

Human health risk assessment of aluminium via consumption of contaminated vegetables

B. Ghasemidehkordi¹, H. Nazem¹, A.A. Malekirad^{2,3}, M. Fazilati¹, H. Salavati⁴ and M. Rezaei^{2,5,6*}

¹Department of Biochemistry, Faculty of Basic Sciences, Payame Noor University, Ashrafi Esfahani Blvd, P.O. Box 81395-671, Isfahan, Iran; ²Department of Biology, Faculty of Basic Sciences, Payame Noor University, Karim Khan-e Zand Street, P.O. Box 19395-4697, Tehran, Iran; ³Toxicology and Diseases Group, Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Poursina Avenue, P.O. Box 14155-6451, Tehran, Iran; ⁴Department of Chemistry, Faculty of Basic Sciences, Payame Noor University, Karim Khan-e Zand Street, P.O. Box 19395-4697, Tehran, Iran; ⁵Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tehran, Qareeb Street, Azadi Av. P.O. Box 14155-6453, Tehran, Iran; ⁶Department of Food Safety and Hygiene, Faculty of Public Health, Tehran University of Medical Sciences, Enqelab Square, P.O. Box 1417613151, Tehran, Iran; rezaei12177@ut.ac.ir

Received: 19 February 2017 / Accepted: 20 July 2017

© 2018 Wageningen Academic Publishers

REVIEW ARTICLE

Abstract

The third most abundant metal in the earth crust is aluminium (Al). Contamination of vegetables with Al poses a threat to human health, therefore it is essential to measure the amount of this metal in edible parts of vegetables and evaluate the possible health risks to human body through the food chain. Eight types of vegetables that are commonly used in Iranian dishes including spinach, fenugreek, parsley, cress, allium, radish, tarragon and coriander were collected from agricultural sites of Markazi province, Iran. These vegetables were oven dried and acid digested and then they were analysed by inductively coupled plasma-optical emission spectroscopy for the Al content. The results indicated that the average concentration of Al was between $3,719.73 \pm 1,652.6$ and 166.42 ± 24.62 in Spinach and Fenugreek respectively. Based on transfer factor of Al, it can be inferred that soil condition has an important effect on uptake of metal by plants. Although the soil was contaminated with Al and vegetables can uptake this metal from soil, there was no significant health risk associated with the contaminated vegetables.

Keywords: aluminium, food safety, green leafy vegetables, health risk

1. Introduction

Green leaves of vegetables are edible parts of the plants that are consumed by human. Vegetables can be eaten raw, frozen, cooked, canned or as dried powder, which play an important role in human nutrition and human health. Green leaves are the main sources of vitamins especially vitamin C, A and folate, minerals and fibre but they are low in fat and carbohydrate. Dark and leafy vegetables are the main parts of a healthy diet but accumulation of some heavy metals or toxic elements can reduce their beneficial impacts. The entry of toxic metals to the water, air and soil and the subsequent transfer of them to the food chain causes adverse effects on biological and biochemical human functions (Haiyan and Stuanes, 2003).

Vegetables are known as good absorbers of metals from soil and water. Al like other heavy metals is discharged from industries and entered to the nature through pathways of waste water, gas and solid waste. Because of its unique properties such as being soft, light in weight, resistant and non-corrosive, this metal is used in different industrial equipment, kitchen tools, packaging and building materials (Kramer and Heath, 2014).

Al is the third most abundant element in the earth's crust after oxygen and silicon, the percentage of Al in the earth's crust is about 8.8% (Zhang *et al.*, 2014). Al and its compounds can be found in food, drinking water, vegetables, fruits, deodorants, air and medicines. food is the major intake source of Al. Al enters human body

through drinking water (Krewski *et al.*, 2007). Al release from acidified and alkalified soils was the main way of Al absorption by plants. It has been estimated that nearly 30% of the world's potential food producing areas are covered with acidic soil (Horbowicz *et al.*, 2011). In acidic aqueous solutions with pH<5, the Al ion exists as $[Al(H_2O)_6]^{3+}$ and Al solubility in acidic pH rapidly increases and Al changes into soluble aluminium cation., while by increasing pH, in alkaline solutions, a series of Al hydroxyl complex from successive steps of deprotonation of $[Al(H_2O)_6]^{3+}$ occur to yield $Al(OH)^{2+}$, $Al(OH)_2^+$ and soluble $Al(OH)_3$, in this situation the number of water molecules are decreased (EFSA, 2007). These conditions increase the level of Al in food chain. Al toxicity of acidic soils is an important problem in plant cultivation (Foy *et al.*, 1978).

