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Assessment of the Heavy Metal Accumulation of Various Green Vegetables Grown in Nevşehir and their Risks Human Health

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Abstract This study aimed to investigate the accumulation of heavy metal in five different leafy green vegetables grown in 10 different agricultural lands in the province of Nevşehir, Turkey, and determine the human health risks that may arise as a result the consumption of such metals. The heavy metal concentrations found in the soil samples taken from the agricultural lands were as follows: manganese (Mn) > lead (Pb) > arsenic (As) > nickel (Ni) > copper (Cu) > cadmium (Cd). These concentrations were found to be well above the permitted limits imposed by the World Health Organization/ Food and Agricultural Organization (WHO/FAO) and the United States Environmental Protection Agency (USEPA). Also, particularly the concentrations of Pb and As were found to be dangerous levels in the soil. According to their accumulation in the vegetables, the detected heavy metal concentrations were listed as Mn > Zn > Cu > Ni > As > Cd. The estimated daily intake amount of the heavy metals was also found to be higher than the limits determined by FAO/WHO. Furthermore, the target hazard quotient of the metals was Mn > As > Cu > Zn > Ni > Cd > Pb. It was found to be > 1 for the sampling areas, except for Cd and Pb. As a result of the study, it was determined that almost all of the annual heavy metal intake amount that the population living in this region should receive was met as a result of the consumption of leafy vegetables grown in the stations designated for this study. It is vital to effectively monitor the heavy metal in the soil and vegetables to reduce metal concentrations in the studied area and to investigate its effects on human health. By doing so, acute and chronic health problems due to the heavy metal exposure in this region can be prevented

Keywords Heavy metals · Leafy vegetables · Target hazard quotient · Risk assessment · Food Contamination · Turkey · Nevşehir

Introduction

Heavy metal contamination in agricultural lands is one of the most important environmental problems encountered worldwide. The intense use of agrochemicals, the untreated wastewater, and irrigation water of agricultural lands and atmospheric deposition are the main

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sources of heavy metals in nature (Ali and Al-Qahtani 2012). The intake of heavy metals, which enter the food chain through plants grown on farmlands that are contaminated with such metals, negatively affects human health, even when consumed at very low levels. Heavy metal intake through the food chain is responsible for 90% of total heavy metal contamination and is much more effective than heavy metal intake by inhalation and dermal contact (Gupta et al. 2008).

Heavy metals are considered to be the most dangerous pollutants as they have a long biological half-life, are non-biodegradable, and do not have the potential to be accumulated at different levels by different body tissues (Radwan and Salama 2006). These features enable heavy metals to become permanent in the environment and cause long-term effects on the living organisms in the environment. Heavy metal contamination can lead to a wide range of health problems including impaired psychosocial behaviors, endocrinal disorders, genomic disorders, immune system problems, and neurotoxic and carcinogenic effects (Dyer 2007).

Even though some heavy metals are necessary for plants, the excessive accumulation of these metals by plants grown on contaminated farmlands is an indication of an increase in pollution load (Naser et al. 2012).

The vegetables that grow in agricultural areas where heavy metal contamination occurs accumulate these materials beyond the range permitted by the WHO. Various studies have reported a correlation between the high concentration of metal detected in vegetables and the concentration of metal in the soil (Ji et al. 2018).

For the benefit of human health, the concentrations of heavy metals in green vegetables, which have a significant role in human nutrition, should not exceed the permitted limits. Thus, it is vital to regularly monitor the accumulation of heavy metals in agricultural lands. Green vegetables have a higher ability to accumulate heavy metals compared to grains and other vegetables, making them more influential in humans' exposure to heavy metals (Salehipour et al. 2015)

The calculations and limits of heavy metals, including the daily intake and total risk indices set by international organizations such as the United States Environmental Protection Agency (USEPA) and WHO, encourage scientists to assess heavy metal intake through food consumption and its risk factors (Barakat et al. 2019; Leblebici and Kar 2018).

