Research Paper:
Health Risk Assessment and Determination of Heavy Metal Contamination in Barley Grains in Khuzestan Province, Iran

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ABSTRACT

Background & Aims of the Study: Heavy metal pollution has become a global problem, and their entry into the food chain is considered a threat to humans and other organisms. This study aimed to assess the risk of metals (chromium, nickel, arsenic, copper, zinc, cadmium, and lead) in edible barley grains grown in Khuzestan Province, Iran, in 2019.

Materials and Methods: In this analytical study, five farms of edible barley grains were selected. Then, four stations were selected in each farm. After sampling, barley seeds were prepared by acid digestion method and read by inductively coupled plasma–mass spectrometry. The amount of metal contamination in the grains was estimated by the crop pollution index based on the classification of the US Environmental Protection Agency (USEPA), Hazard Index (HI), and the risk of carcinogenic risk in children and adults.

Results: The highest amounts of heavy metals in barley grains belonged to zinc and the lowest to chromium. According to the USEPA classification, the potential non-carcinogenic risk for children was higher than for adults, and the HI in all study areas was at level 3 for adults and children and level 4 in some stations for children. In the case of barley samples, the average carcinogenic risk for arsenic was 2 per 10000 people for adults and 4 per 10000 for children, and cadmium was 1 per 10000 people for adults and 2 per 10000 for children in the population of the province. These figures were estimated to be at a safe level.

Conclusion: Barley is one of the foods of the people of the region. The use of its contaminated amounts due to the biological accumulation of heavy metals can create health risks for its consumers in the long run.

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1. Introduction

Cereals, especially wheat, rice, and barley are among the main products and an integral part of the human diet. They play a vital role in human growth by providing carbohydrates, proteins, minerals, and micronutrients [1]. Consumption of wheat grains and other grains is safe when the accumulation of metals is within the permissible limits [2]. Khuzestan Province in Iran has long had many opportunities in the agricultural sector. Geographical features (fertile soil, abundance of permanent and seasonal rivers, water and land roads, etc.) have made this province one of the agricultural hubs of Iran [3]. The resistance and stability of heavy elements in the soil are much longer than other contaminants, and soil contamination by heavy metals is almost permanent [4]. Iron and steel industries, mining, road transport, power plants, and waste incineration are among the causes of heavy metals entering soil and water in surface ecosystems [5-7].

Generally, as the concentration of heavy metals in the soil increases, the amount of bioavailability of these elements increases [8]. Excessive absorption of these elements by plants and entering the food chain threatens human health [9-12]. These metals in high amounts can cause morphological disorders, growth retardation, increased mortality, and genetic effects in humans [13, 14]. In Iran, 50% of the most common cancers are related to the gastrointestinal tract, of which gastric cancer is more common. Research has shown that environmental pollution (from industrial areas or agricultural fertilizers) and heavy metals in the environment and the food chain can be among the most influential and essential factors in causing this type of cancer [3, 5].

Prolonged consumption of foods with high concentrations of heavy metals may increase the risk of disease in humans [15]. However, when these metals are consumed for a long time during their lifetime, they can have destructive effects even in safe doses [16]. Therefore, to assess the risk of a particular element, the period of consumption should also be considered [17].

Khuzestan Province has a high potential for the production of diverse, strategic, and industrial crops. However, the province is affected by many industrial activities such as refineries, petrochemicals, steel industries, sugarcane factories, oil and gas companies, and improper use of fertilizers, pesticides, and unhealthy irrigation. Thus, these issues double the importance of this study [18]. Accordingly, the main purpose of this study was to determine the level of heavy metal contamination of chromium, nickel, arsenic, copper, zinc, cadmium, and lead in barley grains grown in Khuzestan Province and to assess the health risk of their consumption in the age groups of adults and children.

