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Potentially harmful elements in lebanese fattoush salad

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Abstract The estimation of potentially harmful element availability in Fattoush ingredients cultivated in Lebanon is essential for evaluating impending risks for human and ecosystem health. In this study and for the first time, the selected plant species were the ingredients of the traditional Lebanese salad, Fattoush, composed of lettuce, cucumber, tomato, onion, purslane, radish, lemon and sumac in order to fulfill all the requirements for the assessment of the contamination levels in vegetables from soils with potentially harmful elements. The major physicochemical properties of topsoil including pH, organic matter content and texture showed that studied soils were almost neutral pH of 7.0, very poor in organic matter (organic matter percent <0.05 %) and sandy respectively(based on soil texture triangle). Concentration ranges of As, Cu, Fe, Zn, Mn, Ni, V, Cd, Co, Cr, Pb, P, Sn and Al in soils and edible parts of plants collected from urban allotments in the South, Damour, Ghazir and Akkar areas were determined and assessed by Inductively Coupled Plasma Optical Emission Spectroscopy following microwave assisted digestion by HNO_3/H_2O_2. Also, weak acetic acid digestion and aqua regia digestion were similarly measured following microwave assisted digestion. Transfer factors from sandy soils to vegetables because of their health risk were calculated accordingly. Results showed that concentrations of most of studied elements in soil and plant samples were recorded above the permissible limits set by World Health Organization especially in purslane, lemon and sumac. For this reason, amounts of accumulated Cu, Fe, Zn, Mn, Ni and V in the studied plant – soil systems (mg/kg) were separately shown. Furthermore, the sumac, purslane, lemon and lettuce plants were found to be “heavy metal hyperaccumulating plants”. Finally, soil to plant transfer is the major path way of human exposure to PHEs contamination and safety measurements should be strictly applied.

Keywords: pseudototal, mobilizable, assisted digestion, aqua regia, transfer factor

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Introduction

The concerning in soil-plant relationships about potentially harmful elements loading from soils to plants has increased over the past few decades as awareness of metal contamination in human food sources has increased (Sarma, 2011). Vegetables are common diet taken by populations throughout the world especially in the Middle-East where salads are of high interest, being rich sources of essential nutrients, antioxidants and metabolites. It is known that both essential and toxic elements are absorbed by vegetables from the soil (Hajeb *et al.*, 2014). The ingestion of heavy metal contaminated vegetables may expose humans to many health risks; therefore the heavy metal contamination of food is one of the most important aspects of food quality (Khan *et al.*, 2008). Bioavailability of heavy metals in soils is critically dependent on the chemical speciation of the metals and plants respond only to the fraction that is "phytoavailable". The readily soluble fraction of heavy metals in soil is generally considered to be phytoavailable, but there is growing awareness that the various methods for assessment of "soluble" and "phytoavailable" fraction need reevaluation. It is generally known that there are variations in the rates of soil to plants transfer between different plant species but also between the same plant species from different areas (McLaughlin *et al.*, 2011). Lebanese diet is characterized, as in most of the Mediterranean regions, by a dominating contribution of fruits and vegetables (42 %), cereals (34 %: bread 14 %; pastries 5 %) and legumes (7 %) in the daily food ration. This fact makes us sure that salads are highly present at the Lebanese table such as the famous 'Fattoush' made up of many vegetables cultivated in Lebanese soils which may expose the consumers to many threats (Tueni *et al.*, 2012).

Fattoush is a salad made from toasted or fried pieces of Arabic flat bread mixed with vegetables and herbs according to season, region and taste. The vegetables are cut into relatively large pieces compared to Tabbouleh which requires ingredients to be finely chopped. Sumac is the herb usually used to give Fattoush its sour taste (Ariel, 2012). The Fattoush salad mixture is essentially made from a garniture of several vegetables, more precisely, a net hundred gram of "Fattoush" plate (DW basis) is made of the following quantities of vegetables: 20 g of cucumber, 5 g of onion, 20g of tomato, 10g of radish, 25 g of lettuce, 15g of purslane, 2.5g of sumac and 2.5 g of lemon.

