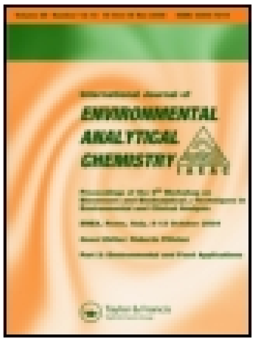

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International Journal of Environmental Analytical Chemistry

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/geac20

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To cite this article: Toraj Ahmadi-Jouibari, Hadi Ahmadi Jouybari, Kiomars Sharafi, Mahtab Heydari & Nazir Fattahi (2021): Assessment of potentially toxic elements in vegetables and soil samples irrigated with treated sewage and human health risk assessment, International Journal of Environmental Analytical Chemistry, DOI: 10.1080/03067319.2021.1893704

To link to this article: https://doi.org/10.1080/03067319.2021.1893704

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INTERNATIONAL JOURNAL OF ENVIRONMENTAL ANALYTICAL CHEMISTRY
https://doi.org/10.1080/03067319.2021.1893704

Assessment of potentially toxic elements in vegetables and soil samples irrigated with treated sewage and human health risk assessment

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ABSTRACT

In this work, a new microextraction approach termed as vortex-assisted liquid phase microextraction based on deep eutectic solvent (VALPME-DES) combined with graphite furnace atomic absorption spectrometry (GFAAS) has been developed for the extraction, pre-concentration and determination of potentially toxic elements (PTEs) in vegetables and soil samples irrigated with treated sewage from two different regions of Iran. The new DES was prepared by mixing a 1:1 molar ratio of choline chloride and citric acid monohydrate. Some effective parameters on extraction were studied and optimised. Under the optimum conditions, the repeatability and reproducibility of the VALPME-DES coupled with ETAAS for 5.0 µg L^{-1} of As(III) and 0.50 µg L^{-1} of Pb and Cd were determined to be 2.7–4.3 and 3.8–6.2%, respectively. The correlation coefficient (r^2) of the calibration curves was in the range of 0.995–0.998. The limit of detections was in the range of 0.03 and 0.1 µg kg^{-1} for different metal ions. Linear range of 0.3 – 100 µg kg^{-1} for As(III) and, 0.03–200 µg kg^{-1} for Cd and Pb were obtained. The results showed among the target metals, the highest impact on the total value of non-carcinogenic risk was related to arsenic. Furthermore, the non-carcinogenic risk value for all vegetable types was lower than the permitted level. We also found that the risk of arsenic carcinogenicity was higher than the acceptable levels in all four types of vegetables. According to the findings, interventions to reduce arsenic should be used, especially in cultivated soils.

ARTICLE HISTORY
Received 2 November 2020
Accepted 11 February 2021

KEYWORDS
Potentially toxic elements; soil analysis; vegetable analysis; health risk assessment

1. Introduction

The water scarcity crisis is one of the challenges that the world faces today. Water resource constraints have drawn the attention of researchers to the principle use of unconventional waters, such as saline water, urban and industrial wastewater. Due to

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Supplemental data for this article can be accessed here.

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the development of cities and increasing water consumption, a large amount of raw sewage and wastewater from wastewater treatment is produced daily. Proper use of urban wastewater in agriculture, in addition to expanding cultivation levels and increasing crop production, also prevents environmental contamination [1]. Despite the useful aspects of using urban wastewater in agriculture as a rich source of nutrients, and the presence of some heavy and toxic metals in it, long-term use of wastewater in agricultural soils will be problematic, resulting in excessive accumulation of these elements in the soil [2]. Soil contamination with heavy and toxic elements causes them to enter the food chain through plant intake and toxicity. Although some of these elements are necessary for plant growth in small amounts, their concentration may be slightly above the threshold for plant and animal life [3]. Therefore, one of the major issues that should be considered when using urban wastewater in agricultural lands is possible of heavy and toxic metals' accumulation in the soil.

In our country, much of the water used in metropolises is converted into sewage, and due to the lack of water resources, it is used for irrigation of agricultural lands after treatment. High concentrations of heavy and toxic metals in agricultural soils irrigated with wastewater not only reduce the quality of crops but also endanger human health [4]. The heavy and toxic metals found in agricultural soils are absorbed by vegetables and accumulate in its edible parts [5]. Since the quality of agricultural products is one of the most important factors affecting human health, it is important to provide sensitive and low-cost techniques for the determination of heavy and toxic metals in these products.

Electrothermal atomic absorption spectrometry (ETAAS) [6,7], inductively coupled plasma mass spectrometry (ICP-MS) [8], atomic fluorescence spectrometry (AFS) [9], inductively coupled plasma-optical emission spectrometry (ICP-OES) [10] and flame atomic absorption spectrometry (FAAS) [11] have been used for the measuring low amounts of metal ions. ETAAS is still used because of its simplicity, short analysis time low sample volume requirements, low detection limit, and low cost. However, this technique has a problem due to the small amounts of toxic and heavy metals as well as the complexity of the matrix. Accordingly, a proper preconcentration step is required before identifying the tool. Until now, various methods have been used to extract metal ions in different matrices, including liquid–liquid extraction (LLE) [12], solid-phase extraction (SPE) [13,14], cloud point extraction (CPE) [15], solid–phase microextraction [16], dispersive liquid–liquid microextraction (DLLME) [17–19] and dispersive liquid–liquid microextraction based on solidification of a floating organic drop (DLLME–SFO) [20,21]. The advantages and disadvantages of these techniques have already been discussed [18].

Green Chemistry is looking for new solvents to replace with toxic organic solvents. Deep eutectic solvents (DESs), which are a mixture of two or three compounds, are a good alternative to older organic solvents, which are often toxic and hazardous. DES is fully in line with the principles of green chemistry and have recently developed extensively in analytical chemistry. The use of DESs as an extractant in the DLLME for the extraction of various organic and inorganic species has increased dramatically in recent years [22–25]. In this research, a new DES was used to the preconcentration of Cd, Pb and As in the vegetables and soil samples irrigated with treated sewage from two different regions of Iran before their analysis by electrothermal atomic absorption spectrometry (ETAAS). The new DES was prepared by mixing 1:1 molar ratio of choline chloride and citric acid monohydrate. Determination of PTEs in vegetables and soil samples by VALPME–DES procedure and GFAAS detection was compared with other methods to extract metal ions from different samples. The results showed the relative standard deviations (RSDs) of the proposed method are better than other methods, except for conventional DLLME, which possess lower LODs than the corresponding values obtained in this work. The proposed method has several advantages over conventional DLLME. In contrast to conventional DLLME, there is no need for disperser solvent, so less organic solvent is used. The LODs and linear range of the VALPME–DES method are better than other methods. The extraction of analytes from solid or semisolid samples is usually time-consuming because it needs one-step sample preparation before the extraction procedure. In the VALPME–DES method, the extraction time is shorter than other extraction methods. Hence, the proposed method would be a good alternative for the determination of ultra-traces of heavy metals in different matrices.

