

Fe, Zn, Cu, Pb and Cd in Tea Grown and Marketed in Kenya; A Quantitative Assessment

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Abstract- Accurate quantitation of levels of essential and non-essential elements in tea is of great importance as they are directly related to health and disease. In this study, levels of Iron (Fe), Zinc (Zn), Copper (Cu), lead (Pb) and Cadmium (Cd) in tea grown and marketed in Kenya were quantified by Flame Atomic Absorption Spectroscopy (FAAS). In unprocessed tea, the levels were found to range between 54.6 and 123.3 µg/g for Fe, 15.4 and 37.5 µg/g for Zn, 10.3 and 14.8 µg/g for Cu, 0.12 and 0.28 µg/g for Pb and 10.0 and 27.1 µg/kg for Cd whereas in black tea, the levels ranged between 81 and 369 µg/g for Fe, 17.1 and 44.9 µg/g for Zn, 9.0 and 17.8 µg/g for Cu, 0.12 and 0.41 µg/g for Pb and 9.1 and 40.0 µg/kg for Cd. The general accumulation pattern of these elements was established to be Fe > Zn > Cu > Pb > Cd in both unprocessed and black tea. All tea samples had metal contents within the Maximum Permissible Concentrations (MPC) set for tea, hence safe for consumption.

Keywords- Essential Elements; Non-Essential Elements; FAAS; Black Tea

I. INTRODUCTION

Kenya is a tropical East African country with varied climatical and geographical regions (Gesimba, Langat, Liu and Wolukau, 2005). She is a major black tea producer, her tea industry employing over four million people directly and indirectly. The tea sub-sector is her leading foreign exchange earner and export commodity contributing over 26% and 4% of her foreign exchange earnings and Gross Domestic Product (GDP), respectively (TBK, 2011, 2012).

The chemical composition of tea leaves is very complex (Jha, Mann and Balachandran, 1996). Research has indicated that they contain a myriad of compounds including flavonoids, proteins, amino acids, enzymes, vitamins and a number of trace elements such as Fe, Zn, Cu, Pb and Cd (Li, Wang and Wang, 2005; Sahito, Kazi, Jakharani, Kazi, Shar and Shaikh, 2005). A portion of these compounds are water soluble and lixiviate into the tea liquor during the tea brewing process. Therefore, tea consumers gain by being furnished with essential elements such as Fe, Zn, and Cu which are required for various biological processes (Saud, 2003). However, the consumed liquor also contains traces of some nutritionally non-essential elements such as Pb and Cd (Zerabruk, Chandravanshi, and Zewge, 2010; Tedayon and Lahiji, 2011) that have been associated with the etiology of a number of diseases in humans even at very low concentrations (WHO, 1995; Steenland and Boffetta, 2000). This is because they are not biodegradable and as such bioaccumulate, leading to harmful side effects (Jarup, 2003).

The main source of trace elements to plants including tea is their growth media (Somer, 1974). For instance, tea garden soils are normally acidic (pH 4.5 to 5.5), a condition attributable to the continuous use of nitrogenous fertilizers, NPKS, to boost tea yields (Owuor, Gone, Onchiri and Jumba, 1990). These fertilizers produce hydrogen ions (H⁺) via the reaction;



which is induced by bacteria present in the soil (Ishibashi, Matsuo, Baba, Nagafuchi, Imato, and Hirata, 2004). Therefore, fertilizer application increases the rate of nitrification during which, inorganic nitrogen is converted to nitrate yielding H⁺, hence subsequent acidification of the soil. Though, high acidity enhances the bioavailability and subsequent uptake of some elements by the tea plant, it has been reported that it diminishes for some and remains unaffected for others. For example, extractable aluminium (Al), Zn, and fluoride (F⁻) decreases whereas extractable calcium (Ca), Cu, potassium (K), magnesium (Mg), sodium (Na) and phosphorous (P) increases when the soil pH is raised from 3 to 6 (Fung and Wong, 2002). Agro inputs such as insecticides, herbicides and fertilizers (Fwu-Ming and Hong-Wen, 2008), industrialization (Sobukola, Awonorin, Idowu and Bamiro, 2008) and substandard machinery have also been shown to influence the element content in plants. Research has also indicated that the plant element content is conditional, the levels being affected not only by the soil chemistry, but also by the ability of the plants to selectively uptake and accumulate certain elements as well as other local environmental factors hence regional variations (Moreda-Pineiro, Fisher, and Hill, 2003).

