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Levels of Essential and Non-Essential Metals in Commercially Available Ethiopian Black Teas

By

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LEVELS OF ESSENTIAL AND NON-ESSENTIAL METALS IN COMMERCIALLY AVAILABLE ETHIOPIAN BLACK TEA

By Daniel Woldegebriel

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Abstract:

Essential (K, Ca, Mg, Mn, Fe, Zn, Cu and Co) and non-essential (Cd and Pb) metal contents of commercially available three brands of Ethiopian black tea (Wush Wush, Gumero, and Black Lion) and their infusions were analyzed by flame atomic absorption spectrometry (FAAS). In black tea leaves K was found to be the highest amount followed by Ca and then Mg. The heavy metals in black tea leaves were found in lower amount than K, Ca and Mg. Co and the non-essential heavy metals (Cd and Pb) were too low to be detected by the available method (FAAS). Generally, in black tea leaves the order was found as K > Ca > Mg > Mn > Fe > Zn > Cu. The black tea infusions prepared from the black tea leaves were also analyzed for their essential and non-essential metal content. It was found that K was found in highest amount followed by Mg in contrast to the black tea leaves. Co, Cd and Pb were found below their detection limit. In black tea infusion the order in the metal content was found to be as follows: K > Mg > Ca > Mn > Fe > Zn > Cu. Generally, the metal contents of the black tea were found to be higher than those of tea infusion. The extraction efficiency of these metals from black tea leaves to their infusion was also calculated and it was found that highest percent was found for K (47 %) and lowest percent for Ca (12.5 %). Except K the essential metal contents of Ethiopian black tea and their infusions were found to be comparable with that of the black tea and their infusions from the rest of the world. While Ethiopian black tea and their infusions were found to be free from the non-essential (Cd and Pb) metals.

Key words: Tea (*Camellia Sinensis*), Black tea, Tea infusion, Essential elements, Non-essential elements.

1. INTRODUCTION

1.1 History of Tea Plant

Tea, one of the most popular beverages in the world, is an infusion of the leaves of the tea shrub, *Camellia sinensis* plant and it is consumed for centuries [1-3]. This tea is different from fruit and herbal infusions which don't come from the *Camellia sinensis* plant, thus they are not true tea.

Legends, and most sources, acknowledge the Chinese Emperor Shen Nung (28th century B.C.) with the discovery of tea. The tea plant originated in South-East Asia, probably in the region incorporating sources and high valleys of the Brahmagutra, the Irrawaddy, the Salween and the Mekong rivers at the border separating India, China and Burma [4]. The Chinese were familiar with camellia leaves, which they used in vegetable relishes and quite probably as part of medicinal compounds. But until the emperor's discovery, the leaves had never been considered an ingredient of a hot, refreshing drink. News of the emperor's discovery spread quickly throughout China, and soon everyone was trying the beverage. Before long, tea (known as Ch'a) became an important part of Chinese culture.

The Western world was not introduced to the enjoyment of tea until about the 17th century, when the Dutch East India Company first began transporting the tea leaves to Holland and beyond; Russia also began importing tea using caravans of camels on the Silk Road [5].

The cultivation of tea plant in other countries was started at different time. The first cultivation introduced to various countries was Indonesia in 1684, India in 1780, Russia in 1833, Sri Lanka in 1839, Malawi in 1875, Iran in 1900, Kenya in 1903, and Turkey and Argentina in 1924. In the late 17th century, tea became a popular beverage served in numerous tea houses in London. From then on, growth of its popularity was rapid and today it is the most widely consumed beverage in the world [6].

1.2 Botanical Classification of Tea Plant

The tea shrub is a perennial evergreen plant. It is under the Theaceae family and the Camellia species (*Camellia sinensis*). *Camellia sinensis* consists mainly of two varieties, Camellia sinensis variety sinensis (The China Tea Plant) and Camellia sinensis variety assamica (The Assam tea plant). In fact there are other varieties in addition to the previous two types of varieties. These varieties differ in the height of the tea bush, the number of stems and characteristics of their leaves [4]. In nature, tea trees can have a height of 20-30 m but usually the plant is kept as an evergreen shrub by pruning, pruned when they are around 1.5-2.0 meters. Old tea plants with age of 1500 years old are still thriving in their original forests of Yunnan Province in the Southwestern China [6].

1.3 Tea Plant in Ethiopia

The production of tea-leaves in Ethiopia has got a history of more than half a century. Nevertheless, it is only three decades since the consolidated and organized development of tea production began. The production of tea-leaves was begun in 1984 with the support of the state and up to 1996 not more than 2,000 hectares were produced.

In the 2005/2006 fiscal year, tea plantation on seven household farms was executed and a contract with tea plantation development organizations was made. Data obtained from the plantation of Wush-wush, Gumero, and Chewaka varieties show that the land covered by the tea plantations covered in 2004/2005 exceeds that of the 2002/2003 by 190 hectares, and the yield correspondingly increased by 390 tons. Compared to that of 2003/2004, land covered by plantation surpassed by 75 hectares and production likewise exceeded by 302 tons. Tea is one of the cash crops in Ethiopia [7].

In Ethiopia, tea is mostly grown in the highland dense forest regions where the land is fertile and thus the use of fertilizer is very minimal. Moreover, the availability of abundant and cheap labor in the country has made the use of manual weeding, instead of chemical weeding, possible. Because of this mostly organic cultivation, Ethiopian tea is increasingly sought for its aroma and natural flavors. Ethiopia exports teas regularly to different countries [8].

1.4 Economic Importance of Black Tea

The economic importance of tea backs to a very long year. A Chinese document published in 347 A.D. states that people in Southwest China used teas for paying tribute to the Chinese emperors as early as 1066 B.C. In the essay "Tong Yue", written by a country landlord Wang Bao and published in 59 B.C., there is mention of the making and sale of tea. It showed that tea was commercially available in the local country market, suggesting that tea processing and marketing as early as 59 B.C. in Southwest China [6].

Tea is the second most consumed beverage in the world with an estimated 18-20 billion cups consumed daily [9]. The principal teas produced and consumed in the world are black and green teas, with small amount of other types [6]. Black tea represents approximately 78% of total consumed tea in the world, whereas green tea accounts for approximately 20% [10]. The major producers of tea are India, China, Sri Lanka, and Kenya, while the major consumers are India, China, Turkey, and Japan. During the 1990s, the world production and consumption of tea has increased steadily with occasional fluctuation in some years. Thus, *Camellia sinensis* has become a very important agricultural and commercial product, with unique horticultural and processing methods [6]. It is easy to see how tea is commercially important. For instance, tea is the leading export crop in Kenya, which places Kenya to be the third largest producer of black tea after India and Srilanka and large amount of money is earned [11].

1.5 Various Types of Tea

There are several major categories of tea, which are distinguished by different processing methods and, consequently, different concentrations of the chemical components in tea. The main types of tea are green tea, Oolong tea and black tea. The fermentation process involves an enzymatic oxidation of polyphenols, leading to the formation of chemical compounds that generate both the aroma and color of black tea [9].

The Green tea is a yellow to green color which is preferably manufactured from the *Camellia sinensis* var. Sinensis. Its chemical constituents are not altered by fermentation and the enzyme is inhibited by tea.

The Oolong tea is a semi-fermented tea which is manufactured by letting the tea leaf to undergo incomplete fermentation. The Oolong tea, with a large twisted leaf, brownish in color with white tips, produces a light green, slightly coppery infusion. Its chemical composition is in between the green tea and the black tea [4]. Oolong tea contains monomeric catechins, theaflavins, and thearubigins. Some characteristic components, such as epigallocatechin esters, theasinensins, dimeric catechins, and dimeric proanthocyanidins, are also found in Oolong tea [12].

The Black tea is a completely fermented tea leaves. Fresh tea leaves are rich in polyphenolic compounds known as catechins. When tea leaves are intentionally broken or rolled during processing catechins become oxidized through the action of polyphenol oxidase enzymes present in the tea leaves. The oxidation of catechins, known as fermentation in the tea industry, causes them to polymerize and to form larger, more complex polyphenols known as theaflavins and thearubigins. This brings a difference in the color, aroma and chemical composition of the tea leaf as compared to green tea and oolong tea [12].

Infact there are other two types of tea, the white and yellow teas that have been regarded as two subclasses of green tea. These two types of tea are different from green tea due to differences in variety, processing, geographical and traditional distributions [6].

1.6 Manufacturing of Black Tea

The green, glossy leaves and young new shoots are plucked and immediately processed for optimal freshness [5].

The first step in the manufacturing of black tea is withering, which removes a large proportion of the water from the fresh leaf by evaporation. The leaves become limp and suitable for rolling and undergoing fermentation.

Rolling consists of twisting or breaking up the leaves so that preparing them for fermentation and transforming them into particles corresponding to the type of commercial tea required.

Fermentation is the most important stage in the manufacture of black tea. It involves the enzymatic oxidation (fermentation) of the polyphenols which are converted into theaflavines and thearubigines, with the leaves turning from green to coppery brown color. In this process, the monomeric flavan-3-ols undergo polyphenol oxidase-dependent oxidative polymerization leading to the formation of bisflavanols, theaflavines, thearubigines. These chemical compounds possess benzotropolone rings with dihydroxy or trihydroxy substitution systems, which give the characteristic color and taste of black tea [12].

Firing will follow the fermentation step which stops the fermentation and reduce the water content of the tea which makes handling and transportation easy. The firing leads to the destruction of the polyphenol-oxidases. Then the tea will be packed after sorting, which consists of extracting the fibers with the aid of winnowing machines and grading the tea by size and volumetric weight.

1.7 Chemical Composition of Black Tea

The chemical composition of a black tea may vary depending on different parameters such as the variety of leaf, growing environment, application of fertilizers, manufacturing, particle size of ground tea leaves and infusion preparation. In Table 1 the average values for the different constituents present in black tea are given [10].

Table 1 Principal components of black tea

Constituent	% weight of extract solids
Catechins	3-10
Theaflavins	3-6

Thearubigens	12-18
Flavonols	6-8
Phenolic acids and depsides	10-12
Amino acids	13-15
Methylxanthines	8-11
Carbohydrates	15
Protein	1
Mineral matter	10
Volatiles	<0.1

Though there is personal difference, among the characteristics of a good cup of black tea are a red or rosy color and briskness without bitterness. The chemical entities in tea that are generally believed to cause variations in color and bitterness are chiefly the theaflavins and thearubigins. The quantities of each, and the ratio of their quantities, are said to determine both the color characteristics of the tea beverage (tea infusion). For example, a tea with a high level of theaflavins and a low level of thearubigins would tend to give a beverage with a yellow-orange color and a high degree of briskness. Conversely, a tea with a low level of theaflavins and a high level of thearubigins would be expected to give a tea infusion with a brown color and little briskness (a soft tea). A tea with an optimum level of each of these chemical groups may give a tea infusion with a rosy color and appropriate briskness [13].

About 10-20% of the dry weight of black tea is due to thearubigens, which are even more extensively oxidized and polymerized, have a wide range of molecular weights, and are less well characterized [12].

1.8 Health Benefits of Black Tea

The chemical components in black tea have a health benefit to human. Although the oxidization process modifies the type of flavonoids present, the total level and their overall antioxidant activity, is similar in both green tea and black tea. Research is now suggesting that antioxidants, such as those found in both green and black tea may have a protective role to different diseases.

