

Research Paper:

Lead and Cadmium Contaminations in Fruits and Vegetables, and Arsenic in Rice: A Cross Sectional Study on Risk Assessment in Iran



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How to cite this paper Tajik R, Alimoradian A, Jamalian M, Shamsi M, Moradzadeh R, Ansari Asl B, Asafari M. Lead and Cadmium Contaminations in Fruits and Vegetables, and Arsenic in Rice: A Cross Sectional Study on Risk Assessment in Iran. Iranian Journal of Toxicology. 2021; 15(2):73-82. <http://dx.doi.org/10.32598/ijt.15.2.784.1>.

doi <http://dx.doi.org/10.32598/ijt.15.2.784.1>



Article info:

Received: 20 Dec 2020

Accepted: 09 Mar 2021

Online Published: 01 Apr 2021

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ABSTRACT

Background: High levels of heavy metals in food are general concerns including carcinogenic effects. According to studies, the accumulation of heavy metals in crops and consumption of these products in diet, has led to serious health concerns. This study investigated the concentrations of lead and cadmium in popular agricultural products.

Methods: In this descriptive study, some fresh agricultural products (leafy vegetables, tubers, cucurbits and seeds) were collected in the winter and summer. The samples were transferred to the laboratory and stored in a cold room. After the preparation of the samples, the lead and cadmium contents were determined by atomic absorption spectrometry. The data were analyzed statistically on SPSS v. 26 software.

Results: The average concentrations of lead and cadmium in the winter was 37.23 ± 4.7 and 34.77 ± 0.5 while they were 44.12 ± 0.02 and 56.83 ± 0.01 $\mu\text{g/g}$ in the summer. The highest amount of lead content was reported in spinach at an average of 71.25 $\mu\text{g/g}$ and the lowest content was found in watermelon at 30.67 $\mu\text{g/g}$. We observed a significant rise in the concentrations of the pollutants in leafy vegetables during the summer, which was also linked to the farms' locations ($P < 0.05$).

Conclusion: The results showed that the highest amount of lead accumulation was found in leafy vegetables and that of the cadmium was at permissible levels in all produces as recommended by WHO. The risk of non-cancerous diseases was also low. Future studies are warranted to assess the risk of heavy metal toxicity in people, especially in children, the elderly and pregnant women.

Keywords: Lead, Cadmium, Heavy metals, Health risk, Fruits, Vegetables, Toxicity levels

Introduction



Vegetables account for important foods in human nutrition. Fruits and vegetables are the important components of a healthy diet and increases in their

consumption are considered taking the right step toward improving the individuals health status and reducing the risk of diseases [1, 2]. The nutritional and dietary recommendations from the World Health Organization and the Food and Agriculture Organization (WHO/FAO) for the prevention of heart disease, cancer, diabetes and

obesity, among others, call for the consumption of at least 400 g of fruits and vegetables per day per person, excluding potatoes and starchy vegetables [3, 4]. Indeed, the consumption level of agricultural foods is the most realistic representation of the health of a community [5], especially in less developed countries.

Among the environmental pollutants, heavy metals, including lead and cadmium, are of special concerns due to their lack of degradation and adverse effects on humans and animals [6]. Contamination of agricultural products with heavy metals due to the surrounding soil and air is a serious threat to the quality and safety of food products [6]. Both lead and cadmium have shown to be carcinogenic [7]. Based on statistical and epidemiological studies, cancer is the third most common cause of death after heart diseases and accidents in Iran [8]. Mo-haghegh, et al., reported that the rate of cancer incidence in Markazi Province was higher than the national rate [9]. According to a report from the FAO, the permissible weekly intake of lead and cadmium is 0.4-0.6 mg per person. Similarly, human exposure to arsenic causes diabetes, cancer, and diseases of the liver, cardiovascular system and the skin. According to FAO, the maximum allowable arsenic level in plants is 0.1-5 $\mu\text{g/g}$ [10].

