

## Health risk assessment of selected heavy metals in some edible fishes from Gorgan Bay, Iran

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### Abstract

The objective of this study was to assess the bioaccumulation of heavy metals (Cd, Pb, Cr and Fe) in the muscles of five fish species (*Sander lucioperca*, *Liza auratus*, *Alosa caspia*, *Cyprinus carpio* and *Liza saliens*) from Gorgan Bay in the south-eastern Caspian Sea, in order to determine the value daily intake of heavy metals by consumption of fish and human health risk assessment. The concentration of metals was estimated using graphite furnace atomic absorption spectrometer. Potential health risk assessments based on estimated daily intake (EDI) values and target hazard quotient (THQ) indicated that the intakes of metals by consuming these fish species do not result in an appreciable hazard risk for the human body. The hazard index (HI) calculated was lower than 1 for all the species. However, the results indicate that the high concentrations of Pb (in the muscle of *L. auratus*) and Fe in all fish is alarming.

**Keywords:** Heavy metal, Fish, Gorgan Bay, EDI, THQ

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## Introduction

Pollution of heavy metals in the environment is growing at an alarming rate and has become an important worldwide problem. Heavy metals enter into an environment, both as a result of natural processes and as pollutants from anthropogenic activities, and accumulate in the tissues and organs of living organisms, thereby affecting the normal processes of the body (Budambula and Mwachiro, 2005). Metals like Cr and Fe are essential for fish metabolism, while others, such as Pb and Cd have no function in biological systems (Fernandes *et al.*, 2008). The amount of metal in fish is dependent on the concentration levels of these metals in the food and the habitats of the fish, and the detoxification rate of the metals (Uysal *et al.*, 2009). These metals may accumulate to a very high toxic level and cause severe impacts on the aquatic organisms without any visible signs (Cai *et al.*, 2012). When metals enter into the environment, they may accumulate in the food chain and cause serious ecological damage and also pose carcinogenic and other adverse effects on human health due to biomagnification over time (Malik *et al.*, 2010).

For most people, diet is the main route of exposure to metals, so the assessment risks of these elements to human via dietary intake is important (Zheng *et al.*, 2007). Fishes are one of the main aquatic organisms in the food chain and may often accumulate large

amounts of certain metals (Zauke *et al.*, 1999; Alipour *et al.*, 2013). Fish have been found to be good indicators of the heavy metal contamination levels in aquatic systems because they occupy different trophic levels (Uysal *et al.*, 2009). Fishes are a major part of the human diet because of their high protein content, Adipose tissues contain unsaturated fatty acids and also omega fatty acids known to support good health, but there is concern that heavy metals accumulated in edible fish may represent a health risk, especially for populations with high fish consumption rates (Alipour *et al.*, 2014).

Studies on metal bioaccumulation in fish are now widespread, but in recent years, risk factor calculations for the population have become of great importance, because although sometimes the contaminants exceed the legal limits set by FAO/WHO regulations for food, they do not always represent a risk to human health (Copat *et al.*, 2012; Alipour *et al.*, 2014).

Different methods have been proposed to estimate the potential risks of toxic metals on human health assessment. To estimate the potential risk for human health derived from ingesting contaminated fish, we have evaluated: the weekly and daily intake, comparing them with the provisional tolerable weekly intake (PTWI) recommended by the FAO/WHO (2010) and the target hazard quotient (THQ) provided in the USEPA Region III Risk-based concentration table (USEPA, 2015). THQ have been

recognized as one of the reasonable indexes for the evaluation associated with the intake of heavy metals by consuming the contaminated foods (Li *et al.*, 2013). A THQ below 1 means the exposed population is unlikely to experience obvious adverse effects, whereas a THQ above 1 means that there is a chance of noncarcinogenic effects, with an increasing probability as the value increases (Saha and Zaman, 2012; Alipour *et al.*, 2014).

