ASSESSMENT OF THE LEVELS OF MICRONUTRIENTS IN BLACK TEA FROM DIFFERENT REGIONS OF EAST AFRICA AND CHANGES IN THEIR LEVELS DUE TO AGRONOMIC PRACTICES

BY

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ABSTRACT

Inadequate supply of micronutrients causes human health complications and is a worldwide problem now referred to as hidden hunger. Beverages from *Camellia sinensis* are claimed to be the most widely consumed fluids after water. However it is not known if they contain adequate levels of micronutrients to alleviate hidden hunger problems. The levels of micronutrients in East African teas are unknown and factors controlling their absorption from the soil remain obscure. The objective of this study was to assess levels of micronutrients in East African teas, local market teas and their infusions, establish if their levels vary with nitrogen fertilizer rates and plucking intervals and determine if there are variations in micronutrient level in the black tea of tea clones planted in various geographic locations. The micronutrients Mn, Fe, Zn, Cu and Se levels from 42 factories in East Africa and effects of grading on the micronutrients levels in four tea grades were assessed in a completely randomized design and two factor completely randomized design respectively. The effects of nitrogenous fertilizer and plucking intervals on micronutrient content of clone 6/8 planted in Timbilil, Sotik Highlands and Changoi were assessed in a three factor randomized complete block design. The levels of these minerals were also assessed in black tea of different clones planted in Kangaita, Kipkebe and Timbilil in a factorial two design randomized in a complete block design replicated three times under similar agronomic practices. The black tea samples were ashed, acid digested and extracted for analysis using the AAS. Mn levels were highest while Se levels were lowest. Mean levels of other micronutrients were in the order Fe>Zn>Cu. Large particle size grades had more ($p \leq 0.05$) micronutrients. Teas from the local market had higher levels of micronutrients than exported teas and the levels varied significantly ($p \leq 0.05$) among the grades. About 41% and 82% Mn and Cu was extracted in hot water infusion respectively while Fe extracted was only 17%. A cup of tea from 2.0g tea can contribute 57%, 1.03%, 0.65%, 2.0% and 2.85% of Mn, Fe, Zn, Cu and Se respectively of daily minimum requirement. Thus consumption of more than two cups of tea per day can supply daily requirements of Mn. However adequate daily supply of other micronutrients must be supplied by other foods. All the micronutrients significantly ($p \leq 0.05$) varied with location of production. Mn and Se levels were not significantly ($p \leq 0.05$) affected by increasing rates of nitrogenous fertilizer, Fe and Zn significantly ($p \leq 0.05$) increased while Cu levels significantly ($p \leq 0.05$) reduced. Plucking intervals did not significantly ($p \leq 0.05$) affect the micronutrient content of the resultant black teas. Different clones showed varied ($p \leq 0.05$) micronutrient content when planted in a single location under similar agronomic practices and did not follow a similar pattern when the clones were planted in different locations. To increase micronutrient levels in tea, it is necessary to optimize nitrogen fertilizer rates and use suitable clones for different geographic locations.

CHAPTER ONE

1.0: Introduction

1.1: Background information

Many elements in trace amounts play vital roles in metabolic processes and are essential for the general well being of human beings. The essential elements can be classified into several categories which include bulk elements like hydrogen, carbon, oxygen and sulphur; macro-minerals like sodium, potassium, magnesium and calcium; trace elements like iron, zinc, copper and selenium among others. The deficiency or excess of any of these elements may cause diseases and/or be deleterious to human health (O'Dell and Sunde, 1997). The world has come a long way in understanding the nature, magnitude and range of solutions to micronutrient malnutrition often called 'hidden hunger'. Hidden hunger is a situation where one is satisfied in terms of quantity of food but still lacks the vital micronutrients (Eileen *et al.*, 1996). Despite abundant global food supply, widespread malnutrition still prevails in form of hidden hunger. Promotions of breast feeding and dietary modifications like improving food availability and micronutrient bioavailability have been advocated to alleviate the problem in children (Eileen *et al.*, 1996). The determination of trace elements levels in foods and related products is essential for understanding their nutritive importance. Tea beverages are defined as brews arising from infusion of products of young tender shoots of *Camellia sinensis*. L .O. Kuntze (ISO 3720, 1986). The beverages are the oldest most popular, non-alcoholic beverage in the world (Mondal *et al.,* 2004) and are prepared from dried young tender leaves of the tea plant (Mokgalaka *et al.*, 2004). It has been claimed the tea beverages are the most widespread consumed fluids after water (Gardner *et al.,* 2007). Tea can therefore contribute immensely to solutions of the hidden hunger problem if it contains the necessary micronutrients in appropriate levels (Al-Oud, 2003; Cao *et al.*, 1998).

The tea plants primarily absorb the essential elements from the soil (Cao *et al.*, 1998). Copper rich soils may supply high amounts of the nutrient to the tea plant. Iron is widely used in various forms in the modern households today. Iron scrapings and rusting of iron (Evans and Leigh, 1991) make this element to be present in the soils. Equally iron is found naturally in the soil (Evans and Leigh, 1991). Although zinc is also present in the soil, deficiency of the element is corrected through forliar application of zinc oxide (Hartlers, 1973a). Selenium naturally occurs in the soil and is infused in some of the rocks/salts (Ip, 1998) like magnate (copper selenide) and antimony selenium sulphide. All these beneficial heavy metals may end up being absorbed by the tea plant although the factors affecting their absorption remain obscure. Some of these micronutrients play vital roles in human lives including in the immune system (Arthur, 1991), as cancer protective agent by protecting cells from oxidative stress thus slowing progression of diseases (Lee *et al.*, 1995). The distribution and level of availability of these micronutrients in East African tea has not been assessed.

The tea drinking habits are spread worldwide and many producer countries make different brands and grades of tea to meet the increasing demands (Yemane *et al.*, 2008). East Africa produces mainly crush, tear and curl (CTC) black tea for export and blending of different grades (Table 1) for domestic consumption. The grades range from broken pekoe (BP), pekoe fannings (PF) and dust (D) samples (Hartlers, 1973b) with the pekoe fannings being the grade produced in largest amounts by all factories in East Africa (Anon, 2009). In all the East African tea factories BP, PD, PF and D are produced as the main grades (Anon, 2009)

Table 1. Different grades of CTC black teas.

Kind of Tea	Grade Name	Nomenclature
Broken	PEK	Pekoe
	BP	Broken Pekoe
	BOP	Broken Orange Pekoe
	BPS	Broken Pekoe Souching
	BP 1	Broken Pekoe One
	FP	Flowery Pekoe
Fannings	OF	Orange Fannings
	PF	Pekoe Fannings
	PF 1	Pekoe Fannings One
	BOPF	Broken Orange Fannings
Dust	PD	Pekoe Dust
	D	Dust
	CD	Churamani Dust
	PD 1	Pekoe Dust One
	D 1	Dust One
	CD 1	Churamani Dust One
	RD	Red Dust
	FD	Fine Dust
	SFD	Super Fine Dust
	RD 1	Red Dust One
	GD	Golden Dust
	SRD	Super Red Dust

Source: Hartlers, 1973a

Tea beverages are a cheap part of daily dietary intake and frequent consumption may increase nutritional health if it has substantial amounts of the necessary nutrients. The micronutrient content of the black teas from East Africa has not been studied. It is therefore not known if consumption of high amounts of East African teas can alleviate the hidden hunger problem. Again it is not known if the levels of these micronutrients vary with grades of tea. The local tea market absorbs only 5% of the total production with the teas is sold in packets, tea bags and instant teas (Kinyili, 2003). It remains unknown the micronutrient content of these teas hence their value in addressing hidden hunger remains obscure.

Standardization and recommendation on safe nutrients levels in foods is based on total contents of the nutrients (NAS, 1980). However tea beverages are consumed as infused liquors. Consequently nutrients may be in the black tea; however such nutrients may not be infused/ extracted into hot water solutions in the making of tea brews. Indeed the amount of infused or dissolved nutrients from black tea in East Africa has not been documented.

Agronomic inputs influence the soil chemical parameters (Othieno, 1992) leading to changes in the absorption and hence variations in the chemical composition of the harvested leaf (Bonheure and Willson, 1992). Nitrogen, phosphorous and potassium are the main nutrients for tea. They are usually supplied in compound nitrogenous fertilizers (Bonheure and Willson 1992). The nitrogenous fertilizer assists in plant growth and alters the absorption of the micronutrients by the tea plants from the soil. Increasing rates of nitrogenous fertilizer increases soil acidity (Owuor *et al.*, 1990; Kebeney *et al.*, 2010; Kamau *et al.*, 2005) and this may cause variations in micronutrient availability in the soils. Thus it is necessary to determine effects of nitrogenous fertilizer rates on the micronutrients in the black tea. In Kenya, tea grows at different rates in different locations. This causes variations in growth, yields (Obaga *et al.*, 1988, 1989; Squire *et al.*, 1993; Ng'etich and Stephens, 2001a, 2001b; Ng'etich *et al.*, 2001) and black tea quality (Owuor *et al.*, 2009, 2010a, 2010b). The effects of growing tea in different locations on the black tea micronutrient content is however unknown. Plucking is the most expensive agronomic input in tea production. Incorrect plucking reduces both tea yields and quality (Owuor *et al.*, 1997, 2000) especially if the plucking standard is varied (Owuor *et al.*, 1987a; Mahanta *et al.*, 1988). Recommended plucking standard in Kenya is two leaves and a bud (Othieno, 1988), although some producers use coarse plucking standards. Plucking intervals has been documented to change the chemical composition and hence quality of tea (Owuor *et al.*, 2009, 1997). However it is not known if the human health beneficial micronutrients levels in black tea are influenced by the harvesting intervals.

Tea is grown under varying environmental conditions causing changes in growth, yields (Wachira *et al.*, 2002; Wickremaratne, 1981) and quality (Gulati and Ravichranath, 1996;

Fernandez *et al.*, 2002; Moreda- Pineiro *et al.*, 2003; Owuor *et al.*, 2008; Peterson *et al.*, 2004). Such changes were attributed to many factors including non-use of single cultivar and different agronomic inputs. However when the studies on single cultivar were used under same agronomic inputs, quality (Owuor *et al.*, 2009, 2010a, 2010b) and yield (Wachira *et al.*, 2002, Owuor *et al.*, 2009, 2010b) variations persisted. It is not known if the micronutrients levels in black tea of same cultivar grown in different regions vary. Different clones are known to have different abilities to absorb micronutrients from the soil when they are under similar agronomic practices (Yemane *et al.,* 2008). In studies on clones grown in a single site (Owuor *et al.*, 1988, 1987b, 1987c) or same clones grown in different environments (Owuor *et al.*, 2010a), variations in black tea biochemical composition were observed. It is not known if such variations are also exhibited in black tea micronutrients levels.

1.2: Statement of the research problem

Hidden hunger is a worldwide problem. It can only be overcome by consuming foods rich in micronutrients. Tea beverages are popular and widely consumed. It is not known if the beverages can supply adequate levels of the micronutrients. It is therefore necessary to establish the quantities of essential micronutrients in tea. Various companies package tea for the local market. The levels of the micronutrients in locally consumed teas have not been assessed. It is therefore unknown if tea consumption is mitigating the effects of micronutrients deficiency in Kenya. Though tea may contain micronutrients, tea beverages are consumed as liquor infusions. The quantities of micronutrients that are infused into the tea liquor have not been documented. Several agronomic inputs influence chemical composition of black tea. But how these agronomic inputs influence micronutrient levels have not been documented.

1.4: Objectives of the study.

1.4.1: Broad objective

The broad objective of the study was to establish the distribution of the micronutrients Mn, Fe, Zn, Cu and Se in the black tea produced in East Africa, how their levels are influenced by grading and some agronomic inputs and to establish the levels of the micronutrients that are infused into tea liquor.

1.4.2: Specific objectives

The specific objectives are to:-

1. Determine the levels of the micronutrients (Mn, Fe, Zn, Cu and Se) in black tea and produced in East Africa.
2. Assess the influence of tea grading on the micronutrients levels.
3. Determine the levels of these micronutrients in locally packaged tea.
4. Determine the percentage of micronutrients infused in tea liquors.
5. Determine the variation of the micronutrients in clone TRFK 6/8 with location of production, fertilizer application rates and plucking interval.
6. Determine whether the levels of the micronutrients in the black tea of several clones planted in single location vary and whether these variations follow the same pattern in the clones planted in different locations.

1.5: Null hypotheses

1. The black tea sold and produced in East Africa may not contain adequate levels of the micronutrients Mn, Fe, Zn, Cu and Se.
2. The concentrations of the micronutrients are not affected by tea grading.
3. The locally packaged tea may not contain adequate amounts of the micronutrients.
4. The amounts of the micronutrients infused into the liquors are not significant.
5. The concentrations of the micronutrients in clone TRFK 6/8 cultivar do not differ with location of production, fertilizer application rates and the plucking interval.
6. The levels of the micronutrients do not differ in different clones even when planted in the same location and do not follow the same patterns in the clones planted in different locations.

If the null hypothesis shall not be accepted then the alternative shall be adopted.

1.6: Justification of the research problem

East African teas may be having high levels of micronutrients and thus contribute to the alleviation of 'hidden hunger'. Assessment of the levels of the micronutrients from different parts of East Africa may establish the teas that can alleviate hidden hunger, thus enhancing the value, income to farmers and improvement of human health. Although tea may have appreciable amounts of the micronutrients, what is important to human life is the amounts that can be extracted into the tea liquor. Agronomic inputs influence chemical composition and quality of tea. Nitrogenous fertilizer application and harvesting are the most expensive but indispensable field agronomic inputs in tea production (Anon, 2002; Othieno, 1988). It is necessary to understand how these agronomic inputs influence black tea micronutrients levels. However the responses to the agronomic inputs may vary with geographical region of production. Generating data on variations of the micronutrients with area of production will help in developing region specific agronomic recommendations leading to production of black tea with optimal micronutrient levels. Identification of cultivars with potential to alleviate the hidden hunger in human beings will help farmers cultivate high value crops with better economic returns.

1.7: Significance of the study

This study will quantify the contribution the East African teas can make towards the alleviation of hidden hunger problem. Indeed the study will quantify the amounts of micronutrients in black tea that are actually consumed in the black tea brews, hence establish the significance of black tea consumption as a method of alleviating hidden hunger. It will also give an insight on how key agronomic inputs can be optimized to enhance levels of the relevant micronutrients in tea.

CHAPTER TWO

2.0: Literature review

2.1: Importance of tea to the economy

Tea, *Camellia sinensis* L. O. Kuntze, is an important commodity crop in East Africa. It is a major forex earner for East Africa and Kenya is the third leading producer of tea in the world after China and India (Anon, 2009). In 2010, Kenya produced 9.8% of world tea and commanded 25% of the world tea export (Anon 2011). The tea industry and its allied activities employ directly over 500,000 families, each on the average supporting 6 members (Ogola and Kibiku, 2004). Thus, tea and allied industries/activities is estimated to support over 5 million Kenyans, most of them being smallholder farmers living in rural areas where intensive economic activities are low. Multinational tea companies also contribute to the economic performance by creating job opportunities for the local population working in the factories and in the estates. Tea is now the single highest foreign exchange earner for Kenya (Anon, 2009). Tea therefore contributes to poverty reduction, promotes infrastructure development in the rural areas and earns the government foreign exchange and revenue. There could be additional benefits in terms of higher incomes to the farmers and the country if other beneficial effects of East African teas can be proved. The investigation of the ability of tea to address the hidden hunger problem is therefore necessary.