Soil in Lebanon is young and is characterized by fragility, poor consistency and shallowness especially on slopping terrains (Fadel *et al.*, 2017). Average elemental concentrations in Damour urban allotments were published low (Fadel *et al.*, 2017). The heavy metal concentrations in the bed sediments of the Lower Litani River Basin in the South were published remarkably high

(Nehme *et al.*, 2014) while in Oustouan River in Akkar region publications referred an enrichment of dissolved salts throughout the course of the river from the source to the mouth (Bouaoun and Nabbout, 2016).

The selected plant species were the ingredients of the Lebanese Fattoush salad (lettuce, cucumber, tomato, onion, purslane, radish, lemon and sumac) collected from allotments in South Lebanon (Bourj Al Moulouk and Deirmimas), Damour, Ghazir and Akkar areas in order to fulfill all the requirements for the assessment of the pseudototal and mobilizable concentrations of the same elements in the rhizosphere soil of the edible parts of collected plants and the assessment of the contamination levels in soils and vegetables with potentially harmful elements (zinc, chromium, arsenic, manganese, cadmium, barium, aluminum, iron, cobalt, nickel, copper and lead).

Materials and methods

Field sampling and chemical analysis

Soil and plant samples were collected in the spring of 2017. The samples were collected from four communal allotments whereof vegetables were grown organically: site A (Borj Al Mlouk town, 85 km south of Beirut), site B (Damour town, 24 km south of central Beirut), site C (Ghazir town, 27 kilometers north of Beirut) and site D (Akkar, 110 km north of Beirut) as shown in Figure 1.

In the laboratory of soil sciences of the Faculty of Agriculture in the Lebanese University, plants were thoroughly washed three times with deionized water and air dried at room temperature. The edible parts of the plants were separated before drying, chopped manually with a cutter into very thin pieces and kept for further analysis. All plant and soil samples were ground in an agate mill (less than 2 μm diameter) in the Faculty of Geology and Geoenvironment, Athens-Greece and concentrations of heavy metals (zinc, chromium, arsenic, manganese, cadmium, barium, aluminum, iron, cobalt, nickel, copper and lead) were measured by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) in the University of Portsmouth, UK following microwave digestion by HNO_3/ H_2O_2, 6:1 v/v. All samples were oven dried at 40^oC for 3 days in a thermostatically controlled oven and kept for further analysis. Pseudototal heavy metal concentrations in soil (less than 2 μm diameter) were measured by ICP-OES following digestion by a mixture of HNO_3, H_2O_2 and HCl (US-EPA, 2002). Acetic acid (0.43 M) extractable concentrations of heavy metals were also measured by the same analytical technique after mixing 1g of the soil samples (less than 2 μm diameter) with 40 ml acetic acid and shaking for 16 h at room temperature in an overhead shaker. All utensils which were used during laboratory work were thoroughly cleaned between the samples in order to avoid cross contamination. Analytical quality control procedures included the performance of duplicate analysis, the inclusion of blank solutions and certified reference materials of soils (NIST SRM 2709 and NIST SRM2711a for the total analysis and BCR-483 and BCR-484 for the acetic acid extraction) at random positions within the analytical batches. The obtained results from analytical control were found within acceptable limits for all geochemical results.

