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⇥ Research Article

Health Risk Assessment of Heavy Metals (Arsenic and Cadmium) in Rice (Oryza sativa L.) Brands Imported to Iran: Using Monte-Carlo Simulation

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Abstract

Background: Rice contamination with heavy metals is one of the most common cases of environmental pollution. The purpose of this study was to investigate the concentration of heavy metals (arsenic and cadmium) in the most widely consumed rice brands imported to southern Iran and to assess the health risk of exposure to them for consumers.

Methods: A total of 103 rice samples were selected from 10 brands imported from India during 2014-2018. Heavy metal concentrations were measured by dry ash method using atomic absorption spectrometer (GBC model SavantAA). After determining the concentration of heavy metals in rice samples, health risk assessment was performed using the Monte-Carlo simulation technique.

Results: The concentrations of arsenic and cadmium were 94.3 ± 34.1 and 11.3 ± 6.5 mg/kg, respectively. The values of non-carcinogenic risk index (hazard quotient) of cadmium and arsenic were 0.017 and 0.489, respectively. The average carcinogenic risk index for arsenic was 1.7E10-4, which is higher than the standard range (10-4 to10-6).

Conclusion: Consumption of imported rice carries a risk of arsenic-induced carcinogenesis. Consumption of contaminated rice with heavy metals, especially arsenic, can pose potential health risks to the consumer population. Therefore, special attention should be paid to contaminated rice and special interventions should be made to reduce arsenic in imported rice.

Keywords: Heavy metals, Rice, Health risk assessment, Monte-Carlo simulation

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Background

In many parts of the world, especially in Asia, rice (*Oryza sativa* L.) is a major part of people's diet and because rice supplies 30% of the world's energy and 20% of its protein, it is the most widely consumed cereal grain (1, 2).

The Food and Agriculture Organization (FAO) also reports that the per capita consumption of global rice in 2016-2017 is equivalent to 402 million tons, which is 54.1 kg per person and about 40 kg in Iran (3-5). The growing trend of the population in Iran has increased the demand for rice consumption and, a significant portion of consumed rice is imported from other countries, especially India and Pakistan (3, 6, 7). In Iran, 21.2%, 6.3%, and 5.7% of the consumed rice is imported from India, Pakistan, and other countries (8).

Rice contamination with heavy metals is one of the most common cases of environmental pollution. Therefore, investigation and detection of rice contaminated with heavy metals has always been a very important issue (8). There have been reports of contamination in rice produced in Southeast Asian countries above the permissible limits (6, 9, 10). Cadmium (Cd) causes kidney damage and affects reproduction and has mutagenicity and carcinogenicity characteristics. Arsenic (As) also causes diseases such as skin cancer, lung cancer, and osteoporosis (7, 11).

In order to protect human health and the environment, a program called Risk Assessment was established by the FAO and World Health Organization (WHO). Risk assessment is a scientific process for estimating the potential effects of risk factors on humans or other sections of the environment and includes risk identification, exposure assessment, dose-response relationship assessment, and determining risk characteristics (12, 13).

Hormozgan province, despite its multiple ports and customs, is one of the entry points for various types of imported rice. Imported rice enters the country under the supervision of the Ministry of Health and after health approval and permission. However, part of the rice is imported illegally and without the approval of the Ministry of Health. Therefore, measuring the concentration of heavy metals in rice in Bandar Abbas indicates the amount of these metals in the country (14). Therefore, because of the importance of maintaining food health and necessity of investigating the concentration of heavy metals in rice, we aimed to measure the concentration of heavy metals (As and Cd) in rice brands imported to Bandar Abbas and to evaluate their carcinogenic and non-carcinogenic risk.

Objectives

The purpose of this study was to investigate the concentration of heavy metals (As and Cd) in the most widely consumed rice brands imported to southern Iran and to assess the health risk of exposure to them for consumers.

Material and Methods
Standards and Reagents

Standard solutions of Cd and As with a mass concentration of 1000 ppm, hydrochloric acid (HCl, 37 wt%), nitric acid (HNO_3, 65 wt%), magnesium nitrate, magnesium oxide were purchased from Merck, Germany. Standard calibration solutions for measuring Cd metal were obtained by diluting a certain concentration of stock solution in 0.1 M nitric acid. While standard As calibration solutions were prepared by diluting a certain volume of stock As solution and pre-reduction solution (5 g potassium iodide and 5 g ascorbic acid dissolved in 100 mL distilled water) with hydrochloric acid.

