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COMPARATIVE STUDY OF SOME TRACE ELEMENTS IN IRAQI VEGETABLES AS A SCREEN FOR ENVIRONMENTAL POLLUTION

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ABSTRACT

Trace elements known as micronutrients which are essential for metabolic pathways, physiological and chemical reactions of human and other living organisms. However, these elements can cause potential health risk in high concentrations, especially of toxic elements. Increase of pollution sources is the main cause of accumulation of trace elements in plants. The aim of this study is to determine of some trace elements in celery, dill, parsley, and cress samples collected from two different areas of Iraq. The obtained results revealed that Cu, Zn, Mn, Ni, Pb, and Cd concentrations in either leaves or stalks of most samples were lower than the permissible limits set by WHO/FAO and Eu. The total mean values (leaves plus stalks) of Cu, Zn, Mn, Ni, Pb, and Cd were 3, 8, 5, 2, 2 and 0.4 mg/Kg respectively. According to total mean values, both of Cu, Zn, Mn, and Ni were lower than the permissible limits. While, Cd and Pb concentrations were appeared to be higher than permissible limits. These results also show that samples collected from Al-Amirea district (in Baghdad) was more polluted than other collected from Fallujah (in Anbar). However the high levels of Pb and Cd in studied samples, calculated human risk index did not show a serious threats for consumers health.
***Keywords* :** Trace elements, pollution, daily intake of elements, health risk index.

Introduction

Vegetables and fruit are very important components of the human diet that comprise vital nutrients and trace elements. They are considered a good source of vitamins, fiber, and minerals which are beneficial for human health (Slavin & Lloyd, 2012; Wallace *et al*., 2020; Yahia, García-Solís, & Celis, 2019). However, these vegetables and fruit may also contain both toxic and essential elements, such as high concentrations of heavy metals that accumulated in plants. Heavy metals are considered the main risk that threats the health of living organisms because of their non-degradable persistence and toxicity that enable them to move into different chemical structures and consequently increasing of their solubility and availability for uptake by plants (Al Mahmud *et al*., 2019; Esposito *et al*., 2019; Kumar *et al*., 2020; Sihlahla, Mouri, & Nomngongo, 2019). Some of heavy metals are classified as essential micronutrients for metabolic pathways, physiological and chemical reactions of human and other living organisms such as (Fe, Cu, Mn, Mo, Ni and Zn) which in excessive quantities are more risk to plants than to animal. Other heavy metals are categorized as an indicator for pollution of plants, soil, water, and air such as (As, Ba, Cd, Co, Cr, Hg, Ni, Pb, Sb, Sn, Sr, Tl, U and V) and these in low concentrations are more harmful to animal and human than plant (Esposito *et al*., 2019; Page & Feller, 2015; R H Jasim, 2012; Wu *et al*., 2020; Zwolak, Sarzyńska, Szpyrka, & Stawarczyk, 2019). These heavy metals are mainly participated in oxidation/reduction processes of plant metabolism (Page & Feller, 2015).They are also play a vital role in the oxidant/antioxidant balance where at high levels these elements can cause an increased in the production of reactive oxygen species (ROS) in plant cells through enhancing of imbalance in the oxidation/reduction metabolism (Ishtiyaq, Kumar, Varun, Kumar, & Paul, 2018).On the other hand, they may be participated in the enzymatic detoxification of ROS (Al Mahmud *et al*., 2019; Hasanuzzaman *et al*., 2017; Page & Feller, 2015).

There are many sources which have been contributing for a significant rising of heavy metals levels in the agricultural environments such as fertilizers and pesticides, transportation, and industrial emissions (Mandal & Kaur, 2019; Ni & Ma, 2018; Sandeep, Vijayalatha, & Anitha, 2019). It has been also reported that the agriculture region differences and cultivar are the main factors which effect on the accumulation of trace elements in vegetables and fruit (Wu *et al*., 2020). Plants absorb these essential and toxic elements, especially Cu, Cr, Cd, Pb, Ni, Mn and Zn, from the polluted soils and water in addition to the air of the contaminated areas (Esposito *et al*., 2019; Sihlahla *et al*., 2019).

