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Heavy metals contamination in silver, common and grass carp caught from Zarivar Lake, western Iran

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Abstract

The aim of the present study is to evaluate the level of contamination of three heavy metal (Cd, Hg and As) concentrations in the muscle tissue of three important hunting species including *Hypophthalmichthys molitrix*, *Cyprinus carpio* and *Ctenopharyngodon idella* caught from the Zarivar Lake during 2013. Concentrations of Cd and As were measured using atomic absorption spectrometry and concentration of Hg was measured using a direct mercury analyzer after acid digestion. Metal levels measured in silver carp were in the following ranges ($\mu g\ g^{-1}$): Cd 0.056-0.091, Hg 0.009-0.019 and As 0.003-0.013. Metal ranges in common carp were ($\mu g\ g^{-1}$): Cd 0.044-0.093, Hg 0.03-0.011 and As 0.004-0.006. Metal level ranges measured in grass carp were ($\mu g\ g^{-1}$): Cd 0.004-0.012, Hg 0.025-0.041 and As 0.009-0.013. Significant differences in metal concentrations were found among fish species. The results presented on metal contents in the examined species 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 and FAO/WHO. Therefore, their contribution to the total body burden of these heavy metals can be considered as negligibly small.

Keywords: metal levels, food safety, carp, Zarivar Lake

Introduction

Metal pollution of the sea affects human health intensively and extensively via the food chain (Emami Khansari *et al*, 2005). Seafood, which is very important in the human diet all over the world, represents a main source of protein, but it can also accumulate trace metals (Plessi *et al*, 2001). The toxic nonessential metals Cd, Hg, As and Pb are characterized as having no demonstrated biological requirement in humans, and exposure is associated with recognizable toxicity (Storelli *et al*, 2007; Boadi *et al*, 2011). Also, severity of toxicity increases with increases in dosage. Although there may be some lower limit of exposure at which toxicity may not be detected (threshold), there may be no level at the molecular level that does not have an adverse effect (Goyer, 1994). Toxicological and environmental studies have prompted interest in the determination of toxic elements in food. While Hg, Cd and Pb can be tolerated at extremely low levels, at certain concentrations they are exceptionally toxic to humans. Cd accumulates in the human body and may induce kidney disfunction, skeletal damage and reproductive deficiencies. Also, it cannot be excluded that it acts as a human carcinogen (Suppin *et al*, 2005). Fish accumulate substantial concentrations of Hg in their tissues and thus can represent a major dietary source of this element for humans. Fish are the single largest sources of Hg and As for man. Hg is a known human toxicant and the primary sources of Hg contamination in man are through eating fish. Biotransformation of Hg and methyl mercury formation constitutes a dangerous problem for human health (Inskip and Piotrowsiki, 1985). Arsenic exposure has been related to the appearance of cancers in humans, including lung, liver, skin and bladder cancer (Kapaj *et al*, 2006).

Zarivar Lake (ZL) is fresh water body with an area of about 750 ha and average water depth of 4-5 meters in far west of Iran located in 35°-30' to 35°-35' North

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and 46°-06' to 46°-09' East in the North of Kurdistan province, Iran (Figure 1) (Jalali and barzegar, 2006). Zarivar Lake is a typical ecosystem of great importance in regard to biodiversity and to aesthetic value. The fish species found most commonly in the lake are *Cyprinus carpio*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Capoeta damascina*, *Pseudorasbora parva*, *Chalcalburnus* sp, *Carassius auratus*, *Gambusia affinis* and *Mastacembelus mastacembelus*. Previous research showed that the pollutants transferred to ZL and regarding the intensity of pollution production, the non point source pollution related to agricultural activities was first rank among other pollutant as community wastewater, solid waste, grassland pollution and forest. These pollutions are transferred directly to wetland and threaten the biological systems of ZL (Ghaderi and Ghafouri, 2006).

