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Original Article

Study on Trace Metals Levels and Health Risk Assessment of *Silago Sihama* in Hormozgan Province

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ABSTRACT

Background and Objectives: Despite the increasing impact of heavy metal pollution in Persian Gulf due to urban growth and agricultural and petroleum activities, few studies have focused on the behavior and relationships of these pollutants in the biotic and abiotic components of aquatic environments. In this study, the amount of some metals (copper, iron, lead, cadmium, zinc and nickel) was determined in the tissues of fish from three locations (Qeshm, Khamir port and Laft port) of Persian Gulf.

Materials and Methods: Fish samples were caught in Aug 2015. After bioassay, the samples of short fish (*Silago Sihama*) were prepared through acid digestion method, and the amount of metals was measured using atomic absorption device (Scientific Equipment GBS).

Results: There were significant variations among heavy metal accumulation levels ($P<0.05$). The heavy metal concentrations found in the tissues varied (Cu: 0.402–0.642, Zn: 2.21–4.20, Ni: 0.10- 0.33, Fe: 8.30–18.25, Cd: 0.02- 0.07 and Pb: 0.007– 0.019 μg g^{-1}wet weight). The heavy metal concentrations of short fish were higher in Qeshm than those in the short fish from other locations in Persian Gulf.

Conclusions: The research results showed that heavy metal concentrations in the muscles of investigated species were also lower than the maximum levels set by law. Therefore, food risk assessment of the case study species indicated that consumption of short fish with the current consumption rate causes no danger to consumers from the viewpoint of zinc, nickel, cadmium, lead, copper and iron.

Keywords: Trace metals, Health risk assessment, Hormozgan province, Pollution

Introduction

The Expanding growth of cities near aquatic environment in the developing countries mixed with the poor control of wastewater and the uncontrolled increase in industrial and agricultural activities has caused serious worries about the generation of pollutants like heavy metals (1). Heavy metals are natural part of the environment, but the increase in their concentrations is because of different human activities (e.g. industry and oil extraction) that have been reported in nearly all coastal environments (2). Due to the capability of metals to bio accumulate in organic tissues, the risk of indirect metal contamination via feeding is of worldwide concern, in fact, this has been considered as the main route of exposure to heavy metals for human beings (3). Heavy metals in marine systems are a global problem, because continuous exposure of marine organisms to their low concentrations may result in bioaccumulation, and subsequent transfer to man through the food chain (4). Trace metals in the marine environment may begin with natural or anthropogenic sources. Trace metals are normal part of the marine environment and some of them are vital for marine organisms; all metals are toxic above the threshold level (5). A fundamental characteristic of trace metals is their lack of biodegradability (4). Once introduced into the aquatic environment, trace metals are duplicated throughout the water column, deposited or accumulated in sediments and consumed by biota (5). Heavy metals can be classified as potentially toxic (arsenic, cadmium, lead, mercury, nickel, etc.), probably essential

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(vanadium and cobalt), and essential (copper, zinc, iron, manganese and selenium) (6). Fish may concentrate large amounts of some metals from the water; they are often at the top of aquatic food chain (7). Fish are among the most indicative factors in fresh water system for estimation of trace metals' pollution and risk potential of human usage (8, 9). In Hormozgan Province, people consume a considerable amount of fish. Based on the information obtained from the Directorate General of Fisheries, Hormozgan Province, the total amount of fish consumed by people (per person in year) in Hormozgan Province was estimated to be 10.6 kg/person/year in 2011(10).

