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# Levels of essential and non-essential metals in leaves of the tea plant (*Camellia sinensis* L.) and soil of Wushwush farms, Ethiopia

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**Abstract**

Five tea clones of the *Camellia 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. Both the tea leaves and the soils of the study farms showed similar accumulation patterns in their contents of the studied macronutrients. Among the macronutrient metals, K was the most abundant element in the tea leaves (17.7–24.8 mg/g) and the soils (7.14–9.73 mg/g). Mn was the predominant micronutrient heavy metal in the tea leaf tissues ranging between 501 and 1281 mg/kg. Level of Fe (29.6–100 mg/kg) in the leaf tissue was found to be the second most abundant micronutrient next to Mn whereas concentrations of Cu and Co were relatively lower both in the soil and tea samples. The toxic heavy metals Pb and Cd in the leaf tissues were present at levels too low to be detected by the analytical technique used in this study. The soils were found 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 traces of 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.
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*Keywords:* Tea clones; Tea leaves; Macronutrients; Micronutrients; Toxic metals; Organic matter; Atomic absorption flame emission spectroscopy

## 1. Introduction

The tea plant, *Camellia sinensis* (L.) O. Kuntze, family *Theaceae*, is a small evergreen, perennial, cross-pollinated plant and grows naturally as tall as 15 m. However, under cultivated conditions, a bush height of 60–100 cm is maintained for harvesting the tender leaves (Mondal, Bhattacharya, Laxmikumaran, & Ahuja, 2004). Tea is the oldest, most popular, non-alcoholic caffeine-containing beverage in the world (Mondal et al., 2004), and it is prepared from the dried leaves of the tea plant (Mokgalaka, McCrindle, & Botha, 2004).

The cultivated taxa of tea comprise of three main natural hybrids. They are: *C. sinensis* (L.) O. Kuntze or China type, *Camellia 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. The nature of the leaf is the main criterion by which three types of tea are classified (Mondal et al., 2004). The chemical composition of tea leaves and manufactured tea is very complex and consists of tannin substances, flavonols, alkaloids, proteins and amino acids, enzymes, aroma forming substances, vitamins, minerals and trace elements (Kumar, Nair, Reddy, & Garg, 2005).

Various reports have discussed the potential health implications of trace metals in tea, particularly since the tea bush is known to accumulate trace metals (Bosque,

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Schuhmacher, & Domingo, 1990). Very recent research findings indicate the positive and negative effects of drinking tea on the health (AL-Oud, 2003). 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 rats (Lee, Kim, & Hee, 1995).

A number of papers have been published regarding the determination of the metal content of tea (Mokgalaka et al., 2004). 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 the level of a few μg/g (Cao, Zhao, Yin, & Li, 1998). 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 source of Mn if one takes a few cups daily (Heydorn, 1988). 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 to range widely with the 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 narrower range, 371–758 μg/g with a mean value of $575 \pm 96$ μg/g (Kumar et al., 2005).

In Indian tea brands, Na content varies widely (21–118 μg/g), with a mean value of $53.5 \pm 27.4$ μg/g. On the other hand, Na content in US tea brands found over an even wider range, 114–796 μg/g with a mean value of $338 \pm 286$ μg/g. The much higher Na content in US brands may be partly due to flavoring additives. However, K contents in Indian and US tea brands are present in a narrow range, 17.7–24.0 and 13.1–23.7 mg/g with mean contents of $21.1 \pm 2.0$ and $18.1 \pm 3.5$ mg/g, respectively (Kumar et al., 2005). 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 (Wang, Ke, & Yang, 1993). Thus, K content in tea leaves is not only higher, by an order of magnitude compared to Na, but is also independent of the brand. However, Na content shows large variability (Kumar et al., 2005).

Cu contents in Indian and US tea brands vary over wide range, 1.60–35.0 and 4.4–17.3 μg/g, with mean values of $14.8 \pm 8.2$ and $12.3 \pm 4.8$ μg/g, respectively, which are comparable. However, Cu content in Indian tea brands is present in a much wider range compared to that in US brands (Kumar et al., 2005). It is reported that Cu content is in the range 9.6–20.9 μg/g in three Chinese tea brands (Wang et al., 1993).

