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ORIGINAL ARTICLE

Permissible Consumption Limits of Mercury, Cadmium and Lead Existed in *Otolithes Rubber*

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ABSTRACT

The study ahead aims at determination of permissible consumption limit without harmful impacts caused by mercury, cadmium and lead in *Otolithes ruber* fishing from Mahshahr Port situated in northern part of the Persian Gulf as one of highest consumption center of the region fish. Also, it focuses on comparison of the obtained results with available health standards to examine the mentioned heavy metals impact on citizens' health. For this purpose, 36 samples of *Otolithes ruber* were randomly caught from Musa Cove located in Mahshahr in the form of tree group sizes (small, medium and big). After isolating of the muscle tissue, the concentration of mercury existed in the tissue was measured using advanced mercury analyzer (Model, Leco 254 AMA). Beside, the cadmium and lead concentration accumulated in the muscle tissue were determined by means of Graphite furnace atomic absorption spectrometry (GFAAS) (model: 220z, Varian, Australia). Total mercury mean (standard error±mean), cadmium and lead were respectively measured equal to 36.4 ± 456.53 (μgkg^{-1}w.w), 4.47±28.99 (μgkg^{-1}w.w), 18.8±126.95 (μgkg^{-1}d.w) in summer and 29.1±305.69 (μgkg^{-1}w.w), 19.12 ±2.8 and 94.82±15.4 (μgkg^{-1}d.w). Regarding to the EPA and WHO permissible consumption limit for *Otolithes ruber* as a carnivore species, due to high accumulation of mercury in its muscle tissue, there is a sever consumption limit particularly for big longitudinal group in summer. Considering permissible limit of mercury determined by World Health Organization (WHO), for an adult person weighted 60 kg, the maximum permissible consumption limit of *Otolithes ruber* in big longitudinal group in summer and winter are tantamount to 52 and 73g severally. And in accordance with the limit set by the EPA maximum permissible consumption limit for the same person in both summer and winter, are 31 and 44 g per week.

Key words: *Otolithes ruber*, mercury, cadmium, lead, permissible limit

Introduction

Heavy metals in marine habitats, due to stability and tendency to accumulate in the tissues of marine organisms as well as harmful effects caused by their toxicity and biomagnifications through food chain have been considered [28,34]. Fish is a food source for human beings as well as an important source of protein for lots of people around the world. Considering the importance of fish on health, its consumption has been increased. Whereas, fish containing omega-3 which has unsaturated fatty acids provide low-cholesterol healthy source of protein and other nutrients. Therefore, fish consumption is recommended two or three times a week [6]. In terms of a wide range of pollutants, which continuously discharge in to the marine environment, fish are contaminated as well. On the other hand, regarding bioaccumulation of toxic materials in muscle and other tissues of fish, eating such contaminated fish is seriously threatened human health [36]. Age, height, weight, gender, dietary habits, ecological needs, metals concentration in water and sediment, retention duration of fish in the aquatic environment, fishing season and the physical and physicochemical properties of water (salinity, pH,

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hardness, and temperature) are among factors affected heavy metals accumulation in various organs of fish [7]. Even it seems fat tissue can also be an important factor in the accumulation of various pollutants in different parts of fish body such as bone, brain, muscle, gill, gonad and liver [13]. Therefore, accumulation of metals in fish can be considered as a metal pollution indicator in aquatics bodies [22,39], which would be a useful tool for studying biological part of the current metals in high concentrations in fish [11]. The amount of toxic elements in fish depends on concentration of these elements in their food, fish habitat and the disposal percentage of these toxic metals from their body [43,44]. Heavy metals such as mercury, lead and cadmium, even at very low concentrations in marine organisms will be very dangerous for humans if they are used for a long-term. Now days, researches on the interaction between marine organisms and heavy metals have been intensified due to the ever increasing rate of such metals caused by human activity and their release into aquatic environment. Cadmium and lead including among unnecessary heavy metals are entered into the natural environment through industrial processes and mining byproducts [38]. Among the acutest form of cadmium poisoned lot of human beings incidence of Itai-itai disease can be named [27]. Also lead entering the body may leads nutrient losses in humans, especially children, which such lesions cause the failure of the nervous system. Mercury is a stable, unnecessary heavy metal with high toxicity and without biological variability remained persistent during the transition from different trophic levels in food chain through bioaccumulation processes [47]Nowadays, mercury and cadmium poisoning incident through fish and shellfish in Minamata (Japan), caused intensified researched in case of heavy metals accumulation in aquatic ecosystems [4,24,25,42]. Mercury absorption through nutrients is of the most major entrance and accumulation ways of this substance in fish body. Hunter fish considered as the last ring in the aquatic food chain have the highest amount of metals in their different organs [22]. Gulf and coastal areas as regeneration and nursery ground for a lot of non - invertebrates and fish species should be protected. Benthic and semi-benthic species are mainly exposed to the pollutants existed in sediments [16]. The Persian Gulf which is an important source for the extraction of food resources has been invaded by various pollutants during recent years. It is clear that because of low depth, water rotation, salinity, high temperature formed characteristics of the northern part of the Persian Gulf, pollutants effects on the aquatic environment is very significant. Musa Cove is a deep natural canal branching from the Persian Gulf in which with its sub-branches seems like a claw went into the land. Considering Musa Cove is one of the important biological areas in the Persian Gulf ecology in which spawning of many aquatics related the area is occurred, its maintaining is so important for the aquatic environment conservation. Unfortunately, at present, due to discharging high volume of various types of petrochemical industries wastewater as well as wastewater resulting from washing of ships and tankers, the estuary environment is severely compromised. Currently, three petrochemical manufacturers, Bandar-e Emam, Razi and Farabi discharge their effluent into the sea and the estuary [26].