The province of Nevşehir is located in the center of the region of Cappadocia. Nevşehir is known for its fairy chimneys, which are located across a wide area and protected by the state. Thus, areas that can be used as agricultural land in this region are limited. In these limited agricultural areas, mostly leafy vegetables are grown, especially because the product is obtained in a short period. Therefore, the detection of heavy metal pollution in these regions and the possible risk factors are of great importance in terms of public health.

In this study, heavy metal concentrations in soil and plant samples taken from 10 different agricultural lands in the province of Nevşehir were measured. The bioconcentration factors of the heavy metals, estimated daily metal intake (EDI), and target hazard risk quotient (THQ) indices were also calculated to assess whether they pose a risk to human health.

Methods and Materials

In this study, five different vegetable samples, namely parsley (*Petroselinum crispum*), spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*), onion (*Allium cepa*), leek (*Allium porrum*), and soil samples were of 10 different settlement areas in the province of Nevşehir were taken

Sample Preparation

The vegetable samples collected from the study area were brought to the laboratory environment and washed with double-distilled water (ddH_2O) to remove the superficial pollution. All vegetable samples were dried in a drying-oven at 80 °C for 24 h. The moisture-free samples were ground in a mortar and transferred to bags to homogenize them.

Sample Digestion and Heavy Metal Analysis

The samples were digested in a microwave using 10-ml pure HNO_3. After the digestion process, the volume of the samples was completed to 10 ml with ddH2O. Element standard solutions are measured applying high precision ICP-MS (ICP MS/MS Agilent 8800 Triple quad) and are directly traceable to the corresponding NIST SRM® (NIST: National Institute of Standards and Technology, Gaithersburg, USA). Multimetal solution specification and standard reference material list given in Table 1. (ICP Ms/MS Agilent 8800 Triple quad). All trials were conducted

Table 1 Multimetal solution specification and standard reference material list

Element	Specification (μg/ml)	NIST SRM
Cd	100.0 ± 0.6	3108
Cu	100.0 ± 0.6	3114
Zn	100.0 ± 0.6	3168a
Ni	100.0 ± 0.6	3136
As	100.0 ± 1	3103
Pb	100.0 ± 1	3128
Mn	100.0 ± 1.5	3132

three times. All of the chemicals used were analytically grade (Merck, Darmstadt, Germany).

The soil samples were brought to the laboratory dried in a drying oven at 80 °C for 24 h and homogenized using a 2-mm sieve. The samples were digested in a microwave instrument using a mixture of 10 ml of pure HNO_3/HCl. After the digestion process, the volume of the samples was completed to 10 ml with ddH_2O. Heavy metal concentrations in samples were determined using the ICP-MS device.

Bio-transfer factor

The bio-transfer factor (BTF) of the samples was calculated to determine the heavy metal transfer capability and yield of the vegetables depending on the concentration of metals in the soil. The following formula was used for BTF calculation: TF = Cv/Cs (Rahmani and Sternberg 1999), where Cv is the metal concentration detected in the vegetables and Cs is the metal concentration in the soil.

Estimated Daily Intake

The estimated daily intake (EDI) of heavy metals among adults with an average life span living in Nevşehir was calculated using the formula EDI = (DcxMc)/W (Chungu et al. 2019). According to this formula, Dc is Mc is the concentrations of metals detected in the vegetables and W is the average weight of an adult. “In this study, the average weight of an adult was determined to be 75 kg and the average consumption of green vegetables per person was taken as 350 g/day as declared by the WHO.

Target hazard quotient

The target hazard quotient (THQ) is the ratio between the reference dose and exposure (USEPA 2001). According to a report published by USEPA, if the THQ index of a plant is greater than 1, the consumption of these plants poses a risk to human health. The calculation of the THQ was based on the formula given below which was provided by the USEPA and has been used in the literature (Chungu et al. 2019; USEPA 2001).

THQ = 0.001 ((Ef × Ed × Dc × Cm)/(RfD × W × T)). Where Ef is exposure time and was determined as 365 days, Ed is the average life span and was considered to be 65 years in the field of study. Dc is the amount to be consumed daily. RfD is the daily oral dose and was determined as 0.003, 0.001, 0.04, 0.04, 0.3, 0.002, and 0.001 in As, Cd, Pb, Cu, Zn, Ni, and Mn, respectively, and T is the average lifetime exposure time (365 days a year × Ed).

