ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

LEVELS OF ESSENTIAL AND NON-ESSENTIAL METALS IN LEAVES OF THE TEA PLANT (*Camellia sinensis* L.) AND SOILS OF WUSHWUSH FARMS, ETHIOPIA

BY

MICHAEL YEMANE BEKHIT

JULY 2006

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MICHAEL YEMANE BEKHIT

DEPARTMENT OF CHEMISTRY

FACULTY OF SCIENCE

Approved by the examining board: SIGNATURE

1. Prof. B. S. CHANDRAVANSHI _____
   Research Advisor
2. Dr. TADDESE WONDIMU _____
   Research Advisor
3. Prof. HAILEMICHAEL ALEMU _____
   Examiner
4. Dr. NIGUSSIE WODAJO _____
   Examiner

To my mother, father and brothers

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Levels of Essential and Non-Essential Metals in Leaves of the Tea Plant (*Camellia sinensis* L.) and Soils of Wushwush Farms, Ethiopia

By: Michael Yemane Bekhit

Advisors: Prof. B. S. Chandravanshi and Dr. Taddese Wondimu

Abstract

Five tea clones of the *C. assamica* variety grown in Wushwush tea plantation farms, Ethiopia were analyzed for their contents of essential, non-essential and toxic metals (K, Ca, Mg, Fe, Mn, Cu, Zn, Na, Cd and Pb) by atomic absorption flame emission spectroscopy. Among the macronutrient metals, K was the most abundant element in the tea leaves and soils. Both the tea leaves and the soils showed similar accumulation pattern in their contents of the studied macronutrients.

Mn was the predominant micronutrient heavy metal in the tea leaves tissue. Level of Fe in the leaf tissue was found to be the second abundant micronutrient next to Mn whereas concentrations of Cu and Co were relatively lower both in the soil and tea samples. Fortunately, the toxic heavy metals Pb and Cd in the leaf tissues were too low to be detected by the analytical technique used in this study.

The soils were observed to be acidic (pH 5.04 - 5.49) with high organic matter (5.48 - 6.02%). Fe was the most abundant metal followed by Mn, Na and Zn in the soils. Unlike the tea leaves, the soils were found to contain the toxic metal, Cd (0.02 - 1.10 mg/kg). The levels of most of the metals determined in this study compared well with those reported for tea leaves from some other parts of the world.

Key words: Tea clones, Tea leaves, Macronutrients, Micronutrients, Toxic metals, Organic matter, Atomic absorption flame emission spectroscopy.

1. INTRODUCTION

Origin and Distribution of Tea

The original home or 'the primary center of origin' of tea was South-East Asia, i.e. at the point of intersection between the 29^{o} N (latitude) and 98^{o} E (longitude) near the source of the Irrawaddy river at the confluence of North-East India, North Burma, South-West China and Tibet provinces. Tea thrives well within the latitudinal ranges between 45^{o} N to 34^{o} S that cross about 52 countries [1].

Tea is the oldest, most popular, non-alcoholic caffeine-containing beverage in the world [1]. It is prepared from the dried leaves of the tea plant *Camellia sinensis* L. - the evergreen tropical shrub [2]. Chinese were the first to use tea as medicinal drink, later as beverage and have been doing so for the past 3000 years [3].

From the earliest times tea was renowned for its properties as a healthy, refreshing drink. The modern term "tea" derives from early Chinese dialect words such as *Tchai*, *Cha* and *Tay* used both to describe the beverage and the leaf. Tea was introduced to Japan in 805 AD as a medicine by Zen Buddhist missionaries because of its meditation enhancing properties. In 1484, the sacred Japanese Tea Ceremony was introduced [4].

In the 1500s, tea arrived in Portugal, as the Portuguese were the first to establish trade relations with China. In the 1600s, the French and the Dutch served tea in restaurants and introduced tea to America by exporting through the Dutch colonists. Ironically, the British who are known as a great nation of tea drinkers were the last of the seafaring nations to be introduced to tea drinking [4].

It was after its well distribution in Asian countries that tea was introduced to Africa. It was first successfully introduced to Nyasaland (now Malawi) in 1886 and the first estate was planted in 1891 [5].

Tea was introduced to East Africa at the beginning of the 20th century which led to commercial production in the 1920's and 1930's in Kenya, Tanzania, and Uganda. Briefly, it was first

planted in East Africa in 1900 at Entebbe in Uganda. The growth of the tea industry was first slow and by 1925 there were only 130 ha in East Africa. In that year, however, a rapid expansion of estates begun when international tea companies bought large hectares of land [5].

Botanical Classification of Tea

The tea plant, *Camellia sinensis* (L.) O. Kuntze, family *Theaceae*, is naturally a small evergreen, perennial, cross-pollinated plant and grows naturally as tall as 15 m. However, under cultivated condition, the bush height of 60 - 100 cm is maintained for harvesting the tender leaves (Figures 1a & 1b), which continues even more than 100 years [1].

(a)

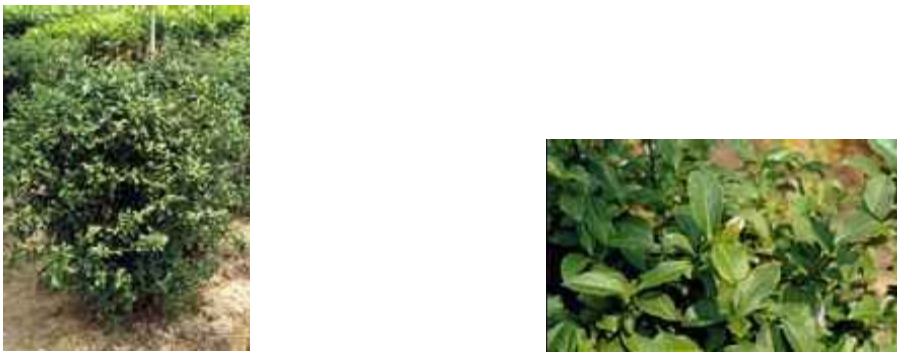
(b)

Figure 1. (a) A single pruned tea plant under cultivation. (b) Pluckable tender leaves of the tea plant.

Leaves of the tea plant are leathery, glossy on the upper surface, elliptic to obovate or lanceolate, 3 - 12.5(- 30) cm long, margin serrate, and apex acute to acuminate [6]. The flowers are white in colour and born singly or in pairs at the axils. The fruits are green in color with 2 - 3 seeds and start bearing within 5 - 6 years after planting [1].

The cultivated taxa of tea comprise of three main natural hybrids. They are: *C. sinensis* (L.) O. Kuntze or China type, *C. assamica* (Masters) or Assam type and *C. assamica* sub spp. *lasiocalyx* (Planchon ex Watt.) or Cambod or Southern type. Two types, which are well known, are the China and Assam: less common is the Cambod [1]. Alternatively, the China type may be described as *C. sinensis* var. *sinensis* and the Assam type as *C. sinensis* var. *assamica* [7]. The nature of leaf is the main criterion by which three types of tea are classified [1].

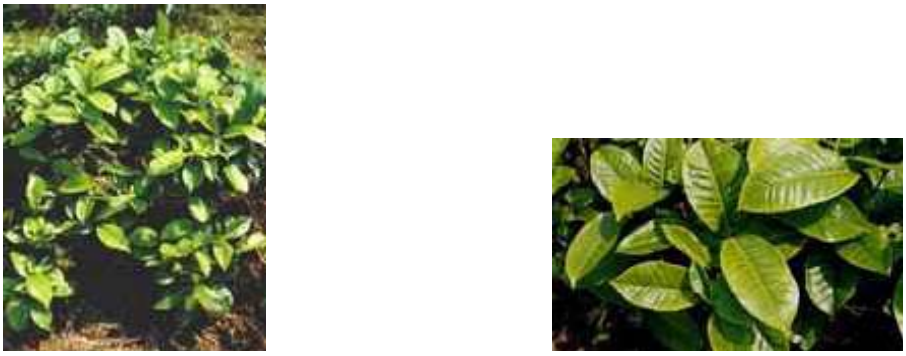
Camellia sinensis L. or the China tea plant is a big shrub, 1 - 2 m tall with many virgate stems arising from the base of the plant near the ground. The leaf is hard, thick and leathery; surface matt, marginal veins indistinct and appear sunken in lamina. Leaf blade is elliptic with obtuse or broadly obtuse apex; leaf base is cuneate, margin bluntly serrulate to sinuate-serrulate with more or less incurved teeth, glabrous above and villose below when young, becoming sparsely villose as the leaf ages. Young leaves are garnet-brown through ox-blood to purple in colour [8] (Figure 2).



(a) (b)

Figure 2. The bush (a) and leaves (b) of the *C. sinensis* variety or the China tea plant [8].

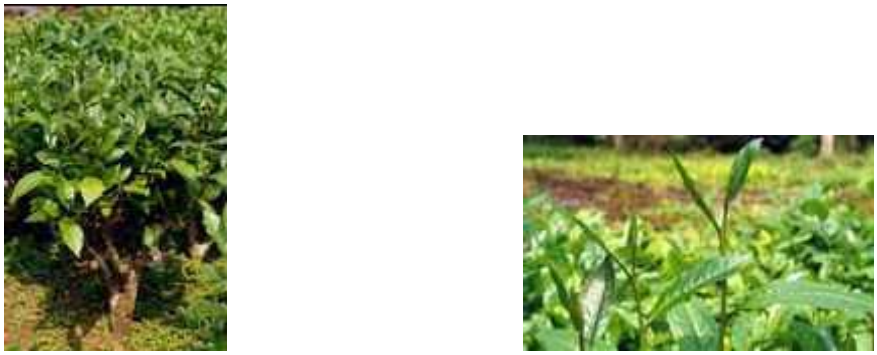
Camellia assamica (Masters) or the Assam tea plant is a small tree, 10 - 15 m tall with a trunk sometimes up to one third of its height, possesses a robust branch system. In typical plants, leaf is dependent, thin, and glossy with more or less acuminate apex and distinct marginal veins. Leaf blade is usually broadly elliptic, 8 - 20 cm long and 3.5 - 7.5 cm wide, leaf base is cuneate, margin obscurely denticulate to bluntly wide-serrulate, glabrous or persistently hairy on the midrib below. Flowers are single or in pair on the cataphyllary axils, pedicels are with scars of 3 caduceus bracteoles, smooth and green. Number of sepals is 5 - 6 and unequal, leathery, and persistent. Petals are 7 - 8, white, occasionally with pale yellow pigmentation at the base of petals. Stamens are numerous as in *C. sinensis* [8] (Figure 3).



(a) (b)

Figure 3. The bush (a) and leaves (b) of the *C. assamica* variety or the Assam tea plant [8].

Camellia assamica sub spp. *lasiocalyx* (Planchon ex Watt.) or the Cambod or Southern form of tea is a small fastigiated tree, 6 - 10 m tall, with several upright, almost equally developed branches. Leaf is more or less erect, glossy, and yellowish-green when young, light-green at maturity changing to coppery-yellow or pinkish-red from autumn till the end of the season. Petiole is pinkish-red at the base. Leaf size is intermediate between *sinensis* and *assamica*, broadly elliptic, marginal veins are not very prominent. Number of ovary is 3 - 4, sometimes 5-locular, style 3 - 5, free nearly up to half the length, ad pressed, straight with apical or linear stigma. On the other floral characters, it resembles the Assam plant, with the difference that 4 or more bracteoles are found on the pedicel of flowers [8] (Figure 4).



(a) (b)

Figure 4. The bush (a) and leaves (b) of the *C. assamica* sub spp. *lasiocalyx* or the Cambod or Southern type [8].

Categories of Tea

Tea has green, shiny leaves with jagged edges. Only the top two leaves and the unopened leaf bud of the tea plant are used to make good quality tea because they have tender, young part with the best flavor. Tea bushes begin to return a viable crop after eight years and can keep producing for more than 100 years [4].

The principal categories of tea – green, black and oolong – originate from a single tea plant, *Camellia sinensis* L. a white-flowered evergreen [9]. The method of processing the leaf distinguishes the three types [10].

Green tea is the type which keeps the original color of the tea leaves without fermentation during processing [11]. It is made by steaming or otherwise heating the leaves immediately after plucking to prevent the fermentation that makes black tea. Then the leaves are rolled and dried [10].

Black tea, also known as "red tea", is the category which is fermented before baking; it is a later variety developed on the basis of the green tea [11]. The traditional method of producing black tea begins with withering. The plucked leaves are placed on shelves called withering racks, where excess moisture is removed. They are then rolled in special machines that release the leaves' enzymes and juices, which give tea its aroma and taste. Next, the leaves ferment in a room with controlled temperature and humidity; finally they are dried in ovens [10].

Oolong tea is fermented only partially to a point between black and green. While the leaves wilt naturally, enzymes begin to ferment them. Processors interrupt the fermentation by stirring the leaves in heated pans, then rolling and drying them [10].

Tea Production in the World

The world total tea growing area in 2003 was 2.41 million ha with an annual production of a total of 3.21 million tons of tea. In the same year, countries with percentages of world tea growing area coverage were India (18%), China (37%), Sri Lanka (9%), Kenya (6%), and

others (30%) and the leading tea producing countries, with percentage of world tea production were India (28%), China (25%), Sri Lanka (9%), Kenya (9%), and others (29%) [12].

The principal types of tea produced and consumed in the world are black and green tea, with small amounts of other types. Major tea producers are India, China, Sri Lanka, and Kenya, while major consumers are India, China, Turkey, Japan, Russia and United Kingdom. The largest annual consumption per capita (kg $head^{-1}$ $year^{-1}$) is Ireland (2.71), followed by Libya (2.65), Kuwait (2.29) and United Kingdom (2.28). Other countries consuming more than 2 kg per capita per year are Iraq, Qatar and Turkey [12].

Major tea producing countries in Africa include Kenya, Malawi, Tanzania, Zimbabwe, and South Africa producing about 25% of world exports amounting to some 250,000 tons of tea per year [13]. Most recently, Eastern Africa has emerged as a major force among tea growers producing excellent teas which are used for blending all over the world [14].

In Ethiopia, in the year 2004-2005, a total of 5,387 tons of black tea was produced which covers only 0.14% of world tea production. From the 5,387 tons of tea produced, 56.7% was consumed domestically while 43.3% was exported for world market [15].

Climatic and Soil Requirements of Tea

Tea developed in the understorey of a dense tropical rain forest south of the Himalayan mountain range and in a less heavily forested environment North of the Himalayas. These give an indication of the conditions natural to the species. These include high rainfall, evenly spread over a large proportion of the year. Temperature would be lower than tropical ambient temperature at low altitude, because of shading in the forest, and humidity would be fairly high [7].

Soil temperature would not be excessive, as the surface would be shaded. Chemically, the soil would be highly leached, due to the high rainfall, and therefore be acidic, with a low availability of base elements. The China variety, indigenous to north of the Himalayas and to higher altitudes, is more resistant to low temperatures than the Assam or Cambod types [7].

Climate

The need for temperature below tropical ambient at low altitude has dictated that, in Equatorial regions, tea is usually planted at high altitudes (1000 - 3000 m). The optimum altitude drops as the distance from the Equator increases, and tea is grown close to sea level at the extremes ranging from latitude of 42^{o}N (Georgia) and 27^{o}S (Argentina) [7].

The minimum annual rainfall considered adequate for the successful cultivation of tea is about 1200 mm without irrigation. Whether other climatic factors are favorable or not, tea, like any other plants, does not grow when temperatures are either too low or too high [5]. The optimum air temperature for its vegetative growth is 18 - 30 ^{o}C. Leaves should be in a temperature above 21 ^{o}C. Growth virtually stops at air temperatures below 13 ^{o}C or over 35 ^{o}C [7].

Solar radiation is important. The amount of radiation reaching a tea canopy at high altitude in Equatorial regions can be up to 600 W m^{-2}. Healthy plants absorb most of the incident radiation, which improves the quality of the leaves. The canopy structure will affect the response to shade, with the Assam types benefiting from the shade to a greater extent than the China type, due to their differences in leaf angle [7].

Tea plant needs a high amount of humidity. The average optimum relative humidity of the atmosphere must not be less than 70 - 75% and during vegetative period 75 - 80%. Very low relative humidity of the atmosphere has a negative influence on shoot growth [5]. The critical value of atmospheric pressure below which shoot growth is inhibited is 2.3 kPa, i.e. relative humidity of 28% at 25 ^{o}C and 45% at 30 ^{o}C [7].

Excessive wind has a negative influence on tea plants. Dry and hot winds decrease the relative humidity of the atmosphere and increase the transpiration rate on the surface of the leaves. This causes lack of moisture in the plant especially in the lower branches and brings about disturbance of physiological processes. Windbreaks, therefore, are essential to prevent the high evapotranspiration and water stress which can occur in unprotected tea [5].

Soils

Tea grows in soils of diverse origins [5]. Tea soils have been formed from a wide range of parent rocks: sedimentary from gneiss or granite, flat alluvium, drained peat and volcanic ash are the most common types. All soils should have a high capacity for retention of water but also be free-draining [7]. Good tea soils, however, are those of volcanic origin. It thrives best on well-drained, permeable deep and fertile soils with a minimum of 2 m depth [5].