2.2: Tea and micronutrient supply

The main sources of essential elements in plants are their growth media, agro inputs and soil (Subbiah *et al*., 2007). Plants take up the elements from the soil and under certain conditions, high levels can be accumulated in the leaves (Lasheen *et al*., 2008). The mineral constituents of tea leaves normally differ according to the geological source (Marcus *et al*., 1996). A number of papers regarding the determination of the mineral contents of tea have been published (Mokgalaka *et al*., 2004; Mondal *et al*., 2004; Cao *et al*., 1998). Several elements such as Ca, Na, K, Mg and Mn are present in mg/g quantities while elements such as Cr, Fe, Co, Ni, Cu, Zn, Se and Cd are present in µg/g level (Cao *et al*., 1998; Mokgalaka *et al*., 2004). However such data have not been generated for East African teas and it is not known how agronomic inputs especially fertilizers, harvesting, cultivars and geographical location influence their levels.

2.3: Black tea grading

Tea grades commonly refer to leaf size and location on the tea bush (Segal, 1996). Tea grading is primarily used by the factory producers for segregating various teas during the manufacturing process and this process is not defined or standardized and therefore is not a good indicator of quality teas or flavor (Hartlers, 1973b). The grades range from broken pekoe which is a medium grade that consists of small leaves and pieces of large leaves, the fannings is a lower grade and consists mainly pieces of tea leaves while the dust is the lowest particle size grade and consists tiny remnants created in sorting and crushing processes (Hartlers, 1973b) often used in tea bags to infuse rapidly and make strong robust brew and the pekoe dust is a grade whose particles are slightly larger than the dust but smaller than the fannings (Segal, 1996). Though tea grading is not a measure of the quality of tea, variations have been observed in the black tea chemical quality parameters but it remains unknown if particle size affects the micronutrient content of the black teas.

2.4: Local market teas.

East Africa supplies substantial amounts of black tea with Kenya supplying 9.8% of world tea and contributes 25% of the world tea export (Anon, 2011). The Kenyan local market absorbs only 5% of the total production and this is sold in packets of tea, tea bags and others prepared as instant teas (Kinyili, 2003). The teas are sold in local shops and supermarket within Kenya. It is not known if the local teas contain adequate amounts of the micronutrients than can aid in alleviation/reduction of human health hidden hunger problem.

2.5: Tea infusions (liquors)

Tea beverages are consumed as infused liquors, but most recommendations on safe metal levels in foods is based on the total contents of the nutrients (NAS 1980). These recommendations may therefore not be applicable to tea crops. The black teas produced in East Africa may contain high levels of micronutrients, but address hidden hunger, only the percentage that ends up in the infusions should be considered. In recent studies Al, Ca, K, Mg, Mn, P and S contents were found to be very high in both infusions and tea particles from Turkey (Gezgin *et al.*, 2006) while As, Cd, Cr, Li, Pb and Se contents of the infusions were very low. Generally, mineral contents of tea particles were higher than those of tea infusions (Gezgin *et al.*, 2006). Some elements were almost completely extracted into the tea infusions such as Br and K, some elements were well extracted into infusions such as Cu, F, Ni, Cr, Mn, Mg, S and Co while others were only partially extracted like Fe and Pb (Chen 1990). Cu infused highly into hot water, Mn and Zn were moderately infused while Fe infusion into hot water was low (Lasheen *et al.*, 2008). None of these studies describe the amounts of micronutrients from the local teas that end up in the infusions.

2.6: Nitrogenous fertilizer rates.

Nitrogenous fertilizer application is the second most expensive agronomic input in tea production (Ellis and Grice, 1981) after harvesting. Although nitrogenous fertilizer plays a major role in plant growth, it alters the absorption of micronutrients by the plants from the soil. That is because increasing rates of nitrogenous fertilizer generally increase soil acidity (Owuor *et al*., 1990; Wanyoko *et al*., 1990; Bonheure and Willson, 1992) and this may cause variations in availability of some of the micronutrients. The recommended rate of fertilizer application in Kenya, that is also widely used in East African countries is 100 to 250 kg N/ha/year as NPKS 25:5:5:5 or NPK 20:10:10 (Othieno, 1988; Anon, 2002), with the actual rate being dependent on level of production. But the optimal rates for different regions vary (Owuor *et al.,* 2010b). Nitrogenous fertilizer application influences the yield through variations in rate of shoot extension, individual shoot weight and density (Odhiambo, 1989; Owuor *et al,* 1997). Appropriate use of nitrogenous fertilizers leads to increase in tea production (Wanyoko, 1983; Willson, 1975; Owuor and Wanyoko, 1996) but the high rates of fertilizer application reduce black tea quality (Owuor *et al,* 1997, 2000) and increase the fatty acids in tea leaf (Okal, 2011). Despite the lowering of quality, application of nitrogenous fertilizer to tea is mandatory since it enhances yields (Othieno, 1988; Bonheure & Willson, 1992). These variations may be an indicator that other nutrients might also be changing due to rates of nitrogenous fertilizer application. The optimal rate of fertilizer for adequate levels of these essential minerals is not known. It is therefore necessary to establish nitrogen fertilizer rates that could enhance availability of the micronutrients to address the hidden hunger in different tea growing areas in Kenya.

2.7: Harvesting (plucking) intervals

Plucking is an important step in tea production. During the plucking operation, young leaves are removed (Willson, 1992) for processing into various tea beverages. The recommended plucking standard is two leaves and a bud that gives desirable good black quality teas (Owuor *et al*., 1987a, 1997, 2000) and acceptable yields (Othieno, 1988; Willson, 1992). Tea grows at different rates in different locations (Obaga *et al*., 1988, 1989; Squire *et al*., 1993; Ng'etich and Stephens, 2001a, 2001b; Ng'etich *et al*., 2001), leading to the achievement of recommended two leaves and a bud (Othieno, 1988) after different time lengths. Recommended plucking interval in Kenya varies from 7 to 14 days (Othieno, 1988). Short plucking intervals remove the leaves when the pluckable shoots are still young and are mostly two leaves and a bud (Odhiambo, 1989; Owuor and Odhiambo, 1994). Short plucking intervals reduce "breaking-back" (Mwakha and Anyuka, 1984), a process that reduces yields, but improves quality (Owuor *et al*., 2000). Aroma quality precursors, especially fatty acids whose degradation products reduce tea quality were demonstrated to increase with longer plucking intervals (Okal, 2011). The changes in the various quality attributes may also suggest there may be variations in the micronutrient content of tea with varying plucking intervals. Such variations have not been established in different tea growing areas. It is necessary to establish plucking intervals leading to optimal micronutrient levels in different regions.

2.8: Locational effects

The effect of region on growth is a known factor in determining the micronutrient content in tea (Mokgalaka *et al.*, 2004). The level of micronutrient in teas has been demonstrated to change with locations in India (Kumar *et al.*, 2005). In recent studies, tea quality parameters (Jondiko, 2010; Owuor *et al.*, 2008, 2009, 2010a, 2010b) and quality precursors (Okal, 2011) were demonstrated to change with geographical area of production. Indeed even the yields (Owuor *et al.*, 2009, 2010a; Wachira *et al.*, 2002) and quality (Li *et al.*, 2007; Owuor *et al.*, 2010a) vary with geographical area of production, the variation occurring in unpredictable patterns. The variation of micronutrients in tea with geographical area of production has not been documented.

2.9: Tea clone cultivars.

Tea is largely out crossing and inherently self infertile. Every plant produced is therefore unique. Clonal tea plants are derived from one bush (a mother bush) through vegetative propagation. Cultivars {culti (vated) + var (iety)} are plants that have been purposely selected and maintained through cultivation. Cultivars are normally registered and protected under law (Kamau, 2008). Several clones have been developed and released to farmers (Anon, 2002: Othieno, 1988). Several studies have demonstrated wide response in yield (Ng'etich *et al.*, 2001; Wachira *et al.*, 1990; Wackremaratne, 1981), yield partitioning (Ng'etich *et al.*, 2001), growth (Ng'etich and Stephens, 2001a, 2001b), shoot population density (Balasuriya, 1999) and dry matter partitioning (Ng'etich and Stephens, 2001b) of tea genotypes to different environments (Carr and Stephens, 1992; Wachira *et al.*, 1990; Wachira *et al.*, 2002) including water stress (Carr, 1997), temperature (Tanton, 1982a) and altitude (Obaga *et al.*, 1989; Squire *et al.*, 1993). Such variations occur even within 10-km radius (Ng'etich and Stephens, 2001a, 2001b; Ng'etich *et al.*, 2001; Obaga *et al.*, 1989; Squire *et al.*, 1993). In terms of the black tea quality, the black tea aroma (Aisaka *et al.*, 1978; Owuor and Obanda, 1996), volatile flavor compounds composition (Horita and Owuor, 1987; Yamanishi *et al.*, 1968) and black tea plain quality parameters (Owuor *et al.*, 1986a, 1986b) varied widely with geographical area of production. Previous studies assumed that large differences in climate are necessary for significant quality differences to be observed. As a result many tea-growing countries have centralized their clonal selection/breeding programmes in single locations. It has been thought that a superior genotype selected in one location maintains its desirable attributes within the country. However tea plants selected in one location and planted in other locations have usually not matched the performance at the site of selection (Wachira *et al.*, 1990, 2002). One such reason for such difference has been altitude, which affects rates of growth, even when other agronomic/cultural practices are similar. All these observations are indicators that there might be variations in the micronutrient content of the resultant black teas due to clones in different environments. Again different clones are known to have different abilities to absorb micronutrients from the soil when they are under similar agronomic practices (Yemane *et al.,* 2008). Region specific cultivars that have the ability to extract optimal amounts of the essential micronutrients have not been identified.

2.10: Micronutrients and the human health

An element is termed as an essential or a micronutrient when; a physiological deficiency is relieved by addition of that element to the diet or a specific beneficial biological function is associated with the element (NAS, 1980). Some elements such as Fe, Zn, Cu and Se termed as micronutrients are essential for the growth and development of human beings within certain permissible limits. Heavy metals, in contrast to most pollutants, are non-biodegradable and undergo ecological cycles (Tam and Wong, 1995). Metal bioaccumulation leads to possible health hazards to the human consumers (Vos and Hovens, 1986). Bioconcentration and bioaccumulation of toxic metal residues in the food chain can put terrestrial consumers including humans at risk (Ansari *et al.*, 2007) but some of the heavy metals like Mn, Fe, Zn, Cu and Se are essential for the human health. Such micronutrients can be adequately supplied through human diet (Eileen *et al.,* 1996). Tea beverages are the second most consumed fluids after water. If they have adequate supply of the micronutrients, they can have enormous contribution to hidden hunger alleviation. It is therefore necessary to establish quantities of the essential elements the tea beverages can supply. Every essential element follows a dosage response curve where at lowest dosages the organism does not survive and in a deficiency region the organism exists with less than optimal function and after optimal dosage region there are high dosages which cause toxicity in organisms and eventually leading to lethality (NAS, 1980). This can be elaborated using the dose-response curve in Figure 1 (NAS, 1980).

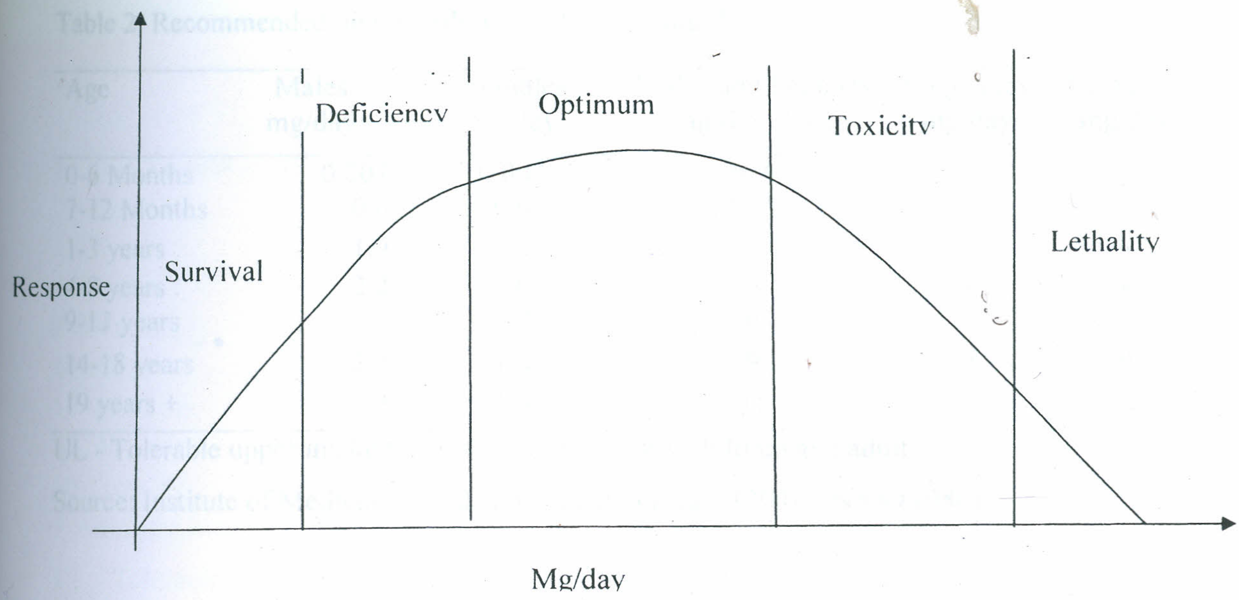


Fig 1.General Dosage Response curve for essential elements. (NAS 1980)

2.10.1. Manganese (Mn)

Mn is a trace mineral that is present in very small amounts in the human body. The human body contains around 20mg of manganese and most of it is found and concentrated in the bones, kidneys, liver and pancreas (Institute of Medicine, Food and Nutrition Board, 2001). Mn serves many functions in the human body. It primarily works as a coenzyme that facilitates various metabolic processes (Willis *et al.*, 2005). Again it is involved in bone formation of connective tissues, sex hormone function, calcium absorption, blood sugar regulation, immune function and in fat and carbohydrate metabolism (NAS 1980). Deficiency in Mn leads to various health problems which include bone malformation, eye and hearing problems, high cholesterol levels, hypertension, infertility, weaknesses, heart disorders, memory loss, muscle contraction, tremors and seizures (NAS 1980). Deficiencies are rare considering that they are naturally abundant in foods, but research has estimated about 37% of the population to be deficient in Mn (Institute of Medicine, Food and Nutrition Board, 2001) and this is caused by improper diet and eating habits.

To maintain Mn health, one needs to maintain good sense of Mn nutrition. Mn is abundant in natural sources but the Mn concentrations in black teas from East Africa remains unknown despite the fact that this commodity is widely available and affordable. Table 2 gives the recommended dietary allowance for manganese according to gender and different age limits.