Various studies have been carried out worldwide on the metal contamination in different edible fish species. Also, based on the THQ values, several studies on the potential risk assessment of dietary intake of heavy metals via the consumption of fish have been reported (Storelli, 2008; Türkmen *et al.*, 2009; Li *et al.*, 2013; Saha and Zaman, 2012; Copat *et al.*,

2012, 2013; Alipour *et al.*, 2014).

The objective of this study was to determine heavy metal concentrations (Cd, Pb, Cr and Fe) in the muscles of five fish species (*Sander lucioperca*, *Liza auratus*, *Alosa caspia*, *Cyprinus carpio* and *Liza saliens*) from Gorgan Bay in the south-eastern Caspian Sea and to estimate the value of daily intake of heavy metals by consumption of fish and human health risk assessment.

## Materials and methods

### *The study area*

The Gorgan Bay, strategically located in the southeastern part of the Caspian Sea, is 60 km long and 12 km wide (Ghorbanzadeh Zaferani *et al.*, 2016). It is a semi-enclosed basin, as it receives no wave energy from the Caspian Sea (Fig. 1).



**Figure 1: The study of area (Gorgan Bay, Iran).**

This area is best known for its economical and high ecological importance because of its appropriate biological conditions for aquatic animals (Bastami *et al.*, 2012; Saghali *et al.*, 2014). The Gorgan Bay is a very important spawning and nursery area for economically important species in the Caspian Sea fishery. On the other hand, the agricultural and industrial (Port of Amir Abad) activities and increasing numbers of tourists in Gorgan Bay have increased during the past two decades which have caused pollution of the Gorgan Bay and its adjacent environment.

#### Chemical analyses

A total of 50 samples (10 of each species) were collected randomly from the Gorgan Bay in June 2013 using Beach seine and then transported to the laboratory. The fishes were washed with distilled water and the scales were removed. Of the muscle tissue samples, 1 g each was accurately weighed into 25 mL Erlenmeyer flasks, 5 mL nitric acid (65%; from Merck, Germany) was added to each sample, and the samples were left overnight to be slowly digested (Ebrahimpour *et al.*, 2011). Thereafter, 2.5 mL perchloric acid (72%; from Merck, Germany) was added to each sample. Digestion was performed in a sand bath on a hot plate at 150 °C for 6 h or until solutions were clear and near to dryness. After cooling, mixtures were diluted to 25 mL in polyethylene bottles with deionized water. Then the solution was filtered

using 0.45 µm nitrocellulose membrane filters (Alipour *et al.*, 2014). The metal determinations were carried out using a flame atomic absorption spectrometer, (Model 97GFS, Thermo). Results for Pb, Cd, Cr and Fe gave a mean recovery of 98%, 97%, 99.5% and 99%, respectively. The concentrations of metals in muscle tissue samples are presented as mg kg<sup>-1</sup> wet weight.

#### Estimated daily intake (EDI)

The estimated daily intake of each heavy metal was found in the following way:

$$EDI \text{ (mg kg}^{-1} \text{ day}^{-1}) = \frac{EF \times ED \times FIR \times C}{WAB \times ATn}$$

#### Target hazard quotient (THQ)

THQ was calculated (USEPA, 2015) by the following equations:

$$THQ = \frac{EF \times ED \times FIR \times C}{RfD \times WAB \times ATn} \times 10^{-3}$$

where: EF = exposure frequency (360 days year<sup>-1</sup>); ED = exposure duration (70 years for adults), equivalent to the average lifetime; FIR=fish ingestion rate (kg person<sup>-1</sup> day<sup>-1</sup>), (0.02kg person<sup>-1</sup> day<sup>-1</sup> for adults); C=metal concentration in fish (mg kg<sup>-1</sup>); RfD=oral reference dose (mg kg<sup>-1</sup> day<sup>-1</sup>); WAB=average body weight (kg), (70 kg for adults); ATn=average exposure time for noncarcinogens (365 days year<sup>-1</sup>×ED)

#### Hazard index (HI)

A total HI was employed by summing all the calculated THQ values of heavy metals as described.

$$HI = \sum_{i=1}^n THQ$$

### Statistical analyses

Data analyses were performed using the statistical package SPSS (version 19). The Kolmogorov–Smirnov test was accomplished to analyze the normality of data distribution. One way-ANOVA, followed by Tukey's test ( $p < 0.05$ ) was used to evaluate differences between species.

