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Contamination of Selective Vegetables of Hamadan With Heavy Metals: Non-carcinogenic Risk Assessment



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Abstract

The current study was undertaken to determine the concentration of lead, cadmium copper, and zinc (Pb, Cd, Cu, and Zn) in three types of collected green leafy vegetables irrigated with contaminated water compared with those irrigated with the fresh water of Hamadan province, Iran using the inductively coupled plasma mass spectroscopy (ICP-MS) technique. Twenty samples of vegetables such as basil, leek, and lettuce irrigated with contaminated water, and twenty samples from five different adjacent areas irrigated with fresh water as control were analyzed to determine heavy metals (HMs). The highest mean concentration of Pb, Cd, Cu, and Zn, regardless of the kind of vegetables irrigated with contaminated water, was 0.95, 0.32, 3.03, and 13.58 mg/kg fresh weight, respectively. Moreover, metals uptake differences by the vegetables were recognized to vegetable differences in tolerance to HMs. The human health risk assessment indicated that non-carcinogenic values of Pb and Cd were higher than the threshold value of 1, and ingestion was the main exposure pathway of HMs to both children and adults. It suggested that all receptors (especially basil and lettuce) in Hamadan province might have significant and acceptable non-carcinogenic risk because of exposure to Pb and Cd. The significant amount of these HMs in some plants may be due to agricultural uses for the irrigation of the vegetable lands of untreated sanitary and industrial wastewater. The findings revealed that vegetables imply the total health risk on local people, and regular monitoring of HMs is strongly recommended in this region.

Keywords: Heavy metals, Leafy vegetables, Non-carcinogenic risk assessment, Hamadan province

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1. Introduction

In recent years, toxicological studies on biological monitoring have been more important for the estimation of human health risk considering environmental pollutions. These pollutions such as contamination by heavy metals (HMs) can affect the quality and safety of vegetables and herbs, which are remarked as the most commonly used plants in daily life (1). HMs are nonbiodegradable, have a long biological half-life, and are able to accumulate in different parts of the plants (2). HMs are divided into group I such as zinc (Zn), iron, copper (Cu), and manganese that are somehow necessary for human health and mercury, cadmium (Cd), and lead (Pb) which not only are essential for humans and animals but also may have adverse effects on humans and the environment. Some of these metals are toxic and harmful to humans or animals even in small quantities (3). Toxic HMs are released into the air, soil, water, and food through

various pathways including anthropogenic activities such as agriculture, industrial activities, mining, draining of sewage, electroplating, smelting, and vehicular traffic, pesticides, and fertilizers (4). In this context, HMs may be accumulated through the consumption of agricultural products grown in the metal-contaminated soil and finally absorbed by humans (5). Generally, vegetables are widely designated as "protective foods" in the human diet due to their varied health benefits. The ability to absorb HMs from the environment by vegetables and herbs depends on different parameters such as the soil, pH, electrical conductivity, clay content, organic matter content, and physical and mechanical characteristics of the soil type, the climate, atmospheric depositions, irrigating water quality, irrigation period and the nature of the vegetables and crops (1,6). Due to fresh water resource scarcity, the use of industrial or municipal wastewater (WW, untreated and treated) in agriculture has long increased and become

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a viable option for farmers in arid and semi-arid regions (7). The irrigation of the agricultural land with polluted water can pose a severe risk to the health, including exposure of farmers and consumers to pathogens such as bacterial, parasitic, and viral infections, soil stabilization, as well as reducing the buffering capacity for HMs in the soil and may infiltrate into the groundwater aquifers or be uptaken by plants (8,9). In addition, considering the consequences of the dietary intake of HMs through vegetables, the health risk assessment by exposures to metals in food is necessary for developing countries. Health risk assessment is an effective approach for determining the risk to human health quantitatively posed by various contaminants through different exposure pathways (10). The health risk assessment of any potentially toxic metal is usually articulated as cancer and non-cancer risks (11). Currently, there is limited information about the level of HMs in the soil, water irrigation, and vegetables in Hamadan, Iran. Therefore, a comprehensive study about integrating the status of metal accumulation and probabilistic risk estimation of HMs in Hamadan is valuable for highlighting HMs for further source apportionment, and finally, designing strategies for reducing contamination and human exposure with HMs. Thus, the present study aimed at determining the concentrations of Pb, Zn, Cu, and Cd in crop products collected from different WW- and tube well water (TWW)-irrigated fields as well as analyzing and comparing the measured concentrations with the World Health Organization/Food and Agriculture Organization (WHO/FAO) recommended levels. Further, the study sought to calculate and evaluate non-carcinogenic potential health risks associated with the food chain contamination of HMs routing from irrigation with WW in comparison to TWW in adults and children. These results are expected to provide a basis for the development of remediation strategies aimed at reducing HM exposure to protect human health.