Different health benefit of black tea is reported [14]. For instance, black tea polyphenols are antioxidants in cancer chemopreventive [15]. The tea polyphenols may reduce oxidative stress through one of several mechanisms that relate to their structural chemistry. For example, the tea flavanoids directly scavenge free radical species through hydrogen/electron donation [14]. Black tea also has antigenotoxic effect [16]. It also represents a promising tool for the prevention and treatment of cardiovascular disorders [17]. It has also been reported that the aqueous extract of black tea prevents chronic ethanol toxicity [18].

The amount of intake of black tea infusion gives health benefit. For instance, one to six cups per day for significant increases in plasma antioxidant capacity and less than eight cups of tea per day for the avoidance of adverse effects on hydration and iron status [19].

Researchers established the effect of black tea extract on salmonella growth, and 42.19% of salmonella typhi strains were inhibited by this extract. Teas are also used in folk medicine for headaches, body aches and pains, digestion, diuretics, enhancement of immune defences, and detoxification, as an energizer, and to prolong life [5].

In addition to the organic components, different minerals and trace metals are present in tea extracts [2].

Many elements, in trace amounts, play a vital role in metabolic processes and are essential for the general well being of humans and their deficiency or excess may cause disease and/or be harmful to health [20]. In addition to their nutritional value trace metals have also been associated with the flavouring characteristics of tea [5].

The accurate determination of the trace element content of tea is thus very important in assessing any potential implications for health [21].

1.9 Requirements for the Growth of Tea Plant

Climate and soil characteristics are the most important ecological factors in growing tea. The variety of the tea plant is also another factor in growing tea. For instance, the Assam variety is less hardy than the China variety, which can tolerate a longer dry season or lower temperature [4].

It grows under a variety of climate conditions. Tea plants are grown in a wide range of latitudes in the world, from 45 °N (Russia) to 30 °S (South Africa), and longitudes from 150 °E (New Guinea) to 60 °W (Argentina). In tropical countries, tea leaves are harvested all year around. In temperate countries, harvesting is seasonal. There are many different kinds of products of different quality arising from different cultivation practices, growing conditions and processing methods [6]. The typical climate for the growing of tea plant is annual rain fall greater than 1500 mm, average temperature of 18 to 20 ^{0}C, 70 to 90 % atmospheric humidity and 5 hour sunshine. The tea plant requires an acidic soil with a pH between 4.5 and 5.5.

The pH of the soil affects the amount of soluble metals absorbed by the plant. For instance, the water-soluble Mn content of soil of the tea field increased with decreasing soil pH [13].

Application of fertilizer is also important for the growth of the tea plant and the quality of the black tea leaves. For instance, an increase in the potassium application rate increases the polyphenol and amino acid content of the tea. The failure to apply fertilizer resulted in depletion of the organic matter status of the soil [20]. Other literature indicated that potassium and magnesium nutrition has effect on the quality components of different types of tea. The results show that potassium and magnesium fertilizer application increased the contents of free amino acids and caffeine of the various tea types with the maximum increase found in the treatment including both nutrients [21].

1.10 Essential and Non-Essential Elements

The elements found in living organisms may be essential (like K, Mg, Ca, Mn, Fe, Co, Cu, and Zn) and they are very important for growth and health, or they may be nonessential (like Cd and Pb), reminders of our geochemical origins or indicators of environmental exposure. Studies conducted on human and animal originally showed that optimal intakes of elements such as sodium, potassium, magnesium, calcium, manganese, copper, zinc, and iodine could reduce individual risk factors, including those related to cardiovascular disease. The increasing use of highly refined foods, which are low in minerals, vitamins, etc., contributes to health problems. Nutritional deficiency may lead to diseases and disease may also lead to nutritional deficiencies. There is growing interest in the trace elements in the area of medicinal science. It is believed that the great majority of these elements act as key components of essential enzyme systems or other protein, for instance, the hemoprotein hemoglobin, which performs vital biochemical functions [22].

All trace elements are toxic if consumed at sufficiently high levels for long enough periods. For example, 0.1 ppm of Se is beneficial while 10 ppm may be carcinogenic. The difference between toxic intakes and optimal intakes to meet physiological needs for essential trace elements is great for some elements but is much smaller for others [23].

1.11 Metal Contents of Black Tea Leaves

There is a variation in the mineral composition due to different origins of the plant. The metal content of the tea is influenced by the soil composition and local environmental factors [2].

Different papers have been published on the determination of level of metals in tea. For instance, aluminum, which is associated with a number of health conditions, is accumulated in high concentrations by the tea bush**.** The aluminum content of plucked tea leaves ranges from 200 to l000 ppm (in fact other studies range it up to 20000 ppm [21]) range compared with 50 to 100 ppm in other plant species. In addition to the health benefit, Al affects the quality of tea. The effect on quality depends on how much aluminum is actually extracted and the amount of theaflavin and thearubigin in the

extract. Preliminary work on the aluminum tea reactions showed that mainly theaflavins were involved in the formation of red compounds [13].
Studies have also shown that tea drinking is a potentially major source of dietary manganese (Mn) [24, 25]. Research has shown that Mn levels of certain black tea infusions 0.51 ± 0.11 mg/100 g (mean ± s.d.) in agreement with previous findings.

Tea is a valuable source of different elements. Several elements, such as Ca, K, Mg and Mn, are present at mg/g level, whereas elements such as Fe, Co, Ni, Cu, Zn and Cd are present at a few mg/g. Also some rare earth elements have been reported at the ng/g level [20, 21].

For instance, the quality of tea brands and herbs available in the retail market in the Kingdom of Saudi Arabia were assessed based on contents of heavy metals in their tissues. All tested brands of tea and herbs possess considerable amounts of the eight tested heavy metals, Mn, Fe, Zn, Cu, Co, Pb and Cd. Among tested heavy metals, Mn was the most abundant one in tea leaves (390-900 μg per g) whereas Fe was the predominance one in herb leaves (326-1755 μg per g). Fortunately, toxic heavy metals, Pb and Cd, had the lowest contents in both tea and herb leaves. Concentrations of tested heavy metals in tea and herb beverage were markedly lower than their total contents. The concentrations of toxic heavy metals, Pb and Cd were too low to be detected in beverage [26].

Some teas and their infusions in Turkey were studied and the Al, Ca, K, Mg, Mn, P, and S contents were very high in both infusions and tea (*i.e.*, pieces of the tea plant). The As, Cd, Cr, Li, Pb, and Se contents of infusion and tea were found to be very low. The level of K of all samples is higher than those of other minerals. Generally, mineral contents of tea were found to be higher than those of tea infusions [4].

Some times a toxic trace element or a metal with more than the recommended amount may be found in tea. Studies have shown that in Chinese tea, lead concentrations in 1225 tea samples collected in China between 1999 and 2001 varied from < 0.2 to 97.9 mg kg^{-1}

dry weight, with 32% of the samples exceeding the national maximum permissible concentration of 2.0 mg kg^{-1} dry weight and a significant difference between tea types [27].

1.12 Metal Contents of Black Tea Infusions

Usually the tea beverage (brew or infusion) is consumed by man kind. Therefore, the amount of metals in tea beverage should be considered to see its nutritional and health impact. The amount of metal found in the tea beverage is less than the total metal content in the tea leave. For instance, studies have shown that the solubility of the studied heavy metals tea infusion extracts varied widely and ranged from 0.0-48% [26].

Clearly the type of tea, including its geographical origin, degree of fermentation prior to drying and leaf size, as well as type and temperature of water, length of brewing time and strength of tea brew will all impact upon the concentration of the nutrients present in the tea infusion. Literature indicated that Mg, K, Ca and Mn are major elements in tea infusion [28-30].

Other investigations have also indicated that it is possible to determine speciation of the metals in tea infusion, for instance, Mg, Mn and Rb are present in tea infusion as cations, which are probably not associated with organic material. Ca, Fe, Co, Ni, Cu, Zn, Sr and Ba appear to be present mainly in cationic form, but there is in addition a certain non-cationic fraction. For Fe, Ni, Cu and Zn, the non-cationic species may be metal–organic complexes [31]. Manganese speciation was also made in tea infusion and the result indicate that 30 % of manganese was extracted from the tea leaves in the form of Mn(II) and 2.5 % of Mn was distributed in the organic bound [32].

The metals extracted from black tea leaves were classified as highly extractable element, moderately extractable element, and poorly extractable element [33].

In one paper the transfer ratio of copper, iron, nickel and chromium has been investigated for different kinds of tea at different infusion temperatures and it was seen that the transfer ratio of each metal into the infusion appeared to be dependent on both temperature and strength of infusions as well as the kind of tea [34].

1.13 Role of Metals in Human Health

Here below the biological importance of the metals of interest are briefly mentioned.

Potassium (K)

Potassium is found in human body abundantly. Usually it has biological function as that of sodium. Deficiency of potassium is implied by irregular heart beat, loss of appetite and muscle cramps. A very high amount of potassium intake could be toxic, leading to slowing down o heart bit and failure of kidney [35, 36].

Calcium (Ca)

It has a very important role in bone and tooth structure, in blood clotting, muscle contraction. If there is deficiency of Ca, it increases the risk of bone loss. In contrast, excess intake of Ca may reduce mineral absorption [35, 36].

Magnesium (Mg)

Magnesium is a co-factor for many coenzymes, also affects metabolism of K and Ca. Its deficiency is related to high blood pressure, pregnancy problems, and poor heart function [35, 36].

Iron (Fe)

Iron has part in carrying of oxygen as a critical component of the hemoprotein hemoglobin, myoglobin and the cytochromes and it is also a co-factor for some enzymes. Its deficiency causes anemia [37].

Manganese (Mn)

Mn is an essential trace element [38, 39] for a number of key enzymes including liver pyruvate carboxylase, arginase and, most notably, mitochondrial or Mn-dependent superoxide dismutase (MnSOD), one of the key antioxidants in the body [30]. It is called the "maternal mineral" because manganese deficiency in females causes a reduced maternal caring for her young [38].

Copper (Cu)

Copper is a nutritionally essential metal and is widely distributed in nature [40]. At low concentrations, copper plays an important role in carbohydrate and lipid metabolism [41-43]. Above trace levels, however, copper has many biological effects both as an essential and a toxic element. Toxic doses affect seriously the blood and kidneys [44]. The deficiency of Cu is also associated with increase risk of hypertension [45, 46].

Zinc (Zn)

Zinc is cofactor of many enzymes, e.g. superoxide dismutase, and they assist in the formation of antioxidative reactions. Its deficiency increases the risk of hypertension [45, 46]. Deficiency of zinc causes inadequate growth, loss of apetite [35, 36].

Cadmium (Cd)

Cadmium is a toxic metal with sterilizing, teratogenic and carcinogenic effects [47]. It may also lead to cardiovascular diseases. It is an inhibitor of the enzymes with sulphydryl groups and disrupts the pathways for the oxidative metabolism [37]. Its toxicity affects many target tissues such as appetite and pain centers (in brain), brain, heart and blood vessels, kidneys, and lungs. This toxicity may causes anemia, dry and scaly skin, emphysema, fatigue, hair loss, heart disease, depressed immune system response, hypertension, joint pain, kidney stones or damage, liver dysfunction or damage, loss of appetite, loss of sense of smell, lung cancer, pain in the back and legs [48].

