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**ADDIS ABABA UNIVERSTIY  
RESEARCH AND GRADUATE PROGERAMS**

**Determination of levels of selected metals in the leaves ‘Tena Adam’ (*Ruta chalepensis* L.)  
collected from four selected areas around Addis Ababa**

**By:**

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**Advisor:**

**Professor B.S. Chandravanshi**

**April 2018**

**Addis Ababa, Ethiopia**

**Determination of levels of selected metals in the leaves ‘Tena Adam’ (*Ruta chalepensis* L.)  
collected from four selected areas around Addis Ababa**

**A thesis submitted to Department of Chemistry in Partial fulfillment of requirements for  
the Degree of Master of Science in Chemistry**

**By:  
Solomon Admasu Mersha  
April 2018**

**School of Research and Graduate Programs Addis Ababa University**

**DECLARATION**

I, the undersigned, declare that this research work is my original work under the supervision of my advisor in the Faculty of Science, Department of Chemistry, AAU in the academic year 2017/2018 and it has not been submitted in this or any other university. All sources of ideas and materials used for the research work are honestly acknowledged.

Name: Solomon Admasu Mersha

Signature: \_\_\_\_\_

This research work has been submitted for examination with my approval as university advisor.

Advisor's Name: Prof. B. S. Chandravanishi

Signature: \_\_\_\_\_

Place and date of submission: School of Research and Graduate Programs, April, 2018

Addis Ababa University

ADDIS ABABA UNIVERSITY  
RESEARCH AND GRADUATE PROGRAMS

**Approval**

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## **DEDICATION**

This thesis work is dedicated to my wife Almaz Argaw and my newly born daughter Yemariam Solomon as well as my parent and my wife's parent for their love and support throughout my life and during the preparation of this thesis paper.

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## **Lists of Acronyms and Abbreviations**

<b>EC</b>	Electrical Conductivity
<b>WHO</b>	World Health Organization
<b>FAO</b>	Food and Agriculture Organization
<b>ANOVA</b>	Analysis of Variation
<b>%RSD</b>	Percentage Relative Standard Deviation
<b>%R</b>	Percent recovered
<b>SD</b>	Standard Deviation
<b>NR</b>	Not reported
<b>NDR</b>	Not Determined
<b>BDL</b>	Below Detection Limit
<b>PMT</b>	Photo multiplier tube
<b>MTL</b>	Maximum Tolerable limit
<b>FAAS</b>	Flame Atomic Absorption Spectrometry
<b>AAA</b>	Addis Ababa Administration
<b>SNNPR</b>	South Nations and Nationality Peoples Region
<b>CRM</b>	Certified Reference Material
<b>Df</b>	Degree of freedom

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## **Abstract**

In this research, leaf samples of Tena Adam were randomly collected from four selected areas around Addis Ababa. After proper sample pretreatment, the volumes of reagents used, digestion temperature and digestion time were optimized and using the optimized conditions the levels of metals were determined by flame atomic absorption spectrometry. The accuracy and precision of the optimized procedure was evaluated by analyzing the digest of the spiked samples and the percentage recoveries obtained varied from 96.7 to 96.9%. The levels of metals determined in mg/kg dry weight were in the ranges Ca (1872–3077), Zn (44.5–64.0), Cu (ND–10.8), Cr (ND–3.10) and Cd (ND–2.87). Ca (3077 mg/kg) and Cr (1.20 mg/kg) were found to be the highest and the least concentration among the metals, respectively and metals Ni and Pb were below the detection limit of the instrument in all sample areas. ANOVA at 95% confidence level indicated that there is a significant difference in the level of Ca and no significant difference in the levels of Cr, Cu and Zn in all the samples and the results of Pearson correlation revealed that there is weak and/or moderate positive correlation between metals with each other. The results indicate that the content of most of the metals are comparable to the permissible amount for medicinal plants which form the raw materials for the finished products set by WHO and FAO and other literature.

**Keywords:** *Ruta chalepensis* L., Rue, Tena Adam, Metals, Flame atomic absorption spectrometry, Ethiopia.

# 1. INTRODUCTION

## 1.1. Background

### 1.1.1. Origin and geographic distribution

*Ruta chalepensis* L. (Tena Adam) is an herbaceous perennial, originally native to the Mediterranean region and the Canary Islands. It has yellow flowers (Figure 1). This plant is a hardy, evergreen shrub of up to 1m tall, with a characteristic grayish color and a sharp unpleasant odor. The leaves are small, oblong, deeply divided, and pinnate; and the stems are ~~ra~~red. It belongs to the family *Rutaceae* and is a shrubby plant [1].



Figure 1: Picture of Tena Adam

It is now cultivated in many parts of the world. It is cultivated in the tropics as a potherb or medicinal plant and has widely become naturalized. In tropical Africa it has been introduced in several countries, including the Cape Verde Islands, Sudan, Ethiopia, Somalia and Southern Africa (including South Africa), where it is mostly cultivated in herbal gardens. It has also been naturalized in peninsular Arabia, India, Malaysia, Vietnam and Java. It has furthermore been naturalized in the United States, Mexico, Cuba and Chile. In Africa, the morphologically and chemically related *Ruta graveolens* L. seems only to be present in South Africa [2].

### **1.1.2. Botanical information**

*Ruta* comprises about 8 species. The botanical identity of *Ruta* grown in tropical Africa is not always clear. The presence of the related *Ruta graveolens* L. in tropical Africa is based on misidentifications, certainly so in Ethiopia. The chemical composition of both species is almost identical. *Ruta graveolens* is a polyploid. The medicinal and culinary uses in South Africa are similar to those of *Ruta chalepensis* [2].

### **1.1.3. Ecology and genetic resources**

Tena Adam grows well in well-drained sandy or rocky limestone soils and prefers an open sunny position. In Ethiopia it is cultivated at 1500–2000 m altitude. In Ethiopia and South Africa, the flowers and fruits are commonly planted in home gardens. Therefore, it is not threatened by genetic erosion. Plants growing at lower altitudes in tropical Africa do not often flower, so the plants are vegetatively multiplied and the genetic variation might therefore be on the low side for these plants [2].

### **1.1.4. Naming in different countries**

Descriptive of the smell and taste of the plant, “*Ruta*” is an old Latin name for rue, which literally means bitterness or unpleasantness. This bitterness arises from the rutin constituent of the plant. The specific epithet “*chalepensis*” is derived from name of the Syrian town of Chalep, which today is called Haleb or Aleppo. In Ethiopia, Tena Adam is the common local name of

*Ruta chalepensis*, which means health of Adam because of its medicinal applications. Herb of Grace and Garden Rue are the common English names for Tena Adam. *Ruta chalepensis* is usually confused with a closely related species, *Ruta graveolens*. The latter is not known to grow in Ethiopia. It is originated in Europe, and is cultivated in many parts of the world. However, the two species share many similar chemical constituents and morphological features. In folk medicine, they also have many intersecting uses [3].

*Ruta chalepensis* is sold under the Arabic names fidjeli and fidjla in drug markets of Algiers (Algeria) and under the names ruta and rutsa in Rabat (Morocco) drug markets. It is also known by the names Syrian rue, Aleppo rue and Rue d'Alep. Interestingly, another plant *Peganum harmala* is also sold in these markets under the same name, Syrian rue. Common names can sometimes be misleading. For example, the name Syrian rue (Persian rue, wild rue) is used to refer to both *R. chalepensis* (family: Rutaceae) and *Peganum harmala* (family: Zygophyllaceae), which are two entirely different plants. The latter is also called African rue, which has hallucinogenic and intoxicant alkaloids. It is said the hallucinogenic seed alkaloids of *Peganum harmala* inspired the concept of “flying carpets” [3].

## **1.2. Chemical composition of Tena Adam**

The compounds isolated from *Ruta chalepensis* and *Ruta graveolens* are essentially the same, although quantitative differences are observed. However, these differences are of the same magnitude as those observed within the same species collected from different sources. Qualitative and quantitative differences are also found for the different parts of the plants. Both species are characterized by the presence of alkaloids (acridone-, quinolone-, furoquinolone-type alkaloids, and quaternary furoquinolines), (furano-) coumarins and essential oils. The essential oils from aerial parts of *Ruta chalepensis* plants harvested at different stages of growth in northern India contained 19 components, representing 85.4–93.3% of the oil. The major components were 2-undecanone (41.3–67.8%), 2-nonanone (5.2–33.6%), 2-nonyl-acetate (2.8–15.3%) and 2-dodecanone (<0.1–11.6%) [2].

Major compounds isolated from the roots of *Ruta chalepensis* are the furoquinolin alkaloids kokusaginin, skimmianin and graveolin, the acridone alkaloids 1-hydroxy-N-methylacridone and

choloridon, and the furanocoumarin chalepensis. From the dried aerial parts, major compounds isolated include the furoquinolin alkaloids kokusaginin, skimmianin, graveolin,  $\gamma$ -fagarin and dictamnin, the acridone alkaloid arborinin, and the (furano-) coumarins bergapten (or 5-methoxypsoralen) and chalepensis [2].

### **1.3. Culinary and medicinal uses of Tena Adam**

Tena Adam is cultivated in several countries in tropical Africa where it is used for cooking and medicinal purposes. The medicinal and culinary properties are attributed to the presence of essential oils which are contained in all parts of the plant. The tops of the fresh shoots are the most active and should be gathered before the plant flowers [2]. It is known that herbal and medicinal plants have been used for treatment of diseases, especially in the developing countries. For example, Tena Adam has been in popular herbalism in various countries to treat a variety of ailments. A decoction of the plant has been used in the treatment of paralysis, coughs, and stomachaches. The leaves have been heated and then placed inside the ear to treat earache. Moreover, it was reported that the methanolic extract of Tena Adam has antibacterial activity, and the essential oil of Tena Adam has antioxidant activities. Thus, Contamination of edible medicinal plants by toxic metals is a threat for human health [4].

In Ethiopian folk medicine, it is used to treat colicky babies, diarrhea, earache, heart pain, hemorrhoids, influenza symptoms and intestinal disorders. The dried and ground fruits are boiled and taken by mouth for diarrhea. The juice from the crushed leaves is mixed with water and administered to colicky babies. The ground plant material is made into an ointment to be used for hemorrhoids. The boiled plant is used to treat influenza symptoms [5].

In addition to medicinal applications; the leaves of Tena Adam are used to flavor sour milk and cheese and used commonly as spice or dipped/stirred during a traditional Ethiopian coffee and tea ceremony (Figure 2). They are also used to flavor “kuti” which is used as a hot beverage brewed from coffee leaves. The fruits are used as ingredients of the local “berbere” (Figure 3) and “mitten shiro” spice mix. The volatile aromatic constituents may be responsible for the flavor of the plant [6].



Figure 2: Tena Adam in Ethiopian coffee Ceremony



Figure 3: Freshly made Ethiopian Berbere

In North Africa, an infusion of Tena Adam is used for colds, earaches and intestinal problems. Due to its strong smell, the fresh plant is used as a scorpion repellent. In Algiers, the infusion of the plant is used as nose drops to treat vomiting and fevers in children [7].

#### 1.4. Production and international trade

Commercial production of essential oil from *Ruta chalepensis* and *Ruta graveolens* is centered in the Mediterranean region. In Ethiopia either dried fruits or fresh or dried twigs with leaves, flowers and fruits are found commonly in the local markets [3].

#### 1.5. Adverse effects of Tena Adam

The oil of Tena Adam, on repeated application, can cause blisters and reddening of the skin. When taken in large doses, it can lead to toxic effects, such as abortion in pregnant women, confusion, convulsive twitches, severe epigastria pain, gastroenteritis and vomiting. The medicinal use of the plant seems to be restricted due to these very secondary effects [5].

