Applied Innovative Research
Vol. 2, September 2020, pp. 226-230

Estimation of some essential, non essential toxic and toxic elements in some Indian black tea sold in market by 'ICP-MS' and their risk assessment.

Vishal Rohilla[a*], Don Biswas[a], Prashant Thapliyal[a], Gambheer Singh Kathait[a] & Surendra Singh[b]
[a] Department of Instrumentation Engineering-USIC, H N B Garhwal University, Srinagar (Garhwal) - 246 174, India
[b] Department of Physics, H N B Garhwal University, Srinagar (Garhwal) – 246 174, India

Received 7 September 2020

Tea *'Camellia sinensis'* is the most consumed non essential, non alcoholic liquid in the world. Black Tea finds its place in Indian culture as well as a part of diet of Indian population. Tea is known for its relaxing effect and therapeutic use as it contains bioactive *polyphenols, amino acids, caffeine, theophylline* and volatile organic compounds. Significant research efforts have been carried out on Tea for its health benefits. However, some ill effects also have been reported. Present work has been carried out to determine the essential, non essential toxic and toxic elements namely Iron (Fe), Copper (Cu), Cobalt (Co), Chromium (Cr), Aluminum (Al), Arsenic (As) and Lead (Pb); health risks associated with them among adult population. For this, the twelve well known and most selling (most consumed) Tea brands available in local Indian market were selected and analyzed by sophisticated instrument ICP-MS.

Keywords: ICP-MS; Tea; Trace elements; Aluminum; Black Tea; Alzheimer's; Dementia

Introduction

Tea with botanical name *'Camellia sinensis'* is the most consumed non essential, non alcoholic liquid in the world. It is now the part of routine habits and diet of the people, while looking in the prospect of Indian population. They consume it without knowing its health benefits or adverse effects. It is now become an integral part of culture also, one can observe that Tea being offered as the welcome drink on the arrival of guests in any Indian family home. This formal use of Tea in Indian culture was come in the period of colonial rule[1]. Use of Tea was different in ancient time, about 5000 years back Chinese people find its use as a traditional medicine. Indian people were known about the medicinal values of it which can be found in *Ayurveda* known as *Rasayanas*[2,3]. Researchers in past had put their efforts to determine the health benefits of Tea and found out that it contains bioactive *polyphenols, amino acids, caffeine, theophylline* and volatile organic compounds[4,5]. Due to its rich bioactive and anti oxidant contents, it prevents aging effects; anticancer activity; prevents cardiovascular diseases and cure several skin disorders[3]. Besides the organic contents, it contains inorganic elements too out of which some are trace elements those considered as essential, some are non essential elements which upon accumulation in body produce disease and highly toxic elements that produce permanent tissue damage or unwanted growth of tissues[6]. In present work, we have determined four essential trace elements Iron (Fe), Copper (Cu), Cobalt (Co) and Chromium (Cr); one non essential harmful Aluminum (Al) and toxic Arsenic (As) and Lead (Pb). Occurrence of them in Tea must be known so that, the daily intake can be estimated and controlled. For this the twelve well known and most selling (most consuming ≥ 1 million people) Tea brands available in local Indian market were selected and analyzed by sophisticated instrument ICP-MS. and the health risks associated with them were assessed.

*Corresponding author (E-mail: mail.vishal.pd@gmail.com)

Materials and Methods

Sample preparation

For analysis, analytical reagent grade Nitric Acid [HNO_3] and deionized MilliQ water having resistivity ≥ 18.2 MΩ were used. Glassware were avoided during whole analysis, all the utensils and materials were first washed with deionized MilliQ water and left soaked for 20 h. in 5% v/v Nitric Acid [HNO_3]. Prior to use they were rinsed with deionized MilliQ water five times.

Twelve different Black Tea pickings' of most selling brands were purchased from local grocery shops. Which were in packing of 100 gm to 250 gm weight. Samples were digested by single acid [Nitric Acid] method for organic and food items on hot water bath.