The results of previous studies have shown that the declared standards for the amount of metals in various foods in Iran have a good level of protection. This is due to the fact that the aforementioned standards have not been formulated in accordance with Iranian indigenous conditions (with regard to per capita food consumption, consumer body weight, exposure period, etc.) and mainly adapted international guidelines [26,27]. In the present study, to solve this problem, we evaluated the possible health risk based on native variables for each Iranian individual. In addition, other innovations in this study were that, unlike most similar studies, there was a risk or non-risk attributed to each of the metals for each vegetable type as well as the degree of influence of each of the independent variables is judged more reliably by the uncertainty analysis method.

2. Experimental

2.1. Reagents and solutions

The standard solutions of Pb and Cd were produced by diluting a stock solution of 1000mgL^{-1} of the Pb and Cd supplied by Sigma Chemical Co. (St Louis, MO, USA). Stock standard solutions of As(III) and As(V) were prepared by dissolving appropriate amounts of As_2O_3 and $Na_2HAsO_4.7H_2O$, respectively (Merck, Darmstadt, Germany). Working standard solutions were obtained daily by diluting the stock solutions with ultrapure water. A mixture of $Pd(NO_3)_2$ (1000 mg L^{-1}) and $Mg(NO_3)_2$ (300 mg L^{-1}) solutions, both from Merck (Darmstadt, Germany) were used as chemical modifiers. Diethyldithiophosphoric acid (DDTP) as a chelating agent was purchased from Merck. Citric acid monohydrate and choline chloride (purity≤99%) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). To determination of total As, As(V) was reduced to the As(III) using $Na_2S_2O_3$ and KI (both Merck).

2.2. Instrumentation

Metal ions were analyzed using a Model nov AA 400 AAS (Analytik Jena AG, Jena, Germany), equipped with auto-sampler MPE-60. Pyrolytically coated graphite tubes were used. Different hollow cathode lamps, operated at a current and wavelength depending on each metal was used. Argon 99.999% obtained from Air Products (West Sussex, UK) as sheath gas. Peak area was employed for quantification of the signal. The instrumental parameters for the ETAAS are listed in Table S1. The digestion of samples was done using a Microwave Multiwave 3000 (Anton Paar, Germany). For centrifugation of samples, the Hettich Zentrifugen Model EBA20 (Tuttlingen, Germany) was employed.

2.3. Sampling and sample preparation

In this research sampling was done as follows: 4 soil samples from different fields (2 from northeast of Kermanshah and 2 from south of Tehran) were collected at a depth of 5 to 20 cm, randomly. Four vegetable cultivars including spinach (*Spinacia oleracea*), coriander (*Coriandrum sativum*), basil (*Ocimum basilicum*) and radish (*Raphanus sativus*) were collected from the same locations simultaneously with the soils. The vegetable edible parts were weighed and then oven-dried at 75–85°C for overnight to eliminate moisture. Dried samples were ground with a mortar and were passed through a sieve óf 0.2 mm.

From each soil sample, 0.5 g was weighed by the scale and placed in a 20-ml digestive tube. Then, 8 ml of concentrated nitric acid (superior) was added and the samples were digested using the MARS X-Press Microwave (CEM Corporation, NC, USA) as follows: First step: Power = 850 W; Ramp time = 1 min; Hold Time = 2 min; Temperature = 80°C and Second step: Power = 850 W; Ramp time = 1 min; Hold Time = 3 min; Temperature = 90°C. After cooling the sample in the microwave, remove it and add 12 ml of perchloric acid at a concentration of 70% and the resulting mixture is slowly boiled until thick white smoke appears. After cooling the sample to room temperature, the contents are transferred to a centrifuge tube and centrifuged at 5000 rpm for 4 min. The above solution is transferred to another clean tube and adjusted to pH 3 using 2.0 mol l^{-1} sodium hydroxide. 10 ml of the resulting solution was used for the proposed extraction method. For the digestion of the vegetable samples, 1 g of each sample was carefully weighed and digested using 2 ml perchloric acid at a concentration of 70% and 8 ml concentrated nitric acid (superior). After cooling, the resulting solution was filtered using Whitman No.42 filter paper and was reached to a volume of 20 ml by distilled water. 10 ml of the resulting solution was used for the proposed extraction method.

2.4. Hydrophobic DESs preparation

To obtain a suitable DES, different imidazolium chloride salts (HBA) and citric acid monohydrate (HBD) were mixed in a certain ratio and was placed on the shaker at a speed of 600 rpm at 70°C. After discoloration and full transparency, the mixture was then placed in the desiccator. The final DES was cooled to room temperature and was used as the extractant in the extraction procedure.

2.5. VALPME–DES procedure

An aliquot of 10.0 mL of distilled water spiked with target metal ions or sample solution is placed in a 20-ml glass test tube and 50.0 µl DES (extraction solvent) containing 10.0 µL DDTP (chelating agent) is injected into the aqueous solution. The mixture is vortexed for 5 min and finally centrifuged at 5000 rpm for 5 min. Then, the fine DES droplets float at the top of the glass test tube. The test tube is put in the freezer for a few minutes to solidify the extraction phase, and the solid phase is transferred to a conic tube with a spatula.

After melting at room temperature, 30.0 µL of the extract is injected into the GFAAS using an auto-sampler and is subjected to the temperature programme, listed in Table S1.

2.6. Risk assessment

2.6.1. Comparison with standards

The mean levels of cadmium and lead in the studied vegetables were compared with the national standard of Iran and the WHO/FAO guidelines, but no standard has been stated for arsenic by the two organisations [27,28]. In addition, the mean of all three metals studied in farm soils was also compared with EU standards [29].

2.6.2. Assessment of possible risk of non-carcinogenesis

Non-carcinogenic risk assessment was performed using the target hazard quotient (THQ) calculation method provided by the EPA [30]. In this method, the estimated daily intake (EDI) was calculated according to Equation (1), and then taking the oral reference dose, the THQ was calculated based on Equation (2).

$$\mathrm{EDI} = \frac{(EF \times ED \times IR \times MC)}{(BW \times AT)} \tag{1}$$

$$\mathrm{THQ} = \frac{EDI}{Rfd} \tag{2}$$

Finally, the total target hazard quotient (TTHQ) is obtained from the total THQ for each metal, calculated based on Equation (3). If the THQ or TTHQ is less than 1 (as considered by the US Environmental Protection Agency), this result is assumed to be an acceptable risk for chronic systemic effects, whereas if the above two parameters are greater than 1, the risk of non-carcinogenicity is unacceptable.

$$\mathrm{TTHQ} = THQ_{As} + THQ_{Pb} + THQ_{Cd} \tag{3}$$

In this study, two scenarios were considered to assess the health risk attributed to these four types of vegetables, and radish vegetable was also classified as foliar vegetables.

