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LEVELS OF METALS IN COMMERCIALLY AVAILABLE ETHIOPIAN BLACK TEAS AND THEIR INFUSIONS

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ABSTRACT. K, Ca, Mg, Mn, Fe, Zn, Cu, Co, Cd and Pb contents of commercially available Ethiopian black tea (Wushwush, Gumero, and Black Lion) and their infusions were analyzed by flame atomic absorption spectrometry (FAAS). In black tea leaves the order of metal content was found to be: K (11503–13780 mg/kg) > Ca (3821–4419 mg/kg) > Mg (3219–3538 mg/kg) > Mn (1242–1421 mg/kg) > Fe (319–467 mg/kg) > Zn (20.2–21.6 mg/kg) > Cu (9.1–11.5 mg/kg). Co, Cd and Pb were too low to be detected by the method (FAAS). In tea infusion, prepared in a laboratory condition following Ethiopian tea making procedures, the range of metal contents was found as follows: K (110–124 mg/L) > Mg (14.5–16.3 mg/L) > Ca (10.0–10.7 mg/L) > Mn (8.5–13.4 mg/L) > Fe (0.98–1.56 mg/L) > Zn (0.098–0.100 mg/L) > Cu (0.038–0.063 mg/L). The metal contents of the black tea leaves were higher than those of tea infusions. The extraction was highest for K (47.1%) and lowest for Ca (12.5%).

KEY WORDS: Tea (*Camellia sinensis*), Black tea, Tea infusion, Heavy metals, Flame atomic absorption spectrophotometer (FAAS)

INTRODUCTION

Tea, one of the most popular beverages in the world, is an infusion of the leaves of the tea plant, *Camellia sinensis* which is under the Theaceae family and the Camellia species (*Camellia sinensis*). *Camellia sinensis* consists mainly of two varieties, Camellia sinensis variety sinensis (The China tea plant) and Camellia sinensis variety assamica (The Assam tea plant) [1]. There are three major categories of tea which are distinguished by different processing methods and, consequently, different concentrations of the chemical components in tea. The main types of tea are green tea, oolong tea and black tea. The black tea is a completely fermented (oxidized) tea leaves.

Tea beverage is consumed for centuries [2-4] and it is the second most consumed beverage in the world with an estimated 18–20 billion cups consumed daily. The economic importance of tea backs to a very long years and it is a source of income for many countries including Ethiopia. For instance, it is the leading export crop in Kenya, India and Sri Lanka [5].

The chemical components in black tea have a number of health benefits [6-8]. In addition to the organic components, different minerals and trace metals are present in black tea leaves and their infusions [3]. Many elements, in trace amounts, play an important role in metabolic processes and are essential for our health and their deficiency or excess may cause disease and be harmful to health [9]. In addition to their nutritional value trace metals have also been associated with the flavoring characteristics of tea [10]. Usually the tea beverage (infusion) is consumed by mankind. Therefore, the accurate determination of the trace element content of tea infusion is thus very important in assessing any possible implications for health.

There is a variation in the mineral composition due to different origins of the plant [11]. The metal content of the tea is influenced by the soil composition and local environmental factors

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[3]. For instance, the water-soluble Mn content of soil of the tea field increased with decreasing soil pH.

Several elements, such as Ca, K, Mg and Mn, are present at mg/g level, whereas elements such as Fe, Co, Ni, Cu, Zn and Cd are present at a few µg/g [12]. The quality of tea brands available in the retail market in the Kingdom of Saudi Arabia was assessed based on contents of heavy metals and they possess considerable amounts of heavy metals and Mn was the most abundant in tea leaves (390–900 µg/g) [13].

