A preliminary risk assessment of the trace metals accumulated in the farmed Beluga sturgeon (Huso huso) caviar from Caspian Sea

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ABSTRACT
Beluga sturgeon (Huso huso), the source of beluga caviar, is so depleted that they were included on the list of endangered species by the convention on international trade in endangered species (CITES), and it is estimated that overfishing for meat and caviar will soon cause global extinction on the remaining natural wild population. The concentration of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Se and Zn as essential metals, were determined in caviar of farmed Beluga sturgeon. Co and Mn levels in the samples were less than 0.01 mg kg⁻¹ wet weight. The mean concentration of Cu and Zn in the caviar samples were under the permissible limits proposed by the UK’s ministry of agriculture, fisheries and foods (MAFF). The caviar maximum allowable daily consumption rate was calculated. However, the health risks from caviar consumption are uncertain because the amount of caviar consumed by heavy users is not known.

Keywords: Beluga sturgeon; Caviar; Essential metals; Seafood safety.

1. Introduction
The Caspian Sea is well-known for its sturgeon species (Persian sturgeon, Acipenser persicus; Russian sturgeon, Acipenser gueldenstaedtii; Sterlet, Acipenser ruthenus; Stellate sturgeon, Acipenser stellatus; Ship sturgeon, Acipenser nuidiventris; and Beluga sturgeon, Huso huso) which accounted for about 85% of the world’s population at its peak in the mid-1980s (Wang, Batterman, Chernyak, & Nriagu, 2008; Wirth, Kirschbaum, Gessner, Krüger, Patrice, & Billard, 2000). This provides more than 90% of the world’s caviar; but overfishing of sturgeon for caviar and meat, has led to large decline in sturgeon population (IUCN, 2012). Therefore, sturgeon were included on the list of endangered species by the convention on international trade in endangered species (CITES), and the Caspian littoral countries as parties to the convention, committed to strengthen national legislation to better regulate fishing and trade of sturgeon. The decision by the CITES in April 2006, restricted caviar exports to protect stocks of fish and specially band export from most Caspian nations (Wang et al., 2008). Some restocking programs are ongoing. However the programs do not compensate for the loss of natural reproduction and the populations continue to decline (IUCN, 2015); soit seems caviar production from aquaculture is the only conservation method for reducing the fishing pressure on the wild stock and saving the endangered sturgeon species (Birstein, Bauer, & Kaiser-Pohlmann, 1997; Gessner, Würtz, Kirschbaum, & Wirth, 2008). Among sturgeons, beluga sturgeons (H. huso) have been the most intensively fished of the Caspian Sea species and have been severely depleted in their natural habitats. The species is listed as Critically Endangered for the first time along with all of the other commercially important Caspian Sea species.
which are the main producers of wild caviar (IUCN, 2010). Caviar of Beluga sturgeon is the most coveted of all caviar (Speer, Lauck, Pikitch, Boa, Dropkin, & Spruill, 2000).

However some metals like Cu, Mn and Zn are essential components of the hydrosphere and are necessary for normal metabolism of organism in low concentration, but also they can produce side effects when their accumulation is excessive (Hosseini et al., 2008; Mashroofeh, Riyahi-Bakhtiari, & Pourkazemi, 2012). Contaminant levels in caviar have been investigated in numerous studies (Gessner et al., 2008; Hosseini, Hosseini, Monsef Rad, Mobinifar, & Regenstein, 2013; Mashroofeh et al., 2012; Wang et al., 2008; Wirth et al., 2000).

Some methods have been proposed for potential risks estimation to human health due to heavy metals in food. These risks may be divided into two groups, including carcinogenic and non-carcinogenic impacts (Yi, Yang, & Zhang, 2011). Risk assessment is one of the fastest methods, which is required to investigate the effect of the hazards on human health and to determine the level of treatment, which tends to solve the environmental issues that occur in daily life (Amirah, Afiza, Faizal, Nurliyana, & Laili, 2013).

The objectives of the present study were to evaluate the concentration of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Se and Zn in the caviar samples of farmed beluga sturgeon and compare the results obtained in other studies regarding metal levels; In addition, the potential human health risk associated with caviar consumption is evaluated.

### 2. Materials and Methods

#### 2.1. Sample collection

A total of nine farmed female Beluga sturgeon (mean weight= 64.1±6.8 kg) was collected from a private sturgeon rearing center (Talesh, Iran). Then fish samples were processed (washed and gutted) on site with hygienic conditions being maintained. Afterward, 65g of roe (“caviar”) free of the egg sack membrane were collected from each female, placed into separate 100 ml plastic containers with lids and immediately transported to the laboratory in an insulated box with a suitable quantity of flaked ice to completely cover the containers and kept frozen at -40 °C in a freezer (Philver Co., Tehran, Iran) until analyzed.