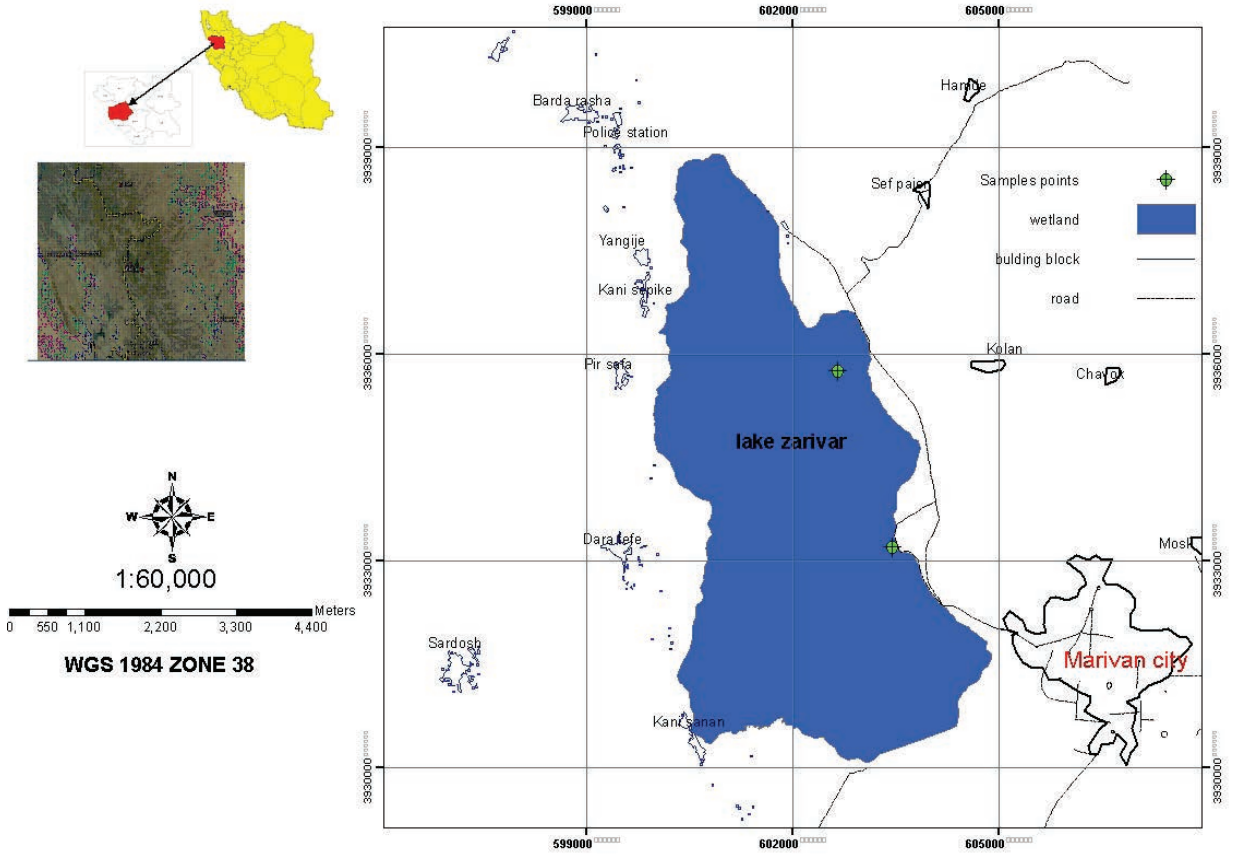

Figure 1. Location of Zarivar Lake in Marivan City, Western Iran.

The aim of the present study is to evaluate the level of contamination of three heavy metal (Cd, Hg and As) concentrations in the muscle tissue of three important hunting species includes silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*).

Materials and Methods

Fresh fish samples were caught from Zarivar Lake in Kurdistan province, Iran during 2013. Specimens collected during the sampling period were separated into three fish species: 25 silver carp, 34 common carp and 37 grass carp. Following collection, specimens were frozen in prewashed polyethylene bags, brought to the laboratory, and stored frozen at -18 °C prior to analysis.

All reagents were of analytical reagent grade, HNO_3, H_2O_2 and HCl (Analytical Grade, Merck, Germany). Double deionised water was used for all dilutions. All plastic and glassware were cleaned by soaking in diluted HNO_3 (1/9, v/v) and rinsed with distilled water prior to use. Calibrations were prepared with element standard solutions of 1000 mg l^{-1} of each element supplied by Perkin Elmer. Stock solution was diluted in HNO_3 (0.2%). As matrix modifiers in each atomization for Cd, 0.005 mg $Pd(NO_3)_2$ and 0.003 mg $Mg(NO_3)_2$ (Perkin Elmer, USA) were used. The hydride technique for mercury determinations 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 AS 800 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. Hg 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 (Boadi *et al*, 2011; Bilandzˇic´ *et al*, 2011).