### Results

Concentrations of Cd, Pb, Cr and Fe in the muscles of the analyzed five fish species are presented in Table 1 as mean (SD) and range. There were vast differences among the heavy metal concentrations in the muscles of different fish species. In general, mean

concentrations of metals in muscle of *C. carpio*, *S. lucioperca* and *A. caspia* followed a trend where  $Cd < Pb < Cr < Fe$ , whereas in *L. auratus* and *L. saliens* muscle the following trend was observed  $Cd < Cr < Pb < Fe$ . Table 1 shows the significant differences in the accumulation levels of metals in the muscle of species ( $p < 0.05$ ). The heavy metal levels recorded for muscles of five fish species from Gorgan Bay in this study and in other literature are listed in Table 2. Data from the existing literatures showed that metal contents in the fish muscles varied widely depending on where and which species were caught.

**Table 1: Mean metal concentrations (mg kg<sup>-1</sup> ww) in the muscles of five fish species from Gorgan Bay (n=10).**

Fish species		Cd	Pb	Cr	Fe
<i>C. carpio</i>	Mean (SD)	0.26(0.09) <sup>a</sup>	0.43(0.14) <sup>a</sup>	6.4(0.27) <sup>a</sup>	501.65(69.98) <sup>a</sup>
	Range	0.14-0.41	0.23-0.66	6.01-6.83	412.5-660.5
<i>S. lucioperca</i>	Mean (SD)	0.09(0.12) <sup>bcd</sup>	0.53(0.6) <sup>a</sup>	5.56(0.26) <sup>b</sup>	455.6(53.19) <sup>ac</sup>
	Range	0.05-0.45	0.43-0.62	5.08-5.98	377.5-559.5
<i>L. auratus</i>	Mean (SD)	0.25(0.05) <sup>a</sup>	8.6(0.58) <sup>b</sup>	0.93(0.09) <sup>c</sup>	914.6(73.93) <sup>b</sup>
	Range	0.2-0.37 <sup>-</sup>	7.57-9.53	0.79-1.07	802.5-1017.5
<i>L. saliens</i>	Mean (SD)	0.09(0.008) <sup>cd</sup>	1.7(0.35) <sup>c</sup>	1.3(0.1) <sup>d</sup>	380.65(47.32) <sup>c</sup>
	Range	0.08-0.11	1.3-2.4	1.31-1.63	311-472
<i>A. caspia</i>	Mean (SD)	0.09(0.008) <sup>d</sup>	0.34(0.09) <sup>a</sup>	9.15(0.62) <sup>e</sup>	1181.25(128.84) <sup>d</sup>
	Range	0.08-0.11	0.22-0.5	8.01-10.01	974-1343.5

a, b, c, d, e: Means with the same letters in each column for each metal are significantly different according to Tukey's test. Significant differences at  $p < 0.05$ .

Daily and weekly intake values of heavy metals (Cd, Pb, Cr and Fe) for an adult via the consumption of five fish from Gorgan Bay are shown in Table 3, and the data of PTDI suggested by the FAO/WHO (2010) are also listed. The daily consumption of Cd, Pb, Cr and Fe in all fish species in this study ranged from  $257 \times 10^{-7}$ - $742 \times 10^{-7}$ ,  $97142 \times 10^{-9}$ - $2457 \times 10^{-6}$ ,  $2657 \times 10^{-7}$ - $2614 \times 10^{-6}$  and 0.108757-0.3375 mg/day/person,

respectively. The average daily intake of metals through muscle of *C. carpio*, *S. lucioperca* and *A. caspia* consumption can be ordered as follows:  $Fe > Cr > Pb > Cd$ , whereas in *L. auratus* and *L. saliens* the following trend was observed  $Fe > Pb > Cr > Cd$ . Table 4 shows the THQs and HI for Cd, Pb, Cr and Fe caused by consuming the investigated fish species collected from the Gorgan Bay.

