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Estimation of Heavy Metals in Some Indian Black Tea Leaves by Inductively Coupled Plasma Mass Spectrometer (ICP-MS) and Associated Health Risks

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ABSTRACT

Background: Some of the heavy metals are considered beneficial, if intake of them is within permissible trace amount and some other are highly toxic even in trace amount. So, heavy metals intake is one of the major concerns. Heavy metals enter into body via air, water, food, clothing and some of them gets accumulate in body organs this leads to many disorders and causes life threatening diseases including cancer. All of our food comes from plants directly or indirectly. Tea the highly consuming beverage after water comes from the leaves of plant/shrub *Camellia Sinensis*.

Methods: Aim of the present work is to determine the elements present in Tea brew (Chai, boiling the Tea leaves in water) made from black Tea leaves of major brands available in local Indian market, more specifically heavy metals Cobalt (Co), Arsenic (As), Silver (Ag), Cadmium (Cd), Indium (In), mercury (Hg) and Lead (Pb). Total eleven samples were investigated with the instrument Perkin Elmer NexIon-300x Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

Result: Results have shown that Cobalt, Arsenic, Cadmium, Indium and Lead were present in all samples. Cobalt concentration varies from lowest 71.9 µg/kg to highest 214.3 µg/kg; Arsenic concentration varies from lowest 1.2 µg/kg to highest 53.7 µg/kg; Cadmium concentration varies from lowest 15.4 µg/kg to highest 75.03 µg/kg; Indium concentration varies from lowest 0.054 µg/kg to highest 0.70 µg/kg; Lead concentration varies from lowest 61.0 µg/kg to highest 2404.3 µg/kg. Mercury was present in two samples only in S9 and S11 with concentration 6.2 µg/kg and 1.4 µg/kg respectively; Out of eleven samples only seven samples have shown the presence of Silver its concentration varies from lowest 10.3 µg/kg to highest 193.0 µg/kg; Samples S2, S7, S8 and S10 were shown the absence of Silver and Mercury; Lead was found to be most abundant with concentration 2404.3 µg/kg and Indium was found to be least abundant with concentration 0.054 µg/kg.

Key words: Carcinogen, Heavy metals, ICP-MS, Indian black tea.

INTRODUCTION

Heavy metals intake is one of the major concerns in human health perspective these days. Idris *et al*. (2013) and Dabanović *et al*. (2016) have proposed the definition of heavy metals as "Heavy metals have the relative atomic weight greater than element Sodium (Na) and density of the elements greater than 5 g cm^{-3}", Moreover, all these metals have an atomic number greater than 20 Rajeswari *et al*. (2014) proposed that, the determination of heavy metals depends on the atomic number, atomic weight, density and position in the periodic table. Very low content of highly toxic heavy metals such as Arsenic, Cadmium, Lead and Mercury, may cause undesirable effects, because of the accumulation effect according to study conducted by Olivier *et al*. (2009) showed that the rate of excretion of heavy metals by the kidneys is very low. Heavy metals enter into body via air, water, food and clothing. All of our food comes directly or indirectly from plants. Environmental exposure to heavy metal pollution is due to increased industrial activities, burning of MSW, vehicular exhaust, irrigation with industrial effluents, fertilizers, chemical waste and different type of dye colors. Most of these organic and inorganic pollutants found in top layer of the soil, Prasad and Hitinayake (2012). It is evident from the study conducted by G.K. Saikia *et al*. (2014), that several types of pests and insects affect the Tea crop and as a preventive measure farmers use different types of insecticides and pesticides. Manju Mahurpawar (2015) in her study she had proposed that, heavy metals in gaseous state can spread many miles away from the sources by wind. Since, Tea is most consuming nonalcoholic beverage in India and the production of tea has become stagnant, Indian tea brands import it from rest of the tea producing countries Sabi Gogoi *et al*. (2016). Anil Kumar Singh (2018) and Tanmoy Karak, *et al*. (2011) had presented the review on the elemental contents in Tea and Tea infusion and their detection method. So, the aim of the present work

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is to determine how much the elements present in Tea brew (Chai, boiling the Tea leaves in water) made from black Tea leaves of major brands available in local Indian market (more than 1 Million consumers), more specifically heavy metals Cobalt (Co), Arsenic (As), Silver (Ag), Cadmium (Cd), Indium (In), mercury (Hg) and Lead (Pb) by most accurate and sensitive analytical technique "*Inductively Coupled Plasma Mass Spectroscopy*" (ICP-MS).