Tea infusions are tea beverages prepared by brewing tea leaves in hot water. Generally, mineral contents of tea leaves are higher than those of tea infusions. Type of tea, type and temperature of water and length of brewing time have impact on the concentration of the nutrients extracted into the tea infusion [14]. The metals extracted from black tea leaves into tea infusion were also classified as highly extractable, moderately extractable, and poorly extractable [15]. The solubility of heavy metals in tea infusion extracts varied widely and ranged from 0.0–48% [13] and concentrations of Pb and Cd were too low to be detected in tea beverage [13]. K, Ca, Mg and Mn contents are very high in both tea leaves and infusions. Other studies have shown that tea drinking is potentially major source of dietary manganese [16-18].

Some times a toxic trace element or a metal with more than the recommended amount may be found in tea. For instance, Pb concentrations in 1225 tea samples collected in China between 1999 and 2001 varied from < 0.2 to 97.9 mg kg^{-1} dry weight, with 32% of the samples exceeding the national maximum permissible concentration of 2.0 mg kg^{-1} dry weight [19].

Other investigations have also indicated that it is possible to determine speciation of the metals in tea infusion [20]. Mg, Mn and Rb are present as cations and Ca, Fe, Co, Ni, Cu, Zn, Sr and Ba present mainly in cationic form and a certain non-cationic fraction. For Fe, Ni, Cu and Zn, the non-cationic species may be metal-organic complexes [21].

The metal contents of Ethiopian black teas have not been studied and there are no reports that are published so far. The aim of the present study was to determine the levels of K, Ca, Mg, Mn, Fe, Cu, Zn, Co, Pb, and Cd in commercially available Ethiopian black tea using flame atomic absorption spectrophotometer (FAAS) and correlate the amount of metals in the black tea with that extracted into tea infusion. Even though black tea beverage is consumed widely in Ethiopia not much has been said about the metal contents and the health impact of Ethiopian black teas and their infusions. This is the first study on Ethiopian black teas and their infusions and its primary concern is to determine the levels of the metal contents of Ethiopian black teas and the solubility of the metals into their infusion and notice their dietary aspect and implied health impact on tea beverage consumers.

EXPERIMENTAL

Reagents and samples

Three different types of Ethiopian black tea samples (Wushwush, Gumero, and Black Lion) were collected from local market. The Wushwush and Gumero black teas are processed from the Wushwush and Gumero tea plantation, respectively, by Ethio-Coffee & Tea Plantation and Marketing Pvt. Ltd. Co, Ethiopia. The Black Lion black tea is packed by East African Group (Ethiopia) Pvt. Ltd. Co. from the Chewaka tea plantation. These three Ethiopian black tea samples were selected for the study because (i) they are widely consumed and most available in the local market of Ethiopia and (ii) the three tea brands represent the three different tea plantations found at three different locations in Ethiopia.

HNO_3 (69–72%, Fine-Chem Mumbai-391780, India) and 70% $HClO_4$ (A.C.S. reagent, Aldrich, UK) were used for the digestion of both the black tea leaves and black tea infusion samples. Lanthanum nitrate hydrate, 99.9% (Aldrich, USA) was used to avoid the chemical

interference on Ca and Mg in the sample solution. Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Mg, Mn, Pb, K, Ca, Fe, Zn, Cu, Co, Cd (Buck Scientific Puro-Graphic™, USA) were used for preparation of calibration standards and in spiking experiments. Distilled and deionized water was used throughout the experiment for rinsing of the glassware and dilution of sample solution.

Equipment and instrumentation

Electronic blending device (Moulinex, France) was used for grinding and homogenizing the black tea leaf samples. Round bottom flasks (250 mL) fitted with reflux condenser were used in Kjeldahl digestion block (Gallenkamp, England) for the digestion of both the black tea leaves and tea infusion samples. Flame atomic absorption spectrophotometer (Buck Scientific Model 210VGP AAS, East Norwalk, USA) with air-acetylene flame was used for the analysis of the analyte metals.

Pretreatment of black tea leaf samples and infusions

For each of the black tea brands three packages of 100 g each were mixed thoroughly and grinded to fineness using an electronic blender. Three samples from each of the three ground black tea brands were then used for the determination of the total metal content in the black tea leaves.

