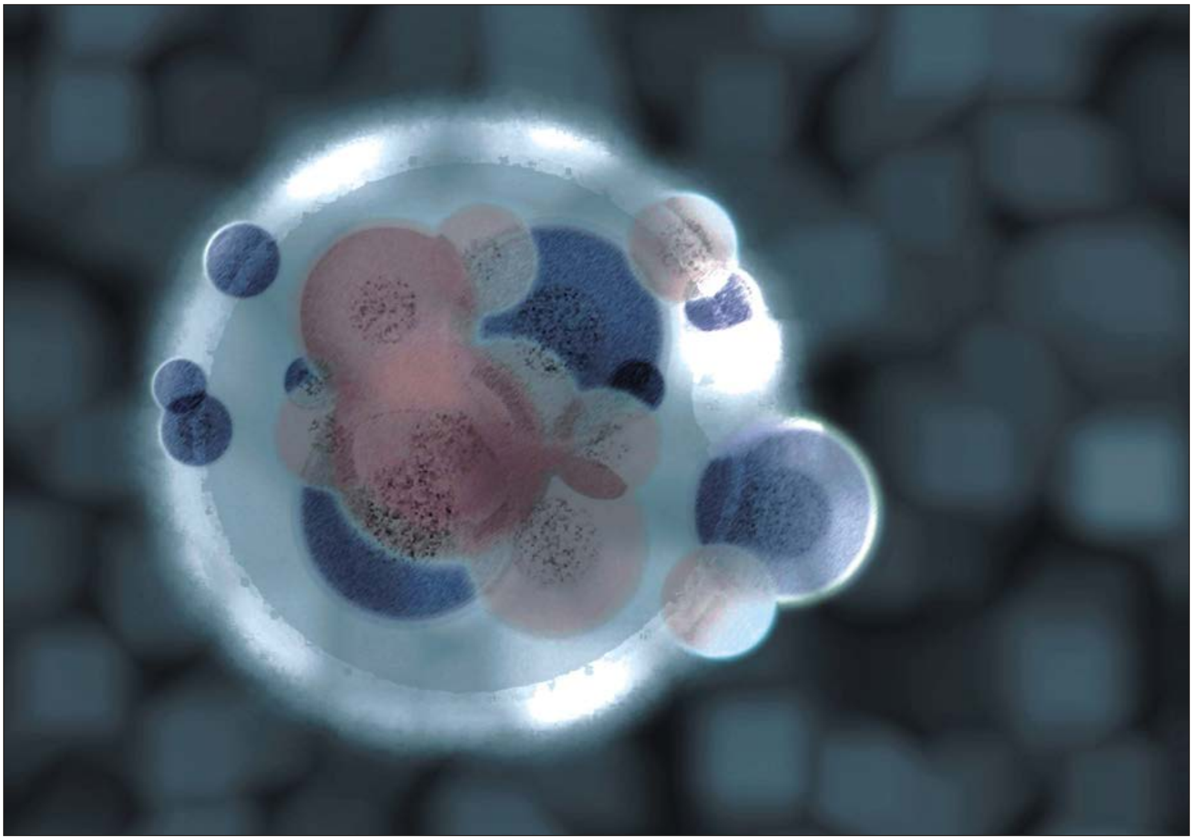

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Heavy Metals in Edible Green Vegetables Grown Along the Sites of the Zanjanrood River in Zanjan, Iran

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Abstract: The study investigated the levels of five different heavy metals (Cd, Pb, Zn, Cr and As) in various vegetables including roots and leaves of radish (*Raphanus sativus* L.), leek (*Allium ampeloprasum* L.), sweet basil (*Ocimum basilicum* L.) and parsley (*Petroselinum crispum*) cultivated along the bank of river passing through the city of Zanjan. The contributions of the vegetable to the daily intake of the heavy metals from the vegetables were determined. Atomic absorption spectrometry was used to estimate and evaluate the levels of these metals in the vegetables. The results of this survey showed the following ranges (mg kg^{-1}): 3.89-32.94, 3.15-27.68, 43.61-223.10, non-detectable and non-detectable for lead, cadmium, zinc, chromium and arsenic, respectively. Some vegetables contaminated high levels beyond the levels given by FAO and WHO for human consumption. When the mean levels of Lead and Cadmium (10.65 and 9.22 mg kg^{-1}) were taken into account the daily intake contribution of the metals was found to be 2.32 and 2 mg for Lead and Cadmium. Increase in vegetable consumption by community the situation could worse in the future.

