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Research Article

# Evaluation of Lead, Cadmium, Arsenic and Mercury Heavy Metal Residues in Fish, Shrimp and Lobster Samples from Persian Gulf [1]

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## Abstract

Severe discharge of sewage and industrial effluents into the Persian Gulf causes the deposition of various types of heavy metals and especially lead, cadmium, arsenic and mercury in the muscles of marine animals. The present study was carried out to evaluate the concentration of lead, cadmium, arsenic and mercury in the fish *(Scomberomorus commerson)*, shrimp *(Fenneropenaeus indicus)* and lobster (Panulirus homarus) samples from Persian Gulf. All of the samples were collected from the shopping centers in the Boushehr city. Weight and length of samples were measured and recorded. Concentrations of lead, cadmium, arsenic and mercury in fish, shrimp and lobster samples were analyzed using atomic absorption spectrophotometer. Average length and weight of collected samples were 46.9±2.68 cm and 642.237±52 g for fish, 18.88±1.67 cm and 45.779±4.51 g for shrimp and 22.3±2.13 cm and 203.098±20 g for lobster. Heavy metals concentrations in fish samples were 91.67±9.21 for lead, 49.00±4.77 for mercury, 60.37±7.07 for cadmium and 101.33±9.85 µg/g for arsenic. Lead, mercury, cadmium and arsenic concentration ranges were 64-93, 104-135, 18-34 and 211-265 µg/g in shrimp and 260-390, 71-130, 114-348 and 118-318 µg/g in lobster, respectively. ANOVA test showed significant statistically differences ($P<0.05$) between the type of seafoods and concentration of heavy metals. However, the levels of toxic elements were less than allowable concentrations but consumption of the contaminated seafoods with low levels of heavy metals may be harmful for human health.

***Keywords:*** *Arsenic, Lead, Mercury, Cadmium, Persian Gulf, Seafood*

# Basra Körfezindeki Balık, Karides ve Istakoz Örneklerinde Kurşun, Kadmiyum, Arsenik ve Civa Ağır Metal Seviyelerinin Değerlendirilmesi

## Özet

İran Körfezine aşırı miktarda lağım ve endüstriyel atığın salınması çeşitli ağır metallerin ve özellikle de kurşun, kadmiyum, arsenik ve cıvanın sucul canlıların kaslarında birikmesine neden olmaktadır. Mevcut çalışma, İran Körfezindeki balık *(Scomberomorus commerson)*, karides *(Fenneropenaeus indicus)* ve ıstakoz (Panulirus homarus) örneklerinde kurşun, kadmiyum, arsenik ve cıva ağır metal konsantrasyonlarının belirlenmesi amacıyla gerçekleştirildi. Tüm örnekler Boushehr şehrindeki alışveriş merkezlerinden toplandı. Örneklerin ağırlık ve uzunlukları ölçülerek kaydedildi. Balık, karides ve ıstakoz örneklerinde kurşun, kadmiyum, arsenik ve cıva ağır metal konsantrasyonları atomik absorpsiyon spektrofotometre ile belirlendi. Ortalama uzunluk ve ağırlıklar sırasıyla balıkta 46.9±2.68 cm ve 642.237±52 g, karideste 18.88±1.67 cm ve 45.779±4.51 g ve ıstakozda 22.3±2.13 cm ve 203.098±20 g olarak kaydedildi. Balık örneklerindeki ağır metal seviyeleri kurşun için 91.67±9.21, cıva için 49.00±4.77, kadmiyum için 60.37±7.07 ve arsenik için 101.33±9.85 µg/g olarak tespit edildi. Kurşun, cıva, kadmiyum ve arsenik konsantrasyonları karideste sırasıyla 64-93, 104-135, 18-34 ve 211-265 µg/g ıstakozda ise 260-390, 71-130, 114-348 ve 118-318 µg/g değerleri arasında belirlendi. ANOVA testi deniz ürünleri ve ağır metal konsantrasyonları yönünden istatistiksel olarak anlamlı farlılıkların olduğunu gösterdi ($P<0.05$). Toksik maddelerin seviyeleri izin verilen seviyenin altında olmasına rağmen düşük düzeyde ağır metal ile kontamine deniz ürünlerinin tüketilmesi insan sağlığı açısından tehlikeli olabilir.