Non-nutrient heavy metals in both tea and herb leaves are Ni (2.46–8.90 μg/g), Pb (0.03–14.8 μg/g), Co (nil–2.35 μg/g), and Cd (nil–0.37 μg/g), although these are present at rather low concentrations. Among tested tea brands, Chinese has the highest contents of studied heavy metals, Mn, Fe, Zn, Cu and Pb. The level of Pb (14.8 μg/g) in Chinese tea was very high which tea consumers should consider (AL-Oud, 2003).

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 (Mokgalaka et al., 2004). The 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 study is, thus, intended to assess levels of essential, non-essential and toxic metals in leaves of the tea plant that are grown in Wushwush farms and in the soils using atomic absorption flame emission spectrophotometry. The study is expected to deliver preliminary data on the levels of metals in tea plants grown in Ethiopia and provide useful information for future studies which will be conducted on agronomy and physiology of the tea plant, fertilizer applications, nutritional, medical and toxicological effects in relation to the tea leaves grown in Ethiopia.

## 2. Materials and methods

### 2.1. Study site description

Tea leaf samples were collected from Wushwush tea plantation farms located in the southwestern highlands of Ethiopia (7°19′N and 36°07′E). The altitude of the area is 1900 m above sea level. Mean annual temperature is 18 °C with an average annual rainfall of 1800 mm. Soils of the area are classified as Plinthic Alisols, with clayey texture and dark reddish brown color (Solomon, Lehmann, Tekalign, Fritzsche, & Zech, 2001).

The tea plantation farms were 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 were BB 35, 6/8, 11/4, 11/56, and 12/38. All of the five tea clones were grown in the fields of unit farms 01, 02, and 04 while four tea clones (BB 35, 6/8, 11/4, 11/56) were grown in unit farm 03.

### 2.2. Sample collection and preparation

A total of 19 (about 500 g each) tea leaf samples (a bud and two leaves) 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. The tea leaf samples were air dried and ground to fineness to pass through a 0.5 mm sieve and stored in polyethylene bags prior to analysis.

At the spots where the tea leaf samples were plucked, a total of 19 (about 500 g each) corresponding soil samples (20 cm depth) were collected, air dried and ground to pass through a 2 mm sieve and stored in clean card boxes prior to analysis.

### 2.3. Instrumentation

A stainless steel soil sampling auger (Oakfield Apparatus Company, USA) was used to collect all the soil samples. The soil samples were ground using a ceramic mortar and pestle. The tea leaf samples were ground using an electric motor grinder (Retsch, Germany).

All of the tea leaves and soil samples were weighed on a digital analytical balance (Mettler Toledo, Switzerland) with $\pm 0.0001$ g precision. Round bottom flasks (100 ml) fitted with reflux condenser and Kjeldahl digestion block (Kjeldatherm, 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 the tea leaves and soil samples were determined by atomic absorption flame emission spectrophotometer (Shimadzu, Japan) using an air-acetylene flame and connected to a personal computer. A potentiometric digital pH meter (Hanna, Portugal) was used to determine the pH of soil samples after stirring by a magnetic stirrer (Jenway Ltd, UK). A water deionizer (Termoacqua Tecnologie S.R.L., Italy) was used to produce demineralized water.

### 2.4. Reagents and chemicals

An acid mixture of 5:1 concentrated $HNO_3$(69–70.5%, AnalaR, BDH) and concentrated $HClO_4$ (70%, Riedel-de Haën) was used for the digestion of the tea leaf samples. Concentrated HCl (36–38%, Hopkin & Williams) and $HNO_3$ (69%, Riedel-de Haën) were used for the digestion of soil samples and for the preparation of stock standard solutions. A solution of 0.1% $LaCl_3 \cdot 7H_2O$ (AnalaR, BDH) was used in the determination of Ca and Mg. Solutions of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ and $K_2Cr_2O_7$ (Merck), concentrated orthophosphoric acid (Hopkin & Williams), and 1,10-phenanthroline ferrous sulfate (Merck) solutions were used to determine soil organic matter. Demineralized water was used throughout the experiment to prepare all the solutions.