The farmed fish were reared in concrete tanks with constantly overflowing freshwater and commercial diets without any added minerals.

#### 2.2. Determination of trace elements

Each sample was analyzed three times for Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Se and Zn by inductively coupled plasma-optical emission spectrophotometer (ICP-OES) (Optima 2100DV, Perkin Elmer Inc., Waltham, MA, USA). The analytical procedure used for determined metal concentrations have been described previously (Türkmen, Türkmen, Tepe, Töre, & Ates, 2009). Briefly, about 5g of homogenized caviar (using a blender Panasonic, MJ-W176P, Osaka, Japan) was mixed with 50 ml of ultrapure concentrated HNO3. The mixture was heated on a lab digital heater (IKA, Staufen, Germany) to 100-150 °C for about 2h until the tissue had dissolved and the solution had evaporated to near dryness. All organic material in each sample was completely digested by repeating the digestion twice more. After cooling, 5 ml of 1 N HNO3 was added to the digested residue. Afterward it was transferred to a 25 ml volumetric flask and brought to level with by MilliQ purified water with conductivity under 0.05 μS (Millipore system, Eschborn, Germany). The recoveries of the metals were assessed by adding increasing amounts of each element to samples (spiking method) which were then taken through the digestion procedure. The resulting solutions were analyzed for their metal concentrations. Recoveries of the metals ranged from 96.8-102%. In this study, metal concentrations were determined as mg kg⁻¹ wet weight of roe.

### Tables 1

<table>
<thead>
<tr>
<th>Elements</th>
<th>Average (SD) metal levels (µg g⁻¹ wet weight) in caviar of farmed Beluga sturgeon (Huso huso, n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>57.85(0.43)</td>
</tr>
<tr>
<td>Co</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cr</td>
<td>0.27(0.027)</td>
</tr>
<tr>
<td>Cu</td>
<td>1.55(0.18)</td>
</tr>
<tr>
<td>Fe</td>
<td>76.24(0.42)</td>
</tr>
<tr>
<td>K</td>
<td>5357.67(13.12)</td>
</tr>
<tr>
<td>Mg</td>
<td>335.62(9.6)</td>
</tr>
<tr>
<td>Mn</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Se</td>
<td>1.30(0.076)</td>
</tr>
<tr>
<td>Zn</td>
<td>21.27(0.31)</td>
</tr>
</tbody>
</table>

### 2.3. Risk assessment

There is not information about caviar consumption; so the maximum allowable daily consumption rate (MADCR) for each metal was used for chronic non-cancer health risk assessment. The MADCR (g day⁻¹) for caviar consumption was calculated using the following equation (Wang et al., 2008):

\[
\text{MADCR} = 106 \times HQ \times \text{RfD} \times \frac{\text{BW}}{\text{C}}
\]

Where the hazard quotient (HQ) = 1; RfD is the reference dose (mg kg⁻¹ day⁻¹) set by US EPA (2011); BW is the body
weight of 70 and 14.5 kg for an adult and child, respectively; C is the metal concentration in the caviar samples (mg kg⁻¹), and the constant adjusts for concentration conversions. Moreover, metal levels in caviar samples were compared with the guidelines proposed by MAFF (2000).

3. Results and Discussion

3.1. Metal concentration

Mean concentration of essential metals in caviar of farmed beluga are shown in Table 1. Among the measured metals, K (5357.6±13.12 µg g⁻¹) and Mg (335.6±9.60 µg g⁻¹) had the highest, and Se (1.3±0.076 µg g⁻¹) had the lowest levels; also the averages of Co and Mn were below detection limits in the samples.

In comparison to our result, Gessner, Wirth, Kirschbaum, Krüger and Patriche (2002) found average concentration of Cu and Zn 1.69 and 10.45 µg g⁻¹ wet weights, respectively, in caviar samples of Acipenser baeri from aquaculture. Sadeghifard, Amini-Ranjbar, Arshad and Joshide (2005) also found Cu and Zn concentration of 2.2-7.9 and 32.9-92.1 µg g⁻¹ wet weight, respectively, in caviar samples of wild Stellate sturgeon from southern Caspian Sea that were higher than the present study. Levels of Cu (0.7-1.6 µg g⁻¹ wet weight), K (1017-1603 µg g⁻¹ wet weight), Mg (230.1-266.0 µg g⁻¹ wet weight) and Zn (16.8-24.0 µg g⁻¹ wet weight) were detected by Wang et al. (2008) in Eurasian caviar that were lower than in this study, and concentration of Ca (56.8-173.9 µg g⁻¹ wet weight), Co (5.2-23.9 ng g⁻¹ wet weight), Mn (0.8-1.4 µg g⁻¹ wet weight) and Se (1.0-2.1 µg g⁻¹ wet weight) that were higher, and Fe levels (21.6-155.1 µg g⁻¹ wet weight) that was comparable. In current finding, Cu, K and Mg levels were higher than the results were reported by Wang et al. (2008) in caviar of beluga sturgeon from southern Caspian Sea (Cu: 0.8-1.0; K: 1327-1603 and Mg: 215.2-259.7 µg g⁻¹ wet weight). Our findings also showed Cu and Zn concentration were lower than the dietary guidelines for fish, as represented by MAFF (2000) (Standard: Cu: 20 µg g⁻¹ww; Zn: 50 µg g⁻¹ww).

