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Analysis of Heavy Metal Contents and Non-carcinogenic Health Risk Assessment through Consumption of Tilapia Fish (*Oreochromis niloticus*)

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ABSTRACT: Due to the fish are often at the top of the aquatic food chain and may accumulate large amounts of heavy metals from the water, this study was conducted to determine of Cd, Cr, Pb and Ni contents in the muscle of imported tilapia fish marketed in the city of Hamedan in 2017. In so doing, totally, 27 muscle samples from nine different brands of tilapia fish were randomly collected from the market basket of the study area. After preparation and processing the samples in the laboratory, the concentration of metals, was determined using inductively coupled plasma-optical emission spectrometer. The results showed that the mean concentrations (mg/kg) of Cd, Cr, Pb, and Ni in samples were 0.26 ± 0.09, 1.54 ± 0.15, 0.55 ± 0.11, and 0.67 ± 0.20 respectively. Also, the mean contents of Cd and Pb were higher than the maximum permissible levels (MPL) established by the World Health Organization (WHO). The computed health risk index values showed that no potential health risk for adults and children via consuming the muscle of tilapia fish at the current consumption rate for the study area. Based on the results, due to the mean contents of Cd and Pb in the muscle samples of tilapia fish were higher than the MPL, therefore, serious attention to the reduction of the discharge of hazardous substances in the aquatic ecosystems and also periodic monitoring of chemical residue particularly toxic heavy metals in the high-demand food is recommended.

Keywords: hazardous substances, risk assessment, tilapia, water pollution, Iran.

INTRODUCTION

The rapid development of urbanization, industrialization and agronomic activities has resulted in toxic heavy metals pollution, which is significant environmental hazard for all creatures including plants, invertebrates, and vertebrates especially humans (Uluturhan & Kucuksezgin, 2007; Sobhanardakani et al., 2011; Nasrabadi et al., 2015). Nowadays, the anthropogenic and natural sources such as the domestic sewage of the inhabitants, effluents discharged by various industries, storm runoff, leaching from landfills, shipping, harbor activities and atmospheric deposits are the main source of aquatic ecosystem pollution (Zhou et al., 1998; Hosseini et al., 2013a). In this regard, the untreated inorganic and organic waste running into the aquatic systems lead to the degradation of the inland and coastal water systems and also sediments (Wong et al.,

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1996; Zhou et al., 1998). Therefore, in recent decades there has been a growing interest in analyzing the content of toxic metals in aquatic environments with additional emphasis on the measurement of their contamination levels in the food supply, especially fish (Wong et al., 1996).

Many elements such as Cu, Fe, Mn, Ni, and Zn that are present in the human diet are essential for human life at low contents but can be toxic at high levels. Others such as As, Cd, Cr, Hg, Pb, and Sn have no known essential function in living organisms and are toxic even at low contents when enters the body over a long time (Sobhanardakani, 2017a). Generally, heavy metals can cause a variety of adverse effects on human health such as physiological, behavioral, and cognitive effects (Hosseini et al., 2013b).

Cadmium is toxic and non-essential for human health and can be accumulated principally in the liver and kidney tissues. It is necessary to mention that potential toxic effects of Cd are mitigated by binding to the metallothioneins both in the liver and kidney tissues, even though this element could be stored over a very long time in the renal tissue of marine mammals (Klaassen & Liu, 1997; Teigen et al., 1999).

Chromium is an essential mineral that is widely distributed in human tissues in extremely low and variable contents. This element can help the body use protein, lipid, and also carbohydrate metabolism (Hosseini et al., 2013a; Stancheva et al., 2014). Hexavalent chromium (VI) compounds are toxics and known human carcinogens, whereas Cr (III) is known an essential element (Kabata-Pendias, 2010).

Lead as the most environmental toxic agent can result the most common environmental health risk. Humans are exposed to this element mainly through inadvertent ingestion of Pb-based paint, inhalation of traffic exhaust fumes, and also consumption of Pb contaminated foods (Adekunle & Akinyemi, 2004; Şireli et al., 2006; Sobhanardakani & Jafari, 2014).