Lead has a large spectrum of physical effects, such as neurological and gastro-intestinal distress and oncogenic effects [11]. This metal is a neurotoxin that can affect almost every organ or system in the human body, including bones, teeth, kidneys, and reproductive system. It can also cause chronic health illnesses such as abdominal pain, anemia, neural damage, lung and stomach cancers, irritability, headache, behavioral disorders, and impaired long-term memory [12]. Cadmium exposure may cause severe health effects including lung cancer, and disorders of the reproductive, gastrointestinal, prostate, endocrine and cardiovascular system. The cadmium toxicity further causes bone fracture, hypertension, anemia, central nervous system damages, and liver diseases [13]. The ingestion of cadmium through contaminated foods or crops is associated with the potential risk of post-menopausal breast cancer [12].

Arsenic causes a variety of adverse health effects in humans after acute and chronic exposures. The major pathologies include integumentary alterations, such as pigmentation, hyperkeratosis, and ulcerations. Further, this toxic metal is involved in almost every organ system's pathological conditions due to its genotoxic, mutagenic and carcinogenic effects. However, future research is warranted to improve upon the risk assessment of arsenic at low levels [14, 15].

The average age of cancer has declined because the preventive strategies are of the priorities in the healthcare system [9]. A study conducted in Tehran Province concluded that the cadmium contents in agricultural products and the lead contents in tomatoes and carrots were found to be lower than the limit set by both the Iranian Standards Organization, FAO and WHO [16]. However, the lead content was higher than the permissible limits in lettuce and cucumber in the summer [16]. Further, Bevarsad, et al. [10] investigated the potential risk of heavy metals in some agricultural produces in Isfahan Province versus human health. They evaluated both the hazard index and target hazard quotient, and reported the possibility of non-cancerous diseases occurring in humans due to the consumption of wheats contaminated with arsenic [17]. Multiple routes of exposure to heavy metals exist, including unintentional soil ingestion, intake of contaminated foods, and inhalation of soil particles [18].

The safety of agricultural products has been threatened in recent decades due to the indiscriminate application of herbicides, pesticides and hormones. In addition, the industrial developments have led to an increase in mortality and reduced life expectancy in populations worldwide due to a variety of diseases secondary to environmental contaminants [16]. Thus, this study investigated the lead and cadmium contents of agricultural products commonly consumed in Arak, Iran, a major city in Markazi Province in Iran.

Materials and Methods

Sampling: This study was designed to collect samples on a nationwide basis. Based on the study's protocol, we made monthly visits to the main fruits and vegetables market in the City of Arak, Iran, and listed the produce that were available for the season. Given the study's objectives, we collected and examined the produce that were available to the public. We compiled 18 produce, including fruits, tubers, cucurbits, seeds and leafy vegetables. These included lettuce, spinach, cabbage, potatoes, carrots, onions, sugar beets, beetroots, eggplants, watermelons, melons, tomatoes, mushrooms, pinto beans, soy protein and rice. Samples were collected over nine sessions, based on consignment notes supervised by an official representative of the Iranian Standard Organization in the summer and winter. Each produce sample weighed about 1-3 kg, containing at least 10 pieces of a given produce.

Sample preparation: The collected samples were brought to the Marjaan Khatam Laboratories in Tehran, Iran, on a monthly basis. We stored them in a cold room

at optimal conditions with each produce group bearing an identification tag and number.

Cereals & legumes: To prepare the samples, the non-edible parts were removed and the rest were washed thoroughly, dried and powdered. We weighted 0.5 g of each powdered sample and added 3mL nitric acid and 1 mL oxygenated water, and warmed up the mixture in a Sineo microwave (model # MDS-15; Shanghai, China) to complete digestion. The digested mixture was transferred to a 10 mL volumetric flask was brought up to 10mL by adding deionized water to the mixture.

Preparation of vegetables: We first wash the cucumbers, tomatos, onions, potatoes, eggplants, lettuce and leafy vegetables in distilled water and a slice was cut from each and homogenized in a blender. Then, 3 cc nitric acide was added to one gram of the samples and the mixture was digested in the same microwave unit. For fruit samples with inedible skin, they were peeled before the digestion procedure.