Statistical analysis

Mean and standard deviation values were calculated for heavy metal concentrations in soil and vegetable samples. We perform the kurtosis and skewness tests to verify the normality; subsequently, the Kolmogorov–Smirnov test and Levene’s test were used to ensure the normality assumption and the homogeneity of variances, respectively. One-way ANOVA and post-hoc Duncan and Kruskal-Wallis and post-hoc Tamhane tests performed were used to determine whether there was a statistically significant difference in five metal accumulations in plants. Pearson’s correlation coefficient was also calculated to illustrate the relationship between the concentration of metals in the soil and the amount of metal concentration accumulated in vegetables. All statistical analyses were carried out in the SPSS 22 program.

Results

The accumulations of the heavy metal, transfer factors, correlations, EDI, and THQ were detected in five different leafy vegetables and soil samples collected from 10 different stations.

The average metal concentrations detected in the vegetables ranged from 0.03 to 41,698 μg g^{-1}. The metals determined in the vegetables, Zn and Mn, in particular, were in very high concentrations. The highest metal concentrations of As (2.06 μg g^{-1}) and Cu (6.61 μg g^{-1}) was found in leeks; the highest , Ni (2.47 μg g^{-1}), and Mn (51.64 μg g^{-1}) in parsley; and the highest Zn (48.05 μg g^{-1}), Cd (0.16 μg g^{-1}) and Pb (0.67 μg g^{-1}) in onions respectively. The kurtosis and skewness values of data were found between " + 2 and − 2" except for Ni and Mn. According to normality tests, it was determined that only Cu showed normal distribution. To test the difference in Cu accumulation between vegetables, one-way ANOVA and Duncan test were applied as post-hoc. Kruskal-Wallis test and Tamhane test as post hoc were performed to all other data. As a result, no significant difference was found between As, Ni, and Mn concentrations in vegetables. However, the Cd concentration in lettuce differs from parsley. Also Cd metal in onion is statistically different from both leek and parsley. Likewise, Zn metal accumulation in onion differs from lettuce and leek (Kruskal-Wallis) (p < 0.05).

According to the ANOVA test in copper, metal accumulation in all vegetables is different from each other (ANOVA) ($p < 0.05$) (Table 2).

Values are expressed as mean ± SEM of three replicates ($n = 10$). *Permissible limits (mg/kg) were adopted from USEPA (2011), EU (European Union 2002), and WHO (2000). There is a statistical difference between the values having different letters (ANOVA) ($p < 0.05$).

The the highest metal concentration among the stations was Mn (1821 μg g^{-1}) collected from Eskiyaylacik station, the lowest concentration −1 was Cd (0.07 μg g^{-1}) collected from the Alkan station (Table 3).

Values are expressed as mean ± SEM of three replicates ($n = 50$). *Permissible limits (mg/kg) were adopted from USEPA (USEPA 2011), EU (European Union 2002), and WHO (WHO 2000)

When the correlation between the concentration of metals in soil and the accumulation of metals in vegetables was investigated, the strongest and most positive correlation was determined as As, which was found to be the highest in lettuce, parsley, and onions. The correlation was not statistically significant *(P> 0.05)* (Table 4).

According to the transfer factors from soil to metal, the highest transfer factor was determined as 0.96 for Zn in onions, while the lowest transfer factor was found to be in Pb in spinach (Table 5)

When the EDI values were observed, it was determined that the highest metal intake was Mn as a result of parsley consumption, while the lowest metal intake was Cd, which was found to be 0.14 in parsley.

However, according to the THQ, the most hazardous metals were determined as Mn > As > Cu > Zn > Ni > Fr > Pb (Table 6).

