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SOME FACTORS INFLUENCING THE FREE FLUORIDE CONTENT IN BLACK TEA INFUSIONS

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ABSTRACT

Fluoride is an essential micro-nutrient owing to its role in the prevention of dental caries. Chronic exposure to high levels of the ion (F^-) results in both dental and skeletal fluorosis. A study was carried out to determine free fluoride content in tea (*Camellia sinensis*) infusions of different grades of black Cut, Tear and Curl (CTC) tea. Tea infusions, 1% w/v of 112 teas of different grades and origins, were prepared using boiling distilled water and their free fluoride contents quantified using a Fluoride Ion Selective Electrode (FISE) method. The free fluoride content of the teas was 0.37±0.04 µg F^- ml^{-1}. Infusions of tea grades BP1 and BMF gave the highest (0.40±0.22 µg F^- ml^{-1}) and lowest (0.32±0.10 µg F^- ml^{-1}) free fluoride contents, respectively. There being no national and international Maxima Residue Limit (MRL) for fluoride in tea, all the teas analysed may be regarded to be safe for consumption with regard to their free fluoride contents based on the 1.5 µg F^- ml^{-1}, World Health Organisation's (WHO) guideline for fluoride in drinking water. Further, the effects of tea grades and area of production, on the fluoride content in tea infusions were significant ($P<0.05$), hence the need to enact safety guidelines and monitor continuously the fluoride levels for all types of tea products to prevent excessive intake.

Key Words: Agronomic practices, *Camellia sinensis,* regional variations, tea grades

RÉSUMÉ

Le Fluor est un micro-élément essentiel lorsque consommé en dessous de certains seuils eu égard à son rôle dans la prévention des caries dentaires. Par ailleurs, lorsqu'utilisé en excès, les ions (F^{--}) entrainent une fluorose des dents et du squelette . Une étude était menée pour déterminer la concentration en fluor libre des infusions de thé (*Camellia sinensis*) de différentes classes de the noir de la catégorie CTC. Les infusions de thé, 1% p/v de 112 thés de différentes classes et origines étaient préparées en utilisant une eau bouillante distillée et leurs concentrations en fluor libre quantifiées par la méthode dite de Fluoride Ion Selective Electrode (FISE). La concentration en fluor libre dans les thés était de 0.37±0.04 µg F^-ml^{-1}. Les classes de thé BP1 et BMF ont présenté respectivement des concentrations les plus élevées de (0.40±0.22 µg F^-ml^{-1}) et moins elevées de (0.32±0.10 µg F^-ml^{-1}) du fluor libre. Etant donné que sur le plan national et international il n'ya pas de Limite Maximale de Résidus (MRL) pour le fluor dans le thé, tous les échantillons des thés analysés pourraient être considérés comme appropriés à la consommation eu égard à leur concentrations en fluor libre sur base de 1.5 µg F^-ml^{-1} tel que recommandé par l'Organisation Mondiale de la Santé (OMS) pour l'eau potable. En plus, les effets des classes du thé et les régions productrices sur la concentration du fluor dans les infusions de thé étaient significatives ($P<0.05$), d'où la nécéssité de mettre sur pied des mesures de protection et faire le suivi régulier des niveaux du fluor pour tous les types de produits de thé afin d'éviter une consommation excessive du fluor.

Mots Clés: Pratiques agronomiques, *Camellia sinensis*, variations régionales, classes de thé

INTRODUCTION

A moderate amount of fluoride has been confirmed to be effective for reducing dental caries in humans (Levi *et al.,* 1983; Pehrsson *et al.,* 2011). The ion (F^-) interacts with hydroxyapatite, replacing the hydroxyl ions (OH) to form fluoroapatite, a more crystalline phase that is more resistant to erosion by plaque acid and demonstrates a lower surface energy which makes plaque adhesion more difficult. However, excessive intake of fluoride results in both dental and skeletal fluorosis (Cao *et al.,* 1995; WHO, 2002). Jin *et al.* (2000) attributed the endemic fluorosis in Tibet, to heavy consumption of foodstuffs prepared with brick tea.

