

Metals Analysis in Common Carp (*Cyprinus Carpio*) from Shirinsu Wetland, Hamedan Province, Iran

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Background & Aims of the Study: Concentrations of Cd, Hg and As were measured by atomic absorption spectrometry (AAS) in muscle, liver and gill tissues of common carp from the Shirinsu Wetland during February to March 2013.

Methods: Fish samples (*Cyprinus carpio*) were caught from Shirinsu Wetland, western Iran from February to March 2013. Specimens were frozen in prewashed polyethylene bags and frozen samples brought to the laboratory in ice chests. Samples (2 g) were digested with 5 ml of HNO₃ (65% v/v), 1 ml of H₂O₂ (30% v/v) with a microwave oven. A blank digest was carried out in the same way. The digestion program began at a potency of 1200W then ramped for 10 min, after which samples were held for 10 min at 1200W. The second step began at a potency of 0W and held for 15 min. All metal concentrations were determined on a wet weight basis as µg g⁻¹.

Results: Metal levels measured in muscle tissue were in the following ranges (µg g⁻¹): Cd 0.007-0.011, Hg 0.006-0.01 and As not detected. In liver tissue were (µg g⁻¹): Cd 0.035-0.043, Hg and As not detected. In gill tissue were (µg g⁻¹): Cd 0.31-0.55, Hg 0.002-0.004 and As 0.001-0.003. The results presented on metal contents in the examined tissues give an indication of the environmental conditions. Concentrations of Cd, Hg and As obtained were far below the established values by the European Community Regulations. However, Cd level found in gill tissue was higher than the recommended legal limits for human consumption and as such may cause a human health issue.

Conclusion: Analytical data shows that the metal concentrations for the fish tissues were generally within the FAO/WHO, ASTDR and EEC recommended limits for fish. Therefore there is no serious health risk associated with the consumption of the three studied metals in the muscle and liver tissues analyzed

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Background

The pollution of water resources due to the disposal of heavy metals has been an increasing worldwide concern for the last few decades (1). Heavy metals from geological and anthropogenic sources are increasingly being released into natural waters (2,3). Contamination of aquatic ecosystems with heavy metals has seriously increased worldwide attention, and a lot of studies have been published on the analysis of heavy metals in the aquatic environment (4,5). Under certain

environmental conditions, heavy metals may accumulate to toxic concentrations and cause ecological damage (6).

Heavy metals such as Fe, Cu, Zn and Mn, are essential metals since they play an important role in biological systems whereas non-essential metals, such as Cr, As, Hg and Cd are toxic even in trace amounts. The essential metals can also produce toxic effects at high concentrations. Only a few metals, of proven hazardous nature are to be completely excluded in food for human consumption. Thus, only three metals, namely Pb, Cd and Hg, have been

included in the regulations of the European Union for hazardous metals (7) while the USFDA has included further three elements, namely, Cr, As and Ni in the list (8,9). The genetic and epigenetic effects of these elements are associated with an increased risk of different types of cancer (10).

Fishes are often at the top of the aquatic food chain and may concentrate large amounts of some metals from the water (11). Furthermore, fish is one of the most indicative factors in freshwater systems, for the estimation of trace metals pollution and risk potential of human consumption (12,13). Heavy metals are taken up through different organs of the fish because of the affinity between them. In this process, many of these heavy metals are concentrating at different levels in different organs at the fish body (14). Hence, it is important to determine the concentration of heavy metals in commercial fishes in order to evaluate the possible risk of fish consumption (15).

Shirinsu Wetland is a fresh water lake located in 35°-30' to 35°-45' North and 28°-25' to 40°-

48' East in the Northwest of Hamedan province, Iran. Area of Shirinsu Wetland is about 300 ha. Shirinsu wetland survival is mostly dependent upon the water quantity entrance through the natural springs and seasonal river near the mentioned lake. Shirinsu Wetland ecosystem has a great biodiversity and aesthetic value. Every year in winter, many aquatic and wading birds migrate to this lake such as *Gelochelidon nilotica*, *Anas platyrhynchos*, *Ciconia ciconia*, *Phalacrocorax carbo*. Also *Cyprinus carpio* are most commonly fish species founded in this wetland. Because the metal pollution in aquatic environments can be harmful to human health, it is necessary to understand and control the hazard levels of heavy metal pollution in fish and seafood. Therefore, this study aimed to determine the levels of Cd, Hg and As in the muscle, liver and gill of the different species from Shirinsu Wetland, and to assess the public health risks associated with consuming fish harvested from this area (16).

