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ASSESSING HEALTH RISKS ASSOCIATED WITH TRACE ELEMENTS IN MARKET VEGETABLES IN HALABJA PROVINCE, IRAQI KURDISTAN

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Abstract

Trace elements are released into the environment from natural and anthropogenic activities and accumulated in soil and vegetables through various pathways which ultimately affects the human health. The purpose of this study was to assess health risks from trace elements (TEs) found in market vegetables. The present study was carried out to assess concentration of six TEs, (Fe, Zn, Se, As, Cd and Pb) in representative samples of highly consumed 22 vegetables in (leafy vegetables, fruit vegetables and tuber vegetables) groups were collected from three different central mini markets (gwllan, sara and bazar) in Halabja city. The range of various total concentration TEs were ranged from (27.3-558 for Fe, 7.33-103 for Zn, 0.009-0.284 for Se, 0.005-0.339 for As, 0.015-1.341 for Cd and 0.012-0.535 for Pb) mg/kg dw, in different types of the vegetables. The obtained results showed that their total concentrations exceeded the permissible limits set by FAO/WHO for Fe in chard and celery and Zn in watermelon and Cd in (chard, spinach, lettuce, okra, carrot, radish and turnip) and Pb in celery among the vegetables which analysed. To evaluate the effect of the heat treatment on total concentration of TEs, the following experimental variables were selected type of thermal processing. The concentration of TEs in all cooked vegetable samples was found to be lower than the raw vegetables. Assessing health risk of TEs in vegetables was determined by Hazard Quotient (HQ) calculation. In this study the values of HQ of Zn, Se, As, Cd and Pb were lower than one HQ<1 in vegetables, suggesting no health hazards posed to adult population, However, HQs of As was higher than one in raw celery and raw carrot which were (1.908) and (1.148) through consumption by child, indicating health risks to child.

Key words: Trace elements, Vegetables, Risk assessment and Hazard quotient

Introduction

Vegetables are very efficient to promote human health especially to maintain body weight (Arós *et al.*, 2013). Eating vegetables are recommended by numerous organizations (Food and Agriculture Organization-FAO, World Health Organization-WHO, European Food Safety Authority-EFSA and United States Department of Agriculture-USDA) as an important for maintain healthy life style requirement (Ragaert *et al.*, 2004). Vegetables considered to have low calories (because of high water and low-fat). They are mainly consisting of carbohydrate, mineral, fiber and micronutrients include vitamins, minerals, carotenoids and polyphenol (Yahia 2010). Trace elements (TEs) are recognized as trace minerals are chemical composites which can be found in soil, plant, wildlife in small degrees (Mehri and Marjan 2015). Soils are the key ground for the elemental composition of plants. The absorption of ingredients in earth is profoundly different which is based on the nature of parent rocks, mineralization and soil processes. Mainly, trace elements (ingredients) are delivered from the earth to the root and then must pass through both cellular and organellar membranes while they are distributed through the plant (Guerinot 2000). The accumulation of TEs in the soil is a matter of concern because they can easily be transferred to the human food chain through food crops and vegetables (Biogeochemistry *et al.*, 2017). Intakes of TEs are expressed as a recommended dietary allowance (RDA) or an adequate amount, the upper limits is the amount of nutrients that are considered to cause no harmful effects on healthy individuals (Tako 2019). Some of them are beneficial for the development and metabolic functioning such as better cell metabolism, good immune function and healthy reproduction of humans (Mehri and Marjan 2015). They also play an important role in different enzymes, hormones, vitamins etc. Lack of some TEs will result in emergence of specific clinical problem, such as lack of iron level is resulting in iron deficiency anemia, copper deficiency responsible for hair changes, zinc deficiency cause age and skin changes, selenium deficiency cause cardiomyopathy and cobalt deficiency cause vitamin b12 deficiency (Dutta and Mukta n.d.). in contracts, presence of some of TEs in diet may pose risk for the consumers (Klevay 2000).

Arsenic exposure may cause dermatitis or it may have carcinogenic effects (Assessment n.d.). Although lead is harmful to the nervous system, it causes blood disorders (Morgano *et al.*, 2011). The population health risks associated with concentricity of trace elements in diet have attracted more interest worldwide. Even when small concentrations of TEs like Hg, As and Pb are well renowned to cause a danger to human health (Zhou *et al.*, 2016). The concentration of TEs in diet is a major public health concern because of the increasing risk of pesticide, TEs or toxins in the food (Hefnawy 2010). With increased emission of pollution, there may be significant impacts on local agriculture, as TEs can enter and accumulate in agricultural soil anywhere and may increase the risk of trace elements contamination of vegetables and their transport to the market for consumers (Yang *et al.*, 2011). Vegetables from street vendors contain good levels of essential minerals and lower concentrations of toxic minerals, so they can be consumed freely to promote good health. It is also freely consumed to promote good health (Bati, Mogobe and Masamba 2017).

The purpose of the study was to assess the human health risks from trace elements in market vegetables. In this approach the main objective would be: Determine the total concentrations of trace elements Fe, Zn, Se, As, Cd and Pb in market vegetables, Evaluate the effect of heat treatment on element concentration in vegetables and quantify potential health risks of selected toxic elements in vegetables in the local population.

Materials and Methods

Sampling collection and preparation and multi elemental analysis

In October-November 2018, twenty-two selected most frequently eaten vegetables in the Halabja population, were randomly collected in three central market and stores from three districts (Gwllan, Sara and Bazar). The samples were in three groups (leafy vegetables (LVs): chard, spinach, lettuce, celery, leek and spring onion. Fruit vegetables (FVs): tomato, cucumber, eggplant, courgette, watermelon, melon, pepper, chili pepper and okra. Tuber vegetables (TVs): potato, onion, beetroot, carrot, radish, turnip and garlic). All the vegetable samples were washed in tap water as would be done during normal food preparation, the inedible part of samples was removed and the samples were homogenized, after that they were washed with ultrapure water, selected part of the samples were dried with filter papers to get fresh weight (GFW) and oven-dried at 70°C for 48 h to get dry weight (GDW) and packed in closable polyethylene bags and stored for digestion. The oven dried samples then ground to powder by high speed multi-functional Crusher, MODEL-100.

Boiling process

To evaluate the effect of the heat treatment on concentration of trace elements, the following experimental variables were selected type of thermal processing (cooking samples in the 1000 ml of boiling distilled water in the beaker at 100°C for 10 to 20 min according to the type of samples. After the heat treatment, the cooked samples were dried in oven at 70°C for 48 hours (Yin *et al.*, 2017).

Total Digestion

Sample preparation for the determination of total analyte concentration involved acid decomposition using a Multiwave 3000 (Microwave Sample Preparation Platform System) in London, United Kingdom. Therefore, 1g, milled and previously dried sample, were directly weighed in a digestion tube, to which 10 mL of Scharlau nitric acid 69.5% analytical grade were added. Digestion started by gradually increasing the temperature, maintaining this temperature for 20 minutes and 10 min for cooling. At the end of digestion, due to the presence of particulate material, the obtained solution was filtered through a quantitative filter paper medium to 100 mL volumetric flasks with ultrapure water.

Sample analysis by ICP-MS

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) measurement conditions were using the built-in Plasma Lab software procedure. Samples of the vegetable extracts were analysed by ICP-MS Optima 2100 DV using an external calibration technique. Internal standard (10 mg/L) were added to all samples, blanks and standard solutions. A blank was analysed with each analytical batch. All data was reported in trace elements concentration (mg/kg, dry weight). The high temperature and high ion density in the plasma provide an ideal ionizing spray of the elements for all types of samples and matrices offered by a variety of specialized devices. Outstanding properties such as relative salt tolerance, compound-independent element response, high sensitivity and highest quantitative accuracy lead to unparalleled performance of ICP MS which reliable to detection, identification and quantification of trace elements (Ammann 2007).

$$Metal(mg/kg) = \frac{[conc.of\ metals(mg/L)x\ volume\ of\ sample(mL)]}{[sample\ weight\ (kg) \times 1000]} \quad \text{Eq(1)}$$

Hazard Quotient (HQ) Method

The potential health risks of trace elements consumption through vegetables were assessed based on the hazard quotient (HQ) approach, which was described in detail by the United States Environmental Protection Agency (Gruszecka-kosowska 2019).

$$HQ = \frac{C \times FIR}{RFD \times BW} \quad \text{Eq(2)}$$

FIR is the food ingestion rate (vegetable consumption values for adults and children are 0.342 and 0.232 g/ person/day, respectively), C is the metal concentration in the edible parts of vegetables (mg/kg), RFD is the oral reference dose (Zn, Se, As, Cd and values were 0.3, 0.005, 0.0003, 0.003 and 0.0035 mg/kg/day, respectively) (US EPA 1989), BW is the average body weight (70 kg for adults and 16.2 kg for child). If the HQ value is greater than 1, the exposure is likely to cause obvious adverse effects.

Statistical Analysis

A one-way ANOVA was used to evaluate the differences among vegetable species. Prior to ANOVA. Duncan's test was used to detect the significant differences between the means of different vegetables classifications. The criterion for significance in the procedures was set at $p < 0.05$ (significant). All data were presented as arithmetic means with standard error attached. All statistical analyses were conducted using the software Excel 2010.