Table 2: Recommended dietary allowance for Mn (mg/day)

Age	Males mg/day	Females mg/day	Males and Females mg/day (UL)	Pregnancy mg/day	Lactation mg/day
0-6 Months	0.003	0.003	N/A	-	-
7-12 Months	0.6	0.6	N/A	-	-
1-3 years	1.9	1.6	2	-	-
4-8 years	2.2	1.6	3	-	-
9-13 years	2.3	1.8	6	-	-
14-18 years	2.3	1.8	9	9	9
19 years +	2.3	1.8	11	11	11

UL - Tolerable upper intake levels for Mn in healthy children and adults

Source: Institute of Medicine, Food and Nutrition Board (2001); NAS (1980)

2.10.2: Iron (Fe)

Fe is an essential mineral and thus a component of several co-factors including haemoglobin and cytochrome. It is a major control element and many enzymes require Fe for their activity in carbohydrate metabolism e.g. with aconitase (Beinert and Kennedy, 1989). Many enzymes that are involved in secondary metabolism require Fe for their activity (Da Silva and Williams, 1991). Fe is virtually found in every food, with higher concentrations in animal tissues than in plant tissues (Hammond and Beliles, 1980). The main role of Fe in the body is in the red blood cells where it combines with a protein to form haemoglobin. On breathing, oxygen in the lungs is attracted to the Fe in haemoglobin and combines with it to form oxyhaemoglobin. This is then transported around the body by the blood cells and oxygen is released whenever it is needed to allow the conversion of carbohydrates (sugars) into energy (Stokinger, 1981). Lack of Fe in the body also known as Fe deficiency can be due to inadequate amounts of Fe in the diet or insufficient number of blood cells caused by blood loss leading to anemia. The Table 3 provides the Reference Dietary Intake (RDIs) showing the recommended dietary allowance (RDA), adequate intake (AI) and tolerable upper intake levels for iron in healthy children and adults (UL) of the Fe for healthy children and adults. (Institute of Medicine, Food and Nutrition Board, 2001; NAS, 1980).

Table 3: Recommended dietary allowance for Fe for healthy children and adults.

Age	Males and females (mg/day)		Males and females (mg/day) (UL)	Pregnancy (mg/day)	Lactation (mg/day)
Birth- 6 Months	0.27*	0.27*	40	–	–
7-12 months	11	11	40	–	–
1-3 Years	7	7	40	–	–
4-8 Years	10	10	40	–	–
9-13 Years	8	8	40	–	–
14-18 Years	11	15	40	27	10
19 -30 Years	8	18	40	27	9
31-50 Years	8	18	45	27	9
50 Years +	8	18	45	27	9

* Adequate Intake (AI) for iron in infants below 7 months.

UL - Tolerable upper intake levels for iron in healthy children and adults

Source: Institute of Medicine, Food and Nutrition Board (2001); NAS (1980)

Soils contain different amounts of the element Fe depending on the geographical area and agronomical practices of the area. Studies on tea farms in Ethiopia classified soils under the category of Plinthic Alisols with clayey texture and dark reddish brown colour (Solomon *et al.*, 2001) which is indicative of the presence of excess amount of haematite Fe_2O_3 (Tan, 1996). Soils with low pH contain high amounts of Fe oxides (Hu *et al.*, 2002). Thus Fe is the predominant metal within the concentration range of 18.8-23.5 mg/g in these soils. In plants Fe is a constituent of many enzymes and is a catalyst for respiration, photosynthesis and reduction of sulphates and nitrates. It also plays an important part in the formation of chlorophyll without entering into the constitution of this substance. Fe is usually available in acid tea soils so deficiencies are uncommon and toxicities are rare. Deficiencies are likely to occur only in soils that have a high pH. Being a vital element data is missing on how much tea contributes to the human health. It is therefore necessary to assess whether tea can be a major contributor of Fe in human diet since this tea is the second most consumed fluid after water (Gardner *et al.*, 2007) and to establish how the levels are influenced by agronomic and cultural practices.

2.10.3: Zinc (Zn)

Zn is a vital mineral to healthy living, as deficiency can cause health problems. It is required for catalytic activity of approximately 100 enzymes (Sandstead, 1994; Institute of Medicine, Food and Nutrition Board, 2000) and also plays a role in immune function (Solomon, 1998; Prasad, 1995), wound healing (Heyneman, 1996), DNA synthesis (Institute of Medicine, Food and Nutrition Board, 2001; Prasad 1995) and cell division (Prasad, 1995). Zn supports normal growth and development during pregnancy, childhood and adolescence (Simmer and Thompson, 1985; Manet and Sandstead, 2006). Zn is required for proper sense of taste and smell (Prasad *et al.*, 1997). A daily intake of Zn is required to maintain a steady state because the body has no specialized Zn storage system (Rink and Gabriel, 2000). Intake recommendations for Zn are provided in the Dietary Reference Intakes (DRIs) developed by Food and Nutrition Board at the Institute of Medicine Food and Nutritional Board (2001). Table 4 gives recommended dietary allowances for Zn in healthy children and adults.

Table 4: Recommended dietary allowances for Zn in healthy children and adults.

Age	Male (mg)	Female(mg)
Birth – 6 months	2*	2*
7 months – 3yrs	3	3
4 – 8 Years	5	5
9 – 13 Years	8	8
14 – 18 Years	11	9
19 Years +	11	8

*The indicated Adequate Intake (AI)

Source: Institute of Medicine, Food and Nutrition Board (2001); NAS (1980).

Zn deficiency is characterized by growth retardation, loss of appetite, and impaired immune function (Shankar and Prasad, 1998) and the body requires Zn to develop active T-lymphocytes (Beck *et al.*, 1997). In more severe cases, Zn deficiency causes hair loss, diarrhea, delayed sexual maturation, impotence, hypogonadism in males and eye and skin lesions (Prasad, 2004; Wang and Busbey, 2005). Weight loss, delayed healing of wounds, taste abnormalities and mental lethargy can also occur (Heyneman, 1996; Prasad *et al.*, 1997). Other diseases associated with Zn deficiency include malabsorption syndrome, chronic liver and renal diseases, sickle cell disease, diabetes, malignancy and other chronic illness (Prasad, 2003). Chronic diarrhea leads to excessive loss of Zn (Prasad, 2004). Pregnant women particularly those starting their pregnancy with marginal Zn status are at increased risk of becoming Zn deficient due, in part to high fetal requirements of Zn. Lactation can deplete maternal Zn from the body. Zn helps maintain the integrity of skin and mucosal membranes. The Zn deficiency can lead to common cold. Zn has the ability to prevent the colds or shorten the duration of the colds (Caruso *et al.*, 2007). Thus it is necessary to find out how much Zn is taken in from tea and how can the levels be optimised by agronomic inputs in different locations.

High Zn intakes can inhibit copper absorption, sometimes producing copper deficiency and associated anaemia (Bruon *et al.*, 1990; Willis *et al.*, 2005). Acute adverse effects of high Zn intake include nausea, vomiting, loss of appetite, abnormal cramps, diarrhea and headaches (Institute of Medicine, Food and Nutrition Board, 2001). The Institute of Medicine, Food and Nutrition Board (2001) has established ULs for Zn. Long term intakes above the upper limits increase the risk of adverse health effects. Table 5 gives the Tolerable upper intake levels for Zn in healthy children and adults.

Table 5: Tolerable upper intake levels (ULs) for Zn in healthy children and adults

Age	Male(mg)	Female (mg)	Pregnant(mg)	Lactation(mg)
0 – 6 months	4	4	_	_
7– 12 months	5	5	_	_
1 – 3 Years	7	7	_	_
4 – 8 Years	12	12	_	_
9 – 13 Years	23	23	_	_
14– 18 Years	34	34	34	34
19 Years+	40	40	40	40

Source: Institute of Medicine, Food and Nutrition Board (2001); NAS (1980)

Zn is essential for the plant growth. As a major constituent of some enzymes, it plays an important part in the formation of nucleic acid and proteins and catalyses the utilization of nitrogen and phosphorous. Shuvalov and Kontridze (1987) studied the distribution of zinc in the tea plant and showed that the levels ranged between 32 µg/g to 39 µg/g. Zn is not readily absorbed from the soil by the tea plant and that deficiencies occur frequently which cannot be readily corrected by ground application of Zn compounds. Hartlers (1973b) reported the benefits of Zn application on an estate scale and introduced aerial application of zinc oxide. Malenga (1986) found significant responses to aerial foliar application of Zn as zinc oxide to mature seedling tea leading to significant and economic yield responses in young clonal tea. There is no information on the levels of zinc in East African black tea and how the levels are influenced by agronomic and cultural practices.

2.10.4: Copper (Cu)

Cu is an essential trace element to man and all vertebrates but the body uses it in small amounts. It is a component of vital enzymes including tyrosine, cytochrome oxidase (Piscator, 1977; USEPA; 1980). Cu is largely combined with serum albumin and alpha globulin ceruloplasmin, which serve to transport and regulate Cu in the body (Stokinger, 1981). The element is especially essential in the electron transfer process which also involves iron in haemoglobin, photosynthesis in plants and the terminal step of mitochondria respiration. Thus, it influences life support functions like production of red blood cells and carbohydrate synthesis (Malmstrom, 1979). As a transition element Cu has a particular chemical suitability of having a functional role in electron transfer and oxygen transport proteins as it has two common valence states; Cu (I) and Cu (II). The oxidation reduction potential governing the relative predominance of these two valence states is sensitive to the nature and steric arrangement of the surrounding ligand potentials (Reinhammar, 1979). Cu (I) in certain complexes can bind oxygen and carbon monoxide (CO) reversibly (Lontie and Vanquickenborne, 1974) despite the fact that oxygen is a neutral symmetric molecule.

Table 6 shows recommended dietary allowance for copper for healthy children and adults.

Table 6: Recommended dietary allowance for Cu for healthy children and adults.

Age	Males and Females (µg/day)	Males and Females (µg/day) (UL)	Pregnancy (µg/day)	Lactation (µg/day)
Birth-6 Months	200*	_	_	_
7- 12 Months	200*	_	_	_
1-3 Years	340	1000	_	_
4-8 Years	440	3000	_	_
9-13 Years	700	5000	_	_
14-18 Years	890	8000	1000	1300
19 years +	900	10,000	1000	1300

* Adequate Intake (AI) for copper for the infants.

UL - Tolerate upper intake levels for Copper in healthy children and adults

Source: Institute of Medicine, Food and Nutrition Board (2001); NAS (1980)

At high doses, Cu is one of the common metallic elements toxic to humans found in polluted environments (Purves, 1977). Excessive consumption of Cu may lead to gastrointestinal distress or liver damage (USEPA, 1980; Prasad and Oberleas, 1976). A condition known as

Wilson's disease is also due to inability to utilize Cu and is a heredity metabolic disorder whereby accumulation of Cu in some organs of the body like the brain and liver (USEPA, 1980; Prasad and Oberleas, 1976). Acute Cu poisoning causes hemolytic anemia (Finelli *et al.*, 1981).

Cu is actively absorbed in the stomach and duodenum. But Cu levels can be decreased by competition with Zn and binding by ascorbic acid and other compounds. Cu deficiency is not common because it is found in a variety of foods but people who take large doses of vitamin C, Zn or Fe supplement may require more Cu. Cu can be also excreted and the major route for the absorbed Cu is the bile while other routes of excretion of Cu include sweat, urine and saliva (USEPA, 1980; Stokinger; 1981).

In tea Cu is an essential constituent of the enzyme polyphenol oxidase which is vital for fermentation (Clowes and Mitini-Nkhoma, 1987; Ramaswamy, 1960). Cu deficiency in tea causes poor fermentation which reduces the quality of black tea. Foliar application of a Cu compound improves its absorption by the plant. Clowes and Mitini-Nkhoma (1987) demonstrated the relationship between poor fermentation and a low Cu content in Malawi and confirmed that a foliar application of a Cu salt alleviates the deficiency. The finer-textured mineral soils generally contain the highest amount of Cu. Cu contents in tea are in the range 9.6-20.9 µg/g in three Chinese tea brands (Wang *et al.*, 1993). There is no information on the levels of Cu in East African tea. It is therefore necessary to assess the status of Cu in East African tea samples and determine how the levels are influenced by agronomic and cultural practices.

2.10.5: Selenium (Se)

Se is a vital trace element essential for good health in humans and other organisms (Ellis and Salt, 2003). A deficiency or excess of selenium can have serious effects to human health (Diaz-Alarcon *et al.*, 1994; Mejuto-Marti *et al.*, 1998). It is incorporated into proteins to make selenoproteins which are important antioxidant enzymes. The antioxidant properties of selenoproteins help prevent cellular damage from free radicals. The free radicals are natural by-products of oxygen metabolism that may contribute to the development of chronic diseases like cancer and heart diseases (Mackenzie *et al.*, 1998; Lavender, 1997). Evidence has been adduced for Se as a cancer protective agent (Combs, 1997). Epidemiological evidence suggests that a low Se intake may increase the risk of cardiovascular disease (Hu *et al.*, 2001a). Se deficiency may contribute to development of a form of heart disease, hypothyroidism and weakened immune system (Combs, 2000; Zimmerman and Kohle 2002). Other selenoproteins help regulate thyroid function and play a role in the immune system

(Arthur, 1991). Usually Se deficiency does not cause illness by itself, rather it makes the body more susceptible to illness caused by other nutritional biochemical or infection stresses (Beck *et al.*, 2003). Three specific diseases that have been associated with Se deficiency in China are the Keshan disease which results in an enlarged heart and poor heart functioning majorly in children, Kashin-Beck disease which results in osteorthropathy and the myxedematous endemic cretinism that result in mental retardation (Foster and Sumar, 1997; Lavender and Beck, 1997). Se affects cancer in two ways (Combs *et al.*, 2001); first as an anti-oxidant where it helps protect the body from damaging effects of free radicals and secondly it prevents or slows tumor growth by enhancing immune cell activity and suppressing development of blood vessels in the tumour (Combs *et al.*, 2001). Individuals with rheumatoid arthritis, chronic diseases that causes pain, stiffness, swelling and loss of function in joints have reduced Se level in their blood (Kose *et al.*, 1996; Heliovaara *et al.*, 1994). In addition some individuals with arthritis have a low Se intake (Stone *et al.*, 1997). HIV/AIDS malabsorption can deplete levels of many nutrients including Se thus increased diseases progression and high risk of death in the HIV/AIDS population (Anseth *et al.*, 1998; Look *et al.*, 1997). The antioxidant nutrients like Se help protect cells from oxidative stress thus slowing progression of the disease (Romeo-Alvira and Roche, 1998). Se status maybe a significant predictor of those infected with HIV (Baum and Shor-Posner, 1998). However high blood levels of Se (greater than 100 µg/dl) can result in a condition called selenosis (Koller and Exon, 1986) whose symptoms include gastrointestinal upsets, hair loss, white blotchy nails, garlic breath odour, fatigue, irritability and mild nerve damage.

Recommendations for Se are provided in the Dietary Reference Intake (DRIs); developed by the Institute of Medicine, Food and Nutrition Board, (2000). The DRIs are used for planning and assessing nutrient intake for healthy people. Three important types of reference values included in the DRIs are; recommended dietary allowance (RDA); adequate Intake (AI); and tolerable upper intake levels (UL). The RDA recommends the average daily intake level that is sufficient for healthy individuals in each age and gender group; an AI is set when there is insufficient scientific data available to establish the RDA while the UL is the maximum daily intake unlikely to result in adverse health effect (Institute of Medicine, Food and Nutritional Board, 2001). The average human body contains around 14 mg of Se (Mackenzie *et al*, 1998; Pennington and Young, 1991). Table 7 shows the three important types for reference values.