Therefore, Fishes are good indicators for the long-term monitoring of metal accumulation in the marine environment (11). As fish constitute an important part of human diet, it is not surprising that the quality and safety aspects of fish are of particular interest. Over the past several decades, the concentrations of heavy metals in fish have been extensively studied in various places around the world. The diet is the main route of human exposure to heavy metals (12). Velusamy et al. (2014) studied the accumulation of heavy metals in the 17 species of commercially important fish harbor of Bambaei in India. The results showed that the maximum concentration of heavy metals in Europe and species studied by the FAO/ WHO recommendations, and the fish in this area were considered safe for human consumption (13). Taweel et al. (2013) studied the concentrations of heavy metals in Tilapia (*Oreochromis niloticus*) fish in the river and Lake Langat (Malaysia). The results showed that concentrations of heavy metals in fish and different locations varied between different organs. All concentrations of heavy metals were within the permitted range, and tilapia for consumers in these regions with the amount of 160 grams per day for person with average weight of 64 kg, does not cause any problems. (14). Turkmen et al. (2005) reported the concentrations of heavy metals in fish species *Saurida undosquamis*, *Sparus aurata* and *Mullus barbatus* by evaluating their commercial value in the Gulf of Eskanderun, the North East Mediterranean Sea in Turkey. The results showed that a concentration of heavy metals in edible portions of fish at the limit for human consumption was not high enough to cause any problems (15). Qiao-qiao et al. (2007) examined the accumulation of cadmium, lead, copper, zinc and chromium in four fish species *Cyprinus carpio Linnaeus*, *Carassius auratus Linnaeus*, *Hypophthalmichthys molitrix* and *Aristichthys nobilis* from Lake Tayho in China. The results showed that the highest and lowest levels of heavy metals in the liver and muscle of the studied fish were safe for human consumption, but the dosage is being controlled by China's food hygiene standards (16). The Persian Gulf is considered as one of the most highly anthropogenically impacted regions in the world. It is estimated that N40% of the coasts of the Gulf has been developed (17). The water quality of Persian Gulf is influenced by different industrial and urban outputs such that the wastewater directly goes into the sea arrivers (17). Persian Gulf has been exposed to different additional contaminants because of the adjacent countries' inputs and pollution through river (in Iran, Iraq, Kuwait, Saudi Arabia, and the Emirates, Bahrain, Qatar and Oman).

The aim of the present study was to determine the concentrations of nickel, iron, copper, cadmium, zinc and lead in the muscular tissue of short fish from the Persian Gulf in Hormozgan Province (Iran). Additionally, a recommendation for the maximum weekly ingestion for short fish is proposed.

Materials and Methods

Sampling: Samples were taken during April and May 2015 from three major stations in the Hormozgan Province; "Qeshm" (geographic coordinates: 26° 46.854′ N and 55° 43.472′ E), "Khamir port" (geographic coordinates: 26° 55.233′ N and 55° 35.080′ E) and "Laft port" (geographic coordinates: 26° 56.075′ N and 55° 45.350′ E) beach located" in Persian Gulf including crap. A total of 90 freshly caught marine fish (n = 30 individuals per species) were included. Muscle tissues were collected, stored in plastic zip-lock bags and frozen at−20°C until being analyzed. Total length and body weight were determined for all the organisms sampled (18) (Table1).

Table1. Biometrics data (mean ± SD) of short fish from the coastal waters of the Persian Gulf.

Station	n	Total Length (cm)	Body Weight (g)
Qeshm	30	10.03± 2.12	8.61 ± 5.48
Khamir port	30	8.56 ± 1.75	6.82± 3.27
Laft port	30	7.73 ± 2.22	6.11 ± 2.3

Digestion and Metal Determination: Before analysis, we thawed fish and took out muscular tissues from dorsal, abdominal and tail regions of each fish and homogenized them. We took 4g of homogenized muscles (without skin) from each specimen and put them in 300 ml digestion tubes. A digestion mixture containing 6.0 ml of high purity nitric acid (Merck), 2 ml of hydrochloric acid (10 M) and 4 ml of hydrogen peroxide (35%) was added to each tube (19). The samples were heated at 130 ˚C by heating digester until we had a clear solution. The digested portions were filtered through Whitman filter paper (No. 42) and diluted to a final volume of 50 ml using deionized water. The analytical technique used to determine heavy metal levels in all samples was thermo element Solar S4 Atomic Absorption Spectroscopy (International Equipment Trading Ltd, USA).

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Statistical Analysis: All samples were collected and analyzed in duplicate and the duplicate tests were statistically similar in the paired-samples t-test at 95% significance. The average results were used to represent the data. Minitab 16.0 for Windows was used to test two-way analysis of variance (ANOVA) at 95% significance to investigate the effect of seasons and different fish species on variation of the metal concentrations in the studied fishes. One-way (ANOVA) was used to compare the heavy metals concentrations between different species in single organ (significant values, $P<0.05$). Heavy metal concentrations in the fish muscles were checked for normality using the Kolmogorov–Smirnov' s test. Other calculations were performed by Microsoft Excel 2010. The results of quality control checks, limit of detection and recovery are shown on Table 2.