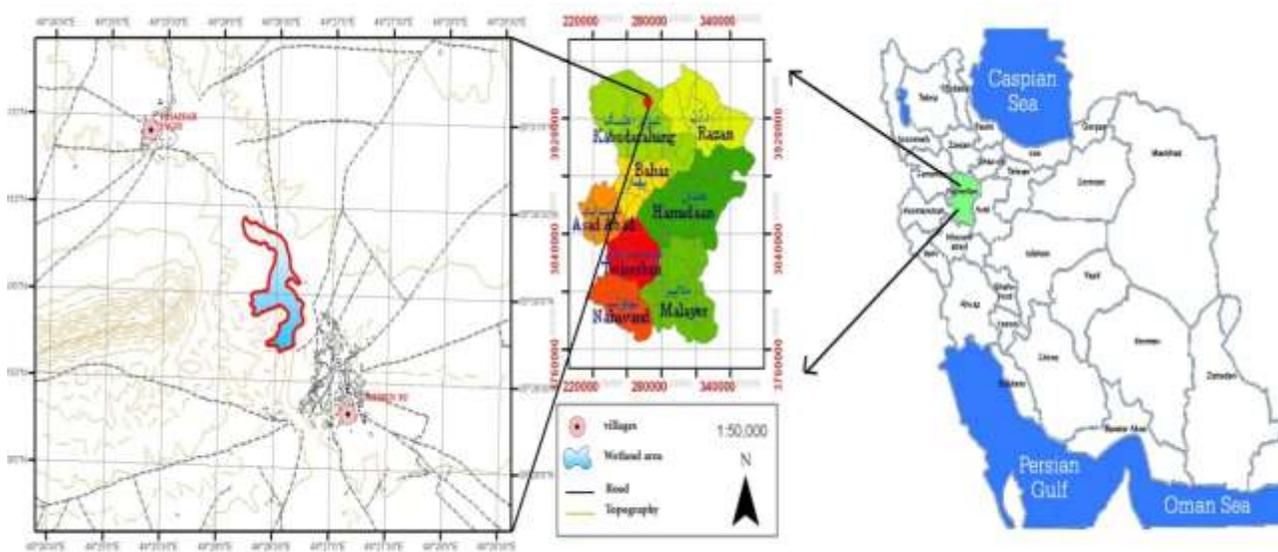


Figure 1) The position of Shirin Su in Hamedan province

Materials & Methods

Sampling:

28 Fish samples (*Cyprinus carpio*) were caught from Shirinsu Wetland, western Iran from February to March 2013. Specimens were frozen in prewashed polyethylene bags and frozen samples brought to the laboratory in ice chests. Approximately 1 g sample of muscle, liver and gill from each fish were dissected, washed with distilled water, weighed, packed in polyethylene bags and stored at -18°C until the performance of chemical analysis.

Reagents:

All reagents were of analytical reagent grade, HNO₃, H₂O₂ and HCl (Merck, Darmstadt, Germany). Deionized water was used for all dilutions. All plastic and glassware were cleaned by soaking in diluted HNO₃ (1/10, v/v) and rinsed with distilled water prior to use. Each element supplied by Perkin Elmer. Generation Pd(NO₃)₂ and 0.003 mg Mg(NO₃)₂ (Perkin Elmer, USA) were used. The hydride technique for Hg determination involves the reaction of acidified aqueous samples (3% v/v HCl) with a reducing agent 0.2% sodium borohydride in 0.05% NaOH.

Analyses of Cd and As were conducted by graphite furnace atomic absorption spectroscopy using an AAnalyst 4110 ZL atomic absorption spectrometer (Perkin Elmer, USA) equipped with an autosampler (Perkin Elmer, USA). For graphite furnace measurements, argon was used as the inert gas. Pyrolytic-coated graphite tubes with a platform were used. The atomic absorption signal was measured in peak area mode against a calibration curve. Mercury was analyzed by the cold vapour technique with a flow injection system coupled using direct mercury analyzer (DMA-80). Microwave closed system Multiwave 3000 (Anton Paar, Germany) was used for digestion of samples.

Microwave digestion:

Samples (2 g) were digested with 5 ml of HNO₃ (65% v/v), 1 ml of H₂O₂ (30% v/v) with a

microwave oven. A blank digest was carried out in the same way. The digestion program began at a potency of 1200W then ramped for 10 min, after which samples were held for 10 min at 1200 W. The second step began at a potency of 0W and held for 15 min. Digested samples were diluted to a final volume of 50 ml with double deionized water. All metal concentrations were determined on a wet weight basis as $\mu\text{g g}^{-1}$. Detection limits were determined as the concentration corresponding to three times the standard deviation of ten blanks. All specimens were run in batches that included blanks, a standard calibration curve, two spiked specimens, and one duplicate (17).

Statistical analysis:

To test the differences between average concentrations of heavy metals in evaluated tissues, one-way ANOVA was performed (Tukey post-hoc). The mean levels of heavy metals were compared with international standard using a one-sample test. Probabilities less than 0.05 were considered statistically significant ($p < 0.05$). All statistical analyses were performed using the SPSS 15.0 (SPSS Inc., Chicago, IL, USA) statistical package.

