

## Heavy Metal Concentration of Wheat Cultured in Golestan Province, Iran and Its Health Risk Assessment

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### ABSTRACT

**Introduction:** Exposure of grain products in polluted soil lead to adverse effects on human health. In this study, concentrations of HM (As-Cr-Hg) were analyzed in wheat grain cultured in Gonbad-e-Kavus City, Golestan province, Iran. Furthermore, its potential health risk was evaluated among residents.

**Materials and Methods:** The sampling sites were located in arable lands. After separating the wheat grains and cleaning them, the seeds were collected in plastic bags for analysis by ICP/MS method. Digestion of samples was performed with Multi wave PRO microwave apparatus.

**Results:** The mean concentrations of Arsenic (As), Chromium (Cr), Mercury (Hg), and Nickel in wheat seeds were  $0.186 \pm 0.08$ ,  $0.9 \pm 0.07$ ,  $0.021 \pm 0.019$ , and  $0.5 \pm 0.17$ , respectively. The results showed that concentrations of HM in wheat were as follow: Cr > Ni > As > Hg. The Hazard Quotient (HQ) was significantly different among various HMs. The largest HQ was related to As ranging from 0.33 to 13.3. The lowest HQ was attributed to Cr, which may be related to its high RfD =  $1.5 \text{ mg kg}^{-1}$ .

**Conclusion:** Different HMs varied largely in terms of their HQ. Regarding the exposed people, As and Hg had the highest contributions to the aggregate risks of HMs, while Cr had the lowest contribution. Although the findings showed low environmental concentrations of the studied elements and implied no danger to human health, it should be considered that many non-cancerous conditions weaken the immune system and prone the human beings to cancerous diseases.

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### Introduction

Environmental contamination is a major threat to arable lands, water resources, and food chain. This contamination is originated from various natural sources as well as human activities including combustion process, industries, and mining<sup>1,2</sup>. Heavy metals (HMs) are the most hazardous pollutants for the environment and human health. Once excessive HMs enter the environment, such as soil, water, or air, they can

cause harm to human beings' health by consuming food products grown in these contaminated environments<sup>3,4</sup>. Consumption of contaminated food is one of the main ways through which HMs enter the human body<sup>5</sup>. However, extreme retention of HMs in the environment has increased the risk to human health. For example, HMs have toxic effects on human white blood cells. In this regard, Arsenic (As), Chromium (Cr), lead (Pb), and Mercury (Hg) are endocrine-disrupting

chemicals. Heavy metals can be absorbed by foods from soil and transfer to the higher food chain<sup>6,7</sup>. Many studies investigated the storage and transfer of HMs in the soil–wheat system<sup>7-12</sup>. Wheat plant is susceptible to many harmful HMs that transfer from roots and branches to seeds<sup>13</sup>. Wheat germ contamination to HMs inhibits germination and increases the root length<sup>14</sup>. Human health can be endangered by dietary exposure to toxic metals by food consumption, especially in developing countries. As a result, the risk of environmental pollutants in foods should be assessed. The study goal was to determine concentrations of HMs in wheat seeds cultivated in different locations of Gonbad-e-Kavus and examine the risk of adverse health effects caused by HMs (Hg, As, Cr) through consumption of wheat with the risk assessment for human health.