Tests & laboratory methods: After the digestion process, the lead, cadmium and arsenic contents were read on an atomic absorption spectrometer. The lead, cadmium and arsenic concentrations were read at 283.3, 228.8 and 193.7 nm, respectively, using a Varian graphite furnace atomic absorption spectrometer with the the gap width set at 0.5. The chemicals included 65% ultra-pure nitric acid and hydrogen peroxide (Merck, Germany) and the deionized water was prepared with a millipore purifier. All chemicals were of pure and analytical grade.

To analyze the products, we grouped them separately based on their type and the farm locations. The farm geographic areas were divided into five groups. They consisted of **Central:** including Alborz, Dezful, Isfahan, Hamedan, Markazi, and Qazvin Provinces; **Western:** Khorramabad and Ahwaz; **Eastern:** Bojnourd and Mashhad; **Northern:** Mazandaran; and **Southern:** Bandar Abbas, Shiraz and Chabahar. The products were also grouped into the following categories: **Leafy Products:** lettuce, vegetables, spinach and cabbage; **Cucurbits:** watermelons, melons, tomatos, eggplants and edible mushrooms; **Beans & Grains:** beans, soy beans, rice and wheat; and **Tubers:** onions, potatoes, carrots, sugar beets and beetroots.

Risk assessment: The formula provided by the U.S. Environmental Protection Agency [19] and similar studies were used to calculate the risk of individuals with non-cancerous diseases [17, 20]. First, the amount of contaminants absorbed through the foods per kilogram

of body weight per day was calculated using the following formula:

$$EDI=(CF\times IR\times FI\times EF\times ED)/(BW\times AT)$$

with the formula components defined as follows:

EDI= amount of daily contaminant absorption;

CF= concentration of contaminants in food;

IR= daily consumption;

FI = amount of pollutants absorbed by body through food. This coefficient varies between 0.25 and 0.40 [17].

EF = Consumption per year,

ED = Number of years that this food is used and in this study is considered 33.2.

BW = Body weight, which in this study is considered 55.9 kg for adults and 15 kg for children [21].

AT = product of ED in number of days of the year

The equation (THQ = EDI/RFDi) was used to determine the risk of non-cancerous diseases, where RFD is the equivalent of maximum tolerance.

Statistical analyses: Due to the abnormal distribution of lead and cadmium variables, non-parametric statistical tests were used. For each product, the amounts of lead and cadmium in the winter and summer were compared by Mann-Whitney test and this parameter was further compared among the products using Kruskal-Wallis test.

Results

Lead & cadmium concentrations: Figure 1 reflects the amounts of lead and cadmium in the agricultural products for different seasons with the limits permissible by the Iranian Standards Institute. As seen in Figure 1, it was evident that the cadmium and lead contents were higher in the summer than in the winter (Cd, P<0.017; Pb, P<0.0001), which were less than the limits permissible by the Iranian Standards Institute (P<0.05).

The results of measuring lead and cadmium in the agricultural products from the fruits market in Arak are shown in Table 1 based on the limits permissible by the Iranian Standards Institute, and at 95% confidence level.

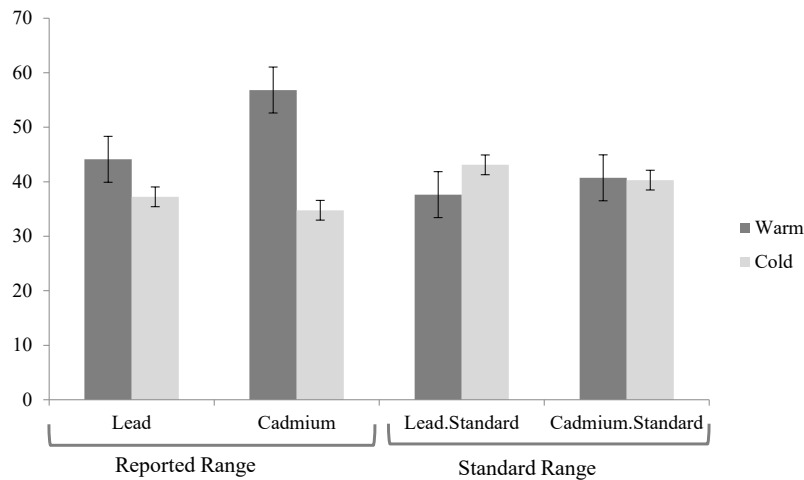


Figure 1. Comparison of lead and cadmium contents in the agricultural products in different seasons

