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The concentration and health risk of potentially toxic elements in black and green tea—both bagged and loose-leaf

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Abstract

The concentration of potentially toxic elements (PTEs), including lead (Pb), cadmium (Cd), arsenic (As), zinc (Zn), and copper (Cu), among 160 samples of black and green tea—both bagged and loose-leaf—in Iran was determined using a graphite furnace atomic absorption spectrophotometer (GF-AAS). Besides, the transfer rate of PTEs from made tea into tea infusion was investigated, and the related health risk for consumers was assessed. According to the results, the content of the PTEs is dependent on the type of tea (black or green), the place of cultivation (Iran or India), and the supplied form (both bagged and loose-leaf). The concentration of Pb, Cd, and As in green tea was significantly lower than black tea (P-value < 0.05). On the other hand, the contents of Zn and Cu in green tea were higher than the corresponding values for black tea. The mean concentration of Pb, As, and Zn in bagged tea samples was significantly higher than those of loose-leaf tea, while the Cd and As levels in Iranian tea samples were significantly lower than the Indian samples. Generally speaking, the mean concentration of Pb, Cd, As, Zn, and Cu were 0.59 ± 0.12, 0.12 ± 0.06, 0.16 ± 0.12, 14.23 ± 4.90, and 11.10 ± 2.49 mg kg^{-1}, respectively. The mean transfer rates of Pb, Cd, As, Zn, and Cu were 7.78, 6.29, 9.27, 12.91, and 13.08%, respectively. The estimated daily intake (EDI) and noncarcinogenic quotient (target hazard quotient [THQ]) due to the ingestion of PTEs besides the carcinogenic risk of As and Cd were considered as acceptable.

Keywords: PTEs; Iran; green tea; risk assessment; black tea

Introduction

Tea prepared from *Camellia sinensis* leaves is the most popular nonalcoholic beverage among the global population (Atasoy *et al.*, 2019). Tea is cultured in many countries, and China and India are the leading producers of tea (Karak and Bhagat, 2010). Moreover, the black and green tea variants are the most consumed (Pourramezani *et al.*, 2019; Sun *et al.*, 2019). It is assumed that 18–20 million cups of tea are consumed globally each day (Chang, 2015). The annual per capita consumption of tea in Iran is 1.3 kg (3.56 g day^{-1}) (Falahi and Hedaiati, 2013). Tea consumption is noticeably increasing due to the reported positive effects on human health, including the reduction in blood cholesterol, improvements in immune system, antiaging properties, and the prevention of several types of diseases, such as cardiovascular diseases, Alzheimer's disease, diabetes, and different types of cancers (Khan and Mukhtar, 2019; Prasanth *et al.*, 2019; Xu *et al.*, 2020). Tea consumption offers some antioxidant

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compounds, such as flavonols and polyphenolic compounds, with therapeutic properties (Abass *et al.*, 2019; Amiri *et al.*, 2018; Benjakul *et al.*, 2018; Hussain *et al.*, 2019; Szymczycha-Madeja *et al.*, 2012).

Recently, the contamination of tea by some of the potentially toxic elements (PTEs), such as lead (Pb), cadmium (Cd), and arsenic (As), has attracted global attention (Brzezicha-Cirocka *et al.*, 2016; Falahi and Hedaiati, 2013; Hu *et al.*, 2014; Zhang *et al.*, 2018a). Exposure to Pb may affect the central nervous system, resulting in memory disorder and delay in response time (Abass *et al.*, 2019). Chronic exposure to As may lead to cancer of the kidney and the lung, and skin lesions (Heshmati *et al.*, 2017, 2020). Cd is a very toxic element that can accumulate in the kidney and the bone (Saini and Dhania, 2020).

The accumulation of PTEs in tea depends on some factors, including the methods used for production, processing, and storage. At the same time, the natural occurrences of PTEs in different soils and geographical areas; the type of pesticides and fertilizers utilized in tea production; and anthropogenic sources, such as mining, traffic emissions, and industrial activities, are also among the crucial factors that have a bearing on the concentrations of PTEs in the different types of tea (Gu *et al.*, 2014; Pourramezani *et al.*, 2019; Zhang *et al.*, 2018a).

The concentration of PTEs in tea consumed in Iran and other countries were investigated (Falahi and Hedaiati, 2013; Karimi *et al.*, 2008; Lisia *et al.*, 2019; Martín-Domingo *et al.*, 2017; Nkansah *et al.*, 2016; Polechońska *et al.*, 2015; Shekoohiyan *et al.*, 2012; Shi *et al.*, 2007; Zhang *et al.*, 2018b). However, very little information was found about the concentration of PTEs in black and green tea—both loose-leaf and bagged. Therefore, the main aims of the current study were to measure and compare the concentrations of Cd, As, Pb, Zn, and Cu in green and black tea, supplied as loose-leaf and bagged, and to assess the potential health risks for consumers due to ingestion of PTEs via tea consumption.

Material and methods

Tea samples

A total of 160 tea samples were collected from different supermarkets located in Hamadan city, Iran, between December 2017 and March 2018. Tea samples were divided into two groups, that is, black and green. Samples of each tea were supplied in two forms, that is, bagged and loose-leaf. As for the location of cultivation, tea samples were produced in Iran or imported from India.

