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 (µg g⁻¹): Cd 0.056-0.091, Hg 0.009-0.019 and As 0.003-0.013. Metal ranges in common carp were (µg 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 (µg 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 Piotrowski, 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
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 pollutants 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, HNO₃, H₂O₂ and HCl (Analytical Grade, Merck, Germany). Double deionised water was used for all dilutions. All plastic and glassware were cleaned by soaking in diluted HNO₃ (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 HNO₃ (0.2%). As matrix modifiers in each atomization for Cd, 0.005 mg Pd(NO₃)₂ and 0.003 mg Mg(NO₃)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 spectrosco-
py using an AAnalyst 4110 ZL atomic absorption spectrometer (Perkin Elmer, USA) equipped
with an AS 800 autosampler (Perkin Elmer, USA). For graphite furnace measurements, ar-
gon 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 mer-
cury analyzer (DMA-80). Microwave closed sys-
tem Multiwave 3000 (Anton Paar, Germany) was
used for digestion of samples (Boadi et al, 2011;
Bilandžić et al, 2011).

Samples (2 g) were digested with 5 ml of HNO₃
(65% v/v), 1 ml of H₂O₂ (30% v/v) with a micro-
wave oven. A blank digest was carried out in the
same way. The digestion program began at a poten-
cy of 1200W then ramped for 10 min, after which
samples were held for 10 min at 1200 W. The sec-
ond step began at a potency of 0W and held for 15
min. Digested samples were diluted to a final vol-
ume of 50 ml with double deionised water. All met-
al concentrations were determined on a wet weight
basis as µg 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 stan-
dard calibration curve, two spiked specimens, and
one duplicate.

Statistical analysis was performed using SPSS
15.0 version (SPSS Inc., Chicago, IL, USA) sta-
tistical 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 assum-
tions 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. Possibili-
ties 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 de-
termined. Table 1 shows the mean concentra-
tions of three elements (geometric means and
range) in the muscle of fish species (Hypo-
phthalmichthys molitrix, Cyprinus carpio
and Ctenopharyngodon idella). The results indicate
that Cd concentration in fish species ranged
from 0.004 to 0.093 µg g⁻¹ whereas Hg con-
centration ranged from 0.009 to 0.11 µg g⁻¹.
Levels of As in fish species ranged from 0.003 to
0.013 µg g⁻¹ and all samples were below the de-
tection limit for Cd, Hg and As. The results in-
dicated that the average concentrations of evalu-
ated 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 sig-
ificant differences (p<0.05).

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Cd</th>
<th>Hg</th>
<th>As</th>
</tr>
</thead>
<tbody>
<tr>
<td>silver carp (Hypophthalmichthys molitrix)</td>
<td>25</td>
<td>Geometric mean Range (min–max)</td>
<td>0.003ᵇ</td>
<td>0.05ᵃ</td>
</tr>
<tr>
<td>common carp (Cyprinus carpio)</td>
<td>34</td>
<td>Geometric mean Range (min–max)</td>
<td>0.002ᵃ</td>
<td>0.07ᵇ</td>
</tr>
<tr>
<td>grass carp (Ctenopharyngodon idella)</td>
<td>37</td>
<td>Geometric mean Range (min–max)</td>
<td>0.005ᶜ</td>
<td>0.08ᶜ</td>
</tr>
</tbody>
</table>

Table 1. Metal concentrations (geometric mean µg g⁻¹ wet wt and range) in the muscle tissues of the silver, common and grass carp from Zarivar Wetland, Iran.
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 0.005 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 and the Croatian legislation (Bilandzic 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 (Uluzlu 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 Kaattupalli 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 (Bilandzic 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 demeral 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.

References


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