# Assessing the effect of grading on the *in vitro* availability of Fe, Zn, Cu, Pb and Cd from CTC black tea

**Moseti, K.O.[1]*; Kinyanjui, T.[2]; Wanyoko, J.K.[1] and Wachira, F.N.[3]**

[1]*Tea Research Institute, P.O. Box 820-20200, Kericho, Kenya.*

[2]*Department of Chemistry, Egerton University, P.O. Box 536-20115, Egerton, Nakuru, Kenya.*

[3]*Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA), P.O. Box 765 Entebbe, Uganda.*

**Corresponding Author Email: kmoseti@tearesearch.or.ke or kelvinmoseti@gmail.com*

## ABSTRACT

*In vitro* analyses of trace element content in biological systems play an increasingly important role in assessing the environmental impact on health. A quantitative study of five trace elements namely Fe, Zn, Cu, Pb and Cd in cut, tear and curl (CTC) black Tea of different grades randomly sampled from four tea growing countries in East Africa, viz., Kenya, Uganda, Rwanda and Tanzania was carried out. The total element content as well as the water extractable content of the tea samples was done by Atomic Absorption Spectroscopy (AAS). Data obtained was analyzed using Graph-pad Prism statistical software for Windows, version 5.0 and $P < 0.05$ considered to be statistically significant. The significance of the difference between means for black tea and tea infusions was determined by one way ANOVA and the least significant difference test was used for mean separation where significant differences were recorded among group means. It was observed that tea contains the studied elements and only a very small portion of the total element content lixiviates into tea liquor during tea making process. The general extractability pattern of the elements studied was in the order Fe > Zn > Cu > Pb > Cd, indicating that tea is an important dietary source of Fe, Zn and Cu. Taking into account the high tea to water ratio used in preparing the analysed tea extracts, these data is best regarded as the extractability potential of the tea liquor and does not reflect the actual concentrations contained in the tea liquor that we actually consumed. However, the need to enact safety guidelines with regard to the liquor element composition is imperative.

**Key words: Effect,** ***In vivo,*** **Tea liquor, Tea samples, Trace elements.**

## INTRODUCTION

Heavy metals are elements that belong to an ill-defined sub-set of elements, with the more acceptable definition being, elements with a specific gravity greater than 5.0 gcm$^{-3}$ (Jarup, 2003). Most heavy metals are non-biodegradable, have long biological half-lives and thus persist in the environment and different body organs, a phenomenon referred to as bioaccumulation, where they eventually lead to undesirable side effects (Jarup, 2003). Such elements are toxic to humans even at very low concentrations and include Pb (lead) and Cd (cadmium) which have been associated with the aetiology of a number of diseases by the medical community including cardiovascular, kidney, nervous as well as bone diseases (Sttenland and Boffeta, 2000).

The human body requires some metallic and non-metallic elements for healthy growth, development (Ahmad *et al.*, 2012) and proper functioning of the body (Soomro *et al.*, 2008), as they play an important role when present even in small quantities (Youssef *et al.*, 2012). However, these elements should be within certain permissible concentrations (Ahmad *et al.*, 2012), with the optimum values required varying widely from element to element, age and sex, among other factors. When these threshold values are surpassed, some of the elements become harmful to human health and several countries have imposed food laws to restrict the levels of the same in food and beverages (Ambadekar *et al.*, 2012). The maximum permissible concentrations (MPCs) set for tea by the Kenya Bureau of Standards (KEBS) and other international standardization bodies and countries is summarized in Table 1.

**Table 1: Maxima residue limits (MRLs) of selected elements in tea.**

| Concentration in µg/g (ppm) | | | | | |
|---|---|---|---|---|---|
| Element | *KS 65:2009 | EU* | **PFA Act, 1954 | China | SLTB* |
| Pb | 0.1 | 1.0 | 10.0 | 5.0*** | 2.0 |
| Cd | 0.02 | 0.1 | - | - | 0.2 |
| Zn | 50.0 | 50.0 | - | - | 100.0 |
| Cu | 30.0 | 150.0 | 150.0 | 60.0*** | 100.0 |
| Fe | - | - | - | - | 500.0 |
| F e Fillings | 50.0 | - | - | - | - |

**MPC's based on black tea; **MPC as set in the Prevention of Food Adulteration Act of India; ***MPC as set by the Chinese Ministry of Health in 2005; KS 65:2009 – Kenyan Standard for black tea as set by the Kenya Bureau of Standards (KEBS); EU – European Union; SLTB – Sri-Lanka's standard for black tea.*

The chemical composition of tea is very complex as it contains several constituents including flavonoids, proteins, amino acids, enzymes, vitamins, and trace elements (Jha *et al.*, 1996; Kumar *et al.*, 2005; Li *et al.*, 2005; Sahito *et al.*, 2005; Seenivasan *et al.*, 2008). The main sources of trace elements to plants, including tea, are their growth media (Narin *et al.*, 2004; Somer, 1974). Other sources include insecticides, herbicides and fertilizers that may be absorbed through the leaves and roots of the tea plant (Fwu-Ming and Hong-Wen, 2008) as well as rainfall in polluted atmospheres largely arising from high traffic density and industrialization (Lozak *et al.*, 2002; Sobukola *et al.*, 2008) and sub-standard machinery during transportation and processing.

