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Original Article

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Levels of Mercury in Persian Gulf Frozen Fish Species

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Abstract

Severe discharge of sewage and industrial effluents into the Persian Gulf leads to the deposition of various types of heavy metals, especially lead and mercury, in the muscles of fish. Total mercury and methylmercury contents were determined in the edible parts (muscle tissue, fillet) of two different most popular frozen fish species from the Persian Gulf to ascertain whether the concentrations exceeded the maximum level fixed by the European Commission or not. During the period from October 2015 to June 2016, a total of 150 frozen fish packaged samples were randomly collected from the recognized supermarkets in Tehran province, Iran. The mercury (Hg) concentration of samples was determined by atomic absorption spectrophotometer using a mercuric hydride system (MHS 10) and also by direct mercury analyzer (DMA). High concentration of total Hg was found in a Carcharhinus dussumie brand ($0.91 \pm 0.12\ \mu g/g$) while the lowest level was detected in Pomadasys furcatus ($0.29 \pm 0.02\ \mu g/g$). Mean Hg levels in fish samples were $0.79 \pm 0.11\ \mu g/g$ which means the frozen fish studied in this study, Persian Gulf had mean mercury concentrations above $0.5\ \mu g/g$, which is the maximum standard level recommended by Joint FAO/WHO/Expert Committee on Food Additives (JECFA). In 13% of Pomadasys furcatus and in 47.2 % of Carcharhinus dussumie fish samples total mercury concentrations exceeded the maximum level fixed by the European Commission. All samples had also mean Hg concentrations that exceeded EPA's established safety level of $0.3\ \mu g/g$.

Keywords: Frozen Fish, Mercury Contamination, Risk Assessment, Carcharhinus dussumie.

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INTRODUCTION

One of the most important issues in the 21st century is that the industrial wastewater has been discharged into the rivers and seas. The sources of heavy metals are mostly chemical waste waters. As heavy metals such as mercury, Arsenic, Lead, and Cadmium can be accumulated in different organs of fish and other marine animals, ultimately affecting the human food chain (Has-Schon et al., 2008; Yildirim et al.,2009; Saei-Dehkordi et al., 2011). Severe discharge of sewage and industrial effluents into the Persian Gulf causes the deposition of various types of heavy metals and especially lead and mercury in the muscles of marine animals (Rahimi et al.,2015). Fish is a food low in fat and high in protein, which administers a variety of health benefits. Fish are lower in fat than any other sources of animal protein, and oily fish are high in omega-3 fatty acids. As our body does not make the representative supply of these nutrients, they consider as an essential source (Al-Hossainy et al.,2012). Unfortunately today most fish have lost their nutritional values caused by environmental pollution. The encounter with fish pollution and the probable health implications of eating contaminated fish are a considerable concern for the responsible governmental organizations and the general public. Fish consumes a lot of toxic wastes which are polluted materials discharged into their surrounding environment. Industrial wastes, sewage, pesticides and heavy metals such as mercury and cadmium are the usual and most common toxic wastes. Lead absorption may constitute a serious risk to public health. Lead in children may lead to reduced cognitive development and intellectual performance and in adults elevated blood pressure and cardiovascular diseases. Mercury pollution in most parts of the districts around the world could be caused by the release of metallic mercury, treated in the resumption of gold by the crude amalgamation method, into the environment. At present, in countries such as Tanzania, Indonesia, Brazil, Ghana, Philippines, Vietnam, and China, approximately over 10 million people are estimated to be elaborated in these activities(WHO. 2002; Francisco. 1996; Akagi et al.,1996; Myers et al., 2000). The toxicity of methylmercury has been researched by all scientists around the world since the first outbreak reported in Minamata, Japan, in 1956 (Myers et al.,2000). Plenty of review articles and reports were published conferring these issues. Myers et al. (2000), in the earliest publications, as has been cited by other publications by the other researchers from the University of Rochester investigating on assessment of exposure and the ramification of exposure to various forms of mercury on experimental animals (Myers et al., 2000). Later, Swedish investigators discovered the methylation process of inorganic mercury by organisms in the aquatic sediments and in fish that probably intensify methylmercury directly through the water or indirectly through constituents of the food chain (Myers et al.,2000). Recent scientific publications have centralized on the contents of methylmercury contamination in seafood, and mostly in fish (Denton et al., 2006; Guerin et al., 2011; Ikem et al., 2005; Mendil et al., 2010; Turkmen et al., 2005: Yilmaz et al., 2010), human exposure, and its pertinent health stuffs (Burger et al., 2009; Castro-Gonžalez et al., 2008; Morgano et al., 2010; Myers et al., 2000; Ahmad et al., 2015). Mercury itself and its compounds are extremely toxic. Acute health effects have been reported on kidney failure after exposure to high concentrations of inorganic mercury. Contact with mercury can lead to allergic skin reactions which also has been reported. Mercury vapor causes erosive bronchitis and bronchiolitis with interstitial pneumonitis. These symptoms were probably associated with its effect on the CNS, such as tremor or increased excitability. Acute Mercury-exposure could exhibit chest pain, dyspnea, cough, hemoptysis and interstitial pneumonitis in workers (Wheatley et al.,1995; Ziarati. 2012; Miranda