## 1.6. Level of trace metals in plants

Trace metals are minerals present in living tissues in small amounts. Some of them are known to be nutritionally essential and the remainders are considered to be non-essential. Trace elements function primarily as catalysts in enzyme systems; some metallic ions, such as iron and copper, participate in oxidation-reduction reactions in energy metabolism. Iron, as a constituent of hemoglobin and myoglobin, also plays a vital role in the transport of oxygen. All trace elements are toxic if consumed at sufficiently high levels for long enough periods. For example, high levels of zinc can result in a deficiency of copper, another metal required by the body. The difference between toxic intakes and optimal intakes to meet physiological needs for essential trace elements is great for some elements but is much smaller for others [8-10].

Heavy metals are stable elements (meaning they cannot be metabolized by the body) and bio-accumulative (passed up the food chain to humans). These include: mercury, nickel, lead, arsenic, cadmium, aluminum, platinum, and copper (the metallic form versus the ionic form required by the body). Most of the heavy metals have no function in the body and can be highly toxic [10]. Nutritionally essential metals may cause adverse health effects at some levels below or beyond the level required for optimum nutrition [11]. Injury to vegetation caused by trace metal has been well recognized because of many botanical and chemical investigations during the past 100 years. More than 60 elements in various parts of the human body have been detected. Among these, at least 25 elements are essential to human health and out of which, 14 are termed as trace elements [12].

Environmental pollution, especially with heavy metals, poses serious problem on the quality of medicinal plants and their products. Thus, the element content assessment is important for specifying the relevance of application to produce drugs [4].

Food is the major intake source of toxic trace elements by human beings. Vegetables, fish, meat, grains, soft drinks, condiments, etc. are used as staple part of food. Various studies investigated the concentration of major, minor, and trace elements from the different items of food.

Vegetables [13-15], food condiments [16], water [17], bread [18], fish [19] are some among others.

Recently, some studies have been reported on the levels of metals in food [20-28], vegetables [29-35], fruits [36-37], spices [38-42] and beverages [43-47] consumed in Ethiopia. However, only one study has been reported on the levels of metal concentration of Tena Adam species by Massadeha *et al.* [4] in Jordan, investigated heavy metals such as Pb, Cu, Zn, Cd, Ni, and Fe by atomic absorption spectrometry (AAS) from different parts of Tena Adam. There is no reported data about the level of selected metals found in Tena Adam medicinal plant grown in Ethiopia. Thus, this study aims to evaluate the concentrations of some selected metals in leaves of Tena Adam and to study the distribution of metals in four different areas of Ethiopia and its relationship with metal absorption by a medicinal plant. This research also compares the acquired concentrations of elements in leaves of Tena Adam plant with toxic levels provided in literature.

## **1.7. Roles played by some essential and non-essential metals and their toxicity**

General roles played by some essential and non-essential metals analyzed in this study such as Ca, Cr, Cu, Zn, Cd, Ni and Pb are presented in the following sections.

### **1.7.1. Calcium**

It is one of essential macro-nutrient element. Calcium, the structural element, is found mainly in our bones. Most individuals are aware of the benefits of calcium supplementation. It is important for bone and teeth formation and structure. Calcium also appears to play a role in maintaining normal blood pressure. In addition to this, it is required by the body for blood clotting, muscle contraction and nerve transmission. This is because, it (calcium) is a component of enzymes that contribute to blood clotting, muscle activity and nerve function. It also regulates cell membrane permeability to control nerve impulse transmission and muscle contraction. It regulates hormonal secretion and cell division. Calcium may play a role in triglyceride and cholesterol reduction due to its fat binding properties within the gastrointestinal tract. Good food

sources of calcium are dairy products such as cheese and yogurt, fortified bread and flower, yoghurt, tofu, cereals and dark green vegetables, milk, sardines, egg yolks, almonds, sesame seeds, and seaweed [48]. It is also known that over dose consumption of minerals may upset the normal functioning of the body. Toxicity of calcium occurs with increased intake of calcium. Calcium competes with a number of minerals for absorption therefore supplementation with a multi-mineral may be necessary to prevent decreased levels of other minerals due to calcium. Calcium supplements are usually the cause for the overdose. Bones and teeth are the main storage tissues of calcium in the body [48].

### **1.7.2. Zinc**

Zinc makes up about 75 ppm of the Earth's crust, making it the 24<sup>th</sup> most abundant element with a density of 7.14 g/cm<sup>3</sup>. Zn is normally found in association with other base metals such as Cu and Pb in ores and has a low affinity for oxygen and prefers to bond with sulfur and occurs as ores such as sphalerite (ZnS), calamite (ZnCO<sub>3</sub>) and zincite (ZnO). Zn forms alloys such as brass and bronze and has been used in construction of buildings, roofing and cladding. Other uses of Zn include making circuit boards, photocopiers, dry cell batteries and its compounds are used in chemical and pharmaceutical industries such as paints, medicines and nutritional supplements [49]. The toxicity of Zn is as a result of excessive absorption which suppresses copper and iron absorption while free Zn<sup>2+</sup> ion in solution is highly toxic to plants, invertebrates, and even fish. Zinc salts are intestinal irritants and can cause nausea, and abdominal pain [50]. Prolonged exposure to high intakes of Zn results in copper deficiency and subsequent anemia [49]. There is also a condition called the zinc shakes or "zinc chills" that can be induced by the inhalation of freshly formed Zn oxide formed during the welding of galvanized materials. It has been reported that zinc is able to damage nerve receptors in the nose, which can cause anosmia and recommended that consumers should stop using zinc based intranasal cold products and ordered their removal from store shelves [51].

### **1.7.3. Copper**

Copper plays important roles in normal carbohydrate and lipid metabolisms. Copper and iron are essential to life because they play major roles in blood building and the functioning of critical enzyme systems. Copper is essential for good health but very high intake can cause adverse health problems, such as liver and kidney damage [28].

### **1.7.4. Chromium**

Chromium is micronutrient element for animals. Trivalent chromium is required for maintaining normal glucose metabolism. Evidence shows that chromium improves glucose tolerance; diabetes and coronary heart disease are associated with low chromium concentrations in human tissue. The chemical forms of chromium in foods are not known with certainty, but the bioavailability of chromium compounds has been found to be high in brewer's yeast, shellfish, whole wheat bread and mushrooms [28].

### **1.7.5. Cadmium**

Cadmium has oxidation state of +2 and forms a number of inorganic compounds such as sulfates, chlorides and acetates most of which are water soluble. Cd is a by-product of mining and smelting of Pb and Zn and is used in nickel-cadmium batteries and paint pigments. Cd can be found in soils under agriculture from insecticides, fungicides, sludge and commercial fertilizers. Ingestion of Cd can rapidly cause feelings of nausea, vomiting, abdominal cramp and headache, as well as diarrhea and shock. Itai-itai disease in Japan was identified among people living in Cadmium-polluted areas where rice was irrigated. Target organs include liver, placenta, kidneys, lungs, brain and bones [49].

### **1.7.6. Nickel**

Nickel metal and its compounds are used in multiple applications as stainless steel and other alloys, castings, catalysts, batteries, electronics, ceramics, pigments and even coins. Although not

an element extensively released to the environment, Ni can present a risk to human health. The main danger of Ni to man besides the carcinogenicity is related to the ability to cause sensitive reactions. The most common harmful health effect in the general population is allergic contact dermatitis elicited by prolonged skin contact of sensitized individuals with Ni. On the molecular level the toxic Ni species responsible for severe health effects, as allergic contact dermatitis and respiratory tract cancer, has been suggested to be caused by Ni<sup>2+</sup>. Ni affects the following organ systems: cardiovascular (heart and blood vessels), dermal (skin), immunological (immune system), respiratory (from the nose to the lungs) [48].

### **1.7.7. Lead**

Lead has a density of 11.3g/cm<sup>3</sup> atomic number 82 and is obtained from its sulfide mineral galena, carbonate cerussite, and sulfate anglesite. The ores are frequently found in combination with other recoverable metals such as Cu, Zn and Cd. Lead exists in various oxidation states (0, I, II and IV), which are of environmental importance with oxidation +2. Lead was placed position 2 on the Agency for Toxic Substances and Disease Registry's (ATSDR) top 20 list of most dangerous heavy metals and it accounts for most of the cases of pediatric heavy metal poisoning. Lead has been used in pipe making, drains and soldering materials as well as battery manufacture, plumbing, ammunition, fuel additives, paint pigments and pesticides [52]. Some effects of Pb poisoning include deficiency in cognitive function due to destruction of the central nervous system, abdominal pain and discomfort, formation of weak bones as Pb replaces calcium and causes anemia due to reduction of enzymes concerned with synthesis of red blood cells. Lead also leads to decreased fertility, causes cancer and other minor effects like vomiting, nausea, and headache [53]. Exposure to high Pb levels can severely damage the brain and kidneys, cause miscarriage in pregnant women, damage the organs responsible for sperm production in men and it may ultimately cause death [52].

## **1.8. Statement of the problem**

There is no reported data about the level of selected metals found in Tena Adam medicinal plant grown in Ethiopia. Thus, this research aims to evaluate the concentrations of some selected

metals in leaves of Tena Adam and to study the distribution of metals in four different areas of Ethiopia and its relationship with metal absorption by a medicinal plant. This research also compares the obtained concentrations of elements in leaves of Tena Adam plant with toxic levels provided in literature.

## **1.9. Objectives of the study**

### **1.9.1. General objective**

The overall objective of this research is to determine selected metals in Tena Adam (*Ruta chalepensis* L.) plant cultivated in four selected areas of Ethiopia.

### **1.9.2. Specific objectives**

The followings are specific objectives of this research:

1. To develop and validate sample preparation procedure (digestion procedure) for the extraction of selected trace metal ions from Tena Adam (*Ruta chalepensis* L.) for their subsequent determination by using flame atomic absorption spectrometry (FAAS).
2. To determine some selected trace metals found in Tena Adam (*Ruta chalepensis* L.) by using flame atomic absorption spectrophotometry (FAAS).
3. To compare the levels of the identified metals in Tena Adam (*Ruta chalepensis* L.) in four different areas of Addis Ababa, Ethiopia, and
4. To compare the levels of the identified metals in Tena Adam (*Ruta chalepensis* L.) in four different areas of Addis Ababa, Ethiopia with that of the data in literature.

### **1.10. Significance of the problem**

The major significance of this research is to provide information about the level of essential and non-essential metals in Tena Adam cultivated in four selected areas around Addis Ababa, Ethiopia. This research is believed to give a clue for further studies in Tena Adam cultivated in different parts of the country. Also, it will give information for the concerned institutions and organizations to take some necessary actions for the well-being of the society and also to provide some helpful information as a stepping stone for other researchers.

## **2. Experimental**

### **2.1. Instrumentation and apparatus**

Ceramic pestle and mortar were used for grinding and homogenizing the dried Tena Adam leaves; digital analytical balance (Mettler Toledo, Model AT250, Switzerland) with  $\pm 0.0001$  g precision and polyethylene plastic bags were used for weighing and drying the samples in open air, respectively. Quick-fit round bottom flasks (250 mL) fitted with reflux condenser were used in Kjeldahl apparatus hot plate to digest the samples. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the samples ready for analysis till determination. 2 mL, 5 mL, and 10 mL pipettes and a micropipette (DRAGONMED, Shanghai, China) were used for measuring different amounts of acids and standard solutions and for spiking of known concentration for recovery test. 25 mL and 100 mL volumetric flasks were used to dilute sample solutions and prepare standard solutions. ZEEnit 700p scientific model Analytikjena (Germany) Flame Atomic Absorption Spectrometry equipped with deuterium arc background corrector using air-acetylene flame was used for analysis of the metals (Ca, Cr, Cu, Zn, Ni, Cd and Pb).