Table 7: Recommended dietary allowance for Se for healthy children and adults.

Age	Males and Females (μg/day)	Males and Females (μg/day) (UL)	Pregnancy (μg/day)	Lactation (μg/day)
0-6 Months	15*	45	_	_
7-12 Months	20*	60	_	_
1-3 years	20	90	_	_
4-8 years	30	150	_	_
9-13 years	40	280	_	_
14-18 years	55	400	60	70
19 years +	55	400	60	70

* Adequate intake levels for infants

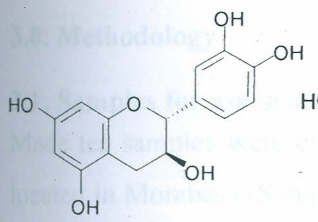
UL - Tolerate upper intake levels for Selenium in healthy children and adults

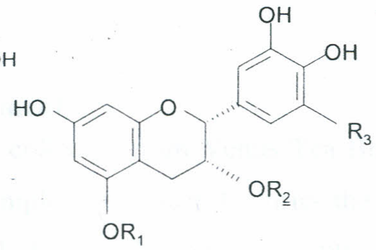
Source: Institute of medicine, Food and Nutrition Board (2001); NAS (1980)

Se has been identified as a constituent of glutathione peroxidase (GSH-Px) (Hou *et al,* 1993; Rotruck *et al.,* 1973) which shows anti-oxidative activity in higher plants and can reduce the active oxygen free radical oxidation (Xu, 1996). The Se level in agricultural products depends on the Se level in the soil where the products are grown. The plants take up Se from the soil and propagate it through food chain. The Se contents in the soil depend on the geographical region and agronomical practices in the area (Penningtone and Schoen, 1996). The factors that influence Se levels in East African teas remain obscure. Different tea cultivars have varied abilities to absorb the micronutrients from the soils (Mokgalaka *et al.,* 2004) in different locations. Thus, it is necessary to asses the levels of Se in the made tea in different parts of East Africa to ascertain agronomic practices that will give optimal levels of the micronutrients.

The sweetness and aroma of green tea extracts is increased as the stringent taste and bitterness is reduced by Se spraying on tea (Xu, 1996). The total amino acids and vitamin C contents of green tea decrease while the ratio of polyphenols to amino acids decrease following Se spraying (Hu *et al.,* 2001b). Foliar application of Se enriched fertilizer of sodium selenite or sodium selenate significantly increased Se and other nutrient content in green tea and such teas exhibited higher antioxidant activity (Juan *et al.,* 2003). This Se application slowed the reduction of tea's major components hence improved preservation qualities of green tea (Price *et al.,* 1998). The contents of tea polyphenols decrease by application of Se (Hu, *et al.,* 2003).Tea leaf contains high levels of polyphenols, dominated by (+)-catechins (C) **[1]**, (-)-Epicatechin (EC) **[2]**, (-)-Epicatechin-3-gallate (ECG) **[3]**, (-)-Epigallocatechin (ECG) **[4]**, (-)-Epigalocatechin-3-gallate (EGCG) **[5]** i.e. flavan-3-ols

(Balentine *et al.*, 1998). The structures (Robertson, 1983) of the catechins are summarized:





1

	R_1	R_2	R_3
2	H	H	H
3	H	gallate	H
4	H	H	OH
5	H	gallate	OH

Fig 2: The flavan-3-ols (catechins) in fresh tea leaves (Robertson, 1983)

Tea also contains phenolic acids mainly caffeine, quinic and gallic acids. It is also a source of methylxanthines primarily in the form of caffeine. Theanine is an amino acid found only in tea leaves. These components are preserved with Se enrichment as their reduction is slowed. Thus it is necessary to determine the distribution and levels of this nutrient in East African teas and how the levels are influenced by agronomic and cultural practices. This can lead to good agronomic practices encouraging the preservation of the major components and hence improving quality. Little attention has been paid to Se role on the growth and yield of plants. Recently it was demonstrated that Se protects plants like ryegrass (*Lolium perenne. L*) and lettuce (*Lactuca sativa. L.)* against UV induced oxidative stress and promotes the growth of plants subjected to high energy light (Hartikainen and Xue, 1999). The application of Se influences the yield and quality of green tea leaves (Hu *et al.*, 2001b). The number of sprouts and the yield were higher by application of Se (Juan *et al.*, 2003). Se at < $20 mgkg^{-1}$ enhanced the contents of total amino acids in spirulina platensis and endivia (*Cichorium endivia*) (Quiao and Shang, 2000; Lee and Park, 1998). Optimum Se increases the yield and quality of tea thus there is need to determine its levels and recommend agronomic practices that will optimize its levels in East African tea.

CHAPTER THREE

3.0: Methodology

3.1: Samples for assessment.

Made tea samples were collected from Venus Tea Brokers and Tea Brokers of East Africa located in Mombasa. Samples were from factories the two brokerage firms offer their teas to the auction. From a total of 42 gardens/markets, 200g of the PF1 grade replicated three times were obtained. From 8 of the gardens/markets, 200g each of grades BP1, PF1, PD and D1 grades of black tea were obtained each in triplicate.

3.2: Samples from the local market.

Tea samples from the local market were obtained from Tuskys, Nakummatt and Ukwala supermarkets in Kisumu, Kenya in triplicate for each brand. A total of 12 brands of tea comprising of sachet teas, instant teas and tea bags were assessed.

3.3. Preparation of infusions

The 12 brands of locally consumed tea and 12 randomly sampled black teas from the tea brokers were used in this experiment. The method of Lasheen *et al.*, (2008) was used in the preparation of black tea infusions. In this method, 100 ml of hot distilled water was added to 2 g of black tea particles. The mixture was left to infuse for 4 minutes and cool at room temperature and then filtered through a whatman filter paper number 40. The residues were oven dried at 105^0C, re-weighed and then subjected to analysis of the micronutrients by the Atomic Absorption Spectrophotometer. Analysis of each sample was repeated three times. The amount of micronutrients infused was calculated as difference between micronutrients in tea leaves before and after infusion.

3.4: Location, Nitrogen fertilizer rates and plucking intervals trials.

Trials were set on clone 6/8 plantations that are uniformly managed and with known past cultivation history planted at Changoi in lower Kericho, Timbilil Estate (Tea Research Foundation of Kenya) in upper Kericho, and Sotik Highlands (Arrocket) Tea Estate in Sotik whose altitude, latitude, longitude and year of plantation are given in Table 8.

Table 8: Site locality and history for clone 6/8 cultivar.

Site Locality/history	Changoi	TRFK Timbilil	Sotik Highlands (Arrocket)
Altitude (m above sea level)	1982	2198	1767
Latitude Longitude	0^0 31'S 35^0 16'E	0^0 22'S 35^0 21'E	0^0 36'S 35^0 4'E
Year planted	1989	1986	1974
Plantation age*	22	25	37

* As at year 2011.

Source: Individual/ estates records.

At each site, the experiment was set as a factorial two arrangement laid out in a randomised complete block design with five fertilizer rates (0, 75, 150, 225 and 300 kgN/ha/yr) and three plucking frequencies (7, 14 and 21 day rounds) and treatments replicated three times. Each effective plot comprised of 48 plants surrounded by a line of tea bushes that served as guard rows (Appendix 1). On the day the three plucking intervals coincided in each experiment, a mass of one kilogram of tea was harvested from each plot and taken for miniature black tea processing (Owuor and Reeves, 1986). Unsorted black tea samples were subjected to analysis of the essential trace elements.

3.5: Genotypes to different environments trials (GxE).

This experiment was incorporated on an ongoing GxE experiment. Twenty widely cultivated (commercial) genotypes of tea clones, TRFK 7/9, TRFK 303/259, TRFK 303/1199, TRFK 54/40, TRFK 31/8, BBK 35, TRFK 6/8, TRFK 31/27, TRFK 12/12, TRFK 303/909, APH S15/10, TRFK 57/15, TRFK 56/89, TRFK 12/19, TRFK 11/26, STC 5/3, TRFK 7/3, TRFK 303/577, EPK TN 14-3 and TRFK 2x1/4 planted in Kangaita Tea Farm East of the Great Rift Valley, Timbilil Estate in Kericho and Kipkebe Estate in Sotik, whose altitude, latitude, longitude and year of plantation are given in Table 9, were used in this trial. At each site, plots were arranged in a randomized complete block design with three replicates (Wachira *et al.,* 2002). The tea was planted in a 122cm by 61 cm rectangular spacing (Anon, 2002). Nitrogen inform of NPKS 25:5:5:5 compound fertilizer at a single dose of 120 kg/ha was applied during the year of plantation and 200 kg/ha/year in subsequent years. Plucking was done at 10-14 days intervals depending on leaf availability. The plants were under uniform management and agronomic practices. One kilogram of leaf was plucked from each plot and miniatured black tea processed (Owuor and Reeves, 1986). The unsorted black tea samples from these clones were also analyzed for the essential trace elements.

Table 9: Site locality and history for the different clones.

Site Locality/history	Kipkebe	TRFK Timbilil	Kangaita
Altitude (m)	1872	2178	2100
Latitude Longitude	$0^0 45'$S $35^0 5'$E	$0^0 22'$S $35^0 21'$E	$0^0 30'$S $37^0 16'$E
Year planted	1997	1986	1991
Plantation age*	14	25	20

* As at year 2011.

Source: Individual/ estate records.

3.6: Analysis of black tea for essential elements.

A modified standard procedure described in AOAC (2000) was followed for the preparation of samples for analysis of essential minerals. The made tea samples were weighed on a digital analytical balance (Mettler Toledo, Switzerland) with $\pm$ 0.0001g precision. Accurately weighed 1.0000g black tea for analyzing Mn, Fe, Zn and Cu while 2.0000g black tea for Se analysis were transferred into ashing tubes and kept in a muffle furnace for ashing at 460 ^oC for 12 hours. The ashed samples were digested using double acid (concentrated hydrochloric and nitric acids in a1:1 ratio) and hydrogen peroxide in the ratio of 2:3. Care was taken to ensure that all ash came into contact with the acid. All the chemicals (Aldrich chemicals) used were of analytical grade obtained through Kobian Chemists. Further the crucible containing acid solution was kept on a hot plate and evaporated to dryness. The final residue was dissolved in 0.05 M hydrochloric acid solution for extraction and made up to 25 mL for Mn, Fe, Zn and Cu analysis and to 10 mL for Se analysis. Working standard solutions were prepared by diluting the stock solution with 0.05M hydrochloric acid. The Mn, Fe, Zn, Cu and Se in made tea samples was analyzed using atomic absorption spectrophotometer (Shimadzu AA-6200 Model, Japan) under standard instrumental conditions (Table 10 and Appendix 2).

Table 10. Atomic Absorption flame emission Spectrophotometer (Shimadzu AA-6200) experimental parameters

Element	Mn	Se	Cu	Zn	Fe
Lamp current (mA)	10	23	10	6	8
Wavelength (nm)	279.5	196.0	324.8	213.9	243.3
Slit width (nm)	0.2	0.7	0.7	0.7	0.7
Mode	BGC-D_2	HVG-1	BGC-D_2	BGC-D_2	BGC-D_2
Flame Type	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2
Fuel flow (L/min)	2.0	1.8	2.0	2.0	1.8
Prespraytime	3 sec	3 sec	3 sec	3 sec	3 sec
Intergration time t	5 sec	5 sec	5 sec	5 sec	5 sec
Callibrations (ppm)	0.5-2.0	0.2-3.2	0.8-3.2	0.2-1.2	1.0-8.0
MDL	0.06ppm	0.20ppm	0.04ppm	0.011ppm	0.08ppm

Key: MDL – machine detection limit; BGC-D_2 – Deterium background correction (compensates for matrix interferences)

3.7: Statistical analysis

The data from the East African tea samples was subjected to statistical analysis to give the means, LSDs and standard deviations. The effect of grading on micronutrient content of the black teas was statistically analyzed using a two factor completely randomized design. Micronutrient levels in teas from the local market were analyzed in a completely randomized design with brands as the main factor while in the infusion data Microsoft Excel was used to calculate the means, standard deviation and percent infusions. Data from location, nitrogen fertilizer rates and plucking intervals trial were analyzed using a randomized complete block design in a 3x5x3 factorial design with location as main treatments, nitrogen NPKS 25:5:5:5 fertilizer rates as sub-treatments and harvesting intervals days as sub-sub treatments while the data from clonal trials were analyzed using a randomized complete block design in a 2-factorial arrangement, with sites as the main treatments and clones as sub-treatments. MSTAT-C statistical package (Michigan State University, MI) was used for ANOVA, while column charts and linear regression were performed using Ms-Excel statistical package.

CHAPTER FOUR

4.0 Results and Discussion.

4.1 Concentration of essential elements in black teas from East Africa.

The variation in the essential micronutrients with factory of production is presented in Table 11. (Appendix 3). Mn had the highest concentration, followed by Fe, Zn, Cu then Se. For each micronutrient, the ranges were large, demonstrating that large variations in factors affecting the distribution of these micronutrients in different regions. Generally the levels of selenium in the black tea were very low compared to other elements analyzed. The results show that East African black teas accumulate reasonable levels of beneficial micronutrients Mn, Fe, Zn, Cu and to a lesser extent Se.

Table 11. Concentrations (μg/g) of micronutrients in East African black tea (PF1 grade).

Statistical analysis*	Mn	Fe	Zn	Cu	Se
Min	341.00	81.67	33.67	2.33	0.27
Max	814.67	536.00	76.67	23.00	6.23
Mean	666.28	169.33	46.47	8.69	2.25
SD	121.44	129.71	13.46	4.62	1.28
Median	677.84	137.5	44.67	8.17	2.13

*No. of samples=42

The comparisons of micronutrient levels from East African teas to teas from other regions is given in Table 12. Ansari *et al.*, (2007) reported the range of Mn, Fe, Cu, and Zn to be 155.2-214.2 μg/g with mean of 182.9 μg/g, 17.2-194.0 μg/g with mean of 92.6 μg/g, 19.6-36.7 μg/g with mean of 29.3 μg/g and 34.1-47.4 μg/g with mean 40.3 μg/g respectively for Iranian black teas. For Indian teas (Kumar *et al.*, 2005) Mn and Cu levels were in the same range of 371-758 μg/g with mean 575$\pm$96 μg/g and 1.6-35.0 μg/g with mean 14.8$\pm$8.2 μg/g respectively as East African teas. The levels of micronutrients in East Africa are similar to the levels in Indian, Japan, Saudi Arabia and Turkey teas but the levels in Spain tea were very high while the levels of Iranian teas were relatively lower. These differences could be due to differences in agronomic inputs, cultivars or geographical area of production.

There was significant ($p \leq 0.001$) differences in concentration of the micronutrients in the 42 different factories in East Africa (Table 13 and appendix 3). These differences demonstrate that although the East African black teas can supply the micronutrients, the ability to supply changes from factory to factory or region to region. It is therefore important that in the use of tea to alleviate/reduce the hidden hunger problems, assessments of the teas are first done to establish the ability of individual source as adequate. Mn was the most abundant micronutrient in East African black tea similar to studies on tea from other regions like India (Kumar *et al.*, 2005), Iran (Ansari *et al.*, 2007) and Pakistan (Al-Oud, 2003). The levels of the micronutrients in Pakistan tea (Al-Oud, 2003) were however lower than the levels of the micronutrients in East African teas except for copper.

