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Original Research

Heavy Metal Contamination and Health Risk Assessment of Green Leafy Vegetables Irrigated with Municipal Wastewater in Gaziantep, Türkiye

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Abstract

The study was conducted to evaluate the heavy metal contents (As, Fe, Cd, Cr, Cu, Hg, Ni, and Pb) of soil and some green leafy vegetables irrigated with municipal wastewater in Gaziantep (Türkiye) and their possible health risks. For this purpose, the samples were collected from random plots that could best represent the area during the harvest period. The mean values of heavy metals analyzed in the soil were generally lower than the maximum permissible limits (MPL) recommended by WHO/FAO. However, the mean Fe (556 mg/kg), Cd (4.13 mg/kg), Cu (11.7 mg/kg), and Ni (7.7 mg/kg) contents in the mint samples were high for human consumption, according to WHO/FAO. It was determined that the mean As in parsley was at the level of MPL, but the Pb contents in mint, parsley, and dill were higher than the MPL. The highest transfer factor (TF) value in mint was in Cd (1.34), followed by Cu (0.36) and Cr (0.15). The TF in parsley was Cd>As>Pb>Cr>Cu>Ni>Fe. On the other hand, it was Cu>Pb>As>Cr>Fe in dill. The daily intake of metal (DIM) of the leafy vegetables was lower than the permitted maximum tolerable daily intake (PMTDI) values. The hazard coefficient and hazard index were <1. Thus, it showed that consumption of the leafy vegetables grown with municipal wastewater did not pose a health risk related to the selected heavy metals.

Keywords: heavy metals, green leafy vegetables, health risk, municipal wastewater

Introduction

Agricultural activities use around 70% of all freshwater, meaning water scarcity can limit food production and supply [1]. Thus, it is a common practice to irrigate agricultural lands with municipal wastewater, especially in developing countries, in order to meet the increasing human population and food demand. Using wastewater treated for agricultural irrigation can reduce the need for fertilization [2]. However, when wastewater is used for irrigation purposes, it can pose a problem for human health as it may contain heavy metals, polycyclic aromatic hydrocarbons (PAHs), personal care products, and endocrine-disrupting chemicals [3]. It has been reported that using fertilizers and pesticides, in addition to irrigation with wastewater, is the source of heavy metals in vegetables [4-6]. Wastewater use raises heavy metal levels in receiving soils, and as a result, their

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uptake by vegetables can affect agricultural production [7]. In addition to natural resources, the concentration of many heavy metals such as Hg, Pb, As, Cu, Zn, Cd, Ni, and Cr entering the environment due to anthropogenic activities has been increasing in recent years [7, 8].

They are also a serious risk for plants and other living organisms, as they are highly persistent and exist in the soil ecosystem for a long time. These metals can accumulate in living organisms, and their concentration increases as they move from lower to higher trophic levels. The transfer of heavy metals from soil to plants is an important pathway for these metals to enter food chains. Therefore, the bioaccumulation of these elements in the biota leads to the contamination of food chains [9]. Ultimately, heavy metals have the most negative impact on agricultural lands worldwide, and their accumulation in plants due to their presence in soil, mostly due to human activity, affects plant growth and poses a health risk to consumers [10].

Vegetables are important in the human diet, but since they are known to absorb large amounts of metals from the soil, they contain both essential and toxic components in a wide range of concentrations. Studies show that heavy metals such as Cd, Cr, and Pb are among the important pollutants that cause vegetable pollution [11, 12]. Due to irrigation with wastewater, the soil becomes polluted, and heavy metals can accumulate in the edible parts of vegetables, thus creating a potential risk to human health. Therefore, this study aimed to evaluate the heavy metal contents (As, Fe, Cd, Cr, Cu, Hg, Ni, and Pb) of edible parts of green leafy vegetables mint (*Mentha* L.), parsley (*Petroselinum crispum* (Mill.), and dill (*Anethum graveolens* L.) grown in agricultural lands irrigated with municipal wastewater in Gaziantep province, a developed industrial city, and their possible human health risks.