Chemical reagents

All chemical reagents, including PTEs, nitric acid, and other analytical grade chemicals, were purchased from Darmstadt, Germany. The plastic vessels were washed several times with tap water and were then soaked in 20% (v/v) nitric solution for the whole night. Subsequently, samples were washed with double distilled water to remove physical contamination.

Infusion preparation

Tea infusion samples were prepared according to common procedures in Iran for tea brewing; 2.00 g of each tea sample was added to 100 mL of boiling distilled water. After 10 min of brewing, the tea infusion was filtered by Whatman No. 42 filter paper to obtain a final clear solution for further processing. The concentration of PTEs in tea infusion was measured according to the described method. The transfer rate percentage (T) of each PTE from made tea samples into the infusion tea was estimated using the following equation (1):

$$T\% = \frac{C_1 \times 0.1 \times 100}{C_2 \times 0.002} \qquad \text{Equation (1)}$$

where T is the transfer rate (%); C_1 and C_2 present the level of the metal in the brewed tea (μg L^{-1}) and made tea (mg kg^{-1}), respectively.

Sample digestion

Before analyzing the PTEs, in order to optimize digestion conditions, oven-dried tea samples were powdered. Then 2.00 g of each sample was poured into a digestion vessel. Afterward, 25 mL of the mixture (3:1 v/v) of HNO_3 (65%) and H_2O_2 (35%) was added. The sample digestion was carried out with the aid of a microwave digestion system (Topex, Preekem Scientific Instruments Co., Ltd., Shanghai, China).

The digestion consists of five stages—stage 1 (2 min at 250 W), stage 2 (2 min at 0 W), stage 3 (2 min at 250 W), stage 4 (5 min at 400 W), and stage 5 (5 min at 600 W). Thus, the extracted sample was dissolved in 10 mL of distilled water; after dilution, it was filtered using filters of 0.45 μm pore size before analysis by an atomic absorption spectrophotometer with a graphite furnace (Thermo Scientifics' CE 3300, Waltham, MA, USA). All digestions were repeated in triplicate. The used experimental conditions for graphite furnace atomic absorption spectrophotometer (GF-AAS) were demonstrated in Table 1. The data were presented as the average of three repeated measurements.

Table 1. The used Graphite furnace atomic absorption spectroscopy conditions.

Parameter	Pb	Cd	As	Zn	Cu
Wavelength (nm)	217	228.8	193.7	213.9	324.8
Slit width (nm)	0.5	0.5	0.2	0.2	0.5
Sensitivity (μg ml^{-1})	2.5	0.8	6	0.25	2.5
Lamp current (mA)	10	8	12	6	5

Quality assurance and quality control for the data analysis

Quality assurance and quality control (QA/QC) were ensured based on previous studies (Heshmati *et al.*, 2017; Martín-Domingo *et al.*, 2017), and different factors, including recovery, the limit of detection (LOD) of the method, the limit of quantification (LOQ) of the method, and linearity were measured. The amount of LOD and LOQ were estimated using equations of 3 s/m and 10 s/m, respectively, where "s" expressed the standard deviation of five replicate detections of the blank sample, and m defined the slope of the standard curve for the studied PTEs. The recovery was determined by spiking the tea samples with the known concentrations of 1 mg kg^{-1} for Pb, 0.1 mg kg^{-1} for Cd, 0.15 mg kg^{-1} for As, 25 mg kg^{-1} for Zn, and 50 mg kg^{-1} for Cu, with analytical standards into the pre-analyzed tea samples (Nkansah *et al.*, 2016). The calibration curves, linearity equations, regression coefficient (R^2), LOD, LOQ, and recovery of studied PTEs in made tea and infusion are indicated in Table 2.

The LOQ of Pb, Cd, As, Zn, and Cu were 0.930, 0.510, 0.600, 5.101, and 5.001 μg kg^{-1} in made tea, and 0.046, 0.020, 0.040, 0.255, and 0.075 μg L^{-1} in tea infusion, respectively. The average recovery was in the range of 93.21–108.91% in made tea and 93.21–113.61% in tea infusion. The RSD percentage estimated for PTEs was lower than 2%. While the LOQ of PTEs was lower than the permissible values in tea, the validated method was sensitive and suitable to determine the PTEs in made tea and also the tea infusion.

Health risk assessment

Calculation of noncarcinogenic risk assessment

For noncarcinogenic health risk assessment, the estimated daily intake (EDI) (mg kg^{-1} bw day^{-1}), the target hazard quotient (THQ), and the hazard index (HI) were determined according to the following equations (Heshmati *et al.*, 2019; Nkansah *et al.*, 2016; USEPA, 2001):

$$EDI = \frac{C1 \times M \times T}{bw}, \qquad \text{Equation (2)}$$

where C_1, M, T, and bw are the mean PTE concentration (mg kg^{-1}) of made tea, the mean daily consumption of tea (3.56 g day^{-1}), the transfer rate (%) of PTE from made tea to infusion, and the average body weight of an adult (60 kg), respectively.

$$THQ = \frac{EDI}{RfD} \qquad \text{Equation (3)}$$

In this equation, reference dose (RfD) is the standard RfD suggested by the US Environmental Protection Agency (USEPA) (USEPA, 2001). Rfd of Pb, Cd, As, Zn, and Cu were 0.0036, 0.001, 0.0003, 0.3, and 0.04 mg kg^{-1} day^{-1},

Table 2. The calibration curves, linear regression equations, regression coefficient (R^2), the limit of detection (LOD), the limit of quantification (LOQ), and recovery of studied metals in tea.

