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Evaluation of heavy metals (lead, cadmium and chromium) and nitrates in some vegetables cultivated in Ahvaz, Iran

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ABSTRACT

Vegetables are an important part of a healthy and balanced diet. High concentrations of heavy metals and nitrates in vegetables may pose a risk to human health. In the present study, concentrations of nitrates and three heavy metals lead, cadmium, and chromium in some vegetables cultivated in the suburbs of Ahvaz city were determined. Also, the daily intake of these metals through consumption of vegetables was calculated. 100 samples of five kinds of vegetables (leek, sweet basil, radish, potato, and tomato) were collected randomly during the cultivation season between December 2013 and April 2014. The samples were taken twice a day, in the morning and afternoon, for observation of the nitrate concentration. After preparation of the samples, atomic absorption spectrometry (AAS) was used to determine the heavy metals and spectrophotometer for determining the nitrate levels. The mean ± SD concentrations of Pb, Cd, and Cr in these vegetables were 6.13 ± 0.47, 2.53 ± 0.43, and 41.73 ± 2.19 mg/kg, respectively. High nitrate concentration was detected in radish (409.83 ± 5.97), while tomato had the lowest nitrate concentration (7.38 ± 0.16) mg/kg. Nitrate concentration in the afternoon samples was less than the morning samples. The daily intake of Pb, Cd, and Cr through consumption of these vegetables was 0.14, 0.06, and 0.99 mg/day, respectively. The mean concentrations of Pb, Cd and Cr in these vegetables were above the permissible levels recommended by food and agriculture organization/world health organization (FAO/WHO). The daily intake of Pb, Cd, and Cr through consumption of these was 65%, 100% and 165% of provisional tolerable daily intake (PTDI), respectively. The average of nitrate content in all samples was less than the standard limits.

KEYWORDS: heavy metal, vegetables, nitrate, daily intake

INTRODUCTION

Humans are frequently exposed to hazardous pollutants in the environment through air, water and soil [1]. Natural processes such as biological and anthropogenic activities lead to an increase in the concentration of metals in the environment [2]. In general, these metals are not easily metabolized, have long half-lives and accumulate in different parts of the body [3, 4]. Cadmium is one of the heaviest metals, which exerts toxic effects on kidney, skeleton and respiratory system, and it has been classified as carcinogenic. Further, high concentrations of lead affect the nervous system, liver and cardiovascular system, and also cause blood disease [5].

Chromium (VI) is also toxic and leads to allergic dermatitis, respiratory inflammation, stomach and intestinal damage, kidney failure, and lung cancer [6]. Lead, cadmium, chromium, and arsenic are the most important contaminants for vegetables [7].

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Vegetables are an essential part of human nutrition as they are valuable sources of vitamins, protein, minerals, and trace elements. Besides, they have antioxidant activities. The contamination of vegetables by the heavy metals may be related to irrigation with contaminated water, polluted soils and the use of fertilizers and pesticides [8].

Vegetables are the major source of nitrates for humans, contributing to 72-94% of the total daily intake [9]. The usage of synthetic fertilizers and livestock manure has increased these days and hence vegetables and drinking water may have higher nitrate concentration. In human organism, 25% of ingested nitrate is secreted in saliva and 20% of the secreted salivary nitrate is reduced to nitrite by the tongue microorganisms [10]. This nitrite binds to the amines derived from proteins to form N-nitroso compounds, which are toxic and carcinogenic for humans [11]. In addition, nitrates react with hemoglobin and form methemoglobin, and as a consequence oxygen delivery to tissues is impaired [12]. In view of the benefits of vegetables to health and the importance of safety of this food source, a series of experiments were conducted in this study. The main aim of this research was to determine the concentration of heavy metals and nitrates in some vegetables cultivated in suburbs of Ahvaz, Iran and to estimate their contribution to their daily intake.

MATERIALS AND METHODS

Sample collection

The study was carried out in the main farmland in Hamidieh region located in the west of Ahvaz city in Iran. In this region, five farms were selected at random. 100 samples of five kinds of vegetables namely leek, basil, radish, tomato, and potato were collected four times randomly from different parts of these farms during the cultivation season between December 2013 and April 2014. These four-time collections were divided into two groups namely morning and afternoon samples.

