Comparative assessment of copper, iron and zinc contents in selected Indian (Assam) and South African (Thohoyandou) tea (*Camellia sinensis* L.) samples and their infusion: A quest for health risks to consumer

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Abstract

The current study aims to assess the infusion pattern of three important micronutrients namely copper (Cu), iron (Fe) and zinc (Zn) contents from black tea samples produced in Assam (India) and Thohoyandou (South Africa). Average daily intakes and hazardous quotient were reported for these micronutrients. Total content for Cu, Fe and Zn varied from 2.25 to 48.82 mg kg^{-1}, 14.75 to 148.18 mg kg^{-1} and 28.48 to 106.68 mg kg^{-1} respectively. The average contents of each of the three micronutrients were higher in tea leaves samples collected from South Africa than those from India while the contents s in tea infusions in Indian samples were higher than in South African tea samples. Results of this study revealed that the consumption of not more than 600 mL tea infusion produced from 24 g of made tea per day may be beneficial to human in terms of these micronutrients content. Application of nonparametric tests revealed that most of the data sets do not satisfy the normality

assumptions. Hence, the use of both parametric and nonparametric statistical analysis that subsequently revealed significant differences in elemental contents among Indian and South African tea.

Keywords: Black tea; health risk assessment; meta-analysis; micronutrients; repeated infusions

1. Introduction

Heavy metals accumulation by plants and their subsequent transfer to human beings either through food or beverages is gaining tremendous global relevance [1]. Young shoots (comprising of two young leaves and one bud) from tea (*Camellia sinensis* L.) plant are often used for preparation of made tea. Approximately 67% of the total world population consume tea infusion (i.e. water extract of made tea) as healthy and non-alcoholic refreshing drink [2]. Among several teas, black tea accounted for around 80% of global tea production [2] and is manufactured following the five steps starting from plucking of young shoots:

Plucked fresh tea leaves (i.e. young shoots) ➡withered ➡rolled ➡fermented ➡dried

The black tea (referred as tea in this paper) is not only considered as a refreshing agent but also has neutracutical values [3]. Therefore, its wide acceptability as fillips in the global market is attested by about 18 to 20 billion cups of tea that are consumed daily in the world [4]. The total tea producing area in the world is 3.52 million ha of which India and South Africa contribute only 16.02% and 0.02% respectively [5]. According to FAO, estimation of global total tea production in the year 2013 has been reported as 5.346 million tonnes [5]. India total tea production in 2013 was 1.21 million tonnes representing 25.16% of the world tea production; and was the second largest tea producer in the world after China [5]. Amongst the 48 tea producing countries in the world, South Africa is ranked 18 producing only 2500 tonnes and contributing 0.05% of the total global tea production [5]. Commercial tea production in South Africa is still practised, albeit in smaller quantities [6].

The infusion of micronutrients such as Cu, Fe and Zn through leaching from tea leaves into water may also serve as a dietary source of these essential elements to the consumers [2]. These trace elements are considered essential for the living system as micronutrients, and are required for a variety of biomolecules to maintain the normal structure, function and proliferation of cells [7]. Nevertheless, excessive consumption of these trace elements can have toxic effects on the human body through interference with the body's physiological processes such as absorption and enzyme function thereby creating stress, including certain genetic disorders that can be considered as ecological toxins [2].

Tea is one of the major sources of Cu, Fe and Zn for human beings. However, earlier studies have demonstrated that excess amount of trace elements in tea plant, due to its acidophilic nature where trace elements get solubilized and make them potentially available to tea plants [8]. Therefore, it is of paramount importance to quantify the total amount of trace elements in made tea and their transfer in tea infusion. Hence, detail information on elemental analysis of tea leaves and tea infusion is important due to the global tea drinking habit and the increasing demand for tea [8]. Generally, the presence of micronutrients in tea infusion can contribute to the provision of adequate and reliable source of micronutrients supplement to human. However, repeated and regular consumption of tea beyond the dietary requirement may lead to accumulation of some trace elements in the body with significant health implications [9]. Several studies have highlighted that high amounts of Cu, Fe and Zn are present in tea leaves and made tea but these elements are not transferred in high amounts to humans through tea infusion [10, 11]. The amount of trace elements in tea infusion is dependent on several factors such as tea cultivars, type of tea, origin, elements content, speciation of elements and extraction methods such as repeated infusion [12, 13]. Several earlier studies have documented that trace elements in made tea as well as tea infusion are statistically different based on their origin. For example, Szymczycha-Madeja et al.[8]

characterised 10 tea samples that originated from Sri Lanka, China, India and Kenya but marketed in Poland through simplified-multivariate classification and reported that variation of trace elements in these tea samples is related to their origin. McKenzie et al. [14] similarly characterised tea leaves according to total mineral content in different tea types by means of probabilistic neural networks.

Although controversy regarding the beneficial and/or harmful effects of tea consumption is continually being debated, it however, remained as a good source of different micronutrients if one drinks a few cups daily [15]. The concern around the trace element contents in tea infusion, calls for the analysis of trace elements in tea and establishment of the importance of their nutritional value so as to guard against any probable ill effects they may cause to human health [12]. Although the comparative analyses of Cu, Fe and Zn contents in made tea and tea infusion among different tea growing countries of the world are available in literature, such study between Indian and South African tea products including assessment of the potential risks associated with high exposure through large consumption have not been studied so far. These determinations are particularly important considering the increasing habitually drink of tea infusions as beverage among Indian and South African people so as to ascertain both the safety of this product and its nutritional value [3]. Furthermore, many prior studies have attempted to statistically quantify relationships between a tea and its country of origin [2, 14] but such comparison between Indian and South African tea is currently very scanty. Although available ~~statistic~~ information regarding South African tea relative to the global tea production statistic appears marginal, several initiatives from government and the tea Industry through increase private partner participation abound aim at bringing about significant increase in tea local production and availability. Increase product availability in the market to meet local demand for consumption is also being facilitated through importation from other tea growing countries like India supported by the two countries' bilateral trade agreement to cater for the current low production level while also working towards increase local production. Therefore, this study aims to assess and compare the levels of Cu, Fe and Zn in made tea and three sequential tea infusions from Indian and South African teas. It further reports on the associated exposure and health risk assessment pattern upon consumption so as to provide useful information for better management through improved agronomic practices used for tea cultivation.

2. MATERIALS AND METHODS

2.1 Sample description

Twelve crush, tear and curl (CTC) tea samples were procured in February 2015 from the local market of Assam, India (sample ID: IND 1 to IND 6) and a tea producing factory at Thohoyandou, South Africa (sample ID: SA 1 to SA 6) that are widely consumed in both countries and used for this study. All the black tea samples were produced from different tea cultivars however, the manufacturing process was similar.

