ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

STUDIES OF ENSET (*ENSET VENTRICOSUM)*

FOR MAJOR, MINOR AND TRACE ELEMENTS

BY

AYALEW DEBEBE

JULY 2006

STUDIES OF ENSET / *ENSET VENTRICOSUM*/

FOR MAJOR, MINOR AND TRACE

ELEMENTS

A Graduate Project Submitted to
the School of Graduate Studies of
Addis Ababa University

In Partial Fulfillment of the Requirements
For the Degree of Master of Science
in Chemistry

AYALEW DEBEBE

JULY 2006

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Studies of Enset/ Enset Ventricosum/ for Major, Minor and Trace Elements

By

Ayalew Debebe

Department of chemistry

Approved by the examining board

Prof. B. S. Chandravanshi ----------

Advisor

Dr. Taddesse Wondimu ----------

Advisor

Prof. Hailemichael Alemu ----------

Examiner

Dr. Mesfin Redi ----------

Examiner

ACKNOWLEDGEMENTS

First I would like to express my deepest thanks to my God for his extra ordinary gifts, uninterrupted support and genuine guide.

I would like to express my deepest gratitude to my advisors Prof. B. S. Chandravanshi and Dr. Taddese Wondimu for their unreserved cooperation, incredible suggestions, supervision and remarks, appreciable encouragement and fatherly hood consultancy.

I am thankful to Dr. Gizachew Alemeyehu and Dr. Yonas Chebude who helped in different ways. I am grateful to Ato Hailegabrel Mengesha, W/o Woinshet Gebeyehu and W/t Amakelech for their cooperation when their help were necessary.

I am thankful to the Oromia Educational Bureau for their fully scholarship for my M.Sc. study. I also acknowledge School of Graduate of Addis Ababa University for the financial support on this study and Department of Chemistry of Addis Ababa University for laboratory facilities.

I am deeply indebted to my family for their love, encouragement and financial support through out the program.

Finally, I am also thankful to all of the persons who gave their cooperation when their helps were necessary

TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

ABSTRACT

TITLE: STUDIES OF ENSET (*ENSET VENTRICOSUM)* FOR MAJOR, MINOR AND TRACE ELEMENTS

By

Ayalew Debebe

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

This paper presents the concentration of K, Ca, Mg, Fe, Zn, Co, Cr, Pb, Cd, Cu, Ni, and Mn in *Enset ventricosum.* Samples of enset were collected from Wolliso (Oromiya region) and Wolkite (SNNPRG) in March 2006. Total acid (4 mL mixture of HNO_3 and $HClO_4$) digestion method was employed and determination was made by flame atomic absorption spectrometry. The percentage recoveries of the metals were in the range of 85% to 120%. The ranges of the concentrations (µg/g) of the metals on dry weight basis are: Ca 36,100 – 39,100; Mg 24,900 – 26,900; K 14,100 – 32,200; Zn 11.9 – 42.3; Cu 1.5 – 5.2, Co 2.8 – 10.5; Cr 5.8 – 7.6; Fe 18.2 – 54.4; Mn 2 ‾ 5; Ni 1 – 4 and Cd 0.6 ‾ 1.8.. The result obtained implies the plant is rich in calcium, magnesium and potassium and has small concentration of non-essential trace elements.

Key words: Enset, *Enset ventricosum*, Acid digestion, Major elements, Minor Elements, Trace Elements, Flame atomic absorption spectroscopy

.

CHAPTER ONE

INTRODUCTION

Eating the right foods is an important part of maintaining a healthy lifestyle. A single day's intake of nutrients may affect the body's organs only slightly but over years and decades the affects of unhealthy diet compounds into disease, shortened lifespan, and a less active lower productive life. The body is constantly restructuring, we replace millions of muscle, bone, skin, and blood cells every day. The nutrients that we intake today will become part of us tomorrow. The cliche, “we are what we eat,” is very true. Understanding this allows us to make decisions, which are healthy for today, and counteract the possibilities of disease in the future.

The nature and composition of what we eat as determines our future, enset as a food would have its own influential impact on million of peoples in Ethiopia. Therefore, studying the mineral composition of enset is very necessity.

1.1. Botanical Classification and Distribution of Enset

Enset belongs to the order *scitamineae*, the family *Musaceae*, and the genus *Enset*. Banana is in the same family as enset, but in the genus *Musa* [1- 3]. In 1947 about 20 species were recognized in the genus *Enset* but in 1962 *E. ventricosum* was placed in the genus *Enset* together with only 5 other species, namely: *E. gilletti*, *E. homblei*, *E. perrieri*, *E. glaucum* and *E. superbum*, and noted that *E. glaucum* and *E. ventricosum* perhaps should be treated as a single species [3, 4].

E. superbum and *E. glaucum* grow in Asia, whereas *E. ventricosum*, *E. gilletti* and *E. homblei* occur in sub-Saharan Africa and *E. perrieri* in Madagascar. *Enset ventricosum* grows wild also in a number of countries in central and eastern Africa including Congo, Mozambique, Uganda, Tanzania, and Zambia [4].

In Ethiopia, wild *E. ventricosum* occurs in the highlands (1100 – 3100 m above sea level) of the country. The distribution is very restricted; the wild populations grow mainly around the city of Bonga (Kefficho administrative region) and in a smaller area by the Omo River (Gamo-Gofa administrative region), in habitats ranging from dense forests to open shrub land, or along riverbanks. There are usually about 10 - 200 plants in a population. Although areas where wild enset grow are often not suitable for human settlements, human interference still prevails through the raising of domestic animals and cutting of trees and shrubs. By contrast, cultivated enset grows in a wider area comprising the central, south and southwestern parts of Ethiopia, but mainly at higher altitudes ranging from 1500 - 3100 m. Enset cultivation in Ethiopia may have started around the 15th century, south of Ghibe River. However, historical evidence regarding the origin and domestication of crops is seldom completely trustworthy [4].

In spite of availability of different species and extensive distribution of enset, it is only in Ethiopia that the plant has been domesticated. Wild enset propagates naturally by seed and domesticated enset vegitatively, and recognized more than 50 different variaties, colons, or landraces [4]. Domesticated enset is also classified taxonomically as *Enset ventricosum*. In Ethiopia, vernacular names of domesticated enset include enset (Amhara), asat (Gurage), weise (Kambata), and wassa (Sidama),

among others [1-6]. Enset plants grown in different parts of the world have similar physical appearance and the domesticated plants subjected to similar processing for consumption in Ethiopia.

1.2. Enset and Its Part

Enset looks like a large, thick single-stemmed banana plant. Both enset and banana have underground corm, a bundle of leaf sheaths that form the pseudo-stem, and large leaves. Enset, however, is usually larger than banana, with the largest plants up to 10 meters tall and with a pseudo-stem up to one meter in diameter. The leaves are more erect than those of a banana, have the shape of a lance head, and may be five meters long and nearly one meter wide. Banana plants normally form suckers or clusters of plants at the base, but enset doesn't.

The stem has three parts; the upper-most portion is the pseudo-stem, which is made of a system of tightly clasping leaf bases or leaf sheaths. The pseudo-stem may be two to three meters tall and contains an edible pulp and quality fiber. The under ground corm is really an enlarged lower portion of the stem. A short section of stem near the soil line, between the pseudo-stem and corm, is the true botanical stem [1]. Parts of the enset plant are shown in Figure 1.