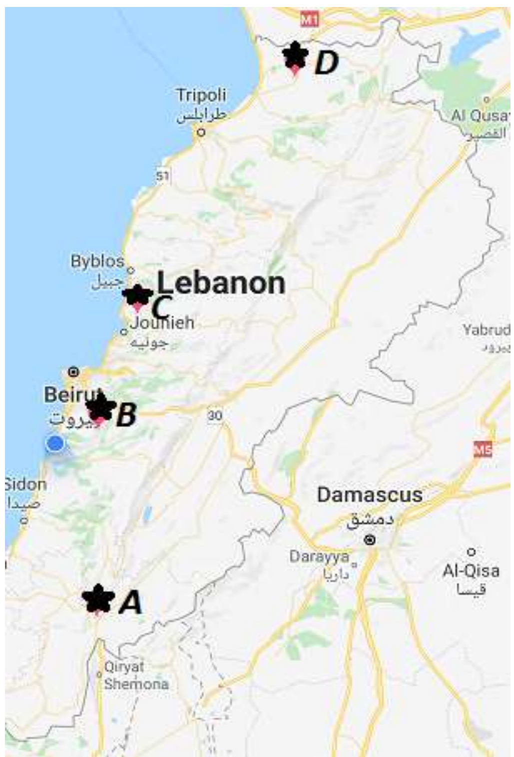

Figure legend:
Navigation *Scale* 1: 2000000
: Capital of Lebanon
: Sampled allotment
: International boundary

Figure 1. The locations of sampled allotments (with named Site A, B, C and D with its related coordinates degrees and decimal minutes) on the Lebanese Map (Scale 1:200 000) with site A (Borj Al Mlouk town: 33.324432, 35.563296), site B (Damour town: 33.731625, 35.453765), site C (Ghazir town: 34.011270, 35.668439) and site D (Akkar: 34.544370, 36.081058)

Measurement of physicochemical parameters of soil

The determination of major soil physicochemical properties has been assessed including pH, electrical conductivity, organic matter content and soil texture (sand, silt, clay). The pH was measured in a solid -to-liquid ratio of 1:2.5 by a calibrated pH meter (ISO, 1994). Organic matter content of the soil samples was estimated by the loss-on-ignition (LOI) method by heating 1 g of each sample to 450 ^{0}C for 4 hours in a furnace oven(US-EPA, 2002). Since the method determines the organic matter content in the soil, a conversion factor of 1.724 has been used to convert organic matter to organic carbon based on the assumption that organic matter contains 58% organic C (i.e., g organic matter/1 .724 = g organic C). The grain size distribution in the sand, silt and clay fractions or the soil texture technique (triangle) was determined using the Bouyoucos Hydrometer Method (Bouyoucos, 1962).

Statistical treatment

Statistical treatment of vegetable ingredients of Fattoush salad and soil data from South region of Lebanon, Damour, Ghazir and Akkar areas was carried out using Microsoft Excel (Means±standard deviation) and Minitab 18 (Coefficient of variation) statistical software. Descriptive statistics of each of the studied potentially harmful element and of their extractability percentages were estimated and were presented in terms of the ISO 9001: 2015 in accordance with the requirements of the quality assurance system. Water Certified Reference Material or Water (CRM), Plant CRM (Recovery percentage range ~100%), Aqua Regia Soil Digestion (Recovery percentage range ~100%) were assessed with a significance level <0.05. The transfer Factor (TF) was calculated. The Coefficient of variation (CV) was also calculated. Analytical quality control procedures included the performance of duplicate analysis, the inclusion of blank solutions and certified reference materials of soils and plants samples at random positions within the analytical batches.

Results

The famous “Fattoush salad” is made up of several mixed ingredients and the amount of potentially harmful elements have been assessed in this study showing measurable concentration of the potentially harmful elements (As, Cu, Fe, Zn, Mn, Ni, V, Cd, Co, Cr, Pb, P, Sn, Al) after plants acid digestion and determined by microwave assisted digestion. A net hundred gram of “Fattoush” plate (DW basis) is normally made of the following quantities of vegetables: 20 g of cucumber, 5 g of onion, 20g of tomato, 10g of radish, 25 g of lettuce, 15g of purslane, 2.5g of sumac and 2.5 g of lemon. The “Table 1” showed the amount of potentially harmful elements in each used vegetable in 100 g of Fattoush mixture. The maximum value of potentially harmful element (Zn, Cu, Ni, Cr, Co, Mn, Fe, Ba, Zn, Pb, Cd, As, Al and V) concentration (mg/100 g DW) in every Fattoush salad ingredient (sumac, lemon, cucumber, tomato, lettuce, onion and radish) was shown in the figure below (Figure 2).