The need to monitor the safety status of teas with respect to their element content is thus a necessity. This study was intended to provide such information by assaying the levels of selected elements of interest in Kenyan teas.

II. MATERIALS AND METHODS

A. Sample Collection

Twenty-four (24) unprocessed tea samples were collected in triplicates from 24 tea factory catchment areas in Kenya. Also, 72 black CTC tea samples of the three primary grades (24 BP1, 24 PF1, 24 PD) were collected from the same (24) factories. Three replicates of the samples were obtained, each being picked from a different drier. In addition, 12 black tea samples from four tea producing countries in East Africa (Tanzania, Rwanda, Uganda and Kenya) were obtained from Venus Tea Brokers (VTB) and Tea Brokers of East Africa (TBEA) at the Mombasa tea auction.

B. Sample Pre-treatment

The tea samples, were placed in clean, well labeled paper bags and transported to the Tea Research Foundation of Kenya (TRFK) laboratories situated at Kericho, Timbilil estate (latitude 0° 22'S, longitude 35° 21'E, altitude 2180m a.s.l) where they were oven dried (Memmert, 854 Schwabach, Germany) for 12 hours at a temperature of 103°C. Once dry, the samples were finely ground using an electric blending device (Moulinex AR1043, China) to reduce the sample particle size and homogenize the sample.

C. Glassware and Reagents Quality

All reagents used in this work were of analytical grade and were procured from Sigma Aldrich, UK. All glassware were soaked in 50% nitric acid (HNO₃) for 24 hours, thoroughly cleaned and subsequently rinsed with distilled water and oven dried before use. Distilled water was used for sample preparation all dilutions of both the samples and calibration solutions.

D. Sample Analysis

An appropriate amount of a commercial single-element (Fe) working solution (Sigma Aldrich, UK) and 5 ml of 0.05 N HCl were transferred into a clean and dry 100 ml volumetric flask and diluted to the mark with distilled water. The calibration solutions were prepared over a concentration range of 1.0 and 8.0 µg/ml Fe as given in Table I. To determine the levels of Fe in the tea samples, the ground, oven dried (103°C) tea samples were allowed to cool in a desiccator for one hour. Then, 1.0 g of each sample was accurately weighed using an analytical balance (BL-3200 HL, Shimadzu, Japan) into clean and dry specimen tubes and ashed in a muffle furnace (Gallenkamp, England) at a temperature of 460°C for twelve (12) hours. The ashed samples were then allowed to cool followed by wet digestion using an acid-peroxide reagent.

TABLE I FAAS OPERATING CONDITIONS, CONCENTRATIONS OF THE CALIBRATION SOLUTIONS, CORRELATION COEFFICIENTS OF THE CALIBRATION CURVES AND THE DETECTION AND QUANTITATION LIMITS

Parameter	Analyte				
	Fe	Zn	Cu	Pb	Cd
Wavelength (nm)	248.0	213.9	324.0	217.0	228.8
Slit width (nm)	0.2	1.0	0.5	1.0	0.5
Oxidant type	Air-C ₂ H ₂				
Oxidant (Flow rate, Lmin ⁻¹)	11.54	12.98	11.30	13.50	11.38
Fuel (Flow rate, Lmin ⁻¹)	1.50	2.45	1.50	2.00	1.60
Lamp current (mA)	5.0	5.0	3.5	5.0	3.5
Concentrations of intermediate calibration solutions (µg/ml)	1.0, 2.0, 3.0, 4.0 & 8.0	0.2, 0.4, 0.6, 0.8 & 1.2	0.8, 1.6, 2.4 & 3.2	2.0, 4.0, 6.0, 8.0 & 10.0	0.2, 0.4, 0.6, 0.8 & 1.0
*r ²	0.9983	0.9918	0.9999	0.9998	0.9997
LOD (µg/g)	0.070	0.003	0.003	0.010	0.002
LOQ (µg/g)	0.233	0.010	0.010	0.033	0.007