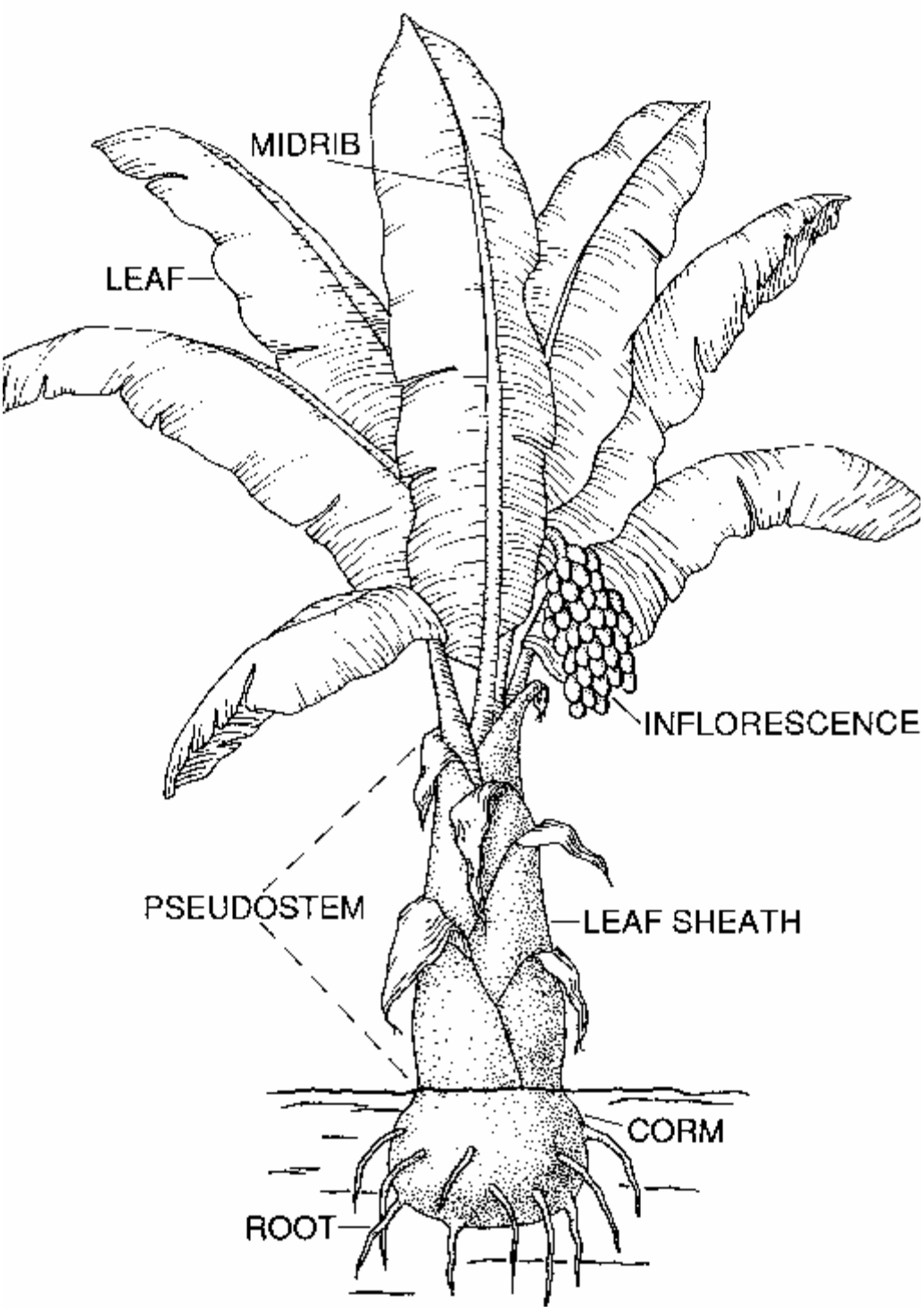


Figure 1. Parts of the enset plant.

1.3. Major Enset Growing Areas in Ethiopia

About 700 years ago, with the coming of domesticated livestock, a settled culture evolved around the use of enset products as the major food in Sidama and Gurage and co-stapled food in Gamo, Kambata, Hadiya, Wolayta, Gedio, Jemjem and Ari among other groups of the Southern Nations, Nationalities, and People's Regional Government (SNNPRG) [3, 7]. Enset is a perennial root crop that is only domesticated in Ethiopia [8].

The SNNPRG is the major enset growing regional state in Ethiopia. The zones in which clones were collected represent the major enset-based farming systems and agro-ecological zones in this state. In total enset is cultivated on an estimated 110 000 hectares in the SNNPR, which is two-thirds of the total area under enset in the country, the remainder being located in the neighboring states of Oromiya and Gambella [9] (Figure 2). Despite large variation in agro-ecological conditions among regions, only 4.8% of the total genetic variation was found between regions, whereas 95.2% was found within regions [7].

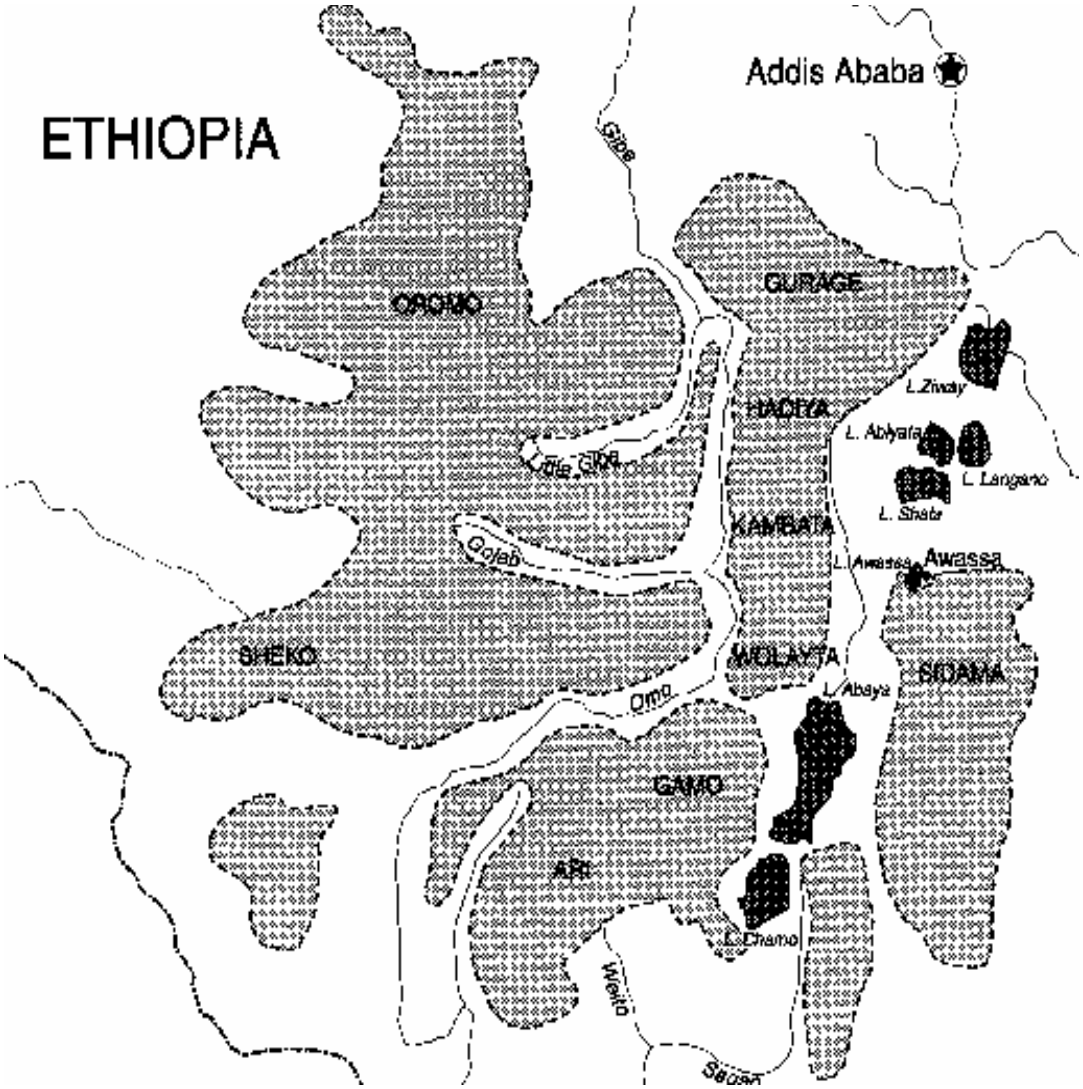


Figure 2. Major enset growing areas of Ethiopia.

1.4. Food and Non-food Uses of Enset

Enset is an important co-staple crop for >20% of the Ethiopian population and, the edible parts are formed by the pseudo-stem and the underground corm rather than by the fruit [10].

The major foods obtained from enset are kocho, bulla and amicho. Kocho is the bulk of the fermented starch obtained from the mixture of decorticated (scraped) leaf sheaths and created corm (underground stem base) [1]. Bulla is the small water-soluble starchy product that may be separated from kocho during processing by squeezing and decanting the liquid. Amicho is the fleshly inner portion of the enset corm, which may be cooked and eaten separately, tasting similar to potato [11, 12].

Enset provides fiber as a byproduct of decorticating the leaf-sheaths. In addition to this, parts of enset plants are used medicinally for humans and livestock to cure bone fractures, broken bone, childbirth problems (i.e. assisting to discharge the placenta), diarrhea and birth control (as an abottifacieant).

Fresh enset leaves are used as bread food wrappers, serving plates, and pit liners to store kocho for fermentation and future use. During enset harvesting enset leaves are used to line the ground where processing and fermentation take place.

The dried petioles and midribs are used as fuel, and to make mats and tying materials for house construction. The dried leaf sheaths are used as cattle feed and wrapping materials. The pulp from the dried leaf sheaths, petioles, and midribs is used as cleaning rags and brushes, baby cushions/diapers, and cooking pot stands [1, 6].