MATERIALS AND METHODS

Materials

Black Tea of different companies and different brands were bought from a local market store that is being consumed by more than 1 million peoples. Total eleven types of Tea bought were in different packages of different weight ranging 100 gm and 250 gm.

Prior to analysis to avoid any contamination of previously present heavy metals all glassware and plastic ware were soaked in 2% HNO_3 solution for 24 h and boiled for 1 h and washed 3 times with 2% HNO_3 solution.

Sample Preparation

Samples were prepared in the same manner in which the Tea brew (Chai) is made in India. *i.e* boiling the Tea leaves in water rigorously for 2-3 times, so that the experimental brew become same as the real composition that people take in Tea brew. Tea packets were opened just before making the Tea brew. Tea brew were prepared by boiling 500 mg of Tea leaves from each packet in 50 ml of 5% HNO_3 solution, taken in platinum coated stain less steel vessel for five minutes on induction cooking plate operating at 1000 W power, the solution reduced to 15 ml. HNO_3 used was of AR grade and water was distilled deionized Millipore water ($\dot{\rho}$ = 18.2 M&Ω). The reduced solution is then directly filtered into 15 ml vials of high density polyethylene (HDPE) with the help of funnels of same HDPE material and Filter paper (Watman No. 42). Solution is then diluted in 2% HNO_3 in the ratio of ten is to one and analyzed in Perkin Elmer Nexlon-300x Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

Instrument and method

Instrument used was Perkin Elmer Nexlon-300x Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The Instrument operating conditions were as follows:

RF power/ W	1500 W
Plasma gas flow	18 L/min
Auxiliary gas flow	1.2 L/min
Integral time	15 s
Sweeps	20
Reading	1
Replicates	3
Scanning mode	Peak hopping
Method	Total quant
Calibration type	External
Measurement time per sample	15 min (approx.)

All the Tea samples were diluted in 2% HNO_3 solution made by distilled deionized Millipore water. 1 ml of each sample is mixed in 10 ml of 2% HNO_3. Instrument was calibrated using external standard 200 µg/L mix of 13 elements in 1% HNO_3 obtained from Perkin Elmer. Three replicates of measurement were done for each sample and statically computed average of readings is presented in Table 1. Semiquantitative method (Total Quant) of analysis is chosen because of it is easy, rapid and accurate. Its accuracy has been reported as it has relative percentage error within 10% and reproducibility (relative standard deviation lower than 5%) at concentrations equal to or greater than 10 times the detection limit (DL) by Antonio *et al*. (2003).

RESULTS AND DISCUSSION

Table 1 is representing the results. Different nomenclature S1, S2 and so on has been adopted for tea names and their brands to avoid any conflict. Average of three measurements at three different times is presented along with standard deviation in measured values. Results have shown that, Cobalt, Arsenic, Cadmium, Indium and Lead were present in all samples. Cobalt concentration varies from lowest 71.9 µg/kg in sample S2 to highest 214.3 µg/kg in sample S8; Arsenic concentration varies from lowest 1.2 µg/kg in sample S2 to highest 53.7 µg/kg in sample S9; Cadmium concentration varies from lowest 15.4 µg/kg in sample S2 to highest 75.03 µg/kg in sample S1; Indium concentration varies from lowest 0.054 µg/kg in sample S9 to highest 0.70 µg/kg in sample S2; Lead concentration varies from lowest 61 µg/kg in sample S6 to highest 2404.3 µg/kg in sample S1. Mercury concentration was detected in two samples only in S9 and S11 with concentration 6.237 µg/kg and 1.386 µg/kg respectively, for all other samples S1-S8 and S10 it was found below the detection limit of instrument; Similarly, the Silver was found below the detection limit of instrument in samples S2, S7,S8 and S10 other seven samples have shown the Silver concentration varies from lowest 10.3 µg/kg in sample S5 to highest 193µg/kg in sample S9; Lead was found to be most abundant with concentration 2404.3 µg/kg and Indium was found to be least abundant with concentration 0.054 µg/kg.

Daily intake of heavy metals via Tea is calculated on the fact that, an adult Indian population consumes three cups of tea daily. Results are presented in Table 2.

Cobalt (Co)

Cobalt has properties similar to those of Iron and Nickel. Cobalt is one of the constituent of vitamin B12 or Cyanocobalamin, Pratt (1972). Vitamin B12 is essential for good health in animals and humans, Strachan (2010) and *ATSDR* (2004). Effects of Cobalt on health have been discussed by Paustenbach (2013) and Unice K.M. (2014). When Cobalt combined with Tungsten it causes cancer, *IARC* (2006) and Wild *et al*. (2009). General public comes in exposure to cobalt through food and drinks, Manju Mahurpawar (2015). Highest concentration of cobalt was

Table 1: Concentration of elements in samples values and deviation.

