LEVELS OF SELECTED HEAVY METALS AND FLUORIDE IN TEA (*Camellia sinensis*) GROWN, PROCESSED AND MARKETED IN KENYA

MOSETI O. KELVIN

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DECLARATION AND RECOMENDATION

DECLARATION

This thesis is my original work and has not, wholly or in parts, been presented in any other university for an academic award.

Moseti O. Kelvin
SM11/2479/09

Signature: …….. Date: ……

RECOMMENDATION

This thesis is the candidate's original work and has been prepared with our guidance and assistance and is being submitted with our approval as the supervisors.

Dr. Thomas Kinyanjui
Egerton University, Njoro.

Signature: ……. Date: …….

Dr. John Wanyoko
Tea Research Foundation of Kenya, Kericho.

Signature: ……. Date: ……....

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DEDICATION

....to my wonderful parents and siblings

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ABSTRACT

Tea-drinking is a habit that has over time spread globally. The chemical composition of tea is very complex and is currently a subject of broad medical and toxicological scientific studies. Thus, the accurate quantification of the levels of both essential and non-essential elements in tea is very important in assessing it's standard and quality as they are directly related to health and disease. This study examined the levels of Iron (Fe), Zinc (Zn), Copper (Cu), Lead (Pb), Cadmium (Cd) and fluoride in tea from various regions in Kenya as well as other tea producing countries in East Africa (Rwanda, Uganda and Tanzania). The levels of these heavy metals were quantified using Flame Atomic Absorption Spectroscopy (FAAS) whereas the fluoride levels were determined potentiometrically using a Fluoride Ion Selective Electrode (FISE) method. The levels of heavy metals in unprocessed tea were found to be in the range 54.6 - 123.3µg/g for Fe, 15.4 - 37.5µg/g for Zn, 10.3 - 14.8µg/g for Cu, 0.12 - 0.28µg/g for Pb and 10.0 - 27.1µg/kg for Cd. For black tea, the levels were in the range 81 - 369µg/g for Fe, 17.1 - 44.9µg/g for Zn, 9.0 - 17.8µg/g for Cu, 0.12 - 0.41µg/g for Pb and 9.1 - 40.0µg/kg for Cd whereas the concentrations were in the range 2.2 - 12.5µg/ml for Fe, 1.5 - 5.9µg/ml for Zn, 0.7 - 3.0µg/ml for Cu, 0.02 - 0.08µg/ml for Pb and below detectable limit (BDL) - 7.0µg/L for Cd in black tea liquors. The general accumulation pattern and extractability of the elements in the unprocessed, black tea and tea liquors was in the order Fe > Zn > Cu > Pb > Cd and the levels of these metals in the tea liquors were proportional to the respective total levels in unprocessed and black tea. The fluoride levels in tea liquors were found to range from 0.11 to 1.35µg/ml. These results confirm that tea consumption is indeed an important dietary source of Fe, Zn, Cu and fluoride. Based on their heavy metal and fluoride contents, Kenyan teas were comparable with those from Uganda, Rwanda and Tanzania, and all the samples analysed conformed to international standards for tea. However, regional variations in heavy metal and fluoride contents were evident.

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ABBREVIATIONS AND ACCRONYMS

ANOVA	Analysis of variance
BDL	Below detectable limit
BP1	Brocken Pekoe 1
CAC	Codex Alimentarius Commission
CTC	Cut, tear and curl
EATTA	East Africa Tea Traders Association
GMP	Good Manufacturing Practices
IAEA	International Atomic Energy Agency
ITC	International Tea Committee
KTDA	Kenya Tea Development Agency
KTGA	Kenya Tea Growers Association
LOD	Limit of detection
LSD	Least significant difference
MPC	Maximum Permissible Concentrations
PD	Pekoe dust
PF1	Pekoe fannings 1
TBEA	Tea Brokers of East Africa
TBK	Tea Board of Kenya
TRFK	Tea Research Foundation of Kenya
UPASI	United Planters Association of Southern India
VTB	Venus Tea Brokers

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Tea is the oldest, most popular non-alcoholic drink globally (Chen *et al.,* 2003; Gulati *et al.,* 2003; Yanagimoto *et al.,* 2003; Saud, 2003; Seenivasan *et al.,* 2008; Gebretsadik and Bhagwan, 2010) second only to water (Wheeler and Wheeler, 2004; Thangapazham *et al.,* 2007; Fwu-Ming and Hong-Wen, 2008; Zerabruk *et al.,* 2010). It is widely consumed globally as a beverage and also due to its medicinal qualities (Tanmoy and Bhagat, 2010). It is obtained from the processing of young shoots of the plant *Camellia sinensis* (L.) that belongs to the plant family Theaceae. It consists of three distinct varieties namely; China (*C. sinensis* var. *sinensis* (L.)), Assam (*C. sinensis* var. *sinensis* (Masters)) and Cambod (*C. sinensis* var. *assamica* ssp *Lasicalyx* (Planchon ex Watt)), a hybrid between China and Assam varieties (Wight, 1962; TRFK, 2002; Mondal *et al.,* 2004).

The tea plant is indigenous to forests of South-East Asia and has over time been introduced to many areas of the world including Europe, North and South America, Africa and Australia and it is cultivated commercially for use both as a beverage and medicinal purposes (Tanmoy and Bhagat., 2010). Depending on the variety, the tea plant can thrive in a wide variety of geological and climatic conditions (Bonheure, 1990) and naturally grows as tall as 15m (Mondal *et al.,* 2004; Yemane *et al.,* 2008). However, for economic production, the tea plant requires deep well drained soils (Othieno, 1992) whose optimum pH values should lie between 4 and 6. However, studies by the United Planters Association of Southern India, UPASIs (1987) revealed that the upper acidity limit varies with the nature of the soil, especially the organic matter content, and is considered as 5.6 in East Africa and 6.0 in Southern India. The growth and development of the tea plant can be described by four stages: the seedling or cutting stage, the frame formation stage, the mature/commercial stage and the degraded or moribund stage (Zeiss and DenBraber, 2001; TRFK, 2002). The duration of each growth stage is determined by the genotype and growing conditions.

Kenya is a tropical East African country with varied climatical and geographical regions (Gesimba *et al.,* 2005) where tea is the leading foreign exchange earner and export commodity, (Gebretsadik and Bhagwan, 2010; TBK, 2012). It is among the leading producers and exporters of tea worldwide. For instance, in 2011, Kenya produced 377 million kilograms of processed tea from which the export earnings amounted to KShs 109 billion. This was much higher than the total earnings of KShs 97 billion recorded in 2010 and KShs 69 billion in 2009 (TBK, 2012). The major importers of Kenyan tea in 2011 were Pakistan

(80.8 million kilograms of processed tea) and Egypt (79.9 million kilograms of processed tea). Other export destinations were the United Kingdom (UK), Afghanistan and Sudan. Generally, the export earnings have doubled in the last five years (TBK, 2012).

The Kenyan tea industry is a rural based enterprise where over 62% of the crop is produced by highly successful small-scale farmers. This offers a direct source of livelihood to over 4 million people, about 10% of the country's population (TBK, 2008). The industry has heavily contributed towards infrastructural development and consequently poverty eradication in the rural areas. It also contributes to environmental conservation through enhanced water infiltration, reduced surface erosion, and mitigation of global warming through carbon sequestration.

The chemical composition of unprocessed tea (two leaves and a bud), processed tea and the tea liquor are very complex. Examples of the compounds present are flavonoids, proteins, amino acids, enzymes, vitamins, minerals and trace elements (Jha *et al.,* 1996; Kumar *et al.,* 2005; Li *et al.,* 2005; Sahito *et al.,* 2005; Seenivasan *et al.,* 2008). The regular consumption of tea can thus contribute to the daily dietary requirements of both the nutritionally essential elements such as Fe, Zn and Cu (Saud, 2003; Seenivasan *et al.,* 2008) and the nutritionally non-essential elements such as Pb and Cd (Mohammed and Sulaiman 2009; Gebretsadik and Bhagwan, 2010; Zerabruk *et al.,* 2010; Tedayon and Lahiji, 2011). These elements, or some form of them occur naturally in the ecosystem with large variations in concentration. However, presently, rampant natural and anthropogenic environmental degradation has seen these concentrations increase markedly.

According to the International Tea Committee, ITC (2009) and the Kenya Tea Development Agency, KTDA (2011), Kenya mainly produces black Cut, Tear and Curl (CTC) tea. This type of tea has the advantage of quicker brewing, makes mores cups per kg, and a large proportion of it is sold in bulk in the export market (KTDA, 2011). However, in the recent years, the world tea prices have declined, hence diminishing returns to the tea farmers. This is a major challenge that should be addressed in a multidisciplinary approach by researchers, processors, promoters and other stakeholders in the agricultural sector. In order to counter this decline, the tea industry stakeholders are currently championing for value-addition as well as product diversification to enable our tea to compete favorably in the global tea market.

1.2 Statement of the Problem

Kenyan teas are grown in different regions that differ in both their edaphic (soil) characteristics and environmental conditions and their heavy metal and fluoride composition has not been established. In the recent past, there has been increased importation of teas (for blending purposes) from different tea producing countries worldwide whose heavy metal and fluoride content is unknown. Thus, in order to compete favorably with the other tea producing nations globally, there is need to asses and ascertain the safety status of our tea with respect to heavy metal and fluoride contamination.

1.3 Objectives

1.3.1 General Objective

To study the status of selected heavy metals and fluoride in tea in Kenya at the different levels of prcessing.

1.3.2 Specific Objectives

1. To determine the levels of Fe, Zn, Cu, Pb and Cd in unprocessed, black tea and tea liquors and establish whether they conform to the maximum permissible concentration's (MPC's) set for tea.
2. To determine the levels of fluoride in black tea liquors and establish whether they conform to the World Health Organization's (WHO) limit for drinking water.
3. To compare the levels of Fe, Zn, Cu, Pb and Cd in unprocessed, black tea and tea liquors.
4. To compare the Fe, Zn, Cu, Pb, Cd and fluoride contents of Kenyan teas with those of teas from Tanzania, Uganda and Rwanda.

1.4 Null Hypotheses (H_o)

1. The levels of Fe, Zn, Cu, Pb and Cd in unprocessed, black tea and tea liquors do not conform to the MPC's set for tea.
2. The levels of fluoride in black tea liquors do not conform to the WHO's limit for drinking water.
3. The levels of Fe, Zn, Cu, Pb, Cd and fluoride in unprocessed, black tea and tea liquors are not significantly different from each other.
4. The Fe, Zn, Cu, Pb, Cd and fluoride contents of Kenyan teas are not significantly different from those in teas from Tanzania, Uganda and Rwanda.

1.5 Justification

Globally, consumers, tea consumers included, are keen about food safety regarding heavy metals and fluoride. Heavy metals as well as fluoride can be introduced in tea through a number of pathways during the various stages of production, most important being the growth media (soil and fertilizers) and during processing (substandard machinery). It is well known that the chemical characteristics of soils from various regions and consequently their heavy metal and fluoride content are different. However, scarce data is available on the levels of heavy metals and fluoride in tea from the different growing areas in Kenya. Also, Kenyan tea is high grown, pesticide free and is of high quality. For this reason, it is usually blended with low quality teas from other tea producing nations to enhance their quality, and subsequently marketed as Kenyan teas fetching high prices. Most of these teas are from countries that use pesticides to control pests and diseases. This implies that consumers buy very little of Kenyan tea and as such, Kenyan farmers loose on sales of their product. Also, there is no mechanism in place to check the safety status of the teas used for blending, hence the risk of Kenyan teas being blacklisted for not conforming to the MPC's for heavy metals and other toxic elements such as fluoride. For these reasons, there is need to analyze Kenyan tea to ensure that the levels of heavy metals and fluoride meet the set local and international food safety standards.

CHAPTER TWO
LITERATURE REVIEW

2.1 Tea Growing and Harvesting

Young tea plants are raised from cuttings obtained from mother bushes and are carefully tendered in nursery beds until they are 12 to 15 months old. They are then planted out in tea fields with a spacing of between 1.0 to 1.5m (KTDA, 2011). Unlike most herbs that only need to be dried, commercial tea must be "processed". Tea is harvested manually or mechanically, with each pluck taking only the flush (two leaves and a bud), and these tender and succulent fresh growth is the raw material from which a number of tea products are processed. High standard fine plucking is the first step towards superior quality tea manufacture. The interval between plucking and delivery is kept as short as possible and great care is taken when transporting green leaf to the factory (KTDA, 2011).

There are several types of tea products based on the method of manufacture and consequently, their chemical composition (Reeves *et al.,* 1987) which includes green, oolong and black tea. The nature and quality of a given tea product is mainly dependent on the chemical composition of the unprocessed tea and the reactions they undergo during the manufacture process. The techniques of manufacture of the mentioned tea products (green, oolong and black) may be orthodox or non-orthodox and vary considerably with regard to their impact on the formative and degradative patterns of the various cellular components (Mahanta and Hemanta, 1992; Wilson and Clifford, 1992). In the present study, black CTC tea was used as it is the main product processed, marketed and consumed in the study area (East Africa).

2.2 Tea Processing

The general steps in the manufacture of black CTC tea are briefly mentioned below;

Withering; Tea leaves are loaded into troughs fitted with powerful exhaust fans that draw the moisture and carry the humid air out. This may take 10 to 20 hours and reduces the moisture content of the fresh leaves to between 65 and 67%, making them amenable to subsequent processing steps (KTDA, 2011). During withering, diverse biochemical changes occur (Robinson and Owuor, 1992; Costa *et al.,* 2002) and include changes in proteins, caffeine, sugars, organic acids, polyphenol oxidase activity, chlorophyll, minerals, volatile components, and permeability of cell membranes (Dev Choudhury and Bajaj, 1980).

Maceration; This step is also referred to as rolling and is accomplished by CTC machines where the cell structures are disrupted, exposing the cell contents to atmospheric

oxygen (KTDA, 2011), bringing various enzymes into intimate contact with their substrates in this case the polyphenols. The chemical and biochemical reactions initiated during withering proceed at an accelerated rate during and after the rolling (Hara *et al*., 1995).

Fermentation or Aeration; Important reactions that occur during this stage are the development of colour, strength and quality of tea by the production of non-volatile compounds through the enzymatic oxidation of catechins and their gallates and the production of volatile compounds responsible for the characteristic aroma of black tea (Tombs and Mashingaidze, 1997). These chemical and biochemical reactions make fermentation the most critical step in black tea manufacture (KTDA, 2011).

Drying or Firing; This step is primarily intended to cause cessation of enzyme activity and reduce the moisture content to about 3% of the dry mass (KTDA, 2011). However, other changes other than removal of moisture that occur during this step include a significant loss of volatile compounds, an increase in the levels of amino acids, the binding of polyphenols to other tea components and an increase in carboxylic acids and Maillard reactions (Hara *et al*., 1995).

Grading or Sizing; After drying the teas are then sorted into primary and secondary grades, the criteria being the size of the tea leaves and their fibre content, where whole, large tea leaves gain a higher grading (KTDA, 2011). The dry tea is exposed to static electricity-charged PVC rollers that pick up the fibres and the open leaf. Tea grading facilitates the international trade in tea and is the central component in the assessing of a money value for the various types of tea. Also, it is an important tool for tea experts in making evaluations and comparisons between the different varieties of tea grown and manufactured world over. In the current study, three primary grades of tea (BP1, PF1 and PD) were considered.

2.3 The Kenyan Tea Industry

The tea plant is said to have been brought to Kenya by the Caine brothers who imported the dark leafed "Manipuri" hybrid seed from Assam in 1904 and 1905 and established a plantation at Limuru, Central Kenya (Matheson, 1950). Tea planting expanded rapidly and by 1929 there were a total of 2,162 hectares of tea in the country (Greenway, 1945), which by the year 1963 had risen to 21,448 hectares while in 2008, the acreage stood at 157,720 hectares. Statistics released in 2009 by ITC (2009), showed Kenya being the third largest producer of tea in the world after China and India and specialized in the processing and export of black CTC tea.

Several studies in Kenya have revealed wide response ranges in tea yield with respect to climate (Ngetich *et al*., 2001). The tea-growing areas in Kenya can be divided into two main regions defined by the Great Rift Valley. To the East of the Rift are the cool Aberdare highlands, the snow-capped Mt. Kenya and the Nyambene hills and to the West of the Rift lie the Nandi Hills, highlands around Kericho, Mt. Elgon and the Kisii highlands. It is on the slopes of these highlands within the altitudes of between 1500 and 2700m above sea level that tea is grown (TRFK, 2002).

According to the Tea Board of Kenya, TBK (2007), tea production in Kenya is characterized by two sectors; large-scale sub-sector (estate plantations) with production units larger than 20ha, and small-scale sub-sector with smaller units averaging 0.25ha per farmer. The commercial cultivation in Kenya started in 1924 by the large-scale sub-sector, under the umbrella of Kenya Tea Growers Association (KTGA). The small-scale sub-sector, managed by the Kenya Tea Development Agency (KTDA) was started in 1964 after Kenya gained her independence (TBK, 2007). The large-scale sub-sector has large plantations which are managed by trained personnel whereas the farmers in the small-scale sub-sector rely on agricultural extension officers who offer advisory service in managing their tea fields (Ogola and Kibiku, 2004).

2.4 Tea and Health

Based on extensive animal experiments and available epidemiologic data, the medical community recognizes tea as a beverage that may offer several health benefits (Moreda-Pineiro *et al.,* 2003; Naithani and Kakkar, 2005). Such health benefits that have been ascribed to the regular consumption of tea include; the reduction of serum cholesterol, decreased risk of cancer and cardiovascular diseases (Zuo *et al.,* 2002; Chung *et al.,* 2003), prevention of a number of diseases, including skin cancer (Katharine, 2001), Parkinson's disease (Richard, 2001), myocardial infarction (Cheng, 2003), and coronary artery disease (Hirano *et al.,* 2003).

These health benefits have been attributed to the strong antioxidant activity of catechins, tea phenolic compounds (Zuo *et al.,* 2002; Karori *et al.,* 2007) that protect the body against free radical-induced oxidative stress (Pourmorad *et al.,* 2006). In addition, polyphenols, a biologically active group of tea components have been associated with amelioration of inflammation (Karori *et al.,* 2008), inhibition of diabetes (Vinson *et al.,* 2001; Sabu *et al.,* 2002), prevention of intestinal damage and anti-diarrhoea properties (Asfar *et al.,* 2003), enhancement of oral health (Wu and Wei, 2002) and the potential to improve spatial

cognitive learning ability (Haque *et al.*, 2006). Other compounds in tea that are beneficial to human health include; fluoride, caffeine and essential minerals such as Fe, Cu and Zn (Cabrera *et al.*, 2003).

2.5 Heavy Metals and Health

Although there is no clear definition of what a heavy metal is, density is in most cases taken to be the defining factor. Heavy metals are thus commonly defined as those having a specific density of more than 5g/cm^3 (Jarup, 2003). The main sources of the heavy metals in plants including tea are their growth media, i.e., the soil (Somer, 1974; Narin *et al.,* 2004). Other sources include agro-inputs such as insecticides, herbicides and fertilizers that may be absorbed through the leaves, roots and the barks of the plant (Fwu-Ming and Hong-Wen, 2008) as well as rainfall in atmospheric polluted areas due to high traffic density, and industrialization (Lozak *et al.,* 2002; Sobukola *et al.,* 2008) and substandard machinery during transportation and processing.

Research has also indicated that the content of 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 metals (Divrikli *et al.,* 2006). Also, most heavy metals are not biodegradable, have long biological half-lives and thus persist in different body organs, a phenomenon called bioaccumulation, where they eventually lead to unwanted side effects (Jarup, 2003; Sathawara *et al.*, 2004). Such elements include Pb and Cd and are toxic to humans even at very low concentrations and have been associated with the etiology of a number of diseases especially cardiovascular, kidney, nervous as well as bone diseases (WHO, 1996; Steenland and Boffetta, 2000; Jarup, 2003). The biological importance, toxicological information and possible sources of selected heavy metals are briefly mentioned below.

2.5.1 Iron (Fe)

Fe is an essential mineral and an important component of proteins involved in oxygen transport (hemoglobin, myoglobin and the cytochromes) and metabolism. It is also an essential co-factor in the synthesis of neurotransmitters such as dopamine, norepinephrine, and serotonin. About 15% of the body's Fe is stored for future needs and mobilized when dietary intake is inadequate. The body usually maintains normal Fe status by controlling the amount of Fe absorbed from food. Its deficiency causes anemia (Tortora, 1997).

2.5.2 Zinc (Zn)

Zn is cofactor of many enzymes such as superoxide dismutase involved in a number of metabolic processes in humans (Tedayon and Lahiji, 2011). Zn deficiency may result due to inadequate dietary intake, impaired absorption, excessive excretion or inherited defects in Zn metabolism (Colak *et al.,* 2005) and results in growth retardation, loss of appetite (Ensminger *et al.,* 1995; Wardlaw and Insel, 1996), skin changes and immunological abnormalities (Tedayon and Lahiji, 2011).

2.5.3 Copper (Cu)

Cu is a nutritionally essential element that is widely distributed in nature and plays an important role in carbohydrate and lipid metabolism at low concentrations (Brun *et al.,* 2001; de Moraes Flores *et al.,* 2001; Kenduzler and Turker, 2003). However, it is highly phytotoxic at high concentrations (Brun *et al.,* 2001); seriously affecting blood and kidneys (Hajjar and Kotchen, 2003), inducing Fe deficiency, lipid peroxidation and destruction of membranes (Zaidi *et al.,* 2005). Also, a number of pathogenic characteristics have been attributed to Cu toxicity and include; non-Indian childhood cirrhosis (Zietz *et al.,* 2003) and Wilson's disease (Verissimo *et al.,* 2005). Therefore, overconsumption of Cu from food and beverages is detrimental to human health (Kawada *et al.,* 2002), hence the importance to trace and control the Cu content in water, food and beverages on a daily basis.

2.5.4 Lead (Pb)

Pb is one of the most widely distributed contaminant in the environment worldwide (Hafen and Brinkmann, 1996; Chena *et al.,* 2005) and is highly toxic to both man and animals (Fifield and Haines, 1997). Pb toxicity affects the brain, heart, kidneys, liver, nervous system, and pancreas. It may cause many signs and symptoms such as abdominal pain, anaemia, anorexia, anxiety, bone pain, brain damage, confusion, constipation, convulsions, dizziness, drowsiness, fatigue, headaches and hypertension, as well as diminishing the intelligence quotient (IQ) in children (Marcus and Schwartz, 1987; IAEA, 1994; WHO, 1996). According to Ellen (1996) leaded petrol caused more exposure to Pb in man than any other single source. These findings led to the close monitoring of Pb in food and drinking water and the enacting of strict regulations that led to the eventual stoppage of the use of leaded petrol. In fact, the WHO and most countries have since stipulated recommendations for the maximum allowable levels of Pb in food, drinking water and beverages (Needleman *et al.,* 1990; Rosen, 1992; WHO, 1996; CAC/FAO, 1999).

2.5.5 Cadmium (Cd)

Cd is a toxic metal with sterilizing, teratogenic and potent, multi-tissue animal carcinogenic effects that may also lead to cardiovascular diseases (Waalkes, 2000; Satarug *et al.,* 2002; Kocak *et al.,* 2005). It is an inhibitor of enzymes with sulphydryl groups and disrupts the pathways for oxidative metabolism (Tortora, 1997). Its toxicity affects many target tissues such as appetite and pain centers in the brain, heart and blood vessels, kidneys and lungs. Its toxicity is characterized by anaemia, dry and scaly skin, emphysema, fatigue, hair loss, heart disease, depressed immune system response, hypertension, joint pain, kidney stones or damage, liver dysfunction or damage, loss of appetite, loss of sense of smell, lung cancer and pain in the back and legs (Taher, 2003). The phytotoxicity of Cd is also well documented (Fodor, 2002) and is manifested as inhibition of plant growth (Titov *et al.,* 1995), nitrate assimilation (Hernandez *et al.,* 1997) and photosynthesis (Barcelo *et al.,* 1988; Larbi *et al.,* 2002), as well as disturbances in plant ion (Wallace *et al.,* 1992) and water balances (Barcelo and Poschenrieder, 1990).