***Anahtar sözcükler:*** *Arsenik, Kurşun, Cıva, Kadmiyum, İran Körfezi, Deniz ürünü*

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## INTRODUCTION

Seafoods such as fish, shrimp and lobster provide essential nutrients for the human nutrition. They contain complete proteins, vitamins and minerals which are considered to be major dietary compounds for the health, in particular, including high levels of polyunsaturated fatty acids such as omega-3 and omega-6. Tens of millions of people consume these products daily. This situation makes the quality of the seafoods much more important for the public health. Otherwise, the seafoods may be potential reservoirs for the pathogens and harmful chemical pollutants, leading to the seafood-related diseases [1-3].

One of the most important issues in the present century is the discharge of industrial waste water into the rivers and seas. Chemical waste waters are usually the sources of heavy metals. Unfortunately, heavy metals can be accumulated in different organs of fish and marine animals and may ultimately affect the human food chain [4-6]. Consumption of seafoods contain consider levels of heavy metals may lead to several disorders. Heavy metals can easily be accumulate in organs such as the liver and the kidney in long time periods. Nervous system and kidneys are the most common targets of lead and cadmium [7,8]. Cardiovascular collapse, acute paralytic syndrome and loss of brain function are the main disorders caused due to the consumption of foods containing high levels of arsenic [9]. Long-term ingestion of arsenic increases the risk of skin, bladder, and lung cancers [9].

Mercury is an element of special concern because its inorganic form is biologically transformed in aquatic environments into methylmercury (MeHg), which is a lipophilic organic compound that bioaccumulates and biomagnifies as it moves up the aquatic food chain [10]. Methylmercury may mimic biologicals and be transported by amino acid or organic anion transporters. Further, the generation of reactive oxidative species is often induced by metals in their ionic form, resulting in oxidative modification of DNA or proteins, including aberrant gene expression and carcinogenesis [11,12].

According to the uncertain status of contamination of seafoods of Persian Gulf with dangerous heavy metals, the present study was carried out to evaluate the concentration of lead, cadmium, arsenic and mercury heavy metals in the fish, shrimp and lobster samples of Persian Gulf.

## MATERIAL and METHODS

### *Samples Collection*

During the period of October 2013 to May 2014, a total of 200 samples (80 fish, 60 lobster, and 60 shrimp) from the supermarkets were randomly collected in the Boushehr province of the Persian Gulf, Iran. All samples were caught recently from the Persian Gulf. 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 46.9±2.68 cm and 642.237±52 g for fish *(Scomberomorus commerson)*, 18.88±1.67 cm and 45.779±4.51 g for shrimp *(Fenneropenaeus indicus)* and 22.3±2.13 cm and 203.098±20 g for lobster *(Panulirus homarus)*. The muscle samples were maintained in −20°C freezer prior to analysis.

### *Devices, Reagents and Materials*

All glassware was soaked overnight in 10% (v/v) nitric acid (Merck, Germany), followed by washing with 10% (v/v) hydrochloric acid (Merck, Germany) and rinsed with double distilled water and dried before using.

A Varian Model 220 atomic absorption spectrophotometer (Varian AA 220FS Atomic Absorption Spectrometer System, United States) equipped with a deuterium background corrector was used for the determination of heavy metals. Lead and cadmium concentrations were determined by a graphite furnace atomic absorption spectrophotometer (GFAAS, Analytik Jena AG, Germany, AAS ZEEnit 650) 110 employing pyrolytic platform graphite tubes (Agilent Tech, Santa Clara, California) [13]. Hydride generation was with a Varian model 77 with quartz tubes.

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% $HNO_3$ (Merck, Germany) for diluting lead and cadmium solutions, 1 M HCl (Merck, Germany) and 5% $H_2SO_4$ (Merck, Germany) for diluting mercury solution, 7 M HCl 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 HCl. The solution was boiled for about 5 min, cooled, and nitrogen bubbled through it to expel any mercury impurities.

