Arch. Pol. Fish. (2016) 23: 99-105
DOI 10.1515/aopf-2016-0011

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RESEARCH ARTICLE

Determination of metals (As, Cu, Fe, and Zn) in two fish species from the Miankaleh wetland

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Received – 19 December 2015/Accepted – 20 June 2016. Published online: 30 June 2016; ©Inland Fisheries Institute in Olsztyn, Poland
Citation: Alipour H., Pourkhabbaz A., Hassanpour M. 2016 – Determination of metals (As, Cu, Fe, and Zn) in two fish species from the Miankaleh wetland – Arch. Pol. Fish. 24: 99-105.

Abstract. The objective of this study was to determine the concentrations As, Cu, Fe, and Zn in the livers, gills, and muscles of vobla or Caspian roach, *Rutilus caspicus* (Yakovlev), and bighead goby, *Neogobius gorlap* (Iljin), from the Miankaleh wetland. The results showed that metal accumulation in the liver tissues was higher than in the gills and muscles for all metals, and Fe concentration was higher than Zn, Cu, and As in all the tissues studied. The concentrations of As, Cu, Fe, and Zn in the livers, gills, and muscles of bighead goby were higher than in vobla tissues, except for concentrations of As in the muscles. Fe, Cu, and Zn concentrations in the muscles were below the maximum permissible limit of the WHO and the FAO for both species, but the concentration of As in the muscles of both species exceeded the permissible limit proposed by the FAO; thus, human consumption should be limited. The levels of the other metals (Fe, Cu and Zn) were completely safe.

Keywords: metals, bioaccumulation, fish tissues

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Introduction

The contamination of aquatic habitats with heavy metals from various industrial and mineral mining sources has been a problem for many years (Di Giulio and Hinton 2008). Among several contaminants, metals pose some of the greatest threats to organisms because of their persistence and possible bioaccumulation and biomagnification in the food chain (Ebrahimpour et al. 2011, Rožič et al. 2013). Some metals, such as Fe, Cu, and Zn, are essential nutrients since they play an important role in living organisms, while As, Pb, and Cd are non-essential metals and have no biological role (Wood et al. 2012).

Fish have been used for many years as indicators of environmental pollution status, and, thus, they are regarded as good indicators of metal pollution in marine environments (Marcus et al. 2013). Demand for food is rising because of population growth and changing diets that include more sea products. Fish are a major part of the human diet thanks to their high protein content and the levels omega fatty acids they contain, which are known to support good health. There is concern, however, that the heavy metals accumulated in edible fish can pose a health risk, especially for populations that have high fish consumption rates (Alipour et al. 2014). In this study, livers, gills, and muscles were chosen as the target tissues for assessing metal accumulation. The gills are regarded as an important site of direct uptake from the water, because concentrations of metals in gills reflect the concentrations of metals in water (Alipour et al. 2013). Muscles were selected because of their importance for human consumption, and studying this tissue is a tool for protecting public health (Ebrahimpour and Mushrifah 2010). The liver plays an important role in basic metabolism, and high concentrations in livers represent the metal storage capability of fish (Roméo et al. 1999).

The Miankaleh wetland was selected as the site for the metal assessment in fish because important spawning and nursery grounds for various fish species are located there (Alipour et al. 2013). The main sources of heavy metals contamination in the Miankaleh wetland are industrial enterprises located in the multi-purpose port of Amirabad, and also agriculture. A few studies have evaluated metal levels in fish from this area. This manuscript provides, for the first time, information on metal concentrations in the livers, gills, and muscles of Caspian roach (vobla), *Rutilus caspicus* (Yakovlev), and bighead goby, *Neogobius gorlap* (Iljin), from the Miankaleh Wetland. The aim of this study was to determine the concentrations As, Cu, Fe, and Zn in the livers, gills, and muscles of vobla and bighead goby from the Miankaleh wetland.

Materials and Methods

Study Area

The Miankaleh wetland is a long, narrow peninsula in Mazandaran Province in the north of Iran situated in the extreme southeastern Caspian Sea (Fig. 1). The long, narrow peninsula is 48 km long, and from 1300 to 3200 m wide. The Miankaleh wetland was designated as a Ramsar Site on June 23, 1975 and an international UNESCO Biosphere Reserve in June 1976. On the national level, it is protected by Wildlife Refuge status by the Iranian Department of the Environment.