Materials and Methods

Study Area and Sample Collection

Gaziantep is located between 36°28’ and 38°01’ east longitudes and 36°38’ and 37°32’ north latitudes (Fig. 1a, b). The southern parts of the city, which are located at the transition point of the Mediterranean and continental climates, are under the influence of the Mediterranean climate but are generally hot and dry in summers and cold and rainy in winters. Precipitation occurs mostly in winter and spring. Gaziantep, which is a developed industrial city, has a high capacity in the food, agriculture, and farming sectors. Since water resources are insufficient in some parts of the city, agricultural production is carried out in areas under the influence of municipal wastewater. Thus, in this study, samples were taken from leafy vegetables and their soils grown in areas affected by municipal sewage. The irrigation type of the sampled agricultural areas is generally surface irrigation. The ellipsoid indicates the sampling area (Fig. 1c). The samples were collected from random plots that could best represent the area during the harvest period (2021-2022). Approximately 100 g of each vegetable was collected for heavy metal analysis. Soil samples taken from 0-20 cm depth were brought to the laboratory and separated from stones and plant particles in clean polyethylene containers. For this purpose, soil samples were obtained by mixing 6 samples collected from each station to obtain a single sample.

Analyses

Physico-chemical Analysis of Soil and Wastewater

The air-dried soils were sieved to <2 mm, and then the amounts required for analysis were put in polyethylene containers and labeled. The texture analysis was done according to Bouyoucos [13]. The pH analysis was measured potentiometrically using a pH meter. The electrical conductivity (EC) was determined by measuring the extract obtained from the saturated sludge with the aid of a conductivity device [14]. Lime ($CaCO_3$) was determined using a Scheibler calcimeter [15]. The modified Walkley-Black method was used to determine the soil’s organic matter (OM) content [16]. The available P content was determined according to Olsen et al. [17]. According to Soil Survey Staff, the extractable K and Ca were determined by extracting soil samples with ammonium acetate solution [18].

The wastewater samples’ temperature, pH, dissolved oxygen, and conductivity were measured *in situ* (8405 Combo Water Meter).

Heavy Metal Analysis

Before analysis, samples were washed with double distilled water, and non-edible parts were removed. For heavy metal analysis, only the edible parts of green leafy vegetables were taken and dried in an oven at 65-70ºC. The homogenized sample was thoroughly dried and weighed approximately 0.5 g. Soil samples were also dried in an oven at 65-70ºC. Then, for all samples, 8 mL of 65% nitric acid and 2 mL of 30% hydrogen peroxide were added to the containers and kept for about 10 minutes. At the end of the time, the containers were tightly closed and placed in the microwave container system properly (Cem Mars 6). It is thoroughly checked that all containers are at the same level. The container system of the microwave was placed inside the device, and the following classic method digestion conditions were applied (Table 1).

After the process, the containers were cooled to room temperature, and the samples were taken into a flask and filled with ultrapure water to 25 mL. Heavy metal analyses were performed using an atomic absorption spectrometer (Agilent 240 FS AA; Agilent GTA 120 graphite furnace). Hydride system equipment (Agilent VGA 77 AA) was used for mercury analysis. Quality assurance and control measures were taken