1.5. Mineral Contents of Enset

The mineral content of enset varies widely due to numerous factors, such as diseases, number of transplantation, sanitation, soil contamination and others [1-16]. The general chemical composition of enset as reported by Agren and Gibson [17, 18] indicates that the products are high in carbohydrate and low in protein and fat.

The elemental compositions in 100 g edible portion of bulla and kotcho are 82 mg Ca, 70 mg Ca, 3.7 mg Fe, and 7.9 mg Fe, respectively [17]. As enset is a plant that grows in most parts of Ethiopia, draught resistant plant and edible as staple and co-staple food studies on major, minor and trace elements are necessary to assess its role as source of essential nutrients.

The importance of optimal intakes of essential mineral elements to maintain peak health is widely recognized. Inadequate intake of mineral elements has been observed to be a major nutritional problem in our environment [15]. Since enset is the tree against hunger, the knowledge of its mineral concentration is of particular interest. However, information on the contents of major, minor and trace elements in enset is scarce in the literature.

1.6. Accumulation of Metals in Plant

In higher plants, the elements enter the root of the plant from the soil and move across the root, either through or between cells, until they reach specialized conducting cells of the vascular system. The cells of the vascular system are generally tubular, and connect end to end with similar tubular cells in a network reaching throughout the plant. In many instances trace elements move through biological membranes in the form of complexes of chelates with organic ligands [19].

The life processes of every living cell are conditioned by its content of trace elements. In some instances, this trace element control over life processes is dramatically reflected in human health, the success of agriculture in a region, or in shifts of the equilibrium between organisms in ecological competition.

The major reason of interest of studying the elements in plants is that plants transfer elements from the soil to animal and man. Thus, the mineral content of human and animal diet is frequently dependent upon the mineral content of plants.

1.7. Classification and Uses of Elements Used in Nutrition

Of the mineral elements phosphorous, calcium, magnesium, potassium, sulfur, sodium and chlorine are sometimes called the “macro-nutrients”. The first five are essential to both plants and animals and the last two to animals alone. Of the “micro-nutrients” minerals, iron, copper, zinc and manganese are necessary to both plant and animal life. Boron, molybdenum, silicon, questionably and possibly gallium are deemed necessary to plants but not to animals, while cobalt and iodine are necessary to animals but

have never been proved to be essential to plants. Aluminum and fluorine, although found in both plants and animals, are of questionable necessity for either [20].

Nicholas categorizes the essential nutrients for plants as follows: (a) the major elements including N, P, K, Mg, Ca, and S; (b) the minor elements, or trace elements, including Fe, Cu, Zn, Mn, B, Na, and Cl; and (c) an ultra micro group including Co, Mo, and V. On the other hand to the soil scientist, the trace elements may be all of the elements found in the soil and plants except for N, P, K, C, H, O, S, Mg, and Ca [19].

Potassium is absolutely essential for plant and can't be entirely replaced by any other element; Potassium is essential for the movement of sugars within the plant and for starch formation. It is necessary for the opening and closing of stomata by guard cells, controlling water use by the plant. It encourages root growth and helps build disease resistance. It is involved with photosynthesis and enzyme activity. It helps regulate metabolic activity and is involved in protein synthesis. Potassium promotes larger and better quality fruits and grains. In some plants, more potassium is required than any other nutrient. However, without sufficient available potassium in the soil, plants become reduced in vigor, are more susceptible to disease, and fail to develop normally [20, 21]. But, in animal it is used for helping muscles and nerves function properly, maintain the proper electrolyte and acid-base balance in the body, and help lower the risk of high blood pressure [22].

Calcium is one of the most important of the biological mineral elements and is necessary to the growth of all animals and all green plants, with the exception only of some of the lower algae. Calcium taken up by the plant is probably used for the neutralization and precipitation of the acids in the plant sap and

germination of seed [20]. It is used for human being to maintain healthy, strong bones, support proper functioning of nerves and muscles and for blood clot [23].

Copper is a catalyst for respiration and an activator of several enzymes. It is important for carbohydrate and protein synthesis. It may also play a role in carotene production. Symptoms of copper deficiency include: (a) stunted growth, (b) dieback of terminal shoots in trees, (c) poor pigmentation, (d) wilting and eventual death of leaf tips, and (e) formation of gum pockets around central pith in oranges [20]. Whereas, in animal it is used for helping the body utilize iron, reduce tissue damage caused by free radicals, maintain the health of bones and connective tissues, produce the pigment called melanin, keep the thyroid gland to function normally, and preserve the myelin sheath that surrounds and protects the nerves [24].

Zinc is a micro mineral needed, the symptoms associated with chlorosis and necrosis of the leaf, such as scant foliage, shortened internodes, reduced fruit and insufficient leave production are due to deficiency of zinc [20]. But, in animal mainly this element is used for regulation of genetic activity and balance of carbohydrate metabolism and blood sugar [25].

Magnesium is essential to both plants and animals but is usually present in plants in smaller amounts than calcium. It is relatively more abundant in the parts of plants concerned with vital processes, such as seeds and foliage, than in storage parts such as stems and roots. The only metal contained in chlorophyll is magnesium and when this element is deficient, chlorosis results [20]. Magnesium is usually referred to as a "macro-mineral" and is sometimes regarded as a "smoothie" mineral, since it has the ability to relax our muscles [26].

Manganese is present in all plants and animals and is an essential element in nutrition. In addition to the stunted growth and failure to produce, the absence or scarcity of manganese in plant results in a

disturbed carbohydrate metabolism, a decrease in the content of ash and in many plants a peculiar motted type of chlorosis [19]. Where as, in human it participates in many enzymes functions, which are responsible for the utilization of several key nutrients including biotin, thiamin, ascorbic acid, and choline [27].

Iron is an essential constituent of plant and animal protoplasm. Plants require iron in larger amounts than any other micronutrient. It is used in chlorophyll synthesis; in oxidation-reduction during respiration; and as a constituent of various enzymes and proteins. It also serves as an activator for nitrogen fixation. Symptoms of iron deficiency include: (a) inter veinal chlorosis—a yellowing of the leaves between the veins, (b) twig dieback and (c) death of entire limbs or plants. Although the amount of iron present in animals is small, it is of vital importance in the living process concerning with the transport of oxygen and the metabolism of food material within the cell [19, 20].

Exposure to cadmium can cause a number of harmful health effects. Eating food or drinking water with high levels of cadmium can severely irritate or bother the stomach and cause vomiting and diarrhea. Breathing high doses of cadmium can irritate and damage the lungs and can cause death. The lower and long-term exposure to cadmium through air or through diet can cause kidney damage. Although the damage is not life threatening, it can lead to the formation of kidney stones and affect the skeleton, which can be painful and debilitating. Lung damage has also been observed [28].

Lead is present in plant and soil in very small amount, and under ordinary circumstances the quantity consumed by animals has no effect on health. However, since lead is a cumulative poison, small quantities, which in them cause no harm, may become dangerous when taken constantly. For this reason, the accumulation of lead in the soil from the spray residues may be of sufficient magnitude to

raise the lead content of food plant to a point where their ingestion in quantity would be dangerous [18].

Chromium is not essential for plants but is essential for animals and may be deficient in human food. Because plants do not easily absorb chromium, regulations allow for higher concentrations of chromium in soils than occur naturally. It is required for carbohydrate metabolism as it potentiates the action of insulin. However, Chromium deficiency should also be considered as one of several nutritional factors that influence three recognized risk factors for public health problems of cardiovascular disease, impaired glucose tolerance, elevated circulating insulin levels, and elevated serum cholesterol. In addition, inadequate maternal chromium intake is known to cause premature birth and intra-uterine growth retardation and in males it has been linked with infertility [20].

Nickel is required for the enzyme urease to break down urea to liberate the nitrogen into a usable form for plants. Nickel is required for iron absorption. Seeds need nickel in order to germinate. Plants grown without additional nickel will gradually reach a deficient level at about the time they mature and begin reproductive growth. If nickel is deficient plants may fail to produce viable seeds [19, 20].

Cobalt is a core element of vitamin B12. Cobalt contributes to resistance against parasites and infection, in concert with other trace elements such as copper, zinc and iron. Cobalt is actually a plant "bio-stimulant," similar to molybdenum, because it is required by nitrogen-fixing bacteria, especially on the root nodules of legumes. It is required for nitrogen fixation in legumes and in root nodules of non-legumes. The demand for cobalt is much higher for nitrogen fixation than for ammonium nutrition. Deficient levels could result in nitrogen deficiency symptoms [19, 20].