*Correlation coefficient of the calibration curve obtained

The acid-peroxide reagent was prepared by thoroughly mixing equal volumes of a mixture of 1:1 HNO₃ (nitric acid) to H₂O (water) and 1:1 HCl (hydrochloric acid) to H₂O and a peroxide solution (200 ml of 30 % hydrogen peroxide, H₂O₂, diluted to 1 litre with distilled water) in the ratio 2:3. 0.5 ml of this acid-peroxide mixture was then added to each of the ashed samples in the sample tubes and heated on an electric hot plate and evaporated to near dryness. 25 ml of 0.05 N HCl solution was then, added into each sample tube, corked, shaken thoroughly and allowed to stand for 4 hours before analysis. Twelve blank solutions were prepared parallel with the samples.

A Spectra AA-880 (Varian) FAAS, equipped with a sample preparation system (SPS-5, Varian), a deuterium lamp for background correction and hollow cathode lamps was used for the analysis. The instrumental parameters such as the wavelength (λ), burner height, slit-width, lamp current, oxidant and fuel flow rates were adjusted according to the manufacturer's recommendation as given in Table I. The blank and standard solutions were run from the most dilute to the least dilute to avoid the memory effect. The absorbance's obtained for the standard series were used to construct a calibration

curve which was used to determine the concentration of Fe in the tea samples based on the absorbance recorded for each under the stated conditions. The absorbances of the blank solutions were used in the estimation of the method detection and quantitation limits. The above procedure was repeated for the determination of the concentrations of the other analytes of interest (Zn, Cu, Pb and Cd) in the tea samples. The instrument parameters used in the quantitation of each of the five analytes are given in Table I.

E. Statistical Analysis of Data

All the metal content determinations were carried out in triplicates, and the data obtained were subjected to analysis of variance (ANOVA) using GraphPad Prism version 5.0 statistical analysis package for windows. Statistically significant differences were evaluated at $p \leq 0.05$. Graphical representation of these data was done using the same software.

III. RESULTS AND DISCUSSION

A. Quality Control

1) Linearity of the Calibration Curves:

A plot of the machine responses (absorbances) against the concentrations of the analyte calibration solutions ($\mu\text{g/ml}$) gave calibration curves that were used to determine the analyte concentrations in the tea samples. The calibration curves obtained for each analyte demonstrated adequate linearity, with all having r^2 values greater than 0.99 (Table I).

2) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

LOD and LOQ are fundamental elements of method validation, a necessity in ensuring the reliability of a given analytical procedure. LOD is the smallest mass of analyte distinguishable from the statistical fluctuations in a blank (Butcher, and Sneddon, 1998; Armbruster, and Pry, 2008). It is expressed as a concentration derived from the smallest measure detectable with reasonable certainty for a given analytical procedure (Thomsen, Schatzlein, and Mercurio, 2003). LOQ on the other hand is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of test. For this study, the above limits were estimated using the equations;

$$\text{LOD} = 3_{s/b} \text{ and } \text{LOQ} = 10_{s/b} \quad (n=12)$$

where s is the standard deviation of the blank analyses, b is the slope of the calibration curve and n is the number of replicate analyses. The limits obtained for each analyte are given in Table I.

B. Analyte Concentrations in the Tea Samples

1) Iron (Fe):

Fe levels in the unprocessed tea samples ranged widely between 55 and 203 $\mu\text{g/g}$ whereas the levels in black tea ranged between 118 and 329 $\mu\text{g/g}$ (Fig. 1). From the data, it is evident that the levels of Fe in black tea were much higher than those in unprocessed tea from the same region. On the other hand, Fe levels were found to range between 151 and 369 $\mu\text{g/g}$ for the black tea samples from the Mombasa tea auction as shown in Fig. 2. Black tea from Uganda had the highest levels (mean; 344 $\mu\text{g/g}$), followed by Rwanda (mean; 262 $\mu\text{g/g}$) and Tanzania (mean; 195 $\mu\text{g/g}$). Kenyan tea samples had the lowest levels of Fe (mean; 158 $\mu\text{g/g}$). The levels of Fe in unprocessed tea have not been frequently documented. However, for black tea, Gebretsadik and Chandravanshi (2010) reported Fe levels between 319 and 467 $\mu\text{g/g}$ in commercially available Ethiopian black tea analysed by FAAS. These results agree with the findings of the current study. Regional variations were evident as samples from the different tea factory catchments (tea growing regions) had varying Fe levels, an observation attributable to the differences in soil characteristics in the different tea growing regions considered in the current study.