Scenario 1: In this scenario, it was assumed that a total of 58 g of vegetables per day would be allocated to only one type of vegetable (spinach, coriander, basil or radish). In this scenario, the contamination concentration of that vegetable was considered to assess the health risk attributed to each vegetable.

Scenario 2: This scenario is assumed that a total of 58 g of vegetable consumption per day is allocated to the sum of these four types of vegetables. In this scenario, to assess the health risk attributed to the vegetables in question, the overall mean concentration of the contaminants associated with all four types of vegetables was considered.

2.6.3. Carcinogenic risk assessment

Incremental lifetime cancer risk (ILCR) was used for carcinogenic risk assessment according to Equation (4) [31,32]. The parameters related to this equation are defined in Table S2.

$$ILCR = \mathrm{EDI} \times \mathrm{CSF} \tag{4}$$

According to the US Environmental Protection Agency's guidelines, a carcinogenic risk of less than $10^{-4}$is considered acceptable (tolerable), whereas if the risk is greater than 10^{-4}, it is considered a carcinogenic risk [33].

2.6.4. Uncertainty analysis

In this study, in addition to point estimating of EDI, THQ, TTHQ, and ILCR using Equations (1)–(4), again using the Monte–Carlo simulation method by oracle crystal ball software (Ver.11.1.2.4), the above parameter values were calculated 10000 times with respect to the amount of independent variables (BW, MC, IR, etc.) [34]. Then, by comparing the uncertainty upper bound (95th percentile) and the uncertainty lower bound (5th percentile) with the allowed values (one for THQ, TTHQ and 10^{-4} for ILCR), the final comment on the health risks of each one of the metals was presented in different vegetable types. The 95th percentile and the 5th percentile are the estimated 9500 and 500 possible states for Equations (1)–(4), respectively.

2.6.5. Statistical analysis

Error bar graph was used to graphically describe the results. To compare the mean of heavy metals of vegetables or soil between different farms and cities, an independent two samples t-test was used at a significant level (α = 0.05). In addition, the component analysis was used to investigate the clustering of heavy metals.

3. Results and discussion

3.1. Optimisation of VALPME–DES parameters

3.1.1. Effect of DES type

For the choice of the best DES, citric acid as HBD was mixed with different imidazolium chloride as HBA in a molar ratio of 1:1. According to the results in Figure 1(a), [DMIM]Cl in combination with citric acid has a better analytical signal than other imidazolium chloride salts. [OMIM]Cl gives an acceptable recovery compared to other imidazolium chloride salts, but due to its high solubility in the aqueous phase, the volume of the extraction phase decreases, causing systematic errors and increasing standard deviation. Thus, [DMIM]Cl was chosen as HBA.

3.1.2. Selection of HBA to HBD molar ratio

In this study, to prepare a DES, the citric acid monohydrate (HBD) was mixed with choline chloride (HBA) in different ratios including 1:1 (DES11), 1:2 (DES12), 1:3 (DES13), 2:5 (DES25), and 3:7 (DES). According to Figure 1(b),

The mixture of the above two compounds is gelatinised in a molar ratio of 1 to 3 and cannot be dispersed in the aqueous phase. The mixture of the above two compounds in other ratios has a positive effect on the recovery of the metal ions. However, when two compounds with a 1:1 molar ratio are mixed together, better extraction recoveries and lower standard deviations are obtained for all metal ions. So, the molar ratio of 1:1 was selected.