Tea samples, each of 500mg were poured into 30ml capacity PTPFE vials with cap, 10 mL of concentrated Nitric Acid was added and all samples were left for 20 h with loose caps. Semitransparent color of the solution was observed after 20 h. Further samples were then placed in hot water bath at temperature $90^0 \pm 2^0$C, approximately after 4 h. All samples were turned into colorless transparent solution. Samples were then left to get cooled to room temperature and filtered with Whatman filter paper of 42 grade, 1 mL of aliquot from each filtered sample is then diluted in 100 mL of 2% v/v Nitric Acid.

Instrument and calibration

ICP-MS instrument model Perkin Elmer NexIon-300x was used and calibrated with stocked standard solution, Perkin Elmer Pure Plus Multi-element Calibration Standard part No. N9300233: Fe, Cu, Co, Cr, Al, As and Pb all elements were in 10 µg/mL. Operating parameters of instrument were: RF Power 1500W; Plasma gas flow 18 L/min; Auxiliary gas flow 1.2 L/min; Integral Time 15s; Sweeps 20; Reading 1; Replicates 3; Scanning Mode Peak hopping, Method Quantitative; Calibration Type External ; Measurement Time 15 min.

Method validation

Method was validated by measuring the trueness, recovery, precision, repeatability, Limit of Detection and Limit of Quantification for each element to be detected.

Instrument was calibrated with different solution of concentration of elements in increasing order; regression line was obtained with a high coefficient of correlation 0.991. Trueness, precision of measured value was verified by measuring the known concentration value solution for each element ten times on consecutive three days. Experiment was repeated ten times again for each element, deliberate addition of known concentration of elements and percentage recovery was observed for increasing concentration. LOD and LOQ were calculated as 3* σ and 10* σ of ten measurements on blank solution. Values of percentage recovery, LOD and LOQ for each element are listed in Table 1.

Results

Table 2 shows the results of analysis. Concentration of elements is presented here in µg kg^{-1}. Brand names samples are avoided here. Different naming is done from sample S1 to S12. Results indicate that most abundant is Aluminum highest concentration 538832.7 µg kg^{-1} is present in sample S4 and lowest 125995.3 µg kg^{-1} in sample S2; least abundant is Arsenic, highest concentration 9.1 µg kg^{-1} is present in sample S11 and lowest in sample S2 below the detection limit of instrument 0.01 µg kg^{-1}.

Risk Assessment

While assessing the risks, it has assumed that most of the adult Indian population including men [average body weight 67.4 kg] and women [average body weight 64.9 kg] consumes three cups of Tea daily.

Table 1 — Values of LOD, LOQ and percentage recovery, for each element.

Element	LOD [µg kg^{-1}]	LOQ [µg kg^{-1}]	Precision % [coefficient of variation]	Average recovery % in 4 concentrations
Fe (Iron)	10.0	33.33	12	114 ±15
Cu (Copper)	0.10	0.3288	4	94±8
Co (Cobalt)	0.70	2.331	10	85±3
Cr (Chromium)	0.01	0.056	15	88±4
Al (Aluminum)	0.48	1.7088	14	110±8
As (Arsenic)	0.01	0.06	5	93±3
Pb (Lead)	10	3.58	4	108±7

Table 2 — Concentration of elements measured µg kg^{-1}; * below detection limit of instrument.

Element	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Al	135875.4	125995.3	363703.6	538832.7	474916.5	312894.3	351526.5	281787.8	343456.4	468271.6	178788.2	451832.8
Cr	0.858	N. D.*	8.567	6	1.23	3.313	2.24	4.3	3	5.36	13.4	4.61
Fe	55732.4	45506.7	35013.6	36372.5	21786.5	81345.7	74564.8	67513.8	88556.3	22025.7	20243.8	15743.8
Co	331.2	190.3	450.2	210.6	223.4	128.6	119.5	495.5	110.32	213.5	187.5	147.6
Cu	13147.4	21025.6	21448.2	11347.5	17245.6	13266.4	15678.3	17725.6	43484.3	25683.6	29745.5	27893.7
As	0.46	N. D.*	0.01	0.41	3.6	1.87	1.56	4.97	2.31	2.3	9.1	3.17
Pb	2.41	2.02	3.1	7.46	2.13	8.23	2.11	9.3	4.42	4.32	2.3	3.63

First, on early morning (Bed Tea), second with the breakfast and third in the evening (evening Tea). Each cup (75 ml) of Tea is made by boiling approximately 500 mg of Tea with water. Table 3 represents elements that have maximum occurrence in any tea brand. Daily intake has been calculated, according to assumption.