2. Materials and Methods

2.1. Study Area and Sampling

This cross-sectional study was conducted in Hamadan, Western Iran from May to October 2019. Hamadan is believed to be among the oldest cities of Iran and one of the oldest ones in the world. According to an investigation conducted in the peri-urban sites, vegetables and crops grown on nearby agricultural lands are usually irrigated with WW generated from various urban activates. For this study, two main sampling zones, namely, WW and TWW irrigation area were selected to assess possible HM contamination. Based on the type and intensity of vegetables grown in Hamadan, five vegetable growing sites were chosen for evaluating the HM contamination level of vegetables in these areas (Fig. 1).



Fig. 1. The Study Area and Sampling Stations.

2.2. Vegetables and Crop Samples

Forty samples of Allium ampeloprasum ssp. iranicum, Ocimum basilicum, and Lactuca sativa were collected into the polyethylene bags and taken to the laboratory, and then washed and rinsed with deionized water and primarily air-dried at room temperature. Next, the fresh and dry weight of vegetable samples were respectively recorded before and after oven-drying at 60°C for 24-48 hours depending on the vegetable type. Dried vegetable samples were ground to powder for further analysis. A 0.5 g of the dry sample was digested in a polyvinyl-fluoride crucible with a (5:1, v/v) acid mixture of concentrated HNO₃ (65%) -H2O₂ (30%) and then heated at 120°C for 10 hours until preparing a clear transparent digest (12,13). The blank samples were consumed and digested similarly to the original one. The solutions were filtered into Whatman No. 42 filter papers, and the volumes were diluted to 25 mL with de-ionized distilled water (12). The inductively coupled plasma mass spectroscopy (ICP-MS) method (Agilent 7500Cs, USA) was used to measure the concentrations of the target HM (12,13). ICP-MS has an excellent limit for detection, high specify, and multicomponent capability in a large variety of applications (1). The experimental conditions are presented in Table 1.

2.3. Chemicals and Reagents

All chemicals and standards or stock solutions in the analytical grade were obtained from Merck (Darmstadt, Germany), and the standard solutions of the elements were prepared by their dilution. Double-deionized water was used in all dilutions.

2.4. Limits of Detection and Limits of Quantification

The limit of quantification (LOQ) and limit of detection (LOD) are two of the main performance characteristics of each measurement at low concentrations in method validation. According to Shrivastava et al (14), LOQ refers to the smallest concentration or mass of the analyte in a sample, which can be quantitatively measured with appropriate precision, and LOD refers to the minimum amount of an analyte concentration that can be reliably detected by a given method (Table 2). The LOD and LOQ can be calculated using the following equation:

$$LOD = 3S_{b}/m \text{ and } LOQ = 10 S_{b}/m$$
 (1)

where S_b and m represent the standard deviation (SD) of the response and the slope of the calibration curve, respectively.

The SD of the response can be estimated by the SD of either y-residuals or y-intercepts or regression lines (14,15). The standard solution concentrations of 0, 1, 2, 4, 6, 8, and 10 ppm for Pb, Cd, Cu, and Zn were used based on the sample content of the element. All HMs displayed linear relationships of the instrumental response and

solutions containing the metals with insignificant intercepts and correlation coefficients of 0.994 or higher.

2.5. Statistical Analysis

The results of this research were analyzed using SPSS (SPSS Inc., Chicago, IL, version 22) to obtain a mean value, SD, and the maximum and minimum. One-way ANOVA was used to compare all results with each other, and parametric statistical analysis (P < 0.05) was applied given data normality.

2.6. Human Health Risk Assessment

The health risk assessment of any potentially toxic metal is usually determined by the amount of carcinogenic and non-carcinogenic risk to human health. The potential health risks were assessed for adult groups. The reference dose (RfD) for non-carcinogenic effects is one important risk factor for estimating the risk (12,16).