Research has indicated that the content of both essential and non-essential elements in plants is conditional; the content being mainly affected by the characteristics of the soil and the ability of plants to selectively accumulate some elements (Dvrikli *et al.*, 2006). Also, the metallic composition of tea leaves normally varies with the type of tea (green, oolong or black) (Jha *et al.*, 1996), as well as the manner of processing and packaging of the tea leaves (Hussain *et al.*, 2006). Therefore, regular consumption of tea can contribute to the daily dietary requirements of the essential elements including Fe (iron), Zn (zinc) and Cu (copper) (Saud, 2003), and dietary non-essential elements such as Pb and Cd (Mohammed and Sulaiman, 2009). Therefore, the quantification of the element contents in tea is of uttermost importance and currently is a subject of study by researchers world over as they are directly related to health and disease.

In the present study, the contents of Fe, Zn, Cu, Pb and Cd in black CTC tea hot water extracts (liquors) have been determined by FAAS. The objective of this research was to establish the potential quantitative profile in terms of essential and non-essential elements in hot water black tea extracts, hence the potential dietary exposure that would result from the regular consumption of tea.

# MATERIALS AND METHODS

## Collection of tea samples

A simple random sampling technique was used to select 29 tea factories from the small and large-scale tea sub-sectors in Kenya from where tea samples were collected in 2011 and 2012. From each factory, black CTC tea samples of the three primary grades (BP1, PF1 and PD) were collected in triplicates. Further, 25 tea samples of different grades (BP1, PF1, PD, D1, D2, FNGs and BMF) were sourced in triplicates from Kisigo, Kibwele, Lugoda, Mufundi, Njombe and Kibena tea factories managed by Unilever tea Tanzania limited. In total, 33 BP1, 35 PF1, 35 PD, 6 D1, 1 D2, 1 FNGs and 1 BMF black CTC Tea were collected. They were oven-dried (Memmert, 854 Schwabach, Germany) to a constant weight at 103 ± 2 °C and stored in desiccators before analysis.

## Preparation of tea infusions and analysis

Standard tea liquors of the black tea samples were prepared as described by Reeves *et al.* (1985), using 375 g of boiling distilled water and 9 g of the dry tea leaves and agitated on a mechanical shaker for 10 minutes. The mixture was then filtered and the filtrate obtained (2.4% w/v) allowed to cool to room temperature prior to analysis. In addition, 12 blank solutions were prepared alongside the sample solutions. The calibration solutions for each of the five elements studied were prepared by diluting appropriate amounts of commercial single-element working solutions obtained from Sigma Aldrich, UK. A Varian Spectra AA-880 FAAS, equipped with a sample preparation system (SPS-5, Varian) and hollow cathode lamps as the radiation sources, was used for the analysis. The instrumental parameters, such as the flame composition and flow rates, lamp currents, and burner height were adjusted according to the manufacturer's recommendation as shown in Table 2.

**Table 2: Instrumental parameters for the FAAS determination of Fe, Zn, Cu, Pb and Cd.**

| | Element | | | | |
|---|---|---|---|---|---|
| Parameter | Fe | Zn | Cu | Pb | Cd |
| Wavelength, λ (nm) | 248.0 | 213.9 | 324.0 | 217.0 | 228.8 |
| Slit width (nm) | 0.2 | 1.0 | 0.5 | 1.0 | 0.5 |
| Flame type | Air–$C_2H_2$ | Air–$C_2H_2$ | Air–$C_2H_2$ | Air–$C_2H_2$ | Air–$C_2H_2$ |
| 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 |
| Working range (µg/ml) | 1.0-8.0 | 0.2-1.2 | 0.8-3.2 | 2.0-10.0 | 0.2-1.0 |
| Limit of detection (µg/ml) | 0.020 | 0.001 | 0.010 | 0.002 | 0.002 |

The calibration solutions were run from the most to the least dilute to avoid the memory effect followed by the blank and sample solutions. The absorbances obtained for the calibration solutions were used to construct calibration curves for each element, whose equations were then used to quantify the metal contents in the blank and sample solutions. In order to determine the limits of detection (LOD) for the water extractable metal contents for the element(s) of interest of the analytical procedure outlined, the standard deviation of the 12 blank determinations was multiplied by 3, a numerical factor usually chosen depending on the confidence level desired and added to the mean of the blank determinations (Butcher and Sneddon, 1998; Thomsen *et* *al.*, 2003). These values give the lowest concentrations that could be determined with reasonable certainty for the given analytical procedures. The same sample solution was used for the determination of the water extractable levels of all the elements of interest in the tea samples, using the instrumental conditions given in Table 2.