Rice Sampling

Rice samples were obtained from the customs of southern Iran during 2014-2018. For this purpose, 103 samples of rice from 10 brands imported from India were randomly selected. One hundred grams was taken from each sample and stored in polyethylene bags, after transfer to the laboratory, Cd and As concentrations were determined for each rice sample. All measurements were repeated three times.

Preparation of Samples and Measurement of Heavy Metals

Before testing, all equipment were placed in 0.1 M nitric acid for 24 hours and then washed with distilled water three times. First, 10 g of rice was burned in a flame and after preliminary ashing, it was placed in a muffle furnace at 450°C for 8 hours. Then to measure Cd in rice samples, 5 mL of 6 M hydrochloric acid and 20 mL of 0.1 M nitric

acid were added to the ash and heated on the heater for 5 minutes. After 2 hours, the contents of the crucible were transferred to a 50 mL flask and brought up to a certain volume with 0.1 M nitric acid (15, 16). Finally, the Cd heavy metal content of the samples was measured with an atomic absorption spectroscopy device equipped with a graphite furnace (GBC model SavantAA) with an accuracy of ± 0.001. To measure As in the samples, 1 g of rice sample, 10 mL of ash aid (which includes 20 g of magnesium nitrate and 2 g of magnesium oxide and brought up to the volume to 100 mL of distilled water) and 5 mL of Nitric acid was added nitric acid 32% and placed on the heater. The crucibles were then placed in a muffle oven at 425 ± 25 °C for 12 hours. Finally, 1 mL of distilled water and 5 mL of 6M hydrochloric acid were added to the contents of the crucible and after 30 minutes it was brought up to the volume with 6M hydrochloric acid. Total As was measured in the samples with a GBC HG 3000 Hydride production machine (17). The limits of detection (LOD) for As and Cd were 0.0105 and 0.003, respectively, while the limit of quantification for these metals was 0.035 and 0.01, respectively. In addition, our study findings showed a stable measurement process and accurate data for the studied heavy metals. Recovery percentages for As and Cd were 99.92% and 91.32%, respectively.

### Health Risk Assessment
Non-carcinogenic Risk Assessment

Non-carcinogenic risk assessment of Cd and As was performed according to the method presented in a previous study (5). For this purpose, the estimated daily intake (EDI) and the hazard quotient (HQ) were calculated according to the following equations:

$$EDI = \frac{(EF \times ED \times FI \times MC)}{(BW \times AT)} \qquad (1)$$

$$HQ = \frac{EDI}{RfD} \qquad (2)$$

In these equations, EDI estimates daily consumption (mg/kg-d), EF id the frequency of exposure (days/year), ED is the exposure duration for adults (years), FI refers to food intake (g/n-d), RfD is the oral reference dose (mg/kg-d), AT is the average time (days), BW is the average weight of consumers (kg) and MC is the concentration of heavy metals in rice samples (mg/kg dry weight), that the unobserved concentrations of the samples were considered equal to half of the LOD (5, 18, 19). Statistical characteristics of risk parameters for calculating HQ and lifetime carcinogenic risk (LTCR) of As and Cd are shown in Table 1 and its shape is shown in Figure 1.

