

## Heavy Metals in the Vegetables Collected from Production Sites

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| ARTICLE INFO  | ABSTRACT   |
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| <b>Article type:</b><br>Original Article  | <p><b>Background:</b> Contamination of vegetable crops (as an important part of people's diet) with heavy metals is a health concern. Therefore, monitoring levels of heavy metals in vegetables can provide useful information for promoting food safety. The present study was carried out in north-west of Iran (Tabriz) on the content of heavy metals in vegetable crops.</p> <p><b>Methods:</b> Samples of vegetables including kurrat (n=20) (<i>Allium ampeloprasum</i>ssp. <i>Persicum</i>), onion (n=20) (<i>Allium cepa</i>) and tomato (n=18) (<i>Lycopersicon-esculentum</i> var. <i>esculentum</i>), were collected from production sites in west of Tabriz and analyzed for presence of Cd, Cr, Cu, Ni, Pb and Zn by atomic absorption spectroscopy (AAS) after extraction by aqua regia method (drying, grounding and acid digestion).</p> <p><b>Results:</b> Mean <math>\pm</math> SD (mg/kg DW) concentrations of Cd, Cu, Cr, Ni and Zn were <math>0.32 \pm 0.58</math>, <math>28.86 \pm 28.79</math>, <math>1.75 \pm 2.05</math>, <math>6.37 \pm 5.61</math> and <math>58.01 \pm 27.45</math>, respectively. Cr, Cu and Zn were present in all the samples and the highest concentrations were observed in kurrat (leek). Levels of Cd, Cr and Cu were higher than the acceptable limits. There was significant difference in levels of Cr (<math>P &lt; 0.05</math>) and Zn (<math>P &lt; 0.001</math>) among the studied vegetables. Positive correlation was observed between Cd:Cu (<math>R = 0.659</math>, <math>P &lt; 0.001</math>) Cr:Ni (<math>R = 0.326</math>, <math>P &lt; 0.05</math>) and Cr:Zn (<math>R = 0.308</math>, <math>P &lt; 0.05</math>).</p> <p><b>Conclusion:</b> Level of heavy metals in some of the analyzed vegetables, especially kurrat samples, was higher than the standard levels. Considering the possible health outcomes due to the consumption of contaminated vegetables, it is required to take proper actions for avoiding people's chronic exposure.</p> |
| <b>Article history:</b><br>Received: Jun 15 2013<br>Accepted: Aug 17 2013<br>e-published: Dec 31 2013       |  |
| <b>Keywords:</b><br>Heavy metals,<br>Vegetables,<br>Contamination,<br>Food safety,<br>Iran                  |  |
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**Citation:** Taghipour H, Mosaferi M. Heavy Metals in the Vegetables Collected from Production Sites. Health Promot Perspect 2013; 3(2): 185-193.

### Introduction

According to chemical properties, heavy metals are elements that exhibit metallic properties and are defined based on density, atomic number or atomic weight, chemical properties or toxicity<sup>1</sup>. The reason of heavy metal emission in the environment and food chain has both anthropogenic (e.g. industry, transport, manure and herbicides) and natural (e.g. soil, seawater, dust, volcano gas and forest fire) origins<sup>2</sup>. Heavy metals are closely

connected with deterioration of the environment and life quality and chronic exposure to low level of heavy metals can lead to severe health effects that in excess will result in acute poisoning<sup>3</sup>. Health risks<sup>4, 5</sup> and effects of heavy metals (e.g. As, Cd, Cr, Hg, Pb) have been well known and documented since past years<sup>6-8</sup>. Humans can be exposed to these metals through different paths such as air, water and food<sup>9-13</sup> and exposure to the

food chain has been widely reported throughout the world<sup>14, 15</sup>. There is a growing concern about exposure to heavy metals around the world; so, measurement of those elements in the media like air, water, soil<sup>2</sup>, food<sup>16</sup> and crops<sup>17-22</sup> has gained more importance. Because of their special nature, heavy metals tend to accumulate<sup>23</sup> in the environment and enter the food chain<sup>2, 3</sup>. As a result, uptake and bioaccumulation by plants and vegetables are one of the important aspects of heavy metals, which depend on many factors such as species and nature of different vegetables in absorbing metals, etc<sup>25-27</sup>.