### Statistical analysis of data

Statistical comparisons of the various groups of data were carried out by ANOVA using GraphPad Prism version 5.0 statistical analysis package for windows at $P < 0.05$. The least significant difference (LSD) test was used in mean separation where statistically significant differences were recorded.

## RESULTS AND DISCUSSION

### Method detection limits

The detection limits obtained for the current analytical procedure were as given in Table 2. These values characterise the analytical performance of a given test, thus helping in understanding their capability and limitations. This in turn enables the analyst to ensure that they are "fit for purpose" (Armbruster and Pry, 2008), in this case the determination of the presence or absence of the respective elements in the water extractable portions in the tea liquors that we consume.

**Table 3: Water extractable Fe, Zn, Cu, Pb and Cd in Kenyan black CTC tea of three primary grades (BP1, PF1 and PD) from different tea factory catchments.**

| | | Elemental Concentrations (Mean ± SD*) | | | | |
|---|---|---|---|---|---|---|
| Factory | Grade | $^{a}$F | $^{a}$Z | $^{a}$Cu | $^{a}$Pb | $^{b}$Cd |
| Changoi | BP1 | 6.8 ± 0.6 | 4.3 ± 0.3 | 2.0±0.2 | 0.05 ± 0.007 | 3.0 ± 1.0 |
| | PF1 | 5.5 ± 1.1 | 3.8 ± 0.2 | 1.5±0.3 | 0.03 ± 0.002 | 4.3 ± 1.5 |
| | PD | 4.6 ± 4.6 | 4.0 ± 0.3 | 1.8±0.2 | 0.05 ± 0.007 | 3.3 ± 3.1 |
| Chemomi | BP1 | 6.4 ± 0.9 | 4.5 ± 0.6 | 1.7±0.3 | 0.05 ± 0.007 | 3.7 ± 2.2 |
| | PF1 | 5.8 ± 0.0 | 4.5 ± 0.4 | 1.7±0.4 | 0.06 ± 0.011 | BDL** |
| | PD | 6.7 ± 0.6 | 3.6 ± 0.3 | 1.6±0.2 | 0.07 ± 0.005 | 3.7 ± 0.5 |
| Kapsumbeiwa | BP1 | 5.9 ± 0.6 | 4.0 ± 0.2 | 2.1±0.2 | 0.04 ± 0.008 | 5.3 ± 1.2 |
| | PF1 | 5.8 ± 0.9 | 3.6 ± 0.3 | 2.0±0.2 | 0.05 ± 0.007 | 4.3 ± 0.2 |
| | PD | 5.8 ± 0.5 | 3.9 ± 0.3 | 1.8±0.2 | 0.06 ± 0.005 | 4.0 ± 1.0 |
| Kaimosi | BP1 | 7.5 ± 0.8 | 3.6 ± 0.4 | 1.9±0.1 | 0.05 ± 0.003 | BDL |
| | PF1 | 6.2 ± 0.9 | 3.9 ± 0.3 | 1.6±0.2 | 0.02 ± 0.002 | BDL |
| | PD | 6.8 ± 0.5 | 3.7 ± 0.3 | 2.3±0.2 | 0.03 ± 0.005 | BDL |
| Koiwa | BP1 | 7.7 ± 0.7 | 3.4 ± 0.3 | 1.4±0.1 | 0.08 ± 0.008 | 5.7 ± 1.5 |
| | PF1 | 7.6 ± 0.9 | 3.9 ± 0.6 | 2.0±0.1 | 0.06 ± 0.005 | 5.0 ± 1.7 |
| | PD | 6.9 ± 0.7 | 3.4 ± 0.3 | 2.0±0.1 | 0.06 ± 0.006 | 4.3 ± 1.5 |
| Cheboswa | BP1 | 7.7 ± 0.5 | 4.5 ± 0.2 | 1.5±0.2 | 0.02 ± 0.003 | 6.7 ± 1.5 |
| | PF1 | 6.1 ± 0.8 | 3.8 ± 0.4 | 1.5±0.4 | 0.05 ± 0.004 | 5.7 ± 1.2 |
| | PD | 7.6 ± 1.3 | 4.7 ± 0.3 | 1.5±0.1 | 0.05 ± 0.005 | 7.7 ± 1.2 |
| Mettarora | BP1 | 7.8 ± 0.2 | 3.2 ± 0.4 | 1.6±0.2 | 0.03 ± 0.003 | 6.7 ± 1.1 |
| | PF1 | 7.1 ± 0.8 | 2.9 ± 0.3 | 1.3±0.1 | 0.04 ± 0.005 | 5.7 ± 1.5 |
| | PD | 7.8 ± 0.7 | 3.9 ± 0.3 | 1.5±0.3 | 0.03 ± 0.011 | 5.3 ± 1.5 |