Nickel is micronutrient in human body and trace amounts of this element act as activator of some enzyme systems. It should of course be noted that Ni toxicity at higher levels can cause bronchial failure and also is carcinogenic (Hussein & Khaled, 2014).

Fish are known to be nutritious, having important contents of polyunsaturated fatty acids especially omega-3, essential proteins, and liposoluble vitamins. Fishes are often at the top of the aquatic food chain and may accumulate large levels of some toxic heavy metals from the water or foods (Hosseini et al., 2013b). The accumulation of metals varies greatly between both fish species and/or fish tissues (Kagi & Schaeffer, 1998).

Tilapia (*Oreochromis niloticus*) is omnivores which have a diversified food spectrum including debris, crustaceans, vascular plants, microalgae and mud (Wong et al., 1996). Also, because this species resistance to disease is strong, their respiratory demands are slight so that they can tolerate high ammonia and low dissolved oxygen contents, they can survive at adverse environmental conditions (Zhou et al., 1998).

The human health risk assessment requires identification, collection and integration of information on hazardous chemicals, their exposure to humans and also the relationship between exposure, dose and adverse health effects (Sobhanardakani, 2017a,b). In this regard, some parameters such as average body weight, intake factor, chronic daily intake, exposure time, frequency and duration for a whole lifetime are known as the important factors for quantitative risk and hazard analysis (Nasrabadi & Shirani Bidabadi, 2013).

Since the large amounts of toxic heavy metals can accumulate in muscle tissue of fish, in view of the concern for food safety, this study was carried out for analysis of the contents and non-carcinogenic risk assessment of Cd, Cr, Pb, and Ni in the muscle of imported tilapia fish marketed in city of Hamedan in 2017.

MATERIAL AND METHODS

In the current study, totally, twenty seven muscle samples from nine different brands of imported tilapia fish were purchased from Shilate Khalij Fars Novin Company from the market basket of city of Hamedan in 2017.

For samples preparation and analysis, 0.5 g of each muscle tissue was inserted directly into acid-washed Teflon digestion vessels. Then, 10 ml of ultra-pure nitric acid (Merck, Germany) was added into each vessel. The samples were heated to 100 ºC using the pretreatment heater to the point that almost all NO_2 was emitted. Thereafter, 4 ml aliquot of concentrated HNO_3: HF (1:1 v/v) acid mixture was added before microwave digestion. One reagent blank for each digestion was included as a representative standard reference, homogeneity and efficacy of process in sample replicated. The digested sample was transferred to a marked flask post-cooling after filtered using Whatman 42 filter paper (Yi et al., 2011). Finally, all muscle samples were analyzed for Cd, Cr, Pb and Ni using an inductively coupled plasma-optical emission spectrometer at wave length 226.50 nm for Cd, 267.72 nm for Cr, 220.35 nm for Pb, and 231.60 nm for Ni. ICP-OES model used in the current study was Varian 710-ES, from Australia. In the current study, the accuracy of the method was assessed by means of analyzed elements determination in the certified reference material DORM-4 fish protein for heavy metals (National Research Council, Canada) (Sobhanardakani et al., 2018). The results showed good accuracy, with recovery rates for analyzed elements between 94.7 and 102.2 for tilapia. the Limits of Detection (LOD) and Limits of Quantification (LOQ) were assessed according to the method introduced by Sobhanardakani (2017b) and both are presented in Table 1.

For potential health risk assessment at the first step the human health risks posed by chronic exposure to the toxic heavy metals were assessed through Equations 1 and 2 for adults and children, respectively (Guo et al., 2016):

$$DIMfish-ing = \frac{Cg-fish \times Cfactor \times IRFa}{BW\ reswa} \quad (1)$$

$$DIMfish-ing = \frac{Cg-fish \times Cfactor \times IRFa}{BW\ reswc} \quad (2)$$

where $DIM_{fish\text{-}ing}$ is the average daily intake of metal through fish ingestion (mg), $C_{g\text{-}fish}$, C_{factor}, IRF_a, are the heavy metal levels in analyzed muscle tissue (mg/kg), conversion factor (0.085) and fish consumption rate (5.0×10^{-3} kg per person per day) (Abdi et al., 2015) respectively. Also, BW_{reswa} and BW_{reswc} are the average body weight that equal 70.0 kg for adults and 15.0 kg for children, respectively (Falco et al., 2006).