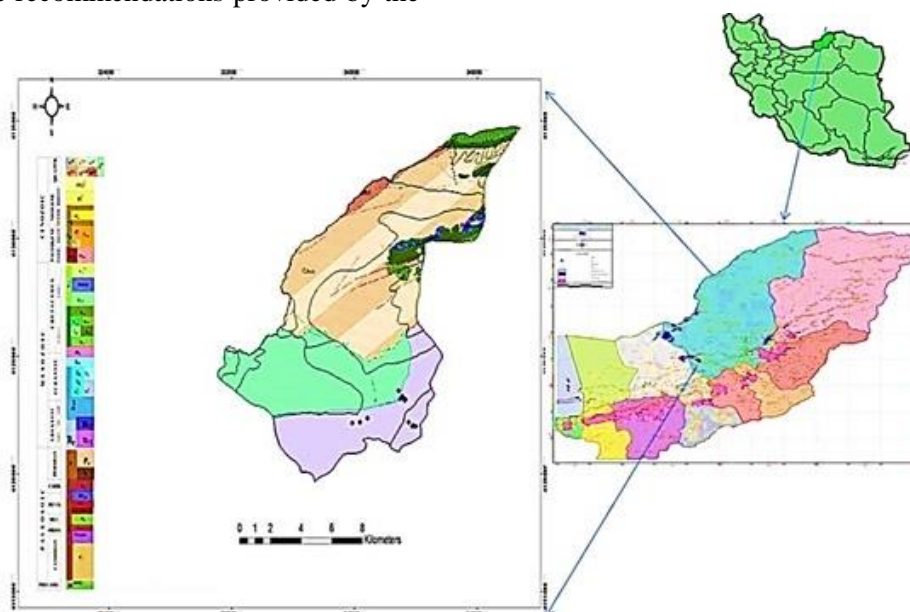
## Materials and Methods

### Study site and sampling

The city of Gonbad-e-Kavus is located in 55° 18' longitude and 37° 17' latitude in the northern and central parts of Golestan province (Figure 1). This area is mostly covered by volcanic plains. In order to conduct the study, the sampling sites were selected based on geological water studies and according to the recommendations provided by the

regional water authorities (Figure 1). Sampling was carried out from the selected wheat agricultural lands in June 2016. Random sampling method was applied among all collected samples from Gonbad-e-Kavus City and its three surrounding villages with the highest incidence of cancer. As a result, three samples were taken from each village and one sample was selected from Gonbad-e-Kavus City, which made a total of 10 samples. Samples were sent to the laboratory, their stems and leaves were isolated from seeds and the concentration of HMs was measured.

Before the wheat harvest time (May 2016), seeds of winter wheat were collected (Figure 1). Considering the soil type and land, the sampling sites were located on the agricultural land by a Global Positioning System tracker with an interval of approximately 4 km. Each site consisted of three to six sub-samples that were located in about 300 m<sup>2</sup>. Wheat samples were collected, put in plastic bags, and taken to the laboratory. Later, the wheat seeds were washed completely using tap water; then, they were rinsed by deionized water, and oven-dried at 70 °C to the constant weight. In the next step, these samples were ground to pass through a 0.149 mm mesh and stored in sealed polyethylene bags for heavy metal analysis<sup>15</sup>.



**Figure 1:** Sampling sites of wheat seeds in Gonbad-e-Kavus

### Wheat grain analyses

After separating and cleaning the wheat grain, the seeds were kept in plastic bags for analysis by ICP/MS method<sup>16</sup>. Digestion of samples was performed by Multi wave PRO apparatus. In this method, 0.5g of the homogenized sample was added into the microwave vessels followed by adding nitric acid and hydrochloric acid. In the next stage, the digestion was performed by adjusting the temperature and pressure. After digestion and cooling, the sample acid was evaporated and diluted with deionized water. Next, concentration of HMs was measured by inductively coupled plasma-mass spectroscopy (Germany, Spectro Genesis) with a Silicon drift detector. The device was adjusted to create 1400 watt radio-frequency, a plasma gas flow rate of 12 liters per minute, auxiliary gas flow rate of 0.8 liters per minute, and a nebulizer dispenser gas flow rate of 0.8 liters per minute. In this method, about 45 elements were simultaneously determined and recorded by the device. Later, concentration of HMs and elements were compared with other highly concentrated elements.

### Risk Assessment Methods

Health risk is defined as the probability of dangerous effects of the environmental contamination on human health. In this research, the health risk assessment model, generated by United States Environmental Protection Agency (U.S. EPA), was applied to investigate the health risk of HMs in adults. This risk assessment method was also employed by other researchers (EPA, 1992)<sup>17</sup>. The health risk assessment consisted of hazard identification, dose-response assessment, exposure assessment, and risk characterization,

In evaluating the risk of elements with potential toxicity in humans, two factors of estimated daily intake (EDI  $\text{mgkg}^{-1} \text{day}^{-1} \text{BW}$ ) and Target Hazard Quotients (THQ) were considered<sup>15, 18</sup>. In estimating the daily intake, elemental concentrations in food and daily food consumption are important. Therefore, the human body weight has an impact on the tolerance of pollutants. This index is calculated by the following formula:

$$EDI = \frac{CF \times IR \times EF \times ED}{BW \times AT}$$

Where, CF is the median concentration of HMs in wheat seed ( $\text{mg kg}^{-1}$ ); IR is the consumption rate of wheat seed ( $\text{kg person}^{-1} \text{day}^{-1}$ ); EF is the exposure frequency (365 days  $\text{year}^{-1}$ ); ED is the exposure duration equal to the average lifetime (70 years for adults and 6 years for children were assumed in this study)<sup>19</sup>; BW is the average body weight (61.6 kg for adults<sup>20</sup> and 18.7 kg for 0–6 year-old children<sup>21</sup>); and AT is the average exposure time for non-carcinogenic effects ( $ED \times 365 \text{ days year}^{-1}$ ). In the case that the EDI exceeds this threshold (i.e., if HQ exceeds unity), potential non-cancer effects may be expected. In other words, greater values of HQ show greater levels of concern<sup>22</sup>.

The potential non-cancer risk for individual HMs is calculated by THQ and can be calculated as follows:

$$THQ = \frac{EDI}{RfD}$$

Where, RfD is the reference oral dose that represents an estimation of the human beings' daily exposure without any appreciable risk of deleterious effects during life time.

In this paper, RfD were based on  $3 \times 10^{-4}$ ,  $3 \times 10^{-4}$ , and  $1.5 \text{ mgkg}^{-1} \text{day}^{-1}$  for Hg, As, and Cr (III), respectively<sup>23</sup>.

To investigate the total potential for non-carcinogenic effects from more than one heavy metal, the Hazard Index (HI) was developed according to the Guidelines for Health Risk Assessment to Chemical Mixtures of EPA<sup>17</sup> as follows:

$$HI = \sum THQ$$

If either TQH or HI exceeds unity, a high risk of non-carcinogenic effects is implied.

### Ethical issues

This study was conducted with approval of Golestan University of Medical Sciences. Research Ethics Code was IR.GOUMS.REC.1398.034.

### Results

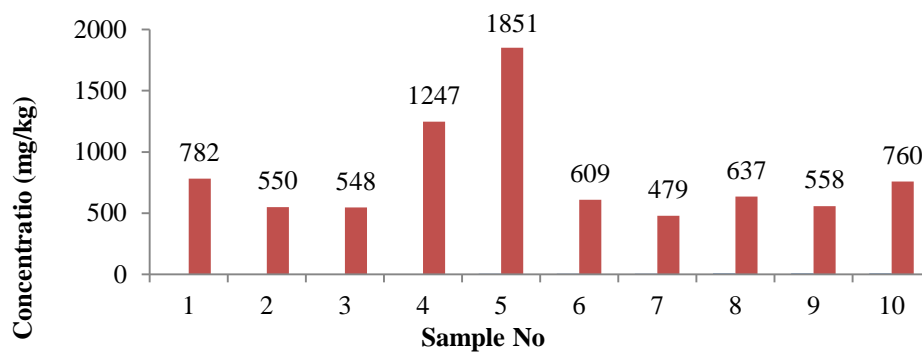
#### Concentration of heavy elements in samples

Concentrations of HMs in wheat seeds collected from the study area are presented in Table 1.

Concentration of phosphate (mg kg<sup>-1</sup> dry wt.) in wheat seed in Gonbad-e-Kavus from the study area are presented in Figure 2.

**Table 1:** Concentrations of HMs (mg kg<sup>-1</sup> dry wt.) in wheat seed in Gonbad-e-Kavus (N = 10)

Metal	Minimum	Maximum	Mean	Std. Deviation
As	0.06	0.36	0.186	0.08695
Cr	0.78	1.02	0.9	0.07483
Hg	0	0.05	0.021	0.01912
Cu	4.8	7.2	5.88	0.88
Se	0.3	0.42	0.36	0.04
Fe	36.0	118.8	62.1	24.73
Mn	28.8	52.2	40.1	8.16
Mo	0.48	1.02	0.76	0.18
Zn	18.0	36.6	25.5	5.8
Ni	0.00	1.92	0.5	0.17



**Figure 2:** Concentration of phosphate (mg kg<sup>-1</sup> dry wt.) in wheat seed in Gonbad-e-Kavus (N = 10)

The means of As, Cr, and Hg in wheat seeds were 0.186 ± 0.08, 0.9 ± 0.07, and 0.021 ± 0.019, respectively. The highest and lowest concentrations of metals in wheat were related to iron and mercury, respectively. The concentration of metals from high to low was as follows:

Fe > Mn > Zn > Cu > Cr > Mo > Ni > Se > As > Hg

Results of HQ for HMs are shown in Table 2-4. Potential of Non-Carcinogenic Risk associated with exposed people was females > males > children. These results indicate that the risk of non-cancerous diseases is high in people consuming wheat.