As shown in Figure 1, the concentrations of As and
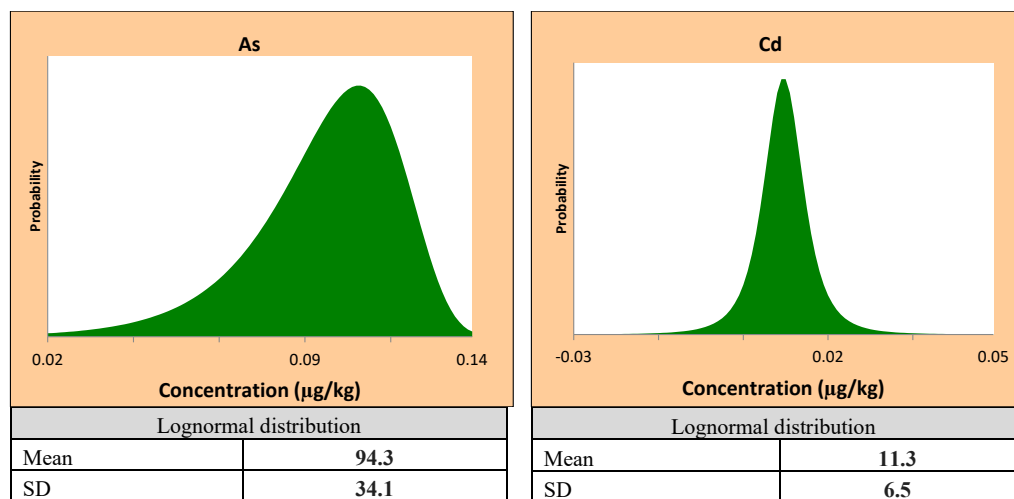
Figure 1. Statistical Distribution of Heavy Metal Concentration Applied for Calculation of HQ and LTCR. Abbreviations: LTCR, lifetime carcinogenic risk; HQ, hazard quotient.
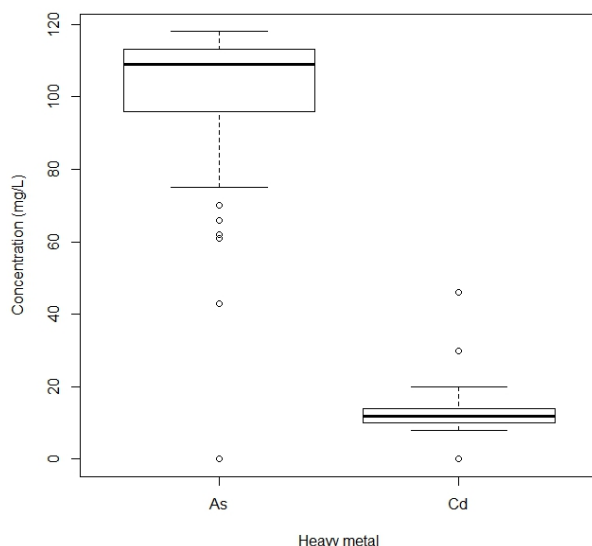
Figure 2. Box Diagram of Cd and As Concentrations in Rice Samples.

Cd follow the statistical distribution of Lognormal; the mean ± SD As and Cd concentrations were 94.3 ± 34.1 and 11.3 ± 6.3, respectively.

Carcinogenic Risk Assessment
As carcinogenic risk assessment was calculated according to equation 3 (20, 21).

$$L\mathit{T}CR = EDI \times SF$$

EDI is the estimated daily intake (mg/kg-d) and SF is the slope factor of the carcinogen (mg/kg day)-1. The amount of carcinogenicity slope factor according to Environmental Protection Agency reports was 1.5 (mg/kg-d)-1 (22).

Because of the uncertainty and variability related to the constant values of the parameters in the equation of risk assessment index, HQ calculation was done using Monte-Carlo simulation with 10 000 repetitions with the Oracle Crystal ball software (version 11.1.2.4, Oracle, Inc., USA). The Monte-Carlo technique selects the parameter values within the specified range and according to the distribution of each variable, and then calculates the risk. This process is repeated several times and as a final result, it calculates average, minimum, maximum, standard deviation values, different percentile values and some other statistical indicators. These iterations show the uncertainty and variability of the parameter values. Therefore, the obtained results are more confident and more valuable than the results calculated with constant values of input parameters.

Results and Discussion
Concentration of Heavy Metals in Consumed Rice
Cadmium
Box diagram was used for comparison between mean and testing normality of data and also determining data related to Cd and As in rice samples (Figure 2). As shown, the mean concentration of Cd in the evaluated rice samples was 11.3 ± 6.5 mg/kg, while the acceptable limit for Cd in consumed rice are 0.06 μg/g and 0.1 μg/g according to Institute of Standards and Industrial Research of Iran (ISIRI) and FAO/WHO standards, respectively (5, 23).
Cadmium is one of the most toxic heavy metals that leads to kidney problems, bone lesions, high blood pressure, and cancer in humans (24). Jafari and colleagues reported that the concentration of Cd in imported rice brands to Iran was 0.16 ± 0.08 mg/kg (25), while in another study, Cd concentration in collected rice samples from shiraz supermarkets were 0.29 ± 0.48 mg/kg (12).