At the second step, the health risk index (HRI) for the local population through the consumption of muscle of tilapia fish was assessed in accordance with Equation 3 (Guo et al., 2016):

$$HRI = \frac{DIMfish-ing}{RfD} \quad (3)$$

where $DIM_{fish\text{-}ing}$ and RfD are daily intake of metal (mg) and reference dose of metal (mg/kg/day), respectively. The oral reference doses are 0.001, 1.50, 0.0035, and 0.02, for Cd, Cr, Pb, and Ni respectively. An HRI < 1 means the exposed population is assumed to be safe (Xue et al., 2012; Liang et al., 2015; Sobhanardakani, 2017a,b).

The total HRI (THRI) of heavy metals for the muscle of tilapia fish was calculated through Equation 4 (Guo et al., 2016):

$$THRI = HRI(toxicant1) + HRI(toxicant2) + \ldots + HRI(toxicant\ n) \quad (4)$$

The statistical analysis of the obtained results consisted in a first with Shapiro-Wilk test for normality followed by the study of the variance homogeneity using an ANOVA parametric test with a DMS post hoc and Duncan multiple range test. The mean levels of heavy metals were compared with maximum permissible limits using a one-sample t test. Finally, to study a correlation between the metals in the different muscle of tilapia fish, 2-tailed test of Pearson correlation was performed. Also, all statistical analyses were performed using the SPSS statistical package (version 19).

RESULTS AND DISCUSSION

The contents of Cd, Cr, Pb, and Ni in the analyzed fish samples are presented in Table 1. Data in Table 1 indicates that, among the analyzed muscle samples, Cd was detected in amounts ranging from 0.14 mg/kg to 0.37 mg/kg, Cr was detected in amounts ranging from 1.39 mg/kg to 1.80 mg/kg, Pb was detected in amounts ranging from 0.40 mg/kg to 0.71 mg/kg, and also Ni was detected in amounts ranging from 0.31 mg/kg to 0.89 mg/kg.

Based on the results of independent one-sample t-test comparing the heavy metal contents in muscle of tilapia fish with the Maximum Permissible Limits (0.05 mg/kg for Cd, 8.0 mg/kg for Cr, 0.2 mg/kg for Pb and 1.0 mg/kg for Ni) established by FAO/WHO (Hosseini et al., 2013a,b; Hosseini et al., 2015), shows that the mean contents of Cd and Pb determined in all samples were higher than the MPL.

The Pearson's correlation analyses were performed between metal concentrations in muscle samples of tilapia fish to understand the relationships between them. Based on the results, there was found negative significant correlations between Pb and Cr ($r = -0.692$, $P < 0.010$), between Pb and Ni ($r = -0.784$, $P < 0.010$), and also between Cd and Ni ($r = -0.533$, $P < 0.010$). Also, positive significant correlations was found between Cr and Ni ($r = 0.513$, $P < 0.010$).

In addition, by assuming that the per capita consumption of fish in the study area is the only tilapia, all the calculated HRI values of Cd, Cr, Pb and Ni in adults and children were within the safe limits (HRI < 1) in the current study (Table 3). Furthermore, the THRI values, in muscle of tilapia fish which varied from 1.76×10^{-3} to 3.59×10^{-3} for adults and from 8.21×10^{-3} to 1.68×10^{-2} for children, and were also within the safe limit (THRI < 1) in this study.