Maximum allowable level of heavy metals in food (edible parts of different vegetables in mg/kg FW) for Cd, Cu, Pb and Zn is 0.05, 10, 0.2 and 20, respectively<sup>28</sup>. Besides, European legislation limit values for Cd and Pb (in mg/kg of fresh weight) for some foodstuff are as Table 1<sup>29</sup>.

**Table1:** European legislation limit values for Cd and Pb (in mg/kg of fresh weight) for some foodstuff

| Vegetable type | Cd   | Pb  |
|----------------|------|-----|
| Carrots        | 0.1  | 0.1 |
| French beans   | 0.05 | 0.2 |
| Leeks          | 0.1  | 0.1 |
| Lettuces       | 0.2  | 0.3 |
| Potatoes       | 0.1  | 0.1 |
| Radish leaves  | 0.2  | 0.3 |
| Radish roots   | 0.1  | 0.1 |
| Tomatoes       | 0.05 | 0.1 |

There is no national standard limits for heavy metals in vegetables; so, in most of published studies, international guidelines like EU have been used for comparison of results. Vegetable crops are an important part of people's diet in Iran. Moreover, food health and safety are a matter of growing concern, especially in urban areas. In 2011, Cd and Pb concentrations were determined in some vegetables in five regions of south of Tehran. Accordingly kurrat (leek) samples had the highest contamination with respect to Cd and Pb and, in comparison with EU guidelines; the concentrations were higher

than the permissible level for human consumption<sup>30</sup>. In Shahroud, Iran, Cr, Cd and Pb levels were above the standard limits considered by FAO and WHO<sup>31</sup>. It was also concluded that urban and industrial wastewaters were the main causes of this problem<sup>31</sup>. Aro-ra et al. (2008) assessed levels of some heavy metals in the vegetables irrigated with water from different sources and demonstrated a substantial increase of heavy metals in the vegetables irrigated with wastewater. Ranges of copper (Cu) and zinc (Zn) were 5.2–16.8 and 22–46 mg/kg, respectively, which were the highest in carrot samples<sup>27</sup>. There are very limited published data on concentrations of heavy metals in vegetables<sup>32, 33</sup>, especially in north-west of Iran.

In Tabriz, the vegetables are coming from farms of the city itself or neighboring or far away cities. In daily diet of the studied area, vegetables, tomato and onion had different uses as raw vegetables or were cooked in soup, omelet, sauce and salads. As a result, contamination of these foods with heavy metals could play an important role in exposure of consumers via the food chain which indicated the importance of determining heavy metals in the food crops.

The objective of the present study was to determine heavy metals of Pb, Cd, Cu, Zn, Ni and Cr in 3 types of highly consumed vegetables (kurrat, onion and tomato) and compare the results with available standard levels.

## Material and Methods

### Studied area

The study was conducted in Tabriz as the fourth largest city located in north-west of Iran with population of more than 1.5 million people [2006] and area of 45,481 km<sup>2</sup> (Fig. 1).

The city is one of Iran's most important industrial, economic cultural and political and commercial centers. The region has undergone heavy urbanization and development in recent decades. The largest industrial areas are located in the suburbs, particularly in western suburb of the city.



Fig.1: Location of the study area (Tabriz)

Unfortunately, surface water from the city (run off) and some wastewaters from some suburban industrial areas are being discharged into natural drainages without enough treatment and are then used by farmers in urban and peri-urban areas of the city for producing different types of vegetables and other commonly consumed crops due to the limited availability of safe water

for irrigation. Total area of studied region is about 200,550 and 240 ha, for kurrat, onion and tomato, respectively.