et al., 1997)
Since the symptoms of Mercury toxicity are comprehensive and public awareness is less than necessary (Ser et al., 2012), most patients experience symptoms resulting from high mercury exposure. These people may be tested and/or treated for more prevalent diseases with similar presentations. Also, there are currently only case reports correlating high Hg seafood consumption with health outcomes such as fatigue. The goal of the current study was to determine total mercury and methylmercury contents in the edible parts (muscle tissue and fillet) of two different most popular frozen fish species from the Persian Gulf to identify whether the concentrations exceeded the maximum level fixed by the WHO and European Commission or not.

Experimental Section

Sample Collection

From October 2015 to June 2016, a total of 140 frozen fish packaged samples from the recognized supermarkets were randomly collected in Tehran province, Iran. All samples were frozen, caught recently from the Persian Gulf (not more than 1 month of packaged). All samples were maintained in cold box and transferred to the laboratory at 4°C. Total length (cm) and body weight (g) of the samples were measured and recorded before dissection. Average length and weight of collected samples were 26.78 ±2.68 cm and 583.33±7.11 g for fish. The muscle samples were maintained at –20°C in a freezer prior to analysis.
Frozen fish samples were thawed, washed with distilled water, and then they were allowed to attain room temperature in desiccators before dissection. The skin and muscle were removed following Tru-cut method by Baker et al(2012), thin layer skin was removed from the dorsal region on the first side of the fish. The notched needle was Sterilized and the outer barrel was inserted into the fish muscle tissue at an oblique angle (to minimize penetration depth). The notched needle was then extended into the flesh and the containment cover was slide over to cut the tissue and placed in a specially labeled plastic dish. However, at the other side, the skin was cut off firmly making sure that no part of the muscle was attached and placed in its labeled drying dish.

Devices, Reagents, and Materials

All glassware was soaked overnight in 10% (v/v) nitric acid (Merck, Germany), followed by washing with 10% (v/v) Perchloric acid (Merck, Germany) and rinsed with double distilled water and dried before using. All reagents used were of analytical reagent grade (Merck, Germany). Standard stock solutions of mercury, arsenic, cadmium, and lead were prepared from Titrasol (1000 mg/l) (Merck, Germany) and were diluted to the corresponding metal solution. The working solution were freshly prepared by diluting an appropriate aliquot of the stock solutions using 10% HNO3 (Merck, Germany) for diluting lead and cadmium solutions, 1 M HClO4 (Merck, Germany) and 5% H2SO4 (Merck, Germany) for diluting mercury solution, 7 M HClO4 for diluting arsenic solution and 5% HCl for diluting tin solution. Stannous chloride, for mercury analysis, was freshly prepared by dissolving 10 g in 100 ml of 6 M HClO4. The solution was boiled for about 5 min, cooled, and nitrogen bubbled through it to expel any mercury impurities. Mercury analyses were determined by direct mercury analyzer (DMA).