**Table 2: Comparison of heavy metal levels (mg kg<sup>-1</sup> ww) in the muscles of five fish species from Gorgan Bay with concentrations taken from the literature.**

Sample area	Fish species	Cd	Pb	Cr	Fe	References
WHO	-	1	2	50	100	WHO (1989)
FAO	-	0.5	0.5	-	-	FAO (1983)
Kayseri, Turkey	<i>S. lucioperca</i>	2.52	BDL	BDL	-	Yildirim <i>et al.</i> 2009
Danube River, Serbia	<i>S. lucioperca</i>	0.005	-	0.043	17.97	Subotić <i>et al.</i> , 2013
Danube River, Serbia	<i>C. carpio</i>	0.005	-	0.01	19.62	Subotić <i>et al.</i> , 2013
Beyşehir Lake, Turkey	<i>S. lucioperca</i>	2.17	1.62	12.21	2.03	Özparlak <i>et al.</i> , 2012
Beyşehir Lake, Turkey	<i>C. carpio</i>	2.17	2.84	12.11	3.03	Özparlak <i>et al.</i> , 2012
Khomse Coast, Libya	<i>L. saliens</i>	0.043	0.012	-	-	Metwally and Fouad, 2008
Porsuk Lake, Turkey	<i>C. carpio</i>	-	-	-	4.42	Uysal, 2011
Miankaleh Wetland, Iran	<i>R. rutilus</i>	0.26	0.67	0.08	28	Alipour <i>et al.</i> , 2014
Kayseri, Turkey	<i>E. aeneus</i>	0.85	1.20	5.97	122.8	Duran <i>et al.</i> , 2014
Alexandria, Egypt	<i>T. thynnus</i>	0.05	0.67	0.86	165.17	Hussein and Khaled, 2014
Gorgan coast, Iran	<i>C. carpio</i>	0.09	0.16	0.05	-	Tabari <i>et al.</i> , 2010
Caspian Sea, Iran	<i>L. saliens</i>	0.019	4.55	-	3.97	Ebrahimzadeh <i>et al.</i> , 2011
Gorgan Bay, Iran	<i>S. lucioperca</i>	0.09	0.53	5.56	455.6	In this study
	<i>L. auratus</i>	0.25	8.6	0.93	914.6	
	<i>A. caspia</i>	0.09	0.34	9.15	1181.25	
	<i>C. carpio</i>	0.26	0.43	6.4	501.65	
	<i>L. saliens</i>	0.09	1.7	1.3	380.65	