2.2 Reagents

Analytical grade reagents procured from Merck Millipore India Pvt. Ltd (Mumbai, India) were used throughout the investigation. High quality water (18.2 MΩ/cm resistivity) obtained from a Milli-Q system (Millipore, USA) was also used. Copper (1000 mg L^{-1} Cu) standard solution traceable to SRM from NIST $Cu(NO_3)_2$ in 0.5 mol L HNO_3; Fe (1000 mg L^{-1} Fe) standard solution traceable to SRM from NIST $Fe(NO_3)_3$ in 0.5 mol L HNO_3 and zinc (1000 mg L^{-1} Zn) standard solution traceable to SRM from NIST $Zn(NO_3)_2$ in 0.5 mol L HNO_3 that were all procured from CertiPUR® AAS Standards, USA were used for the preparation of calibration graph in atomic absorption spectrophotometer (AAS) to measure Cu, Fe and Zn in samples, respectively. All the standard solutions were prepared by successive dilutions to the required concentration. Soil SRM-2710 (Montana soil) and sediment BCR-144 (sewage sludge) were used as standard reference materials for quality control.

2.3 Digestion of tea samples

For the analysis of total Cu, Fe and Zn in tea samples, the tri-acid (concentrated HNO_3:H_2SO_4:$HClO_4$:10:4:1) mixture was used as described by Karak et al [12]. In brief, 0.2 g of tea sample was placed into 100 mL Erlenmeyer flask and 5 mL of tri-acid mixture was added to it. This mixture was heated for 45 minute at 85°C on a hot plate until the the sample was completely digested. The digested sample solution was allowed to cool down under room temperature, diluted with distilled water and filtered through Whatman no. 1 filter paper (Whatman Ltd., England) into 100 mL polycarbonate volumetric flask. The volume was adjusted to 100 mL by adding distilled water. A blank digest was carried out in the same way. The contents of Cu, Fe and Zn were determined by AAS (model: AA240, Agilent, Malaysia).

2.4 Preparation of tea infusion

Standard infusion methodology commonly used in India and South Africa was used for the preparation of tea infusion. Two gram (2 g) of dried tea sample was infused by adding 50 mL boiled distilled water in 100 mL porcelain beaker and it was kept covered with the lid for 5 minutes with intermittent shaking for proper wetting. After 5 minutes, the solution was filtered using filter paper (Whatman No. 1) and decanted into 50 mL volumetric flask and volume was adjusted to 50 mL using same water that was used for the tea infusion. Subsequently, the tea residue left after the first infusion was used for making the second and third infusions following the protocol described by Mehra and Baker [16] but 50 mL boiled distilled water was used to make the tea infusion. The volume of filtrate was made up to 50 mL by adding distilled water and was stored in air-tight container in refrigerator by adding 0.05 mL analytical grade chloroform to prevent microbial growth. Tea infusion was prepared to estimate the actual amount of Cu, Fe and Zn that can reach the human body through drinking tea infusion. Consumption of tea infusion though repeated infusion procedure is a common practice in India as well as in South Africa, therefore, repeated infusion was done for this present experiment. The contents of Cu, Fe and Zn in tea infusion were determined by AAS. The three standards for each of Cu, Fe and Zn as earlier mentioned ~~were~~ used in the study for the preparation of calibration curve for Cu, Fe and Zn were prepared from the stock standards for each ~~metal~~ element ion including a blank. The ion concentrations for each element therefore were 0, 0.2, 0.4, 0.6 and 0.8 µg/g. All results described in the present study are on the basis of dry mass. For ~~metal~~ the determination of each element, two of the three replicates of all ~~the~~ samples of made tea and tea infusion were analysed. The third replicate sample was analysed only if the results of the two replicates were not within an acceptable range of precision (i.e. relative error of <5% for high contents and <15% for low contents).

2.5 Quality control

In order to ascertain the accuracy of analytical results, ~~the~~ standard reference materials (SRM-2710 and BCR-144) were analysed following the same digestion and analytical procedure as those of samples. The obtained values were in good agreement with the certified values. Recoveries from soil sample SRM-2710 were good with an average value of 99.6% for Fe (21.71 mg kg^{-1}), 109.8% for Cu (3245 mg kg^{-1}), and 99.3% for Zn (6903.34 mg kg^{-1}). The BCR-144 also presented good recoveries with an average of 98.6% for Fe (1.81 mg kg^{-1}), 103.8% for Cu (311.40 mg kg^{-1}), and 101.2% for Zn (930.03 mg kg^{-1}).

2.6 Parameters for risks assessment

2.6.1 Average daily intake (ADI) of Cu, Fe and Zn

The ADI of Cu, Fe and Zn was calculated to assess the daily exposure of individual, which is dependent on both the micronutrient content in the infusion and the volume of tea consumed. Average daily intake was calculated following the equation [17]:

$$ADI = Ci \times Ai / Az$$

where: Ci is the content of Cu, Fe and Zn in tea infusion, Ai is the average daily intake rate of tea and Az is the average body weight (BW) of men and women. The calculation was based on the assumption that the average body weight of men and women is 67.4 kg and 64.9 kg, respectively while Ai of tea infusion was 0.6 L d^{-1} [12].

2.6.2 Hazardous Quotient

Hazardous quotient (HQ) was used to explain whether or not, excessive and regular consumption of tea infusion containing micronutrients possesses carcinogenic health risk potential ~~or not~~. Hazardous quotient was calculated by the following equation [18]:

$$HQ = ADI/RfD$$

where: ADI is the average daily intake of the micronutrient in mg $kg^{-1}d^{-1}$ and RfD is the reference dose of a particular micronutrient measured in mg $kg^{-1}d^{-1}$. The RfD values for Cu, Fe and Zn are 0.04 mg kg^{-1} BW d^{-1}; 0.7 mg kg^{-1} BW d^{-1} and 0.3 mg kg^{-1} BW d^{-1}, respectively [19].

2.7 Statistical Analysis

Both parametric and non-parametric tests were performed to evaluate the significant difference in Cu, Fe, Zn contents of made tea and the three consecutive infusions prepared from the Indian and South African tea samples collected. Test for the homogeneity of variances was also performed using both F- and t-tests. However, since the homogeneity of variance assumption of the three metals in the tea samples and their infusions was violated, t-test with Cochran approximation was performed to compare the two different tea sources.

Testing normality of the data was performed following Kolmogorov-Smirnov test originally developed by Cramér [20] and Mises [21] and further adapted by Anderson and Darling [22] and Watson and Freeman [23]. The original test statistic, W^2, Anderson's A^2, and Watson's U^2 are:

$$W^2 = n \int_{-\infty}^{\infty} \left[F_1(x) - F_0(x)\right]^2 d\,F_0(x)$$

$$A^2 = n \int_{-\infty}^{\infty} \frac{\left[F_1(x) - F_0(x)\right]^2}{F_0(x) - \left[F_0(x)\right]^2} \, d\,F_0(x)$$

$$U^2 = n \int_{-\infty}^{\infty} \left[F_1(x) - F_0(x) - W^2\right]^2 d\,F_0(x)$$

The hypothesis regarding the distributional form is rejected at the chosen significance level if the test statistics, W^2, A^2 and U^2 are greater than the critical values.

The equality of variance test, hypothesis testing with two independent samples, the Mann - Whitney U test and the Kruskal-Wallis test [24] ~~have been~~ were performed for testing the equality of variances of two normal populations. All the statistical analyses of data including those described below were performed with SAS software version 9.3.

