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Analysis of Caffeine and Heavy Metal Contents in Branded and Unbranded Tea Available in Pakistan

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Abstract. In the investigation of caffeine and heavy metal contents in four branded and six unbranded tea samples collected from local markets of Lahore, Faisalabad and Peshawar, the amount of caffeine and heavy metals in all the branded tea samples were in agreement with the international standards. In unbranded tea samples, though the amount of caffeine was within standard limits but two of the samples collected from Peshawar had high concentrations of lead being, 13.69 and 15.78 mg/kg, consumption of which can lead to serious problems.

Keywords: tea, caffeine, heavy metals

Introduction

Tea (*Camellia sinensis*) is one of the most popular beverages all over the world. According to an estimate, 2.5 million metric tonnes of dried tea is manufactured annually, 75% of which is processed as black tea and consumed in different countries. In UK, on an average, one litre of tea is consumed per person per day (Al-Oud, 2003). Different brands of tea are manufactured to meet the increasing demands of consumers worldwide. Positive and negative effects of tea on the health have been investigated by many researchers, recently (Yao *et al*., 2006a).

Caffeine ascribes quality characteristics to tea, such as briskness, taste etc., and has been considered an important quality parameter in the evaluation of tea quality (Yao *et al*., 2006b). Caffeine is a pharmacologically active substance and, depending on the dose, can be a mild nervous system stimulant. Caffeine does not accumulate in the body and is normally excreted within several hours of consumption (Mumin *et al*., 2006; Obanda *et al*., 1999). Human body requires both metallic and non-metallic elements for healthy growth and development within certain permissible limits. The optimum concentration needed for this purpose varies widely from one element to another, from infant to childhood to adult and from male to female (Atta, 1995). Determination of these elements in beverages, water, food, plant and soil is thus of utmost important. Tremendous research has been rendered on finding tolerance limits for daily intake of nearly all essential elements needed for healthy growth and sound physiological changes in human body. There is a fairly narrow gap between the essential and the toxic levels of metals and essential trace elements that can otherwise accumulate in bone, hair and soft tissues such as liver, kidney, brain or lungs (Tautkus *et al*., 2004).

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Materials and Methods

Caffeine. ***Preparation of tea solution.*** Two grams of tea were added to boiling water, (200 mL) in a 250 mL conical flask placed on a hot plate at 90 °C while stirring for 10 min by a magnetic bar. Then the tea solution was filtered through cotton wool and the residue was washed thrice with distilled water (10 mL). The tea solution was cooled to room temperature and washings were diluted to 250 mL with distilled water. The sample was analyzed in duplicate.

Measurement. To 10 mL of tea solution, 5 mL HCl (0.9 mL of 36% HCl was diluted to 1000 mL with distilled water) and 1 mL lead acetate solution (100 g of lead acetate was dissolved in small quantity of water and diluted to 200 mL with distilled water) were added and diluted upto 100 mL with distilled water. The solution was then filtered through Whatman filter paper # 42. Filtrate 25 mL and 0.3 mL sulphuric acid (167 mL of 98% H_2SO_4 was diluted to 1000 mL with distilled water) were placed in a volumetric flask and diluted to 50 mL with distilled water. The solution was filtered using the same type of filter paper. Absorbance of the filtrate was measured using spectrophotometer (Spectronic Unicam) at 274 nm. Readings were taken in duplicate.

Standard curve. Caffeine stock solution (20 mg caffeine/10 mL, w/v distilled water) was diluted to 200 mL with distilled water. Next, 0, 10, 20, 30, 40 and 50 mL of the diluted caffeine solution were separately mixed, each with 4 mL HCl in a volumetric flask and diluted to 100 mL with distilled water. Thereafter, the measuring steps were repeated as described above. Readings of the absorption of the standard solution against its concentration were used to prepare the standard curve. Following formula was used to calculate the caffeine contents:

$$\text{Caffeine (\%)} = (E/1000)\times V_o \times (100 \times V_1) \times (50/25) / W$$

or

$$= 0.2\, EV_o / V_1 / W$$

where:

E is caffeine (mg) from the standard curve against the reading of the spectrophotometer

E/1000 is used to convert 'mg' into 'g',

V_o is the total volume of tea solution (250 mL),

V_1 is the volume used for the measurement (10 mL),

$100/V_1$ indicates 10 mL tea solution that were diluted to 1000 mL,

50/25 shows another dilution from 25 mL filtrate made to 50 mL in the measurement and

W is the dry weight of the tea sample.