### **2.2. Chemicals, reagents and standard solutions**

For digestion of Tena Adam leaf samples, 69.5%  $\text{HNO}_3$  (Scharlau AC 1600, Spain) and 70%  $\text{HClO}_4$  (Research—lab fine chem industries, Mumbai, India) were used. 1,000 mg/L stock standard solutions of the metals Ca, Cr, Cu, Zn, Ni, Cd and Pb were prepared as nitrates for each element in 2%  $\text{HNO}_3$  and used for the preparation of calibration curves for the determination of metals in the samples. Distilled-deionized water for preparation of standard solutions, dilution and for cleaning (rinsing) purpose was used. Reagents that were used in the analysis were all analytical grade and all standard solutions are CRM (certified reference material).

## 2.3. Working procedures

### 2.3.1. Areas of the study

The leaf samples of Tena Adam were collected randomly from four selected areas around Addis Ababa. These are Kality, Alem Bank, Holeta and Worabe.

The selection of sample collection areas were based upon mainly accessibility for sampling and potential area for the plant Tena Adam cultivation.

Table 1: Geographical description of sample collection sites

No.	Sample sites	Region	Approximate geographical locations				
			Latitude	Longitude	Altitude above sea level (m)	Distance from Addis Ababa (km)	Direction from Addis Ababa
1	Kality	AAA	9°01'29" N	38°44'48" E	2405	14.2	East
2	Holeta	Oromia	9°03'N	38°30'E	2391	36.8	North West
3	Alem Bank	AAA	9°01'29" N	38°44'48" E	2405	3.5	West
4	Worabe	SNNPR	8°01'N	38°20'E	2113	169	South

### Short descriptions about sampling areas

#### i. Akaki Kality

Akaky Kaliti, also spelled Akaki Kality, is one of the 10 sub-cities of Addis Ababa, the capital of Ethiopia.

## **ii. Holeta**

Holeta is a town and separate woreda in central Ethiopia. Located in the Oromia Special Zone Surrounding Finfinne of the Oromia Region, it has  $9^{\circ}3'N$  latitude,  $38^{\circ}30'E$  longitude and an altitude of 2391 meters above sea level.

## **iii. Alem Bank**

Alem Bank, also known as World Bank, is one of the communities found in Kolfe Keraniyo sub-city in Addis Ababa, Ethiopia. This community named World Bank because of most the houses around there were built by the money obtained from World Bank.

## **iv. Worabe**

Worabe is a town in south-central Ethiopia. Official sources locate this town in the Gurage Zone of the Southern Nations, Nationalities and Peoples Region, although it is reported that at a referendum in 2000 the Silte people unanimously voted to form their own Zone, Silte, which includes Worabe. The town has  $8^{\circ}1'N$  latitude,  $38^{\circ}20'E$  longitude with an elevation of 2113 meters above sea level.

### **2.3.2. Collection of samples**

Leaves of Tena Adam were collected by using random sampling technique from the study areas mentioned above. All the leaf samples collected from sampling areas were stored in clean polyethylene plastic bags independently, labeled as TK-1 for Kality, TAB-2 for Alem Bank, TH-3 for Holeta, TW-4 for Worabe and transported to the laboratory and stored there prior to sample preparation, digestion and analysis.

### **2.3.3. Apparatus cleaning**

Since this research focused mainly on determination of trace metals in trace level, cleaning of any apparatus to be used is of great importance. Hence, to prevent contamination, all glassware used for the analytical methods such as pipettes, volumetric flasks, measuring cylinder and digestion flasks were washed with detergents and tap water, rinsed with deionized water, soaked in dilute nitric acid (10% HNO<sub>3</sub>) for 24h, rinsed with deionized water, dried at room temperature and kept in dust free place until needed for use and sterile disposable powder-free plastic gloves were worn when handling the Tena Adam sample during the sampling and analysis stages. The digested solutions were also kept in the refrigerator until analysis.

### **2.3.4. Pretreatment of the plant and sample preparation for elemental analysis**

The leaves of the plant were washed well with the running tap water and rinsed first with distilled water and then with deionized water to remove earthy impurities, allowed to dry in air for ten days, chopped and grounded to powder with recommended, acid washed mortar and pestle. Then the powdered samples were screened with recommended sieve size and stored in dry, clean and closely packed polyethylene plastic bag until digestion. Finally, 0.5 g aliquot was taken from each sample for digestion and a solution for final metal determination was prepared.

### **2.3.5. Optimization of digestion procedure**

To select an optimum procedure for digestion, parameters like digestion time, volume ratio of reagents, and digestion temperature were optimized by varying one parameter at a time and keeping the others constant. Parameters giving clear solution at lower temperature, requiring minimum reagent volume and digestion time were selected as an optimum procedure for digestion of Tena Adam sample. Finally, the optimum procedure was chosen on the basis of these criteria requiring three hours for complete digestion of 0.5 g Tena Adam sample with 3.0 mL of 69.5% HNO<sub>3</sub> and 1.0 mL 70% HClO<sub>4</sub> as given in Table 2.

Table 2: Optimization of digestion procedure

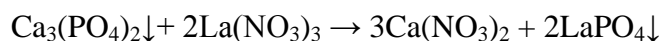
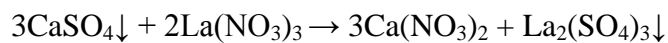
No	Amount of Tena Adam (g)	Volume of reagents (mL)	Digestion temperature (°C)	Digestion time (h)	Results after filtration and dilution
1	0.5	2:2 mL (HNO <sub>3</sub> :HClO <sub>4</sub> )	240	3	Yellow solution with residue
2	0.5	2:2 mL (HNO <sub>3</sub> :HClO <sub>4</sub> )	270	3	Pale yellow clear solution
3	0.5	2:2 mL (HNO <sub>3</sub> :HClO <sub>4</sub> )	300	3	Pale yellow clear solution
4	0.5	2.5:2.5 mL (HNO <sub>3</sub> :HClO <sub>4</sub> )	300	3	Pale yellow clear solution
5	0.5	3:3 mL (HNO <sub>3</sub> :HClO <sub>4</sub> )	300	3	Pale yellow clear solution
6	0.5	3:3 mL (HNO <sub>3</sub> :HClO <sub>4</sub> )	300	3.5	Colorless clear solution
7	0.5	3:1 mL (HNO <sub>3</sub> :HClO <sub>4</sub> )	240	3.5	Yellow solution with residue
8	0.5	3:1 mL (HNO <sub>3</sub> :HClO <sub>4</sub> )	270	3.5	Pale yellow clear solution
9	0.5	3:1 mL (HNO <sub>3</sub> :HClO <sub>4</sub> )	300	3.5	Pale yellow clear solution
<b>10</b>	<b>0.5</b>	<b>3:1 mL (HNO<sub>3</sub>:HClO<sub>4</sub>)</b>	<b>300</b>	<b>3</b>	<b>Colorless clear solution (Optimum)</b>

**Note:** Bold (No. 10) indicate optimized volume, temperature and time.

### 2.3.6. Digestion of leaves of Tena Adam samples

Applying the optimized procedure given above, 0.5 g of powdered rue sample was weighed on analytical digital balance and placed in a 250 mL round bottom flask. To this, 3 mL of HNO<sub>3</sub> (69.5%) and 1 mL of HClO<sub>4</sub> (70%) was added. The round bottom flask was fitted to a reflux condenser and heated on a Kjeldahl apparatus hot plate for 3 h at a temperature of 300°C. The digest was allowed to cool for 10 min without dismantling the condenser and then further cooled to room temperature for 20 min by dismantling the condenser. The mixture then diluted with 10 mL of distilled-deionized water and filtered with Whatman filter paper No. 42 into a 25 mL volumetric flask. The round bottom flask was further rinsed with 5 mL of distilled-deionized water and added to the filtrate. Then 1% of hydrated La(NO<sub>3</sub>)<sub>3</sub>.6H<sub>2</sub>O was added to the flask containing the filtrate the flask was filled to the mark with deionized water.

The main reason why hydrated  $\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$  was added was to prevent the precipitation of  $\text{Ca}^{+2}$  with the  $\text{SO}_4^{-2}$  and  $\text{PO}_4^{-3}$  if they were present in the samples or the reagents used in the process. i.e., on the addition of  $\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$  to the sample solution the  $\text{SO}_4^{-2}$  and  $\text{PO}_4^{-3}$  were precipitated so that the  $\text{Ca}^{+2}$  became free for atomization. The reaction chemical equation can be represented as follows:



For each sample the digestion was done in triplicate. Triplicate blank samples, a mixture of 3 mL of  $\text{HNO}_3$  and 1 mL of  $\text{HClO}_4$  were digested following the same procedure as the samples. Finally, all the digests were kept in refrigerator until FAAS analysis.

### **2.3.7. Calibration of the instrument and determination of the metals by FAAS**

#### **A) Calibration of the instrument**

10 mg/L intermediate standard solutions of metals of interest were prepared from the atomic absorption spectroscopy standard stock solutions that contained 1000 mg/L. These secondary standards were diluted with deionized water to obtain four working standards of each metal, i.e. Ca, Cr, Cu, Zn, Cd, Ni and Pb. The absorbance's of the working standard solutions were measured and the calibration curves for each of the analyte metal (Ca, Cr, Cu, Zn, Ni and Pb) were constructed. The operating conditions for FAAS employed for each analyte were given in Table 3 and 4.

Table 3: Instrumental operating conditions for determination of metals in Tena Adam samples by using FAAS

Metal	Wavelength (nm)	Method detection limit (mg/L)	Energy (eV)	PMT (V)	Slit width (nm)	Lamp current (mA)
Ca	422.7	0.025	87	300	1.2	3
Cr	357.9	0.05	76.9	326	0.2	4
Cu	324.8	0.035	75.2	293	1.2	2
Zn	213.9	0.012	100	461	0.5	2
Cd	228.8	0.012	92.9	259	1.2	2
Ni	232	0.07	84.8	375	0.2	3
Pb	283.3	0.3	74.9	262	1.2	2

Table 4: Working standard concentrations, absorbance, correlation coefficient and equation of the calibration curves for determination of metals using FAAS

No.	Metal	Working standard concentrations (mg/L)	Absorbance	Correlation coefficient (R <sup>2</sup> )	Equation of the calibration curves
1	Ca	0.15	0.00589	0.999	$y = 0.027x + 0.001$
		0.25	0.00882		
		0.50	0.01573		
		1.00	0.02947		
2	Cr	0.50	0.00583	0.997	$y = 0.0138x - 0.0006$
		1.00	0.01383		
		1.50	0.01979		
		2.00	0.02681		
3	Cu	0.25	0.00441	0.998	$y = 0.0192x - 3 \times 10^{-5}$
		0.50	0.00958		
		1.00	0.01991		
		2.00	0.03800		
4	Zn	0.25	0.02552	0.997	$y = 0.087x + 0.0032$
		0.50	0.04453		
		0.75	0.06977		
		1.00	0.09025		
5	Cd	0.25	0.01031	0.993	$y = 0.0503x - 0.0013$
		0.50	0.02546		
		0.75	0.03520		
		1.00	0.04942		
6	Ni	0.5	0.00344	0.998	$y = 0.0069x - 0.0007$
		1.5	0.00891		
		3.00	0.01987		
		6.00	0.04090		
7	Pb	0.40	0.00127	0.996	$y = 0.0037x - 0.0003$
		0.80	0.00252		
		1.20	0.00420		
		1.60	0.00577		