| | | **Elemental Concentrations (Mean ± SD*)** | | | | |
|---|---|---|---|---|---|---|
| Factory | Grade | [a]F | [a]Z | [a]Cu | [a]Pb | [b]Cd |
| Sotik Tea | BP1 | 8.6 ±0.9 | 2.7 ± 0.3 | 1.9 ± 0.7 | 0.03 ± 0.007 | BDL |
| | PF1 | 7.0 ± 0.6 | 3.0 ± 0.4 | 2.0 ± 0.4 | 0.04 ± 0.006 | 7.0 ± 1.0 |
| | PD | 6.5 ± 0.3 | 2.5 ± 0.4 | 2.8 ± 0.7 | 0.03 ± 0.006 | 5.3 ± 1.2 |
| Kangaita | BP1 | 10.0 ± 0.5 | 3.3 ± 0.2 | 1.4 ± 0.2 | 0.03 ± 0.004 | 5.7 ± 0.6 |
| | PF1 | 9.3 ± 2.1 | 3.4 ± 0.2 | 1.6 ± 0.2 | 0.04 ± 0.003 | 6.0 ± 1.0 |
| | PD | 11.1 ± 0.6 | 2.8 ± 0.3 | 1.7 ± 0.4 | 0.04 ± 0.005 | BDL |
| Michimikuru | BP1 | 6.0 ± 0.3 | 2.6 ± 0.2 | 1.3 ± 0.2 | 0.05 ± 0.005 | 5.3 ± 1.1 |
| | PF1 | 6.9 ± 1.0 | 3.6 ± 0.1 | 1.7 ± 0.2 | 0.04 ± 0.006 | 5.0 ± 1.0 |
| | PD | 6.6 ± 0.5 | 2.9 ± 0.7 | 1.7 ± 0.2 | 0.05 ± 0.004 | 3.7 ± 1.2 |
| Kapsara | BP1 | 8.3 ± 0.9 | 2.8 ± 0.7 | 1.0 ± 0.2 | 0.09 ± 0.005 | 3.3 ± 1.9 |
| | PF1 | 6.4 ± 0.3 | 2.4 ± 0.2 | 1.2 ± 0.1 | 0.04 ± 0.004 | 4.3 ± 0.6 |
| | PD | 6.8 ± 0.3 | 2.4 ± 0.2 | 0.7 ± 0.2 | 0.07 ± 0.005 | 2.7 ± 2.5 |
| Tegat | BP1 | 9.5 ± 1.3 | 3.2 ± 0.3 | 1.5 ± 0.2 | 0.05 ± 0.006 | BDL |
| | PF1 | 7.1 ± 1.1 | 3.6 ± 0.2 | 1.6 ± 0.2 | 0.05 ± 0.009 | 3.0 ± 1.0 |
| | PD | 7.0 ± 1.4 | 3.7 ± 0.6 | 1.1 ± 0.3 | 0.07 ± 0.008 | 4.3 ± 1.5 |
| Nyansiongo | BP1 | 11.0 ± 1.4 | 3.2 ± 0.6 | 1.7 ± 0.3 | 0.05 ± 0.003 | 6.0 ± 1.0 |
| | PF1 | 7.0 ± 0.4 | 3.2 ± 0.9 | 1.6 ± 0.4 | 0.05 ± 0.005 | BDL |
