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Original Article

Assessment of toxic metals in commonly used herbs and spices in Turkey

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ABSTRACT

Background and Aims: The levels of cadmium (Cd) and lead (Pb) were analyzed in frequently used herbs including ginger, liquorice, nutmeg and turmeric in Turkey.

Materials and Methods: The levels of Cd and Pb in commonly used herbs were analyzed by inductively coupled plasma optical emission spectrometry after a closed microwave-assisted digestion.

Results: The concentration ranges for the Cd and Pb were found to be 0.25-0.78 mg/kg and 3.04-6.45 mg/kg, respectively. While Pb levels were below the maximum permissible limits, high Cd levels were detected in herbs which would not pose any health risk for consumers regarding an exposure assessment.

Conclusion: It is important to implement regular monitoring of heavy metal content in herbs, including medicinal plants, to assess their potential risks to human health in consideration of the potential for multiple exposure via other sources.

Keywords: Cadmium, lead, ginger, liquorice, nutmeg, turmeric

INTRODUCTION

Herbs are commonly used as flavoring agents, spices, and confectionery additives in the food industry. Moreover, their use as remedies in traditional medicine has increased dramatically worldwide. Thus, a critical evaluation of the safety and quality of herbs and herbal products is important for health authorities (WHO, 2007). It is well known that herbs may be contaminated by natural and chemical contaminants, including pesticides and heavy metals, which may be harmful to consumers (WHO, 2007). In addition to the natural existence in water and soil, the common sources of heavy metal pollution in the environment are anthropogenic activities such as industrial production processes, household waste and waste materials (Järup, 2003). Heavy metals are considered to be significant potential hazards to human, animal and plant health due to their widespread presence, toxicity and persistence in the environment (Järup, 2003). The accumulation of heavy metals in the harvestable parts of plants occurs by root uptake, foliar absorption, and decomposition of specific compounds (Haider, Naithani, & Barthwal, 2004; Kishan, Bhattacharya, & Sharma, 2014; Sarma, Deka, & Deka, 2011). While heavy metals such as copper, iron, zinc, and manganese play an essential role in the structural and biochemical function of the plants, toxic heavy metals such as lead (Pb) cadmium (Cd), mercury and arsenic can cause harmful effects in plants (Nagajyoti, Lee, & Sreekanth, 2010). In addition, an accumulation of toxic heavy metals in plants could also cause adverse effects for consumers (WHO, 2007).

Cd and Pb are two of the major heavy metals that are biologically non-essential and exhibit toxicity, according to health authorities (WHO, 2007, WHO 2011, EFSA 2011). Pb causes significant alterations in various biological processes including cell adhesion, intra- and inter-cellular signaling, apoptosis, ionic transportation, enzyme regulation, calcium homeostasis and oxidative stress re-

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sulting in toxic effects (Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014). Cd binds to metallothionein, cysteine-rich protein, which is accumulated in the renal tissue causing nephrotoxicity (Jaishankar et al., 2014). Available data indicates cause for concern regarding these heavy metals because they are known human carcinogens (Järup, 2003). On the basis of occupational studies, Cd has been classified as a human carcinogen-group 1 (IARC, 1993) and inorganic Pb has been classified as probably carcinogenic to humans-group 2A (IARC, 2006) by the International Agency for Research on Cancer. The World Health Organization (WHO) established the maximum permissible limits for Cd and Pb as 0.3 mg/kg and 10 mg/kg in herbal materials, respectively (WHO, 2007).

Different parts of plants are rich in phytochemicals and antioxidants and have been used as food commodities, medicinal plant, and dietary supplement. Liquorice, roots and extracts of *Glycyrrhizia glabra L.*, have been commonly used as medicinal plant, dietary supplement and food commodities. It has been shown to be beneficial in the treatment of respiratory diseases, gastrointestinal diseases, endocrine disorders, skin diseases, immunodeficiency and cancer (Karahan, Avsar, Ozyigit, & Berber, 2016; Sharma, Katiyar, & Agrawal, 2018). Ginger, the root of *Zingiber officinale*, has been widely used as a food condiment and dietary supplement all over the world due its high content of minerals, vitamins, and phytochemicals. Ginger has been used in medicine as anti-microbial, anti-pyretic, analgesic, anti-inflammatory, hypoglycaemic, anti-ulcer, anti-emetic, anti-hypertensive, hypolipidemic effects (Shahrajabian, Sun & Cheng, 2019). Nutmeg, the dried seed kernel of *Myristica Fragrans*, has been used as spice which exhibits many pharmacological activities such as anti-inflammatory, analgesic, antioxidant, antibacterial, antidiabetic and anticancer activities (Ha, Vu, Tran, Kim, Woo, & Min, 2020). Turmeric, the rhizome of *Curcuma longa*, has been used as a spice and medical herb due to its antioxidant, anti-inflammatory, antimutagenic, antimicrobial, and anticancer properties (Hewlings, & Kalman, 2017).

