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Determination of Mercury in Some Freshwater Fish Species from Chahrmahal va Bakhtyari Province, Iran and Potential Limits for Human Consumption

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Abstract Concentrations of mercury in four freshwater fish species from Gandoman and Sooleghan Lagoons and Beheshtabad River were determined by Cold Vapor Atomic Absorption Spectrometry. Concentrations of mercury in muscle of 90 fish ranged from 21 to 31 $\mu g\ kg^{-1}$ (mean = 26 $\mu g\ kg^{-1}$). Statistical analysis showed no statistical relationship between mean mercury concentration and fish species, although concentration of mercury in different seasons and habitats was statistically different ($p < 0.05$). The results indicated that fish from Gandoman and Sooleghan Lagoons and Beheshtabad River have concentrations well below the maximum permissible levels of mercury according to international standards with no health risk for consumers.

Keywords Mercury · Fish · Gandoman Lagoon · Beheshtabad River

Mercury is a metal that occurs naturally at low levels in soil and generally enters the aquatic environment through atmospheric deposition, erosion of geological matrices or due to anthropogenic activities caused by industrial effluents including coal-fired power plants, boilers, steel production, incinerators, cement plants, agricultural sewage and mining wastes (Oze et al. 2006). Under anoxic aquatic conditions, mercury can become methylated and this methylmercury form can bioaccumulates in organisms at the bottom of the food chain and experience biomagnifications up the food chain reaching its highest concentrations in top predator fish (Zhu et al. 2011). It is generally accepted that the consumption of contaminated seafood is one of the major sources of mercury exposure for humans (Hutcheson and Smith 2008). In recent years, increasing agricultural activities and the use of sewage sludge as fertilizer around the Gandoman and Sooleghan Lagoons and the Beheshtabad River has resulted in the release of metals and pesticides into these aquatic habitats. Hence, it is important to investigate the levels of mercury in fish from aforementioned places to assess whether the concentration is within the permissible level and will not pose any hazard to the consumers. The aim of this study was to determine the levels of mercury in fish from the Gandoman and Sooleghan Lagoons and the Beheshtabad River in Iran and assess the potential risk of Hg exposure associated with the consumption of fish from these regions.

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Materials and Methods

A total number of 90 freshwater fish including common carp (*Cyprinus carpio*, n = 27), Prussian carp (*Carassius auratus gibelio,* n = 18), bleak (*Alburnus alburnus*, n = 27) and *Capoeta damascina* (n = 18), referred to locally as black fish, from Gandoman and Sooleghan Lagoons and Beheshtabad River were studied. Fish were equally caught from the three studied areas. The fish samples were immediately transported to the laboratory in plastic containers filled with crushed ice. The size of each fish was measured and dorsal muscle samples (10–20 g) were dissected from the fish (next to the dorsal fin) and were stored at −18°C before the analysis.

All the plastic and glassware were soaked in nitric acid for 15 min and rinsed with deionized water before use. All reagents and solvents were of analytical reagent grade (Merck, Germany). ASTM® Type I water (from an ELGA® filtration system-ELGA LLC, USA) acidified to 1 % nitric acid was used to make the calibration blank and standards. The stock solution of mercury (1,000 mg/L) was obtained by dissolving appropriate metal salt (Merck, Germany) in double distilled water. The working solution was freshly prepared by diluting an appropriate aliquot of the stock solution using 1 M HCl and 5 % H2SO4 for diluting mercury solution. Stannous chloride was freshly prepared by dissolving 10 g in 100 ml of 6 M HCl. The solution was boiled for about 5 min, cooled, and nitrogen bubbled through it to expel any mercury impurities (Voegborlo et al. 1999). In the laboratory, the moisture content of the tissue samples was determined according to AOAC method in triplicate (WHO 1993). Samples were homogenized using a bench-top mixer (Buchi-Mixer B-400) and subsequently wet digested following the techniques outlined by Oze et al. (2006). Mercury concentrations in sample homogenates were determined according to the International Organization for Standardization (ISO) method ISO 12846: 2012. Mercury was determined in all the digests using Cold Vapor Atomic Absorption Spectrophotometer (Elmer Analyst 4100 model AAS) flow injection mercury/hydride analyzer (FIAS 4100, Perkin Elmer) equipped with hollow cathode mercury lamp at a wave length of 253.7 nm. The atomic absorption signal was measured as a peak height mode against an analytical curve. The recovery rate was determined by adding increasing amounts of mercury to the samples and taking it through the digestion procedure. The Standard Reference Material (SRM) used in this study was dogfish (*Squalus* sp.) muscle, certified by the National Research Council of Canada (NRCC) as DORM-2.