Table 1. The amount of PHEs of each vegetable used in a 100g dry weight “Fattoush” plate

PHEs	Cucumber (mg/20g)	Onion (mg/5 g)	Tomato (mg/20 g)	Radish (mg/10 g)	Lettuce (mg/25 g)	Purslane (mg/15 g)	Sumac (mg/2.5 g)	Lemon (mg/2.5 g)	Total mg/100 g
Zn	0.54	0.12	0.92	0.44	0.725	27.82	0.73	0.71	31.465
Cu	0.10	0.01	0.24	0.31	0.225	3.34	0.18	0.08	4.485
Ni	0.38	0.06	0.06	0.16	0.095	2.00	0.07	0.01	2.835
Cr	#*	0.09	#*	0.12	#*	2.20	0.03	0.00	2.44
Co	0.22	0.47	0.1	0.25	0.53	0.16	0.00	0.00	1.73
Pb	0.01	0.004	0.008	0.006	0.025	0.51	0.21	0.12	0.893
Mn	0.39	0.0815	1.28	0.16	1.82	26.05	0.81	0.17	30.7615
Fe	9.7	2.80	11.52	9.34	16.2	1139.9	14.32	1.15	1204.93
As	-	-	-	-	-	#*	#*	#*	#*
V	-	-	-	-	-	1.99	0.06	#*	2.05
Cd	-	-	-	-	-	0.04	#*	#*	0.04
Sn	-	-	-	-	-	#*	#*	#*	#*
Al	-	-	-	-	-	725.83	38.00	35.18	799.01
				Total					2080.64

#* Concentration below the detection limit (0.1 mg/kg DW)

The selected data for purslane, lemon and sumac was presented in a separated table because of its extremely high rate of potentially harmful elements found in Fattoush salad (Table 2).

No Arsenic nor Tin traces detected in the tissues of three studied plants which is quite satisfactory since. As is very toxic for humans when consumed. The highest concentration of cupper was registered in purslane (222.75 mg/kg) compared to a much lower value in lemon (34.41 mg/kg).

Iron concentration was excessively high in purslane 75986.55 mg/kg, high in sumac 5729.18 mg/kg and acceptable in lemon crop 460.29mg/kg.

The highest concentration of Zn was found in purslane (1854.59mg/kg) and the lowest one in lemon (284.42mg/kg) that strongly exceeded the acceptable limits.

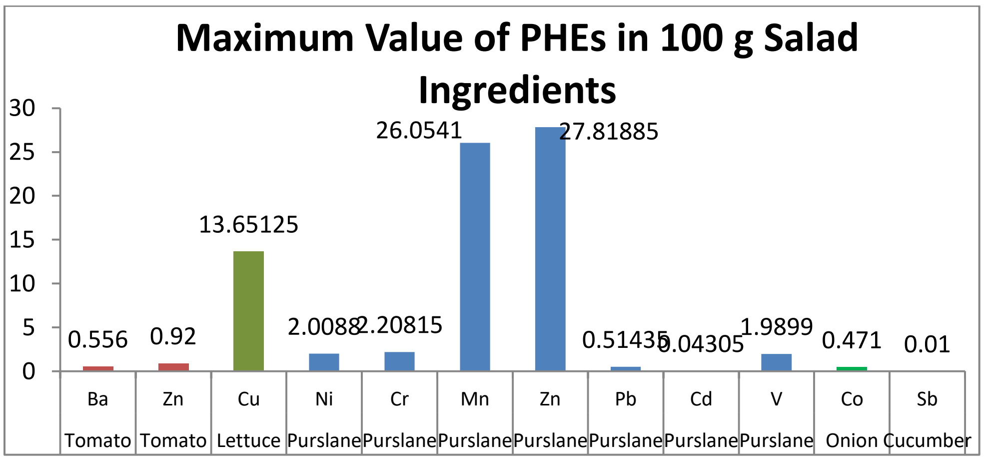

Figure 2. Potentially harmful elements (Zn,Cu, Ni, Cr, Co, Mn, Fe, Ba, Zn, Pb, Cd, As, Al and V) concentration (mg/100 g DW) in Fattoush Salad ingredients (Sumac, Lemon, Cucumber, tomato, lettuce, onion and radish)

Manganese recorded extremely high levels in purslane (1736.94 mg/kg) even though high levels were registered in sumac (324.38 mg/kg) and lemon (69.53mg/kg) crops and strongly exceeded the acceptable limits.