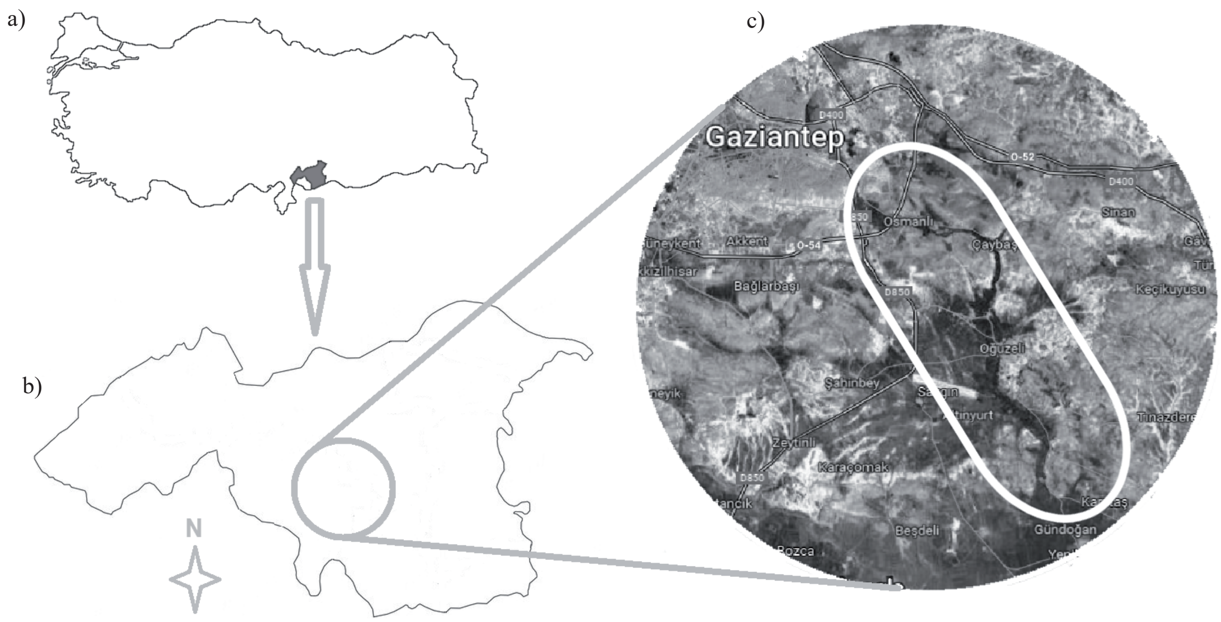

Fig. 1. Map of Türkiye a) showing the study area (b and c).

into account during the sample preparation and analysis processes to ensure the reliability of the results. The limits of detection (LOD) for each metal were estimated using the standard deviation and slope of the calibration curve.

Data Analysis

Transfer factor (TF) was calculated with the following formula [19]:

$$TF = C_M / C_S \quad (1)$$

where C_M is the heavy metal concentration in the vegetable (mg/kg dw), and C_S is the metal concentration in the soil (mg/kg dw).

The daily intake of metal (DIM) is determined as follows [20]:

$$DIM = (C_M \times C_F \times D_I) / B_W \quad (2)$$

where C_M is the heavy metal concentration in the vegetable (mg/kg dw), C_F is the conversion factor, 0.085, to convert fresh vegetable weight to dry weight [21], D_I is the daily intake of vegetables, and B_W is the average body weight (71.5 kg for adults; Turkish Statistical Institute [22]).

Table 1. Conditions for digestion of samples.

Stage	Ramp Time (min)	Hold Time (min)	Temperature (ºC)
1	10	5	140
2	15	15	190

The hazard quotient (HQ) is expressed as follows [23]:

$$HQ = DIM / RfD \quad (3)$$

where DIM represents the daily metal intake of vegetables per day (mg/person/day), and R_fD depicts the oral reference dose of the heavy metal (mg/kg/day). The R_fD values of As, Fe, Cd, Cr, Cu, Hg, Ni, and Pb were reported as 0.0004, 0.007, 0.001, 0.003, 0.004, 0.00016, 0.02, and 0.0035, respectively [24]. The HQ value <1 is assumed to have no carcinogenic risks. When the value is >1, it is considered to have the possibility of notable health hazards.

The hazard index (HI) is expressed as follows [25]:

$$HI = \sum HQ \quad (4)$$

The HI value >1 is not safe for human health [26].

IBM SPSS V22.0 was used for statistical analysis. Pearson correlation analysis was conducted to investigate the soils' heavy metal content.