Al has no specific biological function in the human body (De Sole *et al.*, 2013) and there isn't any evidence about Al participation in human metabolism. The gastrointestinal tract is a major route of Al absorption. Al can be transferred to the blood plasma and interact with transferrin (Exley, 2013). The excess amount of Al can deposit in bones, lungs, muscles and liver. Al will have a long term persistence in different organs and tissues. This accumulation causes neurodegenerative disease, neurotoxicity, Chronic disease, morbidity and mortality through various mechanisms (Verstraeten *et al.*, 2008). Increased level of Al increases the amount of pro inflammatory cytokines, chemoattractant chemokines, proteinase and growth factor in the body fluid (Ligi *et al.*, 2015). Besides, excess amount of Al is related to neurodegenerative diseases such as Alzheimer's disease because, *in vivo* studies revealed that high levels of Al in the body increase the accumulation of both tau protein and B-amyloid in the brain (Kandimalla *et al.*, 2016). More than 95% of Al is excreted by kidneys and ~2% in bile (Brown *et al.*, 2008).

As a consequence, the aim of this study was to determine the dry matter level of Al in some commonly used vegetables by the means of inductively coupled plasma-optical emission spectroscopy (ICP-OES). The collected data were analysed and compared with the recommended standard intake levels

based on World Health Organization (WHO)/Food and Agriculture Organization of the United Nations (FAO) and EFSA safety limit. Also some safety factors like estimated daily intake (EDI), transfer factor (TF), daily intake of metal (DIM), target hazard quotients (THQ), hazard quotient (HQ) and health risk index (HRI) were measured.

2. Materials and methods

Experimental site and its description

This study was conducted randomly in agricultural-industrial sites of Markazi province. This region was selected because it is considered as the main agricultural site of producing vegetables in Iran. Geographic coordinates of Markazi province is 34°05'30" N latitude and 49°41'20" E longitude in west part of Iran. The study sites are exposed to different degrees of industrial pollution. Many mother industries such as petrol, Petrochemicals, Rail Company, Al companies are concentrated in this province.

Sampling

Plant sampling

Vegetables, explained in Table 1 such were gathered randomly from ten agricultural sites in five parts of Markazi province during spring season. In each agricultural site, eight types of vegetables (spinach, fenugreek, parsley, cress, allium, radish, tarragon and coriander), three soil samples and three water samples were collected (120 types of vegetables, 30 water samples and 30 soil samples). All vegetable samples were collected using vinyl gloves and were packed in polyethylene bags as soon as possible to reduce the absorption of air pollution. All vegetable samples were transferred to the laboratory and green leaves of each of them were separated. Green leaves were washed with continuously running tap water to eliminate airborne pollutants and then 3 times with deionised water to remove remaining contamination on leaves (Abbas *et al.*, 2010; Cao *et al.*, 2010; Fishburn *et al.*, 2012; Nouri *et al.*, 2009) and then rewashed with distilled water to remove

Table 1. Vegetable samples characteristics.

Common name	Latin binomial	Family	Harvest time
Cress	<i>Lepidium sativum</i> L.	Brassicaceae	Sometimes ten days after germination
Spinach	<i>Spinacia oleracea</i> L.	Amaranthaceae	6 to 8 weeks
Fenugreek	<i>Trigonella foenum-graecum</i> L.	Fabaceae	20 to 60 days
Tarragon	<i>Artemisia dracunculus</i> L.	Asteraceae	4 to 8 weeks
Parsley	<i>Petroselinum crispum</i> (Mill.) Nyman Consp.	Apiaceae	4 to 6 weeks
Coriander	<i>Coriandrum sativum</i> L.	Apiaceae	4 to 6 weeks
Radish	<i>Raphanus sativus</i> L.	Brassicaceae	2 to 3 weeks
Leek (Allium)	<i>Allium ampeloprasum</i> L.	Amaryllidaceae	3 weeks to 3 years (perennial plant)

all unwanted contaminations. Samples were cut in to small pieces. Each sample was oven dried at 60-70 °C for 24 h. After drying, vegetable samples were powdered and stored in polyethylene container for next step (Bortey-Sam *et al.*, 2015).

Soil sampling

Soil around vegetable samples in different parts of agricultural sites was collected randomly by digging a monolith of 10×10×15 size (Mahmood and Malik, 2014). Non soil particles such as rock, gravel, stones and so on were removed from soil. A part of soil was soaked in water for 24 h then the pH value was measured by pH meter. Soil sample was oven dried (70° C for 24 h) and then crushed and passed through a 2 mm sieve, afterwards they were stored in the labelled polyethylene bags for analysis.

Water sampling

Water samples, used for irrigation, were collected in pre cleaned polyethylene bottle which was washed by 10% HNO₃ over-night and rewashed by deionised water then dried before use. The water samples were transferred to the laboratory. Water samples were filtered and acid stabilised. Approximately 20 ml HCL was added to the bottle and then the sample was heated without boiling, So that most of the water would evaporate and reach the highest concentration (Nham, 1991).

Chemical and reagent

All chemicals and standards were obtained from Merck (Darmstadt, Germany). The purity of argon as a carrier gas was 99.999% (grade 5), with a flow rate of 0.7 l/min for supplementary and Modified Lichte nebulizer and 13 l/min for coolant flow. The speed of the 4 channel peristaltic pump was 60 rpm for 45 s in pre-flush condition and 30 rpm for analysis. The power level was adjusted to 1,400 KW.

Inductively coupled plasma-optical emission spectroscopy

Liquors from the first and second infusions were analysed for Al, Cd, As, Pb and Hg by ICP-OES (Varian Vista-MPX, Spectroacros, Kleve, Germany).