Tea will grow well on soils of almost any texture, although extreme types may create problems. Heavy clays can be slow to drain and the surface will become waterlogged in heavy rain. Light, sandy soil may have a lower capacity so that tea may run short of water in drought [7].

Some times tea can be grown on marginal soils. In China, it grows on hillsides. In Northeast India, the soil type ranges from the lightest of sand to the stickiest of clay. In Sri Lanka, the soils are red and consist largely of sand. In East Africa, tea soils are described as tropical red earths derived from granite and recent volcanic deposits. Such tropical red earths are found on higher altitudes ranging from 900 - 2000 m above sea level and they are friable and liable to erosion [5].

Soil temperature is also related to growth, with an optimum range, over which there is a linear relationship, of 19 - 22 ^{o}C. A good depth of soil ensures that water which has drained to a lower level can remain with in reach of tea. Tea roots have been noted growing to over 15 m depth in suitable soil [7]. Lack of soil and atmospheric moisture decreases the growth of lower branches and leaves become very hard and tend to produce a number of sleeping “banjhi” buds. Consequently, yield and quality will decrease [5].

The Tea Plant in Ethiopia

Tea is an exotic commodity crop grown in Ethiopia, which is gaining momentum to become important to the country’s economy. The tea plant was introduced to Ethiopia through different routes at the beginning of the 20th century [5].

Father George of the Netherlands Catholic Mission at Bonga town was the first man to bring tea seeds to Ethiopia from Kenya in 1927. He planted them in the mission's compound at Bonga town, Kafa region [5]. The following year, in 1928, a British consul stationed at Gore town brought about 500 tea plant nurseries from India as per the request of the governor of Ilubabor province. The nurseries were distributed to farmers around Gumero [16].

In 1955, a Belgian investor continued tea production widely on 33 hectares of land at Gumero locality where Gumero Tea plantation is established as a commercial tea estate. In 1973, Wushwush Tea plantation was initiated by Agricultural and Industrial Development bank. But, in 1978, the tea plantations were owned by Tea Plantation Corporation under the Ministry of Coffee and Tea Development and begun its production under government authority [16].

Presently, the commercial tea in Ethiopia, which is black type, is processed from the *C. assamica* variety or the Assam type of *Camellia sinensis* L. grown in Wushwush, Gummero and Chewaka tea plantations located in Southwestern part of the country at 469, 633 and 579 kms from Addis Ababa, respectively. The three plantations cover a total of 2,680 hectares of land. Presently, the three tea plantations in the country are under private investments. Wushwush and Gummero are owned by Ethio Agri-CEFT private limited company while Chewaka is under East Africa Business Group PLC [15].

The Wushwush tea development, which is under Ethio Agri-CEFT private limited company, is the largest tea producing organization among the tea agro industries found in the country. It is located in the Southern Nations, Nationalities and Peoples (SNNP) regional state, Kafa zone, Gimbo *woreda*, 20 kms from Bonga town. It covers a total of 1,250.68 hectares of land located at 1,900 m above sea level with high rainfall suitable for growing the tea plant. The Wushwush tea development processes the tea plant in its two factories to produce black tea for blending [17].

Chemical Composition of Tea

Like fruits and vegetables, brewed tea (whether consumed as hot and iced, regular or decaffeinated) contains natural compounds called flavonoids, which are antioxidants. Research

suggests that dietary antioxidants can neutralize free radicals, helping to maintain healthy cells and tissues in the body. Free radicals, which can be naturally produced in the body, can cause oxidative damage to cells which in turn may contribute to chronic conditions such as heart disease and cancer [18].

The most commonly known antioxidants are vitamins C, E and β-carotene, found in fruits, vegetables, cereals and some vegetable oils. The amount and type of flavonoids in tea depends on the variety, the amount of tea used in the pot or cup, and brewing habit [19]. The chemical composition of tea leaves and manufactured tea is very complex and consists of tanning substances, flavonols, alkaloids, proteins and amino acids, enzymes, aroma forming substances, vitamins, minerals and trace elements [20].

Various reports have discussed the potential health implications of trace metals in tea, particularly since the tea bush is known to accumulate trace metals [21]. Very recent research findings indicate the positive and negative effects of drinking tea on health [22]. It was pointed out that some of the beneficial effects of drinking tea are prevention of chronic and cardiovascular disease, cancer, antioxidative detoxification and removal of cadmium in administered rates [23].

To date, few negative effects have been reported from drinking green tea. One is insomnia due to the fact that it contains caffeine [24]. On the other hand, tea leaf contains approximately 8% toxic substances. The toxic elements in tea secretly and gradually affect the body parts and permanently damage them [25]. The toxic effects of most metals can be traced to their ability to disrupt the function of essential biological molecules, such as proteins, enzymes and DNA. In some cases this involves displacing chemically related metal ions that are required for important biological functions such as cell growth, division and repair [26].

1.1. Objective of the Present Study

The chemical composition of tea and tea leaves is the subject of broad medical and toxicological scientific studies. The accurate determination of the metal content of tea is thus very important in assessing the standard and quality of tea as well as any potential implications to health [2].

Tea-drinking habit is worldwide spread and many countries cultivate different brands of tea to meet the increasing demands. In Ethiopia, the *C. assamica* variety of the tea plant is fermented and dried to produce black tea for export and blended in different brands for domestic consumption. Since tea is a beverage which is a part of our daily dietary intake and frequently consumed, assessment of the nutrient composition specially the essential, non-essential and toxic metals in tea plants grown in Ethiopia is of great importance from quality and standards, nutrition, health, and pollution perspectives.

The metal contents of tea plants grown in Ethiopia have not been studied and there are no reports or papers that are published so far. This research is, thus, planned to assess the metal contents in the leaves of the tea plant that are grown in Wushwush farms and to correlate with the levels of metals in the soils on which the tea plants are grown. It is also expected to deliver a base line data on the levels of metals in tea plants grown in Ethiopia and provide useful information for future studies which will be conducted on nutritional, medical and toxicological effects in relation to the tea leaves of Ethiopia.

The specific objectives of the present study are:

(i) To develop a working procedure for the digestion and analysis of tea leaves for their essential, non-essential (Na, K, Ca, Mg, Mn, Fe, Cu, Zn, Co) and toxic metal (Pb, Cd) contents by atomic absorption flame emission spectrophotometry.

(ii) To determine essential, non-essential and toxic metals in the tea leaf samples collected from Wushwush tea plantation farms using atomic absorption flame emission spectrophotometry.

(iii) To determine the amount of these metals in the soils of Wushwush farms where tea is grown.

2. LITERATURE REVIEW

2.1. Mineral Nutrients in the Soil

Soil is a heterogeneous material which may be considered as consisting of three major components: a solid phase, a liquid phase and a gaseous phase. All three phases specifically influence the supply of plant roots with nutrients. The solid phase may be regarded as the main nutrient reservoir. The inorganic particles of the solid phase contain cationic nutrients such as K, Na, Ca, Mg, Fe, Mn, Zn, and Co whilst the organic particles of this phase provide the main reserve of N and to a lesser extent also P and S. Colloidal soil particles are mostly negatively charged. The negative charge on the clay mineral surfaces arises largely because of isomorphous replacement of cations in the crystalline lattices where trivalent cations are substituted by divalent cations. The negatively charged surfaces of these various soil particles attract cations such as Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} as well as $Al^{3+}$and Mn^{2+} [27].

Plant growth involves the interaction of soil and plant properties. Soil is the normal medium for plant root growth. The plant's roots absorb nutrients and water from the soil and are an anchor to support the shoot. Maximum plant growth depends on the soil having the biological, chemical and physical conditions necessary for the root system to maximize the plant's required absorption of nutrients and water and to enable the biochemical reactions that occur in the root. The plants rate of absorption of nutrients involves processes going on in both the plant's root and the soil. Each of these processes is important in providing nutrients for use by the shoot [28].

Soil properties are greatly influenced by its pH. Soil pH values can differ widely from values of about 3 to as high as 10, being very low in in acid sulphate and podzolic soils and being rather high in calcareous and alkali soils. In alkali soils in particular very high pH values may occur as the soil solution contains weak acids (HCO_3^{-}) and strong bases (Na^{+} or K^{+}). The H^{+} concentration of the soil solution has a pronounced effect on a number of soil constituents and especially on the soil minerals, soil microorganisms and plant roots. High H^{+} concentration favour the weathering of minerals resulting in a release of various ions such as K^{+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Cu^{2+}, and Al^{3+} [27].

The soil organic matter, another important soil property, is the organic fraction derived from living organisms. It includes the living organisms, partly decomposed and decomposed plant and animal residue. The decomposed organic fraction is usually called humus [29]. Organic matter binds mineral particles in to a granular soil structure that is largely responsible for the loose, easily managed condition of productive soils. It is a major source of plant nutrients. As soil organic matter decays, the nutrient elements, which are present in organic combinations, are released as soluble ions that can be taken up by plant roots [30].

Nutrients in the soil can be transported by two different mechanisms: by mass flow and by diffusion. Mass flow occurs when solutes are transported with the convective flow of water from the soil to plant roots. The amount of nutrients reaching the root is thus dependent on the rate of water flow or the water consumption of the plant and the average nutrient concentration of the water. The level of a particular nutrient around the root may be increased, decreased or remain the same depending on the balance between the rate of its supply to the root by mass flow and the rate of uptake by the root. Diffusion occurs when an ion is transported from a higher to a lower concentration by random thermal motion. Diffusion comes into effect when the concentration at the root surface is either higher or lower than that of the surrounding solution. It is directed towards the root when the concentration at the root surface is decreased and away from the root when it is increased [27].

2.2. Essential and Non-essential Plant Nutrients

The essential nutrients required by green plants are exclusively of inorganic nature. In this respect green plants differ fundamentally from man, animals and a number of microorganisms, which additionally need organic compounds as foodstuffs [27].

An essential element may be defined as one which is required for the normal life cycle of an organism and whose functions cannot be substituted by other chemical compounds. In addition, the element must be shown to be directly involved in nutrition, as for example as a constituent of an essential metabolite or the element must be required for the action of an essential enzyme system. Based on this definition, the following chemical elements: Carbon (C), Hydrogen (H), Oxygen (O), Nitrogen (N), Phosphorous (P), Sulfur (S), Potassium (K), Calcium (Ca),

Magnesium (Mg), Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn), Molybdenum (Mo), Boron (B), Chlorine (Cl), [Sodium (Na), Silicon (Si), and Cobalt (Co)] are known to be essential for higher plants. Sodium, silicon and cobalt have not been established as essential elements for all higher plants [27].

The plant nutrients may be divided into macronutrients and micronutrients. Macronutrients are found and needed in plants in relatively higher amounts than micronutrients. Following this classification based on the element content in plant material, the following elements may be defined as macronutrients: C, H, O, N, P, S, K, Ca, Mg, (Na, Si). The micronutrients are: Fe, Mn, Cu, Zn, Mo, B, Cl. This division of the plant nutrients into macro- and micronutrients is somewhat arbitrary. The Fe or Mn content of plant tissues for example is sometimes nearly as high as the content of S or Mg. The content of the micronutrients is often in excess of physiological requirements, which is true for Mn for example [27].

According to their biochemical behavior and physiological functions, plant nutrients may be divided into four groups. The first group includes the major constituents of the organic plant material: C, H, O, N and S. Carbon is taken up in the form of CO_2 from the atmosphere and possibly in the form of HCO_3^- from the soil solution. The assimilation of C is also accompanied by the simultaneous assimilation of O, for not only C but CO_2 or HCO_3^- is incorporated. Hydrogen is taken up in the form of water from the soil solution or under humid conditions from the atmosphere. Nitrogen is taken up by the plant in the form of NO_3^- or NH_4^+ ions from the soil solution or as gaseous N_2 from the atmosphere. Sulfur is not only taken up from the soil solution in the form of SO_4^{2-}, but can also be absorbed as SO_2 from the atmosphere [27].

The second group constitutes P, B and Si, which show similarities in biochemical behavior. All are absorbed as inorganic anions or acids. P, B and Si are taken up by plants in the form of phosphates, boric acid or borate, and silicate from the soil solution respectively [27].

The third group of plant nutrients is made up of K, Na, Ca, Mg, Mn, and Cl. These elements are taken up from the soil solution in the form of their ions. In the plant cell they are present in the free ionic state or are adsorbed into indiffusible organic anions, as for example the adsorption

of Ca^{2+} by the carboxylic groups of the pectins. Magnesium may also occur strongly bound in the chlorophyll molecule being chelated by both valence and coordinate bonds [27].

This group of elements can have non-specific ionic cellular functions such as establishing osmotic potential in cell organelles or maintaining ionic balance. The cationic nutrients in this group also specifically activate some enzyme systems. This is particularly true for K^{+}, $Mg^{2+}$and Mn^{2+} which are required by numerous enzymes. They have a role in bridging of coenzymes with enzymes and balancing indiffusible and diffusible anions [27]**.**

The fourth group of elements are Fe, Cu, Zn and Mo. These elements are predominantly present as chelates in the plant and, with the exception of Mo, are often taken up by plants as chelate complexes. The most important naturally occurring plant chelates are those of the haem group and chlorophyll. The haem group is an iron porphyrin. The Fe present in the haem moeity can change in valency from Fe^{2+} to Fe^{3+}. This enables the transfer of electrons, the principal function of this prosthetic group [27].

2.3. Mineral Contents of Plant Material

The material of living plants consists of organic matter, water and minerals. The relative amounts of these three components may vary, but for green plant material water is always present in the highest proportion and the minerals in the lowest. The percentage distribution of these three components is in the following order of magnitude: 70% water, 27% organic material, and 3% minerals. The minerals make up only a comparatively small proportion of dry matter. They are nevertheless of extreme importance because they enable the plant to build up organic material (photosynthesis). The mineral content of plants and plant organs is therefore of physiological and practical significance [27].

The main factor controlling the mineral content of plant material is the specific, genetically fixed nutrient uptake potential for the different mineral nutrients. This accounts for the fact that the N and K content of green plant material is about 10 times higher than that of P and Mg which in turn is about 100 - 1000 times higher than the content of the micronutrients. This general pattern occurs in all species of higher plants. The second factor controlling the mineral

content of plant material is the availability of plant nutrients in the nutrient medium. The concentration of a particular mineral or plant nutrient in the plant increases in the form of a saturation curve as its availability in the nutrient medium increases [27].

Mineral contents differ considerably between plant organs. Generally the vegetative parts of the plant such as leaves, stems and roots vary to a higher extent in their mineral composition than fruits, tubers and seeds. The mineral content of plants is also very much dependent on age. Young plants and young plant tissues have high contents of N, K and P, whereas in older plants and more mature plant parts, higher contents of Ca, Mn, Fe and B are often observed, provided the mineral content is expressed on a dry matter basis [27].

The analysis of plant material presents a type of approach in determining the nutrient availability of a soil. It is based on the concept that the content of a particular nutrient in the plant is greater the higher its availability in the soil. In principle, plant nutrients present in the plant must originally have been available in the soil. Unfortunately, however, it has drawbacks, as the mineral content in the plant not only depends on nutrient availability in the soil, but it is also affected by other factors such as the kind of plant organ or tissue, the age of the plant and the supply of the plant with other plant nutrients [27].

In most cases analytical data obtained from the analysis of leaf or tissue correlate fairly well with soil tests, however, it also reflects conditions of uptake. Leaf or tissue analysis provides a particularly useful means of assessing nutritional status of perennial plants such as fruit trees, vines, tea, forest trees various plantation crops [27].

Potassium (K)

The average K content of the earth's crust is in the order of about 2.3%. By far the greatest part of this K is bound in primary minerals or is present in the secondary clay minerals which largely make up the clay fraction of the soil. For this reason soils rich in clay are also generally rich in K and clay soils may often have excess of 4% total K [27]. Potassium is an essential element for all living organisms. In plant physiology it is the most important cation not only in

regard to its content in plant tissues but also with respect to its physiological and biochemical functions [27].

Plants absorb large amounts of potassium, all of it in the form of the K^+ ion. The positive charges of the potassium cations help to maintain electrical neutrality in both soil and plants by balancing the negative charges of nitrates, phosphates, and other anions. Plants require relatively large amounts of potassium and often can use more than the soil can supply. Potassium is the third most likely element to limit plant growth [31].

Potassium is present in plants in the form of organic and inorganic salts. It does not form an integral part of the structure of any known organic compound in plants. Nearly all potassium salts are soluble and highly ionized in solution. The K^+ ions are mobile within the plant but will not leach out of healthy living plant tissue. They are readily leached from dead plant tissue [31].

A deficiency in potassium affects processes such as respiration, photosynthesis, chlorophyll development, and water content of leaves. The best-known function of K is its role in stomatal opening and closing. The highest concentrations of potassium are found in the meristematic regions of the plant. Potassium is essential as an activator for enzymes involved in the synthesis of certain peptide bonds [32]. It exerts a specific role in the conformation of the enzyme protein, thus enabling the alignment of appropriate active cites [27]. Potassium also can act as an activator for several enzymes involved in carbohydrate metabolism [32]. Potassium aids in the uptake of other nutrients and in their movement within the plant. The presence of potassium and other ions in solution helps to maintain the osmotic concentration necessary to keep the cells turgid [31].