2. Materials and Methods

Study area

Khuzestan Province, with an area of 64236 km2 is located between the latitudes of 29° 57› and 33° 0› N and between the longitudes of 47° 40› and 50° 33› E of the Greenwich meridian in southwestern Iran. The province›s total population is 4710509 people, equivalent to 5.89% of the country›s population. The geographical location of the study area is shown in Figure 1. Karun river, as the largest river in Iran, passing through large areas of Khuzestan Province, has contributed significantly to the region›s agriculture. However, along the way, the river is closely related to a group of the most important industries such as metallurgy, petrochemicals, and oil and is also in contact with an essential part of municipal and agricultural wastewater. So the presence of any metal elements, especially heavy metals, in wastewater to this river can be considered a potential cause of pollution and danger [19].

Study sampling

The present study is an analytical study. In the present study, to estimate the risk of barley consumption in Khuzestan Province, 40 farms in 10 cities of the province were designated for sampling. These farms were selected for barley from Hindijan, Hamidiyeh, Azadegan, Shadegan, and Shushtar counties.

Selected cities are marked with an asterisk in Figure 1. In each city, four farms were designated for sampling. After selecting the farms, the sampling was done during the barley harvest season in 2019. Considering a total of 5 areas, four stations in each area, and three replications, we prepared 60 samples. The samples were placed in polyethylene plastics and transported to the laboratory.

Analysis and preparation of samples

Barley samples were washed with distilled water and dried in the air. Then, they were ground using a mill and weighed 1 g of each with a digital scale and were reduced to ashes at 550°C for 5 h. Plants containing barley ash were placed in a desiccator to cool. In the next step, 2.5 mL of 6.0M HNO_3 was added to dissolve the
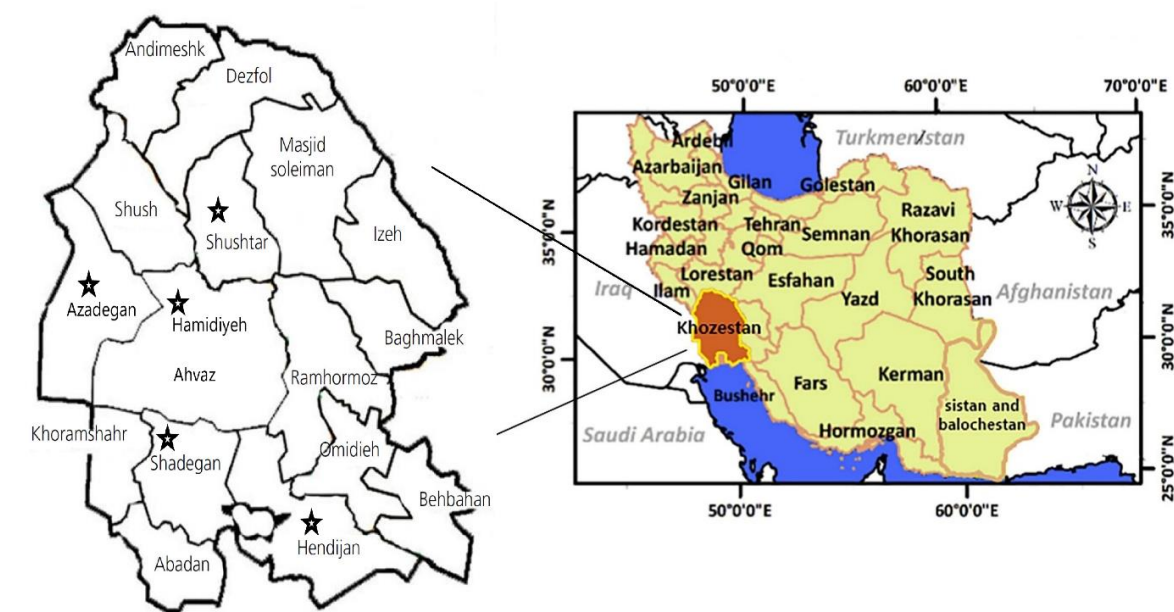
Figure 1. Geographical Location of the Study Area

samples [20]. All required test dishes were washed with 0.30 nitric acid, rinsed with deionized distilled water, and dried in an oven [21]. The dissolved samples were then passed through a paper filter and volumized in a 20-mL flask. The resulting solution was analyzed by Inductively Coupled Plasma–Mass Spectrometry (ICP-MS) to determine the concentration of heavy metals in barley samples [22]. The samples were analyzed in Zarazma Mineral Studies Laboratory in Tehran. Data analysis was performed using SPSS software, and graphs were drawn using Excel software.