### *Sample Preparation and Digestion*

Each sample was homogenized thoroughly in a food blender with stainless steel cutters. A sample were then taken and digested promptly as follows: 2 g of the homogenized sample was weighed into a 0.5 l glass digestion tube, and for mercury, 10 ml of concentration of $HNO_3$ (Merck, Germany) and 5 ml of concentration of $H_2SO_4$ (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 $SnCl_2$ (Merck, Germany) were used. For arsenic determination 2 g of homogenized sample was weighed after pre-digestion. Then, $HNO_3$ mixed with 4 ml of $MgNO_3$ 20% (Merck, Germany) as ashing aid, dried on a hot plate and ashed in a 450°C furnace. The ashes were dissolved in 7 ml of HCl 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 concentration of $HNO_3$ were added. The beaker was covered with a watch glass and, after most of the sample had dissolved by standing overnight, heated on a hot plate with boiling until any vigorous reaction had subsided. The solution was allowed to cool in room temperature, transferred into a 50 ml volumetric flask and diluted to the mark with distilled water.

Aquatic samples were spiked with various concentrations of heavy metals for the recovery repeatability tests and for verifying the analytical methodology. For each run, triplicate samples, spiked samples and blanks were carried through the digestion reaction. The results are shown in *Table 1*.

### *Chemical Analysis*

Mercury and arsenic were determined by the hydride generation system. The manufacturer operation procedure involves continuous addition of reductant, consisting of 0.3% $NaBH_4$ (Merck, Germany), 0.5% NaOH (Merck, Germany) for mercury and 0.6% $NaBH_4$ (Merck, Germany), 0.5% NaOH, 10% KI for arsenic. The manufacturer's operating procedure consists of adding sample, reductant and acid, with the aid of argon gas, to a reaction coil; then any vapour generated is swept into the absorption quartz cell, and heated for arsenic detection. Cells were aligned in the light path of the hollow cathode lamp where the absorption was measured. Cadmium and lead concentrations were determined by graphite furnace atomic absorption spectrophotometry, employing pyrolytic platform graphite tubes (Agilent Tech, Santa Clara, California), ascorbic acid and palladium for matrix modification and using the method of additions for quantification. Graphite Tube Atomizer (GTA) was equipped with an auto sampler and the analysis was done according to the manual instruction, optimized conditions and the method of peak area [13].

**Table 1.** *Evaluation of the recovery rates of lead, cadmium, mercury and arsenic in fish, shrimp and lobster*

**Tablo 1.** *Balık, karides ve ıstakozlarda kurşun, kadmiyum, cıva ve arsenik gerikazanım oranları*

| Heavy Metals | Added Concentration ($\mu g\ g^{-1}$) | Achieved Concentration ($\mu g\ g^{-1}$) (Average±SD) | % Recovery |
|---|---|---|---|
| Lead | 50 | 47.66±1.15 | 95.32 |
| Cadmium | 50 | 48.33±2.00 | 96.66 |
| Arsenic | 50 | 51.00±1.00 | 102.00 |
| Mercury | 50 | 45.33±1.52 | 90.60 |

### *Statistical Analysis*

The results of the mercury, arsenic, cadmium and lead concentration in fish, lobster and shrimp were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 16.0 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 $P<0.05$.

## RESULTS

The results of the present investigation showed that the aquatic food samples of Persian Gulf were contaminated with lead, mercury, cadmium and arsenic heavy metals. Concentration of heavy metals in each studied samples is shown in *Table 2*. Heavy metals concentrations in fish samples were 91.67±9.21 for lead, 49.00±4.77 for mercury, 60.37±7.07 for cadmium and 101.33±9.85 µg/g for arsenic. Lead, mercury, cadmium and arsenic concentration ranges were 64-93, 104-135, 18-34 and 211-265 µg/g in shrimp and 260-390, 71-130, 114-348 and 118-318 µg/g in lobster, respectively. Significant statistically differences were seen for the concentration of lead between shrimp and lobster ($P=0.015$), concentration of mercury between fish and shrimp ($P=0.029$), concentration of cadmium between shrimp and lobster ($P=0.024$) and finally concentration of arsenic between fish and shrimp ($P=0.023$) and lobster and shrimp ($P=0.027$).