Sample Collection and Analysis

Vobla and bighead goby were caught with beach seines and then transported to the laboratory in June 2012. Fish samples were washed with distilled water, and the scales were removed. In this study, the tissues used were the muscles, gills, and livers. The bones were removed from all samples. One gram each of muscle and gill and 0.5 g of liver were accurately weighed into separate 25 mL Erlenmeyer flasks, and 5 mL nitric acid (65%) (As HNO_3; from Merck) was added to each sample. The samples were left overnight to be digested slowly (Ebrahimpour et al. 2011). Thereafter, 2.5 mL perchloric acid (72%) (As $HClO_4$; from Merck) was added to each sample. Digestion was performed on a hot plate (sand bath) at 150°C for about 6 h or until solutions were cleared and nearly dry. After cooling, the solution was quantitatively transferred to 50 mL polyethylene bottles and made up to 25 mL with distilled water. Then the solution was filtered using a 0.45 μm nitrocellulose membrane filter (Ebrahimpour et al. 2011). Determination of Zn, Fe, As, and Cu in the fish tissues was carried out using a graphite furnace atomic absorption spectrometer, (Thermo, Model 97GFS). The limits of detection were as follows: Zn (0.005), Fe (0.05), As (10), and Cu (0.01) μg g^{-1}. The concentrations of metals in fish tissues are reported in μg g^{-1} wet weight.

One way-ANOVA followed by Tukey's test ($P < 0.05$) were used to evaluate differences among the tissues. The *t* test was used to evaluate differences between *R. caspicus* and *N. gorlap*. All statistical calculations were performed with SPSS version 19.

Results

The mean concentrations (μg g^{-1}wet weight) of the metals found in the liver, gill, and muscle tissues of *R. caspicus* and *N. gorlap* are presented with statistical analysis in Table 1. The highest concentrations of As, Cu, Fe, and Zn were measured in the liver of *N. gorlap* at 246.6, 63.27, 18.11, and 1.08 μg g^{-1}, while the lowest concentrations of Fe, Cu, Zn, and As were

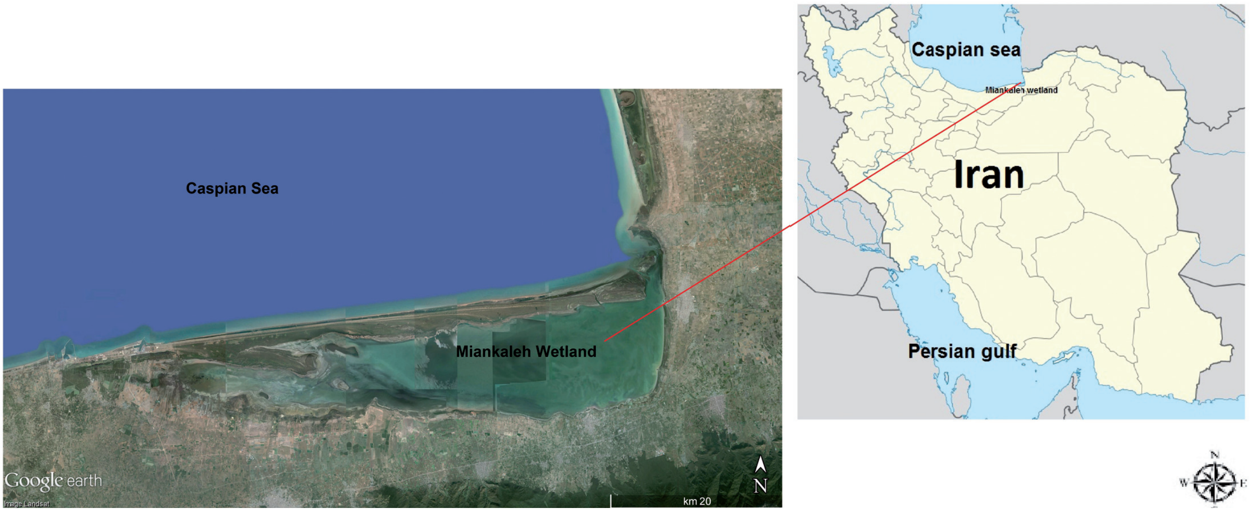

Figure 1. A screenshot of Google Earth™ showing the study area (left part) and map of Iran (right part).

found in muscle of *R. caspicus* at 5.38, 0.69, 7.15, and 0.25 μg g^{-1}, respectively. The distribution patterns of As, Cu, Fe, and Zn in the tissues of both species was as follows: liver>gill>muscle. In general, mean concentrations of metals in the liver of *R. caspicus* and *N. gorlap* followed the trend of Fe> Cu> Zn >As, whereas in the gills of *R. caspicus* and *N. gorlap* the following trend was observed Fe> Zn > Cu >As; the mean concentrations of metals in the muscle of *R. caspicus* and *N. gorlap* followed the trend of Zn > Fe > Cu >As. The *t* and P values of the data are presented in Table 1. In general, it was observed that the benthic fish species (*N. gorlap*) accumulated higher concentrations of metals than did the pelagic species (*R. caspicus*).