Preparation and treatment of samples

About 1 g of each vegetable sample was digested by adding 15 ml of tri acid mixture (HNO₃ (70%), H₂SO₄ (65%), and HClO₄ (70%) in 5:1:1 ratio) to the test tubes. The capped test tubes were placed in oven with 80 °C for 3 h. After cooling, the digested samples were filtered by Whatman No. 42 filter paper (Sigma-Aldrich Chemi GmbH, Monich, Germany) and then centrifuged at 2,800 rpm for ten min

the lower acid aqueous layer was withdrawn and then was adjusted to 25 ml by adding deionised water and then was loaded to the ICP-OES for analysing metals such as Al (Shakya and Khwaounjoo, 2013). Soil samples from each site were collected by excavating a 1×1 m² area. Stones, rocks and gravel were discarded. In the laboratory soil samples were oven dried at 70 °C for 24 h after which a portion of the soil sample was pulverised using a mortar and pestle, passed through a 5 mm mesh sieve and packed in clean polyethylene bags until analysed. The powdered soil was digested with 6 mol/l HNO₃ (Merck, Darmstadt, Germany) solutions and rinsed with deionised water then injected to ICP-OES.

Evaluation methods

Definitions and measurements of some safety indexes related to aluminium consumption in diet

To access the human health with the ingestion of vegetables or other nutrients everyone must be familiar with special index level. Standard index level of each metal helps people to be safe in their lives. Some indexes are explained below and summarised in Table 2.

Transfer factor

TF or plant concentration factor is an indicator to measure the content of heavy metals absorbed by plants, it also indicates bioavailability of metals. The amount of TF is related to some physiochemical and physical features of soil and plant species (Kachenko and Singh, 2006). High levels of TF or TF≥1 shows higher absorption of metals by pants from soil and lower values indicate minimum absorption and safety level for human consumption. This parameter is calculated as a ratio of specific metal concentration in plants to the concentration of the same metal in soil (Jolly *et al.*, 2013a).

$$TF = \frac{C_{\text{plant}}}{C_{\text{soil}}}$$

Estimated daily intake of aluminium through vegetable consumption

EDI is an index to calculate the level of contamination in the food we eat, in the air we breathe and in the water we drink or use in agriculture. The results were compared with tolerable daily intake (TDI) and also tolerable weekly intake which was set by the European Food Safety Authority (EFSA) expert committee (EFSA, 2007). EDI is measured by the following formula (Chamannejadian *et al.*, 2013):

Table 2. Results of analysis and assessment of different indexes according to national Iranian standard.^{1,2}

Vegetables	EDI	THQ	HQ	DIM	HRI	TF
Spinach	3.08	3.08	3.08	0.26	0.26	0.00240
Radish	0.29	0.29	0.29	0.03	0.03	0.00023
Tarragon	0.32	0.32	0.32	0.03	0.03	0.00024
Allium	0.35	0.35	0.35	0.03	0.03	0.00027
Parsley	0.19	0.19	0.19	0.02	0.02	0.00014
Cress	0.43	0.43	0.43	0.04	0.04	0.00033
Fenugreek	0.14	0.14	0.13	0.01	0.01	0.00010
Coriander	0.38	0.38	0.37	0.03	0.03	0.00029

¹ All measurement of aluminium concentration in formula were based on $\mu\text{g}/\text{kg}$ (ISIRI, 2010).

² EDI = estimated daily intake; THQ = target hazard quotients; HQ = hazard quotient; DIM = daily intake of metal; HRI = health risk index; TF = transfer factor.

$$\text{EDI} = \frac{C \times C_{\text{ons}}}{\text{BW}}$$

C is the concentration of the heavy metals in contaminated vegetables (mg/kg). C_{ons} stands for the daily average consumption of vegetables in the study region (Iranian standard) (ISIRI, 2010). BW represents the average body weight of an individual in kg, sometimes it is considered as 70 kg.

Target hazard quotients

This formula is used for carcinogenic and non-carcinogenic risks related to vegetables, fruits, and other different foods (Amirah *et al.*, 2013).

$$\text{THQ} = \frac{\text{Efr} \times \text{ED}_{\text{tot}} \times \text{FIR} \times C}{\text{RfD} \times \text{BW} \times \text{ATn}} \times 10^{-3}$$

Efr is exposure frequency (365 days/year). ED_{tot} is the exposure duration 70 years, average lifetime. FIR: food intake rate (58 g/day) according to the Iranian standard. C: mean heavy metal concentration in vegetable samples (mg/kg). RfD: Reference dose of individual metal as the oral reference dose ($\text{mg}/\text{kg}/\text{day}$). Reference doses (RfD) for each metal were given by the US EPA (2000) (Al: 1.0 mg/kg bodyweight/day). BW is an average adult body weight (70 kg). ATn is the averaging exposure time for non-carcinogens (365 days a year \times number of exposure years, assuming 70 years): 25,550.