Sodium (Na)

The total sodium content of normal soils is approximately 0.63%. High soluble Na contents are very harmful to the physical condition of soils. Sodium tends to disperse the soil colloids. A dispersed soil is puddle when wet and hard when dry. Sodium is not considered an essential nutrient for plant growth. Sodium compounds are widely distributed in nature. The major

mineral source is Na aluminum silicates, also called sodic feldspars or plagioclase, eg. albite, $NaAlSiO_3O_8$. Sodium can also occur in nature as chloride, sulfate and borate compounds. The dissolution of sodium from albite is attributed to hydrolysis reactions, which is a weathering process [29].

The chemistry of sodium is quite similar to that of potassium, but its behavior in the soil is somewhat different [31]. Sodium is not required by plants but can replace part of the K^+ requirement of some species. It is toxic to some plants at high concentrations. High soil pH usually accompanies Na^+ accumulations in soils [33].

Sodium occurs in feldspars but not in micas. The sodium feldspars weather a little more rapidly than the potassium feldspars. Sodium ions that have been released to the soil solution are not subject to fixation and are less tightly held to cation-exchange sites than potassium, magnesium or calcium. Sodium is therefore the easiest basic cation to leach from the soil. This accounts for the sodium content of soils gradually decreasing with time while the potassium content remains nearly constant. Sodium salts sometimes become concentrated in soils of arid regions because of lack of leaching. This can lead to the formation of sodic soils [31].

Calcium (Ca)

Calcium is a divalent alkaline earth cation. It is the fifth most plentiful element in the earth's crust, which has an average calcium concentration of 3.6%. Calcium is the dominant exchangeable cation in many soils. In general, only alkaline soils containing sodium, acid soils containing large amounts of hydrogen and aluminum, and serpentine-derived soils high in magnesium have cations other than calcium as the dominant exchangeable cation. Exchangeable calcium is in equilibrium with soil solution calcium. The relative strength of bonding controls the equilibrium between soluble and exchangeable calcium. Bonding depends on the nature of the cation exchange site, degree of calcium saturation of soil-exchange sites, complementary cations present, and the anion content of the soil solution [28].

The exchangeable calcium in a soil has an important relation to soil pH and to the availability of several nutrient elements. The amounts of calcium and other basic cations present in a soil

decline as a soil becomes more acid and increase a it becomes more alkaline. An excess of calcium causes calcium carbonate to precipitate and buffer the pH to a value near 8. Excess calcium usually results in the solubility of phosphorus, iron, manganese, boron, and zinc and sometimes causes deficiencies of one or more of these essential plant nutrients [31].

Calcium dissolved in the soil solution can move by mass flow and by diffusion, but exchangeable calcium has a very low mobility. It is reported that plant roots will not enter soil layers that are devoid of calcium even though other conditions are favorable for growth and calcium is available in other layers. Monovalent ions such as Na^{+} and K^{+} are more mobile because they are less strongly attracted to cation-exchange sites than Ca^{++} ions [31].

Calcium occurs in plant tissues as free Ca^{2+}, as Ca^{2+} adsorbed to indiffusible ions such as carboxylic, phosphorylic and phenolic hydroxyl groups. It is also present in Ca oxalates, carbonates and phosphates [27].

Calcium is a structural component of cell walls and is therefore vital in the formation of new cells. Furthermore, calcium is so integrated into cell walls that it cannot be removed from old cells to form new cells. Plants deficient in calcium are stunted because they produce fewer and smaller cells. They have weak stems because their cell walls are less than normal thickness [31].

A calcium shortage restricts the growth of roots as well as stems, leaves, etc. The inability of calcium-deficient roots to elongate rapidly handicaps the plant for exploiting new portions of the soil volume to obtain water and nutrients. Restricted root growth could produce or aggravate other nutrient deficiencies as well [31].

Calcium is normally abundant in plant leaves. A calcium deficiency prevents the growth and unfolding of new leaves. It also prevents the growth of the margins of existent leaves and therefore results in curled leaves [31].

Magnesium (Mg)

The Mg content of most soils generally lies in the range of between 0.05% for sandy soils and 0.5% for clay soils. Magnesium in the soil is a constituent of many soil minerals; it is present as exchangeable magnesium on the cation-exchange complex and in soil solution as the soluble magnesium ion. Small amounts of magnesium may also be combined in the soil's organic fraction [28].

Plant roots absorb soluble and ultimately exchangeable magnesium, which is assumed to go into solution before absorption by the root. Exchangeable calcium plus magnesium usually accounts for more than 60% of the exchangeable cations on soils with pH 5.5 or higher. Exchangeable hydrogen, aluminum, potassium, and sodium occupy the majority of remaining exchange sites. Exchangeable magnesium is almost always present in smaller quantities than calcium, though on serpentine-derived soils, magnesium may be the dominant exchangeable cation [28].

Magnesium in the soil that can move rapidly to the root and be absorbed includes both exchangeable magnesium on the cation-exchange sites and the smaller quantity in solution. Exchangeable magnesium is assumed to go into solution before movement to, and absorption by, the root. The quantity in solution is usually from 1 to 10% of the amount of exchangeable magnesium. The measure of exchangeable magnesium plus that in solution reflects the total available for movement to the root [28].

Magnesium is generally taken up by plants in lower quantities than Ca^{2+} or K^{+}. The content of Mg in plant tissues is usually in the order of 0.5% of the dry matter. In plant tissues a high proportion of the total Mg, often over 70%, is diffusible and associated with inorganic anions and organic acid anions such as malate and citrate. Magnesium is also associated with indiffusible anions including oxalate and pectate. The most well known role of Mg is its occurrence at the center of the chlorophyll molecule. Besides its function in the chlorophyll molecule Mg^{2+} is required in other physiological processes. One major role of Mg^{2+} is as a

cofactor in almost all enzymes activating phosphorylation processes. Magnesium is therefore important throughout the metabolism [27].

2.4. The Micronutrients

A micronutrient is an element that plants must have to complete their life cycles but need only in a small amount. These elements have often been called *trace elements* or *minor elements,* but micronutrient is the preferable term [31]. The terms *micronutrient* and *trace element* must not be construed to imply that these nutrients are some how less important than the macronutrients. To the contrary, the effects of micronutrient deficiency can be very severe in terms of stunted growth, low yield dieback, and even plant death. By the same token, where they are needed, very small applications of micronutrients may produce dramatic results [30].

Each plant requires only a tiny amount of each micronutrient, yet it must have that tiny amount. Clearly they cannot serve as building blocks of major plant components; the amounts involved are inadequate for such a role. Some micronutrients function in the enzyme systems of plants. A very small amount of such an element is all that is required to make enough enzymes to catalyze an essential plant process. Cation-forming elements such as copper are more likely to serve as coenzymes that activate an enzyme but are not an integral part of the molecule [31].

Some micronutrients function in oxidation-reduction processes of plant metabolism. Elements such as iron, copper, and manganese can change valences and thus enter into oxidation-reduction reactions. Several of the micronutrients are involved in the production of chlorophyll. Deficiencies of these elements can appear very similar to the pale green condition characteristic of nitrogen deficiency [31].

Iron (Fe)

Iron is present in soils in higher concentrations than any other nutrient. The lithosphere contains 5.1% iron, which forms compounds with sulfur and oxygen [29]. The greatest part of soil Fe usually occurs in the crystal lattices of numerous minerals. Iron minerals commonly found in soils include goethite, (FeOOH), hematite (Fe_2O_3), lepidocrocite, (FeOOH), and

magnetite (Fe_3O_4). Hematite gives a red color to soils, while geothite imparts a yellow color. Amorphous iron as $Fe(OH)_3$ is probably the most significant form in supplying iron for uptake by the plant [28].

In spite of the large amount of iron in the soil and the low quantities needed for plant growth, iron deficiencies occur because so little of the element is in an available form. Soils vary in total iron content from 0.02 to 10%, depending on their origin [28]. The content of soluble iron in soils is extremely low in comparison with the total Fe content. Soluble inorganic forms include Fe^{3+}, $Fe(OH)_2^+$, $FeOH^{2+}$ and Fe^{2+}. In well-aerated soils, however, Fe^{2+} contributes little to the total soluble in organic Fe except under high soil pH conditions [27].

The amount of iron present as Fe^{2+} and Fe^{3+}, in the soil solution depends on the hydroxide forms present in the soil, which in turn, depends on pH and *pe,* a parameter related to the redox potential. Iron solubility is largely controlled by the solubility of the hydrous Fe(III) oxides, which give rise to Fe^{3+} and its hydrolysis species [$Fe(OH)_3$]. The equilibrium is very much in favour of $Fe(OH)_3$ precipitation and is highly pH dependent, the activity of Fe^{3+} falling with increasing pH. At higher pH levels Fe^{3+} activity in solution decreases 1000 fold for each pH unit rise. The soluble Fe level reaches a minimum in the pH range of 6.5 - 8.0. Acid soils are thus relatively higher in soluble inorganic Fe than calcareous soils where levels can be extremely low [27].

Iron forms stable complexes with organic compounds that occur in both the soil's solid phase and soluble organic compounds. Simpler organic compounds are citrate and oxalate. Iron compounds are more stable than combinations with most other nutrients, so that iron can replace them in the chelate except where mass-action displacement occurs due to high concentrations of other ions present. Iron chelates have high stability constants, so that at pH levels below 7.0, iron is frequently the dominant cation in the chelate [28].

Iron may be supplied to plant roots as Fe^{2+}, Fe^{3+} or as Fe chelates. Absorption appears to be dependent on the ability of the roots to reduce Fe^{3+} to Fe^{2+}. The uptake of iron is considerably influenced by other cations. Competitive effects on iron uptake have been observed with Mn^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, K^+ and Zn^{2+}. Heavy metals, in particular Cu and Zn are also known to

displace Fe from chelate complexes forming corresponding heavy metal chelates. Iron uptake is particularly depressed by high pH, high phosphate and Ca^{2+} concentration in the nutrient medium. High pH levels as well as good aeration conditions favour the oxidation of Fe^{2+} to Fe^{3+} and thus the precipitation of Fe(III) salts occurs [27].

The tendency for Fe to form chelate complexes and its ability to undergo a valency change are the two important characteristics which underlie its numerous physiological effects [27]. Iron has a number of important functions in the overall metabolism of the plant. Although iron is frequently taken up in the ferric state (Fe^{3+}), the ferrous state (Fe^{2+}) is generally accepted as the metabolically active form of iron in the plant [32].

The most well known function of iron is in enzyme systems in which haem or haemin function as prosthetic groups [27]. Iron is necessary for the formation of chlorophyll and functions in some of the enzymes of the respiratory system [31]. Iron is incorporated directly into the cytochromes, into compounds necessary to the electron transport system in mitochondria, and into ferredoxin [32].

An iron deficiency results in the younger leaves being small and pale green or yellow in color. This shortage of chlorophyll is called *chlorosis.* The younger leaves are more affected than the older leaves because iron is relatively immobile inside the plant. Often the veins remain green while the areas between veins turn yellow from iron chlorosis [31]. Primarily, this is because of the relative immobility of iron in the plant. Thus the younger leaves cannot withdraw iron from the older leaves [32].

Manganese (Mn)

Manganese is the eleventh most common element in the earth's crust, with an average concentration of 0.09%, or 900 mg/kg. Manganese is present primarily as oxides and sulfides; it often occurs in association with iron. Soils have manganese concentrations that are usually in the range of 20 to 3000 mg/kg, with an average of 600 mg/kg. Soil manganese exists in three oxidation states - Mn^{2+}, Mn^{3+}, and Mn^{4+}. Manganese absorbed by plant roots is primarily as Mn^{2+}. Oxidation-reduction reactions in the soil influence the amount of each oxidation state

present. The predominant oxidation states in most soils are Mn^{2+}, and Mn^{4+}, with much more as Mn^{4+} than Mn^{2+} in aerated soils [28].

Total soil manganese may be divided into mineral manganese, organically complexed manganese, exchangeable manganese, and solution manganese. Manganese in solution may be either Mn^{2+} or manganese combined with soluble organic compounds. The equilibrium of manganese between these forms is influenced greatly by soil pH and redox conditions [28].

Exchangeable manganese is held by the soil cation-exchange sites and measured by displacement with ammonium acetate. It exists essentially as $Mn^{2+.}$ A wide range of values can be obtained, with very acid soils having values above 1000 mg/kg, while organic soils of high pH may have values less than 0.1 mg/kg [29]. Manganese availability is higher in acid soils due to the higher solubility of Mn compounds under low pH conditions. Soluble Mn^{2+} decreases 100 fold for each unit increase in pH. Under high soil pH conditions Mn availability can thus be inadequate to meet plant demand [27].

Manganese is an essential element in respiration and nitrogen metabolism; in both processes it functions as an enzyme activator. However, in many cases, especially with reactions in respiration, manganese can be replaced by other divalent cations, such as Mg^{2+}, Co^{2+}, Zn^{2+}, and Fe^{2+} [32]. Manganese functions in chlorophyll development and in the enzyme systems of plants. Its various valences make it possible for manganese to be either a metallic coenzyme or a part of an organic molecule [31]. Manganese is also in some way involved in the oxidation-reduction processes in the photosynthetic electron transport system [27].

Manganese, like iron, is a relatively immobile element in plants. Deficiency symptoms appear on the younger leaves of the plant first. The symptoms vary with the plant but include a pale color similar to iron chlorosis between the veins of broad-leaved plants. The similarity between manganese and iron causes a form of competition between the two elements. Symptoms of iron toxicity correspond to symptoms of, manganese deficiency, and symptoms of manganese toxicity correspond to those of iron deficiency [31]. Manganese deficiency also appears to have a marked effect on the chloroplast. The chloroplasts lose chlorophyll and starch grains, become yellow green in color, vacuolated, and granular, and finally disintegrate [32].

Copper (Cu)

The average copper concentration in the earth's crust is approximately 70 mg/kg. Copper concentration varies with the type of rock; basalt may contain as much as 100 mg/kg, while granite contains only approximately 10 mg/kg. Sedimentary rocks also vary in copper concentration. Limestone, sandstone, and shale average approximately 4, 30, and 45 mg/kg of copper, respectively [28].

Copper occurs as Cu^{++} ions in most soils and as Cu^{+} ions where the oxidation level is low. Copper ions are held so tightly by cation-exchange sites that they are even less mobile than Ca^{++}. The concentration in solution is only a few part per million. Copper is most soluble in acid soils, and its solubility decreases as the pH rises [31].

Copper can also be substituted isomorphously for manganese, iron, and magnesium in various minerals. Copper oxides, carbonates, and sulfates are not present in most soils, because the copper concentration found in soil solution is much lower than would be maintained by the solubility of these minerals. Copper in soil can occur in soil solution, both ionic and complexes, as an exchangeable cation on the exchange complex, as a specifically adsorbed (non exchangeable) ion, in organic matter, in occluded oxides, and in minerals [28].

Copper is taken up by the plant in only very small quantities. It is thus about one-tenth of the Mn content. Copper uptake appears to be a metabolically mediated process and there is evidence that Cu strongly inhibits the uptake of Zn and vice versa [27].

There is little doubt as to the necessity of copper for normal plant metabolism. Copper acts as a component of phenolase, lactase, and ascorbic acid oxidase, and its role as a part of these enzymes probably represent an important function of copper in plants [32]. Copper is important as a coenzyme that is needed to activate several plant enzymes. It is also involved in chlorophyl formation. Copper uptake seems to be inversely related to iron uptake. Too little copper causes iron to accumulate in plants. Too much copper causes chlorotic symptoms similar to those of iron deficiency [31].

Copper is not readily mobile in the plant although it can be translocated from older to younger leaves [27]. Copper deficiency, symptoms occur mostly on new growth because copper is relatively immobile in plants [31]. Copper deficiency causes a necrosis of the tip of young leaves that proceeds along the margin of the leaf and gives it a withered appearance. Under more severe conditions, the leaves may be lost, and the whole plant may appear wilted [32].

Zinc (Zn)

The mean zinc concentration in the lithosphere is 80 mg/kg. Total soil zinc ranges from 10 to 300 mg/kg. Zinc is present in the soil in only the divalent form. Organic matter forms coordination complexes with zinc; they may be present in both the soil organic matter and soluble organic complexes in soil solution. Zinc that may become available for plant uptake is present as Zn^{2+} in the soil solution, exchangeable zinc on the cation-exchange sites, organically complexes zinc in solution and in the soil solid phase [28].

The levels of Zn in plant materials are low, generally in the order of up to 100 ppm in the dry matter [27]. Zinc participates in the metabolism of plants as an activator of several enzymes [32]. In its function in some enzyme systems, Zn^{2+} resembles Mn^{2+} and Mg^{2+} in that it brings about the binding configuration between enzyme and substrate. Zn is required in the synthesis of tryptophane. As tryptophane is also a precursor of indole acetic acid the formation of this growth substance is also directly influenced by Zn [27]. Zinc is needed for protein metabolism and appears to be involved somehow in the production of chlorophyll [32].

Plants suffering from Zn-deficiency often show chlorosis in the interveinal areas of the leaf. These areas are pale green, yellow, or even white. Zn deficiency is closely related to the inhibition of RNA synthesis. The deficiency prevents the normal development of chloroplast, grana and vacuoles are developed in them [27].