Total Arsenic
The mean ± SD concentration of As in rice samples was 94.3 ± 34.1 mg/kg (Table 1). Arsenic is one of the most important environmental metals in food products,

Table 1. Risk Parameters Applied for Calculation of HQ and LTCR for As and Cd in Rice Imported to Iran (5)

Parameters	Statistical Distribution	Values	Reference
As concentration	Lognormal	Mean: 94.3, SD: 34.1	This study
Cd concentration	Lognormal	Mean: 11.3, SD: 6.5	This study
Exposure frequency (day/year)	-	365	4
Exposure duration (year)	-	54	4
Rice consumption rate (g/d)	-	165	4
Reference dose (mg/kg-d)	-	0.0003 for As, 0.0001 for Cd	5
Body weight (kg)	-	77.45	5
Averaging time (day)	-	19710*, 25550**	6

Abbreviations: LTCR, lifetime carcinogenic risk; HQ, hazard quotient.
*Averaging exposure time (days) for non-carcinogens= (54 years) × 365 days per year.
**Averaging exposure time (days) for carcinogens= (70 years) × 365 days per year.

especially rice (26). This metal can be seen in volcanic ashes and stones, clay, iron oxides, and mineral and organic materials (27). Use of chemical products such as fungicides and herbicides in fertilizers is main cause of increased concentration of elements in soil and agricultural products. Serious health effects of As include lung, bladder, kidney, skin and prostate cancers, melanose, hyper keratosis, limited lung disease, vascular disease, gangrene, diabetes, high blood pressure, and ischemic heart disease (28).

In one study the concentration of As metal in Pakistani, Iranian, and Indian rice were 0.063 ± 0.042, 0.067 ± 0.044, and 0.108 ± 0.088 mg/kg, respectively (5). Another study showed that the concentration of As in collected rice samples from Malaysian supermarkets was 0.087 mg/kg (29).

Health Risk Assessment as a Result of Rice Consumption
Non-carcinogenic Risk Assessment
For non-carcinogenic risk assessment, the EDI and HQ was measured for each element (Table 2). Also, distribution amounts of EDI and HQ of the observed elements was simulated according to the Monte-Carlo technique (Figures 3 and 4). The results showed that the average EDI and HQ of Cd was 1.71E-05 mg/kg-d and 0.017. Also, the content P_{10} for EDI and HQ were 7.83E-06 mg/kg-d and 0.008 and content d90 were 2.64E-05 mg/kg-d and 0.026. EDI and HQ for As in this study were 1.13E-04 mg/kg-d and 0.489, respectively. Also, content P10 for EDI and HQ were 8.10E-05 mg/kg-d and 0.350 and content P90 for EDI and HQ were 1.40E-04 mg/kg-d and 0.607, respectively.

As shown in Table 1, the amounts of HQ for As and Cd was less than 1. Therefore, it can be suggested that

human exposure with these heavy metals in the observed samples, has no non-carcinogenic health hazard. Sharafi and colleagues reported that HQ for As and Cd in rice samples in most consumed brands in Tehran city were 1.8E-04 and 1.2 E-04 mg/kg-d, respectively (5). In another survey in Iranshahr, the HQ amount for heavy metals As, lead (Pb) and Cd in rice samples were 5.23, 0.15 and 0.14. The amounts of HQ for As was higher than 1, meaning that consumption of rice in Iranshahr may lead to non-carcinogenic health hazard in humans (4).

Carcinogenic Risk Assessment
According to United States Environmental Protection Agency (USEPA) guidelines, As is a carcinogenic element in A group and is classified as carcinogenic factor in humans (4). In our study, the average amounts of carcinogenic risk factor (LTCR) for As was 1.70E-4 (Table 1). Distribution amounts for LTCR simulated for As is shown in Figure 5. Acceptable hazard limit for cancer by heavy metals in humans is between 1E−6 until 1E−4 (4). Therefore, it can be inferred that consumption of imported rice to Bandar Abbas leads to cancer. Also, the amounts of P_{10} and P_{90} for carcinogenic As risk were 1.22E-4 and 2.11E-4 , respectively. Fakhri et al performed carcinogenic risk assessment and found that the EDI for As and lead in rice for the 15-24 year-old age group was 5.501E-02 and 0.00009 mg/kg-d and 2.961E-03 and 0.00088 mg/kg-d, respectively (30). In a similar study, the results showed that the incremental lifetime cancer risk for As in rice was 2.7E-04 , in which P_{10} and P_{90} were 1.2E-04 and 4.0E-04 , respectively (5). In another study in Brazil, the LTCR of As and lead in the collected rice samples were bigger than 1E-4 (31).