## DISCUSSION

The results of the present study showed that the fish, shrimp and lobster samples of Persian Gulf have been

**Table 2.** *Average and standard deviation of the concentration of the lead, mercury, cadmium and arsenic heavy metals in fish, shrimp and lobster*

**Tablo 2.** *Balık, karides ve ıstakozlarda kurşun, cıva, kadmiyum ve arsenik konsantrasyonlarının ortalama ve standart sapmaları*

| Samples | Concentration of Heavy Metals ($\mu g\ g^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Lead | | Mercury | | Cadmium | | Arsenic | |
| | Average±SD | Range of Contamination | Average±SD | Range of Contamination | Average±SD | Range of Contamination | Average±SD | Range of Contamination |
| Fish | 91.67±9.21 | 63-121 | 49.00±4.77 | 19-98 | 60.37±7.07 | 18-87 | 101.33±9.85 | 62-148 |
| Shrimp | 75.67±8.33 | 64-93 | 115.67±10.86 | 104-135 | 26.00±2.00 | 18-34 | 237.67±22.01 | 211-265 |
| Lobster | 316.67±29.58 | 260-390 | 98.33±7.74 | 71-130 | 131.46±12.57 | 114-348 | 105.28±10.21 | 118-318 |

contaminated with considerable levels of lead, cadmium, arsenic and mercury heavy metals. However, the levels of these toxic elements were entirely less than their allowable concentrations but consumption of the contaminated seafoods even lower than their permissible levels may be harmful for human health. One of the most important point to evaluate the levels of lead, cadmium, arsenic and mercury heavy metals in seafood is comparison of the permitted extent and acceptance daily intake of heavy metals with the amount obtained. The acceptance limits recommended of mercury, lead, cadmium and arsenic is 0.5 mg/kg [14-16], 0.5 mg/kg [17], 0.5 mg/kg [14-17] and 6 mg/kg [18], respectively. The levels of detected lead, cadmium, arsenic and mercury in our study were 47.66, 48.33, 51.00 and 45.33 µg/g, respectively. The levels of detected elements were lower than the acceptable limits recommended.

Average daily lead intake through diet was about 114 microg/day for adults and 50 microg/day in children and tolerable limit is 250 microg/day for adults and 90 µg/day for children. Acceptance daily intake of cadmium is 3.0 µg/kg body weight per day (2-7 µg/kg body weight per day) and the tolerable weekly intake of this element 15 µg/kg body weight. Tolerable weekly intake of mercury is 1.6 µg/kg body weight. Average daily arsenic intake is 3.0 µg/kg and its tolerable weekly intake is 15 µg/kg body weight [19-21].

Similar studies have been done of the determination of heavy metals such as copper, lead, cadmium, zinc, mercury and arsenic in the seafood samples of the Persian Gulf [6,22-24]. Previous study [6] reported that the obtained range of heavy metals in the fish species of Persian Gulf were 0.024-0.111 µg/g for cadmium and 0.057-0.471 µg/g for lead which was entirely lower than our results. Agah et al.[22] reported that the concentration range of lead was 0.2-25 ng/g in various species of fish caught from the Persian Gulf. Reissy et al.[23] showed that the heavy metals concentrations in lobster samples of Persian Gulf were 32-73 µg/kg for mercury, 118-275 µg/kg for arsenic, 379-1120 µg/kg for lead and 101-401 µg/kg for cadmium which was entirely lower than our results except lead concentration. In a study which was conducted on Vietnam [24], the lead, cadmium, and mercury concentration ranges in shellfish were 0.008-0.083, 0.013-0.056, and 0.028-0.056 mg/kg, respectively. Islam et al.[25] reported that the concentrations of mercury, arsenic, cadmium and lead varied between 0.24±0.007 - 0.01±0.001, 44.54±5.69 - 1.23±0.20, 0.13±0.05 - ND (not detected), 1.32±0.47 - 0.09±0.02 and 0.74±0.28 - 0.05±0.03 mg/kg, respectively.

One possible explanation for the higher presence of heavy metals in our results is the fact that the fish, shrimp and lobster samples of our study are in close contact with contaminant sources like oil tankers and industrial wastewaters. Besides, differences in the races of studied seafoods of our investigation with those of other studies is another reason for the higher prevalence of heavy metals in our research. Considerable differences in the concentration of heavy metal between various races of seafoods have been reported previously [6,22,26,27]. The season which the seafood samples were collected and analyzed for presence of heavy metals is another determinative factor in the concentration of toxic elements. It seems that different seasonal dependent conditions such as water temperature, dietary factors and growth and reproductive cycles are effective on heavy metal fluctuations [28-31]. The higher metal content in winter might be a result from considerable rainfall which washed down the wastes [28-31]. Therefore, variation in the seasons of sampling may has the direct effect of the concentration of heavy metals. Large differences in the levels of heavy metals in the seafood samples of our study may be related to the variation in the weight and length of samples. There were no significant differences between the concentrations of heavy metals and average length and weight of seafood samples ($P>0.05$). Average length and weight of collected samples were 46.9±2.68 cm and 642.237±52 g for fish, 18.88±1.67 cm and 45.779±4.51 g for shrimp and 22.3±2.13 cm and 203.098±20 g for lobster. Fish samples had the highest length and weight but had the lowest concentrations of mercury and arsenic. Petroody et al.[32] reported the significant relationships between lengths and lead concentrations ($P<0.01$), but no significance correlation between length and cadmium concentrations were observed. In a study which was conducted by Jafarzadeh Haghighi et al.[33] concentrations of cadmium in the fish samples was positively correlated with length and weight.