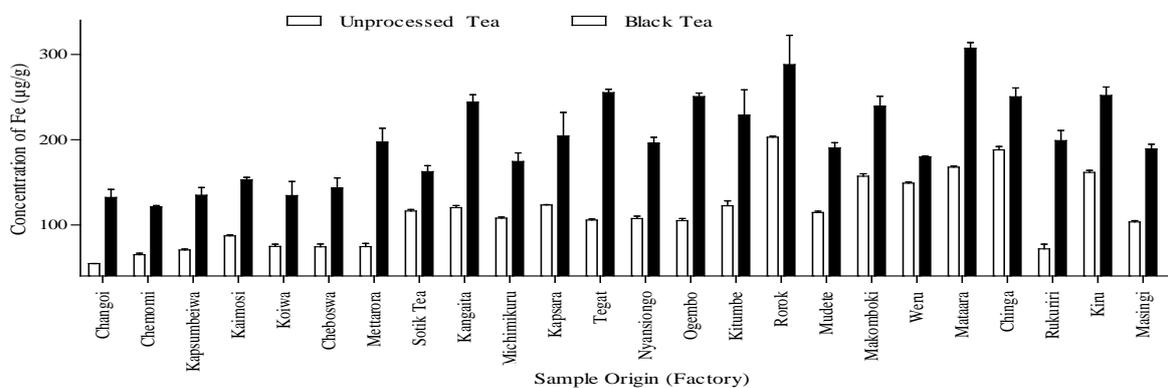


Fig. 1 Levels of Fe in unprocessed and black tea sourced from 24 Kenyan tea factory catchment areas

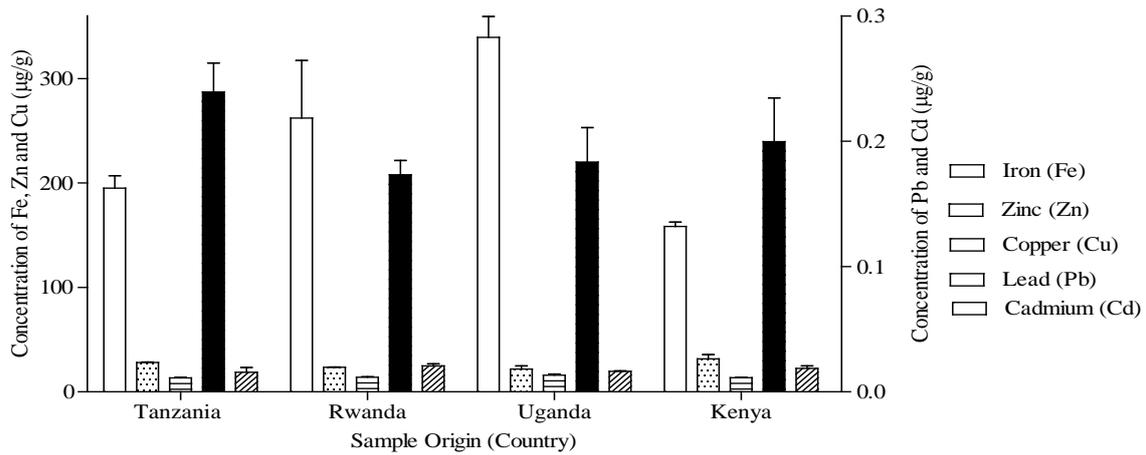


Fig. 2 Levels of Fe, Zn, Cu, Pb and Cd in black tea from four countries (Tanzania, Rwanda, Uganda and Kenya)

