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Potential human health risk of some heavy metals in the commercially tea leaves and tea infusion

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ABSTRACT

This study has been conducted to measure the heavy metal concentrations in different trademarks of tea leaves and tea infusion sold in the markets in Babylon Province, Iraq. The Cd, Pb, and Ni concentrations were quantified by atomic absorption spectrometry. The study recorded that cadmium concentrations in dry samples of tea leaves or its hot drink were 2.43 ppm, while the lowest value was 0.2 ppm in T-5 sample as dry granules to gain colour. The samples of T-1 were grains for taste which recorded 0.2 ppm. It is within the normal limits allowed by the World Health Organization, but close to the limits allowed by the British Pharmacopoeia. On the contrary, the lead concentrations were outside the permissible limits for both. Statistical analysis did not show significant differences between cadmium concentrations in dried tea leaves and tea infusion. The lead concentrations in both groups were the highest: 17.24, 16.64, 15.46, and 14.27 ppm in T-1, T-2, T-3, and T-6 respectively. While the lowest value was recorded at 0.02 ppm in T-5. In the hot tea drink, Pb concentrations decreased (7.15 and 0.04 ppm) in T-3 and T-5 respectively. It showed significant differences that exceeded the permissible limits of WHO. Based on the results of the current study, the bioaccumulation of these elements in the leaves of the plant will contribute significantly to increase their level of accumulation in people who are accustomed to drink tea constantly, especially in the types with a high content that was grown in soils contaminated with toxic heavy metals. Then, the non-carcinogenic risks, quantifying the health risk to consumers, the Hazard Quotient (HQ) and Hazard Index (HI) of the examined heavy metal ingestion in the tea were determined. The THQs for individual metals were less than 1, which has been approved for human consumption. However, it is necessary to measure the toxic metals in all foodstuffs entering the country as part of quality inspection and food safety processes.

Keywords: Heavy metals, Tea infusion, Health risk assessment, Hazard Quotient.
Article type: Research Article.

INTRODUCTION

Tea is one of the most popular beverages in most countries of the world. *Camellia sinensis* is a perennial plant, belonging to the Theaceae family. The Food and Agriculture Organization (FAO) estimated that more than ~4.8 million tons of teas were consumed around the world in 2012. Kenya, China, Sri Lanka, and India are major exporters (FAO 2015). The tea drink is considered the most consumed and preferred by the Arabian people, with about 3-4 cups daily for adults. Several studies have reported that the tea plant accumulates many toxic heavy metals, such as lead, cadmium, nickel, chromium, mercury, and others (Li *et al.* 2015; de Oliveira *et al.* 2018; Zhang *et al.* 2018a; Zhang *et al.* 2018b; Sun *et al.* 2019). Therefore, ingestion of food and these beverages is considered as the main pathways of human health risks (Das *et al.* 2017). Therefore, the presence of heavy elements in tea leaves and essential elements extracted during the tea infusion process, could convey an effective quality of tea and some adverse health effects for consumers (Dambiec *et al.* 2013, Zhang *et al*. 2018.). So, one of the most important markers in determining the quality of tea is the presence of heavy metals in the tea leaves, brewing, and infusions. In Asia, many studies were conducted to determine the toxic elements concentrations in tea leaves. Ning *et al*. (2011) found the Pb content at 0.26-3.2 mg kg^{-1}, and Cd at 0.0059-0.085 mg kg^{-1}