**Table 2:** HQ and EDI of individual HMs in peoples’s exposure to As

No of sample	EDI As			HQ As Non-Carcinogenic Risk			HQ As Carcinogenic Risk		
	Childs	Adults		Childs	Adults		Childs	Adults	
		Males	Females		Males	Females		Males	Females
G-1	0.0008	0.003	0.004	2.6	10	13.3	0.0012	0.0045	0.006
G-2	0.0004	0.001	0.002	1.3	3.3	6.6	0.0006	0.0015	0.003
G-3	0.0005	0.002	0.003	1.6	6.6	10	0.00075	0.003	0.0045
G-4	0.0002	0.001	0.001	0.66	3.3	3.3	0.0003	0.0015	0.0015
G-5	0.0002	0.001	0.001	0.66	3.3	3.3	0.0003	0.0015	0.0015
G-6	0.0001	0.0006	0.0007	0.33	2	2.3	0.00015	0.0009	0.00105
G-7	0.0005	0.002	0.003	1.6	6.6	10	0.00075	0.003	0.0045
G-8	0.0004	0.001	0.002	1.3	3.3	6.6	0.0006	0.0015	0.003
G-9	0.0005	0.002	0.003	1.6	6.6	10	0.00075	0.003	0.0045
G-10	0.0002	0.001	0.001	0.66	3.3	3.3	0.0005	0.0015	0.0015

**Table 3:** HQ and EDI of individual HMs in peoples's exposure to Hg

No of sample	EDI Hg			HQ Hg Non-Carcinogenic		
	childs	Adults		childs	Adults	
		Males	Females		Males	Females
G-1	0.0001	0.0005	0.0006	0.33	1.6	2
G-2	0.00007	0.0003	0.0003	0.23	1	1
G-3	0.0001	0.0005	0.0006	0.33	1.6	2
G-4	0.00002	0.0001	0.0001	0.06	0.33	0.33
G-5	0	0	0	0	0	0
G-6	0.00004	0.0002	0.0002	0.13	0.66	0.66
G-7	0	0	0	0	0	0
G-8	0.00007	0.0003	0.0003	0.23	1	1
G-9	0.00004	0.0002	0.0002	0.13	0.66	0.66
G10	0	0	0	0	0	0

**Table 4:** HQ and EDI of individual HMs in peoples's exposure to Cr

No of sample	EDI Cr			HQ Cr Non-Carcinogenic		
	childs	Adults		childs	Adults	
		Males	Females		Males	Females
G-1	0.0022	0.0101	0.012	0.014	0.067	0.08
G-2	0.0019	0.0088	0.0108	0.012	0.058	0.072
G-3	0.0018	0.008	0.0101	0.012	0.053	0.067
G-4	0.00211	0.0094	0.011	0.014	0.062	0.073
G-5	0.0022	0.0101	0.012	0.014	0.067	0.08
G-6	0.0019	0.0088	0.0108	0.012	0.058	0.072
G-7	0.00211	0.0094	0.011	0.014	0.062	0.073
G-8	0.0019	0.0088	0.0108	0.012	0.058	0.072
G-9	0.0024	0.0107	0.013	0.016	0.071	0.086
G10	0.0022	0.0101	0.012	0.014	0.067	0.08

## Discussion

The mean of As concentration was  $0.18 \text{ mg kg}^{-1}$  ( $0.06$  to  $0.36 \text{ mg kg}^{-1}$ ). The skewness rate of As was  $0.608$ , which showed a fairly uniform distribution.

Arsenic compounds are generally adsorbed to plant species with arsenite and arsenate. Moreover, high concentrations of as in either arsenite or arsenate species are dangerous for the plant and poison it<sup>24</sup>. Arsenic, a non-essential element for plants, enters the environment through chemical and industrial activities and is in competition with chemical phosphate absorption<sup>25</sup>.

One of the determinants of as uptake in plants is the concentration of phosphate in the soil solution, where the two elements may compete for adsorption to the soil and root surface. The uptake of arsenate and phosphate by the plant root is significantly dependent on their concentration in

the environment and their physical and chemical similarity. In this regard, it can be said that in soil samples with high phosphate, As can be less absorbed by plants or wheat (Figure 2).