Results and Discussion

Physico-Chemical Properties of Soil and Wastewater

The physico-chemical properties of the soils are given in Table 2. The textures of the soils ranged from sandy to loamy. The pH values of the soils ranged between 7.17-7.61 (average 7.43), and the soils were slightly alkaline-neutral. EC values ranged from 0.85 to 1.66 (mean 1.24 dS/m). They were non-saline [27].

Table 2. Physico-chemical properties of soils.

Properties	Range	Mean
Texture (%)	30-47	33.5
pH	7.17-7.61	7.43
EC (dS/m)	0.85 -1.66	1.24
$CaCO_3$ (%)	14.5-36.3	32.7
OM (%)	2.43-5.35	3.37
Available P (mg/kg)	54-125	84.6
Extractable K (mg/kg)	163-349	275.7
Extractable Ca (mg/kg)	3470-4549	4052

Approximately 83% of the sampling areas were determined to have high calcareous content. Medium (2.43%) to high (5.35%) OM values were found. However, the overall OM was at the desired level. On the other hand, the soils contained very high available P and high extractable K. Extractable Ca was at a good level [28, 29]. Meanwhile, mean values for pH, conductivity, temperature, and dissolved oxygen of wastewater samples were measured as 7.59 (range 7.42-7.69), 976 µS/cm (range 940-1044 µS/cm), 24.8ºC (range 23.5-26.3ºC), and 7.9 mg/L (range 5.9-8.9 mg/L), respectively.

Heavy Metal Concentrations in Soils

The concentrations of heavy metals ranged from 0.2 to 28000 mg/kg (Table 3). The mean concentration of heavy metals in soil samples was listed as follows: Fe>Pb>Ni>Cu>Cr>As>Cd>Hg. There are studies about the heavy metal contents of soils irrigated with wastewater. For example, Avci and Deveci reported the concentrations of Cd (range 0.3-1 mg/kg, mean 0.7 mg/kg), Cr (range 26-149 mg/kg, mean 84 mg/kg), Cu (range 11-72 mg/kg, mean 41 mg/kg), Ni (range 38-200 mg/kg), and Pb (range 5-46 mg/kg, mean 19 mg/kg) in soils irrigated with wastewater in Gaziantep (Türkiye) [30]. According to Kabata-Pendias and Pendias, heavy metals, except As and Cr, were higher than the stated value for uncontaminated soils [31]. On the other hand, although high values were found in Cu (up to 224.7 mg/kg), other heavy metals were below the recommended values for soils irrigated with wastewater, except for Ni [31]. In addition, the mean values of other analyzed metals, except Ni, were lower than the MPL values recommended by the Food and Agricultural Organization (FAO)/World Health Organization (WHO) [32, 33].

Pearson analysis determined the relationships between heavy metals (Table 4). Except for Hg (+; $p<0.05$), As showed a negative insignificant correlation with Fe, Cd, Cr, Cu, Ni, and Pb ($p>0.05$). Positive and insignificant relationships were determined between Fe and other heavy metals ($p>0.05$), except for As and Pb (-; $p>0.05$). Hg and Cd were generally negatively correlated with the other heavy metals ($p>0.05$). However, there was a strong and positive correlation between Cr-Ni, Cr-Pb, and Ni-Pb ($p<0.01$). In addition, a positive and significant relationship was determined between Cu and Ni ($p<0.05$).

Heavy Metal Contents in the Leafy Vegetables

Leafy vegetables have the potential to accumulate many more heavy metals than other vegetables [34]. Therefore, determining the heavy metal content of green leafy vegetables is important for consumers' health. Among the analyzed vegetables, As could not be determined in mint, and Cd and Ni in dill. Also, Hg could not be determined in all vegetables (Table 5). On the other hand, the highest As concentrations were

Table 3. Heavy metal contents of soils.