2.6 Fluoride

2.6.1 Occurrence of Fluoride

Fluorine (F) occurs in a number of minerals in the earth's crust including; cryolite (Na_3AlF_6), fluorspar (CaF_2), chiolite ($Na_5Al_3F_{14}$) and apatite ($CaFPO_3$) (Wong *et al.,* 2003). Due to its reactivity, fluorine occurs in an anionic form, fluoride, which is found dissolved in sea water, lakes and rivers. Also, appreciable amounts of fluoride are found in plants, with the actual amounts being dependent on the species of the plant, part and age of the plant. Mature plants generally tend to contain higher levels of fluoride than young ones due to accumulation (Leone *et al.,* 1956).

Fluoride is widely used in various branches of industry and some fluoride compounds are formed as by-products in certain processes. For instance, fluorides have been reported to be discharged into the atmosphere by aluminium and fertilizer production plants (Garrec *et al.,* 1977). Therefore, excessive amounts of fluoride in the form of different compounds can enter the human body by means of polluted air, water and food. Additional sources of fluoride for humans are fluoridated toothpaste which contains fluoride in various forms such as NaF, SnF_2 and Na_2PO_3F, fluoridated water (Zerabruk *et al.,* 2010) and fluoride supplements in form of gels, rinses and tablets.

2.6.2 Fluoride Uptake by the Tea Plant

Studies have demonstrated that the tea plant (*Camellia sinensis*) is capable of selectively taking up fluoride from the soil and air and accumulate it in its leaves (Hidekazu and Toshiyuki, 1977; Duckworth and Duckworth, 1978; Fung *et al*., 1999; Lee *et al.,* 2003; Weinstein and Davison, 2004; Sha and Sheng, 1994; Shyu *et al*., 2009). The mobility of the ion (F^-) in the soil is influenced by a number of factors, most importantly the quantity of the minerals present, soil pH, adsorption to positively charged complexes, concentrations of Fe, Ca, and Al in the soil among others (Fung *et al.,* 1999).

The pathway of uptake of fluoride from tea soils to the tea plant has been well established (Wong *et al.,* 2003). Tea garden soils are usually acidic with pH values generally less than 5.5. Under this conditions (acidic), the aluminium-fluoride-halide complexes present in the soil decompose into aluminium ions (Al^{3+}) and fluoride ions (F^-) which correspond to high mobility (solubility) and hence the availability of the anion (F^-) for uptake by the tea plant. The free fluoride ion is then absorbed by tea roots and transported to and stored in the tea plant's leaves (Ruan and Wong, 2001), a phenomenon that has led to the tea plant to be referred to as a fluoride accumulator (Cao *et al.,* 1997).

2.6.3 Fluoride and Health

A moderate amount of fluoride, has been confirmed to be an effective way of reducing the incidence of dental caries (Levi *et al.,* 1983). This is because it interacts with hydroxyapatite on the tooth enamel, 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. It also increases the rate of enamel remineralization, so that calcium (Ca) and phosphate ions (PO_4^{3-}) are protected and not lost during demineralization. In addition, fluoride may reduce oral concentration of cariogenic bacteria or reduce the metabolism of bacteria in plaque. Therefore, the intake of fluoride is needed to promote good dental and oral health. However, excessive intake of fluoride results in both dental and skeletal fluorosis (WHO, 2002). Thus, keeping a safe threshold for fluoride exposure to avoid probable dental and skeletal fluorosis is an important issue in health care.

2.7 Soil pH and Mineral Uptake

Higher acidity of soils in tea farms has been attributed to the continuous application and increasing rates of nitrogenous (NPKS) fertilizers (Owuor *et al.,* 1990). Ishibashi *et al.*

(2004) argues that nitrogenous fertilizers produce hydrogen ions (H^+) via the following reaction which is induced by bacteria present in the soil:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$$

This implies that, fertilizer application increases the rate of nitrification during which inorganic nitrogen is converted to nitrate, yielding H^+, hence subsequent acidification of the soil. This condition enhances the bioavailability and uptake of some minerals by the tea plant. However, it has been reported that the bioavailability and hence the uptake of some minerals by the tea plant diminishes with increasing pH whereas others are not affected. For instance, Fung and Wong (2001), report that soil extractable Al, fluoride and Zn levels decrease whereas extractable Ca, Cu, K, Mg, Na and P levels increase when the soil pH is raised from 3 to 6. Regional variations in the mineral composition have also been well documented (Kumar *et al.,* 2005). Based on these findings, it is therefore safe to assume that, the metal content of tea, depends on the soil composition as well as other local environmental factors (Moreda-Pineiro *et al.,* 2003).

2.8 Food Safety Standards

Food safety standards are a set of limits that define the MPC's of metal residues and other chemical substances that are safe for human consumption. These guidelines are determined by bodies such as the Kenya Bureau of Standards (KEBS), the Food and Agriculture Organization (FAO), WHO among others and their aim is to protect the consumers and ensure fair practices in food trade. They deal with detailed requirements related to a food or group of foods, the operation and management of production processes and the operation of government regulatory systems for food safety and consumer protection.

The Codex Alimentarius is a collection of standards, codes of practice, guidelines and other recommendations adopted by the Codex Alimentarius Commission (CAC) which was established by the FAO and the WHO, in the 1960's. It is the single most important international reference point for developments associated with food standards. Table 1, gives a list of the MPC's set for the heavy metals of interest and fluoride in tea both locally and internationally. A critical examination of the table clearly reveals that the Kenyan standard for black tea (KS 65: 2009) is very strict. For instance, the MPC for Cd in tea in the Kenyan standard is 0.02ppm, a value 10 times less than the one for Sri-Lanka which is 0.2ppm. For fluoride, there is no MPC set for tea. However, according to the WHO (2011), the allowable concentration for fluoride in drinking water is 1.5µg/ml.

Table 1: MPC's for Fe, Zn, Cu, Pb, Cd and fluoride in tea

	Concentration in parts per million (ppm)		
Component	**KS 65:2009***	**SLTB***	**EU***
Fe fillings	50.0	-	-
Fe	-	500	-
Zn	50.0	100	50.0
Cu	30.0	100	150.0
Pb	0.1	2.0	1.0
Cd	0.02	0.2	0.1
Fluoride	-	-	-

*MPC's are based on black tea; **KS 65:2009 -** Kenyan standard for black tea; **SLTB -** Sri-Lanka's standard for black tea; **EU -** European Union.

2.9 Analytical Methods

2.9.1 Heavy Metal Determination

Due to the importance of some elements in healthy growth and development, several studies have been carried out globally using different methods and techniques in order to determine their levels in tea. These include Inductively Coupled Plasma Atomic Emission Spectrometry, ICP-AES (Fernandez *et al.,* 2002), Inductively Coupled Plasma Mass Spectrometry, ICP-MS (Matsuura *et al.,* 2001), capillary electrophoresis (Feng *et al.,* 2003), total reflection X-ray fluorescence (Xie *et al.,* 1998), and Atomic Absorption Spectroscopy, AAS (Seenivasan *et al.,* 2008). Flame Atomic Absorption Spectrophotometry (FAAS) has been widely selected and used as the technique of choice owing to its high sensitivity, simplicity, reproducibility, wide dynamic concentration range and its low cost for these determinations.

2.9.2 Fluoride Determination

The fluoride ion-selective electrode has been a very successful potentiometric technique for the determination of fluoride in aqueous solutions largely replacing the colorimetric methods previously used (IUPAC, 1987), which were limited by lack of sensitivity, reproducibility, sensitivity and susceptibility to interferences by presence of various ions (Kokot and Kupcewicz, 1998). This was employed as the method of choice in fluoride determinations in the current piece of work since it is quick, simple, accurate and economical. However, the development of ion chromatography (IC), with its advantage of

simultaneous multi-ion determination is introducing a new phase into fluoride analysis (IUPAC, 1987).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Sample Collection

The study area involved four countries in East Africa, that is, Tanzania, Rwanda, Uganda and Kenya. Twenty four (24) unprocessed tea samples were collected in triplicates from 24 tea factory catchment areas in Kenya from both the large and small-scale tea sub-sectors, selected to give a good regional representation. The selected factories were Masingi, Kitumbe, Rorok, Tegat, Changoi, Chemomi, Kapsumbeiwa, Kaimosi, Koiwa, Cheboswa, Nyansiongo, Ogembo, Mudete, Kapsara, Kangaita, Weru, Chinga, Kiru, Mataara, Makomboki, Michimikuru, Rukuriri, Metarrora and Sotik tea. Also, 72 black CTC tea samples of three primary grades (24 BP1, 24 PF1 and 24 PD) were collected from the same factories with each replicate sample of each grade being picked from a different drier. In addition, 12 black tea (4 BP1, 4 PF1 and 4 PD) samples from Tanzania, Rwanda, Uganda and Kenya were obtained in triplicates from the Venus Tea Brokers (VTB) and Tea Brokers of East Africa (TBEA) at the Mombasa tea auction. Standard tea samples A and B were provided by the Tea Research Foundation of Kenya (TRFK).

3.2 Sample Pretreatment

The unprocessed and black tea samples, were placed in clean, well labeled paper bags and transported to the TRFK laboratories situated at Kericho, Timbilil Estate, where they were oven dried (Memmert, 854 Schwabach, Germany) for 12 hours at a temperature of 103ºC. Once dry, the samples were finely ground using an electric blending device (Moulinex AR1043, China) to reduce the particle size and homogenize the sample.

3.3 Sample Analysis

3.3.1 Heavy Metal Determination

For the determination of the total metal contents, the ground, oven dried (103ºC) unprocessed and black tea samples were allowed to cool in a desiccator for an hour. Then, 1.0g 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. The acid-peroxide reagent was prepared by thoroughly mixing equal volumes of a mixture of 1:1 HNO_3 (nitric acid) to H_2O (water) and 1:1 HCl (hydrochloric acid) to H_2O and a peroxide reagent (200ml of 30% hydrogen peroxide, H_2O_2, diluted to 1 litre with distilled water) in the

ratio 2:3. 0.5ml of this acid-peroxide mixture was then added to each of the ashed samples in the specimen tubes and heated on an electric hot plate and allowed to evaporate to near dryness. 25ml of 0.05N HCl solution was then, added into each specimen tube, stoperred, shaken thoroughly and allowed to stand for four (4) hours before analysis. To evaluate the water extractable metal contents, standard tea liquors of the tea samples were prepared as described by Reeves *et al*. (1987), using 375ml of boiling distilled water and 9g of the dry tea leaves and agitated on a mechanical shaker for 10 minutes. These mixture was then filtered and the filtrate obtained (2.4% w/v) allowed to cool to room temperature prior to analysis. In addition, 10 blank solutions were prepared alongside the sample solutions for both the total and water extractable metal contents.

The calibration curves for the determination of the total and water extractable metal contents in the tea samples by FAAS were established using the following procedure; Appropriate amounts of commercial single-element working solutions of Fe, Zn, Cu, Pb and Cd and 5ml of 0.05N HCl were transferred into a clean and dry 100ml volumetric flasks and diluted to the mark with distilled water. The concentration ranges of the calibration solutions varied from one element to the next and were as tabulated in Appendix III.

A Varian Spectra AA-880, flame atomic absorption spectrometer (FAAS), equipped with a sample preparation system (SPS-5, Varian) and hollow cathode lamps was used for the analysis. The instrumental parameters such as wavelength, slit width, lamp current, flow rates of the fuel and oxidant among others were adjusted according to the manufacturer's recommendation depending on the type of analyte as given in appendix II. The blank and calibration solutions were then run from the most dilute to the least dilute (starting with the blank) to avoid the memory effect, followed by the sample solutions prepared earlier. The absorbances obtained were recorded.

The absorbance's obtained for the calibration solutions were used to construct calibration curves for each analyte, the equations (appendix III) of which were then used to evaluate the metal contents in the sample and blank solutions. In order to determine the limits of detection (LOD) for the total and water extractable metal contents for the analytes of interest of the analytical procedures outlined, the standard deviation of the ten 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.

3.3.2 Fluoride Determination

Due to difficulties in access to the appropriate equipment and reagents for the quantitative separation of fluoride from the tea (plant) matrix, only the water extractable fluoride content of the black tea samples collected as described in section 3.1 above were quantified. The tea liquors were prepared by dissolving 1g of black tea in 100ml boiling water, agitated for 10 minutes on a mechanical shaker and filtered. The filtrate (1% w/v) obtained was allowed to cool to room temperature before analysis. In addition, ten blank samples were prepared alongside the tea liquor samples. Fluoride calibration solutions were prepared by serially diluting a commercial fluoride stock solution (1000ppm). A Total Ionic Strength Adjustment Buffer (TISAB) prepared as described by Shyu *et al.* (2009) was used to stabilize the ionic strength condition of the blank, calibration and sample solutions and to chelate various cations such as Al^{3+} and Fe^{2+} which would interfere with the analysis of fluoride through complex formation.

A flow plus fluoride ion selective electrode (EDT, direct-ion 5221) was then immersed in the blank series and tea liquor samples and readings (potential) on the ion analyzer (EDT, direct ion, DR359TX) in millivolts were recorded. The standards, which were ten-fold concentration apart and were used to check the instrument slope as well as prepare the calibration curve, whose equation was used to compute the sample concentrations. The method detection limit for this analytical procedure was computed using the mean and standard deviation of the blank determinations as described in section 3.3.1.

3.4 Statistical Analysis of Data

Statistical comparisons of the results of heavy metal levels for the various groups of samples was carried out by ANOVA using MSTAT statistical analysis package for windows version 5.4.2.0 by JSoftware, Inc. Statistically significant differences were evaluated at $p \leq 0.05$.

3.5 Results and Discussion

3.5.1 Analytical Method Detection Limits

LOD is the smallest mass of analyte that can be distinguished from statistical fluctuations in a blank (Butcher and Sneddon, 1998; Armbruster and Pry, 2008). It is expressed as a concentration or quantity derived from the smallest measure that can be detected with reasonable certainty for a given analytical procedure (Thomsen *et al.,* 2003). This value usually corresponds to the mean of the blank measures, added the standard deviation of the blank measures multiplied by a constant value k (Butcher and Sneddon,

1998). The constant, k, is a numerical factor chosen according to the confidence level desired (Thomsen *et al.*, 2003) and in the current study it was chosen to be 3. The detection limits obtained for the analytical procedures for unprocessed, processed tea and tea liquor, for the five analytes were as tabulated in appendix IV. These values characterise the analytical performance of a given test, thus helping in understand their capability and limitations and as such making it possible for 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 analytes in tea samples and more importantly the water extractable portions in the tea liquors that we consume.

3.5.2 Iron (Fe)

Figure 1 shows the levels of Fe in the Kenyan unprocessed, black tea and tea liquors. The plotted values for each factory for the black teas and their liquors are means of the values obtained for the three grades of tea (BP1, PF1 and PD) used. The levels of Fe in unprocessed tea have not been frequently documented, however, the levels of Fe in the 24 unprocessed tea samples were found to range widely between 55 and 203µg/g (Appendix VI), where the highest Fe level (203µg/g) was obtained for the samples from Rorok tea factory and the lowest (55µg/g) was for the sample from Changoi tea factory. Black tea samples had mean Fe levels between 118 and 329µg/g where the samples from Chemomi had the lowest Fe content (mean; 121µg/g) whereas those from Mataara had the highest (299µg/g). Tea liquors prepared from these black tea samples had Fe levels tabulated in Appendix VI. The lowest extractable Fe level obtained was for the sample sourced from Changoi (mean; 5.6µg/ml) whereas the highest concentration was obtained in the sample sourced from Mataara (mean; 11.2µg/ml). The letters S and L in brackets following the factory names stand for small and large-scale tea sub-sectors respectively.

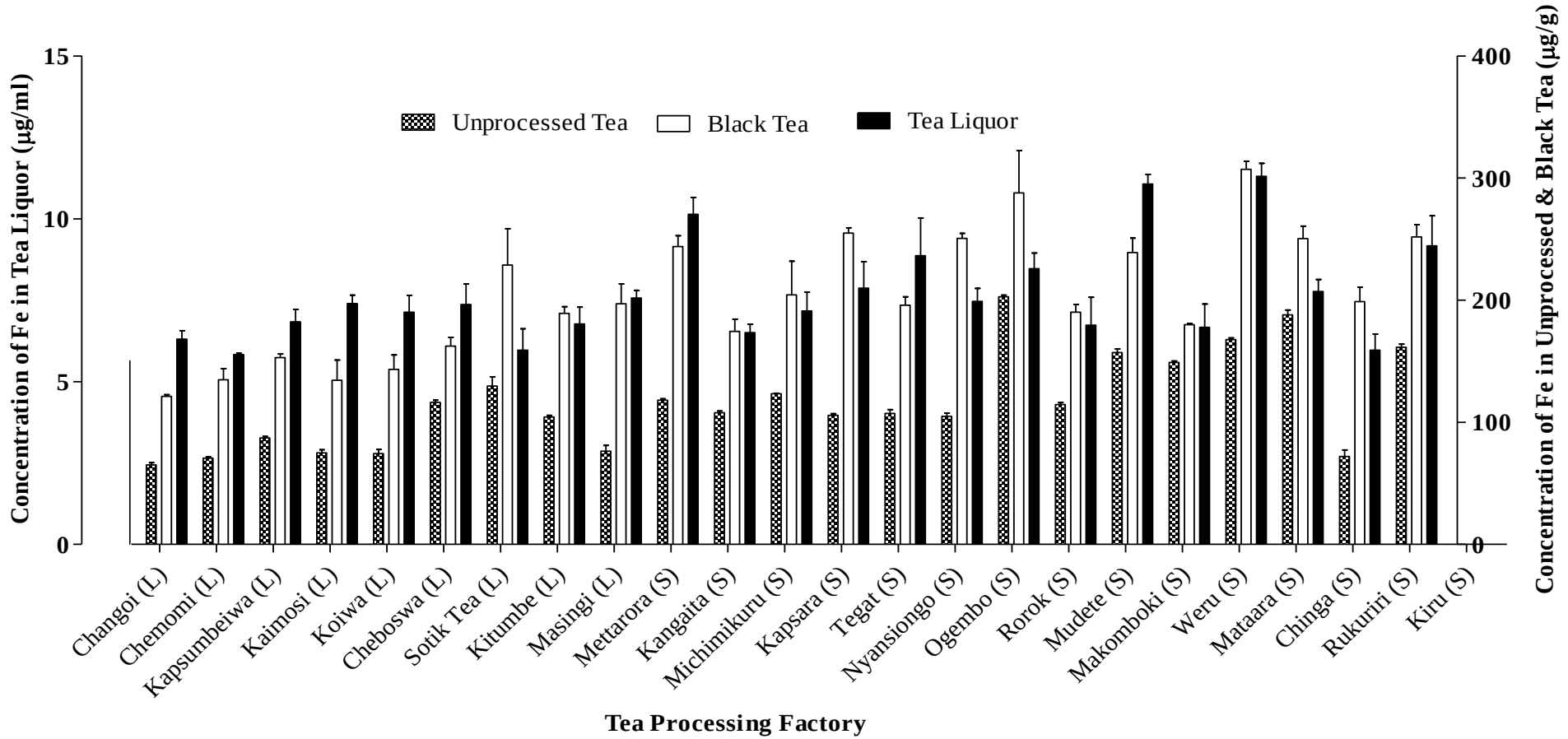

Figure 1: Fe in tea from 24 tea factory catchments in Kenya

Fe levels obtained for the black tea samples from Tanzania, Rwanda, Uganda and Kenya sourced from the Mombasa tea auction were as tabulated in appendix VII. The levels ranged widely between 151 and 369µg/g as can be seen in figure 2. Black tea from Uganda had the highest Fe content (mean; 344µg/g), followed by Rwanda (mean; 262µg/g) and Tanzania (mean; 195µg/g). Kenyan black tea had the lowest levels of Fe (mean; 158µg/g) and evidently, Fe contents of the tea samples from the four countries are statistically different ($p \leq 0.05$). The tea liquors prepared from these black tea samples gave values of Fe tabulated in appendix VI. The highest and lowest Fe levels obtained were 5.9 and 12.5µg/ml respectively and the mean Fe levels for each grade were 8.3µg/ml (BP1), 9.2µg/ml (PF1) and 8.5µg/ml (PD). Tea liquors of the tea samples from Uganda had the highest Fe content (11.4µg/ml) while those from Kenya had the lowest (7.0µg/ml). A graphical representation of this data is given in figure 3.

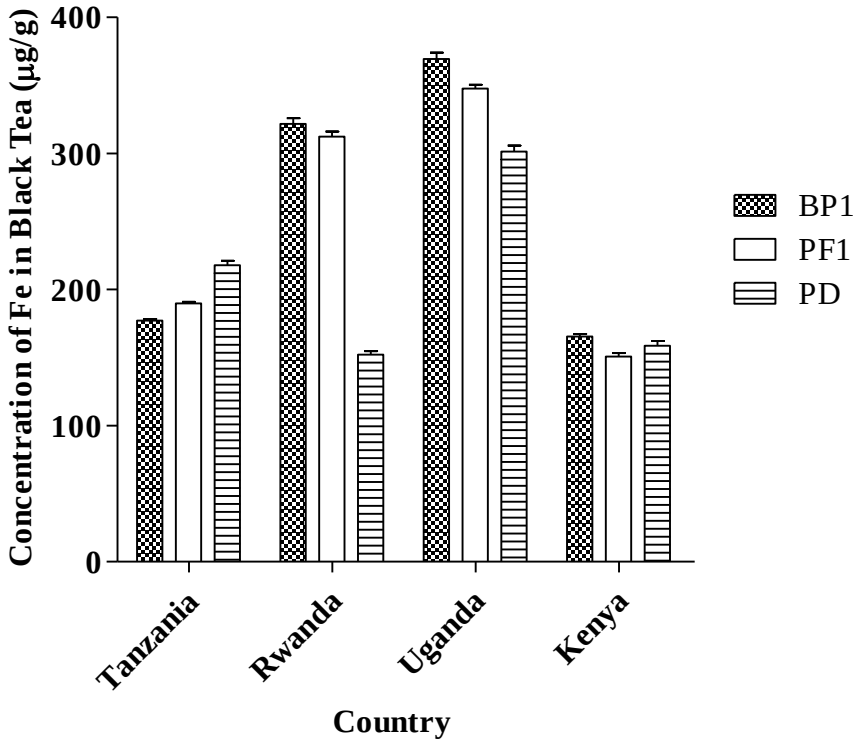

Figure 2: Fe in black tea from Tanzania, Rwanda, Uganda and Kenya

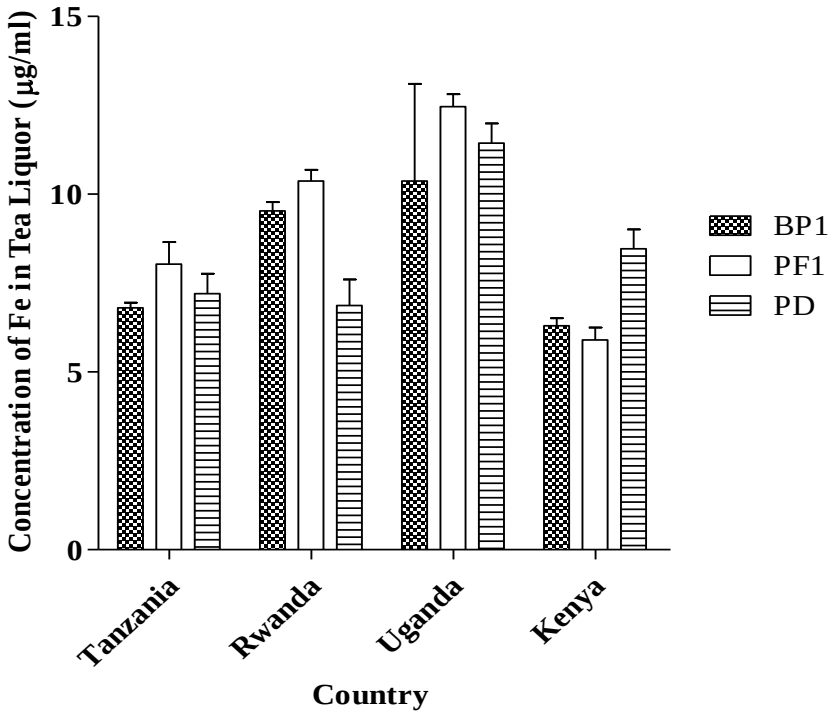

Figure 3: Fe in tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

This data revealed that the levels of Fe in unprocessed, black tea and tea liquors were statistically different ($p \leq 0.05$) as can be clearly seen in figures 1 and 4. The levels of Fe in the tea liquors are only a very small proportion of the total Fe contents in the black teas. This implies that only a portion of the total Fe content is water soluble, with the larger portion being insoluble. This further suggests that, a larger portion total Fe content in black tea is organically bound. It was expected that Fe levels in unprocessed and black tea from the same regions to be the same, however this was not the case as the Fe levels in black tea were much higher than those in unprocessed tea. These implies that iron, probably as iron fillings is introduced during the manufacture process especially so during the CTC process as a result of wear and tear of machinery.