Product crop pollution index

Heavy metal contamination levels in crops are assessed using the product pollution index (CPI). In this study, CPI is obtained by dividing the concentration of heavy metals determined in barley samples by the standard value of each metal. The CPI is calculated from Equation 1.

Equation 1) $CPI = \dfrac{pi}{Spi}$

In Equation 1, Pi represents the measured concentration of element i in the plant, and Spi represents the standard allowable value of each element in the plant seed. For this purpose, we used the value of the standard proposed by FAO/WHO and the Chinese national standard (because it covers more complete elements than JECFA) [23].

Heavy metal health risk assessment

To assess exposure to heavy metals, we calculated the estimated daily intake index (EDI, mg/kg/d) according to Equation 2 provided by the US Environmental Protection Agency.

Equation 2) $EDI = \dfrac{Cmetal \times EF \times ED \times IR}{BW \times TA}$

In Equation 2, EDI is the rate of chronic daily uptake of heavy metals (mg/kg/d). Cmetal is the rate of concentration of heavy metals in the crop consumed (mg/kg). IR refers to the average daily consumption of barley crops (g/d). ED denotes the exposure period (60 years for adults and 12 years for children). EF is the exposure frequency (365 days). TA refers to the mean exposure time (days) multiplied by EF in ED (for non-carcinogenic risk) and for carcinogenic risk (365 × 70 years). Finally, BW is the average body weight in kg (15 kg for children and 70 kg for adults) [28, 29].

The Hazard Quotient (HQ) indicates the potential non-carcinogenic risk for each heavy metal. HQ is defined as the EDI rate (mg/kg/d) to the reference dose (RfD, mg/kg/d) and is an estimate of the daily exposure of the human population that is improbable to indicate a high risk of life-threatening side effects. The method of calculating HQ is given in Equation 3.

Equation 3) $HQ = \dfrac{EDI}{RfD}$

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Table 1. Statistical summary of studied heavy metal concentrations in barley grain and crop contamination index (CPI) for this plant (N= 60)

Contamination Index in Crops (Barley)	Cr	Pb	Cd	As	Cu	Zn	Ni
Average concentration of metals in barley (mg/kg)	0.03	0.29	0.059	0.031	4.29	18.46	0.36
Standard deviation of metal concentration	0.013	0.131	0.025	0.021	1.86	5.25	0.16
The least	0.01	0.06	<LOD	<LOD	1.2	7.5	0.11
The most	0.057	0.63	0.1	0.09	8.3	28.3	0.69
World Food Safety Standard * (mg/kg)	0.02	0.2	0.1	0.126	4.7	24	0.34
Internal standard ** (mg/kg)	-	0.15	0.06	0.15		-	-
Average CPI for each metal in wheat grain	1.50	1.45	0.57	0.24	0.91	0.77	1.06
CPI standard deviation	0.62	0.59	0.24	0.13	0.37	0.22	0.41
Number of infected samples based on CPI index	33	42	0	0	27	6	26
Percentage of infected samples based on CPI (%)	55	70	0	0	45	10	43.3

* [24-26], **[27]

LOD for As: 0.005, Cu: 0.013.

In Equation 3, the RfD values for selected heavy metals in different exposure pathways are determined by the US Environmental Protection Agency (USEPA). According to the assessment of the overall potential risk imposed by more than one heavy metal, HQ can be added to generate a hazard index to estimate the consolidated risk (Equation 4).