2.5. Further Elements of Importance

The elements K, Ca, Mg, Fe, Mn, Cu and Zn, with the possible exception of Na, are essential plant nutrients without which the plant would be unable to complete its life cycle. In addition to

these nutrients are group of elements which can have a beneficial effect on plant growth. Two well-known elements of this type are Co and Si [27].

Cobalt (Co)

The Co concentration in the dry matter of plants grown in soil normally lies between 0.02 to 0.5 ppm. In soils the content is usually much higher and levels from 1 to 40 ppm are common although many values in excess of 40 ppm have been reported [27].

In the soil Co occurs primarily in the crystal lattices of ferromagnesian minerals and as such is unavailable to plants. After release from these minerals by weathering, Co^{2+} is held largely in exchangeable form or as organo mineral complexes. Exchangeable Co^{2+} is very firmly bound and like Cu^{2+} the concentration in the soil solution is very low. Cobalt deficiency occurs in highly leached sandy soils, soils derived from acid igneous rocks or in highly calcareous or peaty soils. It is favored if the soil pH is neutral to alkaline [27].

Co behaves like other heavy metals. In similar way to Fe, Mn, Zn and Cu it tends to form chelate compounds. It can also displace other ions from physiologically important binding sites and can thus decrease the uptake and mode of action of other heavy metals. Plants may take up Co through the leaves, however, Co taken up in this way is practically immobile. Cobalt taken up by the roots primarily follows the transpiration stream so that there is an enrichment of Co at the leaf margins and tips [27].

It is now well established that Co is essential for microorganisms fixing molecular N_2. It is still in question whether in addition to its requirement in symbiotic N_2 fixation, Co is essential for higher plants. It is clear, however, that low concentrations of Co can have a favorable effect on plant growth and there is some indication that there might be a Co requirement. Cobalt is also of importance in animal nutrition. It is well established that Co is a metal component of vitamin B_{12} which is essential in N-metabolism [27].

2.6. Elements with More Toxic Effects

There is no clear division between elements which are toxic to plants and those which have a beneficial or even essential effect. The effect of any element on the plant depends not only on its chemical properties but also on its concentration and the presence and concentrations of other elements. Some elements such as Fe, Mn, Cu, B, Zn are essential at low concentrations but are toxic at higher levels. In the case of the heavy metals Pb and Cd, toxicity is induced by mimicking of lighter essential elements in uptake and biochemical behavior [27].

Cadmium (Cd)

Cadmium, a heavy metal, causes toxicity in humans in very small amounts when consumed. Cadmium occurs naturally in only trace concentrations in agricultural soils. Contamination of agricultural soils with Cd is derived from sources, such as phosphatic fertilizers manufactured from rock phosphates high in Cd and by the application of sewage sludge to a greater extent and by the pesticides and gypsum to lesser extent. Zinc smelters in the vicinity of agricultural soils can also be significant contributors to soil contamination with Cd. Food crops grown on contaminated soils may take up substantial amounts of Cd and this could result in Cd entering the food chain of animals and humans when consumed [34].

There is now general concern that under certain conditions the Cd content of plants may be raised and thus become hazardous to man. The sources of soil Cd are varied. Cadmium is added to soils in very small amounts in phosphate fertilizers. Along with other heavy metals, it is also present in sewage sludge. Levels from about 10 to as much as 1500 ppm Cd have been observed in the dry matter of sewage sludge, which is being used more and more on agricultural land [27].

There is considerable current interest in Cd in plant nutrition. Normal Cd levels in plant material are in the range of 0.1 - 1.0 ppm. Although the roots of several species can take up large quantities of Cd from solution, the movement of Cd through the plant is restricted. Cadmium appears to be held in the roots on exchange sites, and can be replaced by Ca^{2+}, Mn^{2+},

and Zn^{2+}. As Ca^{2+} is normally the dominant cation in soil solution it may substantially affect the uptake of Cd from the roots to the tops is particularly depressed by phosphate [27].

Cadmium and Zn are chemically very similar. Cadmium is thus able to mimic the behavior of the essential element Zn in its uptake and metabolic functions. Unlike Zn, however, Cd is toxic both to plants and animals. The basic cause of toxicity probably lies in the much higher affinity of Cd for thiol groupings (SH) in enzymes and other proteins. The presence of Cd therefore disturbs enzyme activity [27].

Most recently, interest in Cd has been directed at progressive accumulation in biological systems at low levels at which Cd generally occurs environmentally [35]. Toxic effects in man have been observed from the regular consumption of plants in excess of 3 ppm [27]. Continued exposure to small amounts of Cd leads to accumulation, in human and animal liver and kidney tissues resulting in damage and malfunction of these organs [35]. It disturbs the metabolism of Ca and P and cause bone disease, which is very painful, and causes excessive demineralization and embrittlement of the skeleton [27].

Lead (Pb)

The total Pb content of agricultural soils lies between 2 - 200 ppm. Soils with levels in excess of this are limited to a relatively few regions where Pb mineral deposits occur. Lead airborne contamination in soils is usually restricted to the top few cm of the soil profile. This retention in the upper part of the soil profile probably relates the strong adsorption of Pb^{2+} to organic and clay colloids as well as to the formation of insoluble Pb chelates with organic matter. The availability of soil Pb is usually low. A high soil pH may precipitate Pb as hydroxide, phosphate, or carbonate as well as possibly promoting the formation of Pb organic matter complexes [27].

Lead is a major chemical pollutant of the environment, and is highly toxic to man. No other pollutant than Pb has accumulated in man to average levels so close to those which are potentially clinically poisonous. Lead is toxic because it mimics many aspects of the metabolic

behavior of Ca, and inhibits many enzyme systems. In animals, Pb toxicity interferes with Fe metabolism and the formation of heam [27].

Lead reaches soil and plant cover as an aerial deposit and in precipitation, irrigation water, mine drainage, leaf litter, or ground dust blown in from elsewhere. Pb is also added to to soil as pesticide, such as Pb arsenate, or as an impurity in certain fertilizers such as limestone and superphosphates. Two pathways are available for Pb to enter plants: uptake by the roots and uptake by the foliage. Once inside the system, Pb seems to be retained by cell membranes, mitochondria, and chloroplasts [36].

Lead enters man by inhalation and ingestion. Absorbed and carried by the blood, it is accumulated in liver, kidney, and bone up to about the fifth decade of life [36]. Pb causes brain damage particularly to the young. There is evidence that Pb pollution can induce aggressive behavior in animals which can also occur in humans [27].

2.7. Minerals and their Roles in the Tea Plant

Potassuim is a vital nutrient in the tea plant. Tea suffers severely when it cannot absorb sufficient potassium. Application of potash fertilizer to tea showing symptoms of potassium deficiency produces dramatic improvement very quickly. As acid soils do not contain large quantities of available K, application of potash is essential to almost every field of high-yielding tea [7].

Potassium has a marked effect on recovery from pruning. Potassium and calcium are antagonistic in tea. When there is an excessive amount of calcium in the soil, this is absorbed in above-normal uptake of potassium and blocks uptake of potassium as a result severe potassium deficiency results [7].

Potassium deficiency is a cause for tea leaves to develop marginal scorch and may turn reddish bronze. Old leaves discolor first and become loosely attached to the stems; shaking the bush will make many leaves fall. Growth and bud break slow up and many banjhi shoots with small leaves are formed from the upper sections of stems. Bark becomes very white, while brown

blight and gray blight appear on leaves. Luck of bud break lower in the bushes results in plants not spreading after pruning [7].

Magnesium is an essential element for the tea plant, particularly as a constituent of chlorophyll, but the proportion in tea leaves is relatively small. Magnesium is very mobile within the plant, so that it is moved to younger leaves from older ones when supplies arc deficient. Deficiency symptoms therefore appear initially on the oldest leaves and the effect on crop yield is marginal, as the oldest leaves do not [7].

Calcium in small amount is essential for tea. A deficiency of it is very rare, as even highly acid soils contain a little calcium. Large amounts of calcium inhibit potassium uptake and a deficiency results. The application of lime to acid tea soils usually depresses crop yield. In some cases where very acid soil contains substantial amounts of potassium, lime can be applied occasionally and improves the pH, with no adverse effect on crop yield. Where calcium is present in great excess, as in soils with a high pH, growth slows down. Internodes shorten, while leaves do not reach full size before yellowing and curling backwards. Leaf edges blacken and leaves distort, crack and fall off [7].

Iron in small quantity is vital. It is usually freely available in acid soils. So deficiencies are almost unknown. With iron, Mn seems to be involved in chlorophyll formation without being included therein. It is usually freely available in acid soils, from which tea absorbs large quantities. The amount in leaves must reach a very high level before any toxic effect on growth is seen. Manganese deficiency is rare. It occasionally occurs on high-pH soils. Leaves become pale to yellow on the edges, with a mottling of red-brown spots on the lamina. Toxicity can develop with very high levels of manganese in leaves, which harden, turn dull brown and become very brittle [7].

In addition to taking part in the synthesis of chlorophyll and vitamins copper is a constituent of polyphenol oxidase in the tea plant which catalyses the fermentation process in tea. When copper is deficient, tea will not ferment properly leaf becoming dark brown rather than bright orange and the quality of the product is reduced. A mild deficiency is quite common but can be rectified by foliar, application of a copper compound [7].

Zinc, a vital element takes part in many essential processes, particularly in the hormones and auxins that control plant growth. Deficiencies of zinc are common in tea with high yield potential. Two or more small buds form in place of one to form a 'rosette'. Leaves elongate and twist to a sickle shape and may develop a way edge and yellow margins. On new stems, the internodal distance becomes very small [7].

2.8. Levels of Metals in the Tea Plant

A number of papers have been published regarding the determination of the metal content of tea [2]. Several elements, such as Ca, Na, K, Mg, and Mn, are present at mg/g level, whereas elements such as Cr, Fe, Co, Ni, Cu, Zn, and Cd are present at a few μg/g levels [37].

Tea leaves have been reported to contain 350-900 μg/g of Mn, an essential element for plants, microorganisms and higher animals including man. The recommended range of daily dietary intake for an adult is 2-5 mg of Mn. Although intake of tea has good and bad effects it can be a good Mn provider if one takes a few cups daily [38]. Manganese deficiency may cause degenerative bone changes and altered pancreatic functions, although its deficiency in humans is unusual [20].

Mn concentration in the tea leaves of US brands, which were derived from India, Sri Lanka, and China, including herbal infusions with several flavoring additives, were found in a much wider range with lowest (79 μg/g) and highest (768 μg/g) values for Refresh and Zen brands respectively. On the other hand, Mn content in various Indian tea brands is in a much narrow range, 371 - 758 μg/g with a mean value of 575 ± 96 μg/g [20].

In Indian tea brands, Na content is in wide range 21 - 118 μg/g, with mean value of 53.5 ± 27.4 μg/g. On the other hand, Na content in US tea brands is in a much wider range, 114 - 796 μg/g with a mean value of 338 ± 286 μg/g. The much higher Na content in US brands may be partly due to flavouring additives. However, K contents in Indian and US tea brands are in a narrow range, 17.7 - 24.0 and 13.1 - 23.7 mg/g with mean contents of 21.1 ± 2.0 and 18.1 ± 3.5 mg/g respectively [20]. It is reported that Na and K contents in three Chinese tea samples were in the range 29.4 - 78.1 μg/g and 16.9 - 20.3 μg/g respectively [39]. Thus, K content in tea leaves is

not only higher, by an order of magnitude compared to Na, but is independent of the brand. However, Na content shows large variability [20].

Cu contents in Indian and US tea brands are in a large range, 1.60 - 35.0 and 4.4 - 17.3 μg/g, with mean values of 14.8 ± 8.2 and 12.3 ± 4.8 μg/g, respectively, which are comparable. However, Cu content in Indian tea brands is in a much wider range compared to that in US brands [20]. It is reported that Cu content is in the range 9.6 - 20.9 μg/g in three Chinese tea brands [39]. It has been reported that copper toxicity or excessive copper levels have been associated with mental fatigue, depression and other mental problems like learning disabilities, hyperactivity and mood swings (sometimes violent, criminal or psychotic behavior) [40].

Contents of non-nutrient heavy metals in both tea and herb leaves are Ni (2.46 - 8.90 μg/g), Pb (0.03 - 14.84 μg/g), Co (Nil - 2.35 μg/g), Cd (Nil - 0.37 μg/g) indicating rather low concentrations. Among tested tea brands, Chinese one has the highest contents of studied heavy metals, Mn, Fe, Zn, Cu and Pb. The level of Pb (14.84 μg/g) in Chinese tea was too high which tea consumers should consider [22].

2.9. Study site description

In this study, tea leaf samples were collected from Wushwush tea plantation farms, at the southwestern highlands of Ethiopia. It is located at 7º19´N and 36º07´E. The altitude of the area is 1900 m above sea level. Mean annual temperature is 18 ºC with an average annual rainfall of 1800 mm. Geologically, the area is associated with Jimma volcanics with abundant rhyolites and trachybasalts. Soils of the area are classified as Plinthic Alisols, with clayey texture and dark reddish brown color [41].

The tea plantations (*Camellia sinensis* O. Kuntze) are more than 25 years old [41]. They are divided into four unit farms (unit farm 01, 02, 03, and 04). There are five different tea clones of the *C. assamica* variety that are grown in the different fields of the four unit farms. The five tea clones are BB 35, 6/8, 11/4, 11/56, and 12/38. All of the five tea clones are grown in the fields of unit farms 01, 02, and 04 while four tea clones (BB 35, 6/8, 11/4, 11/56) are grown in unit farm 03.

3. EXPERIMENTAL

3.1. Instrumentation

Stainless steel soil sampling auger (Oakfield Apparatus Company, Oakfield, Wisconsin, USA) was used to collect all soil samples. Soil samples were ground using ceramic mortar and pestle. The tea leaf samples were ground using an electric motor grinder (Retsch, GmbH & Co. KG Type ZM 1, Hann 1, Germany).

All of the tea leaves and soil samples were weighed on a digital analytical balance (Mettler Toledo, Model AG204, Switzerland) with ± 0.0001 precision. Round bottom flask (100 mL) fitted with a reflux condenser and Kjeldahl digestion block (Kjeldatherm, Gerhardt GmbH & Co. KG, Type KB 40 S, Bonn, Germany) were used for the total digestion of all the samples.

The concentrations of Na, K, Ca, Mg, Fe, Cu, Mn, Zn, Co, Cd, and Pb in both tea leaves and soil samples were determined by atomic absorption flame emission spectrophotometer (Shimadzu, Model AA-6200, Japan) using an air-acetylene flame and connected to SAMSUNG personal computer. A potentiometric digital pH meter (HANNA, Microprocessor pH meter, Model HI 9017, Portugal) was used to determine the pH of soil samples after stirring by a magnetic stirrer (Jenway LTD, Hotplate & Stirrer, Model 1103, U.K.). A water deionizer (Termoacqua Tecnologie S.R.L., Model DEMA/30, Milan, Italy) was used to produce demineralized water.

3.2. Reagents and Chemicals

An acid mixture of 5:1 conc. HNO_3 (69 to 70.5%, AnalaR, BDH Laboratory Supplies, Poole, England) and conc. $HClO_4$ (70%, Riedel-de Haën AG, Seelze-Hannover, Germany) was used for the digestion of the tea leaf samples. Concentrated HCl (36 - 38%, AnalaR, Hopkin & Williams LTD, Essex, England) and HNO_3 (69%, Riedel-de Haën AG, Seelze-Hannover, Germany) were used in the digestion of soil samples and in preparation of stock standard solutions. A solution of 0.1% $LaCl_3 \cdot 7H_2O$ (AnalaR, BDH Laboratory Supplies, Poole, England) was used in the determination of Ca and Mg. $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ and $K_2Cr_2O_7$ (Merck, Darmstadt, Germany), conc. orthophosphoric acid (AnalaR, Hopkin & Williams LTD,

Essex, England), and 1,10-phenanthroline ferrous sulfate (Merck, Darmstadt, Germany) solutions were used to determine soil organic matter.

Stock standards of 1000 mg/L and intermediate standard solutions of 100 mg/L of each metal were prepared. Demineralized water was used throughout the experiment to prepare all the solutions.

Standard stock solutions of Na and K were prepared from NaCl (Riedel-de Haën AG, Seelze-Hannover, Germany) and KCl (Merck, Darmstadt, Germany), respectively, after drying at 105 ^{o}C in an oven for 2 hours, cooled in a desiccator and dissolved in deionized water. Ca standard stock solution was prepared after drying $CaCO_3$ (Riedel-de Haën AG, Seelze-Hannover, Germany) at 105 ^{o}C in an oven for 2 hours, cooled in a desiccator, dissolved in deionized water and after dropwise addition of a minimum volume of HCl (approximately 10 mL) to effect complete dissolution of the $CaCO_3$ and diluted to 1 liter with deionized water.