There is no MPC's set for Fe in the Kenyan standard as can be seen from table 1. However, Sri-Lanka's Tea Institute gives their upper limit to be 500.0ppm, a comparison to which reveals that all the tea samples analysed are in conformity. A limit for Fe fillings is given to be 50.0ppm the Kenyan standard, KS: 65 2009, but reference to this can only be made once the source of the additional Fe content in black tea (after processing) as compared to the levels in two leaves and a bud (before processing) is confirmed to be Fe fillings, an investigation that was considered in the current piece of work.

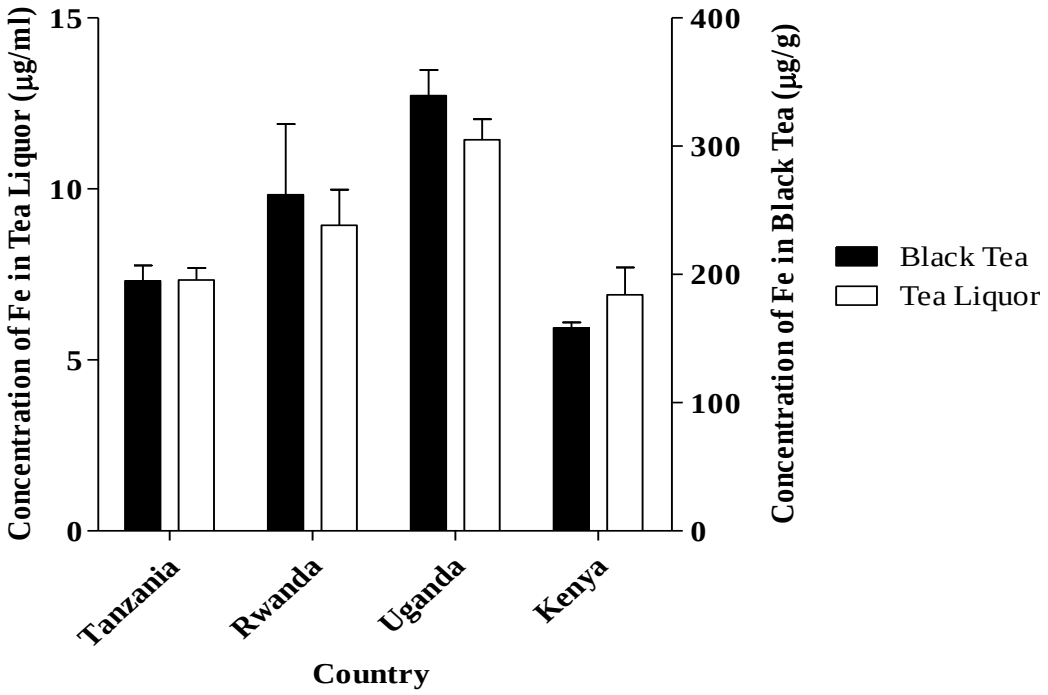

Figure 4: Fe in black tea and tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

Gebretsadik and Bhagwan, (2010) reported Fe levels between 319 and 467µg/g in commercially available Ethiopian black tea analysed by FAAS. These results corroborate the findings of the current study. The current data however reveals geographical variations in the Fe content evidenced by samples from different tea growing regions having different Fe contents. This is in line with the expected because of the differences in soil characteristics in the different tea growing regions considered as well as other agro-climatic factors. Figure 5 gives a representation of five main regions from which the tea samples were obtained as defined in appendix XVII. It is therefore safe to assume that, the Fe content of in tea, depends on a number of factors, most importantly the soil composition (Moreda-Pineiro *et al.,* 2003) as well as other local environmental factors.

Differences in agronomic practices in the large and small and large-scale tea sub-sectors in the Kenyan tea industry are evident and include heavy and regular fertilizer application (both soil and foliar applied), in a bid to maximize on the tea yields. Therefore, an attempt was made to see if this affects the residual levels of the metals in teas from the two sub-sectors. Figure 6 indeed showed that the Fe contents in tea from the small and large-scale tea sub-sectors were statistically different ($p \leq 0.05$), whereby the levels of Fe in unprocessed tea, black tea and consequently the liquors prepared using the black teas from the large scale tea sub-sector being higher than those obtained for the small-scale tea sub-sector.

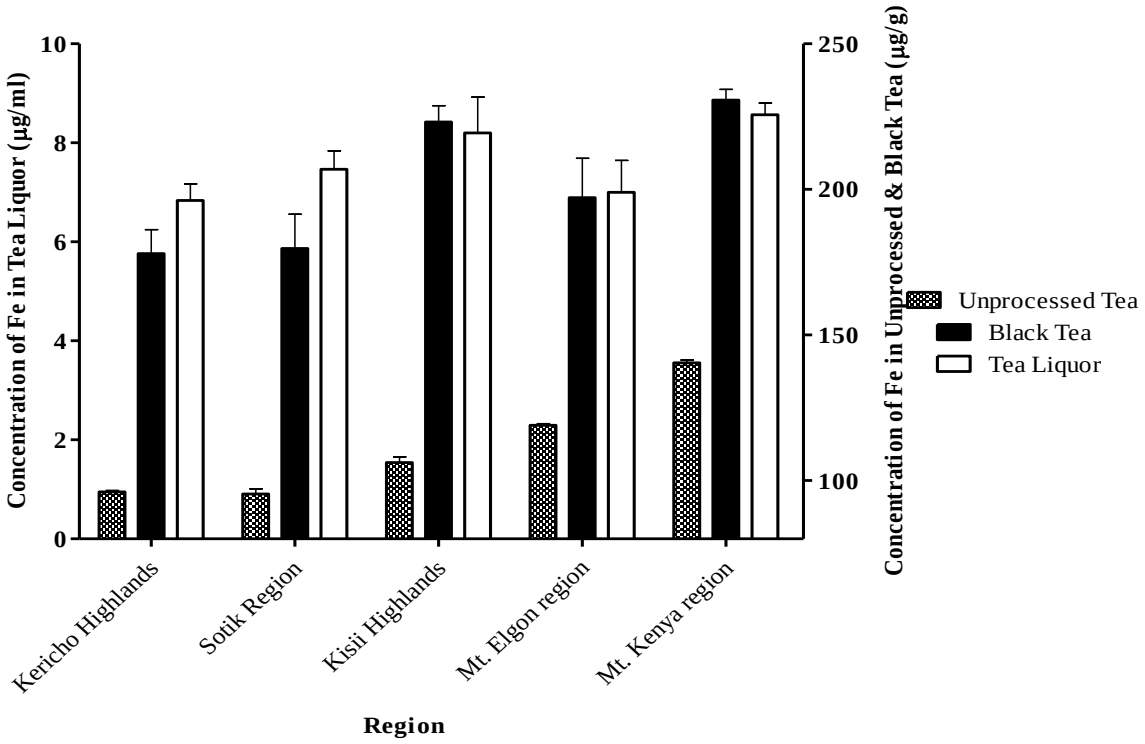

Figure 5: Regional differences of Fe levels in tea

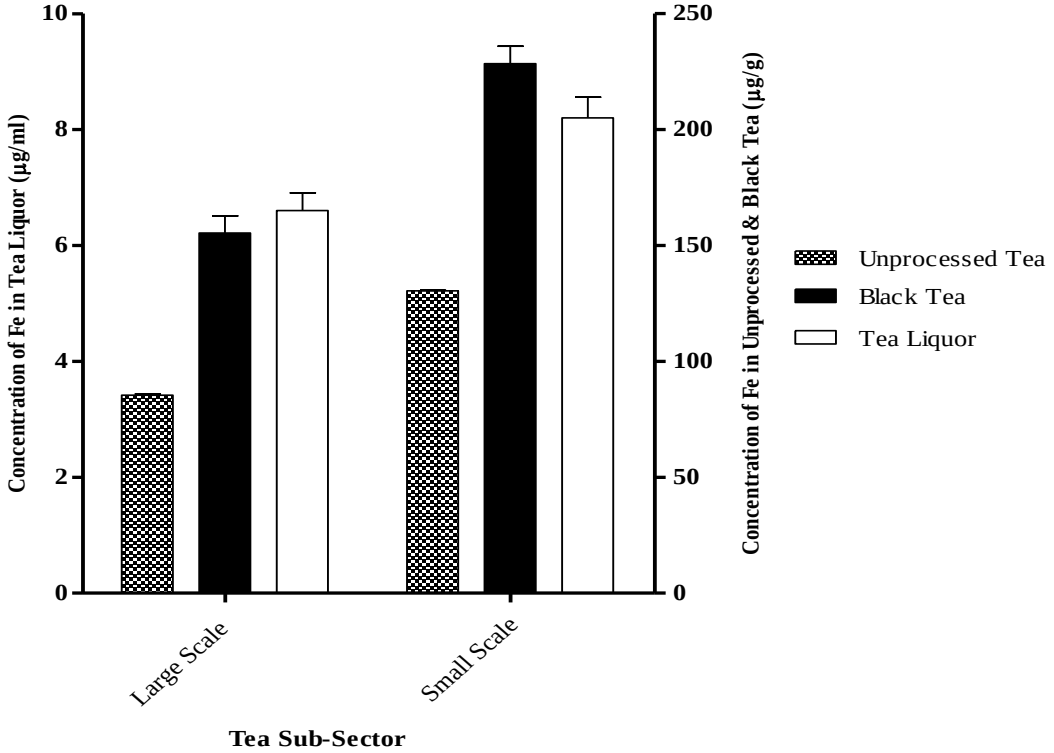

Figure 6: Fe in tea from the small and large-scale tea sub-sectors in Kenya

3.5.3 Zinc (Zn)

Figure 7 is a representation of the levels of Zn in the Kenyan unprocessed tea, black tea and black tea liquors. Similarly, the plotted values for each factory for the black teas and their liquors are mean Zn levels of the three grades of tea (BP1, PF1 and PD) and the liquors obtained from the same. The Zn levels in the unprocessed tea ranged between 15.4 and 32.6µg/g as tabulated in appendix VIII. The highest Zn levels were recorded for the unprocessed tea sample from Cheboswa tea factory whereas the lowest level was for the unprocessed tea sample from Kitumbe. Black tea samples had Zn levels ranging between 18.8 and 44.9µg/g with Kitumbe factory samples having the lowest mean Zn content (19.4µg/g) whereas Chemomi factory had the highest concentration (40.8µg/g). Consequently, the tea liquors prepared from these Kenyan black tea samples had an average Zn content of 3.5µg/ml (Figure 7).

Data obtained for the Zn levels in the black teas from the Mombasa auction for Tanzania, Rwanda, Uganda and Kenya were as tabulated in appendix IX. The Zn levels ranged between 17.1 and 38.9µg/g with Kenyan tea having the highest mean Zn levels (32.7µg/g) as shown in figure 7. The mean Zn levels for the three grades were 27.2µg/g for BP1, 24.3µg/g for PF1 and 28.2µg/g for PD. An average Zn concentration of 4.8µg/ml was recorded for liquors prepared from these tea samples with the highest and lowest concentrations being 5.9 and 4.3µg/ml respectively (Appendix IX). The mean Zn levels for the three grades were 4.7µg/ml (BP1), 4.6µg/ml (PF1) and 5.0µg/ml (PD). A mean of 5.8µg/ml was obtained for Rwanda samples, 4.4µg/ml for Tanzania samples, 4.6 µg/ml for Uganda samples and 4.2µg/ml for Kenya samples.

It is evident from this data that the Zn concentrations in the tea samples from different countries were statistically different ($p \leq 0.05$), an observation in agreement with the data obtained for Fe in the preceding section. This confirms regional variations in the metal contents in black tea. From figures 7 and 10, it is clear that the levels of Zn in unprocessed and black tea were statistically different from the Zn levels in black tea liquor samples from the same region. A slight increase in the Zn content during manufacture is evident. However, this varies from one factory to the next with appreciable increments being observed in tea samples from Kitumbe, Rukuriri and Kapsara tea factories (Figure 7), but the increments were not statistically significant as was the case for Fe levels in the same tea samples.

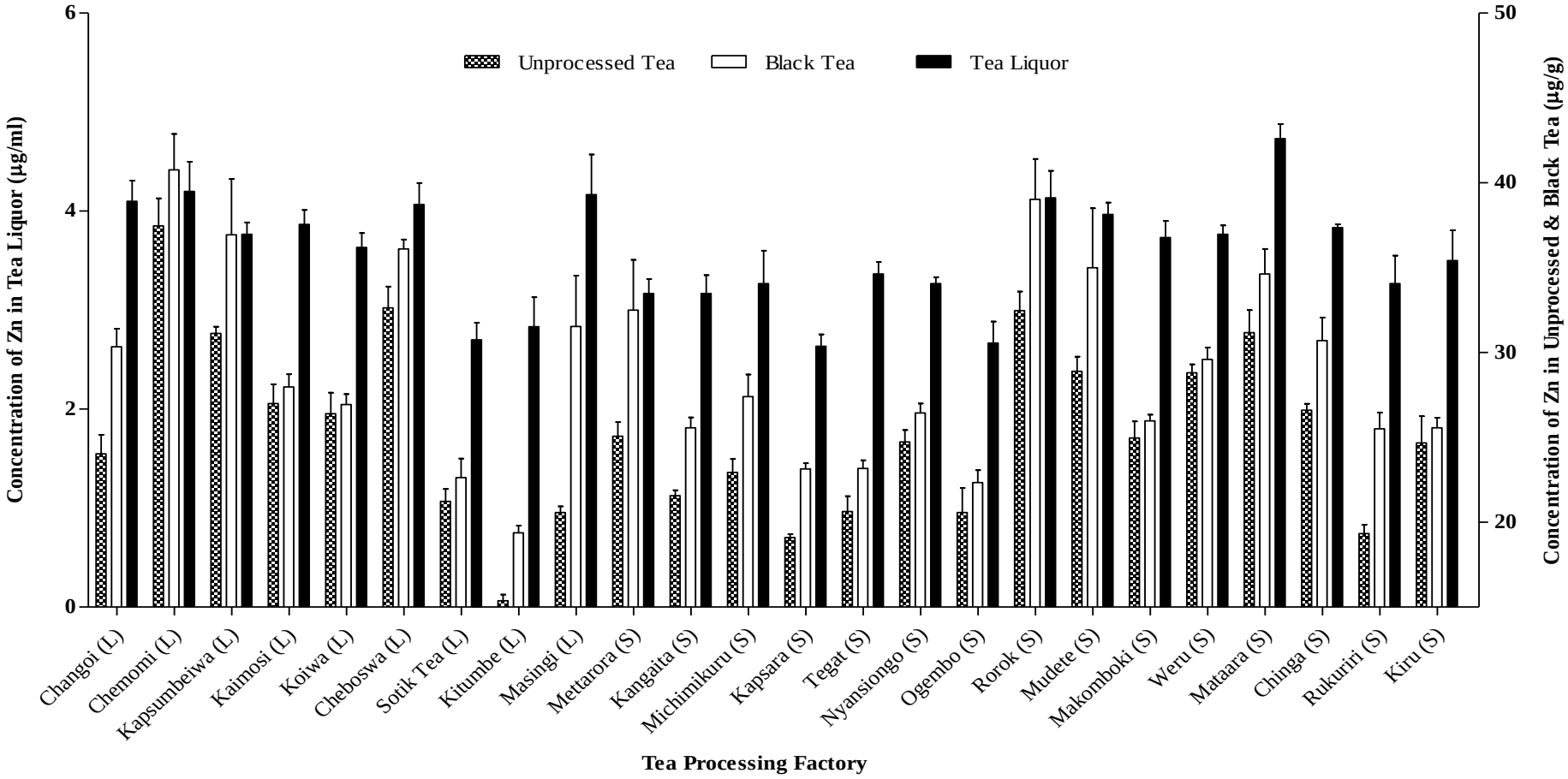

Figure 7: Zn in tea from 24 tea factory catchments in Kenya

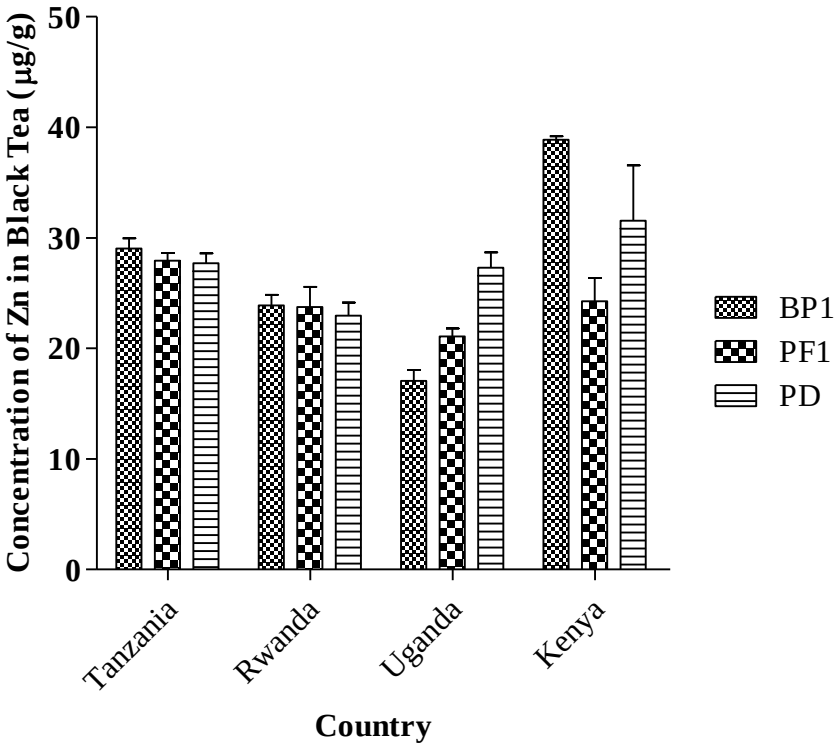

Figure 8: Zn in black tea from Tanzania, Rwanda, Uganda and Kenya

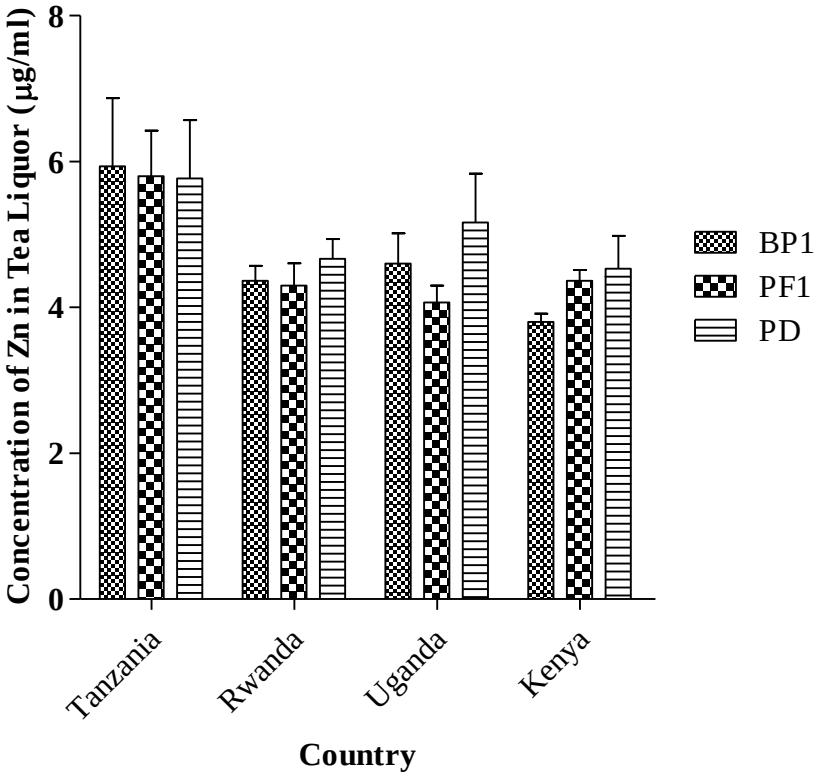

Figure 9: Zn in tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

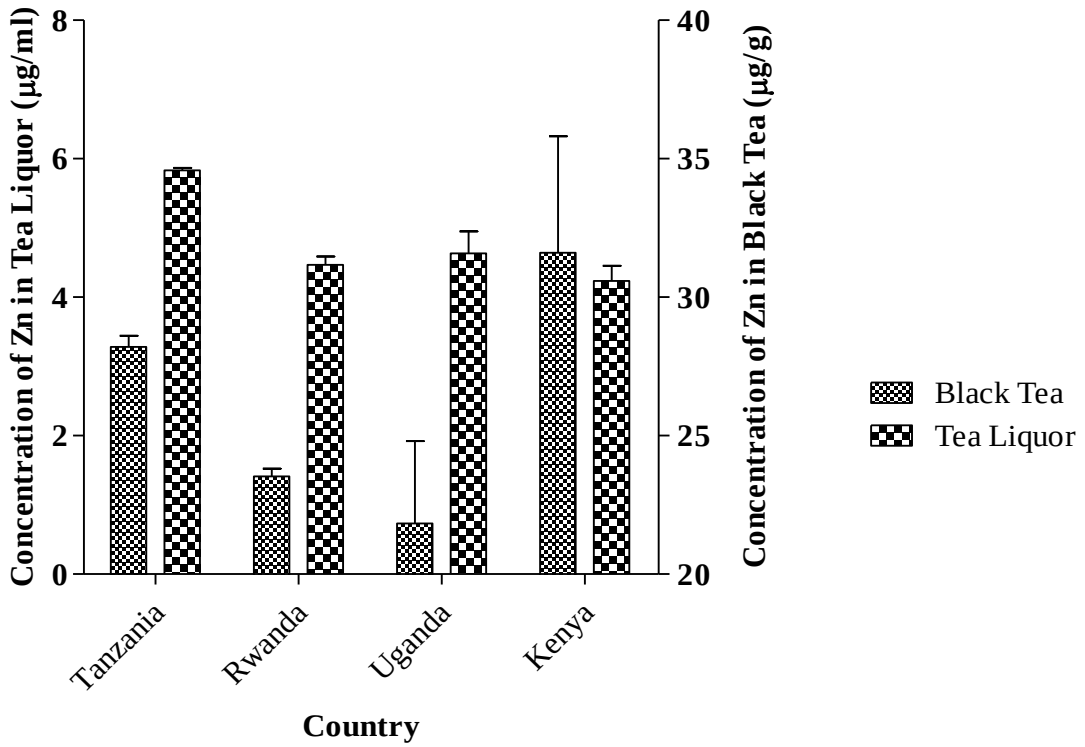

Figure 10: Zn in black tea and tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

Figure 11 clearly shows that the Zn contents of tea vary with the source of the sample. The lowest Zn levels in unprocessed tea were recorded for the samples from Kisii highlands whereas the highest were for the samples from Kericho highlands. This can be primarily attributed to differences in soil characteristics though agronomic practices such as intercropping and varying rates of fertilizer application could also be a major contributing factor. For the small and large-scale tea sub-sectors, the Zn levels were slightly higher in the large-scale than small-scale tea sub-sector as was the case for Fe (Figure 12). Zn levels in tea liquors have not been frequently reported. However, none of the samples (unprocessed and black tea) analysed surpassed the MPC set for Zn (50 ppm; KS 65: 2009) by the Kenya Bureau of Standards (KEBS). It should be noted that the standards for heavy metals are based on processed dry tea and thus it is rather confusing if compared to the tea liquor concentrations.

