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Evaluation of Cadmium, Lead and Mercury Contents in Some Commercially Valuable Fish Species of Caspian Sea and Persian Gulf

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Abstract

BACKGROUND: Fish and fish products are consumed in many countries as a considerable source of nutrients. The heavy metals contents are known to increase drastically in the marine environment.

OBJECTIVES: The present study investigated the contents of cadmium (Cd), lead (Pb), and mercury (Hg) in four commercially valuable fish species of the Caspian Sea (*Rutilus frisii kutum*) and Persian Gulf (*Parastromateus niger*, *Pomadasys kaakan*, and *Scomberomorus commerson*).

METHODS: A total of 200 samples were collected randomly from fresh fish. A microwave-assisted digestion method was conducted to prepare fish samples and atomic absorption spectroscopy was used for determining heavy metals.

RESULTS: The ranges obtained for heavy metals were 0.013-0.038, 0.127-0.352, and 0.007-0.067 mg/kg for Cd, Pb, and Hg, respectively. No fish species overpassed the standard concentrations of metals set by the national or international standards, except for the mean level of Pb in *Parastromateus niger*.

CONCLUSIONS: Results of the present study indicated that Pb, Cd, and Hg were found in Iranian fish species exclusively in trace levels except for the Pb content of the black pomfret of the Persian Gulf. The concentrations of these elements did not exceed the legal limits of the European Commission or the Institute of Standards and Industrial Research of Iran.

KEYWORDS: Cadmium, Fish, Heavy metals, Lead, Mercury

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Introduction

Fish and seafood products are consumed in many countries by people of any age as an integral component of a well-balanced diet providing an important source of proteins, minerals, vitamins, and unsaturated essential fatty acids, especially omega-3 (Pieniak *et al.*, 2010). Unfortunately, in the last few years, environmental contamination by diverse chemical compounds generated due to human industrial activities is elevating considerably throughout the world. These contaminants accumulate in soil and water and transfer through the food chain to the human body. Some groups of people, such as pregnant women, breastfeeding mothers, infants, and children are threatened by these contaminations leading to severe health disorders (Duran *et al.*, 2014).

Among environmental contaminants transferred to food, heavy metals are public health hazards recognized worldwide with a wide spread in the environment, including the aquatic environment. These toxic metals in water and sediments might be absorbed by marine organisms through exposure or may bioaccumulate along the food chain.

Accumulation of heavy metals occurs in the tissues of aquatic animals and may become toxic for fish and consumers when reaches a substantially high level. The severity of harmful health effects depends on the type of heavy metal, its chemical form in the tissue, consumed and accumulated dose in the body, and the period of consumption (Tchounwou *et al.*, 2012). It is established that in aquatic environments, the inorganic form of mercury (Hg) is biologically transformed into methyl mercury (MeHg), which is a lipophilic organic compound that bioaccumulates and biomagnifies as moving up the aquatic food chain and exerts neurotoxic impacts on consumers (Ramon *et al.*, 2011). Exposure to the high concentrations of organic or inorganic forms of Hg leads to damages in the kidneys, brain, and DNA causing arrhythmias, angina, cardiomyopathies, digestive disorders, malfunction of intestinal flora, inflammatory bowel disease, oxidative stress, as well as the disrupted function of pituitary, adrenal, thyroid, and pancreas (Mohammad Nasiruddin *et al.*, 2018; Rice *et al.*, 2014). Exposure to cadmium (Cd) can result in chronic kidney disease, growth inhibition, testicular tumors, renal malfunction, the different types of cancers, hypertension, arteriosclerosis, reproductive disorders, and neurological dysfunction. Lead (Pb) is highly toxic and has the potential to cause retarded physical and mental growth in infants and learning disorders and anemia in children (Mohammad Nasiruddin *et al.*, 2018). In addition, Pb exposure can result in health problems, such as sleeplessness, tiredness, hearing loss, and weight loss. The accumulation of Pb in the hepatic and renal parenchyma causes kidney and liver disease (Mohammad Nasiruddin *et al.*, 2018).