Based on non-parametric tests, the relationship between the amounts of the heavy metals measured in the products per the harvesting seasons, the type of produce and the farm location are shown in Tables 2-4, respectively. Since arsenic was only measured in rice, it could not be generalized to other samples and the data.

As seen in Tables 2, the data indicated that there was significant relationships between lead and cadmium contents in the products and the harvesting season. Clearly, the heavy metal contents in the products were higher in the warm than in the cold season.

The data in Table 3 demonstrate that there was a significant relationship among the various products and the lead contents. Leafy vegetables (61.62), cereals (35.58), tubers (32.68) and finally cucurbits (31.11) had the highest lead contents.

Based on the data presented in Figure 2, spinach (71.25), fresh herbs (64.60) and lettuce (62.86) had the highest lead contents the top three products. The onions (19.71), tomatoes (25.06) and watermelons (30.67)

ranked the lowest, respectively, for their lead contents. Spinach (64.50), rice (55.67), wheat (52.38) had the highest levels of cadmium, while soy beans (15.50), onions (25.21) and tomatoes (26.11) contained the lowest levels, respectively.

The data in Table 4 show that there was a significant relationship among the farm areas and the contents of heavy metals in the products. The highest amount of lead was found in the central regions and the highest cadmium content in the northern regions. Qazvin and Dezful (66.50), Karaj (58.60), and Markazi Province (49.38) had the highest levels of lead contents while Bojnourd, Chabahar, Ahwaz (9.50), Bandar Abbas (27.35) and Khorramabad (33) had the lowest levels in their products. Also, cadmium was reported to be the highest in the products grown in Markazi Province (71), Karaj (56.60), Qazvin (56) and the least level was in Dezful, Bojnourd, Khorramabad, Ahwaz (15.50), Shiraz (29.25), and Isfahan (37.54). Also, the Mean±SD of lead in the groups of leafy vegetables, tubers, cucurbits, legumes and cereals were 90.75±32.52; 0.023±0.022; 0.021±0.020; and 0.078±0.010; while

Table 1. Permissible and standard limits of lead, cadmium and arsenic in the agricultural products

| Heavy Metal | Mean±SD | Min | Max | Z | Sig. |
|--------------------|-------------|-----|------|-------|---------|
| Lead | 47.87±8.56 | 0 | 368 | 1.600 | 0.114 |
| Cadmium | 0.02±0.01 | 0 | 0.22 | 3.984 | <0.0001 |
| Arsenic | 0.05±0.03 | 0 | 0.09 | 4.830 | 0.001 |
| (Standard) Lead | 126±47.66 | 0 | 300 | -7.41 | <0.0001 |
| (Standard) Cadmium | 55.28±21.34 | 0 | 100 | -7.86 | <0.0001 |
| (Standard) Arsenic | 150±0.001 | 150 | 150 | -2.66 | <0.0001 |

Table 2. Relationship among the Pb, Cd and Ar contents in products for different seasons

| Season/Heavy Metal | N | Mean Rank | λ^* | Sig. |
|--------------------|----|-----------|-------------|---------|
| Pb | | | 24.16 | <0.0001 |
| -Warm season | 38 | 44.12 | | |
| -Cold season | 42 | 37.23 | | |
| Cd | | | 8.44 | <0.017 |
| -Warm season | 38 | 56.83 | | |
| -Cold season | 42 | 34.77 | | |
| Ar | | | -1.55 | 0.120 |
| -Warm season | 3 | 7 | | |
| -Cold season | 6 | 4 | | |

λ =Mann Whitney test

those for the cadmium contents were 0.012 ± 0.0100 ; 0.004 ± 0.002 ; 0.010 ± 0.006 ; and 0.050 ± 0.027 . Interestingly, no arsenic was found in any of the samples, with the exception of rice. Based on the descriptive data, the amount of arsenic found in rice samples was 0.05 ± 0.03 , which did not exceed the limit permissible by the Iranian Standards Institute.