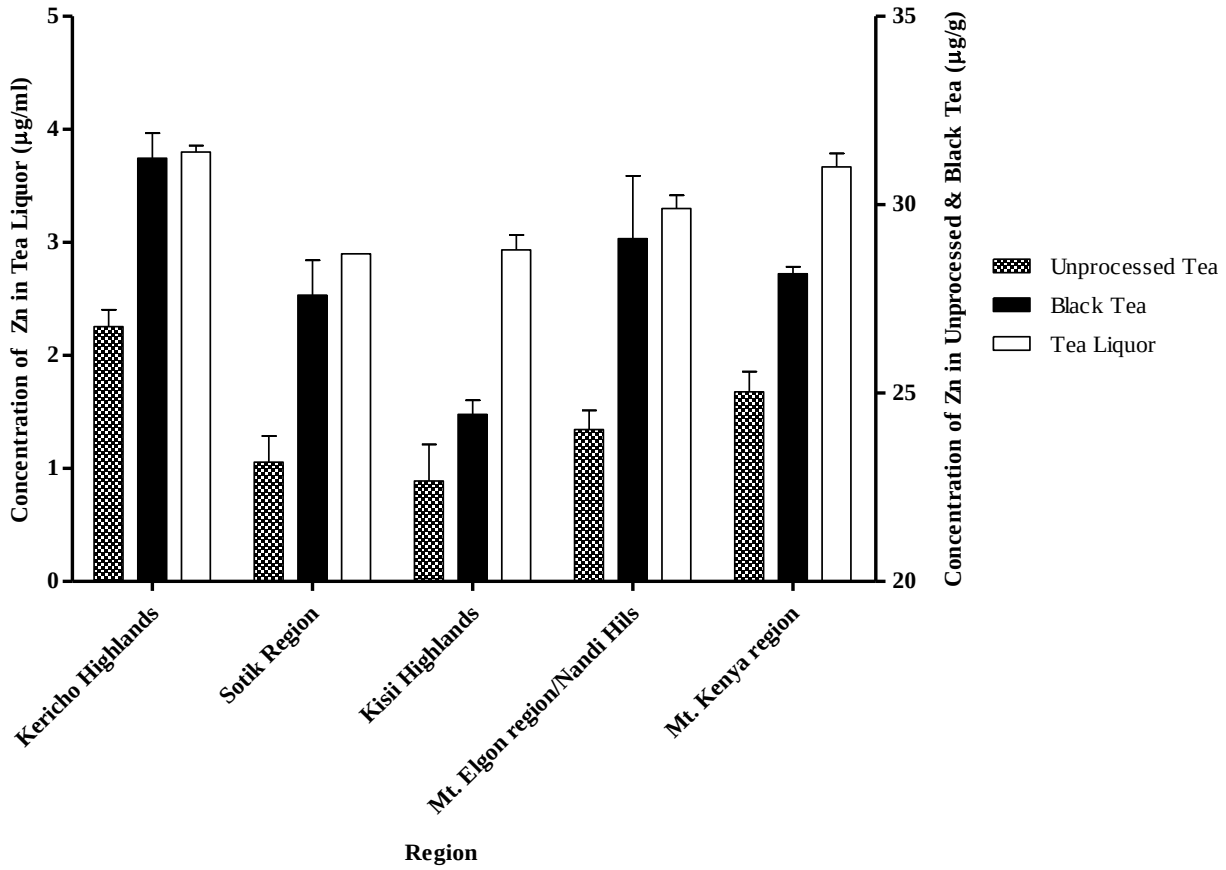

Figure 11: Regional differences of Zn levels in tea

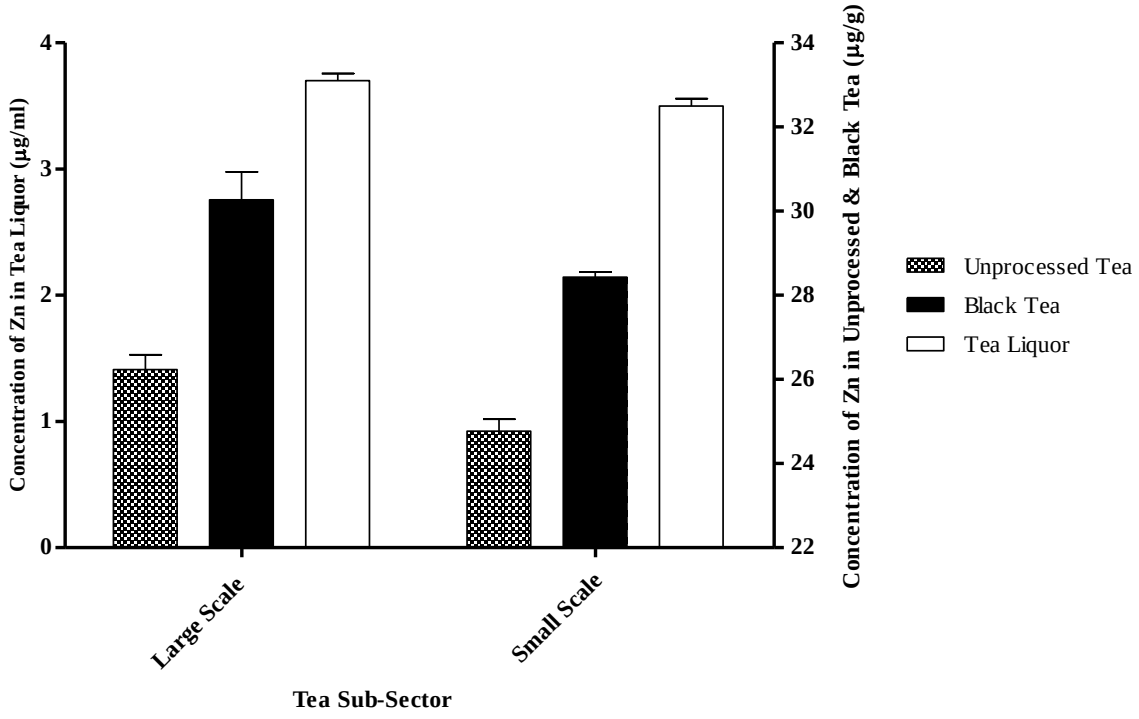

Figure 12: Zn in tea from the large and small-scale tea sub-sectors in Kenya

3.5.4 Copper (Cu)

Figure 13 is a representation of the Cu levels obtained for the unprocessed, black tea and tea liquors sourced from different factory catchments in Kenya. The Cu levels were found to range between 8.4 and 15.1µg/g (Appendix X) and the highest Cu levels recorded for the unprocessed tea samples was from Weru whereas the lowest level was recorded for the unprocessed tea sample from Kiru. Black tea Cu levels ranged between 9.0 and 17.5µg/g (Appendix X) with the lowest mean Cu levels being obtained for the samples sourced from Kiru tea factory (10.0µg/g) and the highest for the samples sourced from Ogembo factory (16.6µg/g) as presented in figure 13. Tea liquors prepared from these black tea samples had an average Cu content of 1.8µg/ml.

The Cu levels in the black tea from Tanzania, Rwanda, Uganda and Kenya sourced from the Mombasa tea auction were as shown in appendix XI. The lowest and highest recorded Cu levels were 12.3 and 17.8µg/g respectively (Figure 14). The samples of Ugandan origin had the highest mean Zn levels (16.0µg/g) whereas those of Tanzanian origin had the lowest Cu levels (13.3µg/g) with the mean Cu levels for the three grades being 14.0µg/g for BP1, 14.6µg/g for PF1 and 14.0µg/g for PD. The Cu levels in the three grades for a given country were not statistically different at $p \leq 0.05$. However, the levels in a given grade of tea in different countries were statistically deferent ($p \leq 0.05$). Tea liquors prepared from these black teas had Cu levels ranged between 1.5 and 2.2µg/ml (Appendix XI) and the means for the three grades were 2.0µg/ml (BP1), 2.0µg/ml (PF1) and 1.9µg/ml (PD) as shown in figure 15.

Data from the current study agrees with the values that have been reported above for tea produced and consumed in other countries world over. For instance, results published by Seenivasan *et al.* (2008) on analysis of Cu levels in made tea samples from South India showed mean Cu levels to be ranging between 15.9 and 32.2µg/g. Kumar *et al.* (2005) report Cu levels ranging between 1.60 to 35.0 and 4.4 to 17.3µg/g for Indian and US tea brands. Wang *et al.* (1993) reported Cu levels to be in the range of 9.6 to 20.9µg/g in Chinese tea brands whereas Han *et al.* (2005) reported Cu contents in Chinese tea samples to range from 2.0 to 447.5µg/g and Ramakrishna *et al.* (1986) reported that 10.0 to 25.0µg/g Cu was found in tea samples from Sri Lanka.

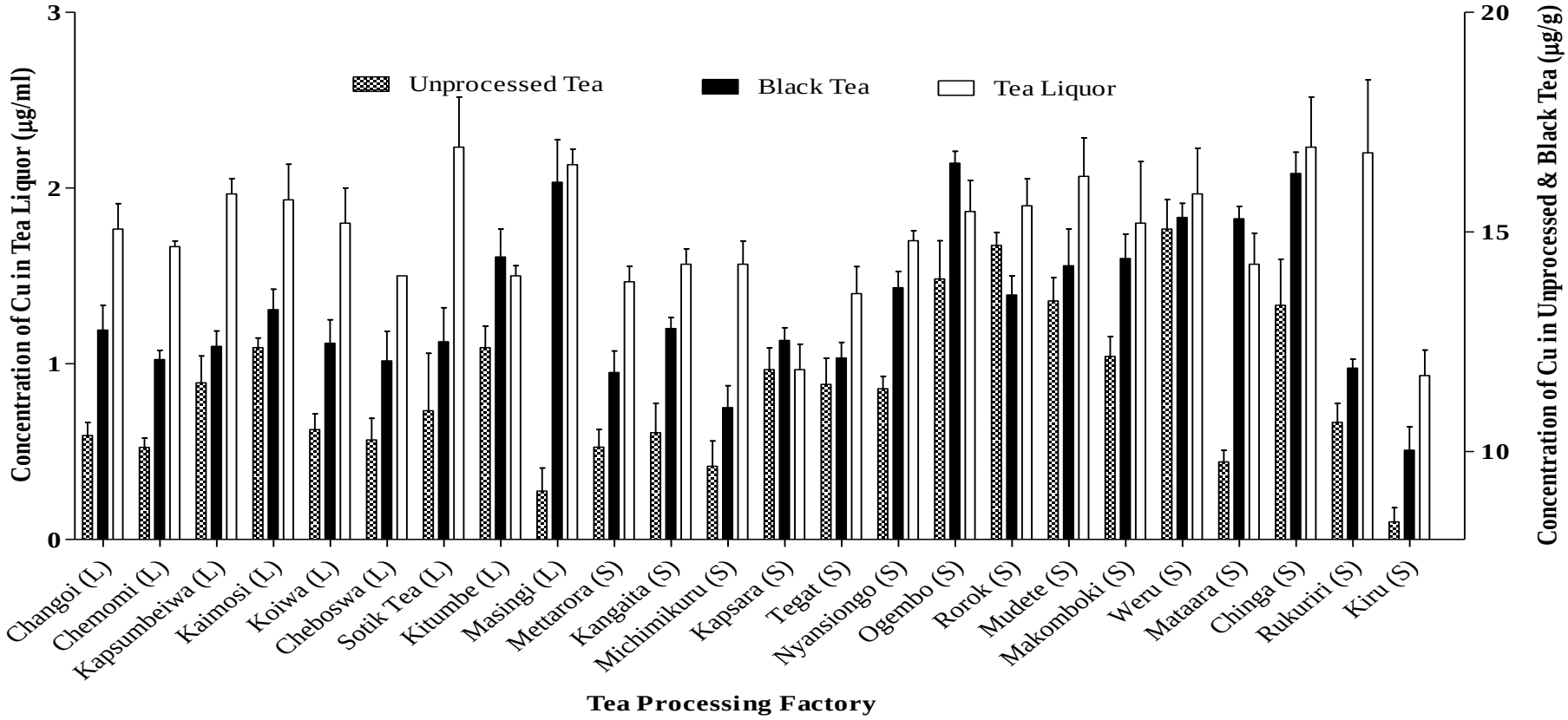

Figure 13: Cu in tea from 24 tea factory catchments in Kenya

Gallaher *et al.* (2006), argue that tea liquors contain 0.05µg/ml Cu. Reports have indicated that this value is dependent on a number of factors such as the duration of extraction (Mehra and Baker, 2007). Powell *et al.* (1998), report Cu levels in 2 min liquors from 12.4g black tea in one litre water to be 0.05µg/ml Cu. These values do not agree with those obtained in the current study (0.02 to 0.08µg/ml). This can be attributed to the differences in the liquor concentrations used, which in the current study was 2.4% W/V (9g of tea in 375ml distilled water) and in that study the concentration was 1.24% W/V (12.4g black tea in one litre water). The infusion durations were also different (10 min in the current study against 2 min in the reported work) among other factors which include agitation using mechanical means in the current study, which would affect the extraction efficiency of the element into solution.

Similarly, the Cu contents at the different levels of productions as well as in the tea liquors are different. Figures 13 and 16 clearly show these differences where only a small proportion of the total Cu content ends up in the tea liquors, as was the case for Fe and Zn discussed above. The levels of Cu in black tea increased from those obtained in unprocessed tea (figures 13 and 17) as was observed for Fe and Zn, though to a lesser extent, and as was the case for Zn, these increments were not significant statistically.

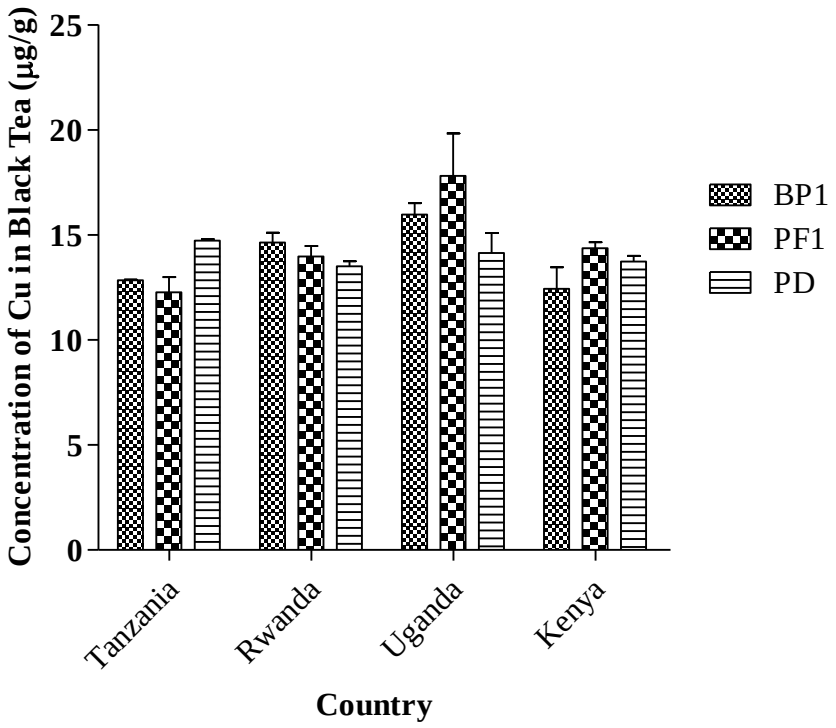

Figure 14: Cu in black tea from Tanzania, Rwanda, Uganda and Kenya

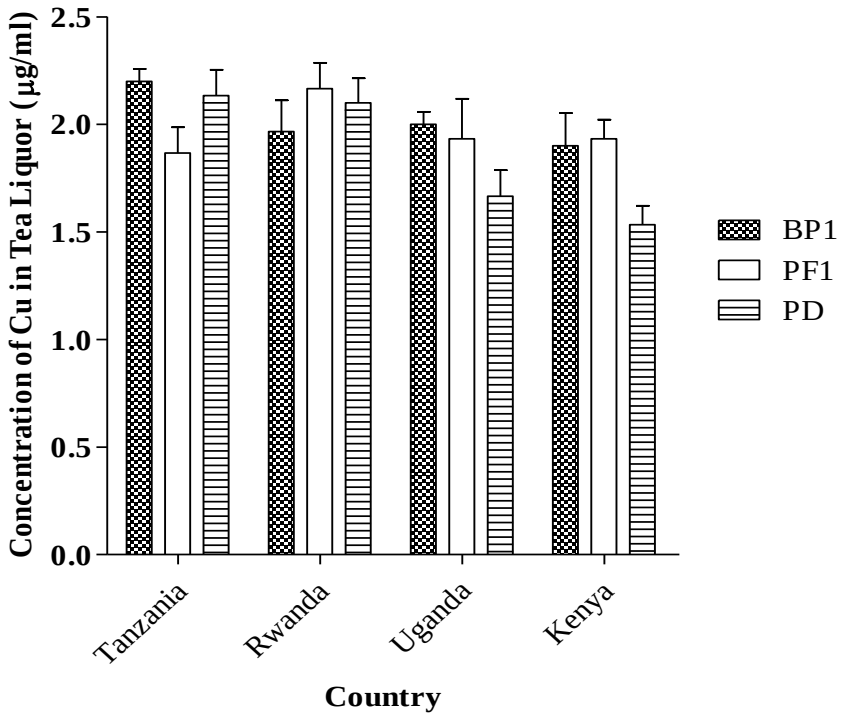

Figure 15: Cu in tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

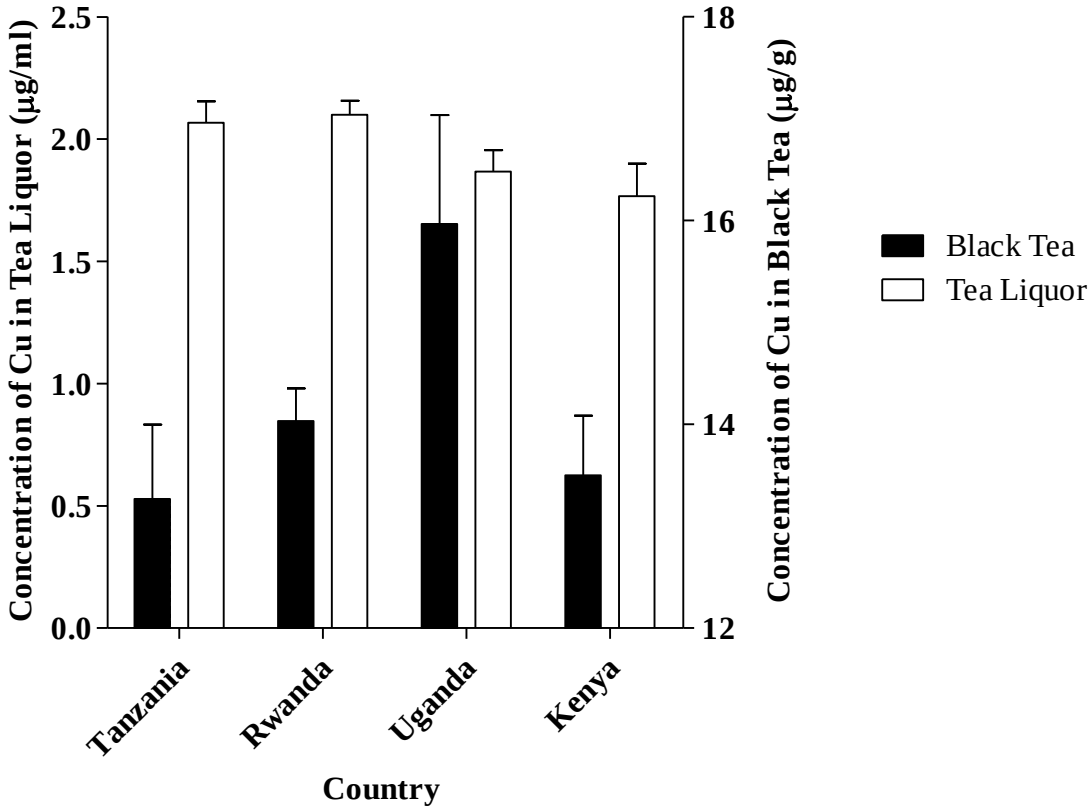

Figure 16: Cu in black tea and tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

Based on this data, regional variations in the Cu content in the tea samples were evident as well (figure 17) with Sotik region teas (Appendix XVIII) recording the lowest Cu content. For the two tea sub-sectors in Kenya, the Cu contents were significantly statistically different at $p \leq 0.05$ (Figure 18). However, it is evident from these findings that the Cu contents in tea produced and marketed in Kenya is comparable not only with tea from tea growing nations in East Africa but globally. Also, the levels are well below the permissible limit of 30µg/g as set by the Kenya Bureau of Standards (KS 65: 2009). Therefore, the Cu concentrations in the processed dry tea leaves grown and marketed in Kenya are acceptable from the perspective of the consumer health.