Metal	Range of linearity[1]	Regression linear equation	Regression coefficient	LOD[1]	LOQ[1]	Recovery ± RSD
Made tea						
Pb	2.5–1000	Y = 0.00826x + 0.0146	0.9673	0.310	0.930	96.84 ± 1.34
Cd	2.5–1000	Y = 0.02021x − 0.0161	0.8966	0.170	0.510	108.91 ± 1.16
As	2.5–1000	Y = 0.00972x − 0.0056	0.9947	0.200	0.600	93.21 ± 1.28
Zn	2.5–1000	Y = 0.01487x − 1.3228	0.9991	1.703	5.101	98.43 ± 1.62
Cu	2.5–1000	Y = 0.04476x + 0.0015	0.9426	1.501	5.001	103.06 ± 1.92
Tea infusion						
Pb	0.1–50	Y = 0.00733x + 0.0126	0.9681	0.015	0.046	100.84 ± 2.32
Cd	0.1–50	Y = 0.01043x − 0.0151	0.8943	0.008	0.020	98.21 ± 1.51
As	0.1–50	Y = 0.00512x − 0.0051	0.9921	0.011	0.040	107.00 ± 1.52
Zn	0.1–50	Y = 0.03467x − 1.3028	0.987	0.085	0.255	93.21 ± 1.31
Cu	0.1–50	Y = 0.02473x + 0.0115	0.9433	0.025	0.075	113.61 ± 1.22

[1]μg kg^{-1} for made tea and μg L^{-1} for tea infusion.

respectively (Antoine *et al.*, 2017; USEPA, 2001; Zhang *et al.*, 2018a)

$$HI = \sum_{i}^{j} THQ_{i} \qquad \text{Equation (4)}$$

HI shows the sum of each of the THQs of the whole PTE analyzed in each product (Antoine *et al.*, 2017). If THQ and HI were lower than 1, the level of health hazard was considered acceptable for health human (Qin *et al.*, 2015).

Carcinogenic risk assessment

The International Agency for Research on Cancer considered As and Cd as potential carcinogenic PTEs, while Zn, Cu, and Pb were considered to be noncarcinogenic (IARC, 2011; Zeng *et al.*, 2015). Carcinogenic risk (CR) of As and Cd was computed according to the following equation (Saha and Zaman, 2013; USEPA, 2017):

$$CR = EDI \times SF, \qquad \text{Equation (5)}$$

where CR is the carcinogenic rate per lifetime, and SF is the slope factor of cancer. According to the USEPA guidelines, CR in the range of 10^{-6} to 10^{-4} is acceptable. SF for Cd and As was 15 and 1.5 mg kg^{-1} day^{-1}, respectively (Zeng *et al.*, 2015).

Statistical analyses

In this study, the results are shown as mean ± SD. The statistical investigation was conducted using SPSS version 21 (SPSS Inc., Chicago, IL, USA). A sample *t*-test was employed to compare the mean concentrations of PTEs in different tea samples with the allowable limits of the Iranian National Standardization Organization (INSO, 2014a). The difference among the mean heavy metals of various samples was determined by one-way ANOVA, and the post-doc test was done using the Duncan's new multiple range test. Statistically, a P-value of < 0.05 was considered as statistically significant.

Results

The comparison between black and green tea with regard to heavy metals

The results obtained for each of the PTEs in tea samples are shown in Tables 3 and 4. The concentration of Pb, Cd, and As in green tea was significantly lower than that in black tea (P-value < 0.05). On the other hand, the concentrations of Zn and Cu in green tea were higher than that in black tea, although the difference was not significant (P-value < 0.05).

The comparison of metals between bagged and loose-leaf tea

The results indicated that the concentration of PTEs differed among tea samples supplied in the form of a tea bag or loose leaf, while the mean concentrations of Pb, As, and Zn in bagged tea samples were significantly higher than that in loose-leaf tea (Table 3).

The comparison of PTEs between Indian and Iranian tea

The levels of Cd (0.10 ± 0.07 mg kg^{-1}) and As (0.13 ± 0.06 mg kg^{-1}) in Iranian tea samples were significantly lower than that in Indian samples (Cd: 0.14 ± 0.04 mg kg^{-1} and As 0.19 ± 0.16 mg kg^{-1}). However, no significant differences were noted in Pb, Zn, and Cu contents between Iranian and Indian tea (Table 3).

The transfer rate of PTE based on the infusion

In this study, the transfer rates of Pb, Cd, As, Zn, and Cu ranged from 5.82–9.32, 5–7.27, 7.98–11.00, 10.17–17.79, 7.14–19.33%, respectively, while the means were 7.78, 6.29, 9.27, 12.91, and 13.08%, respectively (Table 4). The level of Pb, Cd, As, Zn, or Cu in brewed tea samples differed, while this index is dependent on the initial level

Table 3. The PTEs level (mg kg^{-1}) among analyzed tea samples.