### 2.3.7.1. Calibration curves of working standard concentrations of metals versus absorbance

#### A) For calcium

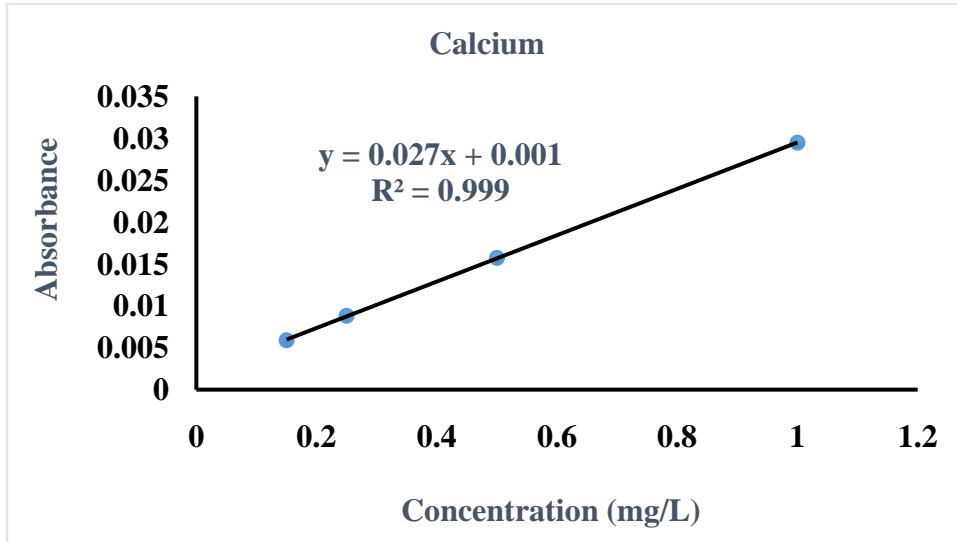


Figure 4: Calibration curve for calcium determination

#### B) For chromium

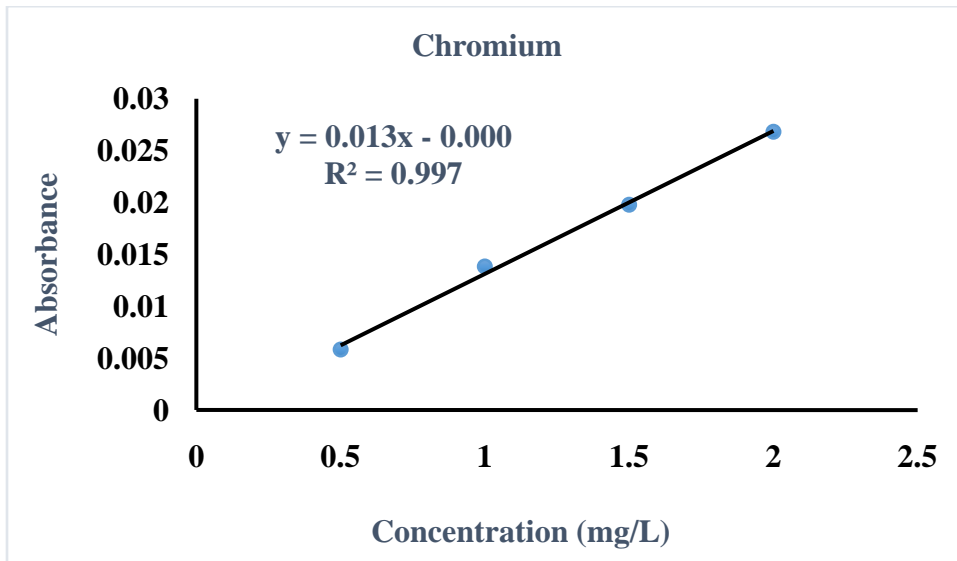


Figure 5: Calibration curve for chromium determination

C) For copper

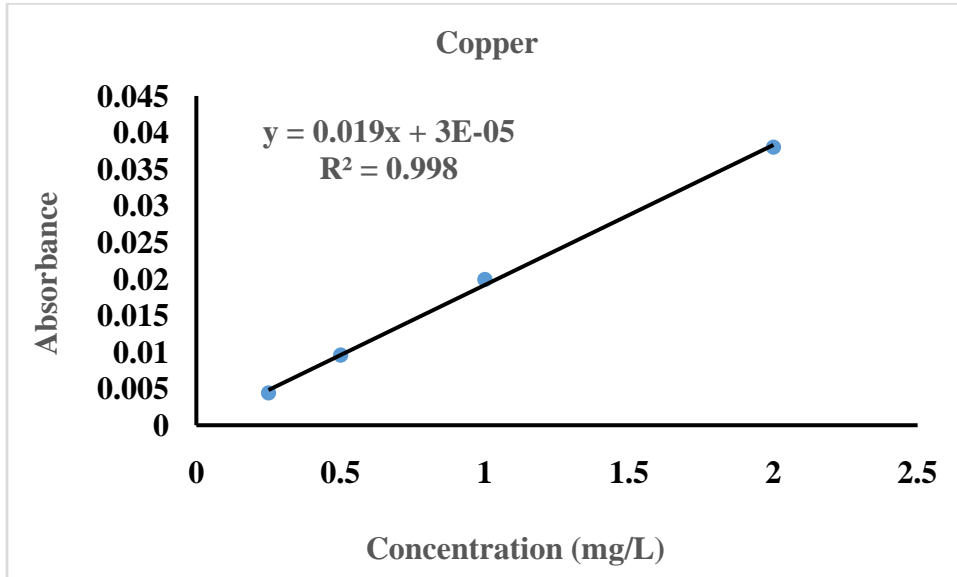


Figure 6: Calibration curve for copper determination

D) For zinc

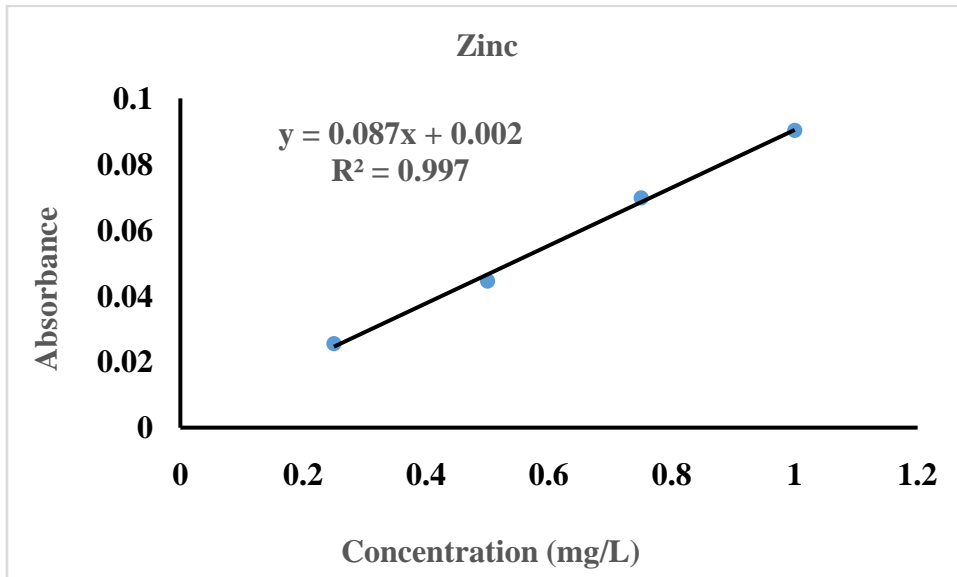


Figure 7: Calibration curve for zinc determination

**E) For cadmium**

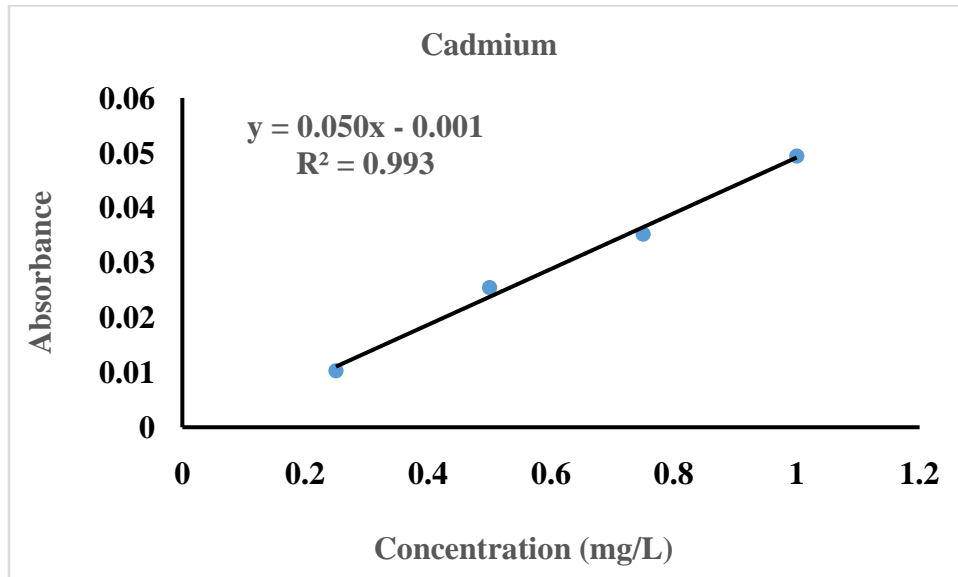


Figure 8: Calibration curve for cadmium determination

**F) For nickel**

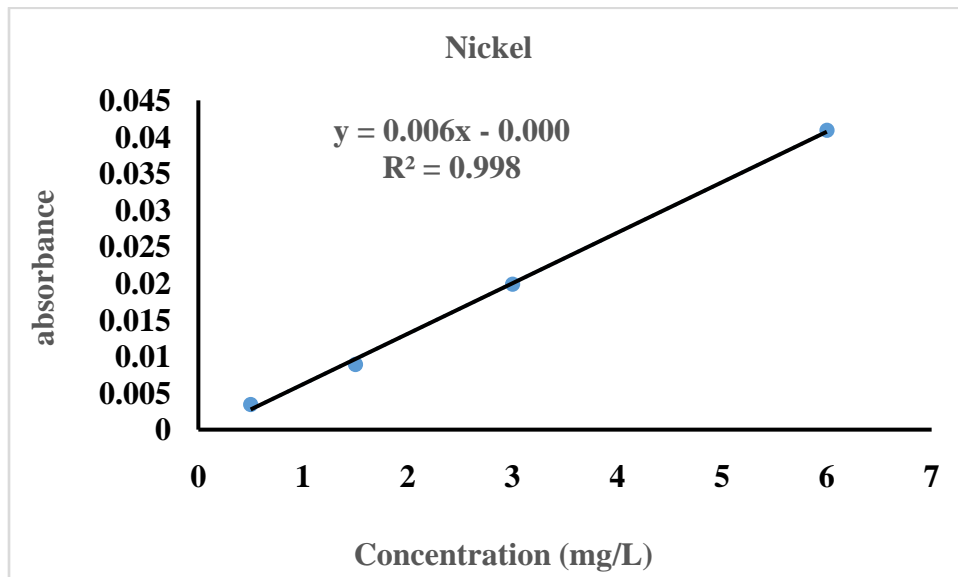


Figure 9: Calibration curve for nickel determination

### G) For lead

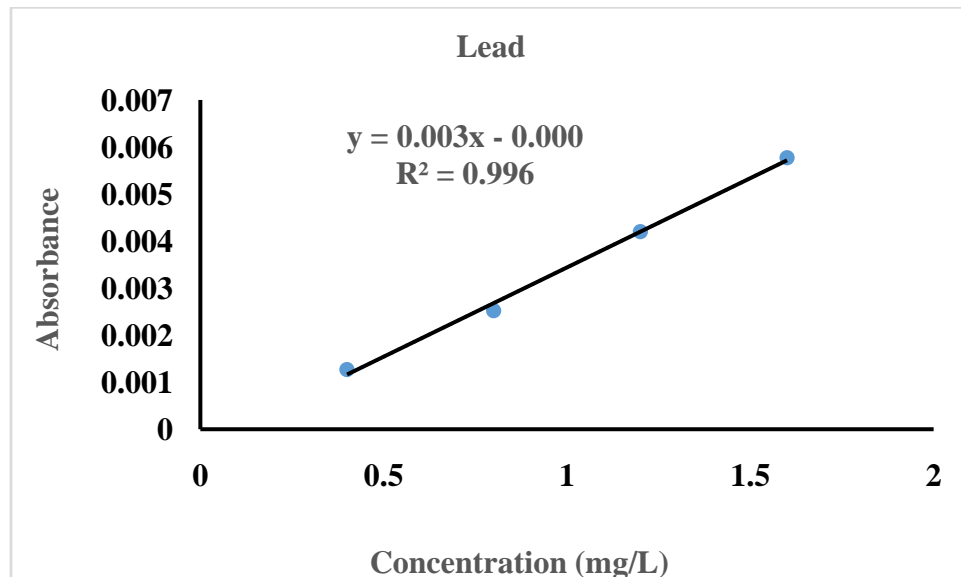


Figure 10: Calibration curve for lead determination

From the correlation coefficients in Table 4 and their corresponding calibration curves in Figures 4–10 for each metal it is possible to say that, the change in absorbance with concentration is in good positive correlation and are linearly fit.