In order to avoid toxicity in humans, it is important to monitor the levels of toxic metals present in every step of the food chain (Jeong et al., 2012). Up to now, there have been a number of studies on the heavy metal contamination levels of herbal materials, including herbal medicines in Turkey (Başgel & Erdemoğlu, 2006; Bilgic Alkaya, Karaderi, Erdoğan, & Kurt Cücü, 2015; Karahan, Ozyigit, Saracoglu, Yalcin, Ozyigit & Ilcim, 2020; Leblebici, Bahtiyar, & Ozyurt, 2012; Ozcan, 2004; Ozcan, Ünver, Uçar, & Arslan, 2008; Ozcan & Akbulut, 2007; Ozturk, Altay, & Karahan, 2017; Ozyigit et al., 2018; Sekeroglu, Ozkutlu, Kara, & Ozguven, 2008; Tercan, Ayanoglu, & Bahadirli, 2016; Görür, Keser, Akçay, Dizman, & Okumuşoğlu, 2011; Divrikli, Horzum, Soylak, & Elci, 2006; Ozkutlu, Sekeroglu, & Kara, 2006; Ozden & Ozden, 2018; Tokalıoğlu, 2012). In the previous study, it was reported that Cd and Pb were found in the range of 0.324-0.524 mg/kg and 3.123-6.487 mg/kg, respectively, in linden, chamomile and sage teas (Ozden & Ozden, 2018). The aims of the present study were (i) to determine levels of Cd and Pb in frequently used herbs including ginger, liquorice, nutmeg, and turmeric (ii) to evaluate their potential hazards to human health.

MATERIAL AND METHODS

Reagents

Standard solutions of 1000 µg/mL of Cd and Pb in nitric acid were obtained from Merck (Darmstadt, Germany). Nitric acid (65%) was purchased from Merck (Darmstadt, Germany). All of the reagents used were of analytical grade.

Sample collection

A total of 54 herbs including liquorice, ginger, turmeric, and nutmeg samples were randomly collected from local markets in İstanbul and the botanical identification was carried out by Hakan Ozden. Scientific name and the part of the plant used were shown in Table 1.

Sample preparation

Each herb sample was milled using a Waring Blender (Conair Corp., Stamford, CT, USA) and stored in clean polyethylene packages at 2-8°C until analysis. The digestion and extraction processes of Pb and Cd were carried out using a closed microwave system (Berghof MWS-4 device, Berghof instruments, Eningen, Germany) equipped with Teflon containers. 0.1-0.3 g of dried and homogenized herbs were placed in the Teflon containers and wet-digested at 150-190ºC with 8 mL of 65% nitric acid. After cooling down to room temperature, the suspensions were diluted to 25 mL with deionized water. Blanks were also prepared using the same method. The samples were passed through syringe-type filters (Chromafil PET-45/25, Macheerey Nagel, Düren, Germany), then analyzed by ICP-OES instrument.

ICP-OES analysis

Analyses of Cd and Pb were conducted in herb samples using the inductively coupled plasma optical emission spectrometry (ICP-OES, Pelkin Elmer, Waltham, MA, USA) with optima 7000

Table 1. Classification of herb samples by scientific name and used part.

Common name	Scientific name	Used part	No. of samples analyzed
Ginger	*Zingiber officinale*	Dried rhizome	15
Liquorice	*Glycyrrhizia glabra L.*	Dried root	12
Nutmeg	*Myristica fragrans*	Dried seed kernel	12
Turmeric	*Curcuma longa*	Dried rhizome	15

Table 2. Contamination of heavy metals in the analyzed herbs.

Herbs	No. of samples	Positive (%)[a]	Mean of contamination[b] (mg/kg)±SD*	
			Cd	Pb
Ginger	15	15 (100)	0.43±0.09	5.46±0.92
Liquorice	12	9 (75)	0.34±0.07	3.51±0.38
Nutmeg	12	9 (75)	0.51±0.07	4.25±0.72
Turmeric	15	14 (93.33)	0.67±0.08	5.64±0.81
Totally	54	47 (87.04)	0.47±0.13	4.89±1.09

*SD: standard deviation, [a]Percentage of samples tested that were contaminated by Cd and Pb, [b]Mean contamination of positive samples

DV model. The specifications of the instrument were as follows: RF generator power 1.3 kW, gas flow rate 0.2 L/min and nebulizer flow rate 0.8 L/min. The emission wavelengths were 226 nm for Cd and 220 nm for Pb.

