

# Heavy Metal Levels and Potential Health Risk Assessment in Honey Consumed in the West of Iran

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## Abstract

Since the worldwide research on content of heavy metals in honey is narrow, this study was carried out to analyze heavy metals (Cd, Cr, Ni, and Zn) in honey and assess its associated health risk in Hamadan City, Iran, in 2015. Totally, 15 freshly ripened samples of honey were analyzed for heavy metals using ICP-OES with three replications after burning to ash and digesting with dilute nitric acid. The potential health index was then calculated. The results showed that the mean levels of Cd, Cr, Ni, and Zn in the honey samples were  $63.18 \pm 43.39$ ,  $58.05 \pm 30.32$ ,  $56.15 \pm 54.32$ , and  $684.43 \pm 190.43 \mu\text{g kg}^{-1}$ , respectively. Also, the health risk assessment showed no potential risk for children and adults in relation to the consumption of honey in the studied region. According to the results of the current study, although there is no adverse effect for the local population through the consumption of honey, due to the increased discharge of pollutants especially heavy metals into the natural environment, it is very important to assess foodstuff before release to the consumer market.

**Keywords:** Heavy Metals, Honey, Health Risk, Food Safety, Iran

## 1. Background

Honey is a natural supersaturated sugar solution. As a viscous semi-liquid and sweet product, honey is produced by the honeybee from nectar plants. It is known as one of the oldest sources of nutrient for human beings and is popular all over the world (1-5). The properties and composition of honey are affected by several factors particularly the floral sources utilized by the honeybees as well as regional and climatic conditions (6). Honey varies greatly in quality with different locations. Since heavy metals content in honey is an important function of environmental pollution, honey is useful for assessing the role of environmental conditions as an excellent indicator of pollution (2, 4, 7).

Heavy metals have long been known as a major environmental problem. Generally, these elements are not biodegradable and can enter into human body via food, water and air. Therefore, they can accumulate in human vital organs especially liver and kidney leading to progressive toxic effects through different mechanisms such as interference with essential metals, oxidative stress, and interaction with cellular macromolecules (8). Iron, Cu, Zn, and Mn are essential nutrients for human growth. However, As, Cr, Pb, Cd, Co, and Hg can be toxic, even in relatively trace concentrations and therefore, pose adverse health effects on both human and animals. Although the effects of chronic exposure to trace amounts of some toxic met-

als are not well-understood, the results of previous studies have proven the seriousness of high levels of exposure to these metals, especially As, Cd, Cr, Hg, Ni, and Zn (9, 10).

Cadmium as a non-essential element is highly toxic for human body and accumulates principally in vital organs especially kidney and liver (4). Chromium (VI) compounds are well-known human carcinogens due to their toxic effects, whereas Cr (III) is an essential element (11). Nickel plays several important roles in body such as enzyme functioning (4, 12, 13). Zinc is one of the important elements for normal growth and development in human body (13, 14).

Risk assessment, as a part of risk analysis process, involves scientific analyses, the results of which are qualitative or quantitative explanations of the likelihood of harm associated with exposure to a hazardous chemical. In this regard, the assessment of human health risk requires identification, collection, and integration of information on health hazards of chemicals, exposure to toxins in polluted environment and relationships between exposure duration, dose, and adverse health effects (15). On the other hand, a human potential health risk assessment includes some steps such as hazard identification, dose-response analysis, exposure assessment, and risk characterization (16).

Since the heavy and toxic metal pollution affects the quantity and quality of crops, it can threaten the health of human beings via the food chain. Therefore for the safe

consumption of honey, the presence of toxic metals in this product and the associated health risks need to be studied. According to a few researches that have analyzed the metal levels of honey (17, 18), and because of the lack of studies assessing the health risk of heavy metals from honey in Iran. The aim of the current study was to conduct the potential health risk assessment of Cd, Cr, Ni, and Zn levels in freshly ripened honey in Hamadan city, Iran.