### B) Determination of the metals by FAAS

The levels of each metal in all the samples were determined with FAAS after the instrumental operating conditions were optimized for maximum signal intensity of the instrument. Triplicate determinations were carried out on each rue samples. Hallow cathode lamp for each metal (Ca, Cr, Cu, Zn, Cd, Ni and Pb) operated at the manufacturer's recommended conditions were used at its corresponding primary source line. The acetylene and air flow rates were managed to ensure suitable flame conditions. The same analytical procedure was employed for the determination of elements in the digested blank solutions.

### 2.3.8. Precision and accuracy

The precision of an analytical procedure expresses the closeness or agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [28]. Most of the common statistical methods applied in analytical chemistry are the standard deviation, variance, coefficient of variance, relative standard deviation and range of series of measurements [54]. For this particular study the results of the measurements are expressed as the mean of the measurements together with the standard deviation of the triplicate samples with triplicate measurements of each sample.

### 2.3.9. Validation of the optimized procedure

Spiking experiments were performed to validate the optimized procedure. For this purpose, standard solutions of 1,000 mg/L each metal were used and intermediate standards of 100 mg/L of each metal (Ca, Cr, Cu, Zn, Cd, Ni and Pb) were prepared from 1,000 mg/L primary standard solution. For recovery measurement the sample from Worabe was selected and the spiking was done in two groups in triplicate.

That is, in the first group 64  $\mu$ L of Zn was spiked and added into 250 mL round bottomed flask containing 0.5 g sample and 4 mL of acid mixture (3 mL HNO<sub>3</sub> and 1 mL of HClO<sub>4</sub>) and in the second group 3077  $\mu$ L of Ca was spiked and added into round bottomed flasks containing the same amount of sample and acid. Then, the round bottomed flasks containing the mixtures were fitted with the condenser and digested simultaneously with un-spiked samples on a hot plate Kjeldahl apparatus by applying the optimized digestion procedure used in sample analysis. Then, the percentage recoveries of the analytes were calculated by the use of the equation:

$$\% R = \frac{(C_M \text{ in the spiked sample }) - (C_M \text{ in the non-spiked sample })}{C_M \text{ added for spiking}} \times 100 \%$$

Where:  $C_M$  is concentration of metal of interest.

Finally, the results of recovery analysis are listed in Table 5 and the percentage recoveries lies within the range 96.7 – 96.9%. Therefore, the percentage recoveries for Tena Adam samples are within the acceptable range for all the metals.

**N.B.:** The percentage of recovery is the amount of element left or recovered from the original sample after the digestion process, and is therefore related to the accuracy.

Table 5: Recovery test using optimized procedure of metals in the leaves of Tena Adam sample

Metal	Concentration of metal in un-spiked sample (mg/L)	% spiked	Amount spiked (mg/L)	Concentration of metal in spiked sample (mg/L)	% R ( $\bar{X}\%R \pm SD$ )
Ca	61.4	20	12.3	73.3	96.7 ± 5.60
Zn	1.3	20	0.26	1.53	96.9 ± 9.70

### 3. Results and Discussion

#### 3.1. Levels of metals in the leaves of Tena Adam samples

The developed FAAS method was applied for the determination of the levels of seven metals (Ca, Cr, Cu, Zn, Cd, Ni and Pb) in leaves of Tena Adam samples collected from four selected areas around Addis Ababa, i.e., Kality, Holeta, Alem Bank and Worabe. The mean values were determined from triplicate analysis of each sample and the accuracy and precision of the results were checked by the aid of different statistical methods (listed under sub topic 2.3.8) after the determination of the levels of metals in the Tena Adam samples. Results obtained for each sample in terms of mg/kg dry weight basis together with values for standard deviation are shown in Table 6.

Table 6: Mean concentration and standard deviation ( $\bar{X} \pm SD$ , mg/kg) of selected metals in each sample analyzed by FAAS

No.	Metal	Sample sites, concentration (mean $\pm$ SD) in mg/kg				Range of metal concentration (mg/kg)
		Kality	Holeta	Alem Bank	Worabe	
1	Ca	2409 $\pm$ 1.0	1872 $\pm$ 2.0	2443 $\pm$ 2.0	3077 $\pm$ 1.0	1872 – 3077
2	Cr	3.10 $\pm$ 0.04	1.20 $\pm$ 0.02	1.70 $\pm$ 0.03	ND	ND – 3.10
3	Cu	5.80 $\pm$ 0.06	ND	10.8 $\pm$ 0.02	ND	ND – 10.8
4	Zn	49.1 $\pm$ 0.06	44.5 $\pm$ 0.02	60.8 $\pm$ 0.5	63.9 $\pm$ 0.10	44.5 – 63.9
5	Cd	2.90 $\pm$ 0.03	ND	ND	ND	ND – 2.90
6	Ni	ND	ND	ND	ND	ND
7	Pb	ND	ND	ND	ND	ND

### 3.2. Distribution patterns of metals in the samples

As indicated in the Table 7 metals Ca and Zn were found in all the samples and metals Ni and Pb were below detection limit of the instrument in all the samples. Cr was found in all samples except in the sample from Worabe, Cu was found in Kality and Alem Bank Tena Adam leaves and Cd was found only in Tena Adam leaves from Kality.

The results of this study showed that the metal contents of Tena Adam leaves varied with the geographical origin in which the Tena Adam plant grows. There are industrial activities in samples collected from Addis Ababa Administration, especially Kality areas. Because of this Cr, Cu and Cd were determined in the areas which are not determined sampling areas out of Addis Ababa like Worabe. The natural weathering of rocks, agricultural activities like using fertilizer, herbicide, and water for irrigation could contribute to these concentrations of metals determined in Tena Adam leaves. A comparison of the metal contents in the Tena Adam leaves in this study showed that Ca contents are in higher amounts in the samples compared to the other heavy metals. A relatively higher amount of Ca in the leaves from all areas could be due to its higher natural abundance in the soil and the nature of Tena Adam. In comparison to other sampling areas, Worabe is more exposed to human activity and the farms were used for long time, which could have significant contribution to gradual accumulation of metals in Tena Adam farms through agricultural activities. This could be suggested as one of the reasons for the higher concentration of Ca determined in Worabe than others.

Metals uptake by plants may occur through different and complex biochemical processes. This uptake varies based on the ability of the plants to absorb metals from the soil, the availability of the mineral elements in soluble and absorbable forms, the abundance of specific metals at the specified site, the contamination level of the soil with heavy metals, etc. The variation of metal levels in soil, especially samples collected around Addis Ababa arises because of increasing industrialization and associated pollution of the biosphere, use of different types of fertilizers, pesticide treatment, and others are the main contributors. The use of sewage sludge, pesticides, irrigation of waters and fertilizers on agricultural land has made some of that land of questionable quality for production of food for humans and animals. The distribution and

accumulation of metals in leaves of Tena Adam are the reflection of the mineral composition of the soil and environment in which Tena Adam plant grows. Therefore, the actual metal content of Tena Adam varies considerably according to geographic origin, the use of fertilizers with different chemical compositions and other characterizing features such as water used for irrigation. Since plants accumulate metals from the soil and environment in its different parts. However, this study focuses the level of metals in leaves of Tena Adam because it is the leaves that are commonly consumed by users.

### **3.3. Concentration of metals in leaves of Tena Adam in four sampling areas**

Metals absorbed by plants from different sources are accumulated in different parts of the plant's body, like roots, stems, leaves, seeds and other parts. The amount of metals accumulated in the plants' body parts is variable, i.e. some of them are higher in the roots, some others in the seeds and others in stems and other parts of the plants.

However, the focus of this project is on the level of metals in leaves of Tena Adam plant peoples commonly use and the contents of the metals analyzed in Tena Adam leaves collected from four different sample sites are discussed in detail in the following sections.

#### **3.3.1. Concentration of metals in Tena Adam leaves collected from Kality**

As shown in Table 6 and Figure 11, Ca is in the highest amount with concentration of  $2409 \pm 1$  mg/kg followed by Zn ( $49.1 \pm 0.06$  mg/kg), Cu ( $5.76 \pm 0.06$  mg/kg), Cr ( $3.1 \pm 0.04$  mg/kg) and Cd ( $2.87 \pm 0.03$  mg/kg) with the least concentration whereas, metals Ni and Pb are observed below detection limit. These results showed that the essential trace metals are found at higher amount whereas the non-essential in a lower amount or none. This is beneficial from the health point of view. Generally, the order of level of metals in Tena Adam leaves collected from Kality is:  $Ca > Zn > Cu > Cr > Cd$ .

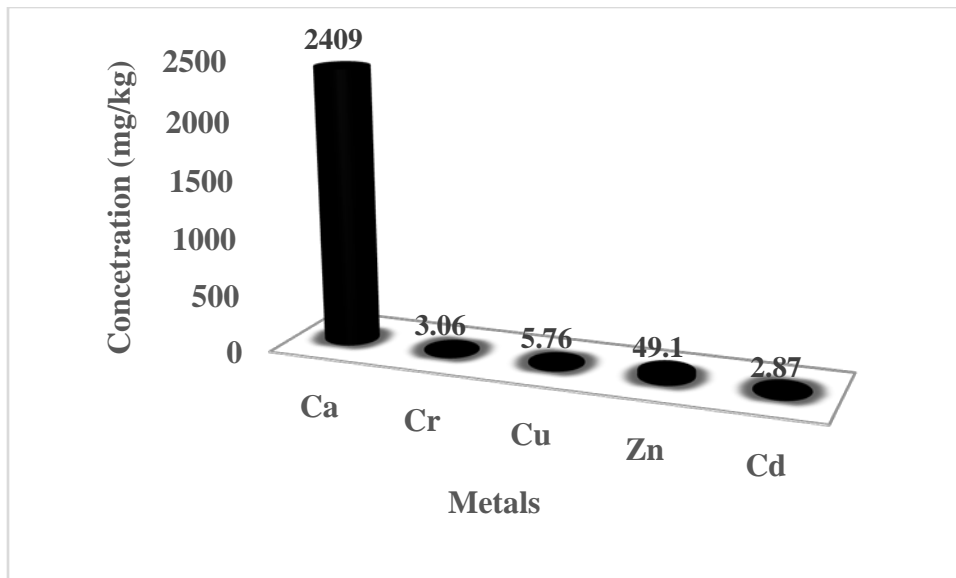


Figure 11: Bar graph of Kality sample

### 3.3.2. Concentration of metals in Tena Adam leaves collected from Holeta

In the Holeta samples, Ca is also in the highest amount with concentration of  $1872 \pm 2$  mg/kg followed by Zn ( $44.5 \pm 0.02$  mg/kg) and Cr ( $1.2 \pm 0.02$  mg/kg) with least concentration and the order of level of metals were found to be  $Ca > Zn > Cr$  in the Tena Adam sample from Holeta. But, the other four metals Cu, Cd, Ni and Pb were observed below detection limit of the instrument and the results are indicated in the Figure 12.