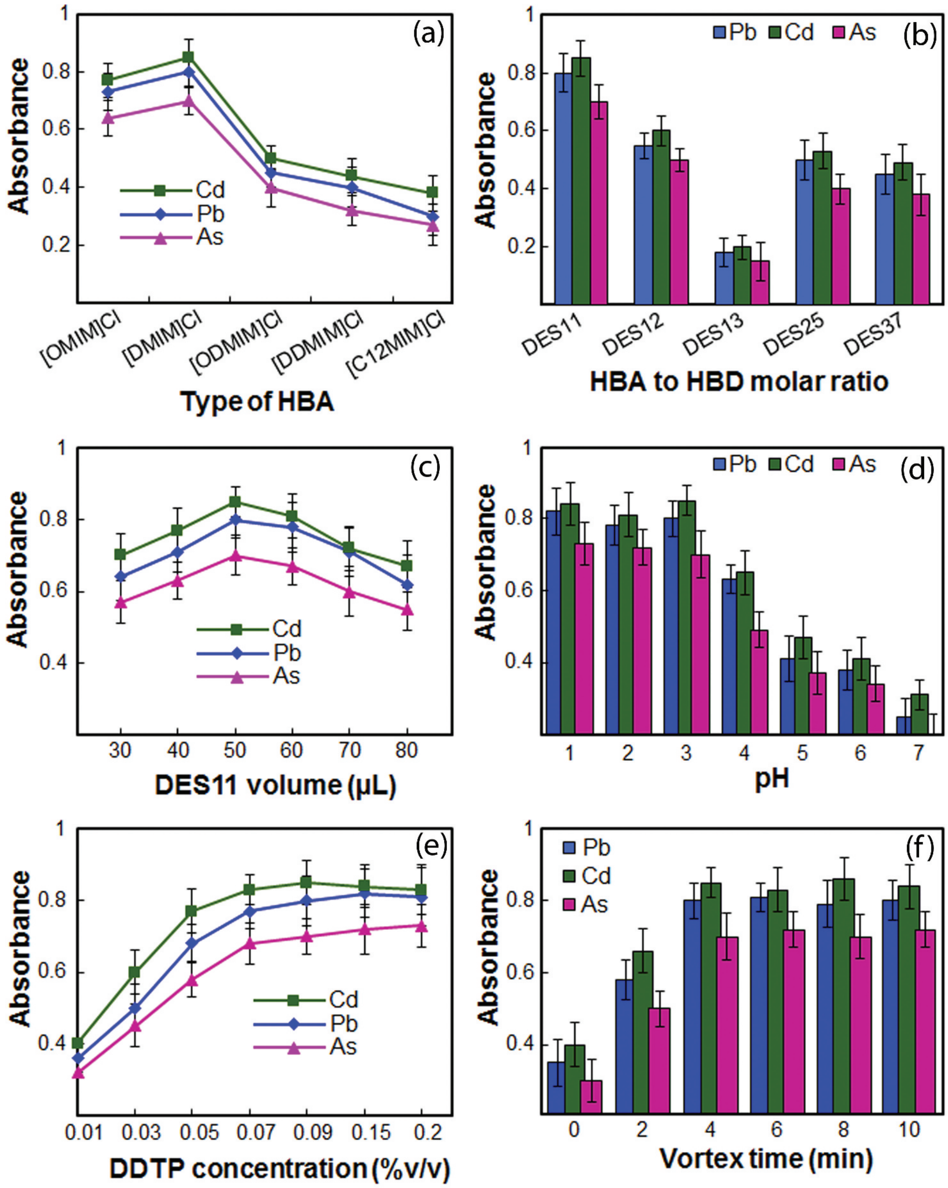

Figure 1. Effect of the different types of HBA (a), molar ratio of HBA to HBD (b), volume of DES11 (c), sample solution pH (d), concentration of DDTP (e) and vortex time (f) on the absorbance of the target metal ions.

3.1.3. *Selection of extraction solvent volume*

To evaluate the effect of DES volume on extraction recoveries of metal ions, additional experiments were performed using different volumes of DES from 30 to 80 μL (with a 10 μl increase per experiment). The results in Figure 1(c) show that by increasing the DES volume to 50 μl, the analytical signal of the metal ions and as a result enrichment factor is gradually increased, and in volumes greater than 50 µl, the enrichment factor decreases. Therefore, 50 µl of DES was chosen as the best volume.

3.1.4. Selection of the pH of sample solution

The DDTP itself is acidic and to investigate the effect of pH, it must first be converted to ammonium salts with the help of ammonia. In this study, the effect of pH in the range of 1 to 7 was investigated. The results in Figure 1(d) show that by increasing the pH from 1 to 3, the adsorption intensity of the studied metals is almost constant and shows the highest possible amount. Since the DDTP solution is acidic (pH = 2.2 in 10.0 ml aqueous solution), therefore, no acid is added to the medium to adjust the pH.

3.1.5. Selection of the concentration of DDTP

An appropriate concentration of DDTP is required for complete complexation of metal ions and increase of their extraction efficiency. The DDTP concentration should be high enough to increase the complexation and extraction efficiency, but if it is too high it may have a quenching effect on the ion analysis. For this purpose, different experiments were done with different concentrations of DDTP in the range of 0.01% to 0.50% (v/v). The results in Figure 1(e) show that with increasing DDTP concentration up to 0.09% (v/v), the absorption of metal ions increases and with increasing DDTP concentration, the absorption remains constant and in some cases slightly decreases. So, 0.1% (v/v) was chosen as the best concentration of DDTP.

3.1.6. Selection of the vortex time

In the proposed method, since the dispersive solvent is not used for dispersing the extraction phase in the sample solution and rapidly transferring the analytes into the extraction phase, a vertex is required for an appropriate time. Preliminary tests showed that in the absence of vertex, the extraction phase did not disperse well in the sample solution. For this purpose, vertex time was studied in the range of 0 to 10 min. The results are shown in Figure 1(f). According to the results, with increasing vertex time from 0 to 4 min, the analytical signal increases and with increasing vertex time, the signal intensity remains constant. Therefore, the vertex time of 4 min was selected as the optimal time.

3.1.7. Effect of coexisting ions

For the investigation of the selectivity of VALPME–DES, recovery of 5 µg l^{-1} As(III) solution and 0.5 µg l^{-1} Cd and Pb solution in the presence of various amounts of interfering ions were treated according to the recommended procedure. The tolerance level is defined as: the highest intrusive ion concentration of less than 5% causes a change in the degradation signal when compared to the signal of 5 µg l^{-1} As (III) and 0.5 µg l^{-1} Cd and Pb solution. The results obtained are given in Table S3.

3.1.8. Figures of merit

The characteristics of calibration curves including repeatability (intraday), reproducibility (interday), limit of detections (LODs), linear dynamic ranges (LRs) and enhancement factor (EF) were obtained under optimised conditions. The repeatability (intra-day) and reproducibility (inter-day) of the VALPME–DES coupled with ETAAS for 5.0 µg L^{-1} of As(III) and 0.50 µg L^{-1} of Pb and Cd were determined to be 2.7–4.3 and 3.8–6.2%, respectively. The

LODs were in the range of 0.03 and 0.1 μg kg^{-1} with r^2 better than 0.995 for different metal ions. LRs of 0.3 – 100 μg kg^{-1} for As(III) and, 0.03–200 μg kg^{-1} for Cd and Pb were obtained. The EF, obtained from the slope ratio of calibration graph after and before extraction, was between 112 and 133.

3.1.9. *Real sample analysis*

The efficiency of the proposed approach in the measurement of metal ions was investigated by analysing various vegetables and soil samples. All samples were selected from two different regions of Iran (two farms in Tehran and two farms in Kermanshah). The results are listed in Tables 1 and 2. It should be noted that the arsenic reported in this study is total inorganic arsenic (t-iAs). To obtain t-iAs, As (V) was reduced to As (III) with $Na_2S_2O_3$ and KI and then As (III) which was equivalent to total inorganic arsenic was measured.

The samples (two soils and four vegetables) were spiked with a standard solution of metal ions at different concentration levels to assess matrix effects. The results showed that the relative recoveries of Cd, Pb and As were in the range of 90.4–107.0, 92.45–105.2 and 89.6–108.0%, respectively. Moreover, the accuracy of the VALPME-DES procedure was also evaluated by determining the amounts of the metal ions in two standard reference materials (GBW10014 cabbage and GBW10015 spinach).

3.2. *Toxic metal level and human health risk assessment*

The mean of heavy metals in all vegetables was not significantly different between the studied farms in Kermanshah ($P < 0.05$) while between the two farms in Tehran, there was

Table 1. Mean amount of toxic metals in soil and vegetables harvested from two Kermanshah farms.