Sample digestion for the extraction of heavy metals

The samples were first washed with tap water and then with distilled water. Then they were dried in an oven at 105 ºC for 24 hours. The dried samples were milled and 2 grams each were placed in a crucible. The dry ash digestion was performed in an electric furnace at a temperature of about 550 ºC for 6 hours. The white ash obtained was kept in desiccators and dissolved in dilute nitric acid. The suspension was filtered with Whatman No. 42 and transferred to 10 ml volumetric flask and filled up to the mark with 65% nitric acid [13].

Determination of heavy metals

The stock solutions of lead, cadmium, and chromium (1000 ppm) from Merck Company were used. The working solutions of Pb, Cd and Cr solutions were prepared by dilution of the standard stock solutions. Analyses for the heavy metals were carried out by AAS model Perkin-Elmer 3500.

Sample preparation for nitrates

The analysis of nitrates in samples was carried out according to Iranian standard protocols (No: 4106). The samples were washed with tap water and then with distilled water. They were sliced into small pieces and the fresh weight of them was determined. Then they were dried in oven at 65 ºC temperature for 24 hours. Dried samples were powdered and 1 g of each was weighed. Hot deionized water (80 °C) and 5 ml saturated tetra borate were added to each sample and the samples were heated on a water bath for 15 min. This solution was clarified with potassium hexacynoferrate and zinc acetate and then transferred to 200 ml volumetric flask, diluted by deionized water and filtered.

Determination of nitrate concentration

10 ml from the filtered solution was mixed with 5 ml buffer and cadmium. Then, chlorosulfonyl amide and n-(1-naphtyle) ethylene diamine dihydro chloride were added to the solution. The absorbance of the obtained purple compound was measured by spectrophotometer (538 nm wave length).

RESULTS

The mean ± SD concentrations of three heavy metals in the vegetable samples and their permissible limits as per FAO and WHO are summarized in table 1. The maximum levels of Pb and Cd were detected in potato and minimum in leek. While tomato had the highest concentration of Cr, leek had the lowest concentration.

Table 1. Mean concentrations of heavy metals in vegetable samples (mg/kg) and recommended maximum levels for vegetables.

Metals	Leek	Basil	Radish	Tomato	Potato	Recommended maximum levels for vegetables [14]
Pb	5.52 ± 0.089	5.80 ± 0.46	6.40 ± 0.89	6.09 ± 0.42	6.84 ± 0.23	**0.3**
Cd	1.98 ± 0.86	2.20 ± 0.50	2.80 ± 0.84	2.55 ± 0.95	3.15 ± 0.20	**0.2**
Cr	39.56 ± 0.76	40.45 ± 0.22	41.06 ± 0.77	45.29 ± 0.60	42.33 ± 1.61	**2.3**

Table 2. Mean concentration of nitrates in vegetable samples (mg/kg) and maximum levels for nitrate in vegetables.

Vegetables	Moisture percent (%)	Nitrate in dry weight (mg/kg)	Nitrate in fresh weight (mg/kg)	Maximum levels for nitrates in vegetables [15] (mg/kg fresh weight)
Leek	95	6338.7 ± 30.25	316.93 ± 1.51	**2000**
Basil	94	3045 ± 16.43	182.74 ± 0.98	**2000**
Radish	94	6823 ± 103.69	409.83 ± 5.97	**3500**
Potato	88	827.52 ± 15.12	104.70 ± 1.81	**150**
Tomato	94	122.97 ± 3.78	7.37 ± 0.16	**250**

Table 2 shows the nitrate concentration levels of all samples and maximum levels for vegetables. Among these vegetables, radish had the highest concentration of nitrates and tomato had the minimum levels.

DISCUSSION

Modern technologies and industrial activities have brought large quantities of pollutants into the environment [16]. The findings of this research show that there are high contaminations of heavy metals in the examined vegetables. In all vegetable samples, the trend in metal content was Cr> Pb> Cd (Table 1). Also, the results of T-test demonstrated that there is significant difference between the mean concentration of Pb, Cd and Cr and permissible limits given by WHO/FAO ($P < 0.05$). The existence of the elevated levels of heavy metals in vegetables may be due to the pollutants in irrigation water, soil, and emission of the pollutants from the high traffic ways [17]. The farms in the studied region are irrigated by the Karkheh River. Human activity, industrial operations along the river and natural processes (high concentration of heavy metals in river sediments) [18] can be indicated as the probable sources of contamination.