2) Zinc (Zn):

Zn levels in the Kenyan unprocessed tea samples ranged between 15.4 and 32.6 µg/g as shown in Fig. 3, with the highest Zn content being recorded for the sample from Cheboswa and the lowest being that of the sample from Kitumbe. Black tea had Zn levels ranging between 18.8 and 44.9 µg/g, with black tea from Kitumbe having the lowest Zn content (19.4 µg/g) and that from Chemomi having the highest (40.8 µg/g). The levels in the black tea from Kenya were slightly higher than those in unprocessed teas from the same region. However, these differences were statistically insignificant ($p > 0.05$).

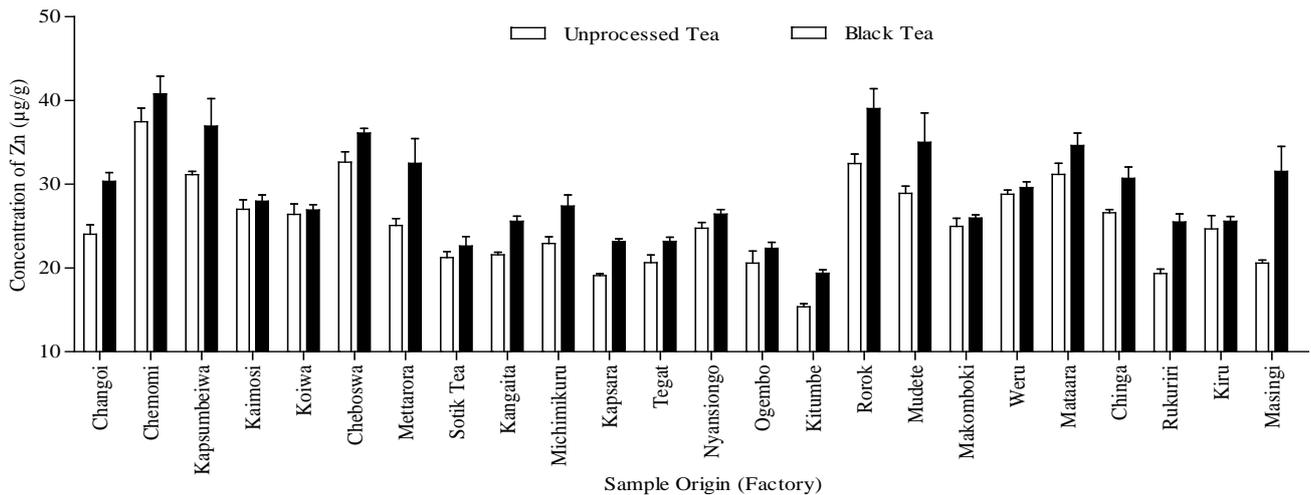


Fig. 3 Levels of Zn in unprocessed and black tea sourced from 24 Kenyan tea factory catchment areas

Data obtained for the Zn content in the black tea from the Mombasa auction were as given in (Fig. 2) and the Zn content ranged between 17.1 and 38.9 µg/g with Kenyan tea having the highest mean Zn levels (mean; 32.7 µg/g). The MPC set for Zn in tea by the Kenya Bureau of Standards (KEBS) is 50 ppm (µg/g) as given in the Kenya Standard KS 65: 2009 and none of the samples analysed surpassed this limit. Zn levels in Kenyan black tea compared well with those in black tea from other tea producing countries in East Africa (Rwanda, Uganda and Tanzania) as shown in Fig. 2.

3) Copper (Cu):

Fig. 4 gives the Cu levels obtained for the Kenyan unprocessed tea (8.4 to 15.1 µg/g). The highest Cu level was recorded for the unprocessed tea from Weru while the lowest level was recorded for the sample from Kiru. Kenyan black tea had Zn contents that ranged from 9.0 to 17.5 µg/g as shown in Fig. 2. The lowest Cu content was obtained for the sample from Kiru (10.0 µg/g) whereas the highest was for the sample from Ogembo (16.6 µg/g). There were no significant ($p > 0.05$), differences between the levels of Cu in unprocessed and black tea. For the black tea samples from the Mombasa tea auction, the lowest and highest Cu levels were 13.3 µg/g (Tanzania) and 16.0 µg/g (Uganda), respectively. All the samples analysed conformed to the MPC for Cu (30 ppm, µg/g) in black tea as given in (KS 65: 2009) by KEBS.