## 2. Methods

### 2.1. Apparatus

To determine metals content in honey samples, a Varian 710-ES inductively coupled plasma-optical emission spectrometer (ICP-OES) (Agilent Technologies, Inc., USA) was used.

### 2.2. Chemicals and Reagents

Standard stock solutions of Cd, Cr, Ni, and Zn ions at the concentration of 1000 mg L<sup>-1</sup> were used to prepare working solutions after appropriate dilution. Standard solutions were of analytical grade (Merck, Darmstadt, Germany). Distilled deionized water was used in all dilution procedures.

### 2.3. Sample Collection and Analysis

In this study, according to the Cochran's sample size formula, 15 freshly ripened unpasteurized honey samples, were donated by beekeepers located in Hamadan city and surrounding villages, from August to September 2015. The samples were taken to a laboratory in clean glass jars. About 5.0 g of each honey sample was placed in porcelain crucibles and heated for at least 12 hours in a hot plate at 70°C and then burned to ash in muffle ovens at 450°C. The white ashes obtained from this procedure were then dissolved in 5% nitric acid to a volume of 50 mL. After digestion process, the samples were filtrated through Whatman No. 42. The filtrates were collected in 50 ml Erlenmeyer flasks and analyzed by ICP-OES. The metal concentrations in the honey samples were quantified against standard solutions of known contents which were analyzed concurrently (2, 4). All the instrumental conditions applied for Cd, Cr, Ni, and Zn content determination were set in accordance with general recommendations (wave length for Cd, Cr, Ni and Zn: 214.4 nm, 359.3 nm, 232.9 nm, and 206.2 nm, respectively) (4, 17). For quality assurance, honey samples were digested in triplicate along with blanks to minimize error. The instrument was calibrated with a series of standard solutions supplied by Merck (Germany). Also, all metal content determinations were performed with three replications.

### 2.4. Statistical Analysis

The statistical analysis of the obtained results was first carried out using Shapiro-Wilk test for normality, followed by the study of variance homogeneity using an ANOVA parametric test according to the Duncan multiple range test. Finally, to study the correlation of the metal concentration in the honey samples, Pearson's correlation was employed (2).

### 2.5. Potential Health Risk Assessment

In this study, for computing potential health risk, the average daily intake of metal (DIM) was calculated according to the Equation 1 (19-22):

where  $C_{\text{metal}}$  indicates the heavy metal concentration in honey (mg kg<sup>-1</sup>),  $C_{\text{factor}}$  represents the conversion factor (0.085),  $D_{\text{food intake}}$  indicates the daily intake of honey, and  $B_{\text{average weight}}$  represents the average body weight (70.0 kg for adults and 15.0 kg for children). The  $C_{\text{factor}}$  is used to convert fresh weight into dry weight (21). The average daily intake (ADI) of honey for adults and children is considered to be 0.001 kg per person per day (17).

The health risk index (HRI) for the affected consumers via the consumption of honey was assessed using the Equation 2 (20-22):

$$HRI = DIM / RfD \quad (2)$$

In the equation 2, DIM represents daily intake of metal and RfD indicates oral reference dose of metal (which was 0.001, 0.003, 0.02, and 0.30 mg kg<sup>-1</sup> day<sup>-1</sup> for Cd, Cr, Ni, and Zn, respectively). An HRI of < 1 implies the exposed population is assumed to be safe (22, 23).

Also, the total health risk index (THRI) of heavy metals due to honey consumption was calculated according to Equation 3 (24):

## 3. Results

### 3.1. Evaluation and Method Validation

The results of limits of detection (LOD) and quantification (LOQ) are presented for each metal in Table 1. In this regard, LOD was calculated as  $3 \times \text{stdv.}$  of 5 measurements in blank matrix and LOQ as  $3 \times \text{LOD}$ . Following the determination of elemental content in four standard samples (50, 100, 200, and 500  $\mu\text{g L}^{-1}$ ), a known amount of the specific metal was added to each sample, and the whole procedure was repeated to calculate recoveries (17).