Daily lead & cadmium intakes in diet: The consumption of vegetables has an important and undeniable role on the health of consumers. The daily consumption of

vegetables in the household food basket is shown in Table 5. Also, the estimated daily intake of heavy metals in the diet in the two groups of children and adults is presented separately in Table 6. The standard daily tolerable levels of lead and cadmium are 0.0036 and 0.001 (mg/kg), respectively. The daily intake of lead in all of the examined products was higher than the provisional tolerable daily intake. Except for tuberous vegetables and potatoes, the rest of the products were more tolerable than the daily intake for cadmium.

Table 3. Investigation of Pb, Cd and Ar contents in the products

| Products/Heavy Metal | N (P) | Mean Rank | λ^* | Sig. |
|----------------------------|-------|-----------|-------------|---------|
| Pb | | | 24.16 | <0.0001 |
| Leafy vegetables | 21 | 61.62 | | |
| Cucurbits | 22 | 31.11 | | |
| Legumes and cereals | 18 | 35.258 | | |
| Tuber vegetables | 19 | 32.68 | | |
| Cd | | | 8.44 | <0.038 |
| Leafy vegetables | 21 | 61.62 | | |
| Cucurbits | 22 | 31.11 | | |
| Legumes and cereals | 18 | 35.258 | | |
| Tuber vegetables | 19 | 32.68 | | |
| Ar | | Mean | T** | Sig. |
| Legumes and cereals (rice) | 9 | 9 | 4.8 | 0.001 |

λ : Kruskal–Wallis Test. **T: One-sample test

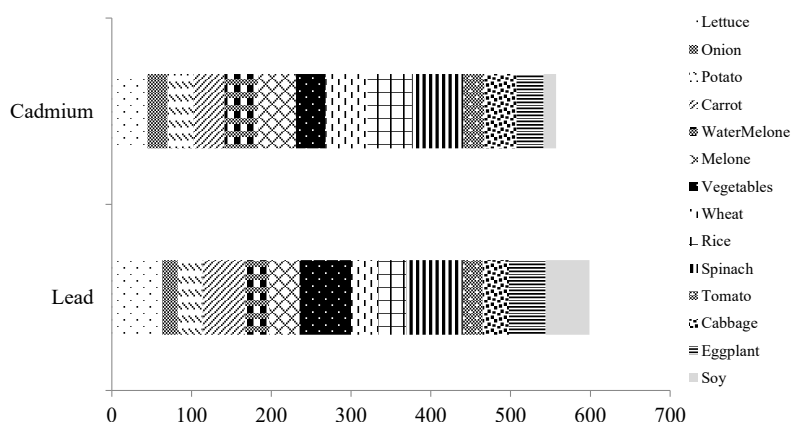


Figure 2. Cadmium and lead contents in the agricultural products

Hazard Index (HI): The hazard index for non-cancerous diseases caused by the entry of heavy metals into the body were 0.0490 for adults and 0.212 for children. The total amount of hazard was obtained by summing up the risks for the heavy metals. When the non-cancerous HI reaches the value of 1, it represents high risk of non-cancer diseases in the consumers [9]. In this study, the target hazard quotient of either lead or cadmium content in the products was less than one. This indicated that there was no risk of non-cancerous diseases posed by any of the heavy metals by consuming the products for the residents in Arak. Evidently, lead is more dangerous

than cadmium because of the potential hazard of nutrients from food intakes.