		Kermanshah farms								
		Farm 1		Farm 2		Total		Standards (μg/kg)		
Soil/ vegetables	Toxic metals	NS	Mean ±SD (μg/kg)	NS	Mean ±SD (μg/kg)	NS	Mean ±SD (μg/kg)	WHO/FAO, 2004	EU, 2002	ISIRI, 2010
Soil	As	3	185.3 ± 4.5	3	144.0 ± 5.0	6	164.7 ± 23.1	-	2E+04	-
	Cd	3	243.0 ± 7.6	3	117.0 ± 5.6	6	180.0 ± 69.3		3E+03	
	Pb	3	369.4 ± 11.1	3	303.7 ± 6.5	6	336.5 ± 36.9		3E+05	
Spinach	As	3	114.7 ± 3.1	3	90.7 ± 4.2	6	102.6 ± 13.5	-	-	-
	Cd	3	12.7 ± 1.5	3	7.3 ± 0.8	6	9.9 ± 3.2	200	-	100
	Pb	3	51.3 ± 3.5	3	25.6 ± 2.1	6	38.5 ± 14.3	300	-	200
Coriander	As	3	51.7 ± 3.0	3	103.0 ± 4.0	6	77.3 ± 28.3	-	-	-
	Cd	3	9.8 ± 1.0	3	15.3 ± 1.5	6	12.6 ± 3.2	200	-	100
	Pb	3	28.7 ± 2.5	3	11.3 ± 1.5	6	20.0 ± 9.7	300	-	200
Basil	As	3	206.0 ± 4.0	3	130.3 ± 4.1	6	168.2 ± 41.6	-	-	-
	Cd	3	13.7 ± 2.5	3	8.1 ± 1.0	6	10.8 ± 3.5	200	-	100
	Pb	3	35.3 ± 1.5	3	27.3 ± 2.2	6	31.3 ± 4.7	300	-	200
Radish	As	3	207.0 ± 8.9	3	180.7 ± 7.5	6	193.83 ± 16.2	-	-	-
	Cd	3	21.3 ± 2.1	3	5.7 ± 0.6	6	13.5 ± 8.7	200	-	100
	Pb	3	49.0 ± 2.0	3	36.0 ± 2.0	6	42.5 ± 7.3	300	-	200
Total Vegetables	As	12	144.8 ± 68.3	12	126.7 ± 36.4	24	135.5 ± 54.6	-	-	-
	Cd	12	14.4 ± 4.7	12	9.1 ± 3.9	24	42.5 ± 7.3	200	-	100
	Pb	12	41.1 ± 10.0	12	25.1 ± 9.4	24	33.1 ± 12.5	300	-	200

NS: Number of samples
SD: Standard deviation
EU: European Union
ISIRI: Institute of Standards and Industrial Research of Iran.

Table 2. Mean amount of toxic metals in soil and vegetables harvested from two Tehran farms.

		Tehran farms						Standards (μg/kg)		
		Farm 1		Farm 2		Total				
Soil/ vegetables	Toxic metals	NS	Mean ±SD (μg/kg)	NS	Mean ±SD (μg/kg)	NS	Mean ±SD (μg/kg)	WHO/FAO, 2004	EU, 2002	ISIRI, 2010
Soil	As	3	242 ± 6.5	3	272.3 ± 7.7	6	257.2 ± 17.8	-	2E04	-
	Cd	3	211.3 ± 4.5	3	180.3 ± 3.1	6	195.8 ± 17.3		3E03	
	Pb	3	408.3 ± 13.0	3	386.3 ± 10.6	6	397.4 ± 16.1		3E05	
Spinach	As	3	157.0 ± 6.2	3	82.0 ± 4.6	6	119.5 ± 41.4	-	-	-
	Cd	3	21.0 ± 2.0	3	20.7 ± 1.4	6	20.8 ± 1.6	200	-	100
	Pb	3	65.3 ± 1.5	3	34.7 ± 2.1	6	50.0 ± 16.9	300	-	200
Coriander	As	3	105.3 ± 4.5	3	182.0 ± 7.9	6	143.7 ± 42.4	-	-	-
	Cd	3	14.4 ± 1.5	3	35.7 ± 1.5	6	25.0 ± 11.8	200	-	100
	Pb	3	43.7 ± 2.5	3	21.3 ± 2.1	6	32.5 ± 12.4	300	-	200
Basil	As	3	221.0 ± 7.1	3	103.7 ± 3.1	6	162.3 ± 64.3	-	-	-
	Cd	3	31.0 ± 1.9	3	20.3 ± 1.5	6	25.7 ± 6.1	200	-	100
	Pb	3	55.3 ± 2.3	3	49.3 ± 1.5	6	52.3 ± 4.3	300	-	200
Radish	As	3	283.2 ± 10.6	3	201.6 ± 6.3	6	242.5 ± 45.4	-	-	-
	Cd	3	15.3 ± 1.5	3	11.1 ± 1.0	6	13.2 ± 2.6	200	-	100
	Pb	3	75.4 ± 2.5	3	54.7 ± 1.5	6	65.0 ± 11.4	300	-	200
Total Vegetables	As	12	191.5 ± 70.2	12	142.3 ± 53.1	24	167.0 ± 65.8	-	-	-
	Cd	12	20.4 ± 7.1	12	21.9 ± 9.3	24	21.2 ± 8.2	200	-	100
	Pb	12	59.9 ± 12.4	12	40.0 ± 13.8	24	49.9 ± 16.4	300	-	200

NS: Number of samples
SD: Standard deviation
EU: European Union
ISIRI: Institute of Standards and Industrial Research of Iran.

a significant difference in Pb-Soil, Cd-Spinach, Pb-Basil (P > 0.05). The amount of heavy metals in some vegetables was significantly different between Kermanshah and Tehran farms (P < 0.05) (Table 3 and Figures 1–3). The results showed that all three toxic metals studied are in a cluster (Table 4 and Figure 4), which means that the metals detected in the vegetables and soil studied belong to a similar source of external contamination (Figure 5).

Table 3. Statistical comparison of the mean of heavy metals studied between different soils, vegetables, crops and cities.

		Between farms					
		Kermanshah		Tehran		Between cities	
Soil/ vegetables	Toxic metals	Farm 1	Farm 2	Farm 1	Farm 2	Kermanshah farms	Tehran farms
Soil	As	>0.001		0.006		>0.001	
	Cd	>0.001		0.001		0.608	
	Pb	0.002		0.086		0.008	
Spinach	As	0.001		>0.001		0.380	
	Cd	0.005		0.830		>0.001	
	Pb	>0.001		>0.001		0.232	
Coriander	As	>0.001		>0.001		0.011	
	Cd	0.007		>0.001		0.032	
	Pb	0.001		>0.001		0.080	
Basil	As	>0.001		>0.001		0.857	
	Cd	0.022		0.002		0.001	
	Pb	0.006		0.074		>0.001	
Radish	As	0.017		>0.001		0.047	
	Cd	>0.001		0.015		0.931	
	Pb	0.001		>0.001		0.003	