Results

The table 1 showed the mean proportion and standard deviation of trace elements (TEs) in edible portion of vegetables. The values for Fe were varied from (26.3-558 mg/kg dw) which in onion and celery. The Fe concentration significantly increased in leafy vegetables (LVs) sample in order of (celery 558 > chard 492 > spinach 341 > lettuce 331 > leek 314 > spring onion 233) mg/kg dw, which was significantly higher than other types of vegetables. The Fe concentration was significantly decreased in order of (onion 26.3 < cucumber 27.3 < watermelon 28.3) mg/kg dw, which significantly lower than other types of vegetables. The concentration of Fe in the chard and celery were (452 and 558 mg/kg dw) which was above the permissible limit (450 mg/kg dw). The values for Zn were varied from (7.33-103 mg/kg dw) which in onion and watermelon. The concentration of Zn significantly increased in order of (watermelon 103 > okra 64.7 > cucumber 42.8) mg/kg dw, compared to other vegetables. On the other hand, the concentration of Zn significantly decreased in order of (onion 7.33 < melon 9.63 < garlic 10.4) mg/kg dw, compared to other vegetables. The Zn concentration in watermelon was (103 mg/kg dw) which slightly above the permissible limit (99.4 mg/kg dw FAO/WHO). The values for Se were varied from (0.009-0.284 mg/kg dw) which in okra and leek. The Se concentration significantly increased in order of (leek 0.284 > eggplant 0.208 > celery 0.190) mg/kg dw compared to other vegetables. The concentration of Se significantly lower in okra which was 0.009 mg/kg dw compared to other vegetables. On the other hand, the values for As were varied from (0.005-0.339 mg/kg dw) which in melon and celery. The concentration of As significantly increased in order of (celery 0.339 > turnip 0.233 > radish 0.223) mg/kg dw compared to other vegetables. However, As concentration significantly decreased in order of (melon 0.005 < watermelon 0.006 < courgette 0.006 < eggplant 0.007) mg/kg dw compared to other vegetables. The values for Cd were varied from (0.015-1.341 mg/kg dw) which in courgette and lettuce. The Cd concentration significantly increased in order of (lettuce 1.341 > spinach 1.321 > chard 0.575) mg/kg dw compared to other vegetables. Cd concentration significantly decreased in order of (courgette 0.015< onion 0.020 < spring onion 0.033) mg/kg dw compared to other vegetables. The Cd concentration in chard, spinach, lettuce, okra, carrot, radish and turnip were above the permissible limit (0.2 mg/kg dw FAO/WHO). The values for Pb were varied from (0.031-0.535 mg/kg dw) which in onion and celery. The Pb concentration significantly increased in order of (celery 0.535 < leek 0.277 < chard 0.253) mg/kg dw compared to other vegetables. The concentration of Pb in onion was 0.031 mg/kg dw which significantly lower than other vegetables. The Pb concentration in celery was 0.535 mg/kg dw which above the permissible limit (0.3 mg/kg dw FAO/WHO).

The Fig. 1 showed the concentration of Fe, Zn, Se, As, Cd and Pb in the leafy vegetables (LVs) (chard, spinach, celery, lettuce, spring onion and leek) at raw and cooked situation Overall the concentration of TEs in raw samples relatively higher than concentrate of same TEs in corresponding cooked samples. The Fe concentration in celery and chard were found significantly higher than other leaf vegetables which were (559 and 491) mg/kg dw, respectively. The concentration of Fe in spring onion was 234 mg/kg dw, which significantly lower

Table 1: Mean trace element concentrations (mg/kg dw) in edible portion of vegetables.

Sample		Fe	Zn	Se	As	Cd	Pb
Chard	Mean	492[b]	21.5[ghi]	0.144[f]	0.181[d]	0.575[c]	0.253[c]
	± SD	3.74	3.33	0.002	0.004	0.01	0.007
Spinach	Mean	341[c]	29[de]	0.177[e]	0.111[g]	1.321[b]	0.221[d]
	± SD	3.27	3.73	0.004	0.007	0.005	0.006
Celery	Mean	558[a]	29.6[de]	0.190[c]	0.339[a]	0.235[g]	0.535[a]
	± SD	3.29	3.68	0.005	0.005	0.01	0.006
Lettuce	Mean	331[d]	30.5[de]	0.179[e]	0.118[fg]	1.341[a]	0.227[d]
	± SD	3.68	2.55	0.003	0.005	0.007	0.004
Spring Onion	Mean	233[f]	14[jk]	0.091[h]	0.14[e]	0.033[m]	0.161[f]
	± SD	3.3	2.45	0.005	0.004	0.007	0.005
Leek	Mean	314[e]	26.7[efgh]	0.284[a]	0.121[f]	0.175[h]	0.277[b]
	± SD	3.68	2.87	0.004	0.004	0.007	0.005
Pepper	Mean	74.7[i]	29[de]	0.04[k]	0.073[i]	0.141[i]	0.079[ij]
	± SD	3.3	2.94	0.006	0.004	0.005	0.006
Okra	Mean	53.7[k]	64.7[b]	0.009[l]	0.01[klm]	0.343[d]	0.102[g]
	± SD	3.68	3.3	0.001	0.002	0.006	0.002
Tomato	Mean	33.3[mn]	27.4[defg]	0.179[e]	0.01[klm]	0.291[f]	0.068[k]
	± SD	3.3	3.68	0.005	0.002	0.008	0.002
Cucumber	Mean	27.3[mn]	42.8[c]	0.189[cd]	0.101[h]	0.081[kl]	0.181[e]
	± SD	3.68	2.08	0.004	0.004	0.005	0.003
Eggplant	Mean	34[m]	22.3[fghi]	0.208[b]	0.007[lm]	0.102[j]	0.081[i]
	± SD	3.74	2.49	0.005	0.002	0.005	0.005
Courgette	Mean	64.7[j]	33.7[d]	0.081[j]	0.006[lm]	0.015[n]	0.101[g]
	± SD	3.68	3.68	0.004	0.005	0.006	0.003
Chilli Pepper	Mean	74.3[i]	28.5[def]	0.044[k]	0.07[i]	0.144[i]	0.078[ijk]
	± SD	3.3	3.67	0.003	0.005	0.006	0.005
Watermelon	Mean	28.3[mn]	103[a]	0.119[g]	0.006[lm]	0.091[jk]	0.071[jk]
	± SD	2.05	2.62	0.004	0.004	0.005	0.003
Melon	Mean	33.3[mn]	9.63[kl]	0.082[ij]	0.005[m]	0.071[l]	0.091[h]
	± SD	2.05	1.19	0.004	0.003	0.005	0.003
Potato	Mean	93.6[h]	20.3[hij]	0.085[hij]	0.018[k]	0.281[f]	0.081[ij]
	± SD	2.58	2.49	0.004	0.005	0.006	0.003
Onion	Mean	26.3[n]	7.33[l]	0.015[l]	0.066[i]	0.020[n]	0.031[m]
	± SD	2.05	1.25	0.004	0.005	0.005	0.004
Beetroot	Mean	95.9[gh]	18.7[ij]	0.09[hi]	0.016[kl]	0.291[f]	0.091[h]
	± SD	2.12	2.87	0.005	0.004	0.006	0.004
Carrot	Mean	102[g]	18.3[ij]	0.18[e]	0.23[bc]	0.34[d]	0.071[jk]
	± SD	1.25	3.4	0.005	0.005	0.006	0.005
Garlic	Mean	41.3[l]	10.4[kl]	0.198[c]	0.027[j]	0.231[g]	0.012[l]
	± SD	3.68	2.05	0.005	0.003	0.005	0.002
Radish	Mean	101[g]	17.3[ij]	0.181[de]	0.223[c]	0.344[d]	0.077[ijk]
	± SD	3.68	2.48	0.005	0.005	0.006	0.005
Turnip	Mean	99.3[gh]	18.9[ij]	0.19[c]	0.233[b]	0.325[e]	0.08[ij]
	± SD	2.87	3.27	0.004	0.005	0.006	0.002
FAO/WHO*		450	99.4	0.35	0.43	0.2	0.3

Different letters indicate significant differences at $p < 0.05$ as calculated by the least significant difference (ANOVA) test. *Recommended maximum concentration.

than other LVs. The Fe concentration in all LVs show that in raw situation were significantly higher than in cooked situation except for lettuce. The Zn concentration was significantly higher in lettuce which was 31.5 mg/kg dw, but in spring onion Zn concentration was 14.1 mg/kg dw, which significantly lower than other LVs. The Zn concentration in all LVs show that in raw situation were significantly higher than in cooked situation except for leek. The Se concentration in leek was 0.284 mg/kg dw, which significantly higher than other LVs. The Se concentration in spring onion was 0.09 mg/kg dw, which significantly lower than other LVs. The Se concentration in all LVs show that in raw situation were significantly higher than in cooked situation except for spring onion. On the other hand, the As concentration in celery was 0.34 mg/kg dw, which significantly higher than other LVs. The As concentration in spinach was significantly lower than other LVs which was 0.11 mg/kg dw. The As concentration in all LVs show that in raw situation were significantly higher than in cooked situation except for lettuce. The Cd concentration in lettuce and spinach were significantly higher than other LVs which were 1.34 and 1.32 mg/kg dw respectively. The Cd concentration in spring onion was 0.03 mg/kg dw which significantly lower than other LVs. The Cd concentration in all LVs show that in raw situation were significantly higher than in cooked situation except for spring onion. The Pb concentration in celery was 0.534 mg/kg dw, which significantly higher than other LVs. The concentration of Pb in spring onion was 0.16 mg/kg dw, which significantly lower than other LVs. The Pb concentration in all LVs show that in raw situation were significantly higher than in cooked situation except for chard.