3. Results and Discussion

3.1 Copper ~~(Cu)~~ in made tea

A wide variation in total Cu content (2.3±0.01 to 48.8±4.02 mg kg^{-1}) between South African and Indian tea was observed (Table 1). Moreover, the ~~copper~~ Cu content varied significantly ($p<0.05$) between the two countries. Copper content in Indian tea varied from 35.0±2.02 (IND 5) to 48.8±4.02 (IND 3) mg kg^{-1} with mean content of 40.22±1.28 mg kg^{-1} while in South African tea, it ~~was~~ varied from only 2.3±0.01 (SA 1 and SA 3) to 4.5±0.03 (SA 4) mg kg^{-1} with mean content of 2.96±0.27 mg kg^{-1}. The measured Cu values in Indian tea were higher than the range of between 10.0 and 25.0 mg kg^{-1} reported in Sri Lankan tea by Ramakrishna et al. [25], while the reverse was true for the South African tea. Data reported in the present study ~~is~~ are comparable with the reported values described by Kumar et al. [26] for Indian and US tea.

None of the 12 tea samples has Cu content that exceeded the prescribed upper limits in tea reported from different countries such as India, Australia, the United Kingdom and the United States (150 mg kg^{-1}; [26]), China (60 mg kg^{-1}; [27]), Japan (100 mg kg^{-1}; [28]) and Turkey (120 mg kg^{-1} [29]). However, the Indian tea samples (IND 1 to IND 3) exceed the permissible limit of Cu in made tea prescribed in Germany (40 mg kg^{-1}; [30]), USEPA's health criteria (30 mg kg^{-1}; [31]), WHO Provisional guideline limit (2 mg kg^{-1}; [32]) and Kenya (30 mg kg^{-1} as set by the Kenya Bureau of Standards KS 65: 2009). The significantly lower Cu contents in tea samples collected from South Africa could be due to non-application of chemical fertilizers and copper based fungicides over the last ten years at the tea garden in Thohoyandou (Estate Manager, personal communication). Based on the aforesaid, it could be stated that the higher Cu contents ~~of Cu~~ in Indian tea may be related to the phosphatic fertilizers and possible over application of copper fungicides that play a crucial role ~~of~~ in Cu accumulation in tea plants [2, 33].

3.2 Iron ~~(Fe)~~ in made tea

Among the three micronutrients reported in the present study, Fe was found to be the most abundant in all the tea leaf samples followed by Zn and Cu (Table 1). The content of Fe ranged between 91.4±8.02 (IND 2) and 148.2±11.2 (IND 4) mg kg^{-1} with an average of 106.55±16.72 mg kg^{-1} in Indian tea samples but ~~ranged~~ varied from 14.8±7.12 (SA 3) to 140.0±16.02 (SA 2) mg kg^{-1} with an average of 98.88±22.85 mg kg^{-1} for the South African tea samples. The abundance of Fe in tea has also been reported by Görür et al. [34]. The mean ~~Fe~~ content of Fe measured in all the tea samples was lower than the permissible limit in tea or other foods that were earlier reported from various countries. For instance, Australia and New Zealand (110 mg kg^{-1}; [35], Australian Legal Requirements (350 mg kg^{-1}; [36]), Brazil (150 mg kg^{-1}; [36]) India (150 mg kg^{-1}; [13]), Sri Lanka (150 mg kg^{-1}; [13]), Thailand (133 mg kg^{-1} ; [36]) and WHO (50-150 mg kg^{-1}; [37]) but higher than some developed countries (100 mg kg^{-1}; [38]) and Turkey (20 mg kg^{-1};[36]). The present results are in line with Street et al. [39] who reported a mean content of 146.9 mg Fe kg^{-1} in Indian tea.

Comparatively higher amount of Fe in Indian than South African tea could be due to such factors as the age of the tea leaves, differences in soil conditions, rainfall, altitude, genetic makeup of the plants as well as manufacturing practices in India. Desideri et al. concluded that the variation ~~of~~ in Fe content among tea samples is mainly attributed to the differences in the degree of fermentation and the plant available Fe content in the soil on which the plants were cultivated [1]. Furthermore, Fe content in tea ~~quality~~ was found to vary with varied realm of production and manufacturing as reported in different studies [40].

3.3 Zinc ~~(Zn)~~ in made tea

Total Zn content in made tea samples (Table 1) differed significantly ($p<0.05$) between the two countries. The range and mean of Zn contents in Indian tea samples were from 54.7±0.01 (IND 1) to 106.7±0.02 (IND 4) mg kg^{-1} and 74.2±0.78 mg kg^{-1}, respectively. For the South African tea samples, the range and mean values of Zn measured were from 28.5±0.01 (SA 2) to 34.3±0.01 (SA 5) mg kg^{-1} and 31.47±1.28 mg kg^{-1}, respectively. The total Zn content in all tea samples was higher than the reported permissible limit of Zn in tea or in other food samples from China (20 mg kg^{-1}; [41]) , International Turkish Standard (50 mg kg^{-1}; [36]) but lower than the maximum permissible level (250 mg kg^{-1}) established by Brazilian Ministry of Health [36], Ministry of Public Health, Thailand (667 mg kg^{-1}; [36]), Australian Legal Requirements (750 mg kg^{-1}; [36]) and World Health Organisation (200-500 mg kg^{-1}; [32]). Comparatively lower Zn content (36.6 ± 0.7 mg kg^{-1}) in various black tea leaf samples was reported by Matsuura et al. [42].

The ~~S~~significant variation in Zn content in tea samples observed in present study can be attributed to several factors such as the age of the plant when harvested, the genetics of the plant, soil conditions, rainfall and altitude [12]. Results from current study are in agreement with the findings from the work by Görür et al. [34] where the total Zn in made tea collected in Turkey was reported to range from 23.59 to 120.46 mg kg^{-1}. On the other hand, ~~a~~ much higher Zn content~~s~~ of 147.5 mg kg^{-1} ~~have been~~was reported by Narin et al. [29].

■ **TABLE 1** ➢

3.4 Elements content (Cu, Fe and Zn) in tea infusion

Made tea is not consumed as a whole but as a water extract that is often referred to as tea infusion. Hence, tea infusion often represents a major source of essential and nonessential minerals for the human body. The contents of Cu, Fe and Zn in tea infusions are shown in Figs. 1A, B and C, respectively.

3.4.1 Copper in tea infusion

Fig 1A showed that the amount of Cu in different infusion steps differs significantly ($p<0.05$) irrespective of tea samples. The present findings are in agreement with previous different reports that revealed 0.96 to 22% of total Cu was released through tea infusion [43, 44]. Similarly lower amount of Cu extraction from made tea had been reported by Chand et al. [45] where the content of Cu dissipation from tea to infusion ranged from 0.2 to 1.8%. Notwithstanding, the infusions produced from South African tea provided better source of Cu than Indian tea.