Therefore, the determination of heavy metal content in marine foods is one of the most significant monitoring items that should be taken into consideration in the safety control process. Several studies have investigated the levels of heavy metal contaminants in fishery products with some indicating that fish and shellfish products were safe for the average consumer. However, a potential risk cannot be neglected for the regular or excessive consumers of particular fish species (Chahid *et al.*, 2014; Olmedo *et al.*, 2013) and some authors reported a high content of these contaminants in marine products (Mohammed and Mohammed, 2017).

In the present research, the levels of Hg, Cd, and Pb contamination was assessed in four commercially valuable fish species of Iran. The species were selected according to the Statistical Yearbook of Fisheries Organization (Ghorbanzadeh and Nazari, 2017) and included *Rutilus frisii kutum* (Caspian kutum), *Parastromateus niger* (black pomfret), *Pomadasys kaakan* (Javelin Grunter), and *Scomberomorus commerson* (narrow-barred Spanish mackerel) caught from the Caspian Sea in the North of Iran and the Persian Gulf in the South of Iran. We assumed that our work is novel, particularly because we studied the fishes from both seas of Iran, which are the most used fishes and are also commercially valuable.

Materials and Methods

Sample Collection

A total of 200 samples from fresh fishes, including 50 samples from each species were collected onboard from fishing boats (Charan and Biswas, 2013). Caspian kutum samples were caught from different cities of Mazandaran province in the North of Iran and black pomfret, Javelin Grunter, and narrow-barred Spanish mackerel from Hormozgan province, South of Iran. Based on the information concerning the biology and immigration behavior of Caspian kutum from sea to coast and river estuary, winter was selected as the sampling time. Consequently, all specimens were collected during January 2017-March 2017. All samples were put in plastic bags and transported to the laboratory in an icebox on the same day and were stored at -18ºC until analysis. The fish samples were gutted, heads and tails were removed, and the muscles were washed three times with distilled water. The muscles were sliced into small fillets with a stainless steel knife and were washed three times with clean demineralized water.

Reagents

All the used reagents were purchased from Merck Co. (Germany). Aqueous solutions of standards and reagents were prepared using a Milli-RO 12 plus Milli-Q purification system for water (Millipore, Bedford, MA). Standard stock solutions of Hg, Cd, and Pb were made from the stock solutions with the concentration of 1000 mg/L and were diluted as serial dilutions to the intended concentrations. The fresh working solutions were made by diluting the aliquots from the stock solutions using HNO3 (1 M) for diluting Hg solution and HNO3 (10%) for diluting Cd and Pb solutions.

Chemical Analysis

A microwave-assisted digestion method was conducted to prepare fish specimens for measuring Cd and Pb. Tissues were homogenized (IKA, Germany) and 0.5 g dry weight of the samples was digested using 5 mL of HNO3 for Pb and Cd assessment in glass vessels in a microwave oven (Titan MPS Microwave Sample Preparation System, N3130110, USA). A microwave digestion program was set according to Chahid *et al.* (2014). Briefly, the settings entailed the pressure of 200 psi, temperature of 185ºC, ramp time of 25 min, maximum power of 300 W, and holding time of 20 min. Afterwards, 2 mL of hydrogen peroxide 30% was added to each digested sample to degrade the organic compounds that might have remained untouched in the acidic treatment. The same abovementioned heating procedure was applied again.

For measuring Hg, 27.5 mL of HNO_3 and 1 mL of H_2O_2 were gradually added to 5 g of sample in a Soxhlet extraction tube. The mixture was distilled for 4 h while the valve was open. After this time, the valve was closed but heating was continued until about 20±1 mL liquid was obtained in the extractor tube. Next, heating was cut off and the flask was left to become cool. The flask was separated from the extractor tube and 20 mL water was added to the digestion residue and boiled for a few minutes followed by letting the flask cool down. The solution was transferred to a 100 mL volumetric flask and was diluted with distilled water up to the marker line. The same procedure was performed for the control and 5 mL of water was utilized instead of the specimen. The calibration curve was plotted using atomic absorption spectrometry (AAS) to measure the absorbance of calibrator solutions at the wavelength of 253.7 nm. The absorbance of the specimens was determined in the same conditions as the calibrator solutions and the results were compared with the calibration curve previously plotted (Iran Standard, No. 5346). All metal levels were measured based on wet weight as mg/kg. All the analyses were carried out in triplicates.