Therefore, intake of vegetables and fruit which grown in the polluted environment can cause several health effects in our bodies through food chain processes (Mandal & Kaur, 2019). A recommendation about the minimum daily intake value of vegetables and fruit has been recently reported by FAO/WHO to be 400 g per day (except starchy tubers such as potatoes) to avoid of many diseases such as cancer, diabetes, chronic diseases, and obesity and to reduce the effect of essential elements deficiency (World Health Organization, 1–3 September 2004; Zwolak *et al*., 2019).

It has been reported that cadmium and lead are cumulative toxicant and environmental health hazards. Therefore, maximum levels for cadmium that consumption from various foods, such as vegetables and fruit, have been setting due to its possibility to induce a negative effects on bones and renal function (Esposito *et al*., 2019; Godt *et al*., 2006). The allowable limits for lead were also set, where the developing of neurotoxicity in young children, nephrotoxicity and cardiovascular symptoms in adults as a result of lead intake have been demonstrated (Esposito *et al*., 2019). Furthermore, both of As, Cd, and Pb have been classified by International Agency for Research on Cancer (2012) as a carcinogenic elements which can induce a harmful health disorders such as cardio-vascular impacts, kidney and liver diseases, malnosis, and nerve damage (Godt *et al*., 2006; International Agency for Research on Cancer, 2012; Wu *et al*., 2020). Chromium has also been listed as a toxic metal which in case of severe exposure to Cr can cause cancer and acute injury in kidney, liver, and stomach(Duran, Tuzen, & Soylak, 2011; Wu *et al*., 2020).So, it has been recommended to reduce the consumption of vegetables and fruits that are growing in the contaminated areas. The toxicity degree of some trace elements on the living organisms has been arranged in the following order

Hg > Cu > Zn > Ni >Pb> Cd > Cr > Sn > Fe >Mn> Al (Filipiak-Szok, Kurzawa, & Szłyk, 2015; Pueyo, Lopez-Sanchez, & Rauret, 2004; Wang, Cui, Liu, Dong, & Christie, 2003; Zwolak *et al*., 2019).

However, all trace elements are threats of human health when consuming in food with high levels. For instance, intake of high zinc levels in food can interfere with physiological pathways. There have been few studies relating to intake limitation and toxicity data of other metals in food. Recently, study of Esposito *et al.* (2018) provided by a new data about the trace elements levels in two general types of vegetables and fruit (Esposito *et al*., 2019).

In the last decade, industrialization and urbanization have been rapidly growing through anthropogenic activities caused significant environmental challenges in the producing regions, which led to differences in trace elements of foods, particularly in toxic elements (Gupta *et al*., 2019; Sandeep *et al*., 2019; Srinivas, Rao, & Kumar, 2009). For example, Gan *et al.* (2017) investigate the levels of six trace elements in vegetables and they found there concentrations in urban periphery (Cd 0.04, Cr 0.19, Cu 1.0, Ni 0.4, Pb 0.08, and Zn 4.3 mg/kg) were more higher than those in the rural region (Cd 0.03, Cr 0.17, Cu 0.6, Ni 0.3, Pb 0.04, and Zn 3.5 mg/kg) (Gan *et al*., 2017). Therefore, rapid urbanization led to more rising in the spatial variability of trace elements in the world, which directly reflected on the distribution of metals in food (Wu *et al*., 2020).

From the above view, heavy metals contamination can be threats both of urban and farmland environment and consequently the purpose from the current study is to investigate and compare of Cu, Zn, Mn, Pb, Ni, Cd levels in group of vegetables include celery, dill, pursley, and cress from two Iraqi district (Al-Amirea in the west of Baghdad and fallujah in the east of Anbar province.

Material and Methods

Sample collection

Vegetables samples (celery, dill, parsley, and cress) were randomly collected from two different areas (Al-Amirea and Fallujah). Samples for each vegetable species were separated into two groups (leaves and stalks). After that, samples were washed with tap water and then with de-ionized water and placed in the oven for drying at 50°C.

Sample preparation

Samples were crushed using mortar and then digested using 10 mL of the nitric-perchloric acid digestive mixture in a 3:1 ratio (nitric acid (ACS reagent 70%): perchloric acid (ARISTAR® PLUS)) and heated at 190°C. The solution was left under these conditions until a translucent solution had formed. Sample solution was transferred to a 25 ml volumetric flask, and the volume was completed with de-ionized water.