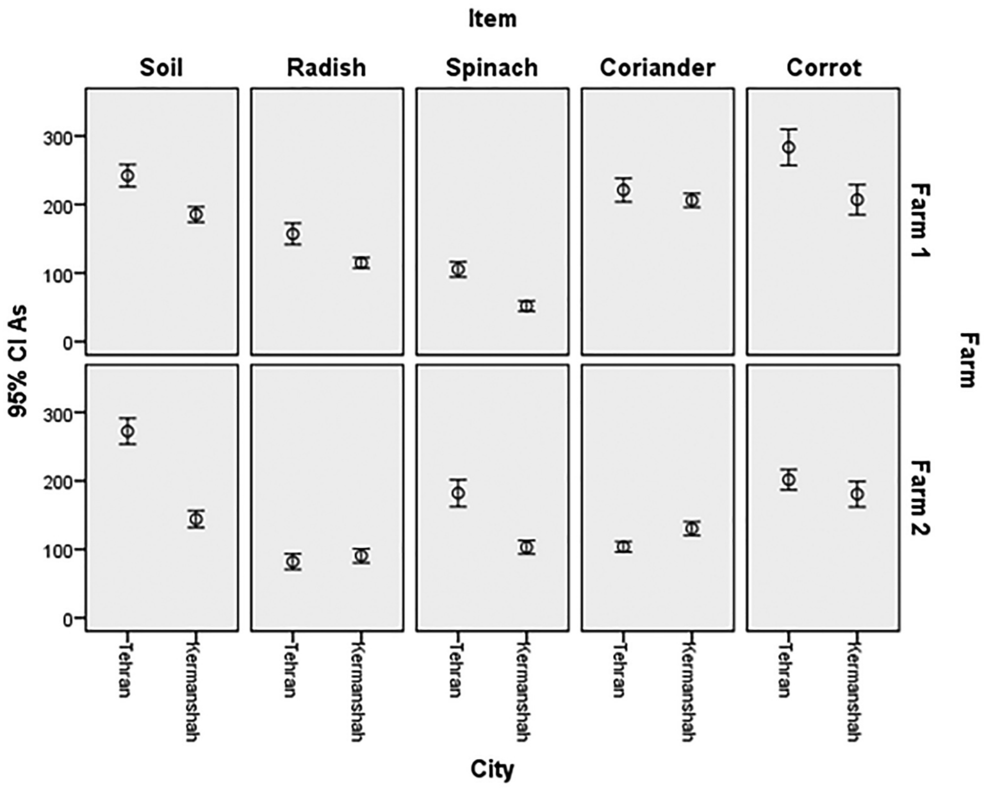

Figure 2. Graphic comparison of arsenic metal based on different farms, cities, soils and vegetables.

The mean levels of lead and cadmium in all four vegetables grown in all studied farms were lower than the national standard of Iran and WHO/FAO guidelines, and in addition, the mean of all three metals in soil was lower than the EU standard (Tables 1 and 2). In Kermanshah farms, the highest EDI was in arsenic, cadmium, cadmium and lead in Spinach, Coriander, Basil and Radish vegetables, whereas in Tehran farms the highest EDI was in arsenic, lead, lead, and cadmium, respectively (Table 5). The reason of the difference in EDI is due to the difference in the concentration of metals measured in each vegetable. Cheshmazar et al. (2018) in Iran showed that the amount of cadmium and lead in Spinach irrigated with treated wastewater was 1001 and 300 µg/kg, respectively [35]. Whereas in the present study, the overall mean of cadmium and lead for Spinach grown in Kermanshah farms were 9.9 ± 3.2 and 38.5 ± 14.3 µg/kg, respectively, and for Tehran farms were 20.8 ± 1.6 and 50.0 ± 16.9 µg/kg, respectively. Demirezen and Aksoy (2006) in Turkey showed that the levels of cadmium and lead in the studied vegetables were exceeded by the FAO/WHO [36]. In a study by Wilberforce and Nwabue (2013) in Nigeria, it was found that vegetables grown in farms adjacent to lead mine have higher levels of lead, arsenic and cadmium than the acceptable limit [37]. Gupta et al. (2012) showed that lead, zinc and cadmium concentrations in some samples of spinach and radish were higher than the standard limit.

The difference between the results of the quality of vegetable production in different countries in terms of heavy metals, in previous studies together and also in the present

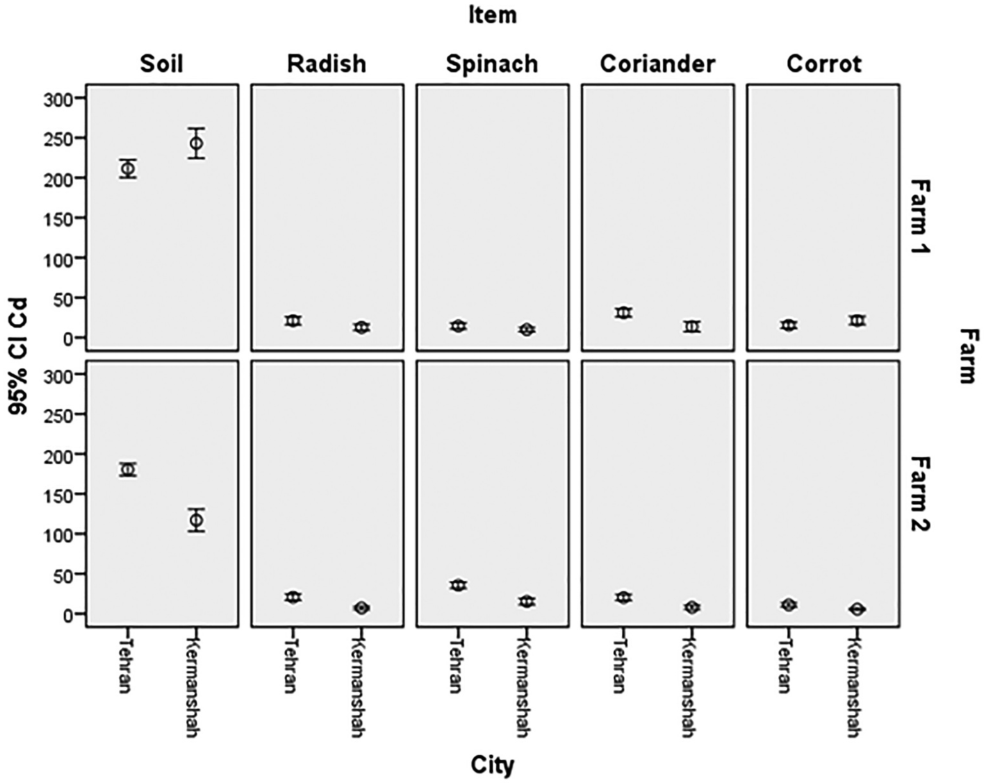

Figure 3. Graphic comparison of cadmium metal based on different farms, cities, soils and vegetables.