AAS

A WFX-210 AAS (Beijing Rayleigh, China) equipped with a flame and graphite furnace, argon as an inert gas, and a D_2 lamp background correction was used for measuring Hg, Pb, and Cd. Pyrolytic graphite tubes were applied and the signals were determined at a peak height. The samples were injected into the furnace utilizing an automatic autosampler.

Statistical Analysis

Levels of heavy metals in the fish samples were non-normally distributed. Metals concentrations were compared between the four fish categories by the Kruskal-Wallis test. Moreover, the levels of four groups were compared with Iranian standard limits using one-way analysis of variance (ANOVA). Statistical analysis was performed utilizing the SPSS software version 22 (IBM, Chicago, Ill., USA). P-value<0.05 was considered statistically significant.

Results

The mean levels of Cd, Pb, and Hg in all fish species have been summarized in Table 1. The ranges obtained for Cd, Pb, and Hg in fish samples were found as 0.013-0.038, 0.127-0.352, and 0.007-0.067 mg/kg, respectively.

Table 1. **Average concentration (mg kg-1) of Cd, Pb and Hg in fish samples (January to March 201, Iran)**

Fish species	Cd	Pb	Hg
Rutilusfrisii kutum	[*a]0.013±0.005	[a]0.196±0.02	[a]0.021±0.008
Parastromateus niger	[b]0.038±0.004	[b]0.352±0.01	[b]0.067±0.000
Pomadasys kaakan	[a]0.017±0.002	[c]0.131±0.015	[c]0.007±0.003
Scomberomorus commerson	[b]0.038±0.000	[c]0.127±0.05	[a]0.018±0.000
Mean	0.026±0.003	0.201±0.014	0.028±0.003
Minimal	0.013	0.127	0.007
Maximal	0.038	0.352	0.067
ISIRI allowed level	0.05	0.3	0.3
EC allowed level	0.1	0.3	0.5

*Different letters in the columns indicate statistically significant difference (P<0.05). Data are means ± SD.

In the present study, the mean concentration of Cd content was reported as 0.026 mg/kg in the fish samples. Black pomfret and mackerel showed the highest concentration of 0.038 mg/kg for Cd followed by Javelin Grunter (0.017 mg/kg) and Caspian kutum (0.013 mg/kg). It is worthy to note that no fish species exceeded the maximum levels of 0.05 and 0.1 mg/kg Cd for muscle meat set by the Institute of Standards and Industrial Research of Iran (ISIRI, 2002) and European Commission regulation No. 629-2008 (EC, 2008), respectively. The lowest Cd concentration was observed in Caspian kutum from the Caspian Sea (0.013 mg/kg).

The mean concentration of Pb in fish samples was 0.201 mg/kg with the maximum concentration of 0.352 mg/kg in black pomfret followed by Caspian kutum (0.196 mg/kg), Javelin Grunter (0.131 mg/kg), and narrow-barred Spanish mackerel (0.127 mg/kg). The level in black pomfret was significantly higher than the permitted value of 0.3 mg/kg in fish muscle set by both the ISIRI and EC No. 629-2008 (P<0.05).

In the present study, the mean Hg concentration was found to be 0.028 mg/kg in fish muscles. Black pomfret had the highest content of Hg (0.067 mg/kg) followed by Caspian kutum (0.21 mg/kg), narrow-barred Spanish mackerel (0.18 mg/kg), and Javelin Grunter (0.007 mg/kg). The results showed that no fish species overpassed the maximum levels set by the ISIRI (0.3 mg/kg for fish muscle) and EC No. 629-2008 (0.5 mg/kg for fish muscle). The lowest levels of 0.007 mg/kg for Hg were reported in Javelin Grunter.