Even though there are different ways of preparing tea infusion around the world, for this study we used the common practice for serving tea beverage in Ethiopia which is direct boiling of the black tea leaves in boiling water. The black tea infusion was prepared as follows: 100 mL of distilled and deionized water was boiled in a 400 mL Pyrex glass beaker on a hot plate and 2 g of black tea leaves (unground) were added in to the boiling water and allowed to boil for 5 min, then cooled for 5 min and filtered to obtain the pure black tea infusion. Distilled and deionized water was added to make it 100 mL in a volumetric flask. Three such black tea infusions were prepared from each of the three black tea brands and used for the determination of their total metal content.

Digestion of black tea leaf samples

To prepare a clear colorless sample solution that is suitable for the analysis using FAAS different black tea leaves digestion procedures were assessed using the HNO_3 and $HClO_4$ acid mixtures by varying parameters such as volume of the acid mixture, digestion time and digestion temperature. The choice of the optimized digestion procedure was made by observing clarity and colorlessness of the final solution. In addition, the digestion procedure should use the smallest volume of the reagents so that risk of contamination and cost decrease [22]. Among the different digestion procedures, digestion of 0.5 g black tea leaves with 6 mL of 5:1 mixture of concentrated HNO_3 and concentrated $HClO_4$ heated at 300 °C for three hours gave a clear colorless solution and this procedure was chosen for the digestion of black tea leaves throughout this study.

Exactly 0.5 g of black tea leaf sample was transferred quantitatively into a 250 mL round bottom digestion flask. Exactly 6 mL of freshly prepared 5:1 mixture of concentrated HNO_3 and concentrated $HClO_4$ was added to the sample. The sample was swirled gently to homogenize, fitted to a reflux condenser and digested continuously for three hours at a maximum temperature of 300 °C on a Kjeldahl digestion block. The digest was quantitatively transferred to a 50 mL volumetric flask and made up to the mark with distilled-deionized water. Digestion was made in triplicate and hence a total of nine digests were made for the three brands. Digestion of a reagent blank was performed in parallel with the tea leaf samples keeping all digestion

parameters the same. For the analysis of the black tea leaf samples 12 reagent blanks were prepared. All the digested samples were stored in refrigerator until analysis of the 10 elements by the FAAS.

Digestion of black tea infusion samples

Though some papers report digestion of tea infusion with HNO_3 followed by filtering [15], complete digestion was achieved with HNO_3-$HClO_4$ mixture. Among different digestion procedures, digestion of tea infusion sample with 5 mL of 5:1 mixture of concentrated HNO_3 and concentrated $HClO_4$ at 300 °C for two and half hours gave a clear colorless solution and this optimized procedure was chosen.

About 25 mL of the black tea infusion was transferred quantitatively into the 250 mL round bottom flask and heated to reduce volume by evaporation The residue was allowed to cool before the addition of the acid mixture to avoid explosion due to contact between the organic matter and concentrated $HClO_4$. Exactly 5 mL of 5:1 HNO_3 and $HClO_4$ was added, fitted to a reflux condenser and digested continuously for two and half hours at a maximum temperature of 300 °C on a Kjeldahl digestion block. The clear colorless digest was quantitatively transferred to a 50 mL volumetric flask and made up to the mark with distilled-deionized water. A reagent blank was also prepared by digesting 5 mL of 5:1 HNO_3 and $HClO_4$ mixture for two and half hours at 300 °C. Digestion was made in triplicate and hence a total of nine digest for the three brands of black tea infusion samples. All the digested samples were stored in refrigerator until analysis of the 10 elements by FAAS.