Jafarian (2013) investigated heavy metals in vegetables in Isfahan, Iran and reported that high levels of Pb, Cd and Cr in the vegetables could be attributed to contaminants that enter into the Zayandehrood River [7]. The levels of Pb, Cd and Cr in this study were higher than Maleki (2013) study carried out in Sanandaj, Iran and lower than the results of Gupta (2008)'s studying in India [19, 20]. Maleki *et al.* [19] stated that the heavy metals in ground water that is used for the irrigation of vegetables were lower than the standard levels. Moreover, Gupta *et al.* [20] indicated that irrigation of the areas of Titagar located in West Bengal, India with wastewater was the main reason for the contamination of the vegetables. The mean concentration of cadmium in this study was similar to Nazemi (2010)'s research in Shahroud, Iran and Kumar (2007)'s study in India [21, 22]. The results showed that there was significant difference in the levels of these heavy metals among the vegetables. The difference in metal contents in vegetables depends on the physical and chemical nature of the soil and metal absorption capacity of the plants, which were affected by the environmental changes and human factors [22]. Among the five vegetables, the maximum and minimum levels of

heavy metals were found in tomato and leek, respectively (Figure 1).

Comparison of the nitrate concentration and standard limits are given in Table 2. The results indicated that the mean concentration of nitrates in vegetable samples was lower than the maximum levels for nitrate ($p > 0.05$). This finding was consistent with the results of Shokrzadeh *et al.* (2007) and Fakhreddin's study (2014) [23, 24]. The statistical analysis showed that the mean concentration of nitrates in five vegetables was different. Nitrate accumulation in vegetables depends on genetic factors (plant species), environmental factors (light intensity, temperature, humidity) and agricultural factors (use of nitrogen fertilizers and herbicides) [11]. The highest concentration of nitrates was observed in radish, followed by basil, then leek, potato and tomato. In Shabazzadegan's study (2010), concentration of nitrates was reported in leek (323 mg/kg), radish (428 mg/kg), potato (97 mg/kg), and basil (184 mg/kg), which were similar to the findings of the present study [25]. The nitrate levels of radish and tomato in this study are consistent with the findings of the Pirsaheb (2010)'s study in Kermanshah, Iran [26]. He found nitrate concentrations in radish to be 510.4 mg/kg and in tomato 7.7 mg/kg. The nitrate concentrations in leek and radish in Mor's study [27] was 40 mg/kg and 3428 mg/kg, respectively, and they are in agreement with the results of the present study.

The comparison of the average concentration of nitrates in the morning and afternoon samples showed that nitrate levels in the afternoon were less than the morning samples (Figure 2). There was significant difference between nitrate concentrations in morning and afternoon samples in tomato ($p = 0.002$), radish ($p = 0.0001$) and potato ($p = 0.0001$) (p means p value). Light intensity has an inverse correlation with the nitrate content of plants. Under high light intensity, the activity of nitrate reductase and protein synthesis increases, and as a result the nitrate content in plant organs decreases [28]. Hence the time of harvesting can help in reducing the nitrate concentration.

Estimation of daily intake of heavy metals through consumption of the vegetables

The daily intake of heavy metals through vegetables was calculated according to this equation:

Daily intake of heavy metals (mg/day) = [mean concentration of heavy metals in vegetables (mg/kg) x (0.085) x average daily vegetable consumption (gm/day)];

0.085 = converting factor for the fresh weigh of vegetables to dry weight [29].

It has been reported that the average daily consumption of vegetables is 278 gm for each person in Iran [30]. Hence, the daily intake of Pb, Cd and Cr through consumption of vegetables would be 0.14, 0.06 and 0.99 mg/day, respectively (Table 3).