| | PD | 8.6 ± 0.9 | 3.8 ± 0.3 | 1.8 ± 0.1 | 0.05 ± 0.008 | 3.0 ± 1.6 |
| Ogembo | BP1 | 7.7 ± 0.5 | 2.4 ± 0.3 | 1.6 ± 0.2 | 0.05 ± 0.007 | 5.3 ± 1.2 |
| | PF1 | 6.7 ± 1.5 | 2.5 ± 0.3 | 2.2 ± 0.3 | 0.02 ± 0.002 | 4.3 ± 1.2 |
| | PD | 8.0 ± 1.2 | 2.8 ± 0.2 | 1.8 ± 0.2 | 0.03 ± 0.004 | 4.0 ± 1.0 |
| Kitumbe | BP1 | 6.3 ± 0.9 | 2.4 ± 0.3 | 1.5 ± 0.4 | 0.05 ± 0.004 | 3.0 ± 1.2 |
| | PF1 | 1.3 ± 6.9 | 2.7 ± 0.3 | 1.6 ± 0.5 | 0.05 ± 0.004 | BDL |
| | PD | 4.7 ± 1.4 | 2.8 ± 0.5 | 1.4 ± 0.4 | 0.08 ± 0.006 | 5.7 ± 0.5 |
| Rorok | BP1 | 9.4 ± 0.4 | 4.5 ± 0.4 | 1.6 ± 0.2 | 0.06 ± 0.005 | 3.0 ± 1.0 |
| | PF1 | 7.8 ± 0.9 | 4.3 ± 0.3 | 2.0 ± 0.2 | 0.05 ± 0.004 | 4.3 ± 1.5 |
| | PD | 8.2 ± 1.0 | 3.6 ± 0.3 | 2.1 ± 0.2 | 0.06 ± 0.004 | 3.7 ± 1.5 |
| Mudete | BP1 | 8.0 ± 0.6 | 4.2 ± 0.3 | 1.9 ± 0.1 | 0.04 ± 0.005 | 6.0 ± 1.0 |
| | PF1 | 7.1 ± 0.8 | 3.9 ± 0.3 | 2.5 ± 0.5 | 0.06 ± 0.007 | BDL |
| | PD | 5.1 ± 0.9 | 4.0 ± 0.2 | 1.8 ± 0.2 | 0.05 ± 0.004 | 3.0 ± 1.0 |
| Makomboki | BP1 | 10.5 ± 0.9 | 3.4 ± 0.3 | 1.4 ± 0.1 | 0.04 ± 0.006 | 6.7 ± 1.5 |
| | PF1 | 11.5 ± 0.4 | 3.9 ± 0.3 | 1.5 ± 0.1 | 0.07 ± 0.003 | 5.7 ± 1.2 |
| | PD | 11.2 ± 1.0 | 3.6 ± 0.4 | 2.5 ± 0.3 | 0.07 ± 0.003 | 7.7 ± 1.2 |
| Weru | BP1 | 7.7 ± 0.7 | 3.8 ± 0.5 | 2.4 ± 0.2 | 0.03 ± 0.003 | BDL |
| | PF1 | 5.3 ± 0.8 | 3.9 ± 0.6 | 1.5 ± 0.4 | 0.03 ± 0.005 | 3.0 ± 0.9 |
| | PD | 7.0 ± 1.4 | 3.4 ± 0.3 | 2.0 ± 0.1 | 0.04 ± 0.002 | 4.3 ± 0.5 |