Samples (2 g) were digested with 5 ml of HNO_3 (65% v/v), 1 ml of H_2O_2 (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 deionised water. All metal concentrations were determined on a wet weight basis as $\mu g\ g^{-1}$. 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.

Statistical analysis was performed using SPSS 15.0 version (SPSS Inc., Chicago, IL, USA) statistical package. Data were grouped according to species. One-way analysis of variance was used to test for differences in tissue metal concentrations. Data were log-transformed to improve normality before analysis to meet the underlying assumptions of the analysis of variance; the values given are therefore geometric means. The differences between the metal concentrations in different species were analyzed using the t-test. Possibilities less than 0.05 were considered statistically significant ($p < 0.05$).

Results

In the present study levels of Cd, Hg and As in muscle tissue of three fish species caught from Zarivar international wetland in were determined. Table 1 shows the mean concentrations of three elements (geometric means and range) in the muscle of fish species (*Hypophthalmichthys molitrix*, *Cyprinus carpio* and *Ctenopharyngodon idella*). The results indicate that Cd concentration in fish species ranged from 0.004 to 0.093 $\mu g\ g^{-1}$ whereas Hg concentration ranged from 0.009 to 0.11 $\mu g\ g^{-1}$. Levels of As in fish species ranged from 0.003 to 0.013 $\mu g\ g^{-1}$ and all samples were below the detection limit for Cd, Hg and As. The results indicated that the average concentrations of evaluated elements in this study are significantly lower than the adverse level for the species themselves and for human consumption with FAO/WHO, ASTDR and EEC permissible limits (WHO, 1996; EEC, 2001; ASTDR, 2003; FAO/WHO, 2009). Therefore, their contribution to the total body burden of these metals can be considered as negligibly small. Statistical grouping of the concentrations of each element in the different fish species by ANOVA and Tukey test are shown in table 1. The results indicated that there were significant differences within and between all of the evaluated brands ($p<0.05$).

Vertically, letters a, b and c show statistically significant differences ($p<0.05$).

Table 1. Metal concentrations (geometric mean $\mu g\ g^{-1}$ wet wt and range) in the muscle tissues of the silver, common and grass carp from Zarivar Wetland, Iran.

Species	N		Cd	Hg	As
silver carp (Hypophthalmichthys molitrix)	25	Geometric mean Range (min–max)	0.003^b 0.056-0.091	0.05^a 0.009-0.019	0.45^a 0.003-0.013
common carp (Cyprinus carpio)	34	Geometric mean Range (min–max)	0.002^a 0.044-0.093	0.07^b 0.03-0.11	0.69^b 0.004-0.006
grass carp (Ctenopharyngodon idella)	37	Geometric mean Range (min–max)	0.005^c 0.004-0.012	0.08^c 0.025-0.041	1.12^c 0.009-0.013

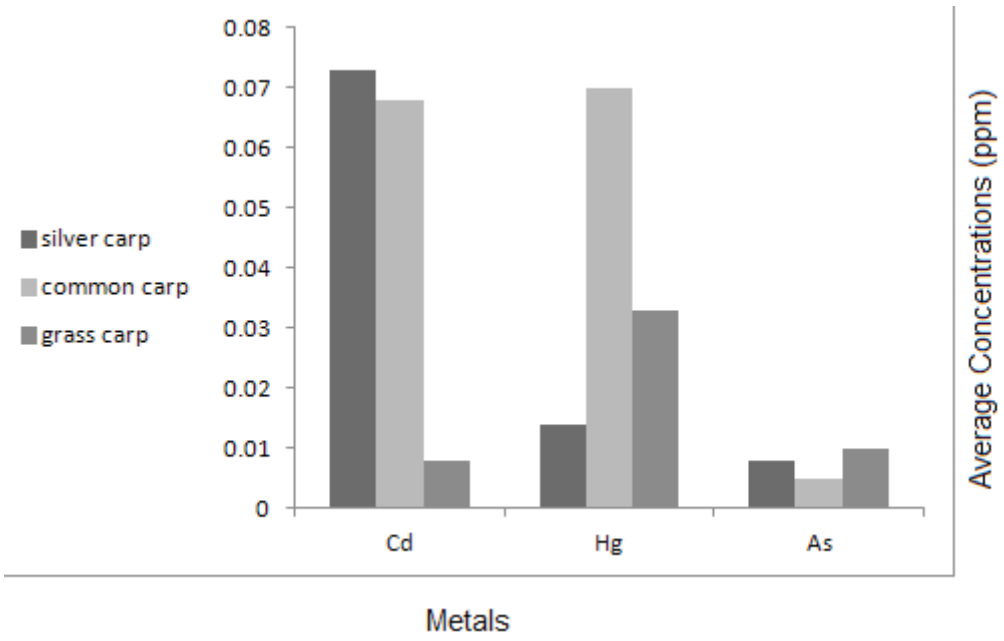

Figure 2. Comparative levels of selected heavy metals in fish species.