Accordingly, at present study, measuring of metals concentration (mercury, cadmium and lead) in *Otolithes ruber* muscle tissue were discussed on the basis of the following reasons:

1- To study the effects of Petrochemical Complex of Mahshahr Port regarding heavy metal contamination on aquatics of Musa Cove.
2- Considering the important role of Otolithes ruber fisheries economy
3- Lack of sufficient studies in Iran regarding confidence of the consumption health of this fish in terms with investigation of various pollutants rate including heavy metals in different tissues of the fish.

Considering the important role of *Otolithes ruber* fisheries economy

Lack of sufficient studies in Iran regarding confidence of the consumption health of this fish in terms with investigation of various pollutants rate including heavy metals in different tissues of the fish.

Beside, the mean concentrations of elements were compared with permissible consumption limits determined by international standards (Table 1) and weekly permissible consumption limit of the considered fish muscle tissue were examined within both summer and winter using three length groups of humans.

Material and methods

2.1. The study area:

The research was conducted at Musa Cove located on the northern part of the Persian Gulf. The area is located between latitudes 21°30'-31°30'N and longitudes 52°48'-49'E. Musa Cove has an outfall with a width of 40 km and a variable depth between 20 to 50 meters. The approximate area of the cove equals to 1350 km^2 during the ebb-tide time (Fig. 1) [7].

2.2. Sampling method:

To do the current research, *Otolithes rubber* was selected as a statistical society. For this purpose fish in the area were sampled within two months January and July of years 2009 and 2010. Accordingly, 36 samples of fish in the form of three different longitudinal sizes including 1-25 cm (small fish), 30-34 cm (medium fish), and 43-47 cm (big fish) were collected as waiting-fishing from Musa Cove in Mahshahr Port. Afterward, fish were putted into a plastic bag and coded. Fish samples after being completely freezed were placed into a clean Ice Box and transferred to the laboratory within the least possible time and stored in temperature of 30 °C until analysis getting started.

2.3. Technique of muscle tissue sampling and device analysis:

Initially, bioassay operation was performed and then their sexuality was determined using sex gonadals. 10 grams of muscle tissue was carefully isolated and placed in to the cleaned laboratory dishes and subsequently, putted into freeze-drying device (IL shin) at temperature of -52°C for 48 hours. After ensuring of samples dryness they were removed and powdered using ceramic mortar. Subsequently, of each dried tissue sample, the amount of50 mg were separated and its total concentration of accumulated mercury were measured using advanced mercury device (Model; Leco 254 AMA) and ASTM, D-6722 standard in terms of ngg $^{-1}$ dry weight [18]. To measure the concentration of lead and cadmium in muscle tissue of the considered fish, 1 gr of powdered sample were poured into a Teflon container, 10ml of nitric acid and Perchloric acid ($HCLO_4$+ HNO_3) was added to it for digestion. The mentioned mixture was placed into a heater at a temperature of 40°C for one hour, and then it was stored for 3 hours at 140 °C. After being isothermal with the environment, the sample was passed through a 150 mm filter paper, poured into a 25 ml Jujeh Balloon and brought to volume using D.I. (deionized) water. Ultimately, samples were transferred to the lidded polyethylene containers to injection in to the device. Afterward, they were measured with Graphite furnace atomic absorption spectrometry (GFAAS) (model: 220z, Varian, Australia) in terms of µg 1^{-1}. To control experimental process and verification of device IAEA-407 reference standard was used. Meanwhile, to achieve a higher efficiency and ensure of data accuracy, each sample was analyzed three times, for every 18 samples, one control sample was analyzed [30].