Appreciable amounts of fluoride are found in plants with the actual concentrations being dependent on the species, part and age of the plant (Leone *et al.,* 1956). The mechanism of uptake of fluoride from acidic (pH < 5.5) soils by the tea plant is well understood (Wong *et al.,* 2003). The mobility of the fluoride ion in the soil is influenced by a number of factors, including the quantity of the minerals present, soil pH and adsorption of positively charged complexes (Fung *et al.,* 1999). Under acidic conditions, aluminium-fluoride-halide complexes present in the soil decompose into aluminium and fluoride ions, enhancing their availability to tea plants. The free fluoride ion is then absorbed by tea roots and transported to and stored in the plant's leaves (Ruan and Wong, 2001). The fluoride level in tea leaves has been shown to increase with the maturation of the leaves (Lu *et al.,* 2004; Yi and Cao, 2008), observations that have resulted in the tea plant being referred to as a fluoride accumulator (Pehrsson *et al.,* 2011).

Tea is consumed by over two thirds of the world's population, owing to its medicinal, refreshing and mild stimulating effects (Karak and Bhagat, 2010). However, chronic consumption of large volumes of tea has been reported to result in skeletal fluorosis (Shu *et al.,* 2003; Izuora *et al.,* 2011; Kakumanu, and Rao, 2013); hence, the need for a safe threshold for fluoride exposure.

Kenyan teas are grown in different regions that differ in soil and other environmental characteristics (Moseti, 2013). Major growing areas in the country include the Aberdare highlands, Mt. Kenya region, Nyambene hills, Nandi Hills, highlands around Kericho, Mt. Elgon region and Kisii highlands (TRFK, 2002). Kenya's tea industry is characterised by two sub-sectors; the large-scale sub-sector (estate plantations) with production units larger than 20 ha; and the small-scale farmers with smaller production units averaging 0.25 ha per farmer (TBK, 2007). Tea plantations of the large-scale sub-sector are managed by well trained personnel; whereas the farmers in the small-scale sub-sector rely on agricultural extension officers for advice (Ogola and Kibiku, 2004). However, the extension officers available to the small-scale tea farmers are not adequate to serve them effectively, hence, low adoption of technologies for optimum yields from their tea farms.

Kenya predominantly produces black CTC tea, about 95% of which is sold in the export market in bulk; accounting for over 20% of the global market (International Tea Committee, 2009). This type of tea has the advantage of quicker brewing and makes mores cups per kg (KTDA, 2011). The nature and quality of a given tea product is mainly dependent on the chemical composition of the fresh tea leaves and the reactions they undergo during the manufacture process. Significant differences in the free fluoride content in infusions of Kenyan black tea due to variations in soil characteristics in the different areas of production, agronomic practices in the small and large-scale sub-sectors, as well as the grain sizes of the grades of black CTC tea, have been reported (Moseti *et al.,* 2013). The objective of this study was to establish the free fluoride content in infusions prepared from black CTC tea sourced from different areas. Data obtained could be an important source of information with regard to quality and standards, nutrition and contamination.

MATERIALS AND METHODS

Collection of tea samples. A simple random sampling technique was used to select 29 tea factories from the small (14) and large-scale (15) tea sub-sectors in Kenya 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 teas were collected. They were oven-dried (Memmert, 854 Schwabach, Germany) to a constant weight at 103 ºC and stored in desiccators before analysis.

Preparation of tea infusions. Tea infusions were prepared using a tea to water ratio of 1% w/v as described by Moseti *et al.* (2013); where 1.0 g of oven-dried tea was added into 100 ml of boiling distilled water and agitated for 10 minutes on a mechanical speed variable reciprocal shaker (100 oscillations per min; Gallenkamp Flask Shaker, England). The mixture was filtered through a Whatmman No. 1 filter paper (Fung *et al.,* 1999) and the filtrate allowed to cool to room temperature. For analytical method validation purposes, two control samples, A and B, were spiked with known analyte concentrations for recovery studies whereas sample blanks were prepared as described by Moseti *et al.* (2013).