When it comes to Ni the levels in the three crops exceeded the acceptable limits especially in purslane and sumac crops where amounts of 133.92 mg/kg and 29.63 mg/kg were recorded respectively. Chromium registered levels that strongly exceeded the limits in the three studied crops with purslane accumulating the highest amount (147.21 mg/kg).

Total absence of V was recorded in lemon crop, whereas in purslane an amount of 132.66 mg/kg was recorded followed by a level of 25.66 mg/kg in sumac plant that strongly exceeded the acceptable limits. Usually the range of V in vegetables is 6.7–78.9 mg/kg which makes the content in purslane higher than normal.

Cadmium as well as cobalt uptake were high only in purslane crop and recorded higher values (2.87mg/kg Cd and 10.7 mg/kg Co) than the acceptable limits (0.24 mg/kg Cd and 0.5 mg/kg Co).

When it comes to lead, sumac showed the highest uptake with an amount of 85.8 mg/kg that strongly exceeded the acceptable limits (2.11 mg/kg).

Table 2. Distribution of potentially harmful elements concentrations (means ± standard deviation in mg/kg DW) in purslane, sumac and lemon plant sample samples.

PHE	Purslane	Sumac	Lemon
As (mg/kg)	#*	#*	#*
Cu (mg/kg)	222.75 ±4.67	74.23 ±0.35	34.41 ±0.08
Fe (mg/kg)	75986.55 ±152.2	5729.18 ±2.1	460.29 ±0.26
Zn (mg/kg)	1854.59 ±29.70	295.24 ±11.13	284.42 ±1.32
Mn (mg/kg)	1736.94 ±18.47	324.38 ±2.2	69.53 ±2.84
Ni (mg/kg)	133.92 ±6.8	29.63 ±0.92	3.85 ±0.02
V (mg/kg)	132.66 ±0.3	25.66 ±0.43	#*
Cd (mg/kg)	2.87 ±0.11	#*	#*
Co (mg/kg)	10.73 ±0.06	0.02 ±0.00	0.10 ±0.00
Cr (mg/kg)	147.21 ±12.1	14.14 ±3.1	1.52 ±0.08
Pb (mg/kg)	34.29 ±1.26	85.80 ±5.2	50.37 ±0.21
Sn (mg/kg)	#*	#*	#*
Al (mg/kg)	48383.98 ±56.7	15203.71 ±42.1	14075.82 ±100.2

#*Concentration below the detection limit (0.1 mg/kg DW)

Al high content is clearly shown in this study.

One approach to assess the mobility of potentially harmful elements by plants is to calculate the transfer factor (TF), as defined in the following equation (Chojnacka *et al.*, 2005):

$$TF = \frac{C\ plant}{C\ total - soil}$$

where C_{plant} is the concentration of an element in the plant material (dry weight basis) and C_{total} is the total concentration of the same element in the soil (dry weight basis) where the plant was grown. The higher the value of the TF, the more mobile/available the element is. The TF values of the elements for the plants studied are presented in Table 3.

If the ratios >1, the plants have accumulated elements, the ratios around 1 indicate that the plants are not influenced by the elements, and ratios < 1 show that plants exclude the elements from the uptake (Olowoyo *et al*., 2010).

Table 3. The Transfer Factor (TF) values of potentially harmful elements in purslane, sumac and lemon plant samples

PHE	**Samples**		
	Purslane	**Sumac**	**Lemon**
As	-	-	-
Cu	1.05	0.95	0.97
Fe	0.85	0.93	0.84
Zn	1.06	0.94	0.97
Mn	0.96	0.99	1.00
Ni	1.26	1.30	0.84
V	0.83	0.95	-
Cd	0.75	-	-
Co	0.97	2.15	0.1
Cr	0.99	0.96	0.86
Pb	0.16	2.63	1.08
Sn	-	-	-
Al	0.95	1.01	0.99

TF value of Cu in plants was the highest in purslane (1.05) with sumac and lemon showing approximately the same value for TF (0.95 and 0.97). As for Fe a TF less than 1 was recorded for the three crops with sumac recording the highest TF value (0.93). When it comes to Zn, purslane accumulated the highest amount with a TF of 1.06 followed by lemon (0.97) and sumac (0.94). Mn uptake was the highest in lemon with a TF value of 1.