Table 12. Comparison of micronutrient levels (μg/g) in East African teas with those from other regions in the world.

	East African Tea[g]	Iran[a]	India[b]	Japan[c]	Spain[d]	Saudi Arabia[e]	Turkey[f]
Mn	666.3±121.67	182.9±123.40	575+112.78	503±7	1004.1	750.9±185.34	806.0±34.2
Fe	169.3±129.33	92.6±89.68	NA	134±48	946.2	250.5±199.23	NA
Zn	46.5±13.47	40.3±13.89	NA	36.6±0.7	43.2	65.7±31.30	140.9±9.1
Cu	8.7±4.67	29.3±6.37	14.8±3.43	27.7±0.7	31.5	18.1±6.94	24.8±1.4
Se	2.3±1.23	NA	NA	NA	NA	NA	NA

a- Ansari *et al.* (2007)

b- Kumar *et al.* (2005)

c- Matsuura *et al.* (2001)

d- Pedro *et al.* (2001)

e- Waqar and Mian, (2008)

f- Narin *et al.* (2004)

g- This study

NA- Not analysed

Table 13. Analyses of variances for the changes in micronutrients in tea from different factories in East Africa.

Micro-nutrient	K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Mn							
	2	Factor A	41	1816072.86	44294.46	436.92	0.0000
	-3	Error	82	8313.10			
		Total	125	1824622.86			
Coefficient of Variation (%)			1.51				
Fe							
	2	Factor A	41	2068996.71	50463.33	4180.26	0.0000
	-3	Error	82	989.889	12.072		
		Total	125	2070077.37			
Coefficient of Variation (%)			1.92				
Zn							
	2	Factor A	41	14767.968	360.194	44.0470	0.0000
	-3	Error	82	670.556	8.178		
		Total	125	15445.302			
Coefficient of Variation (%)			5.89				
Cu							
	2	Factor A	41	3234.381	78.887	39.5125	0.0000
	-3	Error	82	163.714	1.997		
		Total	125	3401.714			
Coefficient of Variation (%)			14.69				
Se							
	2	Factor A	41	199.322	4.862	227.2397	0.0000
	-3	Error	82	1.754	0.021		
		Total	125	201.455			
Coefficient of Variation (%)			6.50				

The large variations compared to teas from other regions (Ansari *et al.*, 2007; Kumar *et al.*, 2005) are attributed to different agro-climatic region and possible differences in the varieties of teas and agronomic inputs used. The variations of the micronutrients in black tea from different factories in East Africa in the present study might be due to the differences in agronomic, agro-climatic, soils, cultural factors and varieties. Indeed the nitrogenous fertilizer application rates (Bonheure and Willson, 1992), plucking standards (Owuor *et al.*, 1987a), plucking intervals (Owuor *et al.*, 1997, 2000) and cultivars (Wachira, 2002) have been demonstrated to cause large yield and/or quality differences.

4.2. Effect of grading on micronutrient content of black tea.

Grading is based on the size of the black tea particles which is determined by their ability to fall through mesh screens ranging from 8-30μm mesh size (Segal 1996) following drying process. Broken pekoe is a medium grade that consists of small leaf particles of leaves, the fannings is a lower grade and consists mainly smaller pieces while the pekoe dust is lower particle size grade and consists remnants that are created in sorting and crushing processes (Hartlers, 1973b) and when further refined it gives the dust which is the smallest particle size grade (Segal, 1996). Different grades of black tea from different factories showed significant ($p \leq 0.05$) differences in micronutrients content (Table 14 and appendix 4). All the micronutrients significantly ($p \leq 0.05$) varied with factories of production as noted in Table 13. Again Mn had the highest concentration while Se had the least concentration in all the studied black tea grades. The concentrations of Mn, Fe, Zn, Cu and Se followed the BP1>PF1>D1>PD pattern in all the grades but Zn was higher in PD grade than in D1 grade of black tea. The variation in the distribution of the micronutrients in various grades is not strange although the grades originated from same leaves. In a previous study similar variations had been shown in the black tea chemical quality parameters (Owuor *et al.*, 1987b). The difference in the chemical quality parameters (Owuor *et al.*, 1987b) and micronutrients concentrations in the black tea grades are attributed to the different parts of the leaf the grade originated. The East African black teas are CTC processed. Generally the grade distribution varies from factory to factory even when plucking standards and/or leaf age is the same. This is due the setting of the CTC machines. When the CTC rollers are set very tightly, there is usually more extensive leaf cell matrix destruction leading to more dust of finer particle sizes than when the CTC rollers are more loosely set. Thus the significant variations observed may be due to the differences in CTC roller settings during the black tea processing and differences caused by other growth factors. However, the extent of change in concentrations varied from factory to factory leading to significant ($p \leq 0.05$) interaction effect between grades and factory. It is therefore not possible to predict the pattern of distribution of the micronutrients in the different factories.

Table 14. Levels (µg/g) of micronutrients from different black tea grades from East Africa.

Element	Grade	Factory of production								Mean grade
		1	2	3	4	5	6	7	8	
Mn	BP1	696.67	691.33	694.33	672.33	718.67	734.00	710.67	715.33	704.17
	PF1	692.00	693.00	678.67	660.33	674.67	681.00	682.00	708.67	683.79
	D1	680.67	644.67	667.67	643.67	665.00	655.33	659.00	705.33	665.17
	PD	623.33	650	624.67	623.67	635.00	624.33	641.67	618.33	630.13
	Mean factory	673.17	669.75	666.33	650.00	673.33	673.67	673.33	686.92	
	C.V %					1.69				
	LSD(P≤0.05)					10.96				10.43
	Interactions					19.28				
Fe	BP1	170.00	119.67	91.33	163.67	101.00	85.33	124.67	78.00	116.71
	PF1	116.67	81.67	114.67	82.33	115.67	75.33	115.00	75.67	97.13
	D1	100.33	73.33	74.33	99.33	86.33	75.67	74.00	70.67	81.75
	PD	46.33	45.33	45.00	91.33	83.00	36.00	18.67	42.67	51.04
	Mean factory	108.33	80.00	81.33	109.17	96.50	68.08	83.08	66.75	
	C.V %					3.02				
	LSD(P≤0.05)					2.53				2.40
	Interactions					4.44				
Zn	BP1	41.00	45.67	33.00	44.00	42.67	46.33	42.33	46.00	42.63
	PF1	40.00	43.00	42.67	46.00	42.33	39.00	36.67	39.33	41.13
	D1	32.33	26.00	27.00	43.00	38.33	38.00	31.00	21.33	32.13
	PD	42.00	56.33	26.33	43.67	48.67	34.33	22.67	33.33	38.42
	Mean factory	38.83	42.75	32.25	44.17	43.00	39.42	33.17	35.00	
	C.V %					5.92				
	LSD(P≤0.05)					2.20				1.82
	Interactions					3.88				
Cu	BP1	15.00	17.00	19.67	17.33	16.67	20.33	17.33	13.67	17.13
	PF1	18.67	14.00	16.00	12.00	15.33	15.33	13.00	17.00	15.17
	D1	7.67	11.33	10.33	15.00	12.00	15.00	15.33	13.00	12.46
	PD	7.67	11.67	9.00	12.33	11.67	7.00	11.67	5.33	9.54
	Mean factory	12.25	13.50	13.75	14.17	13.92	14.42	14.33	12.25	
	C.V %					12.30				
	LSD(P≤0.05)					1.61				1.53
	Interactions					2.84				
Se	BP1	2.40	13.97	10.30	10.23	8.57	1.08	4.37	2.53	6.68
	PF1	3.11	3.93	3.63	4.17	3.43	4.23	3.97	4.53	3.88
	D1	3.43	2.40	1.40	4.43	2.43	3.67	1.50	2.67	2.74
	PD	3.20	0.50	2.43	2.37	3.10	1.63	1.43	3.13	2.22
	Mean factory	3.04	5.20	4.44	5.30	4.38	2.65	2.82	3.22	
	C.V %					14.47				
	LSD(P≤0.05)					0.54				0.52
	Interactions					0.95				

4.3: Levels of the micronutrients in teas from the local market.

The levels of the micronutrients in several brands of tea in the local market are presented in Table 15. (Appendix 5). Mn had the highest concentrations followed by Fe, Zn, Cu and Se respectively. The Mn, Fe and Zn levels were much higher than those observed from the factories (Table 15). It had been speculated that the quality of tea offered for the local market could be lower than those offered for export market. It is possible that the teas generally offered in local market are from coarser plucking standards where the third and mature leaves may be ending up for manufacture, leading to low black tea quality (Owuor *et al.*, 1987b) and high Zn and Fe contents. The observation suggests that the quality of teas offered in the local market could be different from those offered in the export market. On the other hand the instant teas had significantly ($P \leq 0.05$) lower levels of the micronutrients than the teas in sachets and tea bags which were comparable. Instant teas are made from concentrates of liquor infusions. The result demonstrates that a lot of micronutrients were left in the residues. Fahari ya Kenya tea had the highest concentration of Mn, Melvins Tangawizi tea had highest Fe concentration, Eden tea had the highest Zn concentration, Baraka Chai and Melvins Tangawizi tea had the highest Cu concentrations while Sasini tea had the highest Se concentration. Tea quality (Odhiambo *et al.*, 1988) and levels of tea leaf nutrients (Wanyoko and Njuguna, 1983) are known to vary with several agronomic practices. Generally, high Zn and Fe levels are associated with mature leaf (Wanyoko and Njuguna, 1983). The significant ($p \leq 0.05$) differences in the micronutrients imply that the ability of the brands to supply the micronutrients varies. It is therefore important to determine the levels of the nutrients in tea before using specific brands for alleviating the hidden hunger. However in all the brands, the levels of the micronutrients were high suggesting that irrespective of the brand, all the teas were sources of the micronutrients. Although considered low quality the teas offered in the local markets had higher ability to supply the micronutrients to human diets than teas offered for sale in export markets.

Table 15. Concentrations levels of micronutrients in brands of tea from the local market.

Tea Brands	Micronutrients				
	Mn	Fe	Zn	Cu	Se
Sachets					
Baraka chai	2784.67	290.00	41.33	21.00	3.20
Sasini tea	2844.00	332.00	34. 00	8. 00	3. 90
Fahari ya Kenya tea	3066.67	351.33	36.33	11. 00	3. 20
Aberdare tea	2463.33	273.67	37.33	10. 00	2. 30
All time Kenya tea	2557.33	310.00	44.33	8. 00	2.57
Melvins Tangawizi tea	2640.33	471.33	44.33	20. 00	2.30
Eden tea	2358.33	222.33	45.33	11.33	3. 40
Kericho Gold tea	2558.33	244.00	43.67	8.67	2.97
Instant teas					
Alitea instant tea	216.00	36.33	14.00	2.33	0.60
Finlays instant tea	165.67	27.00	12.00	2.67	0.37
Tea bags					
All time Kenya tea	2368.33	214.33	35. 00	5.00	2.57
Ketepa Pride	2658.67	342.00	45.67	3.67	2.43
C.V (%)	1.25	4.28	9.68	13.15	10.02
L.S.D ($P \leq 0.05$)	49.99	19.96	6.28	2. 20	0.45

4.4. Contribution of black tea to the requirements of micronutrients for humans

The black teas from East Africa had reasonable amounts of micronutrients (Table 11, 14, 15). Tea is consumed as liquor infusion. Thus unlike foods wholly consumed, critical levels of nutrients should be based on the amounts infused into the tea liquor. Despite the reasonable levels of micronutrients observed in Tables 11, 14 and 15, the amounts that are infused could be a lot less. The amounts of micronutrients infused in tea liquors are presented in Table 16. Some elements were well extracted into the infusion such as Mn, Cu and Zn while Fe and Se were only partly extracted (Table 16). Chen, (1990) reported complete extraction into the tea infusion of Br, K, good extraction of Cu, F, Ni, Zn, Cr, Mn, Mg, S and Co while Fe, Se, Pb and Ca could only be partly extracted. These findings are similar to the findings of this study. The micronutrients concentration in black tea was more than what goes into the infusion, as also observed by Lasheen *et al.* (2008). It is therefore not possible to predict from the total micronutrients in whole tea, the actual contribution of tea beverages to the alleviation of hidden hunger. However it can be concluded that black tea from local markets can be a major source of Mn to alleviate/reduce hidden hunger related to Mn deficiency. The contribution of tea to alleviating/reducing human health hidden hunger of the other micronutrients is very minimal. Indeed, Powell *et al.,* (1998) and Chen, (1990) demonstrated that tea is not a rich dietary source of essential metals for humans except manganese.

In the preparation of a cup of tea 2g of black tea particles or one tea bag is used which is approximately 2g (Lasheen *et al.*, 2008). Thus the extent of contribution of tea to partial alleviation of shortage of the micronutrients depends on the actual number of cups consumed per day (Table 16). Consumption of about two cups per day of the local black teas and at least four cups of the export teas will supply adequate Mn. Very many number of cups would be required to alleviate other micronutrient deficiencies. Tea is therefore not a suitable nutrient supply for these micronutrients and can only supplement other sources.

Table 16. Contribution of East African tea to the requirements micronutrients for humans.

Levels (μg/g) of one cup of local market teas (2g of black tea infused)					
ement	Mn	Fe	Zn	Cu	Se
nount in black tea	5222.16±180.32	602.16±64.80	82.84±4.67	26.58±8.08	6.30±1.63
nount in infused tea residue	3081.08±123.34	499.80±45.89	43.79±3.37	4.78±1.67	4.25±0.45
nount extracted during infusion	2141.08±46.56	102.36±12.54	48.05±4.33	21.80±0.21	2.05±0.13
mean Extraction	41.00±5.33	17.00±7.67	58.00±4.67	82.00±3.47	32.00±2.67
mparisons (% Extractions)					
Chen, (1990)	33-36	< 10	36-56	70-80	8-24
Lasheen *et al.*, (2008)	36	24	50	75	-
Powell *et al.*, (1998)	45.8	<0.02	0.44	0.91	-
nimum requirements (NAS, 1980)	2300	8000	8000	900	55
ntribution to hidden hunger (%)	87.00	1.20	0.60	2.40	3.73
mparison (% of required amount)					
Chen, (1990)	60-100	<1-1.6	1.3-4.0	10-30	1.0-8.0
Levels (μg/g) of one cup of Export market teas (2g of black tea infused)					
ount in black tea	1412.32±123.67	233.34±24.80	78.84±4.67	25.58±8.08	6.87±1.63
ount in infused tea residue	833.27±67.34	193.67±5.89	33.12±3.37	4.58±1.67	4.68±0.45
ount extracted during infusion	579.05±46.56	39.67±6.54	45.72±4.33	21.00±0.21	2.19±0.13
nean Extraction	41.00±5.33	17.00±7.67	58.00±4.67	82.00±3.47	32.00±2.67
ntribution to hidden hunger (%)	25.18	0.50	0.57	2.33	3.98

*No. of samples = 12 different brands of local teas and 12 samples from export market.

4.5. Effects of nitrogenous fertilizer rates, plucking intervals and location of production on the micronutrient levels of the black tea.