2.4. Statistical analysis:

In this study, the SPSS (ver.17) Software was applied for statistical analysis and Microsoft Office Excell 2007 was used to draw diagrams. Shapiro-wilk test and normal distribution were used to determine nationality of data. To compare the amount of elements concentration with global standards within two seasons (summer and winter) One Sample T-Test was applied which results are given in Table 2 separately.

Result and discussion

A major amount of heavy metals have accumulated in various tissues including muscles and contained a significant proportion in human diet [23]. The total mean of mercury, cadmium and lead (standard error± mean) accumulated in muscle tissue of three longitudinal groups of *Otolithes ruber* within summer and winter is shown in Table 2.

Lots of emphasis have been expressed regarding adjustment of heavy metals receiving especially mercury, cadmium and lead in connection with food consumption. So that, the maximum amount of these metals which a person can be received through nutrition without suffering from complications caused by its accumulation, is calculated using the following equation:

$$a = \frac{b \times c}{w}$$

a=permissible consumption limit per weak based on WHO and EPA (µg/kg)
b=mercury concentration in tissue (µg/kg)
c= maximum permissible consumption limit per weak (gr)
w= the weight of the body (kg)

Since, people's weight in calculation of permissible consumption limit is accounted as an important factor, in this research people were divided into four weighing groups as follows:
1 - Infant people weighing 15 kg
2 - Young people weighing 30 kg
3 - Adults weighing 60 kg
4 - Obese individuals weighing 75 kg

Considering the weight of people and concentration of mercury, cadmium and lead in tissues, the maximum permissible consumption limit for each age group was calculated according to WHO and EPA permissible limits.

3.1. Comparison of mercury concentrations in Otolithes ruber with international standards and determination of permissible consumption limit:

Based on comparison of mean concentration of mercury with WHO and EPA standards all three longitudinal groups were beyond the permissible limit determined by EPA while all the mentioned groups were lower than permissible limits considered by WHO but the big one. Beside, in winter, all longitudinal groups contain mean concentration of mercury below the permissible limit of EPA excluding the big longitudinal group meanwhile its concentration at all three longitudinal groups were lower than WHO standard permissible limit (Figure 2).

Fig. 1: the under investigation area, Mahshahr Port- the Persian Gulf

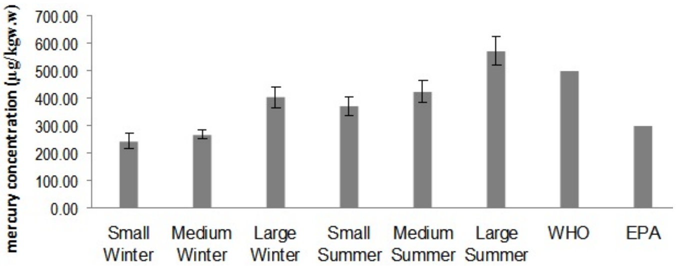

Fig. 1: Comparison of mercury concentrations in *Otolithes rubber* with international standards in summer and winter of 2009-2010

Table 1: heavy metal standards determined by WHO and EPA

Standards	Resource	Cadmium	Lead	Mercury
WHO	19	100	500	500
EPA	46	———	———	300

*cadmium and lead metal concentrations have been reported in terms of micrograms per kg of dry weight while mercury has been measured based on micrograms per kg of wet weight

Table 2: Mean concentration of elements mercury (µg / kg ww), cadmium and lead (µg / kg dw) in muscle tissue of *Otolithes ruber* caught from Mahshahr Port in three longitudinal group within summer and winter of 2009-2010.

Season Heavy metal Group	Winter Hg	 Pb	 Cd	Summer Hg	 Pb	 Cd
Small	244.13±27.2	147.50±21.9	27.60±4.5	373.23±33.5	194.80±7.1	44.90±2.3
Medium	267.02±17	84.80±5.8	18.95±1.6	425.94±40.5	116.91±11.7	25.69±4.5
Large	405.90±38.7	52.89±5.2	10.81±2.8	570.42±52.2	69.13±7.5	16.37±1.4
Total means	305.89±29.1	94.82±15.4	19.12±2.8	456.53±36.4	126.95±18.8	28.99±4.47

According to the results obtained from the study and the permissible limit determined by EPA (300 ngg^{-1}w.w), the mercury concentration in big longitudinal group were beyond the permissible limit within two seasons. Accordingly, there is a sever consumption limit for big longitudinal group within two mentioned seasons rather than the medium and small ones. So that, maximum permissible weekly consumption of *Otolithes rubber* big group muscle tissue for a fifteen kilogram person within two seasons of summer and winter are respectively equal to 7 and 11 gram per week. While, this person can consume 12 and 18 grams per week of *Otolithes rubber* small longitudinal group in summer and winter respectively. Whereas, based on the results of this study mercury concentration was only beyond WHO standard (500 ngg^{-1}w.w) in big longitudinal group in the summer.