The TF values of Ni were high in both sumac (1.3) and purslane (1.2). V also accumulated in purslane and sumac crops while Cd accumulated only in purslane. The lowest accumulation for Co was in lemon with a TF value of 0.1 compared to a much higher accumulation in sumac with a TF value of 2.1. The TF values of Cr were below 1 for the three crops while Pb was transferred to the crops in higher quantities since the TF obtained for sumac was 2.6 and for lemon 1.08.

The coefficient of variation (CV) which is the ratio of the standard deviation to the mean was calculated (Figure 3). Purslane CV values ranged from 0.1% for Al to 8.2% for Cr. When it comes to sumac samples, the highest CV was registered for Pb with a 6.0% value and lowest one was for Fe with a 0.03% value. Moreover, Cr registered the highest CV for lemon crop with a percentage of 5.2 while P had the lowest CV with a value of 0.1%.

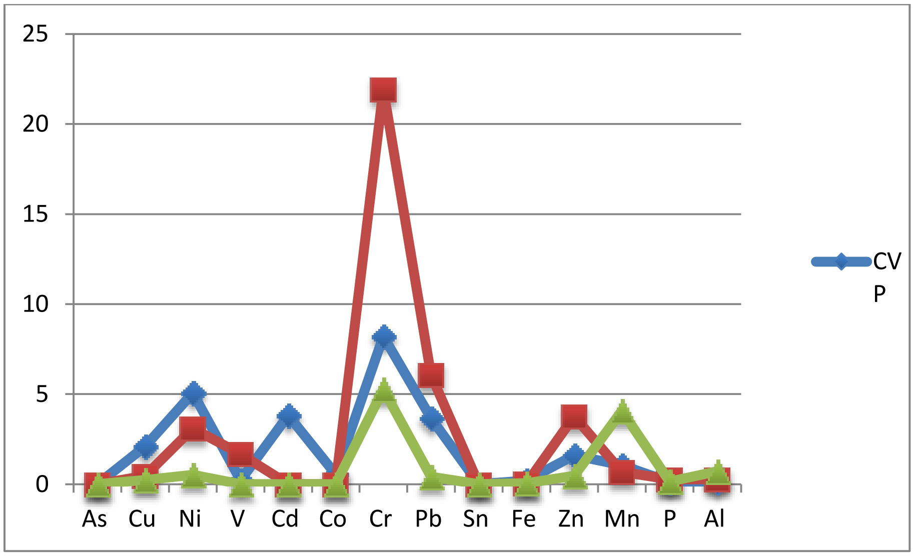

Figure 3. Variation Coefficient for PHEs in Plant Samples

Discussion

"Fattoush salad" case is alarming and leads us to posing many questions about the nature and the quality of many other consumed foods since the daily requirements of the trace elements range between 50 micrograms and 18 milligrams (Bini and Besh, 2014). The studied salad contains approximately an amount of 2080.64 mg/100g of harmful elements equivalent to the amount 4.08g which seems not acceptable and way higher than the required or acceptable daily needs. These obtained values are shocking and clearly indicate that the soils mainly in the South region are really poor in organic matter and extremely rich to a toxic extent in harmful elements that are causing contamination to the cultivated vegetables. The highest concentration of cupper in purslane compared to that found in lemon could be linked to the studied soils containing high levels of Cu that strongly exceeded the acceptable limits (4 mg/Kg). The recommended values of iron concentration by WHO/FAO are 400-500 mg/kg. In general, it is a common belief that most of the vegetables are enriched with Fe and the same was recorded here (Demirbas, 2010). Adsorption rate of Zn differed between the crops and similar results were obtained by (Zhang *et al*., 2010). Cadmium as well as cobalt uptake were high only in purslane crop and recorded higher values (2.87mg/kg Cd and 10.7 mg/kg Co) than the acceptable limits (0.24 mg/kg Cd and 0.5 mg/kg Co). Such values strongly exceeded the acceptable limits. Some published studies linked the accumulation of Cd with that of Zn (Cherif *et al.*, 2011). Al high content may be due to the application of high levels of fertilizers (Yanling, 1989).