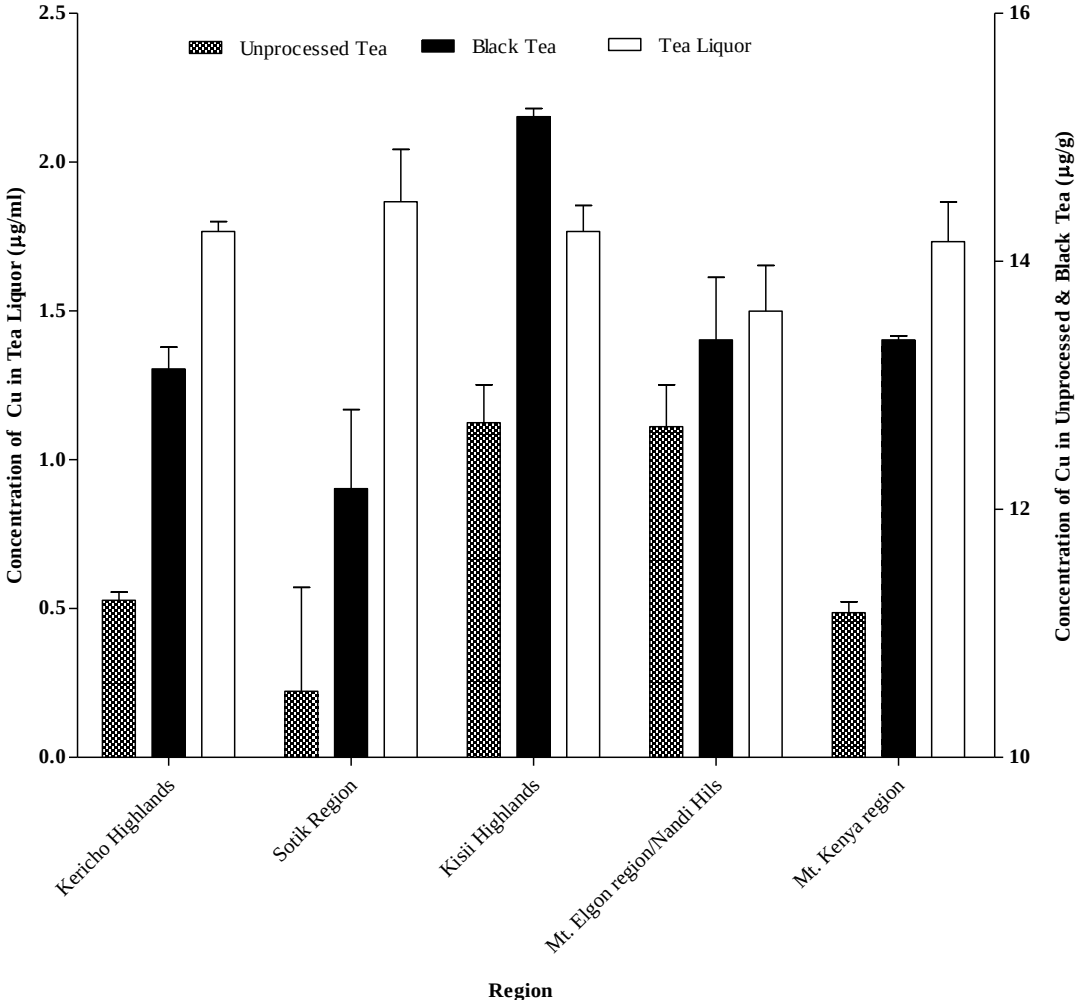

Figure 17: Regional differences of Cu levels in tea

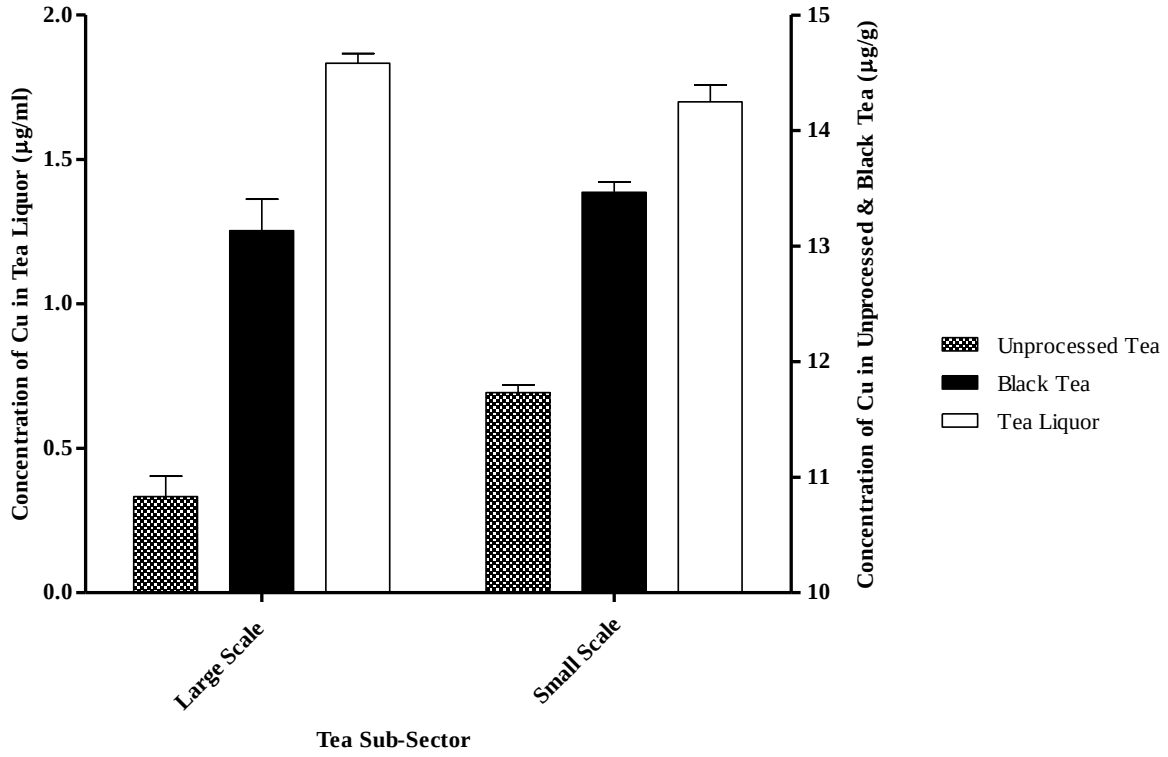

Figure 18: Cu in tea from the small and large-scale tea sub-sectors in Kenya

3.5.5 Lead (Pb)

Figure 19 is a graphical representation of the levels of Pb, in unprocessed, black tea and tea liquors. The Pb levels in unprocessed tea ranged from 0.10µg/g (Mudete) to 0.30µg/g (Tegat) as tabulated in Appendix XII. For black tea, Pb levels were found to range between 0.12 and 0.35µg/g with the lowest mean Pb level recorded for the samples sourced from Weru and Cheboswa (0.14µg/g) and the highest level being for the sample sourced from Tegat (0.32µg/g). Tea liquors prepared from these black teas had Pb levels tabulated in Appendix XII. The lowest Pb level obtained was 0.02µg/ml whereas the highest level was 0.08µg/ml. Samples from Tiluet had the lowest mean Pb level (0.02µg/ml) while the samples obtained from Kapsara had the highest (0.07µg/ml).

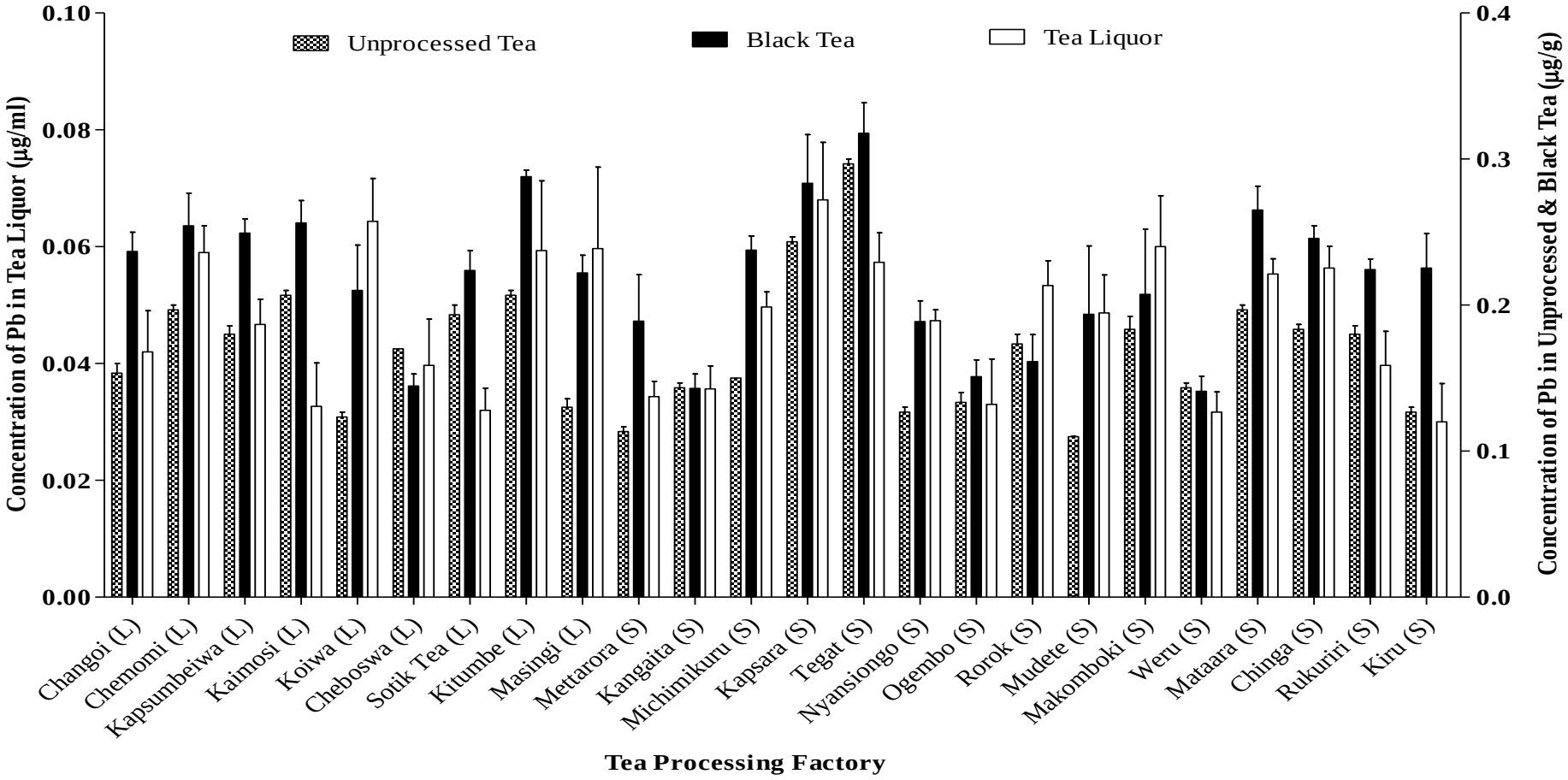

Figure 19: Pb in tea from 24 tea factory catchments in Kenya

Black tea from Tanzania, Rwanda, Uganda and Kenya obtained from the Mombasa tea auction had Pb levels ranging between 0.13 and 0.27µg/g as indicated in appendix XIII. Kenyan black tea had the lowest mean Pb levels (0.19µg/g) whereas Tanzanian black tea had the highest (Figure 20). For the three grades, the mean Pb levels were 0.20µg/g for BP1, 0.20µg/g for PF1 and 0.18µg/g for PD. Tanzanian black tea Pb contents were significantly different ($p \leq 0.05$) from the Pb contents of the samples from the rest of the countries. On the other hand, tea liquors prepared from the black tea from the Mombasa tea auction had Pb levels that ranged between 0.02 and 0.08µg/ml (Figure 21), with the Rwandan black tea giving the lowest mean Pb levels (0.02µg/ml) and Tanzanian samples giving the highest (0.04µg/ml). The means Pb levels for the three grades were 0.05, 0.04 and 0.04µg/ml for BP1, PF1 and PD respectively as tabulated in appendix XIII.

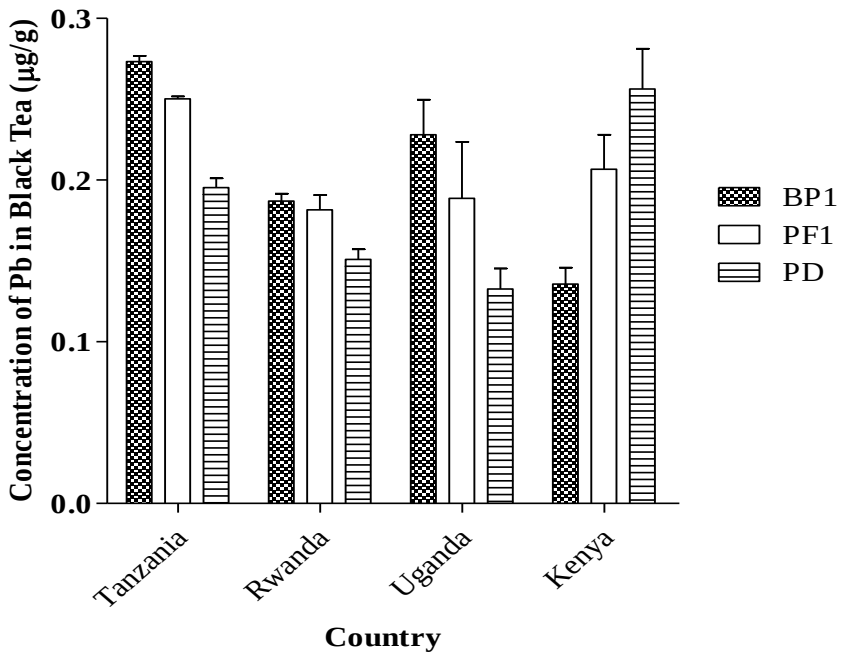

Figure 20: Pb in black tea from Tanzania, Rwanda, Uganda and Kenya

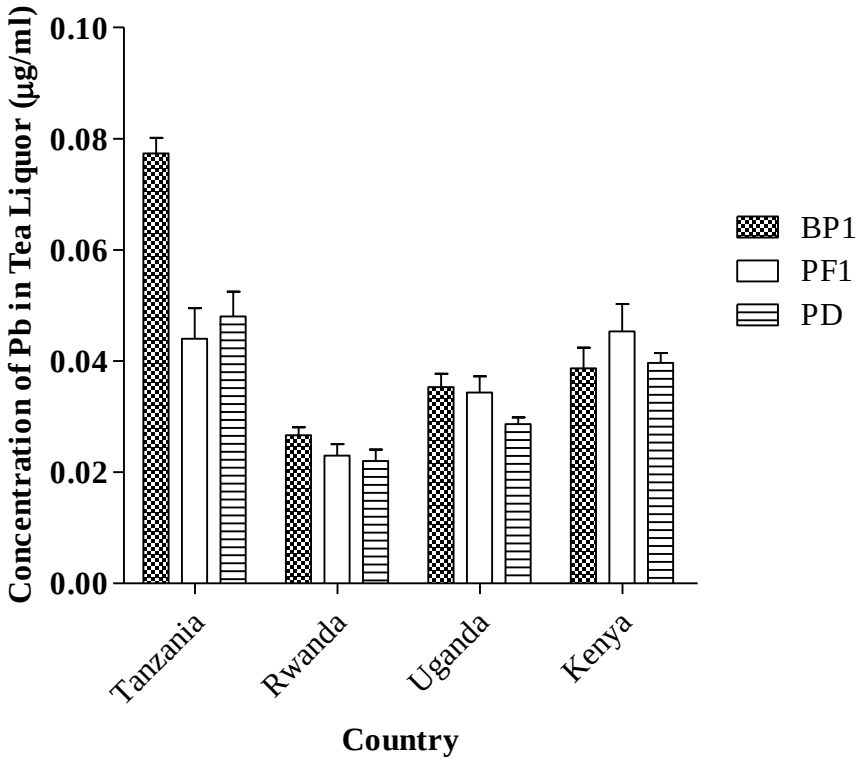

Figure 21: Pb in tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

Evidently, the levels of Pb in unprocessed tea were slightly lower than those in black tea, with the tea liquors of the black teas having very low concentrations (Figures 19 and 22).

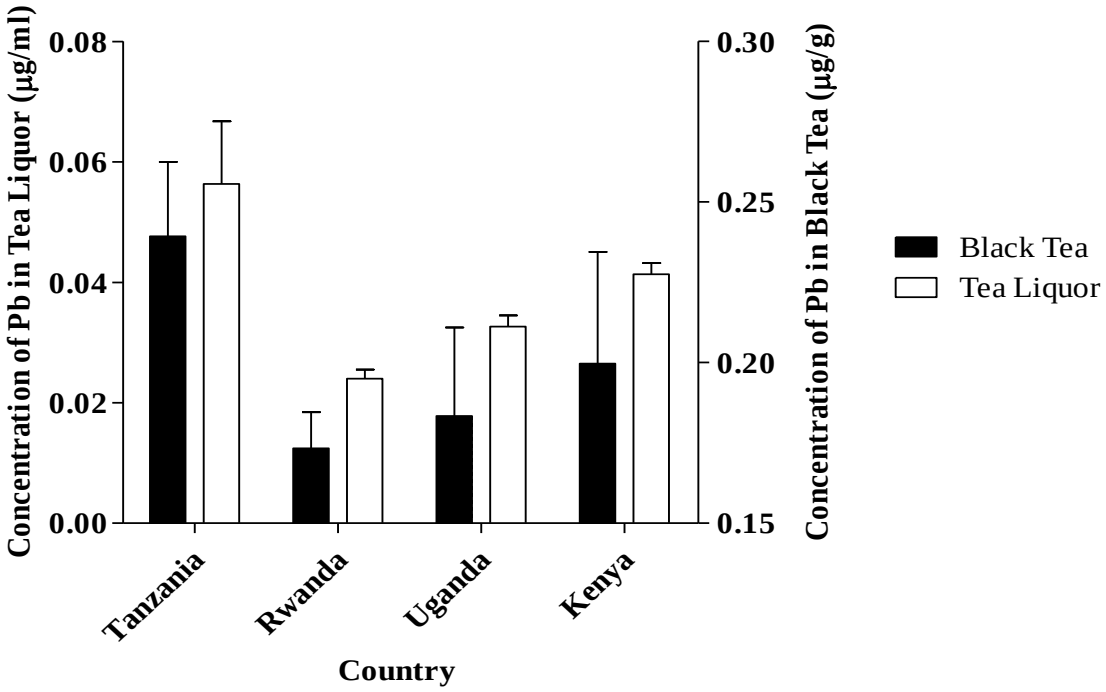

Figure 22: Pb in black tea and tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

The tea plant can uptake Pb from the soil, a portion of which can be transported to and accumulated in the plant's leaves (Jin *et al.,* 2005). This observation is well supported by the results of the present study which further corroborate Chen *et al.* (2009) findings who reported the Pb content of different Chinese tea cultivars to be between 2.3 and 5.6µg/g. Further work that corroborate with the findings of the current study are Seenivasan *et al.* (2008) who reported Pb contents of between 0.04 and 1.36µg/g in tea, Ramakrishna *et al.* (1986) who reported Pb contents in tea produced in Sri Lanka to vary between 0.19 and 0.56µg/g, Ashraf and Mian (2008) who reported black tea in Saudi Arabia to have Pb contents that ranged between 0.3 and 2.2µg/g, Tsushida and Takeo (1977) who reported the concentration of Pb in Japanese tea to be between 0.11 to 1.93µg/g, Narin *et al.* (2004) who found the maximum Pb content in Turkish tea 27.3µg/g and Chen *et al.* (2009) who reported Pb contents in tea ranging between 2.3 and 5.6µg/g. However, the current findings do not agree with those of Yemane *et al.* (2008) who reported that, five clones of Ethiopia tea (*Camellia sinensis* var. *assamica*) did not show either the ability to uptake and accumulate Pb or the Pb content in the tea samples were too low to be detected by the analytical techniques used.

Based on the five regions as defined in Appendix XVIII, the Pb contents in the tea samples were not statistically different as can be seen in figure 23.

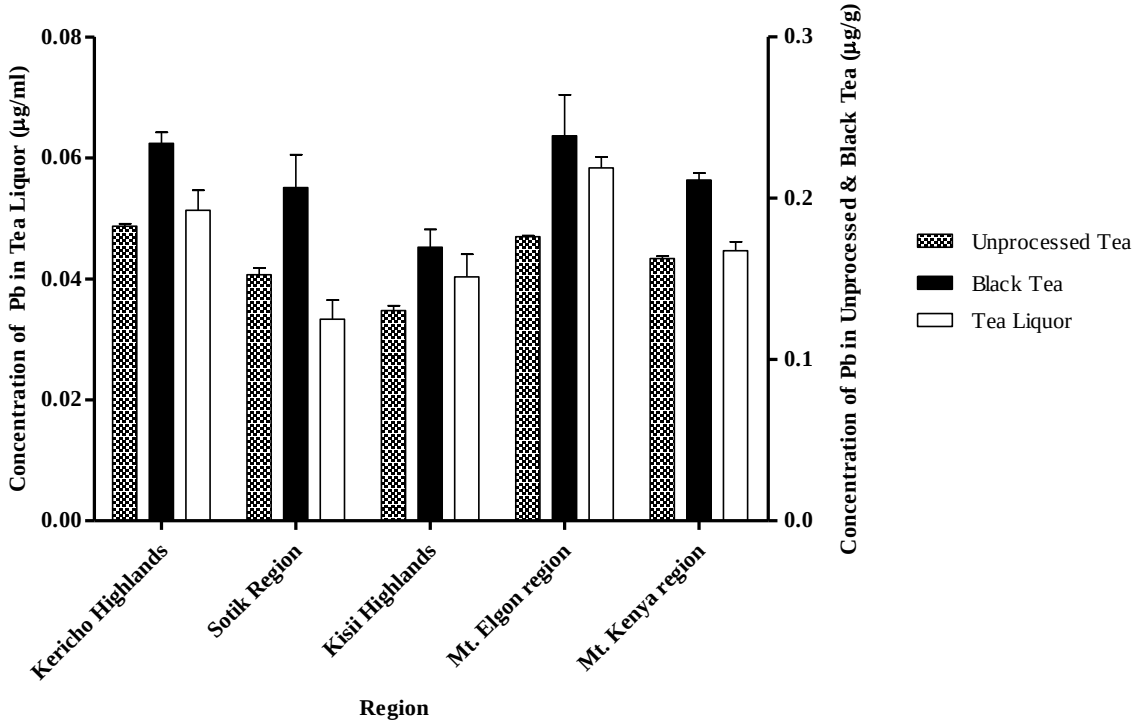

Figure 23: Regional differences of Pb levels in tea

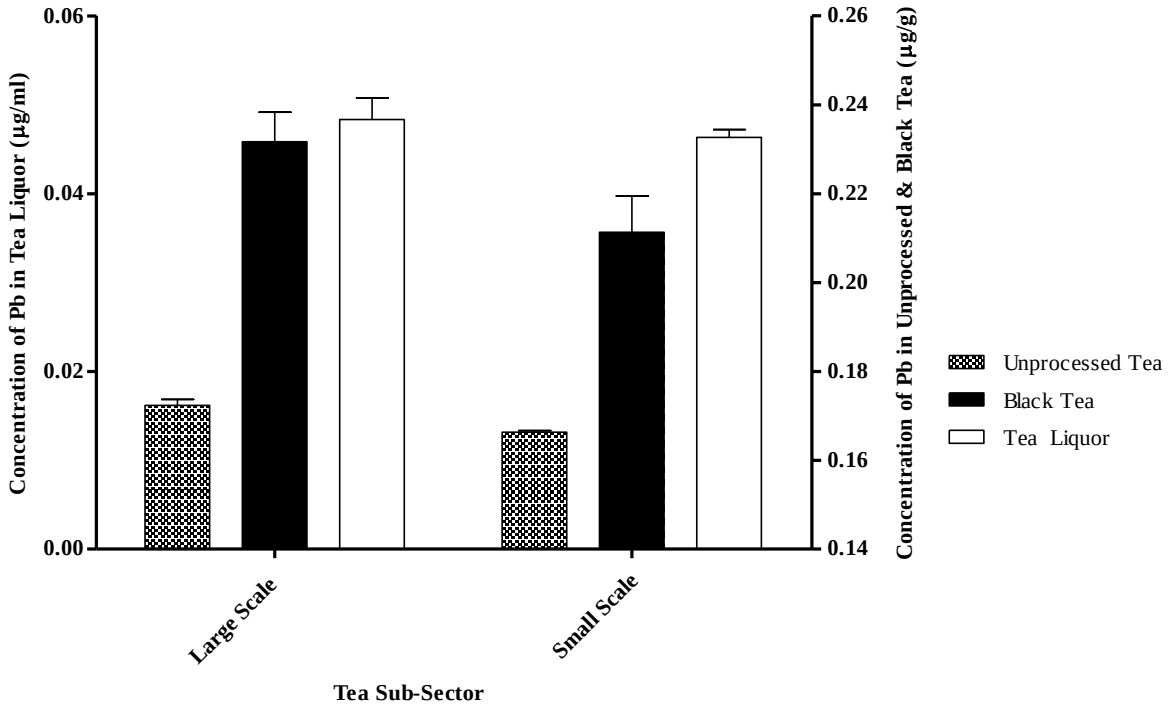

Figure 24: Pb in tea from the small and large-scale tea sub-sectors in Kenya

The Pb content in most of the samples was above 0.1µg/g, the maximum permissible limit for Pb in tea in Kenya (KS 65: 2009). However, none of the samples exceeded 0.5µg/g and as such, all the samples analysed were below the international MPC's for Pb in tea (1.0µg/g). It should however be noted that the Kenyan standard is up to times stricter than the international one. However, water extractable Pb concentrations in some tea liquors such as those prepared from the samples from Chemomi, Koiwa, Kapsara, Tegat, Kitumbe, Makomboki, Mataara, Chinga and Masinga in the present study exceed the 0.05µg/ml limit set for drinking water by WHO (2003).