| | | Elemental Concentrations (Mean ± SD*) | | | | |
|---|---|---|---|---|---|---|
| Factory | Grade | [a]F | [a]Z | [a]Cu | [a]Pb | [b]Cd |
| Mataara | BP1 | 11.7 ± 1.3 | 4.5 ± 0.8 | 1.9 ± 0.7 | 0.06 ± 0.006 | 6.0 ± 1.0 |
| | PF1 | 10.5 ± 0.6 | 5.0 ± 0.4 | 1.3 ± 0.1 | 0.05 ± 0.002 | BDL |
| | PD | 11.7 ± 1.4 | 5.1 ± 0.3 | 1.5 ± 0.3 | 0.05 ± 0.002 | 3.0 ± 2.6 |
| Chinga | BP1 | 8.5 ± 0.6 | 3.8 ± 0.4 | 1.9 ± 0.7 | 0.06 ± 0.003 | 5.3 ± 1.2 |
| | PF1 | 7.3 ± 0.4 | 3.8 ± 0.4 | 2.0 ± 0.4 | 0.06 ± 0.007 | 4.3 ± 0.6 |
| | PD | 7.5 ± 0.3 | 4.4 ± 0.3 | 2.8 ± 0.7 | 0.05 ± 0.003 | 4.0 ± 1.0 |
| Rukuriri | BP1 | 6.3 ± 0.9 | 2.7 ± 0.3 | 1.6 ± 0.2 | 0.03 ± 0.005 | 3.0 ± 0.6 |
| | PF1 | 6.6 ± 1.2 | 3.6 ± 0.3 | 2.0 ± 0.5 | 0.05 ± 0.005 | BDL |
| | PD | 5.0 ± 0.6 | 3.6 ± 0.4 | 3.0 ± 0.2 | 0.05 ± 0.003 | 5.7 ± 1.3 |
| Kiru | BP1 | 11.0 ± 0.9 | 3.7 ± 0.6 | 0.9 ± 0.1 | 0.04 ± 0.005 | 3.3 ± 0.9 |
| | PF1 | 8.5 ± 0.7 | 3.9 ± 0.4 | 1.2 ± 0.1 | 0.02 ± 0.002 | 4.3 ± 0.6 |
| | PD | 8.0 ± 0.3 | 2.8 ± 0.3 | 0.7 ± 0.1 | 0.03 ± 0.005 | 3.3 ± 1.0 |
| Masingi | BP1 | 7.7 ± 0.9 | 3.5 ± 0.3 | 2.3 ± 0.3 | 0.03 ± 0.005 | 6.3 ± 2.5 |
| | PF1 | 6.7 ± 0.9 | 4.9 ± 0.2 | 2.1 ± 0.2 | 0.08 ± 0.006 | 6.0 ± 2.6 |
| | PD | 5.9 ± 0.3 | 3.9 ± 0.6 | 2.0 ± 0.4 | 0.06 ± 0.004 | 5.3 ± 1.1 |
| Kaisugu | BP1 | 7.1 ± 1.1 | 3.3 ± 0.2 | 2.0 ± 0.3 | 0.03 ± 0.002 | 5.3 ± 0.6 |
| | PF1 | 7.0 ± 1.4 | 2.3 ± 0.3 | 2.1 ± 0.2 | 0.06 ± 0.008 | 6.0 ± 1.0 |
| | PD | 7.7 ± 0.1 | 3.1 ± 0.6 | 2.4 ± 0.7 | 0.03 ± 0.005 | 4.7 ± 1.2 |
| Kiptagich | BP1 | 10.8 ± 1.5 | 2.4 ± 0.3 | 2.3 ± 0.2 | 0.04 ± 0.005 | 3.0 ± 0.4 |
| | PF1 | 9.0 ± 0.4 | 2.2 ± 0.2 | 1.7 ± 0.4 | 0.03 ± 0.003 | 4.3 ± 0.6 |
| | PD | 6.5 ± 0.6 | 2.6 ± 0.2 | 1.9 ± 0.4 | 0.04 ± 0.004 | 5.0 ± 1.7 |
| Tiluet | BP1 | 8.4 ± 1.3 | 2.9 ± 0.5 | 1.2 ± 0.3 | 0.02 ± 0.003 | 5.0 ± 1.7 |
| | PF1 | 7.9 ± 0.3 | 3.1 ± 0.4 | 1.8 ± 0.2 | 0.03 ± 0.001 | BDL |
| | PD | 10.1 ± 0.5 | 3.2 ± 0.3 | 1.5 ± 0.5 | 0.02 ± 0.001 | BDL |
| Bondet | BP1 | 8.1 ± 1.7 | 3.7 ± 0.4 | 1.3 ± 0.3 | 0.02 ± 0.004 | 6.7 ± 2.1 |
| | PF1 | 8.1 ± 0.9 | 3.9 ± 0.2 | 1.0 ± 0.2 | 0.03 ± 0.001 | 5.0 ± 1.7 |
| | PD | 8.3 ± 1.3 | 4.2 ± 0.3 | 3.0 ± 0.4 | 0.03 ± 0.005 | 3.0 ± 0.5 |
| Sisiba | BP1 | 8.1 ± 1.9 | 3.8 ± 0.2 | 3.0 ± 0.4 | 0.04 ± 0.005 | BDL |
| | PF1 | 8.0 ± 0.9 | 3.3 ± 0.4 | 1.6 ± 0.3 | 0.03 ± 0.003 | 5.7 ±1.2 |
| | PD | 8.0 ± 0.8 | 3.2 ± 0.2 | 2.9 ± 0.4 | 0.04 ± 0.005 | 7.0 ± 1.0 |
| | CV (%) | 11.5 | 10.4 | 16.6 | 11.3 | 39.3 |
| | Grade | 0.4 | NS | 0.2 | 0.003 | NS |
| LSD ($P = 0.05$) | Factory | 0.6 | 0.4 | 0.3 | 0.005 | 2.0 |
| | Interactions | 1.4 | 0.6 | 0.5 | 0.008 | 3.4 |