Table 1

As, Cu, Fe, and Zn concentrations (SD) in tissues (μg g^{-1}, wet weight) and *t* test statistical analysis of concentrations in *R. caspicus* and *N. gorlap*

		Vobla (n=15)		Bighead Goby (n=15)			
Metals	Tissue	Mean (SD)	Range	Mean (SD)	Range	*t* value	P
Iron	Liver	185 (29.52)^{a}	137.2-250.08	246.6 (5.11)^{a}	191.14-361.25	4	<0.05
	Gills	28.42 (11.60)^{b}	11.06-47.08	13.4 (4.05)^{bc}	7.21-19.09	4.7	<0.001
	Muscles	5.38 (2.29)^{c}	1.99-10.09	7.88 (2.64)^{c}	3.1-12.1	2.7	>0.05
Copper	Liver	23.69 (9.31)^{a}	14.61-46.3	63.27 (21.28)^{a}	28.55-95.13	6.5	<0.05
	Gills	1.62 (0.62)^{bc}	0.95-3.11	6.48 (1.97)^{bc}	3.31-9.85	9.1	<0.05
	Muscles	0.69 (0.4)^{c}	0.25-3.11	2.37 (1.01)^{c}	1.01-4.18	5.9	<0.05
Zinc	Liver	10.1 (3.03)^{a}	5.43-15.5	18.11 (3.68)^{a}	9.95-24.2	6.5	>0.05
	Gills	9.5 (1.21)^{a}	7.13-11.05	14.28 (3.7)^{b}	9.77-23.17	4.7	<0.05
	Muscles	7.15 (1.5)^{b}	4.97-9.32	10.21 (2.55)^{c}	4.33-13.55	3.9	>0.05
Arsenic	Liver	0.92 (0.22)^{a}	0.55-1.23	1.08 (0.44)^{a}	0.56-2.23	1.2	>0.05
	Gills	0.31 (0.2)^{bc}	0.09-0.81	0.66 (0.33)^{b}	0.24-1.40	3.6	>0.05
	Muscles	0.25 (0.08)^{c}	0.11-0.41	0.24 (0.09)^{c}	0.12-0.42	0.2	>0.05

Mean values in the same column with different letter indexes differ significantly statistically according to Tukey's test at a level of significance of $P \leq 0.05$

Discussion

Fe is an essential element required for growth and survival. Fish is a major source of Fe in the human diet, and it is reported that Fe deficiency causes anemia (Biswas et al. 2012). The range of Fe levels of the livers, gills, and muscles of *R. caspicus* were 137.2-250.08, 11.06-47.08, and 1.99-10.09 μg g^{-1}, and concentrations of Fe in the livers, gills, and muscles of *N. gorlap* ranged from 191.14-361, 7.21-19.09, and 3.1-12.1 μg g^{-1}, respectively. Fe concentrations in livers were 6.5 and 34 times higher, respectively, than those in gill and muscle tissues of *R. caspicus*. There were significant differences in Fe among the livers and the gills and muscles ($P < 0.001$). Fe concentrations in the gills of *R. caspicus* were approximately three-fold higher than in the muscles ($P < 0.05$). Fe accumulation in the liver of bighead goby was approximately 18- and 31-fold higher than in the gills and muscles, respectively. There were significant differences in Fe concentrations between the liver and the gills and muscles ($P < 0.001$). The Fe concentration in the gills of *N. gorlap* was higher than that of the muscles, but no significant differences were found ($P > 0.05$).