**Table 3: Estimated daily and weekly intakes for muscles of five fish species from Gorgan Bay consumed by adult.**

Metals	Cd*	Pb*	Cr**	Fe*
PTWI (mg kg <sup>-1</sup> bw/w)	0.007	0.025	0.0233	0.08
PTWI (mg kg <sup>-1</sup> bw/w)	0.49	1.75	1.631	5.6
PTDI (mg kg <sup>-1</sup> bw/d)	0.07	0.25	0.233	0.8
EWI ( <i>C. carpio</i> )	52×10 <sup>-5</sup>	86×10 <sup>-5</sup>	128×10 <sup>-4</sup>	1.003303
EDI ( <i>C. carpio</i> )	742×10 <sup>-7</sup>	123×10 <sup>-5</sup>	1828×10 <sup>-6</sup>	0.143329
EWI ( <i>S. lucioperca</i> )	18×10 <sup>-5</sup>	106×10 <sup>-5</sup>	1112×10 <sup>-5</sup>	0.9112
EDI ( <i>S. lucioperca</i> )	257×10 <sup>-7</sup>	16×10 <sup>-5</sup>	1588×10 <sup>-6</sup>	0.130171
EWI ( <i>L. auratus</i> )	5×10 <sup>-4</sup>	172×10 <sup>-4</sup>	186×10 <sup>-5</sup>	1.829198
EDI ( <i>L. auratus</i> )	714×10 <sup>-7</sup>	2457×10 <sup>-6</sup>	2657×10 <sup>-7</sup>	0.261314
EWI ( <i>L. saliens</i> )	18×10 <sup>-5</sup>	34×10 <sup>-4</sup>	26×10 <sup>-4</sup>	0.761300
EDI ( <i>L. saliens</i> )	257×10 <sup>-7</sup>	486×10 <sup>-6</sup>	371×10 <sup>-6</sup>	0.108757
EWI ( <i>A. caspia</i> )	18×10 <sup>-5</sup>	68×10 <sup>-5</sup>	183×10 <sup>-4</sup>	2.3625
EDI ( <i>A. caspia</i> )	257×10 <sup>-7</sup>	97142×10 <sup>-9</sup>	2614×10 <sup>-6</sup>	0.3375

PTDI permissible tolerable daily intake.

PTWI provisional tolerable weekly intake.

EWI estimated weekly intake.

EDI estimated daily intake.

\*FAO/WHO (2010)

\*\*Zaidi *et al.* (2012)**Table 4: Estimated THQ, HI and RfD (mg/kg/day) of metals due to consumption of five fish species from Gorgan Bay.**

Metals	Cd*	Pb*	Cr**	Fe*	HI
RfD (mg kg <sup>-1</sup> day <sup>-1</sup> )	0.001	0.002	1.5	0.7	
THQ (mg kg <sup>-1</sup> bw/d) ( <i>C. carpio</i> )	742×10 <sup>-7</sup>	614×10 <sup>-7</sup>	121×10 <sup>-8</sup>	204×10 <sup>-6</sup>	340×10 <sup>-6</sup>
THQ (mg kg <sup>-1</sup> bw/d) ( <i>S. lucioperca</i> )	257×10 <sup>-7</sup>	757×10 <sup>-7</sup>	105×10 <sup>-8</sup>	185×10 <sup>-6</sup>	287×10 <sup>-6</sup>
THQ (mg kg <sup>-1</sup> bw/d) ( <i>L. auratus</i> )	714×10 <sup>-7</sup>	122×10 <sup>-5</sup>	177×10 <sup>-9</sup>	373×10 <sup>-6</sup>	166×10 <sup>-5</sup>
THQ (mg kg <sup>-1</sup> bw/d) ( <i>L. saliens</i> )	257×10 <sup>-7</sup>	242×10 <sup>-6</sup>	248×10 <sup>-9</sup>	155×10 <sup>-6</sup>	422×10 <sup>-6</sup>
THQ (mg kg <sup>-1</sup> bw/d) ( <i>A. caspia</i> )	257×10 <sup>-7</sup>	485×10 <sup>-7</sup>	174×10 <sup>-8</sup>	482×10 <sup>-6</sup>	557×10 <sup>-6</sup>

\* USEPA (2015)

\*\* USEPA (2002)

## Discussion

### Cadmium

Cd is generally classified as a toxic trace element and there is no evidence indicating its essentiality to humans. Cd occurs naturally at low levels in the environment. Nevertheless, industrial processes can increase the concentration of Cd in the environment (Ahmed *et al.*, 2015). Research has shown that Cd has the ability to be bioaccumulated in aquatic organisms. In the present study, Cd had the lowest concentrations of all metals in the muscle of fish tissues that were analyzed. The concentrations of Cd in the samples analyzed ranged from 0.05 to 0.45 mg kg<sup>-1</sup>. The highest amount of Cd was found in the muscle of *C. carpio* (0.26 mg kg<sup>-1</sup>) and the lowest amount of Cd concentrations were observed in the muscle of *S. lucioperca* (0.09 mg kg<sup>-1</sup>), *L. saliens* (0.09 mg kg<sup>-1</sup>) and *A. caspia* (0.09 mg kg<sup>-1</sup>).