Variable		Pb	Cd	As	Zn	Cu
Tea type	Green	0.54 ± 0.12	0.07 ± 0.04	0.10 ± 0.05	14.66 ± 5.74	11.96 ± 2.79
	Black	0.65 ± 0.10	0.17 ± 0.01	0.22 ± 0.15	13.80 ± 4.76	10.25 ± 2.18
	P-value	0.033	0.015	0.038	0.084	0.068
Tea production origin	Iran	0.59 ± 0.09	0.10 ± 0.07	0.13 ± 0.06	14.84 ± 6.92	12.74 ± 1.40
	India	0.61 ± 0.17	0.14 ± 0.04	0.19 ± 0.16	13.62 ± 2.68	9.46 ± 2.31
	P-value	0.092	0.043	0.023	0.081	0.07
Consumed form	Loose leaf	0.69 ± 0.08	0.12 ± 0.06	0.22 ± 0.14	16.08 ± 5.51	10.17 ± 2.79
	Bagged	0.50 ± 0.07	0.12 ± 0.04	0.10 ± 0.07	12.39 ± 4.08	12.04 ± 2.09
	P-value	0.013	1.15	0.043	0.012	0.064

Table 4. Level of PTEs in made tea and tea infusion.

Heavy metal type	Tea type	Culture place	Form	Mean ± SD (mg kg^{-1}) in made tea	Range (mg kg^{-1}) in made*	No. upper of Iranian standard (%)	Mean ± SD (µg L^{-1}) in tea infusion	Transfer rate (%)
Pb	Black	India	Bagged	0.79 ± 0.4^{a**}	0.12–1.51	3 (15)	2.5 ± 0.21^{a}	5.82 ± 0.12
			Loose leaf	0.53 ± 0.4^{b}	0.02–1.21	3 (15)	0.82 ± 0.01^{ab}	7.74 ± 1.21
		Iran	Bagged	0.69 ± 0.2^{a}	0.42–1.32	2 (10)	1.1 ± 0.22^{a}	7.79 ± 0.89
			Loose leaf	0.58 ± 0.3^{b}	0.04–1.08	3 (15)	0.76 ± 0.02^{ab}	8.64 ± 1.02
	Green	India	Bagged	0.69 ± 0.43^{a}	0.22–2.00	6 (30)	1.31 ± 0.02^{a}	8.23± 2.12
			Loose leaf	0.41 ± 0.34^{c}	0.03–1.20	3 (15)	0.52 ± 0.11^{b}	6.43 ± 0.68
		Iran	Bagged	0.59 ± 0.27^{b}	0.10–1.03	3 (15)	1.1 ± 0.10^{a}	9.32 ± 1.45
			Loose leaf	0.48 ± 0.33^{bc}	0.03–1.06	2 (10)	0.78 ± 0.09^{ab}	8.13 ± 1.35
	Total	–	–	0.59 ± 0.12	0.02–2.00	24 (15)	1.11 ± 0.61	7.78 ± 0.98
Cd	Black	India	Bagged	0.18 ± 0.23^{a}	0.21–0.35	13(65)	0.21 ± 0.01^{a}	5.53 ± 0.87
			Loose leaf	0.16 ± 0.09^{a}	0.07–0.39	10 (50)	0.22 ± 0.02^{a}	6.88 ± 1.05
		Iran	Bagged	0.11 ± 0.09^{a}	0.03–0.38	6 (30)	0.35 ± 0.03^{a}	5.91 ± 0.35
			Loose leaf	0.04 ± 0.01^{b}	0.01–0.09	0 (0)	0.08 ± 00.20^{b}	6.25 ± 0.81
	Green	India	Bagged	0.17 ± 0.11^{a}	0.02–0.37	14 (70)	0.1 ± 0.01^{b}	5.00 ± 0.28
			Loose leaf	0.15 ± 0.08^{a}	0.05–0.33	10 (50)	0.22 ± 0.5^{a}	6.47 ± 1.20
		Iran	Bagged	0.11 ± 0.09^{a}	0.02–0.35	8 (40)	0.21 ± 0.01^{a}	7.00 ± 1.03
			Loose leaf	0.03 ± 0.03^{b}	0.01–0.09	–	0.16 ± 0.09^{ab}	7.27 ± 0.84
	Total		–	0.12 ± 0.06	0.01–0.39	61 (76.25)	0.19 ± 0.08	6.29 ± 0.45
As	Black	India	Bagged	0.42 ± 0.5^{a}	0.10–1.83	10 (50)	0.67 ± 0.01^{a}	7.98 ± 0.59
			Loose leaf	0.20 ± 0.3 ^{b}	0.07–1.21	3 (15)	0.36 ± 0.03^{ab}	9.00 ± 1.56
		Iran	Bagged	0.20 ± 0.3^{b}	0.05–1.32	2 (10)	0.44 ± 0.01^{a}	11.00 ± 1.32
			Loose leaf	0.07 ± 0.04^{c}	0.11–0.16	2 (10)	0.14 ± 0.01^{b}	9.50 ± 1.65
	Green	India	Bagged	0.10 ± 0.02^{bc}	0.04–0.17	4 (20)	0.21 ± 0.02^{ab}	10.00 ± 1.85
			Loose leaf	0.05 ± 0.01^{c}	0.03–0.09	–	0.09 ± 0.01^{c}	9.00 ± 0.96
		Iran	Bagged	0.17 ± 0.21^{bc}	0.03–1.09	3 (15)	0.28 ± 0.07^{ab}	8.24 ± 0.58
			Loose leaf	0.09 ± 0.02^{c}	0.06–0.13	–	0.17 ± 0.01^{b}	9.44 ± 0.23
	Total		–	0.16 ± 0.12	0.03–1.83	24 (0.3)	0.3 ± 0.19	9.27 ± 1.09
Zn	Black	India	Bagged	11.52 ± 1.90^{bc}	8.34–15.00	–	23.44 ± 1.61^{c}	10.17 ± 2.01
			Loose leaf	15.16 ± 3.03^{b}	12.08–22.00	–	41.35 ± 2.61^{b}	13.64 ± 2.21
		Iran	Bagged	8.76 ± 3.21^{cd}	4.70–16.50	–	22.49 ± 3.01^{c}	12.84 ± 1.68
			Loose leaf	19.77 ± 4.50^{a}	11.8–25.00	–	48.10 ± 0.13^{b}	12.16 ± 1.52
	Green	India	Bagged	11.20 ± 2.70^{bc}	8.30–18.80	–	37 ± 0.22^{b}	16.09 ± 2.41
			Loose leaf	16.60 ± 2.93^{ab}	14.10–25.60	–	58 ± 0.05^{a}	17.79 ± 2.05
		Iran	Bagged	9.02 ± 1.24^{cd}	8.14–12.22	–	12.2 ± 0.14^{d}	16.29 ± 2.03
			Loose leaf	21.82 ± 4.50^{a}	16.4–34.01	–	62.2 ± 0.01^{a}	14.27 ± 2.12
	Total	–	–	14.23 ± 4.90	4.70–34.01		38.10 ± 17.80	12.91 ± 1.59
Cu	Black	India	Bagged	7.5 ± 3.5^{bc}	2.2–13.2	–	23.14 ± 1.21^{bc}	14.43 ± 2.01
			Loose leaf	9.7 ± 1.4^{ab}	7.4–12.6	–	30.05 ± 0.01^{b}	11.90 ± 2.45
		Iran	Bagged	12.6 ± 1.2^{a}	9.3–16.9	–	37.76 ± 0.06^{a}	12.92 ± 2.00
			Loose leaf	11.2–2.5^{ab}	6.6–15.8	–	28.33 ± 0.4^{b}	11.28 ± 1.67
	Green	India	Bagged	8.02 ± 4.90^{b}	2.2–16.00	–	29.12 ± 3.41^{b}	19.33 ± 2.13
			Loose leaf	12.63 ± 3.15^{a}	8.21–19.50	–	25.18 ± 2.13^{bc}	12.83 ± 2.63
		Iran	Bagged	12.56 ± 4.38^{a}	6.6–19.80	–	33 ± 3.31^{ab}	14.73 ± 2.08
			Loose leaf	14.61 ± 2.55^{a}	10.05–19.20	–	18 ± 1.15^{c}	7.14 ± 0.65
	Total	–	–	11.10 ± 2.49	2.2–19.80	–	28.07 ± 6.06	13.08 ± 1.11