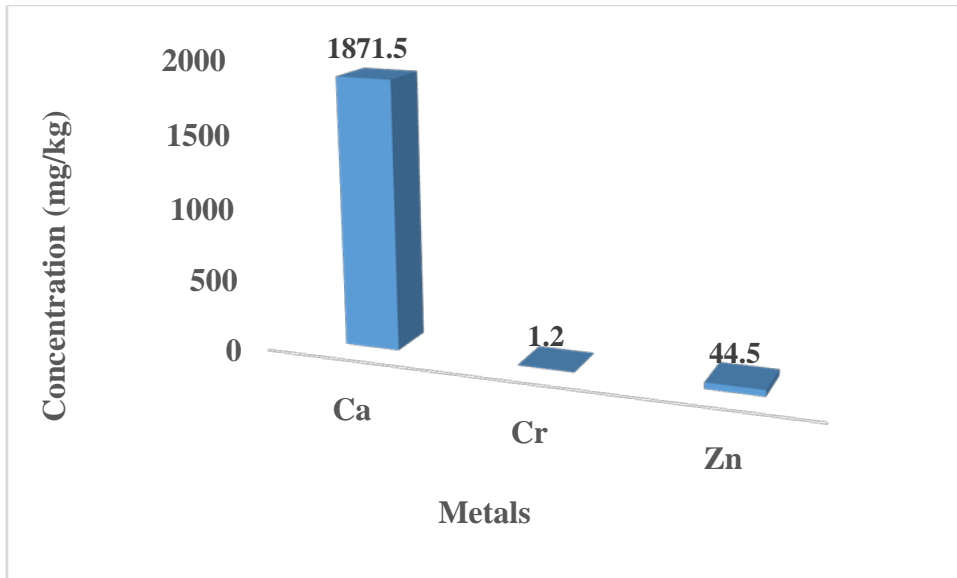


Figure 12: Bar graph of Holeta sample

### 3.3.3. Concentration of metals in Tena Adam leaves collected from Alem Bank

As the concentrations are displayed in Figure 13, Ca is in the highest amount with concentration of  $2443 \pm 2$  mg/kg followed by Zn ( $60.8 \pm 0.5$  mg/kg), Cu ( $10.8 \pm 0.02$  mg/kg) and Cr ( $1.7 \pm 0.03$  mg/kg) and the order of the metals were found to be  $Ca > Zn > Cr$  in the Tena Adam leaves collected from Alem Bank. However, the concentrations of metals such as Cd, Ni and Pb were below detection limit of the instrument.

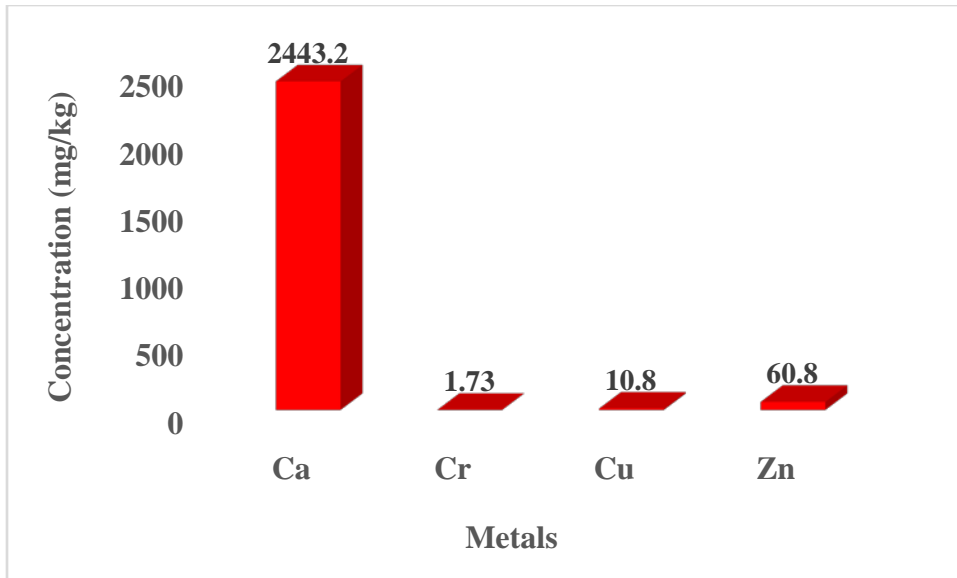


Figure 13: Bar graph of Alem Bank sample

#### 3.3.4. Concentration of metals in Tena Adam leaves collected from Worabe

The last Tena Adam leaf sample analyzed was collected from Worabe. Like the previous samples Ca is in the highest amount with the concentration of  $3077 \pm 1$  mg/kg followed by Zn with concentration of  $63.9 \pm 0.1$  mg/kg. But, in this sample the concentrations of Ca and Zn are higher than those of the other three samples. In contrast to other sample sites, all the other five metals Cr, Cu, Cd, Ni and Pb are below detection limit of the instrument. The concentration of the metals is indicated in the Figure 14.

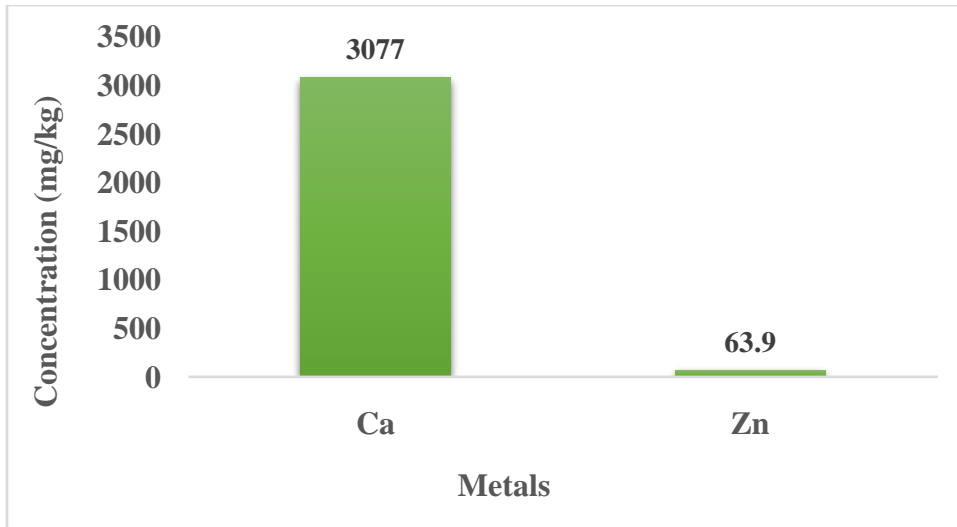


Figure 14: Bar graph of Worabe sample

### 3.3.5. Comparison of the concentration of metals in four different Tena Adam samples

Figure 15 and 16 shows the concentrations of metals in leaves of Tena Adam collected from Kality, Holeta, Alem Bank and Worabe.

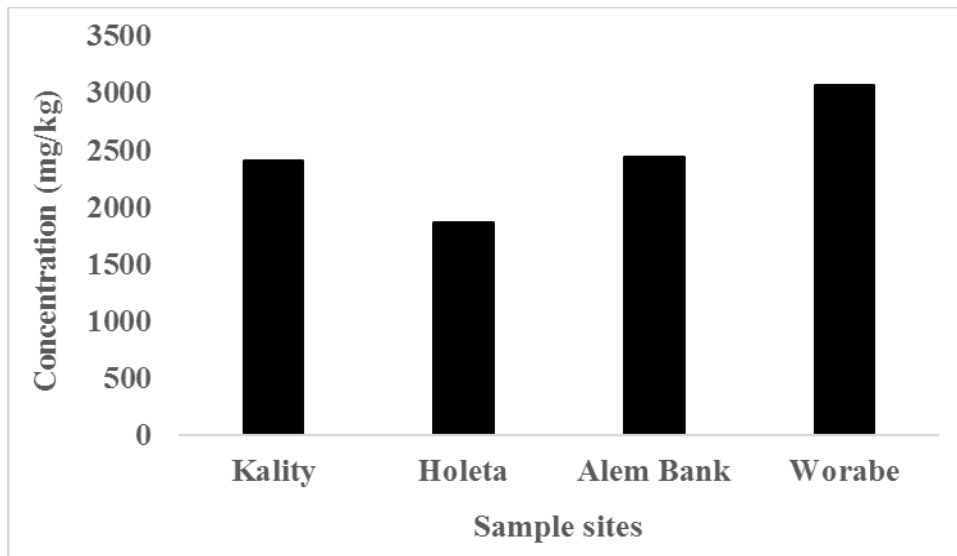


Figure 15: Bar graph summary of concentration of Ca in Kality, Holeta, Alem Bank and Worabe samples

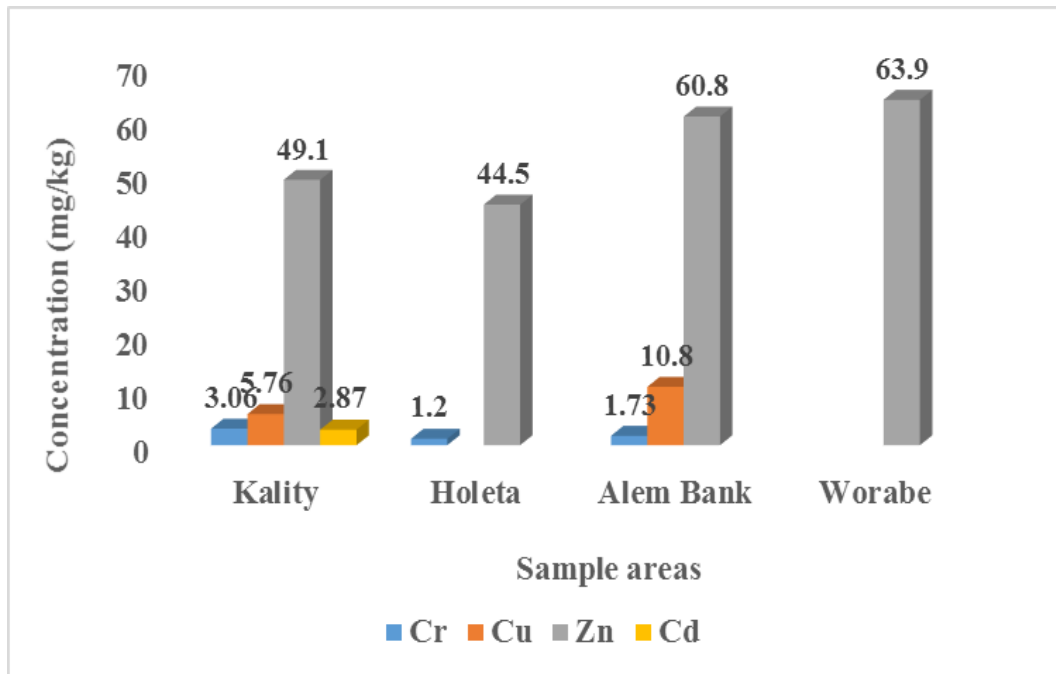


Figure 16: Bar graph summary of concentrations of Cr, Cu, Zn and Cd in Kality, Holeta, Alem Bank and Worabe samples

From Figure 15 and 16 we can see that the trend of levels of metals concentration especially metals Ca and Zn in each sample is similar. Other metal contents in each sample are found to be quite different.

In general, from the figures and tables discussed above we can summarize that Tena Adam sample from Worabe contains the highest amount Ca (3077 mg/kg) and Zn (63.9 mg/kg) essential macro and micro nutrient, respectively, followed by Alem Bank, Kality and Holeta with the least concentration. Ni and Pb were found too low to be detected in all Tena Adam samples, Cd was found too low to be detected in the three samples except samples from Kality and in contrast to other three sample sites Cr was found too low to be detected only in one sample site, i.e. Worabe.