Standard stock solutions of Mg, Mn, Zn, Cd (AnalaR, Hopkin & Williams LTD, Essex, England) and Fe, Cu, Co (Riedel-de Haën AG, Seelze-Hannover, Germany) were prepared from metal powders of each element. Pb standard stock solution was prepared from lead nitrate, $Pb(NO_3)_2$ (Riedel-de Haën AG, Seelze-Hannover, Germany). Standard stock solutions of Mg, Zn, Cd and Co were prepared from metal powders of each element after dissolving in a minimum volume of concentrated HCl (1:1 HCl:H_2O) and diluted to1 liter with 1% (v/v) HCl. Standard stock solutions of Mn and Fe were prepared from metal powders of each elements after dissolving in a minimum volume of concentrated HNO_3 (1:1 HNO_3:H_2O) and diluted to 1 liter with 1% (v/v) HCl and with deionized water respectively. Standard stock solutions of Cu and Pb were prepared from Cu metal powder and lead nitrate, $Pb(NO_3)_2$, respectively, after dissolving in a minimum volume of (1:1) concentrated HNO_3 (1:1 HNO_3:H_2O) and diluted to 1 liter with 1% (v/v) HNO_3.

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 (100 mg/L).

3.3. Sample Collection

The tea leaf and soil samples were collected from the four unit farms of Wushwush Tea Development. The tea plantation farms are the largest of all tea farms in hectare coverage (1,250.68 ha) and the tea plants in these farms are representative of tea clone types grown in the country.

3.3.1. Tea Leaf Sample Collection

A total of 19 (about 500 g each) tea leaf samples were used in this study. The parts of the tea plant plucked for sampling were young shoots of one apical bud with recently matured two youngest true foliage leaves. The tea leaf samples were collected randomly from five different tea clones of the *C. assamica* variety grown in different fields of the four unit farms of Wushwush Tea Development: five tea clones (BB 35, 6/8, 11/4, 11/56, and 12/38) from unit farms 01, 02, and 04 and four tea clones (BB 35, 6/8, 11/4, 11/56) from unit farm 03. All of the samples were collected in clean paper bags.

3.3.2. Soil Sample Collection

At the spots where the tea leaf samples were plucked, a total of 19 (about 500 g each) corresponding soil samples were collected for this study. The soil samples were taken at 50 cm canopy radius of each tea plant in 20 cm depth using stainless steel soil sampling auger. The samples were collected in clean polyethylene bags.

3.4. Sample Preparation

3.4.1. Tea Leaf Sample Preparation

To remove surface contaminants such as dust and herbicide spray residues, the tea leaf samples were washed with tap water followed by rinsing with distilled water. In order to facilitate grinding, samples were dried in an open air at room temperature until constant dry weight was obtained. The dried samples were ground to fineness using an electric motor grinder to pass 0.5 mm sieve and stored in polyethylene bags for analysis.

3.4.2. Soil Sample Preparation

All soil samples were air dried; friable fragments (> 2 mm) such as shells, organic debris, schist, weathered rocks, calcareous nodules and other concretions of pedogenic nature, gravel, stones etc., were removed. The dried soils were ground manually using ceramic mortar and pestle to pass a 2 mm sieve and stored in clean card boxes until analysis.

3.5. Analysis of Samples

3.5.1. Optimization of Working Procedure

Different procedures for tea leaf sample digestion were assessed based on reagent volume, digestion time and digestion temperature. By varying the three variables, tea leaf samples were digested to obtain clear, colorless solution without residue and suspended matter.

3.5.2. Analysis of Tea Leaf Samples

Exactly 0.5 g of the dried and ground tea leaf sample was accurately weighed on a digital analytical balance and transferred quantitatively into a 100 mL round bottom flask. 8 mL of a freshly prepared 5:1 mixture of conc. HNO_3 (69 to 70.5%) and conc. $HClO_4$ (70%) was added to the sample. The samples was swirled gently to homogenize, fitted to a reflux condenser and digested continuously for 3 hours at 300 °C on a Kjeldahl digestion block. After completion of digestion, a clear colorless solution with no residue and suspended matter was obtained.

The clear digest was cooled and quantitatively transferred to a 100 mL volumetric flask and made up to the mark with deionized water. Each tea samples was digested in triplicate. Digestion of a reagent blank was also performed in parallel with the samples. All the solutions were stored in tightly capped polyethylene bottles and stored in a refrigerator until analysis.

The solutions were used to determine concentrations of Na, K, Ca, Mg, Fe, Mn, Cu, Zn, Co, Pb and Cd by atomic absorption flame emission spectrophotometer (AAFES). Concentrations of Na and K were determined in emission mode of the spectrophotometer. For each tea leaf

sample, three repeat measurements were performed by the AAFES. Therefore, as three acid digests were prepared from each tea sample, the element concentration values used in the study were obtained from the mean of nine measurements for each sample.

3.5.3. Analysis of Soil Samples

3.5.3.1. Soil pH

Soil pH was measured potentiometrically by a pH meter in a suspension of a 1:1 soil:water mixture. About 10 g of air-dried soil (< 2 mm) was weighed and transferred in to 100 mL beaker and 10 mL of water was added. The sample was stirred by a magnetic stirrer and the pH was measured after allowing the suspension to stand for 1 h at room temperature [42].

3.5.3.2. Soil Organic Matter

Soil organic matter is oxidized under standard conditions with potassium dichromate in concentrated sulfuric acid. A measured amount of $K_2Cr_2O_7$ is used in excess of that needed to destroy the organic matter and the excess is determined by titration with ferrous ammonium sulfate or ferrous sulfate solution, using 1,10-phenanthroline ferrous sulfate indicator to detect the first appearance of unoxidized ferrous ion [42].

Exactly, 0.1 g of soil was weighed and transferred in to 500 mL Erlenmeyer flask. A reagent blank was included. 10 mL of 1 N $K_2Cr_2O_7$ solution was added to both samples and blank. 20 mL of conc. H_2SO_4 was carefully added with measuring cylinder in a fume cupboard. The flask was swirled and allowed to stand for 30 min. Then 200 mL distilled water was added and allowed to cool. 10 mL of conc. orthophosphoric acid was added and just before titration, 5 drops of 1,10-phenanthroline ferrous sulfate indicator solution was added. Both the sample and blank were titrated with 0.5 N ferrous sulfate solution until the color changes to purple, then ammonium ferrous sulfate solution was added drop by drop until the color flashes to green then continued slowly to a light green end point [42].

3.5.3.3. Total Metal Analysis of Soils

1.0 g of soil was accurately weighed and transferred in to 100 mL round bottom flask. 10 mL of 5:2 mixture of conc. HNO_3 and HCl was added to the flask and digested on a Kjeldahl digestion block under reflux condenser for 3 h at 370 °C. The digest was left to stand for 30 min to cool down to room temperature then about 60 mL deionized water was added to the flask, filtered through Whatman No. 41 filter paper in to a 250 mL volumetric flask, and made up to the mark with deionized water. The solutions were used for the analysis of the total soil metal concentrations for Na, K Ca, Mg, Fe, Cu, Zn, Mn, Co, Pb and Cd by atomic absorption flame emission spectrophotometer (AAFES). Concentrations of Na and K were determined in emission mode of the spectrophotometer [43].

3.6. Recovery Tests

To check the efficiency of the procedure, 50 µL of 1000 mg/L Ca, Mg, Cd, Co and Mn were spiked at once in to a tea sample and 50 µL of 1000 mg/L Na, K, Fe, Zn and Cu into another digestion flask containing the same tea sample. Each sample was determined for their respective spiked metals by atomic absorption flame emission spectrophotometer (AAFES). Recovery test was also performed for soil samples using the same procedure. Each recovery test for both samples was performed in triplicate.

3.7. Method Detection Limit

Seven blank samples were digested and each of the samples were determined for the elements, metal concentrations for Na, K Ca, Mg, Fe, Cu, Zn, Mn, Co, Pb and Cd by atomic absorption flame emission spectrophotometer (AAFES). The standard deviations for each element were calculated from the seven blank measurements to determine method detection limit of the instrument.

4. RESULTS AND DISCUSSION

4.1. Instrument Calibration

The instrument was calibrated using standard working series of solutions of each of the metals. The standard working solutions of each metal were prepared freshly by appropriate dilution of the intermediate standard solutions. Concentrations of the intermediate standards, working standard solutions and values of correlation coefficients of the calibration graph for each of the metals are presented in Table 1.

Table 1. Concentration values of working standard solutions and correlation coefficients of calibration graph.

Metal	Conc of intermediate standard (mg/L)	Conc of standard series (mg/L)	Correlation coefficient of calibration graph
Na	100	2.0, 4.0, 6.0, 8.0	0.9987
K	100	2.0, 4.0, 6.0, 8.0	0.9973
Ca	50	0.5, 1.0, 2.0, 4.0	0.9984
Mg	50	0.5, 1.0, 2.0, 4.0	0.9999
Fe	50	0.5, 1.0, 2.0, 4.0	0.9975
Mn	50	0.5, 1.0, 2.0, 4.0	0.9986
Cu	50	0.25, 0.5, 1.0, 2.0	0.9999
Zn	50	0.25, 0.5, 1.0, 2.0	0.9968
Co	10	0.05, 0.1, 0.2, 0.4	0.9979
Cd	10	0.05, 0.1, 0.2, 0.4	0.9982
Pb	10	0.05, 0.1, 0.2, 0.4	0.9999

4.2. Optimization of Working Procedure

Different procedures for tea leaf sample digestion were assessed and the procedure that consumed smaller reagent volume, took smaller digestion time and produced clear solutions with no residue and suspended matter was selected for the routine digestion of the samples. In this study, alternative 3 was used for routine analysis of the tea samples (Table 2).

Table 2. Different methods tested during the optimization of procedures.

Method	Wt of tea sample (g)	5:1 conc HNO_3 and $HClO_4$ (mL)	Digestion temp. (^oC)	Digestion time (min)	Results
1	0.5	10	300	180	Clear, colorless solution with no residue
2	0.5	10	300	150	Slightly yellowish solution with no residue
3	0.5	8	300	180	Clear, colorless solution with no residue
4	0.5	8	300	150	Light yellow solution with suspension
5	0.5	7	300	180	Yellowish solution with residue

4.3. Analytical Method Detection Limit

In general terms, detection limit of an analytical method refers to the minimum concentration of a substance that can be reported with 99% confidence to be greater than zero [44]. In statistical terms of analytical chemistry, the limit of detection (LOD) is the smallest mass of analyte that can be distinguished from statistical fluctuations in a blank, which usually corresponds to the standard deviation of the blank absorbance times a constant. The limit of detection is most commonly defined as the mass of analyte that gives a signal equal to three times the standard deviation on the blank [45].

In this study, the detection limit for the methods was calculated by multiplying the standard deviation of seven blank signals each determined in triplicate by three. The calculated LOD for tea leaf and soil samples are given in Table 3. The method detection limits are generally comparable with that of instrument for both tea leaf and soil samples.

Table 3. Method detection limits for tea leaf and soil samples.

Metal	Instrument detection limit (mg/L)	[a]Method detection limit for tea leaf (mg/g)	[a]Method detection limit for soil (mg/g)
Na	0.006	0.002	0.003
K	0.012	0.003	0.002
Ca	0.070	0.006	0.004
Mg	0.004	0.004	0.006
Fe	0.080	0.034	0.022
Mn	0.028	0.002	0.004
Cu	0.040	0.022	0.002
Zn	0.011	0.012	0.005
Co	0.060	0.008	0.008
Cd	0.012	0.001	0.001
Pb	0.280	0.036	0.020

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

4.4. Evaluation of Analytical Method

Recovery tests using the proposed method were performed for both tea leaf and soil samples using non-spiked and spiked samples, and each sample was determined in triplicate. As shown in Table 4, the results of percentage recoveries for the studied metal nutrients in tea leaves were all between 90 to 104%. The results of the recovery tests for tea leaf samples were within the acceptable range verifying the validity of the proposed method for tea leaf analysis.

Table 4. Recovery test results for tea leaf samples.

Metal	[a]Conc in sample	Amount added (mg)	[a]Conc in spiked sample	Amount recovered (mg)	[b]Recovery (%)
Na	116.593 ± 0.02 mg/kg	0.05	116.644 ± 0.15 mg/kg	0.051	102 ± 0.88
K	21.870 ± 2.31 mg/g	0.05	21.917 ± 1.32 mg/g	0.047	94 ± 1.46
Ca	0.717 ± 0.01 mg/g	0.05	0.762 ± 0.02 mg/g	0.045	90 ± 0.86
Mg	2.646 ± 0.64 mg/g	0.05	2.697 ± 0.46 mg/g	0.051	102 ± 1.15
Fe	85.527 ± 0.37 mg/kg	0.05	85.575 ± 0.39 mg/kg	0.048	96 ± 0.57
Mn	995.447 ± 2.78 mg/kg	0.05	995.493 ± 1.25 mg/kg	0.046	92 ± 1.78
Cu	19.147 ± 0.02 mg/kg	0.05	19.196 ± 0.26 mg/kg	0.049	98 ± 0.82
Zn	67.880 ± 0.25 mg/kg	0.05	67.932 ± 0.02 mg/kg	0.052	104 ± 1.39
Co	1.210 ± 0.01 mg/kg	0.05	1.255 ± 0.11 mg/kg	0.015	90 ± 0.31
Cd	nil[c]	0.05	0.049 ± 0.50 mg/kg	0.049	98 ± 0.56
Pb	nil	0.05	0.045 ± 0.71 mg/kg	0.045	90 ± 1.25

[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.

Table 5. Recovery test results for soil samples.

Metal	[a]Conc in sample	Amount added (mg)	[a]Conc in spiked sample	Amount recovered (mg)	[b]Recovery (%)
Na	963.680 ± 0.57 mg/kg	0.05	963.728 ± 1.44 mg/kg	0.048	96 ± 1.27
K	7.347 ± 0.02 mg/g	0.05	7.392 ± 0.35 mg/g	0.045	90 ± 0.43
Ca	0.445 ± 0.01 mg/g	0.05	0.490 ± 0.42 mg/g	0.045	90 ± 0.50
Mg	1.423 ± 0.11 mg/g	0.05	1.472 ± 0.03 mg/g	0.049	98 ± 0.21
Fe	24.554 ± 0.14 mg/g	0.05	24.605 ± 2.51 mg/g	0.051	102 ± 1.69
Mn	1.796 ± 0.01 mg/g	0.05	1.841 ± 0.16 mg/g	0.045	90 ± 0.53
Cu	7.780 ± 0.18 mg/kg	0.05	7.829 ± 0.30 mg/kg	0.049	98 ± 0.12
Zn	68.120 ± 0.13 mg/kg	0.05	68.167 ± 0.05 mg/kg	0.047	94 ± 0.17
Co	21.140 ± 0.02 mg/kg	0.05	21.187 ± 0.09 mg/kg	0.047	94 ± 0.08
Cd	0.440 ± 0.12 mg/kg	0.05	0.490 ± 0.07 mg/kg	0.050	100 ± 0.57
Pb	nil[c]	0.05	0.049 ± 0.11 mg/kg	0.049	98 ± 0.63

[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.

The recovery tests for soil samples were performed using the procedure presented in section 3.5.3.3. The values of percentage recoveries for the studied macro- and micronutrient and the toxic metals in soil samples were within the range of 90 to 102% (Table 5).

4.5. Important Soil Properties

Plants normally derive their mineral components from the soil. The elements essential for plants can be subdivided into those required in relatively large amounts, the macronutrients (H, C, N, O, Mg, P, S, K, and Ca) and those required in small amounts, the micronutrients (B, Cl, V, Mn, Fe, Cu, Zn, Co and Mo) [33]. Soil pH and its organic matter content are among those major factors that greatly affect the availability of macro- and micronutrients and even toxic elements in soils and their uptake by plant roots [30].

4.5.1. Soil pH

The degree of soil acidity or alkalinity, expressed as soil pH, is a master variable that affects a wide range of soil properties - chemical, biological, and indirectly even physical. This chemical variable greatly influences the availability for root uptake of many elements, including both nutrients and toxins [30].

Soils supply plants with the inorganic mineral nutrients in the form of dissolved ions. To be taken up by a plant, a nutrient element must be in a soluble form and must be located at the root surface which is in such intimate contact with soil particles that a direct exchange may take place between nutrient ions adsorbed on the surface of soil colloids and the H^+ ions from the surface of root cell walls [30].

Most plants grow best in slightly acidic soils (pH 6.0 - 7.0). In this pH range, nearly all plant nutrients are available in optimal amounts for plant growth. Soils with a pH below 6.0 are more likely to be deficient in some available nutrients. Calcium, Mg and K are especially deficient in acid soils [29].

Soil pH, especially in well-aerated soils, has a decided influence on the availability of all the micronutrients except chlorine. Under acid conditions, most micronutrient cations are soluble and freely available some times at toxic levels [30]. In strongly (pH 4.0 - 5.0) and very strongly (pH 3.0 - 4.0) acid soils, Al, Fe, and Mn may exist in very high amounts because of their increased solubilities. If phosphates are present, these elements react with the phosphates to form insoluble phosphates. In alkaline soils (pH > 7), Fe, Mn, Zn, and Cu become unavailable for plant growth [29].

4.5.2. Soil Organic Matter

Soil organic matter consists of a wide range of organic (carbonaceous) substances, including living organisms (the soil biomass), carbonaceous remains of organisms that once occupied the soil, and organic compounds produced by current and past metabolism in the soil [30].

This organic fraction of the soil is constantly undergoing physical and chemical changes as a result of decomposition and mineralization processes. The end result of these processes is the productions of CO_2, H_2O, nutrients, and organic and inorganic acids. Upon decomposition of organic matter, inorganic nutrients in the plant tissue, N, P, K, Ca, Mg, Fe, Cu, Zn and Mn etc., are released to the soil [29].