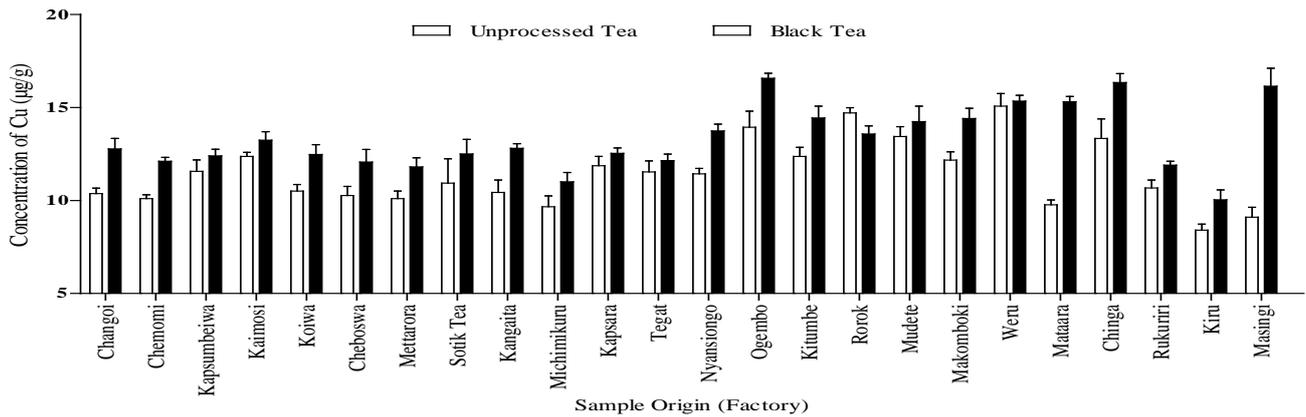


Fig. 4 Levels of Cu in unprocessed and black tea sourced from 24 Kenyan tea factory catchment areas

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Levels of Cu in tea have been widely reported globally and the levels obtained for black tea in the present study (9.0 to 17.8 µg/g) agree with those that have been reported world over. For instance, Kumar, Nair, Reddy and Garg (2005), report Cu levels in Indian and US tea brands to vary widely between 1.6 and 35.0 µg/g and 4.4 and 17.3 µg/g, respectively. Also, Qin and Chen (2007), report Cu levels in commercial tea samples in China, to vary from 1.7 to 21.2 µg/g for green tea, 8.2 to 28.7 µg/g for scented tea, 12.1 to 48.2 µg/g for oolong tea and 8.7 to 28.9 µg/g for black tea. Jin, Zheng, He, Zhou, and Zhou (2005), reported Cu levels in Chinese tea (Yuyao County), ranging from 8.1 to 33.5 µg/g. However, when compared with Turkey (120µg/g; Narin, Colak, Turkoglu, Soylak, and Dogan, 2004), the levels of Cu in Kenyan black tea are far much lower and as such acceptable from the perspective of consumer health.

4) Lead (Pb):

Jin, Zheng, He, Zhou, and Zhou (2005), argue that the tea plant can uptake Pb from the soil, a portion of which is transported to and accumulated in its leaves. Findings of the present study agree with this observation where the Pb content in unprocessed tea ranged from 0.10 µg/g (Mudete) to 0.30 µg/g (Tegat) while those in Kenyan black tea ranged between 0.14 µg/g (Weru and Cheboswa) and 0.32 µg/g (Tegat) as shown in Fig. 5. The Pb content in black tea was slightly higher compared to that in unprocessed tea from the same catchment area. These differences were however not statistically significant (p>0.05). Rwandan black tea from the Mombasa auction had the lowest Pb levels (0.17 µg/g) whereas black tea from Tanzania had the highest mean Pb levels (0.17 µg/g) as shown in Fig. 2.