For each run, a duplicate sample, spiked samples and two blanks were carried through the whole procedure. SRM was analyzed once for every 3 fish samples.

Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, Washington, USA) for analysis. SPSS 18.0 statistical software (SPSS Inc., Chicago, Illinois, USA), was used for ANOVA and Student's t test analysis; differences were considered significant at values of $p < 0.05$.

Daily Consumption Limits were calculated according to the following Eq. 1 (EPA 2000). It shows allowable daily consumption of mercury contaminated fish based on a contaminant's carcinogenicity, expressed in kilograms of fish consumed per day:

$$CR_{lim} = \frac{RfD \times BW}{C_m} \qquad (1)$$

Calculation of maximum allowable fish consumption rate

CRlim = maximum allowable fish consumption rate (kg/day)

RfD = reference dose (0.1 µg/kg-day for mercury)

BW = consumer body weight (kg)

Cm = measured concentration of chemical contaminant *m* in a given species of fish (µg/kg).

The consumption limit was also determined in part by the size of the meal consumed based on 0.227 kg meal size was assumed (EPA 2000). The Eq. 2 was used to convert daily consumption limits to the number of allowable meals per month:

$$CR_{mm} = \frac{CR_{lim} \times T_{ap}}{MS} \qquad (2)$$

Calculation of maximum allowable fish consumption rate

CRmm = maximum allowable fish consumption rate (meals/mo)

CRlim = maximum allowable fish consumption rate (kg/day)

MS = meal size (0.227 kg fish/meal)

Tap = time averaging period (365.25 days/12 month = 30.44 days/month).

Results and Discussion

Biometric characteristics, age and sex of the studied fish are mentioned in Table 1.

The method detection limit for Hg was determined to be 0.0006 µg/g. The relative standard deviations were less than 10 % for mercury. The accuracy of the method and spike recoveries was calculated by means of mercury determination in SRM. The achieved results were in good agreement with certified values. The mean recovery value of mercury was 96.33 % (Table 2).

The concentrations of mercury in studied samples and the permissible limit are presented in Table 2. The results indicated that the concentration varied from 21 to 31 with mean of 26.6 µg kg^{-1} (Table 3).

Mean concentrations of mercury in three locations were lower than the maximum allowed levels according to international standards. Statistical analysis showed no statistical relationship between mercury concentration and fish species, age and weight, although concentration of mercury in different seasons was statistically different (Table 4). There was a statistical difference in mercury content of fishes from different habitats ($p < 0.05$), as concentration of mercury in fish from Gandoman Lagoon was higher than it in Sooleghan Lagoon and Beheshtabad River (Table 3). The obtained results show that the variation of mercury concentration among studied fish species is relatively low. The relative lack of variability of Hg

Table 1 Age, sex, total length, weight and number of studied fishes

Fish species	Age (year)		Sex		Length (cm)		Weight (g)	
	Range	Mean	Male	Female	Range	Mean	Range	Mean
Cyprinus carpio	1–3	2.1	16	11	9.5–21	14.2	23.7–178	74.5
Carassius auratus gibelio	1–3	2.2	11	7	10.6–18.4	13.1	35.5–212	60.8
Alburnus alburnus	1–3	2.2	13	14	8.3–14.7	10.8	17.7–45.5	43.2
Capoeta damascina	1–3	2.1	8	10	11.5–19.3	14	41–196.4	69.9

concentration among the different species of fish collected at each location, and also among the different locations may be because of the high ecological similarity between the studied areas, the low distances between lagoons and the river, and having the same contaminating resources that are generally resulted from agricultural activities. In addition, the studied fish species belong to the same trophic level and same age and size range which make it more possible to accumulate the same content mercury from the environment, although it needs more study.

Concentrations of mercury were compared in the same species from different habitats. The Analysis of Variance test showed a statistical difference in mercury concentration for all four studied species ($p < 0.05$).

Estimates of the health risks associated with consumption of Hg contaminated fish are presented according to daily (kg/day) and monthly (meals/month) limits for the 3–75 year old population demographic (Table 5). According to the results, maximum allowable fish consumption rate ranged between 233 and 321 g/day and 31 and 43 meal/month for an adult person with mean 71.5 kg body weight in the studied area.