2.4. Health - Risk Assessment of Fish Consumption

Many online databases gave information about how much fish do people eat. Here, we chose the U.S. Environmental Protection Agency methodology based on the estimation of risk-based consumption limits expressed in terms of real meals. We estimated the consumption limits for adults for a meal size of 227 g and a body weight (BW) of 70 kg USEPA, 2000 (20). The estimated daily intake per meal size of seafood (EDI) was made according to Eq. (1):

$$EDI = \frac{C \times MS}{BW} \qquad (1)$$

Where MS is the meal size, C is the metal concentration (mg kg−1 (w.w.)) and BW is the body weight. Based on the USEPA, 1989 Guidance (21), Cooking has no effect on the contaminants because the in egestion does is as same as absorbed dose (22).

The risks for fish consumption were assessed based on target hazard quotients (THQ). Target hazard quotients (THQ, Equation 2) provide the ratio between exposure and the reference doses. Calculations were made using the standard assumption for an integrated USEPA risk analysis USEPA, 1989 (21).

$$THQ = \frac{EF \times ED \times MS \times C}{BW \times RfD \times AT} \qquad (2)$$

THQ value is above 1 means that THQ is higher than the reference dose, and thus systemic toxic effects may occur. In Eq. 2, EF is the exposure frequency, or the number of exposure events per year of exposure (from 365 days per year for people who eat fish seven times a week to 52 days per year for people who eat fish once a week); ED is the exposure duration (70 years in adults and 6 years in children), MS is the food meal size (0.227 kg day^{-1} for adults and 0.114 kg day^{-1} for children), C is the metal concentration in fish (μg g^{-1}, (w.w)), RfD is the oral reference dose (μg g^{-1} day^{-1}), BW is the body weight (adults 70 kg; children 16 kg), and AT is the averaging time (equal to EF × ED). EF, ED, MS, BW and AT are default data provided by the USEPA (20, 21), for consumption limits calculations.

Table 2. Comparison of the average concentration control reference and material concentration with a detection limit wavelength for each metal.

Element	Wavelength nm	LOD (Limit Of Detection) μg l^{-1}	*Measured concentration μg g^{-1}	*Certified concentration μg g^{-1}	Recovery
Zn	213.9	1	181.02±3	180.00±6	100.5±2
Fe	280.5	1	178.2±5	179 ± 8	99.5±2
Cu	324.8	1	104.1±4	106.00±10	98±5
Ni	232.0	2	21.22±0.14	20.50±0.19	103.50±1
Cd	228.6	2	25.13±0. 4	26.70±0.6	95±6
Pb	217.0	3	0.33±0.04	0.35±0.13	94±7

***** Reference material: Lobster with certain concentrations of various heavy metals standardization was made by the Canadian company TORT-2. Then the concentrations of heavy metals were reread by atomic absorption spectrometry laboratory reference material (model were reread CONTRAA700) and the reference materials were compared with standard concentrations. Concentrations were achieved in a favorable range.

Results

The concentrations of Cu, Zn, Fe, Ni, Cd and Pb in the muscles of short fish of the analyzed three locations are presented in Table 3 by mean values and standard errors. All results are expressed as μg/g wet weight. Also the average daily intake of metals per person was estimated and is presented in Table 4. The daily consumption of Cd, Cu, Fe, Ni, Pb and Zn in all locations in this study was in the range 0.009-0.024, 0.21–0.29, 4.81- 7.67, 0.17–0.26 and 1.23– 2.31 μg/day/person, respectively.

Table 3. Comparison of the results of the average of the elements nickel, lead, iron, cadmium, zinc and copper in the muscle tissue of short fish in Qeshm, Khamir port and Laft port (mean ± SD), (n=30).

Index	Locations amount (μg g^{-1})		
heavy metal	Qeshm	Khamir port	Laft port
Ni	0.33±0.03	0.25±0.05	0.10±0.03
Pb	0.019±0.002	0.014±0.002	0.007±0.001
Zn	4.20±0.05	2.80±0.04	2.21±0.03
Fe	18.25±0.21	15.88±0.36	8.30±0.19
Cu	0.642±0.025	0.521±0.004	0.402±0.027
Cd	0/07±0/02	0/05±0/01	0/02±0/01

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Table 4. Daily intake of trace elements through marine fish consumption by people in Hormozgan Province, Iran.