Discussion

According to the findings of the current study, the lowest level of Cd was found in Caspian kutum from the Caspian Sea (0.013 mg/kg). The obtained data were almost similar to the results of Shahryari *et al.* (2010), who reported 0.017 mg/kg of Cd content in the muscles of Caspian kutum samples caught during 2006-2007. On the other hand, the results were lower than the findings of Hosseini *et al.* (2015) and Naghipour *et al.* (2016), who obtained 0.038-0.211 and 0.05 mg/kg of Cd, respectively, in kutum fish samples collected from Mazandaran province, Iran. Furthermore, the Cd concentration of black pomfret specimens in the present investigation was significantly (P<0.05) lower than the results of 0.05 and 0.041 mg/kg of Cd in the muscles of the pomfrets of the Persian Gulf in the studies conducted by Oryan *et al.* (2010) and Sadeghi *et al.* (2011), respectively. The Cd contents of 0.017 mg/kg for Javelin Grunter and 0.038 mg/kg for narrow-barred Spanish mackerel in our study were significantly (P<0.05) lower than the reports of 0.105, 0.118, and 0.75 mg/kg by Obeidi *et al.* (2018), Pazira and Khosravifard (2015), and Dehghani (2016), respectively. Several investigations in the US, China, India, Iran, and the Mediterranean Sea found that the Cd content in fish tissue was low and in *S. commerson* muscle was lower than 0.1 µg/g of wet weight (Rodriguez *et al.*, 2015). Saei-Dehkordi and Fallah (2011) researched the Persian Gulf fish species and reported considerable differences between Cd levels in the muscles of *S. commerson* during cold and hot seasons with the concentrations of 0.102±0.048 and 0.053±0.035 µg/g of wet weight, respectively. It seemed that the high Cd contents in muscles in winter are due to metal precipitation in the sediments in rainy seasons. Our results in narrow-barred Spanish mackerel are consistent with the abovementioned studies. In general, the results demonstrated that the Cd content of the Persian Gulf species was higher than the Caspian Sea species. It is believed that the differences between the Cd levels of fish species could be attributed to the occurrence of this metal in deeper waters, including the habitat of the Persian Gulf species. Moreover, the concentrations decrease in areas closer to the surface and are absorbed by living organisms in these regions, such as planktons and plants (Storelli and Marcotrigiano, 1999). Therefore, fishes closer to the water surface, namely *Caspian kutum* are not highly exposed to Cd. It should be mentioned that Cd content is not dependent on the weight and size of fish (Canli and Atli, 2003).

The Pb content of black pomfret in the current study was higher than the studies performed by Oryan *et al.* (2010) and Sadeghi *et al.* (2011), who reported 0.29 and 0.323 mg/kg lead contamination, respectively. The Pb level in *Caspian kutum* samples was higher than the level of 0.08 mg/kg revealed by Shahryari *et al.* (2010) and was within the range of 0.068-0.415 mg/kg Pb accumulation observed by Hosseini *et al.* (2015). In the present investigation, the lowest Pb levels were found in narrow-barred Spanish mackerel and Javelin Grunter, which are lower than the mean Pb of 0.349 mg/kg reported for narrow-barred Spanish mackerel (Pazira *et al.*, 2016) and 0.043 and 0.45 mg/kg observed in Javelin Grunter (Sadeghi *et al.*, 2015; Dehghani, 2016). Furthermore, Saei-Dehkordi and Fallah (2011) reported the Pb range of 0.158-0.367 µg/g wet weight in narrow-barred Spanish mackerel during a year, which was higher than the results of the present study. It is noteworthy that in recent years, some corrective practices, such as reducing Pbed-gasoline to diminish the emissions of Pb in the environment, have decreased the concentration of this element in the food chain and it is no longer present as the previous decades (Perello *et al.,* 2014).

The mean Hg content detected for Javelin Grunter in the present study was found to be less than the mean Hg level of 0.042-1.027 mg/kg reported for Javelin Grunter from other parts of the world (Chung *et al.,* 2015; Tremlová *et al.,* 2017). Moreover, the mean Hg concentration in the present study for *Caspian kutum* was lower than the means of 0.383, 0.894, and 0.19 mg/kg reported by Hosseini *et al.* (2011), Frooghi *et al.* (2007), and Anan *et al.* (2005), respectively. There is no report about the Hg level of narrow-barred Spanish mackerel of the Persian Gulf. However, studies from the Atlantic countries showed the mean of 0.05 mg/kg Hg in narrow-barred Spanish mackerel samples (FDA, 2017), which is significantly higher than the concentrations reported in the present study for this species in Iran (P<0.05). The highest Hg content of 0.067 mg/kg belonged to black pomfret in the present work. The latter level was significantly (P<0.05) lower than the mean reported for Hg in black pomfret (0.330 mg/kg) by Kordestani *et al.* (2013). The Hg has a considerable tendency to accumulate in high quantities in fish species feed through marine organisms and it is noteworthy that Hg shows a great capacity to bioaccumulate in the food chain. Moreover, the half-life of methyl-Hg in fish meat is long reaching even two years in some species. The other factor that affects the Hg content is the life-longevity and age of the species (Monteiro and Lopes, 1990). Consequently, one of the reasons for the discrepant results of various studies is the age, weight, and size of the studied species.