The Fig. 2 showed the concentration of Fe, Zn, Se, As, Cd and Pb in the fruit vegetables (FVs) (pepper, okra, tomato, eggplant, courgette and chilli pepper), at raw and cooked situation. Overall the concentration of TEs in raw samples relatively higher than concentrate of same TEs in corresponding cooked samples. The Fe concentration in pepper and chilli pepper was significantly higher than the rest of other FVs which were (74.7 and 74.3) mg/kg dw respectively. The concentration of Fe in tomato

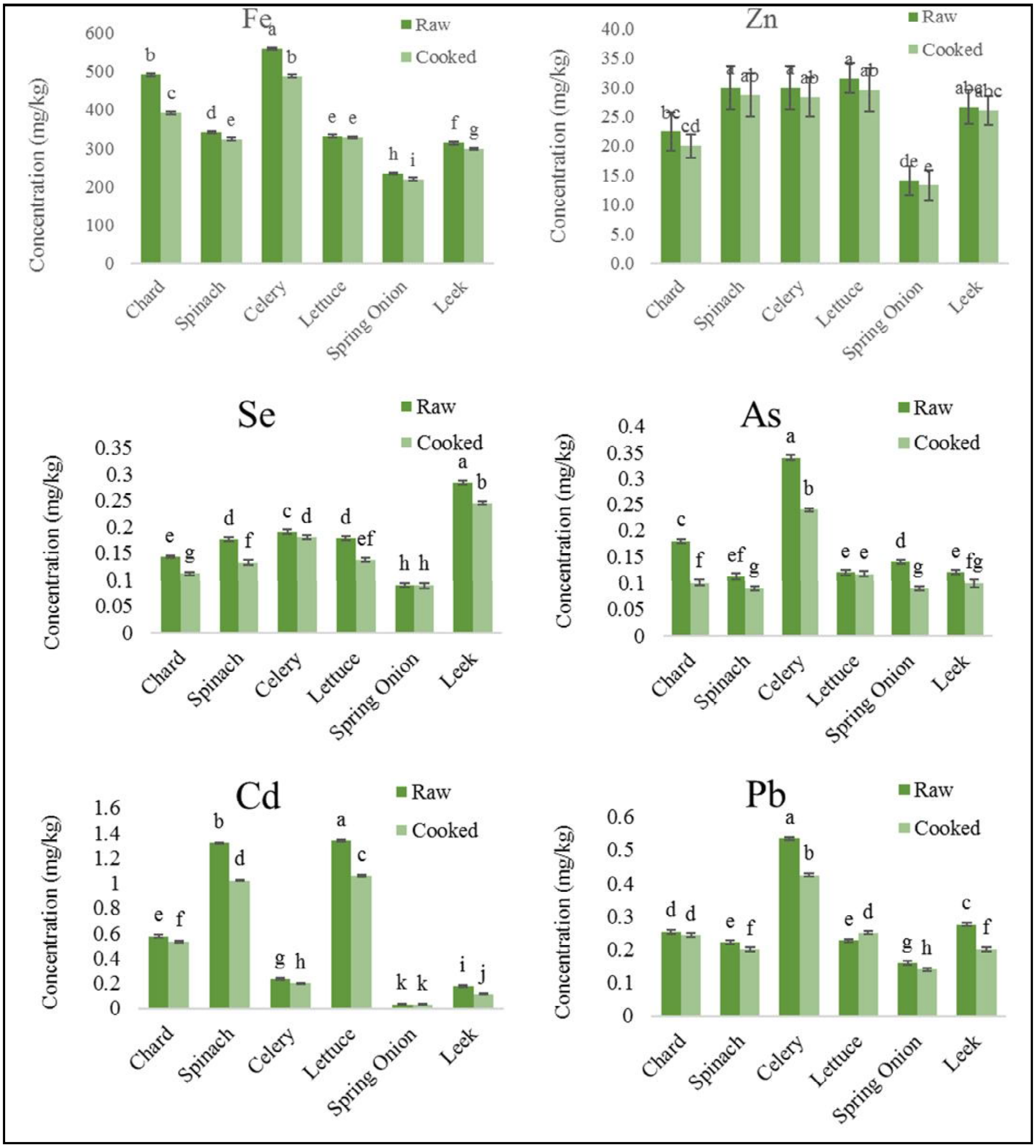

Fig. 1: A comparison Effects of cooking on the concentration of (Zn, Se, As, Cd and Pb) in the leafy vegetables (LVs) (Chard, Spinach, Celery, Lettuce, Spring Onion and Leek), respectively. Different letters indicate significant differences at $p<0.05$ as calculated by the least significant difference (ANOVA) test.

and eggplant was significantly lower than the rest of other FVs which were (33.3 and 34) mg/kg dw respectively. The Fe concentration in pepper, courgette and chilli pepper show that in raw situation were significantly higher than in cooked situation except for okra, tomato and eggplant. The concentration of Zn in all FVs had relatively similar concentration, but concentration of Zn in okra was 64.7 mg/kg dw, which significantly higher than other FVs. The Zn concentration in eggplant was 22.3 mg/kg dw which significantly lower than other FVs. The Zn concentration in all FVs show that in raw situation were significantly higher than in cooked situation except for okra. The Se concentration in eggplant was 0.208 mg/kg dw which significantly higher than other FVs. The concentration of Se in okra was 0.009 mg/kg dw, which significantly lower than other FVs. The Se concentration in tomato, eggplant and courgette show that in raw situation were significantly higher than in cooked situation except for pepper, okra and chilli pepper. On the other hand, the As concentration in both pepper and chilli pepper were significantly higher

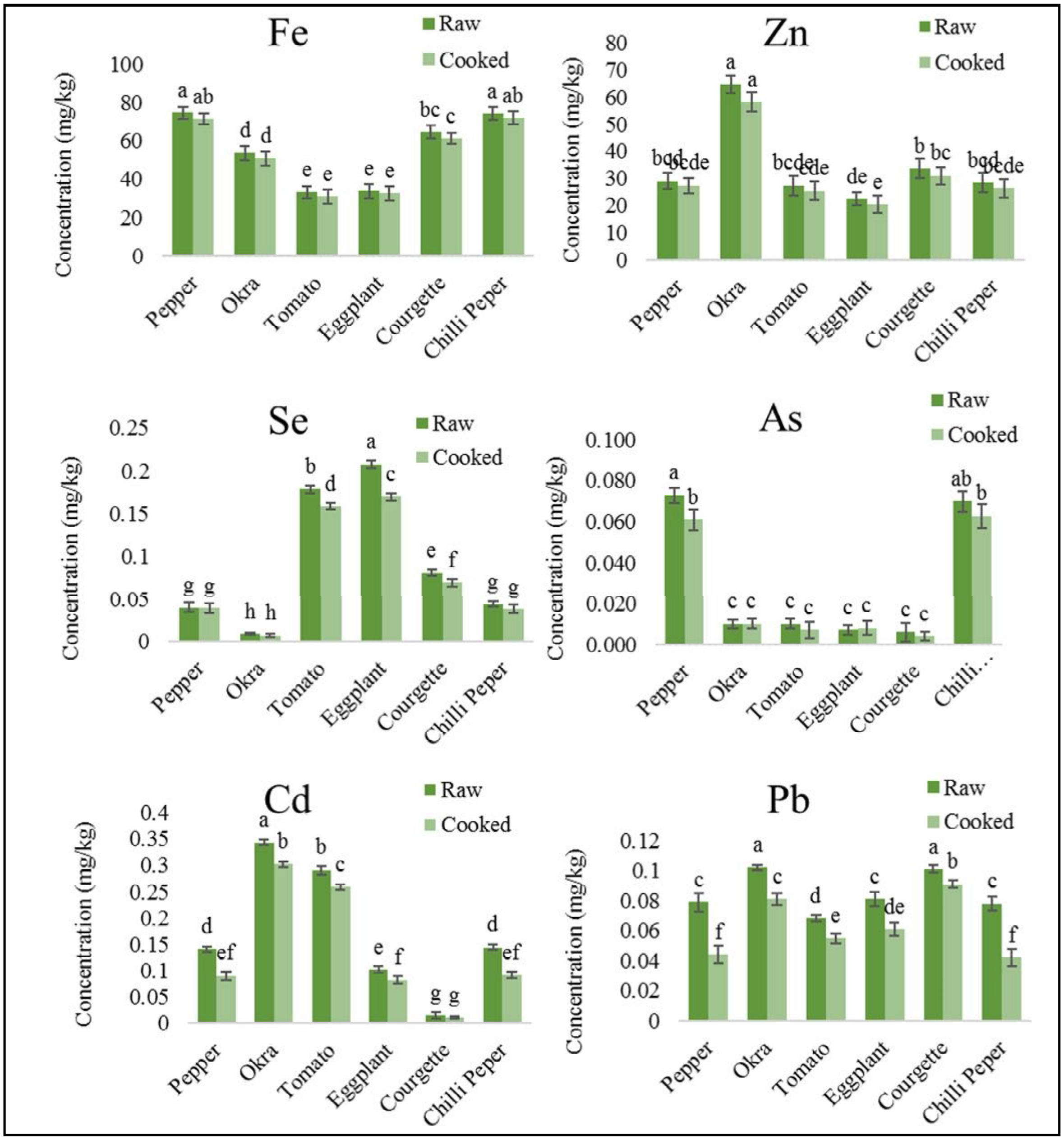

Fig. 2: A comparison Effects of cooking on the concentration of (Zn, Se, As, Cd, Pb) in the fruit vegetables (Pepper, Okra, Tomato, Eggplant, Courgette and Chilli Pepper), respectively and different letters indicate significant differences at $p < 0.05$ as calculated by the least significant difference (ANOVA) test.

than rest of the FVs which were (0.073 and 0.070) mg/kg dw respectively. The As concentration in other FVs were relatively almost had similar concentration in both raw and cooked situation. The As concentration in all samples show that in raw situation were significantly higher than in cooked situation just for pepper and chilli pepper. The Cd concentration in okra was significantly higher than other FVs which was 0.343 mg/kg dw, but Cd concentration in courgette was 0.015 mg/kg dw, which significantly lower than other FVs. The Cd concentration in all FVs show that in raw situation were significantly higher than in cooked situation except for courgette. The Pb concentration in okra and courgette were (0.102 and 0.101) mg/kg dw respectively, which significantly higher than other FVs. The Pb concentration in pepper and chilli pepper, were (0.079 and 0.078) mg/kg dw respectively, which significantly lower than other FVs. The Pb concentration in all FVs show that in raw situation was significantly higher than in cooked situation.