Location	Metals	C (µg g^{-1} ww)	EDI (µg/kg/day)	Rfd (µg/kg bw/day)	Hazard quotient
Qeshm	Ni	0.33	0.09	50	0.004
	Cd	0.07	0.021	1	0.0002
	Fe	18.25	5.47	700	0.010
	Cu	0.642	0.19	40	0.004
Khamir port	Zn	4.20	1.26	300	0.004
	Ni	0.25	0.07	50	0.003
	Cd	0.05	0.015	1	0.0001
	Fe	15.88	4.76	700	0.009
	Cu	0.521	0.15	40	0.003
	Zn	2.80	0.84	300	0.002
Laft port	Ni	0.10	0.03	50	0.001
	Cd	0.02	0.006	1	0.0001
	Fe	8.06	2.49	700	0.004
	Cu	0.402	0.12	40	0.003
	Zn	2.21	0.66	300	0.002

EDI, estimated daily intake; Health Organization Expert Committee on Food Additives (30).
RfD, reference doses of trace metals as established by the US EPA (2000); Hazard quotient = EDI/ RfD. If the ratio is <1, there is no obvious risk.

Discussion

There were vast differences among the heavy metal concentrations in the muscles of different locations. The highest concentrations were for iron, and the lowest were for lead and cadmium. Calculation of the overall average concentrations of Fe, Zn, Ni, Cu, Mn, Pb, and Cd in the muscles of the three locations showed the following results: Fe 14.14, Zn 3.07, Ni 0.22, Cu 0.521, Pb 0.013 and Cd 0.04. This leads to the following ranking: Fe>Zn> Ni> Cu > Cd >Pb. Cadmium can accumulate in the human body and may cause kidney dysfunction, skeletal damage, and reproductive deficiencies (23). The highest Cd level in Qeshm was 0.07 µg g^{-1} for short fish, while the lowest level was 0.02 µg g^{-1} in Laft port. The mean level was 0.05 µg g^{-1} with the following decreasing order: Qeshm> Khamir port> laft port. However the Cd levels in the present study were lower than the reported international standards, including: Turkish guidlines 0.1 µg g^{-1} wet weight (24), and FAO/WHO limits 0.5 µg g^{-1} wet weight (25). Lead poisoning can cause reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease (CVDs) in adults (26, 27). The maximum concentration of Pb observed was 0.019 µg g^{-1} in the Qeshm island of Persian Gulf. Maximum Pb content allowed is 2000 and 500 ng g^{-1} for the World Health Organization (WHO) and FAO, respectively (25). Pb levels in fish from the Persian Gulf were generally lower than legal limits. The maximum level of Ni was detected in Qeshm and the lowest was for Laft port. Ni concentrations recorded in short fish from the Qeshm and Laft port were 0.33 µg g^{-1}, and 0.10 µg g^{-1}respectively. There is no limit of Ni for fish in international standards. WHO recommends a daily intake of 100,000–300,000 ng Ni (25). The highest concentrations of Cu, Zn, and Fe were all found in Qeshm. Maximum concentrations of Cu, Zn and Fe were 0.642, 4.20, and 18.25 µg g^{-1}, respectively. Copper is an essential trace mineral, but very high intake can cause adverse health problems such as liver and kidney damage (28). Zinc is involved in most metabolic pathways in humans, and deficiency can result in loss of appetite, inhibition of growth, skin changes, and immunological abnormalities (29). Iron deficiency causes anemia, and fish is a major source of Fe for adults and children. Cu, Fe and Zn are essential for human nutrition and good health but very high intakes can cause health problems. Maximum allowable limits by FAO are: Fe 50 µg g^{-1}, Cu 30 µg g^{-1} and Zn 30 µg g^{-1} (25), and all the fish samples had lower heavy metal concentrations than these limits. The levels of Cu, Fe and Zn determined in the muscles of the short fish in three locations (Qeshm, Khamir port and Laft port) from the Persian Gulf were lower than the levels issued by FAO and Turkish legislation.

The average daily intake of metals through fish consumption can be ordered as follows: Fe>Zn> Ni ≈ Cu> Cd. EDI values for the examined fish samples were below the recommended values (25, 30), indicating that health risk associated with the intake of studied heavy metals through the consumption of examined fish samples was absent. The HQ values of Cd, Ni, Cu, Zn and Fe were all less than 1. HQ could not be calculated for Pb because

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there are no reported RfD values. THQ values for Fe, Cu, Ni and Zn for both adults are not likely to cause any adverse effects during lifetime in any population. Bioaccumulation of heavy metals in seafood is a major health concern worldwide. Here, we conducted a large-scale investigation of heavy metals (Pb, Ni, Cu, Zn and Fe) in short fish in the Persian Gulf. Detected mean metal concentrations in short fish (in decreasing order) were: Fe>Zn> Ni> Cu > Cd > Pb. Heavy metal concentrations in the analyzed fish species were lower than the acceptable limits. Human health risk assessment, however, indicated that the human health risks of heavy metal exposure through consumption of these marine wild fish are negligible.

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