Key words: Heavy metals, contaminants, edible green vegetables, daily intake, Zanjanrood River

INTRODUCTION

Vegetables constitute essential components of the diet by contributing protein, vitamins, iron, calcium and other nutrients which are usually in short supply (Thompson and Kelly, 1990). They also act as buffering agents for acid substances obtained during the digestion process. However, these plants contain both essential and toxic elements over a wide range of concentrations. Chronic low level intakes of heavy metals have damaging effects on human beings and other animals, since there is no good mechanism for their elimination. Metals such as lead, mercury, cadmium and copper are cumulative poisons. These metals cause environmental hazards and are reported to be exceptionally toxic (Ellen *et al*., 1990). Vegetables take up metals by absorbing them from contaminated soils as well as from deposits on parts of the vegetables exposed to the air from polluted environments (Zurera *et al*., 1989). A number of factors influence the concentration of heavy metals on and within plants. These factors include climate, atmospheric deposition, the nature of soil on which the plants is grown, application of fertilizers and irrigation with wastewater (Anyanwu *et al*., 2004; Khairiah *et al*., 2004; Itanna, 2002; Madyiwa *et al*., 2002; Denkota and Schmidt, 2000; Frost and Ketchum, 2000). The water of rivers can be polluted by heavy metals include Pb, Cu, Zn, Fe, Cr, Cd and Hg. The major sources of heavy metals are industrial effluents and indiscriminate disposal of domestic or sewage drainage directed to the rivers untreated or partially treated. Heavy metals, in general, are not biodegradable, have long biological half-lives and have the potential for accumulation in the different body organs leading to unwanted side effects (Jarup, 2003; Sathawara *et al*., 2004). Lead and cadmium are among the most abundant heavy metals and are particularly toxic.

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The excessive content of these metals in food is associated with etiology of a number of diseases, especially with cardiovascular, kidney, nervous as well as bone diseases (WHO, 1992 and 1995; Steenland and Boffetta, 2000; Jarup, 2003). In suburban areas, the use of industrial or municipal wastewater is common practice in many parts of the world (Sharma *et al.*, 2007) including Zanjan City. Zanjan City is located in the north-west of Iran. In Iran, Zanjan City in particular, it has been a common practice to cultivate vegetables along the banks of the rivers passing through the city. Due to the population increasing as well as rapid development of agriculture and industry and lack of strict legislation and regulation, heavy metals, such as Cu, Zn, Pb, Cd, Cr, As and Hg are emitted into environment in large quantities through atmospheric deposition solid waste emissions and wastewater irrigation. It is therefore anticipated that plants grown along the banks of the river Zanjanrood is not free from heavy metal pollution and may likely result in adverse health effects to the population of Zanjan City. The aim of the this study is determination of heavy metals in selected green vegetables grown along the banks of the Zanjanrood river and to estimate the daily intake of these metals.

MATERIALS AND METHODS

Sample collection: Vegetable samples of roots and leaves of radish (*Raphanus sativus* L.), leek (*Allium ampeloprasum* L.), sweet basil (*Ocimum basilicum* L.) and parsley (*Petroselinum crispum*) were randomly collected along the cultivated banks of the river zanjanrood (Fig. 1). The samples were collected from these growing areas over a period of four months during the dry season (July-October) of the year 2004. A total of 40 samples of five vegetables collected. All samples were collected and stored in polythene bags according to their type and brought to the laboratory for preparation and treatment.