Results

In the present study levels of Cd, Hg and As in muscle tissue of three fish species caught from Shirinsu Wetland in were determined. Table 1 shows the mean concentrations of three elements (geometric means and range) in the muscle, liver and gill of fish *Cyprinus carpio*. The results indicate that Cd concentration in fish tissues ranged from 0.007 to 0.55 $\mu\text{g g}^{-1}$ whereas Hg concentration ranged from 0.002 to 0.01 $\mu\text{g g}^{-1}$. Levels of As in different tissues ranged from 0.001 to 0.003 $\mu\text{g g}^{-1}$ and all samples were below the detection limit for Cd, Hg and As. The results indicated that the average concentrations of evaluated metals in the edible part of common carp are significantly lower than the adverse level for the species themselves and for human consumption with

FAO/WHO, ASTDR and EEC permissible limits (6,18-21). Therefore, their contribution to

the total body burden of these metals can be considered as negligibly small.

Table1) Metal concentrations (geometric mean $\mu\text{g g}^{-1}$ w.w and range) in the muscle, liver and gill tissues of the common carp from Shirinsu Wetland, Iran.

Tissue		Cd	Hg	As
Muscle	mean±SD	0.003±0.001 ^{a*}	0.06±0.02 ^b	nd
	Range (min–max)	0.007-0.011	0.006-0.010	nd
Liver	mean±SD	0.004±0.001 ^b	nd	nd
	Range (min–max)	0.035-0.043	nd	nd
Gill	mean±SD	0.006±0.002 ^c	0.02±0.009 ^a	1.01±0.32
	Range (min–max)	0.31-0.55	0.002-0.004	0.001-0.003

** The letters (a, b, c, d) represent the statistical differences among different samples ($p < 0.05$)

* nd= Not Detectable

Table 2) Comparison of present mean values in fish tissues with other studies result

Fish Species	Area	Tissue	Cd ($\mu\text{g g}^{-1}$)	Hg ($\mu\text{g g}^{-1}$)	As ($\mu\text{g g}^{-1}$)
Common Carp (<i>Cyprinus carpio</i>)	Present Study	muscle	0.003±0.001	0.06±0.02	nd
		Liver	0.004±0.001	nd	nd
		gill	0.006±0.002	0.02±0.009	1.01±0.32
<i>Triglia lucerna</i>		muscle	-	-	1.38
		Liver	-	-	1.01
<i>Lophius budegassa</i>	Turkey ¹	muscle	-	-	0.98
		Liver	-	-	1.32
<i>Solea lascaris</i>		muscle	-	-	1.74
		Liver	-	-	1.98
Anchovy			0.002	0.001-0.52	0.01-54.8
Red Mullet			0.002-0.006	0.001-2.07	0.01-70.9
mackerel	Croatia ²	muscle	-	0.001-0.78	0.01-36.4
picarel			-	0.001-2.07	0.01-54.6
<i>Dicentrarchus labrax, Sparus aurata, Mugil cephalus</i>	Turkey ³	muscle	0.03±0.03	-	-
		Liver	0.1±0.02	-	-
		gill	0.7±0.57	-	-
red mullet	Croatia ⁴	muscle	0.007- 0.029	-	-
red mullet			0.001-0.01	0.14-0.36	15.39-17.77
anchovy			0.001-0.02	0.08-0.09	3.93-5.42
hake	Spain ⁵		0.005-0.01	0.12-0.29	-
mackerel			0.003-0.01	0.06-0.15	1.73-7.47
<i>Saurida undosquamis, Sparus aurata, Mullus barbatus</i>	Turkey ⁶	muscle	0.01-4.16	-	-
<i>Leuciscus cephalus, Lepomis gibbosus</i>	Turkey ⁷		0.010-0.084	-	-
<i>Sarda sarda, Mulus barbatus ponticus, Trachurus trachurus, Merlangius merlangus</i>	Turkey ⁸		0.09-0.48	-	-
<i>Otolithes rubber, Pampus argenteus, Parastrumateus niger, Scomberomorus commerson, Onchorynchus mykiss</i>	Iran ⁹	muscle	0.001-0.45	0.001-0.26	0.007-0.04
		Liver	0.002-0.58	0.034-0.41	0.014-0.26
		gill	0.004-0.09	0.012-0.47	0.01-0.21
Milk fish (<i>Chanos chanos</i>)	India ¹⁰		0.039-0.153	-	-
<i>Solea vulgaris, Anguilla Anguilla, Liza aurata</i>	Spain ¹¹	muscle	-	0.01- 0.023	0.52-3.96
<i>Mullus barbatus</i>	Turkey ¹²		-	0.014-0.50	-