Discussion

Based on the WHO’s standards [23, 24], the permissible limits for cadmium and lead contents detected in the products were 0.1 and 0.3 (mg/kg), respectively. Also, according to the Iranian Standards Institute, the permissible limits of lead and cadmium in leafy vegetables, cucurbits, tubers and cereals were 0.1, 0.2, 0.1, 0.05 and 0.05, 0.1, 0.05, 0.3 and 0.6 mg/kg [22], respectively. As shown in Figure 1, the average concentrations of lead

Table 4. Relationships among the farm areas, and the lead and cadmium contents in the products

| Descriptive Statistics Growing Location | N (P) | Mean Rank | λ^* | Sig. |
|---|-------|-----------|-------------|--------|
| Lead | | | 13.08 | <0.011 |
| Center | 56 | 46.601 | | |
| South | 8 | 13.29 | | |
| North | 9 | 34.78 | | |
| East | 3 | 18.67 | | |
| West | 4 | 15.38 | | |
| Cadmium | | | 15.33 | <0.004 |
| Center | 56 | 42.85 | | |
| South | 8 | 28.88 | | |
| North | 9 | 55.67 | | |
| East | 3 | 15.50 | | |
| West | 4 | 15.50 | | |

λ^* =Kruskal-Wallis test

Table 5. Consumption of daily vegetables [22]

| Product | Garden Vegetables | Leafy Vegetables | Legumes | Root Vegetables (Except Potatos) | Rice | Wheat | Potatos | Mushrooms |
|--------------|-------------------|------------------|---------|----------------------------------|------|-------|---------|-----------|
| Daily intake | 109 | 58 | 19 | 39 | 110 | 334 | 68 | 0.42 |

Table 6. The amount of daily absorption of cadmium and lead (mg/kg)

| Daily Absorption in Age Categories | | Garden Vegetables | Leafy Vegetables | Root Vegetables Except Potato | Potato | Wheat | Rice |
|------------------------------------|---------|-------------------|------------------|-------------------------------|--------|---------|--------|
| Adults | Lead | 0.0155 | 13.49 | 0.0064 | 0.0111 | 0.167 | 0.0550 |
| | Cadmium | 0.004 | 0.004 | 0.001 | 0.001 | 0.064 | 0.021 |
| Children | Lead | 0.0581 | 50.297 | 0.0239 | 0.0417 | 0.6234 | 0.2053 |
| | Cadmium | 0.0174 | 0.0154 | 0.0041 | 0.0072 | 0.24048 | 0.7919 |

and cadmium in the products during the warm season were 44.12 and 56.83, respectively, both of which were more than those permissible for the winter.

The findings from two earlier studies [16, 25] were consistent with our findings, and the amounts of heavy metals in the products were significantly higher than those in the winter. The reduction was likely due to the frequent rainfall in the winter season. Also, only 2.5% of the products contained lead at higher amounts than the permissible limit. Also, the detected cadmium content was always below the permissible limit in the products. Our findings were also consistent with those of the two previous studies [26, 27] that reported low levels of cadmium contents but higher lead levels in most of the similar products to those tested in the current study. Based on the reviewed literature, the proximity of farm lands to factories and the presence of lead in the air, vehicles' smoke, wind direction and heavy metal sediments from the air on plants have been reported as the major causes of lead contamination in agricultural crops [7]. Also, the use of chemical fertilizers is another reason for lead contamination in popular agricultural products [28].

In this study, the highest amount of lead was detected in lettuce (14.28%) followed by other vegetables (10%). Our findings contradict the findings of two earlier Iranian studies that reported very low concentrations of heavy metals in lettuce [10, 16]. Based on the data shown in Table 3, we found the highest amounts of heavy metals in leafy vegetables, which were consistent with those reported by earlier studies [29, 30]. The sources of cadmium contamination in agricultural soils have been suggested to be human activities and excessive use of chemical fertilizers, especially phosphates, which enter

the soil and is absorbed by the products over time [7, 31, 32]. Fytianos et al. reported that the concentrations of lead and cadmium in the soil around industrial areas were higher than those of the urban areas [33, 34]. Also, the presence of lead in the soil may be associated with painted buildings and their age, since old structures might be covered with lead-based paints, especially those dated before 1975 [35].