### 3.4. Comparison of concentrations (mg/kg) of selected metal levels in leaves of Tena Adam obtained in the present study with literature and permissible values from FAO/WHO

Comparison of analytical data with reference material is a common practice in analytical chemistry to validate the results. Although various chemical analysis target to a similar objective, there may be a difference in sampling, sample preparation and analysis techniques [28]. Considering all these, the result of the present study can be compared to the findings of other authors and standard reference material given by WHO. Since, no detail studies were made on the levels of metal composition on Tena Adam in Ethiopia, this study was compared with the literature obtained from Jordan. The comparative levels of metals in Tena Adam found in this study with the levels reported in the literature is indicated in Table 7.

Table 7: Comparison of selected metal concentrations (mg/g, dry weight basis) in leaves of Tena Adam samples of this study with reported values from literature

Metal	Range of concentration of metals (mg/kg)		
	Ethiopia	Jordan	FAO/WHO
Ca	1872-3077	NR	NR
Cr	ND-3.1	NR	2
Cu	ND-10.8	5.55-9.38	20
Zn	44.5-63.9	10.31-23.36	50
Cd	ND-2.9	0.32-0.86	0.3
Ni	ND	0.45-3.17	1.5
Pb	ND	14.17-16.44	10
Reference	This study	[4]	[57]

As indicated in Table 7 the concentrations of Ca and Cr were not reported in Jordan Tena Adam samples, hence they are not compared. The concentration of Cu is in the range of the values from Jordan (5.55-9.38 mg/kg) with the sample from Kality (Ethiopia) (5.8 mg/kg) is in good agreement. However, the concentration of Cu in the sample from Alem Bank (Ethiopia) (10.8 mg/kg) is slightly higher than the range of concentration of Cu from Jordan (5.55-9.38 mg/kg)

and its concentration is not detected in two sample sites in Ethiopia, which is different from the values from Jordan. The level the other metal Zn (44.5-63.9 mg/kg) from Ethiopia was found higher than the range of the reported data in Jordan (10.31-23.36 mg/kg). Except Tena Adam samples from Kality the concentration of Cd was below detection limit in the other sample sites in Ethiopia, but its concentration (2.9 mg/kg) was found slightly higher than the reported data from Jordan (0.32-0.86 mg/kg). The concentrations of Ni and Pb were found below detection limit and this is different from the results reported from Jordan.

Generally, most of concentrations of metals in Tena Adam samples from Ethiopia differ slightly from concentration of Tena Adam samples reported from Jordan. This might need further studies on the geographical origin to help in finding out possible sources of heavy metal pollution and vegetation of the area from where the Tena Adam was originated.

The Zn concentration in the leaves of all samples of Tena Adam in this study ranges from 44.5-63.9 mg/kg and WHO's recommended limit of Zn in plants is 50 mg/kg [55]. This indicates that the Zn concentrations in the leaves of Tena Adam are below the permissible limit in Kality and Holeta samples but above the permissible limit in Alem Bank and Worabe samples.

The range of concentration of Cu in this study is ND-10.8 mg/kg, which is below the permissible limit of Cu for plants recommended by WHO, i.e. 10 mg/kg [56] and also below the permissible limits for Cu in medicinal plants according to WHO, i.e. 20 mg/kg [57].

For Cr, the proposed limit set by WHO is 2 mg/kg [57]. Thus, the concentration of Cr is above the proposed limit set by WHO in Kality (3.1 mg/kg) sample and is below the proposed limit set by WHO in the other three samples.

For medicinal herbs the permissible limit for Cd set by WHO is 0.3 mg/kg [57]. Thus, Cd levels in the leaves of Tena Adam in Ethiopian sample (ND-2.9 mg/kg) were below the permissible limit in the three samples collected from Holeta, Alem Bank and Worabe and above the permissible limit only in the sample collected from Kality.

Metals Ni and Pb were not detected in all four Tena Adam samples from Ethiopia. Therefore, the levels of Ni and Pb in the leaves Tena Adam in all samples in this study were below WHO's prescribed limit of Ni (1.5 mg/kg) and Pb (10 mg/kg) in medicinal herbs [57].

Generally, the level of most of selected metals found in the four Tena Adam samples collected from four selected areas around Addis Ababa were found comparable to the maximum tolerable limits proposed by WHO. Thus, they are in safety baseline levels for human consumption.

### **3.6. Statistical analysis**

In analytical work two or more mean results are compared to check whether there is a significant difference between them or not. The variation between the means of different samples can result from random and controlled sources of error. This kind of variation and the variation due to the difference in the original sample type can be separated and estimated by a powerful statistical technique known as analysis of variance (ANOVA) [58]. In this research, a one-way ANOVA and Pearson correlation coefficient was used to decide the variation between samples analyzed was significant or not for the mean concentrations of each metal in triplicate analysis.

### **3.7. Analysis of variance (ANOVA)**

The variation in samples means come from different sources such as experimental procedure or heterogeneity among the samples (i.e. difference in mineral contents of soil, pH of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides, etc. or other variations in cultivation procedures) [39]. In this study the variation in samples means of the analyte was tested whether they have significant difference or not by using ANOVA of single factor of Microsoft excel (Table 9). From Table 9 we can see that there is no significant difference among the sample means at 95% confidence level in mean concentration for metals Cr, Cu and Zn, since F-calculated is less than the critical value (at  $p \geq 0.05$ ). While there is significant difference among the sample means for Ca, since F-calculated is more than the critical value (at  $p \leq 0.05$ ). This is due to variations in the other factors than in the experimental procedure. The source for this significant difference between sample means may be the

difference in mineral contents of soil or pH of soil which predict the extent of mineral absorption by Tena Adam plant. ANOVA of Ni and Pb and Cr were not done, since they were below detection limit of the instrument and found only in one sample respectively. Based on this description we can conclude that most of metals analyzed in this project were not significantly different except Ca.

Table 8: Analysis of variance (ANOVA) between and within Tena Adam samples at 95% confidence level

Metal	Comparison	Df	Fcal	Fcrit	p value	Significance
Ca	Between samples	3	135	4.1	$3.41 \times 10^{-7}$	There is significant difference among the sample means
	Within samples	8				
	Total	11				
Cr	Between samples	2	0.9	5.1	0.5	There is no significant difference among the sample means
	Within samples	6				
	Total	8				
Cu	Between samples	1	0.7	7.7	0.5	There is no significant difference among the sample means
	Within samples	4				
	Total	5				
Zn	Between samples	3	1.95	4.1	0.2	There is no significant difference among the sample means
	Within samples	8				
	Total	11				

### 3.8. Pearson correlation of metals within Tena Adam samples

In this research, to correlate the effect of one metal concentration on the concentration of the other metal, the Pearson correlation matrices using correlation coefficient (r) for the samples were employed for metals determined in the samples and presented in Table 9. The results of Pearson correlation in Table 9 show that negative correlations are observed in the case of Ca

(with Cr, Cu and Cd) and Zn with Cd. These weak correlations indicate that the presence or absence of one metal affects the other metal in a lesser extent.

As indicated in Table 9 Ca with Zn; Cr (with Cu, Zn and Cd) and Cu (with Zn and Cd) show positive correlation. These strong correlations may arise from common anthropogenic or natural sources as well as from similarity in chemical properties. In general, relatively good positive correlation is observed between Cu and Zn. In contrast, weak correlation is observed between Ca and Cr.

Table 9: Pearson correlation matrices for metals in leaves of Tena Adam samples

Parameters	Ca	Cr	Cu	Zn	Cd
Ca	1				
Cr	-0.343	1			
Cu	-0.082	0.428	1		
Zn	0.530	0.078	0.555	1	
Cd	-0.061	0.292	0.185	-0.260	1

#### 4. Conclusion and recommendations

This study focused on determination of the levels of metals in Tena Adam collected from four selected around Addis Ababa such as Kality, Alem Bank, Holeta and Worabe and Tena Adam leaf samples collected from the above sample sites were analyzed for their contents of Ca, Cr, Cu, Zn, Cd, Ni and Pb using flame atomic absorption spectrophotometer.

The optimized wet acid digestion method for the analysis of Tena Adam was found efficient for all the metals and it was evaluated through the recovery test and a good percentage recovery ranges from 96.7 to 96.9% was obtained for all the metals identified.

The levels of metals in Tena Adam samples investigated in this research can be expressed in the following order: Ca (1872–3077 mg/kg) > Zn (44.5–63.9 mg/kg) > Cu (ND–10.8 mg/kg) > Cr (ND–3.1 mg/kg) > Cd (ND–2.9 mg/kg). The levels of metals in Tena Adam samples determined and assessed for its quality comparing with permissible limits given by various agencies and organization and levels are in safety baseline levels for human consumption. Since, the concentration of Ca was higher; we can recommend that peoples who have deficiency of Ca can use Tena Adam products in different ways.

The levels of Ni and Pb were found to be below the detection limits of the instrument. Cd was found in only one sample with small concentration and this is in a very good agreement with most of the results reported in other country. Since concentration of non-essential toxic heavy metals Ni and Pb were found below the detection limit of the instrument in this study and determined in other country, we recommend to do further investigations on determination of metals by using different analytical methods to minimize the gap.

Statistical analysis by using one-way ANOVA indicates that there is significant difference in mean concentration of Ca in Tena Adam in the four sampling sites. This may be attributed to differences in soil composition, use of different fertilizers, pesticides, etc. and there is no significant difference among the sample means at 95% confidence level in mean concentration for metals Cr, Cu and Zn, which could be attributed to having similar factors mentioned above.

Also, the results of Pearson correlation revealed that there is weak and/or moderate positive correlation between metals with each other.

Based on the results of this study, it can be concluded that Tena Adam contains higher levels of essential metals Ca and Zn. But highly toxic metals like Pb was below detection limit of the instrument and Cd was also below detection limit of the instrument except in the sample from one sample site. The content of most of toxic metals are less than and comparable with permitted limits set by WHO and safe for human consumption.

## 5. References

1. Moerman, D. **1998**. *Native American Ethnobotany*, Timber Press, Portland, USA.
2. Matu, E.N., **2011**. *Ruta chalepensis* L. In: Schmelzer, G.H. and Gurib-Fakim, A. (Editors). *PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale)*, Wageningen, Netherlands.
3. Asfaw, N.; Demissew, S. *Aromatic Plants of Ethiopia*. Shama Books: Addis Ababa, Ethiopia, **2009**; pp 162-164.
4. Massadeh, A.M.; Abdul-Wahab, O.; El-Rjoob, A.-W.O.; Mohammad, N. Al – Omari, M.N. Assessment of heavy metals in different parts of *Ruta chalepensis* L. Rutaceae medicinal plant and soil samples collected from three selected zones in Jordan, *Soil and Sediment Contamination: An International Journal*, **2016**, 25(6), 587-596, DOI: 10.1080/15320383.2016.1184618.
5. Fullas F. *Spice Plants in Ethiopia: Their Culinary and Medicinal Applications*. Reference Publications, Inc., Sioux City (USA), **2003**; pp 133-137.
6. Tena Adam. Available at: [http://www.behance.net/gallery/65063/Tena Adam](http://www.behance.net/gallery/65063/Tena-Adam); Accessed on 15 Oct **2017**.
7. Boulos L. *Medicinal Plants of North Africa*. Reference Publications, Inc., Michigan (USA), **1983**; p 158.
8. Derby, A.; Chandravanshi, B.S. Concentration levels of selected metals in the leaves of different species of thyme (*T. schimperi* and *T. vulgaris*) grown in Ethiopia. *Biological Trace Element Research*, **2011**, 141, 317-328.
9. The National Academy of Sciences (**1989**) *Trace elements: diet and health: implications for reducing chronic disease risk*. Available at [www.nap.edu/openbook.php?isbn=0309039940&page=367](http://www.nap.edu/openbook.php?isbn=0309039940&page=367). Accessed 16 Oct **2017**.
10. Health effects of exposure to heavy metals (**2009**). Available at [www.bewellrx.com/articles/health-effects-of-exposure-to-heavy-metals/](http://www.bewellrx.com/articles/health-effects-of-exposure-to-heavy-metals/). Accessed 16 Oct **2017**.
11. Goyer, R.; Golub, M.; Choudhury, H.; Hughes, M.; Kenyon, E.; Stifelman, M. (2009) Issue paper on *the human health effects of metals*. Available at

[www.epa.gov/raf/publications/pdfs/humanhealtheffects81904.pdf](http://www.epa.gov/raf/publications/pdfs/humanhealtheffects81904.pdf). Accessed 16 Oct 2017.