Lead (Pb)

It is highly toxic to man and animal [49]. Lead toxicity influences brain, heart, kidneys, liver, nervous system, and pancreas. It may cause many signs and symptoms such as abdominal pain, anemia, anorexia, anxiety, bone pain, brain damage, confusion, constipation, convulsions, dizziness, drowsiness, fatigue, headaches and hypertension. It also diminishes IQ in children [48].

Many black tea leaves and their tea infusion from different countries such as India, US, England, were investigated for their metal contents. The results have shown that the tea plant accumulates large amount of certain metals such as Al, Mn, Mg, Ca, K, and Na. The levels of the metals in the tea infusion have also been investigated and the results indicated that tea beverage is a rich dietary source of metals. In some tea brands it has been shown that there are toxic metals in the black tea mainly due to being grown in industrialized areas.

There has been one thesis made on Ethiopian tea. The study reports the level 11 metals in the green leaves of Ethiopian tea indicating the ability of the plants to accumulate relatively higher amounts of K and Mn. The levels of the toxic metals such as Cd and lead were very small to be detected.

It is, there fore, necessary to determine the metal contents of Ethiopian black tea leaves to check whether the manufacturing process affects the level of the metals or not. The level of the metals in Ethiopian black tea infusion should also be investigated to see whether the toxic metals are consumed by the consumers or not.

In Ethiopia, like the other countries, tea is consumed largely. Since tea is the most consumed beverage next to water, in our daily life, determination of the level of the minerals and trace metals (the essential and non-essential metals) is very important. The result then could be a source of information with regard to quality and standards, medicine, nutrition and pollution.

This research project is intended to analyze the levels of metal contents in commercially available Ethiopian black tea and their tea infusion so that the total metal content of the black tea and amount of the metal obtained from the tea infusion will be compared. Then, the results obtained might initiate further studies on nutritional, medicinal and toxicological effects of the commercially available Ethiopian tea.

1.14 Objectives of the Present Study

1.14.1 General objective

The main objective of this project is to determine the levels of essential and non-essential metals in commercially available Ethiopian black tea and their tea infusion.

1.14.2 Specific objectives

1. To develop a working procedure for the digestion and analysis of black tea for their essential and non-essential metal contents by flame atomic absorption spectrometer.
2. To develop a working procedure for the analysis of tea infusions for their essential and non-essential metal contents by flame atomic absorption spectrometer.
3. To determine essential (K, Ca, Mg, Mn, Fe, Cu, Zn, Co) and non-essential (Pb, Cd) metals in the commercially available Ethiopian tea using flame atomic absorption spectrophotometer (FAAS).
4. To correlate the amount of metals in the black tea with that present in the tea infusion.

2. EXPERIMENTAL

2.1 Instrumentation and Apparatus

Electronic blending device (Moulinex, France) was used for grinding and homogenizing the black tea leave sample to determine the total metal content of the black tea leaves. Analytical digital balance was used to weigh the black tea leave sample. Micropipettes (DRAGONMED, Shanghai, China, 100-1000 μL) were used for measuring different amounts of acid mixtures and standard solutions. 25, 50 and 100 mL volumetric flasks were used to dilute sample solutions and prepare standard solutions. 250 mL round bottom flask with reflux condenser were used in Kjeldahl digestion block (Gallenkamp, England) apparatus for the digestion of both the black tea leave sample and black tea infusion. Flame atomic absorption spectrophotometers (BUCK SCIENTIFIC MODEL 210VGP AAS, East Norwalk, USA) with air-acetylene flame was used for the analysis of the analyte metals (K, Mg, Ca, Mn, Fe, Cu, Zn, Co, Cd and Pb) in the black tea leaves and black tea infusion sample solutions. K was analyzed in the emission mode of the instrument.

2.2 Reagents and Chemicals

Analytical grade chemicals and reagents were used in the analysis of the black tea leaves and black tea infusion samples. 69-72 % HNO_3 (FINE-CHEM MUMBAI-391780, India) and 70% $HClO_4$ (A.C.S. REAGENT, Aldrich UK) were used for the digestion of both the black tea leaves and black tea infusion samples.

Lanthanum nitrate hydrate, 99.9% (Aldrich, USA) was used to avoid the chemical interference on Ca and Mg in the sample solution.

Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Mg, Mn, Pb, K, Ca, Fe, Zn, Cu, Co, Cd (BUCK SCIENTIFIC PURO-GRAPHIC^{tm}) were used for preparation of calibration standards and in the spiking experiments. Calibration standards of 1000 mg/L and intermediate standard solutions of 10 mg/L of each metal (K, Mg, Ca, Mn, Fe, Cu, Zn, Co, Cd and Pb) were prepared to plot the calibration curves for their respective metals. Standard working solutions were prepared freshly from the standard stock solutions (1000 mg/L) of each of the metals by appropriate dilution of the intermediate standard solution (10 mg/L).

Deionized water (water chemically pure: 1.5 µs/cm and below) was used throughout the experiment for rinsing of the glassware and dilution of sample solution.

2.3 Sample Collection

2.3.1 Black Tea leaf sample collection

Three different types of commercially available Ethiopian black teas were used in this study. The three types of black teas used in the experiment were Wush Wush , Gumero, and Black Lion Tea, each of them are in a 100 g package, are obtained from market. The Wush wush and Gumero black teas are processed from the Wush wush and Gumero tea plantation, respectively. The Black Lion black tea is packed after blending black tea from the Chewaka plantation and the other. The two plantations found in the country are represented by the two brands, but there is no unblended black tea that represents the Chewaka plantation which has been transferred to the private sector.

2.4 Sample Preparation

2.4.1 Black tea leaves sample preparation

For each of the black tea brands three packages of 100 g were used. The three packages were mixed thoroughly and grinded to fineness using an electronic blender. The grinded black tea leaves was then used for the determination of the total metal content.

2.4.2 Black tea infusion preparation

The black tea infusion was made from each of the black tea types. Though there is no universal agreement on how to prepare tea beverages, it is usual in Ethiopia to prepare it by first boiling the water and adding the required amount of the black tea leaves for a certain time depending on personal interests. For this study the black tea infusion was prepared as follows: 100 mL of water was boiled in a beaker and 2 g of black tea leaves were added in to the boiling water and allowed to boil for 5 min, then cooled for five minutes and filtered to obtain the pure black tea infusion.

2.5 Analysis of Samples

2.5.1 Optimization of working procedures

To prepare a clear colorless sample solution that is suitable for the analysis using AAS different black tea digestion procedures were assessed using the HNO_3 and $HClO_4$ acid mixtures by varying parameters such as volume of the acid mixture, digestion time and digestion temperature.

2.5.2 Digestion of black tea leaves samples

Exactly 0.5 g of the dried and ground black tea leaf sample was accurately weighed on a digital analytical balance and transferred quantitatively in to a 250 mL round bottom digestion flask. 6 mL of freshly prepared 5:1 mixture of conc. HNO_3 and conc. $HClO_4$ was added to the sample. The sample was swirled gently to homogenize then fitted to a reflux condenser and digested continuously for three hours on a Kjeldahl digestion block. The temperature was adjusted with in certain time intervals and to give the maximum temperature of 300 ^{o}C. The digestion gives a clear colorless solution. The digest was quantitatively transferred to a 50 mL volumetric flask and made up to the mark with deionized water. Each black tea leaves sample was digested in triplicate and hence a total of nine digest was made for the three types of black tea leaves samples.

Digestion of a reagent blank was also performed in parallel with the black tea leaves samples keeping all digestion parameters the same. For the analysis of the black tea leaves samples 12 reagent blank samples were prepared. All the digested samples were stored in refrigerator until analysis using AAS. The solutions were used to determine concentration of the 10 elements by AAS. To avoid the ionization interference the concentrations of K was determined in emission mode. To avoid chemical interference in the determination of concentrations of Mg and Ca lanthanum nitrate hydrate is added to the solutions.

For each of the black tea leave samples three repeat measurements were performed. Therefore, for each sample, the results were obtained from the mean of nine measurements.

2.5.3 Analysis of black tea infusion samples

To determine the amount of metals extracted from the black tea leave to the tea infusion (to the hot water) 25 mL of the tea infusion was transferred quantitatively to the 250 mL round bottom flask and heated to evaporate. This was done to avoid dilution of the acid mixture added for digestion. The solution was allowed to cool before the addition of the acid mixture to avoid explosion due to contact between the organic matter and conc. $HClO_4$. 5 mL of 1:4 HNO_3 and $HClO_4$ was added and swirled gently to homogenize then fitted to a reflux condenser and digested continuously for two and half hours on a Kjeldahl digestion block. The temperature was adjusted within certain time intervals and to give the maximum temperature 300 ^oC.

The digestion gave a clear colorless solution. The digest was quantitatively transferred to a 50 mL volumetric flask and made up to the mark with deionized water. Each black tea infusion sample was digested in triplicate and hence a total of nine digest was made for the three types of black tea infusion samples.

Digestion of a reagent blank was also performed in parallel with the black tea infusion samples keeping all digestion parameters the same. For the analysis of the black tea infusion samples 12 reagent blank samples were prepared. All the digested samples were stored in refrigerator until analysis using AAS. The solutions were used to determine concentration of the 10 elements by AAS. To avoid the ionization interference the concentrations of K was determined in emission mode. To avoid interference in the determination of concentrations of Mg and Ca lanthanum nitrate is added to the solutions.

For each of the black tea infusion samples three repeat measurements were performed. Therefore, for each sample, the results were obtained from the mean of nine measurements.

2.6 Recovery Tests

Efficiency of the procedure was checked by spiking 100 μL of 1000 mg/L Ka and Mn and 50 μL of 1000 mg/L Cu, Zn and Pb at once in to a black tea leaves sample and 100 μL of 1000 mg/L Fe, Ca, Mg and 50 μL of 1000 mg/L Co and Cd was added into another digestion flask containing the same black tea leave sample.

Since the concentration of metal in the black tea infusion is lower than that of the black tea leaves, the recovery test for the black tea infusion was done by adding smaller amount of the spiked metals than that of the black tea leaves. 50 μL of 1000 mg/L K and Mn and 10 μL of 1000 mg/L Cu, Zn and Pb at once in to a black tea infusion sample and 50 μL of 1000 mg/L Fe, Ca, Mg and 10 μL of 1000 mg/L Co and Cd into another digestion flask containing the same black tea infusion sample.

For both the black tea leaves sample and black tea infusion sample the recovery test was performed in triplicate. Each of the samples was then analyzed for its respective spiked metals by AAS.

2.7 Method Detection limits

Twelve reagent blank samples were digested and each of the samples were analyzed for metal concentrations of K, Ca, Mg, Fe, Cu, Zn, Mn, Co, Pb and Cd by AAS. The standard deviations for each element were calculated from the twelve blank measurements to determine method detection limit of the instrument.

3. RESULTS AND DISCUSSION

3.1. Optimization of Working Procedure

For both black tea leaves and black tea infusion samples different digestion procedures using the HNO_3 and $HClO_4$ acid mixture were assessed by varying volume of the acid mixture, digestion temperature and digestion time.

Table 2. Different methods tested during the optimization of procedures for black tea leaves samples.