Trace elements analysis

Flame atomic absorption spectrophotometer (Varian, A240FS, Mulgrave, Australia) was used to analyse trace elements (Cd, Cu, Mn, Ni, Pb, and Zn) in vegetable samples by atomization in an air/acetylene flame at the rate of 13.5 L /min/ 2.0 L /min for the elements(Pigozzi, Passos, & Mendes, 2018). Standard solutions were prepared for each element and measured using hollow cathode lamps which are specified for the metal of interest, Table 1.

Table 1: Wavelengths of the hollow cathode lamps used in the analysis of trace elements

	Cd	Cu	Mn	Ni	Pb	Zn
Wavelength	228.8	324.7	279.9	232	283.3	213.9

Health risk analysis

The human health risk index (HRI) of Cd, Cu, Mn, Ni, Pb, and Zn trace elements was assessed for each of celery, dill, parsley, and cress. HRI estimating through determination of the metal level that the human exposure to it by following the path of exposure of pollutant to the human body (Han, Gao, Geng, Li, & Wang, 2018). HRI can be calculated from the ratio of the metal daily intake (DIM) to the oral reference dose (RfD) (Balkhair & Ashraf, 2016; U.S. Environmental Protection Agency (US-EPA), 2006). RfD represents the value/day human body exposure metal that has non-toxic effect (safe) throughout lifetime (U.S. Environmental Protection Agency (US-EPA), 2006). HRI for Cd, Cu, Mn, Ni, Pb, and Zn were calculated according to the following equation 1 (Balkhair & Ashraf, 2016; Han *et al*., 2018).

$$HRI = \frac{DIM}{RfD} \qquad ...(1)$$

Where DIM and RfD represent the daily intake of metals and the reference oral dose, respectively. RfD values were estimated by USEPA to be 0.001,0.04,0.033, 0.02,0.004, and 0.30 mg kg^{-1} bw/day for Cd, Cu, Mn, Ni, Pb, and Zn, respectively (U.S. Environmental Protection Agency (US-EPA), 2006).

DIM was calculated using Equation (2):

$$DIM = \frac{C_{metal} * C_{factor} * D_{intake}}{B_{weight}} \quad ... (2)$$

where C_{metal} is the heavy metal concentrations in the vegetables, C_{factor} is the conversion factor, $D_{intake}$is the daily intake of the vegetables and B_{weight} is the average body weight (Balkhair & Ashraf, 2016).Conversion factor for fresh vegetables to dry weights has been reported by Balkhair and Ashraf (2016) to be 0.085. Also, the mean daily intake of vegetables 1 and the mean body weight for an adult are of 0.527 kg $person^{-1}$ d^{-1} and 60 kg respectively, which were used in the current study for the estimation of HRI (Balkhair & Ashraf, 2016).

Statistical analysis

All data were analysed using Graph Pad Prism 6 software. One-way ANOVA was used to determine if there any significant differences among collected data where differences were showed as significant if $p \leq 0.05$.

Results and Discussion

This study focused on the estimation of some trace elements in group of vegetables that growing in two different areas of Iraq. Trace elements concentrations in leaves and stalks were determined in each of celery, dill, parsley, and cress and then compared with trace elements permissible limits which have been previously reported by European Union (Eu) (2006) and (WHO/FAO) (2007) guidelines (Sihlahla *et al*., 2019; U.S. Environmental Protection Agency (US-EPA), 2006), Table1.

Table 1: Trace elements content in leaves and stalks of four vegetable groups collected from two different Iraqi sites, with permissible limits of Eu and WHO/FAO.