Sample preparation and treatment: For lead, cadmium, chrome, arsenic and zinc analysis vegetable samples were washed with distilled water to eliminate air-borne pollutants. The leafy stalks were removed from all samples and these were sliced and dried on a sheet of paper to eliminate excess moisture. Once dried, each sample was weighed and oven-dried at 60°C to constant weight. Each oven-dried sample was ground in a mortar until it could pass through a 60 mesh sieve. The samples stored in a clean, dry and high density polyethylene bottles, 100 mL

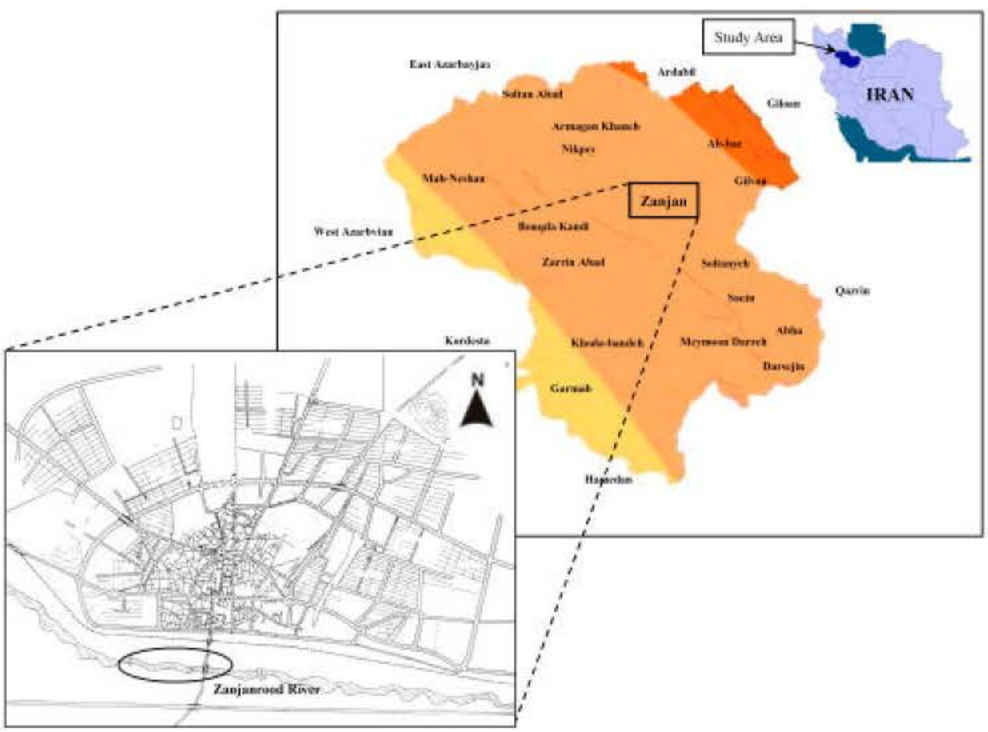

Fig. 1: Map of the studied area

capacity, with screw caps. Bottles were pre-washed with nitric acid, rinsed with de-ionized water, dried and tested for contamination by leaching with 5% nitric acid. The bottles contained no metal liners that can contaminate the samples.

Sample extraction: Samples were precisely weighed (2 g each) and ground in mortar followed by wet digestion with HNO_3:$HClO_4$ (2:1) in the conical flask for 2-3 h on a sand bath. Some 10 mL of HCl was added to solute inorganic and oxides salt. Digested samples were filtered with 0.45 μm pore size cellulose nitrate membrane filter paper (Millipore) and made up to 100 mL with distilled water and bottles were stored until the flame atomic absorption spectrophotometry was performed.

Sample analyzation: The samples were analyzed by atomic absorption spectrophotometer (ALPHA 4, Chem Tech Analytical Co. England) using a nitrous oxide-acetylene flame for As and an air-acetylene flame for four heavy metals Pb, Cd, Cr and Zn, using at least two standard solutions for each metal. The certified standard reference material (Alpha-Line, Chem Tech Analytical, Ltd, England) was used to check the accuracy and the analytical values were within the range of certified values. All the recoveries of the metals studied were over 95%. All the concentration of the metals is expressed in mg kg^{-1} in dry weight.

