Concentrations and Health Risk of Heavy Metals in Tea Samples Marketed in IRAN

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Summary

Tea is one of the most popular beverages in the world. Thus, the chemical components in tea have received great interest because they are related to health. In this study, the concentration of five heavy metals including Al, As, Cu, Hg and Pb were determined by atomic absorption spectrometry on samples collected from Mashhad market. The results showed that the highest (908.30 ± 377.70 µg/g) and the lowest (0.09 ± 0.02 µg/g) amount were related to Al and As, respectively. Generally, metals contents of black tea were found to be higher than those of tea infusions. The percent release of Hg to infusion was 70%, whereas only 2.6% was found for pb. The daily intake of all elements from these tea infusions is within the average daily intake except for Al in some samples. Therefore, it may not produce any health risk for human consumption, if other sources of toxic metals contaminated food are not taken after same time.

Key words: Black tea; Infusion; heavy metal; Atomic absorption; Iran

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Introduction

Next to water, tea (*Camellia sinensis*) is the most widely consumed beverage because of its taste aroma and health benefits. The 75% of the estimated 2.5 million metric tons of dried tea that are manufactured annually processed as black tea which consumed by many countries [1]. In Iran, 1.3 Kg of black tea, in average, is consumed per person per year [2].

Tea is used in folk medicine for headache, digestion, diuresis, enhancement of immune defense, as an energizer and to prolong life [3, 4]. According to pharmacological and epidemiological studies, tea is considered to have beneficial effects on the prevention of many diseases, including cancer [5], Parkinson disease [6], myocardial infarction [7] and coronary artery disease [8].

The intake of food and beverages contaminated by heavy metals is harmful to human health and several countries have imposed laws to restrict the presence of heavy metals concentration in food and beverages. Various reports have discussed the potential health implications of some chemical factors such as heavy metals in tea, particularly since the tea bush is known to accumulate trace metals [9, 10].Tea can be contaminated by heavy metals during growth period and manufacturing processes which might increase the metal body burden in humans. Metallic constituents of tea leaves is normally different according to the type of tea (green or black) and geological source [11]. The main sources of heavy metals in plants are their growth media, nutrients, agro inputs and soil. Other factors may include pesticides and fertilizers [12].

The current study aimed to determine the Aluminum (Al), Arsenic (As), Copper (Cu), Lead (Pb) and Mercury (Hg) concentrations in tea samples collected from Mashhad and to give an overview of the current safety situation of black teas marketed in Mashhad, Iran.

Materials and Methods

Sample collection

Five marked brands of black tea, which commonly consumed in Mashhad city and other Iranian cities were collected from local markets in March 2007. Tea brands include India (brand 1), mixture of several imported tea (brand 2), Sir Lanka (brand 3), Kenya (brand 4) and Iran (brand 5). Three pack of each brand with different production date were obtained. Each sample was analyzed to determine the amount of heavy metals including Lead, Arsenic, Mercury, Copper and Aluminum, in black and infusion tea.

Apparatus

All glassware was soaked over night in 10% (v/v) nitric acid, followed by washing with 10% (v/v) hydrochloric acid and rinsed with double distilled water and dried before using. A Perkin – Elmer 3030 and Shimadzu AA. 6650 atomic absorption spectrophotometeres were used for the determination of heavy metals. Lead concentration was determined by a graphite furnace atomic absorption spectrophotometer 110 employing pyrolytic platform graphite tubes. Hydride generation was with a Perkin – Elmer with quartz tubes.

Reagents

Standard stock solutions of mercury, arsenic and lead were prepared from Titrasol (1000 mg/l) and were diluted to the corresponding metal solution. These reagents used were of analytical reagent grade (BDH, England). Also, A standard copper and aluminum solution were prepared from CuSO4 and $Al(NO3)_3$ respectively, in distilled water and standardized. A 0.3 % solution of $NaBH_4$ and 0.1 % solution of NH_4VO_3 were prepared in 1% NaOH.