The maximum level of Pb, Cd, As, and Cu (in black tea), and Cu (in green tea) according to Iran standard was 1, 0.1, 0.15, 50, and 150 mg kg^{-1}, respectively.
**Different letters indicated a significant difference among average values within each column ($P < 0.05$).

of PTEs in making tea. However, there is significant difference between each PTE transfer rate from various tea samples into the infusion.

The risk assessment of PTE intake through tea infusion

The health risk based on PTE intake (Pb, Cd, As) through tea infusion consumption among the Iranian people is shown in Table 5. The results indicated that the EDI of Pb, Cd, and As was lower than the EDI specified by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA 1993). Besides, THQ and HI amounts calculated for the above-mentioned toxic metals were lower than 1, indicating that the levels of PTEs mentioned in tea samples are acceptable. According to Table 6, the CR of Cd and As was lower than the standard limit (10^{-6}–10^{-4}), indicating that Cd and As toxicity through the consumption of green and black tea samples pose no problem and could be considered as safe for the consumer (JECFA 1993).

Discussion and Conclusions

In this study, the concentration of PTEs in tea samples consumed in western Iran was determined. The type of tea (black or green), the place of cultivation (Iran or India), and the supplied form (loose-leaf or bagged) were among the factors that were investigated.

The mean concentrations of Pb (0.59 ± 0.12 mg kg^{-1}) and Cu (11.10 ± 2.49 mg kg^{-1}) (Table 4) were lower than the maximum allowable limit according to Iranian standards (Pb: 1 mg kg^{-1}, Cu: 50 and 150 mg kg^{-1} in black and green tea, respectively). The mean concentrations of Cd (0.12 ± 0.06 mg kg^{-1}) and As (0.16 ± 0.12 mg kg^{-1}) were higher than the acceptable values (0.1 mg kg^{-1} for Cd and 0.15 mg kg^{-1} for As), while no significant difference was noted (P-value > 0.05).