Statistical analysis: All statistical analyses were performed using the Microsoft EXCEL (version 2003). Analysis of Kruskal-Wallis test were employed to examine statistical significant of differences in the mean concentration of metals between group of families of vegetables using SPSS, version 11.5. A probability level of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The heavy metal concentrations were determined based on vegetable dry weight (Table 1). The results showed that the levels of Pb in all commodities were between 3.89 mg kg^{-1} in parsley and 32.94 mg kg^{-1} in leaves of radish, respectively. Cd contents varied from 3.15 mg kg^{-1} in leek to 27.68 mg kg^{-1} in sweet basil. The levels of Pb and Cd in all vegetables were higher than recommended maximum residues of these heavy metals in edible vegetables by of Codex Alimentarius Commission (Table 1). The contents of some trace elements such as As and Cr were no detectable amount in all analyzed vegetable samples. The highest concentrations of Pb were in radish leaves among selected vegetables. Further, the highest concentration of Cd and Zn were observed in sweet basil and radish leaves. Leafy vegetables appear to contain high levels of Pb and Cd. This trend is similar to those reported in Iran (Samarghandi *et al*., 2000) Egypt (Dogheim *et al*., 2004; Radwan and Salama, 2006), Pakistan (Parveen *et al*., 2003), Greece (Karavoltsos *et al*., 2002; Denkota and Schmidt, 2000) Tanzania (Bahemuka and Mubofu, 1999) Ethiopia (Rahlenbeck *et al*., 1999) USA (Pennington *et al*., 1995). The results of present study showed that the levels of Pb and Cd are higher than that were found in literature. Bahemuka and Mubofu (1999) determined four heavy metals includes cadmium, copper, lead and zinc in some green vegetables cultivated along the sites of the Sinza and Msimbazi rivers in Dares Salaam, Tanzania by Atomic absorption spectrophotometry. They reported that the contributions of the heavy metals from the vegetables were the following ranges (100 mg g^{-1}): 0.01±0.06, 0.25±1.60, 0.19±0.66 and 1.48±4.93 for cadmium, copper, lead and zinc, respectively. Some vegetables contained high levels beyond the permissible levels given by FAO and WHO for human consumption. Pless-Mulloli *et al*. (2001) determined the contamination of vegetables with the heavy metals includes copper, lead and zinc. The examined vegetables were cabbage, carrot, turnip, courgette, beetroot, potato, parsnip and swede. The average level of all heavy metals in all types of vegetables was low. They were also under the recommended guideline values for commercially grown foods and lower or well in line with levels in a UK National Total Diet Study (FSA, 1999).

Samarghandi *et al*. (2000) determined the concentrations of Pb, Ni, Cd and Cr in vegetables which are irrigated with polluted water in Hamadan City, Iran.

Table 1: Concentrations of heavy metals in vegetables cultivated along the sites of Zanjanrood river, in terms of mg kg^{-1} dry weight

			Radish			
Element	Parsley	Leek	Leaves	Roots	Sweet basil	Recommend maximum limits for vegetables[a]
As	ND	ND	ND	ND	ND	0.43
Cd	11.54	8.34	7.64	8.86	9.95	0.20
	(4.3-16.75)	(3.15-19.06)	(4.88-11.98)	(5.72-14.36)	(3.38-27.68)	
Cr	ND	ND	ND	ND	ND	2.30
Pb	7.44	10.21	19.15	6.95	9.46	0.30
	(3.89-9.41)	(7.53-11.98)	(12.41-32.94)	(4.06-11.06)	(7.67-11.96)	
Zn	85.78	72.12	107.55	71.73	101.18	99.40
	(72.51-127.6)	(59.48-101.2)	(72.36-223.1)	(43.61-158.6)	(81.03-139.9)	

ND = Not detected, Levels were below the detection limit, Values in the parenthesizes are minimum and maximum concentration of each element, [a]Source: Codex Alimentarius Commission (2001)

They reported that Pb concentration in vegetables was more than the permissible limitation for human foods. The concentrations of other heavy metals were lower than permissible limitation for human foods. In present study, the levels of Cd and Pb are 9.22 and 10.65 mg kg^{-1} and they are 35 and 46 times above the permissible levels set by FAO and WHO for human consumption, respectively (Fig. 2). However, other amounts were within the acceptable levels. The variations of metal contents in these vegetables can be related to the physical and chemical nature of soil and absorption capacity of each metal by plant. They are altered by innumerable environmental and human factors and nature of the plant (Zurera *et al*., 1989; Sharma *et al*., 2007).