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in 30 different teas from China. In Iran, Parviz *et al*. (2015) found that some heavy metal levels (Al, Pb, Cd, Hg, and As) were close to maximum limits and infusion time had no significant effect on heavy metal contents, except Pb in bulk samples. In addition, the quality of tea samples studied were acceptable for heavy metal contents. They also estimated daily intake of heavy metals as compared with the allowable daily intake (ADI), and found that the means were compared to the limits allowed by national Iranian standards. However, according to Pourramezani *et al*. (2019), the hazard index of Indian and Sri Lankan black tea samples were non-significant for tea consumers, indicating that there was no risk of heavy metal exposure from drinking the tea. In Saudi Arabia, Ashraf & Mian (2008) found that the Pb and Cd concentrations in tea samples from different commercial brands ranged between 0.3 -2.2 and 0.32–2.17 µg g^{-1} respectively, which are safe for human consumption according to acceptable levels of WHO (WHO 2007). Brzezicha-Cirocka *et al*. (2016) and De Oliveira *et al.* (2018) investigated that the metal accumulations in herbal and infusion tea for black, green, and Oolong tea can occur naturally or as a result of manufacturing and agronomic processes as well as tea origin, application of pesticides, fertilizers, and plant uptake from acidic soils where tea plants are cultivated. In general, the sources of contamination of tea leaves with heavy elements include contaminated soil, mining, agricultural runoff, polluted water irrigation, pesticides and fertilizer application as well as manufacturing processes of tea (Karimi *et al.* 2008; Nakansah & Ackumy 2016). Daily excessive drinking of tea that accumulates high levels of toxic metals can result in poisoning and a variety of consequences, including, methemoglobinemia, liver cirrhosis, renal failure and red blood cell formation damage (Mahan & Raymond 2012). Lead and cadmium are extremely toxic to humans, especially for the urinary and nervous systems which lead to nervous, kidney, cardiovascular, and bone diseases. Long-term exposure to high levels of cadmium can result in bone deterioration and chronic renal failure (Järup 2003). The highest acceptable concentration of Pb and Cd set by the WHO is 10 and 5 ppm in all plant parts while European pharmacopeia determined these levels by 5 and 0.5 ppm respectively (European Pharmacopoeia 2008a,b; WHO 2007). It has been estimated that all black tea in the Iraqi market was imported from different countries by the private sector, which imports inferior types with the absence of supervision and control. Therefore, this study was conducted as a part of examining the heavy metal contents of tea infusion and tea leaves in different types of imported tea. So, the objectives of the current study were the evaluation of some heavy metal contents of commercial tea leaves and tea infusions prepared from several commercial brands with excessive drinking of tea daily in public and private different places. The health risk of these heavy metals in Iraqi tea drinkers was also examined using the Hazard Quotient (HQ) and Hazard Index (HI) methodologies to calculate the human health hazard.

MATERIALS AND METHODS

Sample collection and extraction

The tea samples were collected from the markets in Babylon Province, Iraq. They were supplied from different sources of the world with eight brands (India, Sri Lanka, and china) chosen for the investigation of toxic metal levels. All samples (16) were divided into two groups (8 tea powders and 8 tea infusions). In the laboratory, the tea samples were dried in the oven for 24 h under 65 °C, and finally ground into the fine powder, the samples were accurately sieved and weighted, 1 g of each sample was put in a conical flask (50 mL), with 50 mL of deionized water was added, to prepare tea infusions, 1 g from other eight samples with 50 mL deionized water were heated till boiling then filtered through filter paper (Whatman 1) then dried. A 10 mL solution of nitric acid and perchloric acid (5:5 v/v) was added. The contents of the conical flask were heated on a hot plate and digested with nitric acid (5 mL) and perchloric acid (5 mL) as the temperature was raised. All samples were appropriately labelled, packed in plastic bags, and transported to the lab. All of the reagents were of analytical quality. Merck KgaA, Darmstadt, Germany, provided deionized water, nitric acid, perchloric acid, and H_2O_2. Before use, the glassware and equipment were carefully cleaned with 10% HNO_3 and then rinsed with deionized water. To remove any particulates, all glassware was cleaned and rinsed with distilled water. Thereafter, they were immersed in 10% (v/v) nitric acid overnight (Merck). The glassware was then cleansed in distilled water, rinsed, and dried before being used.

Assessment of heavy metals

Heavy metals were measured in the studied samples using the Flame Atomic Absorption Spectrophotometer. In the laboratory of the advisory office of the College of Environmental Sciences, Al-Qasim Green University. After the standard solutions of the examined elements were prepared according to the methods mentioned in (APHA 2012). The concentrations of heavy metals were calculated from the previously prepared calibration curve to making standard curve equations.