1.8. Objectives

1.8.1. General objectives

The main objective of this project was to determine the levels of nutrients: major, minor and trace elements in enset.

1.8.2. Specific objectives

The specific objectives of this project are to:

1. develop suitable digestion method for enset samples;
2. determine the major, minor and trace elements (K, Ca, Fe, Zn, Mn, Cu, Pb, Mg, Co Ni, Cd and Cr) contents of the unprocessed edible part of enset.
3. list mineral composition table of enset;
4. report nutritional status of enset relative to major, minor, and trace metals;
5. suggest means of supplementing deficient essential elements in enset, if any.

CHAPTER TWO

EXPERIMENTAL

2.1. Instrumentation

BUCK SCIENTIFIC MODEL 210VGP (USA) atomic absorption spectrometer equipped with deuterium arc lamp background corrector for the determination of the analyte metals, Selecta model 2001241 (Spain) Oven for drying purpose, Thermo savant: model MODUL YOD- 230, 100 Colin drive, Holbrook NY 11741 was used for freeze drying the enset samples, Kjeldahl apparatus equipped with temperature regulated mantles was used as a hot plate for digesting enset and Metal sieve: Weite 0.5 mm diameter, Ser. no: 811624, Retscn 5657 HAAN. W. Germany was employed for sieving the grinded enset samples, which were done using pestle and mortar.

2.2. Chemicals and Reagents

All dissolutions were prepared using analytical grade pure reagents: nitric acid (70% trace metal grade; spectrosol BDH, England), perchloric acid (70% Analar BDH, England), and lanthanum nitrate hydrate (98% Aldrich, Muwaukee, USA) were used for dissolution of samples and controlling ionization interference in AAS measurements. Stock standard solutions containing 1000 mg element/L, in 2% HNO_3, of the metals K, Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr, Ni, Pb, and Cd (Buck scientific PURO-Graphic^{tm}) were used for preparing a series of calibration standards. Dilute calibration solutions were made using deionized water immediately before use.

2.3. Procedures

2.3.1. Collection and preservation of enset samples

Unprocessed enset samples were collected from four different plants, two from Wolliso (Oromiya region) and the other two from Wolkite (SNNPRG). The relative location of sampling sites is shown in Figure 3. The geographic location and distance from the capital Addis Ababa are given in Table 1. All the samples were collected during March 5-6, 2006, some physical characteristics recorded (Table 2), and transported in sealed plastic bags, which were washed with detergents, rinsed with deionised water and dried completely prior to use. Each enset sample was frozen at –20 ^{0}C and then freeze-dried using Thermo savant freeze dryer. The freeze dried samples were finely ground in a mortar using a pestle and sieved through 0.5 mm sieve.

Table 1. Geographic location and distance from the capital Addis Ababa of sampling sites.

Site	Latitude	Longitude	Distance (km)
Wolliso	8^0 31'N	37^0 58' E	120
Wolkite	8^0 15' N	37^0 47' E	155

Table 2. Some characteristics of enset samples collected from Wolliso and Wolkite.

	Sample number			
Characteristics	Wolliso-1	Wolliso-2	Wolkite-1	Wolkite-2
Height (including the leaf), m	4	5	4.5	6.15
Circumference, m	1.5	1	1	2.10

Age, years	4	6	4	4

Figure 3. Map of Ethiopia indicating the relative location of sampling sites.

2.3.2. Contamination control

Washing procedures for sample containers, the digestion vessels, glassware for the standards and sample tubes for metal determinations followed these procedures. In brief, sample containers and other glassware were cleaned with metal free non-ionic detergent solution, rinsed three times with tap water, and rinsed three times with metal-free deionized water. All sample containers were rinsed again with deionized water prior to use. Blanks, consisting of deionized water and reagents were subjected to a similar sample preparation and analytical procedure. Tips for micropipettes (model No. MT II, capacity 1000 μL) were subjected to the same decontamination procedure. All the analytical procedures were carried out under an environmental hood to avoid dust contamination.

2.3.3. Moisture determination

Accurately weighed aliquots (120 g) of well-homogenized enset samples were placed in (Thermo savant) freeze-drying bottles. The water content of enset was determined by taking weight difference before and after putting the samples in freeze drying unit. The percent loss in weight of samples was calculated as moisture content.

2.3.4. Digestion of enset samples

An accurately weighed aliquot (0.5 g) of powdered enset was placed in 100 mL flat-bottomed flask and 2.0 mL 70% HNO_3 and 2.0 mL 70% $HClO_4$ were added to it. The mixture was heated under reflux for 2.5 h on a micro kjeldahl flask digestion unit (setting the temperature dial at 8) cooled at

room temperature for 5 min, and then diluted partially up to about 40 mL after that it was filtered. The final dilution contained 1% (w/v) lanthanum solution to release calcium and magnesium from their respective phosphates, which are refractory in the determinations by flame atomic absorption spectrometer [29].

The cooled digest and its washing were drawn off and transferred in to a 50 mL volumetric flask after partially diluted up to 40 mL and diluted to the mark with deionized water. The digests were prepared in triplicate for each sample.

A reagent blank containing the same reagents (2.0 mL 70% HNO_3 and 2.0 mL 70% $HClO_4$) and subjected to the same digestion procedure prepared for correcting effect of the blank. Hence reagent blank was prepared for each digested samples.

2.3.5. Determination of major, minor and trace metals

BUCK SCIENTIFIC MODEL 210VGP atomic absorption spectrometer equipped with deuterium arc background corrector and standard air-acetylene burner system was used. A hollow cathode lamp for each metal (K, Ca, Mg, Fe, Zn, Mn, Cu, Co, Cr, Ni, Pb, and Cd) operated at the manufacturer's recommended conditions were used (Table 3). The acetylene and airflow rates were managed to ensure suitable flame conditions. The burner height was adjusted for optimum sensitivity and the nebulizer uptake rate was optimized (6-7 mL/min) to provide optimum absorbance signal in conventional sample aspiration. The spectrometer was operated with the time constant of 0.2 s.

Table 3. Instrument operating conditions for the determination of metals by atomic absorption spectrometer.

No	Element	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Detection limit (mg/L)	Flame type/color
1	Ca	422.7	0.7	2.0	0.01	
2	Cd	228.9	0.7	2.0	0.005	Lean/blue
3	Cr	357.9	0.7	2.0	0.05	Rich/yellow
4	Co	240.7	0.2	4.5	0.05	Lean/blue
5	Cu	324.8	0.7	1.5	0.02	Lean/blue
6	Fe	248.3	0.2	7.0	0.03	Lean/blue
7	K	766.5	0.7	2.0	0.01	Lean/blue
8	Mg	285.2	0.5	4.0	0.001	Lean/blue
9	Mn	279.5	0.2	5.0	0.01	Lean/blue
10	Ni	232.0	0.2	4.0	0.04	Lean/blue
11	Pb	217.0	1.0	5.0	0.1	Lean/blue
12	Zn	213.9	0.7	2.0	0.005	Lean/blue

2.3.6. Digestion of enset samples spiked with standard metals solutions

For the determination of the efficiency of the developed optimized digestion procedure used for the analysis of enset sample, fixed volumes of standard solutions of the metals were added. That is, in spiking 0.1 mL of each element was added from 100 mg/L standard solution of the corresponding metal. However, for cadmium the added 0.1 mL was taken from 10 mg/L of its standard solution. This was done in to two parts; in the first spiking the standards of six elements (Ca, Mg, K, Zn, Cd and Cr) and in the second case another six elements (Fe, Co, Cu, Ni, Pb and Mn) were added. Therefore, for all elements 0.2 mg/L but for cadmium 0.02 mg/L was added for spike. Then, they were digested and diluted to 50 mL volumetric flask based on the procedure of the developed digestion method for enset

CHAPTER THREE

RESULT AND DISCUSSION

3.1. Site Selection for Sampling Enset

Similar to some enset growing areas both Wolkite and Wolliso sites are in the regions where enset is the staple food. The two sites almost have the same weather condition that can avoid the variation based on it. In addition the two sites are more preferable to compare between regions. This enables to have information in which region the plant has more mineral content. Further more; the two sites are so nearer to capital Addis Ababa. Thus, they are convenient to transport the samples to the area where they are processed.

3.2. Selection of Appropriate Part for Analysis

Corm was selected for sample because it is a common part of the plant for: (a) different tribes in SNNPRG that used enset as a source of food, (b) different aged plants that can be harvested, (c) types of foods produced from enset and (d) since *Enset ventricosum* (welw) is grouped in tuber plants.

3.3. Sample Preparation

The wet sample of enset was taken from refrigerator and chopped to small pieces with plastic knife. The measured mass of the wet sample was added to freeze drying flask for moisture content determination. The wet sample was dried in freeze-drying unit until constant mass was obtained. The

dried sample of enset was grounded in a mortar using pestle and kept in tightly tied plastic bag for the analysis.