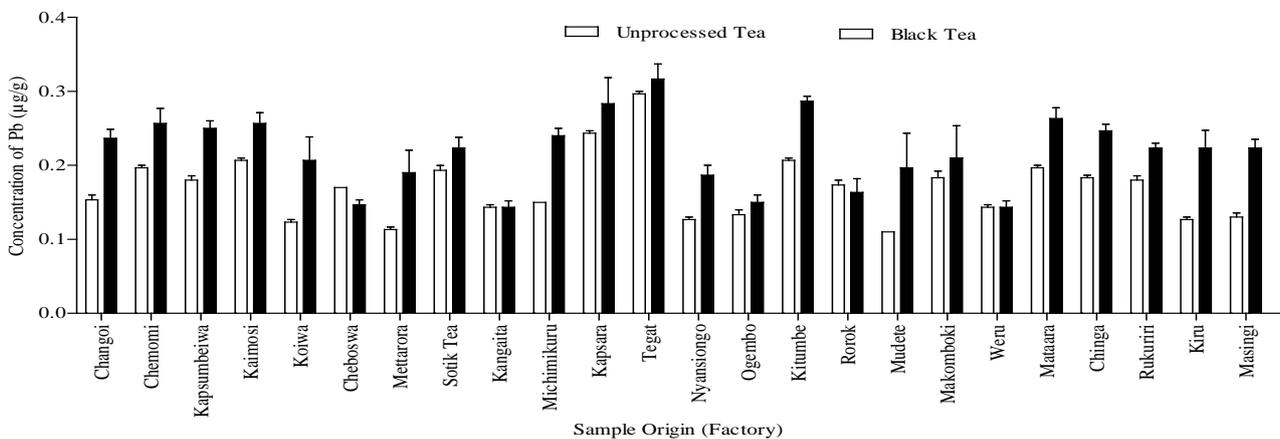


Fig. 5 Levels of Pb in unprocessed and black tea sourced from 24 Kenyan tea factory catchment areas

Findings of the current study corroborate with those of Ramakrishna, Palmakumbura and Chatt (1986) who reported Pb levels in tea produced in Sri Lanka to vary between 0.19 and 0.56 µg/g, Matsuura, Hokura, Katsuki and Haraguchi (2001) who found Pb to be between 0.69 and 0.73 µg/g for black tea samples in Japan and Seenivasan, Manikandan, Muraleedharan and Selvasundaram (2008) who reported Pb levels in tea samples to vary between 0.04 and 1.36 µg/g. However, findings of the present study do not agree with those of Ashraf and Mian (2008) who in a study on black tea in Saudi Arabia found the Pb content to range between 0.3 and 2.2 µg/g and Yemane, Chandravanshi and Wondimu (2008), who reported that five clones of tea grown at in Ethiopia did not show either the ability of Pb accumulation or contained values too low to be detected by the analytical technique used. The EU MPC for Pb in tea is 1.0 µg/g and as such all the samples analysed had Cd contents well within this upper limit.

5) Cadmium (Cd):

Cd was present in the lowest levels of the five elements considered in the current study (Fig. 2 and Fig. 6) with the lowest Cd level for the unprocessed tea samples being 7.0 $\mu\text{g}/\text{kg}$ (Michimikuru) and the highest being 33.0 $\mu\text{g}/\text{kg}$ (Cheboswa). Kenyan black tea had Cd levels that ranged between 9.1 and 40.0 $\mu\text{g}/\text{kg}$ as shown in Fig. 6. For the black tea from the Mombasa tea auction, Cd levels were found to range between 11.0 and 22.0 $\mu\text{g}/\text{kg}$ (Fig. 2).