Calibration procedure and determination of metals

Calibration curves were prepared to determine the concentration of the metals in the sample solution. Calibration curves for each of the metals were made from diluted solutions prepared from stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals K, Ca, Mg, Mn, Fe, Zn, Cu, Co, Cd and Pb. The correlation coefficients of the calibration curve for the entire analytes were higher than 0.999 which assured a linearity of responses for individual analytes. Analytical wavelengths, especially, for analytes of low concentration were chosen in a manner that they give better sensitivity. Determination of the metals in the black tea leaf samples was made by FAAS. To avoid loss through ionization, the concentrations of K were determined in the emission mode. To avoid chemical interference in the determination of concentrations of Mg and Ca lanthanum nitrate was added to the solutions. Similarly, the tea infusion samples were analyzed by FAAS for their metal content. For each of the black tea leaf and tea infusion samples three repeat measurements were performed. Therefore, for each sample, the results were obtained from the mean of nine measurements.

Method detection limits

Detection limit was calculated based on calculation of the standard deviation of twelve replicate measurements of blank solutions. A detection limit three times the calculated standard deviation of the blanks was used for this study. The method detection limits for tea leaf and tea infusion samples are given in Table 1.

Recovery tests

Efficiency of the optimized procedure was checked by spiking 0.5 g ground black tea leaf sample with 100 µL of 1000 mg/L K and Mn and 50 µL of 1000 mg/L Cu, Zn and Pb at once and 100 µL of 1000 mg/L Fe, Ca, Mg and 50 µL of 1000 mg/L Co and Cd was added into

another digestion flask containing the same black tea leaf sample and the same digestion procedure was followed as black tea leaf samples.

Table 1. Analytical wavelengths, correlation coefficients of the calibration curve and method detection limits for black tea leaf and tea infusion samples.

Metal	Wavelength (nm)	Correlation coefficient of calibration curves	MDL for black tea leaves (μg/g)[a]	MDL for black tea infusions (μg/g)[a]
K	766.5	0.9994	0.002	0.0060
Ca	422.7	0.9990	0.005	0.0006
Mg	285.2	0.9999	0.080	0.0060
Fe	248.3	0.9983	0.070	0.0200
Mn	279.5	0.9993	0.003	0.0020
Cu	324.8	0.9999	0.003	0.0100
Zn	213.9	0.9999	0.003	0.0010
Co	240.7	0.9995	0.003	0.0020
Cd	228.9	0.9996	0.003	0.0010
Pb	283.3	0.9998	0.010	0.0020

[a]MDL: Method detection limit.

Since the concentration of metals in the black tea infusion is lower than that of the black tea leaves, the recovery test for the black tea infusion was done by adding smaller amount of the spiked metals than that of the black tea leaves. About 25 mL black tea infusion sample was spiked with 50 μL of 1000 mg/L K and Mn and 10 μL of 1000 mg/L Cu, Zn and Pb at once and 50 μL of 1000 mg/L Fe, Ca, Mg and 10 μL of 1000 mg/L Co and Cd into another digestion flask containing the same black tea infusion sample and the same digestion procedure was followed as black tea infusion samples. For both sample types the recovery test was performed in triplicate. Each of the samples was then analyzed for its respective spiked metals by FAAS.

The recovery values for the black tea samples and black tea infusion samples for the metals were 91–110 % which are within the acceptable range. Mg (110%) and Cu (110%) showed high percentage of recovery and Fe (91%) showed low percentage of recovery. Even though the exact reason is not known it could be due to the loss in precision that arises from the atomization process. Percentage recovery values for individual analytes are given in Tables 2 and 3.

Table 2. Percentage recovery values for black tea leaf samples.