12. Hashmi, D.R.; Ismail, S.; Shaikh, G.H. Assessment of the level trace metals in commonly edible vegetables locally available in the market of Karachi city. *Pakistan Journal of Botany*, **2007**, *39*, 747–751.
13. Oyedele, D.J.; Asonugho, C.; Awotoye, O.O. Heavy metals in soils and accumulation by edible vegetables after phosphate fertilizer application. *Electronic Journal of Environmental, Agricultural Food Chemistry*, **2006**, *5*(4), 1446–1453.
14. Aiwonegbe, A.E.; Ikhuoria, E.U. Levels of selected heavy metals in some Nigerian vegetables. *Trends in Applied Sciences Research*, **2007**, *2*, 76–79.
15. Dagnaw, L.A.; Chandravanshi, B.S.; Zewge, F. Fluoride content of leafy vegetables grown by irrigation water and farmland soil in rift valley and non-rift valley areas of Ethiopia. **2017**, *50*, in press.
16. Nnorom, I.C.; Osibanjo, O.; Ogugua, K. Trace heavy metal levels of some Bouillon cubes and food condiments readily consumed in Nigeria. *Pakistan Journal of Nutrition*, **2007**, *6*, 122–127.
17. Okator, E.C.; Nwajei, G.E. Metal contents in water and aquatic plant (*Macarangahendelotic*) from Ororiner around Nigerian cement factory, Nkalagn. *Research Journal of Biological Sciences*, **2007**, *2*, 85–88.
18. Ghdam, R.; Khanki, J.; Yunesian, M.; Mahvi, A.H.; Nazmara, S. Trace metal contaminants in Iranian flat breads. *Journal of Agriculture and Social Sciences*, **2005**, *1*, 301–303.
19. Gulfraz, M.; Ahmed, T.; Afzal, H. Concentration levels of heavy and trace metals in the fish and Mangla lakes. *Online Journal of Biological Sciences*, **2001**, *1*, 414–416.
20. Debebe, A.; Chandravanshi, B.S.; Wondimu, T. Metallic nutrients in enset (*Ensete ventricosum*) corm cultivated in Wolliso and Wolkite towns in Ethiopia. *SINET: Ethiopian Journal of Science*, **2012**, *35*, 71–80.
21. Aregahegn, A.; Chandravanshi, B.S.; Atlabachew, M. Levels of major, minor and toxic metals in tubers and flour of *Dioscorea abyssinica* grown in Ethiopia. *African Journal of Food, Agriculture and Nutrition Development (AJFAND)*, **2013**, *13*, 7870–7887.

22. Akalu, Z.K.; Chandravanshi, B.S. Levels of essential and non-essential elements in raw and processed *Lupinus albus* L. (White lupin, Gibto) cultivated in Ethiopia. *African Journal of Food, Agriculture and Nutrition Development (AJFAND)*, **2014**, 14, 2015–2035.
23. Mekebo, D.; Chandravanshi, B.S. Levels of essential and non-essential metals in linseed (*Linum usitatissimum*) cultivated in Ethiopia. *Bulletin of the Chemical Society Ethiopia*, **2014**, 28, 349–362.
24. Ayele, E.; Urga, K.; Chandravanshi, B.S. Effect of cooking temperature on mineral content and anti-nutritional factors of yam and taro grown in southern Ethiopia. *International Journal of Food Engineering*, **2015**, 11, 371–382.
25. Abebe, A.; Chandravanshi, B.S.; Debebe, A. Assessment of essential and non-essential metals in popcorn and cornflake commercially available in Ethiopia. *Chemistry International*, **2017**, 3, 268–276.
26. Tegegne, B.; Chandravanshi, B.S. and Zewge F. Levels of selected metals in commercially available rice in Ethiopia. *International Food Research Journal*, **2017**, 24, 711–719.
27. Atlabachew, M.; Chandravanshi, B.S. Levels of major, minor and trace elements in commercially available enset (*Ensete ventricosum* (Welw.) Cheesman) food products (Kocho and Bulla) in Ethiopia. *Journal of Food Composition and Analysis*, **2008**, 21, 545–552.
28. Yohannes, W. Assessment of trace metals and physicochemical parameters of commercially available honey in Ethiopia. *Chemistry International*, **2018**, 4(2), 91-101.
29. Yemane, M.; Chandravanshi, B.S.; Wondimu, T. Levels of essential and non-essential metals in leaves of the tea plant (*Camellia sinensis* L.) and soils of Wushwush farms, Ethiopia. *Food Chemistry*, **2008**, 107, 1236–1243.
30. Weldegebriel, Y.; Chandravanshi, B.S.; Wondimu, T. Concentration levels of metals in vegetables grown in soils irrigated with river water in Addis Ababa, Ethiopia. *Ecotoxicological and Environmental Safety*, **2012**, 77, 57–63.
31. Kitata, R.B.; Chandravanshi, B.S. Concentration levels of major and trace metals in onion (*Allium cepa* L.) and irrigation water around Meki Town and Lake Ziway, Ethiopia. *Bulletin of the Chemical Society Ethiopia*, **2012**, 26, 27–42.

32. Gebre, A.; Chandravanshi, B.S. Levels of essential and non-essential metals in *Rhamnus prinoides* (Gesho) cultivated in Ethiopia. *Bulletin of the Chemical Society Ethiopia*, **2012**, 26, 329–342.
33. Mekonnen, K.N.; Ambushe, A.A.; Chandravanshi, B.S.; Redi-Abshiro, M.; McCrindle, R.I. Assessment of potentially toxic elements in Swiss chard and sediment of Akaki River, Ethiopia. *Toxicological and Environmental Chemistry*, **2014**, 96, 1501–1515.
34. Mehari, B.; Redi-Abshiro, M.; Chandravanshi, B.S.; Combrinck, S.; McCrindle, R. Characterization of the cultivation region of Ethiopian coffee by elemental analysis. *Analytical Letters*, **2016**, 49, 2474–2489.
35. Gure, A.; Chandravanshi, B.S.; Godeto, T.W. Metals in green coffee beans from major coffee-growing regions of Ethiopia. *Chemistry International*, **2017**, 3, 359–369.
36. Aregahegn, A.; Chandravanshi, B.S.; Atlabachew, M. Mineral contents of fruits of cactus pear (*Opuntia ficusindica*) grown in Ethiopia. *Acta Horticulture (ISHS)*, **2013**, 979, 117–126.
37. Yami, S.G.; Chandravanshi, B.S.; Wondimu, T.; Abuye, C. Assessment of selected nutrients and toxic metals in fruits, soils and irrigation waters of Awara Melka and Nura Era farms, Ethiopia. *SpringerPlus*, **2016**, 5:747. DOI 10.1186/s40064-016-2382-3.
38. Endalamaw, F.D.; Chandravanshi, B.S. Levels of major and trace elements in fennel (*Foeniculumvulgari* Mill.) fruits cultivated in Ethiopia. *Springer Plus*, **2015**, 4:5. DOI: 10.1186/2193-1801-4-5.
39. Wagesho, Y.; Chandravanshi, B.S. Levels of essential and non-essential metals in ginger (*Zingiberofficinale*) cultivated in Ethiopia. *Springer Plus*, **2015**, 4:107. DOI 10.1186/s40064-015-0899-5.
40. Mekassa, B.; Chandravanshi, B.S. Levels of selected essential and non-essential metals in seeds of korarima (*Aframomumcorrorima*) cultivated in Ethiopia. *Brazilian Journal of Food Technology*, **2015**, 18, 102–111.
41. Syume, M.; Chandravanshi, B.S. Nutrient composition of niger seed (*Guizotiaabyssinica* (L.f.) Cass.) cultivated in different parts of Ethiopia. *Bulletin of the Chemical Society Ethiopia*, **2015**, 29, 341–355.

42. Hagos, M.; Chandravanshi, B.S. Levels of essential and non-essential metals in fenugreek seed (*Trigonella Foenum-Graecum* L.) cultivated in different parts of Ethiopia. *Brazilian Journal of Food Technology*, **2016**, 19, e2015059.
43. Woldemariam, D.M.; Chandravanshi, B.S. Concentration levels of essential and non-essential elements in selected Ethiopian wines. *Bulletin of the Chemical Society Ethiopia*, **2011**, 25, 169–180.
44. Bekele, D.; Chandravanshi, B.S. Levels of essential and non-essential metals in Ethiopian ouzo. *SINET: Ethiopian Journal of Science*, **2012**, 35, 19–28.
45. Debebe, A.; Chandravanshi, B.S.; Redi-Abshiro, M. Assessment of essential and non-essential metals in Ethiopian traditional fermented alcoholic beverages. *Bulletin of the Chemical Society of Ethiopia*, **2017**, 31, 17–30.
46. Ashu, R.; Chandravanshi, B.S. Concentration levels of metals in commercially available Ethiopian roasted coffee powder and their infusions. *Bulletin of the Chemical Society Ethiopia*, **2011**, 25, 11–24.
47. Gebretsadik, D.W. and Chandravanshi, B.S. Levels of metals in commercially available Ethiopian black teas and their infusions. *Bulletin of the Chemical Society Ethiopia*, **2010**, 24, 339–349.
48. Wilson, L. *Minerals for life, A basic introduction*, The Center for Development, USA; **2012**.
49. Reilly, C. *Metal contamination of food*, Backwell Science Limited, USA; 2002; pp 81–194.
50. FAO/WHO. Joint FAO/WHO food standards programme CODEX Committee on Contaminants in Foods. Fifth Session, The Hague, the Netherlands, **2011**, pp 14–88.
51. Safty, A.E.; Khalid, E.; Mahgoub, S.H.; Neveen, A.M. Zinc toxicity among galvanization workers in the iron and steel industry. *Annals of the New York Academy of Sciences*, **2008**, 1140, 256–262.
52. Agency for toxic substances and disease registry (ATSDR) (2005). Toxicological profile for lead, cadmium, nickel. Atlanta, U.S. Department of Public Health and Services, Public Health Service. Available at: <http://www.atsdr.cdc.gov/toxprofiles>. Accessed on January **2017**.

53. Lars, J. Hazards of heavy metal contamination. *British Medical Bulletin*, **2003**, 68, 167–182.
54. Skoog, D.A., West, D.M., Holler, F.J.; Crouch, S.R. *Fundamentals of Analytical Chemistry*. 8th Ed., David Harries: USA, **2005**; pp 720–723.
55. Shah, A.; Niaz, A.; Ullah, N.; Rehman, A.; Akhlaq, M.; Zakir, M.; Khan, M.S. Comparative study of heavy metals in soil and selected medicinal plants. *Journal of Chemistry*, **2013**, 1–5.
56. Hassan, Z.; Anwar, Z.; Khattak, K.U; Islam, M.; Khan, R.U.; Khattak, J.Z.K. Civic pollution and its effect on water quality of river Toi at district Kohat, North-West Frontier Province (NWFP). *Research Journal of Environmental and Earth Science*, **2012**, 4 (3), 334–339.
57. World Health Organization (WHO). *Quality Control Methods for Medicinal Plant Materials*, Revised, World Health Organization, Geneva; **2005**.
58. Miller, J.N.; Miller, J.C. *Statistics and Chemometrics for Analytical Chemistry*, 6th Ed. Pearson Education Limited: Essex, England; **2010**; p 202.