The mean chromium concentration was  $0.9 \text{ mg/kg}$  and its maximum concentration was  $1.02 \text{ mg/kg}$ , while the minimum chromium concentration was  $0.78 \text{ mg/kg}$ . The skewness of Chrome was zero.

Concentration rate of Hg in wheat seed ranged from  $0.000$  to  $0.05 \text{ mg kg}^{-1}$ , which was lower than that of vegetables<sup>3</sup>.

### *Correlation between elements in wheat collected from the study areas*

In this study, due to the normality of the data to determine the correlation coefficient between the data is based on Pearson correlation coefficient. Copper had a very strong positive correlation ( $r > 0.7$ ) with manganese at the significant level of  $>$



0.01, indicating that it has a common origin and similar geochemical behavior. Copper also had a very strong positive correlation with iron ( $r > 0.7$ ) at the significant level of  $> 0.01$ , which indicates that iron increased with increase of copper. The maximum positive correlation coefficient ( $r = 0.737$ ) was related to iron and copper. The common origin and geochemical behavior of these two elements can be justified by referring to their positive and very strong correlation. Zinc was positively correlated with phosphorus, iron, and copper ( $r > 0.6$ ). Zinc, iron, and copper have almost identical geochemical origin and behavior, but have a positive correlation with zinc and phosphorus. This can be due to the high zinc transfer coefficient in plants, which is more mobile than other elements and reaches the grain easily. Although phosphate fertilizers are highly effective, plants absorb high levels of phosphorus. Mercury had a strong negative correlation ( $r > -0.6$ ) with phosphate at the confidence level of  $> 0.01$ . This indicates that phosphorus accumulates more in plant due to phosphate fertilizer and it can bond with most lithophilic elements since it is a lithophilic element. However, the elemental mercury is siderophilic and almost non-mobile; so, the geochemical behavior of mercury and phosphate is not the same because of their negative correlation. Nickel had a negative significant correlation with molybdenum ( $r > -0.6$ ), representing that nickel has a detrital origin and is originated from outside the sedimentary basin. However, molybdenum has a separate origin and falls into a separate group that can be traced back to industrial and agricultural activities. This results according to Meharg study<sup>25</sup>.

#### **Risk of individual HMs**

For various exposed people, HQs of HMs were above 1. In other words, the daily intake of each individual metal by consuming wheat seeds can cause harmful health effects in Gonbad-e-Kavous residents. This results according to Wang M et al study<sup>26</sup>. They investigated four heavy metals of cadmium, lead, copper and zinc in soil, water and plants in this area. They concluded that the

presence of cadmium and lead in the environment increased the risk of several types of cancer, especially abdominal, esophageal and lung cancers, there was a significant positive correlation between male and female sexes, and mentioned metals showed no correlation with the prevalence of this disease<sup>26</sup>.

The order of HQ in the exposed people was as follow: County adults  $>$  Urban adults, Country children  $>$  Urban children. The highest HQ was related to As, ranging from 0.33 to 13.3. The lowest HQ was attributed to Cr, which can be related to its high RfD ( $1.5 \text{ mg kg}^{-1}$ ). The non-cancer HQ following exposure to Cr was less than 0.009 for all exposed people. Wang et al. also found that Cr had the lowest HQ in consumption of vegetables and fish<sup>3</sup>.

#### **Conclusion**

The concentration rates of HMs in wheat seed were as follows:  $\text{Cr} > \text{Ni} > \text{As} > \text{Hg}$ . Although the median concentrations of HMs in wheat seed were lower than the permissible limits of standards, many wheat seed samples presented high concentrations of HMs. The difference in concentrations of HMs at various locations suggested that the environmental effect differed significantly from other HMs. The possible health risk of these HMs was significant, but the total risk of three HMs for country residents (both children and adults) was higher than 1. This indicates that crucial attention should be paid to the potential health risk of HMs caused by consumption of wheat seeds. Among HMs, As and Hg had the highest concentration, which may be due to the wastewater released from chemical plants in Gonbad-e-Kavous. Although findings showed low environmental concentrations of the studied elements that implied no danger to human health, it should be considered that many non-cancerous conditions weaken the immune system and prone the human beings to cancerous diseases.

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### Conflict of interest

The authors declare that they have no competing interests.

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