Heavy metals. Double distilled water and analytical grade reagents were used in all the experiments. Standard solution (10 ppm) of heavy metals i.e., Zn, Cu, Cd, Co, Pb, Mn and Ni were prepared by taking 1 mL of each heavy metal stock solution (1000 ppm) in a 100 mL volumetric flask and diluted upto the mark with double distilled water. Heavy metal working standard solutions i.e., 0.5, 1.0, 1.5 and 2.0 ppm were prepared by diluting 5, 10, 15 and 20 mL, respectively, standard solution (10 ppm) of each metal made upto 100 mL with double distilled water.

The calibration curve used for the determination of heavy metals in tea samples by flame atomic absorption spectrometry (FAAS) was established using the working standard solutions. For the determination of metals in tea, 3 g of the oven dried (105 °C) samples were preheated for 30 min at 250 °C and burned for one hour at 800-850 °C. The resulting ash was wetted with double distilled water and mixed with 10 mL of diluted HCl (1:1). The mixture was mildly boiled, cooled to room temperature, filtered (if necessary), transferred to 100 mL volumetric flask and diluted with double distilled water.

A Varian (AA 240) flame atomic absorption spectrophotometer equipped with hollow cathode lamps was used for the analysis. The instrumental setup was adjusted according to the manufacturer's instructions.

Results and Discussion

The spectrophotometric method is the most common method used for the determination of caffeine. Flame atomic absorption spectrometry (FAAS) is one of the techniques most extensively used for determining various elements with a significant precision and accuracy. This analytical technique is remarkable for its selectivity, speed and fairly low operation cost. However, in some cases it is rather difficult to determine traces of heavy metals in environmental samples due to unsufficient sensitivity or matrix interferences. Thus, a pre-concentration or/and separation step is necessary.

Caffeine. Caffeine content in different tea samples ranged from 3.41 to 3.74% with a mean of 3.56% of the dry mass (Table 1). Earlier studies showed that caffeine content in black tea was affected by clone of plant, season and stage of plucking of leaves (Obanda and Owuor, 1995). Caffeine content can vary in tea leaves from 24% to 40% depending on the maturity i.e., the young leaves may contain more caffeine than the older tea leaves (Owuor and Orchard, 1992; Owuor *et al*., 1987). The results (Table 1) of the current study showed that the mean caffeine content in tea varieties marketed in Pakistan was

Table 1. Concentrations of caffeine and heavy metals in branded and unbranded tea samples

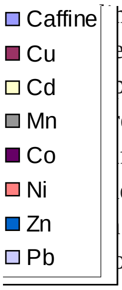
Tea sample	Caffeine	Heavy metals (mg/kg)						
(code)	(%)	Cu	Cd	Mn	Co	Ni	Zn	Pb
Branded (S 1)	3.41	1.20	nd	2.75	0.69	4.58	nd	4.64
Branded (S 2)	3.45	1.05	nd	40.50	0.60	4.58	nd	3.28
Branded (S 3)	3.42	0.75	nd	0.25	0.65	4.59	0.25	4.75
Branded (S 4)	3.46	0.75	nd	39.75	0.61	4.58	1.00	4.28
Unbranded (LHR 1) (S 5)	3.64	0.50	0.25	19.50	0.10	9.71	nd	4.64
Unbranded (LHR 2) (S 6)	3.72	1.0	0.28	15.70	1.29	9.27	0.76	4.59
Unbranded (FSD 1) (S 7)	3.53	0.50	0.25	16.10	1.20	9.98	0.84	4.20
Unbranded (FSD 2) (S 8)	3.49	0.75	0.50	25.75	1.14	8.66	0.25	3.75
Unbranded (PWR 1) (S 9)	3.74	1.45	0.34	25.50	1.02	10.01	nd	13.69
Unbranded (PWR 2) (S 10)	3.73	0.75	0.25	16.50	0.89	9.69	0.75	15.78

nd = not detected.

similar to the values reported in literature, 3.32 to 31.81%, and also that the tea varieties originating from different sources, with differences in clones, seasons and maturity, differ in caffein content (Gulati *et al*., 1999).