Health hazard estimation

To determine non-carcinogenic health concerns in tea drinkers, the Hazard Quotient (HQ) of each metal was calculated. The HQ is determined using the equation stated by (Cao *et al.* 2010). If the HQ value is less than one, this indicates that there is no considerable danger of non-carcinogenic consequences in consumers (Zhang *et al.* 2018). In industrialized countries, heavy metals in contaminated herbs are assessed for health risks; but, in developing countries, nothing is done (WHO 1998). As a result, the goal of this study was to determine the concentration of heavy metals (Cd, pb, and Ni) in regularly used herbal plants in public marketplaces to avoid health risks associated with their use and to assess the health risk measurement situation. All analyses were carried out in triplicate, and the mean values were utilized to determine the results. To calculate the average daily intake (ADI) of heavy metal per person per day (g $person^{-1}$ day^{-1}) of Cd, Pb, and Ni by the human subjects via consumption of herbal teas we used the equation below:

ADI = (C × IR × TR) / (BW × 1000; Zhang *et al.* 2020).

Where:

C = Concentration of metals.

IR = Tea ingestion rate (11.4 g $person^{-1}$ per day; Peng *et al.* 2018).

TR is the metal transfer rate from tea leaves to tea infusion. (Zhang *et al.* 2018a).

BW is bodyweight, 70 kg for adults (de Oliveira 2018).

Target Hazard Quotient (THQ): To calculate Non-carcinogenic risk of heavy metals exposure. The hazard quotient of a single contaminant is determined by the equation below:

THQ = Exposure dose/ Reference dose. (Wang *et al.* 1998).

Where:

Reference dose = 5.0×10^{-4} for Pb; 1.5×10^{-3} for Cd and 2×10^{-2} mg kg^{-1} bw d^{-1}
for Nickel. (respectively (US EPA, 2019; Cai *et al.* 2019).

Hazard Index (HI): The hazard index of the heavy metals is calculated from the equation below to assess the total risk for a combination of heavy metals.

HI = THQ (Cd) + THQ (Pb) + THQ (Ni) (US EPA 2007).

Statistical analysis

Microsoft Excel software was used to calculate and analyse all data. Statistical analysis program (SPSS ver. 16) were used in the Paired T-test at $p < 0.05$ to compare the results obtained for heavy metal concentrations in the tea leaves and tea infusion.

Table 1. Different tea brands were collected from local markets in Babylon, Iraq, with the origin and the date of validation for each sample.Samples.

	Origin	Validation
T-1	Indian	2023/5_2020/6
T-2	China	2020/9/ _ 2023 /8
T-3	Indian	6/2020_12/2032
T-4	Sri Lanka	1/2021_8/2023
T-5	Indian	2023/5_2020/6
T-6	Sri Lanka	2023/1_2021/1/2
T-7	Sri Lanka	2023/1_2020/2
T-8	Sri Lanka	2023_2020

RESULTS AND DISCUSSION

Metal Concentrations in tea leaves and tea infusion

The results of the current study recorded the mean concentrations of Cd, Pb, and Ni (ppm) in the tea leaves and tea infusions in examined samples (Table 2). Cadmium in dry leaves was recorded as 2.43 and 0.18 ppm in T-5 and T-1 as dry granules to gain colour and flavour respectively. However, Cd levels decreased in infusion hot tea to 0.4 ppm in T-8, and 0.02 ppm in T-3, with transfer rate, ranged between 6.5 and 0.011% in T-3 brand and T-2 respectively. The Pb and Ni were recorded with high levels ranged between 17.24-0.02 and 4.4-5.2 ppm in both brands of T-5 and T-1 to gain flavour and colour respectively. In the case of lead concentrations, high concentrations were recorded as 16.64, 15.46 and 14.27 ppm in T-1, T-4 and T-6; In the two last treatments, Pb levels were increased to exceed the limits permitted by WHO. This increasing may be due to the industrial flavour and colour additives containing different levels of some heavy metals. Also these metals decreased in infusion tea to 7.15-0.04 ppm in T-3 and both in T-5 and T8 about Pb. In addition, Ni levels decreased to 4.4-0.008 ppm in infusion tea for T-1 and T-7 respectively. On the other hand, transfer rate (TR) of both metals were recorded as 0.005-2% and (0.002-0.8% for Pb in T-1 and T-5. Furthermore, in T-7 and T-8, the Ni recorded high percentage in a transfer rate from dry leaves to infusion tea. In contrast, heavy metals were not completely transferred from dried leaves