The Fig. 3 showed the concentration of Fe, Zn, Se, As, Cd and Pb in the tuber vegetables (TVs) (potato, onion, beetroot, carrot, garlic, turnip) at raw and cooked situation. Overall the concentration of TEs in raw samples were relatively higher than concentrate of same TEs in

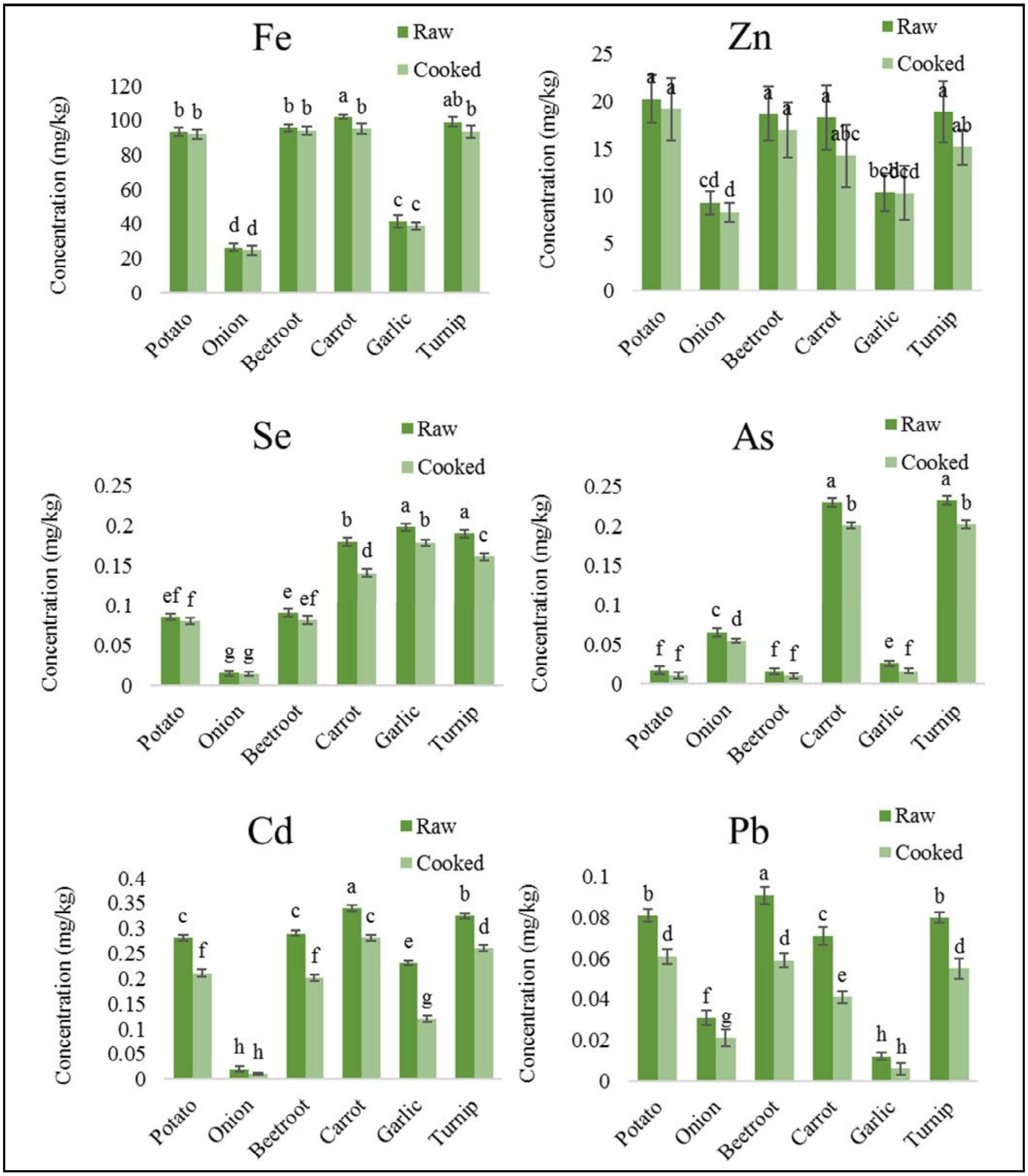

Fig. 3: A comparison Effects of cooking on the concentration of (Zn, Se, As, Cd, Pb) in the tuber vegetables (Potato, Onion, Beetroot, Carrot, Garlic and Turnip), respectively. different letters indicate significant differences at p< 0.05 as calculated by the least significant difference (ANOVA) test.

corresponding cooked samples. The Fe concentration in carrot was 102 mg/kg dw which significantly higher than other TVs, but Fe concentration in onion was significantly lower than other TVs which was 26.3 mg/kg dw. The Fe concentration in in all TVs show that in raw situation were significantly higher than in cooked situation just for carrot and turnip. The Zn concentration in potato was significantly higher than the rest of other TVs which was 20.3 mg/kg dw. The Zn concentration in both onion and garlic were significantly lower than the rest of other TVs which (9.3 and 10.4) mg/kg dw respectively. The Zn concentration in onion, carrot and turnip show that in raw situation were significantly higher than in cooked situation except for potato, beetroot and garlic. The Se concentration in garlic and turnip were higher than other TVs which were (0.198 and 0.190) mg/kg dw, respectively. The concentration of Se in onion was significantly lower than other samples which was 0.015

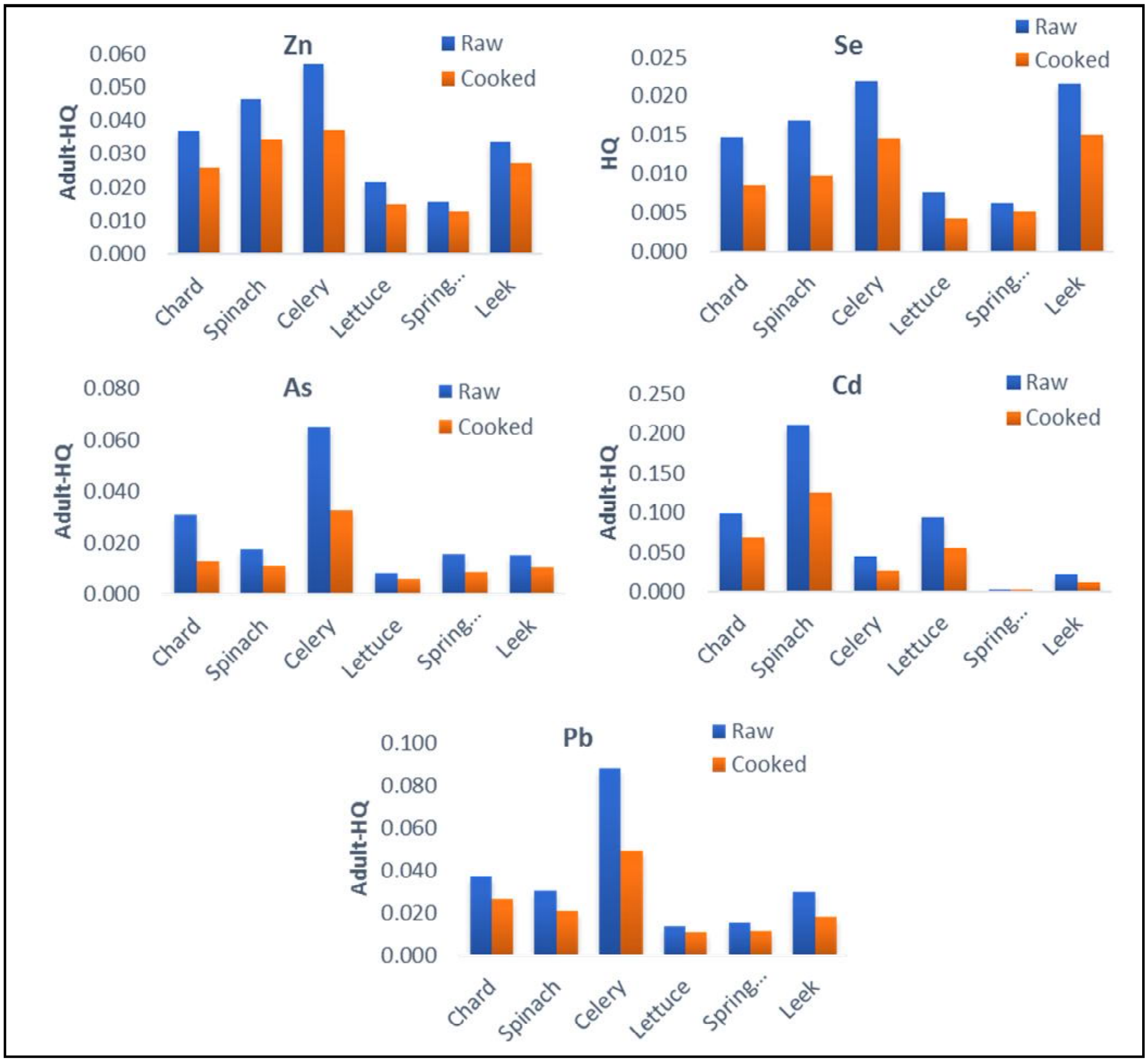

Fig. 4: Total target hazard quotients of the five-trace element (Zn, Se, As, Cd and Pb) via consumption of leaf vegetables (Chard, Spinach, Celery, Lettuce, Spring Onion and Leek) in adult group.

mg/kg dw. The Se concentration in all TVs show that in raw situation were significantly higher than in cooked situation except for onion. On the other hand, the As concentration in both turnip and carrot were (0.233 and 0.230) mg/kg dw respectively, which significantly higher than other TVs, but As concentration in beetroot significantly had lower concentration than other TVs which was 0.016 mg/kg dw. The As concentration in all TVs show that in raw situation were significantly higher than in cooked situation except for potato and beetroot. The Cd concentration in carrot and turnip were (0.34 and 0.325) mg/kg dw which significantly higher than other TVs. The Cd concentration in onion was 0.02 mg/kg dw, which significantly lower than other TVs. The Cd concentration in all TVs show that in raw situation were significantly higher than in cooked situation except for onion. The Pb concentration in beetroot was significantly higher than other TVs which was 0.091 mg/kg dw. The Pb concentration in garlic which was 0.012 mg/kg dw, which significantly lower than other TVs. The Pb concentration in all TVs show that in raw situation were significantly higher than in cooked situation except for garlic.