Accordingly, there is also a sever consumption limit in big longitudinal group rather than small and medium ones within the two seasons, so that maximum permissible weekly consumption rate of *Otolithes rubber* muscle tissue from big longitudinal group for a fifteen kilogram person trough summer and winter are 13 and 18 kg per week respectively. For other age groups such a comparison holds true. The above results are shown in Table 3.

Comparison of cadmium and lead concentrations in *Otolithes rubber* with international standards and determination of permissible consumption revealed that mean concentration of cadmium and lead elements for each three longitudinal group within the summer and winter is lower than the permissible limit determined by WHO (Figure 2 and 3).

Table 3: The maximum permissible consumption of Otolithes rubber muscle tissue without effects caused by mercury (g)

weight group (Kg)	Hg (WHO) Summer			Hg (WHO) Winter		
	Small	Medium	Large	Small	Medium	Large
15	20	17	13	30	28	18
30	40	35	26	61	56	36
60	80	70	52	122	112	73
75	100	88	65	153	140	92
weight group (g)	Hg (EPA) Summer			Hg (EPA) Winter		
	Small	Medium	Large	Small	Medium	Large
15	12	10	7	18	16	11
30	24	21	15	36	33	22
60	48	42	31	73	67	44
75	60	52	39	92	84	55

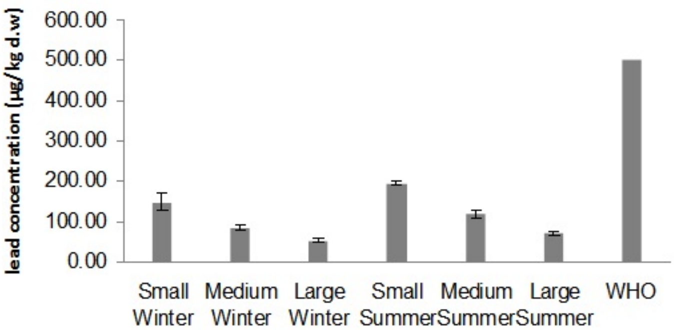

Fig. 2: Comparison of lead concentrations in *Otolithes rubber* with global standards within the summer and winter of 2009-2010

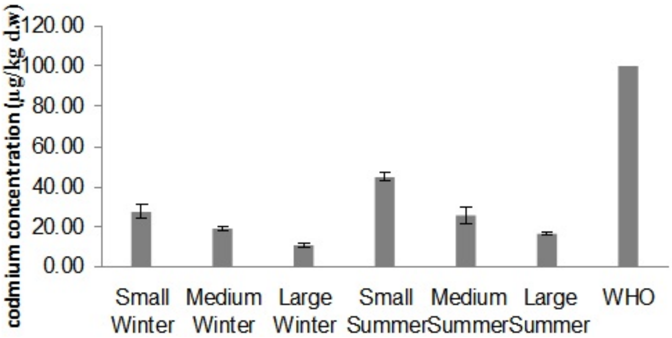

Fig. 3: Comparison of cadmium concentrations in *Otolithes rubber* with global standards within the summer and winter of 2009-2010

Table 4: The maximum permissible consumption of *Otolithes rubber* muscle tissue without effects caused by cadmium and lead (g)

weight group (Kg)	Pb (WHO) Summer			Pb (WHO) Winter		
	Small	Medium	Large	Small	Medium	Large
15	15	25	43	20	35	56
30	30	51	86	40	71	113
60	61	102	173	81	142	226
75	77	128	216	101	178	283

Table 4: Continue

weight group (Kg)	Cd (WHO) Summer			Cd (WHO) Winter		
	Small	Medium	Large	Small	Medium	Large
15	33	58	91	54	79	138
30	66	116	183	108	158	277.
60	133	233	366	217	316	555
75	167	291	458	271	395	693

Based on the obtained results, the highest accumulation of cadmium and lead in muscle tissue of *Otolithes rubber* were observed in small longitudinal group within tow mentioned seasons. Moreover, based on calculations, among all weight groups, weekly consumption limit in small longitudinal group is higher than the small and medium ones. The mentioned results are given in Table 4.