Table 2. Descriptive Statistics of Carcinogenic and Non-carcinogenic Risk Assessment of As and Cd in Imported Rice to Iran

Statistics	AS			Cd	
	EDI (mg/kg.day)	HQ	LTCR	EDI (mg/kg.day)	HQ
Mean	1.13E-04	0.489	1.70E-4	1.71E-05	0.017
Median	1.17E-04	0.507	1.76E-4	1.7E-05	0.017
STD	2.50E-05	0.108	3.75E-5	9.42E-06	0.009
P_{10}	8.10E-05	0.350	1.22E-4	7.83E-06	0.008
P_{20}	9.54E-05	0.412	1.43E-4	1.16E-05	0.012
P_{30}	1.04E-04	0.451	1.57E-4	1.38E-05	0.014
P_{40}	1.12E-04	0.482	1.67E-4	1.55E-05	0.015
P_{50}	1.17E-04	0.507	1.76E-4	1.7E-05	0.017
P_{60}	1.23E-04	0.531	1.84E-4	1.87E-05	0.019
P_{70}	1.28E-04	0.553	1.92E-4	2.04E-05	0.020
P_{80}	1.34E-04	0.578	2.01E-4	2.27E-05	0.023
P_{90}	1.40E-04	0.607	2.11E-4	2.64E-05	0.026

Mean	SD	P_{10}	P_{90}	Mean	SD	P_{10}	P_{90}
1.13E-04	2.50E-05	8.10E-05	1.40E-04	1.71E-05	9.42E-06	7.83E-06	2.64E-05
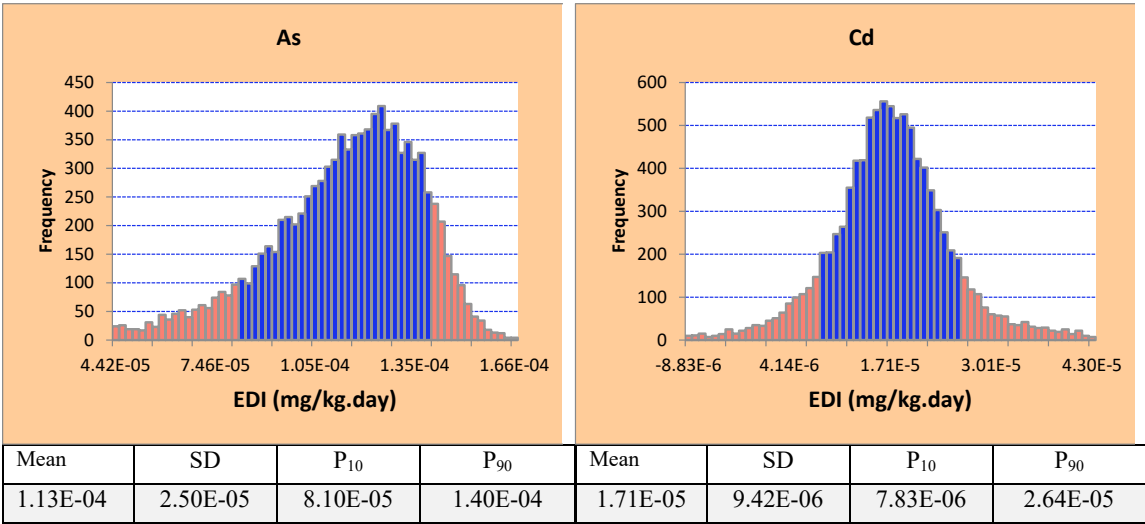
Figure 3. Simulated EDI Values for As and Cd.