3.5.6 Cadmium (Cd)

Cd was present in the lowest levels of the five heavy metals considered in the current study (few µg/kg of sample) as can be seen in Appendix XV. The lowest Cd level obtained was 7.0µg/kg (Michimikuru) whereas the highest obtained concentration was 33.0µg/kg (Cheboswa). Black tea had Cd levels that ranged between 9.1 and 40.0µg/kg as tabulated in appendix XIV. The lowest mean Cd level obtained was for the black tea samples from Kiptagich (15.3µg/kg) while the highest level was for the samples from Makomboki (34.8µg/kg). The means Cd levels for the three grades were 23.1, 23.7 and 24.9µg/kg BP1, PF1 and PD respectively (Figure 25).

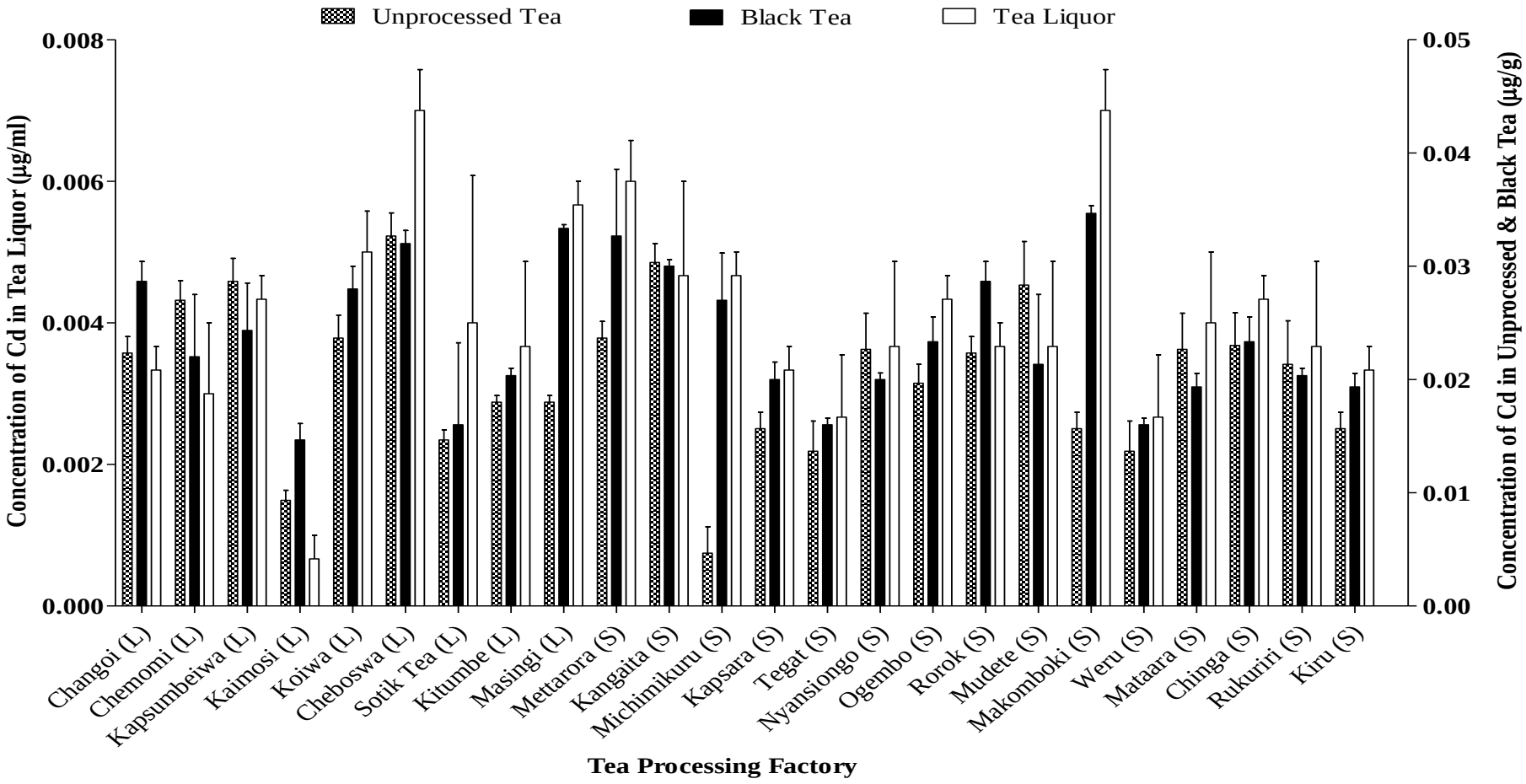

Figure 25: Cd in tea from 24 tea factory catchments in Kenya

In some samples analysed, the Cd levels in the tea liquors (water extractable Cd) could not be quantified as the concentration was too low to be quantified by the analytical method used (Appendix IV). Tea liquors prepared using the black tea from Kaimosi had Cd content values too low to be detected by the current method, whereas Masingi had the highest Cd content of 7.0µg/L (Appendix XIV). The Cd concentration range for the samples was BDL (BP1, PF1 and PD, Chemomi; BP1, Sotik Tea; PD, Kangaita; BP1, Tegat; PF1, Nyansiongo; PF1, Kitumbe; PF1, Mudete; BP1, Weru; PF1, Mataara; PF1, Rukuriri; PF1 and PD, Tiluet and BP1, Sisiba) and 7.0µg/L (BP1, Mettarora; BP1, PF1 and PD, Chaboswa; PF1, Sotik tea; PD, Mettarora; BP1 and PF1, Masingi; BP1, Bondet and PD, Sisiba). The means per grade were all 4.0µg/L for all the three grades.

For the black tea from Tanzania, Rwanda, Uganda and Kenya sourced from the Mombasa tea auction, the Cd levels were found to range between 11.0 and 22.0µg/kg (Appendix XV). The mean Cd levels for the three grades were 15.0µg/kg for BP1, 17.0µg/kg for PF1 and 18µg/kg for PD (Figure 26). Tea liquors prepared from these black teas gave Cd levels given in Appendix XV. The range of the Cd levels for these teas was from below the detectable limit (BDL) to 4.0µg/L (Figure 27).

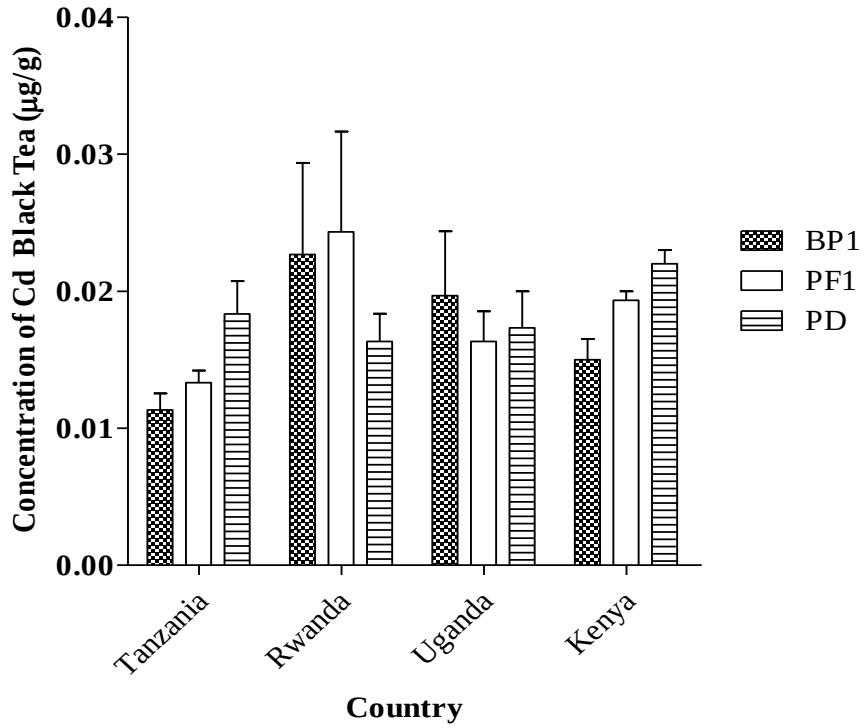

Figure 26: Cd in black tea from Tanzania, Rwanda, Uganda and Tanzania

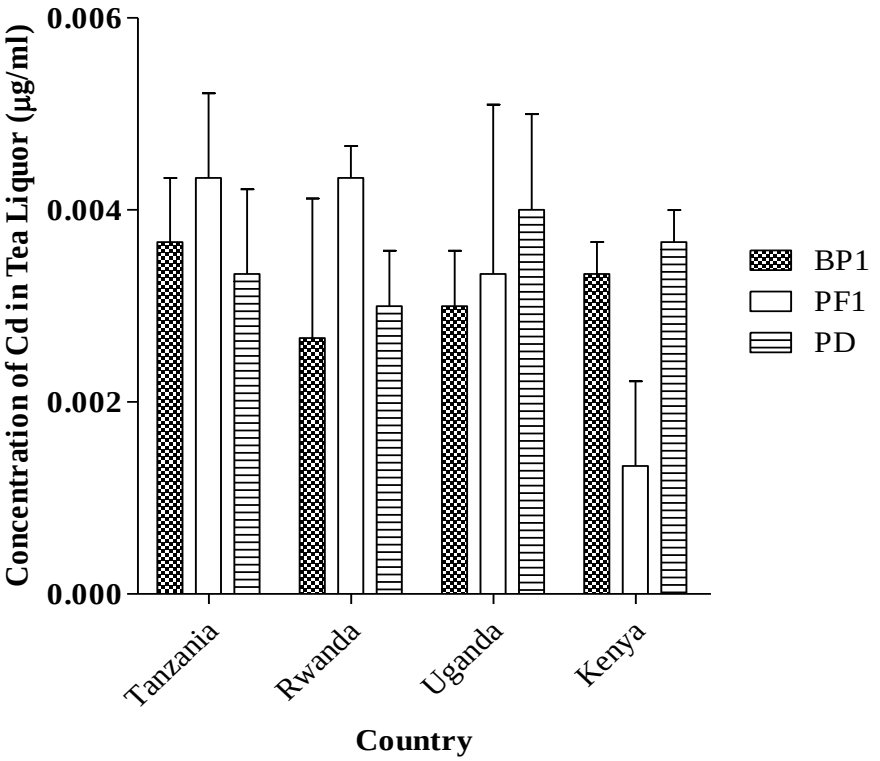

Figure 27: Cd in tea liquor samples from Tanzania, Rwanda, Uganda and Tanzania

The trend of the levels of Cd in tea at the different levels of production is the same as has been observed for the other metals (Fe, Zn, Pb and Cd). The increase in the levels of Cd at the factory level is also evident though the increments are statistically insignificant (Figures 25 and 28).

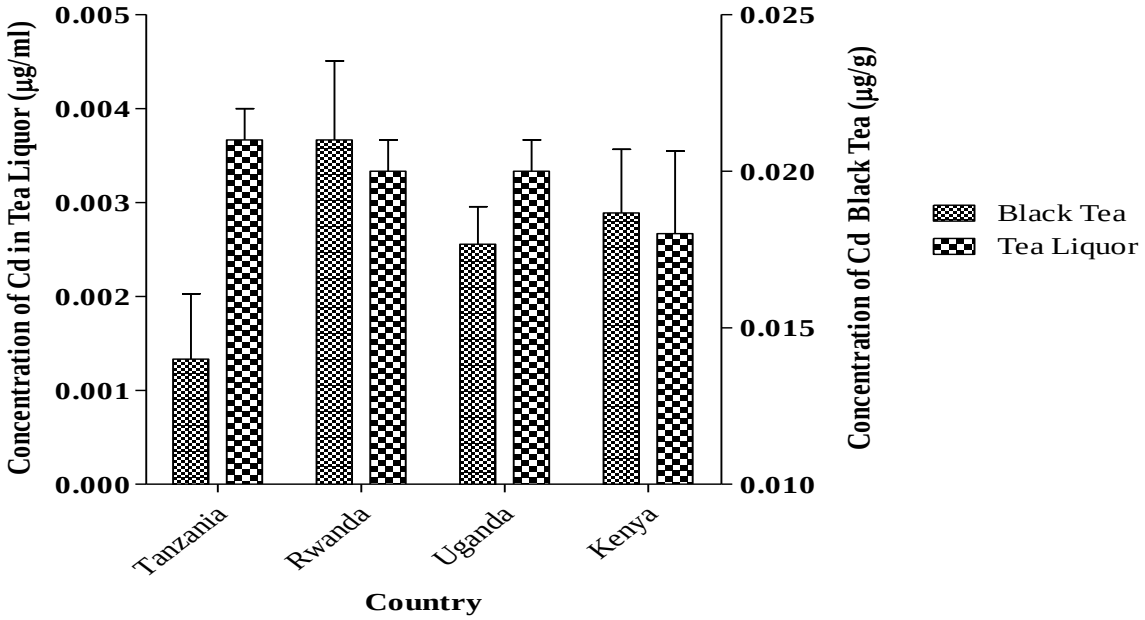

Figure 28: Cd in black tea and tea liquor samples from Tanzania, Rwanda, Uganda and Tanzania

Chen *et al.* (2009), reported levels of Cd in tea leaves that ranged from 0.03 to 0.08µg/g, findings that were in good agreement with those obtained in a previous study by Moreda-Pineiro *et al.* (2003) as well as the present study. As indicated for Pb in the previous section, the level of Cd also exceeded the standard for Kenya but all the samples analysed had their Pb contents well within the international standards. Further work that agrees with the findings of the current study are Tsushida and Takeo (1977) who reported Cd contents Japanese tea to be between 0.11 and 1.93µg/g, AL-Oud (2003) who reported the level of Cd in tea samples marketed in Pakistan to be from BDL to 0.18µg/g, Seenivasan *et al.* (2008) who reported Cd contents in black tea from south India to be between 0.05 and 0.38µg/g, Narin *et al.* (2004) who reported Cd levels in Turkish black tea to be between 1.0 to 3.0µg/g and Zhang and Fang (2007) who reported Cd content of Chinese green tea leaves to be ranging from 0.01 to 0.06µg/g. However, Yemane *et al.* (2008) reported Cd levels in tea leaves of different clones of tea grown in Wushwush tea plantation farms in Ethiopia to be present at levels too low to be detected by the analytical techniques used clones. These results as well as those of Ferrara *et al.* (2001) who reported Cd in tea from different parts of the world to be BDL, do not agree with the findings of the current study.

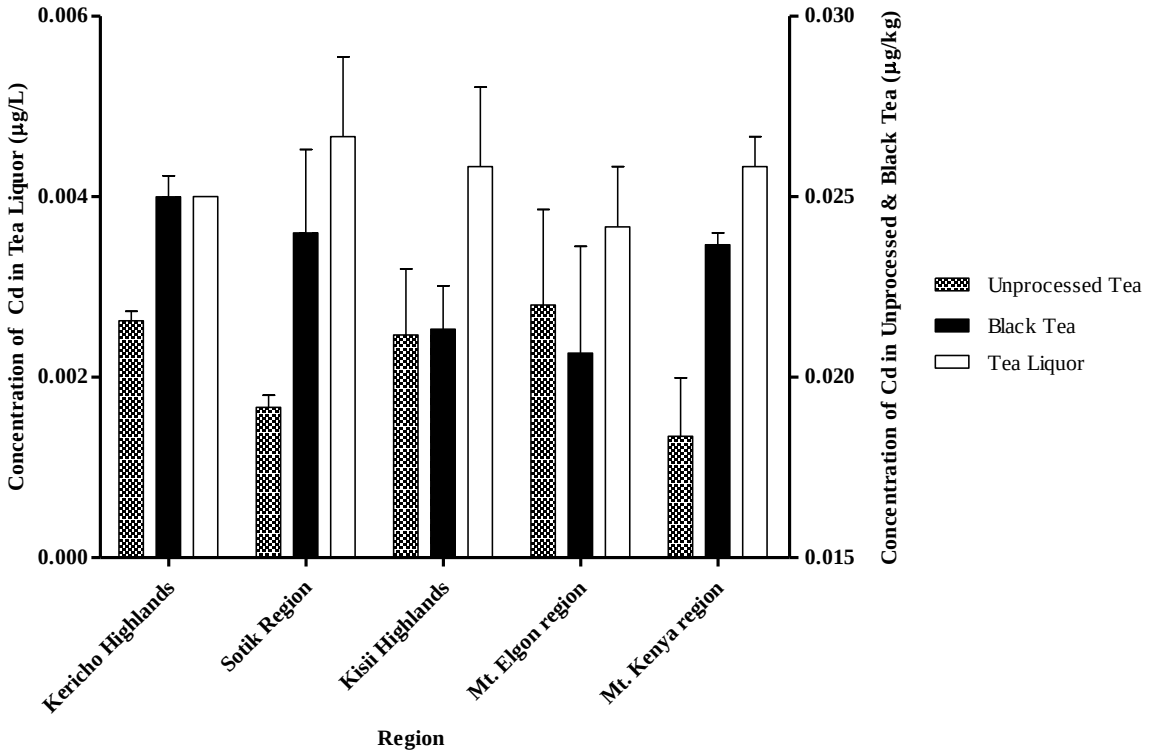

Figure 29: Regional differences of Cd levels in tea

Regional variations were evident as the Cd content of the tea samples from the five regions at the different levels of production were statistically different at $p \leq 0.05$ as seen Figure 29. Figure 30 gives the Cd levels obtained for the tea samples from the two tea sub-sectors in the Kenyan tea industry. Cd contents in the large-scale tea sub-sector are higher than those obtained for the samples sourced from the small-scale tea sub-sector. It is evident from the findings of the present study and the other reports on the same subject show that the tea plant can uptake and accumulate Cd and as such the consumption of tea can serve as an additional dietary source of this undesirable heavy metal.

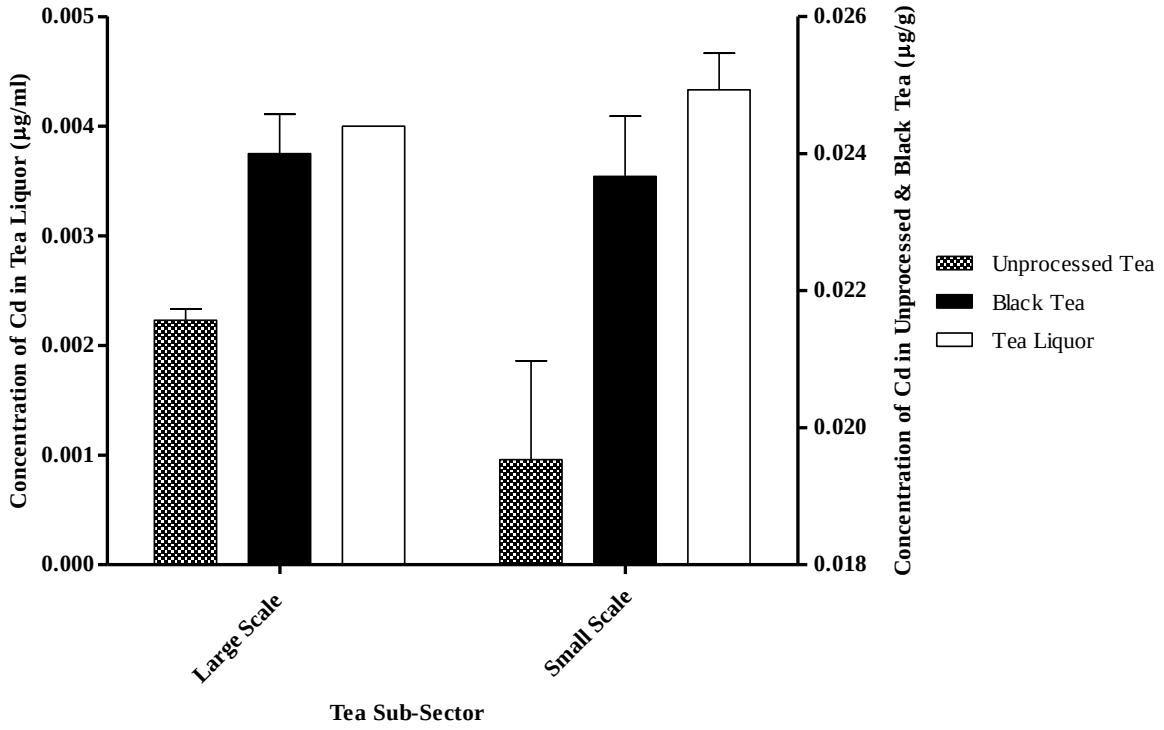

Figure 30: Cd in tea from the small and large-scale tea sub-sectors in Kenya

3.5.7 Fluoride

The Kenyan black tea liquors had a mean fluoride content of 0.37µg/ml as shown in figure 31. The means for the black tea liquors of each of the three grades of tea were 0.40µg/ml (BP1), 0.37µg/ml (PF1) and 0.33µg/ml (PD) as tabulated in Appendix XVI. The tea liquor prepared from the black tea from Kaimosi had the highest fluoride content (0.89µg/ml) whereas that from Tiluet factory recorded the lowest (0.13µg/ml).