Metal	Concentration in the sample (mg/kg)[a]	Amount added (mg/kg)	Concentration in the spiked sample (mg/kg)[a]	Amount recovered (mg/kg)	Recovery (%)[b]
K	12330 ± 23	200	12548 ± 27	217 ± 35	109 ± 18
Ca	4505 ± 19	200	4697 ± 15	192 ± 16	96 ± 8
Mg	3541 ± 27	200	3761 ± 6	220 ± 28	110 ± 14
Mn	1240 ± 6	200	1436 ± 4	196 ± 7	98 ± 3
Fe	439 ± 6	200	654 ± 5	215 ± 6	108 ± 3
Zn	28.4 ± 0.5	10	38.2 ± 0.3	9.85 ± 0.58	99 ± 6
Cu	11.3 ± 0.2	10	22.0 ± 0.5	11 ± 0.54	110 ± 5
Co	< MDL[c]	-	-	-	-
Cd	< MDL[c]	-	-	-	-
Pb	< MDL[c]	-	-	-	-

[a]Concentration values are average of three analyzed samples ± standard deviation. [b]Recovery values are mean ± standard deviation. [c]Concentration values of the studied metals were below method detection limit.

Table 3. Percentage recovery values for black tea infusion samples.

Metal	Concentration in the sample (mg/kg)[a]	Amount added (mg/kg)	Concentration in the spiked sample (mg/kg)[a]	Amount recovered (mg/kg)	Recovery (%)[b]
K	6227 ± 7	100	6329 ± 3	102 ± 7	102 ± 7
Ca	550 ± 3	100	647 ± 4	97 ± 5	97 ± 5
Mg	822 ± 1	100	932 ± 5	110 ± 5	110 ± 5
Mn	454 ± 2	100	547 ± 4	93 ± 4	93 ± 4
Fe	107 ± 1	100	198 ± 5	91.4 ± 5.4	91 ± 5.4
Zn	9.17 ± 0.35	10	18.7 ± 0.3	9.56 ± 0.46	96 ± 4.6
Cu	7.67 ± 0.12	10	17.4 ± 0.2	9.73 ± 2.6	97 ± 2.6
Co	< MDL[c]	-	-	-	-
Cd	< MDL[c]	-	-	-	-
Pb	< MDL[c]	-	-	-	-

[a]Concentration values are average of three analyzed samples ± standard deviation. [b]Recovery values are mean ± standard deviation. [c]Concentration values of the studied metals were below method detection limit.

RESULTS AND DISCUSSION

Levels of metals in black tea leaves

The levels of essential and nonessential metals were quantified by FAAS. The levels of metals in the black tea leaves varied widely. Cd and Pb were found to be below the analytical method detection limit, indicating that the commercially available Ethiopian black tea leaves pose no health risk from toxic elements Cd and Pb. On the other hand, the black tea leaves contain the essential elements in appreciable amounts. The concentrations of the metals in each of the three types of the commercially available Ethiopian black tea brands are given in Table 4.

The metal content of tea leaves varies widely since they depend upon the country and area of origin, the mineral content of the soil, and the part and age of the tea plant analyzed [23]. For instance, it was reported that plant nutrient concentrations such as K and Mg in the tea stands decreased in the order: young leaves (and buds) > mature leaves > branches > stems. Nevertheless, some general trends are well established: for example, the predominance of potassium in tea leaves followed by calcium and magnesium was seen in many investigations [24].

Table 4. Levels of metals in three commercially available Ethiopian black tea brands (mg/kg)[a].

Tea brand	K	Ca	Mg	Mn	Fe	Zn	Cu	Co	Cd	Pb
Wushwush	12402±28	4419±35	3538±6	1242±12	319±25	21.2±0.4	9.1± 0.14	<MDL[b]	<MDL[b]	<MDL[b]
Gumero	11503±79	3821±9	3416±33	1297±4	363±3	21.6±0.3	11.5±0.1	<MDL[b]	<MDL[b]	<MDL[b]
Black Lion	13780±33	4118±6	3219±5	1421±4	467±2	20.2±0.1	10.3±0.12	<MDL[b]	<MDL[b]	<MDL[b]

[a]Concentration values are average of three analyzed samples ± standard deviation. [b]Concentration values of the studied metals were below method detection limit.