Hazard quotient

HQ is an index to show the risk to human health by the intake of contaminated vegetables with heavy or toxic metals. If the level of HQ is calculated less than 1, it has no

adverse or carcinogen effect, while in higher level or greater than 1 the adverse or unwanted effects will be possible. It is calculated by this formula (Sridhara Chary *et al.*, 2008):

$$\text{HQ} = \frac{(\text{Div}) \times (C_{\text{metal}})}{\text{RfD} \times \text{BW}}$$

Div is daily intake of vegetables ($\mu\text{g}/\text{kg}$). C_{metal} is concentration of Al in the vegetables (mg/kg). RfD is the reference dose for metal (mg/kg bw/day).

Daily intake of metal by human being from vegetable consumption

Daily Al intake based on the Al concentration which was determined by ICP-OES for vegetable samples was calculated by this formula:

$$\text{DIM} = \frac{C_{\text{metal}} \times C_{\text{factor}} \times C_{\text{food intake}}}{\text{BW}}$$

C_{metal} is concentration of metal in vegetables (mg/kg). C_{factor} is the conversion factor used to convert fresh green vegetables to dry weight which is 0.085 (Rattan *et al.*, 2005). $C_{\text{food intake}}$: the average vegetable intake was calculated by daily intake of green leafy vegetables in Iranian dishes. BW: The average body weight of human was assumed 70 kg and also it was assumed that each person consumes approximately 0.058 kg vegetables per day based on Institute of Standards and Industrial Research of Iran (ISIRI, 2010).

Health risk index

Health risk of consumers according to the intake of metal contaminated vegetables was assessed using HRI. Value of HRI lower than 1 ($HRI < 1$) is said to be safe for consumer. HRI is calculated by the following equation (Jan *et al.*, 2010).

$$HRI = \frac{DIM}{RfD}$$

Calibration standards

Individual stock standard solutions (10 µg/ml) were prepared. Spiked calibration standards were prepared by addition of 2.5 µl, 5 µl, 10 µl, 100 µl, 200 µl, 300 µl, 500 µl and 1000 µl of mixed standard stock solution respectively to 1 g or 1 ml of blank vegetable, soil and water samples in each case.

Recovery studies

For recovery determination, spiked vegetable, soil and water blank samples at concentration levels of 15, 25, 75, 150, 250, 500 and 750 µg/ml were prepared in triplicates and then treated according to the procedure described in sample preparation. The recoveries were calculated using the spiked calibration curves.

Statistical analyses

Results of this research were analysed by SPSS version 22 (IBM Corporation, Armonk, NY, USA) and Minitab 16 (Minitab Ltd., Coventry, UK) to obtain mean value and standard deviation. By using One Way ANOVA all results were compared with each other. Because all the data were normally distributed, we used parametric statistical analysis ($P < 0.05$).

3. Results and discussion

Method validation

Linearity of the standards in spiked calibration curves

The Al showed linearity. Linear spiked calibration curves for this metal was obtained by correlation factors 0.9992 (Table 3).

Limitation of detection and limits of quantification

The limitation of detection and limits of quantification for the Al in this method were calculated as 0.1 and 0.3 µg/kg, respectively.

Recovery

Table 4 presents the recovery and repeatability for the six concentration levels. The recovery of Al at these six levels was in the range of 97.4-114.3%. In terms of repeatability, Al gave relative standard deviation (RSD) < 10% with $n=3$ at each spiking level.

Aluminium concentration in vegetables, soil and water

Results summarised in Table 5 and Figure 1, indicate that the highest and the lowest values of Al in vegetables varied between $3,719.73 \pm 1,652.6$ in spinach and 166.42 ± 24.62 (µg/kg) in fenugreek. Spinach absorbs and accumulates high level of Al in green leaves but it does not have meaningful statistical differences with other plants ($P < 0.05$). Spinach is considered as the highest accumulator of Al among other vegetables. Spinach as a perennial plant is cultivated in cool seasons of year, especially in early spring and is ready to be harvested after six to eight weeks. This plant is very tolerable to soil conditions, for example it can be grown in alkaline soil (Roy *et al.*, 2014) and easily absorb metals from soil, whereas other plants show some sensitivity to soil conditions. It is noted that mean value of soil pH in different soil samples (measured by pH meter (AZ Instrument Corp, Taichung City, Taiwan)) was between 7.6 and 7.8 that shows alkaline properties.

The results show that the soil (with the mean concentration $1,536,729.58 \pm 129,805.51$ µg/kg) and water (with the mean concentration $16,087.05 \pm 14,931.15$ µg/kg) are polluted

Table 3. Linear equations and regression coefficient of the calibration curves for aluminium.