Table 1. Residual levels of examined elements in muscle of tilapia fish (mg/kg dry weight)

Sample	Metal Concentration			
	Cd	Cr	Pb	Ni
1	0.35 ± 0.05b	1.45 ± 0.03a	0.69 ± 0.09c	0.58 ± 0.08b
2	0.33 ± 0.04b	1.44 ± 0.03a	0.69 ± 0.07c	0.39 ± 0.06a
3	0.35 ± 0.03b	1.43 ± 0.04a	0.68 ± 0.06c	0.32 ± 0.01a
4	0.16 ± 0.05a	1.45 ± 0.05a	0.53 ± 0.01b	0.71 ± 0.11c
5	0.16 ± 0.04a	1.47 ± 0.07a	0.53 ± 0.04b	0.85 ± 0.06d
6	0.15 ± 0.05a	1.41 ± 0.07a	0.53 ± 0.03b	0.75 ± 0.07cd
7	0.28 ± 0.06b	1.74 ± 0.07b	0.43 ± 0.05a	0.87 ± 0.05d
8	0.28 ± 0.01b	1.70 ± 0.06b	0.43 ± 0.05a	0.77 ± 0.02cd
9	0.28 ± 0.04b	1.77 ± 0.12b	0.45 ± 0.02ab	0.83 ± 0.06cd
LOD	0.039	0.075	0.64	0.047
LOQ	0.110	0.230	0.190	0.130
Min.	0.14	1.39	0.40	0.31
Max.	0.37	1.80	0.71	0.89
Mean	0.26	1.54	0.55	0.67
S.D.	0.09	0.15	0.11	0.20

* The letters (a, b, c, and d) represent the significant difference between the mean concentration of metals in tilapia fish samples that computed by One-way ANOVA and Duncan multiple range test ($P = 0.05$)

Table 2. Daily intakes of metals ($DIM_{fish\text{-}ing}$, mg) for individual heavy metal

Sample	Cd	Cr	Pb	Ni
		Adults		
1	2.12×10^{-06}	8.80×10^{-06}	4.19×10^{-06}	3.52×10^{-06}
2	2.00×10^{-06}	8.74×10^{-06}	4.19×10^{-06}	2.37×10^{-06}
3	2.12×10^{-06}	8.68×10^{-06}	4.13×10^{-06}	1.94×10^{-06}
4	9.71×10^{-07}	8.80×10^{-06}	3.22×10^{-06}	4.31×10^{-06}
5	9.71×10^{-07}	8.92×10^{-06}	3.22×10^{-06}	5.16×10^{-06}
6	9.11×10^{-07}	8.56×10^{-06}	3.22×10^{-06}	4.55×10^{-06}
7	1.70×10^{-06}	1.06×10^{-05}	2.61×10^{-06}	5.28×10^{-06}
8	1.70×10^{-06}	1.03×10^{-05}	2.61×10^{-06}	4.67×10^{-06}
9	1.70×10^{-06}	1.07×10^{-05}	2.73×10^{-06}	5.04×10^{-06}
		Children		
1	9.92×10^{-06}	4.11×10^{-05}	1.95×10^{-05}	1.64×10^{-05}
2	9.35×10^{-06}	4.08×10^{-05}	1.95×10^{-05}	1.10×10^{-05}
3	9.92×10^{-06}	4.05×10^{-05}	1.93×10^{-05}	9.07×10^{-06}
4	4.53×10^{-06}	4.11×10^{-05}	1.50×10^{-05}	2.01×10^{-05}
5	4.53×10^{-06}	4.16×10^{-05}	1.50×10^{-05}	2.41×10^{-05}
6	4.25×10^{-06}	3.99×10^{-05}	1.50×10^{-05}	2.12×10^{-05}
7	7.93×10^{-06}	4.93×10^{-05}	1.22×10^{-05}	2.46×10^{-05}
8	7.93×10^{-06}	4.82×10^{-05}	1.22×10^{-05}	2.18×10^{-05}
9	7.93×10^{-06}	5.01×10^{-05}	1.27×10^{-05}	2.35×10^{-05}

Table 3. Health risk index (HRI) for individual heavy metal caused by the tilapia fish