Element	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
Co	213.4±10.1	71.9±3.2	205.7±6.4	84.7±5.2	122.95±7.5	97.5±7.8	117.7±9.5	214. 3±7.1	97.1±12.8	182.6±16.1	111.7±10.8
As	39.8±2.1	1.2±0.5	23.1±3.1	8.6±1.2	15.9±1.5	6.2±1.3	14.8±2.8	42.1±2.4	53.7±5.3	33.1±7.0	21.4±3.1
Ag	152.9±3.5	BDL	22. 5±1.2	44.5±3.4	10.3±1.9	18.1±2.3	BDL	BDL	193.0±6.8	BDL	58.2±1.8
Cd	75.03±4.0	15.4± 1.5	40.5±2.6	16.6±2.0	30.2±2.1	34.1±4.2	38.3±2.4	43.3±2.8	42.8±3.5	53.3±6.3	42.0±3.4
In	5.6±1.5	0.70±0.2	0.21±0.3	0.24±0.9	0.12±0.03	0.32±0.5	0.12±0.9	0.3±0.6	0.054±0.02	0.17± 0.1	0.077± 0.02
Hg	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	6.2±1.7	BDL	1.4±0.13
Pb	2404.3±18.1	168.3±11.2	485.4±12.0	235.5±9.1	856.2±4.5	61.0±5.0	220.1±2.3	733.6± 6.4	912.8±12.5	403.0±9.3	486± 4.8

(in micro gram per Kilogram); BDL below detection limit of instrument.

Table 2: Calculated daily intake of elements via three cups of tea.

Element	Maximum concentration (µg/kg)	Daily intake (µg/kg)
Co	214.3	0.32
As	53.7	0.08
Ag	193	0.29
Cd	75.03	0.11
In	5.6	0.008
Hg	6.2	0.009
Pb	2404.3	3.60

found 214.3 µg/kg. Calculated daily intake via tea 32.0 µg/kg is found below the permissible maximum daily intake.

Arsenic (As)

Arsenic is also a natural semi metal having properties like metals. Arsenic can be found in trace amount in water, rocks, soil. Arsenic generally exists in combination with other elements like oxygen and sulfur, Nrashant Singh, *et al*. (2007) and Aronson (1994). It has been reported that exposure to arsenic in trace amount produces serious health effects like skin cancer, arteriosclerosis, heart disease, chronic lung disease, reproductive system disorder, Nrashant Singh, *et al*. (2007). High arsenic level in drinking water can affect the visual perception of children, Siripitayakunkit *et al.* (2000) and Siripitayakunkit et.al (2000). In West Bengal (India), the arsenic concentration in ground water highly used as drinking water is about 60 to 3700 µg/l, Acharyya (2002). Risk of Arsenic exposure gets increased when peoples use crops that irrigated with arsenic effected water. Highest concentration of Arsenic was found 53.7 µg/kg. Calculated daily intake via tea 0.08 µg/kg is found below the permissible maximum daily intake.

Silver (Ag)

Silver is one of the ornamental metals semiprecious and rare. Naturally exists in trace amount in water, rocks, soil. It occurs mainly in compound form Silver nitrate, Silver Chloride, Silver Sulfide and Silver Oxide. It finds its use in making jewelry, electronic industry, dental fillings and some pesticides. Silver compounds can turn the skin and other body parts in gray or blue gray color called *Argyria*. Exposure to higher level of silver compounds may cause breathing problems, lung and throat irritation and stomach pain, *ATSDR* (1990). At the nano size scale Silver is harmful Thabet *et al*. (2010). To study this Zebrafish has been chosen as model and it has been found that high dose slows down the development of embryo and several other abnormalities may develop, Fako *et al*. (2009). Highest concentration of Silver was found 193.0 µg/kg. Calculated daily intake via tea 0.29 µg/kg is found below the permissible maximum daily intake.