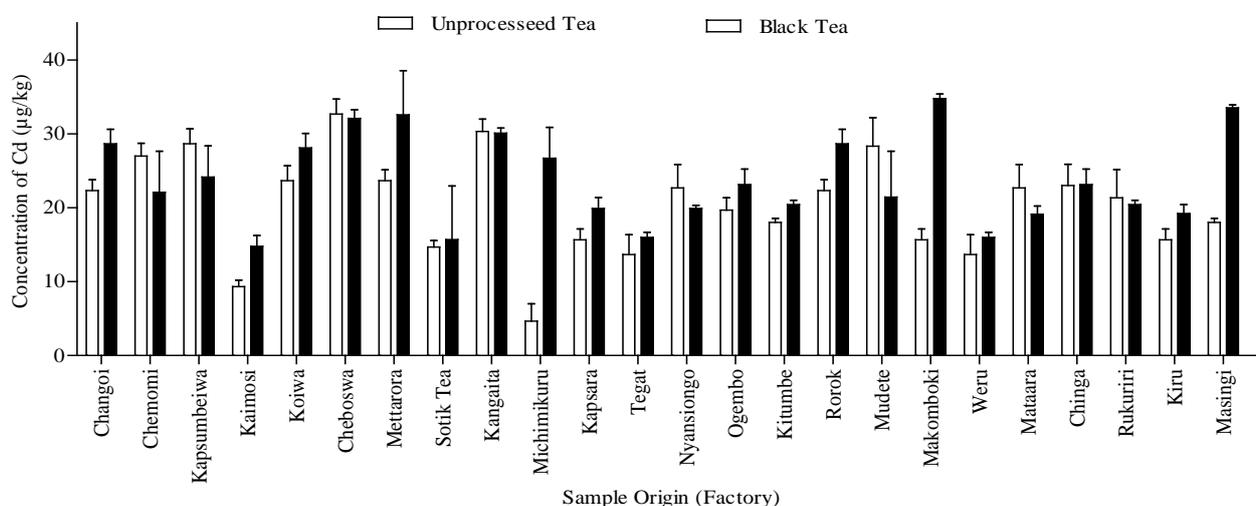


Fig. 6 Levels of Cd in unprocessed and black tea sourced from 24 Kenyan tea factory catchment areas

These findings agree with those of Zhang and Fang (2007) who reported the Cd content in unprocessed tea leaves in China to range from 10 to 60 $\mu\text{g}/\text{kg}$ and Chen, Yu, Xu, Chen and Shi (2009), who reported the Cd content in tea leaves to range from 30 to 80 $\mu\text{g}/\text{kg}$. Further work that agrees with the findings of this study are Al-Oud (2003) who reported the Cd content in tea marketed in Pakistan to range from below the detectable limit (BDL) to 180 $\mu\text{g}/\text{kg}$ and Seenivasan, Manikandan, Muraleedharan and Selvasundaram (2008) who analyzed 100 black tea samples from South India and reported their Cd content to range between 50 and 380 $\mu\text{g}/\text{kg}$. However, analysing tea samples collected from various countries of the world, Ferrara, Montesano and Senatore (2001) found that Cd in tea was below the detectable limit (BDL). This observation is in agreement with the findings of Yemane, Chandravanshi and Wondimu (2008) who analyzed 5 tea clones grown in Ethiopia, and reported the Cd content to be too low to be detected by the analytical techniques used. As was noted for Pb, the level of Cd was also within the EU standard (0.1 $\mu\text{g}/\text{g}$).

IV. CONCLUSIONS

The levels of the studied elements in unprocessed and black tea samples of Kenyan origin compared well to tea grown in Rwanda, Uganda and Tanzania. The levels of the studied metals in unprocessed and black tea in all the groups of samples considered varied widely among the different regions of production. This is largely attributable to the differences in soil characteristics, cultural practices and other agro-climatic conditions in the study area. It was also noted that the Fe content in Kenyan unprocessed tea were lower compared to that in Kenyan black tea from the same catchment area, an observation that suggests possible introduction of additional Fe into black tea during the manufacture process possibly as iron fillings. More work in this area is therefore necessary to determine the actual source of the additional Fe content in black tea. For Zn, Cu, Pb and Cd, unprocessed tea had slightly lower levels compared to black tea but these differences were statistically insignificant. All the tea samples analysed had Fe, Zn, Cu, Pb and Cd levels well within the international MPC's set for tea. Therefore, this study suggests that tea grown and marketed in Kenya is potentially a rich dietary source of some essential minerals including Fe, Zn and Cu.

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