Some studies have reported lower levels of PTEs in green tea than black tea; our findings were in line with these studies (Barone *et al.*, 2016; Podwika *et al.*, 2018). The level of Pb in green tea samples (0.47 ± 0.07 mg kg^{-1}) analyzed by Barone *et al.* (2016) was lower than that of black tea (0.55 ± 0.35 mg kg^{-1}), although de Oliveira *et al.* (2018) reported higher Pb level in green tea (0.76 ± 0.12 mg kg^{-1}) while compared with black tea (0.64 ± 0.11 mg kg^{-1}) (Barone *et al.*, 2016; de Oliveira *et al.*, 2018). Besides, Zhong *et al.* (2018) reported that the Pb value of green tea samples from China (0.48–10.57 mg kg^{-1}) was greater than that of black tea (1.88–5.63 mg kg^{-1}) (Zhang *et al.*, 2018b). It seemed that the transfer of PTEs through the industrial equipment used for black tea manufacturing, dust particles during processing, and also soldering being used in the packaging step could be accounted among the reasons for the higher level of PTEs while compared with green tea (Jin *et al.*, 2008).

Our findings were in agreement with a study conducted by Dambiec *et al.* (2013), which reported that the Pb level of the bagged tea samples was significantly higher than that of loose-leaf tea (Dambiec *et al.*, 2013). The discrepancy between PTE content of bagged and loose-leaf tea was related to the production processes and tea quality that were different for bagged and loose-leaf tea. The bagged tea generally had lower quality, compared with loose-leaf tea (Polechońska *et al.*, 2015).

No significant difference was noted between PTE transfer rates in various kinds of tea, while Dambiec *et al.* (2013) found that the transfer rate of PTEs in bagged tea was higher than the corresponding values associated with loose-leaf tea. These authors believed that the orthodox process was utilized in leaf tea, causing less cellular damage than the CTC (crush, tear, and curl) process frequently utilized for bagged tea. Therefore, the leaves in bagged tea are crushed into tiny pieces, leading to a higher transfer rate of PTEs (Dambiec *et al.*, 2013). The transfer rates of PTEs into infusion depended on the organic or inorganic form, the solubility of each PTE and their binding into tea leaves, brewing time, and temperature (Lin *et al.*, 2006).

The average level of Pb in samples analyzed in our study (black tea: 0.65 ± 0.10 mg kg^{-1}; green tea: 0.54 ± 0.12 mg kg^{-1})

Table 5. The estimated daily intake (EDI), the total hazard quotient (THQ), and hazard index (HI) for heavy metal intake through tea consumption.

Heavy metal	Black tea		Green tea	
	EDI (mg kg^{-1} bw day^{-1})	THQ	EDI (mg kg^{-1} bw day^{-1})	THQ
Pb	3.00E–06	8.33E–04	2.49E–06	6.92E–04
Cd	6.34E–07	6.34E–04	2.61E–07	2.61E–04
As	1.21E–06	4.03E–03	5.50E–07	1.83E–03
Zn	1.06E–04	3.52E–04	1.12E–04	3.74E–04
Cu	7.95E–05	1.99E–03	9.28E–05	2.32E–03
	HI = 7.84E–03		HI = 5.48E–03	

Table 6. Carcinogenic rate (CR) of As and Cd intake through tea consumption.

Heavy metal type	Black tea		Green tea	
	EDI	CR	EDI	CR
Cd	6.34E–07	4.23E–08	2.61E–07	1.74E–08
As	1.21E–06	8.07E–07	5.60E–07	3.67E–07

The means of Cd and As were 0.07 ± 0.0 and 0.10 ± 0.05 mg kg^{-1} in green tea, and 0.17 ± 0.01 and 0.22 ± 0.15 mg kg^{-1} in black tea samples, respectively.