The values of soil pH and organic matter contents of the soils for the selected fields of the four unit farms are presented in Table 6. The soil pH of the four unit farms is with in the range of 5.04 to 5.49, which categorizes the soils under strongly acidic soils [29].

Table 6. Average values of soil pH, OC and OM for selected fields of each unit farm.

Soil	Field No.	pH (1:1 H_2O)	% OC[a]	% OM[b]
Unit farm 01	01, 03, 02, 07, 17	5.04	3.44	5.93
Unit farm 02	02, 01, 04, 14, 12	5.04	3.18	5.48
Unit farm 03	17, 13, 18, 10	5.49	3.29	5.67
Unit farm 04	11, 07, 01, 09, 08	5.20	3.49	6.02

[a] OC – organic carbon.
[b] OM – organic matter.

The higher acidity of soils of the four tea farms is mainly attributed to the continuous application of NPKS 25:5:5:5 fertilizer for several years. Owuor *et al*. [46] reported increasing rates of nitrogenous fertilizers generally increase soil acidity.

According to Ishibashi *et al*. [47], nitrogenous fertilizers are known to produce H^+ by the following reaction, which is induced by soil bacteria:

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

Thus, during application of these fertilizers to the soil, the rate of nitrification is reported to be higher and inorganic nitrogen may be rapidly converted to nitrate producing H^+, which acidifies the soil.

The tea plant under cultivation is normally pruned in order to maintain the plucking table so that the tea bush becomes within reach of pickers with large canopy capable of producing many shoots. The organic matter content of these soils is high (5.48% to 6.02%) due to the accumulation of tea biomass through the incorporation of tea prunings. Dang [48] reported that pruning is an important approach to balance nutrient in plant-soil system in the tea cultivation because it returns to soil a lot of nutrients and organic carbon.

According to Weeraratna *et al*. [49], retention of the pruned biomass after pruning increase soil organic matter content of sub-surface soils as the organic matter is transported from the surface soil to the sub-surface soil by biological activity or leaching. Addition of fertilizers has also increased the organic matter content of sub-surface soils. This is likely to be due to the increased microbial activity brought about by the addition of fertilizers. The incorporation of nitrogen accelerates decomposition of carbonaceous material.

4.6. Levels of Metals in the Soils

Analytical data for nutrient elements, K, Ca, Mg, Fe, Cu, Zn, Na, Co and for the toxic metals Cd and Pb in soils of the four unit farms are given in Table 7 and Figure 5 and 6. Potassium is the third of the major plant nutrients next to N and P [50]. Among the macroelements, K

content of the soils is higher within a range of 7.14 - 9.73 mg/g followed by Mg (0.84 - 1.13 mg/g) and Ca (0.29 - 0.49 mg/g). The much higher K content in the soils may be due to the application of NPKS fertilizer whereas due to the high rainfall (1800 mm) distribution in the area [41], extensive leaching of Ca and Mg occur rendering these metals to exist at lower levels.

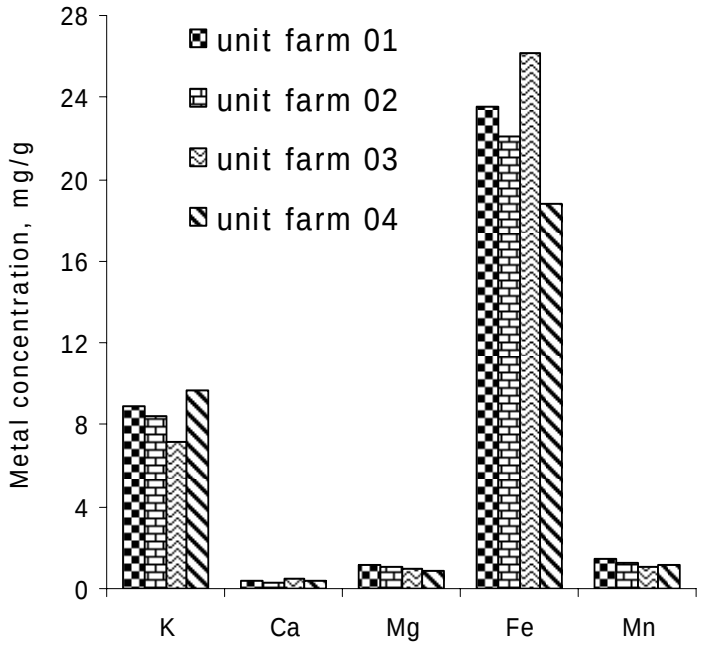


Figure 5. Average metal concentration (K, Ca, Mg, Fe and Mn) in the soils of the four farms (dry mass basis).

Weeraratna *et al*. [49] reported that incorporation of tea prunings and addition of K fertilizers to the soils rapidly increase concentration of available K that could be attributed to mineralization of the organic matter, the solubilization effect of rain water and due to increased chemical and biological fixation of potassium in the presence of fertilizers.

Soils of the unit farms are classed under the category of Plinthic Alisols, with clayey texture and dark reddish brown color [41], which is indicative of the presence of excess amount of hematite (Fe_2O_3) [29]. Soils with low pH contain high amounts of Fe and Al oxides [51]. Thus, Fe is the predominant metal within the concentration range of 18.83 - 23.50 mg/g in these soils whereas Mn content is in the range of 1.03 - 1.47 mg/g.

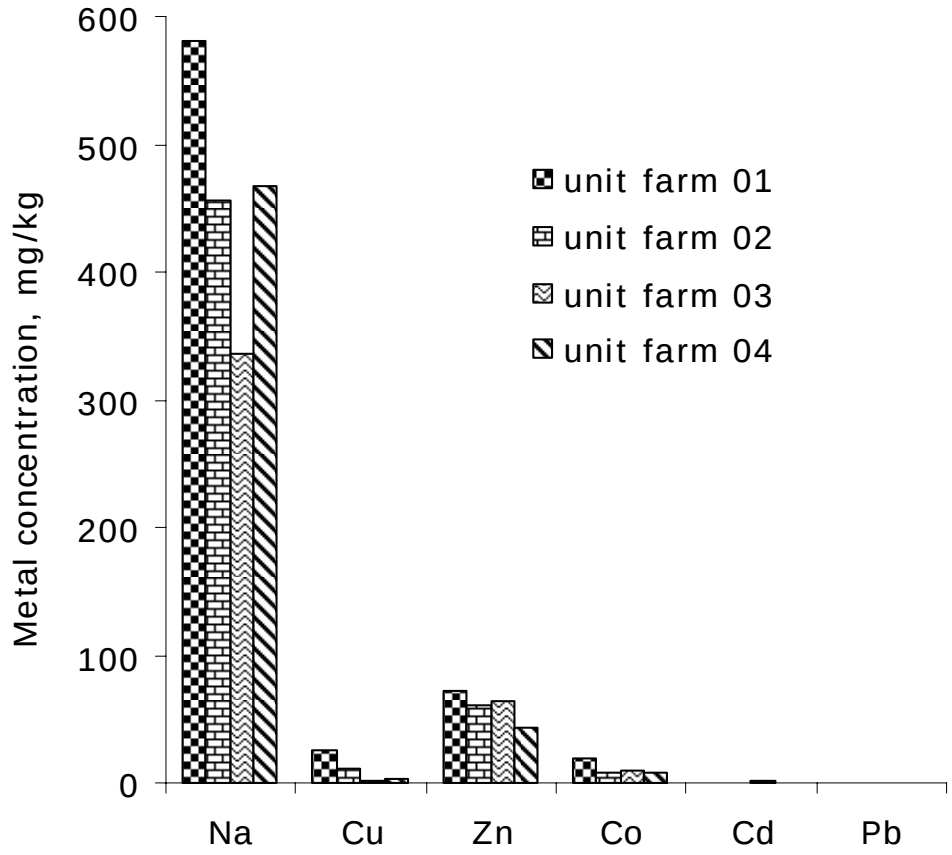


Figure 6. Average concentrations of metals (Na, Cu, Zn, Co, Cd and Pb) in the soils of four farms (dry mass basis).

Concentration of Na (336.04 - 580.71 mg/kg) a non-essential metal in these soils is higher when compared to the micronutrient heavy metals Cu (2.34 - 25.63 mg/kg), Zn (43.96 - 71.27 mg/kg), and Co (7.54 - 19.92 mg/kg). On the other hand, level of the toxic heavy metal Cd ranges from 0.2 mg/kg for unit farms 02 and 03 up to 1.10 mg/kg for soils of unit farm 03. The level of Pb, the other tested toxic metal, in soils of all unit farms was found to be below detection limit of the method used in this study.

Table 7. [a]Average metal concentrations of soils of the selected fields of four unit farms.

	[b]Metal concentrations of soils (dry mass basis)					
Unit farm	K (mg/g)	Ca (mg/g)	Mg (mg/g)	Fe (mg/g)	Mn (mg/g)	
01	8.90 ± 0.02	0.34 ± 0.02	1.13 ± 0.01	23.50 ± 0.07	1.47 ± 0.03	
02	8.39 ± 0.02	0.29 ± 0.01	1.07 ± 0.004	22.11 ± 0.08	1.30 ± 0.03	
03	7.14 ± 0.01	0.49 ± 0.02	0.93 ± 0.003	26.20 ± 0.12	1.03 ± 0.03	
04	9.73 ± 0.02	0.42 ± 0.02	0.84 ± 0.004	18.83 ± 0.11	1.19 ± 0.02	
	Na	Cu	Zn	Co	Cd	Pb
	..... (mg/kg)					
01	580.71 ± 4.99	25.63 ± 0.89	71.27 ± 0.47	19.92 ± 3.77	0.32 ± 0.43	nil[c]
02	456.31 ± 3.43	11.22 ± 1.39	60.94 ± 0.53	8.44 ± 1.72	0.20 ± 0.74	nil
03	336.04 ± 5.92	2.34 ± 1.11	63.80 ± 0.54	9.44 ± 2.37	1.10 ± 0.25	nil
04	467.62 ± 4.11	2.88 ± 0.87	43.96 ± 0.71	7.54 ± 3.81	0.20 ± 0.76	nil

[a] Average values of four soil samples from unit farm 03 and fifteen soil samples from unit farms 01,02 and 04.
[b] Analytical results for all metals expressed as mean ± ts/√N calculated for 9 measurements at 95 % confidence limit ($P < 0.05$).
[c] Concentration of the tested heavy metal below the method detection limit.

4.7. Levels of Metals in the Clonal Tea Leaves

The results of total contents of the studied nutrient and toxic metals in the five clones of *C. assamica* variety show the ability of these plants to accumulate high amounts of both macro- and micronutrient elements. The most abundant metal among the macroelements was K followed by Mg and Ca whereas Mn content of the tea leaves was the predominant among the tested micronutrient heavy metals followed by Fe, Zn, Cu and Co. On the other hand, the content of Na, the non-essential alkali metal in plant nutrition, was found to be higher than all the heavy metals except Mn and clone 11/4 of unit farm 01 for it has highest content of Zn.

4.7.1. Tea Clones of Unit Farm 01

The level of each metal in the five tea clones grown in unit farm 01 is given in Table 8. Concentration of K in the five clones was higher with in the range of 21.87 - 23.73 mg/g, whereas the levels of Mg (2.65 - 3.10 mg/g) and Ca (0.72 - 1.82 mg/g) were much lower. These macronutrient elements could be arranged in descending order according to their levels in the five tea clones: K (21.87 - 23.73 mg/g) > Mg (2.65 - 3.10 mg/g) > Ca (0.72 - 1.82 mg/g) (Figure 7).

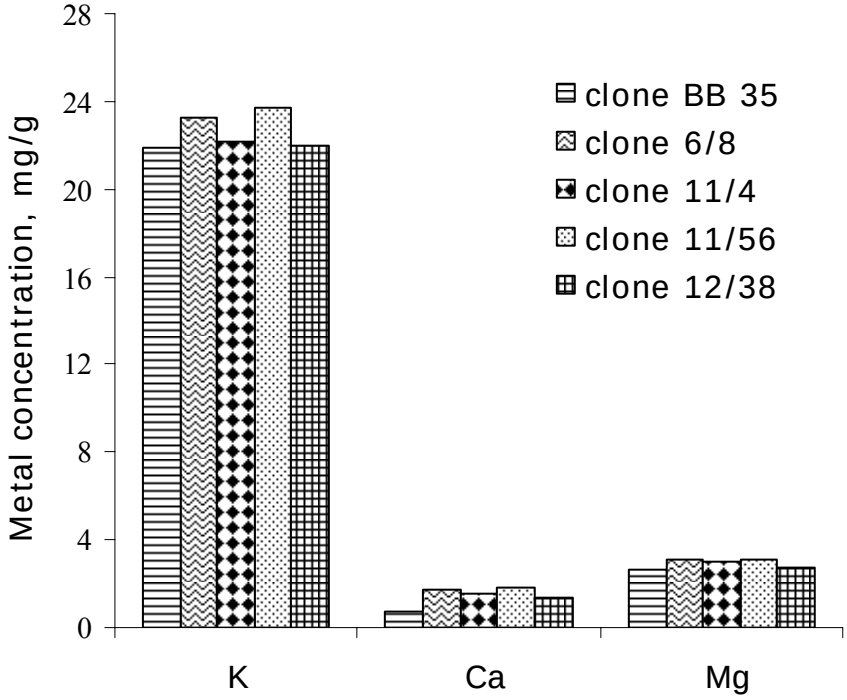


Figure 7. Levels of macronutrients elements (dry mass basis) in five tea clones of unit farm 01.

The results show that among the tested heavy metals Mn (599.04 - 280.55 mg/kg) content of the five clones was predominantly higher. Content of Na, non-essential alkali metal, in the five tea clones varied from 116.59 - 265.07 mg/kg, which was much higher than the essential heavy metals Fe (71.07 - 100.43 mg/kg), Cu (4.55 - 19.15 mg/kg), and Co (1.02 - 2.84 mg/kg). The level of Zn in clone 11/4 was 330.53 mg/kg, whereas the other clones show comparable values for this metal ranging between 57.87 to 86.69 mg/kg.

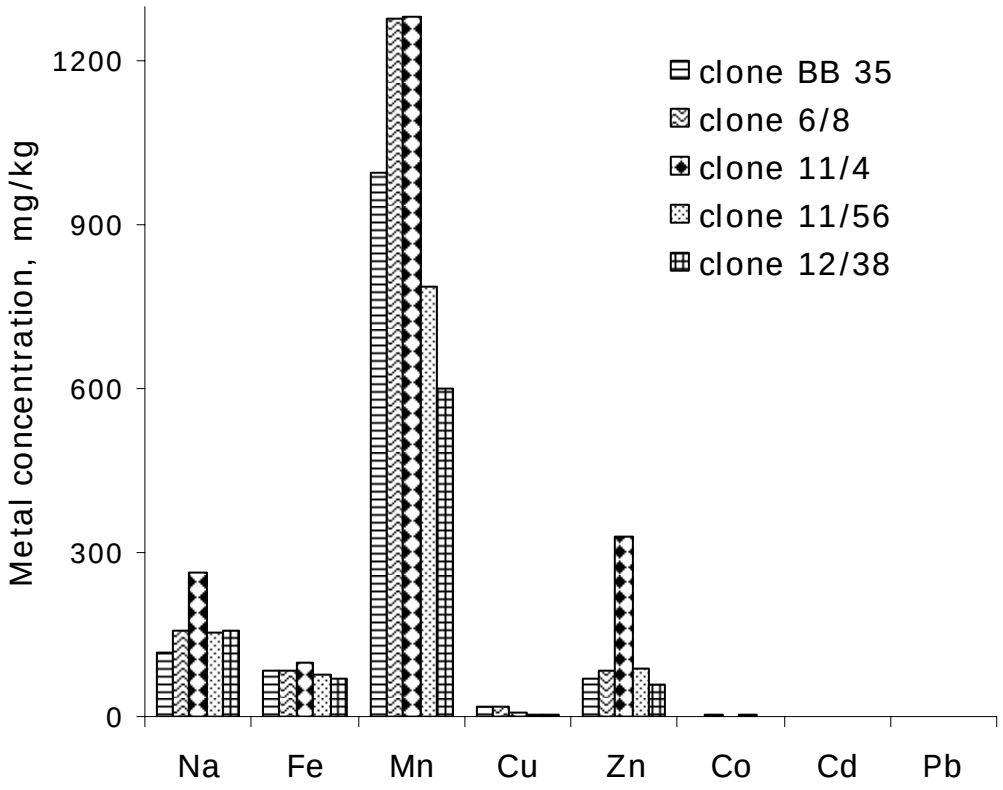


Figure 8. Levels of Na, Fe, Mn, Cu, Zn, Co, Cd and Pb (dry mass basis) in five tea clones of unit farm 01.

The histogram (Figure 8) clearly demonstrates that among the five clones, clone 11/4 contains the highest levels of Na (265.07 mg/kg), Fe (100.43 mg/kg), Mn (1280.55 mg/kg), and Zn (330.53 mg/kg) with exceptions of Cu and Co. The highest level of Cu (19.15 mg/kg) and Co (2.84 mg/kg) were observed in BB 35 and 11/56, respectively. The concentrations of toxic heavy metals, Cd and Pb, in all five clones were too low to be detected using the analytical method used in this study.