### Sampling and pretreatment

In spring and summer of 2009 (Table 2) the vegetables samples including kurrat (n=20) (*Allium ampeloprasum*ssp. *Persicum*), onion (n=20) (*Allium cepa*) and tomato (n=18) (*Lycopersiconesculentum* var. *esculentum*), were collected from production sites in west of Tabriz. Sampling was designed in such a way to cover all of studied area. In this kind of works generally an especial method has not being used for determination of the number of samples [14, 19, 27, 41, 43, 44], so the number of samples was determined according previous experiences and studies in the other part of the world.

The vegetable samples were air-dried for 72 h, crushed, passed through a 2-mm-mesh sieve and stored at ambient temperature before the analysis<sup>21</sup>.

All of the containers used in the analysis were cleaned by a detergent solution, rinsed with tap water, soaked in acid (2+1 HCl) and then rinsed with metal-free water.

Table 2: Sampled vegetables and their characteristics

| Local name | English name | Scientific name                                      | Family     | n  |
|------------|--------------|--|------------|----|
| Tare       | Kurrat*      | <i>Allium ampeloprasum</i> ssp. <i>persicum</i>      | Alliaceae  | 20 |
| Piyaz      | Onion        | <i>Allium cepa</i>                                   | Alliaceae  | 20 |
| Gojeh      | Tomato       | <i>Lycopersiconesculentum</i> var. <i>esculentum</i> | Solanaceae | 18 |

\*Kurrat, or Egyptian leek (*Allium ampeloprasum* var. *kurrat*), is grown in the Middle East for its leaves. It is closely related to elephant garlic and leeks and is generally regarded as being in the same species, though it is also commonly listed as *Allium kurrat* (<http://en.wikipedia.org/wiki/Kurrat>)

### Digestion and analysis

Metals of the samples were extracted by aqua regia. From each dried sample, 1.5 g grounded fine particles was added to a flask containing HNO<sub>3</sub> (4 ml) and concentrated HCl (12 ml; aqua regia). The flask was covered with a watch glass and then was allowed

to stand for at least 12 h. Afterwards the mixture was heated progressively and boiled under reflux for 2 h. After cooling and rinsing with 15 mL of deionized water, the rinse water was recovered in the digestion flask. Then, the ingests were passed through a pre-washed filter (Whatman No. 540) and the

filtrates were used to make a volume of up to 100 ml using ultra 2 M HNO<sub>3</sub>. The prepared samples were refrigerated in acid-washed polyethylene bottles at 4 °C before final analysis of the heavy metals (Pb, Cd, Cu, Zn, Ni and Cr)<sup>34-36</sup> by buck scientific atomic absorption spectroscopy. All of the results were reported in mg/kg Dry Weight (DW) of vegetables.

**Statistical analysis**

Statistical analyses were carried out using SPSS software, version 16. Mean and standard deviation of each heavy metal were calculated and the data were given in mg/kg dry weight of the sample. ANOVA and correlation tests were used for comparing mean concentration of heavy metals between the vegetables and determining their correlation level, respectively.

**Results**

Table 3 illustrates descriptive statistics of the analyzed heavy metals in the selected vegetables. Considering mean concentration, Zn had the highest concentration and Cd had the lowest one. However, considering the maximum level, Cu had the highest level with 221.83 mg/kg. Cd was observed in some of these samples. In kurrat samples, mean concentration was  $0.54 \pm 0.85$ , ranging from zero to 3 mg/kg. In onion samples, mean concentration was  $0.14 \pm 0.28$  which ranged from zero to 1.17 mg/kg and, in tomato samples, mean concentration was  $0.28 \pm 0.36$  with the range from zero to 1.17 mg/kg. Considering the mean values, order of Cd concentration was kurrat > tomato > onion.

**Table 3:** Descriptive statistics of analyzed heavy metals

| Metal (mg/kg) | Minimum | Maximum | Mean  | Std. Deviation |
|---------------|---------|---------|-------|----------------|
| Cd            | 0.00    | 3.00    | 0.32  | 0.58           |
| Cu            | 6.22    | 221.83  | 28.86 | 28.79          |
| Cr            | 0.24    | 10.96   | 1.75  | 2.05           |
| Ni            | 0.00    | 21.25   | 6.37  | 5.61           |
| Zn            | 22.47   | 138.70  | 58.01 | 27.45          |

Cu was present in all the samples. In kurrat samples, mean concentration was  $39.21 \pm 44.13$  which ranged from 18.5 to 221.83 mg/kg. In onion samples, it was  $21.11 \pm 16.04$  with the range from 6.22 to 81.22 mg/kg and, in tomato samples, it was  $25.98 \pm 10.43$ , ranging from 8.44 to 54.56 mg/kg. Considering the mean values, order of Cu concentration was kurrat > tomato > onion.