Discussion

The maximum Cd level permitted by the FAO (1983) is 0.5 μg g^{-1} and 0.2 μg g^{-1} by MAFF (1995) (FAO, 1986; MAFF, 1993). Cd levels in muscles of fish species ranged: 0.056-0.091 μg g^{-1} in silver carp, 0.044-0.093 μg g^{-1} in common carp and 0.004-0.012 μg g^{-1} in grass carp. The mean lowest Cd content 0.002 μg g^{-1} was found in common carp while the highest Cd level was 0.005 μg g^{-1} in grass carp. Cd mean levels in the analyzed fish samples (μg g^{-1}) were below the maximum permissible value indicated by the European Community (EEC, 2001). Bilandzic *et al.* (2011) analyzed Cd concentration in the muscles of red mullet, mackerel and picarel from the Croatian waters of the Adriatic Sea and reported that the mean lowest Cd content 0.002 μg g^{-1} was found in anchovy and red mullet while the highest Cd level was 0.006 μg g^{-1} in red mullet. Cd mean levels in the analyzed fish samples (μg g^{-1}) were below the maximum permissible value indicated by the European Community and the Croatian legislation (Bilandz˘ic´ *et al*, 2011). Kljakovic Gašparic *et al.* (2002) reported that Cd in the muscle tissue of red mullet from the eastern Adriatic Sea, ranging from 0.007 to 0.029 μg g^{-1} (Kljakovic´ Gašparic´ *et al*, 2002). In fish species in Catalonia, Spain, Cd levels were 0.001-0.01 μg g^{-1} in red mullet, 0.001-0.02 μg g^{-1} in anchovy, 0.005-0.01 μg g^{-1} in hake and 0.003-0.01 μg g^{-1} in mackerel (Falco *et al*, 2006). Also, in six fish species caught from the northeast Mediterranean Sea concentrations of Cd in muscle tissue ranged from 0.37 to 0.79 μg g^{-1} (Canli and Atli, 2003). Cd levels reported in different sea locations in different fish species, ranging (μg g^{-1}) from 0.01 to 4.16 (Turkmen *et al*, 2007) and 0.010 to 0.084 and 0.010 to 0.04 in Iskenderun Bay (Yilmaz *et al*, 2010), 0.09 to 0.48, 0.1 to 0.35 and 0.18 to 0.35 in the Black Sea (Mendil *et al*, 2010; Tuzen, 2003), <0.02 to 0.24 off the Black Sea coasts (Topcuoglu *et al*, 2002), 0.45 to 0.80 in the Black and Aegean Seas (Uluozlu *et al*, 2007), 0.03 to 0.12 in Tuzla Lagoon, Mediterranean Sea region (Dural *et al*, 2007), 0.03 to 0.39 in the Aegean and Mediterranean Seas (Turkmen *et al*, 2009), 0.2 to 1.2 in the lakes in Tokrat (Mendil *et al*, 2005), 0.001 to 0.45 (Sobhanardakani *et al*, 2011), and 0.039 to 0.153 in Kaattuppalli Island, India (Rajeshkumar and Munuswamy, 2011).

Hg is known to be a very toxic metal, and fish is the most important source in the human diet (Mol, 2011). The maximum limit for Hg is set by the U.S. FDA as 1.0 μg g^{-1} for fish (FDA, 2001). Similarly, the Turkish Food Codex and the European Commission Regulation 466/2001 recommended 1.0 μg g^{-1} as the limit value for bonitos, but they set a limit of 0.5 μg g^{-1} for other species (Turkish Food Codex, 2002; EU, 2005). Hg levels (μg g^{-1}) in fish muscles in this study ranged from a minimum of 0.009 to a maximum of 0.11 in silver carp and common carp respectively, 0.019 in silver carp and 0.041 in grass carp. However, mean metal levels in the analyzed fish samples (0.05-0.08 μg g^{-1}) were below the maximum permissible value indicated by the European Community, U.S. FDA, Turkish Food Codex and European Commission Regulation 466/2001 (Bilandz˘ic´ *et al*, 2011). In a recent study of fish spe-