into tea infusions. So, TR value depend on infusion time in hot water, metals mobility, and total content of each metal (Zazouli *et al.* 2010). Statistical analysis to compare two independent groups showed that no significant differences between the concentrations of both groups of Cd *t*-value was 0.84 ($p < 0.2$), while showing significant differences for Pb and Ni ($t = 2.74$ and 3.74) respectively ($p<0.05$) exhibiting a significant decrease in the element levels in the infusion tea by the reduced release of heavy elements, in spite of their high concentrations. It may be due to their strong linkage with plant cell components and their bioaccumulation in different parts of the tea leaves, including the thick walls of the cells. Some released elements may have been part of the components of the cytoplasm when preparing tea infusion with hot water under heating which may increase over time and repeated infusion. These results are consistent with De Oliveira (2018) who detected that the contents of the metals such as Cd and Pb in traditional teas were higher than in herbal tea. On the other hand, infusion with boiled water or distillation processes of dried herbs can release some heavy metals much higher from dried leaves (Kohzadi *et al.* 2012). The Pb and Cd contents in tea samples were similar to those from Iranian tea (0.66–15.5and 0.09-1.92 mg kg^{-1}; Zazouli *et al.* 2010).
The difference in the metals contents of the tea may be due to the different pollution levels in agricultural lands or natural content of the earth's crust in the country of the tea production and also total mineral content in different tea types as well as agricultural applications of fertilizers and pesticides, which may contain different levels of some elements. All these factors play important role in the mineral content of tea leaves (Nkansah *et al.* 2016; Soliman 2016). Mortvedt (1995) suggested that the movement of heavy elements from soil to the tea plant through the movement of soil elements occurs naturally in the earth's crust and usually takes place in all ecosystems in trace amounts. Industrial activities also contribute to an elevated heavy metal concentrations in the soil. Similarly, in the present study, the element levels in some examined samples exceeded the levels set by the WHO (2007) including Pb: 10 mg kg^{-1}; Cd: 3 mg kg^{-1}; Ni: 0.02 mg kg^{-1}, while European Pharmacopoeia set the permissible limits for lead, cadmium and Ni at 5, 0.5 and 1.63 ppm respectively (European Pharmacopoeia, 2008a,b).

Estimated heavy metal daily intake

The total content of heavy metals in tea leaves is not completely transferred into tea infusions when preparing tea. As a result, the metal transfer rate (TR) from tea leaves to hot infusions should be considered. The current study involved the leaching of heavy metals into infusions (Zhang *et al.* 2018a). Thus, TR values were also employed to determine each metal's estimated daily consumption. So, a high values of TR in trademarks (T-3, T-5 and T-8) were recorded as 6.5%, 2% and 0.8% for Cd, Pb, and Ni respectively. In other studies, the TR values were 14.18% for Cd, 7.11% for Pb, and 67.71% for Ni (Zhao 2016; Zhang *et al.* 2018a). This variation could be due to the fermentation process used to make black tea, total mineral content, and infusion time, which are all important factors in determining element levels. The high average daily intake (ADI) of these elements was less than one. However, The mean EDI values in black tea, on the other hand, these values were observed in the current study with arranged Pb > Cd > Ni.
This factor is affected by both the metal concentration in the leaves and the total amount of tea consumed. The ADI values of all the metals in all studied brands were found to be less than the reference dose = 5.0×10^{-4} for Pb, 1.5×10^{-3} for Cd and (0.02) mg kg^{-1} bw d^{-1} for Ni. These results are consistent with those published for 15 different commercial tea brands in Pakistan, where ADI values were found to be lower than reference dosage values except for Cu (Idrees *et al*. 2020). Other authors have observed similar results (Cao *et al*. 2010; Peng *et al*. 2018; Zhang *et al*. 2018a). Health risk assessment methods are used to estimate target hazard quotient (THQ) values which are calculated for every metal in black tea and summation hazard of heavy metals to extract IH value (Table 4). The acceptable guideline value for THQ is 1 according to the USEPA (2007). THQ of individual metal and accumulative HI values for Cd and Ni were all less than one for tea infusions from the eight brands with exception for Pb content in three brands (T-2, T-3, T-6) which exhibited that consuming heavy metals in tea regularly will have no negative health consequences for adults. Peng *et al.* (2018) and Zhang *et al.* (2018a) published similar findings, indicating that heavy metal exposure from tea consumption poses no health risk to humans. The mean THQs of each metal is linked to the rate of consumption and overall content of toxic metals. Atmospheric deposition of Pb can be collected in old tea leaves through the stomata of the tea plant's leaf tissue. Moreover, the internal differences of various brands for accumulating metals or manufacturing processes may be caused increasing in lead levels of some samples then a rise in the THQ and IH values (Zhang *et al*. 2020)