Conclusion:

Having information on concentration of heavy metals in fish is important from both natural management and human health aspects. Heavy metal poisoning is generally occurred on different tissues including brain and liver, but may also cause other complications [7]. Various investigations have shown that metal accumulation in a tissue is mainly dependent on metals concentration in water and duration that they are exposed. Some other environmental factors like salinity, pH, hardness and temperature also play an important role in accumulation of metals. Ecological needs, gender and size in aquatics have been identified as an effective factor on accumulation of metals in their tissues [23]. Existence of *Otolithes ruber* in food basket of people, increased spread of pollutants due to high tides as well as establishing a set of petrochemical facilities, industries and oil export jetties in the region are among the main reasons to do the research ahead. U.S. Environmental Protection Agency (US.EPA) knows consumption of fish contaminated with mercury as the most important poisoning factor. Poisoning with mercury in its organic or mineral form leads nerve damages and genetic defects. Damages caused by alkyl mercury compounds on the nervous system are irreversible. Minimata Disease which is a kind of epidemic disease was happened due to consumption of fish contaminated with mercury in Japan in 1959. The obtained results of the current study suggested that, mean concentration of mercury within two seasons, summer and winter in most longitudinal groups was beyond the standards determined by EPA. Beside, according to World Health Organization (WHO) mercury accumulation in big longitudinal group in summer was higher than it in small and medium size longitudinal groups. The reason of increasing accumulation of mercury concentration by increasing the fish size can be known due to very close biocorrelashion of mercury with methyl mercury organic compound having a high biological half-life in the living body. This case has been proven in other researches. Nowadays it is well recognized that mercury accumulates in the body of the marine organisms. Thus, it is getting increased by increasing the age and therefore the size of organism [10,12,35,37]. Cronion et al. through a research showed that the concentration of the mercury is increased in species by increasing the age and body length [9]. High concentration of mercury in muscle tissue of *Otolithes ruber* can be justified by its carnivorous diet [5]. This species feeding from other aquatics contaminated with mercury metal in Mahshahr Region can be a justification for high levels of heavy elements in their body. Heavy elements can accumulate with very high concentrations in the body of molluscs, so fish feeding of these aquatics usually contains high contamination level of heavy metals [15]. Concentration of cadmium and lead compared with standards determined by WHO revealed that the elements mean concentration through all longitudinal groups were lower than the WHO permissible limit. Based on the obtained results, accumulation of heavy metals cadmium and lead in two seasons in *Otolithes ruber* muscle tissue belonged to the small longitudinal group was higher than the others. On this basis, increasing in body length of the fish is accompanied by a reduction in concentrations of cadmium and lead. According to conducted studies, with growth of the fish, its physiological adaptation with the environment is simultaneously developed. This evolution is so essential in removal or neutralization of heavy metals in muscles [32]. According to researchers, in fish liver and muscle, Metallothionein Protein is responsible for removal or neutralization of heavy metals and their toxic effects. Thus, following increscent of weight and length of the fish and consequently compatibility with the environment, the concentration of metals in the muscles is reduced and added to their rates in paunch and viscera [14]. In 2003, Alti and Canli expressed that metabolic activities have an important role in the accumulation of heavy metals in marine organisms. As regards, metabolic activities of young fish are higher than the elders so, heavy metal accumulation in young fish is more than the others [7]. Accumulation of heavy metals, mercury, cadmium and lead in summer was higher than in winter. According to research by Pourang et al in 2004 this seasonal variation may be resulted from internal biological cycles of organisms or changes in availability of metals to organisms in the environment [31]. Some researchers [17,40,11,3] through their investigations expressed that the amount of elements in marine organisms is influenced by seasonal variations. Seasonal variations in the absorption of metals can be attributed to changes in fish growth rate and fat stimulation (dynamic) [13]. Also, Adeyemo *et al.,* [1,48,29,2] were reported similar results. In this study, despite the lack of limitation to consuming *Otolithes ruber* contaminated with elements cadmium and lead, the limiting factor for consumption is high concentration of mercury. Regarding accumulation of mercury beyond permissible limits determined by EPA and WHO, there is a weekly sever consumption limit for *Otolithes ruber* belonged to the big longitudinal group fishing from Mahshahr Port in summer. These results can be a serious warning for consuming this kind of fish in the region.

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