Discussion

High concentrations of Zn and Mn were detected in the soil samples collected taken from the stations. This was thought to be due to irrigation water, in which high-level metals are transported. A similar result was achieved by Harmanescu et al. (2011) in their studies conducted in Romania, where they determined that there was a higher level of accumulation of Zn and Mn compared with the other metals that were found (Harmanescu et al. 2011). In a previous study conducted in the province of Nevşehir, it

Table 2 Heavy metal concentrations (mean ± SD, μg g^{-1}) in green vegetables in Nevşehir Province, Turkey

	As	Cd	Pb	Cu	Zn	Ni	Mn
Lettuce	0.71 ± 0.18	$0.09 \pm 0.03^{*}$	0.45 ± 1.02	4.28 ± 1.04^{a}	$27.42 \pm 6.78^{*}$	1.83 ± 0.56	46.81 ± 11.6
Parsley	1.05 ± 0.26	$0.05 \pm 0.014^{*+}$	0.43 ± 1.35	6.59 ± 1.53^{bc}	36.28 ± 9.05	2.47 ± 0.68	51.64 ± 11.9
Leek	2.06 ± 0.52	0.03 ± 0.003^{x}	0.25 ± 0.04	6.61 ± 1.68^{c}	$30.26 \pm 7.02^{+}$	1.84 ± 0.48	33.58 ± 7.9
Spinach	0.75 ± 0.17	0.03 ± 0.004	0.26 ± 0.06	5.11 ± 1.35^{ab}	31.30 ± 7.65	1.56 ± 0.37	38.67 ± 9.5
Onion	1.19 ± 0.26	$0.16 \pm 0.05^{+x}$	0.67 ± 0.16	$6.58 \pm 1.48b^{c}$	$48.05 \pm 11.25^{*+}$	1.76 ± 0.42	37.79 ± 9.03
Permissible Limits*	0.1	0.1	0.20	3	3	1.5	2

Table 3 Heavy metal concentration (mean ± SD, μgg^{-1}, n = 50 per location) in soils in Nevşehir Province, Turkey

	As	Cd	Pb	Cu	Zn	Ni	Mn
Alkan	10.3 ± 2.04	0.07 ± 0.01	14.02 ± 3.5	6.6 ± 1.1	30.12 ± 5.28	9.69 ± 1.87	400.99 ± 78.5
Altıpınar	12.1 ± 3.06	0.49.08 ± 0.05	20.35 ± 4.5	8.2 ± 2.1	41.13 ± 7.58	54.18 ± 9.54	1082.44 ± 125.36
Civelek	34.0 ± 7.98	0.63 ± 0.11	99.50 ± 15.5	23.1 ± 4.92	125.02 ± 25.6	29.91 ± 6.37	1821.81 ± 890.36
Dadagı	41.63 ± 8.45	0.88 ± 0.15	41.1 ± 8.5	14.99 ± 3.25	58.75 ± 14.3	26.96 ± 5.96	1215.62 ± 49.25
Emiler	25.39 ± 4.85	0.63 ± 0.11	16.3 ± 5.69	24.75 ± 8.24	43.23 ± 7.19	25.97 ± 4.78	581.87 ± 37.26
Eski Yaylacık	10.14 ± 2.25	0.76 ± 0.08	19.03 ± 4.26	13.48 ± 2.36	58.43 ± 10.6	28.8 ± 5.55	488.15 ± 29.54
Gürpınarı	4.85 ± 0.95	0.37 ± 0.07	6.46 ± 1.25	10.91 ± 2.05	32.54 ± 5.28	13.19 ± 2.36	373.42 ± 95.26
Kızılkaya	4.64 ± 1.02	0.36 ± 0.12	4.35 ± 1.36	14.79 ± 2.87	47.75 ± 8.25	12.41 ± 6.25	251.6 ± 83.57
K.ayhanlar	111.38 ± 20.25	0.56 ± 0.14	45.2 ± 8.77	29.11 ± 4.65	73.20 ± 6.25	23.22 ± 4.59	1331.77 ± 65.48
Uchisar	4.23 ± 1.62	0.43 ± 0.09	10.02 ± 2.9	14.34 ± 3.69	63.87 ± 7.25	18.65 ± 8.54	421.3 ± 47.85
Permissible Limits*	0.2	0.3	0.3	75	50	1.5	500

was emphasized that the river water used for irrigation in the r region may cause heavy metal accumulation in the soil (Leblebici and Kar 2018). In the present study, it was determined that the concentration of heavy metals in the soil was high, as most of the agricultural lands from which the samples were taken were irrigated with river water.