The content of Cu in tea infusion depends on infusion time and the solubilities of Cu, which was significantly ($p< 0.01$) higher in the first infusion than in the second infusion and similarly higher significantly in the second infusion than in the third infusion ($p < 0.01$) [16]. However, in the present study we did not find any consistent trend for Cu release pattern in the tea infusions from made tea evaluated. Available literatures reported that Cu level in tea infusion ranged from 0.12 to 0.84 mg L^{-1} with the mean value of 0.42 mg L^{-1} [44], 0.05~~±0.01~~ to 0.21~~±0.02~~ mg L^{-1}[40] and 2.04~~ ± 0.14~~ to 7.93~~ ± 2.18~~ mg L^{-1}[34]. The Cu values in the tea infusions in the current study were in agreement with those reported in the literatures [2, 43]. Presently, the maximum allowable limit of Cu in tea infusion is 1 mg L^{-1} [2]. The Cu content in the first infusion produced from IND 6, SA 4 and SA 5 tea samples as shown in Fig. 1A exceeded the permissible limit of Cu. Therefore, it is advisable that such tea samples with high Cu content in the first infusion should be ~~properly labelled from the factory and~~

consumed with caution since excessive consumption of Cu from food and beverages could be detrimental to human health causing stomach upset, diarrhea, anemia, and can lead to tissue injury[46]. This is particularly important because of the potential presence of other beneficial compounds such as polyphenols and flavonoids in the tea samples that may also be in the first infusion containing high Cu content. Hence, either moderate dilution of the first infusion or the reduction of the infusion time may help to reduce the high Cu ~~concentration~~ content and the potential associated risk.

3.4.2 Iron in tea infusion

The Fe release pattern from the tea samples as contained in the three repeated infusions is shown in Fig. 1B. The amount of Fe in different infusion steps differs significantly ($p<0.05$) irrespective of tea samples. Similar to Cu, there were significant differences ($p<0.05$) in the Fe content of the different infusions as well as the tea origin. Notwithstanding, the first and third infusions from South African tea serve as better Fe source than Indian tea. The amount of extractable Fe in the infusions was comparable to the results obtained by several research findings [2, 13, 33]. Wróbel et al. [47] reported that only 4.3% of the total Fe can be infused from tea despite the high total Fe content in tea samples. This observation is in agreement with the results of the current study where Fe-extractability in the three infusions was about 2.46% and 3.73%, respectively in the Indian and South African tea samples. Furthermore, Lasheen et al. [48] reported that among the seven tea samples from Egypt, Fe content in tea infusion ranged from 3.34 to 20.12 mg L^{-1} when tea infusion was produced from 2 g tea with 100 mL of hot distilled water for 5 min; which is in agreement with the results obtained from the present study. However, the Fe contents in the tea infusions obtained in the present study were higher than the average content~~s~~ of 0.006 ± 0.002 mg L^{-1} in tea infusion consumed in UK as reported by Powell et al. [49].

3.4.2 Zinc in tea infusion

Fig 1C showed that the amount of Zn in the different infusion steps differ significantly ($p<0.05$) irrespective of tea samples. Nookabkaew et al. [44] and Wong et al. [50] reported that the solubility of Zn in the first infusion was significantly ($p<0.01$) higher than that of the second infusion while the solubility in the second infusion was also higher significantly ($p<0.05$) than the third infusion. However no definite trend in Zn release pattern was observed in the present study as the number of infusion increases. Notwithstanding, the first and third infusions produced from South African tea contains better source of Zn than the Indian tea. The Zn contents in the tea infusions were lower than the previously reported mean ranges of 3.35 ~~± 0.919~~ - 8.35 ~~± 1.06~~ mg L^{-1}[10] and 37.7 ~~± 2.1~~ - 47.3 ~~± 2.0~~ mg L^{-1}[51]. The Zn results from the current study are at variance with other documented work in the literature such as 0.12 - 0.60 mg L^{-1}; mean: 0.32 mg L^{-1}[52]; 0.59-4.32 mg L^{-1}[44, 53]; 0.19 ± 0.08 mg L^{-1}[51] and 0.070 ~~± 0.004~~ - 0.441 ~~± 0.006~~ mg L^{-1}[40] where values were almost ten times higher.

In general, the decrease in elemental content in the tea infusions as compared to the total element in tea leaves could be attributed to chelation of these elements with tannic acid and tannins which exudates during the boiling of tea particles. Precipitation of these chelates may have ~~therefore~~ contributed to the noticeable decrease in ~~metal~~ element content in the brew extract [52]. In teas with lower tannin levels, better leaching of elements into infusion was observed [10].

■ **FIG. 1** ➢

3.5 Human risk assessment and statistical interpretation of the micronutrients content in the infusions

The average daily intake (ADI) and hazardous quotient (HQ) values of Cu, Fe and Zn are shown in Figs 2, 3 and 4, respectively. The mean values for Indian and South African teas in the 1st, 2nd and 3rd infusions along with the percentages of each element released into the infusions are also presented in Table 2. It should be noted that the ADI values for Indian and South African teas differ greatly due to variation in the extraction percentages of the analysed trace elements which depend on the tea origin.

■**FIG.2**➢

■**FIG.3**➢

■**FIG.4**➢

■**TABLE 2**➢

The results revealed that the contribution of tea towards the average daily dietary intake values of Fe and Zn are high when compared to Cu, which may be attributed to the high contents of Fe and Zn in the analysed tea samples. Similarly, the results clearly showed that HQ values for Cu, Fe and Zn in the different infusions were generally less than one suggesting that these ~~metals~~ trace element contents in the infusions are safe for humans and do not pose any carcinogenic risk [54]. According to Joint FAO/ WHO Expert Committee on Food Additives [55], the provisional tolerable daily intake (PTDI) values for Cu, Fe, and Zn are 3 mg kg^{-1} BW day^{-1}, 48 mg kg^{-1} BW day^{-1}, and 60 mg kg^{-1} BW day^{-1}, respectively. Therefore, regular consumption of tea such as 600 mL tea infusion produced from 24 g tea per day can potentially contribute about 1 to 5%, 4.6 to 12.3% and 7.8 to 9.1% of the daily dietary requirements of Cu, Fe and Zn, respectively. According to Powell et al. [49], Cu content in 2 min infusion from 12.4 g tea in one litre water provided 2.0% of average daily dietary intake. Mehra and Baker [16] showed that 'available' Cu obtained from drinking 1 L of tea per day provided 2.88% (loose tea) and 2.39% (tea bag tea) of the average daily dietary intake.

The results of normality test for the contents of the three ~~metals~~ trace elements assessed in the made tea samples and their infusions are shown in Table 3 while the outcomes of the F- and t-tests are presented in Table 4. The tests results revealed that the Fe content of tea samples from both countries as well as in the three consecutive infusions followed the non-normal distribution indicating the inappropriateness of t-test.