Nitrogenous fertilizer application rates and harvesting intervals (Owuor *et al.*, 1997, 2000, 2010b) are known to influence the chemical quality parameters of tea. Indeed high rates of nitrogenous fertilizer and long plucking intervals impair black tea quality. Similarly the chemical composition and hence quality of tea has been demonstrated to change with geographical area of production even when one cultivar was used (Owuor *et al.*, 2009, 2010a, 2010b). Using clone 6/8, the influence of these factors was evaluated on micronutrients content. Clone 6/8 used in this study is a popular cultivar grown widely in Kenya (Wachira, 2002) and East Africa in general for manufacture of black tea.

The changes in micronutrients levels in black tea due to location of production are presented in (Tables 17, 18 and appendix 6). All the five micronutrients significantly ($p \leq 0.05$) varied with locations of production, therefore it is not possible to make black teas with similar micronutrient contents in all the three locations. This can be attributed to the differences in the soil pH in the three sites as observed in the earlier studies (Kamau *et al*, 2008a). Some of these micronutrients like Fe, Zn and Cu are known to be more bioavailable in strongly acidic soils (Yemane *et al*, 2008), thus the levels of the micronutrients inversely followed that of pH (Kamau *et al.*, 2008b). Equally mature leaf Fe and Zn levels were high in locations with lower pH (Kamau *et al.*, 2005). The changes in the levels of micronutrients Mn, Fe, Zn, Cu and Se could be due to several factors including temperature (Tanton, 1982a), rainfall and rainfall distribution (Othieno *et al*, 1992), altitude (Obaga *et al*, 1989; Squire *et al*, 1993) and sporadic hail damage experienced in the tea growing locations (Ng'etich *et al*, 2001a; Ng'etich & Stephens, 2001b; Othieno *et al*, 1992) observed in different locations in Kenya. Although these factors were not monitored in the present study, the extents of their variations may be large at the various geographical locations. Equally past crop husbandry and management may also play a bigger role in micronutrient variations in the different geographical locations.

The changes in micronutrient levels in black tea due to application of nitrogenous fertilizer rates are presented in (Tables 17, 19 and appendix 6). Mn levels did not significantly ($p \leq 0.05$) vary with an increase in nitrogenous fertilizer rates. These results were similar to the report by Kamau *et al.* (2005) on the first mature leaf Mn levels where there was no significant ($p \leq 0.05$) difference by increasing nitrogenous fertilizer rates. This was attributed to the high acidic nature of the tea soils which make Mn to be in excess and thus luxurious uptake may be expected ending up with no specific pattern (Kamau *et al.*, 2005).

Table 17: Effects of location of production and nitrogen fertilizer rates on micronutrients.

Micronutrient	Location	N-Rates (kg N/ha/year) 0	75	150	225	300	Mean locations
Mn (µg/g)	Timbilil	652.33	685.89	654.78	660.56	678.11	666.33
	Sotik Highlands	557.56	554.78	586.11	576.56	572.22	569.44
	Changoi	662.22	648.56	655.89	631.44	655.11	650.64
	Mean N-Rate	624.04	629.74	632.26	622.85	635.14	-
	C.V (%)			5.12			
	L.S.D (P≤0.05)			NS			29.22
Fe (µg/g)	Timbilil	110.33	121.89	121.78	148.22	128.44	126.13
	Sotik Highlands	69.89	75.22	71.89	75.33	83.56	75.17
	Changoi	60.67	71.56	70.22	69.67	70.11	68.44
	Mean N-Rate	80.3	89.56	87.96	97.74	94.04	-
	C.V (%)			16.10			
	L.S.D (P≤0.05)			10.93			13.13
	Interactions (SxN)			15.73			
Zn (µg/g)	Timbilil	18.11	25.11	29.00	31.11	29.89	26.64
	Sotik Highlands	19.44	20.78	21.00	22.78	29.22	22.64
	Changoi	20.33	22.22	22.44	24.89	25.22	23.02
	Mean N-Rate	19.30	22.70	24.15	26.26	28.11	-
	C.V (%)			20.60			
	L.S.D (P≤0.05)			3.75			3.51
	Interactions (SxN)			5.40			
Cu (µg/g)	Timbilil	23.67	22.78	21.11	17.44	12.67	19.53
	Sotik Highlands	9.00	7.22	4.56	3.67	3.11	5.51
	Changoi	19.00	15.22	13.33	13.67	14.56	15.16
	Mean N-Rate	17.22	15.07	12.96	11.70	10.11	-
	C.V (%)			24.46			
	L.S.D (P≤0.05)			2.78			2.97
	Interactions (SxN)			3.56			
Se (µg/g)	Timbilil	1.58	1.91	1.56	1.93	1.62	1.72
	Sotik Highlands	1.48	1.46	1.66	1.56	1.60	1.55
	Changoi	2.50	2.03	2.49	1.96	2.28	2.25
	Mean N-Rate	1.86	1.80	1.90	1.82	1.83	-
	C.V (%)			23.60			
	L.S.D (P≤0.05)			NS			0.39

Mn levels are high in strongly acidic soils and that the nutrient can be accumulated highly in the leaves to an extend that application of fertilizer or manure might not affect the levels (Ishibashi *et al*,. 2004). Irrespective of location of production, nitrogenous fertilizer rates does not significantly (p≤0.05) affect the Mn content of black teas. Thus for optimal Mn in black tea for alleviation of hidden hunger nitrogenous fertilizer rates do not dictate the levels of this micronutrient in black teas.

Fe levels significantly ($p \leq 0.05$) increased with the increase in nitrogenous fertilizer rates in the three locations (Tables 17, 19 and appendix 6). Increasing the rates of nitrogenous fertilizer is known to reduce the soil pH (Kamau *et al.*, 2008b) and thus increases the bioavailability of this nutrient. Increasing the rates of nitrogenous fertilizer generally increase soil acidity (Owuor *et al.*, 1990) and this may cause variations in the metal content of teas. The increase in Fe concentrations by increasing the rates of nitrogenous fertilizer is demonstrated in Figure 3 where the increase had a positive gradient with high R^2 values indicating the magnitude of increase was high.

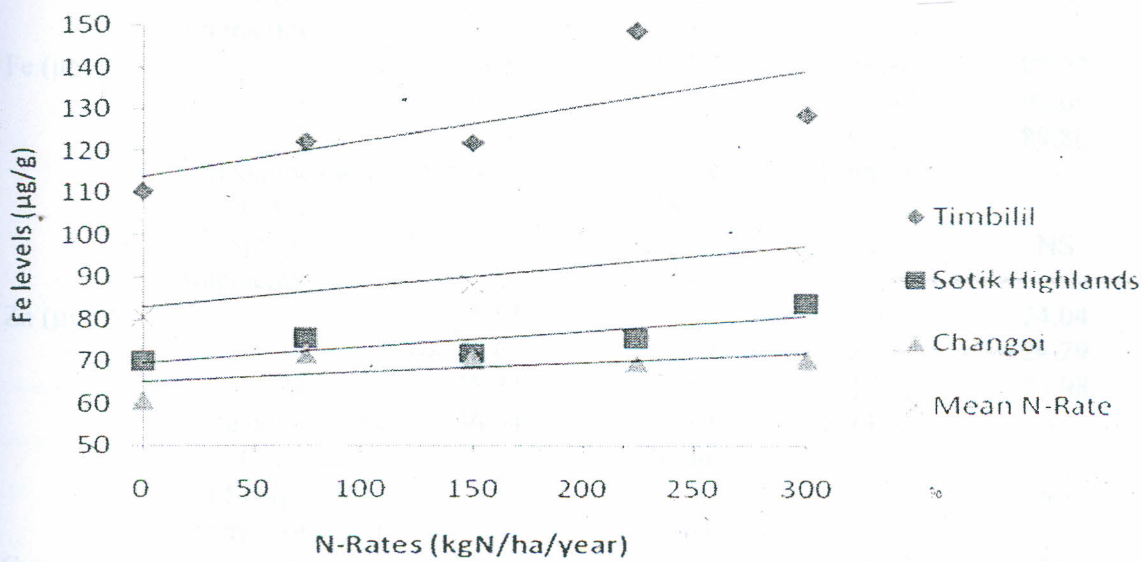


Fig 3. Effects of N-rates on iron levels in the unsorted black teas from different locations.

$Fe_{(Timbilil)} = 0.163x + 107.6$ ($R^2 = 0.916$)

$Fe_{(Changoi)} = 0.049x + 63.04$ ($R^2 = 0.816$)

$Fe_{(Sotik\ Highlands)} = 0.036x + 69.68$ ($R^2 = 0.890$)

$Fe_{(Mean\ N\text{-}Rates)} = 0.074x + 80.78$ ($R^2 = 0.919$)

Thus for increasing the Fe content in black tea, high levels of nitrogenous fertilizer should be used. This is also same with yields where they increase with increased fertilizer application but not exceeding 250 kg N/ha/year (Owuor *et al.*, 2009, 2010a, 2010b). Similar yield responses to rate of nitrogen had been widely recorded for trials conducted on single locations (Bonheure & Wilson, 1992; Kamau, 2008; Kamau *et al.*, 1998; Owuor *et al*, 1997, 2000, 2008; Ranganathan & Natesan, 1987). On the other hand, irrespective of geographical area of production, high rates of nitrogenous fertilizer is deleterious to quality (Owuor *et al.*, 2009, 2010a, 2010b) hence levels of nitrogenous fertilizer rates should be used that is a compromise between yield, quality and micronutrient content of the black teas.

Table 18. Effects of plucking intervals and location of production on micronutrients

		Locations			
Micronutrient	P-Freq (days)	Timbilil	Sotik Highlands	Changoi	Mean P-Freq
Mn (μg/g)	7	658.20	542.00	668.53	622.91
	14	672.80	574.33	641.53	629.55
	21	668.00	592.00	641.87	633.96
	Mean locations	666.33	569.44	650.64	-
	C.V (%)		5.12		
	LSD ($p \leq 0.05$)		29.22		NS
	Interactions (SxP)		32.65		
Fe (μg/g)	7	123.80	75.20	68.80	89.27
	14	129.13	74.47	68.47	90.69
	21	125.47	75.87	68.06	89.80
	Mean locations	126.13	75.18	68.44	-
	C.V (%)		16.10		
	LSD ($p \leq 0.05$)		13.13		NS
	Interactions (SxP)		14.67		
Zn (μg/g)	7	25.53	21.20	25.4	24.04
	14	28.67	22.20	22.00	24.29
	21	25.73	24.53	21.67	23.98
	Mean locations	26.64	22.64	23.02	-
	C.V (%)		20.60		
	LSD ($p \leq 0.05$)		3.51		NS
	Interactions (SxP)		5.03		
Cu (μg/g)	7	18.93	4.73	15.13	12.93
	14	20.53	5.40	17.27	14.40
	21	19.13	6.40	13.06	12.87
	Mean locations	19.53	5.51	15.16	-
	C.V (%)		24.46		
	LSD ($p \leq 0.05$)		2.97		NS
	Interactions (SxP)		3.32		
Se (μg/g)	7	1.51	1.64	2.11	1.76
	14	1.92	1.30	2.36	1.86
	21	1.73	1.71	2.28	1.91
	Mean locations	1.72	1.55	2.25	-
	C.V (%)		23.60		
	LSD ($p \leq 0.05$)		0.39		NS
	Interactions (SxP)		0.44		

Zn levels significantly ($p \leq 0.05$) increased with the increase in nitrogenous fertilizer rates (Table 17, 19 and appendix 6). This was also attributed to the increased acidity of the soils when nitrogenous fertilizers rates are increased (Kamau *et al.*, 2008b) which makes extractable Zn to be more bioavailable (Lasat *et al.*, 1996). This is elaborated in Figure 4

...h has positive gradient with very high R^2 values. Just like the micronutrient Fe, a higher ... of nitrogenous fertilizer maximizes Zn content in black teas.

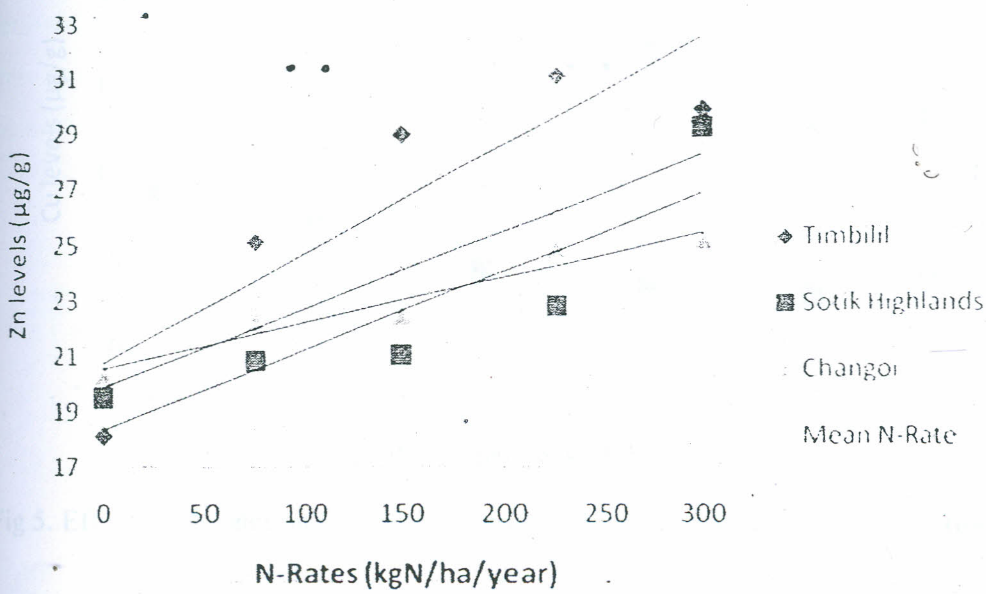


Fig 4. Effects of N-rates on zinc levels in the unsorted black teas from different locations.

$Zn_{(Timbilil)} = 0.052x + 19.73$ ($R^2 = 0.856$)

$Zn_{(Changoi)} = 0.016x + 20.53$ ($R^2 = 0.936$)

$Zn_{(Sotik\ Highlands)} = 0.028x + 18.33$ ($R^2 = 0.878$)

$Zn_{(Mean\ N\text{-}Rates)} = 0.028x + 19.86$ ($R^2 = 0.980$)

Cu levels in black tea significantly ($p \leq 0.05$) reduced with increased rates of nitrogenous fertilizer (Table 17, 19 and appendix 6). High concentrations of Zn causes significant reduction in the rate of Cu transport (Reeves *et al.*, 1996). Thus the rate and kinetics of Cu transport in any cell is affected by concentration of Zn in which the mechanism involves an inhibition of Cu efflux from the cell (Reeves *et al.*, 1996). In the current study the levels of Cu decreased with high rates of nitrogenous fertilizer rates possibly due to increase in soil acidity and rise in Zn levels which might have inhibited Cu absorption by the tea plants. This decrease is illustrated in Figure 5 where there are negative gradients with high R^2 values indicating there is a strong decrease in the Cu levels with increased nitrogenous fertilizer rates. High levels of Cu can be achieved by minimizing the rates of nitrogenous fertilizer but this will reduce the concentrations of Zn and Fe as observed earlier thus fertilizer application should be that which is a compromise between the micronutrient levels in the black teas.