The Fig. 4 showed the hazard quotients (HQ) of five TEs (Zn, Se, As, Cd and Pb) via consumption of LVs (chard, spinach, celery, lettuce, spring onion and leek) in adult. Overall, there was an increase in the all HQ of raw LVs compare to HQ in cooked LVs. The Zn HQ in celery was higher compare to other LVs, whereas the lowest Zn HQ was in spring onion. The Se HQ in celery and leek was higher compare to other LVs. While Se HQ in spring onion was lowest than other LVs. The As HQ in was higher than to other LVs. While As HQ in lettuce was lowest compare to other LVs. The Cd HQ in spinach was higher than other LVs, while Cd HQ in spring

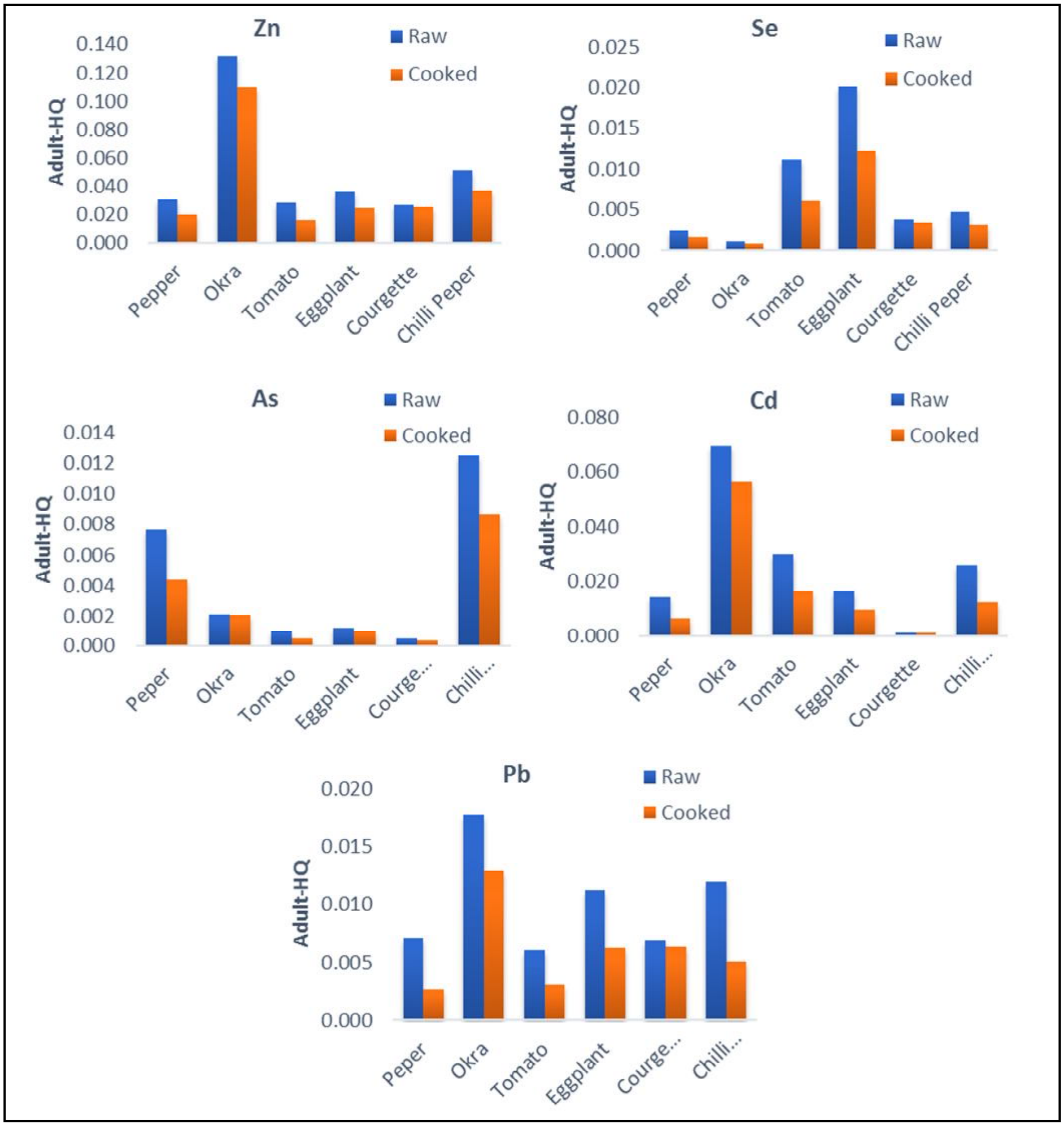

Fig. 5: Total target hazard quotients of the five-trace element (Zn, Se, As, Cd, Pb) via consumption of fruit

onion lower than other LVs. On the other hand, the Pb HQ was higher in celery compared to other LVs. The Pb HQ in lettuce and spring onion were lowest compared to other LVs.

The Fig. 7 show the HQ of five TEs (Zn, Se, As, Cd and Pb) via consumption of LVs (chard, spinach, celery, lettuce, spring onion and leek) in child. Overall, there was an increase in the all HQ of raw LVs compare to HQ in cooked LVs. The Zn HQ in celery was higher than other LVs, whereas the lowest Zn HQ was in spring onion. The Se HQ in celery and leek was higher than other LVs. The Se HQ in spring onion was lower than other LVs. The As HQ in celery was higher than other LVs, especially in raw celery which exceeded permissible limits for children, which is 1. The As HQ in lettuce was lowest compare to other LVs. The Cd HQ in spinach was higher than other LVs, while Cd HQ in spring onion was lower than other LVs. On the other hand, the As HQ was higher in celery compared to other LVs. The Pb HQ in both lettuce and spring onion were lowest than other LVs.

Discussion

Total Concentrations and Effect of Cooking Process

The results of this study indicated that concentrations of Fe, Zn, Se, As, Cd and Pb in vegetables collected from local market of Halabja city are given in table 1.

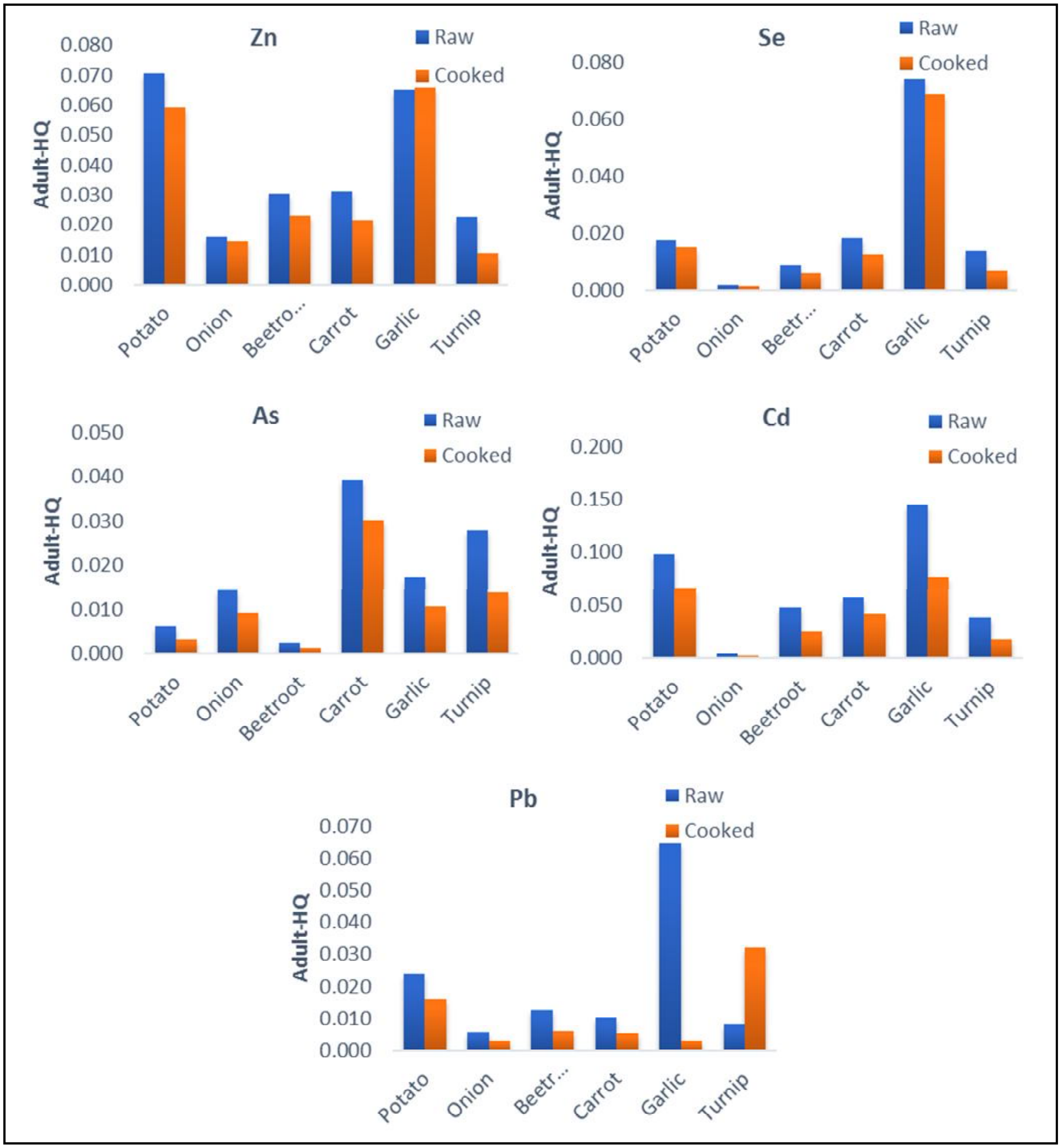

Fig. 6: Total target hazard quotients of the five-trace element (Zn, Se, As, Cd and Pb) via consumption of tuber vegetable (Potato, Onion, Beetroot, Carrot, Garlic and Turnip) samples in adult group.

