Study on heavy metals (Chromium, Cadmium, Cobalt and Lead) concentration in three pelagic species of Kilka (Genus Clupeonella) in the southern Caspian Sea

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Introduction
Environmental pollution by heavy metals has been a matter of growing concern over the last decades. Heavy metals are toxic and tend to accumulate in living organisms (Devier et al., 2005). In many studies, fish has been used as a sampling item to monitor the quality of ecosystems because of two reasons: 1- Fish bio-concentrate and integrate contaminant load both in time and space leading to more representative results compared to water samples, and 2- Fish represent the bio-available fraction of environmental contaminants, unlike water and sediment samples. On the other hand, humans consume fish species which makes attention to these fauna even more important (Danielsson, 2007). Levels of some metals in the water running in Volga River, towards the Caspian Sea (Dumont, 1998), and elevated concentrations of some trace elements have been reported in sediment (Mora et al., 2004). Also, various pollutants have accumulated in the Caspian Sea due to effluents from coastal catchments and leakage from offshore oil production and land-based sources (Karpinsky, 1992). Fish is an important food source for humans and is a key component in many natural food webs. Three pelagic fish species such as common kilka, C. cultriventris, anchovy C. engrauliformis and bigeye kilka C. grimmii are abundant and commercially valuable species in the Caspian Sea (Fazli et al., 2007). Also, kilka species are distributed throughout the Caspian Sea, and comprise important prey items for sturgeons and Caspian seals. It is therefore necessary to examine the geographical variation of trace elements in these fishes and to understand their
trophic transfer in the Caspian Sea ecosystem. The objective of the present study was to investigate the concentrations of chromium (Cr), cadmium (Cd), cobalt (Co) and lead (Pb) in fish muscle in the most commercially important fish species of Kilka (common kilka, *C. cultriventris*, anchovy *C. engrauliformis* and bigeye kilka *C. grimmii*) in the Caspian Sea.

**Materials and methods**

In order to conduct this study samples of common kilka, anchovy and bigeye kilka, were collected in May and December 2012 from the Caspian Sea in Babolsar coastal zone (latitudes 36°N and longitudes 53°E), north of Iran. Samples were gathered from the traditional kilka conical lift-nets equipped with underwater electric lights and the mesh size of 7–8 millimeters between two knots (Fazli *et al.*, 2007).

Fish samples were frozen on board of the fishing vessel and transferred to the laboratory for further work. In the laboratory, the samples were thawed, washed and cleaned with sterile distilled water. After identification the total length (to mm) and weight (to g) of the fishes were measured and recorded. After dissection and evisceration five grams of the whole fish was dried in an oven at 60°C for 48 hours to determine the moisture content. One gram of ground-dried tissue was accurately weighed into 100 ml Erlenmeyer flasks; 5ml perchloric acid and 10 ml nitric acid were added. Digestion was performed on a hot plate (set between 200 to 250 °C) for 4 hours under a hood until solutions were clear of fumes of perchloric acid (Van Loon, 1980). Then contents of the flasks were made up to 50 ml with distilled water. Digested samples aspirated into the flames of the atomic absorption spectrophotometer (UNICAN929) for heavy metal determination (APHA, 1990). Metal concentration in the tissues was expressed as μg·g⁻¹ dry weight and in the water samples as μg·L⁻¹.

The metal accumulation in a tissue can be expressed as Bioaccumulation Factor (BAF) (Ivanciuc *et al.*, 2006). In this study, BAF is the ratio of Pb and Cd concentration in the muscle (CM) of kilka to the concentration in water (CW) at equilibrium given by the equation: BAF = CM / CW × 100. The archival data of Pb and Cd concentration in water of the Caspian Sea recorded in the same region in 2010 (mean±S.E. 1.18±0.26 and 0.55±0.18 μg.L⁻¹, respectively; (Nasrollahzadeh, 2013) were used as input data.

Statistical analysis of the obtained data was carried out using SPSS version 18 software. One-Way ANOVA was employed to find any likely significant differences of heavy metals concentration in three species of fish and the Tukey pair-wise test for multiple comparisons was used to assess differences among the species. Data from the two periods (May and December) were compared using t-test. Finally, a correlation matrix was calculated between the parameters estimated.
Results and discussion

The mean (±S.D.) length and weight of the three species of kilka were 86.6±10.48 mm and 6.6±0.93 g for anchovy, 117.1±9.95 mm and 9.2±0.82 g for bigeye and 88.3±6.21 mm and 6.6±1.48 g for common Kilka (Table 1).

There was no significant differences between the two sexes, for the heavy metals Cr, Cd, Co and Pb in the three species of Kilka (t-test; p>0.05), a combined data analysis for the whole samples was therefore carried out (Table 1). For the three species, concentrations of the metals were in the order: Cr>Co>Pb>Cd (Table 1).

Fig. 1 shows concentrations of the heavy metals studied in the two periods (May and December) for the three species of kilka. Statistically, significant differences were not found between the mean Cr, Co, Cd in the two periods for the three species (t-test; p>0.05). Also, the average Pb was not statistically different between the two periods for bigeye kilka, but for anchovy and common kilka were significantly different (t-test, p<0.05).