Heavy metals	Range (mg/kg)	Mean (mg/kg)	*	**	***
As	0.2-7.1	3.5	10	20	20[a]
Fe	5000-28000	13000	-	-	50000[b]
Cd	0-1.2	1.1	0.5	3	3[b]
Cr	0-98.9	30.4	50	600	100[b]
Cu	5.6-224.7	32.5	20	135	100[b]
Hg	0-0.8	0.2	0.1	1	1[b]
Ni	4.9-359	89.2	25	75	50[b]
Pb	12.2-188	95.8	50	250	100[b]

*; Typical total heavy metal contents of uncontaminated soils (mg/kg) [31].
**; Recommended upper limits for soils heavy metal concentrations after sewage sludge additions (mg/kg) [31].
***; Maximum permissible limits (MPL) of FAO/WHO in soils: a and b were described by Custodio et al. and Khan et al., respectively [32, 33].

Table 4. Pearson correlation coefficient of the metals.

	As	Fe	Hg	Cd	Cr	Cu	Ni	Pb
As	1							
Fe	-0.177	1						
Hg	0.613*	0.096	1					
Cd	-0.444	0.295	-0.292	1				
Cr	-0.361	0.210	-0.243	-0.044	1			
Cu	-0.259	0.017	-0.232	-0.119	0.512	1		
Ni	-0.354	0.083	-0.310	-0.158	0.932**	0.613*	1	
Pb	-0.310	-0.023	-0.348	0.097	0.825**	0.392	0.880**	1

*: P<0.05; **: P<0.01

Table 5. Heavy metal contents in the leafy vegetables and their MPLs.

Heavy metals	Leafy vegetables	Range (mg/kg dw)	Mean (mg/kg dw)	MPL*
As	Mint	-	-	0.2[a]
	Parsley	0.019-0.44	0.2	
	Dill	0.014-0.032	0.021	
Fe	Mint	316-771	556.2	450[b]
	Parsley	0.21-0.56	0.39	
	Dill	28.3-41.3	34.1	
Cd	Mint	1.02-12	4.13	0.20[b]
	Parsley	0.08-0.12	0.1	
	Dill	-	-	
Cr	Mint	1.8-10.9	4.46	2.30[b]
	Parsley	0.04-0.69	0.36	
	Dill	0.018-0.36	0.102	
Cu	Mint	4.3-17.4	11.7	10[b]
	Parsley	0.015-0.23	0.15	
	Dill	0.98-1.6	1.26	
Hg	Mint	-	-	0.1[b]
	Parsley	-	-	
	Dill	-	-	
Ni	Mint	1.2-13.7	7.7	2.70[b]
	Parsley	0.012-0.02	0.016	
	Dill	-	-	
Pb	Mint	3.4-11.3	5.28	0.30[b]
	Parsley	1.23-4.11	2.87	
	Dill	0.89-4.11	2.03	

- ; <LOD

*; Maximum permissible limits (MPL) of heavy metals recommended by WHO/FAO: a and b were described by Rehman et al. and Sultana et al., respectively [35, 36].

determined in parsley. The highest Fe contamination was determined in mint, followed by dill and parsley. The mean Cd content in mint samples was approximately 41 times higher than that of parsley. The highest Cr, Cu, and Pb contamination was determined in mint, followed by parsley and dill. The mint samples' mean Fe, Cd, Cu, and Ni contents were higher than the recommended maximum permissible limits (MPL). It was determined that the mean As in parsley was at the level of MPL, but the Pb contents in mint, parsley, and dill were higher than MPL. The findings may indicate that vegetables grown in polluted areas may not only affect their physiology but also pose a risk to consumers. Using municipal wastewater for irrigation significantly changes soil chemistry and leads to heavy metal accumulation in the soil far above the permitted standards. Thus, it can accumulate high concentrations of heavy metals in the edible parts of vegetables. It has been suggested that using wastewater as an irrigation source could lead to serious health risks for subsequent consumers [37].