The *t*-value of Pb is 2.74. The *p*-value is 0.0079. The result is significant ($p < 0.05$).
The t-value 0f Cd is 0.84. The p-value is 0.2. The result is not significant ($p > 0.05$).
The t-value of Ni is 3.74. The p-value is 0.0011. The result is significant ($p <0 .05$).

Table 2. The concentration of Cd, Pb and Ni metals (ppm) and transfer rate (TR) in samples of tea leaves and tea infusion for studied samples.

Samples	Cd (ppm)		Pb (ppm)		Ni (ppm)		Hazard Index HI
	ADI	THQ	ADI	THQ	ADI	THQ	
T-1	0.00014	0.01	0.001	0.3	0.0007	0.035	0.34
T-2	0.0003	0.0022	0.001	3.9	0.0003	0.001	3.97
T-3	0.0002	0.14	0.001	23.3	0.0004	0.002	23.4
T-4	0.0003	0.0021	0.001	0.3	0.0003	0.019	0.31
T-5	0.0001	0.01	0.006	0.13	0.0006	0.034	0.17
T-6	0.0009	0.007	0.001	2.28	0.0003	0.017	2.3
T-7	0.0003	0.003	0.003	0.65	0.0003	0.0007	0.65
T-8	0.0003	0.2	0.001	0.13	0.0005	0.027	0.35

Table 3. Average daily intake (ADI), Hazard Index (HI) of heavy metals and Target hazard quotients (THQ) from studied tea samples.

Samples	Cd Conc. (ppm)			Pb Conc. (ppm)			Ni Conc.(ppm)		
	Tea leaves	Tea infusion	TR	Tea leaves	Tea infusion	TR	Tea leaves	Tea infusion	TR
T-1	0.18	0.09	0.5	17.24	0.09	0.005	9.3	4.4	0.47
T-2	1.75	0.024	0.011	15.46	1.22	0.08	6.6	0.2	0.03
T-3	1.29	0.2	6.5	16.64	7.15	0.42	6.7	0.25	0.04
T-4	0.2	0.022	0.11	8.9	0.09	0.01	4.5	2.4	0.5
T-5	2.43	0.09	0.037	0.02	0.04	2	5.2	4.24	0.8
T-6	0.3	0.06	0.2	14.27	0.7	0.05	4.2	2.2	0.5
T-7	0.67	0.024	0.036	0.3	0.2	0.66	3.7	0.008	0.002
T-8	1.85	0.4	4.62	0.4	0.04	0.1	4.4	3.4	0.7

CONCLUSION AND RECOMMENDATIONS

This work outlined the importance of monitoring heavy metal content and estimating the impacts of origin plantations' difference on cadmium (Cd), lead (Pb), and nickel distribution in soils and accumulation of these compounds in tea leaves. The process of quality control of food products of all kinds is important in protecting the consumers and preserving their health. However, one aim of the current study was to notify the need to monitor and investigate the tea quality, especially food and beverages, including the most important and most consumed beverages in Iraq. Tea is one of the most popular types of drink around the world, so the quality and validity of this drink is considered important because of its close relationship to health. The results of the current study indicate that there are high concentrations of lead higher than the level of parameters recommended by the World Health Organization and the British Pharmacopoeia, and this suggests that the examined samples contain levels of other trace elements such as chromium, nickel, mercury, copper, iron and others. We also recommend conducting other studies to know and determine the level of bioaccumulation of each element using experimental animals, as well as studying and evaluating the level of health risk caused by these elements by continuing to drink this beverage. Human age, weight and consumption rate are necessary to assess the combined risk of multiple metals (Hazard Index) and estimated daily intake to imported good quality of specific trademarks to ensure the quality of the product and that it is free from the element that threatens public health.

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