Sample	Cd	Cr	Pb	Ni
		Adults		
1	2.12×10^{-03}	5.87×10^{-06}	1.20×10^{-03}	1.76×10^{-04}
2	2.00×10^{-03}	5.83×10^{-06}	1.20×10^{-03}	1.18×10^{-04}
3	2.12×10^{-03}	5.79×10^{-06}	1.18×10^{-03}	9.71×10^{-05}
4	9.71×10^{-04}	5.87×10^{-06}	9.19×10^{-04}	2.15×10^{-04}
5	9.71×10^{-04}	5.95×10^{-06}	9.19×10^{-04}	2.58×10^{-04}
6	9.11×10^{-04}	5.71×10^{-06}	9.19×10^{-04}	2.28×10^{-04}
7	1.70×10^{-03}	7.04×10^{-06}	7.46×10^{-04}	2.64×10^{-04}
8	1.70×10^{-03}	6.88×10^{-06}	7.46×10^{-04}	2.34×10^{-04}
9	1.70×10^{-03}	7.16×10^{-06}	7.81×10^{-04}	2.52×10^{-04}
		Children		
1	9.92×10^{-03}	2.74×10^{-05}	5.59×10^{-03}	8.22×10^{-04}
2	9.35×10^{-03}	2.72×10^{-06}	5.59×10^{-03}	5.52×10^{-04}
3	9.92×10^{-03}	2.70×10^{-05}	5.50×10^{-03}	4.53×10^{-04}
4	4.53×10^{-03}	2.74×10^{-05}	4.29×10^{-03}	1.00×10^{-03}
5	4.53×10^{-03}	2.78×10^{-05}	4.29×10^{-03}	1.20×10^{-03}
6	4.25×10^{-03}	2.66×10^{-05}	4.29×10^{-03}	1.06×10^{-03}
7	7.93×10^{-03}	3.29×10^{-05}	3.48×10^{-03}	1.23×10^{-03}
8	7.93×10^{-03}	3.21×10^{-05}	3.48×10^{-03}	1.09×10^{-03}
9	7.93×10^{-03}	3.34×10^{-05}	3.64×10^{-03}	1.17×10^{-03}

It has been proved that the many factors including the duration of exposure to contaminants in habitat, feeding habit, contents of contaminants in the water column, contamination of fish during handling and processing, sex, age, weight, and season depend upon heavy metal contents in fish tissues (Sobhanardakani, 2017b).

Cadmium is listed as a human carcinogen in Group 1 by the IARC and due to it has an exceptionally long biological half-life this element can be harmful to human. Chronic effects of oral exposure to Cd are decrease in the significant proteinuria, glomerular filtration rate, and an increased frequency of kidney stone formation (Bernard, 2008; Jarup & Akesson, 2009; Kim et al., 2014; Zhang et al., 2017). Also, low-level postnatal exposure to Cd may cause neurotoxic effects in children (Ciesielski et al., 2012; Llop et al., 2013; Rodriguez-Barranco et al., 2013). Based on the results of the current study, the contents of Cd in the muscle of tilapia fish with an average of 0.26 ± 0.09 mg/kg were higher than the MPL. The main source of Cd contamination can be attributed to using the medicines containing cadmium and also residue of the chemical pesticides in the habitat or farming and food of tilapia. Cadmium concentration in the literature were reported between 0.02 mg/kg to 0.03 mg/kg in the muscle of tilapia fish collected from the selected river in Kuantan, Malaysia (Amirah et al., 2013), 0.02 ± 0.01 mg/kg in the muscle tissue of tilapia fish collected from Selangor, Malaysia (Taweel et al., 2011), 0.016 ± 0.01 mg/kg in the muscle tissue of tilapia fish consumed in Nigeria (Edem et al., 2009), and 0.46 ± 0.11 mg/kg in the muscle tissue of tilapia fish collected from inland waters of Hong Kong (Zhou et al., 1998).

Chromium distributed in human tissues in extremely low and variable levels (Hosseini et al., 2013a). Irritation to the lining of the nose, nose ulcers, running nose, asthma, cough, shortness of breath, or wheezing are the effects of breathing high levels of this element. Also, chronic exposure to Cr can cause skin irritation, damage to the kidneys or liver, circulatory, and nerve disorders, as well as (Kabata-Pendias, 2010). The results showed that the contents of Cr in the muscle of tilapia fish with an average of 1.54 ± 0.15 mg/kg were much lower than the MPL. In this regard, the mean value of Cr in the muscle of tilapia fish that collected from some areas of Selangor, Malaysia was found 5.99 ± 0.19 mg/kg (Taweel et al., 2011). Also, the average contents of Cr in the muscle tissue of tilapia fish collected from inland waters of Hong Kong were 0.43 ± 0.13 mg/kg (Zhou et al., 1998).