3.4. Moisture Determination

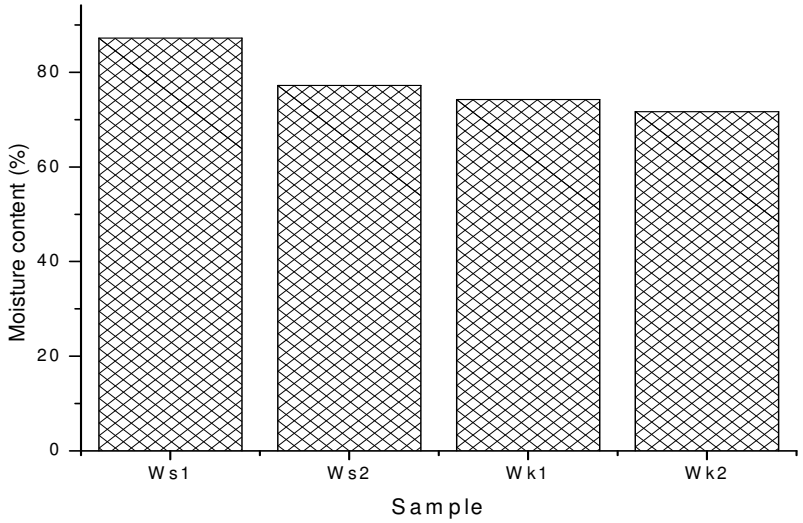
The dry plant sample, rather than the wet weights, provides a more accurate information about the metal load since water content in plants varies with species, age, condition, etc. The water content of enset samples was determined by monitoring the weight loss of the wet samples until constant dry mass was obtained in freeze-drying unit. The moisture content was then calculated as percent loss of mass. The percent loss calculated varied from 71.7% to 87.2% as can be seen from Table 4 and Figure 4.

The variation in water content of enset sample was found to be highly dependent on the time interval of introducing the sample into freeze-drying unit after the samples were collected. The samples which were put in the freeze-dry unit after a short time in the refrigerator from the time of harvesting (the time of sample collecting) (Wk_1 and Ws_1) have good agreement with the moisture content of unprocessed enset [12, 30]. Whereas, the other samples (Wk_2 and Ws_2) which were kept for additional three weeks time in the refrigerator than the first two have good agreement with moisture content of fermented enset [30]. The samples from Wolliso have high amount of moisture content than the samples from Wolkite's. This might be due to species difference either age or type and the type of soil.

$$\% \text{ moisture content} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100$$

Table 4. Percent moisture content of enset samples.

Characteristics	Sample number			
	Wolliso-1	Wolliso-2	Wolkite-1	Wolkite-2
Moisture content (%)	87.2	77.2	74.2	71.7



Ws1= Wolliso -1, Ws2 = Wolliso- 2, Wk1=Wolkite-1, Wk2 = Wolkite-2

Figure 4. Moisture content (%) of corm (edible part) of enset samples.

3.5. The Digestion Procedure

A series of digestion procedures involving changes in reagents volumes, reagent compositions and digestion time were tested during the initial stage of digestion. Accordingly, eight procedures were tested for the digestion of freeze-dried enset samples.

The optimum procedure was selected depending on: minimum reagent volume consumption, digestion time, obtaining clear solution, easy of complexity and simplicity.

The optimal procedure chosen on the basis of these criteria required 2.5 h for complete digestion of 0.5 g freeze dried enset sample with 2.0 mL 70% HNO_3 and 2.0 mL 70% $HClO_4$ as given in Table 5.

Table 5. Digestion procedures tested for decomposing enset ventricosum sample.

Method	Amount of sample	Phase	Reagent	Process	Time	Result before and after filtration and dilution
1	0.5 g	1	2 mL 70% HNO_3 1 mL 70% $HClO_4$	Reflux	2.5 h	pale yellow solution

		In between				
		2				
		Final		Cooling	5 min	
2	0.5 g	1	2 mL 70% HNO_3 2 mL 70% $HClO_4$	Reflux	2 h	Slight pale yellow solution
		In between				
		2				
		Final		Cooling	5 min	
3	0.5 g	1	3 mL 70% HNO_3 1 mL 70% $HClO_4$	Reflux	2.5 h	Slight pale yellow solution
		In between				
		2				
		Final		Cooling	5 min	
4	0.5 g	1	2 mL 70% HNO_3 2 mL 70% $HClO_4$	Reflux	2 .5 h	Clear solution
		In between				
		2				
		Final		Cooling	5 min	
5	0.5 g	1	3.5 mL 70% HNO_3 1.5 mL 70% $HClO_4$	Reflux	2.5 h	Clear solution
		In between				
		2				
		Final		Cooling	5 min	
6	0.5 g	1	2.5 mL 70% HNO_3 1 mL 70% $HClO_4$	Reflux	2.5 h	Cloudy Solution
		In between		Cooling	3 min	
		2	1 mL 70% HNO_3 0.5 mL 70% $HClO_4$	Reflux	2 h	
		Final		Cooling	5 min	

7	0.5 g	1	2.5 mL 70% HNO_3 1 mL 70% $HClO_4$	Reflux	2.5 h	Clear solution
		In between		Cooling	3 min	
		2	1 mL 70% HNO_3 0.5 mL 70% $HClO_4$	Reflux	2 h	
		Final		Cooling	5 min	
8	0.5 g	1	2 mL 70% HNO_3 1.5 mL 70% $HClO_4$	Reflux	2.5 h	Clear solution
		In between		Cooling	3 min	
		2	1 mL 70% HNO_3 0.5 mL 70% $HClO_4$	Reflux	2 h	
		Final		Cooling	5 min	

Note. The attempts 1-6 were done using temperature setting at dial 8 but for 7 and 8 the temperature setting was at dial 9.

The common drawbacks of other tested procedures were their higher chemical composition, longer duration for complete digestion and observation of colors in some of the tested procedures.

Due to the use of acid digestion procedure, the acid used for the digestion might add some amount of metals to the samples to be analyzed since most of the reagents have metals as impurities. So, it is always necessary to prepare acid/reagent blanks for each type of digestion performed. Thus, reagent blanks were prepared with the same reagents and subjected to the same digestion procedure as the samples. Therefore, reagent blank was prepared for each digested samples.

3.6. Calibration of the Instrument

Calibration of the instrument (BUCK SCIENTIFIC MODEL 210VGP) was done by the standards prepared before the determinations were done. The standards were prepared from the 100 mg/L of the elements which were prepared prior by taking 1 mL from the stock standard solutions containing 1000 mg element/L, in 2% HNO_3, of the metals. The correlation coefficients of the elements were determined using prepared standards versus their corresponding absorbances. The standards and their corresponding correlation coefficients are given in the Table 6.

Table 6. Concentrations of the standard solutions used to establish calibration graphs for the determination of metals in the enset samples and their correlation coefficients based on their sequential order.

Element	Standards in mg/ L	Correlation coefficient
Ca, Mg, K	0.5, 1.0, 2.0, 4.0	0.96, 0.99, 0.99
Cr, Co, Cu, Zn, Fe, Mn, Ni, Pb	0.01, 0.05, 0.1, 0.5	0.96, 0.98, 0.97, 0.96, 0.94, 0.99, 0.98, 0.97
Cd	0.005, 0.01, 0.02, 0.04	0.95

3.7. Method Detection and Quantitation Limit

A method detection limit (MDL) is the minimum concentration of a substance that can be measured [31, 32]. The determinative procedures involve digesting and diluting the blank solutions and then analyzing the concentration of each element of the samples in accordance with the optimized procedure. Then, the standard deviation of the triplicate readings of five blanks was calculated. The standard deviation was multiplied by three to give MDL.

Table 7. Method detection limit (in µg/g) of elements ($3\delta_{blank}$, n = 5) determined using the blank solution.

Element	Zn	Cu	Co	Cd	Cr	Ca	Fe	Mn	Mg	Ni	Pb	K
Detection limit	6.9	1.2	2.7	2.4	1.5	96	15	1.5	36	6	7.2	-

Note: The instrument detection limit and the lowest standard concentration of potassium is 0.01 and 0.5 mg/L respectively.

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. A PQL (Practical Quantitation Limit) is normally 3 to 10 times the MDL and is considered the lowest concentration that can be accurately measured, as opposed to just detected [31, 32].

Table 8. Quantitation limit (in µg/g) of elements ($10\delta_{blank}$, n = 5) determined using the blank solution.