In present study, significant differences were found in Pb concentrations between selected edible vegetables grown along the banks of Zanjanrood river ($p = 0.00$, Kruskal-Wallis test). The results showed that there are no significant differences among the Cd concentrations of vegetables. In a research, Hibber *et al*. (1984) reported that the concentrations of cadmium and lead is below the range (0.09 to 0.26 mg 100 g^{-1} of cadmium, and 1.1 to 1.7 mg 100 g^{-1} of lead) for vegetables grown in Metropolitan Boston and Washington DC (Hibber *et al*., 1984). Further, the cadmium and lead levels reported in this study were lower than those reported for vegetables in Nigeria (Ndiokwere, 1984). The National Nutrition and Food Research Institute of Iran have estimated that the average consumption of edible vegetables is 218 g $person^{-1}$ day^{-1}. Present study showed that the mean levels of Pb and Cd is 10.65 and 9.22 mg kg^{-1}, respectively. Therefore, the dietary intake of Cd and Pb can be 2.32 and 2 mg day^{-1}, respectively. Other studies from various countries have reported that the dietary intake for lead in adult is between 54 μg day^{-1} (Debeca *et al*., 1987) and 412 μg day^{-1} (Dick *et al*., 1987). Also, the dietary intake for cadmium is between 10 and 30 mg day^{-1} (Reilly, 1991). It can be concluded that estimation of daily intake for lead in present study is above those reported from other countries, whereas the estimation for cadmium is below the estimations. Also, the estimated daily intake for the Pb an Cd in this study are above that those reported by FAO/WHO (30 μg day^{-1} and 4.67 μg day^{-1}) who has set a limit for heavy metal intake based on body weight for an average adult (60 kg b.wt.) (Codex Alimentarius Commission, 2001). Bahemuka and Mubofu (1999) reported that the daily intake contribution of heavy metals in vegetables in Dares Salaam, Tanzania was found to be 21.60, 858.60, 426.60 and 3.65 mg for cadmium, copper, lead and zinc, respectively.

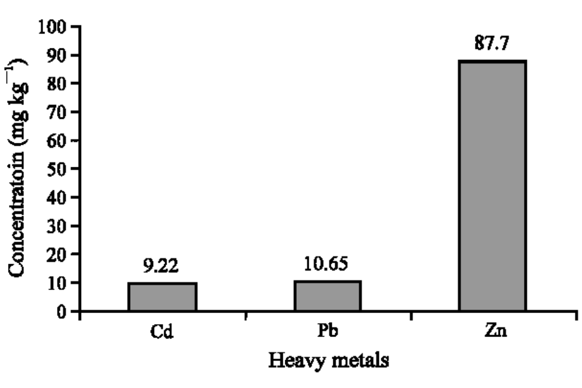

Fig. 2: Average concentration of heavy metals in edible green vegetables

Zinc is an essential element for plants and animals, but the slight increase in its levels may interfere with physiological processes. Sufficient Zn is essential to neutralize the toxic effects of Cd. In this study, the highest and the lowest of zinc were detected 223.10 mg kg^{-1} and 43.61 mg kg^{-1} in leaves and the roots of radish, respectively. The results showed that there is a significant variation in the concentration of Zn among the studied vegetables ($p = 0.005$, Kruskal-Wallis).

CONCLUSIONS

Comparison of the levels of heavy metals in the studied vegetables indicated that the permissible levels must be done for a safe food. The result clearly showed a divergence from the permissible levels by FAO and WHO. High contents of lead and cadmium were found in radish and sweet basil leaves, respectively. Further, there is a potential health hazard posed by the residues of heavy metals in edible vegetables when the high quantity of vegetables is consumed by people. A regulatory law implementation in vegetables products and long term planning is required to do vegetables safety. In addition, there are other items such as training of personnel or current good manufacturing practices and regular monitoring of heavy metals from effluents, sewage and edible vegetables. Monitoring can accompany by regular reevaluation of the acceptable levels must continue, but with the realization that some heavy metals will probably always be found in very low quantities and they are considered to be unavoidable contaminants.

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