Table 4. Results of component analysis to investigate the relationship of heavy metals with each other.

		Toxic metals		
Analytical parameters	Toxic metals	As	Cd	Pb
Correlation	As	1.000	0.419	0.462
	Cd	0.419	1.000	0.967
	Pb	0.462	0.967	1.000
P-value	As	-	<0.001	<0.001
	Cd	<0.001	-	<0.001
	Pb	<0.001	<0.001	-
Component extracted		0.663	0.951	0.963
Component scores		0.292	0.419	0.424

study, can be due to differences in the type of vegetable selected for measurement (even with the same country of cultivation site), type of irrigation source, type of fertiliser (chemical or organic), spraying conditions and type of toxin used, characteristics of farm soil of cultivation site (oxidation-reduction potential, moisture and pH), geographical conditions, etc. [26,38]. Based on the results of Tables 6 and 7, for both scenarios, the estimated possible risk of non-carcinogenic effects (THQ) attributed to lead, arsenic and cadmium separately, as well as the sum of the probable risk attributed to all three metals

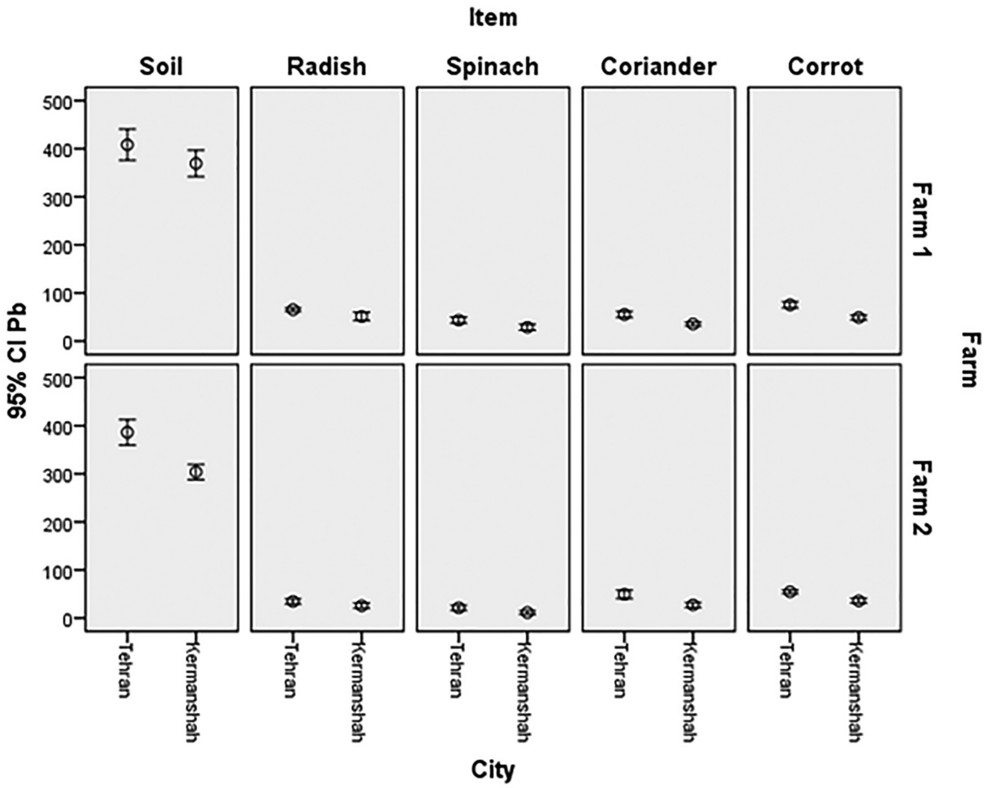

Figure 4. Graphic comparison of lead metal based on different farms, cities, soils and vegetables.

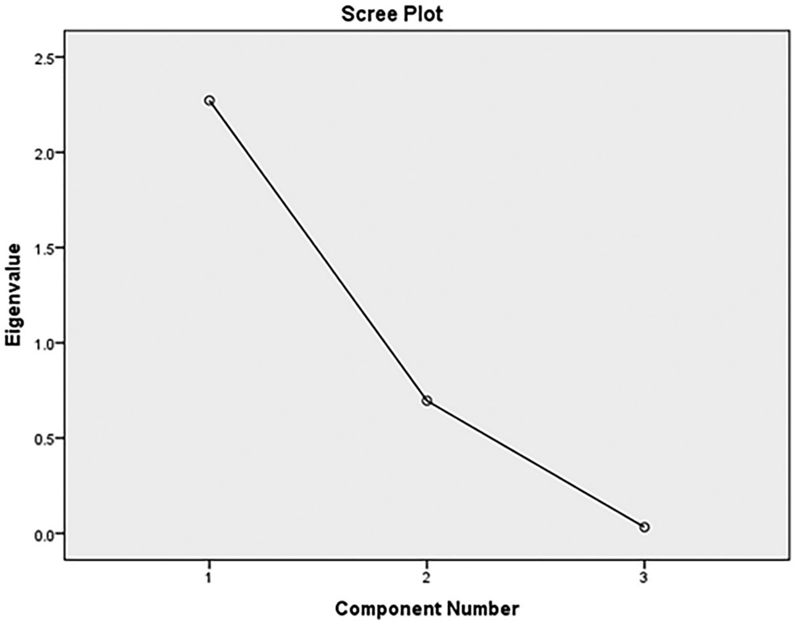

Figure 5. Scree plot of possible components.

Table 5. Chronic daily intake (CDI) of toxic metals (mg/kg.bw.d) through consumption of vegetables planted in the investigated farms.

			Farm location					
			Kermanshah			Tehran		
				Uncertainty analysis			Uncertainty analysis	
Scenario	Vegetables	Toxic metals	Point estimate	P5	P95	Point estimate	P5	P95
First scenario	Spinach	As	7.7E-05	3.5E-05	1.4E-04	9.0E-05	4.7E-05	1.7E-04
		Cd	7.4E-06	2.8E-06	1.2E-05	1.6E-05	0.2E-05	3.6E-05
		Pb	2.9E-05	0.5E-05	4.3E-05	3.8E-05	1.2E-05	5.3E-05
	Coriander	As	5.8E-05	1.7E-05	9.9E-05	1.1E-04	5.9E-05	2.8E-04
		Cd	9.5E-06	5.2E-06	2.1E-05	1.9E-05	9.5E-06	3.4E-05
		Pb	1.5E-05	0.2E-05	3.6E-05	2.4E-05	0.3E-05	3.2E-05
	Basil	As	1.3E-04	0.2E-04	3.1E-04	1.2E-04	0.2E-04	3.3E-04
		Cd	8.1E-06	2.1E-06	0.5E-05	1.9E-05	9.5E-06	3.4E-05
		Pb	2.4E-05	0.3E-05	3.1E-05	3.9E-05	1.4E-05	5.2E-05
	Radish	As	1.5E-04	0.2E-04	3.5E-04	1.8E-04	9.2E-05	3.3E-04
		Cd	1.0E-05	5.8E-06	2.8E-05	9.9E-05	5.6E-05	1.6E-04
		Pb	3.2E-05	0.7E-05	4.8E-05	4.9E-05	0.8E-05	8.1E-05
Second scenario	Total Vegetables	As	1.0E-04	6.1E-05	2.8E-04	1.3E-04	0.2E-04	3.1E-04
		Cd	3.2E-05	0.6E-05	4.7E-05	1.6E-05	0.2E-05	3.8E-05
		Pb	2.5E-05	0.4E-05	3.2E-05	3.8E-05	0.2E-05	4.1E-05

P5: 5th percentile
P 95: 95th percentile.