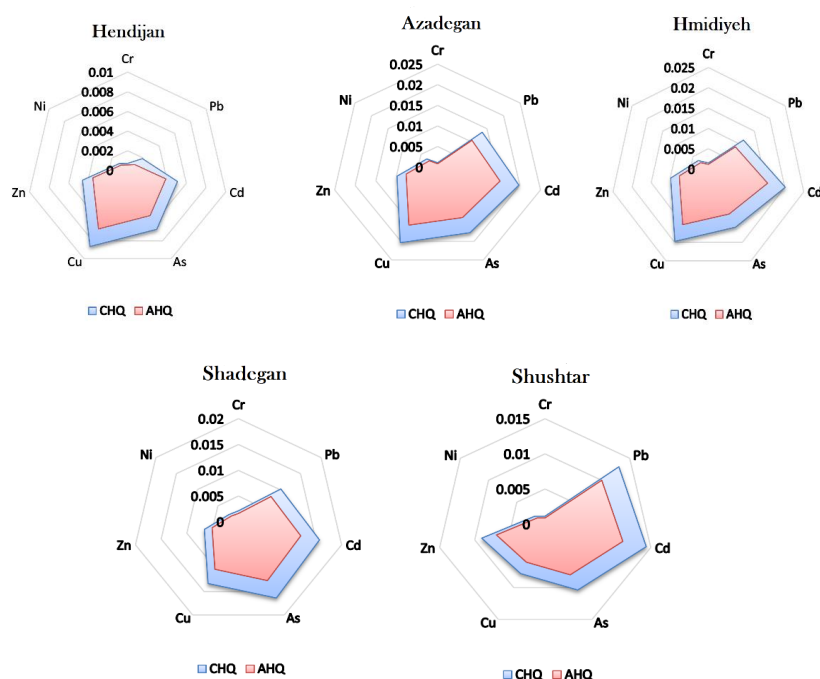$$\text{Equation 4) } Hazard\ Index = \sum_{n=1}^{n} \frac{EDIn}{RfDn}$$

Figure 2. Risk of each heavy metal in HQ for children and adults due to barley consumption over one year

Table 2. Carcinogenic Hazards (CR) of heavy metals (Arsenic and Cadmium) due to barley consumption for adults and children

Content	Risk at Carcinogenicity of Children		Risk at Adult Carcinogenesis	
Heavy metals study stations	Cancer risk (Cd)	Cancer risk (As)	Cancer risk (Cd)	Cancer risk (As)
Mean (CR) of the whole area in the case of barley:	2.8×10^{-6}	6.04×10^{-6}	2.2×10^{-6}	4.6×10^{-6}

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If the HI exceeds 1, there is a chance of non-carcinogenic effects, and the probability increases with increasing value. Otherwise, there is probably no non-carcinogenic effect [30].

For carcinogens, the risk is estimated as an increased likelihood of developing cancer in a person's lifetime due to exposure to potential carcinogens. Potential carcinogenic risk can be assessed using Equation 5:

Equation 5) *Cancer Risk=EDI×SF*

In Equation 5, Cancer Risk (CR) is the risk of carcinogenic risk (without dimensions). It refers to the total risk of carcinogenic risk, and SF is the carcinogenic slope factor of each metal (1/mg/kg/d). The overall risk of carcinogenicity is equal to the total risk of all exposure pathways due to all individual metals. The SF values for selected heavy metals at different exposure paths are determined by USEPA. The permissible total risk range for legislative purposes is 10^{-6} to 10^{-4}. Typically, a CR less than or equal to 10^{-6} indicates relatively effective immunity, and a CR equal to or greater than 10^{-4} indicates a potentially high carcinogenic risk.

3. Results

According to Table 1, the amounts of heavy metals in the studied atmosphere in the five regions of the province are ascending and as follows:

Zn (5.25±18.46) Cu>(4.29±1.86) >Ni (0.36±0.16) Pb>(0.29±0.131)>Cd(0.059±0.025)>As(0.031±0.021)> Cr (0.03±0.13)

The highest concentration is related to zinc, and the lowest to chromium. According to the crop contamination index, the number of infected samples and the corresponding percentage of each are presented in Table 1.