Since the essential trace elements daily requirements range between 50 micrograms to 18 milligrams/ day depending on the element (Bini and Besh, 2014), those amounts are exceeded only in the three studied crops exposing though human beings to many threats. Such results demonstrated that metals pollution is dominating in the studied sites showing also high amounts accumulated in plants (but below the detection limits) grown in agricultural soil in South region of Lebanon (Bourj Al Moulouk and Deirmimas), Damour, Ghazir and Akkar. The presented data showed clearly the highest accumulation of heavy metals in the edible tissues of purslane sumac and lemon. The three crops accumulated high amounts of PHEs and since they are ingredients of the famous Fattoush they may constitute a potential threat to the consumers.

Sumac had a high TF value of Ni (1.3), of Co (2.15) and for Pb (2.6) when compared to other studied plants which can make us consider it as a “hyperaccumulator plant”. A “hyperaccumulator plant” is considered the plant that can take up toxic metal ions at very high levels and should not be cultivated in the South region (Bourj Al Moulouk and Deirmimas). Purslane also accumulated high amounts of harmful elements and may present a threat for human when ingested. Lemon also showed to be an accumulator but less than the other two studied crops.

Similar results were obtained by Antoniadis *et al.* (2015) showing that the metal soil-to-plant transfer coefficient was lower in the contaminated soil than that of uncontaminated soil, thus, indicating slower metal uptake with increased metal concentrations in lettuce, purslane and geranium grown in a Cd and Zn contaminated soil. In all these studied plants, Zn was absorbed in favour of Cd, irrespective of the fact that Cd was more mobile than Zn (judged by metal TC values).

According to results of the present study our crops were planted in harmful elements enriched soils. Harmful elements accumulation varies and depends on many factors such as, soil properties (e.g., soil pH, organic matter, clay content and metal concentration), plant factors (e.g., plant type and panting mode) and other environmental conditions (e.g., atmosphere and industrial pollution) (Xu *et al.*, 2016). Therefore, caution must be taken when making specific agricultural planting plans especially when it comes to crops destined for human or animal consumption.

Conclusion

Potentially harmful element contamination in soil is receiving increasing attention all over the world. The cultivation of healthy edible plants starts by adequate protection, restoration remediation of soil ecosystems contaminated by potentially harmful elements. Concentration of different elements in edible plants depends upon the relative level of exposure of plants to the contaminated soil as well as the deposition of toxic elements in the polluted air by sedimentation. The concentration of potentially harmful elements in studied soils and plants exceeded the acceptable limits. The contamination levels in edible parts of plants were also assessed with the calculation of the transfer factor value (TF) of zinc, chromium, arsenic, manganese, cadmium, barium, aluminum, iron, cobalt, nickel, copper and lead. TF value showed that there are external sources of harmful elements threatening the cultivated vegetables. Consequently, sumac, lemon and purslane are considered “potentially harmful elements accumulating plants” and are considered toxic for consumption, so growing such plants in contaminated soils should be restricted. These values of high TF could be due to long-term use of fertilizers, pesticides application and bad watering practices in agricultural lands. Obtained results indicate clearly that high accumulators are unsuitable to be grown in such contaminated soils.

Therefore, it is recommended that the study of potentially harmful elements in environmental components in the selected studied areas should be often repeated and sources of contaminants should be studied. The remediation of the contamination of soil and plants is necessary not only to preserve soil and plants but also to safeguard ecosystem and protect the humans and animals from the dangers of consumption. In addition, it is mandatory to grow the plants in healthy soils and irrigate with clean water because eating such plants in salads will lead the society to severe health problems.

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