Cd distribution in the muscle of five fish species followed the decreasing sequences: *C. carpio* > *L. auratus* > *S. lucioperca*  $\cong$  *L. saliens*  $\cong$  *A. caspia*. The Cd concentrations in the muscle tissue of all fishes from the Gorgan Bay were below the recommended guidelines of FAO (1983) and WHO (1989).

Mean Cd concentrations in the muscle tissue of all fishes in the present study were found to be lower than the data reported for *S. lucioperca*, in the Kayseri (Yildirim *et al.*, 2009) and Beyşehir Lake (Özparlak *et al.*, 2012), for *C. carpio* in the Beyşehir Lake

(Özparlak *et al.*, 2012), for *E. aeneus* in the Kayseri (Duran *et al.*, 2014) and for *R. rutilus* in the Miankaleh wetland (Alipour *et al.*, 2014) (Except fish *C. carpio*). In the present study, the mean Cd concentrations in the muscle tissue of all fishes were higher than the concentrations found in *S. lucioperca* and *C. carpio*, in the Danube River (Subotić *et al.*, 2013), in *L. saliens* in the Khomse Coast (Metwally and Fouad, 2008), in *T. thynnus* in the Alexandria (Hussein and Khaled, 2014) and in *L. saliens* in the Caspian Sea (Ebrahimzadeh *et al.*, 2011). Cd concentrations in the muscle tissues of *S. lucioperca*, *A. caspia* and *L. saliens* from this study were similar to those reported in muscle of *C. carpio* in the Gorgan coast (Tabari *et al.*, 2010).

### Lead

Pb is not essential for fish, and excessive amounts can cause deficits or decreases in the survival, and growth rates, as well as development and metabolism, in addition to increased mucus formation (Burger *et al.*, 2002). Pb levels found in muscles of fish in the present study ranged from 0.22 to 9.53 mg kg<sup>-1</sup>. The highest amount of Pb was found in the muscle of *L. auratus* (8.6 mg kg<sup>-1</sup>) and the lowest amount of Pb concentrations was observed in the muscle of *A. caspia* (0.34 mg kg<sup>-1</sup>). Pb concentrations decrease in the following order: *L. auratus* > *L. saliens* > *S. lucioperca* > *C. carpio* > *A. caspia*. FAO (1983) and WHO (1989) maximum permissible concentrations

for Pb are 2 and 0.5 mg kg<sup>-1</sup>, respectively. Based on the values obtained Pb concentrations, found in muscles of all fishes are below (except of *L. auratus*) the proposed limit by the WHO (1989) for human consumption of toxic compounds. The Pb concentrations in the muscle tissues of *A. caspia* and *C. carpio* from the Gorgan Bay are below levels of concern for human consumption as defined by the FAO (1983).

Mean Pb concentrations in the muscle tissues of *S. lucioperca*, *A. caspia* and *C. carpio* in the present study were found to be lower than the data reported for the *C. carpio* in the Beyşehir Lake (Özparlak *et al.*, 2012) and *L. saliens* in the Caspian Sea (Ebrahimzadeh *et al.*, 2011). In the present study, the mean Pb concentrations in the muscle tissue of all fishes were higher than the concentrations found in *S. lucioperca*, in the Kayseri (Yildirim *et al.*, 2009), in *C. carpio* in the Gorgan coast (Tabari *et al.*, 2010) and in *L. saliens* in the Khomse Coast (Metwally and Fouad, 2008). The mean Pb concentrations in the muscle of *L. auratus* were higher than the concentrations found in the other areas (Table 2).