Except for the Fe content and its infusions, the two sources of tea are significantly different indicating that as far as Fe content and its infusions are concerned, there is no statistical difference between Indian tea and South African tea. Whereas Cu and Zn contents in the tea samples from the two countries along with their infusions differed significantly. Results of normality test for ADI for men and women carried out are given in Table 5. Whereas the calculated ADI values for Cu based on the first infusion of the Indian tea and the second infusion of South African tea samples as well as the Fe and Zn contents in the first infusion of South African tea samples satisfied the normality assumption, the normality assumption was violated in all other cases. The results of F-test for testing homogeneity of variances and t-test for testing the differences in ADI values of the tea samples from the two countries are reported in Table 6. The homogeneity of variance test for both men and women ADI ~~is~~ are unequal in all other cases except for Cu in the second infusion indicating that the assumption was violated. Hence, the nonparametric tests of Mann Whitney U and Kruskal Wallis were performed with the results shown in Table 7. A perusal of Table 7 reveals that all the pairs are significantly different.

■ **TABLE 3**➢

■ **TABLE 4**➢

■ **TABLE 5**➢

■ **TABLE 6**➢

■ **TABLE 7**➢

Conclusion

~~A w~~Wide range of Cu, Fe and Zn contents in made tea and tea infusions were found in the Indian and South African tea samples evaluated. The difference in micronutrients content in made tea could be attributed to the tea being produced in different tea gardens with variable soil micronutrients content as well as micronutrient application strategies, which resulted in differential micronutrients uptake by tea plants. It is noteworthy that Cu, Fe and Zn contents in the first infusion are significantly higher ($p<0.05$) than those in the subsequent infusions except for Fe content in the Indian tea. The estimated daily intakes for the studied micronutrients based on the body weight of an average adult (67.4 kg and 64.9 kg body weight for men and women, respectively) were below the limits set by FAO/WHO for Cu, Fe and Zn intake. In conclusion, this study provides quantitative data on Cu, Fe and Zn to judge the potential risk assessment of consumer exposure to these expected ~~metals~~elements. The results of this study thus suggest that the consumption of three 200 mL cups of tea infusion per day will not constitute any adverse effect on human health but rather, a benifical effect since only a marginal portion of the average dialy body ~~metal~~ micronutrients dietary requirements are met. However, it is suggested that further studies should be carried out to incorporate more tea samples collected from both countries in the elemental analysis. Such surveys should be carried out regularly to promptly ascertain the health risks of consumers regarding micronutrients and ~~heavy metal~~trace elements toxicity considering changing farmers' practices due to peculiar socio-economic realities and production constraints across the two countries. Human bioavailability studies for specific ~~metals~~ elements may also be useful to characterise their risks to consumers accurately.

Conflict of interest statement

We firmly believe that the present work has not been influenced by any of the financial and personal relationships with anyone or with industries. We declare that there is no conflict of interest.

Acknowledgement

Authors acknowledge the joint financial support for this research provided by the Department of Science and Technology (DST), Ministry of Science and Technology, Govt. of India (DST/INT/South Africa/P-11/2014) and the National Research Foundation (NRF), Republic of South Africa (Grant UID: 90762). Fund received for procurement of AAS by National Tea Research Foundation (NTRF), Tea Board and the Government of India (Project Code No. 151/2011) is herein duly acknowledged. Finally, our sincere thank is extended to the anonymous reviewers for their valuable and constructive comments on this manuscript that have helped us to greatly improve its quality.

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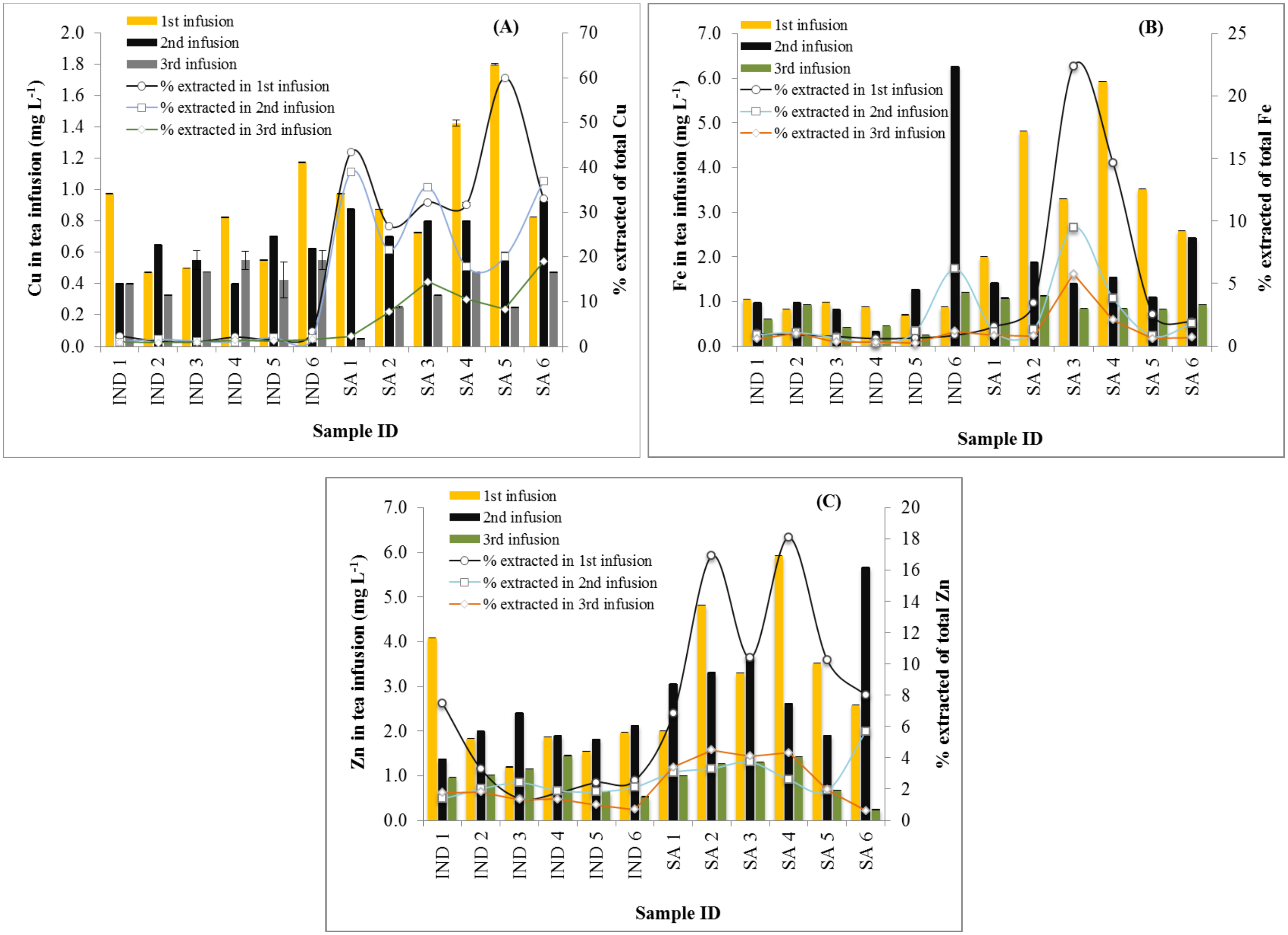
(A)
1st infusion
2nd infusion
3rd infusion
% extracted in 1st infusion
% extracted in 2nd infusion
% extracted in 3rd infusion
Cu in tea infusion (mg L-1)
% extracted of total Cu
Sample ID
IND 1
IND 2
IND 3
IND 4
IND 5
IND 6
SA 1
SA 2
SA 3
SA 4
SA 5
SA 6
(B)
Fe in tea infusion (mg L-1)
% extracted of total Fe
(C)
Zn in tea infusion (mg L-1)
% extracted of total Zn

Fig. 1.