Equation 3 was used to calculate the non-cancer risk of the studied elements for one year. HQ is divided into two age groups of adults and children in 40 stations of 10 study areas and is shown in Figure 2. The non-carcino-

genic risk classification of heavy metals was performed by the US Environmental Protection Agency [31].

According to the USEPA classification, arsenic belongs to group A carcinogens, and the IARC has also introduced cadmium as a carcinogen. The carcinogenic risk of these two elements was calculated with Equation 5 in different regions, and the results are presented in Table 2 separately for adults and children.

4. Discussion

As mentioned in Table 1, the lowest CPI belonged to arsenic (0.24±0.13) mg/kg and the highest to chromium (1.50±0.62) mg/kg. According to Table 1, 10%, 43.3%, 45%, 55%, and 70% of the barley samples for zinc, nickel, copper, chromium, and lead were above the standard, respectively. In the case of cadmium and arsenic, no sample exceeded the standard [32].

According to the study results, in the Ahvaz region, HQ values of cadmium, arsenic, and copper for children are above 1 and at level 3 (medium) chronic risk (chronic). The non-carcinogenic health risk of heavy elements in children increases significantly due to the increase in the intake of the element relative to their body weight [17, 33, 34].

Figure 2 shows the degree of involvement of each heavy element affecting the HQ index for children and adults in each of the five regions (in the case of barley species) separately. In Hindijan, Hamidiyeh, and DashteAzadegan, copper, and cadmium have the highest impact on the potential non-carcinogenic risk index in both children and adults. In Shadegan and Shushtar regions, Lead, arsenic, and cadmium have played the most crucial role in increasing the HQ.

According to Table 2, the carcinogenic risk by consuming barley in the study areas for adults was average and for children was low. Therefore, the consumption of barley in the study areas has little effect on increasing the risk of cancer. Findings of this study were consistent with the research of Djahed et al. in Iranshahr [22], Doabi et al. in Kermanshah [24], Rezapour et al. in West

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Azerbaijan Province [33] in Iran, and other similar studies on the higher risk of carcinogenicity in children compared to adults [35, 36].

5. Conclusion

In areas with high heavy metal content in the soil and high enrichment coefficients (heavy enrichment coefficients) of heavy metals, we can reduce the potential harm to human health by controlling the source of contamination, adjusting cropping practices, setting planting patterns, proper use of fertilizers or even reduce land use change. The presence of nickel and chromium in agricultural soils may be due to anthropogenic resources such as fertilizer application, precipitation due to vehicle traffic, and industrial activities [37]. Chromium and cadmium in agricultural soils may be due to the use of wastewater for field irrigation [38]. On the other hand, the association of nickel with copper in agricultural soils is often due to the use of municipal wastewater for irrigation [39]. The presence of copper and arsenic in the first component reflects the industrial activities or direct use of pesticides for products, which is mainly related to the use of pesticides and fungicides [40]. Copper is considered an indicator of agricultural activities that are specifically related to commercial fertilizers [37].

In this study, the risks of barley consumption (HQ and CR) for children were much higher than for adults. This finding indicates that children are more sensitive to consuming foods contaminated with heavy metals than adults. According to the USEPA assessment, the average Risk of Carcinogenicity (CR) in the province's barley consumption for arsenic is 4 adults per million (low-risk level 2) and 6 children per million (low-risk level 2), and for cadmium, it was estimated at 2 adults per million (low-risk level 2) and 3 children per million (low-risk level 2) in the population of the province, which means that the consumption of barley in Khuzestan Province in the study areas is not endangered during the long life of the consumer.

Ethical Considerations

Compliance with ethical guidelines

This article is a meta-analysis with no human or animal sample.

Authors' contributions

Methodology: Maryam Mohammadi Rouzbahani; Software, Validation, Formal Analysis: Sina Attar Roshan; Writing – Original Draft Preparation:Masoumeh Fouladi; Writing – Review & Editing: All outhors.

Conflict of interest

The authors declared no conflict of interest.

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