was higher than that reported in Italy (black tea: 0.55 ± 0.35 mg kg^{-1}; green tea: 0.47 ± 0.07 mg kg^{-1}) (Barone *et al.*, 2016) and lower than that reported in Slovakia (black tea: 1.387 ± 0.54 mg kg^{-1}; green tea: 0.875 ± 0.59 mg kg^{-1}), China (green tea: 3.04 mg kg^{-1}), Saudi Arabia (black tea: 1.7 ± 0.8 mg kg^{-1}), Turkey (black tea: 8.3 ± 0.1 mg kg^{-1}), Japan (black tea: 0.71 ± 0.02 mg kg^{-1}), China (black tea: 3.30 ± 1.42 mg kg^{-1}), and Iran (black tea:1.41 ± 0.72 mg kg^{-1}) (Árvay *et al.*, 2015; Fernández-Cáceres *et al.*, 2001; Han and Li, 2002; Matsuura *et al.*, 2001; Narin *et al.*, 2004; Salahinejad and Aflaki, 2010). The transfer rate of Pb in this research (7.78%) was lower, compared with those explained by de Oliveira *et al.* (2018) (33% in green tea and 32.5% in black tea). The concentrations of Pb among the tea infusion samples ranged from 0.52 to 2.5 μg L^{-1}, with a mean of 1.11 ± 0.61 μg L^{-1}, which were lower in comparison with the limit (10 μg L^{-1}) set by the World Health Organization for drinking water (WHO, 2011). The diversity observed in the Pb level in different studies may be related to various contaminant sources of Pb in tea crops, including deposits from the unclean air onto the leaves of the tea plants, the plant growth in different soils, and the geographical location (Han and Li, 2002). Cd's range and mean concentration in all tea samples were reported as 0.01–0.0.39 and 0.12 ± 0.06 mg kg^{-1}, respectively. Cd level in 61 (76.25%) samples was higher than the Iranian limit (0.1 mg kg^{-1}) (INSO, 2014a, 2014b). The highest (0.18 ± 0.23 mg kg^{-1}) and the lowest (0.03 ± 0.03 mg kg^{-1}) mean Cd values were found in Indian bagged black tea and Iranian loose-leaf green tea, respectively. In comparison with other studies, the average concentration of Cd in our study (0.12 ± 0.06 1 mg kg^{-1}) was higher than that reported by Han *et al.* (2005) in Japan (0.1 mg kg^{-1}), Hosseni *et al.* (2013) in Iran (0.027 mg kg^{-1}), Li *et al.* (2015) in China (0.05 mg kg^{-1}) (Han *et al.*, 2005; Hosseni *et al.*, 2013; Li *et al.*, 2015) and was lower than that reported by Yousefi *et al.* (2017) in Iran (0.19 mg kg^{-1}), Moreda-Piñeiro *et al.* (2003) in India (2.01 mg kg^{-1}), Saud and Oud (2003) in Japan (1.48 mg kg^{-1}), Narin *et al.* (2004) in Turkey (2.0 mg kg^{-1}), Ashraf and Mian (2008) in Saudi Arabia (0.49 mg kg^{-1} mg kg^{-1}), Sofuoglu and Kavcar. (2008) in Turkey (0.19 mg kg^{-1}), and Tsushida and Takeo (1977) in Japan (0.51 mg kg^{-1}) (Ashraf and Mian, 2008; Moreda-Pineiro *et al.*, 2003; Narin *et al.*, 2004; Saud and Oud, 2003; Sofuoglu and Kavcar, 2008b; Yousefi *et al.*, 2017). When compared with other analyzed PTEs, the transfer rate of Cd (6.29%) was lower, which might be related to the strong connection between Cd and the tea matrix (Matsuura *et al.*, 2001). The results indicated that the mean level of Cd (0.19 μg L^{-1}) in tea infusion was similar to a previous study (Sofuoglu and Kavcar, 2008) and lower than the maximum contaminant level in drinking water, that is, 3 μg L^{-1} (WHO, 2011). It should be mentioned that Cd is a hepatotoxic and nephrotoxic metal, and persistent exposure to it may negatively impact the proximal tubular cells related to the kidneys and lead to renal failure in humans (Byber *et al.*, 2016; Heshmati and Salaramoli, 2015).

In the current investigation, seven (8.75%) green tea samples and 17 (21.25%) black tea samples had higher As content than the maximum limit (0.15 mg kg^{-1}) per the INSO (2014a, 2014b). While the average As in the current study (0.16 mg kg^{-1}) was lower than that demonstrated by de Oliveira *et al.* (2018) in the United States (green tea: 0.18 ± 0.07 mg kg^{-1} in; black tea: 0.22 ± 0.02 mg kg^{-1}), Shi *et al.* (2007) in China (0.30 mg kg^{-1}), Nejatolahi *et al.* (2014) in Iran (0. mg kg^{-1}), Martín-Domingo *et al.* (2017) in Spain (0.20 mg kg^{-1}), and Sofuoglu *et al.* (2008) in Turkey (0.21 mg kg^{-1}), it was higher than the corresponding values reported by Milani *et al.* (2016) in Brazil (0.021 mg kg^{-1}), Nookabkaew *et al.* (2006) in China (0.01 mg kg^{-1}), Karimi *et al.* (2008) in Iran (0.11 mg kg^{-1}), Zhelev *et al.* (2019) in India (0.05 mg kg^{-1}), and Popović *et al.* (2017) in Belgrade (0.04 mg kg^{-1}) (de Oliveira *et al.*, 2018; Karimi *et al.*, 2008; Martín-Domingo *et al.*, 2017; Milani *et al.*, 2016; Nejatolahi *et al.*, 2014; Nookabkaew *et al.*, 2006; Popović *et al.*, 2017; Shi *et al.*, 2007; Sofuoglu and Kavcar, 2008b; Zhelev *et al.*, 2019). As concentration in tea, infusion samples ranged from 0.09 to 0.67 μg L^{-1}, while the recorded transfer rate was between 7.98 and 11.00%. Arsenic levels in all tea infusions were below the MRL (10 μg L^{-1}) in drinking water (WHO, 2011). The mean As content in the tea infusion sample in our study was higher than that analyzed by Szymczycha-Madeja *et al.* (2012) and Sofuoglu and Kavcar (2008), and lower than samples investigated by Schwalfenberg *et al.* (2013) (Schwalfenberg *et al.*, 2013; Sofuoglu and Kavcar, 2008). The discrepancies in different studies may be related to the ratio of pollution in various regions, the type of pesticides and fertilizers utilized in tea culture, the attributes of soils, the tea cultivars, and the brewing methods (Chen *et al.*, 2006; Karak *et al.*, 2016; Tokalıoğlu and Kartal, 2004). The exposure to a high level of As via daily consumption of tea could lead to different side effects, such as skin cancers and internal diseases. There are also reports of a significant association between exposure to As and cancer of the liver, prostate, and bladder (Hong *et al.*, 2014).