Discussion

Iron (Fe)

Iron is important one out of the essential trace elements, it has significant role in biological systems of humans as an ingredient of *Haem* proteins that performs the function of oxygen transport. Types of *Haem* proteins are *haemoglobin, myoglobin*, and the *cytochromes.* Iron is a constituent of some enzymes also, that play a major role in oxygen and food metabolism like *succinic dehydrogenase, mitochondrial NADH-dehydrogenase* and *xanthine oxidase.* Iron occurs as naturally in all food items. Sometimes doctors prescribe an additional intake of Iron in some clinical conditions. Significant loss of Iron during menstrual period occurs in females hence, more Iron is required than males. Provisional Maximum Tolerable Daily Intake [PMTDI] of iron is specified as 0.8 mg per kg body weight[7]. The maximum daily intake of Iron detected in Tea sample is 132.83 µg hence it is well below the PMTDI.

Copper (Cu)

Copper is an essential trace element that helps in formation of blood hemoglobin, melanin which plays important biological role in staying healthy body and skin . It helps in proper functioning of thyroid gland as well as it neutralize free radicals through its antioxidant property. Some other forms of Copper like *Sulfate* and *Nitrites* act as pro-oxidant that may cause Alzheimer's disease[8]. According to Guidelines[9] PMTDI of Copper is specified as 0.5 mg. per kg. body weight. The maximum daily intake of Copper detected in Tea samples is 65.23 µg hence it is below the PMTDI.

Table 3 — Estimated daily consumption.

Element	Maximum concentration[µg kg^{-1}]	Sample name	Daily int [µg]
Al	538832.7	S4	808.2
Cr	13.4	S11	0.02
Fe	88556.3	S9	132.8
Co	495.5	S8	0.74
Cu	43484.3	S9	65.23
As	9.1	S11	0.01
Pb	9.3	S8	0.01

Cobalt (Co)

Cobalt is another essential trace element that is being consumed through diet. Vegetables and water are the main source. It is one of the constituent of vitamin B12 [Cobalamin] and certain enzymes. It has been reported that cobalt keeps nervous system and blood cells healthy. Some researchers have reported that Cobalt [ii] compounds show carcinogenic activity[10]. Provisional Maximum Tolerable Daily Intake [PMTDI] of cobalt is specified as 0.1 to 1 mg. per kg of body weight[11]. The maximum Daily intake of Cobalt detected in Tea samples is 0.74 µg hence it is well below the PMTDI.

Chromium (Cr)

It has been proven that Chromium plays a significant role in regulation of carbohydrate and lipid metabolism as well as insulin activity[12]. Hence, it is an essential trace element, reduce the risk of diabetes and promote healthy cardiac activity. However, higher level of Chromium intake through respiration have been reported as carcinogen[13]. Provisional Maximum Tolerable Daily Intake [PMTDI] of Chromium is specified as 0.1 mg. per kg of body weight[14]. The maximum Daily intake of Chromium detected in Tea samples is 0.02 µg $^{-1}$ hence it is well below the PMTDI.

Aluminum (Al)

Aluminum is non essential for humans. It forms hydroxides that shows a very little solubility and hence shows speciation in body. Several epidemiological studies have shown an increased risk of various mental disorders, including Alzheimer's disease, associated with increased aluminum intake[15, 16]. Patients with renal disorders are at higher risk of aluminum toxicity. Provisional Maximum Tolerable Daily Intake [PMTDI] of aaluminum is specified as 7.0 mg. per kg of body weight[17]. The maximum Daily intake of Aluminum detected in Tea samples is 808.25 µg hence it is well below the PMTDI.