Sample preparation

Digestion

Based on the method described by AOAC [13], 3g of each black tea particles sample were digested using 100 ml of concentrated HNO_3 for 10 min. The mixture was heated using electric heater until nearly dried. The mixture left to cooled at room temperature.

The digested sample was mixed with 60 ml of a mixture (5:1 v/v) of concentrated HNO_3 and $HCLO_4$. The mixture was heated on electric heater until the solution turned white and gives out the white fumes. The digest was transferred into 50 ml volumetric flask and the volume was adjusted to the mark using distilled water. Concentrations of heavy metals (Pb, As, Hg, Cu, Al) were determined in the obtained clear solutions using atomic absorption spectrophotometery.

Infusion

Tea infusion samples were prepared while taking into account Iranian drinking habits. In this method, 100 ml of boiling distilled water was added to 2 g of each black tea samples and for the same conditions incubated in $80^{\hat{c}}$ .The mixture left to cool at room temperature for 10 min and then filtered to obtain the clear solution for further processing.

Validation of methods

Tea (digested) samples were spiked with various concentrations of heavy metals for the recovery repeatability tests and for verifying the analytical methodology. For each run, triplicate samples, spiked samples and blanks were carried through the digestion reaction.

Chemical analysis

Aluminum was determined by direct aspiration of the sample solution into the NO2/acetylene flame. The blanks and calibration standard solutions were also analyzed in the same way as the sample solutions. Mercury and arsenic were determined by the hydride generation system. The manufacturer operation procedure involves continuous addition of reductant, consisting of 0.3%$NaBH_4$, $Kmno_4$,1.5%HNO_3 for mercury and 0.3%$NaBH_4$, $Kmno_4$, 1.5%HCL for arsenic. The manufacturer's operating procedure consists of adding sample, reductant and acid, with the aid of argon gas, to a reaction coil; then any vapor generated is swept into the absorption quartz cell, and heated for arsenic detection. Cells were aligned in the light path of the hollow cathode lamp where the absorption was measured. lead concentration was determined by graphite furnace atomic absorption spectrophotometry, employing pyrolytic platform graphite tubes, 0.1%NaOH and NH_4VO_3 for matrix modification and using the method of additions for quantification.

Statistical analysis

The results are expressed as mean ± SD. Data were analyzed by ANOVA. Sequential differences among means were calculated at the level of $P<0.05$, using Tukey contrast analysis or Dun's test as needed.

Results and Discussion

A recovery test of the total analytical procedure was performed for selected samples by spiking analyzed samples with metal standards according to the original concentrations of spiked samples. Acceptable recoveries were obtained for externally added heavy metals (Table 1).

The heavy metal contents of several black tea materials and infusions are shown in table 2 and 3. Statistical analysis showed significant difference among the concentration of Al and Cu in different brands (table 3). The results of total contents of studied heavy metals in black tea show the ability of this plant to accumulate heavy metals, particularly Al and to a lesser extent Cu.

The highest (908.30 ± 377.70 µg/g) and lowest (0.09 ± 0.02 µg/g) amount were related to Al and As, respectively. The metals could be arranged in descending order according to their total content in black tea as follows: Al > Cu >Pb > Hg > As.

The concentration of As in tea samples varied from 0.08 to 0.12 µg/g and the Cu content in investigated teas ranged from 17.59 to 32.80 µg/g. All samples contained As and Cu at levels below those set as the standard maximum values by Iranian Ministry of Health (As and Cu, 1 and 150 µg/g, respectively). The Cu content in teas was higher by an order of magnitude, as compared with As.

Moreover, the Cu content of all tea samples in the current study were below the upper limits imposed on tea by various countries: China (60 µg/g), Japan (100 µg/g) and Australia, the United Kingdom and the United Stated (150 µg/g). Our results compared well with the literature values reported for tea samples from Turkey, India, China and the United States [14, 15, 16].