Following penetrating the waters, trace elements bioaccumulate in the aquatic sediments depending on various factors, such as the microfloral microorganisms, the reduction and oxidation potential of sediments, water cycle, seasonal variations, and pH. Heavy metal compounds migrate from the aqueous environment into many types of aquatic organisms and are finally introduced into marine food stocks. The difference in the content of heavy metals between fish samples is probably due to their variable concentrations in water, bioavailability, fish feeding, nutritional condition, the region of feeding (Rodriguez *et al.*, 2015), size, gender, age (Agusa *et al.*, 2004), sampling time, region (Ruelas-Inzunza *et al.*, 2008), and analysis method (Wagemann *et al.*, 1997).

Conclusion

The levels of the heavy metals in four species, namely *Rutilus frisii kutum*, *Parastromateus niger*, *Pomadasys kaakan*, and *S. commerson* from several fishing harbors of the North and South of Iran were investigated applying ASS. The data indicated that Cd, Pb, and Hg were detected in the Iranian fish species only in trace concentrations except for the Pb content of black pomfret of the Persian Gulf. The concentrations and Cd, Pb, and Hg did not exceed the standard limits of 0.1, 0.3, and 0.5 mg/kg proposed by the EC, respectively, or even 0.05, 0.3, and 0.3 mg/kg set by the ISIRI, respectively. The results of the present work provide novel information about the distribution of Cd, Pb, and Hg as important heavy metals in fish meat from different fishing stations of the North and South of Iran. The levels of the heavy metals in most of the analyzed samples were within the limits adjusted by food legislation. In conclusion, the fishes caught from these regions are considered generally safe for human consumption.

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Conflict of Interest

The author declared no conflict of interest.

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ارزیابی میزان کادمیوم، سرب و جیوه در برخی گونه‌های ماهیان دارای ارزش تجاری دریای خزر و خلیج فارس

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زمینه مطالعه: ماهی و فرآورده‌های آن در بسیاری از کشورها مصرف می‌شوند زیرا منبع قابل ملاحظه‌ای از مواد مغذی به‌شمار می‌روند. مشخص شده است که محتویات فلزات سنگین در محیط دریایی به شدت در حال افزایش است.

هدف: در این مطالعه میزان کادمیوم (Cd)، سرب (Pb) و جیوه (Hg) در ۴ گونه از ماهیان دارای ارزش تجاری دریای خزر (*Rutilusfrisi kutum*) و خلیج فارس (*Parastromateus niger*، *Pomadasys kaakan* و *Scomberomorus commerson*) مورد ارزیابی قرار گرفته است.

روش کار: در مجموع ۲۰۰ نمونه ماهی تازه به طور تصادفی جمع‌آوری شد. برای آماده‌سازی نمونه‌های ماهی روش هضم با استفاده از مایکروویو و برای تعیین میزان فلزات سنگین طیف سنج جذب اتمی به کار گرفته شد.

نتایج: محدوده میزان فلزات سنگین بر اساس $mg\ kg^{-1}$ به شرح زیر بود: Cd (۰/۰۱۳ – ۰/۰۳۸)، Pb (۰/۱۲۷– ۰/۳۲۵) و Hg (۰/۰۰۷ – ۰/۰۶۷). در هیچ کدام از گونه‌های ماهی میزان فلزات سنگین فراتر از بیشینه مجاز تعیین شده بوسیلهٔ استانداردهای ملی یا بین‌المللی نبود، به جز محتوای سرب در حلوای سیاه.

نتیجه‌گیری نهایی: نتایج نشان داد که کادمیوم، سرب و جیوه در گونه‌های ماهی ایران (به جز میزان سرب در حلوای سیاه در خلیج فارس) در غلظت‌های پایین وجود داشته و از حدود مجاز کمیسیون اروپا و یا استاندارد ملی ایران تجاوز ننموده است.

واژه‌های کلیدی: جیوه، سرب، فلز سنگین، کادمیوم، ماهی

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