The values were varied from Fe (27.3-558), Zn (7.33-103), Se (0.009-0.284), As (0.005-0.339), Cd (0.015-1.341) and Pb (0.012-0.535) mg/kg dw. Over all the results showed that the concentration of Fe in the chard and celery were (452 mg/kg and 558 mg/kg) which was above the permissible limit (450 mg/kg dw FAO/WHO). The Zn concentration in watermelon was (103 mg/kg dw) which slightly above the permissible limit (99.4 mg/kg dw FAO/WHO). The Cd concentration in chard, spinach, lettuce, okra, carrot, radish and turnip were above the permissible limit (0.2 mg/kg dw FAO/WHO). A possible explanation for these results are Cd had major accumulation capacity in edible parts of vegetables than Pb, As and another possible explanation for that the tuber vegetables are more likely to translocate Cd to aerial portion of plants (Zhou *et al.*, 2016). The Pb concentration in celery was (0.535 mg/kg dw) which above the permissible limit (0.3 mg/kg dw FAO/WHO). In general vegetables are able to accumulate TEs from contaminated soil and also surface precipitation resulting from contaminated atmospheric environments may consider one of the factor of vegetable contamination by

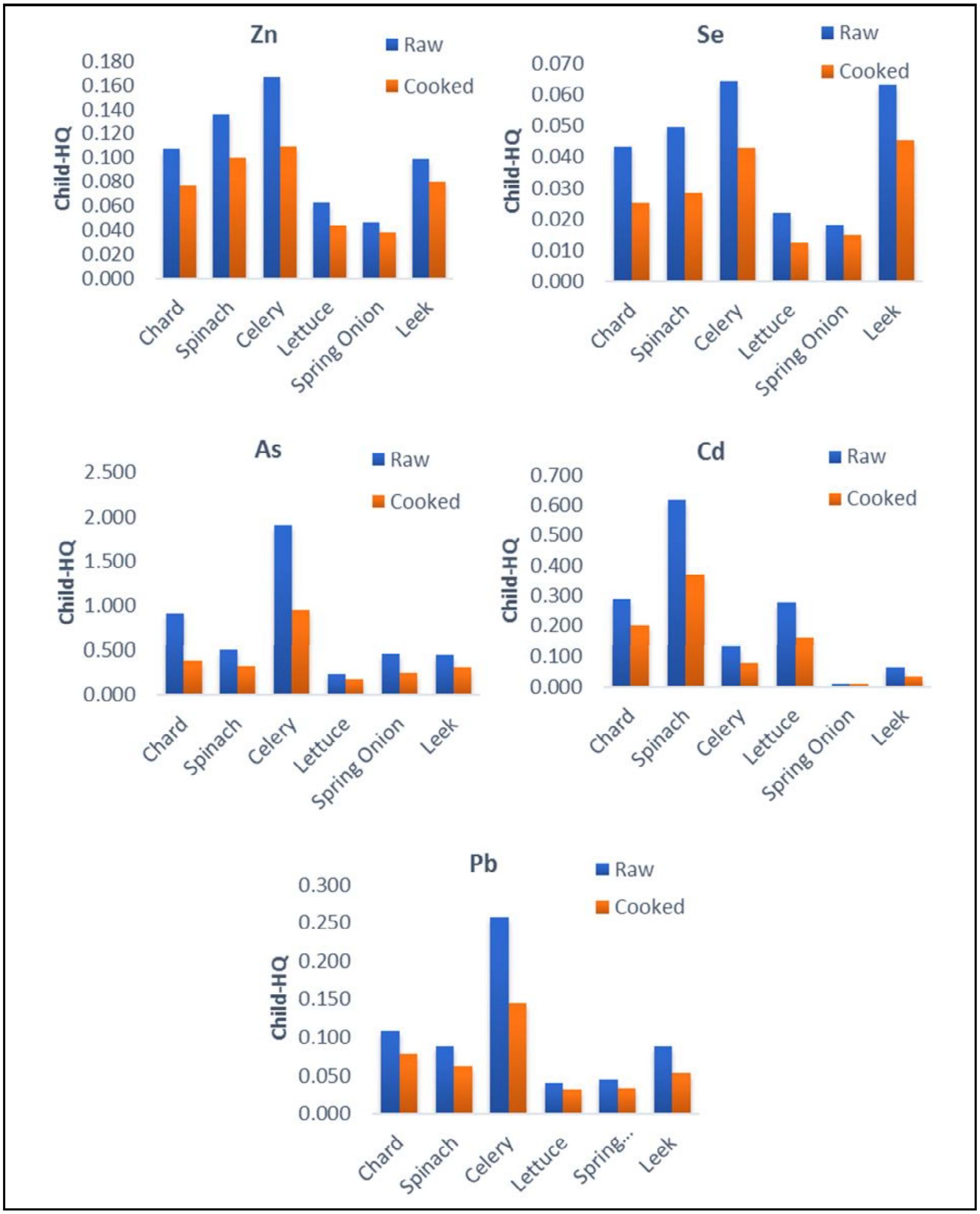

Fig. 7: Hazard quotients of the five-trace element (Zn, Se, As, Cd and Pb) via consumption of leafy vegetables (Chard, Spinach, Celery, Lettuce, Spring Onion and Leek) in child group.

TEs (Kumar, Agrawal and Marshall 2007). The presence of TEs in the air has been reported and significantly impact on total concentrations of TEs in vegetables, especially when they are not completely washed, those factors may explain the relatively good correlation between concentration TEs in soil, air with concentration in vegetables (Publishing and Science 2013). However, this study showed that the concentration of investigated TEs in other vegetables were lower than permissible limit (FAO/WHO).

On other hand, in recent years, many research projects have been undertaken in different countries, to explore the effect of cooking methods on elements in food. A large number of studies recorded a significant decrease in TEs in foods after cooking (Kananke, Wansapala and Gunaratne 2015). the results in Fig. 1, 2

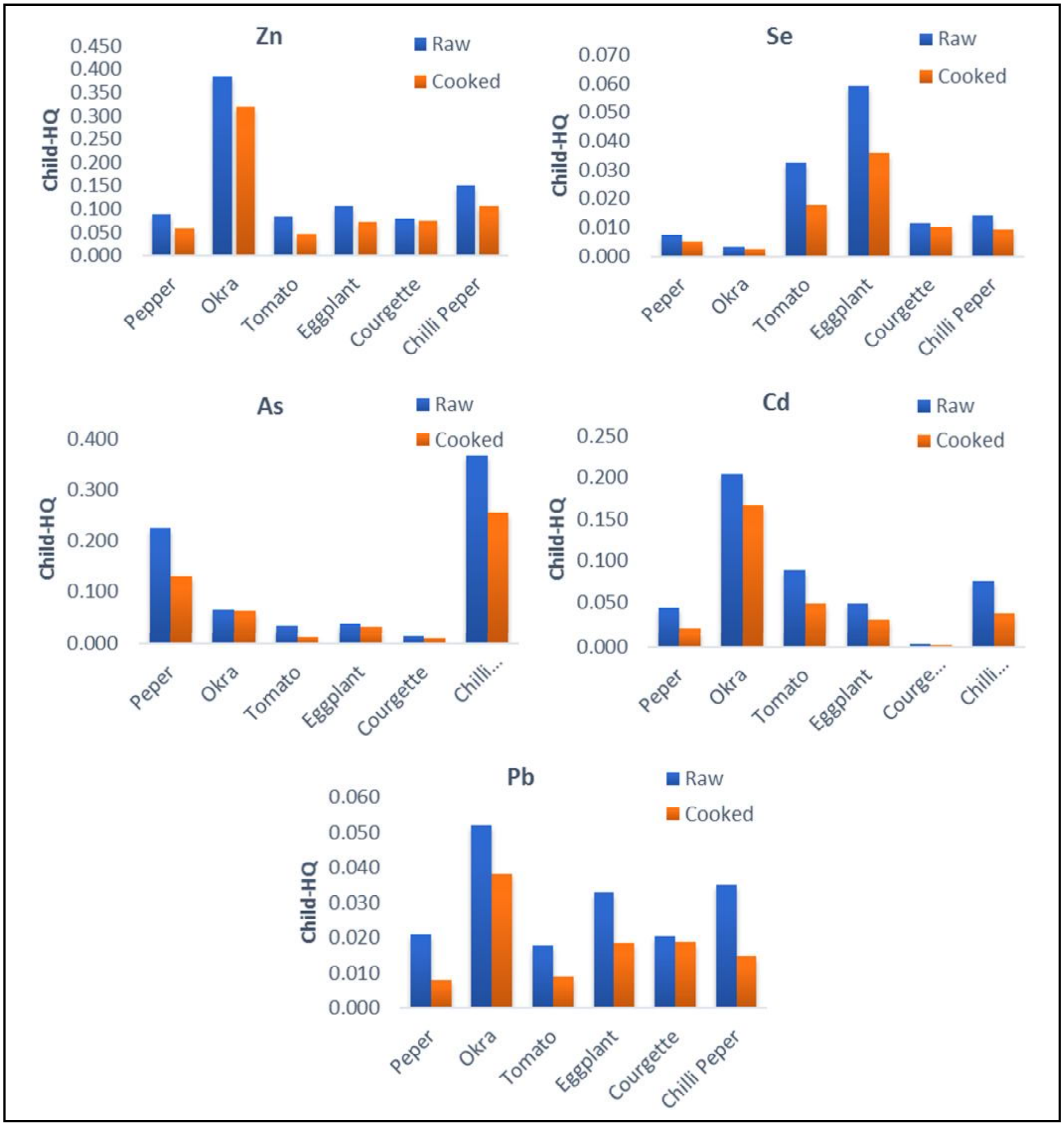

Fig. 8: Total target hazard quotients of the five-trace element (Zn, Se, As, Cd and Pb) via consumption of fruit vegetable (Pepper, Okra, Tomato, Eggplant, Courgette and Chilli Pepper) samples in child group.

and 3 showed the effect of heat on the elemental concentration in vegetable types. The concentration of TEs in all cooked vegetable samples was found to be lower than the raw vegetables. Other studies evaluated that depending on the heat treatment, the internal and bacterial enzymes will be disabled in most condition. This means that endogenous enzymes such as pectinase phytases and cellulase. However, these results are likely to be related that thermal treatments reduce the content of anti-nutritional factors like phenolic compound, phytic acid and tannins of above to 40% (Hemalatha, Platel, and Ã 2007). However, the effect on the fiber portion has not been documented this way through heat treatment, although it can be supposed that some degeneration of the fibers will happen (Hemalatha, Platel and Ã 2007). Cooking methods can change the elements concentration through different means, including volatile components and water evaporation and dissolving the element and also by binding to other large nutrients present in the food element such as carbohydrates, proteins and fats (Kananke, Wansapala and Gunaratne 2015).