### 2.5. Chemical analysis of samples

Exactly 0.5 g of the dried and ground tea leaf sample was digested using 5:1 mixture of concentrated $HNO_3$ (69–70.5%) and concentrated $HClO_4$ (70%) under reflux. The digests 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 the emission mode of the spectrophotometer. Each tea leaf sample was digested in triplicate and three repeat measurements were performed by the AAFES.

Soil pH was measured potentiometrically by a pH meter in a suspension of a 1:1 soil:water mixture. Soil organic matter was determined by oxidation of the sample with excess of $K_2Cr_2O_7$ in concentrated $H_2SO_4$ and by titration with ferrous ammonium sulfate solution using 1,10-phenanthroline ferrous sulfate indicator (Tan, 1996).

Total metal concentrations in the soils were determined by digestion of 1.0 g of the sample using a 5:2 mixture of concentrated $HNO_3$ and HCl. The digests were filtered through Whatman No. 41 filter paper and the filtrate was used for the analysis of Na, K Ca, Mg, Fe, Cu, Zn, Mn, Co, Pb and Cd by AAFES. Concentrations of Na and K were determined in emission mode of the spectrophotometer (Tan, 1996).

### 2.6. Recovery tests

To check the efficiency of the procedure, appropriate volumes of 1000 mg/l Ca, Mg, Cd, Co and Mn were spiked at once into a tea sample and appropriate volumes of 1000 mg/l Na, K, Fe, Zn and Cu into another digestion flask containing the same tea sample. Each sample was analyzed for their respective spiked metals by AAFES. A recovery test was also performed for the soil samples using the same procedure. Each recovery test for both samples was performed in triplicate.

### 2.7. Method detection limit

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

## 3. Results and discussion

### 3.1. Analytical method detection limit

In this study, the detection limit of the methods was calculated by multiplying the standard deviation of seven blank signals each determined in triplicate by three. The calculated limits of detection for tea leaf and soil samples are given in Table 1. The method detection limits are generally comparable with that of instrument for both tea leaf and soil samples.

### 3.2. Recovery tests

Recovery tests using the proposed method were performed for both tea leaf and soil samples using non-

Table 1
Method detection limits for tea leaf and soil samples

| Metal | Instrument detection limit (mg/l) | Method detection limit for tea leaf (mg/g)[a] | Method detection limit for soil (mg/g)[a] |
|---|---|---|---|
| Na | 0.006 | 0.005 | 0.004 |
| K | 0.012 | 0.015 | 0.010 |
| Ca | 0.070 | 0.060 | 0.068 |
| Mg | 0.004 | 0.004 | 0.006 |
| Fe | 0.080 | 0.074 | 0.082 |
| Mn | 0.028 | 0.029 | 0.033 |
| Cu | 0.040 | 0.042 | 0.047 |
| Zn | 0.011 | 0.012 | 0.015 |
| Co | 0.060 | 0.058 | 0.056 |
| Cd | 0.012 | 0.012 | 0.014 |
| Pb | 0.050 | 0.046 | 0.053 |

[a] Values are mean of 3 × standard deviation of seven blank determinations each measured three times.

spiked and spiked samples, and each sample was analyzed in triplicate. As shown in Table 2, the results of percentage recoveries for the studied metal nutrients in tea leaves were all between 90% and 104%. The results of the recovery tests for tea leaf samples are within the acceptable range verifying the validity of the proposed method for tea leaf analysis. The values of percentage recoveries for the studied macro- and micronutrient and the toxic metals in the soil samples were within the range of 90–102% (Table 3).