Irrigation water has a key role in heavy metal accumulation in soil, while the intensive use of agrochemical fertilizers can be a major factor responsible for increasing heavy metal accumulation. Also, the intake of heavy metals by food plants depends on long exposure time, the physicochemical properties of plant species, levels of the element, pH, seasonal changes, and abiotic factors such as the temperature and salinity of the soil (Addis and Abebaw 2017).

High levels of Zn and Fe, the use of organic and chemical fertilizers, and bedrocks that are the source of these metals can also be effective. The result of Barakat et al. (2019) was similar to findings present study (Barakat et al. 2019).

On the other hand, the reason for high levels of heavy metal concentrations in the soil and plant samples can be the result of being increased exposure to dissolved chemicals due to drought and decreased quality of irrigation water (Marvin et al. 2013).

Khan et al. reported that leafy vegetables were also exposed to heavy metals by alternative means as well such as dust and rainwater due to the large surface areas of their leaves and could accumulate more heavy metals than normal plants due to their high growth rates (Khan et al. 2013). In the present study, heavy metal in the green plants was also found to be at very high levels.

The metal concentrations detected in the vegetables used in this study were different from one another. This was thought to be because the plants had different morphologies and physiologies (Tom et al. 2014).

Even though Zn is essential for the human body, prolonged exposure at high concentrations can lead to various imbalances in the human immune system. Similarly, excessive Cu uptake can cause liver disorders (Volpe et al. 2009). In their study, Rahmdel et al. found

Table 4 Correlation coefficients for heavy metal concentrations between green vegetables and soils

	As	Cd	Pb	Cu	Zn	Ni	Mn
Lettuce	0.888**	− 0.089	0.246	− 0.033	0.299	0.822	0.244
Parsley	0.721*	0.38	0.335	− 0.169	− 0.304	0.195	− 0.025
Leek	0.59	− 0.335	− 0.209	0.424	0.298	− 0.304	− 0.315
Spinach	0.218	− 0.239	0.579	0.014	− 0.535	0.75	− 0.329
Onion	0.703*	0.475	− 0.167	0.129	0.336	− 0.09	0.355

Table 5 Transfer factors of metals from soil to vegetables in Nevşehir Province, Turkey

	As	Cd	Pb	Cu	Zn	Ni	Mn
Lettuce	0.0501	0.259	0.032	0.335	0.531	0.087	0.054
Parsley	0.058	0.080	0.023	0.516	0.764	0.121	0.093
Leek	0.090	0.081	0.021	0.476	0.586	0.098	0.072
Spinach	0.049	0.088	0.012	0.394	0.671	0.076	0.101
Onion	0.069	0.388	0.044	0.497	0.962	0.089	0.066

that the highest accumulated metals in the plants were Cu and Zn (Rahmdel et al. 2018). Similarly, in the present study, it was determined that the concentrations of Cu, Zn, and Mn in all of the plants were above the accepted limits and thus dangerous for human health.

Transference from soil to plant is one of the main components of human exposure to heavy metals through the food chain. This study determined that the BTF values between the sampling areas and species differed significantly. The BTF value of all of the metals was found to be low in terms of transfer factors. This may be since the concentration of metals in the soil was higher than the concentrations in the vegetables. Similarly, Khan et al. reported that there was no linear increase in the BTF values of plants as a result of the heavy metal concentration in the soil (Khan et al. 2013).

The awareness of the relationship between diet and human health has led to a very intense consumption of green vegetables. Since green plants are produced and consumed very quickly, food safety and sustainability have become an important issue (Rahmdel et al. 2018). Also, heavy metal contamination in humans can occur from the metals in the air, water, and soil, as well as through nutrients, which are more effective (Caussy et al. 2003). Vegetables can pose a major health risk to people when they are contaminated with heavy metals. The vegetables collected in this study are grown and consumed raw and cooked by the local people and used both raw and cooked, thus, heavily exposing them to the heavy metals in the vegetables. The EDI of heavy metals was calculated according to the average concentration of each heavy metal in the vegetables and their corresponding consumption rates (Santos et al. 2004). The uptake of each heavy metal through the consumption of vegetables was significantly higher than in the previous study (Leblebici and Kar 2018). The amount of EDI assessed in terms of plants was very high. Therefore, the continuous and long-term consumption of these contaminated vegetables greatly increases the likelihood of health problems that may arise due to exposure to all of the determined metals, particularly Ni, Zn, and Mn.