Table 6. Estimated possible non-carcinogenic health risk (THQ) attributed to toxic metals through consumption of vegetables planted in the investigated farms.

			Farm location					
			Kermanshah			Tehran		
				Uncertainty analysis			Uncertainty analysis	
Scenario	Vegetables	Toxic metals	Point estimate	P5	P95	Point estimate	P5	P95
First scenario	Spinach	As	0.257	0.123	0.542	0.300	0.151	0.592
		Cd	0.007	0.002	0.018	0.005	0.001	0.015
		Pb	0.008	0.002	0.022	0.011	0.002	0.041
	Coriander	As	0.194	0.042	0.473	0.361	0.195	0.651
		Cd	0.009	0.002	0.029	0.006	0.002	0.017
		Pb	0.004	0.001	0.014	0.007	0.001	0.017
	Basil	As	0.422	0.251	0.772	0.408	0.228	0.772
		Cd	0.008	0.002	0.022	0.006	0.002	0.017
		Pb	0.007	0.002	0.018	0.011	0.002	0.041
	Radish	As	0.486	0.314	0.819	0.609	0.297	0.882
		Cd	0.010	0.002	0.032	0.003	0.001	0.012
		Pb	0.009	0.002	0.028	0.014	0.003	0.041
Second scenario	Total vegetables	As	0.340	0.175	0.636	0.419	0.278	0.793
		Cd	0.032	0.007	0.094	0.005	0.001	0.015
		Pb	0.007	0.001	0.016	0.011	0.003	0.040

P5: 5th percentile
P95: 95th percentile.

(TTHQ) for each vegetable cultivated in the studied farms was lower than the acceptable risk (THQ or TTHQ = 1). The above results are based on the estimate point, which is consistent with the results of the uncertainty analysis since even the 95th percentile of THQ and TTHQ in all vegetables cultivated in the studied farms was lower allowed risk limit. The results of the estimate point as well as the results of the uncertainty analysis

Table 7. Total possible non-carcinogenic health risk (TTHQ) and incremental lifetime cancer risk (ILCR) attributed to toxic metals through consumption of vegetables planted in the investigated farms.

			Farm location						
			Kermanshah			Tehran			
				Uncertainty analysis			Uncertainty analysis		
Scenario	Vegetables	TTHQ/ILCR	Point estimate	P5	P95	Point estimate	P5	P95	Acceptable maximum level
First scenario	Spinach	TTHQ	0.273	0.127	0.572	0.316	0.154	0.648	1
		ILCR	1.2E-04	0.2E-04	3.1E-04	1.3E-04	0.4E-04	3.4E-04	1.0E-04
	Coriander	TTHQ	0.208	0.045	0.516	0.374	0.198	0.685	1
		ILCR	8.7E-05	3.2E-05	1.4E-04	1.6E-04	0.5E-04	3.9E-04	1.0E-04
	Basil	TTHQ	0.437	0.255	0.812	0.425	0.232	0.830	1
		ILCR	1.9E-04	0.6E-04	4.1E-04	1.8E-04	0.6E-04	4.0E-04	1.0E-04
	Radish	TTHQ	0.505	0.318	0.879	0.625	0.301	0.935	1
		ILCR	2.2E-04	0.7E-04	4.5E-04	2.7E-04	0.9E-04	5.1E-04	1.0E-04
Second scenario	Total vegetables	TTHQ	0.379	0.183	0.746	0.435	0.435	0.848	1
		ILCR	1.5E-04	0.5E-04	3.8E-04	1.9E-04	0.6E-04	4.2E-04	1.0E-04

P5: 5th percentile
P95: 95th percentile.

showed that the probable risk share in TTHQ for Spinach was Cd<Pb<As, while for other vegetables was Cd<Pb<As. In summary, in all vegetables, arsenic metal has the highest impact on the possible health risk.

The results of the estimate point of the ILCR attributed to an arsenic metal show that the vegetable intake for Iranians has an arsenic content that the ILCR attributed to this metal is higher than acceptable risk. The uncertainty analysis confirms the above results because the estimated P95 of the ILCR obtained from this type of analysis also indicates that the ILCR level is higher than the allowed limit. The main strategies to reduce the risk of arsenic-related carcinogenicity in vegetables are to reduce metal concentrations through interventional strategies at the site of cultivation of vegetables, such as quality control of irrigation sources, fertilisers and pesticides used, the soil of the cultivation site, etc. [39,40].

4. Conclusion

In the present research, the new VALPME–DES was coupled to determine the Potentially toxic elements in the vegetables and soil samples irrigated with treated sewage. The new DES was prepared by mixing a 1:1 molar ratio of choline chloride and citric acid monohydrate. The proposed method does not require disperser solvent compared to other different modes of DLLME technique, and the consumption of organic solvent is greatly reduced. Moreover, it can be concluded that in terms of the non-carcinogenic risk of toxic metal ions, for all studied vegetables, the TTHQ was lower than the permissible limit. In terms of the carcinogenic risk of arsenic, the quality of four vegetable samples was estimated unsatisfactory ($ILCR>10^{-4}$), and arsenic has the largest contribution in TTHQ of four vegetable samples. Focusing on reducing arsenic concentrations through interventions seems to be more important than lead and cadmium. Finally, it can be concluded that, to reduce the health risks associated with heavy metals through the consumption of vegetables, first of all, measures should be taken to reduce the amount of heavy metals, especially arsenic, in vegetable fields.

Acknowledgments

The authors gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences (Grant Number: 97638) for financial support. Also, the authors appreciate the Clinical Research Development Center experts at Imam Khomeini and Dr. Mohammad Kermanshahi and Farabi Hospitals for their cooperation in the analysis of samples.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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