Arsenic (As)

Arsenic occurs naturally and has been reported to have tendency to enter into food system via plants, animals and most prominently via fish. Arsenic has found its main use in pesticides and electronic components. Most of the organic arsenic gets excreted through body. This is the inorganic arsenic that causes toxicity since it inactivates more than 200 enzymes that causes cellular activity, DNA repair and synthesis[18]. Provisional Maximum Tolerable Daily Intake [PMTDI] of Arsenic is specified as 0.015 mg. per kg of body weight[19]. The maximum Daily intake of Arsenic detected in Tea samples is 0.01 µg hence it is well below the PMTDI.

Lead (Pb)

Researcher have reported that, Lead is highly toxic and carcinogen as it has tendency to replace other essential elements like Calcium, Phosphorous and Iron etc. that are bound to protein molecules. It also inhibits the activity of *Haem* proteins and enzymes that cause a severe damage to the DNA, tissues and organs[20]. Provisional Maximum Tolerable Daily Intake [PMTDI] of Lead is specified as 0.025 mg. per kg. body weight[21]. The maximum Daily intake of Lead detected in Tea samples is 0.01 µg hence it is well below the PMTDI.

Conclusions

Results have been shown that Tea can be a rich source of essential trace elements. It also contains high levels of non essential toxic Aluminum. Researchers have been shown that Aluminum has a tendency of accumulation in bone, kidney and spinal cord that also increases with aging[22-24]. Recent researches have been shown that, the rate of occurrence of Alzheimer disease and metal toxicity increase rigorously with increasing age[23-24]. An unusual case of *myelopathy* due to aluminum toxicity has been reported[25], authors have diagnosed a 28 year old man suffering from gradual weakness of his limbs and motor ability due to exceptionally high aluminum level in his blood. Both young leaves and older leaves of Tea plant are being utilized to make black tea and green. Researchers have been shown that both types of leaves contain high level of Aluminum[26]. However, the concentration of aluminum estimated during our work does not show an enormous high amount but found in significantly high amount. It can be stated that the regular consumption of more than 3 cups of Tea may significantly increase the accumulation level of Aluminum in body, hence may contribute to consequences of this accumulation. Similar type of inference was observed during literature review, one research group in their review, based on survey of functional foods consumption including tea and coffee, have been reported that Tea consumption was highest in Finland population among the four countries namely Finland, United States, Belgium and Japan[27]. When we put our attention to the situation on Alzheimer's and Dementia in these countries we observed that death rate due to said diseases was highest in Finland among these countries in relation with the population in year 2014[28]. In remaining three countries number of patients suffering from Alzheimer's and Dementia shows increase in trend[29-31]. So, there is a need to assess biologically the intake of Aluminum via Tea and other food items and its contribution to accumulated Aluminum and the health risks associated. Our study has shown the presence of trace elements within the safe limits. But it is evident that the certain chemical forms of detected trace elements including Aluminum and others may cause toxicity. So total elemental determination may not be adequate hence more intense research efforts are required.

Acknowledgement

Researchers of our group are thankful to H N B Garhwal University, Srinagar Garhwal and Dean School of Engineering, Prof. N S Panwar for his significant motivation and providing the access to instrument ICP-MS and facilities.

References

1 Teabox (2017). An overview: Tea Industry in India accessed on 24 June 2019. https://Tea101.Teabox.com/brief-history-indian-Tea-industry.
2 Narender K Jain, Maqsood Siddiqi and John Weisburger, CAB International, p. 9 (2006).
3 Parmar Namita, Rawat Mukesh and Kumar J. Vijay Camellia Sinensis, *Global J Pharmacol,* 6 (2) (2012) 52.
4 Tariq M, Naveed A & Ali K Barkat, *J Med Plants Res,* 4 (19) (2010) 2028.
5 Cabrera C, Gimenez R & Lopez M C, *J Agri Food Chem,* 51 (15) (2003) 4427.
6 Watrak Sandra, et al., *KOSMOS*, 65 (4) (2016) 563.
7 WHO International Programme on Chemical Safety, IPCS Inchem home,WHO food additives series 571 Iron accessed on 30 June 2019. http://www.inchem.org/documents/jecfa/jecmono/v18je18.htm.
8 Osredkar J & Sustar N *J Clinic Toxicol,* S3:001, (2011).
9 WHO International Programme on Chemical Safety, IPCS Inchem home, WHO food additives series 17 551 Copper, accessed on 1 July 2019. http://www.inchem.org/documents/jecfa/jecmono/v17je31.htm.
10 Bhattacharya P T, Misra Satya Ranjan & Hussain Mohsina, *Scientifica Cairo*, 5464373 (2016). doi: 10.1155/2016/5464373.
11 D J Snodin, *Human Exper Toxicolo,* 34 (12) (2015) 1258.
12 Vincent J B, *Acc Chem Res*, 33 (7) (2000) 503.

