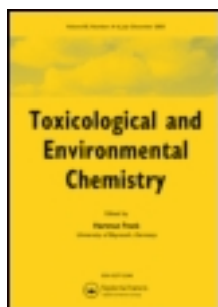


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Concentrations of Cd, Ni, Pb, and Cr in the two edible fish species *Liza klunzingeri* and *Sillago sihama* collected from Hara biosphere in Iran

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The concentrations of four metals (Cd, Ni, Pb, and Cr) were determined in the muscles, gills, and livers of two edible fish species (*Liza klunzingeri* and *Sillago sihama*) caught from the Hara biosphere of Southern Iran. In both fish species, metal concentrations and bioaccumulation factors were in the sequence liver > gill > muscle. Bioaccumulation factors were found to be highest in *S. sihama*. The metal concentrations were descending in the order of Ni > Cr > Pb > Cd, except for muscle samples from *S. sihama* showing an inversion of Pb and Cr. There is a significant negative correlation between the concentrations of the metals in each tissue with length, weight, and age, except for muscle in *L. klunzingeri*. Some metal levels in the muscle exceeded the limits recommended by FAO, WHO, and FEPA.

Keywords: *Liza klunzingeri*; *Sillago sihama*; metals; tissues; bioaccumulation

Introduction

The Hara biosphere, with an area of 85,686 hectares, is located in the Southern Hormozgan province of Iran at the Northeastern coast of the Persian Gulf. It is a unique water body of ecological importance due to its singular mangrove trees, great biodiversity, and for its local economy. Recently, due to extended domestic activities, urbanization, and the continuous industrial growth of the region, the water quality of Hara is changing. Metal pollution is an important factor in decreasing the quality of water in this ecosystem, which may adversely influence the fish health. Fishes are at the top of the aquatic food chain and may accumulate large amounts of some metals from the water, food, sediments, and suspended particles (Fernandes et al. 2007; Yılmaz et al. 2007). Various biotic and abiotic factors control metal bioaccumulation in fish tissues such as life style, feeding habits, fish age, gender, body mass, and physiologic conditions, as well as water temperature, pH value, and dissolved oxygen concentration (Castro-Gonzalez and Mendez-Armenta 2008; Dural, Goksu, and Ozak 2007; Fernandes et al. 2007; Kamaruzzaman, Ong, and Rina 2010).

This study has been conducted to determine the concentrations of Ni, Cd, Cr, and Pb in the gills, muscles, and livers of two edible fish species (*Liza klunzingeri* and

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Sillago sihama) to follow the tissues' tendency for accumulating these metals, and to recognize relationships between fish species with different habitats, ecological characteristics, and metals bioaccumulation. Relationships between size (weight and length), age, and metal concentrations in the tissues were determined. The results were evaluated according to international standards to identify potential public health risks in order to assess their consumption safety.

Materials and methods

Study area

Samples were collected at two stations during the month of November 2010. The first station was located near the Khamir port (26°58'12"N, 55°37'50"E) and the second sampling station was situated near the Laft port (26°51'23"N, 55°44'14"E) in the Hara biosphere (Figure 1).

Water and fish sampling

In this investigation, 114 fish of the species Klunzinger's mullet (*Liza klunzingeri*, Mugilidae) and Silver sillago (*Sillago sihama*, Sillaginidae) were captured by gill net and trawl at two stations. Fish species were labeled, stored in ice, and transported to the laboratory for further treatment and analysis. Prior to the analysis, the length and weight of the fishes were determined. The mean and range of lengths, weights, and ages of the fish species, as well as the numbers of individual fish species, are presented in Table 1.