Sample Preparation and Digestion

Each sample was homogenized thoroughly in a food blender with stainless steel cutters. A sample was then taken and digested promptly as follows: 2 g of the homogenized sample was

weighed and added to a 0.5 l glass digestion tube, and for mercury, 10 ml of HNO3 (Merck, Germany) and 5 ml of H2SO4 (Merck, Germany) were slowly added. The tube was then placed on top of a steam bath unit to complete dissolution. It was then removed from the steam bath, cooled and the solution transferred carefully into a 50 ml volumetric flask; for the reduction of mercury, 5 ml SnCl2 (Merck, Germany) were used. For arsenic determination, 2 g of homogenized sample was weighed after pre-digestion. Then, HNO3 mixed with 4 ml of MgNO3 20% (Merck, Germany) were used as ashing aid. The samples were dried on a hot plate and ashed in a 450°C furnace. The ashes were dissolved in 7 ml of HClO4 and diluted to 50 ml. For the determination of lead and cadmium, about 2 g of homogenized sample were weighed into a 200 ml beaker and 10 ml of HNO3 were added. The beaker was covered with a watch glass and, after most of the sample had been dissolved standing overnight, they were heated on a hot plate boiling until any vigorous reaction had subsided. The solution was allowed to cool to room temperature, transferred to a 50 ml volumetric flask and diluted to the mark with distilled water. For each run, triplicate samples were carried through the digestion reaction. The results are shown in Table 1.

Risk Assessment

Risk assessment in the current study was checked out by considering only the edible part (muscles tissues) to determine daily intake of metal (DIM) and health risk index (HRI). The daily intake of metals (DIM) was calculated to estimate the daily loading of metals into the body system (via the consumption of fish meal specified in this study) of a specified body weight of a consumer. This would entail the relative bioavailability of the studied metals in this study. The daily intake of metals (DIM) was determined by the following (Abdullahi. 2015):

$$DIM = C_{metal} \times D_{fish} \times C_{factor} / B_0$$

Where Cmetal is the concentration of heavy metals in the fish (mg•kg−1), Dfish is the daily nutritional intake of fish (mg•day−1), and Cfactor is the factor for conversion of fresh fish to dry constant weight.

The health risk index (HRI) for the populations through the consumption of contaminated fish was assessed based on the daily intake of metals (DIM) relative to reference oral dose (Rf D) for each metal. This is an index justifying individual's risk of heavy metals. The HRI value of less than one (1) implies safe tread and is considered acceptable; otherwise, the fish may pose heavy metals risk (Abdullahi. 2015).The following formula was used for the calculation of HRI:

$$HRI = DIM / R_f D$$

Statistical Analysis

The results of the mercury, cadmium and lead concentration in two spices fish studied: Carcharhinus dussumie and Pomadasys furcatus were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA), Analysis of Variance (ANOVA) test were used for analysis of the variances. Differences were considered significant at values of $P < 0.05$.

Result & Discussion

The current study indicated a significant association between the spices of fish and mercury concentration deposited in muscles. Results of mercury levels contents in fish samples as compared with the WHO criteria showed that mean levels in Carcharhinus dussumie were heightened by the size and length of fish samples with the recording of the highest level of 0.91 ± 0.12 μg/g for total mercury and 0.74 ± 0.0 5 μg/g for methyl mercury. The mean proportion of Me-Hg to T-Hg in all Carcharhinus dussumie fish samples were in the spectrum

of 53.21 -99.76 % indicating that most of the mercury in the fish sampled were in the form of methylmercury. The Carcharhinus dussumie had registered the highest percentage of methyl mercury content, while the lowest level was detected in Pomadasys furcatus (0.29 ± 0.02 μg/g). Mean Hg levels in fish samples were 0.79 ± 0.11 μg/g which means studied frozen fish in current study from the Persian Gulf had mean mercury concentrations above 0.5 μg/g, which is the maximum standard level recommended by Joint FAO/WHO/Expert Committee on Food Additives (JECFA. 2006). In 13% of Pomadasys furcatus and in 47.2 % of Carcharhinus dussumie fish samples total mercury concentrations exceeded the maximum level fixed by the European Commission. All samples had also mean Hg concentrations that exceeded EPA's established safety level of 0.3 μg/g. Laboratory results were shown in table 1.

Table 1: Frozen fish packaged samples analyzed for Total and Me-Hg

Fish sample	Mean THg (μg/g)	Mean Me-Hg (μg/g)	Mean % Me- Hg
Carcharhinus dussumie	0.79 ± 0.11	78.82 ±0.08	99.76
Pomadasys furcatus	0.29 ± 0.02	15.43 ± 0.04	53.21

Based on the analysis obtained, there was a significant risk of human exposure to Mercury regarding consumption of studied species obtained which was shown in Table 2. Also, the risks from consumption of any fish tissues were high. since the levels of Methyl-Mercury analyzed in the entire tissues were above their corresponding permissible limits recommended by FAO/WHO (2003), CCFAC (2011) , and WHO(2005)