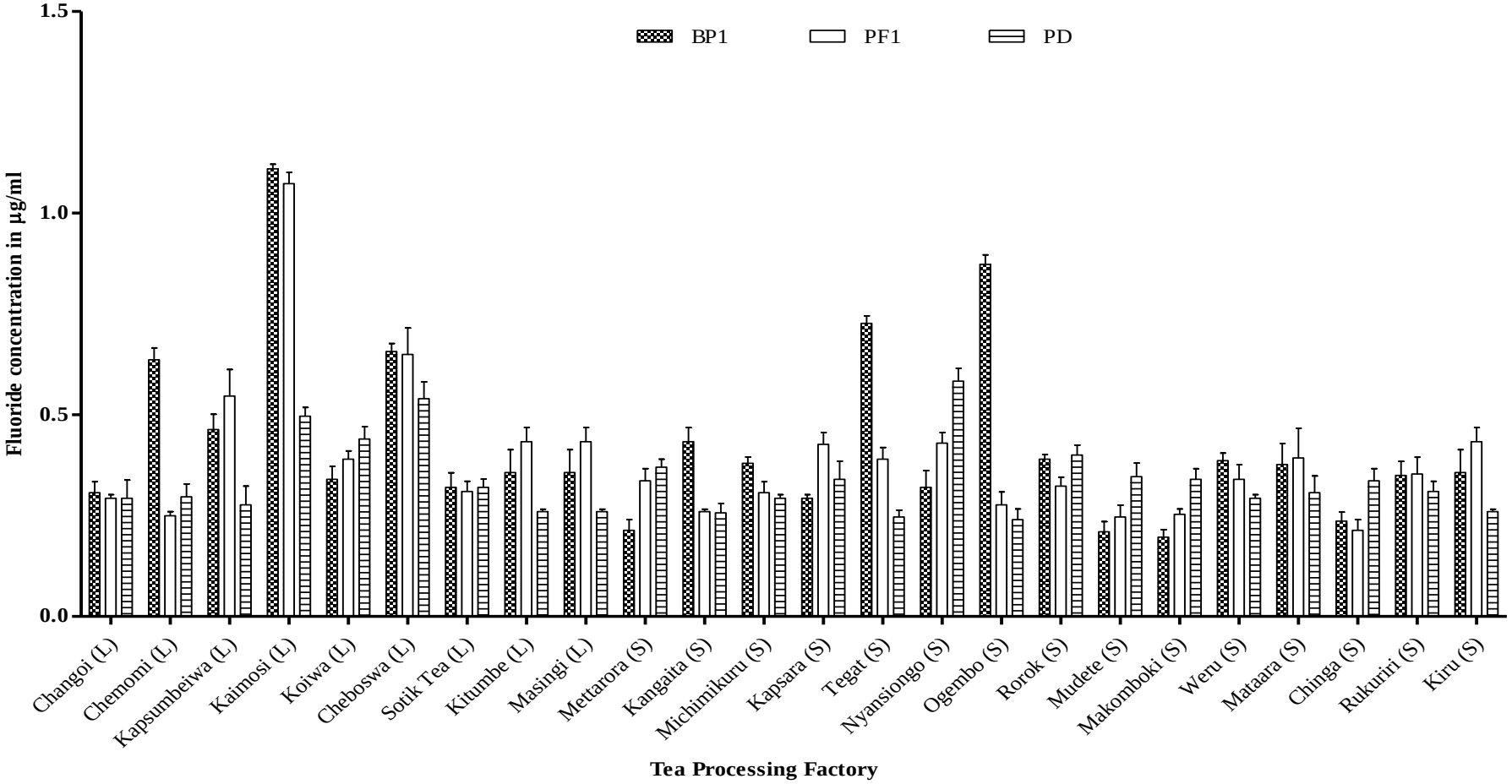

Figure 31: Fluoride in three grades of tea from 24 tea factory catchments in Kenya

The black tea samples from Tanzania, Rwanda, Uganda and Kenya obtained from the Mombasa tea auction gave liquor fluoride levels presented in appendix XVII. The means for each of the three grades were 0.36, 0.54, and 0.42µg/ml for BP1, PF1 and PD respectively as tabulated in appendix XVII (Figure 32). Samples from Uganda and Kenya had the highest mean fluoride concentration of 0.66µg/ml while the lowest concentration was obtained in the samples from Rwanda which had 0.25µg/ml fluoride.

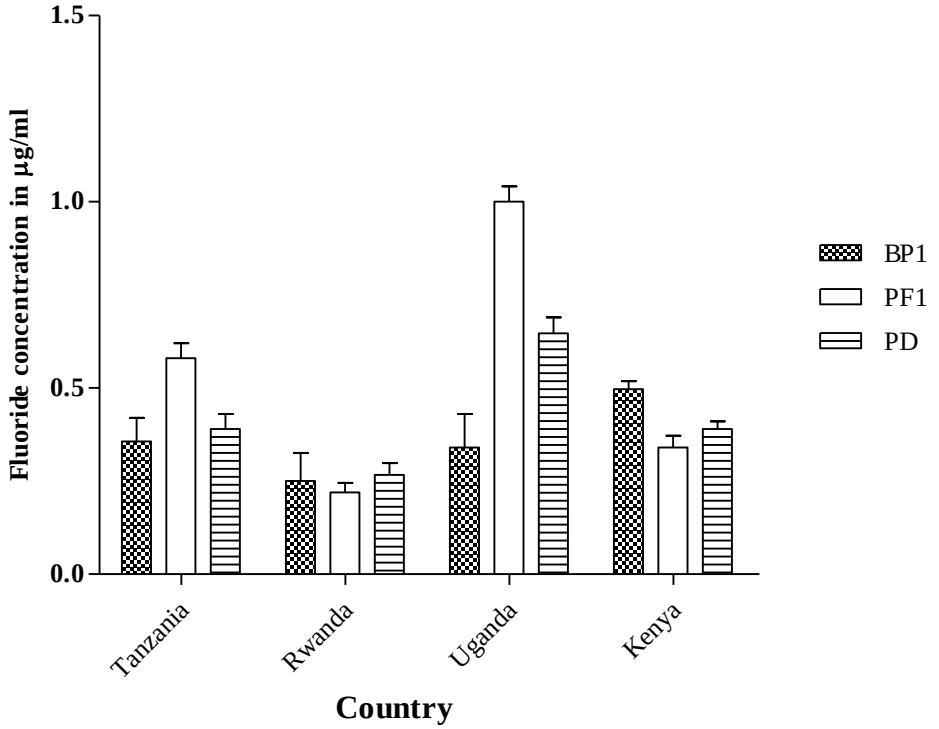

Figure 32: Fluoride in black tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

The fluoride levels in the tea liquors of the black tea samples from the different regions (Appendix XVIII) had different levels of fluoride as shown below (Figure 33). The levels of fluoride for the two tea sub-sectors were not statistically different, indicating that differences in the agronomic practices do not affect the levels of fluoride in tea. The levels obtained were as given in figure 34 below.

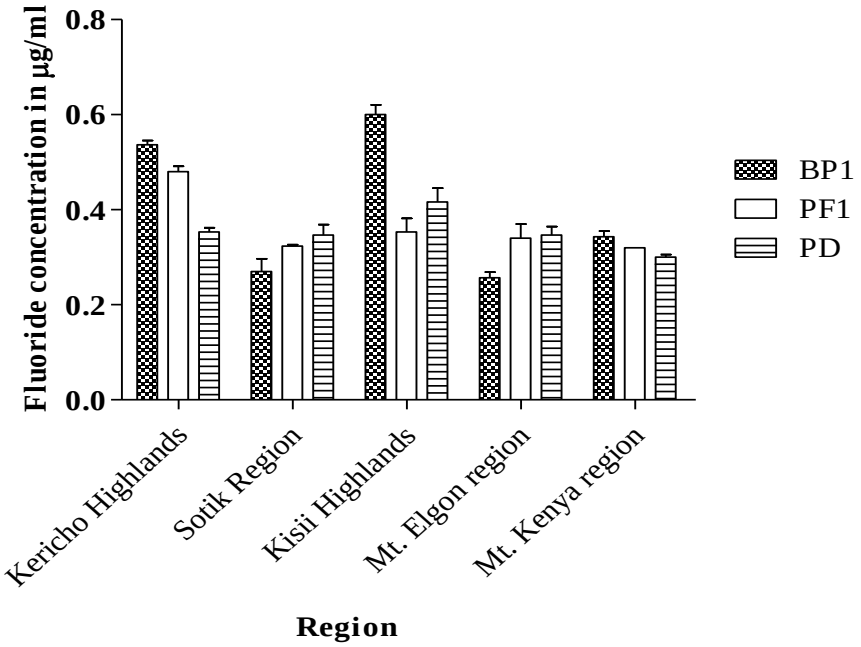

Figure 33: Regional variations of fluoride levels in tea

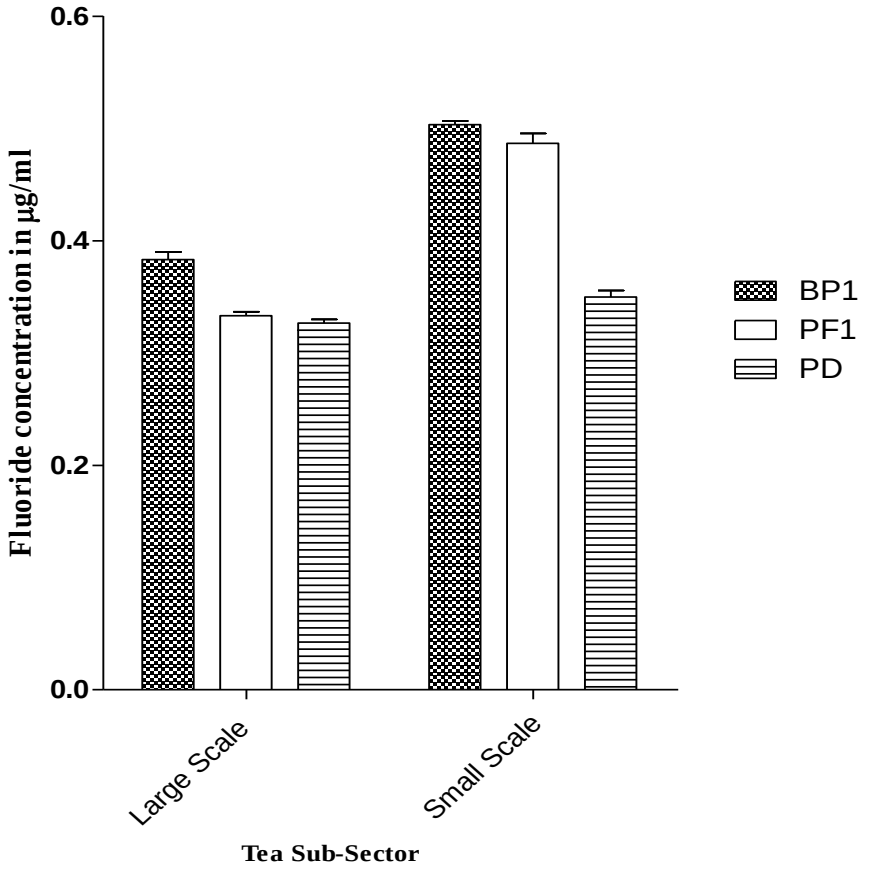

Figure 34: Fluoride in tea liquor samples from the small and large-scale tea subsectors in Kenya

The fluoride levels obtained in the current study were comparable with those reported by researchers elsewhere. For example, the water soluble fluoride levels of Turkish teas was found to range between 55 and 127µg/g (Tokalioglu *et al.*, 2001) which correspond to 0.6 to 1.3µg/ml when converted into liquor concentrations (2g of tea infused in 200ml of H_2O). Kalayci and Somer (2003) measured liquor concentrations of 2.6 and 3.9µg/ml of fluoride with 5 and 20 minutes extraction times respectively in black tea. Hudaykuliev *et al.* (2005) reported a range of fluoride concentrations for Turkish teas as 88 to 289µg/g which corresponds to between 0.8 and 2.6µg/ml liquor concentrations with the assumption of water-soluble fraction as 90%.

CHAPTER FOUR
CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

Based on the findings of the present work, the following conclusions can be drawn;

1. The levels of the studied heavy metals (Fe, Zn, Cu, Pb and Cd) in unprocessed tea, black tea and tea liquors samples of Kenyan origin compared well to tea grown in other East African countries. Also, the levels of fluoride in tea liquors of Kenyan tea compares well to tea grown in other East African countries.
2. The levels of Fe, Zn, Cu, Pb and Cd in unprocessed, black tea and tea liquors of all the groups of samples under study varied widely among the different grades and regions of production. A similar observation was obtained for the levels of fluoride in the liquors of the black teas of the different groups.
3. The levels of Fe in the unprocessed tea from the factories were lower than that of the black tea from the corresponding factories, implying that processing contributed to some Fe in the black tea. Therefore, there is need for strict adherence to Good Manufacturing Practices (GMP) such as the use of high quality machinery. For the other heavy metals (Zn, Cu, Pb and Cd), the unprocessed tea had slightly lower levels compared to the black teas but these differences were statistically insignificant.
4. All the tea samples analysed had Fe, Zn, Cu, Pb and Cd levels well within the international MPC's set for tea. However, some teas were marginally close and in some cases higher than the MPC's in the Kenyan standard (KS 65: 2009) for Pb (0.1ppm) and Cd (0.02ppm). There is no MPC for fluoride in tea.
5. Heavy metal levels in black tea and tea liquors indicated that the levels in the liquor were from 3 to 25 times lower than those in the black teas and therefore there was no risk posed to consumers of tea liquors. However, there are other toxic element such as arsenic and mercury whose levels in tea were not determined and as such this data is not sufficient enough in deciding on the safety status of tea grown and marketed in Kenya.
6. The heavy metal levels of black tea and tea liquors indicate that the MPC's should be based on liquor since the highest potential risk would depend on what is taken in through liquor as opposed to the concentrations in black tea.

4.2 Recommendations

Based on the findings of this study, the following recommendations are made;

1. Results from this work indicate potential for contamination of tea by Fe during processing. More work in this area is recommended to determine the actual source of the high Fe levels in black tea. It is probable that the contaminants are from iron fillings from substandard machinery. This can be verified by the use of powerful magnets to remove iron fillings followed by analysis to determine whether the increased levels are due to iron fillings. In the event that the source is iron fillings, it will be important that the quality of the machinery is guaranteed.
2. The Kenyan standard (KS 65: 2009) is too stringent for Pb and Cd for Kenyan teas and those in the market. Because of this, some Kenyan teas may fail to meet the permissible limits for these two metals and should be revised upwards. It is recommended that the Tea Board of Kenya (TBK) takes the initiative to get the Kenyan standard KS 65: 2009 revised. Suggested maximum permissible limits should be:
    a. Pb $\leq$ 0.50ppm instead of 0.10ppm
    b. Cd $\leq$ 0.05ppm instead of 0.02ppm
3. It is also recommended that the Kenyan standard, KS 65: 2009, should include a limit for fluoride in tea. This is a matter of discussion even at the international level including the Food and Agriculture Organization (FAO) and the Intergovernmental Group (IGG) on tea.
4. MPC's based on liquor should be set for heavy metals and fluoride in tea.

4.3 Suggestions for Further Research

1. Other elements, both essential and non-essential in tea should be evaluated so that the safety status of tea can be comprehensively be evaluated. Such elements include mercury (Hg), arsenic (As and chromium (Cr).
2. The status of roadside contamination by Pb in tea in plantations adjacent to highways and the time effect on such levels should be determined.

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APPENDICES

Appendix I: The KTDA factory regions (zones)

Region	Factory	Region	Factory
Region 1	Kambaa	Region 4	Michimikuru*
	Kagwe		Kiegoi
	Thieta		Kionyo
	Mataara*		Weru*
	Gachege		Igembe
	Kuri	Region 5	Litein
	Njunu		Tegat*
	Ngere		Kapkatet
	Makomboki*		Momur
	Ikumbi		Toror
	Nduti		Rianyamwamu
	Gacharage		Kapkoros
Region 2	Githambo		Mogogosiek
	Kanyenyaini		Kapset
	Gathunguru		Tirgaga
	Kiru*		Rorok*
	Chinga*		Olenguruone
	Iriani	Region 6	Nyansiongo*
	Gitugi		Kebirigo
	Gathuthi		Sanganyi
	Ragati		Tombe
Region 3	Ndima		Nyankoba
	Mununga		Gianchore
	Kangaita*		Kiamokama
	Kimunye		Nyamache
	Thumaita		Ogembo*
	Mungania		Itumbe
	Rukuriri*		Eberege
	Katangariri	Region 7	Chebut
Region 4	Kinoro		Mudete*
	Imenti		Kapsara*
	Githongo		Kaptumo

*Indicates the factory selected from each region; (Source: KTDA)

Appendix II: FAAS operating conditions.

Parameter	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
Flame temperature (°C)	2300	2300	2300	2300	2300
Oxidant type	Air	Air	Air	Air	Air
Oxidant (Flow rate, $Lmin^{-1}$)	11.54	12.98	11.30	13.50	11.38
Fuel type	Acetylene	Acetylene	Acetylene	Acetylene	Acetylene
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

Appendix III: Concentrations of the calibration solutions and correlation coefficients of the calibration curves

Analyte	Concentrations of intermediate Calibration solutions (ppm)	*r^2	Calibration equation
Fe	1.0, 2.0, 3.0, 4.0 and 8.0	0.9983	$y = 0.0492x + 0.0038$
Zn	0.2, 0.4, 0.6, 0.8 and 1.2	0.9918	$y = 0.2801x + 0.0215$
Cu	0.8, 1.6, 2.4 and 3.2	0.9999	$y = 0.0504x - 8 \times 10^{-05}$
Pb	2.0, 4.0, 6.0, 8.0 and 10.0	0.9998	$y = 0.0163x + 0.0064$
Cd	0.2, 0.4, 0.6, 0.8 and 1.0	0.9997	$y = 0.0706x - 0.0002$

*Correlation coefficient of the calibration curve

Appendix IV: Method detection limits for unprocessed, processed tea and tea liquors

Analyte	Unprocessed tea (µg/g)	Black tea (µg/g)	Tea liquor (µg/ml)
Fe	0.070	0.070	0.020
Zn	0.003	0.003	0.001
Cu	0.003	0.003	0.010
Pb	0.010	0.010	0.002
Cd	0.004	0.004	0.002
Fluoride	-	-	0.100

* Values are the mean the ten blank determinations added to thrice the standard deviation of the blank determinations.

Appendix V: A sample of the output from the statistical analysis package (MSTAT)

Data file:Kenyan Made Tea

Title: Iron (Fe)

Function: Factor

Experiment Model Number 8: Two Factor Randomized Complete Block Design

Data case no. 1 to 261.

Factorial ANOVA for the factors:

Replication (Replicates) with values from 1 to 3

Factor A (Factories) with values from 1 to 29

Factor B (Grades) with values from 1 to 3

Variable 4: Fe ppm

Grand Mean = 197.823 Grand Sum = 51631.800 Total Count = 261

T A B L E O F M E A N S

1	2	3	4	Total
1	*	*	198.626	17280.500
2	*	*	197.297	17164.800
3	*	*	197.546	17186.500
*	1	*	132.044	1188.400
*	2	*	121.144	1090.300
*	3	*	134.778	1213.000
*	4	*	152.922	1376.300
*	5	*	134.411	1209.700
*	6	*	143.200	1288.800
*	7	*	197.011	1773.100
*	8	*	162.300	1460.700
*	9	*	243.956	2195.600
*	10	*	174.411	1569.700
*	11	*	204.233	1838.100
*	12	*	255.044	2295.400
*	13	*	195.822	1762.400
*	14	*	250.589	2255.300
*	15	*	228.689	2058.200

*	16	*	287.922	2591.300
*	17	*	190.144	1711.300
*	18	*	238.900	2150.100
*	19	*	179.944	1619.500
*	20	*	299.444	2695.000
*	21	*	250.356	2253.200
*	22	*	198.767	1788.900
*	23	*	251.844	2266.600
*	24	*	189.122	1702.100
*	25	*	175.422	1578.800
*	26	*	204.322	1838.900
*	27	*	191.478	1723.300
*	28	*	178.500	1606.500
*	29	*	170.144	1531.300

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*	*	1	209.470	18223.900
*	*	2	195.790	17033.700
*	*	3	188.209	16374.200

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*	1	1	150.700	452.100
*	1	2	126.767	380.300
*	1	3	118.667	356.000
*	2	1	124.100	372.300
*	2	2	119.100	357.300
*	2	3	120.233	360.700
*	3	1	118.200	354.600
*	3	2	137.167	411.500
*	3	3	148.967	446.900
*	4	1	158.200	474.600
*	4	2	152.533	457.600
*	4	3	148.033	444.100
*	5	1	167.600	502.800
*	5	2	118.000	354.000
*	5	3	117.633	352.900

*	6	1	162.800	488.400
*	6	2	121.700	365.100
*	6	3	145.100	435.300
*	7	1	229.500	688.500
*	7	2	184.367	553.100
*	7	3	177.167	531.500
*	8	1	176.600	529.800
*	8	2	154.967	464.900
*	8	3	155.333	466.000
*	9	1	254.900	764.700
*	9	2	226.667	680.000
*	9	3	250.300	750.900
*	10	1	184.467	553.400
*	10	2	154.267	462.800
*	10	3	184.500	553.500
*	11	1	259.467	778.400
*	11	2	174.167	522.500
*	11	3	179.067	537.200
*	12	1	262.800	788.400
*	12	2	253.700	761.100
*	12	3	248.633	745.900
*	13	1	206.900	620.700
*	13	2	197.600	592.800
*	13	3	182.967	548.900
*	14	1	257.733	773.200
*	14	2	250.733	752.200
*	14	3	243.300	729.900
*	15	1	285.567	856.700
*	15	2	215.600	646.800
*	15	3	184.900	554.700
*	16	1	315.667	947.000
*	16	2	328.833	986.500
*	16	3	219.267	657.800
*	17	1	188.167	564.500

*	17	2	201.800	605.400
*	17	3	180.467	541.400
*	18	1	262.733	788.200
*	18	2	225.767	677.300
*	18	3	228.200	684.600
*	19	1	178.333	535.000
*	19	2	181.900	545.700
*	19	3	179.600	538.800
*	20	1	271.233	813.700
*	20	2	316.400	949.200
*	20	3	310.700	932.100
*	21	1	246.367	739.100
*	21	2	269.933	809.800
*	21	3	234.767	704.300
*	22	1	209.033	627.100
*	22	2	212.267	636.800
*	22	3	175.000	525.000
*	23	1	271.300	813.900
*	23	2	245.433	736.300
*	23	3	238.800	716.400
*	24	1	187.233	561.700
*	24	2	180.767	542.300
*	24	3	199.367	598.100
*	25	1	182.400	547.200
*	25	2	176.033	528.100
*	25	3	167.833	503.500
*	26	1	204.700	614.100
*	26	2	214.000	642.000
*	26	3	194.267	582.800
*	27	1	220.033	660.100
*	27	2	172.867	518.600
*	27	3	181.533	544.600
*	28	1	175.767	527.300
*	28	2	185.933	557.800

*	28	3	173.800	521.400
*	29	1	162.133	486.400
*	29	2	178.633	535.900
*	29	3	169.667	509.000

A N A L Y S I S O F V A R I A N C E T A B L E

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	86.948	43.474	1.0397	0.3558
2	Factor A	28	559675.902	19988.425	478.0352	0.0000
4	Factor B	2	20202.708	10101.354	241.5800	0.0000
6	AB	56	71318.700	1273.548	30.4577	0.0000
-7	Error	172	7191.957	41.814		
	Total	260	658476.215			

Coefficient of Variation: 3.27%

s_y for means group 1: 0.6933 Number of Observations: 87

s_y for means group 2: 2.1555 Number of Observations: 9

s_y for means group 4: 0.6933 Number of Observations: 87

s_y for means group 6: 3.7333 Number of Observations: 3

Appendix VI: Fe in Kenyan unprocessed, black tea and tea liquors

	[a]Unprocessed Tea (µg/g)	[b]Black Tea (µg/g)				[c]Tea Liquor (µg/ml)			
Factory		**BP1**	**PF1**	**PD**	**Mean**	BP1	PF1	PD	**Mean**
Changoi (L)	55	151	127	119	**132**	6.8	5.5	4.6	**5.6**
Chemomi (L)	65	124	119	120	**121**	6.4	5.8	6.7	**6.3**
Kapsumbeiwa (L)	71	118	137	149	**135**	5.9	5.8	5.8	**5.8**
Kaimosi (L)	87	158	126	148	**153**	7.5	6.2	6.8	**6.8**
Koiwa (L)	75	168	118	118	**134**	7.7	7.6	6.9	**7.4**
Cheboswa(L)	74	163	122	145	**143**	7.7	6.1	7.6	**7.1**
Mettarora (L)	75	230	184	177	**197**	7.8	7.1	7.8	**7.6**
Sotik Tea (L)	116	177	155	155	**162**	8.6	7.0	6.5	**7.3**
Kangaita (S)	120	255	227	250	**244**	10.0	9.3	11.1	**10.1**
Michimikuru (S)	108	185	154	185	**174**	6.0	6.9	6.6	**6.5**
Kapsara (S)	123	260	174	179	**204**	8.3	6.4	6.8	**7.2**
Tegat (S)	106	263	254	249	**255**	9.5	7.1	7.0	**7.9**
Nyansiongo (S)	107	207	198	183	**196**	11.0	7.0	8.6	**8.9**
Ogembo (S)	105	258	251	243	**251**	7.7	6.7	8.0	**7.5**
Kitumbe (L)	122	286	216	185	**229**	6.3	1.3	4.7	**6.0**
Rorok (S)	203	316	329	219	**288**	9.4	7.8	8.2	**8.5**
Mudete (S)	115	188	202	181	**190**	8.0	7.1	5.1	**6.8**
Makomboki (S)	157	263	226	228	**239**	10.5	11.5	11.2	**11.1**
Weru (S)	149	178	182	180	**180**	7.7	5.3	7.0	**6.7**
Mataara (S)	168	295	316	311	**299**	11.7	10.5	11.7	**11.3**
Chinga (S)	188	246	270	235	**250**	8.5	7.3	7.5	**7.7**
Rukuriri (S)	72	209	212	175	**198**	6.3	6.6	5.0	**6.0**
Kiru (S)	162	271	245	239	**252**	11.0	8.5	8.0	**9.2**
Masingi (L)	104	187	181	199	**189**	7.7	6.7	5.9	**6.8**
		209	**196**	**188**		**8.2**	**7.3**	**7.4**	