The results of the present study are in accordance with the findings of Ebrahimzadeh et al. (2011), which indicated that concentrations of Fe in the liver were up to two and 14 times higher than those in the gills and muscles of *Liza saliens* (Risso). They were also similar to the findings of Rajkowska and Protasowicki (2013) and Karadede-Akin and Ünlü (2007) where the highest concentrations of Fe were observed in the livers of *Abramis brama* (L.) and *Silurus triostegus* Heckel, respectively. Rajkowska and Protasowicki (2013) reported mean liver, gill, and muscle Fe concentrations of 54.9, 24.8 and 1.5 μg g^{-1} in *A. brama*, from Lake Ińsko, respectively. These values were generally lower than those observed in the present study. Fe levels in the muscle are below the maximum permissible limit (100 μg g^{-1}) of the WHO (1989) for both species.

Cu is also an essential element for all aerobic organisms since its redox potential is utilized by mitochondrial cytochrome *c* oxidase, and it acts as a cofactor for many other enzymes. However, very high intakes can cause adverse health problems (Wood et al. 2012). In our study, the mean concentrations of Cu in the liver, gills, and muscles of *R. caspicus* were 23.69, 1.62, and 0.69 μg g^{-1}, and the average concentrations of Cu in the in the liver, gills, and muscles of *N. gorlap* were 63.27, 6.48, and 2.37 μg g^{-1}, respectively. Cu concentrations in the liver were approximately 10 to 14.5 and 26.5 to 34 times higher than in the gills and muscles tissues of *N. gorlap* and *R. caspicus*, respectively. There were significant differences for Cu between the livers and the gills and muscles ($P < 0.001$) for both species. Cu concentrations in the present study were lower than those reported in *Hypophthalmichthys molitrix* (Val.) (liver – 126, gills – 12.4, and muscles – 4.89 μg g^{-1}) from south China (Zhuang et al. 2013) and higher than those reported for *Esox lucius* L. and *A. brama* from lakes Ińsko and Wisola (Rajkowska and Protasowicki 2012). The WHO (1989) and FAO (1983) maximum permissible concentrations for Cu are 30 and 10 μg g^{-1}, respectively. The Cu concentrations in the fish species examined in the present study were found to be lower than these permissible limits.

Zn is environmentally ubiquitous, and it is an essential trace element for all living organisms, and it can either be deficient or toxic. In this study, the average concentrations of Zn in the liver, gills, and muscles of *R. caspicus* were 10.1, 9.5, and 7.15 μg g^{-1}, and the average concentrations of Zn in the in the liver, gills, and muscles of *N. gorlap* were 18.11, 14.28, and 10.21 μg g^{-1}, respectively. Zn concentrations in the liver were higher than the gill and muscle tissues for *R. caspicus*. There were significant differences in Zn between the livers and the gills and muscles ($P < 0.01$). Zn accumulation in the liver of *N. gorlap* was higher than in the gills or muscles. There were significant differences in Zn concentrations between the livers and the gills and muscles ($P < 0.01$) and gills and muscles ($P < 0.05$). The mean concentrations of Zn in our study were far lower than those reported for *Leuciscus cephalus* (L.) and *Tinca tinca* (L.) from Yeniçağa Lake, Turkey (Saygi and Yiğit

2012). Malik et al. (2010) reported mean liver, gill, and muscle Zn concentrations of 1.03, 0.23, and 0.48 μg g^{-1} in *Labeo rohita* (Hamilton) from the freshwater Lake of Bhopal, respectively. These values were far lower than those observed in the present study. The maximum Zn level permitted for human consumption in fishes is 100 μg g^{-1} according to WHO (1989) and FAO (1983). In the present study, Zn levels in the studied fish species were found to be lower than these permissible limits.

As is a moderately toxic, naturally abundant element with no known nutritional or metabolic roles. As is far less acutely toxic to fish than are most metals, but it is a known carcinogen in humans that causes lung, liver, skin, and bladder cancer (de Rosemond et al. 2008, Wood et al. 2012). The mean concentration of As ranged from 0.55 to 1.23 μg g^{-1} in the liver, 0.09 to 0.81 μg g^{-1} in the gill, and 0.11 to 0.41 μg g^{-1} in the muscle of *R. caspicus* and *N. gorlap*. The average concentration of As ranged from 0.56 to 2.23 μg g^{-1} in the liver, 0.24 to 1.40 μg g^{-1} in the gill, and 0.12 to 0.42 μg g^{-1} in the muscle of *N. gorlap*. As concentrations in the liver were higher than the gill and muscle tissues for *R. caspicus*. There were significant differences for As between the liver and the gills and muscles ($P < 0.001$). As accumulation in the liver of *N. gorlap* was higher than in the gills and muscles. There were significant differences for As between the liver and the gills and muscles ($P < 0.01$) and the gills and muscles ($P<0.01$). As concentrations in our study were higher than those reported in *Salmo macrostigma* (Duméril) (liver – 0.046, gills – 0.032, and muscles – 0.026 μg g^{-1}) from Tunceli, Turkey (Can et al. 2012) and lower than those reported for *L. cephalus* and *T. tinca* from Yeniçağa Lake, Turkey (Saygi and Yiğit 2012). The FAO (1983) maximum permissible concentration for As is 0.1 μg g^{-1}. The concentrations of As in the muscles of both species were above the permissible limit.