Calibration procedure

To assess the linearity of the method, calibration curves were prepared at six different concentrations (0.25-5 mg/L for Cd and 0.4-10 mg/L for Pb) and each injected in triplicates. Blanks were also used during the analysis to check for any possible contamination. For the sensitivity of method, limit of detections (LOD; signal-to noise ratio = 3) and limit of quantifications (LOQ; signal-to-noise ratio = 10) were calculated for Cd and Pb in each of the matrixes. Recovery studies were performed utilizing certified reference materials and were done in triplicate.

RESULTS

Method validation

Cd and Pb recoveries were in the range of 86-104% with a relative standard deviation of 2.15-4.8%. A good linear relationship was observed with correlation coefficients of 0.999 for Cd (with calibration equation of $y = 0.9326x - 0.053$) and 0.9986 for Pb (with calibration equation of $y = 0.9625x - 0.0079$). The levels of LOD and LOQ in herbs were 0.08 mg/kg and 0.25 mg/kg for Cd and 0.13 and 0.4 mg/kg for Pb, respectively.

Pb and Cd levels in herbs

In total we analyzed 54 herbs, including liquorice (12), ginger (15), turmeric (15) and nutmeg (12) from unpackaged samples collected from local markets in İstanbul (Table 1). As shown in Table 2, 9 out of 12 liquorice, 15 out of 15 ginger, 14 out of 15 turmeric and 9 out of 12 nutmeg samples contained both Cd and Pb in the ranges of 0.25-0.78 mg/kg and 3.04-6.45 mg/kg, respectively. Among the Cd-positive samples, only one liquorice (0.25 mg/kg) and one ginger (0.25 mg/kg) sample did not exceed the maximum permissible level of Cd (0.3 mg/kg) set by WHO for herbal products (WHO, 2007), whereas 45 out of 54 herbs contained Cd above the maximum permissible level. In addition, the Pb levels in herbs were lower than the maximum permissible level (10 mg/kg) set by WHO (WHO, 2007). We also observed that all positive samples contained both Cd and Pb.

DISCUSSION

The levels of Pb and Cd were investigated in 54 herb samples. The LOD levels were observed as 0.08 for Cd and 0.13 for Pb by ICP-OES analysis. Alhusban, Ata, & Shraim (2019) reported that the LOD levels for Cd and Pb were 0.15 and 0.10 mg/kg, respectively by ICP-OES which are similar for Pb and higher for Cd compared with our results.

As shown in Table 2, 47 out of 54 herbs contained Cd and Pb in the ranges of 0.25-0.78 mg/kg and 3.04-6.45 mg/kg, respectively. Consistently, in a study from Turkey, the rhizomes of *Glycyrrhiza glabra* contained Cd and Pb at 0.4 and 7.725 mg/kg, respectively (Karahan et al., 2020). In India, Kumar et al. showed that nutmeg contained Cd at 0.46 mg/kg and Pb at 30.07 mg/kg, implicating risks associated with the ingestion of herbal medicines contaminated with high levels of Pb (Kumar et al., 2018). Siriangkhawut, Sittichan, Ponhong, & Chantiratikul (2017) reported different levels of Cd and Pb found in medicinal plants, but they did not detect any in turmeric samples in Thailand. In Pakistan, Idrees et al. (2018) and Alhusban et al. (2019) showed Pb at levels of 4.48 mg/kg and 3.74 mg/kg, respectively, in ginger samples, which are similar to the present results. Gasser et al. also reported Cd and Pb in the concentration ranges of < 0.07-0.64 and < 0.4-4.12 in ginger samples, < 0.07-0.18 and < 0.4-1.45 in liquorice samples and < 0.07-0.21 and < 0.4-0.8 in turmeric samples, respectively (Gasser, Klier Kuhn, & Steinhoff, 2009). In a study from North Carolina, Pb was found in the range of 0.7-1.6 in ginger and 0.1-740 mg/kg in turmeric samples (Angelon-Gaetz, Klaus, Chaudhry, & Bean, 2018). In Saudi Arabia, Pb was found at a rate of 1 mg/kg while Cd was not detected in turmeric samples (Seddigi, Kandhro, Shah, Danish, & Soylak, 2016). In a study reported in Latvia, Reinholds, Pugajeva, Bavrins, Kuckovska, & Bartkevics (2017) showed that mean levels of Cd and Pb were 0.04 and 0.13 mg/kg in nutmeg samples, respectively. In Italy, Bua et al. reported detecting Cd in the range of 0.033-0.294 mg/kg in nutmeg and 0.029-0.092 mg/kg in ginger, while Pb was found in the range of 0.164-1.402 mg/kg in nutmeg and 0.309-1.154 mg/kg in ginger samples (Bua, Annuario, Albergamo, Cicero, & Dugo, 2016). In a study from Ethiopia, Baye & Hymete (2010) showed Cd and Pb at mean levels of 0.34-0.42 mg/kg and 0.17-0.25 mg/kg in ginger samples, respectively, from three different places of collection. In a study from Malaysia, Cd and Pb were found at mean levels of 1.08 mg/kg and 5.54 mg/kg in turmeric and 1.97 mg/kg and 3.15 mg/kg in ginger samples, respectively (Nordin & Selamat, 2013). In Zambia, high levels of Pb and Cd were found at the mean levels of 26.85 mg/kg and 2.39 mg/mg in ginger samples, respectively (Alolga, Chavez, & Muyaba, 2018). Olujimi et al. (2017) did not detect Pb or Cd in ginger samples in Nigeria. In Iran, commercial powder and root samples of turmeric were positive for Cd content (0.11–1.15 mg/kg) and were positive for Pb content (0.11–0.62 mg/kg) (Ahmed, Khaleeq, Huma, & Munir, 2017). Pb and Cd were detected at the levels of 0.093 mg/kg and 0.059 mg/kg in turmeric and 0.039 mg/kg and 0.105 mg/kg in nutmeg, respectively in the Republic of Korea (Shim, Cho, Leem, Cho, & Lee, 2019).