In the above figure also has been showed that the concentration of Fe in leafy vegetable (LVs) was significantly higher than other vegetables. However, the

Fig. 9: Total target hazard quotients of the five-trace element (Zn, Se, As, Cd and Pb) via consumption of tuber vegetable (Potato, Onion, Beetroot, Carrot, Garlic and Turnip) samples in child group.

observed result in celery and chard represents the highest concentration of Fe (558 and 492 mg/kg dw) respectively Table 1. A possible explanation for these results may be high concentration of TEs and the strong ability to accumulate TEs in LVs, this may due to the fact that leaves are the main parts of vegetables used for photosynthesis, consequently high mineral mass flows to the leaves due to strong transpiration (Marchiol *et al.*, 2004). Another explanation leaves are also easily exposed to polluted soil because LVs were generally dwarf plants means leaves are nearer to the ground than other kinds of vegetables. Moreover, the precipitation of TEs in the atmosphere may be one of the cause for the high concentrations of minerals in LVs (Huang *et al.*, 2007). Generally, the Fe concentration was significantly higher in celery and chard in LVs also pepper and chilli pepper in fruit vegetables (FVs) and carrot in tuber vegetables (TVs) than other vegetables but Fe concentration was significantly lower in spring onion in LVs also tomato and eggplant in FVs and onion in TVs than other vegetables Fig. 1, 2 and 3. The Fe concentration in all vegetables show that in raw situation were significantly higher than in cooked situation except for lettuce in LVs, okra, tomato and eggplant in FVs and carrot and turnip in TVs Fig. 1, 2 and 3. The lower concentrations of elements during thermal processes are due to a decrease in protein contents and also to the removal of TEs (such as free salts) with leachate. Moreover, pH changes in the medium are also believed to cause changes in the chemical forms of the elements (Kananke, Wansapala and Gunaratne 2015).

This study showed that Zn concentration has irregular change between various vegetables samples in various parts, the concentration of Zn in FVs samples was found to be higher compared to leaf and tuber vegetable groups specially in watermelon and okra (103 and 64.7 mg/kg dw, respectively) table 1. The results in other study reported that Zn concentration in most vegetables was (3.56–4.592 mg/kg) which among the permissible limits (Kachenko and Singh 2006). On the other hand, other studies reported zinc levels in some vegetables ranged between 14.14 and 76.28 mg/kg (Jassir, Shaker and Khaliq 2005). In general, the current study showed that the zinc concentration was within the permissible limits (99.4 FAO and WHO). Moreover, watermelon was slightly higher than permissible limits table 1. It might be related to presence of high amount of water in watermelon and close proximity to soil compared to other FVs samples (Gupta *et al.,* 2018). The Zn is an essential plant micronutrient and has a long biological half-life in plants. So usually present at adequate levels in plant tissues (Oteef and Fawy 2015). Generally, the Zn concentration was significantly higher in lettuce in LVs also okra in FVs and potato in TVs than other vegetables but Zn concentration was significantly lower in spring onion in LVs also eggplant in FVs and onion and garlic in TVs than other vegetables Fig. 1, 2 and 3. The Zn concentration in all vegetables show that in raw situation were significantly higher than in cooked situation except for leek in LVs, okra in FVs and potato, beetroot and garlic in TVs Fig. 1, 2 and 3.

The concentration of Se in most vegetables is showed table 1 detected that all of them were lower than permissible limits set by FAO and WHO (0.35 FAO/ WHO). It could be suggested that a little fraction of the total concentration of Se ingested during food in the daily diet is absorbed and converted into a biologically active form (Osman and Latshaw 1975). Generally, the Se concentration was significantly higher in leek in LVs also eggplant in FVs and garlic and turnip in TVs than other vegetables but Se concentration was significantly lower in spring onion in LVs also okra in FVs and onion in TVs than other vegetables Fig. 1, 2 and 3. The Se concentration in all vegetables show that in raw situation were significantly higher than in cooked situation except for spring onion in LVs also pepper, okra and chilli pepper in FVs and onion in TVs Fig. 1, 2 and 3.

The concentrations of As in all vegetable samples were less than the permissible limit (0.43 mg/kg dw FAO/ WHO) table 1. Other studies have reported that the concentration of As in the Brinjal was 0.2 mg/kg and 0.01 mg/kg in potatoes based on dry weight, which was lower than current study, this observation may support the hypothesis that concentration of As in our soil higher than other study (Alam, Snow and Tanaka 2003). Generally, the As concentration was significantly higher in celery in LVs also pepper and chilli pepper in FVs and turnip and carrot in TVs than other vegetables but As concentration was significantly lower in spinach in LVs also okra, tomato, eggplant and courgette in FVs and beetroot in TVs than other vegetables Fig. 1, 2 and 3. The As concentration in all vegetables show that in raw situation were significantly higher than in cooked situation except for lettuce in LVs, also okra, tomato, eggplant and courgette in FVs and potato and beetroot in TVs Fig. 1, 2 and 3. According to the results, cooking vegetables with distilled water greatly reduced the As concentration of raw vegetables because heat treatment accelerates the breakdown of the bonds between arsenic and food particles and then helps dissolve them in boiling water (Kananke, Wansapala and Gunaratne 2015).

The highest contents of Cd found of LVs and TVs also in okra table 1. It is believed that the accumulation of Cd in the human body may cause reproductive deficiency, prostate, skeletal damage, breast cancer and impaired kidney function [53] (Fytianos *et al.,* 2001). It is reported that lettuce and spinach cultivated in the soil of the industrial zone in Greece are enriched on Cd. (Jassir, Shaker and Khaliq 2005) also the author reported that Cd levels were higher in the vegetable rocket species in the garden for washed and unwashed samples. In this study, was observed that the Cd concentration was higher than the critical level of 0.2 mg/kg as reported by WHO. Previous studies found that the concentration of Cd was 0.1 mg/kg in potatoes, which these result was lower than current study (Alam, Snow and Tanaka 2003). Generally, the Cd concentration was significantly higher in lettuce and spinach in LVs also okra in FVs and carrot and turnip in TVs than other vegetables but Cd concentration was significantly lower in spring onion in LVs also courgette in FVs and onion in TVs than other vegetables Fig. 1, 2 and 3. The Cd concentration in all vegetables show that in raw situation were significantly higher than in cooked situation except for spring onion in LVs also courgette in

FVs and onion in TVs Fig. 1, 2 and 3. Another study examined the effect of general cooking practices on the toxic elements (Hg, Pb, As and Cd) present in various nutrients. The results confirmed that boiling significantly reduced pollution in vegetables (Kananke, Wansapala and Gunaratne 2015).

The results showed that the concentration of Pb in celery samples was the highest value of 0.535 mg/kg dw table 1. Pb contents in celery were significantly higher than in the other vegetables. A possible explanation for this there is general consensus that Pb is neurotoxic and found in paints, dyes, coloring sets and plastics in bibs. This rather contradictory result may be due to its intoxication can result in disruption of certain cellular signaling processing, the generation of action potentials in certain nerve cells and the function of various enzymes and proteins [54] (Muchuweti *et al.*, 2006). It has been reported that the Pb concentration (6.77 mg/kg) in vegetables irrigated with sewage water mixtures from Zimbabwe is higher than the WHO safe limit (0.3 mg/kg). (Jassir, Shaker and Khaliq 2005) The studied six washed and unwashed green leafy vegetables from Saudi Arabia and noticed the higher concentrations of Pb in coriander and parasol. Other studies showed the mean Pb concentration in potato as 0.5 mg/kg (Alam, Snow and Tanaka 2003) in this case that result higher than current result and lower than 0.02 mg/kg which indicated by (Shaheen *et al.*, 2017) in the current study Pb concentration in potato was 0.081 mg/kg dw. Generally, the Pb concentration was significantly higher in celery in LVs also okra and courgette in FVs and beetroot in TVs than other vegetables but Pb concentration was significantly lower in spring onion in LVs also pepper and chilli pepper in FVs and garlic in TVs than other vegetables Fig. 1, 2 and 3. The Pb concentration in different sections in all vegetables show that in raw situation were significantly higher than in cooked situation except for chard in LVs and garlic in TVs Fig. 1, 2 and 3. The our result such as this results that said: Since toxic trace elements cannot be evaporated or changing into non-toxic elements, the elements separated from nutrients during treatment processing should definitely move to the cooking medium (boiling water) so lead to reducing the total concentration (Kananke, Wansapala and Gunaratne 2015).

Risk Assessment

The hazard quotient HQ is the rate of potential exposure to the substance and the level at which no adverse effects are expected. If HQ is less than 1, no health effects from exposure are expected. If HQ is greater than 1, health effects may occur. The HQ of studied TEs through vegetable consumption by inhabitant (adults and child) from 22 vegetable samples. This study aimed to assessment the non-cancer health risk concerning to the presence of TEs (Zn, Se, As, Cd and Pb) residues in vegetables eaten by adult and child. The obtained results showed that non-cancer health risks appeared the current study because in only HQ of two vegetables out of 22 were above standard limit for As, which was in raw celery and raw carrot in child group Fig. 7 and 9 in appendix. Although most of the calculated individual target HQ was below 1 in all samples in raw and cooked situation for adult and child group, which showed in Fig. 4, 7 and Fig. 5, 6, 8, 9 in appendix, respectively. This means the vegetables are safe to be eaten by the population but still attention should be paid to some high values of HQ that approaches or exceeds 1 for As TEs in raw celery and carrot for a child cause of the high value of HQ which was (1.908 and 1.141) as shown in Fig. 7 and Fig. 9 in appendix, which implies a pronounced adverse effect on health by consuming among children (Health and Environmental Effects NVIRONMENTAL Document for 4-Aminopyridie 1989).

The HQ of the studied TEs showed that consumption of vegetables from the central market in Halabja is almost risk-free because the HQ of Zn, Se, As, Cd and Pb of the vegetables were lower than 1, HQ < 1 for adult consumers in Halabja. Also, for raw and cooked situation which showed in Fig. 4 and 5 and 6. Accordingly, for the purpose of health protection against the toxicity of TEs, it needs to pay attention to levels of consumption of celery and carrot by the child. However, each TEs may have its own toxicity mechanism and hence a different health endpoint (Tchounwou *et al.*, 2012). For example, acute exposure to Cd may lead to pulmonary effects such as alveoli, chronic subacute, emphysema and bronchiolitis inhalation of Cd may also lead to renal effects (Chauhan and Chauhan 2014), While toxicity of Pb disturbance in the functioning of the kidneys, reduces hemoglobin synthesis, chronic damage to the central, peripheral nervous system, joints and cardiovascular systems (Duruibe and Egwurugwu 2016). The HQ which lower than one (H <1) means that the exposed inhabitance was supposed to be safe. However, when HQ is among 1 and 5 there is a potential risk related to the studied elemental in the exposed population (Darjah, Sasaran and Kesihatan 2017).