The contents of Cd, Cr, Ni, and Zn in the analyzed honey samples are presented in Table 2. Data in Table 2 show that, the percentage of metals contamination of honey samples reached 100%. The results showed that the levels of Cd in honey samples were in range of 20.20 - 148.30  $\mu\text{g kg}^{-1}$ , Cr in

$$DIM = (C_{metal} \times C_{factor} \times D_{foodintake}) / B_{averageweight} \quad (1)$$

$$THRI = HRI (toxicant1) + HRI (toxicant3) + \dots + HRI (toxicantn) \quad (3)$$

**Table 1.** Recovery and Limit of Detection (LOD) of Measured Elements in Honey Samples

Heavy metal	Recovery of Metal from Honey Sample (% [mean $\pm$ RSD])	LOD, $\mu\text{g}/\text{kg}$	LOQ, $\mu\text{g}/\text{kg}$
Cd	99.6 $\pm$ 4.1	13.0	40.0
Cr	97.9 $\pm$ 1.8	50.0	150.0
Ni	101.2 $\pm$ 2.7	90.0	250.0
Zn	99.5 $\pm$ 3.1	10.0	35.0

range of 3.70 - 90.40  $\mu\text{g}/\text{kg}$ , Ni in range of 2.40 - 129.20  $\mu\text{g}/\text{kg}$ , and Zn in range of 510.90 - 1112.60  $\mu\text{g}/\text{kg}$ , respectively.

Comparison of the heavy metal contents in analyzed honey samples with the maximum permissible limits (MPL) (200  $\mu\text{g}/\text{kg}$  for Cd, 1500  $\mu\text{g}/\text{kg}$  for Cr, 500  $\mu\text{g}/\text{kg}$  for Ni, and 10000  $\mu\text{g}/\text{kg}$  for Zn.) established by FAO/WHO (5, 25, 26) showed that the mean concentration of Cd, Cr, Ni, and Zn were lower than MPL.

In addition, all the calculated health risk index values of heavy metals were within the safe limits ( $HRI < 1$ ) (Table 3). Furthermore, the total health risk index values, which varied from 2.82E-05 to 2.29E-04 for adults and from 1.31E-04 to 1.07E-03 for children, were also within the safe limit ( $THRI < 1$ ) in this study. Therefore, we can conclude that people might have no potential significant health risk by consuming honey in the studied region. In this regard, Ru et al. (2013) reported that there was no chronic-toxic risk attributed to some heavy metals including Cd and Zn for inhabitants of Zhejiang, China, due to the consumption of honey (19).

Metal industry wastewater and sewage are the most important sources of Cd pollution. Environmental contamination can cause Cd contamination in foodstuff. Acute toxicity due to Cd may adversely affect cardiovascular and skeletal systems (4). The results indicated that the mean content of Cd in honey samples was  $63.18 \pm 43.39 \mu\text{g}/\text{kg}$  which was much lower than the MPL. The Cd content in this study was also much lower than those reported from Turkey (250 and 390  $\mu\text{g}/\text{kg}$  in non-contaminated and contaminated honey samples, respectively) (4), Italy (305  $\mu\text{g}/\text{kg}$ ) (7), Iran (390  $\mu\text{g}/\text{kg}$ ) (17), Egypt (500  $\mu\text{g}/\text{kg}$  in sesame honey) (27), and New Zealand (149  $\mu\text{g}/\text{kg}$ ) (28).

Breathing high amounts of Cr can cause irritation to the lining of nose, nose ulcers, nose running, and breathing problems (cough, shortness of breath, asthma, or

wheezing). Long-term exposure to Cr can cause damage to liver, kidney, and circulatory system, and lead to nerve disorders and skin irritation (11). The results showed that the mean concentration of Cr in honey samples was  $58.05 \pm 30.32 \mu\text{g}/\text{kg}$  that was much lower than the MPL. The Cr content in this study was much lower than that reported from Iran (899.75  $\mu\text{g}/\text{kg}$ ) (5), and much higher than that reported from Turkey (20  $\mu\text{g}/\text{kg}$  in contaminated honey samples) (4).