Element	Zn	Cu	Co	Cd	Cr	Ca	Fe	Mn	Mg	Ni	Pb	K
Quantitation limit	23	3.9	9	8	5	320	50	5	120	20	24	-

Note: The instrument detection limit and the lowest standard concentration of potassium is 0.01 and 0.5 mg/L respectively.

3.8. Determination of Major, Minor and Trace Elements

The pseudostem and corm constitute the most important source of food from enset for humans. Foods derived from enset are protein - poor but contribute large amounts of energy [30]. Enset is a major source of carbohydrate, calcium and iron [2-3, 16-17]. Moreover, it constitutes these essential mineral nutrients (Table 9). For potassium, calcium and magnesium determination the digested samples were diluted 100-times with deionized water before analysis.

Table 9. Mean concentration of major and minor mineral nutrients (X ± SD, n = 3) in enset samples in Wolkite and Wolliso.

Element	Concentration in µg/g			
	Wolliso-1	Wolliso-2	Wolkite-1	Wolkite-2
Calcium	39,100 ± 5,900	39,000 ± 3,500	36,700 ± 2,100	36,100 ± 2,400
Magnesium	26,900 ± 600	24,900 ± 230	25,600 ± 920	25,500 ± 880
Potassium	32,200 ± 6,300	26,200 ± 2,000	17,700 ± 6,100	14,100 ± 1,200
Iron	18.2 ± 5.5	54.4 ± 9.5	29.7 ± 5.8	21.8 ± 2.5
Zinc	11.8 ± 8.9	42.3 ± 8.1	18.2 ± 6.6	21.8 ± 1.1
Copper	5.1 ± 1.5	5.2 ± 1.0	1.5 ± 1.3	2.6 ± 0.3
Cobalt	10.5 ± 0.6	7.9 ± 1.1	5.7 ± 2.8	2.8 ± 1.1
Chromium	7.6 ± 3.0	6.3 ± 2.6	6.9 ± 0.4	5.8 ± 1.6
Cadmium	1.8 ± 0.8*	0.60 ± 0.06*	1.8 ± 1.0*	0.60 ± 0.07*
Manganese	2.00 ± 0.06	4.0 ± 1.0	5.0 ± 0.6	4.0 ± 0.6
Lead	*	*	*	15.3 ± 0.6
Nickel	4.0 ± 3.5*	3.5 ± 4.0*	4.0 ± 0.7*	1.0 ± 0.1*

Note: *Result found, with a statement of its uncertainty; the same status has been described as not detected in other literatures [33]

X is the mean value of the triplicate analysis with triplicate readings of each sample.

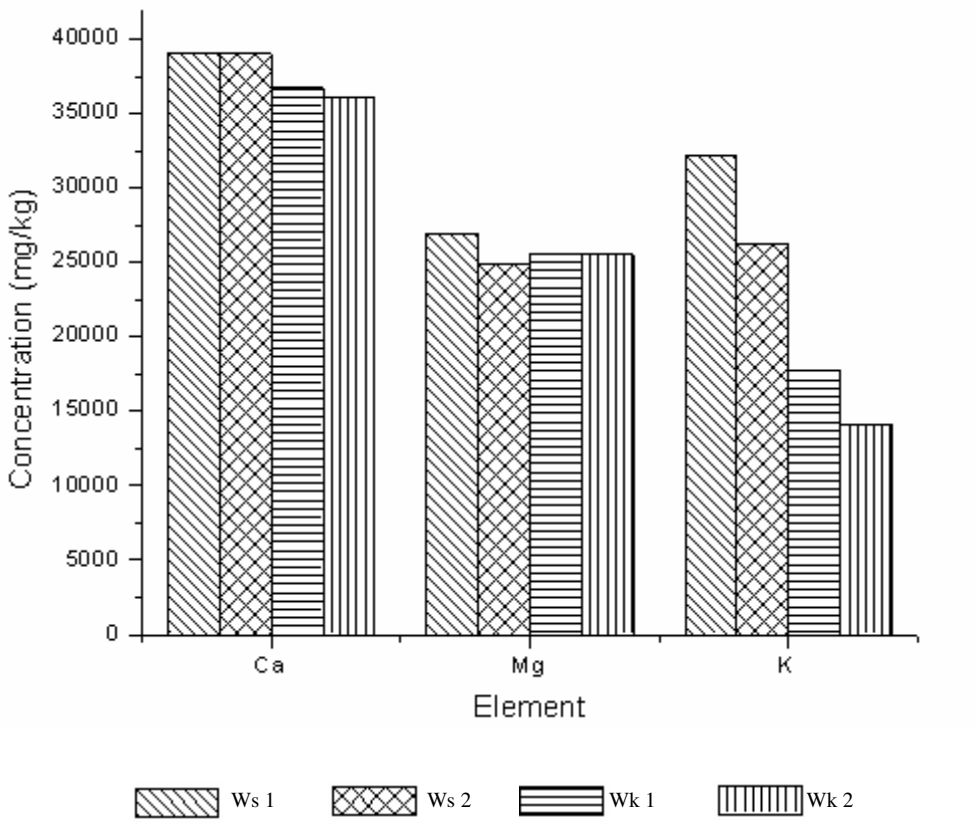


Figure 5. Relative concentration of macronutrients in the enset samples.

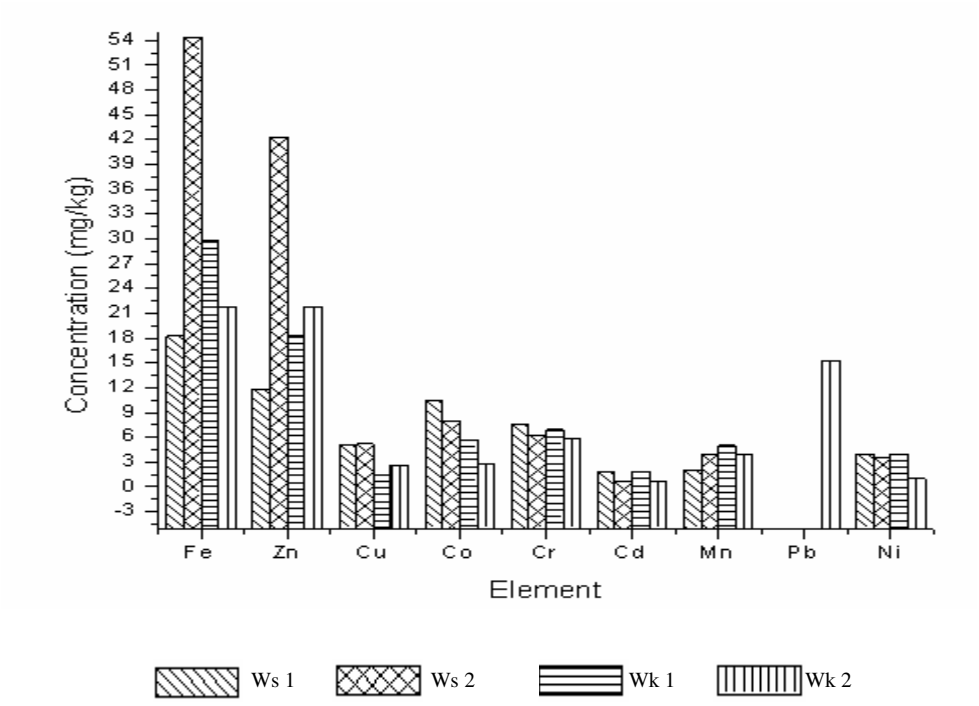


Figure 6. Relative concentration of micronutrients in the enset samples.

Distribution of selected metals in enset

The distributions of selected metals concentrations in the enset samples are given in Table 9. The concentration of the metals (in μg/g) in the samples are: Ca 36,100 – 39,100; Mg 24,900 – 26,900; K 14,100 – 32,200; Zn 11.9 – 42.3; Cu 1.5 – 5.2, Co 2.9 – 10.5; Cr 5.8 – 7.6; Fe 18.2 – 54.4; Mn 2 – 5; Ni 1 – 4 and Cd 0.6 – 1.8.

Area specific distribution

When considering the two sites the distance between the two is not as such large and as a result of this there might be some expectation on which the sites would be able to have identical comparison parameters like weather conditions and others. However, among the metals which were determined using the optimized method almost more than a three fourth of them are found in high concentration in Wolliso relative to Wolkite.

Plant specific distribution pattern

The distribution and accumulation of metals in plant depends on many factors: species, age, root distribution of the plant, physical and chemical nature of the soil, proportion and distribution of the elements, method of cultivation, and climatic conditions [19].

In plant 1 from Wolkite the concentration of the selected metals was lowest for copper and highest for calcium. The trend is Ca > Mg > K > Fe > Zn > Cr > Co > Mn > Ni > Cd > Cu (Table 9).