cies from the Black Sea in Turkey, Hg levels were reported in the range of 0.025 to 0.078 μg g^{-1} (Tuzen, 2009). In Spain, muscle Hg concentrations ranged from 0.14 to 0.36 μg g^{-1} in red mullet, 0.08 to 0.09 μg g^{-1} in anchovy, 0.12 to 0.29 μg g^{-1} in hake, and 0.06 to 0.15 μg g^{-1} in mackerel (Falco *et al*, 2006). In the southern Atlantic coast of Spain, Hg concentration in three fish species ranged from 0.01 to 0.023 μg g^{-1} (Usero *et al*, 2003). Bilandzic *et al*. (2011) reported that Hg in the muscle tissues of anchovy, red mullet, mackerel and picarel from the Croatian waters of the Adriatic Sea in the range of 0.001-0.52, 0.001-2.07, 0.001-0.78 and 0.001-2.07 μg g^{-1} respectively (Bilandz˘ic´ *et al*, 2011), Sobhanardakani *et al*. (2012) reported that Hg in the muscle tissue of five fish species (*Otolithes ruber, Pampus argenteus*, *Parastromateus niger, Scomberomorus commerson, Onchorynchus mykiss)* ranged from 0.001-0.26 μg g^{-1} (Sobhanardakani *et al*, 2012), Kucuksezgin *et al*., 2011 reported that Hg in the muscle tissue of *Mullus barbatus* caught from Izmir Bay, Mediterranean Sea during 1997-2009 ranged from 14.0-500.0 μg kg^{-1} (Kucuksezgin *et al*, 2011).

As concentrations ranged from minimum values of 0.003 μg g^{-1} to: 0.013 μg g^{-1} for silver carp, 0.006 μg g^{-1} for common carp and 0.013 μg g^{-1} for grass carp. The highest and lowest mean As concentration were found 1.12 μg g^{-1} and 0.45 μg g^{-1} in grass carp and silver carp respectively. The maximum As level permitted for marine fish is 2 μg g^{-1}, according to the guidelines of the European Community (EEC, 2001). In this study, the mean As level found in all species were lower than the prescribed limit. Bilandzic *et al*. (2011) reported that As in the muscle tissues of anchovy, red mullet, mackerel and picarel from the Croatian waters of the Adriatic Sea in the range of 0.01-54.8, 0.01-70.9, 0.01-36.4 and 0.01-54.6 μg g^{-1} respectively (Bilandz˘ic´ *et al*, 2011). Falco *et al*. (2006) reported that As in the muscle tissues of anchovy, red mullet and mackerel from Catalonia, Spain in the range of 3.93-5.42, 15.39-17.77 and 1.73-7.47 μg g^{-1} respectively (Falco *et al*, 2006). In three fish species from the southern Atlantic coast of Spain, As concentration ranged from 0.52 to 3.96 μg g^{-1} and the mean metal concentrations in different fish showed significant differences (Usero *et al*, 2003). As levels found were higher than fish muscle concentrations reported in fish from the Black Sea in Turkey, where they ranged from 0.11 to 0.32 μg g^{-1} (Tuzen, 2009). In fish from the Gulf of Mexico, the mean As concentration was 7.0 μg g^{-1} (Lewis *et al*, 2002). As accumulation in different muscle tissues of 10 fish species in Manchar Lake, Pakistan that are commonly consumed by the local population showed significant differences in As concentrations, ranging between 2.0 and 14.8 μg g^{-1} (Shah *et al*, 2009). In fish consumed in Hamedan province, Iran, the range of As concentration was 0.007 to 0.04 μg g^{-1} (Sobhanardakani *et al*, 2012). In muscle tissues of three demersal fish species from Iskenderun Bay, Turkey, the range of As concentration was 0.98 to 1.74 μg g^{-1} (Yilmaz *et al*, 2010). Investigations off 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 (Valette-Silver *et al*, 1999). 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 (Fattorini *et al*, 2008). 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 (Fattorini *et al*, 2004).

Conclusions

The results from this study suggested that significant differences existed in the metal concentrations across muscle tissues of analyzed fishes. Also, analytical data obtained from this study shows that the metal concentrations for the tissue were generally within the FAO/WHO, U.S. FDA and U.S. EPA recommended limits for fish (table 1). Therefore there is no serious health risk associated with the consumption of the fishes analyzed.

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