No.	Weight of tea leaves (g)	Volume of 5:1 HNO_3 and $HClO_4$ (mL)	Digestion temperature (^0C)	Digestion time (min)	Results
1	0.5	7	300	120	Clear colorless solution with no suspended matter
2	0.5	7	300	150	Clear colorless solution with no suspended matter
3	0.5	7	300	180	Clear colorless solution with no suspended matter
4	0.5	6	300	120	yellowish solution with no suspended matter
5	0.5	6	300	150	Yellowish solution with out suspended matter
6	**0.5**	**6**	**300**	**180**	**Clear colorless solution with no suspended matter**
7	0.5	5	300	120	Yellowish solution with no suspended matter
8	0.5	5	300	150	Yellowish solution with no suspended matter
9	0.5	5	300	180	Yellowish solution with no suspended matter
10	0.5	4	300	120	Yellowish solution with suspended matter
11	0.5	4	300	150	Yellowish solution with suspended matter
12	0.5	4	300	180	Yellowish solution with no suspended matter

Optimization of the digestion step for the black tea infusion was also made by varying the parameters used for the tea leaves. The volume of the tea infusion taken was 25 mL but to avoid the dilution of the conc. HNO_3 and $HClO_4$ the volume of the black tea infusion sample was decreased by evaporating to around 5 mL.

Table 3. Different methods tested during the optimization of procedures for black tea infusion samples.

No.	[a] Volume of tea infusion (mL)	Volume of 5:1 HNO_3 and $HClO_4$ (mL)	Digestion temperature (^{0}C)	Digestion time (min)	Results
1	25	6	300	120	Clear colorless solution with no suspended matter
2	25	6	300	150	Clear colorless solution with no suspended matter
3	25	6	300	180	Clear colorless solution with no suspended matter
4	25	5	300	120	yellowish solution with no suspended matter
5	25	5	300	150	yellowish solution with no suspended matter
6	25	**5**	**300**	**180**	**Clear colorless solution with no suspended matter**
7	25	4	300	120	Yellowish solution with no suspended matter
8	25	4	300	150	Yellowish solution with no suspended matter
9	25	4	300	180	Yellowish solution with no suspended matter

[a] The volume of the sample was decreased to around 5 mL by evaporating.

Alternatives No. 6 in Table 2 and alternative No. 6 in Table 3 were chosen for the digestion of the black tea leaves and black tea infusion samples, respectively. The choice was made by noticing that the final solution should be clear and colorless without any suspended matter. In addition, the selected digestion procedure should use the smallest volume of the reagents so that risk of contamination decreases [50].

For the black tea infusion, though some papers report the tea infusion sample preparation for the analysis simply by adding HNO_3 and filtering [33], it seems there should be complete digestion to remove organic components from the solution since they decrease the sensitivity.

3.2. Instrument Calibration

Calibration curves were prepared to determine the concentration of the metals in the sample solution. A series of working standard solutions were prepared from the 10 mg/L intermediate standard solutions of their respective metals. Wavelengths, concentration of the intermediate standards, working standard solutions and the correlation coefficients of the calibration curve for each of the metals are presented in Table 4.

Table 4. Concentrations of working standard solutions and correlation coefficients of the calibration curves.

Metal	Wavelength (nm)	Conc. of intermediate standard solution (mg/L)	Conc. of intermediate standard (mg/L)	Correlation coefficient
K	766.5	10	0.3, 0.6, 1.2, 2.4	0.9994
Ca	422.7	10	0.25, 0.5, 1.0, 2.0	0.9990
Mg	285.2	10	0.25, 0.5, 1.0, 2.0	0.9999
Fe	248.3	10	0.25, 0.5, 1.0, 2.0	0.9983
Mn	279.5	10	0.01, 0.1, 0.5, 1.5	0.9993
Cu	324.8	10	0.02, 0.04, 0.08, 0.16	0.9999
Zn	213.9	10	0.02, 0.04, 0.3, 0.6	0.9999
Co	240.7	10	0.05, 0.1, 0.2, 0.4	0.9995
Cd	228.9	10	0.05, 0.1, 0.2, 0.4	0.9996
Pb	283.3	10	0.3, 0.6, 1.2, 2.4	0.9998

3.3. Analytical Method Detection Limit

The limit of detection is the smallest mass of analyte that can be distinguished from statistical fluctuations in a blank, which usually correspond to the standard deviation of the blank absorbance times a constant. Usually it is defined as the amount of analyte that gives a signal equal to three times the standard deviation on the blank [51].

For both the black tea leaves and black tea infusion sample the method detection limits were calculated by multiplying the standard deviation of twelve blank signals measured in triplicate by three. The method detection limits are generally comparable with that of instrument for both tea leaf and tea infusion samples.

Table 5. Method detection limits for black tea leaves and black tea infusion samples.

Metal	Instrument detection limit (mg/L)	[a] Method detection limit for black tea leaves samples (mg/g)	[a] Method detection limit for black tea infusion samples (mg/g)
K	0.010	0.002	0.0060
Ca	0.010	0.005	0.0006
Mg	0.001	0.080	0.0060
Fe	0.030	0.070	0.0200
Mn	0.010	0.003	0.0020
Cu	0.020	0.003	0.0100
Zn	0.005	0.003	0.0010
Co	0.050	0.003	0.0020
Cd	0.005	0.003	0.0010
Pb	0.100	0.010	0.0020

[a] Values are mean of twelve blank determinations each measured three times.

3.4. Evaluation of Analytical Method

The efficiency of the method was assessed by spiking both black tea leaves and black tea infusion samples with known amounts of metals, and each of the metals were analyzed in triplicate. Recovery was calculated using the equation below [52].

$$\%\ Recovery = \frac{metals\ content\ of\ the\ spiked\ sample - metals\ content\ of\ nonspiked\ sample}{the\ expected\ metals\ content} x100\%$$

The recovery values for the black tea samples and black tea infusion samples are given in Table 6 and 7, respectively. The tables show that the recovery results for the metals lie in the range 93 – 110 %. The results show the validity of the proposed methods for tea leaves and tea infusion analysis.

Table 6. Recovery test results for black tea leaves samples.

Metal	[a]Conc. in sample (mg/kg)	Amount added (mg/kg)	[a]Conc. in spiked sample (mg/kg)	Amount recovered (mg/kg)	[b]Recovery (%)
K	12330 ± 23	200	12548 ± 27.0	217 ± 35	109 ± 18
Ca	4505 ± 19	200	4697 ± 15.0	192 ± 16	96 ± 8
Mg	3541 ± 27	200	3761 ± 6.0	220 ± 28	110 ± 14
Mn	1240 ± 6.0	200	1436 ± 4.0	196 ± 7.0	98 ± 3
Fe	439 ± 6.0	200	654 ± 5.0	215 ± 6.0	108 ± 3
Zn	28.4 ± 0.5	10	38.2 ± 0.3	9.85 ± 0.58	99 ± 6
Cu	11.3 ± 0.2	10	22.0 ± 0.5	11 ± 0.54	110 ± 5
Co	[c] nil	-	-	-	-
Cd	nil	-	-	-	-
Pb	nil	-	-	-	-

[a] Concentration values are average of three analyzed samples ± standard deviation.
[b] Recovery values are mean ± standard deviation.
[c] Concentration values of the studied metals are below method detection limit.

Table 7. Recovery test results for black tea infusion samples.

Metal	[a]Conc. in sample	Amount added	[a]Conc. in spiked sample	Amount recovered	[b]Recovery (%)
K	6227 ± 7	100	6329 ± 3.00	102 ± 7	102 ± 7
Ca	550 ± 3	100	647 ± 4.00	97 ± 5	97 ± 5
Mg	821.9 ± 1.20	100	932 ± 4.69	110 ± 5	110 ± 5
Mn	454 ± 2	100	547 ± 4.00	93 ± 4	93 ± 4.3
Fe	107 ± 1	100	198 ± 5.00	91.4 ± 5.4	91 ± 5.4
Zn	9.17 ± 0.35	10	18.7 ± 0.3	9.56 ± 0.46	96 ± 4.6
Cu	7.67 ± 0.12	10	17.4 ± 0.2	9.73 ± 2.6	97 ± 2.6
Co	[c] nil	-	-	-	-
Cd	nil	-	-	-	-
Pb	nil	-	-	-	-

[a] Concentration values are average of three analyzed samples ± standard deviation.
[b] Recovery values are mean ± standard deviation.
[c] Concentration values of the studied metals below method detection limit.

3.5. Levels of Metals in Black Tea Leaves

The levels of total metal contents of the three black tea brands shows that the black tea leaves could be a source of nutrients in addition to its use as a stimulant. The levels of the essential and non-essential metals in the black tea leaves differ widely. The essential metals are quantified by the available instrumental technique (FAAS). On the other hand the non-essential heavy metals are found to be below the analytical method detection limit, indicating that the commercially available Ethiopian black tea leaves contain only the essential elements in appreciable amounts. The concentrations of the metals in each of the three types of the commercially available Ethiopian black tea brands are reported and discussed in the following subtopics.

3.5.1 Levels of metals in Wush Wush tea leaves

The levels of the metals in the Wush Wush black tea leaves are given in Table 8. The results show that out of the ten analyzed metals the alkali and alkali earth metals were with larger amount compared to the heavy metals. K is with the largest amount with dry weight of 12402 ± 28 mg/kg followed by Ca, 4419 ± 35 mg/kg and Mg, 3538 ± 6 mg/kg was also with appreciable amount next to Ca. The Wush Wush black tea leaves contained heavy metals in smaller amount than the K, Ca and Mg. The amount of Mn in the sample was 1242 ± 12 mg/kg preceding Fe, 319 ± 25 mg/kg. Zn was found in larger amount than Cu, with concentrations of 21.2 ± 0.4 mg/kg and 9.1 ± 0.014 mg/kg. The essential heavy metal, Co, was found to be below the detection limit of the available method. The non-essential heavy metals, Cd and Pb, were also found to be below the detection limit of the instrument. The amount of the analyzed metals in the Wush wush black tea leaves could be arranged in an increasing order of K > Ca > Mg > Mn > Fe > Zn > Cu. Figure 1 and 2 shows the level of K, Ca and Mg and the heavy metals, respectively in decreasing order.

Table 8. Levels of metals in Wush Wush black tea leaves.

Metal	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
Concentration	12402 ± 28	4419 ± 35	3538 ± 6	1242 ± 12	319 ± 25

Metal	Zn (mg/kg)	Cu (mg/kg)	Co (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
Concentration	21.2 ± 0.4	9.1 ± 0.14	nil	nil	nil

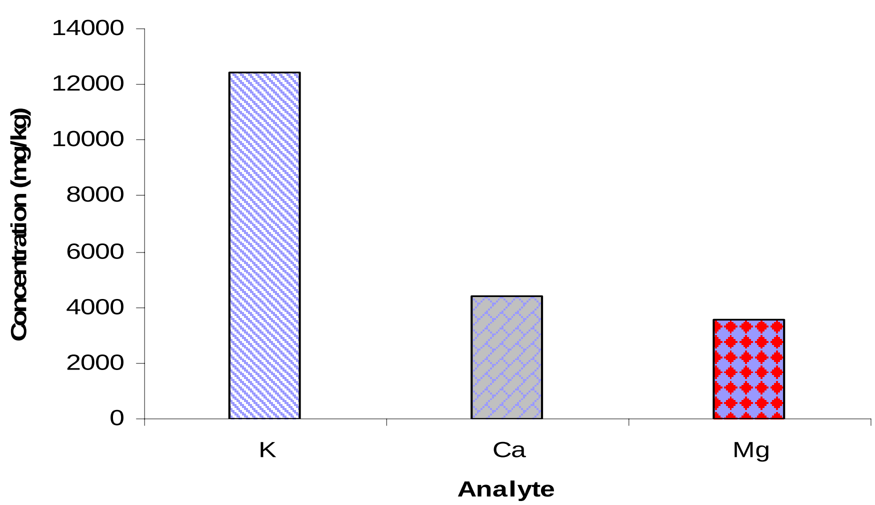

Figure 1. Levels of K, Ca and Mg in Wush Wush black tea leaves.