### 3.3. Soil pH

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 4. The soil pH of the four unit farms was within the range of 5.04–5.49, which categorizes the soils under strongly acidic soils (Tan, 1996). 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, Gone, Onchiri, and Jumba (1990) have reported that the increasing rates of nitrogenous fertilizers generally increase soil acidity. Ishibashi et al. (2004) have reported that 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 the 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 a large canopy capable of producing many shoots. The organic matter content of these soils is high (5.48–6.02%) due to the accumulation of tea biomass through the incorporation of tea prunings. Dang (2005) has reported that pruning is an important approach to balance nutrient in plant-soil system in the tea cultivation because it returns to the soil a lot of nutrients and organic carbon. According to Weeraratna, Watson, and Wettasingha (1977), retention of the pruned biomass after pruning increases the soil organic matter content of sub-surface soils as the organic matter is transported from the surface soil to the sub-surface soil by

Table 2
Recovery test results for the tea leaf samples

| Metal | Concentration in the sample[a] | Amount added (mg) | Concentration in the spiked sample[a] | Amount recovered (mg) | Recovery[b] (%) |
|---|---|---|---|---|---|
| Na | 116.6 ± 0.02 mg/kg | 15.0 | 131.9 ± 0.15 mg/kg | 15.3 | 102 ± 0.9 |
| K | 21.9 ± 1.31 mg/g | 5.00 | 26.6 ± 1.32 mg/g | 4.70 | 94 ± 1.5 |
| Ca | 0.72 ± 0.01 mg/g | 0.10 | 0.81 ± 0.02 mg/g | 0.090 | 90 ± 0.9 |
| Mg | 2.65 ± 0.64 mg/g | 0.50 | 3.16 ± 0.46 mg/g | 0.51 | 102 ± 1.2 |
| Fe | 85.5 ± 0.37 mg/kg | 1.00 | 86.5 ± 0.39 mg/kg | 0.98 | 98 ± 0.6 |
| Mn | 995 ± 2.78 mg/kg | 50.0 | 1041 ± 1.25 mg/kg | 46.0 | 92 ± 1.8 |
| Cu | 19.1 ± 0.02 mg/kg | 1.00 | 20.1 ± 0.06 mg/kg | 0.98 | 98 ± 0.8 |
| Zn | 67.9 ± 0.25 mg/kg | 10.0 | 78.3 ± 0.15 mg/kg | 10.4 | 104 ± 1.4 |
| Co | 1.21 ± 0.01 mg/kg | 0.10 | 1.30 ± 0.02 mg/kg | 0.090 | 90 ± 0.3 |
| Cd | Nil[c] | 0.05 | 0.049 ± 0.01 mg/kg | 0.049 | 98 ± 0.6 |
| Pb | Nil[c] | 0.05 | 0.045 ± 0.02 mg/kg | 0.045 | 90 ± 1.3 |

[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 3
Recovery test results for the soil samples

| Metal | Concentration in the sample[a] | Amount added (mg) | Concentration in the spiked sample[a] | Amount recovered (mg) | Recovery[b] (%) |
|---|---|---|---|---|---|
| Na | 964 ± 0.6 mg/kg | 50.0 | 1012 ± 1.4 mg/kg | 48.0 | 96 ± 1.3 |
| K | 7.4 ± 0.02 | 1.0 | 8.3 ± 0.03 | 0.90 | 90 ± 0.4 |
| Ca | 0.45 ± 0.03 | 0.10 | 0.54 ± 0.04 mg/g | 0.090 | 90 ± 0.5 |
| Mg | 1.4 ± 0.11 mg/g | 0.10 | 1.5 ± 0.03 mg/g | 0.098 | 98 ± 0.2 |
| Fe | 24.5 ± 0.14 mg/g | 5.0 | 29.6 ± 0.35 mg/g | 5.10 | 102 ± 1.7 |
| Mn | 1.8 ± 0.05 mg/g | 0.50 | 2.25 ± 0.12 mg/g | 0.45 | 90 ± 0.5 |
| Cu | 7.8 ± 0.18 mg/kg | 1.0 | 8.78 ± 0.30 mg/kg | 0.98 | 98 ± 0.1 |
| Zn | 68.1 ± 0.13 mg/kg | 10.0 | 77.5 ± 0.05 mg/kg | 9.40 | 94 ± 0.2 |
| Co | 21.1 ± 0.02 mg/kg | 5.0 | 25.8 ± 0.09 mg/kg | 4.70 | 94 ± 0.1 |
| Cd | 0.44 ± 0.02 mg/kg | 0.10 | 0.54 ± 0.07 mg/kg | 0.10 | 100 ± 0.6 |
| Pb | Nil[c] | 0.05 | 0.049 ± 0.01 mg/kg | 0.049 | 98 ± 0.6 |

[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 4
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.