13 Costa Max & Klein Catherine B, *Rev Toxicolo*, 36 (2) (2006) 155-163.
14 Kogan S, Zeng Q, Ash N & Greenes R A, *Proc AMIA Symp*, (2001) 329.
15 Martyn C N, *et al., Lancet* (1) (1989) 59.
16 Forbes W F, Lessard S & Gentleman J F, *Can J Aging*, 14 (1995) 642–656.
17 WHO International Programme on Chemical Safety, IPCS Inchem home, WHO food additives series 24 657 Aluminum accessed on 3 July 2019. http://www.inchem.org/documents/jecfa/jecmono/v024je07.htm.
18 Johnstone R M, Sulfhydryl agents: arsenicals, In: Hochster RM, Quastel JH [eds], Metabolic Inhibitors: A Comprehensive Treatise, Academic Press, New York, 2 (1963) 99.
19 WHO International Programme on Chemical Safety, IPCS Inchem home, WHO food additives series 24 658 Arsenic accessed on 4 July 2019. http://www.inchem.org/documents/jecfa/jecmono/v024je08.htm.
20 Godwin Hilary Arnold, The biological chemistry of lead Current Opinion in Chemical Biology, 5 (2) (2001) 223.
21 WHO. International Programme on Chemical Safety, IPCS Inchem home, WHO food additives series 21 622 Lead, accessed on 5 July 2019. http://www.inchem.org/documents/jecfa/jecmono/v21je16.htm.
22 Lukiw Walter J, *et al*., *J Alzheimers Dis Parkinsonism,* 8 (6) (2018) 457, DOI: 10.4172/2161-0460.1000457.
23 Vas C J *et al*. *Int Psychogeriatr*, 13 (4) (2001) 439.
24 Mathuranath P S, *et al*., *Neurol India*, 60 (6) (2012) 625. doi: 10.4103/0028-3886.105198.
25 Verma R & Sarkar S, *Neurol India*, 67 (3) (2019) 866.
26 Chenery E M, *Plant and Soil*, 6 (1955) 174.
27 Ozen Asli E, Pons Antoni & Tur Josep A, Nutrition Reviews, 70 (8) (2012) 472. doi:10.1111/j.1753-4887.2012.00492.x.
28 Official Statistics of Finland (OSF): Causes of death [e-publication]. ISSN=1799-5078. 2016, 3. Deaths from dementia and Alzheimer's disease are increasing. Helsinki: Statistics Finland [referred: 17.10.2019]. http://www.stat.fi/til/ ksyyt/2016/ksyyt_2016_2017-12-29_kat_003_en.html.
29 2019 Alzheimer's Disease Facts and Figures, accessed on 23 September 2019. https://www.alz.org/media/Documents/alzheimers-facts-and-figures-2019-r.pdf.
30 Alzheimer's association (2019) Alzheimer's and Dementia Resources Alzheimer's and Dementia in Japan accessed on 23 September 2019. https://www.alz.org/jp/dementia-alzheimers-japan.asp.
31 Belgium 2013: The prevalence of dementia in Europe accessed on 23 September 2019. https://www.alzheimer-europe.org/Policy-in-Practice2/Country-comparisons/2013-The-prevalence-of-dementia-in-Europe/Belgium.