As shown in Table 4, Zn was the PTE identified the most in this current study. Zn is considered an essential element and has an important role in most of the metabolic activities (Abass *et al.*, 2019; Heshmati *et al.*, 2019). The insufficiency of Zn daily can lead to diminishing appetite, slowing growth, skin disorders, and immune system abnormalities (Salgueiro *et al.*, 2000). Exposure to high amounts of Zn could lead to nausea, vomiting, diarrhea, and prostate cancer (Plum *et al.*, 2010). Based on the recommended daily consumption (RDI) of Zn for adults (15 mg day^{-1}), the maximum daily Zn intake through tea consumption was estimated at 0.1% of RDI for this element (Salgueiro *et al.*, 2000; Zhang *et al.*, 2018b). The average consumption of Zn (14.23 ± 4.90 mg kg^{-1}) in our study was lower than the value described by Srividhya *et al.* (2011) in green tea (26.39 mg kg^{-1}) of India, Podwika *et al.* (2018) in green tea (24.4 mg kg^{-1}) of Poland, Falahi and Hedaiati, (2013) in black tea (28.8 mg kg^{-1}) of Iran (Falahi and Hedaiati, 2013; Podwika *et al.*, 2018; Srividhya *et al.*, 2011).

Cu is one of the PTEs found in tea, which is a major element present in various polyphenol oxidase enzymes (Zhang *et al.*, 2018b). According to the INSO (2014a, 2014b) , the permitted level for Cu in green and black tea are 150 and 50 mg kg^{-1}, respectively. In the current study, the Cu level of all samples was less than the mentioned limits. Cu is an important element, and its presence in tea can be beneficial for human beings, although a high dose is toxic. Considering the daily consumption of made tea (3.56 g), the maximum Cu level in samples analyzed in the current study (19.80 mg kg^{-1}), and the transfer rate of 13.08%, the daily Cu intake through tea among Iranian consumers was estimated to be 0.0092 mg, which is 0.46% of the RDI (2 mg) for this element in adults (Goldhaber, 2003). The maximum transfer rate of Cu in our study (19.33%) was less than the findings reported by Zhang *et al.* (2018b) in China (24%), Brzezicha-Cirocka *et al.* (2016) in different geographical areas of China (27%), Matsuura *et al.* (2001) in Japan (23%), and Street *et al.* (2006) in tea samples (30%) imported into China (Brzezicha-Cirocka *et al.*, 2016; Matsuura *et al.*, 2001; Street *et al.*, 2006; Zhang *et al.*, 2018b). The discrepancy between the present study data and the previous research was related to the existence of different contaminant sources of heavy metals in tea production areas, tea storage and processing procedures, and packaging (Han *et al.*, 2006).

In the current study, EDI values of the mentioned PTEs in tea samples were reduced in the following order: Zn > Cu > Pb > As > Cd. Soliman (2016) indicated EDI levels of Cu and Pb in tea as 0.7 and 0.007 (mg day^{-1}), respectively, which was higher than our findings (Soliman, 2016). In our study, the THQ level of metals in black tea and green tea was in the order of Pb > Cd > As > Zn > Cu and Pb > Zn > Cd > Cu > As. The THQ associated with PTEs in Sri Lankan and Indian black tea were reported as Cu > As > Pb > Cd > Hg and As > Cu > Pb > Cd > Hg, respectively (Pourramezani *et al.*, 2019). In other investigations carried out by Gruszecka-Kosowska and Mazur-Kajta (2016) and Zhang *et al.* (2018), similar THQ and HI values were reported (Gruszecka-Kosowska and Mazur-Kajta, 2016; Zhang *et al.*, 2018a).

In conclusion, in this study, the concentration of PTEs and the health risk of tea consumed in western Iran were investigated. Pb, Cd, As, Zn, and Cu content were compared between the tea variants (black or green) cultivated in two different places, that is, Iran or India, and supplied as loose-leaf or bagged tea. The mean Pb (0.59 ± 0.12 mg kg^{-1}) and Cu (11.10 ± 2.49 mg kg^{-1}) were lower than the maximum allowable limit according to the INSO (Pb: 1 mg kg^{-1}, Cu: 50 and 150 mg kg^{-1} in black and green tea, respectively). The mean of Cd (0.12 ± 0.06 mg kg^{-1}) and As (0.16 ± 0.12 mg kg^{-1}) was higher than the acceptable value (0.1 mg kg^{-1} for Cd and 0.15 mg kg^{-1} for As), although no significant difference was noted. The statistical data showed that the level of Pb, Cd, and As in green tea was significantly lower than that of black tea (P-value < 0.05). Also, the mean Pb, As, and Zn in bagged tea samples was significantly higher than in loose-leaf tea. Furthermore, the Cd and As levels in Iranian tea samples were significantly lower than the Indian samples. The mean transfer rates of Pb, Cd, As, Zn, and Cu were 7.78, 6.29, 9.27, 12.91, and 13.08%, respectively. An estimate of the non-CR of whole metals and CR for As and Cd indicated that tea consumption posed no health risk to Iranian consumers.

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