The accumulation pattern for these metals, with exception of clone 11/4 for its high Zn content, could be arranged in descending order for most of the clones: Mn (599.04 - 1280.55 mg/kg) > Na (116.59 - 265.07 mg/kg) > Fe (71.07 - 100.43 mg/kg) > Zn (57.87 - 86.69 mg/kg) > Cu (4.55 - 19.45 mg/kg) > Co (1.02 - 2.84 mg/kg).

Table 8. [a]Metal content of tea clones (dry mass basis) in unit farm 01.

Tea clone	K (mg/g)	Ca (mg/g)	Mg (mg/g)	Cd	Pb
BB 35	21.87 ± 0.96	0.72 ± 0.04	2.65 ± 0.10	nil[b]	nil
6/8	23.29 ± 0.42	1.77 ± 0.03	3.10 ± 0.02	nil	nil
11/4	22.18 ± 0.31	1.57 ± 0.07	3.04 ± 0.02	nil	nil
11/56	23.73 ± 0.72	1.82 ± 0.08	3.08 ± 0.04	nil	nil
12/38	21.98 ± 0.50	1.35 ± 0.03	2.75 ± 0.01	nil	nil

	Na	Fe	Mn	Cu	Zn	Co
	…… (mg/kg) ……..					
BB 35	116.59 ± 1.88	85.53 ± 0.37	995.45 ± 18.21	19.15 ± 0.54	67.88 ± 4.18	1.21 ± 0.01
6/8	155.92 ± 3.86	84.72 ± 0.62	1279.80 ± 7.44	17.31 ± 1.75	82.89 ± 1.01	2.16 ± 0.04
11/4	265.07 ± 1.32	100.43 ± 0.12	1280.55 ± 3.44	7.42 ± 0.43	330.53 ± 3.33	1.35 ± 0.02
11/56	155.49 ± 2.00	75.74 ± 6.34	786.53 ± 2.24	4.55 ± 0.73	86.69 ± 4.99	2.84 ± 0.17
12/38	156.04 ± 2.26	71.07 ± 2.76	599.04 ± 3.44	5.24 ± 1.78	57.87 ± 4.59	1.02 ± 0.03

[a] Analytical results for all metals expressed as mean ± ts/√N calculated for 9 measurements at 95 % confidence limit ($P < 0.05$).
[b] Concentrations of the tested heavy metals below the method detection limit.

4.7.2. Tea Clones of Unit Farm 02

Metal contents of tea clones grown in unit farm 02 follow the same pattern as those of unit farm 01 for the macro- and micronutrient elements. As shown in Table 9, Figure 9 and 10, K content in the five clones was in the range of 20.88 mg/g in clone BB 35 up to 24.83 mg/g in clone 12/38. Similarly, levels of Mg were with in a range of 3.05 mg/g in clone BB 35 up to 3.45 mg/g in clone 12/38 whereas Ca levels ranged between 1.02 mg/g to 1.82 mg/g in clone BB 35 and 11/56, respectively.

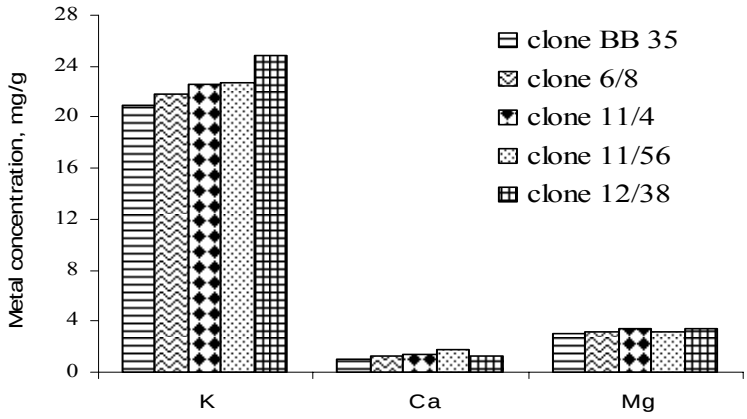


Figure 9. Levels of macronutrients elements (dry mass basis) in five tea clones of unit farm 02.

Clone BB 35 was found to contain highest level of Na (188.88 mg/kg) whereas lowest concentration of Na (109.61 mg/kg) was accumulated in clone 11/56. Among the studied heavy metals, highest concentrations of Mn (1140.35 mg/kg) in 12/38, Fe (83.55 mg/kg) in 11/56, Zn (83.85 mg/kg) and Co (2.65 mg/kg) in BB 35, and Cu (4.22 mg/kg) in 11/4 were observed. On the other hand, Cd and Pb contents of the five clones were below method detection limit.

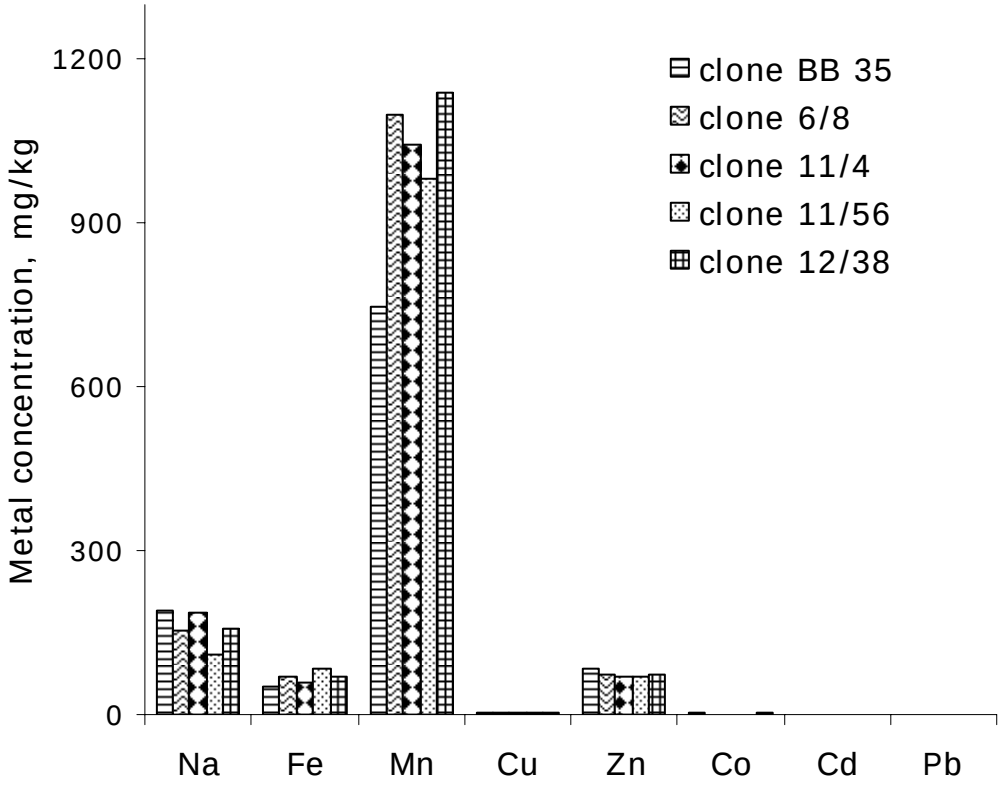


Figure 10. Levels of Na, Fe, Mn, Cu, Zn, Co, Cd and Pb (dry mass basis) in five tea clones of unit farm 02.

The results show that levels of Na (109.61 - 188.88 mg/kg), Fe (51.51 - 83.55 mg/kg) and Cu (2.67 - 4.22 mg/kg) in tea clones of unit farm 02 were generally lower when compared to those of unit farm 01. Mn levels in clone BB 35 (747.91 mg/kg), clone 6/8 (1100.05 mg/kg), and clone 11/4 (1042.87 mg/kg), with exceptions of clones 11/56 and 12/38, were lower than levels in those of unit farm 01. On the other hand, contents of Zn and Co in clone 6/8 (Zn, 72.87 mg/kg; Co, 1.17 mg/kg), clone 11/4 (Zn, 67.81 mg/kg; Co, 0.98 mg/kg), and clone 11/56 (Zn,

70.37 mg/kg; Co, 1.71 mg/kg) were also lower than the corresponding levels of these metals for those clones of unit farm 01.

Table 9. [a]Metal content of tea clones (dry mass basis) in unit farm 02.

Tea clone	K (mg/g)	Ca (mg/g)	Mg (mg/g)	Cd	Pb
BB 35	20.88 ± 0.54	1.02 ± 0.12	3.05 ± 0.14	nil[b]	nil
6/8	21.81 ± 0.81	1.27 ± 0.01	3.13 ± 0.03	nil	nil
11/4	22.61 ± 0.93	1.38 ± 0.07	3.37 ± 0.02	nil	nil
11/56	22.66 ± 0.28	1.82 ± 0.09	3.10 ± 0.15	nil	nil
12/38	24.83 ± 0.67	1.24 ± 0.26	3.45 ± 0.03	nil	nil

	Na	Fe	Mn	Cu	Zn	Co
	(mg/kg)					
BB 35	188.88 ± 7.13	51.51 ± 1.03	747.91 ± 1.61	3.73 ± 0.11	83.85 ± 3.74	2.65 ± 0.14
6/8	153.97 ± 6.47	69.64 ± 1.87	1100.05 ± 1.24	3.28 ± 0.20	72.87 ± 0.78	1.17 ± 0.01
11/4	185.78 ± 6.59	57.17 ± 3.36	1042.87 ± 5.16	4.22 ± 0.47	67.81 ± 2.25	0.98 ± 0.01
11/56	109.61 ± 1.83	83.55 ± 2.06	982.99 ± 6.02	2.96 ± 0.57	70.37 ± 3.94	1.71 ± 0.04
12/38	158.09 ± 3.36	68.03 ± 1.52	1140.35 ± 4.48	2.67 ± 0.05	75.07 ± 2.11	2.51 ± 0.12

[a] Analytical results for all metals expressed as mean ± ts/√N calculated for 9 measurements at 95 % confidence limit ($P < 0.05$).
[b] Concentrations of the tested heavy metals below the method detection limit.

4.7.3. Tea Clones of Unit Farm 03

Tea clones grown in unit farm 03 are only the four types, BB 35, 6/8, 11/4, and 11/56. The level of macronutrient metal, K is highest in clone 11/4 (23.89 mg/g) and lowest in clone BB 35 (19.21 mg/g). Clone 11/4 contained highest concentration of Mg (3.45 mg/g) whereas levels of Ca being highest in clone 11/56 (1.30 mg/g) and lowest in clone BB 35 (0.83 mg/g). The trend of availability of these macronutrients in the four clones was the same as that for unit farms 01 and 02 and could be arranged in decreasing order: K (19.21 - 23.89 mg/g) > Mg (2.86 - 3.45 mg/g) > Ca (0.83 - 1.30 mg/g).

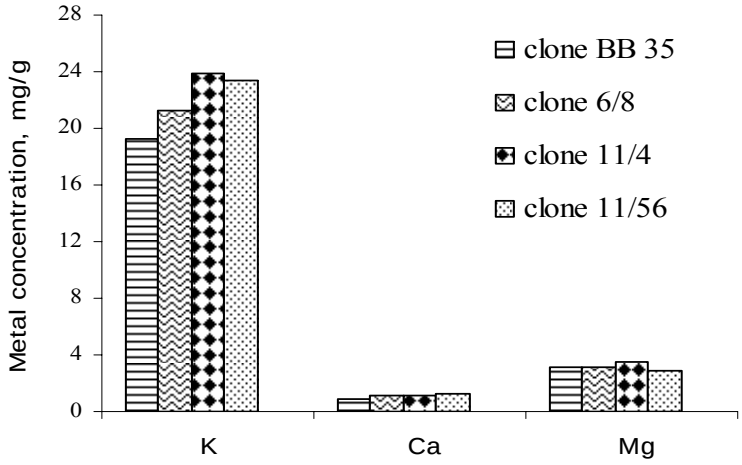


Figure 11. Levels of macronutrient metals (dry mass basis) in five tea clones of unit farm 03.

The content of Na in the four clones varied from 84.92 - 167.78 mg/kg being lowest in clone 11/56 and highest in clone 11/4. Level of the heavy metal, Fe (52.86 - 77.81 mg/kg) was, generally, lower as compared to those of unit farms 01 and 02. Cu level (nil - 4.58 mg/kg) was found to be highest in clone 6/8 whereas the level of this metal was below method detection limit in clone 11/56. Copper levels in clones of this farm should be considered, since tea will not ferment properly if Cu is deficient, leaf becoming dark brown rather than bright orange reducing the quality of the product [7].

Table 10. [a]Metal content of tea clones (dry mass basis) in unit farm 03.

Tea clone	K (mg/g)	Ca (mg/g)	Mg (mg/g)	Cd	Pb
BB 35	19.21 ± 0.99	0.83 ± 0.06	3.09 ± 0.03	nil[b]	nil
6/8	21.28 ± 0.49	1.08 ± 0.06	3.14 ± 0.06	nil	nil
11/4	23.89 ± 0.43	1.17 ± 0.02	3.45 ± 0.02	nil	nil
11/56	23.35 ± 0.59	1.30 ± 0.01	2.86 ± 0.03	nil	nil

	Na	Fe	Mn	Cu	Zn	Co
	…….. (mg/kg) ……..					
BB/35	126.61 ± 0.65	77.81 ± 3.86	794.37 ± 4.75	1.31 ± 0.10	68.10 ± 3.72	1.81 ± 0.08
6/8	135.04 ± 7.42	53.31 ± 2.49	758.41 ± 3.19	4.58 ± 0.20	90.23 ± 5.82	0.03 ± 0.01
11/4	167.78 ± 2.56	61.30 ± 3.10	969.10 ± 4.02	3.97 ± 0.24	69.86 ± 1.48	1.02 ± 0.01
11/56	84.92 ± 2.60	52.86 ± 0.97	749.78 ± 8.44	nil	67.95 ± 5.34	2.11 ± 0.09

[a] Analytical results for all metals were mean ± ts/√N calculated for 9 measurements at 95 % confidence limit ($P < 0.05$).
[b] Concentrations of the tested heavy metals below the method detection limit.

Mn concentrations (749.78 - 969.10 mg/kg) in the four clones were comparable (Table 10) with those values of unit farms 01 and 02, being highest in clone 11/4 and lowest in clone 11/56. However, the maximum Mn concentration (969.10 mg/kg) was relatively lower than those levels for clones of unit farms 01 and 02. Content of Zn in the four clones varied between 67.95 - 90.23 mg/kg. The level of Zn in clone 6/8 (90.23 mg/kg) was the highest of all clones in the four unit farms next to clone 11/4 (330.53 mg/kg) of unit farm 01. Co levels in the four clones ranged between 0.03 mg/kg in clone 6/8 to 2.11 mg/kg in clone 11/56, while levels of Cd and Pb were below method detection limits.

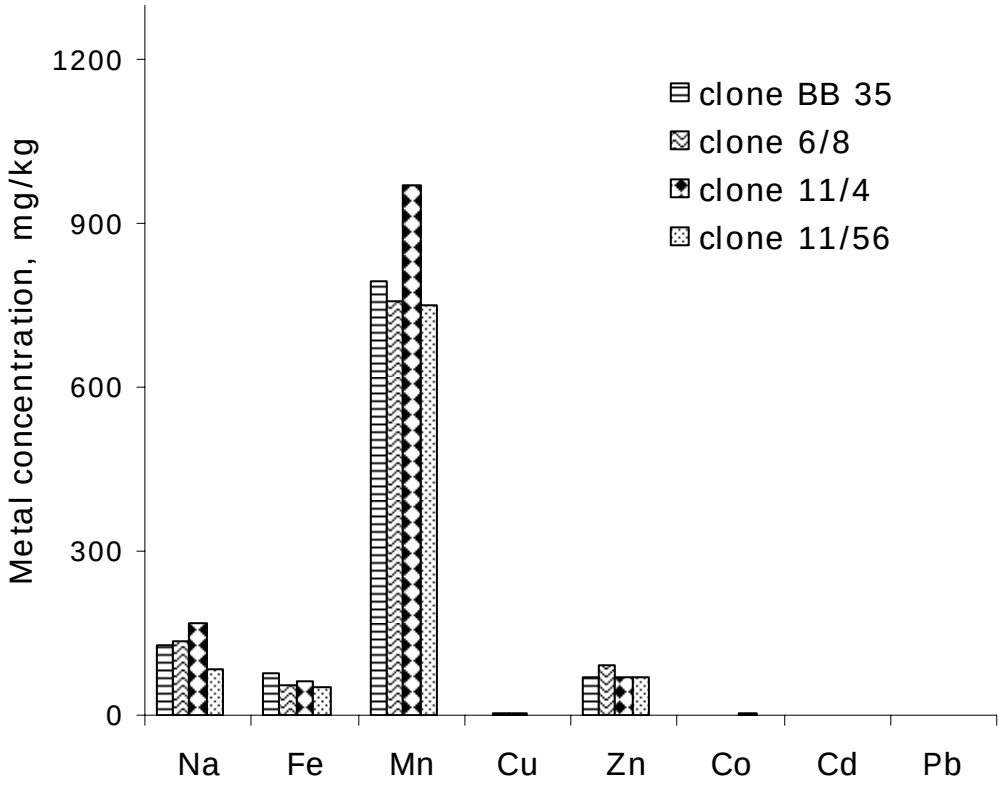


Figure 12. Levels of Na, Fe, Mn, Cu, Zn, Co, Cd and Pb (dry mass basis) in five tea clones of unit farm 03.