Trace amounts of Ni may be beneficial to activate some biological systems. However, its toxicity at higher amounts can cause enzyme inactivation and cancer in nasal cavity (4, 12, 13). The highest accumulation of Ni occurs in the brain and lung. The results indicated that the mean content of Ni in honey samples was  $56.15 \pm 54.32 \mu\text{g}/\text{kg}$  that was not significantly lower than the MPL. The Ni content found in this study was much lower than those reported from Turkey (480 and 1080  $\mu\text{g}/\text{kg}$  in non-contaminated and contaminated honey samples, respectively) (4) and Iran (652  $\mu\text{g}/\text{kg}$ ) (5).

Zinc as an essential, structural, and functional element is responsible for some important biological functions such as the mediation of redox reactions via reversible changes in the oxidation state of metal ions. On the other hand, Zn can damage some physiological processes like breathing (13, 14). The results of the present study indicated that Zn was the most abundant element in honey samples with an average concentration of  $684.43 \pm 190.43 \mu\text{g}/\text{kg}$  although it was much lower than the MPL. The mean concentration of Zn was lower than those found in honey samples from Turkey (12690 and 112760  $\mu\text{g}/\text{kg}$  in non-contaminated and contaminated honey samples, respectively) (4), Iran (1482  $\mu\text{g}/\text{kg}$ ) (5), Italy (3205  $\mu\text{g}/\text{kg}$ ) (7), Iran (2530  $\mu\text{g}/\text{kg}$ ) (17), Iran (4410  $\mu\text{g}/\text{kg}$ ) (18), China (1330  $\mu\text{g}/\text{kg}$ ) (19), New Zealand (1180  $\mu\text{g}/\text{kg}$ ) (28), Poland (7760

**Table 2.** Heavy Metal Contents of the Honey Samples ( $\mu\text{g kg}^{-1}$ , Dry Weight)<sup>a</sup>

Sample	Metal Concentration			
	Cd	Cr	Ni	Zn
1	61.40 <sup>c</sup>	67.60 <sup>c</sup>	14.70 <sup>b</sup>	550.20 <sup>ab</sup>
2	71.10 <sup>d</sup>	79.30 <sup>cd</sup>	12.40 <sup>b</sup>	560.30 <sup>ab</sup>
3	74.20 <sup>d</sup>	83.20 <sup>d</sup>	13.10 <sup>b</sup>	563.30 <sup>ab</sup>
4	20.20 <sup>a</sup>	71.20 <sup>cd</sup>	2.40 <sup>a</sup>	1061.80 <sup>e</sup>
5	27.50 <sup>a</sup>	80.90 <sup>d</sup>	3.70 <sup>a</sup>	908.30 <sup>d</sup>
6	22.30 <sup>a</sup>	72.20 <sup>cd</sup>	2.60 <sup>a</sup>	1112.60 <sup>e</sup>
7	31.80 <sup>ab</sup>	72.30 <sup>cd</sup>	119.20 <sup>de</sup>	685.70 <sup>c</sup>
8	37.50 <sup>b</sup>	84.70 <sup>d</sup>	125.60 <sup>e</sup>	697.30 <sup>c</sup>
9	32.90 <sup>ab</sup>	90.40 <sup>d</sup>	110.30 <sup>d</sup>	643.20 <sup>b</sup>
10	46.70 <sup>bc</sup>	4.60 <sup>a</sup>	18.20 <sup>b</sup>	510.90 <sup>a</sup>
11	57.60 <sup>c</sup>	3.70 <sup>a</sup>	31.80 <sup>c</sup>	548.70 <sup>ab</sup>
12	43.20 <sup>bc</sup>	4.50 <sup>a</sup>	26.30 <sup>c</sup>	525.80 <sup>a</sup>
13	128.90 <sup>e</sup>	48.80 <sup>b</sup>	110.40 <sup>d</sup>	624.70 <sup>b</sup>
14	144.10 <sup>f</sup>	58.10 <sup>bc</sup>	129.20 <sup>e</sup>	643.10 <sup>b</sup>
15	148.30 <sup>f</sup>	49.30 <sup>b</sup>	122.40 <sup>e</sup>	630.60 <sup>b</sup>
<b>Mean concentration <math>\pm</math> S.D.</b>	<b>63.18 <math>\pm</math> 43.39</b>	<b>58.05 <math>\pm</math> 30.32</b>	<b>56.15 <math>\pm</math> 54.32</b>	<b>684.43 <math>\pm</math> 190.43</b>