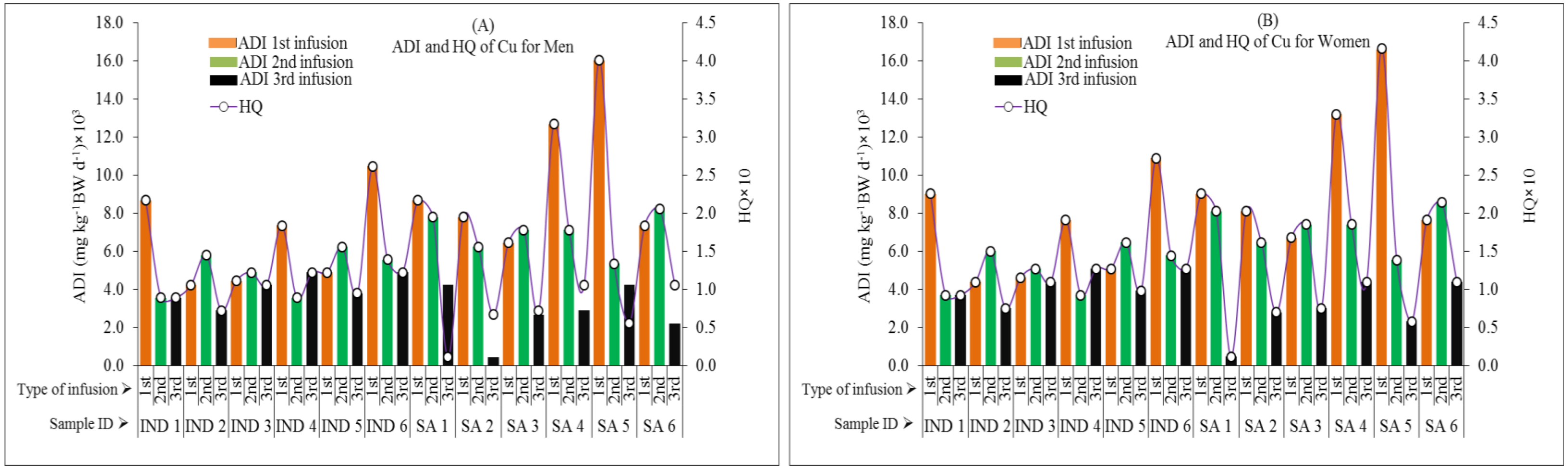
(A)
ADI and HQ of Cu for Men
ADI 1st infusion
ADI 2nd infusion
ADI 3rd infusion
HQ
ADI (mg kg⁻¹BW d⁻¹)×10³
HQ×10
Type of infusion
Sample ID
IND 1
IND 2
IND 3
IND 4
IND 5
IND 6
SA 1
SA 2
SA 3
SA 4
SA 5
SA 6
(B)
ADI and HQ of Cu for Women

Fig. 2.

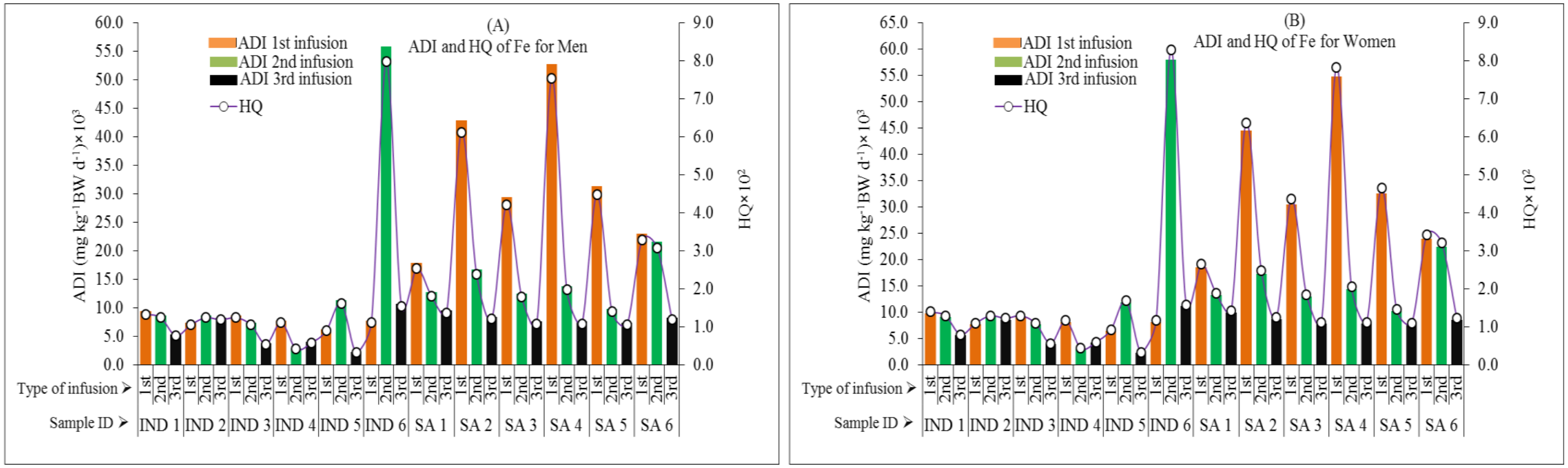
(A)
ADI and HQ of Fe for Men
ADI 1st infusion
ADI 2nd infusion
ADI 3rd infusion
HQ
ADI (mg kg⁻¹BW d⁻¹)× 10³
HQ× 10²
Type of infusion ➢
Sample ID ➢
IND 1
IND 2
IND 3
IND 4
IND 5
IND 6
SA 1
SA 2
SA 3
SA 4
SA 5
SA 6
(B)
ADI and HQ of Fe for Women

Fig. 3.