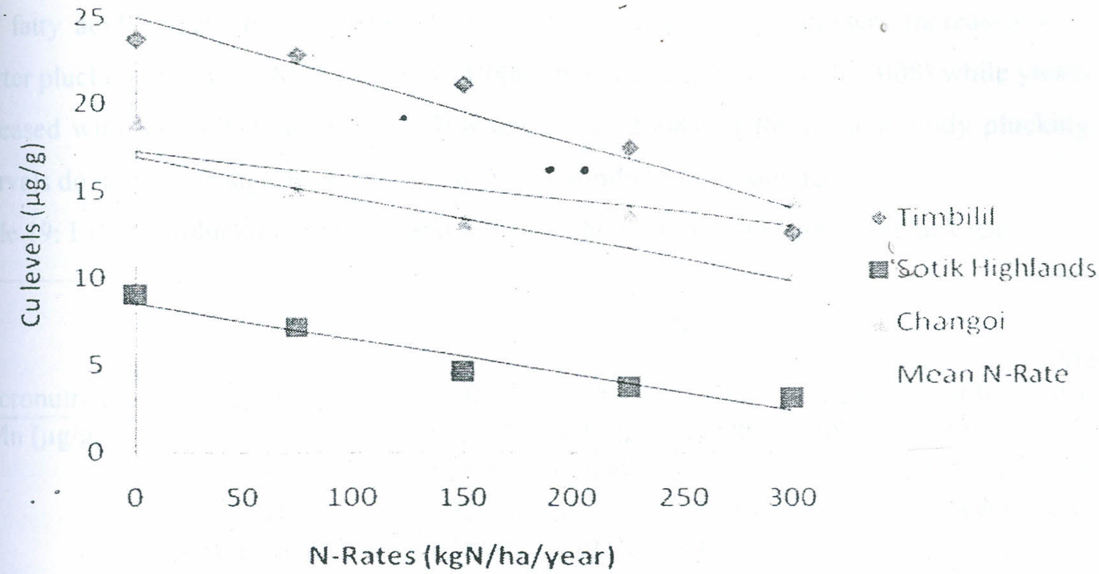


Fig 5. Effects of N-rates on Cu levels in unsorted black teas from different locations.

$Cu_{(Timbilil)} = -0.036x + 25.00$ ($R^2 = 0.915$)

$Cu_{(Changoi)} = -0.021x + 17.84$ ($R^2 = 0.718$)

$Cu_{(Sotik\ Highlands)} = -0.020x + 8.578$ ($R^2 = 0.934$)

$Cu_{(Mean\ N\text{-}Rates)} = -0.014x + 15.58$ ($R^2 = 0.988$)

The Se levels in the black tea do not follow any order meaning that nitrogenous fertilizer rates have no effect on these levels. Se levels were not significantly ($p \leq 0.05$) affected by increasing rates of nitrogenous fertilizer. (Table 17, 19 and appendix 6). To increase Se concentrations in tea then Se-enriched fertilizer like fertilizer of sodium selenite or sodium selenate is to be used (Xu *et al.*, 2003). The Se levels were also very low compared to other micronutrients. It is possible Se levels in the soils are very low or the plant absorbs very little Se.

The changes in micronutrient levels in black tea due to plucking intervals are presented in (Tables 18, 19 and appendix 6). Plucking intervals did not significantly ($p \leq 0.05$) affect the micronutrient content of the black teas (Tables 18, 19 and appendix 6). This implies that it is possible to make black teas with similar micronutrient content with varied plucking intervals. These results are similar to the yield response to plucking intervals which did not vary significantly with plucking intervals (Jondiko, 2010). Contradicting reports on yield responses to plucking intervals have been reported for example in Malawi yields decreased with increased plucking rounds (Palmer-Jones, 1977; Tanton, 1982b), but increased in Kenya with short plucking intervals (Owuor and Odhiambo, 1994). An increase in plucking intervals reduced black tea quality (Owuor *et al.*, 2009, 2010a, 2010b) partly due to increase in green leaf fatty acid content (Okal, 2011). In recent studies quality parameters increased with shorter plucking intervals (Mahanta *et al.*, 1988; Owuor *et al.*, 1997, 2000, 2008) while yields increased with long plucking intervals (Owuor *et al.*, 2008). In the present study plucking intervals do not have a significant effect on the micronutrient of black teas.

Table 19: Effect of plucking intervals and fertilizer rates on micronutrients in black tea

Micronutrient	P-Freq	N-Rates 0	75	150	225	300	Mean P-Freq
Mn (µg/g)	7	623.11	629.22	630.89	608.67	622.67	622.91
	14	626.44	636.44	632.44	613.33	639.11	629.56
	21	622.56	623.56	633.44	646.56	643.67	633.96
	Mean N-rate	624.04	629.74	632.26	622.85	635.14	-
	CV (%)			5.12			
	LSD (P≤0.05)			NS			NS
Fe (µg/g)	7	80.56	89.33	85.44	96.56	94.44	89.27
	14	82.44	86.78	90.33	102.00	91.89	90.69
	21	77.89	92.56	88.11	94.67	95.78	89.80
	Mean N-rate	80.30	89.56	87.96	97.74	94.04	-
	CV (%)			16.10			
	LSD (P≤0.05)			10.93			NS
Zn (µg/g)	7	19.56	19.56	24.89	26.22	30.00	24.04
	14	20.33	23.78	23.56	26.56	27.22	24.29
	21	18.00	24.78	24.00	26.00	27.11	23.98
	Mean N-rate	19.30	22.70	24.15	26.26	28.11	-
	CV (%)			20.60			
	LSD (P≤0.05)			3.75			NS
Cu (µg/g)	7	14.33	12.67	14.44	13.11	10.11	12.93
	14	17.33	17.89	12.89	13.89	10.00	14.40
	21	13.78	13.00	13.33	14.00	10.22	12.87
	Mean N-rate	15.15	14.52	13.56	13.67	10.11	-
	CV (%)			24.46			
	LSD (P≤0.05)			2.78			NS
Se (µg/g)	7	1.87	1.78	1.74	1.64	1.74	1.76
	14	1.76	1.72	1.93	2.00	1.88	1.86
	21	1.93	1.90	2.02	1.80	1.88	1.91
	Mean N-rate	1.86	1.80	1.90	1.82	1.83	-
	CV (%)			23.60			
	LSD (P≤0.05)			NS			NS

There were no significant ($p \leq 0.05$) interactions between location of production and nitrogenous fertilizer application for the micronutrients Mn and Se (Table 17) meaning that the response patterns for these micronutrients occurred in a similar manner. For the micronutrients Fe, Zn and Cu, there were significant ($p \leq 0.05$) interactions between location of production and nitrogenous fertilizer application rates (Table 17) meaning that the response patterns were different at each region. This is also evident from Figures 3, 4 and 5 where the lines showing the micronutrient response to nitrogenous fertilizer rates do not cross each other. There were significant ($p \leq 0.05$) interactions between location of production and plucking intervals for all the micronutrients (Table 18) indicating that the response patterns were different at each site. On the other hand, there were no significant ($p \leq 0.05$) interactions between nitrogenous fertilizer application rates and plucking intervals for all the micronutrients (Table 19) meaning that the responses were not related to the treatments.

4.6. Effect of genotypes in different environments on micronutrient content of black tea.

The clones used here were commercial clones, most of which were initially selected at the Timbilil site. Their selections were based on yield; not micronutrient content. The effect of location of production and cultivars on Mn content of black teas is presented in Table 20. (Appendix 7). The concentration levels of the different clones significantly ($p \leq 0.05$) varied from clone to clone and did not follow any specific pattern when the clones are planted in different geographic locations. This indicates that different clones have different abilities to absorb Mn from the soils. Clone TRFK 11/26 recorded highest mean Mn levels while clone TRFK 303/1199 recorded the lowest levels. The highest levels of Mn in Timbilil, Kipkebe and Kangaita were recorded in clones TRFK 7/9, TRFK 31/27, EPK TN14-3 respectively. But clones TRFK 7/3, STC 5/3 and BBK 35 had the lowest concentrations of Mn in Timbilil, Kipkebe and Kangaita respectively. There were significant ($p \leq 0.05$) differences in mean levels of Mn in the three sites indicating that the clones have different abilities to absorb micronutrients in different locations. The highest mean Mn levels were recorded in Kipkebe while the lowest levels were recorded in Timbilil. There was a significant interaction between the clones and different locations meaning that the responses did not occur in the same pattern. Thus to effectively address the human health hidden hunger problem the clones with highest ability to extract Mn at specific locations should be adopted for these locations.

Table 20: Clonal black tea Mn levels (μg/g) and relative ranking based on Mn levels to growing environments.

Clone	Mn concentrations (μg/g)				Ranking			
	Site			Mean clones	Site			Mean clones
	Timbilil	Kipkebe	Kangaita		Timbilil	Kipkebe	Kangaita	
TRFK 7/9	1466.67	1777.33	720.67	1321.55	1	3	9	2
TRFK 303/259	832. 00	1264. 00	618.33	904.78	16	17	15	19
TRFK 303/1199	550.67	1291.67	731.33	857.89	20	15	7	20
TRFK 54/40	916.67	1438. 00	636. 00	996.89	13	13	14	15
TRFK 31/8	705.33	1534.33	636.67	958.78	18	12	13	18
BBK 35	938. 00	1575.67	483.33	999. 00	12	10	20	14
TRFK 6/8	912. 00	1428.33	660. 00	1000.11	14	14	10	13
TRFK 31/27	760.67	2073.33	530.33	1121.44	17	2	18	7
TRFK 12/12	1123. 00	1261.33	659.33	1014.56	9	18	11	12
TRFK 303/999	1172. 00	1565.33	729.33	1155.56	6	11	8	6
APH S15/10	847.33	1764. 00	745. 00	1118.78	15	4	5	9
TRFK 57/15	1464. 00	1696. 00	653.67	1271.22	2	8	12	3
TRFK 56/89	1164.67	1757.33	568.67	1163.56	7	6	16	5
TRFK 12/19	1107.33	1722.67	528.67	1119.56	10	7	19	8
TRFK 11/26	1446. 00	2356. 00	823. 00	1541.67	3	1	1	1
STC 5/3	1296.67	925. 00	734.67	985.44	4	20	6	17
TRFK 7/3	673.33	1760. 00	544.67	992.78	19	5	17	16
TRFK 303/577	1241.67	1632.67	761. 00	1211.78	3	9	4	4
EPK TN14-3	1156. 00	1244. 00	764.67	1054.89	8	19	3	10
TRFK 2x1/4	1093.67	1269. 00	796. 00	1052.89	11	16	2	11
Mean site	1043.38	1566.82	666.27					
C.V (%)		4.36						
LSD ($P \leq 0.05$)		37.44		47.02				
Interactions		79.46						

Significant ($p \leq 0.05$) differences were observed in Fe levels due to location of production and clones (Table 21). Clone TRFK 6/8 had significantly ($p \leq 0.05$) higher mean levels of Fe than other clones while clone BBK 35 had the lowest levels. The mean levels of Fe varied significantly ($p \leq 0.05$) from clone to clone meaning that the clones have varied abilities to absorb Fe from the soil. There were significant ($p \leq 0.05$) differences in the mean Fe levels in the three locations indicating that the clones have varied abilities to absorb Fe when planted in different locations. In Kipkebe clones TRFK 7/9, TRFK 303/1199, TRFK 54/40 recorded the highest Fe levels while clone TRFK 31/27 had highest Fe levels in Kangaita. Clone

BB35, TRFK 57/15 and TRFK 7/9 had the lowest Fe concentrations in Timbilil, Kipkebe and Kangaita respectively. The results indicate that clones in Kipkebe tea farm had the highest mean Fe levels while Timbilil recorded the lowest levels. There were significant ($p \leq 0.05$) interaction effects between the clones and geographical area of production meaning the responses occurred in different patterns. Thus the use of cultivars that extract high levels of Fe will assist in increasing the Fe content of the resultant black teas.

Table 21: Clonal black tea Fe levels (μg/g) and relative ranking based on Fe levels to growing environments.

Clone	Fe concentrations (μg/g)				Ranking			
	Site			Mean clone	Site			Mean clone
	Timbilil	Kipkebe	Kangaita		Timbilil	Kipkebe	Kangaita	
TRFK 7/9	53.67	77.33	44.67	58.56	8	2	20	13
TRFK 303/259	54.67	69.67	51.67	58.67	5	10	17	11
TRFK 303/1199	53.67	74.33	85.00	71.00	8	4	1	3
TRFK 54/40	57.33	74.00	64.00	65.11	5	5	10	4
TRFK 31/8	44.00	72.00	55.33	57.11	19	6	15	18
BK 35	37.00	63.00	67.33	55.78	20	18	3	20
RFK 6/8	96.67	71.00	64.33	77.33	1	7	7	1
RFK 31/27	73.33	70.00	76.33	73.22	2	9	2	2
RFK 12/12	54.67	68.00	65.00	62.56	5	14	5	5
RFK 303/999	51.33	68.67	53.67	57.89	11	12	16	16
PH S15/10	57.67	70.67	45.67	58.00	4	8	18	14
RFK 57/15	46.33	60.00	66.67	57.67	17	20	4	17
RFK 56/89	52.33	77.00	56.00	61.78	10	3	14	6
RFK 12/19	54.33	69.33	59.00	60.89	7	11	13	8
RFK 11/26	48.00	87.00	45.33	60.11	15	1	19	9
TC 5/3	49.67	68.33	65.00	61.00	12	13	15	7
RFK 7/3	49.67	64.67	64.33	59.56	12	15	7	10
RFK 303/577	45.67	61.00	64.33	57.00	18	19	7	16
PK TN14-3	47.33	63.33	63.33	58.00	16	17	11	14
RFK 2x1/4	48.67	64.00	63.33	58.67	14	16	11	11
ean site	53.80	69.67	61.02					
V (%)			8.85					
D ($P \leq 0.05$)			4.27	5.37				
eractions			9.07					

The concentration levels of Zn significantly ($p \leq 0.05$) varied due to different clones planted in different locations (Table 22). This also implies that it is not possible to produce black teas with similar Zn content from different cultivars planted in a single location or even in different locations. The mean Zn levels significantly ($p \leq 0.05$) varied among the clones significant ($p \leq 0.05$) differences in mean levels of Zn in the different sites indicating that the clones have varied abilities to absorb Zn when subjected to different locations. Higher levels of Zn can be achieved by planting clones TRFK 57/15, TRFK 54/40 and TRFK 303/259 in Timbilil, Kipkebe and Kangaita respectively since there black teas had significantly ($P \leq 0.05$) higher levels of Zn. Clones STC 5/3 and TRFK 2x1/4 recorded significantly ($P \leq 0.05$) lower levels of Zn in Timbilil. In Kipkebe lower levels of Zn were from clones TRFK 7/9, TRFK 56/89, APH S15/10, TRFK 303/999 and STC 5/3. There were significant ($p \leq 0.05$) interactions between location of production and the cultivars meaning the clones absorb Zn differently in the single location and do not follow the same pattern when planted in other locations. The low levels of Zn in the black teas may not optimally aid in alleviation of hidden hunger problems but clones that extract more Zn from the soils need to be planted.