Mashroofeh et al. (2012) found mean concentration of Cu and Zn, 2.05 and 21.48 Mn µg g⁻¹ wet weight, respectively, in caviar sample of Persian sturgeon from southern Caspian Sea that was comparable and mean Mn concentration 1.66 µg g⁻¹ wet weight that was higher than in this study.

Variation observed in metal concentrations among sturgeon species depend on many factors such as habitat, seasonal variations, individual affinity for metal uptake and dietary habits (Papagiannis, Kagalou, Leonaros, Petridis, & Kalfakakou, 2004). Difference of physiological and each tissue position in the fish can also effect on the accumulation of a particular metal (Pourang, Tanabe, Rezvani, & Dennis, 2005). The increased levels of some essential metals such as Ca, Co, Cu, K, Mg and Zn may reflect the elevated requirement of sturgeons for these components (Gessner et al., 2002). Concentrations of essential metals are regulated by physiological mechanisms in fish (Pourang et al., 2005); however they are regarded as potential risks that can endanger both fish and human health (Yılmaz, Özdemir, Demirak, & Tuna, 2007). For example, although Se considered as essential mineral, there is a fine line between toxic and beneficial levels of Se, and its bioaccumulation in egg could be associated with fish reproductive failure (Kruse & Scarneccchia, 2002); so detection of these metal concentrations in fish is very important with regard to ecosystem management and human consumption of fish (Yılmaz et al., 2007).

3.2. Health risks

Table 2 shows the toxicity information and the estimated MADCR. Caviar is a valuable food substance that depending on the fish species, it contains 14-31% of protein, 0.3-15% of oil, and 1.5-2.0% of mineral substance (Ahmerova & Kopylenko, 2010). The rate of caviar consumption is unknown, so the health risks calculation of caviar consumption are not possible; however the MADCR provides an approach to evaluate its potential importance (Tüzen, 2003; Wang et al., 2008). The MADCR shows an

<table>
<thead>
<tr>
<th>Metals</th>
<th>RfD(b)(mg kg⁻¹ day⁻¹)</th>
<th>Children</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr(III)</td>
<td>1.50E+00</td>
<td>81600</td>
<td>389000</td>
</tr>
<tr>
<td>Cu</td>
<td>4.0E-02</td>
<td>380</td>
<td>1810</td>
</tr>
<tr>
<td>Fe</td>
<td>7.0E-01</td>
<td>133</td>
<td>643</td>
</tr>
<tr>
<td>Mg</td>
<td>4.90E+01</td>
<td>212</td>
<td>1020</td>
</tr>
<tr>
<td>Se</td>
<td>5.00E-03</td>
<td>55800</td>
<td>269000</td>
</tr>
<tr>
<td>Zn</td>
<td>3.00E-01</td>
<td>205</td>
<td>987</td>
</tr>
</tbody>
</table>

*Table 2: Maximum allowable daily consumption rate (MADCR) estimates for children and adults using median concentration (mg kg⁻¹ wet weight)*

*a Data arranged by three significant figure

*b Reference doses of metals set by US EPA (2011)

*c ng g⁻¹ wet weight
average daily consumption rate that would not be expected to cause adverse non-carcinogenic health effects (US EPA, 2000). In this study, for adult chronic non-cancer health effects using a HQ of 1, the MADCR is relatively low for Fe and Zn especially for children. In comparison to our result, Wang et al. (2008) estimated the MADCRs for Cr (358x10^4 g day^{-1} for children; 1727x10^4 g day^{-1} for adult) that was higher, Zn (200 g day^{-1} for children; 980 g day^{-1} for adult) that was comparable, and Se (53 g day^{-1} for children; 980 g day^{-1} for adult) that was more restrictive. Because of the difference in body weight, the MADCRs for children are about 5 times lower than for adults; although the same per kg values are used as insufficient work has been done to generate separate values for children.

4. Conclusion

In this study the concentrations of Co, Cr, Cu, Fe, K, Mg, Mn, Se and Zn were analyzed as essential metals in caviar samples of farmed beluga sturgeon. The Caviar consumption rate is unknown, but it is expected to be very low (Wang et al., 2008). With regard to low per capita consumption rates, declining levels and health benefits of consuming caviar, our results suggest that advisories or consumption limits are not warranted.

5. References