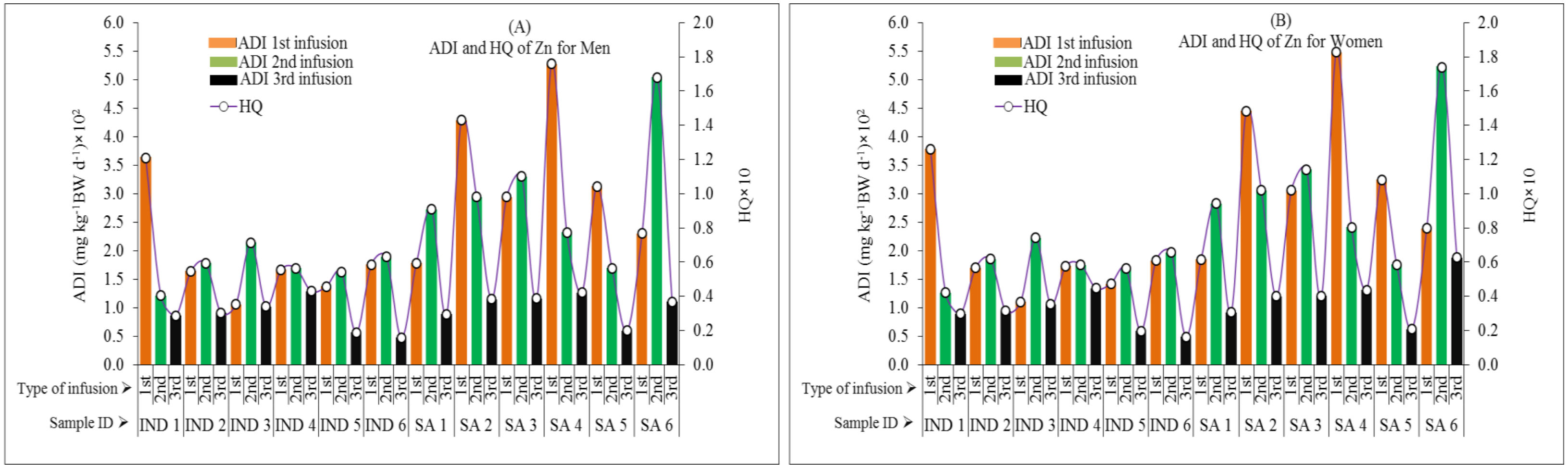
(A)
ADI and HQ of Zn for Men
(B)
ADI and HQ of Zn for Women
ADI 1st infusion
ADI 2nd infusion
ADI 3rd infusion
HQ
ADI (mg kg⁻¹BW d⁻¹)× 10²
HQ× 10
Type of infusion
Sample ID
1st
2nd
3rd
IND 1
IND 2
IND 3
IND 4
IND 5
IND 6
SA 1
SA 2
SA 3
SA 4
SA 5
SA 6

Fig. 4.

LIST OF TABLES

Table 1

Total Cu, Fe, and Zn content (mg kg^{-1}) in tea samples (values represent mean of three replications ±SE, same letter(s) in a particulars column represent non-significant difference between the samples on the basis of DMRT statistical analysis)

Sample ID[*]	Cu[‡**]	Fe[‡‡***]	Zn [‡‡‡****]
IND 1	41.5±1.01[a]	107.5±11.02[a]	54.7±0.01[a]
IND 2	40.5±3.01[a]	91.4±8.02[a]	55.9±0.02[a]
IND 3	48.8±4.02[a]	127.7±9.02[a]	85.7±0.02[b]
IND 4	40.0±1.02[a]	148.2±7.12[a]	106.7±0.02[b,c]
IND 5	35.0±2.02[a]	109.0±8.02[a]	63.8±0.02[a,b]
IND 6	35.6±3.02[a]	101.7±11.02[a]	77.3±0.01[a,b]
Mean IND	40.22±2.04	114.23±8.34	74.00±8.21
SA 1	2.3±0.01[b]	126.8±14.02[a]	29.2±0.01[d]
SA 2	3.3±0.02[b]	140.0±16.02[a]	28.5±0.01[d]
SA 3	2.3±0.01[b]	14.8±0.01[b]	31.8±0.02[d]
SA 4	4.5±0.03[b]	40.5±3.02[c]	32.8±0.01[d]
SA 5	3.0±0.01[b]	137.8±12.01[a]	34.3±0.01[d]
SA 6	2.5±0.01[b]	133.5±11.02[a]	32.4±0.02[d]
Mean SA	2.96±0.35	98.88±22.85	31.47±0.91
Overall mean	21.59±8.07	106.55±16.72	52.73±10.64

[*] SA stands for South Africa and IND stands for India

[‡**]Minimum detection limits of Cu is 2 μg L^{-1}

[‡‡***]Minimum detection limits of Fe is 5 μg L^{-1}

[‡‡‡****]Minimum detection limits of Zn is 10 μg L^{-1}

Table 2

The mean concentration (mg L^{-1}) of Cu, Fe and Zn in the 1st, 2nd and 3rd infusions (values represent mean of three replications ±SE, same letter(s) in a particulars column represent non-significant difference between the samples on the basis of DMRT statistical analysis)

Element	Infusion step	Indian tea (n=6)	South African tea (n=6)
Cu	1st	0.750±0.002[a]	1.104±0.005[b]
	2nd	0.554±0.012[a]	0.783±0.003[b]
	3rd	0.454±0.04 [b]	0.304±0.002[b]
Fe	1st	0.883±0.002[a]	3.692±0.001[b]
	2nd	1.775±0.001[a]	1.629±0.002[b,c]
	3rd	0.642±0.002[a]	0.942±0.001[a,b]
Zn	1st	2.085±0.002[a]	3.692±0.001[b]
	2nd	1.936±0.001[a]	3.376±0.002[b,c]
	3rd	0.958±0.001[a]	0.984±0.003[a]

Table 3

Testing the normality of metal content data in Indian and South African tea.

Goodness-of-Fit Tests for Normal Distribution																
Test	Cu				Percentage 1st infusion				Percentage 2nd infusion				Percentage 3rd infusion			
	India		South Africa		India		South Africa		India		South Africa		India		South Africa	
	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value
K-S	0.118	>0.15	0.204	>0.150	0.147	>0.140	0.322	0.049	0.173	0.050	0.322	0.049	0.095	>0.150	0.155	>0.150
C-V	0.054	>0.25	0.065	>0.250	0.044	0.270	0.101	0.087	0.110	0.082	0.101	0.087	0.043	>0.250	0.027	>0.250
A-D	0.384	>0.25	0.427	0.209	0.314	0.302	0.568	0.082	0.600	0.108	0.568	0.082	0.288	>0.250	0.175	>0.250
	Fe				Percentage 1st infusion				Percentage 2nd infusion				Percentage 3rd infusion			
K-S	0.210	<0.010	0.357	0.017	0.313	<0.010	0.357	0.018	0.350	<0.010	0.313	0.065	0.281	<0.010	0.348	0.022
C-V	0.181	0.009	0.144	0.021	0.670	<0.005	0.135	0.027	0.792	<0.005	0.136	0.025	0.574	<0.005	0.174	0.008
A-D	1.058	0.008	0.763	0.022	3.707	<0.005	0.721	0.029	4.315	<0.005	0.768	0.021	3.175	<0.005	0.947	0.006
	Zn				Percentage 1st infusion				Percentage 2nd infusion				Percentage 3rd infusion			
K-S	0.332	<0.010	0.216	>0.150	0.119	>0.150	0.280	0.143	0.116	>0.150	0.246	>0.150	0.108	>0.150	0.238	>0.150
C-V	0.850	<0.005	0.049	>0.250	0.093	0.136	0.074	0.216	0.072	>0.250	0.045	>0.250	0.040	>0.250	0.076	0.202
A-D	4.529	<0.005	0.286	>0.250	0.690	0.066	0.422	0.216	0.497	0.202	0.265	>0.250	0.261	>0.250	0.494	0.132

Table 4

Testing for equality of variance and differences in metal content in Indian and South African tea