Mercury is recognized as a global environmental pollutant, with high toxicity even at low concentrations. Mercury strongly bioaccumulates in aquatic food, and about 95 % of the methyl mercury in human is originated from aquatic organisms (Raissy and Ansari 2013). Mercury and methyl mercury are neurological toxicants to humans (Commission of the European Communities 2001). Methyl mercury is classified as a group C possible human carcinogen (US and FDA 2004). Accordingly, the tolerable weekly intake (1,600 μg g^{-1} bw) established by the Commission of the European Communities (2006) has led to regulatory guidelines for the mercury concentrations allowed in seafood being established in several countries. The European Commission Decision 1881/2006 sets the maximum limit for mercury in seafood at 500 μg kg^{-1} for fresh food, increasing to 1,000 μg kg^{-1} for the edible parts of some listed species that, for physiological reasons, concentrate mercury more easily in their tissues (Commission of the European

Table 2 Recovery of mercury from fish samples

Metal	Concentration of the metal added (μg g^{-1})	Concentration of the metal recovered (μg g^{-1})[a]	% Recovery[a]
Mercury	0.010	0.0099	99
	0.020	0.019	95
	0.040	0.038	95

[a] Data are mean of three samples of three replicates

Table 4 Mercury concentration in studied samples in different seasons (μg kg^{-1})

Season	No	Mean ± SD
Summer	16	26.8 ± 0.63
Autumn	23	26.3 ± 0.53
Winter	28	24.9 ± 0.38
Spring	23	28.8 ± 0.51
Total	90	26.6 ± 0.52

Differences were considered significant at values of $p < 0.05$

Table 3 Mercury concentration in studied samples (μg kg^{-1})

Sampling area	*Cyprinus carpio*	*Carrasius auratus gibelio*	*Alburnus alburnus*	*Capoeta damascina*	Mean	Range	Permissible amount
Sooleghan Lagoon	$22.3^{a} \pm 0.63$	$22.5^{a} \pm 0.25$	$22.1^{a} \pm 0.55$	$22.1^{a} \pm 0.53$	22.3 ± 0.35	21–23	500[1,2]
Gandoman Lagoon	$30.8^{b} \pm 0.49$	$30.9^{b} \pm 0.24$	$30.7^{b} \pm 0.50$	$30.0^{b} \pm 1.99$	30.7 ± 0.67	26–31	
Beheshtabad River	$26.7^{c} \pm 0.84$	$27.2^{c} \pm 0.98$	$26.2^{c} \pm 0.52$	$26.7^{c} \pm 0.93$	26.8 ± 0.45	26–29	
p value	<0.05	<0.05	<0.05	<0.05	<0.05		

Different letters in each column show statistical difference

[1] Commission of the European Communities (2006), US and FDA (2004)

[2] MAFF (1995)

Communities 2006). The 500 μg kg^{-1} recommended guideline for mercury concentrations in fish for human consumption is effectively a global guideline with North American (USEPA & Health Canada) regulatory agencies enforcing the same limit (US and FDA 2004).

Although it is common that fish is one of the major sources of mercury, the efficiency of metal uptake from contaminated freshwater and food may differ in relation to ecological needs, metabolism and the contamination level of mercury in water, food and sediment as well as

Table 5 Maximum allowable fish consumption rate according to the mercury content

Age (year)	Average body weight for males and females (kg)	Maximum allowable fish consumption rate (kg/d)				Maximum allowable fish consumption rate (meals/mo)			
		Sooleghan Lagoon	Gandoman Lagoon	Beheshtabad River	Mean	Sooleghan Lagoon	Gandoman Lagoon	Beheshtabad River	Mean
3–6	11.6	0.052	0.038	0.043	0.043	7.0	5.1	5.8	5.8
6–9	25	0.112	0.081	0.093	0.093	15.0	10.9	12.5	12.6
9–12	36	0.161	0.117	0.134	0.135	21.6	15.7	18.0	18.1
12–15	50.6	0.227	0.165	0.189	0.190	30.4	22.1	25.3	25.5
15–18	61.2	0.274	0.199	0.228	0.230	36.8	26.7	30.6	30.9
18–25	67.2	0.301	0.219	0.251	0.252	40.4	29.4	33.6	33.9
25–35	71.5	0.321	0.233	0.267	0.268	43.0	31.2	35.8	36.0
35–45	74.0	0.334	0.242	0.278	0.279	44.7	32.5	37.2	37.5
45–55	74.5	0.334	0.243	0.278	0.280	44.8	32.5	37.3	37.6
55–65	73.4	0.329	0.239	0.274	0.275	44.1	32.1	36.7	37.0
65–75	70.7	0.317	0.230	0.264	0.265	42.5	30.9	35.4	35.6

Table 6 Comparison of mercury concentrations in freshwater fish muscles in this study with other researches