Table 1

Recovery of heavy metals from black tea and infusion samples

Metal	Recovery of metal from black tea (mean ± SD)	Recovery of metal from infusion tea (mean ±SD)
Pb	95.13 ± 1.68	85.86 ± 5.53
Hg	91.66 ± 2.88	84.16 ± 3.81
As	87.00 ± 2.64	80.66 ± 2.88
Cu	92.50 ± 2.50	87.50 ± 2.50
Al	92.83 ± 2.02	86.33 ± 3.21

Table 2

Total contents of heavy metals in both black tea and infusion samples

Metal	Black tea ($\mu g. g^{-1}$) mean ± SD	Infusion tea (µg/100 ml) mean ± SD	%Release
Pb	2.31 ± 0.29	0.001 ± 0.0002	2.60
As	0.09 ± 0.02	0.001 ± 0.0002	67.50
Hg	0.61 ± 0.01	0.0008 ± 0.0002	70
Cu	26.49± 6.16	0.26 ± 0.056	49
Al	908.30 ± 377.70	3.50 ± 3.14	19

Table 3

Mean contents of heavy metals in black tea samples available in Mashhad

Heavy metal	Tea	Brand 1 (mean ± SD)	Brand 2 (mean ± SD)	Brand 3 (mean ± SD)	Brand 4 (mean ± SD)	Brand 5 (mean ± SD)
Pb	**Black ($\mu g. g^{-1}$)**	2.21 ± 0.10	2.08 ± 0.41	2.43 ± 0.39	2.28 ± 0.09	2.59 ± 0.15
	Infusion (µg/100 ml)	0.001± 0.0002	0.001 ± 0.0002	0.0014 ± 0.0002	0.0012 ± 0.0004	0.001 ± 0.0002
Hg	**Black ($\mu g. g^{-1}$)**	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.04 ± 0.02	0.06 ± 0.01
	Infusion (µg/100 ml)	0.0008± 0.0002	0.0008 ± 0.0002	0.0008 ± 0.0002	0.001 ± 0.0004	0.0008 ± 0.0002
As	**black ($\mu g. g^{-1}$)**	0.08 ± 0.01	0.12 ± 0.04	0.1 ± 0.01	0.08 ± 0.01	0.09 ± 0.02
	Infusion (µg/100 ml)	0.001± 0.0002	0.0016 ± 0.0002	0.0012± 0.0002	0.0012± 0.0002	0.001 ± 0.0002
Cu	**Black ($\mu g. g^{-1}$)**	22.54 ± 0.92	31.18 ± 0.13	28.36 ± 2.46	17.59 ± 3.09	32.80 ± 3.84
	Infusion (µg/100 ml)	0.23 ± 0.017	0.29 ± 0.013	0.23 ± 0.022	0.22± 0.0036	0.33 ± 0.08
Al	**Black ($\mu g. g^{-1}$)**	931.0 ± 36.54	728.7 ± 59.17	699.0 ± 63.63	932.4±133.75	1549.0 ± 411.74
	Infusion (µg/100 ml)	1.60±0.37	2.33±0.68	1.72±0.41	5.52±1.51	6.41±6.18

The large variation of Cu content in the teas could be attributed to different types, grades and producing areas of the teas. Cu pollution could be mainly from the rolling machine and fungicides [17]. Fertilizers and pesticides may elevate the soil As level [18]. As in soil could be uptake and gets accumulated in plants grown on it [19]. Considering that high As level may occur in tea leaf and also the enormous amount of consumption of tea, it is necessary to assess tea contribution to the daily dietary As intake.