The ability of the tea plant to accumulate heavy metals, particularly Mn and Fe, to a lesser extent Zn and Cu was investigated on different tea brands from the local market of Kingdom of Saudi Arabia and the results showed that Mn (390–900 µg/g), Fe (23.90–513.00 µg/g), Zn (26.69–53.89 µg/g), Cu (22.12–40.66 µg/g), Pb (0.03–14.84 µg/g) > Co (below detection limit–2.35 µg/g) > Cd (below detection limit) [13]. Other study reported that K (21904–26883 mg/kg) and Ca (3370–4823 mg/kg) were also determined in black tea leaves from Turkey [25]. In

another investigation concentration of Cu in black and green tea leaves available in Lithuania was found in the range 19.8–33.9 mg/kg [26]. Ca (3170–7980 μg/g), Mg (2060–2640 μg/g), Mn (790–1620 μg/g) and Zn (26.3–50.4 μg/g) were also found in tea leaves of Ceylon tea blends and Chinese green tea brands in South Africa [27]. There is also a report on African and Asian tea that were found to contain Ca (3511–3581.68 μg/g), Mg (562.61–1567.91 μg/g), Mn (562.61–843.56 μg/g), Fe (153–178.97 μg/g), Zn (25.06–26.29 μg/g), Cu (12.4–19.34 μg/g), Pb (0.18–1.12 μg/g) and Co (0.21–0.23 μg/g) [3].

Table 5. Comparison of concentrations of metals in the Ethiopian black tea leaves to that reported in the literature.

Origin	Concentration of metal in tea leaves (mg/kg)					Ref.
	K	Ca	Mg	Mn	Fe	
Ethiopia	11503–13780	3821–4419	3219–3538	1242–1421	319–467	Present study
India	17700–24000			371–758		[11]
		4249–4486	2171–2433	551–734		[10]
Sri Lanka		4906	2203	310		[10]
Japan	19200	4550	2070	503	134	[15]
		6261	1847	2678		[10]
Turkey				1107–1871		[20]
	22052–26883		3661–4913			[25]
Poland				286–1290		[29]
Saudi Arabia				68.6–305	123.9–262.2	[13]
China		3769–5089	2247–2823	731–881		[10]
		3224	1653.9	720.18	280.75	[3]
USA	13100-23700			79–768		[11]
Africa		3581	1567.9	562	178	[3]
		3263	2124	842		[10]
Origin	Concentration of metal in tea leaves (mg/kg)					Ref.
	Zn	Cu	Co	Pb	Cd	
Ethiopia	20.2–21.6	9.1–11.5	< MDL[a]	< MDL[a]	< MDL[a]	Present study
India		1.6–35				[11]
	31.3–32.8	21.0–28.4				[10]
Sri Lanka	39.5	24.6				[10]
Japan	36.6	27.7	0.506	0.709	0.018	[15]
	26.7	10.1				[10]
Poland	22.3–28					[29]
Saudi Arabia	26.69-39.1	22.12–40.66	< MDL[a]–2.35		< MDL[a]–0.18	[13]
China	27.9–47.8	13.2–25.0				[10]
	27.85	19.06		2.09		[3]
				0.2–97.9		[19]
USA		4.4–17.3				[11]
Africa	25.06	19.14	0.21	1.12		[3]
	26.7	11.6				[10]
Asia	26.29	12.34	0.23	0.18		[3]

[a]Concentration values of the studied metals were below method detection limit.

The results of the present study are consistent with the results reported by different authors, particularly, the increasing trend of the metals concentrations. The order is K (11503–13780 mg/kg) > Ca (3821–4419 mg/kg) > Mg (3219–3538 mg/kg) > Mn (1242–1421 mg/kg) > Fe (319–467 mg/kg) > Zn (20.2–21.6 mg/kg) > Cu (9.1–11.5 mg/kg) and with concentrations of Co, Pb and Cd being too low to be detected by the method (FAAS). In fact the concentration of the toxic heavy metals are expected to be very low in Ethiopian tea since these metals are

usually related to environmental pollution caused by different industrial activities. The amount of heavy metals in tea leaves has been used as an indication for contamination of the environment by heavy metal particles [28]. The comparison of concentrations of metals in the Ethiopian black tea leaves obtained in the present study to that reported in the literature is given in Table 5.