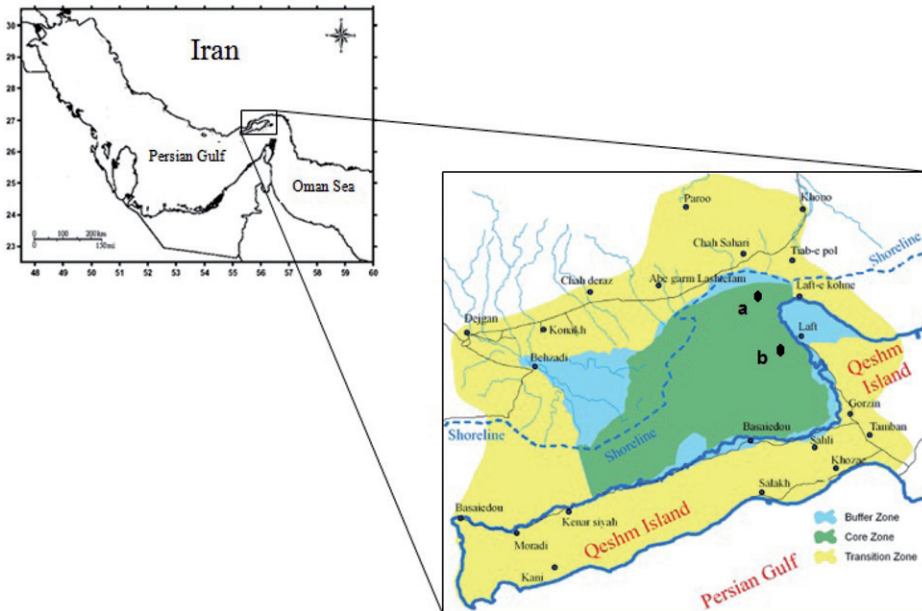


Figure 1. Map of the sampling location (Khamir port (a) and Laft port (b)) in Hara biosphere of southern Iran.

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Water samples were taken from two sites in three replicates from a depth of 30 cm below the water surface and stored in 250 mL polyethylene bottles, which were pre-cleaned with detergents and soaked overnight in 10% nitric acid (Merck, Darmstadt, Germany) and washed with double-distilled water (Ebrahimpour and Mushrifah 2008). Physico-chemical parameters of water were also analyzed *in situ* with Niskin sampler. The mean values of DO, water temperature, pH, salinity, and EC were 6.7 mg L^{-1} , 27°C , 8.4, 3.6%, and 55 mScm^{-1} , respectively.

Analytical procedure

All water samples were immediately brought to the laboratory and were filtered through $0.45 \mu\text{m}$ nitrocellulose membrane filters (Germany). Then, they were kept at 4°C until analysis. Fish samples were delivered to the laboratory where they were sorted by species. Each sample collected was dissected for its otolith bone (for age determination) (Kirby, Maher, and Harasti 2001), muscle, gill, and liver tissues. About 1 g of each organ (wet weight) was weighed out in an open beaker and 8 mL of nitric acid (65%, Merck, Germany) was added. The samples were left overnight. Thereafter, 3 mL of perchloric acid (70%, Merck, Germany) was added to each sample (Storelli and Marcotrigiano 2005). Further digestion was performed at 160°C in a sand bath on a hot plate until the solutions were clear. After cooling, mixtures were diluted to 25 mL in volumetric flasks with de-ionized water. Then they were filtered through $0.45 \mu\text{m}$ nitrocellulose membrane filters and kept in 25 mL plastic bottles at 4°C . Metal analyses were performed using a graphite furnace atomic absorption spectrometer (model AA3030 Perkin Elmer, Germany). The limits of detection were as follows: Cd ($0.05 \mu\text{g kg}^{-1}$), Pb ($0.8 \mu\text{g kg}^{-1}$), Ni ($0.7 \mu\text{g kg}^{-1}$), and Cr ($0.3 \mu\text{g kg}^{-1}$). Two standard reference materials, including DORM-2 for fish (dogfish muscle, National Research Council of Canada) and STD TMDA-70 for water (fortified lake water), were digested in triplicate to support quality control. Moreover, a blank was run for the digestion procedure to correct the measurements. For sets of every 10 samples, a procedure blank and spike sample was ran to check cross-pollutions. Recoveries ranged from 96% to 109%. Results were expressed as milligrams of metal per kilogram wet weight.