Location	Fish species	Number of samples	Mean or range of length (cm)	Mean or range of mercury concentration (μg kg^{-1})	References
Gheshlagh dam, Iran	*Cyprinus carpio* (white muscle)	48	26.5–37 (30.6)	123–458 (233)	Khoshnamvand et al. 2010
	Cyprinus carpio (red muscle)			115–455 (227)	
Ebro River, Spain	*Cyprinus carpio*	68	309–2,050	70–399 (75)	Carrasco et al. 2011
Zarivar Wetland, Iran	*Cyprinus carpio*	10	411.5	400–1,900 (1,100)	Majnoni et al. 2013
Serbia	*Cyprinus carpio*	10	16.9–31.6 (24.4)	630	Skoric et al. 2012
	Carassius auratus gibelio	10	17.2–23.6 (19.7)	790	
Salek Lake, Slovenia	*Cyprinus carpio*	5	45.2	20–40 (30)	Al Sayegh Petkovšek et al. 2012
	C. auratus gibelio	11	30.9	50–310 (140)	
	Alburnus alburnus	5	15.2	70–120 (90)	
Cinca River, Spain	*Alburnus alburnus*	30	6.7–15.6	41–2,362 (545.8)	Raldúa et al. 2007
Gandoman Lagoon	*Cyprinus carpio*	9	12.9–21 (14.8)	29.5–31 (30.81)	This study
	C. auratus gibelio	6	10.6–15.2 (13.3)	30.4–31 (30.90)	
	Alburnus alburnus	9	10.3–14.7 (9.5)	29.7–31 (30.72)	
	Capoeta damascina	6	11.5–16.5 (14.2)	26–31 (30.05)	
Sooleghan Lagoon	*Cyprinus carpio*	9	9.5–16.3 (13.9)	21.1–23 (22.30)	
	C. auratus gibelio	6	11.4–18.4 (13.2)	22.1–22.9 (22.50)	
	Alburnus alburnus	9	9.7–14.6 (11.2)	21–22.6 (22.14)	
	Capoeta damascina	6	12–17 (14.6)	21.4–22.8 (22.15)	
Beheshtabad River	*Cyprinus carpio*	9	11.7–18 (13.7)	26.1–28.8 (26.70)	
	C. auratus gibelio	6	10.8–15.2 (12.5)	26.1–28.3 (27.25)	
	Alburnus alburnus	9	8.3–14 (11.8)	26–27.6 (26.25)	
	Capoeta damascina	6	11.9–19.3 (13.2)	26–27 (26.75)	

physicochemical factors of water such as water pH and hardness (Canli and Furness 1993).

Influence of season and habitat on mercury content of fish has been proven before by Saei-Dehkordi et al. 2010. In this study, habitat and season were found to be effective on mercury residue in fish. According to the results the concentration of mercury in Gandoman Lagoon was significantly higher than it in other places. The mean concentration of mercury in fish from Gandoman Lagoon was higher than for fish from the Sooleghan Lagoon and Beheshtabad River locations. Fish from lakes may be more likely to have higher concentrations than rivers, due to the methylation rates in lake ecosystems versus river ecosystem. The other reason of high concentrations of mercury in fish from Gandoman Lagoon is wide agricultural activities and entering agricultural sewages into the Lagoon compared to two other regions.

There are previous reports on bioaccumulation of mercury in cultured and wild aquatic species in Iran (Zolfaghari et al. 2005; Saei-Dehkordi et al. 2010; Jalilian et al. 2011; Raissy et al. 2011, 2012). At the time of this study, little was known about the mercury level in fish from the studied areas while bioaccumulation of mercury has been widely studied in other places with similar ecological importance. According to table 4, the mean concentrations of mercury in the muscle of common carp from this study are almost the same as those reported from the Salek Lake, Slovenia and Czech Republic, while comparing mean mercury levels in this study demonstrates that our results are lower than the same species in many other places as the mean mercury content of *Capoeta* and *C. carpio* from Kızılırmak River, Turkey and Zarivar Wetland, Iran is near 55 and 37 times higher than mercury content of the same genus from Gandoman Lagoon.

The variability of mercury concentrations in different studies could be attributed to several factors: ecological conditions of habitat, age and body weight and time of the study (Canli and Furness 1993).

The similarity of Hg contamination among the fish species included in this study is likely a consequence of their common trophic level and benthic feeding strategy in addition to the relative proximity of the study habitats (Table 6).

The results presented in this study show that the mean concentrations of mercury in fishes are lower than maximum permitted levels according to international standards and are suitable for human consumption (Commission of the European Communities 2006; MAFF 1995). Maximum suggested fish consumption rate ranged between 233 and 321 g/day and 31 and 43 meal/month for an adult person in the studied area. Fish from the aforementioned locations should be analyzed more often with respect to toxic metals not only from the human consumption point of view but also from the environmental point of view.

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