The toxicity of heavy metals, even at low levels, is of considerable health concerns to humans due to their accumulation in the vital organs over time [36]. Although acute lead poisoning is not believed to be a major cause of death worldwide, an American study has estimated that the annual mortality rate for blood lead levels is 412,000. The accumulation of lead in children's blood may impair the development of their nervous system [37], causing mild to moderate degrees of mental retardation [38]. High doses of lead in children can result in such diseases as colitis, anemia, depression, CNS damage, manifested as seizure, coma, and even death [37].

Cadmium has toxic effects on the renal, skeletal and respiratory systems, and can cause impaired calcium metabolism, osteomalacia and osteoporosis, and is classified as a human carcinogenic agent [39]. Although cadmium is present in small amounts in the environment, it can travel long distances to urban areas with large populations in a variety of ways, the consumption of contaminated foods being the main route [39, 40]. Cadmium in soil and water is absorbed by agricultural products and aquatic organisms, and accumulate in the food chains [39]. The risk assessments conducted by the current study indicated that there was no risk of non-cancerous disease from either lead or cadmium alone due to

the consumption of the popular agricultural products in Arak. These findings were different from those of a similar study conducted in Isfahan Province. The latter study detected cases of noncancerous diseases in the consumers of the agricultural products [17]. Also, due to the presence of arsenic in rice (0.05 ± 0.03 ppb), the amount of target hazard quotient in it was 0.393 and the risk of non-cancerous diseases was 0.0008. Therefore, the role of Ar in the development of non-cancerous diseases was not reported. This was consistent with the results of another study that documented the daily Ar intake through rice being below the tolerable daily limit [41].

The safe levels of heavy metals recommended by WHO for arsenic in drinking water and soil are 0.01 mg/L and 40 mg/kg, respectively [42]. Based on studies conducted in some parts of Iran, the soil and water contaminations with arsenic of terrestrial origin has been reported [42-45]. Therefore, the use of Ar-contaminated well water to irrigate farms increases the concentration of this toxic metal in the soil and its transfer to such crops as rice and eventually to human communities. Ar is toxic to human health, disrupting the nervous system, blood circulation, digestive tracts and skin, which may lead to death [41].

Conclusions

Based on the results of this study, the soil and water may contain large amounts of heavy metals, such as lead, cadmium and arsenic, that do not always meet the required standard limits. Therefore, it is necessary for the regulatory organizations, such as the Iranian Standards Institute, to take the necessary actions. In the current study, we found that the tested vegetables were contaminated with lead, but its levels were not high enough to cause noncancerous diseases in the consumers.

However, due to the small number of studies conducted to date on the subject and the lack of rigorous evaluation of the adverse effects, especially in sensitive age groups, we recommend that further studies be conducted to examine this subject in-depth. We also recommend that the municipal and industrial wastewaters and drinking water be treated appropriately to remove heavy metals while preventing the cultivation of garden vegetables in contaminated soils in Arak. Also, to prevent harmful accumulation of lead and cadmium in the human organs, there should be effective policies drawn and imposed to prevent growing edible fruits and vegetables in areas with high lead and cadmium contents, e.g. near industrial plants and highways. Upon imposing proper policies, we recommend that identification, labeling and tracking systems be

developed to prevent the spread of contaminated agricultural products to unsuspecting urban areas and consumers.

Ethical Considerations

Compliance with ethical guidelines

This work did not involve human participants or animals; therefore, the ethical guidelines did not apply. The study protocol was approved by Arak University Research Council (Registration #: IR.ARAKMU.REC.1399.270).

Funding

This research was supported by the research project set by the Research Council of Arak University of Medical Science.

Author's contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

This study was part of a comprehensive plan to monitor the agricultural products at Arak University of Medical Sciences. The authors express their gratitude to the Research Council, Arak University of Medical Sciences, for funding this research project. We also appreciate Marjaan Khatam Laboratories, Tehran, Iran, for their assistance with the experiments.

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