Statistical analysis

The Kolmogorov–Smirnov test was accomplished to analyze the normality of data distribution. The analysis of variance (ANOVA) was used for the detection of differences in metal concentrations between tissues; Tukey's honest significant difference test was employed. Furthermore, the obtained data were statistically analyzed by the *t*-test for the

Table 1. Age, total length (cm), standard length (cm) and body weight (g) of fishes.

Factor	<i>Liza klunzingeri</i> ($n = 54$)		<i>Sillago sihama</i> ($n = 60$)	
	Range	Mean \pm SD	Range	Mean \pm SD
Weight	37.5–69.7	54 ± 9.2	30.5–76	50.5 ± 15.0
Total length	14.5–21	17.3 ± 2.1	15–30	21.3 ± 4.7
Standard length	12.5–19	15 ± 2.0	13–28.5	19 ± 4.8
Age	2–5.5	3.9 ± 1.1	2–6.5	4 ± 1.5

assessment of variation in metal concentrations between two different species. Pearson's correlation coefficients were used when calculating correlations between fish length, weight, age, and concentration of metals. Statistical analyses were carried out using the SPSS statistical package program. Moreover, bioaccumulation factor between fish and water were calculated using the mean metal concentrations in fish and the corresponding metal concentrations for water (Bu-Olayan, Thomas, and Al-Husaini 2008).

Results and discussion

In the water, the mean concentrations of Pb, Cd, Ni, and Cr were 0.15, 0.04, 0.1, and 0.25 mg L⁻¹. The concentrations in muscles, gills, and livers of *L. klunzingeri* and *S. sihama* are shown in Table 2. Bioaccumulation factors of the various metals were calculated and presented in Table 3.

In general, mean concentrations in the muscles, gills, and livers of *S. sihama* and *L. klunzingeri* were as follows: Ni > Cr > Pb > Cd, except muscles in *S. sihama* (Ni > Pb > Cr > Cd). The concentrations of metals were highest in the livers followed by the gills and the muscles ($p < 0.01$) (Table 2). Content of Ni in the tissues varied from 1.5 to 2.1 mg kg⁻¹ in *L. klunzingeri* and 2.9–3.1 mg kg⁻¹ in *S. sihama*. Our results of Ni content were lower than that reported by Abu Hilal and Ismail (2008) for fish species from the Gulf of Aqaba in the Red Sea (2.5–8.1). However, the concentration of Ni was higher than those given for fishes from Ataturk Dam Lake 0.6–1.1 (Karadede, Oymak, and Unlu 2004). Mean Cr concentrations ranged from 0.5 to 1.3 mg kg⁻¹ in fish species. These results were lower than those reported in the fishes from Gulf of Aqaba 4–14 (Abu Hilal and Ismail 2008) and higher than those given in Tuzla lagoon in the Mediterranean region (Turkmen et al. 2009). The mean concentrations of Pb levels were 0.3–1 mg kg⁻¹ for the analyzed fish samples. These values were lower than those reported by Abu Hilal and Ismail (2008) for the fish samples of Gulf of Aqaba in Red sea (4.4–17.6). Average Cd values in fish species were found to be in the range of 0.2–1 mg kg⁻¹. These results were

Table 2. Metal concentrations in different organs (mg kg⁻¹ wet weight) and *t*-test statistical analysis of concentrations in *L. klunzingeri* and *S. sihama*.