Compound	Range (µg/kg)	Equation	Regression coefficient
Al	2.5-1000	$y = 0.0007x - 0.0023$	0.9992

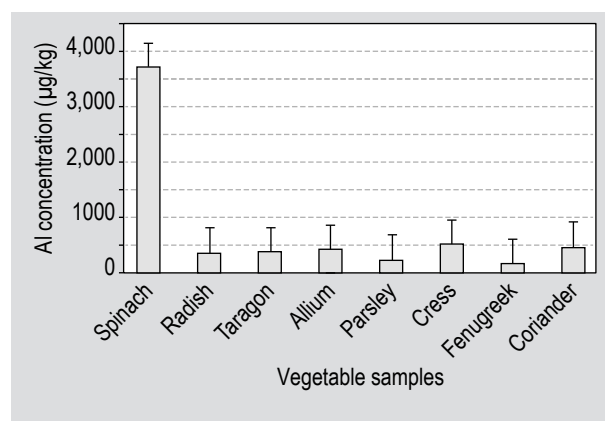
Table 4. Average recoveries (%), relative standard deviations (%) and LOD (µg/kg) obtained by ICP-OES analysis of at 6 spiking levels ($n=3$) in vegetable, soil and water samples.¹

Samples	Recovery ($n=18$)	Range of RSDr ($n=6$)	LOD	LOQ
Vegetable	108.2	4.1-7.2	0.1	0.3
Soil	97.4	5.8-9.4	0.1	0.3
Water	114.3	4.6-6.7	0.1	0.3

¹ LOD = limitation of detection; ICP-OES = inductively coupled plasma-optical emission spectroscopy; LOQ = limits of quantification; RSDr = relative standard deviation of reproducibility.

Table 5. Mean concentration of aluminium (mean and standard deviation (SD)) in different vegetable samples ($\mu\text{g}/\text{kg}$ dry weight basis).

Samples	Mean	Min	Max	SD
Spinach	3,719.73	1,200.25	6,358.40	1,652.64
Radish	355.37	220.45	458.20	75.11
Tarragon	383.87	232.10	483.11	79.15
Allium	428.12	100.55	587.11	114.95
Parsley	225.81	280.20	165.45	35.46
Cress	519.47	298.98	665.25	85.90
Fenugreek	166.43	126.57	199.06	24.62
Coriander	453.76	300.25	557.75	72.21
Soil	1,536,729.58	1,289,820.0	1,815,520.0	129,805.51
Water	16,087.05	973.55	55,709.50	14,931.15

**Figure 1.** Variation of aluminium concentration among different vegetables.

with Al, they have high levels of this metal or in the other words contaminated with Al. This feature (water and soil contamination) can have an effect on food safety and vegetables consumption. The order of vegetables according to Al accumulation are spinach>cress>coriander>allium>tarragon>radish>parsley>fenugreek.

According to WHO/FAO standard limit, the maximum permitted concentration of Al in water is up to 1000 $\mu\text{g}/\text{l}$ (WHO, 2007), the level of this metal in water sample of this study was $16,085.05 \pm 14,931.15 \mu\text{g}/\text{l}$ that is higher than permissible limit. Based on WHO guideline, the amount of Al in soil is between 7 to over 100 g/kg, in this research the level of Al in soil is approximately 1.53 g/kg which is lower than safety limit. The level of Al in vegetables, water and soil samples were compared that shows the meaningful statistical differences among soil and vegetable or water samples ($P < 0.05$).

Index evaluation

All data were analysed and summarised in Table 2.

Assessment of transfer factor

According to the TF, this parameter is an indicator of the plant species ability or tendency to absorb a certain element from the soil, in this project Spinach had maximum amount of TF (0.0024), although this level is less than 1 ($\text{TF} < 1$), it is more than the amount of TF among other vegetables, this study supports the low Al uptake by plants from the contaminated soil. The mobility of Al from soil to plant is a function of chemical, biochemical and physical properties of soil and types of vegetables (Aktaruzzaman *et al.*, 2013).

The TF of Al from soil to vegetable plants are presented in Table 2. The mobility of Al from soil to plant is related to soil condition and vegetable types. Due to previous study, the best soil condition for Al uptake is acidic soil. It has a strong relationship between soil pH and the solubility of various compounds. Compounds such as organic and inorganic matters in the soil can change soil pH. People sometimes use animal manure or animal waste as a plant nutrient source for cultivation in most agricultural sites. Animal manure can increase soil pH, that is related to lime like material like magnesium and calcium in manure (Whalen *et al.*, 2000). Lower soil pH can increase Al exchange between soil and plant and cause Al toxicity in plant (Moir *et al.*, 2013). The use of fertiliser can increase soil pH and interferes with Al absorption by plant.

Assessment according to estimated daily intake, daily intake of metal and comparison with tolerable daily intake

Based on the dietary nutrition intake level survey by institute of standard and industrial research of Iran (ISIRI) vegetables allocate high amount of daily consumption and

the adult residents in the region had an average daily intake of 58 g green leafy vegetables in diet per day. EDI results ($\mu\text{g}/\text{day}/\text{BW}$) show some differences in daily intake of Al between spinach and other vegetables. Based on TDI for adults ($0.10 \text{ mg}/\text{kg}/\text{BW}/\text{day}$) based on EFSA standard (EFSA, 2007) who consume more vegetables in daily diet, the results in this study are less than TDI standard. TDI is an index for measuring the amount of analyte or chemical substance in food or drinking water expressed on a body-mass basis (usually $\text{mg}/\text{kg BW}$) that can be ingested daily by human without any hazardous effects. Researchers in the United States Department of Agriculture (USDA) estimated daily Al intakes $0.10\text{-}0.12 \text{ mg Al}/\text{kg}/\text{BW}/\text{day}$ for adult (25-30 to 70 years-old) males and females. Also it is noted that EFSA established tolerable weekly intake ($1 \text{ mg}/\text{kg}/\text{BW}/\text{week}$) of Al instead of TDI (EFSA, 2007).