Both the liver and gills are considered good indicators in terms of long-term exposure to heavy metals because of the position of these tissues and metal metabolism (Askary Sary and Beheshti 2012). Fish respond to heavy metal exposure by producing metal-binding metallothioneins, particularly in the liver (Filazi et al. 2003). Metallothioneins play an important role in metal homeostasis and in protection against heavy metal toxicity in vertebrates and invertebrates. Bharti and Banerjee (2011) suggested that the reason for increased Fe, Zn, and Cu concentrations in livers might be because this organ is the main site of synthesis of various proteins and other molecules which have high affinities for metal-forming complexes. Dural et al. (2006) note that liver tissue is highly active in the uptake and storage of metals. Bharti and Banerjee (2011) note that increased metal accumulation in the gills is perhaps linked to the increased number in these tissues of chloride cells which can selectively accumulate metal ions. Roy (2010) suggested that metal ions precipitate the mucous secretions of the gills. These precipitates occupy the intralamellar spaces and the movement of the gill filaments become arrested and respiration is prevented. Metal concentrations in the muscles, which is the edible part of the fish, is important, because the muscles constitute the greatest mass that is consumed. Although fish muscles are the most important part used for human consumption, the skin and livers are also consumed to some extent. The results of many studies show that muscles are not an active tissue in the accumulation of metals (Karadede-Akin and Ünlü 2007, Ebrahimzadeh et al. 2011, Rajkowska and Protasowicki 2013, Zhuang et al. 2013).

Previous studies indicate that different fish species contain different metal concentrations in their tissues. Metal concentration differences might be related to the metabolic activities of different fish species, distinct growth rates, and the diet and feeding habits of benthic and pelagic fish species (Kargin 1996, Krishnamurti and Nair 1999, Roméo et al. 1999, Bustamante et al. 2003, Canli and Atli 2003, Chi et al. 2007). Abdolahpur Monikh et al. (2012) suggest that benthic species accumulate higher concentrations of metals thanks to the greater exposure to metals, enriched sediments, and interactions with benthic organisms. In the present study, Cu, Zn, As, and Fe concentrations in liver, gills, and muscles of the bighead Goby, which is pelagic, were higher than in *R. caspicus*, which is benthic, except in concentrations of As in the muscles. Chi et al. (2007) reported that the Cd, Cu, and Zn contents in muscles of the benthic *C. carpio* and *C. auratus* were higher than those in the pelagic *Hypophthalmichthys nobilis* (Richardson) and *H. molitrix*. Krishnamurti and Nair (1999) noted that maximum concentrations of metals (Cu, Zn, Cd, Pb and Ni) were found in benthic feeders as compared to plankton feeders and pelagic carnivores. Roméo et al. (1999) pointed out that Cd, Cu, Mg, and Zn concentrations in the edible muscles of pelagic fish species were lower than those of benthic fish species.

Conclusion

This study was carried out to provide information on metal concentrations (As, Cu, Fe, and Zn) in two fish species (*R. caspicus* and *N. gorlap*) from the Miankaleh wetland. Our results show that metal accumulation in liver tissues was higher than in the gills and muscles for all metals. This is probably because of the metabolic activities of fish. Organs with higher metabolic activities, such as livers and gills, accumulate more metals than tissues with lower metabolic activities, such as the muscles. Our results show that the benthic *N. gorlap* accumulated higher metal levels than did the pelagic *R. caspicus*. Fe, Cu, and Zn levels in the muscles are below the maximum permissible limits of the WHO (1989) and the FAO (1983) for both species, but As concentrations in the muscles of both species were above the permissible limit proposed by the FAO (1983); thus, human consumption should be limited. The levels of the other metals (Fe, Cu, and Zn) are completely safe.

Author contribution. H.A., and A.P. conceived and designed the research; H.A., M.H., and A.P. performed the research; H.A. analyzed the data and wrote the paper.

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