Analytical procedure and determinations. Analysis of the tea infusions for free fluoride extracted from the teas was carried out potentiometrically. The instrumentation consisted of a flow plus fluoride ion selective electrode (EDT, directION 3221, UK) and an ion analyser (EDT, directION, DR359TX, UK). Working solutions in the order of 0.1, 1.0, 10.0 and 100.0 µg F^- ml^{-1} were prepared by serially diluting a commercial fluoride stock solution (1000 µg F^- ml^{-1}; EDT, directION 2133, UK). Equal volumes (25 ml) of a Total Ionic Strength Adjustment Buffer (TISAB, pH 5.30-5.35) prepared as described by Shyu *et al.* (2009) and each of the working and blank solutions, control samples and tea infusions were thoroughly mixed using a magnetic stirrer (Digisystem Laboratory Instruments Inc., Model MS-90, Taiwan) for one minute.

Readings of the above mixtures were taken at room temperature. A standard calibration curve was obtained by plotting the machine response (mV) against the logarithm of the concentrations of the working solutions. A potential change of -59.4 mV per decade of fluoride concentration was recorded with a correlation coefficient (r) of 0.9992; a value in good agreement with the theoretical Nerstian slope (Giljanovic *et al*., 2012). The limits of detection and quantitation were estimated to be 0.02 and 0.07 µg F^-ml^{-1}, respectively. Further, the current method demonstrated good accuracy (96.7 and 102.0%) and precision (0.67 and 2.59) as is evident in the percent recoveries and standard deviations of the triplicate analyses of the control samples A and B, respectively.

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

Free fluoride content of Kenyan black tea infusions. The mean free fluoride content in the 87 tea infusions of Kenyan black CTC tea from the 29 tea growing catchments was 0.39 µg F^- ml^{-1}. Figure 1 presents the mean free fluoride contents of the triplicate determinations. A critical examination reveals that the amount of fluoride released into infusions during the tea making process varies with; (i) the origin of the tea product, in this case the factory; and (ii) the grade of the tea product. Indeed, the differences in the mean free fluoride content in tea infusions from one factory to another as well as from one grade to another were statistically significant ($P<0.05$), consequently resulting in significant interaction effects.

It is evident from this study that tea, if consumed regularly can be an important dietary source of fluoride. Taking into consideration that tea is not the only dietary source of fluoride, continuous monitoring of fluoride levels and enactment of safety guidelines should be done for all types of tea products to prevent excessive intake of fluoride. The current data is comparable with recent findings by Shyu and Chen (2013) in