Finally, There was no statistically significant differences among the three species of kilka, for all heavy metals (ANOVA, P>0.05; Table 1). Therefore, a combined data analysis for the whole samples was carried out. The results showed that, the mean (±S.D.) Cr, Cd, Pb and Co for whole samples were 0.474±0.206, 0.014±0.006, 0.015±0.007 and 0.028±0.007 μg.g⁻¹ dry weight, respectively. Therefore, the concentrations of the metals in the muscle were in the order: Cr>Co>Pb>Cd

There were statistically significant correlation only between Cd-Pb (r = 0.770, n=60, p<0.001), Cd-Co (r = 0.503, n = 60, p<0.001) and Pb-Co (r = 0.350, n = 60, p<0.001).

Regarding BFA, the level of Cd (2.25 g.g⁻¹ DW) was higher than the level of Pb (1.27 g.g⁻¹ DW).

As reported by (FAO, 1983; EC, 2001; FDA, 2001); among the different metals analyzed Co, Cr and Cd are classified as chemical hazards for which maximum residual levels have been prescribed for human. In this study, the maximum concentrations of Cd contents (0.014 μg.g⁻¹) in the samples were less than the permitted level (0.02 – 0.1) (FAO, 1983). Cr concentrations are also much less than the permitted level (Table 2). In the present study, all metal concentrations were below the European Commission report limit while as for the FDA (US Food and Drug Administration, 2001) limit none of the samples contained concentrations above the prescribed limits. For toxicity and serious contamination of foods with Pb and Cd that occurs from time to time during commercial handling and processing, most countries monitor the levels of toxic elements in foods.

Metals can enter the body through the water-permeable skin and the gut and via the blood circulation, accumulate in the liver and other tissues (Papadimitriou and Loumbourdis, 2002).
Therefore, liver as the main organ for homeostasis, becomes a metal storage place. Muscle is one of the ultimate parts for heavy metal accumulation. Several authors reported that the metal concentrations were always lowest in the muscle and highest in the gill and liver (Taghavi et al., 2011). Marzouk (1994) concluded that different accumulation levels in various organs of a fish can primarily be attributed to the differences in the physiological role of each organ.

According to Carvalho et al. (2005), behavior and feeding habits are the factors that influence the accumulation differences in the different organs. All three species of kilka are pelagic species and feed on zooplankton (Prikhod’ko, 1981). Therefore, because of their feeding habits, the concentrations of heavy metals had no significant differences among the three species of Kilka.

Kilka populations are declining in the Caspian Sea. The most obvious factors that may have influenced kilka dynamics negatively are excessive fishing, climate change, seismic activity, and invasion by the exotic Mnemiopsis (Daskalov and Mamedov 2007; Fazli et al., 2009). As cited in Daskalov and Mamedov (2007), earthquake data reveal that, in the first quarter of 2001, the local Absheron seismic plate was active, and water and gas systems in the soil were unstable and indicative of hydro-volcanic events or significant gas blow-outs containing poisonous substances, which most probably contributed to the mass kill (Katunin et al., 2002).
Figure 1: Monthly heavy metal concentrations (μg.g⁻¹ dry weight) in muscle of three species of Kilka from southern part of the Caspian Sea (mean ± SD, n=10).

Table 2: Comparison concentrations (μg.g⁻¹ dry weight) of metals in muscle of kilka with other selected area.

<table>
<thead>
<tr>
<th>Metals</th>
<th>This Work</th>
<th>Literature Values with relevant references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>0.474</td>
<td>1.28-1.60[1], 0.18-0.25[2], 1.9-7.5[3], &lt;0.06-0.24[4], 0.53[7]</td>
</tr>
<tr>
<td>Co</td>
<td>0.028</td>
<td>0.01[2], &lt;0.05-0.40[4]</td>
</tr>
<tr>
<td>Cd</td>
<td>0.014</td>
<td>1.07-1.43[1], 0.02-0.05[2], 1.9-24[3], &lt;0.02-0.24[4], 0.05-0.1[5], 0.5[6], 0.20[7], 0.35[8], 0.18[9]</td>
</tr>
<tr>
<td>Pb</td>
<td>0.015</td>
<td>0.821[7], 0.59[8], 0.28[9], 0.85-1.68[10]</td>
</tr>
</tbody>
</table>

However, the use of biomarkers and histo-pathological studies (Papadimitriou and Loumbourdis, 2002; Stolyar et al., 2008), which can assess the status of kilkas populations as subjects of environmental pressure, are completely lacking. This can be the subject of a new project in the future.

Kilka species are an important food for sturgeons (59.4% by weight of sevryuga diet in the Middle Caspian) and the Caspian seal. Predators consume 590 million kg of the three kilka species which themselves are the main consumers of zooplankton. Kilkas are very important element in the life web of the Caspian Sea (Krylov, 1984). Moreover, Khan and Tansel (2000) reported that the mercury accumulation for liver and kidney were 39.9 × 10⁷ and 32.9 × 10⁷ respectively, for adults and 10.5 × 10⁷ and 9.34 × 10⁷ for juveniles of the Alligator species mississippiensis. They also reported that the BAF increased by 72% in 10 years. This means that the alligator was continuously accumulating heavy metals. That study clearly showed that the BAF should be better applied to long-lived animals. The sturgeons and Caspian seals live more than 20 years, compared with 5–7 years for kilkas. Also, sturgeons and seals, being at the highest trophic level, have a greater likelihood of living for many years.

In conclusion, the results of the present study revealed that the concentrations of heavy metals were not significantly different among the three species of kilka, and were lower than international standards. However, kilka fish species are important food item for sturgeons, Caspian seal and other fish, which are situated in the top position of the Caspian trophic web. Therefore, because of the biomagnifications, the concentrations of heavy metals should be documented in these species (specially, sturgeons and other fish species as a food item for local people) in further studies.

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