2.6.1. Non-carcinogenic Risk Assessment

To determine the health risk associated with long-term exposure to HM contamination in vegetables, total hazard quotients (THQ) in combination with the chronic daily intake (CDI) and oral RfD were calculated as (17,18):

2.6.2. Estimation of THQ

The non-carcinogen risk to the local inhabitants related

Table 1. The Applied Experimental Conditions on Analyzer ICP-MS7500cs in the Present Study

Plasma gas flow rate	15 L/min
Auxiliary gas flow rate	1.5 L/min
Viewing height	12 mm
Pump rate	15 rpm
Nebulizer flow	0.55 L/min
Measurement replicate	3
Sample uptake time (S)	68
Initial stabilization time (S)	Preflush: 21
Frequency of RF generator (MHz)	Resonance frequency: 27.12 MHz

Note. RF: radio frequency.

Table 2. LOQ and LOD Analysis of at 6 Spiking Levels (n=3) Obtained by ICP-MS Analysis

Samples	Unit	Cd	Cu	Pb	Zn
LOD	ppb	0.3	0.1	1.2	0.3
LOQ	ppb	1	0.3	4	1
Wavelength	nm	214.438	324.754	220.353	213.856
Corr. Coef.		0.99943	0.99998	0.99964	0.9995

Note. LOD: Limit of detection; LOQ: Limit of qualification; Cd: Cadmium; Cu: Copper; Pb: Lead; Zn: Zinc; Corr. Coef.: Correlation of coefficient; ICP-MS: Inductively coupled plasma mass spectroscopy.

to the consumption of crops was computed based on the THQ, which is the ratio of a determined dose of a pollutant divided into an RfD. There is no considerable risk of adverse non-carcinogenic effects on health if THQ < 1 while THQ \geq 1 for any metal in crop products indicates the great chance of non-carcinogenic effects on the consumer population (18).

The potential health risks were assessed for the adult group. The THQ was calculated using Eq. (2) as follows (19):

$$THQ = \frac{CDI}{RfD}$$
(2)

where CDI is the chronic daily intake dose of the HM (mg/kg.d) following the consumption of vegetables. In addition, RfD is the oral reference dose of HMs (mg/kg.d) via the oral exposure route, where CDI is calculated using Eq. (3) (18):

$$CDI_{ing}(\frac{EF \times ED \times IngR \times C_{vegetables}}{WAB \times TA}) \times CF$$
(3)

where C denotes the concentrations of Pb, Cd, Zn, and Cu in vegetables or/and herbs (mg/kg fresh weight). The specific and constant applied parameters in Eq. (3) are provided in Table 3.

The total target hazard quotient (TTHQ) for multiple HMs from crops was computed by Eq. (4)(20):

$$TTHQi = \sum_{(=1)} THQi$$
(4)

3. Results and Discussion

3.1. Heavy metal concentrations

3.1.1. Heavy Metal Concentrations in Leafy Vegetables

In this study, the concentrations of Pb, Cd, Zn, and Cu based on the fresh weight (FW) of the sample were determined in most consumed vegetables including basil, leek, and lettuce in Hamadan. The type and number of vegetables were selected based on the availability of samples. Table 4 summarizes the mean \pm SD, the range of the HMs in different vegetable samples, and the threshold limit established by Codex permissible limits

CODEX, and Hu et al (25-27). Based on data in Table 4, the ranking of HM concentration in vegetables was as Zn > Cu > Pb > Cd except for lettuce irrigated with WW. A recent study in the Narayanganj District in Bangladesh by Ratul et al (28) reported a similar variation of HM concentration in vegetables. The bioavailability of HMs in plants is variable, which can be correlated with the unique uptake mechanism of each of the trace elements (29). For instance, the bioavailability and movement of Cd and Cu from the soil to aerial plant parts are high whereas Pb has generally a low tendency for uptake into above-ground tissues because it forms precipitates with phosphates, sulfates, and chemicals in the rhizosphere (30).