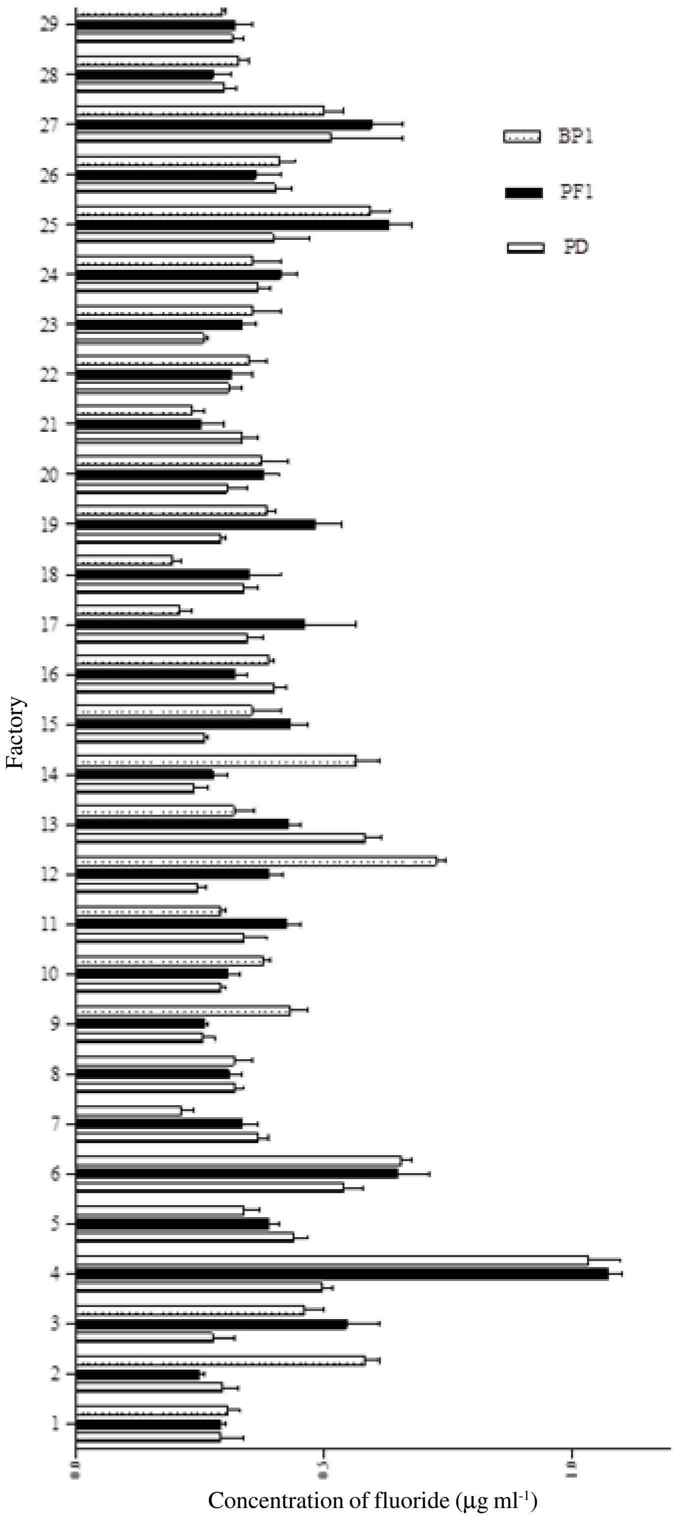

Figure 1. Mean free fluoride contents in tea infusions prepared from black tea sourced from 29 tea growing catchments in Kenya.

Taiwan and Moseti *et al*. (2013) in Kenya, who reported fluoride in tea infusions to range between 0.23 and 3.60 µg F^- ml^{-1} and 0.11 and 1.35 µg F^- ml^{-1}, respectively. The implication of this is that, tea contains fluoride a portion of which is water soluble and is released into tea infusions during the tea making process.

Agronomic practices. Mean fluoride contents extracted from the different grades of tea from the two sub-sectors are given in Figure 2. The differences were significant ($P<0.05$); the mean free fluoride content in infusions prepared from teas from the large-scale tea sub-sector being lower than those from the small-scale sub-sector. These findings suggest that the amount of water soluble fluoride is not only affected by the region of production of the teas but also by the agronomic practices in place.

However, this is not in agreement with our early findings, where the differences in the mean fluoride content in tea infusions prepared from teas form the two sub-sectors were not significant ($P>0.05$) (Moseti *et al.,* 2013). The implication of this is that there are other factors, such as seasonal variations possibly playing a role in the amount of fluoride accumulated in tea leaves, hence, the proportions released into tea infusions during the tea making process. Thus, there is need for extensive studies on this subject to determine the dynamics of the fluoride ion along the food chain and the associated health implications.