Regions	Plant types	Plant species	Plant trace elements					
			Cu (mg/Kg)	Zn (mg/Kg)	Mn (mg/Kg)	Pb (mg/Kg)	Ni (mg/Kg)	Cd (mg/Kg)
Al-Amirea (Baghdad)	Celery	Leaves	1.14 ± 0.036	4.815 ± 0.018	1.371 ± 0.0014	1.497 ± 0.0017	1.032 ± 0.054	0.171 ± 0.03
		Stalkes	0.456 ± 0.042	2.757 ± 0.072	0.831 ± 0.0017	0.924 ± 0.0014	1.245 ± 0.048	0.225 ± 0.042
	Dill	Leaves	1.284 ± 0.048	3.477 ± 0.036	3.765 ± 0.0017	2.451 ± 0.0022	1.371 ± 0.054	0.204 ± 0.042
		Stalkes	1.296 ± 0.078	2.148 ± 0.042	1.437 ± 0.0014	0.9 ± 0.001	0.699 ± 0.042	0.183 ± 0.024
	Parsley	Leaves	1.011 ± 0.054	4.548 ± 0.054	2.679 ± 0.0035	1.452 ± 0.0013	1.257 ± 0.072	0.171 ± 0.03
		Stalkes	0.651 ± 0.042	3.303 ± 0.048	1.932 ± 0.0017	1.38 ± 0.0014	1.425 ± 0.06	0.297 ± 0.051
	Cress	Leaves	1.599 ± 0.048	5.112 ± 0.042	3.42 ± 0.001	1.035 ± 0.002	0.861 ± 0.06	0.177 ± 0.036
		Stalkes	1.239 ± 0.039	4.377 ± 0.054	1.284 ± 0.0022	0.837 ± 0.0034	1.14 ± 0.054	0.18 ± 0.036
Fallujah (Anbar)	Celery	Leaves	1.779 ± 0.048	3.456 ± 0.066	3.708 ± 0.0014	1.095 ± 0.0022	0.792 ± 0.054	0.189 ± 0.036
		Stalkes	1.374 ± 0.039	3.78 ± 0.042	1.917 ± 0.0019	1.5 ± 0.001	1.356 ± 0.042	0.204 ± 0.024
	Dill	Leaves	1.917 ± 0.066	7.869 ± 0.12	3.504 ± 0.0026	1.092 ± 0.0014	1.215 ± 0.057	0.18 ± 0.042
		Stalkes	1.239 ± 0.018	4.02 ± 0.042	1.38 ± 0.001	1.788 ± 0.0019	0.927 ± 0.054	0.192 ± 0.036
	Parsley	Leaves	1.677 ± 0.069	3.6 ± 0.042	2.544 ± 0.002	0.909 ± 0.002	1.737 ± 0.042	0.324 ± 0.075
		Stalkes	1.287 ± 0.063	3.216 ± 0.042	1.263 ± 0.0026	0.903 ± 0.0017	1.032 ± 0.066	0.201 ± 0.024
	Cress	Leaves	1.563 ± 0.072	5.676 ± 0.024	3.483 ± 0.0014	0.879 ± 0.0012	1.257 ± 0.051	0.243 ± 0.051
		Stalkes	1.224 ± 0.042	4.944 ± 0.078	2.439 ± 0.0017	0.945 ± 0.0026	1.041 ± 0.063	0.192 ± 0.036
Trace elements permissible limits		EU	20	50	500	0.43	N/A	0.2
		WHO/FAO	40	60	N/A	0.3	68	0.2

It has been noticed that trace elements were accumulated in leaves with concentrations higher than in stalks and these were observed in most of studied groups. However, only few samples show that concentrations of trace elements were higher in stalks than leaves and this was mainly found for Pb, Ni, and Cd. Therefore, celery, dill, parsley, and cress leaves were rich in trace elements compared to their stalks. Furthermore, concentrations of Cu, Zn, Mn, and Ni in either leaves or stalks alone were quite lower than the maximum permissible limits set by WHO/FAO and EU. Conversely, it was observed that Pb concentration in leaves and stalks higher than maximum permissible limits. Despite, the lower Cd level in most samples of leaves and stalks, there were a few samples which show a high level of Cd compared to permissible limits, Table1.

Whole (leaves plus stalks) trace elements levels were also calculated and compared according to the growing areas and permissible limits of Eu and WHO/FAO, Figure1.