Table 2: Risk Assessment based on Fish consumption frequency

Times/Month fish Consumption	Carcharhinus dussumie	Pomadasys furcatus
None/hardly ever	2%	0.42%
1–2 times/month	2.9%	0.89 %
1–2 times/week	4%)	1.6%
3 times/week	8.6%	2.3 %
4 times/week	14.3%	4.5%

**ANOVA of the Mahaffey reported times of fish consumption in a week and Risk assessment, p-value < 0.0001.*
***ANOVA between our specialty of the kind of consumed fish, p-value = 0.003.*

Total mercury median concentrations varied significantly ($p<0.05$) among different fish species tested. Mercury can be accumulated into fish tissue through the food chain whereby it gets transferred between aquatic plants and aquatic animals, from sediment, as well as from the water environments (Bidone et al. 1997) Methyl-mercury is a major fraction of total mercury concentrations accumulated into studied fish tissue where it ranges from 53.21 to 99.76 % . Highest percentage of mercury as methyl-mercury in Carcharhinus dussumie

specie was found in muscle tissue and accounting to nearly 100 % of mercury present as methyl-mercury.

The indirect bioaccumulation process is a phenomena in which a mercury substance accumulates into fish tissue based on its tropic level in a food chain (Bidone et al. 1997). Tropic level expresses the position of a species in the marine food web, and its estimation requires knowledge of what each species feeds on and in what quantities (Stergiou et al. 2005). Briefly, mercury enters the food chain via phytoplankton and then accumulated as methylmercury by other links in the chain, via tropic transfer (Seixas et al. 2013). These results were similar and comparable to Agah et al (2005). According to their study, for four fish species of Persian Gulf (Pomadasys sp., Haemulidae; Platycephalus sp., Platycephalidae; Epinephelus tauvina and Pampus argenteus) collected in January 2004, the median arsenic concentrations were within the range between 0.2 and 2 lg/g (mean: 0.6 ± 0.4 lg/g of wet weight). Also, Khansari et al. (2005) reported that the canned tuna fish marketed in Iran, had arsenic levels ranging from 0.0369 to 0.269 lg/g (mean ± SD: 1.28 ± 0.081 lg/g). Over the past decade, the levels in food have decreased significantly owing to the awareness that it could lead to health problems and related efforts were done to reduce the emission of lead. The EC concluded in its opinion, that whereas the mean level of Mercury in foodstuffs does not seem to be a cause for alarm, long-term action should be taken with the objective of further lowering the mean levels of lead in food materials.

For public risk assessment, the PTWIs of mercury were compared with their intakes through fish consumption. The estimated daily/weekly intakes (EDI/EWI) for an adult with a 60 kg body weight are demonstrated in Table 2. The daily and weekly dietary exposure estimation by an adult person was carried out on basis of an average 21 and 147 g of fresh fish muscle consumption per day and week in Iran, respectively according to Annual Fishery Statistics of Iran, (2010). The joint FAO/WHO Expert Committee recommended a PTWI concentration under 5 lg/kg body weights for mercury (Torres-Escribano et al. 2010) Therefore the EDI and EWI values revealed that the dietary exposure to mercury could be considered safe. The values were lower than PTDI and PTWI, recommended safety standard limits. Although the EDI and EWI of mercury were lower than the legislated limits, the role of the low capitation consumption of fish per year in Iran should not be overlooked. Its impact on the community, especially the high-risk groups, pregnant women, and children should be highly considered.

Conclusion

The current mercury pollution has been added to the burden of people living in the contaminated sites and even the other cities such as Tehran. The metallic mercury has been used extensively and discharged directly into the environment. So, it is responsible for the methylmercury formation in the natural environment. Ultimately, it has accelerated the bioaccumulation of mercury in human body via the food chain. Thus, there is a need for long-term monitoring to understand the sources, kinetics, environmental behavior, and toxicity of Mercury (both organic and inorganic forms) on the community especially the high-risk groups such as sick people seeking safe food, pregnant women, and children. There is a need to establish a laboratory to undertake a comprehensive inorganic and methylmercury determination in the south area of the country, especially in south provinces, to provide the necessary guidelines for the community, especially high risk groups. Responsible organizations should follow remediation . Mitigation measures in the environment should be undertaken to ensure that the exposure limits to mercury will be kept at a minimum or within permissible limits. We recommend conduct-

ing monitoring on fish especially those with high level of mercury.

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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