The Kenyan tea industry is characterised by two sub-sectors (Large and Small scale), whose agronomic practices differ due to low adoption of recommended technologies for maximisation of tea yields, such as planting of elite tea varieties with high yield and quality potentials, correct plant spacing and fertiliser application frequency and rates (Kagira *et al.,* 2012). This has mainly been attributed to poverty and poor access to information, as a result of poor extension services, by the small-scale farmers among other factors. A total of 15 of the 29 Kenyan factories considered in this study belonged to the large-scale farmers, and the rest were managed by the small-scale farmers.

Grading and fluoride levels in tea infusions. The mean free fluoride content in tea infusions of the seven grades of tea considered in the current study are given in Table 1. Different tea grades gave different levels of fluoride in their infusions; grade BP1 and BMF giving the highest and lowest levels, respectively. During grading, tea granules resulting from the Cut, Tear and Curl operations during the manufacture of black CTC tea are separated into various "groups" based on size. This gives primary (BP1, PF1, PD and D1) and secondary grades (BMF, Fannings and D2) with

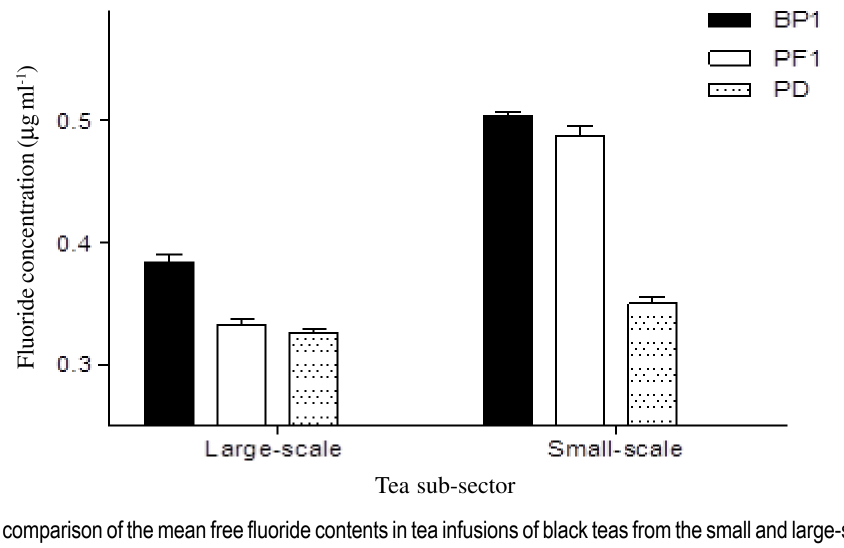

Figure 2. A comparison of the mean free fluoride contents in tea infusions of black teas from the small and large-scale tea sub-sectors.

TABLE 1. Mean fluoride concentration in tea infusions of different grades of tea

Tea grade	Sample size	Fluoride concentration ($\mu g\ F^- ml^{-1}$)		
		Minimum	Maximum	Mean±SD
BP1	33	0.11	0.98	**a0.40±0.22
PF1	35	0.26	0.40	b0.40±0.04
PD	35	0.20	0.95	ab0.39±0.20
D1	6	0.25	0.36	ab0.33±0.05
D2	1	0.37	0.47	ab0.43±0.05
Fannings	1	0.27	0.49	ab0.37±0.11
BMF	1	0.37	0.44	c0.32±0.10
Grand mean		0.26	0.58	0.37±0.04
Coefficient of variation (%)				11
LSD (P=0.05)				0.09

Means preceded with a similar letter are not significantly different (P>0.05)

whole, large tea leaves gaining a higher grading (KTDA, 2011). These help in facilitating the tea trade and is the central component in assessing the monetary value of various types of tea. These results suggest that the fluoride content in infusions of different grades of tea depends on the size of the tea granules; with BP1 the highest grade giving the highest content. However, sample size, especially of the secondary grades of tea, is a major limiting factor and as such, this trend might be misleading. Therefore, extensive studies with better sample sizes are necessary to confirm these findings.