Limited studies have been conducted regarding heavy metal contamination of herbal materials in Turkey (Başgel & Erdemoğlu, 2006; Bilgic et al., 2015; Leblebici et al., 2012; Ozcan, 2004; Özcan et al., 2008; Ozcan & Akbulut, 2007; Ozyigit et al., 2018; Sekeroglu et al., 2008; Tercan et al., 2016; Görür et al., 2011; Divrikli et al., 2006; Ozkutlu et al., 2006; Ozden & Ozden, 2018; Tokalıoğlu, 2012). Among these, only a few studies reported on the heavy metal contamination of turmeric, ginger, nutmeg, and liquorice. Ozkutlu et al. (2006) reported Cd at the mean levels of 0.029 mg/kg in turmeric and 0.072 mg/kg in ginger, while Cd was not detected in nutmeg samples. Studies from Ozcan & Akbulut (2007) and Sekeroglu, Ozkutlu, Kara, & Ozguven (2008) found Cd at mean levels of 0.72 mg/kg and 0.043 mg/kg in liquorice, respectively. In another study, Pb was detected at a mean concentration of 3.01 mg/kg in ginger samples Tokalıoğlu (2012). In general, our data found levels of Cd and Pb in herbs very similar to the values reported in the literature from Turkey and elsewhere. It has been implied that variations in metal accumulation may be a result of differences in species, harvesting times, soil properties, locations and geographic conditions (Özcan et al., 2008; Kumar et al., 2018; Arpadjan, Celik, Taskesen, & Gucen, 2008). As shown in the studies above conducted worldwide, it has been observed that very different levels of Cd and Pb levels are detected in herbs. It is concluded that the result may be due to different geographic locations, agricultural input (fertilizer and pesticides) and industrial activities.

A tolerable weekly intake of Cd of 2.5 µg/kg bw was established by the European Food Safety Authority (EFSA) (EFSA, 2011). In the present study, the weekly intake of Cd (µg/kg bw) was calculated according to our results because the tested herbals contained Cd levels exceeding those proposed by WHO (WHO, 2007). It is considered a mean daily consumption of 2.3 g of herbal teas for the Middle Eastern diet (WHO, GEMS/Food Regional Diets) for an adult with a mean body weight of 70 kg (WHO, 2003). In the present study, we found the maximum Cd level to be 0.78 mg/kg in the turmeric sample, which gives us the highest estimated human weekly intake for Cd as 0.18 µg/kg. Thus, the intake of Cd represents 7.2% of the tolerable weekly intake set by EFSA (EFSA, 2011) which did not represent a risk to human health. Exposure assessment of Cd from the consumption of herbs (such as turmeric) used in tea was estimated for the first time for the Turkish population. Considering the different exposure sources, it is thought that it is important to monitor heavy metals in herbal products.

CONCLUSION

In summary, Cd and Pb were detected in the range of 0.25-0.78 mg/kg and 3.04-6.45 mg/kg in herbs, respectively. We revealed that Cd levels in herbals exceeded the permissible limits, whereas Pb levels fell under the limit recommended by WHO (WHO, 2007). According to the exposure assessment for Cd, the intake of herbs does not represent a risk to human health. However, taking herbs together with other foods contaminated with heavy metals may cause adverse health effects resulting from the accumulative effects of heavy metals. Considering the different factors in geographical conditions and industrial activities, metal levels in herbs should be carefully monitored. In conclusion, regular monitoring of herbal materials, including medicinal plants sold in local markets as well as imported products, is necessary, as is continued consideration of the risks of heavy metal contamination.

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