[a]Coefficient of Variation (CV) in (%) = 4; Least Significant Difference (LSD) = 8: [b]CV (%) = 3.3; LSD (p≤0.05): Factories = 6.2; Grades = 4.2; F*G = 10.5: [c]CV (%) = 11.5; LSD (p≤0.05): Factories = 0.6; Grades = 0.4; F*G = 1.4

Appendix VII: Fe in black tea and tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

	[a]Black Tea (µg/g)				[b]Tea Liquor (µg/ml)			
Country	BP1	PF1	PD	**Mean**	**BP1**	**PF1**	**PD**	**Mean**
Tanzania	177	190	218	**195**	6.8	7.2	8.0	**7.3**
Rwanda	322	312	152	**262**	10.4	9.5	6.9	**8.9**
Uganda	369	361	301	**344**	12.5	11.4	10.4	**11.4**
Kenya	166	151	159	**158**	8.5	6.3	5.9	**6.9**
Mean	**259**	**254**	**208**		**8.2**	**9.2**	**8.5**	

[a]CV (%) = 3.8; LSD (p≤0.05): Countries = 13.7; Grades = 16.0; C*G = 15.4: [b]CV (%) = 15.9; LSD (p≤0.05): Countries = 2.1; Grades = NS; C*G = 2.7

Appendix VIII: Zn in Kenyan unprocessed, black tea and tea liquors

	[a]Unprocessed Tea (µg/g)	[b]Black Tea (µg/g)				[c]Tea Liquor (µg/ml)			
Factory		**BP1**	**PF1**	**PD**	**Mean**	BP1	PF1	PD	**Mean**
Changoi (L)	24.0	31.2	28.2	31.6	**30.3**	4.3	3.8	4.0	**4.1**
Chemomi (L)	37.5	44.9	39.5	37.9	**40.8**	4.5	4.5	3.6	**4.2**
Kapsumbeiwa (L)	31.1	43.5	33.5	33.8	**36.9**	4.0	3.6	3.9	**3.8**
Kaimosi (L)	27.0	27.6	29.4	26.9	**28.0**	3.6	3.9	3.7	**3.7**
Koiwa (L)	26.4	26.0	26.7	28.1	**26.9**	3.4	3.9	3.4	**3.6**
Cheboswa(L)	32.6	37.2	35.4	35.7	**36.1**	4.5	3.8	4.7	**4.3**
Mettarora (L)	25.1	30.6	28.6	38.3	**32.5**	3.2	2.9	3.9	**3.3**
Sotik Tea (L)	21.2	23.5	24.0	20.4	**22.6**	2.7	3.0	2.5	**2.7**
Kangaita (S)	21.6	26.6	25.6	24.5	**25.6**	3.3	3.4	2.8	**3.2**
Michimikuru (S)	22.9	29.5	27.7	25.0	**27.4**	2.6	3.6	2.9	**3.0**
Kapsara (S)	19.1	22.7	23.8	22.9	**23.2**	2.8	2.4	2.4	**2.5**
Tegat (S)	20.6	23.5	22.2	23.8	**23.2**	3.2	3.6	3.7	**3.5**
Nyansiongo (S)	24.7	25.4	26.6	27.3	**26.5**	3.2	3.2	3.8	**3.4**
Ogembo (S)	20.6	23.3	20.9	22.8	**22.3**	2.4	2.5	2.8	**2.6**
Kitumbe (L)	15.4	20.2	18.8	19.1	**19.4**	2.4	2.7	2.8	**2.6**
Rorok (S)	32.5	39.5	42.9	34.7	**39.1**	4.5	4.3	3.6	**4.1**
Mudete (S)	28.9	42.0	31.7	31.3	**35.0**	4.2	3.9	4.0	**4.0**
Makomboki (S)	25.0	25.7	25.5	26.7	**26.0**	3.4	3.9	3.6	**3.6**
Weru (S)	28.8	28.2	30.3	30.3	**29.6**	3.8	3.9	3.4	**3.7**
Mataara (S)	31.2	32.2	37.3	34.4	**34.6**	4.5	5.0	5.1	**4.9**
Chinga (S)	26.6	29.6	29.1	33.4	**30.7**	3.8	3.8	4.4	**4.0**
Rukuriri (S)	19.3	24.0	25.2	27.3	**25.5**	2.7	3.6	3.6	**3.3**
Kiru (S)	24.7	26.3	26.0	24.4	**25.6**	3.7	3.9	2.8	**3.5**
Masingi (L)	20.6	28.9	37.5	28.2	**31.6**	3.5	4.9	3.9	**4.1**
		29.0	**28.7**	**28.2**		**3.5**	**3.5**	**3.5**	

[a]CV (%) = 6.3; LSD = 2.7: [b]CV (%) = 5.6; LSD (p≤0.05): Factories = 1.5; Grades = 1.0; F*G = 2.6: [c]CV (%) = 10.4; LSD (p≤0.05): Factories = 0.4; Grades = NS; F*G = 0.6

Appendix IX: Zn in black tea and tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

	[a]Black Tea (µg/g)				[b]Tea Liquor (µg/ml)			
Country	**BP1**	**PF1**	**PD**	**Mean**	**BP1**	**PF1**	**PD**	**Mean**
Tanzania	29.0	27.9	27.7	**28.2**	5.9	5.8	5.8	**5.8**
Rwanda	23.9	23.7	23.0	**23.5**	4.4	4.3	4.7	**4.4**
Uganda	17.1	21.1	27.3	**21.8**	4.1	4.6	5.2	**4.6**
Kenya	38.9	24.3	34.9	**32.7**	4.5	3.8	4.4	**4.2**
Mean	**27.2**	**24.3**	**28.2**		**4.7**	**4.6**	**5.0**	

[a]CV (%) = 8.5; LSD (p≤0.05): Countries = 3.4; Grades = 3.9; C*G = 4.5: [b]CV (%) = 16.0; LSD (p≤0.05): Countries = 1.1; Grades = 1.4; C*G = 1.5

Appendix X: Cu levels in Kenyan unprocessed, black tea and tea liquors

	[a]Unprocessed Tea (µg/g)	[b]Black Tea (µg/g)				[c]Tea Liquor (µg/ml)			
Factory		**BP1**	**PF1**	**PD**	**Mean**	**BP1**	**PF1**	**PD**	**Mean**
Changoi (L)	10.4	13.6	11.7	13.0	**12.8**	2.0	1.5	1.8	**1.8**
Chemomi (L)	10.1	11.7	12.2	12.4	**12.1**	1.7	1.7	1.6	**1.7**
Kapsumbeiwa (L)	11.6	11.8	13.0	12.4	**12.4**	2.1	2.0	1.8	**2.0**
Kaimosi (L)	12.4	13.3	12.4	14.0	**13.2**	1.9	1.6	2.3	**2.0**
Koiwa (L)	10.5	13.5	11.7	12.2	**12.5**	1.4	2.0	2.0	**1.8**
Cheboswa(L)	10.3	13.4	11.2	11.6	**12.1**	1.5	1.5	1.5	**1.5**
Mettarora (L)	10.1	12.7	11.0	11.7	**11.8**	1.6	1.3	1.5	**1.5**
Sotik Tea (L)	10.9	13.6	11.0	12.9	**12.5**	1.9	2.0	2.8	**2.2**
Kangaita (S)	10.4	12.3	13.0	13.1	**12.8**	1.4	1.6	1.7	**1.6**
Michimikuru (S)	9.7	10.4	10.6	12.0	**11.0**	1.3	1.7	1.7	**1.6**
Kapsara (S)	11.9	13.0	12.6	12.0	**12.5**	1.0	1.2	0.7	**1.0**
Tegat (S)	11.5	11.6	12.0	12.8	**12.1**	1.5	1.6	1.1	**1.4**
Nyansiongo (S)	11.4	14.1	14.1	13.0	**13.7**	1.7	1.6	1.8	**1.7**
Ogembo (S)	13.9	16.2	16.4	17.1	**16.6**	1.6	2.2	1.8	**1.8**
Kitumbe (L)	12.4	13.7	15.7	13.9	**14.4**	1.5	1.6	1.4	**1.5**
Rorok (S)	14.7	14.1	13.9	12.7	**13.6**	1.6	2.0	2.1	**1.9**
Mudete (S)	13.4	14.2	15.7	12.8	**14.2**	1.9	2.5	1.8	**2.1**
Makomboki (S)	12.2	13.3	15.0	14.9	**14.4**	1.4	1.5	2.5	**1.8**
Weru (S)	15.1	15.3	14.8	15.9	**15.3**	2.4	1.5	2.0	**2.0**
Mataara (S)	9.8	15.8	15.3	14.8	**15.3**	1.9	1.3	1.5	**1.6**
Chinga (S)	13.3	17.3	15.8	15.9	**16.3**	1.9	2.0	2.8	**2.2**
Rukuriri (S)	10.7	12.3	11.7	11.7	**11.9**	1.6	2.0	3.0	**2.2**
Kiru (S)	8.4	10.8	10.3	9.0	**10.0**	0.9	1.2	0.7	**0.9**
Masingi (L)	9.1	17.0	14.2	17.2	**16.1**	2.3	2.1	2.0	**2.2**
		13.5	**13.2**	**13.3**		**1.7**	**1.7**	**1.9**	

[a]CV (%) = 8.9; LSD = 1.7: [b]CV (%) = 6.9; LSD (p≤0.05): Factories = 0.9; Grades = Not Significant (NS); F*G = 1.5: [c]CV (%) = 16.6; LSD (p≤0.05): Factories = 0.3; Grades = 0.2; F*G = 0.5

Appendix XI: Cu in black tea and tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

	[a]Black Tea (µg/g)				[b]Tea Liquor (µg/ml)			
Country	**BP1**	**PF1**	**PD**	**Mean**	**BP1**	**PF1**	**PD**	**Mean**
Tanzania	12.8	12.3	14.7	**13.3**	2.2	1.9	2.1	**2.1**
Rwanda	14.6	13.9	13.5	**14.0**	1.9	2.2	2.1	**2.1**
Uganda	15.9	17.8	14.1	**16.0**	2.0	1.9	1.7	**1.7**
Kenya	12.4	14.4	13.7	**13.5**	1.5	1.9	1.9	**1.8**
Mean	**14.0**	**14.6**	**14.0**		**2.0**	**2.0**	**1.9**	

[a]CV (%) = 9.3; LSD (p≤0.05): Countries = 2.0; Grades = 2.3; C*G = 2.6: [b]CV (%) = 10.7; LSD (p≤0.05): Countries = 0.3; Grades = NS; C*G = NS

Appendix XII: Pb in Kenyan unprocessed, black tea and tea liquors

	[a]Unprocessed Tea (µg/g)	[b]Black Tea (µg/g)				[c]Tea Liquor (µg/ml)			
Factory		**BP1**	**PF1**	**PD**	**Mean**	**BP1**	**PF1**	**PD**	**Mean**
Changoi (L)	0.15	0.23	0.22	0.26	**0.24**	0.05	0.03	0.05	**0.04**
Chemomi (L)	0.19	0.22	0.26	0.29	**0.25**	0.05	0.06	0.07	**0.06**
Kapsumbeiwa (L)	0.18	0.26	0.23	0.26	**0.25**	0.04	0.05	0.05	**0.05**
Kaimosi (L)	0.20	0.23	0.26	0.28	**0.26**	0.05	0.02	0.03	**0.03**
Koiwa (L)	0.12	0.27	0.18	0.17	**0.21**	0.08	0.06	0.06	**0.06**
Cheboswa(L)	0.17	0.14	0.14	0.16	**0.14**	0.02	0.05	0.05	**0.04**
Mettarora (L)	0.11	0.13	0.23	0.21	**0.19**	0.03	0.04	0.03	**0.03**
Sotik Tea (L)	0.19	0.20	0.22	0.25	**0.22**	0.03	0.04	0.03	**0.03**
Kangaita (S)	0.14	0.14	0.16	0.13	**0.14**	0.03	0.04	0.04	**0.04**
Michimikuru (S)	0.15	0.23	0.26	0.23	**0.24**	0.05	0.04	0.05	**0.05**
Kapsara (S)	0.24	0.27	0.23	0.35	**0.28**	0.09	0.04	0.07	**0.07**
Tegat (S)	0.30	0.28	0.35	0.32	**0.32**	0.05	0.05	0.07	**0.06**
Nyansiongo (S)	0.13	0.20	0.20	0.16	**0.19**	0.05	0.05	0.05	**0.05**
Ogembo (S)	0.13	0.17	0.14	0.14	**0.15**	0.05	0.02	0.03	**0.03**
Kitumbe (L)	0.21	0.18	0.30	0.28	**0.29**	0.05	0.05	0.08	**0.06**
Rorok (S)	0.17	0.14	0.15	0.20	**0.16**	0.06	0.05	0.06	**0.05**
Mudete (S)	0.11	0.11	0.27	0.21	**0.19**	0.04	0.06	0.05	**0.05**
Makomboki (S)	0.18	0.13	0.22	0.28	**0.21**	0.04	0.07	0.07	**0.06**
Weru (S)	0.14	0.14	0.16	0.13	**0.14**	0.03	0.03	0.04	**0.03**
Mataara (S)	0.20	0.24	0.26	0.29	**0.27**	0.06	0.05	0.05	**0.06**
Chinga (S)	0.18	0.26	0.23	0.25	**0.25**	0.06	0.06	0.05	**0.06**
Rukuriri (S)	0.18	0.23	0.23	0.21	**0.22**	0.03	0.05	0.05	**0.04**
Kiru (S)	0.13	0.27	0.21	0.19	**0.23**	0.04	0.02	0.03	**0.03**
Masingi (L)	0.13	0.20	0.23	0.24	**0.22**	0.03	0.08	0.06	**0.06**
		0.21	**0.22**	**0.22**		**0.04**	**0.04**	**0.05**	

[a]CV (%) = 7.0; LSD = 0.02: [b]CV (%) = 4.8; LSD ($p \leq 0.05$): Factories = 0.01; Grades = 0.01; F*G = 0.02: [c]CV (%) = 11.3; LSD ($p \leq 0.05$): Factories = 0.005; Grades = 0.003; F*G = 0.008

Appendix XIII: Pb in black tea and tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

	[a]Black Tea (µg/g)				[b]Tea Liquor (µg/ml)			
Country	**BP1**	**PF1**	**PD**	**Mean**	**BP1**	**PF1**	**PD**	**Mean**
Tanzania	0.27	0.25	0.20	**0.24**	0.08	0.04	0.05	**0.06**
Rwanda	0.19	0.18	0.15	**0.17**	0.03	0.02	0.02	**0.02**
Uganda	0.21	0.19	0.13	**0.18**	0.04	0.03	0.03	**0.03**
Kenya	0.14	0.17	0.26	**0.19**	0.04	0.04	0.04	**0.04**
Mean	**0.20**	**0.20**	**0.18**		**0.05**	**0.04**	**0.04**	

[a]CV (%) = 12.3; LSD ($p \leq 0.05$): Countries = 0.04; Grades = NS; C*G = 0.05: [b]CV (%) = 13.8; LSD ($p \leq 0.05$): Countries = 0.01; Grades = 0.01; C*G = 0.01

Appendix XIV: Cd in Kenyan unprocessed, black tea and tea liquors

	[a]Unprocessed Tea (µg/kg)	[b]Black Tea (µg/kg)				[c]Tea Liquor (µg/L)			
Factory		**BP1**	**PF1**	**PD**	**Mean**	**BP1**	**PF1**	**PD**	**Mean**
Changoi (L)	22.3	25.7	32.3	28.0	**28.7**	3.0	4.3	3.3	**3.6**
Chemomi (L)	27.0	27.3	11.0	28.0	**22.1**	3.7	BDL*	3.7	**2.8**
Kapsumbeiwa (L)	28.7	27.7	15.7	29.0	**24.1**	5.3	4.3	4.0	**4.6**
Kaimosi (L)	9.3	12.0	15.3	17.0	**14.8**	BDL	BDL	BDL	**-**
Koiwa (L)	23.7	26.0	32.0	26.3	**28.1**	5.7	5.0	4.3	**5.0**
Cheboswa(L)	32.7	32.3	34.0	30.0	**32.1**	6.7	5.7	7.7	**6.7**
Mettarora (L)	23.7	37.3	20.7	39.7	**32.6**	6.7	5.7	5.3	**5.9**
Sotik Tea (L)	14.7	BDL*	27.7	16.7	**22.2**	BDL	7.0	5.3	**4.1**
Kangaita (S)	30.3	29.0	30.0	31.3	**30.1**	5.7	6.0	BDL	**4.7**
Michimikuru (S)	4.7	32.7	28.7	18.7	**26.7**	5.3	5.0	3.7	**4.7**
Kapsara (S)	15.7	17.0	21.0	21.7	**19.9**	3.3	4.3	2.7	**3.4**
Tegat (S)	13.7	17.0	14.7	16.3	**16.0**	BDL	3.0	4.3	**2.8**
Nyansiongo (S)	22.7	19.7	19.3	20.7	**19.9**	6.0	BDL	3.0	**3.8**
Ogembo (S)	19.7	19.0	24.7	25.7	**23.1**	5.3	4.3	4.0	**4.6**
Kitumbe (L)	18.0	21.0	19.3	21.0	**20.4**	3.0	BDL	5.7	**3.7**
Rorok (S)	22.3	25.7	32.3	28.0	**28.2**	3.0	4.3	3.7	**3.7**
Mudete (S)	28.3	27.3	9.0	28.0	**21.4**	6.0	BDL	3.0	**3.8**
Makomboki (S)	15.7	36.0	34.0	34.3	**34.8**	6.7	5.7	7.7	**6.7**
Weru (S)	13.7	17.0	14.7	16.3	**16.0**	BDL	3.0	4.3	**2.8**
Mataara (S)	22.7	19.7	17.0	20.7	**19.1**	6.0	BDL	3.0	**3.9**
Chinga (S)	23.0	19.0	24.7	25.7	**23.1**	5.3	4.3	4.0	**4.6**
Rukuriri (S)	21.3	21.0	19.3	21.0	**20.4**	3.0	BDL	5.7	**3.7**
Kiru (S)	15.7	17.0	21.0	19.7	**19.2**	3.3	4.3	3.3	**3.7**
Masingi (L)	18.0	34.3	33.0	33.3	**33.5**	6.3	6.0	5.3	**5.9**
		23.1	**23.7**	**24.9**		**4.3**	**4.1**	**4.2**	

*BDL = Below detectable limit: [a]CV (%) = 16.4; LSD = 4.5: [b]CV (%) = 15.3; LSD (p≤0.05): Factories = 3.5; Grades = NS; F*G = 6.0: [c]CV (%) = 49.3; LSD (p≤0.05): Countries = 2.0; Grades = NS; C*G = 3.4

Appendix XV: Cd in black tea and tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

	[a]Black Tea (µg/g)				[b]Tea Liquor (µg/ml)			
Country	**BP1**	**PF1**	**PD**	**Mean**	**BP1**	**PF1**	**PD**	**Mean**
Tanzania	11.5	13.3	16.6	**13.8**	4.4	4.6	3.5	**4.2**
Rwanda	18.5	22.1	18.7	**19.8**	3.8	4.1	3.0	**3.6**
Uganda	16.7	16.4	17.8	**17.0**	3.3	3.5	4.2	**3.7**
Kenya	15.7	19.4	22.0	**19.0**	1.8	3.3	4.7	**3.3**
Mean	**15.6**	**17.8**	**18.8**		**3.3**	**3.9**	**3.9**	

[a]CV (%) = 16.0; LSD (p≤0.05): Countries = 4.6; Grades = 5.5; C*G = NS: [b]CV (%) = 26.4; LSD (p≤0.05): Countries = NS; Grades = 2.3; C*G = 1.4

Appendix XVI: Fluoride in black tea liquor samples from 29 Kenyan tea factory catchments

	Concentration of fluoride in µg/ml			
Factory	**BP1**	**PF1**	**PD**	**Mean**
Changoi	0.31	0.29	0.29	**0.30**
Chemomi	0.64	0.25	0.30	**0.39**
Kapsumbeiwa	0.46	0.55	0.28	**0.43**
Kaimosi	1.11	1.07	0.50	**0.89**
Koiwa	0.34	0.39	0.44	**0.39**
Cheboswa	0.66	0.65	0.54	**0.62**
Mettarora	0.21	0.34	0.37	**0.31**
Sotik Tea	0.32	0.31	0.32	**0.32**
Kangaita	0.43	0.26	0.26	**0.32**
Michimikuru	0.38	0.31	0.29	**0.33**
Kapsara	0.29	0.43	0.34	**0.35**
Tegat	0.73	0.39	0.25	**0.45**
Nyansiongo	0.32	0.43	0.58	**0.44**
Ogembo	0.87	0.28	0.24	**0.46**
Kitumbe	0.36	0.43	0.26	**0.35**
Rorok	0.39	0.32	0.40	**0.37**
Mudete	0.21	0.25	0.35	**0.27**
Makomboki	0.20	0.25	0.34	**0.26**
Weru	0.39	0.34	0.29	**0.34**
Mataara	0.38	0.39	0.31	**0.36**
Chinga	0.24	0.21	0.34	**0.26**
Rukuriri	0.35	0.35	0.31	**0.34**
Kiru	0.36	0.43	0.26	**0.35**
Masingi	0.36	0.43	0.26	**0.35**
Kaisugu	0.43	0.34	0.30	**0.36**
Kiptagich	0.34	0.35	0.35	**0.35**
Tiluet	0.12	0.17	0.11	**0.13**
Bondet	0.24	0.21	0.34	**0.26**
Sisiba	0.32	0.33	0.31	**0.32**
Mean	**0.40**	**0.37**	**0.33**	

CV (%) = 15; Factories = 0.05; Grades = 0.04; C*G = 0.09

Appendix XVII: Fluoride in black tea liquor samples from Tanzania, Rwanda, Uganda and Kenya

	Concentration of fluoride in µg/ml			
Country	**BP1**	**PF1**	**PD**	**Mean**
Tanzania	0.36	0.39	0.58	**0.44**
Rwanda	0.25	0.22	0.27	**0.25**
Uganda	0.34	1.00	0.65	**0.66**
Kenya	0.50	0.34	0.39	**0.41**
Mean	**0.36**	**0.54**	**0.42**	

CV (%) = 20; Countries = 0.13; Grades = 0.15; C*G = 0.17

Appendix XVIII: Tea factories grouped into regions

Region	**Factories**
Kericho Highlands	Masingi, Kitumbe, Rorok, Tegat, Changoi, Chemomi, Kapsumbeiwa, Kaimosi, Koiwa, Cheboswa,
Kisii Highlands	Nyansiongo and Ogembo
Mt. Elgon region	Mudete and Kapsara
Mt. Kenya region	Kangaita, Weru, Chinga, Kiru, Mataara, Makomboki, Michimikuru, Rukuriri
Sotik region	Metarrora and Sotik tea