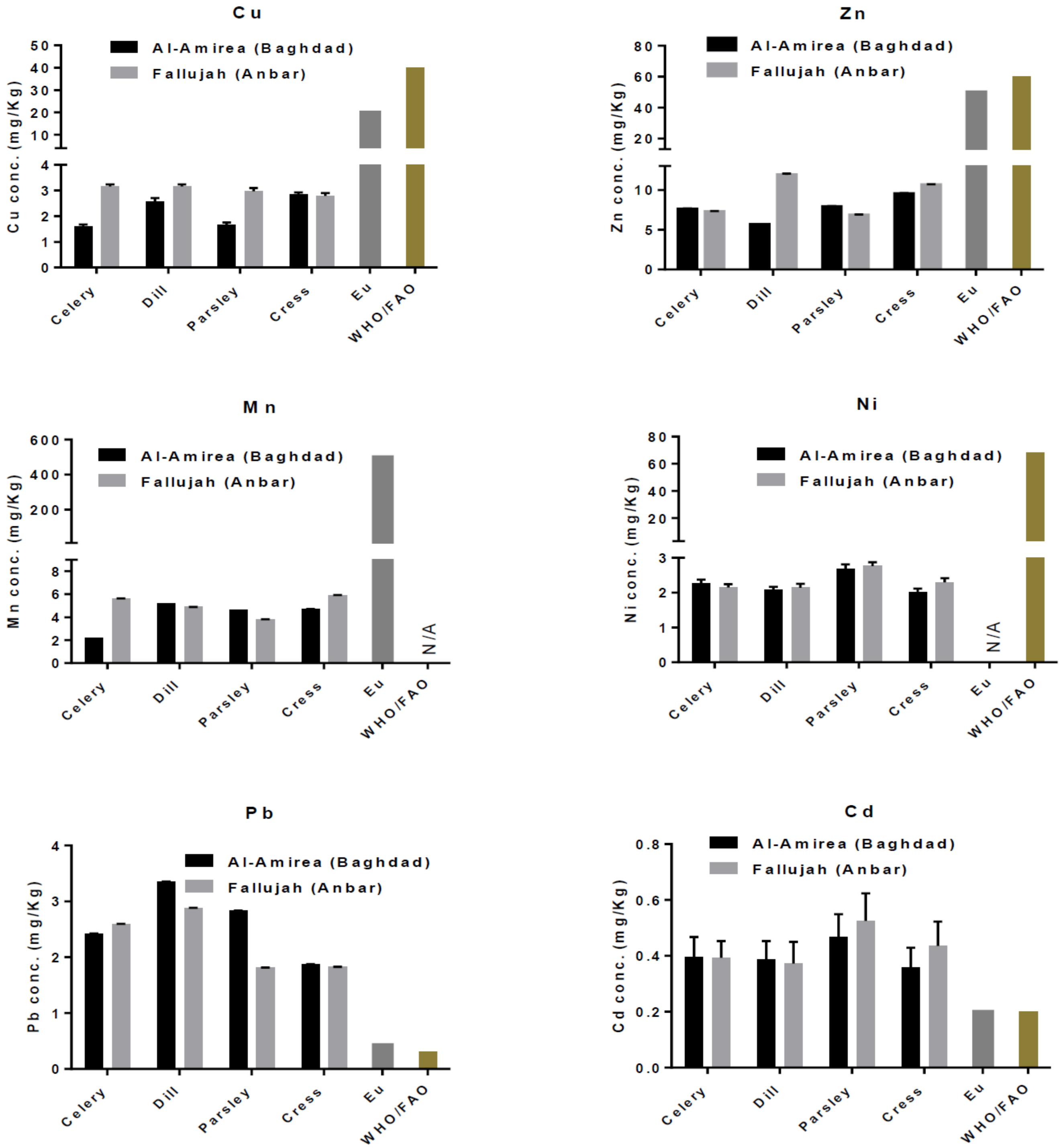

Fig. 1 : Comparison of total concentrations of trace elements (leaves plus stalks) between two different areas of four vegetable groups, where N/A means not available.

The mean values of trace elements concentrations in mg/Kg were decreased in the order

Zn (8) > Mn (5) > Cu (3) > Ni = Pb (2) > Cd (0.4).