However, among the vegetables irrigated with WW, the Pb content in basil and leek exceeded the standard limit level by 85% and 100%, respectively. The Cd content in lettuce was higher than its respective threshold by 66%. The high level of Pb and Cd in some vegetables is stimulated by pH, particle size, and the cation exchange capacity of the soil, as well as root exudation and other physio-chemical parameters. Furthermore, it might be due to the excessive use of inorganic fertilizers, the presence of various pesticides and WW in agricultural fields, a large number of small-scale industries around the agricultural land, and vehicular emissions (32,33). Malan et al (34) also reported a high concentration of Zn, followed by Mn, Cu, Cr, Pb, and Cd in vegetables from Western Cape Province, South Africa. The HM concentrations in leafy vegetables were mostly lower than the FAO permissible limits. The mean concentrations and range of HMs in different vegetables are presented in Table 4. The obtained results of the present study showed that there is a statistical relationship between individual HMs and different vegetables regardless of the type of irrigation. The results in this study indicated that the average levels of Zn (13.57) and Cu (3.03) in basil based on mg/kg-fresh weight were significantly (P < 0.05) higher than those of other vegetables. Conversely, there was a slight difference (P < 0.05) in Zn and Pb concentrations among the types of vegetables. The highest Pb and Cd concentrations were found in Persian leek and lettuce samples irrigated with

Factors		Description	Unit	Adult	Child	Source
С	Exposure-point concentration		mg/kg			This study
IngR	Ingestion rate	Vegetables	kg/day	0.345	0.232	(21,22)
EF	Exposure frequency		Day/year	350	350	(23)
ED	Exposure duration	Vegetables	Year	70	10	(23)
BW	Average body weight		Kg	70	32	(23)
AT	Average time		Day	ED × 365	ED × 365	(23)
RfD	Chronic reference dose	Vegetables	mg/kg-day	,	(Cu), 0.0035 (Pb), (Zn)	(24)

Note. HM: Heavy metal; Cd: Cadmium; Pb: Lead; Cu: Copper; Zn: Zinc.

Vegetables irrigated With Wastewater	Value	Pb	Cd	Cu	Zn
	Mean ± SD	0.44±0.16	0.03±0.04	2.47±0.34	7.31±3.33
	Minimum	0.21	0.01	2.08	3.85
	Maximum	0.68	0.13	3.03	13.58
	Threshold	0.3ª	0.2 ª	10ь	20 ^b
	Exceeded limit (%)	85	0	0	0
Basil	Mean \pm SD	0.70±0.18	0.03±0.01	1.75±0.55	5.33±1.79
	Minimum	0.48	0.02	1.09	3.19
	Maximum	0.95	0.06	2.57	7.64
	Threshold	0.3 ª	0.2 ª	10 ^b	20 ^b
	Exceeded limit (%)	100	0	0	0
Leek	Mean ± SD	0.18±0.05	0.21±0.08	1.06±0.30	4.3±1.74
	Minimum	0.12	0.09	0.78	1.95
	Maximum	0.27	0.32	1.64	6.03
	Threshold	0.3 ª	0.2 ª	10 ^b	20 ^b
Lettuce	Exceeded limit (%)	0	66	0	0
		Pb	Cd	Cu	Zn
Irrigated with tube well	Mean ± SD	0.11±0.03	0.006±0.002	0.62±0.23	2.40±0.66
water	Minimum	0.08	0.002	0.35	1.63
	Maximum	0.17	0.01	0.96	3.35
	Threshold	0.3 ª	0.2 ª	10 ^b	20 ^b
	Exceeded limit (%)	0	0	0	0
Basil	Mean ± SD	0.09±0.03	0.007±0.002	0.47±0.20	1.68±0.70
	Minimum	0.04	0.003	0.3	1
	Maximum	0.14	0.01	0.85	2.79
	Threshold	0.3 ª	0.2 ª	10 ^b	20 ^b
	Exceeded limit (%)	0	0	0	0
Leek	Mean ± SD	0.08±0.02	0±0.036	0.46±0.15	1.21±0.22
	Minimum	0.05	0.001	0.26	0.8
	Maximum	0.12	0.04	0.65	1.46
1	Threshold	0.3 ª	0.2 ª	10 ^b	20 ^b
Lettuce	Exceeded limit (%)	0	0	0	0

 Table 4. The Mean Level, Ranges and Standard Deviation of HMs in Different Vegetables in Various Sites of Hamadan, Iran (mg/kg FW)

Note. ^a Commission Regulation (EC) No 1881/2006 of 19 December 2006 (25,31); ^b Threshold limits introduced by (27); HM: Heavy metal; SD: Standard deviation. Pb: Lead; Cd: Cadmium; Cu: Copper; Zn: Zinc.