Conclusion

This study revealed the concentration of elements in selected most frequently eaten vegetables in Halabja and their effects on health risks by using the hazard quotient (HQ) approach. The concentration of trace elements (TEs) were varied widely in vegetables and their values found to be lower than the maximum permissible concentration, except for Fe in chard and celery, Zn in watermelon, Cd in (chard, spinach, lettuce, okra, carrot, radish and turnip) and Pb in celery. The results showed significant differences in most TEs in vegetables between raw and cooked situations ($P < 0.05$). The concentration of TEs in all cooked vegetable samples was found to be lower than the raw vegetables. In term of THQ calculation, the vegetables are safe to be consumed by the population except for As in celery and carrot which is bigger than 1 meaning children may be exposed to risks if they consumed these two types of vegetables from Halabja markets. However, consuming nutrients with elevated levels of TEs in the long run may lead to a high level of accumulation in the body causing related health disorders. There is a general consensus that the total concentration of TEs is not represent the labile fractions of trace elements but bioaccessbile fractions are more likely to represent the absorbed portion. Therefore, labile fraction of TEs in vegetables would be strongly suggested to be investigated in the future studies. It is also suggested that regular monitoring of TEs in vegetables is necessary to prevent excessive accumulation of these herbs in the human food chain. Moreover, areas contaminated with toxic TEs such as (As, Cd and Pb) should not be encouraged to farm vegetables.

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References

Alam, M.G.M., E.T. Snow and A. Tanaka (2003). "Arsenic and Heavy Metal Contamination of Vegetables Grown in Samta Village, Bangladesh." **308:** 83-96.

Ammann and A. Adrian (2007). "SPECIAL FEATURE/ : Inductively Coupled Plasma Mass Spectrometry (ICP MS): A Versatile Tool." *Mass Spectrometry*: 419-27.

Arós and Fernando *et al.*, (2013). "Primary Prevention of Cardiovascular Disease with a Mediterranean Diet.": 1-12.

Assessment and A. Nutritional. "Dietary Intakes of Minerals, Essential and Toxic Trace A Nutritional Assessment."

Bati, Keagile, Oarabile Mogobe and Wellington R.L. Masamba (2017). "Concentrations of Some Trace Elements in Vegetables Sold at Maun." *Food Research*, **6(1):** 69-77.

Biogeochemistry, The *et al.*, (2017). "Biogeochemistry of Trace Elements in the Environment e-Editorial to the Special Issue." **186:** 127-30.

Chauhan, Geetanjali and Prof. U.K. Chauhan (2014). "Risk Assessment of Heavy Metal Toxicity Through Contaminated Vegetables From Waste." *International Journal of Advanced Technology in Engineering and Science*, **51(02):** 444-60.

Darjah, Penganggaran, Bahaya Sasaran and Risiko Kesihatan (2017). "Estimation of Target Hazard Quotients and Potential Health Risks for Metals by Consumption of Shrimp (Litopenaeus Vannamei) in Selangor , Malaysia." **46(10):** 1825-30.

Duruibe, Joseph and Jude Egwurugwu (2016). "Heavy Metal Pollution and Human Biotoxic Effects." May 2007.

Dutta, Tarun Kumar and V. Mukta. "Trace Elements 6/ : 10." : 353-57.

Fytianos, K., G. Katsianis, P. Triantafyllou and G. Zachariadis (2001). "Accumulation of Heavy Metals in Vegetables Grown in An." May: 423-30.

Gruszecka-kosowska and Agnieszka (2019). "Human Health Risk Assessment and Potentially Harmful Element Contents in the Fruits Cultivated in the Southern Poland." *Environmental Research and Public Health Article*: 1-24.

Guerinot and Mary Lou (2000). "The ZIP Family of Metal Transporters." **1465:** 190–98.

Gupta, Neha *et al.*, (2018). "Trace Elements in Soil-Vegetables Interface/ : Translocation, Bioaccumulation, Toxicity and Amelioration- A Review Science of the Total Environment Trace Elements in Soil-Vegetables Interface/ : Translocation, Bioaccumulation, Toxicity and Amelioration- A Review." *Science of the Total Environment* **651:** (October). https://doi.org/10.1016/j.scitotenv. 2018. 10.047.

"Health and Environmental Effects Invionmental Document for 4-Aminopyridie." 1989. 1-74.

Hefnawy and Magdy (2010). *Advances in Food Protection*. Cairo, Egypt: Cooperation with NATO Emerging Security Challenges Division.

Hemalatha, Sreeramaiah, Kalpana Platel and Ã. Krishnapura Srinivasan (2007). "Influence of Heat Processing on the Bioaccessibility of Zinc and Iron from Cereals and Pulses Consumed in India." **21:** 1-7.

Huang, S.S. *et al.*, (2007). "Survey of Heavy Metal Pollution and Assessment of Agricultural Soil in Yangzhong District, Jiangsu Province, China." **67:** 2148-55.

Jassir, M.S.Al, A. Shaker and M.A. Khaliq (2005). "Deposition of Heavy Metals on Green Leafy Vegetables Sold on Roadsides of Riyadh City, Saudi Arabia." (March): 1020-27.

Kachenko, Anthony George and Balwant Singh (2006). "Heavy Metals Contamination in Vegetables Grown in Urban and Metal Smelter Contaminated Sites in Australia." *Water, Air, and Soil Pollution*, **169(1-4):** 101-23.

Kananke, Thilini, Jagath Wansapala and Anil Gunaratne (2015).

"Effect of Processing Methods on Heavy Metal Concentrations in Commonly Consumed Green Leafy Vegetables Available in Sri Lankan Market Effect of Processing Methods on Heavy Metal Concentrations in Commonly Consumed Green Leafy Vegetables Available in Sri L." *Pakistan Journal of Nutrition,* **14:** (Sep. 2017): 1026-33.

Klevay, Johd D. Bogdne and M. Leslie (2000). *Clinical Nutration of The Essential Trace Elements and Minerals*. New York.

Kumar, Rajesh, Madhoolika Agrawal and Fiona Marshall (2007). "Heavy Metal Contamination of Soil and Vegetables in Suburban Areas of Varanasi, India." **66:** 258-66.

Marchiol, L., S. Assolari, P. Sacco and G. Zerbi (2004). "Phytoextraction of Heavy Metals by Canola (Brassica Napus) and Radish (Raphanus Sativus) Grown on Multicontaminated Soil." 132.

Mehri, Aliasgharpour and Rahnamaye Farzami Marjan (2015). "Trace Elements in Human Nutrition/ : A Review." *International Journal of Medical Investigation,* **2(3):** 115-28.

Morgano, A. Marcelo *et al.,* (2011). "Assessment of Trace Elements in Fi Shes of Japanese Foods Marketed in São Paulo (Brazil)." *Food Control,* **22(5):** 778-85. http://dx.doi.org/10.1016/j.foodcont.2010.11.016.

Muchuweti, M., J.W. Birkett, E. Chinyanga and R. Zvauya (2006). "Heavy Metal Content of Vegetables Irrigated with Mixtures of Wastewater and Sewage Sludge in Zimbabwe/ : Implications for Human Health." **112:** 41-48.

Osman, M. and J.D. Latshaw (1975). "Biological Potency of Selenium from Sodium Selenite, Selenomethionine and Selenocystine in the Chick*." **43210:** 987-94.

Oteef, Mohammed D.Y. and Khaled F. Fawy (2015). "Consumed in Aseer Region , Saudi Arabia Levels of Zinc , Copper , Cadmium , and Lead in Fruits and Vegetables Grown and Consumed in Aseer Region , Saudi Arabia." (November).

Publishing, I.W.A. and Water Science (2013). "Spatial Distribution and Sources of Dissolved Trace Metals in Surface Water of the Wei River , China Li Jing , Li Fadong, Liu Qiang, Song Shuai and Zhao Guangshuai.": 817-23.

Ragaert, Peter, Wim Verbeke, Frank Devlieghere and Johan Debevere (2004). "Consumer Perception and Choice of Minimally Processed Vegetables and Packaged Fruits." **15:** 259-70.

Shaheen, Nazma *et al.,* (2017). "Presence of Heavy Metals in Fruits and Vegetables/ : Health Risk Implications in Bangladesh Chemosphere Presence of Heavy Metals in Fruits and Vegetables/ : Health Risk Implications in Bangladesh." *Chemosphere,* **152:** (March 2016): 431-38. http://dx.doi.org/10.1016/j.chemosphere.2016.02.060.

Tako, Elad. (2019). "Dietary Trace Minerals." *nutrients Editorial*: 10-12.

Tchounwou, Paul B., Clement G. Yedjou, Anita K. Patlolla and Dwayne J. Sutton (2012). *Heavy Metal Toxicity and the Environment*. USA: Clinical and Environmental Toxicology.

US EPA (1989). "Risk Assessment Guidance for Superfund Volume I Human Health Evaluation Manual (Part A)." *Office of Emergency and Remedial Response,* **1(540/R/99/005):** 1-291. https://www.epa.gov/sites/production/files/2015-09/documents/rags_a.pdf%0Ahttps://rais.ornl.gov/documents/HHEMA.pdf.

Yahia, Elhadi M. (2010). *1 The Contribution of Fruit and Vegetable Consumption to Human Health*.

Yang, Qing wei, *et al.,* (2011). "Concentration and Potential Health Risk of Heavy Metals in Market Vegetables in Chongqing, China." *Ecotoxicology and Environmental Safety,* **74(6):** 1664-69. http://dx.doi.org/10.1016/j.ecoenv.2011.05.006.

Yin, Naiyi, *et al.,* (2017). "Investigation of Bioaccessibility of Cu, Fe, Mn, and Zn in Market Vegetables in the Colon Using PBET Combined with SHIME." *Scientific Reports,* **7(1):** 1-7. http://dx.doi.org/10.1038/s41598-017-17901-1.