Promoting the intensive consumption of green vegetables due to the vitamins they contain further increases the risk of heavy metal intake. International institutions have determined relevant upper limits on the metal intake to increase the protection from their dangers. The amount of Pb and Cd detected in the present study was higher than the 1.5 mg/kg daily Pb intake limit determined by the European Food Safety Authority and the 25 mg/kg monthly Cd intake limit determined

Table 6 Daily intake of heavy metals and target hazard quotient values through consumption of green vegetables

	As		Cd		Pb		Cu		Zn		Ni		Mn	
	EDİ	THQ	EDİ	THQ	EDİ	THQ	EDİ	THQ	EDİ	THQ	EDİ	THQ	EDİ	THQ
Lettuce	3.33	11.11	0.42	0.45	2.13	0.05	19.99	0.49	127.96	0.42	8.56	0.42	218.47	44.98
Parsley	4.93	16.43	0.19	0.27	2.04	0.05	30.79	0.76	169.32	0.56	11.56	0.57	241	47.17
Leek	9.61	32.05	0.12	0.14	1.19	0.029	30.87	0.77	141.24	0.47	8.62	0.43	156.73	0.52
Spinach	3.51	11.71	0.09	0.16	1.23	0.03	23.85	0.59	146.10	0.48	7.31	0.36	180.49	0.60
Onion	5.58	18.61	0.68	0.76	3.13	0.07	30.72	0.76	224.2	0.74	8.22	0.41	176.3	0.58

by the Joint FAO/WHO Expert Committee on Food Additives (FAO/WHO 2001). The Codex Alimentarius Commission has set the daily maximum intake limit of Zn and Cu as 1 and 0.5 mg/kg, respectively (WHO/FAO 2007). These values were found to be quite high in the present study. According to the results of this study, the leafy vegetables grown in the study region met almost all of the daily heavy metal intake of the people living in that region. In contrast to this study, Rahmdel et al. reported that vegetables did not contribute to heavy metal intake. Consequently, the environment in which vegetables are grown affects metal accumulation and the exposure of humans accordingly (Rahmdel et al. 2018).

THQ has been approved as an important component of health risk assessment, as it is very useful in assessing the health risk associated with consuming heavy metal–contaminated food crops (Santos et al. 2004). The THQ in the study areas was as follows: Mn > As > Cu > Zn > Ni > Cd > Pb. The THQs of all of the heavy metals investigated in this study were < 1, except for Mn and As. However, when the total THQ of the other metals was examined, it was determined that the health risk indexes of all metals were at an extremely dangerous level. While some of the heavy metals taken into the human tissues can be removed from the body, long-term accumulation of metals due to their properties can harm human health (Dyer 2007). Khan et al. reported in their study that the THQ values of Cr and Ni were higher than 1, which could result in dangerous consequences for public health (Khan et al. 2013). Zhuang et al. also found the THQ index to be greater than 1 in terms of Cd and Pb and emphasized that the risk factor was even more important with both non-essential metals and the high THQ index (Zhuang et al. 2009). In the present study, it was determined that the THQ indexes of not only the non-essential but also the essential metals, such as Cu and Zn, were higher than 1. It was also concluded that consuming the vegetables grown in the study area could cause serious health problems.

Conclusion

In this study, the heavy metal accumulation in soil and green vegetables occurring due to intensive agricultural activities in the province of Nevşehir was investigated. Also, the accumulation of metal in vegetables is at a very critical level according to the criteria published by international organizations.

Consequently, measures may need to be taken to prevent heavy metal contamination in the soil and the reduction of heavy metal translocation from soil to food products in these studied areas. It is vital to effectively monitor the heavy metal in the soil and vegetables to reduce metal concentrations in the studied area and to investigate its effects on human health. By doing so, acute and chronic health problems due to the heavy metal exposure in this region can be prevented.

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