¹Yilmaz et al. (24) ²Bilandzic et al. (29) ³Dural et al. (34) ⁴Kljakovic Gašparic et al. (35) ⁵Falco et al. (36) ⁶Turkmen et al. (37) ⁷Yilmaz et al. (38)

⁸Mendil et L. (30) ⁹Sobhanardakani et al. (39,40) ¹⁰Rajeshkumar and Munuswamy (41) ¹¹Usero et al. (42) ¹²Kucuksezgin et al. (43)

Discussion

The maximum Cd level permitted by the FAO is $0.5 \mu\text{g g}^{-1}$ and $0.2 \mu\text{g g}^{-1}$ by MAFF (22, 23). Cd levels in muscle, liver and gill tissues of common carp ranged: $0.007\text{-}0.011 \mu\text{g g}^{-1}$, $0.035\text{-}0.043 \mu\text{g g}^{-1}$ and $0.31\text{-}0.55 \mu\text{g g}^{-1}$ respectively. The mean lowest Cd content $0.003 \mu\text{g g}^{-1}$ was found in muscle while the highest Cd level was $0.006 \mu\text{g g}^{-1}$ in gill. Cd mean levels in the analyzed fish samples ($\mu\text{g g}^{-1}$) were below the maximum permissible value indicated by the European Community (7). Yilmaz et al (2010) analyzed Cd concentration in the muscles and livers of three demersal fish species (*Triglia lucerna*, *Lophius budegassa* and *Solea lascaris*) from Iskenderun Bay, Turkey and reported that the mean Cd content for muscle tissue of evaluated species 0.01 , 0.02 and $0.04 \mu\text{g g}^{-1}$ and for liver tissue 0.24 , 0.26 and $0.39 \mu\text{g g}^{-1}$ respectively (24). Statistical grouping of the concentrations of Cd in the different tissues of common carp by ANOVA and Tukey test indicated that there were significant differences within and between all of the evaluated tissues ($p < 0.05$) (Table 1). Comparison of the results of this study with other studies is shown in table 2.

Mercury is known to be a very toxic metal, and fish is the most important source in the human diet (25). The maximum limit for Hg is set by the U.S. FDA (26) as $1.0 \mu\text{g g}^{-1}$ for fish. Similarly, the Turkish Food Codex (27) and the European Commission Regulation 466/2001 (28) recommended $1.0 \mu\text{g g}^{-1}$ as the limit value for bonitos, but they set a limit of $0.5 \mu\text{g g}^{-1}$ for other species. Mercury was observed only in muscle and gill tissues at $0.006\text{-}0.01$ and $0.002\text{-}0.004 \mu\text{g g}^{-1}$ respectively, and could not be detected in liver tissue. However, mean metal levels in the analyzed tissues ($0.02\text{-}0.06 \mu\text{g g}^{-1}$) were below the maximum permissible value indicated by the European Community (7), U.S. FDA (26), Turkish Food Codex (27) and European Commission Regulation 466/2001

(28,29). In a recent study of fish species from the Black Sea in Turkey, Hg levels were reported in the range of 0.025 to $0.078 \mu\text{g g}^{-1}$ (30). Comparison of the results of this study with other studies is shown in table 2.

Arsenic concentration ranged from minimum values of $0.001 \mu\text{g g}^{-1}$ to $0.003 \mu\text{g g}^{-1}$ for gill tissue of common carp and could not be detected in muscle and liver tissues. The mean As concentration of $1.01 \mu\text{g g}^{-1}$ was found in gill tissue. The maximum As level permitted for marine fish is $2 \mu\text{g g}^{-1}$, according to the guidelines of the European Community (7). In this study, the mean As level found in common carp was lower than the prescribed limit. Investigations of the US coast suggested that environmental factors such as the seasonal cycle of absorption/solubilization of the element in specific observed areas, local physico-chemical parameters such as temperature, salinity and the nature of sediments might affect the large bioaccumulation of As (31). In fact, the different levels of As measured in mussels sampled from the off-shore districts of the northern and central Adriatic Sea are due to the significant influence of seawater salinity in modulating accumulation of the metal (32). Previous findings have determined the natural origin of As, which was mostly present as arsenobetaine, a non-toxic As compound normally accumulated by marine organisms through diet and not released from anthropogenic activities (33). Comparison of the results of this study with other studies is shown in table 2.

Conclusions

Analytical data obtained from this study shows that the metal concentrations for the fish tissues were generally within the FAO/WHO, ASTDR AND EEC recommended limits for fish (table 1). Therefore there is no serious health risk associated with the consumption of

the three studied metals in the muscle and liver tissues analyzed.

Footnotes

Conflict of Interest:

The authors declare no conflict of interest.

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