*[*] Standard Deviation; **Below Detectable Limit; [a]Mean element contents (μg/ml) of triplicate measurements;*
*[b]Mean element contents (μg/L) of triplicate measurements;*
*NS = Not Significant Water extractable element contents*

Elemental quantification in the tea liquor samples prepared from the black CTC tea samples by FAAS show that indeed tea can be an important source of both essential (Fe, Zn and Cu) and toxic elements (Pb and Cd). The levels obtained in the 2.4% w/v tea solutions for the five elements considered were as given in Tables 3 and Table 4. These results demonstrate large variations in the element concentrations in the different tea liquors. Fe is a vital element in the human body system and an examination of the data obtained (Tables 3 and Table 4) reveals that tea contains water soluble Fe, albeit in varying extents. Water extractable Fe contents between 5.6μg/ml (Changoi) and 11.2μg/ml (Mataara) were obtained for the Kenyan black tea (Table 3). On the other hand, the tea obtained from the Mombasa Tea Auction (market) gave Fe levels between 5.9 and 12.5μg/ml with the mean Fe levels for the three grades considered being 8.3μg/ml (BP1), 9.2μg/ml (PF1) and 8.5μg/ml (PD). Tea samples from Uganda had the highest mean Fe content (11.4μg/ml) while those from Kenya had the lowest (7.0 μg/ml). For Zn, the analytical data obtained reveals that the average Zn content in the Kenyan tea samples was 3.5μg/ml (Table 3), whereas that for the tea from the Mombasa Tea Auction was 4.8 μg/ml (Table 4). These data agrees closely with that reported by Soomro *et al.* (2008).

The average water extractable Cu content for Kenyan black tea was 1.8 μg/ml (Table 3) whereas that for the black tea from the Mombasa Tea Auction was 1.9 μg/ml (Table 4). Cu in tea liquors has not been frequently reported. These results are largely consistent with the results reported by Soomro *et al.* (2008). However, a comparison of the current data with reports on the total Cu content in tea by various researchers world over (10.0 to 25.0μg/g – Ramakrishna *et al.*, 1986; 9.6 to 20.9μg/g – Wang *et al.*, 1983; 1.7 to 48.2μg/g – Qin and Chen, 2007; 15.9 to 32.2μg/g – Seenivasan *et al.*, 2008; 1.6 to 35.0μg/g – Kumar *et al.*, 2005; and, 8.1 to 33.5μg/g – Jin *et al.*, 2008) show that only a small proportion of the total Cu content in tea ends up in the tea liquor we consume. Kenyan tea Pb contents ranged from 0.02 to 0.08μg/ml (Table 3). The black tea from the Mombasa Tea Auction also gave water extractable Pb contents ranging between 0.02 and 0.08μg/ml as shown in Table 4, with Rwandan black tea having the lowest mean Pb content (0.02μg/ml) and Tanzanian tea the highest (0.04μg/ml). The current findings do not conform to those of Soomro *et al.* (2008) who reported Pb level in the tea extracts in all the samples analysed to be below the detectable limit. However, based on these findings and those of the total Pb content reported by researchers globally (0.04 to 1.36μg/g – Seenivasan *et al.*, 2008; 0.2 to 0.6μg/g – Ramakrishna *et al.*, 1986; 0.3 to 2.2μg/g – Ashraf and Mian, 2008; 0.1 to 1.9μg/g –Tsushida and Takeo, 1977; 0.69 to 0.73μg/g – Matsuura *et al.*, 2001; 2.3 to 5.6μg/g – Chen *et al.*, 2009; and, 1.0 to 19.8μg/g – Chen *et al.*, 2010), it is evident that the tea plant can uptake Pb from the soil, a portion of which is be transported to and accumulated in its leaves.

Cadmium (Cd) is a toxic element with no known biological functions in humans, animals and plants (Hussain *et al.*, 2006), and in the current study, the water extractable Cd in some tea samples was found in levels too low to be quantified by the available analytical technique (Table 3) as is the case for the tea samples from the Kaimosi Tea Factory catchment. However, in some tea samples, levels up to 7.0μg/L Cd (Masingi) were obtained with the mean Cd content in all the Kenyan tea samples for all the grades being 4.0μg/L. For the black tea sourced from the Mombasa Tea Auction, the Cd levels obtained were as given in Table 4 (BDL to 4.0μg/L). On the basis of the existing literature (Soomro *et al.*, 2008), and the findings of the current study, it is evident that Cd in tea is generally low. However, the current data suggests that the tea plant can uptake and accumulate Cd in its leaves, though to varying extents and as such the consumption of tea can serve as an additional dietary source of this undesirable element.