The water extractable fluoride content depend on a number of factors including the infusion duration, temperature of infusion and the type of tea (Giljanovic *et al.*, 2012). Since the infusion method used for all the groups of tea samples in this study was the same, then the data obtained demonstrate that tea from different regions, in these case factories, contain different concentrations of fluoride. These differences can be attributed to the geological, soil chemical and physical characteristics of the area of production (Hudaykuliyev *et al.*, 2005). Soils are clearly the main source of fluoride and other trace elements for the tea plant. However, in order to boost tea yields, most tea growers use nitrogenous fertilisers, which may further increase the fluoride content in tea by (a) contributing additional fluoride, and (b) making the soils acidic by producing hydrogen ions (H^+) *via* nitrification ($NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$) induced by bacteria present in the soil (Ishibashi *et al.,* 2004). This in turn increases the mobility and consequently the bioavailability of fluoride for uptake by the plants (Fung and Wong, 2002). Other factors that may influence the fluoride content in tea infusions include leaf age (Shu *et al.,* 2003) as well as genetics factors.

The World Health Organisation (WHO), in light of the associated health effects of fluoride has set the guideline for fluoride in drinking water at 1.5 $\mu g\ F^- ml^{-1}$ (WHO, 2011). However, the upper limit for fluoride uptake from tea is not included in the current Kenyan black tea quality standard (KS 65: 2009). Based on the guideline for drinking water, black CTC tea may be regarded as safe for consumption with respect to fluoride content.

CONCLUSION

Tea contains fluoride, part of which is released into tea infusions during the tea making process. However, in Kenya, tea grades and tea sub-sectors have significant effects on the fluoride content in tea infusions. Thus, regular tea consumption is a potential additional source of dietary fluoride and in light of these findings, inclusion of an upper limit for fluoride exposure from tea by the relevant national regulatory bodies in the Kenyan black tea quality standard is recommended. Further, the effect of leaf age and clonal variations on fluoride content in tea leaves is an aspect that should be considered in future investigations.

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REFERENCES

Cao, J., Zhao, Y., Liu, J.W., Bai, X.S., Zhou, D.Y., Fang, S.L., Jie, M. and Wu, J.S. 1995. Research on ûuorosis caused by drinking brick tea in Sichuan Province. *Chinese Journal of Tea Science* 17:35-38.

Fung, K.F. and Wong, M.H. 2002. Effects of soil pH on the uptake of Al, F and other elements by tea plants. *Journal of the Science of Food and Agriculture* 82(1):146-152.

Fung, K.F., Zhang, Z.Q., Wong, J.W.C. and Wong, M.H. 1999. Fluoride contents in tea and soil from tea plantations and the release of ûuoride into tea liquor during infusion. *Environmental Pollution* 104(2):197-205.

Giljanovic, J., Prkic, A., Bralic, M. and Brkljaca, M. 2012. Determination of fluoride in tea infusions by using fluoride ion selective electrode. *International Journal of Electrochemical Science* 7:2918-2927.

Hudaykuliev, Y., Tastekin, M., Poyrazoglu, E.S., Baspinar, E. and Velioglu, Y.S. 2005. Variables affecting ûuoride in Turkish black tea. *Fluoride* 38:38-43.

International Tea Committee, 2009. International Tea Committee: Annual Bulletin of Statistics. International Tea Committee, Ltd., London, United Kingdom.

Ishibashi, Y., Matsuo, H., Baba, Y., Nagafuchi, Y., Imato, T. and Hirata, T. 2004. Association of manganese eûuent with the application of fertiliser and manure on tea field. *Water Research* 38:2821-2826.

Izuora, K., Twombly, J.G., Whitford, G.M., Demertzis, J., Pacifici, R. and Whyte, M.P. 2011. Skeletal fluorosis from brewed tea. *The Journal of Clinical Endocrinology and Metabolism* 96(8):2318-2324.

Jin, C., Yan, Z. and Jianwei, L. 2000. Fluoride in the environment and brick-tea-type fluorosis in Tibet. *Journal of Fluorine Chemistry* 106(1):93-97.