DIM is related to Al concentration in vegetable samples which are used by people and amount or average food consumption. The estimated DIM of Al, shown in Table 2 reveal the average intake of Al through vegetable consumption, although different segment of population at different times use various amount of vegetables in diet (Jolly *et al.*, 2013b).

Assessment of target hazard quotients and health risk index

The results of THQ showed that this parameter for most of the vegetable samples are generally less than 1 ($\text{THQ}<1$), based on average value of vegetable consumption in Iranian diet it's considered to be safe for consumer and suggest that people would not experience significant health risk from the intake of Al in these vegetables. In this study the calculated THQ was in the same with the amount of HQ that is related to the calculated numbers in the equation, by the reason of in THQ we think people in 365 days of year during average life time (70 years) can use vegetables. Also HRI that is used to evaluate the health risks and quality of dietary diet in relation to health promotion was the same as DIM and that was because of the oral references dose of Al is 1 ($\text{HRI}=\text{DIM}$ for Al concentration).

There are similar studies conducted in other parts of the world to assess the level of Al contamination in fruits and vegetables. Steven Jansen *et al.* (2002) investigated the accumulation of Al in leaves of 127 species of Melastomataceae, they reported the range of Al between $970 \text{ mg}/\text{kg}$ in *Memecylon sp.* and $66,500 \text{ mg}/\text{kg}$ in *Miconia acinodendron*, they concluded that environmental influences specially soil, determine the amount of Al absorption and accumulation in plant species (Jansen *et al.*, 2002). Kröppel *et al.* (2012) measured the amount of Al in various tea powders (green, black, herbal and fruit), they showed statistically significant differences in Al content of tea powders. Green and black tea contain high amount of Al (910 ± 31 and $760\pm 36 \text{ mg}/\text{kg}$ respectively)

while herbal ($230\pm 90 \text{ mg}/\text{kg}$) and fruit ($220\pm 80 \text{ mg}/\text{kg}$) teas show low concentration of Al. It can be inferred that the leaves of green tea can accumulate high amount of Al (Kröppel *et al.*, 2012). Farahani *et al.* (2015) determined the level of Al in eggs, they reported the concentration of Al in the samples was in the range of $0.562\text{-}387.24 \text{ mg}/\text{kg}$, the average being $119.213 \text{ mg}/\text{kg}$ and also EDI of egg for consumer was $4.371 \text{ mg}/\text{kg}$ (Farahani *et al.*, 2015). Parviz *et al.* (2015) investigated the level of some heavy metal like Al in tea plant, they concluded that Al concentration in bulk, packed and tea bag was 528.341 ± 130 , 188.506 ± 184.334 , $201.667\pm 193 \text{ mg}/\text{kg}$ respectively that were contaminated with Al and also EDI of Al was lower than the ADI in all samples (Parviz *et al.*, 2015). López *et al.* (2002) estimated Al concentration in drinking water, soft water and fruit juice. They reported daily dietary intake of Al accounted for approximately $156 \mu\text{g}/\text{person}/\text{day}$ that is more than dietary intake of metal in this research (López *et al.*, 2002). Jorhem and Haeggglund (1992) analysed Al concentration in foodstuff and diets in Sweden, they concluded that the average Sweden daily diet contain about 0.6 mg Al (Jorhem and Haeggglund, 1992). Kyoko Sato *et al.* (2014) reported that Al concentration in un processed food ranged from 0.32 to $0.54 \text{ mg}/\text{kg}$ and also the dairy dietary Al intake in fruit and vegetables consumption are higher than other food categories (Sato *et al.*, 2014) that contribution of Al in vegetables in this study are somehow similar to those of the previous report.

In conclusion, the highest mean concentration of Al was detected in spinach. The mean concentration of Al was within the safety limits of WHO/FAO and USDA for human consumption. The health risk indexes such as THQ and HRI were less than 1, indicating that the high amount of Al in soil and water cannot be easily absorbed by plants because of competition with other metals and ions and also environmental condition that it doesn't have negative impact on human health. Based on transfer of Al from soil to plants and meaningful differences in Al concentration between soil and vegetables it can be inferred that bio-available concentration of Al in agricultural soil are low, which shows that Al is not easily absorbed by plant. The amount of Al in the leafy vegetables, compared with the level of Al in soil and irrigation water is incredibly challenging and may relate to oxidation state.

Deprotonating of Al ions, diversity of plant roots and also the capacity of plants to absorb and accumulate Al and mechanical activities done by human. Although plants can gather low amount of Al in their leaves or edible parts, there is a need to protect the soil and water used for irrigation from contamination through regular monitoring and thus produce a healthy diet.