4.7.4. Tea Clones of Unit Farm 04

The macro- and micronutrients as well as toxic metal contents of all the five clones in unit farm 04 were estimated. The statistically analyzed mean data are presented in Table 11. The content

of K in clone BB 35 (17.66 mg/g) was the lowest of all the studied clones in the four unit farms. Range of levels of K (17.66 - 23.51 mg/g), Mg (2.90 - 3.30 mg/g) and Ca (0.62 - 1.12 mg/g) in tea clones of this farm follows the same trend as the rests of the unit farms. Moreover, values of these macro- elements for each tea clone were more or less similar with the levels obtained for clones of the other unit farms (Figure 13).

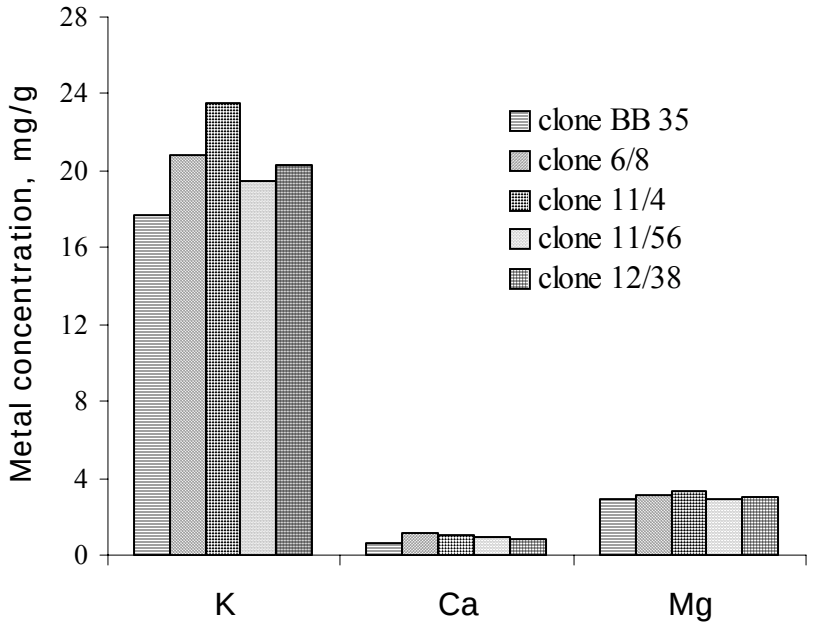


Figure 13. Levels of macronutrient metals (dry mass basis) in five tea clones of unit farm 04.

Among the heavy metals, range of levels of Fe (26.03 - 32.58 mg/kg) in clones of this unit farm was the lowest of all clones of the farms. The range values of the studied metals Na (101.63 - 151.63 mg/g), Mn (500.91 - 986.50 mg/kg), Cu (0.73 - 4.60 mg/kg), Zn (61.43 - 85.35 mg/kg) and Co (0.54 - 2.24 mg/kg) were generally comparable with values in clones of the rest of the unit farms. Clone 11/4 was observed to contain the lowest amount of Fe (26.03 mg/kg) among all clones in the four farms (Figure 14).

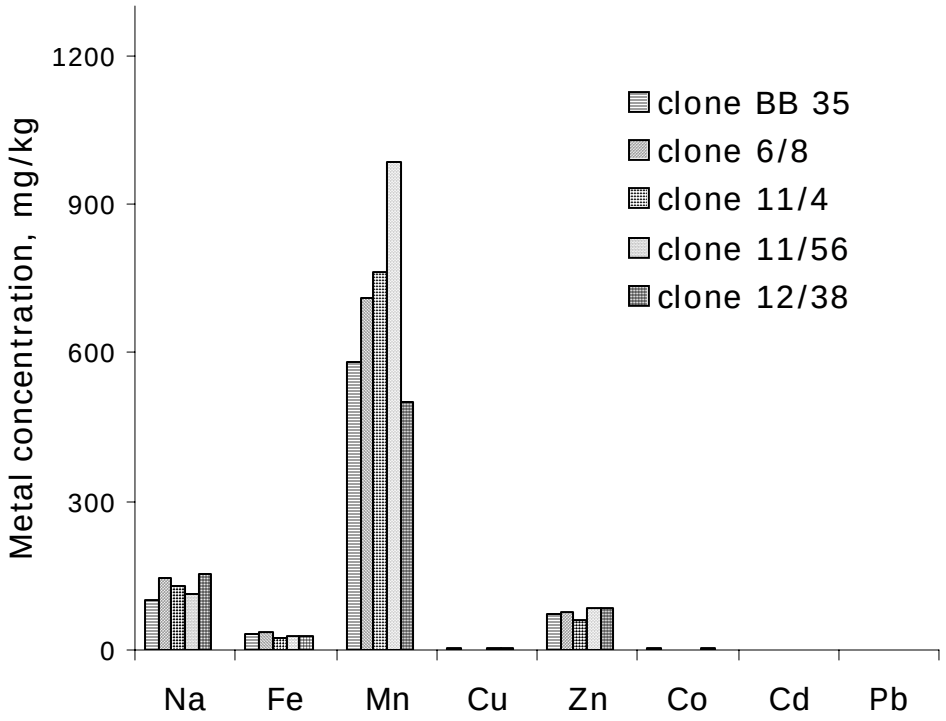


Figure 14. Levels of Na, Fe, Mn, Cu, Zn, Co, Cd and Pb (dry mass basis) in five tea clones of unit farm 04.

The highest amount of Mn (986.50 mg/kg) and Cu (4.60 mg/kg) was observed in clone 11/56, whereas clone 12/38 was found to contain maximum amounts of Na (151.63 mg/kg) and Zn (85.35 mg/kg). Maximum levels of Fe (35.99 mg/kg) and Co (2.24 mg/kg) in clones 6/8 and BB 35 of this unit farm were observed respectively. As observed for all clones of the three unit farms, levels of the toxic metals, Cd and Pb in all clones of this unit farm were below the method detection limits.

It can be deduced from levels of all the metals in studied tea clones of all unit farms, the concentrations of the macro- and the micronutrient metals (Table 12) followed similar trend for all the unit farms. In general, ranges of concentrations of the studied macronutrient metals could be arranged according to their levels in tea plants of all unit farms in the following order:

K (17.66 - 24.83 mg/g) > Mg (2.65 - 3.45 mg/g) > Ca (0.62 - 1.82 mg/g)

Table 11. [a]Metal content of tea clones (dry mass basis) in unit farm 04.

Tea clone	K (mg/g)	Ca (mg/g)	Mg (mg/g)	Cd	Pb
BB/35	17.66 ± 0.23	0.62 ± 0.08	2.90 ± 0.08	nil[b]	nil
6/8	20.84 ± 0.39	1.12 ± 0.04	3.09 ± 0.01	nil	nil
11/4	23.51 ± 0.32	1.07 ± 0.07	3.30 ± 0.03	nil	nil
11/56	19.51 ± 0.35	0.90 ± 0.02	2.94 ± 0.01	nil	nil
12/38	20.32 ± 0.30	0.82 ± 0.07	2.97 ± 0.03	nil	nil

	Na	Fe	Mn	Cu	Zn	Co
	…… (mg/kg) ……..					
BB/35	101.63 ± 0.57	32.58 ± 3.51	579.56 ± 9.07	2.47 ± 0.44	71.09 ± 2.06	2.24 ± 0.16
6/8	145.71 ± 3.61	35.99 ± 2.41	712.29 ± 8.74	0.73 ± 0.14	74.72 ± 1.15	1.47 ± 0.03
11/4	128.57 ± 6.77	26.03 ± 3.48	763.63 ± 2.09	1.60 ± 0.43	61.43 ± 7.46	0.54 ± 0.01
11/56	111.54 ± 5.16	29.88 ± 1.12	986.50 ± 1.31	4.60 ± 0.20	83.15 ± 1.55	1.63 ± 0.07
12/38	151.63 ± 7.19	29.61 ± 0.63	500.91 ± 1.89	2.09 ± 0.24	85.35 ± 1.94	2.07 ± 0.13

[a] Analytical results for all metals were mean ± ts/√N calculated for 9 measurements at 95 % confidence limit ($P < 0.05$).
[b] Concentrations of the tested heavy metals below the method detection limit.

Dang [48] reported that plant nutrient concentrations in the tea plant are highest in young leaves and buds with concentration ranges for the major nutrient elements K (20.9 - 23.6 mg/g), Ca (4.4 - 4.7 mg/g) and Mg (2.0 - 2.3 mg/g). These ranges of values are highly consistent with the levels obtained in the leaves of the studied tea plants. However, Ca concentration range reported by Dang [48] was slightly higher than the value reported in this study. This slight difference in Ca level may be attributed to the lower pH (5.04 - 5.49) of soils of the study farms, which indicates the presence of intensive leaching that availability of Ca is decreased in these soils.

The higher levels of K in the studied tea plants according to Marschner [52] was due to the fact that nutrient elements such as N, P, K, S and Mg are highly mobile in the tea plant tissue and are translocated from old leaves to young leaves. Kumar *et al*. [20] also reported a higher concentration of K and suggested that it may be specifically incorporated with in a binding ligand in the tea leaves.

Among the tested heavy metals, Mn was the most abundant metal in tea clones of all the unit farms ranging between 500.91 mg/kg in clone 12/38 of farm 04 up to 1280.55 mg/kg in clone 11/4 of farm 01. Levels of Fe in all the studied tea clones was with in the range of 29.61 - 100.43 mg/kg being highest in clone 11/4 of farm 01 and lowest in clone 12/38 of farm 04.

Table 12. [a]Range of metal concentrations of all tea clones in each unit farm.

	Range of metal concentrations of tea clones					
Unit farm	K (mg/g)	Ca (mg/g)	Mg (mg/g)	Fe (mg/kg)	Mn (mg/kg)	
01	21.87 - 23.73	0.72 - 1.82	2.65 - 3.08	71.07 - 100.43	599.04 - 1280.55	
02	20.88 - 24.83	1.02 - 1.82	3.05 - 3.45	51.51 - 83.55	747.91 - 1140.35	
03	19.21 - 23.89	0.83 - 1.30	2.86 - 3.45	52.86 - 77.81	749.78 - 794.37	
04	17.66 - 23.51	0.62 - 1.12	2.90 - 3.30	26.03 - 35.99	500.91 - 986.50	
	Na	Cu	Zn	Co	Cd	Pb
	..... (mg/kg)					
01	116.59 - 265.07	4.55 - 19.15	57.87 - 330.53	1.02 - 2.84	nil	nil
02	109.61 - 188.88	2.67 - 4.22	67.81 - 83.85	0.98 - 2.65	nil	nil
03	84.92 - 167.78	nil[b] - 4.58	67.95 - 90.23	0.03 - 2.11	nil	nil
04	101.63 - 151.63	0.73 - 4.60	61.43 - 85.35	0.54 - 2.24	nil	nil

[a] Average values of four soil samples from unit farm 03 and fifteen soil samples from unit farms 01,02 and 04.
[b] Concentrations of the tested heavy metals below the method detection limit.

On the other hand, Cu content ranged between nil to 19.15 mg/ kg in clone BB 35 of farm 01, whereas the concentration range for Zn was found to be 57.87 mg/kg in clone 12/38 up to 330.53 mg/kg in clone 11/4 of farm 01. Wang *et al*. [39] reported Cu content in the range 9.6 - 20.9 mg/kg in three Chinese tea samples, which are comparable with values obtained for the studied tea clones.

AL-Oud [22] suggested the ability of the tea plant to accumulate heavy metals particularly Mn, Fe and Zn, to a lesser extent Cu and reported higher levels of Mn in the tea plant with in a range of 390 - 900 mg/kg. Ranges of levels of Fe, Zn and Cu in the tea leaf were reported to be 123.90 - 513.00 mg/kg, 26.69 - 53.89 mg/kg, and 22.12 - 40.66 mg/kg respectively.

Kumar *et al*. [20] reported higher Mn contents (1100 - 2678 mg/kg) in tea leaves from Turkey and Japan. Mn content of a recently developed tea leaves standard, has been found to be much higher 1585 ± 40 mg/kg. Okamoto and Fuwa [53] also reported certified Mn content of another tea standard reference material (SRM) from Japan to be 700 ± 25 mg/kg.

Higher Mn levels in the studied clonal tea plants may be attributed to the availability of this micronutrient heavy metal in relatively acidic soils of the unit farms. According to Ishibashi *et al*. [47], Mn in soils is typically present in the form of pyrochroite [$Mn(OH)_2$], hausmannite

(Mn_3O_4), manganite (γ-MnOOH), birnessite (δ-MnO_2), and a freshly precipitated form of $MnCO_3$. The chemical forms of Mn present in soil are known to depend on soil pH. Valence electrons in Mn_3O_4 and γ-MnOOH can rearrange themselves spontaneously to give δ-MnO_2 and Mn^{2+} in an acid soil by the reactions shown below.

$$Mn_3O_4 + 4\ \gamma\text{-MnOOH} + 8\ H^+ \rightarrow 3\ MnO_2 + 4\ Mn^{2+} + 6\ H_2O$$

Thus, Mn^{2+} released from soil by H^+, which is produced from NH_4^+, can be readily taken up and accumulated in the tea plant as the age of the tea plantations is increased. Dang [48] also reported that total accumulation of nutrients stored in the standing crop increases with increasing age of the tea plants.

Levels of Na in all of the studied tea clones of the unit farms were within a range of 84.92 - 265.07 mg/kg the highest being in clone11/4 of unit farm 01 and the lowest in clone 11/56 of farm 03. Concentrations of Co for all tea plants ranged between 0.03 - 2.84 mg/kg, the maximum being in clone 11/56 of farm 01 and the lowest in clone 6/8 of farm 03.

Fortunately, the concentrations of toxic heavy metals, Pb and Cd in the studied clonal tea leaves were too low to be detected by the analytical technique used in this study. However, there are reports of the availability of these metals at lower levels in different blended tea leaves. AL-Oud [22] reported level of Cd with in a range of nil - 0.18 mg/kg. On the other hand, level of Pb was reported with in a range of 0.03 - 14.84 mg/kg, the highest value being in Chinese green tea and suggested that the level was too high to be considered by tea drinkers.

The presence of these toxic heavy metals in the blended tea leaves of different brands while their absence in leaves of the standing tea plant may be attributed to the possibility of contamination from these metals in fermentation processes during manufacture of the tea leaves. Moreover, tea grown in roadside soils and plantation farms in the vicinity of industries may be contaminated with Pb. According to Lagerwerff [36], Pb emitted in exhaust fumes of petrol combustion as minute particles of inorganic Pb compounds accounts for about 80 % of the total lead in the atmosphere. About 50 % of this falls somewhere with in the region of 100 m from the road, rendering Pb concentration to be higher in roadside soils and in its vegetation.

5. CONCLUSIONS

The study determined levels of macro- and micronutrient and the toxic heavy metals Cd and Pb in leaves of five clonal tea plants grown in Wushwush farms, Ethiopia. The results showed the ability of these plants to accumulate relatively higher amounts of K and Mn among the determined macro- and micronutrient metals respectively. Heavy metals Cu and Co were found to be comparatively at lower levels in most of the clones.

Plant nutrient concentrations of K, Ca and Mg in the bud and young leaves of clonal tea plants decreased in the order: K > Mg > Ca. With respect to the unit farms, the tea plants showed no significant difference in their pattern of accumulation of the studied metals. Heavy metals and Na contents of all clones followed, generally, similar trend across the unit farms that could be arranged in descending order: Mn > Na > Zn > Fe > Cu > Co.

Cu concentration in the clonal tea leaves is of great importance with respect to quality of tea. Clones 11/56 and BB 35 of farm 03 and clone 6/8 of farm 04 require due consideration as they are relatively deficient in Cu that they will not ferment properly and quality of their products might be severely affected. The levels of toxic heavy metals Cd and Pb in all clones were too low to be detected by the method used in this study. However, their absence in the standing tea plants of the farms may not necessarily guarantee the non-existence of these toxic metals in the different commercial tea brands available in retail markets.

The soils of the study farms were found to contain high levels of Fe followed by K, Mn, Mg, Na and Zn. The level of Cu was relatively lower in the studied soils of unit farms 03 and 04. In all of the soils, level of Pb was below method detection limit. However, unlike the tea leaves, the soils of all the unit farms were found to contain Cd at relatively lower levels. In general, the levels of most of the metals, especially the macronutrients, in the studied soils were found to correlate positively with the levels found in the tea leaves.

Tea is a commercial crop and one of the most popular widely consumed beverage all over the world. Thus, this study may have significance in delivering a preliminary data in the levels of nutrient elements in the tea plants. It might be useful in pointing directions for studies that will be conducted on the nutrient levels of different brands of blended tea produced in the country.

The data may also be helpful for researches that may be conducted on agronomy and physiology of the plant, nutrient requirement and soil-plant nutrient balance of tea crops, fertilizer applications, nutritional and toxicological studies in regard to human health as well as with respect to quality of its products.

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