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 also accelerates decomposition of carbonaceous material.

### 3.4. Levels of metals in the soils

Analytical data for the 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 5. Potassium is the third of the major plant nutrients next to N and P (Salardini, 1978). 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 (Solomon et al., 2001), extensive leaching of Ca and Mg occur rendering these metals to exist at lower levels.

Weeraratna et al. (1977) have reported that incorporation of tea prunings and addition of K fertilizers to the soils rapidly increases the 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 (Solomon et al., 2001), which is indicative of the presence of excess amount of hematite ($Fe_2O_3$) (Tan, 1996). Soils with low pH contain high amounts of Fe and Al oxides (Hu, Pan, & Zhu, 2002). Thus, Fe is the predominant metal within the concentration range of 18.8–23.5 mg/g in these soils whereas Mn content is in the range of 1.03–1.47 mg/g.

Concentration of Na (336–581 mg/kg) a non-essential metal in these soils is higher when compared to the micronutrient heavy metals Cu (2.34–25.6 mg/kg), Zn (43.9–71.3 mg/kg), and Co (7.54–19.9 mg/kg). On the other hand, the level of the toxic heavy metal Cd ranges from 0.2 mg/kg for unit farms 02 and up to 1.10 mg/kg for soils of unit farm 03. The level of Pb, the other tested toxic metal, in the soils of all unit farms was found to be below the detection limit of the method used in this study.

### 3.5. 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 (Table 6). 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, while clone 11/4 of unit farm 01 had the highest content of Zn.

It can be deduced from the levels of all the metals in the studied tea clones of all the unit farms, that the concentrations of the macro- and the micronutrient metals 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 the tea plants of all the unit farms in the following order:

Table 5
[a]Average metal concentrations of soils of the selected fields of four unit farms

| Unit farm | [b]Metal concentrations of soils (dry mass basis) | | | | |
|---|---|---|---|---|---|
| | 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 | 23.5 ± 0.07 | 1.47 ± 0.03 |
| 02 | 8.39 ± 0.02 | 0.29 ± 0.01 | 1.07 ± 0.004 | 22.1 ± 0.08 | 1.30 ± 0.03 |
| 03 | 7.14 ± 0.01 | 0.49 ± 0.02 | 0.93 ± 0.003 | 26.2 ± 0.12 | 1.03 ± 0.03 |
| 04 | 9.73 ± 0.02 | 0.42 ± 0.02 | 0.84 ± 0.004 | 18.8 ± 0.11 | 1.19 ± 0.02 |

| | Na (mg/kg) | Cu (mg/kg) | Zn (mg/kg) | Co (mg/kg) | Cd (mg/kg) | Pb (mg/kg) |
|---|---|---|---|---|---|---|
| 01 | 581 ± 5 | 25.6 ± 0.89 | 71.3 ± 0.47 | 19.9 ± 3.77 | 0.32 ± 0.43 | Nil[c] |
| 02 | 456 ± 3 | 11.2 ± 1.39 | 60.9 ± 0.53 | 8.44 ± 1.72 | 0.20 ± 0.74 | Nil[c] |
| 03 | 336 ± 6 | 2.34 ± 1.11 | 63.8 ± 0.54 | 9.44 ± 2.37 | 1.10 ± 0.25 | Nil[c] |
| 04 | 468 ± 4 | 2.88 ± 0.87 | 43.9 ± 0.71 | 7.54 ± 3.81 | 0.20 ± 0.76 | Nil[c] |

[a] Average values of four soil samples from unit farm 03 and 15 soil samples from unit farms 01, 02 and 04.
[b] Analytical results for all the metals expressed as mean ± $ts/\sqrt{N}$ calculated for 9 measurements at 95% confidence limit ($P < 0.05$).
[c] Concentration of the tested heavy metal below the method detection limit.