Cr was present in all samples. In kurrat samples, mean concentration was  $2.82 \pm 2.81$  which ranged from 0.32 to 10.96 mg/kg. In onion samples, mean concentration was  $1.37 \pm 1.19$ , ranging from 0.3 to 4.8 mg/kg and, in tomato samples, it was  $0.97 \pm 1.25$  with the range from 0.24 to 5.15 mg/kg. Considering the mean values, order of Cr concentration was kurrat > onion > tomato. Like Cd, Ni was present in some of the samples. In kurrat, onion and tomato samples, mean concentrations were  $6.25 \pm$

$6.91$  ranging from non-detectable to 21.25 mg/kg,  $5.75 \pm 5.04$  ranging from non-detectable to 14.17 mg/kg and  $7.18 \pm 4.95$  with the range similar to onion's, respectively. Considering the mean values, order of Ni concentration was tomato > kurrat > onion. In the case of Zn, this element was present in all the samples. In kurrat samples, mean concentration was  $85.25 \pm 28.09$  which ranged from 47.7 to 138.7 mg/kg. In onion samples, it was  $47.13 \pm 11.52$ , ranging from 25.8 to 71.8 mg/kg and, in tomato samples, it was  $39.84 \pm 11.62$ , from 22.47 to 61.13 mg/kg. Considering the mean values, order of Zn concentration was kurrat > onion > tomato. Interestingly, Pb was not detectable in any of the samples.

According to ANOVA test, there was significant difference in levels of Cr ( $P < 0.05$ ) and Zn ( $P < 0.001$ ) among the studied vegetables and positive correlation was observed

between Cd:Cu ( $R=0.659$ ,  $P<0.001$ ) Cr:Ni ( $R=0.326$ ,  $P<0.05$ ) and Cr:Zn ( $R=0.308$ ,  $P<0.05$ ).

Figure 2 provide a comparison of heavy metals concentration in the studied vegeta-

bles. For all the metals, the highest dispersion of concentrations was related to the kurrat samples.

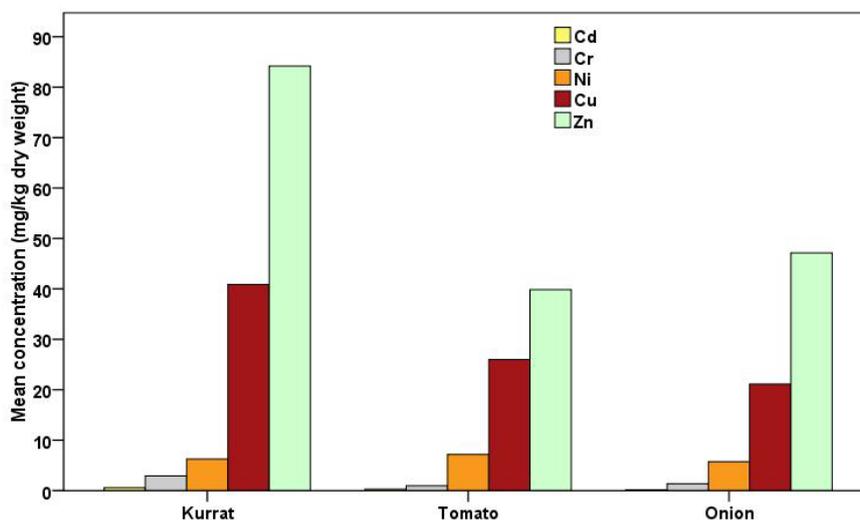


Fig. 2: Comparison of heavy metals concentration in kurrat, tomato and onion samples