Table 22: Clonal black tea Zn levels (µg/g) and relative ranking based on Zn levels to growing environments.

Clone	Zn concentrations (µg/g)				Ranking			
	Site			Mean clone	Site			Mean clone
	Timbilil	Kipkebe	Kangaita		Timbilil	Kipkebe	Kangaita	
TRFK 7/9	27.67	23.67	18.33	23.22	18	19	14	18
TRFK 303/259	59.00	43.67	28.67	43.78	2	2	2	1
TRFK 303/1199	49.00	35.33	30.33	38.22	6	4	1	3
TRFK 54/40	49.00	47.67	24.00	40.22	6	1	4	2
TRFK 31/8	48.00	25.00	23.67	32.22	8	14	9	11
BBK 35	43.67	35.67	24.33	34.56	12	3	5	7
TRFK 6/8	32.00	24.00	21.33	25.78	17	17	11	16
TRFK 31/27	52.67	34.67	24.33	37.22	4	5	5	5
TRFK 12/12	33.67	33.00	23.33	30.00	16	7	10	13
TRFK 303/999	42.00	24.33	21.33	29.22	13	16	11	14
APH S15/10	57.00	24.67	24.00	35.22	3	15	7	6
TRFK 57/15	63.33	32.00	18.00	37.78	1	8	15	4
TRFK 56/89	37.00	23.67	16.33	25.67	15	19	16	17
TRFK 12/19	46.33	25.33	25.33	32.33	10	13	3	10
TRFK 11/26	39.67	25.67	15.67	27.00	14	11	18	15
STC 5/3	26.33	24.00	13.67	21.33	20	17	20	20
TRFK 7/3	46.67	33.33	14.67	31.56	9	6	19	12
TRFK 303/577	44.33	31.33	24.00	33.22	11	9	7	8
EPK TN14-3	52.67	25.67	21.33	33.22	4	11	11	8
TRFK 2x1/4	26.67	26.33	16.00	23.00	19	10	17	19
Mean site	43.83	29.95	21.43					
C.V (%)			10.61					
LSD ($P \leq 0.05$)			2.65	3.32				
Interactions			5.62					

The effect of region of production and cultivars on Cu content of black teas is presented in (Table 23). There were significant ($p \leq 0.05$) variations in the Cu levels among the clones. This indicates that the clones have varied abilities to absorb Cu from the tea soils. Clone TRFK 56/89 recorded the highest mean Cu levels among the studied clones while clone EPK TN14-3 recorded the lowest levels. The mean Cu levels significantly ($p \leq 0.05$) differed in the three locations with Timbilil recording significantly ($p \leq 0.05$) higher levels of Cu while Kangaita tea farm recorded the lowest levels. In Timbilil, clones TRFK 6/8 and TRFK 303/259 had higher Cu levels while EPK TN14-3 had the lowest levels. Clones TRFK 56/89 and EPK TN14-3 had the highest and lowest Cu levels respectively in Kipkebe. In Kangaita, clones TRFK 303/1199, TRFK 31/8 and TRFK 12/99 had significantly ($p \leq 0.05$) higher levels of Cu while lower levels were recorded from clones TRFK 6/8 and TRFK 303/999. There were significant ($p \leq 0.05$) interactions between location of production and clones indicating that the clones behaved differently in the locations of production and that the response patterns were not similar. In addressing hidden hunger tea contributes very minimal amounts of the essential micronutrient Cu to the human body but this levels can be increased by the use of recommended clones that can maximally absorb this nutrient from the soil. Thus it is necessary to determine the source and genotype of the teas that could increase the Cu content in the resultant black teas.

The changes in black tea Se levels due to location of production and clones are presented in (Table 24). The clones showed significant ($p \leq 0.05$) variations in black tea Se levels. This implies that the clones have varied abilities to absorb Se. This was confirmed by the fact that clone TRFK 303/999 had significantly ($p \leq 0.05$) higher mean levels of Se than the other clones while clone TRFK 12/19 recorded the lowest levels. Such variations significantly ($p \leq 0.05$) differed at different locations indicating that the clones have varied abilities to absorb Se when planted in different locations. Clones TRFK 2x1/4, APH S15/10 and TRFK 54/40 had significantly ($p \leq 0.05$) higher concentration levels of Se in Timbilil, Kipkebe and Kangaita respectively. Lower levels were recorded from clones TRFK 12/19, TRFK 2x1/4 and TRFK 7/9 in Timbilil, Kipkebe and Kangaita respectively. These differences are expected since Se is known to be naturally available in the soils for absorption by the tea plants (Ip, 1998) and the different cultivars have varied abilities to absorb this nutrient even when under similar agronomic practices in different locations. There was also significant ($p \leq 0.05$) interaction effect between the cultivars and location of production indicating that the response pattern of the clones was different in the different locations. Thus for increasing the Se content in black teas, cultivars with a high ability to extract Se should be adopted.

response pattern of the clones was different in the different locations. Thus for increasing the Se content in black teas, cultivars with a high ability to extract Se should be adopted.

Table 23: Clonal black tea Cu levels (µg/g) and relative ranking based on Cu levels to growing environments.

Clone	Cu concentrations (µg/g)				Ranking			
	Site			Mean clone	Site			Mean clone
	Timbilil	Kipkebe	Kangaita		Timbilil	Kipkebe	Kangaita	
TRFK 7/9	11.67	11.00	11.00	11.22	14	19	4	12
TRFK 303/259	14.67	15.67	11.00	13.78	5	2	4	3
TRFK 303/1199	13.00	15.00	12.33	13.44	9	6	2	5
TRFK 54/40	13.67	15.33	11.00	13.33	7	3	4	6
TRFK 31/8	12.67	14.67	12.67	13.33	12	7	1	6
BBK 35	15.33	13.67	11.00	13.33	3	10	4	6
TRFK 6/8	14.67	11.33	7.33	11.11	5	18	18	15
TRFK 31/27	13.67	13.67	9.00	12.11	7	10	10	9
TRFK 12/12	9.67	11.67	8.00	9.78	19	16	15	18
TRFK 303/999	13.00	13.00	7.67	11.22	9	13	17	12
APH S15/10	15.00	15.33	11.00	13.78	4	3	4	3
TRFK 57/15	11.00	14.00	8.67	11.22	18	9	13	12
TRFK 56/89	17.67	16.00	9.00	14.22	1	1	10	1
TRFK 12/19	16.33	13.33	12.33	14.00	2	12	2	2
TRFK 11/26	12.00	15.33	8.00	11.78	13	3	15	10
STC 5/3	11.33	13.00	10.00	11.44	17	13	9	11
TRFK 7/3	12.00	14.33	7.00	11.11	13	8	19	15
TRFK 303/577	11.67	12.00	8.67	10.78	14	15	13	17
EPK TN14-3	7.67	10.67	9.00	9.11	20	20	10	20
TRFK 2x1/4	11.67	11.67	6.00	9.78	14	16	20	18
Mean site	12.92	13.53	9.53					
C.V (%)			12.54					
LSD (P≤0.05)			1.18	1.48				
Interactions			2.51					

The results presented herein demonstrate that different clones have varied abilities to absorb micronutrients in single locations and same clone has different ability to absorb the micronutrients in different locations. The genetic makeup of the teas used in these study was not indicated and the noted differences could be in part due to genotypes thus to effectively address hidden hunger then region specific clones should be adopted that can optimally absorb the micronutrients. Similar variations in composition of some chemical constituents of tea from the same country had been observed in other studies. In China, the F and Al (Shu *et al.*, 2003) and Cu (Jin *et al.*, 2008) contents of tea from different farms within Sichuan Province varied. In Turkey, Fe and Mn contents of tea from different growing regions were significantly different (Sahin *et al.*, 1991). These results were similar to recent research done in the Wushwush farms in Ethiopia on the levels of essential and non-essential elements where there were significant variations in the elements of five different clones planted in four unit farms under similar agronomic practices (Yemane *et al.*, 2008). Earlier, large variations had been shown in mature leaf nutrients contents grown at the same location and that the nutrients contents were not related to the yields (Wanyoko and Njuguna, 1983). Recently clone BBK 35 was shown to have different mature leaf nutrient levels when grown in different regions in Kenya under same agronomic inputs (Kamau *et al.*, 2005). Similar variations were recently observed in the plain tea quality parameters (Owuor *et al.*, 2010a, 2010b). Successful cultivars of most crop species, successful tea genotypes should be adapted to a wide range of climatic and edaphic conditions. Tolerance to drought, cold, frost high solar radiation and high pH are among the major environmental factors that affect adaptation and performance of tea in different sites (Wachira *et al.*, 2002). The clones that are grown in a single site (Owuor *et al.*, 1988, 1987b, 1987c) and even in different environments (Owuor *et al.*, 2010a) exhibited variations in their black tea chemical composition. Indeed even the yields significantly differed in the different environments (Wachira *et al.*, 2002). Previous research demonstrated wide response ranges among tea genotypes to different environments (Carr, 1977; Obaga *et al.*, 1988; Tanton, 1982a; Wickremaratne, 1981; Carr and Stephens, 1992). Indeed, dry matter partitioning (Ng'etich and Stephenes, 2001a, 2001b; Magambo and Canell, 1981; Magambo and Waithaka, 1983) and quality (Owuor *et al.*, 2010b) of tea vary with clones and location of production. However, different clones have varying abilities to absorb nutrients from the soil (Yemane *et al.*, 2008; Wanyoko, 1981) leading to clonal variation in mature leaf nutrient levels (Wanyoko and Njuguna, 1983).

The results together with data presented herein demonstrate clear evidence that there is need to generate additional data on widely grown clones in different locations to help in managing the human health hidden hunger problems. It is necessary that clonal and location specific agronomic recommendations are developed that will strike a balance in all the essential micronutrients from black tea in order to effectively alleviate/reduce human health hidden hunger problems by the use of tea.

Table 24: Clonal black tea Se levels (µg/g) and relative ranking based on Se levels to growing environments.

Clone	Se concentrations (µg/g)				Ranking			
	Site			Mean clone	Site			Mean clone
	Timbilil	Kipkebe	Kangaita		Timbilil	Kipkebe	Kangaita	
TRFK 7/9	1. 60	2.37	1. 30	1.76	13	7	19	17
TRFK 303/259	2.37	2.37	1. 60	2.11	5	7	17	12
TRFK 303/1199	2.33	2.03	3.67	2.68	6	11	2	2
TRFK 54/40	1. 40	1.57	4.43	2.47	18	15	1	6
TRFK 31/8	1.47	1.43	3.13	2.01	15	17	4	13
BBK 35	3. 30	1.47	2.97	2.58	2	16	5	3
TRFK 6/8	1.46	1. 70	1.43	1.53	17	13	18	19
TRFK 31/27	2.63	1.36	1. 70	1. 90	4	19	14	15
TRFK 12/12	1.47	2.13	1.17	1.59	15	10	20	18
TRFK 303/999	2.33	2.57	3.33	2.74	6	5	3	1
APH S15/10	2.26	3.53	1.73	2.51	10	1	12	4
TRFK 57/15	1. 60	3.43	2.47	2. 50	13	2	6	5
TRFK 56/89	1. 30	2. 60	2. 10	2. 00	19	4	11	14
TRFK 12/19	1.27	1.43	1. 70	1.47	20	17	14	20
TRFK 11/26	1.73	2.36	2. 40	2.17	11	9	7	9
STC 5/3	2.27	2. 40	1.73	2.13	8	6	12	11
RFK 7/3	1.63	1.73	2. 30	1.89	12	12	8	16
RFK 303/577	2.27	2.93	2.13	2.44	8	3	10	7
PK TN14-3	3.17	1.67	1.63	2.16	3	14	16	10
RFK 2x1/4	3.33	1.33	2.17	2.28	1	20	9	8
lean site	2.06	2.12	2.26					
.V (%)			9.27					
SD ($P \leq 0.05$)		0.16		0. 20				
lteractions			0.33					

CHAPTER FIVE

5.0. Summary, Conclusions, Recommendations and Suggestions for Future Studies.

5.1. Summary

1. This study has revealed that East African black teas contain micronutrients and that Mn was the most abundant micronutrient and other micronutrients decreased in the order of Fe>Cu>Se.
2. The levels of the micronutrients varied with grading of black tea such that the micronutrients decreased in the order of BP1>PF1>PD>D1 among these grades.
3. The local market teas contain higher levels of the micronutrients than teas in the export market.
4. The micronutrient Cu is highly infused to hot water than the other micronutrients but when compared to minimum body requirements tea is a rich source of Mn and that the local teas supply more Mn than the export teas.
5. The study has shown that in a single cultivar the micronutrients content of black teas varied with location of production. Levels of Mn and Se in black tea are not affected with nitrogenous fertilizer rates, Fe and Zn increase while Cu decrease with increasing nitrogenous fertilizer rates. Plucking intervals did not affect the micronutrient contents of the black teas.
6. The micronutrient content of several cultivars planted in a single location under similar agronomic practices varied and that the variation did not follow the same pattern when the clones were planted in different locations.

5.2. Conclusions.

1. Thus for effectively addressing hidden hunger the large particle size grades eg. The BP1 grade of black tea should be used and it is also necessary to know the source of the teas since the nutrient contents vary with location of production.
2. Local teas can effectively contribute to the reduction of hidden hunger related to Mn deficiency to a larger extent than the teas from the export market.
3. The black teas from East Africa can only be a major source of Mn and not other micronutrients in addressing the hidden hunger problem.
4. Nitrogenous fertilizer rates used in the different tea growing locations should be the ones that strike a balance among the micronutrients.
5. Different tea growing locations need to grow suitable clones that maximize the micronutrient levels in the resultant teas.

5.3. Recommendations.

1. East African teas contain appreciable levels of micronutrient Mn thus to effectively reduce the hidden hunger problem associated with Mn deficiency people should use the large particle size grades or the teas from the local market.
2. Consumption of at least two cups of tea from the local market and at least four cups of tea from the export market in a day will alleviate hidden hunger related to Mn deficiency but other food stuffs should be used to provide the other micronutrients.
3. The regional variations in terms of micronutrient content of black tea due to nitrogenous fertilizer rates and plucking rounds for clone 6/8 indicate that the farmers should use fertilizer rates that will strike a balance between the micronutrients. This study recommends fertilizer rates of 150 kgN/ha/year which strikes a balance between Cu and other micronutrients in using black tea to reduce hidden hunger.
4. Different clones have been identified to have different abilities to absorb the micronutrients when planted in same location under similar agronomic practices and the response pattern is not similar when planted in other locations, therefore it is necessary that farmers identify the clones with the highest potential to absorb micronutrients in their given locations in order to optimally address the hidden hunger problem.

5.4. Suggestion for future studies.

1. Since tea cannot supply all the required micronutrients by itself, there is need for research to be done on other food stuffs in order to get a combination that can give all the required micronutrients.
2. This study used an infusion time of 4 minutes, thus its necessary to find out if time is a factor in the infusion process.
3. It is speculated that plucking standards may cause variations in the micronutrient content of the black teas thus there is need for research to be conducted to validate this argument.
4. It is also necessary to find out the levels of the micronutrients in the infusions that end up in the human body through ingestion.
5. Other clones can also be subjected to different rates of nitrogenous fertilizer experiments with an aim of developing region specific agronomic recommendations that maximally absorb the micronutrients to aid in reducing the hidden hunger problems.

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