Lead as one of the major environmental health risk throughout the world can promote serious damage to human health particularly children. Exposure to Pb causes encephalopathic symptoms and irreversible brain damage. Food is the main source of non-occupationally exposure to this element, so that about 80-90% of daily doses of Pb absorbed into the human body caused by food consumption (Krejpcio et al., 2005; Liu et al., 2010). In the present study the contents of Pb in the muscle tissue of tilapia fish with an average of 0.55 ± 0.11 mg/kg were higher than the MPL. The main source of Pb contamination can be attributed to transfer of lead from contaminated water and sediment caused by discharge of industrial or agricultural effluents into the aquatic environment and also feeding tilapia fish from contaminated food. Lead content in the literature were reported between 0.10 mg/kg to 0.18 mg/kg in the muscle of tilapia fish collected from the Malaysia (Taweel et al., 2011). In the other studies, Edem et al. (2009) reported that the mean value of Pb in muscle of tilapia fish consumed in Nigeria were 0.058 ± 0.006 mg/kg (Edem et al., 2009). Also, the mean contents of Pb in muscle of tilapia fish consumed in Malaysia were reported 0.18 ± 0.05 mg/kg (Amirah et al., 2013). While, the mean levels of Pb in the muscle of tilapia fish collected from Hong Kong were found 1.61 ± 0.28 mg/kg (Zhou et al., 1998).

Nickel plays an important role in the synthesis of red blood cells. Trace amounts of Ni do not damage biological cells, but exposure to a high dose of this element for a longer time may decrease body weight, damage cells, and also damage the liver and heart. Nickel poisoning may cause reduction in cell growth, nervous system damage and cancer (Wong et al., 2000). Based on the results Ni was detected in all examined muscle samples. Also, the results showed that the mean contents of Ni in the muscle of tilapia fish were 0.67 ± 0.20 mg/kg and was lower than the MPL. The result was against the findings of Taweel et al. (2011) who reported that the contents of Ni in the muscle of tilapia fish caught from Selangor, Malaysia were varied from 2.70 mg/kg to 3.20 mg/kg (Taweel et al., 2011). Also, the mean levels of Ni in the muscle of tilapia fish consumed in Hong Kong were found 0.72 ± 0.10 mg/kg (Zhou et al., 1998).

As shown in Table 3, the HRI values of Cd, Cr, Pb and Ni for adults and children were less than 1. Here, the average HRI value in muscle of tilapia fish was 6.86×10^{-4} for adults and 3.20×10^{-3} for children. Therefore, it can be concluded that target population might have no potential significant health risk through only consuming imported tilapia fish under the current consumption rate. However, the non-carcinogenic risks were greater for children than for adults. In this regard Amirah et al. (2013) after analyzed the heavy metals in the muscle of tilapia fish caught from selected river in Kuantan, Malaysia, reported that Target Hazard Quotient (THQ) values of Cd, Cu and Pb were less than 1 and represents that a daily exposure at this level of heavy metals through consumption of tilapia fish is unlikely to cause any adverse effects during a person lifetime.

It should be noted that heavy metals may accumulate over lifetimes of individuals and their cumulative effects, may be additive and/or interactive (Sobhanardakani, 2017b).

CONCLUSION

The element levels increase according to the following descending order for all tilapia samples: Cr > Ni > Pb > Cd. Based on the results, the mean contents of Cd and Pb were higher than the MPL. Also, despite there are no potential health risk for adults and children through consumption of muscle of imported tilapia fish marketed in city of Hamedan at the current consumption rate, but due to human health effects of heavy metals and also lack of adequate information about processing conditions, adjacent of aquaculture farms to industrial and agricultural areas, regular monitoring of chemical pollutants content specially toxic heavy metals in foodstuffs are recommended to maintain human health.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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