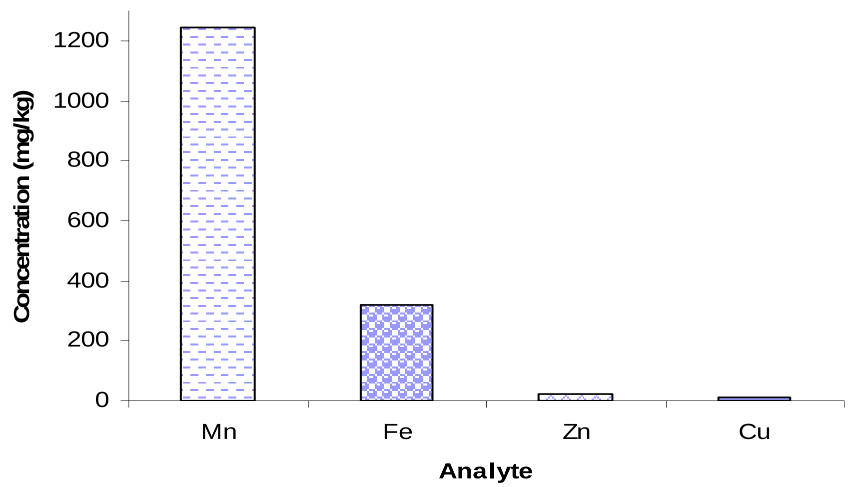

Figure 2. Levels of Mn, Fe, Zn and Cu in Wush Wush black tea leaves

3.5.2 Levels of metals in Gumero black tea leaves

Table 9 presents the level of the analyzed metals in Gumero black tea leaves. The results show that K, 11503 ± 79 mg/kg was the largest of the nine metals. Ca and Mg were found in larger amount next to K with values of 3821 ± 9 mg/kg and 3416 ± 33 mg/kg, respectively. Out of the heavy metals, Mn, 1297 ± 4 mg/kg was found to be in larger amount preceding Fe, 363 ± 3 mg/kg. Zn and Cu were found to be 21.6 ± 0.3 and 11.5 ± 0.1, respectively. Co and the other two nonessential heavy metals were found to be below the detection limit. The increasing trend was found to be the same as the Wush Wush black tea leaves. That was K > Ca > Mg > Mn > Fe > Zn > Cu. Figure 3 and 4 show the level of the alkali and alkali earth metals and heavy metals, respectively in decreasing order.

Table 9. Levels of metals in Gumero black tea leaves.

Metal	K (mg/kg)	Ca(mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
concentration	11503 ± 79	3821 ± 9	3416 ± 33	1297 ± 4	363 ± 3

Metal	Zn(mg/kg)	Cu(mg/kg)	Co (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
concentration	21.6 ± 0.3	11.5 ± 0.1	nil	nil	nil

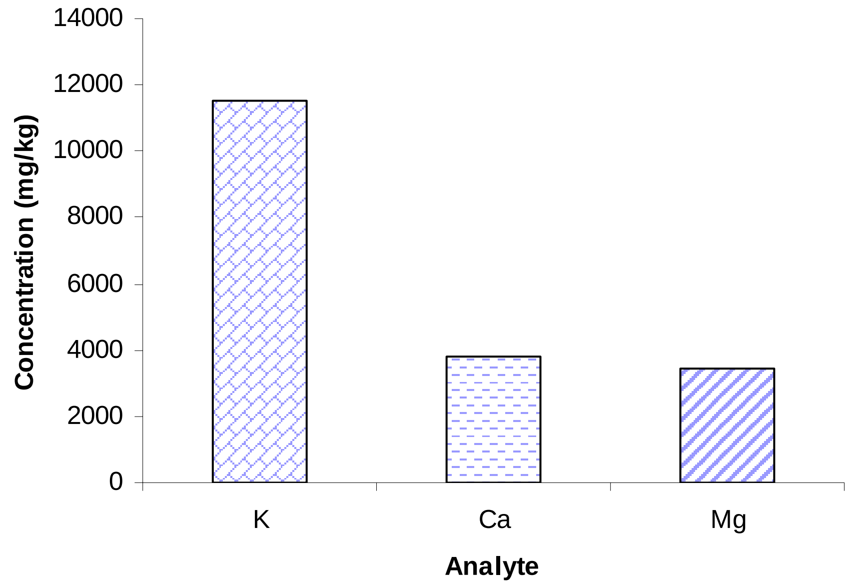

Figure 3. Levels of K, Ca and Mg in Gumero black tea leaves.

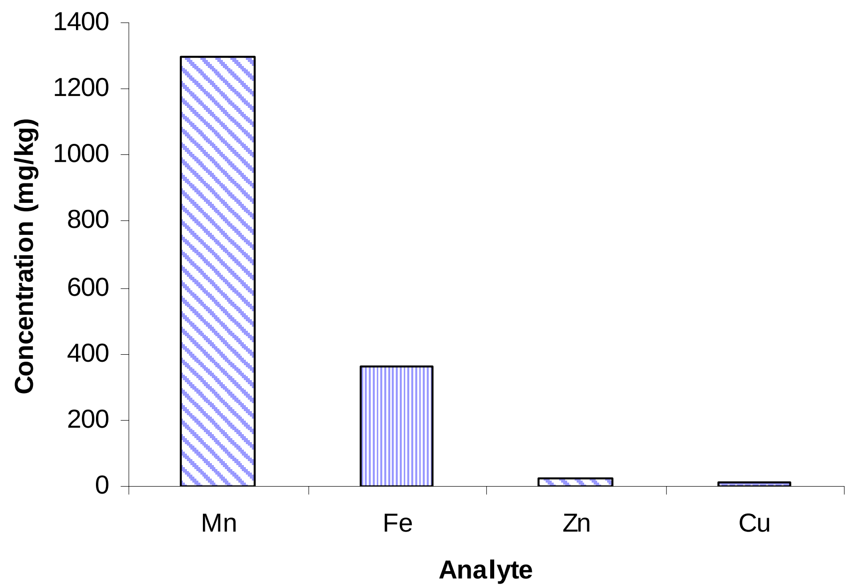

Figure 4. Levels of Mn, Fe, Zn and Cu in Gumero black tea leaves.

3.5.3 Levels of metals in Black Lion black tea leaves

The third type of the black tea brand (Black Lion black tea) was also analyzed for its total metal content and the levels of the metals found are given in Table 10. Like the previous two black tea brands, Black Lion black tea also was found to contain the largest amount of K, 13780 ± 33 mg/kg. Ca, 4118 ± 6 mg/kg and Mg, 3219 ± 5 mg/kg were found to be next to K. All the heavy metals were found in lesser amount than K, Ca and Mg. Mn, 1421 ± 4 mg/kg was found to be larger than Fe, 467 ± 3 mg/kg. Zn, 20.2 ± 0.1 mg/kg was found to be around two fold of Cu, 10.3 ± 0.1 mg/kg. Figure 5 and 6 shows the level of K, Ca and Mg and the heavy metals in Black Lion black tea leaves, respectively.

Table 10. Levels of metals in Black Lion black tea leaves.

Metal	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
concentration	13780 ±33	4118 ± 6	3219 ± 5	1421 ± 4	467 ± 2

Metal	Zn (mg/kg)	Cu (mg/kg)	Co (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
concentration	20.2 ±0.1	10.3 ± 0.12	nil	nil	nil

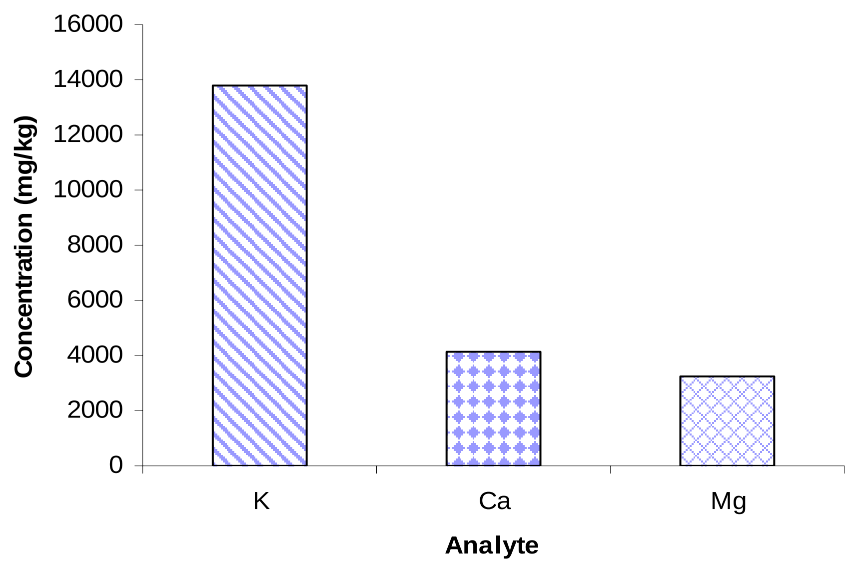

Figure 5. Levels of K, Ca and Mg in black Lion black tea leaves.

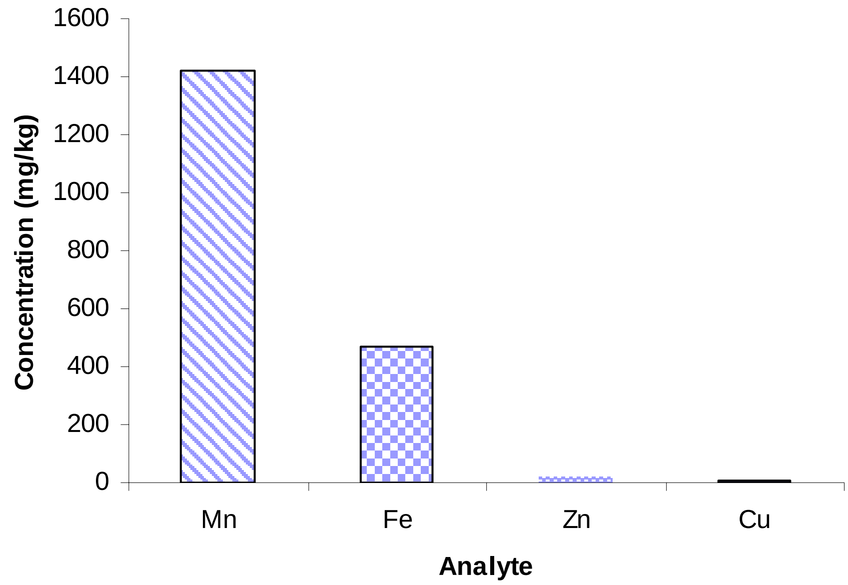

Figure 6. Levels of Mn, Fe, Zn and Cu in Black Lion black tea leaves

3.5.4 Comparison of the levels of metals in the black tea leaves of the three black tea brands

From the results of the three types of black tea leaves it was found that the total metal content lie in certain range for each of the metals as it is presented on Table 11.