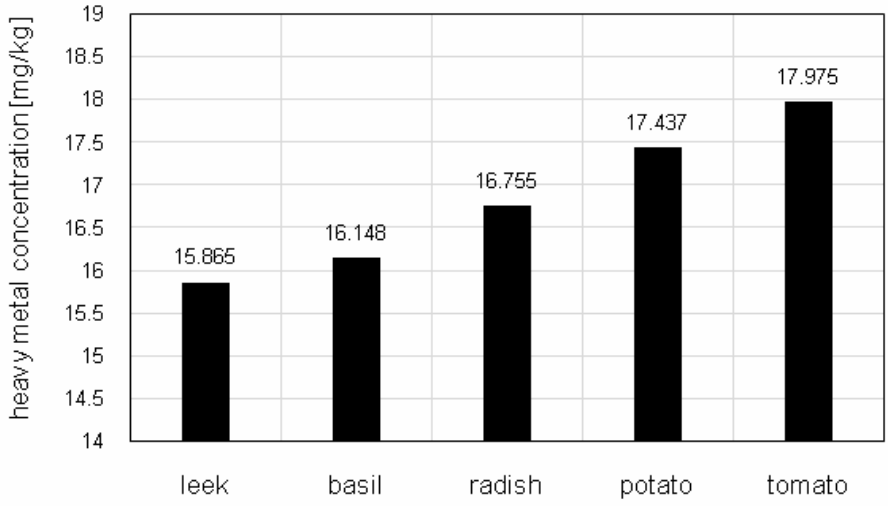

Figure 1. Comparison of five vegetables for accumulation of the three heavy metals.

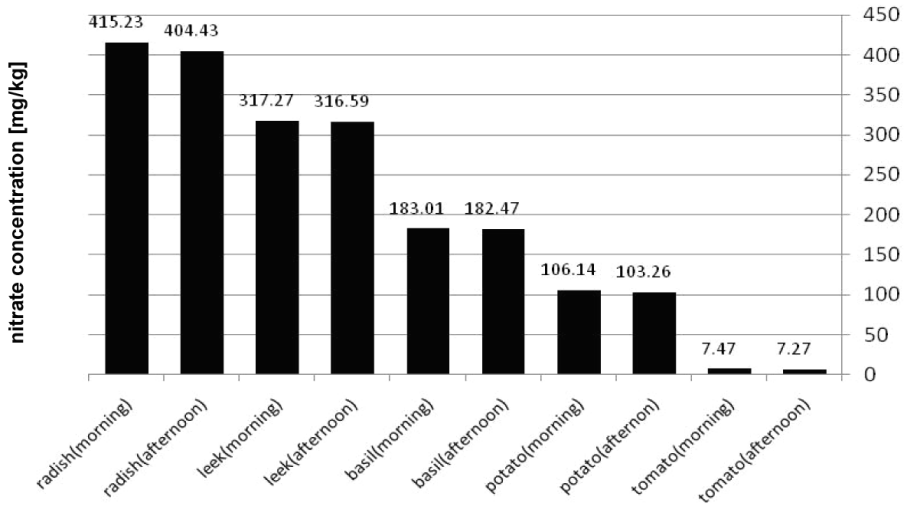

Figure 2. Comparison between nitrate levels in the morning and afternoon samples.

Table 3. Estimation of daily intake of heavy metals through consumption of vegetables.

Heavy metals	Mean concentration (mg/day)	Daily intake (mg/day)	PTDI (WHO/FAO)
Pb	6.13	0.14	0.21
Cd	2.53	0.06	0.06
Cr	41.73	0.99	0.06

Exposure of consumers to health risks are usually expressed as a reference value set by FAO/WHO for provisional tolerable daily intake (PTDI), which gives a limit for heavy metal intake based on body weight for an average adult (60 kg body weight). The PTDI for Pb, Cd and Cr are 0.214, 0.06 and 0.06 mg/day, respectively [31].

It is concluded that the contributions from these vegetables to daily intake of Pb, Cd and Cr are 65%, 100% and 165% of PTDI, respectively. PTDI refers to metal intake from various sources such as food, water, and air considering the maximum tolerable levels of metal that the body can handle and excrete before causing any signs of toxicity. Thus we conclude that a large daily intake of these vegetables is likely to be hazardous for the consumer's health.

CONCLUSION

The findings of this study show that the amounts of Pb, Cd and Cr are higher than the allowable levels recommended by WHO/FAO. It is suggested that regular monitoring of heavy metals should be carried out in order to prevent excessive build-up of these metals in the human food chain. Nitrate concentration in all samples was lower than standard limits and hence there is no hazard for consumers.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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