 0.05$ for Cd; $t = 2.4$, $p < 0.05$ for Cu; $t = 6.8$ and $p < 0.001$ for Zn; $t = 2.8$ and $p < 0.05$ for Pb). Concentration of Cd, Zn, Pb and Cr in the liver tissue, $t = 5.1$ and $p < 0.001$ for Cd; $t = 6.6$ and $p < 0.001$ for Zn; $t = 3.0$ and $p < 0.01$ for Pb; $t = 2.3$ and $p < 0.05$ for Cr. Concentrations of Cd, Cu and Cr in the intestine tissue, $t = 3.6$ and $p < 0.01$ for Cd; $t = 3.5$, $p < 0.01$ for Cu; $t = 2.4$ and $p < 0.05$ for Cr. It has been observed that fish feeding piscivorous species accumulated the highest concentrations of metals than zooplankton feeder species, especially in the kidney and liver tissues.

Since accumulation of metals in the biological system is dangerous to both human beings and other living organisms, there is a need for sequential or seasonal monitoring of heavy metals concentrations in the aquatic environments to acquire data for environmental protection and conservation measures (Asuquo et al. 2004). In this study metals concentrations in the muscle of both fish species were used to investigate possible transfer of metals to human populations via fish consumption. For the purpose of this comparison, the concentrations have been expressed mostly on a wet weight basis. A wet weight-dry weight conversion factor of 0.2 can be assumed. The FAO (1983) maximum permissible concentrations are for Cd: 0.5 $\mu g\ g^{-1}$, Pb: 0.5 $\mu g\ g^{-1}$, Cu: 30 $\mu g\ g^{-1}$ and Zn: 30 $\mu g\ g^{-1}$. 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).

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