4. Conclusions

TF, EDI, DIM, HQ, THQ and HRI statistical analysis were successfully applied for the assessment of Al concentration and contamination in vegetable samples gathered from Markazi province. Degree of Al contamination in the soil and the water was higher but vegetables could not uptake increased level of metal in green leaves.

Acknowledgements

The authors are grateful to the authorities of medical-biochemical laboratory manager in Tehran university medical science. We would like to thank all biological and biochemical scientific groups and coworkers in Isfahan University, Iran.

References

- Abbas, M., Parveen, Z., Iqbal, M., Riazuddin, M., Iqbal, S., Ahmed, M. and Bhutto, R., 2010. Monitoring of toxic metals (cadmium, lead, arsenic and mercury) in vegetables of Sindh, Pakistan. *Kathmandu University Journal of Science, Engineering and Technology* 6: 60-65.
- Aktaruzzaman, M., Fakhrudin, A., Chowdhury, M., Fardous, Z. and Alam, M., 2013. Accumulation of heavy metals in soil and their transfer to leafy vegetables in the region of Dhaka Aricha Highway, Savar, Bangladesh. *Pakistan Journal of Biological Sciences* 16: 332.
- Amirah, M.N., Afiza, A.S., Faizal, W.I.W., Nurliyana, M.H. and Laili, S., 2013. Human health risk assessment of metal contamination through consumption of fish. *Journal of Environment Pollution and Human Health* 1: 1-5.
- Bortey-Sam, N., Nakayama, S.M.M., Akoto, O., Ikenaka, Y., Fobil, J.N., Baidoo, E., Mizukawa, H. and Ishizuka, M., 2015. Accumulation of heavy metals and metalloid in foodstuffs from agricultural soils around Tarkwa area in Ghana, and associated human health risks. *International Journal of Environmental Research and Public Health* 12: 8811-8827.
- Brown, R.O., Morgan, L.M., Bhattacharya, S.K., Johnson, P.L., Minard, G. and Dickerson, R.N., 2008. Potential aluminum exposure from parenteral nutrition in patients with acute kidney injury. *Annals of Pharmacotherapy* 42: 1410-1415.
- Cao, H., Chen, J., Zhang, J., Zhang, H., Qiao, L. and Men, Y., 2010. Heavy metals in rice and garden vegetables and their potential health risks to inhabitants in the vicinity of an industrial zone in Jiangsu, China. *Journal of Environmental Sciences* 22: 1792-1799.
- Chamannejadian, A., Sayyad, G., Moezzi, A. and Jahangiri, A., 2013. Evaluation of estimated daily intake (EDI) of cadmium and lead for rice (*Oryza sativa L.*) in calcareous soils. *Iranian Journal of Environmental Health Science and Engineering* 10(1): 28.
- De Sole, P., Rossi, C., Chiarpotto, M., Ciasca, G., Bocca, B., Alimonti, A., Bizzarro, A., Rossi, C. and Masullo, C., 2013. Possible relationship between Al/ferritin complex and Alzheimer's disease. *Clinical Biochemistry* 46: 89-93.
- European Food Safety Authority (EFSA), 2007. Safety of aluminium from dietary intake. *EFSA Journal* 754: 1-34.
- Exley, C., 2013. Human exposure to aluminium. *Environmental Science: processes and Impacts* 15: 1807-1816.
- Farahani, S., Eshghi, N., Abbasi, A., Karimi, F., Shiri Malekabad, E. and Rezaei, M., 2015. Determination of heavy metals in albumen of hen eggs from the Markazi Province (Iran) using ICP-OES technique. *Toxin Reviews* 34: 96-100.
- Fishburn, J.D., Tang, Y. and Frank, J.F., 2012. Efficacy of various consumer-friendly produce washing technologies in reducing pathogens on fresh produce. *Food Protection Trends* 32: 456-466.
- Foy, C., Chaney, R.L. and White, M., 1978. The physiology of metal toxicity in plants. *Annual Review of Plant Physiology* 29: 511-566.
- Haiyan, W. and Stuanes, A.O., 2003. Heavy metal pollution in air-water-soil-plant system of Zhuzhou city, Hunan Province, China. *Water, Air, and Soil Pollution* 147: 79-107.
- Horbowicz, M., Kowalczyk, W., Grzesiuk, A. and Mitrus, J., 2011. Uptake of aluminium and basic elements, and accumulation of anthocyanins in seedlings of common buckwheat (*Fagopyrum esculentum* Moench) as a result increased level of aluminium in nutrient solution. *Ecological Chemistry and Engineering*. S 18: 479-488.
- Institute of Standards and Industrial Research of Iran (ISIRI), 2010. ISIRI 12968. Food and feed-maximum limit of heavy metals. ISIRI Press, Tehran, Iran.
- Jan, F.A., Ishaq, M., Khan, S., Ihsanullah, I., Ahmad, I. and Shakirullah, M., 2010. A comparative study of human health risks via consumption of food crops grown on wastewater irrigated soil (Peshawar) and relatively clean water irrigated soil (lower Dir). *Journal of Hazardous Materials* 179: 612-621.
- Jansen, S., Watanabe, T. and Smets, E., 2002. Aluminium accumulation in leaves of 127 species in *Melastomataceae*, with comments on the order Myrtales. *Annals of Botany* 90: 53-64.
- Jolly, Y.N., Islam, A. and Akbar, S., 2013a. Transfer of metals from soil to vegetables and possible health risk assessment. *SpringerPlus* 2: 385.
- Jolly, Y.N., Islam, A. and Akbar, S., 2013b. Transfer of metals from soil to vegetables and possible health risk assessment. *SpringerPlus* 2: 1.
- Jorhem, L. and Haeggglund, G., 1992. Aluminium in foodstuffs and diets in Sweden. *Zeitschrift für Lebensmittel-Untersuchung und Forschung* 194: 38-42.
- Kachenko, A.G. and Singh, B., 2006. Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. *Water, Air, and Soil Pollution* 169: 101-123.
- Kandimalla, R., Vallamkondu, J., Corgiat, E.B. and Gill, K.D., 2016. Understanding aspects of aluminum exposure in Alzheimer's disease development. *Brain Pathology* 26: 139-154.
- Kramer, M.F. and Heath, M.D., 2014. Aluminium in allergen-specific subcutaneous immunotherapy – a German perspective. *Vaccine* 32: 4140-4148.
- Krewski, D., Yokel, R.A., Nieboer, E., Borchelt, D., Cohen, J., Harry, J., Kacew, S., Lindsay, J., Mahfouz, A.M. and Rondeau, V., 2007. Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide. *Journal of Toxicology and Environmental Health, part B, Critical Reviews* 10: 1-269.
- Kröppl, M., Zeiner, M., Cindrić, I.J. and Stinger, G., 2012. Differences in aluminium content of various tea powders (black, green, herbal, fruit) and tea infusions. *European Chemical Bulletin* 1: 382-386.