Levels of metals in black tea infusion samples

The present study shows that the tea infusion (tea beverage) contains K, Ca, Mg and Mn in appreciable amount. It was also found that the essential heavy metals were extracted from the black tea leaves to tea infusion. The amounts of the metals extracted widely differ. K was the highest of all metals in the three black tea brands. The concentrations of metals in the Ethiopian black tea infusion are given in Table 6.

Table 6. Levels of metals in black tea infusions of the three brands (mg/kg)[a].

Tea brand	K	Ca	Mg	Mn	Fe	Zn	Cu	Co	Cd	Pb
Wushwush	5504±71	533±17	723±27	423±34	56±1	4.89±0.3	2.82±0.36	<MDL[b]	< MDL[b]	< MDL[b]
Gumero	5961±16	510±41	757±8	449±2	49±10	5.0±0.2	1.92±0.8	< MDL[b]	< MDL[b]	< MDL[b]
Black Lion	6197±61	500±29	816±12	672±23	78±6	4.97±0.35	3.17±1.1	< MDL[b]	< MDL[b]	< MDL[b]

[a]Concentration values are average of three analyzed samples ± standard deviation. [b]Concentration values of the studied metals were below method detection limit.

Table 7. Comparison of concentrations of metals in the Ethiopian black tea infusion to that reported in the literature[a].

Origin	Concentration of metal in tea infusion (mg/L)					Ref.
	K	Ca	Mg	Mn	Fe	
Ethiopia[a]	110–124	10.0–10.7	14.5–16.3	8.5–13.4	0.98–1.56	Present study
India		2.61–3.12	6.79–9.86	1.24–2.61		[10]
Japan	305	4.03	19.6	5.16	0.18	[15]
		4.11	4.93	4.72		[10]
Turkey				366–421		[20]
Poland				1.56–6.02		[29]
Saudi Arabia				0.66–3.05	0.132–1.35	[13]
China		2.50–2.75	5.12–6.56	1.30–1.69		[10]
Origin	Concentration of metal in tea infusion (mg/L)					Ref.
	Zn	Cu	Co	Pb	Cd	
Ethiopia[a]	0.098–0.100	0.038–0.063	< MDL[b]	< MDL[b]	< MDL[b]	Present study
India	0.132–0.141	0.058–0.68				[10]
Japan	0.288	0.102	0.506	0.709	0.018	[15]
	0.110	0.017				[10]
Poland	0.107–0.121					[29]
Saudi Arabia	0.057–0.287	0.003-0.175	< MDL[b]		< MDL[b]	[13]
China	0.101–0.141	0.019–0.053				[10]

[a]To make the comparison uniform the expression mg/kg was converted to mg/L accordingly. [b]Concentration values of the studied metals were below method detection limit.

Since different research reports employed different approaches to prepare tea infusion samples, direct comparison of the present study may not be practical. Different papers have been published on metal contents of tea infusions. For instance, in tea beverages the concentrations of toxic heavy metals, Pb and Cd and the essential heavy metal, Co, were too low

to be detected [13]. Concentrations of Mn and Fe in tea infusion range between 68.6–305.6 µg/g and 19.0–135.0 µg/g, respectively. Concentrations of Zn and Cu were also found to be 8.55–28.60 µg/g and 0.7–6.95 µg/g, respectively [13]. Mn levels of 0.51±0.11 mg/100 g in black tea infusions were also reported [16].

The results obtained in the present study are in agreement with papers published on this subject. The comparison of concentrations of metals in the Ethiopian black tea infusions obtained in the present study to that reported in the literature is given in Table 7. To make the comparison uniform the expression mg/kg was converted to mg/L.