Kagira, E.K., Kimani, S.W. and Githii, K.S. 2012. Sustainable methods of addressing challenges facing small holder tea sector in Kenya: A supply chain management approach. *Journal of Management and Sustainability* 2(2):75-89.

Kakumanu, N. and Rao, S.D. 2013. Skeletal fluorosis due to excessive tea drinking. *The New England Journal of Medicine* 368(12): 1140.

Karak, T. and Bhagat, R.M. 2010. Trace elements in tea leaves, made tea and tea infusion: A review. *Food Research International* 43(9): 2234-2252.

KTDA. 2011. From bush to cup (Tea life cycle). Kenya Tea Development Agency Limited. http://www.ktdateas.com/index.php/the-tea/from-bust-to-cup. Accessed on October 4, 2012.

Leone, I.A., Bennan, E. and Daines R.H. 1956. Atmospheric fluoride: Its uptake and distribution into tomato and corn plants. *Plant Physiology* 31:329-333.

Levi, S., Zilberman, L. and Sarnat, H. 1983. Fluoride: An essential or poison element. *Journal of Fluorine Chemistry* 23:447.

Lu, Y., Guo, W.F. and Yang, X.Q. 2004. Fluoride content in tea and its relationship with tea quality. *Journal of Agricultural and Food Chemistry* 52(14):4472-4476.

Moseti, K.O. 2013. Levels of selected heavy metals and fluoride in tea (*Camellia sinensis*) grown, processed and marketed in Kenya. M.Sc. Thesis. Egerton University, Njoro, Kenya.

Moseti, K.O., Wanyoko, J.K., Kinyanjui, T., Wachira, F.N. 2013. Extractability of fluoride into black tea liquors, Kenya. *International Journal of Environmental Protection* 3(5): 14-19.

Ogola, S.O. and Kibiku, P.N. 2004. Smallholder tea growing enterprise: Productivity and profitability survey report. Tea Board of

Kenya. Ministry of Agriculture, Livestock and Fisheries, Government of Kenya.

Pehrsson, P.R., Patterson, K.Y. and Perry, C.R. 2011. The fluoride content of select brewed and microwave-brewed black teas in the United States. *Journal of Food Composition and Analysis* 24(7):971-975.

Ruan, J.Y. and Wong, M.H. 2001. Accumulation of fluoride and aluminium related to different varieties of tea plant. *Environmental Geochemistry and Health* 23(1):53-63.

Shu, W.S., Zhang, Z.Q., Lan, C.Y. and Wong, M.H. 2003. Fluoride and aluminium concentrations of tea plants and tea products from Sichuan Province, PR China. *Chemosphere* 52(9): 1475-1482.

Shyu, T. and Chen, J. 2013. Fluoride levels in different types of tea drinks consumed in Taiwan and daily human exposure from tea drinks. *Journal of Food Agriculture and Environment* 11(1):178-183.

Shyu, T., Chen, J. and Lee, Y. 2009. Determination of fluoride in tea leaves and tea liquors by ion selective electrode. *Journal of Food and Drug Analysis* 17:22-27.

TBK 2007. Fact sheet on Kenya tea. Tea Board of Kenya statistics. Nairobi, Kenya.

TRFK, 2002. Tea Research Foundation of Kenya, Tea Growers Handbook. 5th Ed., Kericho, Kenya.

WHO. 2002. World Health Organisation. Fluorides, environmental health criteria 227. WHO, Geneva, Switzerland.

WHO. 2011. World Health Organisation. Water Sanitation and Health (WSH). Available at: http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/index.html. Accessed on 7th June 2012.

Wong, M.H., Fung, K.F. and Carr, H.P. 2003. Aluminium and fluoride contents of tea, with emphasis on brick tea and their health implications. *Toxicology Letters* 137: 111-120.

Yi, J. and Cao, J. 2008. Tea and fluorosis. *Journal of Fluorine Chemistry* 129:76-81.