## Discussion

Accumulation of heavy metals in plants is particularly dangerous since plants and vegetables are at the bottom of the food chain and are consumed by animals and humans. Consuming the vegetables contaminated with heavy metals has different detrimental effects on human health; therefore, monitoring contamination of heavy metals will allow for avoiding unnecessary exposures<sup>37</sup>. EU standards for the permissible levels of Cd and Cu are 0.2 mg/kg and 20 mg/kg, respectively. For Pb, permissible levels allowed by both EU standards and UK guidelines are 0.3 mg/kg and, for Zn, the guideline value is 50 mg/kg<sup>14, 38-40</sup>. Standard concentrations of Cd, Cr, Cu, Ni, Pb and Zn in vegetables and fruits are normally < 0.5, 0.1-1, 2-20, 1-10, 6-9 and 5-100 ppm, respectively<sup>41</sup>. Results of the present study indicated that, among the analyzed heavy metals, Cr, Cu and Zn were present in all the samples with mean  $\pm$  SD concentrations of  $2.82 \pm 2.81$ ,  $39.21 \pm 44.13$ ,  $85.25 \pm 28.09$  mg/kg, respectively. Cd ( $0.54 \pm 0.85$  mg/kg) and Ni ( $6.25 \pm 6.91$  mg/kg) were present in some

of the samples and Pb was not detectable in any of them. While in Givianradstudy (2011), the mean amount of Cd was determined as 0.1 mg/kg FW in mint and 0.16 mg/kg FW in lettuce. In leek samples, Cd and Pb were 0.15 mg/kg FW and 0.14 mg/kg FW, respectively<sup>30</sup>. In comparison with the previous study, the measured concentration of Cd in the present work was higher. However, it should be considered that results of Givianrad's study were expressed in fresh weight and the present results were expressed in dry weight.

Considering the EU standards, levels of Cd, Cr and Cu were higher than standard limits and kurrat samples showed the highest concentration of heavy metals; in kurrat samples, mean concentration of Cd was higher than 0.5 ppm. Furthermore, in one of the samples, the concentration was 6 times higher than this level, which indicated accumulation of Cd in kurrat. However, in 2 potato samples and 1 onion sample, higher level of Cd was also observed (Fig. 2). Nazemi (2010) reported concentration of Cd in leek samples to be equal to  $6.3 \pm 1.79$  mg/kg DW<sup>31</sup>. The current results for leek samples

were much less than those of Shahroudi's study. Cadmium is used in corrosion-resistant plating on steel and iron, alloys, bearings, pigments and rechargeable batteries. Effects of acute cadmium poisoning in the exposed people are very serious and include high blood pressure, kidney damage, destruction of testicular tissue and destruction of red blood cells<sup>42</sup>.

Chromium is used in metal plating, stainless steel, wear-resistant and cutting tool alloys and chromium chemicals<sup>42</sup>. According to Fig. 2, concentration of Cr indicated heavy contamination of nearly all the samples, especially kurrat and onion, with this metal. Concentrations equal to 10-fold and 5-fold higher than 1 ppm were observed in kurrat and both onion and tomato samples, respectively. High concentration of Cr showed frequency of emission sources of Cr. In the studied area, the number of small plating workshops and tannery industries was considerable. As a result, disposal of effluent of the mentioned industries to the environment and use of the polluted surface run offs as irrigation water might be a source of contamination. Nazemi (2010) reported concentration of Cr in leek samples as  $5.2 \pm 1.55$  mg/kg DW<sup>31</sup> which was higher than the present study's.

Copper is used in electrical conductors, alloys, chemicals and also has many other applications<sup>42</sup>. For Cu, mean concentration of 3 types of vegetables was higher than 20 ppm (Fig. 2). In one of the kurrat samples, this level was 11-fold more than the mentioned concentration; with the exception of this sample, Cu concentration of other samples was 1-3 fold higher than 20 ppm and, for onion and tomato, it was 4- and 2.5-fold, respectively.

For Ni, mean concentration of 3 vegetables was less than 10 ppm. Although tomato samples had the highest mean concentration of Ni, in kurrat samples, the highest concentration was observed (21 ppm).