#### Chromium

Cr is an essential metal in humans and some animals, but the occurrence of excessive levels of it is regarded as a potential hazard which can endanger both fish and human health. In the present study, the mean Cr

concentrations in the muscle of fish species ranged from 0.79 to 10.01 mg kg<sup>-1</sup>. The highest amount of Cr was found in the muscle of *A. caspia* (9.15 mg kg<sup>-1</sup>) and the lowest amount of Cr concentrations was observed in the muscle of *L. auratus* (0.93 mg kg<sup>-1</sup>). Cr concentrations decrease in the following order: *A. caspia* > *C. carpio* > *S. lucioperca* > *L. saliens* > *L. auratus*. Based on WHO (1989), the maximum permissible concentration for Cr is 50 mg kg<sup>-1</sup>. Based on the values obtained, Cr concentrations found in muscles of all fishes are below the proposed limit by the WHO (1989) for human consumption of toxic compounds.

Mean Cr concentrations in the muscle tissues of *S. lucioperca*, *L. auratus* and *L. saliens* in the present study were found to be lower than the data reported for *C. carpio* and *S. lucioperca* in the Beyşehir Lake (Özparlak *et al.*, 2012) and *E. aeneus* in the Kayseri (Duran *et al.*, 2014). In the present study, the mean Cr concentrations in the muscle tissue of all fishes were higher than the concentrations found in *S. lucioperca* and *C. carpio*, in the Danube River (Subotić *et al.*, 2013), *S. lucioperca* in the Kayseri (Yildirim *et al.*, 2009), *R. rutilus* in the Miankaleh Wetland (Alipour *et al.*, 2014), *T. thynnus* in the Alexandria (Hussein and Khaled, 2014) and in *C. carpio* in the Gorgan coast (Tabari *et al.*, 2010).

### Iron

Fe is an essential nutrient to almost all organisms, being involved in oxygen transfer, respiratory chain reactions, DNA synthesis, and immune function (Wood *et al.*, 2012). Fe can be damaging when it accumulates in the tissues and can also produce toxic effects when the metal intake is excessively elevated. Fe showed the highest concentrations in tissues of all the analyzed fish species. Mean concentrations for Fe in the muscle of fish species ranged from 311 to 1343.5 mg kg<sup>-1</sup>. The highest amount of Fe was found in the muscle of *A. caspia* (1181.25 mg kg<sup>-1</sup>) and the lowest amount of Fe concentrations was observed in the muscle of *L. saliens* (380.65 mg kg<sup>-1</sup>). Fe distribution in the muscle of five fish species followed a decreasing sequence: *A. caspia* > *L. auratus* > *C. carpio* > *S. lucioperca* > *L. saliens*. In the present study, the mean Fe concentrations in the muscle tissue of all fishes were higher than the concentrations found in the other areas (Table 2). Also, it was found that accumulations of Fe in the muscle tissue of all fishes were above the permissible limit (FAO, 1983).

### Daily intake of metals

Consumption of fish is a major part of the human diet. For this reason, there is great interest in the estimation of the daily intakes of heavy metals through fish. The daily intake was estimated for economically important fish species consumed by adult people. According

to FAO (2014) reports, the per capita fish consumption in Iran is 24 g person<sup>-1</sup> day<sup>-1</sup> for adults. This is also equivalent to 174 g per person per week. The daily intake values presented in Table 3 were estimated by assuming that a 70 kg person will consume 24 g fish per day.

EWI and EDI values are presented in Table 3. Also, Table 3 compares the estimated PTWI and PTDI to recommended values. PTWI value is an estimate of the amount of contaminant that can be consumed by human over a lifetime without appreciable risk. PTWI is established by the Joint Food and Agricultural Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (Bat *et al.*, 2012; Alipour *et al.*, 2014).