Also, the concentration of the studied Cu, Zn, Mn, Ni, Pb, Cd were in the range of 1.6-3.2, 5.6-11.9, 2.2-5.9, 2-2.8, 1.8-3.4, 0.36-0.53 mg/Kg respectively. Concentrations of these elements were compared with their levels in studies which carried out in some neighbouring countries of Iraq. It was found that Cu, Zn, Mn, Ni concentrations were either equal or less than their values in each of Turkey, Jordan, and Saudi Arabia, except Cd and Pb which their concentrations appeared to be equal or more than their levels in these countries (Ababneh, 2017; Seddigi, Kandhro, Shah, Danish, & Soylak, 2016; Targan, Yelboğa, & Cittan, 2018).

It was also observed that the content of Cu, Zn, Mn, and Ni in both of celery, dill, parsley, and cress were significantly lower than the maximum allowable limits. Therefore, these vegetables are safe source for providing with beneficial elements which are essential for growing of plants. Also, these trace elements play a vital role in the antioxidant systems where they are involving in the antioxidant enzyme to protect from the harmful effects of accumulative ROS in living organisms. For example, Cu is existing in the plastidial plastocyanin, in Cu-Zn superoxide dismutase, in the mitochondrial cytochrome c oxidase, and in many of other proteins (Bueno, Varela, Gimenez-Gallego, & del Rio, 1995; Hänsch & Mendel, 2009; Hasan & Al-Ani, 2010; Marschner, 1995; Page & Feller, 2015; Welch & Shuman, 1995; Yruela, 2009, 2013). Zn is essential for serious of enzymes (such as metalloproteinase, Cu-Zn superoxide dismutase, and carbonic anhydrase) (Delorme, McCabe, Kim, & Leaver, 2000; Hacisalihoglu, Hart, Wang, Cakmak, & Kochian, 2003; Hänsch & Mendel, 2009; Marschner, 1995; Mishra, Dixit, Ray, & Sabat, 2014; Takatsuji, 1998; Welch & Shuman, 1995; Yruela, 2013). Mn is necessary for the generation of oxygen processes in photosystem II and for several of enzymatic reactions (such as superoxide dismutase and phosphoenol pyruvate carboxy kinase) (Filiz & Tombuloğlu, 2015; Hänsch & Mendel, 2009; Lanquar *et al*., 2010; Lidon, Barreiro, & Ramalho, 2004; Marschner, 1995; Welch & Shuman, 1995). Also, Ni is involvement in the urease activity (Campbell, 1999; Hänsch & Mendel, 2009; Marschner, 1995; Polacco, Freyermuth, Gerendás, & Cianzio, 1999; Psaras, Constantinidis, Cotsopoulos, & Manetas, 2000; Sirko & Brodzik, 2000; Welch & Shuman, 1995; Witte, 2011).

Nevertheless, Pb and Cd concentrations were significantly higher than the maximum permissible limits. These Pb and Cd elements were listed as carcinogenic heavy metals by International Agency for Research on Cancer (2012), which when intake with high dose can cause many diseases such as cardio-vascular impacts, kidney and liver diseases, malnosis, and nerve damage (Al-Ani, 2010; International Agency for Research on Cancer, 2012; Wu *et al*., 2020).

According to vegetables groups and their growing areas, it was noticed that the Cu, Mn, and Pb concentrations in celery collected from Al-Amirea was significantly lower than its concentration in Fallujah celery samples, Figure1. However, celery group shows that the levels of Zn, Ni, and Cd which collected from Al-Amirea were slightly higher their levels in Fallujah samples. Dill group shows that both of Cu and Zn concentrations were significantly accumulated in Fallujah samples compared to Al-Amirea samples. Ni concentrations in Fallujah samples were also higher than its concentration in Al-Amirea but the difference was insignificant. Both of Mn and Cd were slightly higher in Al-Amirea dill samples than in Fallujah samples, this was also observed for Pb but the difference was statistically significant. Furthermore, levels of Cu, and Cd were significantly higher in Fallujah samples than Al-Amireain parsley group. Moreover, Ni was slightly higher in Fallujah samples than Al-Amirea. In contrast, parsley group shows Zn, Mn, and Pb elements were significantly higher in Al-Amirea samples compared to Fallujah samples. It was also observed that in cress group the concentrations of Zn, Mn, Ni, and Cd were significantly higher in Fallujah samples compared to Al-Amirea samples. However, Pb of cress group shows a highly accumulated in Al-Amirea samples compared to Fallujah samples, whereas there was no significant difference in Cu level among these samples, Figure1. From the above view, our results revealed that the concentration of Cu and Ni in (dill, parsley, and cress) and also the Cu concentration in celery group were higher in Fallujah samples than their concentrations in Al-Amirea samples. Conversely, Pb concentrations in three of studied groups (dill, parsley, and cress) were higher in Al-Amirea samples compared to Fallujah samples. These results suggesting that Al-Amirea district in Baghdad (capital city of Iraq) is more polluted than Fallujah in Anbar city. In Iraq, Baghdad known as industrial city which recognized by many of pollutant sources such as factories and traffic activities compared to other cities and this might be explain the relatively increased in Cd and Pb in Al-Amirea compared to Fallujah.