**Table 4: Water Extractable Fe, Zn, Cu, Pb and Cd in black CTC tea from Tanzania, Rwanda, Uganda and Kenya obtained from the Mombasa Tea Auction.**

| | | Elemental Concentrations (Mean±SD*) | | | | |
|---|---|---|---|---|---|---|
| Country | Grade | [a]Fe | [a]Zn | [a]Cu | [a]Pb | [b]Cd |
| Tanzania | BP1 | 6.8 ± 0.3 | 5.9 ± 1.6 | 2.2 ± 0.1 | 0.077 ± 0.005 | 4.1 ± 1.0 |
| | PF1 | 7.2 ± 1.0 | 5.8 ± 1.1 | 1.9 ± 0.2 | 0.044 ± 0.010 | 4.3 ± 1.2 |
| | PD | 8.0 ± 1.1 | 5.8 ± 1.4 | 2.1 ± 0.2 | 0.048 ± 0.008 | 3.2 ± 1.2 |
| Rwanda | BP1 | 10.4 ± 0.6 | 4.4 ± 0.4 | 1.9 ± 0.3 | 0.027 ± 0.003 | 3.2 ± 0.9 |
| | PF1 | 9.5 ± 0.4 | 4.3 ± 0.5 | 2.2 ± 0.2 | 0.023 ± 0.004 | 4.0 ± 1.0 |
| | PD | 6.9 ± 1.3 | 4.7 ± 0.5 | 2.1 ± 0.2 | 0.022 ± 0.004 | 3.1 ± 0.6 |
| Uganda | BP1 | 12.5 ± 0.6 | 4.1 ± 0.4 | 2.0 ± 0.1 | 0.035 ± 0.004 | 3.5 ± 1.1 |
| | PF1 | 11.4 ± 1.0 | 4.6 ± 0.7 | 1.9 ± 0.3 | 0.034 ± 0.005 | 3.0 ± 0.3 |
| | PD | 10.4 ± 4.8 | 5.2 ± 1.2 | 1.7 ± 0.2 | 0.029 ± 0.002 | 4.1 ± 0.8 |
| Kenya | BP1 | 8.5 ± 0.9 | 4.5 ± 0.8 | 1.5 ± 0.2 | 0.039 ± 0.007 | 1.3 ± 0.7 |
| | PF1 | 6.3 ± 0.4 | 3.8 ± 0.2 | 1.9 ± 0.2 | 0.040 ± 0.003 | 3.4 ± 0.9 |
| | PD | 5.9 ± 0.6 | 4.4 ± 0.3 | 1.9 ± 0.3 | 0.045 ± 0.009 | 4.2 ± 1.2 |
| | CV (%) | 15.9 | 16.0 | 10.7 | 13.8 | 26.4 |
| LSD (*P*= 0.05) | Grade | NS | 1.4 | NS | 0.01 | 2.3 |
| | Country | 2.1 | 1.1 | 0.3 | 0.01 | NS |
| | Interactions | 2.7 | 1.5 | NS | 0.01 | 1.4 |

*[*]Standard Deviation; [a]Mean element contents (µg/ml) of triplicate measurements; [b]Mean element contents (µg/L) of triplicate measurements;*
*NS = Not Significant*

The solubility (extractability) in water of the studied heavy metals varied widely and despite the high water to tea ratio used in the preparation of the tea liquors, all the samples were well in conformity with the international MPCs set for tea. The concentration of the elements transferred into the hot water extract was determined to be in the order Fe > Zn > Cu > Pb > Cd. Fortunately, toxic heavy metals (Pb and Cd), possibly from the soil, fertilizers and machinery had the least contents in the extracts, with some samples having Cd levels too low to be quantified by the available analytical technique.

MPCs for tea are based on black tea. However, findings of the current study suggest that these should be based on the levels in the tea extract (liquor) that we consume, since the highest potential risk would depend on what is ingested through liquor as opposed to the total metal contents in the tea leaves. However, this may pose various challenges owing to potential differences that can arise as a consequence of brewing methods, water quality, among other factors and the need to adhere to Codex definitions.

## CONCLUSIONS

Despite the limiting sample size of the secondary grades of tea that are rare, findings of the current study strongly suggest that tea grades significantly influence the portions of the "total" element content that lixiviates into the tea brew during the tea making process. Evidently, regular consumption tea is indeed a potential additional source of dietary Fe, Zn, Cu, Pb and Cd. In light of these findings, the inclusion of upper limits for exposure of the same from tea by the relevant national regulatory bodies in the Kenyan black tea quality standard (KS 65: 2009) is highly recommended. However, the current study did not examine the species of the elements of interest in the tea brew as well other factors that have been reported to affect their extractability such as the infusion duration, aspects that will be considered in our future investigations.

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