Metal	Tissue	<i>L. klunzingeri</i>		<i>S. sihama</i>		<i>t</i> -test
		Range	Mean ± SD	Range	Mean ± SD	
Pb	Muscle	0.25–0.39	0.32 ± 0.04 ^a	0.63–0.79	0.71 ± 0.05 ^a	27.11 ^{**}
	Gill	0.3–0.56	0.44 ± 0.08 ^b	0.7–0.93	0.83 ± 0.06 ^b	16.59 ^{**}
	Liver	0.5–0.85	0.67 ± 0.11 ^c	0.84–1.17	0.99 ± 0.09 ^c	9.89 ^{**}
Cd	Muscle	0.05–0.25	0.16 ± 0.06 ^a	0.29–0.59	0.45 ± 0.09 ^a	11.54 ^{**}
	Gill	0.24–0.42	0.32 ± 0.06 ^b	0.47–0.85	0.65 ± 0.11 ^b	11.42 ^{**}
	Liver	0.51–0.74	0.63 ± 0.07 ^c	0.77–1.08	0.91 ± 0.08 ^c	11.32 ^{**}
Cr	Muscle	0.38–0.56	0.5 ± 0.05 ^a	0.42–0.86	0.63 ± 0.12 ^a	3.85 ^{**}
	Gill	0.7–1.05	0.87 ± 0.11 ^b	0.69–1.33	0.99 ± 0.17 ^b	2.53 ^{**}
	Liver	0.85–1.18	1.04 ± 0.1 ^c	0.87–1.62	1.26 ± 0.23 ^c	3.72 ^{**}
Ni	Muscle	1.35–1.68	1.52 ± 0.1 ^a	2.74–2.98	2.87 ± 0.07 ^a	48.21 ^{**}
	Gill	1.65–1.95	1.78 ± 0.09 ^b	2.89–3.07	2.99 ± 0.05 ^b	51.66 ^{**}
	Liver	1.87–2.2	2.06 ± 0.11 ^c	3–3.22	3.12 ± 0.08 ^c	35.17 ^{**}

Notes: ^{a,b,c}Means with the same letters in each column for each metal are not significantly different according to Tukey's test.

**Significant differences at $p < 0.01$.

lower than those reported in fish species of Gulf of Aqaba 0.8–2.2 (Abu Hilal and Ismail 2008). However, the concentration of Cd was higher than those given for fishes from Saricay stream in Anatolia (0.001–0.1) (Yilmaz et al. 2007).

In this study BAF were generally higher in the liver than gills and muscles (Table 3). Metals are mostly accumulated in metabolically active organs such as liver and gills (Dural et al. 2006; Qiao-qiao, Guang-wei, and Langdon 2007). The literature shows that the liver has a significant function in basic metabolism, contaminant storage, and transformation (Agah et al. 2009; Malik et al. 2010). Liver stores metals to detoxify by producing metallothioneins (Yilmaz et al. 2007). Thus, it is an excellent environmental indicator of chronic exposure to metals and water contamination (Agah et al. 2009; Fernandes et al. 2007). In our investigation, gills had the highest concentration of metals after liver. Gills are the first tissues to be exposed to suspended sediment particles, so they can be significant places of interaction with metals (Dural et al. 2006; Fernandes et al. 2007). Also, the reason for high metal concentrations in the gill could be caused by the metal complexation with the mucus that is not possible to be eliminated entirely from it (Bahnasawy, Khidr, and Dheina 2009; Yilmaz 2009). In contrast, results were reported from a number of fish species that the muscle tissues are not considered an active location for metal accumulation (Fernandes et al. 2007; Kamaruzzaman, Ong, and Rina 2010).