Level of arsenic in the seafood samples in the current study was entirely higher than previous studies that reported arsenic concentration in crabs, shrimps, lobster, fishes and bivalves of Persian gulf [23,34-36]. In a study which was conducted by Heidarieh et al.[34] the concentrations of heavy metals was evaluated in crab and shrimp samples of Persian Gulf, Iran. Their results showed that arsenic concentrations in crab and shrimp samples were 21.38±3.31 and 8.28±2.82 µg/g, respectively. In another study which was conducted by Javaheri Babooli and Velayatzadeh [37] the mean concentrations of mercury, arsenic, cadmium and lead in shrimp samples of Persian Gulf was 0.032±0.002, 0.117±0.07, 0.175±0.006, 0.414± 0.012 µg/g which was lower than our results. The main anthropogenic sources of arsenic in Persian Gulf are emissions from coal burning electrical generating facilities, mining and smelting operations, herbicide or algicide applications especially for algae bloom and leaching from hazardous waste facilities and from insecticide [38].

High concentration of mercury was seen in the seafood samples of our investigation. Another Iranian survey showed the mean levels of mercury in fish samples of Persian Gulf was 0.05 µg/g [39]. Agah et al.[40] reported that total mercury concentrations in fish ranged from 0.0123 to 0.0867 mg/kg. Documented data reported that in several coastal areas of the Persian Gulf such as United Arab

Emirates, Iran, Kuwait and Qatar, mercury levels exceeded 0.1 mg/kg in various fish species. Only in the coastal area of Bahrain, the average concentrations of mercury were below 0.1 mg/kg [41]. Mercury toxic effects have been emphasized by some cases of collective poisoning in people who consumed a large amount of fish [42]. It is generally accepted that seafood represents one of the major sources of mercury in the human food chain.

Other important heavy metals studied in our study were cadmium and lead. Our results showed that the mean concentration of lead and cadmium in fish, shrimp and lobster samples of Persian Gulf had the range of 75.67±8.33 to 316.67±29.58 µg/g and 26.00±2.00 to 131.46±12.57 µg/g, respectively. In a study which was conducted by Dadolahi and Nazarizadeh Dehkordi [43] on Sediments from the North of the Strait of Hormuz, Persian Gulf, Iran the mean concentrations of lead and cadmium were 3.00 to 28.00 and 2.96 to 10.11 mg/g, respectively which was entirely lower than our findings. Haghighi et al.[33] reported the lower range of cadmium in two different fish samples of Persian Gulf (0.1±.05 to 0.31±0.09 mg/g). The concentrations of cadmium and lead in the fish samples of Persian Gulf have been studied previously by Mohammadnabizadeh et al.[44]. They showed that the total concentration of cadmium and lead in the fish samples had the ranges of 0.58±0.03 to 1.7±0.2 and 0.22±0.06 to 1.12±0.17 mg/g, respectively.

The main factors which may lead to the pollution of water sources with heavy metals mainly are leakage of industrial sewage from seaside, industrial effluents, agricultural runoff, domestic and municipal sewage and pedogenic background contributions.

In conclusion, the results of the present study indicated that the seafoods of the Persian Gulf were contaminated with low range of lead, cadmium, arsenic and mercury heavy metals. Our results indicated the improvement of local environmental conditions with respect to mercury, lead, arsenic and cadmium concentrations, which is explained by reduced industrial activity in the region (Boushehr seaside), the use of more efficient dust traps and filters in recent years, and, hence, the decreased discharge of these metals into the sea. However, the contents of toxic metals in Persian Gulf were below the permitted levels but more studies are needed to properly assess other sources and even other sites of the Persian Gulf to monitoring the levels of heavy metals.

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