In plant 2 from Wolkite the concentration of the cited metals was lowest for cadmium and highest for calcium. The descending order is Ca > K > Mg > Fe > Zn > Pb > Cr > Mn > Co > Cu > Ni > Cd (Table 9).

Likewise, in the cases of plants 1 and 2 from Wolliso, the highest concentration was observed for calcium and the lowest for cadmium. The sequential order for the highest to least was Ca > Mg > K > Fe > Zn > Co > Cr > Cu > Ni > Mn > Cd (Table 9).

A high accumulation of calcium, magnesium and potassium relative to the others is due to their higher natural abundance in the soil [21]. Whereas, in all the four samples cadmium concentration was the least, this may be due to low natural abundance in the soil, low absorption constant and liable complexion with organic matter in the soil.

In relation to this from the micronutrients the concentrations of iron and zinc are higher than the rest. This is, as a result of plant requires high amount of iron, abundance of iron in the soil and the pH of the soil ranges from 5.6 to 7.3 [3, 21]. For zinc the reason is due to acidic to slightly basic pH of the soil and high abundance of zinc in the region [3, 21].

In general, from the selected elements, cobalt, chromium, potassium, calcium, magnesium, nickel and cadmium in plant 1 and zinc, copper, manganese and iron in plant 2 from Wolliso are found in the highest concentration. This may be due to the age, matrix, the concentration of the corresponding elements in the soil and the nature of the interaction between metals and chelating agents and so on.

Metal specific distribution pattern

Knowing the distribution of the metals in the parts of the plant is very selective and essential to:

- supplement if there is a shortage of nutrients relative to required amounts, which would be obtained from the edible part of the plant;
- recommend it as a source of certain nutrients;
- safe the health of the consumer from the effect of accumulation of non essential nutrients;
- enhance or stop the use of the plant as a food;
- select the plant from others based on its nutritional value; and
- show what is required for the plant based on cultivation to be rich in the needed mineral nutrients and so on.

Calcium, Magnesium and Potassium

All of them as they are macro elements are found in large amount than the rest [21]. The distribution patterns for calcium, magnesium and potassium respectively are: $Ws_1 > Ws_2 > Wk_1 > Wk_2$, $Ws_1 > Wk_1 > Wk_2 > Ws_2$, and $Ws_1 > Ws_2 > Wk_1 > Wk_2$ (Table 9, Figure 5).

In some plants, more potassium is required than any other nutrients [21]. But, for this case it is found in the third rank. Where as, from all of the nutrients, calcium is the leading. This may be due to its high abundance as a result it is held by soil particles more tightly than potassium, magnesium and other exchangeable cations and parent materials from which soil are formed, immobility, major constituent of cell wall and its concentration usually decreases from the top to the bottom of the plant [21, 34]. But, the limited availability of potassium in the soil, mobility of the nutrient, the decrease in concentration of the element from top to bottom [21, 35] and unfamiliarity of using commercial fertilizers for the cultivation of the plant are the major reasons for least amount of potassium from the rest macro nutrients. Similarly, due to mobility, less concentration at the bottom of the plant and the major constituent of chlorophyll magnesium is the second next to calcium in its concentration [21, 36].

Generally, since the soils, which have been used for cultivating the plant, are highly fertilized with manure and organic residues, they were high in available potassium, calcium and magnesium [37]. Hence, the plant has high amount of these metals.

From the distribution patterns in all of them, Ws_1 has the highest but Wk_2 has the least amount of these elements except for magnesium. For calcium and potassium, both plants, which were collected from Wolliso, have higher amounts. Whereas, which were collected from Wolkite have lower amounts. These pattern lead to a conclusion, the result may be due to soil, the type of species and others.

In cases of magnesium and potassium, the patterns reveal that Ws_1 has high but Ws_2 has low concentration. Therefore, this variation may not be based on soil, weather conditions and so on. But, it is due to maturity. Thus, unlike calcium the concentrations of both elements decrease in plant tissue as maturity increases.

Zinc, Copper and Iron

Among the plants, Ws_2 accumulates high amount of zinc, copper and iron while the lowest is for Ws_1 except in the case of copper. The distribution patterns of zinc, iron and copper respectively are: $Ws_2 > Wk_2 > Wk_1 > Ws_1$, $Ws_2 > Wk_1 > Wk_2 > Ws_1$ and $Ws_2 > Ws_1 > Wk_2 > Wk_1$ (Table 9). This indicates the species Ws_2 has high uptake of minerals from the soil as the result of the age, matrix and others (Table 2). But, Ws_1 for zinc and iron has the lowest amount. Since, plants, Ws_1 and Ws_2 were grown around the same areas, where physical and chemical nature of the soil, proportion and distribution of the elements, method of cultivation and other parameters are expected to be the same; the variation may be due to the matrix they have. Whereas for the case of copper, Wk_1 has the lowest amount while Ws_1 and Ws_2 have the highest amount. Since, the two sites do not have a devastating climatic variation; the variation should be as a result of physical and chemical nature of the soil.

Cobalt, Cadmium and Chromium

Among the plants, Ws_1 accumulates high amount cadmium, chromium and cobalt while the lowest is for Wk_2. The distribution patterns for cadmium, chromium, and cobalt respectively are $Ws_1 = Wk_1 > Ws_2 = Wk_2$, $Ws_1 > Wk_1 > Ws_2 > Wk_2$, and $Ws_1 > Ws_2 > Wk_1 > Wk_2$ (Table 9). In every case except Ws_1, which has the highest accumulation, in all patterns there are no identical sequential orders.

Therefore, for cadmium and chromium the variation may be due to the differences in the uptake potential of the plants. But, for cobalt both plants from Wolliso has the leading ranks. This should be as a result of variation in species, soil type, cultivation and others.

In case of cadmium, the other parameters like soil type and variation in cultivation are not observed. Instead, the parameter that can be seen in the pattern is the difference in the type of species. Therefore, Ws_1 and Wk_1 were the two plants, which accumulate more cadmium than Ws_2 and Wk_2.

Nickel, Lead and Manganese

Among the plants, Wk_1 has higher amount of manganese and nickel while the lowest is Ws_2 for manganese and Wk_2 for nickel. The distribution patterns for manganese and nickel respectively are: $Wk_1 > Wk_2 = Ws_1 > Ws_2$ and $Wk_1 = Ws_1 > Ws_2 > Wk_2$. In both cases except Wk_1 has the highest accumulation, in others there is no identical pattern. The reasons for this distribution pattern of manganese in the samples are may be due to the variation in type of species, soil and others. But, for nickel according to the pattern, since the two plants, Wk_1 and Ws_1 have high amount of nickel, the result might be due to high uptake potential of the plants. The concentration of lead in most plants is uncertain as it is given in Table 9 and Figure 6. Even if the calibration was so good, there were fluctuations of absorptions while the analysis was done.

3.9. Recovery Test

The validity of the analytical procedure can be checked by: (a) analyzing a series of samples using two different methods, e.g. the new method and a standard method; (b) analyzing reference or certified reference material; (c) performing standard methods of analysis; (d) comparative analysis with the other experienced laboratories; and (e) analyzing synthetic samples and spiked samples [38].

The efficiency of the optimized method was checked by digesting the 0.5 g enset samples spiked with standard solutions of the metals. In the first experiment 0.5 g enset sample was spiked with six different metals standards (0.1 mL of 100 mg/L of Ca, K, Mg, Zn, and Cr and 0.1 mL of 10 mg/L of Cd) before digestion. In the second experiment 0.5 g enset sample was spiked with the rest six different metals standards (0.1 mL of 100 mg/L of Co, Fe, Ni, Cu, Pb and Mn) before digestion. After this the sample was digested according to the optimized method. Then, the digests were transferred in to 50 mL volumetric flask and diluted to the mark with deionized water. Finally, the solutions were analyzed for each element that was spiked with atomic absorption spectrophotometer.

$$\% \text{ Recovery} = \frac{(\text{Amount after spike} - \text{Amount before spike})}{\text{Amount added}} \times 100$$

Table 10. Percentage recovery of the method.