Equality of Variances and								
Test			Percentage 1st infusion		Percentage 2nd infusion		Percentage 3rd infusion	
F-test	F-test for testing the difference in Indian tea and South African tea							
	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
	Cu							
	51.71	0.0003	Percentage 1st infusion 418.46	<.0001	Percentage 2nd infusion 680.85	<.0001	Percentage 3rd infusion 416.53	<.0001
	Fe							
	4.67	0.0081	135.06	<.0001	9.12	0.0001	12.88	<.0001
	Zn							
	333.83	<.0001	8.17	0.0003	30.87	<.0001	5.93	0.0021
t-test	t-test for testing the difference in Indian tea and South African tea							
	t Value	Pr > t	t Value	Pr > t	t Value	Pr > t	t Value	Pr > t
	Cu		Percentage 1st infusion		Percentage 2nd infusion		Percentage 3rd infusion	
	6.09	<.0001	-7.26	0.0008	-6.91	0.001	-3.9	0.0114
	Fe		Percentage 1st infusion		Percentage 2nd infusion		Percentage 3rd infusion	
	0.51	0.6267	-1.88	0.1189	-1.42	0.2133	-1.51	0.1900
	Zn		Percentage 1st infusion		Percentage 2nd infusion		Percentage 3rd infusion	
	5.05	<.0001	-4.42	0.0060	-4.95	0.0041	-4.66	0.0045

Table 5

Testing normality of men and women ADI

Goodness-of-Fit Tests for Normal Distribution																								
Test	1st infusion								2nd infusion								3rd infusion							
	Men ADI				Women ADI				Men ADI				Women ADI				Men ADI				Women ADI			
	India		South Africa		India		South Africa		India		South Africa		India		South Africa		India		South Africa		India		South Africa	
	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value	test statistic	P-value
	Cu																							
K-S	0.098	0.074	0.290	<0.010	0.097	0.08	0.290	<0.010	0.220	<0.010	0.206	0.042	0.219	<0.010	0.205	0.045	0.141	<0.010	0.188	0.091	0.144	<0.010	0.187	0.092
C-V	0.110	0.085	0.259	<0.005	0.110	0.085	0.259	<0.005	0.580	<0.005	0.094	0.128	0.581	<0.005	0.094	0.126	0.335	<0.005	0.123	0.049	0.333	<0.005	0.123	0.05
A-D	0.814	0.036	1.442	<0.005	0.810	0.036	1.442	<0.005	2.983	<0.005	0.576	0.119	2.987	<0.005	0.578	0.118	1.810	<0.005	0.908	0.018	1.807	<0.005	0.904	0.018
	Fe																							
K-S	0.282	<0.010	0.217	0.023	0.282	<0.010	0.218	0.023	0.280	<0.010	0.239	<0.010	0.283	<0.010	0.232	0.011	0.237	<0.010	0.325	<0.010	0.237	<0.010	0.324	<0.010
C-V	1.658	<0.005	0.114	0.07	1.660	<0.005	0.114	0.069	2.138	<0.005	0.162	0.016	2.138	<0.005	0.158	0.018	1.398	<0.005	0.360	<0.005	1.397	<0.005	0.359	<0.005
A-D	9.354	<0.005	0.708	0.054	9.360	<0.005	0.709	0.053	11.923	<0.005	0.992	0.01	11.923	<0.005	0.973	0.011	8.185	<0.005	2.028	<0.005	8.181	<0.005	2.021	<0.005
	Zn																							
K-S	0.153	<0.010	0.217	0.023	0.153	<0.010	0.21805	0.023	0.142	<0.010	0.221	0.02	0.141	<0.010	0.224	0.018	0.113	0.019	0.246	<0.010	0.113	0.019	0.242	<0.010
C-V	0.269	<0.005	0.113	0.073	0.269	<0.005	0.11296	0.072	0.239	<0.005	0.145	0.024	0.237	<0.005	0.147	0.024	0.143	0.03	0.211	<0.005	0.144	0.029	0.209	<0.005
A-D	2.133	<0.005	0.699	0.058	2.134	<0.005	0.70079	0.057	1.490	<0.005	0.977	0.011	1.477	<0.005	0.981	0.011	0.906	0.021	1.368	<0.005	0.910	0.021	1.354	<0.005

Table 6

Testing for homogeneity of variances and difference in mean ADI in Indian and South African tea

Test	Equality of Variances and											
	1st infusion				2nd infusion				3rd infusion			
	Men ADI		Women ADI		Men ADI		Women ADI		Men ADI		Women ADI	
	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	Pr > F	F Value	Pr > F	Pr > F
F-test	Cu											
	6.03	<.0001	6.01	<.0001	1.18	0.7229	1.19	0.7209	2.76	0.0028	2.76	0.0028
	Fe											
	4.66	<.0001	4.66	<.0001	6.38	0.0001	6.42	<.0001	32.95	<.0001	32.86	<.0001
	Zn											
	3.20	0.0005	3.21	0.0005	12.04	<.0001	12.01	<.0001	3.01	0.0011	3.02	0.0011
t-test	t-test for testing the difference in Indian tea and SA tea											
	t Value	Pr > t	t Value	Pr > t	t Value	Pr > t	t Value	Pr > t	t Value	Pr > t	t Value	Pr > t
	Cu											
	-3.87	0.0011	-3.87	0.0011	-10.51	<.0001	-10.50	<.0001	3.16	0.0049	3.16	0.0049
	Fe											
	-7.78	<.0001	-7.78	<.0001	-2.67	0.0094	-2.67	0.0095	-4.32	<.0001	-4.33	<.0001
	Zn											
	-4.94	<.0001	-4.94	<.0001	-5.84	<.0001	-5.85	<.0001	-1.67	0.1115	-1.67	0.1106

Table 7

Nonparametric test for equality of means in men and women ADI

Elements	Infusion	ADI	Wilk test statistics	Probability	Kruskal-Wallis Test	Probability
Cu	1st	Men	4.24	<0.0001	18.02	<0.0001
		Women	4.25	<0.0001	18.10	<0.0001
	2nd	Men	6.02	<0.0001	36.31	<0.0001
		Women	6.02	<0.0001	36.31	<0.0001
	3rd	Men	-2.99	0.0028	8.95	0.0028
		Women	-2.99	0.0028	8.98	0.0027
Fe	1st	Men	6.21	<0.0001	38.63	<0.0001
		Women	6.21	<0.0001	38.63	<0.0001
	2nd	Men	4.72	<0.0001	22.35	<0.0001
		Women	4.72	<0.0001	22.35	<0.0001
	3rd	Men	4.74	<0.0001	22.48	<0.0001
		Women	4.74	<0.0001	22.48	<0.0001
Zn	1st	Men	4.84	<.0001	23.47	<0.0001
		Women	4.84	<.0001	23.46	<0.0001
	2nd	Men	5.83	<.0001	34.09	<0.0001
		Women	5.85	<.0001	34.25	<0.0001
	3rd	Men	2.36	0.0183	5.59	0.0181
		Women	2.36	0.0183	5.59	0.0181