EDIs of metals (Cd, Pb, Cr and Fe) were compared to the PTDI values based on the reference doses established by the JECFA. The present study showed that the contributions of these fish to daily intake of Cd, Pb, Cr and Fe were 0.1, 0.04, 0.78 and 17.91 % for *C. carpio*; 0.03, 0.06, 0.68 and 16.27 % for *S. lucioperca*; 0.1, 0.98, 0.11 and 32.66 % for *L. auratus*; 0.03, 0.19, 0.15 and 13.59 % for *L. saliens*; and 0.03, 0.03, 1.12 and 42.18 % for *A. caspia*, respectively, of PTDI as suggested by JECFA. In the present study, the EWI and EDI values of Cd, Pb, Cr and Fe concentrations in the muscle tissue of all fishes were lower than the established PTWI and PTDI. The EDI values found in this study are

in agreement with values reported by many researchers for fish. Safiur Rahman *et al.* (2012) reported the daily intake ( $\text{mg day}^{-1} \text{ person}^{-1}$ ) of metals Pb, Cd and Cr from fish in Bangshi River, Bangladesh as: 0.0203, 0.0013 and 0.0049, respectively. Taweel *et al.* (2013) reported the daily intake of Cu, Pb and as for tilapia fish in Cempaka Lake (Bangi, Malaysia) as 4.55, 1.20 and  $0.45 \mu\text{g g}^{-1}/\text{bw}/\text{day}$ , respectively, which were less than the JECFA for studied metals, indicating there is no potential health risk for people who have a high consumption rate.

#### *Target hazard quotient (THQ) and hazard index (HI)*

THQ and HI proposed by USEPA (2015) are parameters for risk assessment which compare the ingestion amount of a pollutant with a standard reference dose and have been widely used in the risk assessment of metals in contaminated foods. The THQ value has been recognized as one of the reasonable parameters for the risk assessment of metals associated with the consumption of contaminated fish (Li *et al.*, 2013). A THQ below 1 means the exposed population is unlikely to experience obvious adverse effects; whereas a THQ above 1 means that there is a chance of no carcinogenic effects, with an increasing probability as the value increases (Saha and Zaman, 2012). In the present study, the THQ values for all the metals in the muscle of fish species were below 1, which indicates that the intakes of

metals by consuming these fish do not result in an appreciable hazard risk on the human body.

Storelli (2008) measured the Hg, Cd, and Pb concentrations in fish from the Adriatic Sea, and reported that the THQs of Cd (0.01–0.04) and Pb (0.002–0.18) from consumption of fish was less than 1, suggesting that the health risk was insignificant. Conversely, mercury THQs values, ranging from 0.08 to 1.87, were of concern. Copat *et al.* (2013) estimated the THQ of metals consumed in fish and shellfish from the eastern Mediterranean Sea, and reported that the THQ values for Cd, Cr, Mn, Ni, V, and Zn were all below 1. That means that there is no risk for developing chronic systemic effects due to the intake of the above named metals, but Arsenic THQ values were above 1. Values for  $\text{THQ} < 1$  were also reported for Cu, Cd, Pb, Hg and Cr in fish from the Eastern Aegean Sea (Yabanli and Alparslan, 2015).

Results show that the HI of metals for species follows the order: *L. auratus* > *A. caspia* > *L. saliens* > *C. carpio* > *S. lucioperca*.

In the present study, the average HI values for all the four fish species (*C. carpio*, *S. lucioperca*, *L. auratus*, *L. saliens* and *A. caspia*) were below 1, which indicates that the intakes of metals by consuming these fish do not result in an appreciable hazard risk for the human body. HI exceeding 1 indicates that the metals are toxic and

present a hazard to human health (Li *et al.*, 2013).

This study has identified the level of metals such as Cd, Pb, Cr and Fe in the muscles of five fish species (*Sander lucioperca*, *Liza auratus*, *Alosa caspia*, *Cyprinus carpio* and *Liza saliens*) caught from the Gorgan Bay in the south-eastern Caspian Sea. Potential health risk assessments based on PTWI values, EDI, and THQ indicated that the intakes of metals by consuming these fish species do not result in an appreciable hazard risk for the human body. The HI calculated was lower than 1 for all the species. However, the results indicate that the high concentrations of Pb (in the muscle of *L. auratus*) and Fe for all fish are alarming and do present an appreciable hazard risk to human health. Nevertheless, this needs to be further examined in future studies.

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