Table 11. Range of the values for the metal contents in the three types of black tea leaves.

metal	range	metal	range
K	11503-13780 mg/kg	Fe	319-467 mg/kg
Ca	3821-4419 mg/kg	Zn	20.2-21.6 mg/kg
Mg	3219-3538 mg/kg	Cu	9.1-11.5 mg/kg
Mn	1242-1421 mg/kg		

Table 12 presents the black tea brands as 1st, 2nd and 3rd showing whether they contain the highest, higher or lower concentrations for each of the metals in their black tea leaves.

Table 12. Three black tea brands ranked as 1st, 2nd and 3rd based on their content of each metal.

Rank	K	Ca	Mg	Mn	Fe
1st	Black lion	Wush Wush	Wush Wush	Black lion	Black Lion
2nd	Wush Wush	Black Lion	Gumero	Gumero	Gumero
3rd	Gumero	Gumero	Black Lion	Wush Wush	Wush Wush

Rank	Zn	Cu
1st	Gumero	Gumero
2nd	Wush Wush	Black Lion
3rd	Black Lion	Wush Wush

The results obtained in the present study could be compared with the results that have been reported by different authors. In fact there is a difference in the sample preparation and analysis techniques.

The metal content of tea leaves span a wide range since they depend upon the country and area of origin, the mineral content of the soil, and the part and age of the tea plant analyzed [53]. For instance, it was reported that plant nutrient concentrations such as K and Mg in the tea stands decreased in the order: young leaves (and buds) > mature leaves > branches > stems [54].

Nevertheless, some general trends are well established: for example, the predominance of potassium in tea leaf followed by calcium and magnesium was seen in many investigations [53].

Different papers reported the total metal contents of tea leaves. For instance, the ability of the tea plant to accumulate heavy metals, particularly Mn and Fe, to a lesser extent Zn and Cu was investigated. It has been shown that Mn (390-900 µg/g), Fe (23.90-513.00 µg/g), Zn (26.69-53.89 µg/g), Cu (22.12-40.66 µg/g), Pb (0.03-14.84 µg/g) > Co (nil-2.35 µg/g) > Cd (nil) [26].

In another investigation concentration of Cu in tea leaves was found in the range 33.9-19.8 mg/kg [55].

Other study reported that K (21,904–26,883 mg/kg) and Ca (3,370–4,823 mg/kg) were also determined in black tea leaves [56].

The amount of heavy metals in tea leaves has been used as an indication for contamination of the environment by heavy metal particles [57].

Ca (3170-7980 μg/g), Mg (2060-2640 μg/g), Mn (790-1620 μg/g) and Zn (26.3-50.4 μg/g) were also found in different tea brands [58].

There was also a report on African and Asian tea that were found to contain Ca (3581.68-3511 μg/g), Mg (562.61-1567.91 μg/g), Mn (562.61-843.56 μg/g), Fe (153-178.97 μg/g), Zn (25.06-26.29 μg/g), Cu (12.4-19.34 μg/g), Pb (0.18-1.12 μg/g) and Co (0.21-0.23 μg/g) [2]. Roughly similar results were also reported [59].

The results reported by different authors are consistent with the results of the present study. Particularly, the increasing trend for the metals concentration is in agreement. The order is K > Ca > Mg > Mn > Fe > Zn > Cu and Co, Pb and Cd are too low to be detected by the available technique. In fact the concentration of the toxic heavy metals are expected to be very low in Ethiopian tea since these metals are related to environmental pollution caused by different industrial activities. In fact K content of the Ethiopian black tea leaves was found to be smaller than those reported by many authors.

The comparison of concentrations of metals in the Ethiopian black tea obtained in the present study with that reported in the rest of the world are given in Table 13.

Table 13. Comparison of concentrations of metals in the Ethiopian black tea with that reported in the rest of the world.

Origin	Concentration of metal in tea leaves (mg/kg)					Ref.
	K	Ca	Mg	Mn	Fe	
Ethiopia	11503-13780	3821-4419	3219-3538	1242-1421	319-467	present
India	18500-24000			371-663		[33]
Sri Lanka		4235-7980	2086-3200	790-1620		[58]
Japan	19200	4550	2070	503	134	[33]
Turkey				1107-1871		[32]
Africa		3581	1567.9	562	178	[2]
				842		[20]
Asia		3511.15	562.61	843	153.19	[2]
Poland				286-1290		[60]
Saudi Arabia				68.6-305	123.9-262.2	[26]

Origin	Concentration of metal in tea leaves (mg/kg)					Ref.
	Zn	Cu	Co	Pb	Cd	
Ethiopia	20.2-21.6	9.1-11.5	nil	nil	nil	present
India		1.6-35				[33]
Sri Lanka	26.3-40.5					[58]
Japan	36.6	27.7	0.506	0.709	0.018	[33]
Africa	25.06	19.14	0.21	1.12		[2]
Asia	26.29	12.34	0.23	0.18		[2]
Poland	22.3-28					[60]
Saudi Arabia	26.69-39.1	22.12-40.66	nil-2.35		nil-0.18	[26]

3.6. Level of Metals in Black Tea infusion Samples

Black tea infusions of the three black tea brands were analyzed to determine the level of the metals extracted into hot water. The results showed that the tea infusion (tea beverage) contains K, Ca and Mg in appreciable amount. It was also found that the essential heavy metals were extracted from the black tea leaves to tea infusion. The amounts of the metals extracted widely differ. K was the largest of all metals in all the three black tea brands.

3.6.1 Levels of metals in Wush Wush black tea infusion

Table 14 shows the levels of metals measured in tea infusion prepared from the Wush Wush black tea leaves. K, 5504 ± 71 mg/kg was found in highest amount. Unlike the black tea leaves, the amount of Mg, 723 ± 27 mg/kg was found to be higher than Ca, 533 ± 17 mg/kg. Mn, 423 ± 34 mg/kg was found to precede Fe, 56 ± 0.8 mg/kg. Zn and Cu were found in very small amounts, 4.89 ± 0.3 mg/kg and 2.82 ± 0.36 mg/kg, respectively. Co and the two non-essential heavy metals were found to be below the method detection limit. Figure 7 and 8 present the level of the metals.

Table 14. Levels of metals in Wush Wush black tea infusion.

Metal	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
Concentration	5504 ± 71	533 ± 17	723 ± 27	423 ± 34	56 ± 0.8

Metal	Zn (mg/kg)	Cu (mg/kg)	Co (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
Concentration	4.89 ± 0.3	2.82 ± 0.36	-	-	-

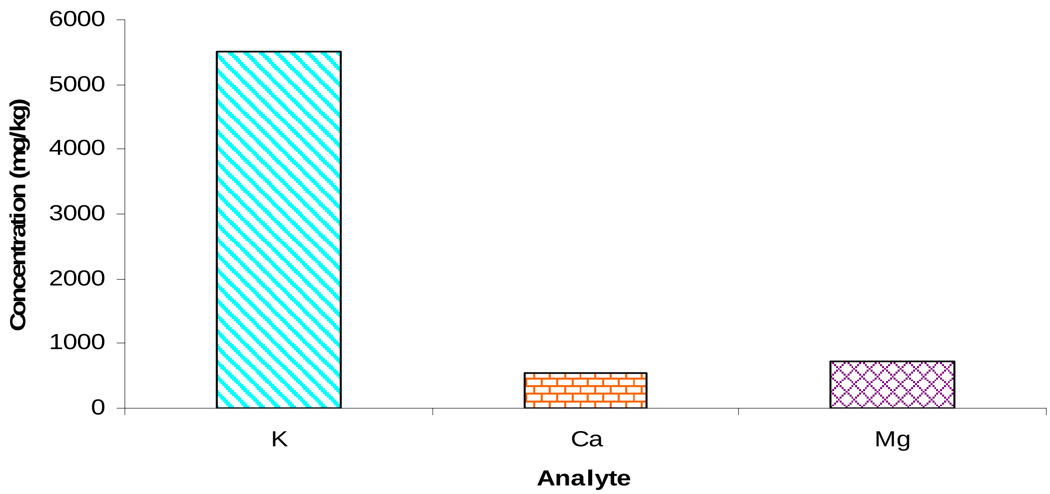

Figure 7. Levels of K, Ca and Mg in Wush Wush black tea infusion.

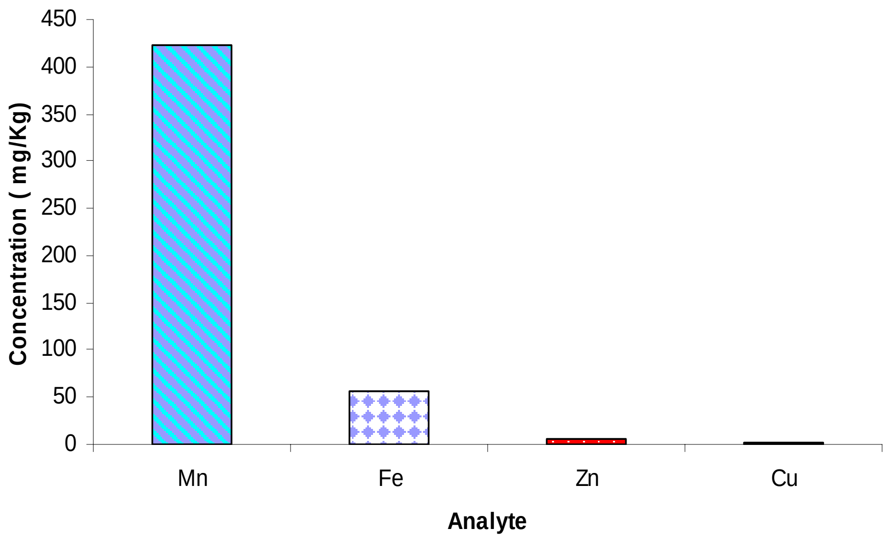

Figure 8. Levels of Mn, Fe, Zn and Cu in Wush Wush black tea infusion.

3.6.2 Levels of metals in Gumero black tea infusion

The tea infusion prepared from Gumero black tea leaves was analyzed and the amounts of the metals extracted were found to be in similar trend with that of Wush Wush black tea infusion. K was found to be 5961 ± 16 mg/kg followed by Mg and Ca, 757 ± 8 mg/kg and 510 ± 41, respectively. The essential heavy metals were also determined in the tea infusion and three of them were below the detection limit. Level of K, Ca and Mg and the heavy metals are plotted in Figure 9 and 10, respectively.