WW with a mean of 0.94 and 0.32 mg/kg-FW, respectively. Contrarily, the lowest Pb and Cd concentrations belonged to Persian leek and lettuce samples irrigated with TWW with the corresponding values of 0.04 and 0.001 mg/kg. Cd is primarily allocated to leaves, and leafy vegetables (e.g., lettuces and endives), and similar horticultural crops have a relatively high potential for Cd uptake and translocation and are considered as Cd accumulators (35). However, compared to the results reported by Seid-Mohammadi et al (36), the levels of Pb, Cd, and Cr in leafy vegetables irrigated with contaminated water were 6.24, 1.57, and 0.15 mg/kg- DW, respectively. The level of Pb and Cd in all leafy vegetables in this survey was higher than the FAO/WHO safe limits.

3.1.2. Heavy Metal Concentration in Vegetables Irrigated With Different Water Sources

Table 4 provides the mean concentrations of all HMs were compared between leafy vegetables imported to Hamadan Province and grown in the province, as well as between crops irrigated with WW and TWW. In the present study, the heavy metal concentrations in wastewater-irrigated vegetables were significantly higher than the vegetables irrigated with tube-well water (P > 0.05), this conclusion was based on the independent sample t-test. The results showed that the average concentration of HMs mg/kg FW among leafy vegetables irrigated with WW and TWW is statistically significantly different (P < 0.05). In addition, the average concentration of the above-mentioned metals

Vegetables Leafy vegetables		N/ I	Dh			~	
		Value	Pb	Cd	Cu	Zn	
Irrigated With Wastewater							
Basil	Adult	CDI	0.0022	0.0002	0.0114	0.0341	
		THQ	0.6226	0.1826	0.2850	0.1137	
Dash	Children	CDI	0.0031	0.0003	0.0171	0.0512	
		THQ	0.8963	0.2628	0.4279	0.1706	
	Adult	CDI	0.0035	0.0002	0.0088	0.0258	
Leek	Adult	THQ	1.0062	0.1947	0.2206	0.0861	
Leek	Children	CDI	0.0051	0.0003	0.0132	0.0388	
	Children	THQ	1.4345	0.2802	0.3312	0.1292	
		CDI	0.0009	0.0010	0.0052	0.0198	
Lattura	Adult	THQ	0.2459	1.0031	0.1293	0.0661	
Lettuce	Children	CDI	0.0012	0.0014	0.0078	0.0298	
		THQ	0.3539	1.4491	0.1941	0.0993	
		Irriga	ted With Tube-Well	Water			
	Adult	CDI	0.0005	0.00003	0.0030	0.0106	
Basil		THQ	0.1447	0.0271	0.0758	0.0354	
Dasii	Children	CDI	0.0007	0.00004	0.0046	0.0160	
		THQ	0.2083	0.0390	0.1138	0.0532	
	Adult	CDI	0.0005	0.00003	0.0022	0.0086	
L l		THQ	0.1309	0.0326	0.0556	0.0288	
Leek	Children	CDI	0.0007	0.00005	0.0033	0.0130	
		THQ	0.1884	0.0469	0.0835	0.0432	
	6 al. 14	CDI	0.0004	0.0001	0.0021	0.0058	
1	Adult	THQ	0.1214	0.0651	0.0523	0.0193	
Lettuce	Children	CDI	0.0006	0.0001	0.0030	0.0084	
		THQ	0.1747	0.0938	0.0754	0.0279	

Note. CDI: Chronic daily intake; THQ: Total hazard quotients; TTHQ: Total target hazard quotients; HM: Heavy metal; Pb; Lead; Cd: Cadmium; Cu: Copper; Zn: Zinc.

in the products of different groups is as WW irrigated area > vegetables TWW irrigated area. Longer irrigation with WW can result in higher aggregations of HMs in the irrigated soil and directly affect the transfer and further accumulation of HMs in vegetables. In our study, HM concentrations were higher in WW-irrigated compared to TWW-irrigated vegetables, which is in line with the findings of Mehmood et al (37). When sewage and industrial WW, which are inappropriate for irrigation, are used to irrigate fields for extended periods, the overall allowable concentration of HMs in edible plants and crops obviously exceeded the maximum permissible limits (36). However, WW composition and characteristics can vary depending on the source type (municipal, industrial, or both) and the applied treatment process. Generally, the frequency and duration of industrial WW have a higher load of HMs in comparison with municipal WW that comparatively can distribute them to the environment in a larger amount (4,38).