Table 6
Range of metal concentrations of all the tea clones in each unit farm[a]

| Unit farm | Range of metal concentrations of tea clones | | | | |
|---|---|---|---|---|---|
| | K (mg/g) | Ca (mg/g) | Mg (mg/g) | Fe (mg/kg) | Mn (mg/kg) |
| 01 | 21.9–23.7 | 0.72–1.82 | 2.65–3.08 | 71.07–100.43 | 599–1281 |
| 02 | 20.9–24.8 | 1.02–1.82 | 3.05–3.45 | 51.51–83.55 | 748–1140 |
| 03 | 19.2–23.9 | 0.83–1.30 | 2.86–3.45 | 52.86–77.81 | 750–794 |
| 04 | 17.7–23.5 | 0.62–1.12 | 2.90–3.30 | 26.03–35.99 | 501–987 |

| | Na (mg/kg) | Cu (mg/kg) | Zn (mg/kg) | Co (mg/kg) | Cd (mg/kg) | Pb (mg/kg) |
|---|---|---|---|---|---|---|
| 01 | 117–265 | 4.55–19.15 | 57.9–330.5 | 1.02–2.84 | Nil[b] | Nil[b] |
| 02 | 110–189 | 2.67–4.22 | 67.8–83.8 | 0.98–2.65 | Nil[b] | Nil[b] |
| 03 | 84.9–167.8 | Nil[b]–4.58 | 67.9–90.2 | 0.03–2.11 | Nil[b] | Nil[b] |
| 04 | 102–152 | 0.73–4.60 | 61.4–85.3 | 0.54–2.24 | Nil[b] | Nil[b] |

[a] Average values of four soil tea samples from unit farm 03 and 15 soil tea samples from unit farms 01, 02 and 04.
[b] Concentrations of the tested heavy metals below the method detection limit.

$$K\ (17.7\text{–}24.8\ \text{mg/g}) > Mg\ (2.65\text{–}3.45\ \text{mg/g}) > Ca\ (0.62\text{–}1.82\ \text{mg/g})$$

Dang (2005) has reported that plant nutrient concentrations in the tea plant are highest in the 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 (2005) 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 (1995) 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. (2005) have 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 mg/kg in clone 12/38 of farm 04 up to 1281 mg/kg in clone 11/4 of farm 01. Levels of Fe in all the studied tea clones was with in the range of 29.6–100.4 mg/kg being highest in clone 11/4 of farm 01 and lowest in clone 12/38 of farm 04.

On the other hand, Cu content ranged between nil and 19.2 mg/kg in clone BB 35 of farm 01, whereas the concentration range for Zn was found to be 57.9 mg/kg in clone 12/38 up to 331 mg/kg in clone 11/4 of farm 01. Wang et al. (1993) 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 (2003) 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 123.9–513.0 mg/kg, 26.69–53.89 mg/kg, and 22.12–40.66 mg/kg, respectively.

Kumar et al. (2005) have reported higher Mn contents (1100–2678 mg/kg) in tea leaves from Turkey and Japan. The Mn content of a recently developed tea leaves standard, has been found to be much higher 1585 ± 40 mg/kg. Okamoto and Fuwa (1987) have also reported certified

Mn content of another tea standard reference material (SRM) from Japan to be $700 \pm 25$ 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. (2004), Mn in soils is typically present in the form of pyrochroite [$Mn(OH)_2$], hausmannite ($Mn_3O_4$), manganite ($\gamma$-MnOOH), birnessite ($\delta$-$MnO_2$), and a freshly precipitated form of $MnCO_3$. The chemical forms of Mn present in soil are known to depend on the soil pH. Valence electrons in $Mn_3O_4$ and $\gamma$-MnOOH can rearrange themselves spontaneously to give $\delta$-$MnO_2$ and $Mn^{2+}$ in an acid soil by the reactions shown below:

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

Hence, $Mn^{2+}$ 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 (2005) 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 84.9–265 mg/kg the highest being in clone 11/4 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 and 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 (2003) has reported level of Cd within a range of nil–0.18 mg/kg. On the other hand, level of Pb was reported within 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 safe 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 (1972), 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 within the region of 100 m from the road, rendering Pb concentration to be higher in roadside soils and in its vegetation.

## 4. Conclusions

This 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 the 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.

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