Table 15. Levels of metals in Gumero black tea leaves

Metal	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
Concentration	5961 ± 16	510 ± 41	757 ± 8	449 ± 2	49 ± 10

Metal	Zn (mg/kg)	Cu (mg/kg)	Co (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
Concentration	5.0 ± 0.2	1.92 ± 0.8	-	-	-

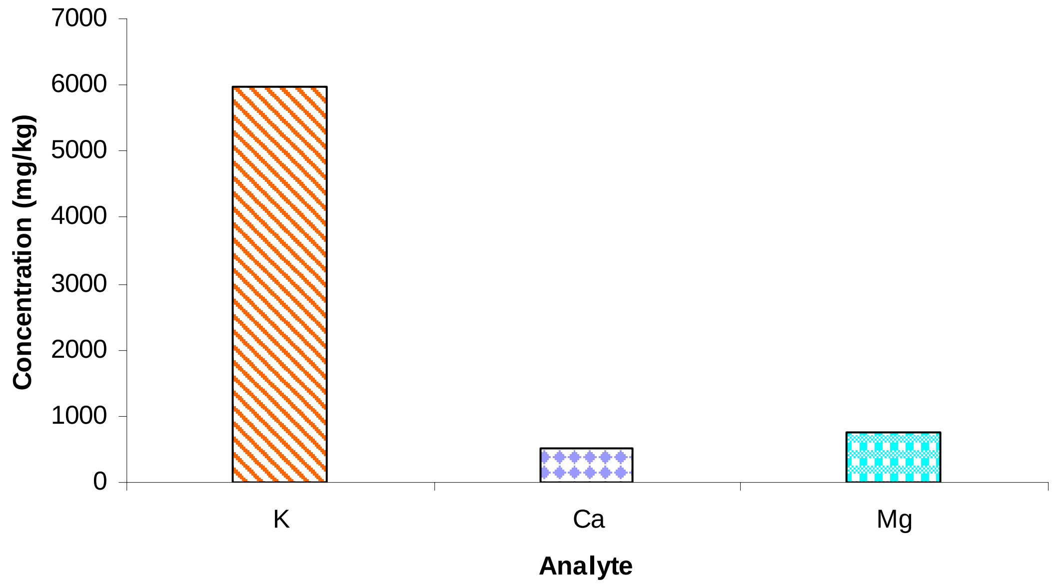

Figure 9. Levels of K, Ca and Mg in Gumero black tea infusion.

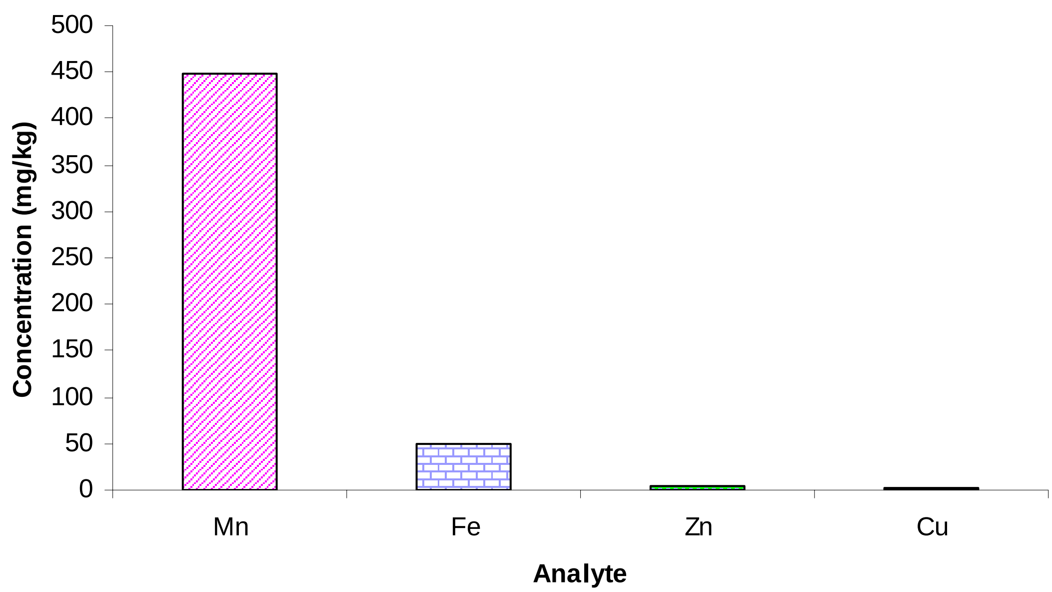

Figure 10. Levels of Mn, Fe, Zn and Cu in Gumero black tea infusion.

3.6.3 Levels of metals in Black Lion black tea infusion

In the tea infusion of the Black Lion black tea the order was changed compared to the previous two brands. Mn, 672 ± 23 mg/kg level was found to be higher than Ca, 500 ± 29 mg/kg. The non-essential heavy metals and Co were too low to be detected by the available method.

Table 16. Levels of metals in Black Lion black tea leaves

Metal	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
Concentration	6197 ± 61	500 ± 29	816 ± 12	672 ± 23	78.4 ± 6.3

Metal	Zn (mg/kg)	Cu (mg/kg)	Co (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
Concentration	4.97 ± 0.35	3.17 ± 1.1			

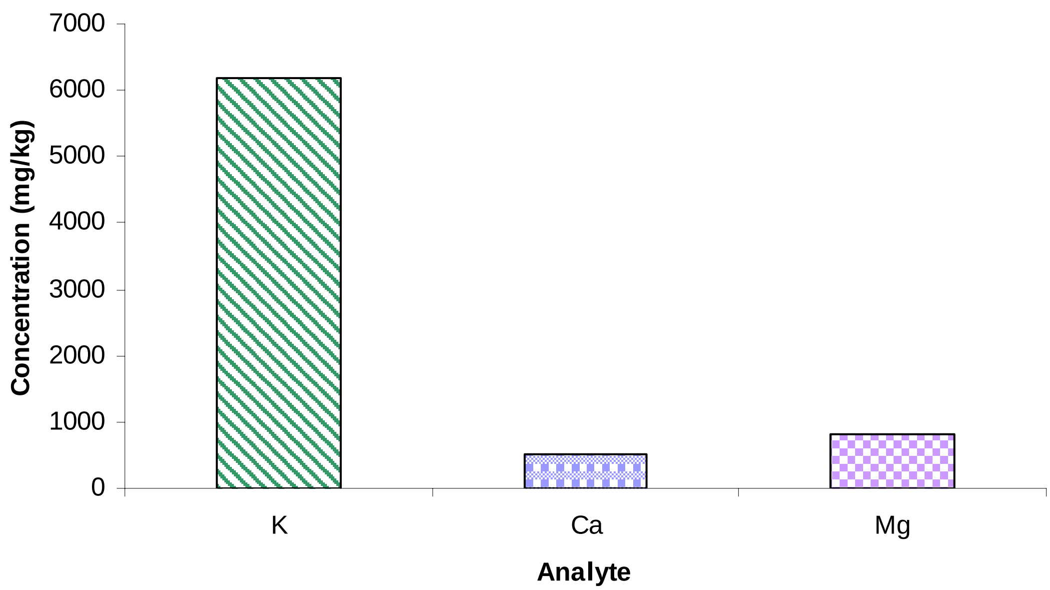

Figure 11. Levels of K, Ca and Mg in Black Lion black tea infusion.

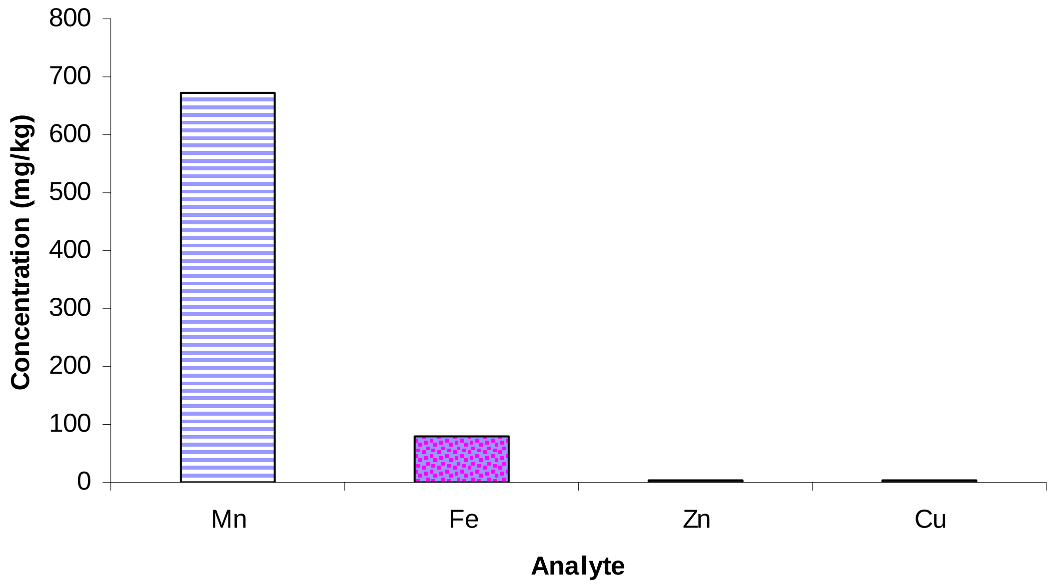

Figure 12. Levels of Mn, Fe, Zn and Cu in Black Lion black tea infusion.

3.6.4 Comparison of the levels of metals in the black tea infusions of the three black tea brands

The levels of the metals extracted from the three black tea brands were found to differ by certain values and lie in the range indicated in Table 17.

Table 17. Range of the values for the metal contents in the three types of black tea infusions.

Metal	Range	Metal	Range
K	5504 - 6197 mg/kg	Fe	49 - 78 mg/kg
Ca	500 - 533 mg/kg	Zn	4.89 - 5.0 mg/kg
Mg	723 - 816 mg/kg	Cu	1.92 - 3.17 mg/kg
Mn	423 - 672 mg/kg		

In Table 18 the black tea brands are ranked as 1st, 2nd and 3rd showing whether they contain the highest, higher or lowest concentrations for each of the metals in their black tea infusions.

Table 18. Three black tea brands ranked as 1st, 2nd and 3rd based on their content of each metal.

Rank	K	Ca	Mg	Mn	Fe
1st	Black Lion	Wush Wush	Black Lion	Black Lion	Black Lion
2nd	Gumero	Gumero	Gumero	Gumero	Wush Wush
3rd	Wush Wush	Black Lion	Wush Wush	Wush Wush	Gumero

Rank	Zn	Cu
1st	Gumero	Black Lion
2nd	Black Lion	Wush Wush
3rd	Wush Wush	Gumero

Different papers have been published on metal contents of tea beverages. For instance, in tea beverages the concentrations of toxic heavy metals, Pb and Cd and the essential heavy metal, Co, were too low to be detected. Concentrations of Mn and Fe in tea infusion range between 68.60-305.60 µg/g and19.00-135.00 µg/g, respectively. Concentrations of Zn and Cu were also found to be 8.55-28.60 µg/g and 0.7-6.95 µg/g, respectively [26]. Mn levels of 0.51± 0.11 mg/100 g in black tea infusions were also reported [28].

In other study considerable amount of minerals were found in tea beverages. For instance K (40-46 mg), Mg (2.7-3.0 mg), Ca (0.48-0.66 mg) and traces of Mn (0.28-0.40 mg), Fe (0.16-0.20 mg) and Cu (12-22 µg) were found in a cup of black tea beverage [61].

The result obtained from the present study was in agreement with most of the papers published on this issue.

The comparison of concentrations of metals in the Ethiopian black tea infusion obtained in the present study with that reported in the rest of the world is given in Table 19.

Table 19. Comparison of concentrations of metals in the Ethiopian black tea infusion with that reported in the rest of the world.

Origin	Concentration of metal in tea infusion (mg/L)					Ref.
	K	Ca	Mg	Mn	Fe	
[a]Ethiopia	55.04 – 61.97	50.0 – 53.3	72.3 – 81.6	42.3 – 67.2	0.49 – 0.78	present
Japan	305	4.03	19.6	5.16	0.18	[33]
Turkey				366-421		[32]
Poland				1.56-6.02		[60]
Saudi Arabia				0.662-3.05	0.132-1.35	[26]

Origin	Concentration of metal in tea infusion (mg/L)					Ref.
	Zn	Cu	Co	Pb	Cd	
[a]Ethiopia	0.0489 – 0.0500	0.0192 – 0.0317	nil	nil	nil	present
Japan	0.288	0.102	0.506	0.709	0.018	[33]
Poland	0.107 0.121					[60]
Saudi Arabia	0.0572-0.2866	0.0033-0.1748	nil		nil	[26]

[a] For Ethiopian tea (present study) the mg/Kg could be changed to mg/L by dividing the value by 100.