Heavy metals. Results of the study show that different tea samples contain the elements Cu, Cd, Mn, Co, Ni, Zn and Pb in various proportions (Table 1 and Fig. 1). Variations in elemental contents from sample to sample can be attributed to the differences in botanical profile as well as the mineral com-position of the soil in which plants are cultivated. Other factors responsible for variation in elemental contents may be pre-ferential absorbability of the plant, fertilizers, irrigation water, climatic conditions and, most of all, differences in the methods of processing and packing of tea leaves. Deposition of various metals from the vehicular emission and other sources on the open tea leaves could contribute to differ-ences in analytical results.

Lead. Lead was found in variable amounts in all tea samples (Table 1). For example high concentration of lead i.e., 13.69 and 15.78 mg/kg were found in two tea samples (S-9 and 10, respectively), while in other samples the amount of Pb varied from 3.28 to 4.75 mg/kg which is lower than the prescribed mit of WHO (1998) for Pb content in herbs i.e. 10 mg/kg. It is elieved that 95% of Pb present in plants is due to foliar ptake from the surroundings. As majority of the tea varieties e imported from other countries, so source plants with fferent origins have different air, water and soil environ-ent. The high concentration of Pb in plants growing above e ground is due to air borne Pb and the surrounding lluted environment.



Cadmium. Cd is another toxic metal not required by any living being. In the four branded tea samples (1, 2, 3 and 4) Cd was not detected while in other samples its value ranged from 0.20 to 0.50 mg/kg (Table 1). It may have been introduced during packaging or the tea plants might have absorbed this pollutant from the surrounding air and the soil. Thus con-sumption of such tea for long period of time is dangerous due to Cd contamination. Cd damages nerve cells; it inhibits the release of acetylcholine and activates cholinesterase enzyme, resulting in hyper activity of the nervous system. By altering calcium and phosphorus metabolism, toxic level of Cd can contribute to arthritis, osteoporosis and neuromuscular diseases. In cardio-vascular system, Cd replaces Zn in the arteries making them brittle and inflexible. Cd accumulates in the kidneys and once accumulated, it stays there and is not removed through excretion, resulting in high blood pressure and kidney diseases.

Copper. In all the tea samples, high Cu concentration was found. In sample-9, concentration of Cu was 1.45 mg/kg (Table 1), while in all other samples, its concentration varied from 0.50 to 1.20 mg/kg. Although Cu is an essential enzymatic element for normal plant growth and development but at excessive levels, it can be toxic. Phytotoxicity can occur if its concentration in plants is higher than 20 mg/kg. Copper accumulation in body can result in a tendency for hyper-activity in autistic children. An excess of Cu can cause stuttering, insomnia and hypertension as well as oily skin, loss of skin tone (due to blocking of vitamin C absorption) and dark pigmentation of the skin, usually around the face. Cu can also make nails brittle and thin and may contribute to hair loss especially in women.

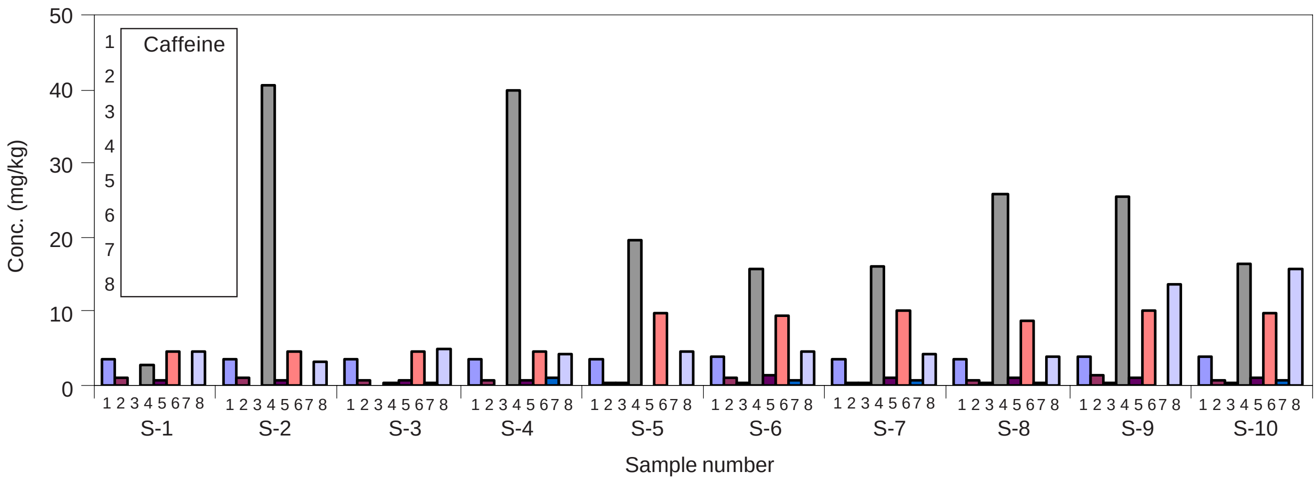


Fig. 1. Concentration of caffeine and heavy metals in different tea samples.