Transfer Factor

It is important to determine the TF to evaluate metal bioavailability in the substrate where green leafy vegetables are grown, which have an important place in human nutrition. The TF, also called the bioconcentration factor (BCF), was calculated using the ratio of the metal concentration of the vegetable to the metal concentration in the soil [38]. Transfer factors (TF) of the leafy vegetables are given in Fig. 2. The highest TF values in mint (ranging from 0.043 to 1.34) were calculated for Cd (1.34), followed by Cu (0.36) and Cr (0.15) (Fig. 2a). The TFs in parsley (ranging from 0.0003 to 0.09) were Cd>As>Pb>Cr>Cu>Ni>Fe (Fig. 2b). On the other hand, it was Cu>Pb>As>Cr>Fe in dill (range from 0.0026 to 0.04) (Fig. 2c). TF values showed differences according to the metals and the vegetables. The heavy metal TF of eight different leafy vegetables, including mint, parsley, and dill, collected from wastewater-polluted areas was determined as Cd>Zn>Pb>Cu>Mn>Ni>Fe [39]. It has been reported that if the TF value is <1, plants respond poorly to metal absorption, while values being ≥1 show higher metal absorption from the soil [19]. Thus, the value was lower than 1 for the other leafy vegetables, except for the TF of Cd in mint. This may indicate that the vegetables bioaccumulate some heavy metals at low levels. Similarly, Cd has been reported to have high bioaccumulation in plants [39]. In this respect, it has been shown that vegetables grown in polluted soils can accumulate high concentrations of heavy metals, such as Cd, causing serious health risks for humans.

Health Risk

The results showed that the DIM ranged from 0.00001-0.00007, 0.0002-0.23, 0.00004-0.002, 0.00004-0.002, 0.00006-0.005, 0.00001-0.003, and 0.0008-0.002, for As, Fe, Cd, Cr, Cu, Ni, and Pb, respectively (Table 6). According to the data, the DIM values of leafy vegetables were lower than the recommended PMTDI values. Moreover, the total DIM of each vegetable was in descending order of parsley <dill<mint. Thus, their consumption may have different effects on human health. Besides, similar results were obtained for HQ values (Table 7). The highest HQ values calculated for heavy metals were found in mint. However, both HQ and HI values were <1. When the value is >1, it is considered to have the possibility of notable health hazards. Since the obtained HQ values are <1, it may be stated that the probability of adverse effects on human health is low. Another parameter related to HQ is the HI. Similarly, HI values were determined to be ranked as parsley<dill<mint. The fact that the obtained values are <1 shows that their consumption is safe for human health [26]. Overall, the results of this study indicate