Zinc is widely used in alloys (brass), galvanized steel, paint pigments and chemicals<sup>42</sup>. In the case of Zn, mean concentration of kurrat samples was 2-fold higher than

those of onion and tomato samples, but less than 100 ppm. In the study by Nazemi (2010), concentration of Zn in leek samples was reported as  $263.51 \pm 15.87$  mg/kg DW<sup>31</sup> which was obviously much higher than the study.

Lead is the 5th most widely consumed metal that is used in storage batteries, chemicals, gasoline and pigments<sup>42</sup>. Despite greatly increased use of lead by industry, evidence from hair samples and other sources has indicated that body burdens of this toxic metal have decreased during recent decades<sup>42</sup>. Acute lead poisoning in humans may cause severe dysfunction of kidney, reproductive system, liver, brain and central nervous system, which leads to sickness or death. Lead poisoning by environmental exposure is thought to cause mental retardation in many children and mild lead poisoning causes anemia<sup>42</sup>. During the recent years in Iran, Pb has been replaced with MTBE in gasoline. As a result, contamination of the environment with Pb has been decreased. In the present study, an important finding was the absence of Pb in all the analyzed samples. There are several possible explanations for this result; e.g., low level of Pb in agricultural soil, limitation of Pb contamination sources and no intake or accumulation of Pb by the studied vegetables.

Among the selected heavy metals, Cr, Cu and Zn were present in all the samples; however, Cd and Ni were present in some of them. This result might indicate source of contamination as naturally (Cr, Cu, Zn) or anthropogenic (Cd and Ni). For chromium, activities of tannery industries in the region and improper disposal of generated wastewaters might have an important role in contamination of the vegetables. Different levels of heavy metals in 3 types of vegetables (kurrat, onion and tomato) could be described by factors such as pH, organic matter, moisture, temperature and nutrient availability and also soil properties which influence absorption and accumulation of heavy metals in the vegetables.

In India transportation and marketing systems of vegetables play a more significant

role in elevating contaminant levels of heavy metals of the vegetables than production sites<sup>21</sup>. Atmospheric depositions could elevate levels of heavy metals in the vegetables during marketing<sup>43</sup>. In China, in the industrial/sewage irrigation vegetable land, the highest concentration of As, Cd, Hg and Zn occurred<sup>44</sup>. In Zimbabwe, studying concentration of heavy metals highlighted the potential risks involved in the cultivation and consumption of vegetables on the plots irrigated with sewage sludge<sup>44</sup>. Concentrations of heavy metals in vegetables from open fields were significantly higher than those grown in greenhouses and locally produced vegetables. All heavy metals, except Zn, were significantly higher than those in the provincial vegetables. In Egypt, market basket of fruits and vegetables showed that average concentrations of detected heavy metals ranged from 0.01 to 0.87, 0.01 to 0.15, 0.83 to 18.3 and 1.36 to 20.9 mg/kg for Pb, Cd, Cu and Zn, respectively<sup>20</sup>. In industrial area, in Lagos City, Nigeria, concentration of heavy metals of vegetables ranged from 1.13 to 1.67 mg/kg for cadmium, 25.08 to 56.84 mg/kg for copper and 1.33 to 2.06 mg/kg for nickel, which was higher than those of the residential areas as a result of contamination<sup>19</sup>. In comparison, measured concentrations of heavy metals during the present study were unfortunately higher than the above mentioned studies.

## Conclusion

Considering the health outcomes of consuming contaminated vegetables by the public, it is necessary for responsible authorities to take proper measures, especially on the sites with very high levels of heavy metals. Regular monitoring of irrigation water quality can be considered an important measure, particularly considering possible application of industrial effluents for irrigation.

## Acknowledgments

The authors would like to thank Research Deputy of Tabriz University of Medical Sci-

ences (TUMS), National Public Health Management Center (NPMC) and Nutrition Research Center of TUMS for their financial support from this research. Besides, we appreciate efforts of all people who cooperated with us in this study.

## Competing interests

The authors declare that there is no conflict of interest.

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