Zinc. Zn is an important metal required by both plants and animals. The highest concentrations of Zn (1.0 mg/kg) was found in one branded sample (sample-4), while in other samples, its amount varied from 0.25 to 0.84 mg/kg (Table 1). In few tea samples, its concentration was very low. Zn is very important for plant and human life. In the blood about 85% of Zn combines with proteins for the transport of the latter after its absorption and its turnover is rapid in the pancreas. Deficiency of Zn causes diabetic hyposmia, hypogensia or coma.

Nickel. In all the studied unbranded tea samples, higher amount of Ni was recorded: 10.01 mg/kg in sample-9, followed by 8.66-9.98 mg/kg in five samples (samples 5, 6, 7, 8 and 10), while equal amounts of Ni (4.58 mg/kg) was found in all the branded samples. Ni plays an important role in the production of insulin in the pancreas. Its deficiency results in disorder of the liver. However, higher concentration, of Ni have adverse effects on health. For example Ni tends to accumulate in kidneys causing kidney damage. Being a common ingredient in fashion jewellery, Ni can cause allergic reactions in some wearers; eczema and even asthma attacks may develop. A steady exposure ot Ni can cause cancer of lungs and nasal sinus.

Cobalt. As shown in Table 1, the highest concentration of Co was found in one sample (sample 6, 1.29 mg/kg), followed by 1.20 mg/kg in sample-7. Other tea samples had a concentration of 0.60 mg/kg to 1.14 mg/kg. Thus as a whole, considerable variations were recorded in all the tea samples which may be due to the differences in the air, soil and water environment of the source plants as well as local activities in their nearby surroundings. Human body needs Co in trace amounts and it is toxic in higher concentrations. Co in the form of vitamin B_{12} is in its physiologically active form.

Manganese. Mn was the most abundant metal found in tea samples in variable amounts (Table 1). For example high Mn concentration was found in two branded samples No.2 and 4, being 40.50 and 39.75 mg/kg, respectively, and 25.75 and 25.50 mg/kg in two unbranded samples, samples 8 and 9, respectively. The least amount of Mn was found in one branded sample (sample 3) being 0.25 mg/kg. The presence of Mn in tea samples may be due to its use as a colouring material for tea leaves.

Daily intake of heavy metals. Due to lack of information about the maximum allowable levels of heavy metals in tea leaves the discussion will be extended to the acceptable daily intake that can be taken into account through foods and drinking water. For intance, the expected calculated intake of Mn in the present study was 121.38 mg/day for tea (Table 2). This value is much higher than the intake through food (0.008) and at the same time nearly equal to 3.8 mg/day through drinking water according to the standards of US Environmental Protection Agency (MAFF, 1997). The calculated overall mean intake of Ni through tea was 49.39 mg/day. This value is in agreement with the human requirements (50 mg/day). However, this value is higher than the average intake in the UK (0.13 mg/day) recorded for the total diet studied (MAFF, 1999). According to MAFF (1998) tea beverage has considerable amount of Ni that could significantly contribute to daily intake of metals. Calculated intake of Cu through tea was 5.22 µg daily or 0.005 mg daily. This value was much lower than that of 1.2 mg daily set by UK and WHO (1998).

Table 2. Daily intake of different heavy metals

Heavy metal	Total (ppm)	Intake (mg/day)
Cu	0.87	5.22
Cd	0.18	1.12
Mn	20.23	121.38
Co	0.91	5.46
Ni	7.56	49.39
Zn	0.38	2.31
Pb	6.36	38.16

The mentioned values were calculated based on the assumption that the average consumption of tea beverage for a single person is three cups a day with one packet of 2 g in a cup, i.e., 6 g of tea particles/day.

The results show (Table 2) that only small part of heavy metal content through tea leaves may be introduced into beverage preparation. All branded and unbranded tea samples were found to contain heavey metal contents within safe levels except for only two unbranded samples collected from Peshawar, which had high concentrations of Pb.

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