Table 2: DIM and HRI Values of Trace elements at different study sites

Trace elements	RfD	DIM (mg Kg^{-1} $person^{-1}$ d^{-1})								HRI							
		Baghdad				Fallujah				Baghdad				Fallujah			
		Celery	Dill	Pursley	Cress	Celery	Dill	Pursley	Cress	Celery	Dill	Pursley	Cress	Celery	Dill	Pursley	Cress
Cu	0.04	0.00040	0.00064	0.00041	0.00071	0.00078	0.00079	0.00074	0.00069	0.00993	0.01605	0.01034	0.01766	0.01962	0.01964	0.01844	0.01734
Zn	0.3	0.00188	0.00140	0.00195	0.00236	0.00180	0.00296	0.00170	0.00264	0.00628	0.00467	0.00651	0.00787	0.00600	0.00986	0.00565	0.00881
Mn	0.033	0.00055	0.00129	0.00115	0.00117	0.00140	0.00122	0.00095	0.00147	0.01661	0.03923	0.03477	0.03547	0.04242	0.03683	0.02871	0.04466
Pb	0.004	0.00060	0.00083	0.00070	0.00047	0.00065	0.00072	0.00045	0.00045	0.15062	0.20848	0.17619	0.11647	0.16145	0.17918	0.11273	0.11348
Ni	0.02	0.00057	0.00052	0.00067	0.00050	0.00053	0.00053	0.00069	0.00057	0.02833	0.02576	0.03337	0.02490	0.02673	0.02665	0.03445	0.02859
Cd	0.001	0.00010	0.00010	0.00012	0.00009	0.00010	0.00009	0.00013	0.00011	0.09855	0.09631	0.11647	0.08884	0.09780	0.09258	0.13065	0.10825

These results were more analysed to calculate the DIM and HRI values. These values are necessary to assess the risk of these trace elements on human health through monitoring the route of transferring of pollutant to human body (Sihlahla *et al*., 2019). Table 2 shows that DIM values for all studied groups were below 1. This means that the consumption of celery, dill, parsley, and cress, which were growing and producing from the studied areas, can provide with essential Cu, Zn, Mn, and Ni instead of threating of our health. This was also observed for Cd and Pb toxic elements which their DIM values were also lower than 1. Furthermore, HRI values which calculated based on DIM were below 1 and this for all essential (Cu, Zn, Mn, Ni) and toxic (Cd, Pb) elements. This indicates that there is no possible future risk from consuming of studied vegetables. Our results suggesting that these vegetables did not significantly effect by the surge in heavy metals contamination. Despite, the concentration of toxic elements (Cd and Pb) were significantly higher than the permissible limits of Eu and WHO/FAO (Table1), the dietary intake levels and health risk assessment are within the acceptable limits (Table 2).

Conclusion

Current study investigates the level of some trace elements collected from two different areas (Al-Amirea and Fallujah). It has been found that Cu, Zn, Mn, and Ni concentrations, which are considered as essential for growing and other physiological reactions, were much lower than the permissible limits of Eu and WHO/FAO. However, other studied elements (Cd and Pb), which classified as a toxic elements, were significantly higher than the allowable limits. These toxic elements were highly accumulated in most of Al-Amirea samples compared to those which were collected from Fallujah. This might be gave an indication that Al-Amirea is more polluted than Fallujah. On the other hand, DIM and HRI data show that all studied elements were lower than DIM and HRI limits. Therefore, we can conclude that although Cd and Pb concentrations were higher than allowable limits, the dietary intake levels and health risk assessment are within the acceptable limits.

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