3.2. Potential Human Health Risk Assessment

The health risk to residents associated with the consumption of individual HMs containing vegetables was assessed with the calculation of CDI, THQ, and TTHQ for children and adults (Table 5 and Fig. 2). The obtained data regarding children's consumption of leafy vegetables revealed the highest CDI and THQ values as compared to adults, and THQ in children was 4.78 times higher than adults due to lower BW, thus children are more substantially exposed to health risks compared to adults (4). Regardless of the age group, the highest CDI for HMs was in the order of Zn > Cu > Pb > Cd. Among leafy vegetables irrigated with WW, the highest CDI (0.051) was found for Zn in basil and then leek (0.038). The difference between the CDI rates of the investigated HMs in different types of vegetables may be related to the



Fig. 2. Comparison of TTHQ Values for Non-carcinogenic Health Risks for Adults and Children in Leafy Vegetables Irrigated With WW (1) and TWW (2)

Note. TTHQ: Total target hazard quotients; Pb; Lead; Cd: Cadmium; Cu: Copper; Zn: Zinc; WW: Wastewater; TWW: Tube-well water.

metal concentration in vegetables, and to body weight and ingestion rate of vegetables in adults and children. The THQ of Zn, Cu, Cd, and Pb from leafy vegetables for adults and children was below the corresponding tolerable limits (THQ < 1), indicating that the risk of metals from vegetables is lower than RfD, presenting a slight health risk (39). Among vegetables irrigated with TWW, the highest THQ was related to Pb in basil and leek (0.208 and 0.188) for children, which was lower than 1. Among vegetables irrigated with WW, the highest THQ due to Cd and Pb in lettuce and basil was 1.449 and 1.444 in adults and children (Fig. 2), which was higher than 1 in the study area. The results indicated that Cd was the major element contributing to the potential health risk and the consumption of vegetables irrigated with WW. The large THQ of metals demonstrated that exposed adults and children were likely to experience detrimental health effects from the leafy vegetable intake, which corroborates with the results of Hu et al (40). The THQ reached 5, indicating that all vegetables had a high potential to cause severe health risks upon ingestion. The results are also consistent with the findings of (41), implying that leaf vegetables posed a higher risk of Cd and Pb effects compared to other vegetables. The overall potential risk posed by a mixture of HMs was assessed by summing the THQ of each HM. Fig. 2 displays the probability distribution of TTHQ for the total HM caused by the consumption of different crops. However, the mean TTHQ for HM exposure caused by the consumption of leafy vegetables was higher than 1 except for leafy vegetables irrigated with TWW. Due to different crop bioavailability rates, further research is required on TTHQ rectification for these four HMs based on crop species and their bioavailability to humans and depends on many factors including the dose of environmental contaminants absorbed by the human digestive system per unit body weight due to behavioural and physiological characteristics such as hand-to-mouth activities for soils, higher respiration rates per unit body weight, and increased gastrointestinal absorption of some substances (42).

4. Conclusion

Recently, the measurement of HMs and toxic elements in vegetables has received much attention. Excessive accumulations of HMs in vegetables can result in systemic health problems in humans. The results of the present study indicated that the Pb and Cd content of leafy vegetables from Hamadan and its outskirts can be a cause of concern because around 100 and 66% of vegetable samples exceeded the permissible limit proposed by FAO/WHO. The CDI of Pb, Cd, Zn, and Cu through the consumption of leafy vegetables was negligible, and these products seemed to pose no health risk to the consumers. The THQ values demonstrated that Pb and Cd contents were higher than the safe level (= 1) for children and adults, indicating that there is non-carcinogenic risk from these metals. Given that the TTHQ was higher than 1 value induced to the green leafy vegetable irrigated with the WW content of Pb, Cd, Zn, and Cu to ingestion, the consumers are at high-level non-carcinogenic risk. Therefore, to decrease HMs in vegetables in the Hamadan province, some projects should be implemented to control and reduce HM levels in soil and agricultural water.

Conflict of Interests

The authors declare that they have no conflict of interests.

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