<sup>a</sup>The letters (a, b, c, d) represent the statistical differences among different samples ( $P < 0.05$ )

**Table 3.** Daily Intake of Metals (DIM, mg) and Health Risk Index (HRI) for Individual Heavy Metal Due to the Honey Consumption

	Cd	Cr	Ni	Zn
<b>Adults</b>				
<b>DIM</b>	7.67E-08	7.05E-08	6.82E-08	8.31E-07
STD	5.27E-08	3.68E-08	6.60E-08	2.31E-07
Min	2.45E-08	4.49E-09	2.91E-09	6.20E-07
Max	1.80E-07	1.10E-07	1.57E-07	1.35E-06
<b>HRI</b>	7.67E-05	2.35E-05	3.41E-06	2.77E-06
STD	5.27E-05	1.22E-05	3.30E-06	7.71E-07
Min	2.45E-05	1.50E-06	1.46E-07	2.07E-06
Max	1.80E-04	3.66E-05	7.84E-06	4.50E-06
<b>Children</b>				
<b>DIM</b>	3.58E-07	3.29E-07	3.18E-07	3.88E-06
STD	2.46E-07	1.72E-07	3.08E-07	1.08E-06
Min	1.14E-07	2.10E-08	1.36E-08	2.89E-06
Max	8.40E-07	5.12E-07	7.32E-07	6.30E-06
<b>HRI</b>	3.58E-04	1.09E-04	1.59E-05	1.29E-05
STD	2.46E-04	5.73E-05	1.54E-05	3.60E-06
Min	1.14E-04	6.99E-06	6.80E-07	9.65E-06
Max	8.40E-04	1.71E-04	3.66E-05	2.10E-05

$\mu\text{g kg}^{-1}$ ) (29), Slovenia (3610  $\mu\text{g kg}^{-1}$ ) (30), Italy (1820  $\mu\text{g kg}^{-1}$ ) (31), and Poland (1760  $\mu\text{g kg}^{-1}$ ) (32).

As shown in Table 3, HRI values of Cd, Cr, Ni, and Zn for children and adults were below 1. Here, the average HRI value was 2.66E-05 for adults and 1.24E-04 for children.

Therefore, it can be concluded that target consumers in the study area might have no potential significant health risk in relation to the consumption of honey. However, the non-carcinogenic risks were greater for children than adults.

The results of Pearson's correlation analysis showed

that there was no significant correlation between content of heavy metals in the studied honey samples.

#### 4. Conclusion

As heavy metals as well as many other chemical pollutants may accumulate over the lifetime of the human (33), the assessment of health risk attributed to these pollutants is important. In this regard, for the first time in Iran, the current study was conducted to determine metal content and estimate exposure-risk of Cd, Cr, Ni, and Zn in some freshly ripened honey samples consumed in Hamadan City to provide some baseline information for further research in this field.

According to the health risk index value, although there was no possible health risk to consumers through the intake of honey under the current consumption rate, due to the increased discharge of the pollutants especially heavy metals into the natural environment, the regular monitoring of hazardous chemicals such as heavy metals and pesticide residues should be performed for honey in Hamadan City. Moreover, a strict management program should also be implemented to reduce the amount of pollutants accumulated in the nectar plants used by honeybees.

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