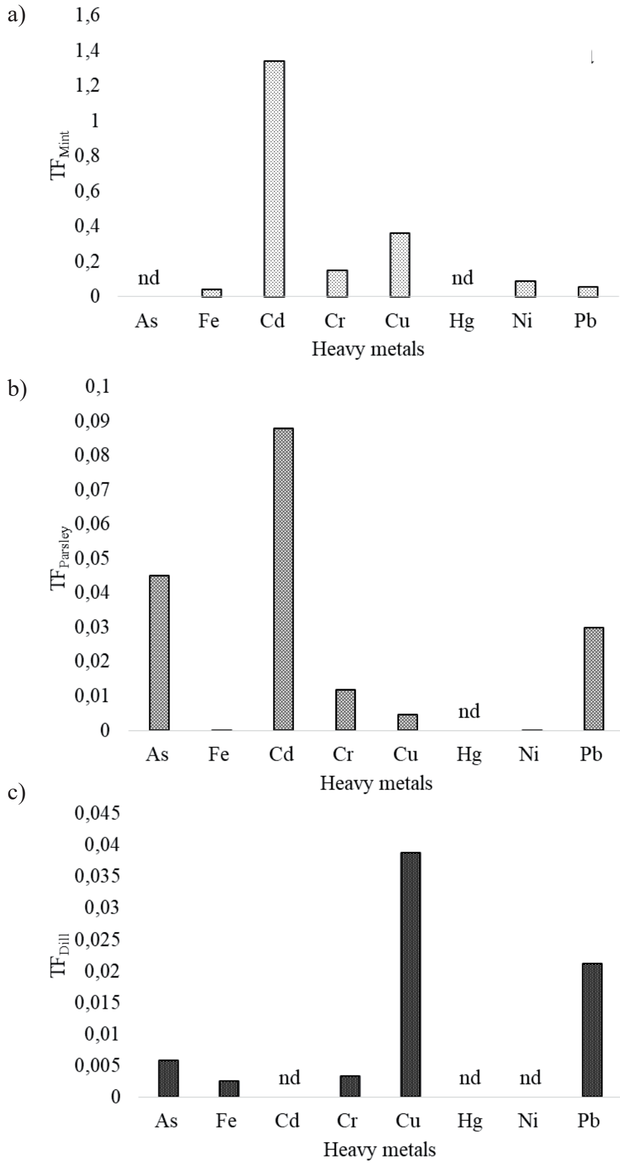

Fig. 2. Metal transfer factors in mint a), parsley b), and dill c).

Table 6. Daily metal intake (DIM) of metals and their PMTDI.

Heavy metals	DIM (mg/person day)			PMTDI*
	Mint	Parsley	Dill	
As	nc	0.00007	0.00001	0.015[a]
Fe	0.23	0.0002	0.01	17.0[b]
Cd	0.002	0.00004	nc	0.046[b]
Cr	0.002	0.0002	0.00004	0.20[b]
Cu	0.005	0.00006	0.0005	2.0[b]
Hg	nc	nc	nc	0.04[b]
Ni	0.003	0.00001	nc	0.30[b]
Pb	0.002	0.001	0.0008	0.21[b]
Total DIM each vegetables	0.24	0.002	0.015	

*; Permitted maximum tolerable daily intake (PMTDI) levels of FAO/WHO: a and b were described by Sultana et al. and Shrivastava, respectively [36, 40].
nc; not calculated

Table 7. HQ and HI of the leafy vegetables.

Heavy metals	HQ		
	Mint	Parsley	Dill
As	nc	2,60E-08	3,40E-09
Fe	1,60E-03	1,10E-06	9,80E-05
Cd	1,70E-06	4,10E-08	nc
Cr	5,50E-06	4,40E-07	1,30E-07
Cu	1,90E-05	2,50E-07	2,10E-06
Hg	nc	nc	nc
Ni	6,30E-05	1,30E-07	nc
Pb	7,60E-06	4,10E-06	2,90E-06
HI of each the vegetables	1,70E-03	6,09E-06	1,03E-04

nc; not calculated

that different leafy vegetables grown in soils irrigated with municipal wastewater accumulate heavy metals in their edible parts, but fortunately, they do not yet pose a health risk.

Conclusions

Plants take up pollutants such as heavy metals from their environment and accumulate them in their structures. Like other agricultural plants, leafy vegetables are also included in the food chain. For this reason, it is very important to determine the levels of heavy metals in vegetables and soils and estimate their possible risks to human health. Since the leafy vegetables collected in this study have different biological properties, the metal concentrations detected differ from the others. The average Fe, Cd, Cu, and Ni contents of green leafy vegetables, such as mint, were higher than the recommended values according to WHO/FAO. It was also determined that Pb content was higher than MPL in all three vegetables. However, the DIM values of leafy vegetables were lower than the PMTDI values. Thus, the total DIM of each vegetable was, in descending order, parsley<dill<mint. The fact that the HQ and HI were <1 showed that consumption of leafy vegetables grown with municipal wastewater did not pose a health risk for the sampling period regarding the selected heavy metals. Heavy metal contents in vegetables may vary depending on many factors such as plant species and growth stages, irrigation type, season, soil properties, etc. Therefore, it would be beneficial to conduct more comprehensive studies in the area to better evaluate the dynamics of metals by taking these factors into account. On the other hand, phytoremediation can be an effective method to solve the problems of areas contaminated with heavy metals due to human activities. Thus, these techniques are recommended to ensure the sustainability of contaminated agricultural lands. It is also recommended that heavy metals in agricultural areas contaminated with municipal wastewater be regularly monitored.

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Conflict of Interest

The authors declare no conflict of interest.

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