Cadmium (Cd)

Cadmium is highly toxic and carcinogenic for humans, IARC (1979). Naturally exist in trace amount in water, rocks, soil. Cadmium readily react with Sulfur and Chlorine these

compounds easily soluble in water. It has been used extensively in electronics industry most common uses are different types of solders due to low melting point, pigments, batteries, in phosphor materials of CRT screens and many more, Hiscock (1983) and Tolcin (2015). Toxicity and effect of Cadmium on human health and plants has been studied extensively by many authors, Prodan (1932). Cadmium reacts with Calcium inside body, this reduces the Calcium level and bones become weak, Dartmouth, *et al*.. Accumulation of Cadmium leads to pancreatic cancer, prostate cancer, kidney and various other types of cancers Vladimir Djordjevic, *et al*. (2017) and David Wallace (2017). Highest concentration of Cadmium was found 75.03 µg/kg. Calculated daily intake via tea 0.11 µg/kg is found below the permissible maximum daily intake.

Indium (In)

Indium is soft and silvery metal it is relatively rare metal. Mostly found with in Zinc, Copper and Iron ores. Naturally it occurs in trace amount in water, rocks, soil. Indium tends to be enriched in Sulûde minerals and in Tin oxide, Smith *et al*. (1978) and Wood *et al* (2006). Indium found its use in alloys as it decreases the melting point, different types of solders, electronic components like transistors, LEDs, Indium Tin Oxide ITO is used for making different type of transparent coating, Jorgenson (2005) and Nakamura, *et al*. (1994). Prolonged exposure to Indium produces interstitial pneumonia, lung damage, liver damage, Homma *et al* (2003) and Chonan *et al*. (2007). Highest concentration of Indium was found 5.6 µg/kg. Calculated daily intake via tea 0.008 µg/kg is found below the permissible maximum daily intake.

Mercury (Hg)

Mercury is a shiny metal due to its liquid state at room temperature it is called as liquid silver. It exists naturally in trace amount in water, rocks, soil. Mercury found in three different forms elemental (or metallic) Mercury, inorganic Mercury compounds (Sulfides, Oxides *etc*.) and organic Mercury compounds (methylmercury, phenylmercuric acetate). Mercury found its use in thermometers, fluorescent light bulbs, fertilizers, pesticides and various industrial activities, Thomas W. Clarkson (1997). When living organisms exposed to elemental or inorganic Mercury they convert it into organic Mercury, which is highly toxic, Farhana Zahir, *et al*. (2005). Mercury causes numerous disorders in humans several studies in past and recent showed this. Mercury affects the nervous system of fetus according to study foetus being more susceptible to methylmercury toxicity, Snyder (1971). In adults it causes Alzheimer's and Parkinson's diseases, Olivieri *et al*. (2002). High dose of mercury can cause lungs, lever to damage, eyesight may loss, immune system modulation *etc*., Tan and Parkin (2000) and Apaydın *et al*. (2016). Highest concentration of Mercury was found 6.2 µg/kg. Calculated daily intake via tea 0.009 µg/kg is found below the permissible maximum daily intake.

Lead (Pb)

Lead is soft, bluish grey metal it occurs naturally in earth crust. Due to human activities it can be found in varying amount all over the environment water, rocks and soil. Lead is an abundant metal mainly found in the form of lead sulfide, lead sulfate and lead carbonate. It founds its extensive use in batteries, paints, gasoline, various types of solders, radiation shielding and many more, Robert, Ziegfeld (1964) and Adrian Demayo, *et al*. (1982). There is no such level of lead that appears to be necessary or beneficial to the body and no "safe" level of exposure to lead has been found, Gagan Flora *et al*. (2012). Exposure to Lead affects the nervous system, Hematopoietic System, Renal dysfunction, Cardiovascular, reproductive system and bone, Kalia Flora (2005) and Guidotti *et al*. (2008). Highest concentration of Lead was found 2404.3 µg/kg. Calculated daily intake via tea 3.06 µg/kg is found below the permissible maximum daily intake.

CONCLUSION

All the samples appears to be different from each other in terms of presence of heavy metals, this may be due to the different region of cultivation, difference in the way of handling and processing. Also, the different regions of India have different levels of pollution of heavy metals in air, water, rocks and soil. High levels of concentration of highly toxic Mercury and Lead have been found in some samples so there is a need strict legal guideline for quality control and routine monitoring. The concentration of all heavy metal and their daily intake is well below the permissible daily intake but the retention of them in body may cause serious problems in later life span. Peoples who drink more than three or four cups of Tea regularly are at higher risk of getting exposure to heavy metal contents of Tea. This may lead to adverse effects on their health and organs. Further, by identifying the origin of Tea and by measuring the heavy metals in Tea leaves and other plants with reference to our experiment it can be conclude that the heavy metal pollutants are present in the environment of that region. The way in which Tea is cultivated, harvested, handled and processed needs to be reviewed.

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