Mean	SD	P_{10}	P_{90}	Mean	SD	P_{10}	P_{90}
0.489	0.108	0.350	0.607	0.017	0.009	0.008	0.026
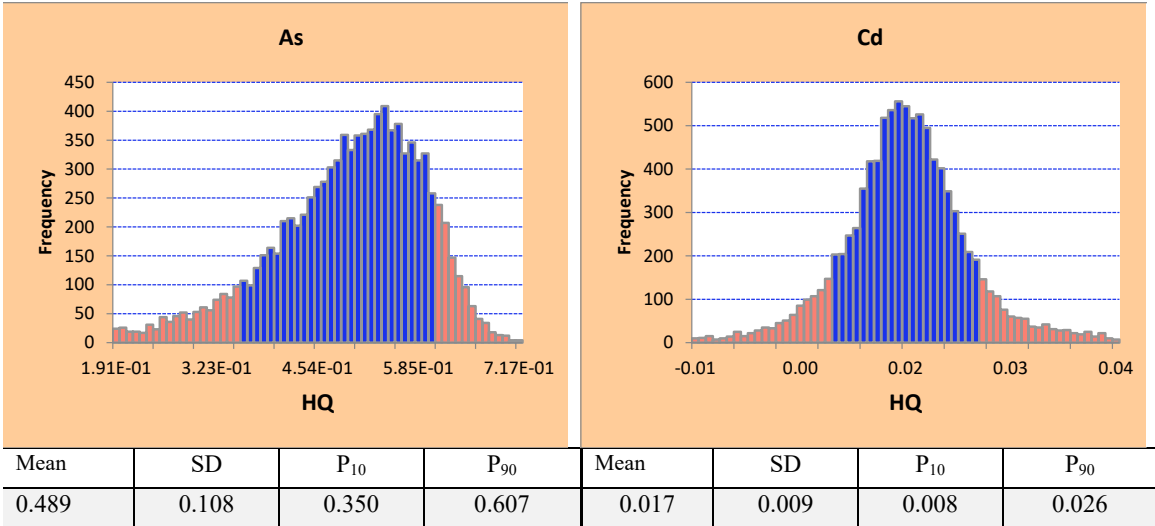
Figure 4. Simulated HQ Values for As and Cd.

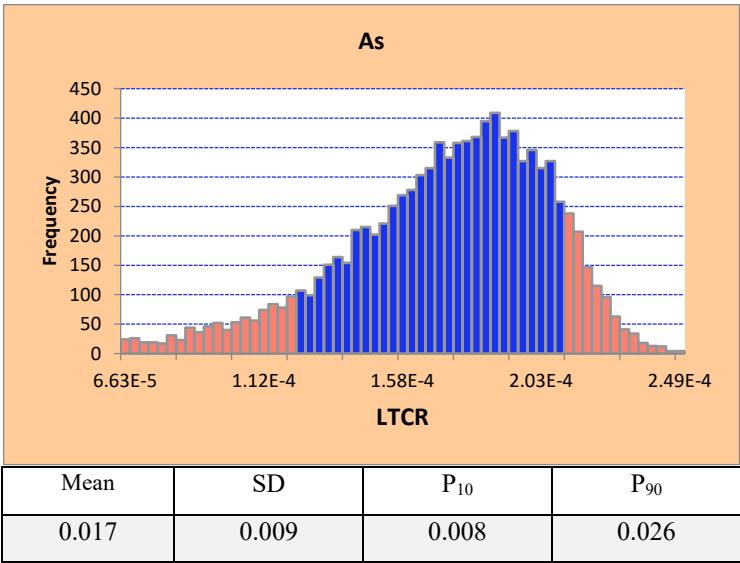| Mean	SD	P_{10}	P_{90}
0.017	0.009	0.008	0.026

Figure 5. Simulated LTCR Values for As.

Conclusion

Foods are the main source of toxic heavy metals for humans and consumption of rice contaminated with heavy metals can be a serious health hazard. Therefore, continued control on imported rice to determine its heavy metals content is a priority priorities (30). We aimed to measure the of concentration of As and Cd in imported rice to Bandar Abbas and perform carcinogenic and non-carcinogenic risk assessment. The concentration of the studied samples was within the ISIRI and FAO/WHO. Also, the amounts of HQ for heavy metals (Cd and As) was less than 1 and the quality of observed rice sample was desirable. Furthermore, the average carcinogenic risk index for arsenic was 1.7E10-4, which was higher than the standard range (10-4 to10-6). Therefore, consumption of imported rice carries a risk of arsenic-induced carcinogenesis. Therefore, interventions such as using phytoremediation technique of heavy metal in soil and expansion of organic agriculture instead of using different chemical products containing heavy metals in farms can decrease the concentration of heavy metals in agricultural products.

Conflict of Interests

The authors declared that they have no competing interests.

Ethical Approval

This study was approved by the ethical committee of Hormozgan University of Medical Sciences (Ethics No. IR.HUMS. REC.1397.320).

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