- Ligi, D., Santi, M., Croce, L. and Mannello, F., 2015. Aluminum induces inflammatory and proteolytic alterations in human monocytic cell line. *Journal of Inorganic Biochemistry* 152: 190-198.
- López, F.F., Cabrera, C., Lorenzo, M.L. and López, M.C., 2002. Aluminium content of drinking waters, fruit juices and soft drinks: contribution to dietary intake. *Science of the Total Environment* 292: 205-213.
- Mahmood, A. and Malik, R.N., 2014. Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arabian Journal of Chemistry* 7: 91-99.
- Moir, J., Moot, D., Black, A. and Lucas, D., 2013. Soil pH and aluminium toxicity challenges in high country. Oral presentation. Department of Agricultural Sciences, Lincoln University, Christchurch, New Zealand.
- Nham, T.T., 1991. Analysis of potable water for trace elements by ICP-AES. Varian Australia Pty. Ltd., Mulgrave, Australia.
- Nouri, J., Khorasani, N., Lorestani, B., Karami, M., Hassani, A. and Yousefi, N., 2009. Accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential. *Environmental Earth Sciences* 59: 315-323.
- Parviz, M., Eshghi, N., Asadi, S., Teimoory, H. and Rezaei, M., 2015. Investigation of heavy metal contents in infusion tea samples of Iran. *Toxin Reviews* 34: 157-160.
- Rattan, R., Datta, S., Chhonkar, P., Suribabu, K. and Singh, A., 2005. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater – a case study. *Agriculture, Ecosystems and Environment* 109: 310-322.
- Roy, S., Afsar, M.Z. and Kashem, M.A., 2014. Nutrient content of Indian spinach in saline soil as affected by different organic manures. *International Journal of Environmental Sciences* 4: 694.
- Sato, K., Suzuki, I., Kubota, H., Furusho, N., Inoue, T., Yasukouchi, Y. and Akiyama, H., 2014. Estimation of daily aluminum intake in Japan based on food consumption inspection results: impact of food additives. *Food Science and Nutrition* 2: 389-397.
- Shakya, P.R. and Khwaounjoo, N.M., 2013. Heavy metal contamination in green leafy vegetables collected from different market sites of Kathmandu and their associated health risks. *Scientific World* 11: 37-42.
- Sridhara Chary, N., Kamala, C.T. and Samuel Suman Raj, D., 2008. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotoxicology and Environmental Safety* 69: 513-524.
- Verstraeten, S.V., Aimo, L. and Oteiza, P.I., 2008. Aluminium and lead: molecular mechanisms of brain toxicity. *Archives of Toxicology* 82: 789-802.
- Whalen, J.K., Chang, C., Clayton, G.W. and Carefoot, J.P., 2000. Cattle manure amendments can increase the pH of acid soils. *Soil Science Society of America Journal* 64: 962-966.
- World Health Organisation (WHO), 2007. Evaluation of certain food additives and contaminants 67th report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series 940. WHO, Geneva, Switzerland.
- Zhang, H., Jiang, Z., Qin, R., Zhang, H., Zou, J., Jiang, W. and Liu, D., 2014. Accumulation and cellular toxicity of aluminum in seedling of *Pinus massoniana*. *BMC Plant Biology* 14: 264.