However, muscle tissue is the principal edible fish section and can directly affect human health (Agah et al. 2009). In this study Cd levels recorded in muscle samples were low, when compared to FAO (0.5 mg kg^{-1}) (Anim et al. 2011), WHO, and FEPA (2.0 mg kg^{-1}) maximum recommended limits in fish food (Obasohan 2007). Pb levels in the *S. sihama* muscles were higher than the maximum acceptable concentrations established by FAO (0.5 mg kg^{-1}) (Anim et al. 2011). But comparing our average values with that of WHO and FEPA (2.0 mg kg^{-1}) (Obasohan 2007), our results showed that the Pb values in two fish species are lower than these guidelines. Lead causes neurological immunological and reproductive impacts (Obasohan 2008), as well as renal failure and liver injury when it exceeds the endurable limit in humans (Zhang et al. 2007). The mean concentration of Ni in the fish muscles was higher than the maximum acceptable limit of $0.5\text{--}0.6 \text{ mg kg}^{-1}$ for food fish, recommended by WHO and FEPA (Obasohan 2007). Ni also shows carcinogenic influence on aquatic organisms and humans when absorbed in high quantity (Malik et al. 2010). Moreover, the mean concentrations of Cr were higher than the acceptable limit proposed by WHO and FEPA (0.15 mg kg^{-1}) (Obasohan 2007). Cr accumulation in fish has been reported to cause damaged respiratory and osmoregulatory duties through structural injury to gill epithelium (Obasohan 2008). Also, several studies have shown that chromium is a human carcinogen (Malik et al. 2010; Zhang et al. 2007). Therefore we can conclude that Pb, Cr, and Ni have presumably posed a threat for the consumption of these fishes. Two selected sampling sites in this study are affected by the

Table 3. Metal bioaccumulation factors.

Species	Tissue	Pb	Cd	Cr	Ni
<i>L. klunzingeri</i>	Muscle	2.2	4	2	15
	Gill	2.9	8	3.5	18
	Liver	4.5	16	4.16	20
<i>S. sihama</i>	Muscle	4.7	11.3	2.5	29
	Gill	5.5	16	4.0	30
	Liver	6.6	23	5.0	31

harbors, some industrial and commercial activities. Release of untreated sewage from various industries close to the sampling sites like cement plant, lead and zinc factory, power plant, aluminum factory, paint industries and also the release of petroleum components from commercial ships, small boats, and a refinery into water make it polluted.

In this study, metal accumulations in all tissues of *S. sihama* were higher than that in *L. klunzingeri* ($p < 0.01$) (Table 2). It is difficult to compare the metal concentrations between the two different species due to different ecological needs (Qiao-qiao, Guang-wei, and Langdon 2007), feeding habits (Malik et al. 2010), differences in the absorptive capabilities and the animals' anatomic considerations (Sia su et al. 2009), and other factors. But we can conclude that benthic fishes (*S. sihama*) which have close relationship with sediment, exhibit comparatively high body concentrations of metals (Bahnasawy, Khidr, and Dheina 2009; Qiao-qiao, Guang-wei, and Langdon 2007).

There were significant negative relationships between all metal levels and weight, length, and age in the liver and gills of *L. klunzingeri* ($p < 0.05$), except Pb, Cr, Cd, and Ni in the muscle and also Cd in the gills ($p > 0.05$). Also, in *S. sihama*, results show negative correlations between all metal levels and biotic factors ($p < 0.01$). In various studies, negative correlations between fish size, age, and metal concentrations were reported due to higher metabolic rates (Pourang et al. 2005); faster growth than metal accumulation (Agah et al. 2009) and short residence time of these metals within the fish (Zhang et al. 2007).

Conclusion

Results showed that the mean concentrations of the metals and also BAF were the lowest in muscles and the highest in livers. Tissues with higher metabolic activities, such as livers, accumulate more metals than tissues with lower metabolic activities, like muscles. The accumulation of metals was more pronounced in *S. sihama* than in *L. klunzingeri*. Several factors like regulatory ability, behavior, and feeding habits can affect the metal accumulation in the different species. The significant negative relationship is observed between fish age, length, weight, and accumulation of metals in most of the tissues. Therefore, younger fishes show higher metal levels than older fishes. The present results showed that, these fishes, based on the higher levels of metal accumulation, could be unsafe for human consumption. Consequently, very close monitoring of heavy metal loads in this vital environment is recommended in view of the possible risks to health of consumers and aquatic organisms.

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