Efficiency of extraction of metals from black tea leaves into tea infusions

The percentage of metals extracted from the black tea leaves to black tea infusion was found to vary widely. For instance, the largest amount of extraction was found for K (47.1%). Mn (38.6%) was the second highly extracted metal followed by Cu (26.1%), and Zn (23.5). The other three metals were also extracted to significant degrees: Mg (23.6%), Fe (15.9%), and Ca (12.5%). The efficiency of extraction of the metals from black tea leaves to black tea infusion is in the following order: K > Mn > Cu > Zn > Mg > Fe > Ca. Table 8 presents the percentage extraction of individual metals from the tea leaves of the three brands into tea infusion.

Table 8. Percentages of metals extracted from black tea leaves into tea infusions.

Metal	Wushwush (%)	Gumero (%)	Black Lion (%)	Average (%)
K	44.4	51.8	45.0	47.1 ± 4.1
Ca	12.1	13.4	12.1	12.5 ± 0.7
Mg	20.4	21.9	25.4	22.6 ± 2.6
Mn	34.0	34.6	47.2	38.6 ± 7.5
Fe	17.5	13.5	16.8	15.9 ± 2.1
Zn	23.0	23.1	24.6	23.6 ± 0.9
Cu	30.9	16.6	30.8	26.1 ± 8.2

Statistical analysis of data (ANOVA)

Statistical analysis of data was made to check whether there was a significant difference in metal contents between the three commercially available black tea leaves and their infusions. Analysis of variance (ANOVA) is a powerful statistical technique which can be used to separate and estimate the different causes of variation. A one-way ANOVA was used for this study and calculations were made using the Mintab software. For each of the analysis six means (three for each tea sample) were used. The degree of freedom for each of the data analysis was 5. For $p > 0.05$ the difference in the levels of each particular metal in the three Ethiopian black tea leaves was not significant.

The difference in the level of both Ca and Cu in Wushwush and Gumero black tea leaves was found to be significant. In Wushwush and Black Lion black teas the level of Mg was significantly different. It was also the case for Mn and Fe in the two black tea leaf samples. The third comparison was made to find out if there is a significant difference in the amount of individual metals in Gumero and Black Lion black tea leaves and it was observed that the Fe content in the two black tea brands differ significantly.

In Wushwush and Black Lion black tea infusions, except Zn and Cu the level of K, Ca, Mg, Mn and Fe differ significantly. Level of Mn in Gumero and Black Lion black tea infusions was with significant difference as it was the case for Fe. The significant variation in the metal contents of each of the three commercially available Ethiopian black tea leaves as well as their

infusions might arise from the specific environmental conditions in which the tea plant was cultivated [3].

CONCLUSIONS

The levels of metals in commercially available three brands of Ethiopian black tea leaves (Wushwush, Gumero, and Black Lion) and their infusions were determined by flame atomic absorption spectrometry (FAAS). The metal contents of the black tea leaves were found to be higher than those of tea infusions. In both black tea leaves and their infusions levels of Co and the toxic heavy metals (Cd and Pb) were too low to be detected by the method (FAAS) assuring that the commercially available Ethiopian black teas pose no health risk from toxic elements Cd and Pb. The concentrations of metals in the three black tea brands were found in the following order: K (11503–13780 mg/kg) > Ca (3821–4419 mg/kg) > Mg (3219–3538 mg/kg) > Mn (1242–1421 mg/kg) > Fe (319–467 mg/kg) > Zn (20.2–21.6 mg/kg) > Cu (9.1–11.5 mg/kg). The general increasing trend is similar with those reported for many other black tea leaves of other countries.

The contents of metals in black tea infusions of Ethiopian black tea brands were found to be comparable with that published in different literatures. This study confirms that the commercially available Ethiopian black tea could be source of dietary minerals and trace metals. The extraction efficiency of the metals into tea infusion varied widely indicating that these metals exist in tea leaves in different forms. Thus, further research for the speciation of the metals in tea leaves may pave the way for better understanding of the nature of occurrence of the metals.

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