The concentration of Pb in samples marketed in Mashhad (Iran) was detectable at high levels (> 2 µg/g). Although this amount is higher than the permissible level (1 µg/kg) given by Iranian Ministry of Health, but compared to limit prescribed by other countries such as China (5 µg/kg), India (10 µg/g) and Thailand (10 µg/g), the samples were acceptable. Previous studies demonstrated that the range for Pb was from 0.03 to 14.84 µg/g in Saudi Arabia, 0.198 to 6.345 µg/g in China and 0.26 to 0.83 µg/g in India [12, 20, 21]. The main sources of Pb in tea samples are their growth media, such as soil. Pb contamination in soil usually can be attributed to industrial activities (smelting process), agricultural activities (application of insecticides) and urban activities (combustion gasoline). Tea plants normally are grown in highly acidic soils where Pb is more bioavailable for root uptake. Deposits from the polluted air into the leaves of the plant can be another source of Pb contamination of tea [22].

The Hg and Al content in our tea samples varied between 0.04 to 0.06 µg/g and 699 to 1549 µg/g, respectively. There is not a permissible level for Al and Hg in Iran. However, the Hg content of the tea samples in current study was below the upper limit (2 µg/g) imposed on tea by Thailand [23].

Tea plants absorb Al from acidic soil by passive diffusion, which are accumulated in the leaves during the plant's life span [24]. In spite of high level of Al in tea samples, bioavailability of Al is very low, probably because not much Al is potentially available for absorption [25]. Therefore, there is insufficient basis for setting a health standard for Al in tea samples, but the high level of Al in tea would be a health concern for people who consume a large amount of tea daily.

The element concentrations in tea infusions were determined to assess the actual amount of exposure to the elements by drinking these beverages. Generally, the metal contents of tea pieces were found to be higher than those of tea infusions (Table 2 and 3). The percent release of the studied metals that calculated as a ratio between infusion extractable metal and total content varies widely among tested metals. The percentage of Pb and Hg which were released from black tea to infusions were 2.6% and 70%, respectively. Our results show that high part of Al, As, Cu and Hg may brought into beverage. Thereby, much care should be paid to metals concentration when daily uptake is considered.

The drop in concentration of metals in infusion could be attributed to chelating of these metals with tannic acid and tannins which exudates during the boiling of tea particles. Precipitation of these chelates leads to the noticed decrease in metal concentration in infusion [20]. Other factors that could effect the metal concentration in infusion are physiological properties or structures of plants, the pH of the water used in tea preparation, the extraction time and temperature and the solubility of these elements in hot water [23, 26]

Because the lack of standard limits for tea heavy metals in nonalcoholic beverages, the daily metal intake from the consumption of tea infusion has been determined to evaluate their potential hazard to health. Also, the total amount of each element in tea infusion was calculated for daily intake. The calculated amounts are based on the concentrations of elements in the infusion and the assumption that the average consumption of black for a single Iranian person is 3.56 g/day.

The data in table 4 show the ranges of calculated results compared to the average daily dietary intakes of each element. The concentrations of all metals for daily intake were below the safety levels for human consumption except for Al in some samples. Due to the lack of a defined permissible level for some of heavy metals like Al and Hg in Iran, a maximum safe concentration should be introduced. It is essential to have good quality control of plant raw materials and to determine the presence of some contaminants, especially toxic elements to avoid overconsumption and their cumulative toxicities in long-tern use.

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Table 4

Comparison of the Average Daily Dietary Intakes of Each Metal.

Metal	Estimated dietary intake (µg/day)	Calculated intake [a] (µg/day)
Lead	7 – 12.6 [b]	0.0024 – 0.005
Mercury	2.1- 9.1 [b]	0.0016 – 0.0038
Arsenic	105 – 406 [b]	0.0021 – 0.0055
Copper	700 – 900 [c]	0.62 – 1.20
Aluminium	4.69-4.76	3. 57 – 40.85

[a]Assumption that the average consumption of black tea is 3.56 g/day [b]Estimated dietary intakes based on 70 kg body weight (Committee on Toxicity, 2003) . [c]Recommended dietary allowance (RDAs)/ adequate daily dietary intake (www.nap.edu).

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