3.7. Efficiency of Extraction from Black Tea Leaves to Black Tea Infusions (to hot water)

Table 20 shows that the percentage of metals extracted from the tea leaves to tea infusion and these values were found to vary widely. For instance, the largest amount of extraction was found for K, 47 %. Mn, 38.6% was the second highly extracted metal followed by Cu and Zn. The rate of extraction of the metals from tea leaves to tea infusion could be put in the following order: K > Mn > Cu > Zn > Mg > Fe > Ca.

Table 20. Percentage of the metals extracted from black tea leaves to the tea infusion.

Metal	Wush Wush (%)	Gumero (%)	Black Lion (%)	Average (%)
K	44.4	51.8	45.0	47.1
Ca	12.1	13.4	12.1	12.5
Mg	20.4	21.9	25.4	22.6
Mn	34.0	34.6	47.2	38.6
Fe	17.5	13.5	16.8	15.9
Zn	23.0	23.1	24.6	23.5
Cu	30.9	16.6	30.8	26

The next three figures show the difference in metal contents for the black tea leaves and their infusion.

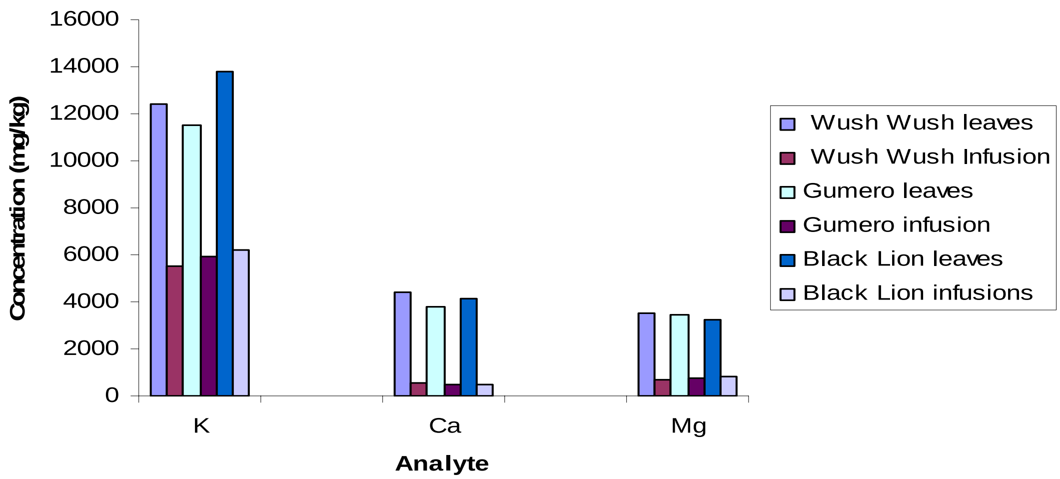

Figure 13. Levels of K, Ca and Mg in three black tea leaves and their infusions.

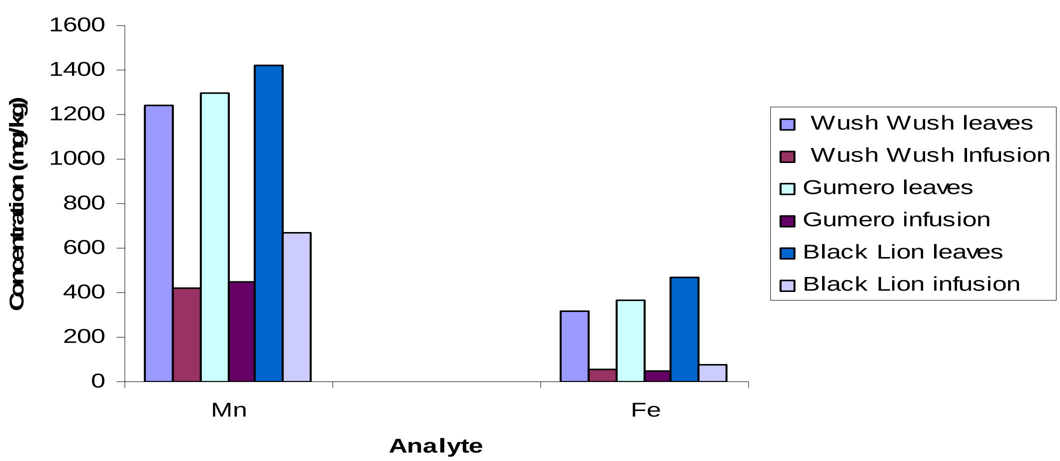

Figure 14. Levels of Mn and Fe in three black tea leaves and their infusions.

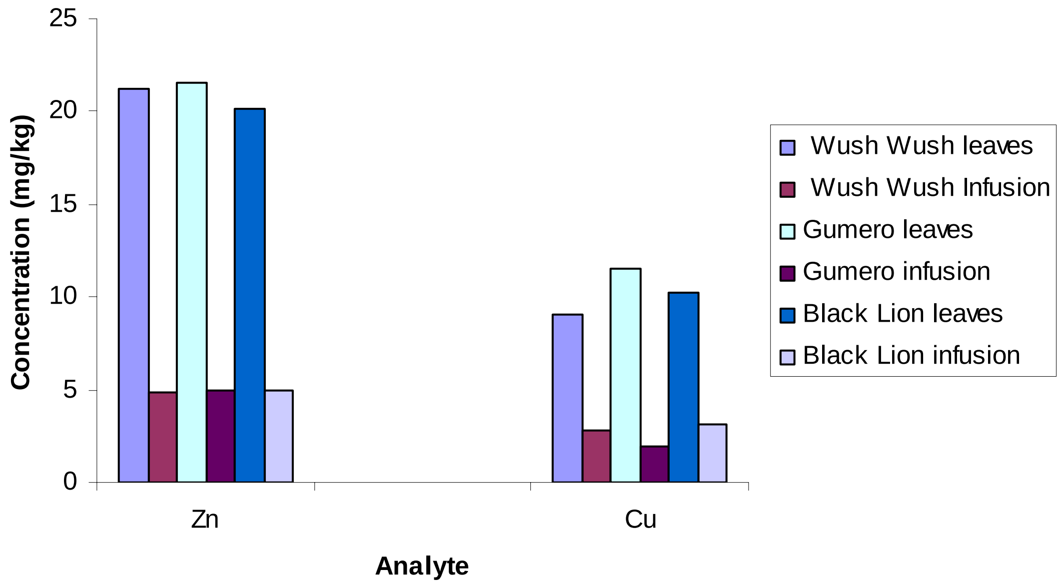

Figure 15. Levels of Zn and Cu in three black tea leaves and their infusions.

3.8. Statistical Analysis of Data (ANOVA)

Statistical analysis of data was made to check whether there was a significant difference in metal contents between the three commercially available black tea leaves and their infusions. Analysis of Variance (ANOVA) is a powerful statistical technique which can be used to separate and estimate the different causes of variation. A one-way ANOVA was used for this study and calculations were made using the Mintab software. For each of the analysis six means (three for each of tea sample) were used. The degree of freedom for each of the data analysis was 5. For $P > 0.05$ the levels of each particular metal in the three Ethiopian black tea leaves are not significant.

The p values for each of three combinations of black tea leaves are given in Table 21. The table shows that the difference in the level of both Ca ($P=0.018$) and Cu ($p=0.008$) in Wush Wush and Gumero black tea leaves was found to be significant. Comparing Wush Wush abd Black Lion black tea leaves, it was possible to see that level of Mg($P=0.018$)in the two black tea brands is significantly different. It was also the case for Mn ($P=0.002$)and Fe($P=0.038$) in the two black tea leaves samples. The third comparison was made to find out if there is a significant difference in the amount of each particular metal in Gumero and Black Lion black tea leaves and it was observed that the Fe content in the two black tea brands was differ significantly.

Table 21. P values of different combination of the three black tea leaves (degree of freedom is 5).

Metal	WushWush with Gumero Black tea leaves	Wush Wush with Black Lion black tea leaves	Gumero with Black Lion tea black tea leaves
	P value	P value	P value
K	0.121	0.366	0.719
Ca	0.018	0.078	0.163
Mg	0.276	0.018	0.183
Mn	0.822	0.002	0.628
Fe	0.459	0.038	0.004
Zn	0.915	0.544	0.778
Cu	0.008	0.199	0.218

Statistical analysis of data was also made for the tea infusions prepared from the three black tea brands. Table 22 presents the P values for each particular metal in each of the three combinations (Wush Wush-Gumero, Wush Wush- Black Lion, Gumero-Black Lion). Amount of Ca in Wush Wush and Gumero black tea infusions differ significantly.

In Wush Wush and Black Lion black tea infusion, except Zn and Cu the level of the K, Ca, Mg, Mn and Fe differ significantly. Level of Mn (P= 0.04) in Gumero and Black Lion black tea infusions was with significant difference as it was the case for Fe (P= 0.007)

The significant variation in the metal contents of each of the three commercially available Ethiopian black tea leaves as well as their infusion might arise from the chemical composition of the leaves it self and the specific environmental conditions in which the tea plant was cultivated.

Table 22. P values of different combination of the three black tea infusions (degree of freedom is 5).

Metal	WushWush with Gumero Black tea leaves	Wush Wush with Black Lion black tea leaves	Gumero with Black Lion tea black tea leaves
	P value	P value	P value
K	0.065	0.012	0.303
Ca	0.005	0.017	0.288
Mg	0.485	0.018	0.182
Mn	0.751	0.000	0.040
Fe	0.134	0.015	0.007
Zn	0.107	0.953	0.491
Cu	0.139	0.777	0.405

4. Conclusions

The level of essential and non-essential elements in commercially available three brands of Ethiopian black tea leaves (Wush Wush, Gumero, and Black Lion) and their infusion was determined by flame atomic absorption spectrometry. The metal contents of the black tea leaves were found to be higher than those of tea infusions. In both black tea leaves and their infusion level of Co and the toxic heavy metals (Cd and Pb) were found to be too low to be detected by the available technique (FAAS) indicating that the commercially available Ethiopian black teas are free from environmental pollution due to non-essential heavy metals which are usually caused by industrial activities. The level of each of the metals in each of the three brands could be put in the following order K (11503-12402 mg/kg) > Ca (3821-4419 mg/kg) > Mg (3219-3538 mg/kg) > Mn (1242-1421 mg/kg) > Fe (319-467 mg/kg) > Zn (20.2-21.6 mg/kg) > Cu (9.1-11.5 mg/kg). The general increasing (or decreasing trend) is similar with those reported for many other black tea leaves of other countries. Nevertheless, the K content of Ethiopian black tea leaves was found to be less than those reported for black tea leaves of other origins. The level of the other metals was found to be comparable with other literature values. Except K the levels of the essential metals in Black tea infusion of commercially available Ethiopian black tea leaves were found to be comparable with that of the rest of the world. This study sows that the commercially available Ethiopian black tea could be source of dietary minerals and trace metals. The extraction efficiency of the metals in to tea infusion varied widely indicating that these metals exist in tea leaves in different forms. Thus, further research for the speciation of the metals in tea leaves should be conducted.

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