Element	Added (mg/L)	Difference (mg/L)	% Recovery
Cadmium	0.02	0.017 ± 0.001	85.0 ± 0.1
Cobalt	0.2	0.172 ± 0.020	86 ± 2
Chromium	0.2	0.185 ± 0.002	92.5 ± 0.2

Copper	0.2	0.20 ± 0.02	100 ± 2
Iron	0.2	0.19 ± 0.06	95 ± 6
Manganese	0.2	0.20 ± 0.02	100 ± 2
Nickel	0.2	0.17 ± 0.02	85 ± 2
Lead	0.2	0.24 ± 0.04	120 ± 4
Zinc	0.2	0.205 ± 0.070	102.5 ± 7
Calcium	0.2	0.21 ± 0.08	105 ± 8
Potassium	0.2	0.17 ± 0.08	85 ± 8
Magnesium	0.2	0.17 ± 0.08	85 ± 8

The recovery of the method ranged from 85 % to 120 %. The results are displayed in Table 10. Good recoveries were obtained for most of the metals, except for lead, especially for manganese, copper, zinc, iron and chromium. The good recovery for most metals indicates the digestion method used for sample preparation is precise and reliable. Thus, the obtained recovery value for lead has direct relationship with the concentration reading of the analyte. That is, as the recovery value became far away from 100, the uncertainty of the reading was too high.

CHAPTER FOUR

CONCLUSION AND RECOMMENDATION

4.1. Conclusion

The optimized procedure used small volumes of reagents, time of digestion and lack of complexity. Moreover, this acid digestion procedure was found to be reliable from the recovery test.

The determination indicated that the concentration of most of metals is above the method detection limit except nickel and cadmium. Moreover, macro and some micronutrients have concentration reading above the quantitation limit of the corresponding elements. Thus, this implies that the data

illustrated for elements are precise and which have concentrations above quantitation limit are more precise.

The time of collecting the samples was a dry weather condition for the areas. Thus, the concentrations of some mineral nutrients were high. This was as a result of good condition for their availability to the plant. That is as soil warms, microbial activity and root proliferation increases, and allowing plants to absorb more mineral nutrients.

Since the soil types of enset growing areas of Ethiopia are moderately acidic to slightly basic with the pH reaction ranges from 5.6 to 7.3 [3, 37], the plant expected to have a better accumulation of micronutrients like iron and zinc. Moreover, *Enset ventricosum* from the investigated results of the concentration of metals, it is rich in calcium, magnesium and potassium. However, due to the unfamiliarity and less advantageous of using artificial fertilizers as for other plants, in enset potassium can't take the first place in concentration order. Any way these results confirm, either knowingly or unknowingly using of the food extracted from the plant for repairing bone or replacing blood, which is recommended by the users for such, cases are right. This implies the plant is rich in calcium and iron.

The plant has better accumulation of zinc; the cadmium concentration it has may not bring a great effect on the consumers due to less absorption of this element as a result of higher zinc presence.

Generally, since the soils, which have been used for cultivating the plant, are highly fertilized with manure and organic residues, they are high in available potassium, calcium and magnesium [37]. Hence, the plant has high amount of these metals and other micro minerals.

4.2. Recommendation

Historically enset is the first plant which has been cultivated early and as it is known around 7-10 millions of Ethiopian peoples are dependent on enset as food. But, till now there was no enough information about the mineral content of enset. Thus, the people fully or partly dependent on enset are highly affected by lack of knowledge about the mineral composition of enset. Therefore, this project is so essential and crucial to give highlights on the point even if the number of samples and sites are limited.

The plant is rich in calcium, magnesium, potassium, zinc and iron. Therefore, introducing the foods that are being extracted from the plant especially for cases when the scarcity of these minerals will occur is needed.

Since different species have various selectivity for peculiar purposes like medicine and others, for mineral composition also, there is variation among plants. Therefore, for future research should be conducted which will have a large number of samples especially from all major enset growing areas of the country to differentiate species which can consist more mineral content than the rest. So, by doing this we may satisfy the mineral need of the people, which are highly dependent on enset.

Additionally, the plant is a drought resistant, to keep the food security, introducing the plant to other part of the country is required. To do this having a food content table is very essential. Therefore, by adding other elements, which were not included in this project, research should be conducted on the mineral composition of the soil, wet weather to compare with dry weather and to confirm the mineral content of the plant.

5. REFERENCES

1. Brand, S.A.; Spring, A.; Hiebsch, C.; Yntiso, G.; Tabogie, E.; Diro, M.; Woldemichael, G.; Tesfaye, S.; McCabe, J.T.; Shigeta, M.; 1997. The Tree Against Hunger: Enset Based Agricultural System in Ethiopia.American Association for the Advancement of Science with Awassa Agricultural Research Center, Koyto University Center of African Area Studies and University of Florida, Directorate for International

Programs,Washington, D.C. pp 1- 41.

2. http://ecoport.org/ep?Plant=5700, accessed 10 June 2006.

3. Bobosha, K., 2003. Characterization of Xanthomonas campestris pv musacearum Isolates: Causal Agent Of Enset Bacterial Wilt Disease, Master of Science in Biology, a thesis summated to Addis Ababa University, Ethiopia.

4. Genet, B.; Hilde, N.; Endashaw, B., 2004. Distniction between wild and cultivated enset (*Enset ventricosum*) gene pools in Ethiopia using RAPD markers Heriditas 140: 139-148. Lund, Sweden.

5. Temesgen, A., 2005. Biology of Enset root mealybug (*catenococcus enset*) Williams and Matile-Ferrero (*Homo petra pseudococidae*) and its Geographical Distribution in the Southern Ethiopia, Master of Science in Agriculture (crop protection / entomology), a thesis submitted to Alemaya University, Ethiopia.

6. Bekalu, M.; Digafe, F.; 1996. Ethiopian village studies: imdibir Haya Gasha, Guraghe, Sociology Department, Addis Ababa University, Ethiopia and Center for the Study of African Economies, Oxford, pp 20.

7. Shank, R.; 1994. The Enset Culture: A Technical Report on Enset venticosume or False'Banana', United Nations Development Programme Emergencies Unit for Ethiopia.

8. Kamar, S.; Baah, F.A.; Pozo, E.; Taye, K.; Africa, Z.; Okwadi, J.; 2000. International Center for Development Oriented Research in Agriculture, Wageningen, The

Netherlands.

9. Almaz, N.; Admasu, T.; Treuren, R.; Visser, B.; 2002. Crop Breeding, Genetics and Cytology: AFLP Analysis of Enset Colonal Diversity in Southwestern Ethiopia. *Crop Science Society of America*, 42: 1105-1111.

10. Almaz, N.; Admasu, T.; Treuren, R.; Visser, B.; 2002. AFLP Analysis of Enset Clonal Diversity in South and Southwestern Ethiopia for Conservation, *Crop Science*, 42: 1105-1111.

11. http://www.africa.upenn.edu/EUE/enset.html, accessed 12 June 2006

12. http://www.sidamaconcern.com/country/wesse_research.htm, accessed 5 June 2006.

13. Deckers, J.; Tessera, M.; Alemu, K.; Abate, T.; Swennen, R.; 2001. Ensete, *Ensete ventricosum* (Welw.) Cheesman; DGCI, Bruxelles (BEL), Ethiopia.

14. Golder, A.; 2001. Health and Nutritional Status of Mother and Children Below 5 years in Bio- Village Area, Wolkite, Ethiopia, Institute of Biological Chemistry and Nutrition, University of Hohenheim, Hohenheim, pp17-24.

15. Aremu, C.Y.; Undoessien, E.I.; 1990**.** *Food Chemistry*, 37, 229-236.

16. Shank, R.; 1996. Mole rats: Another Possible Mode of Spreading Enset Bacteri Wilt, Emergencies Unit For Ethiopia [UNDP-EUE] Development Programme.

17. Agren, G.; Gibbson, R.; 1969. *Food Composition Table for use in Ethiopia*, Almqvist and Wiksell., Stockholm.

18**.** Amede, T.; Stroud A.; Aune; J.; 2004. Advancing human nutrition without degrading

land resources through modeling cropping systems in the Ethiopian Highlands; *Food and Nutrition Bulletin*, 25, 344-353.

19. Morrison, G. H.; 1965. *Trace Analysis Physical Methods*, John Wiley and Sons, New York, pp 67-94.

20. Gilbert, Frank A., 1948. *Mineral Nutrition of Plants and Animals*, University of Oklahoma Press, Oklahoma, USA, pp 8-95.

21. http:// www. socialsciences.ucsc.edu/casfs/instruction/tofg/download/ unit_2.2a_soil_chem.pdf, accessed 31 May 2006.

22. http://www.whfoods.com/genpage.php?tname=nutrient&dbid=90, accessed 5 June 2006.

23. http://www.whfoods.com/genpage.php?tname=nutrient&dbid=45, accessed 5 June 2006.

24. http://www.whfoods.com/genpage.php?tname=nutrient&dbid=53, accessed 5 June 2006.

25. http://www.whfoods.com/genpage.php?tname=nutrient&dbid=115, accessed 5 June 2006.

26. http://www.whfoods.com/genpage.php?tname=nutrient&dbid=75 , accessed 5 June 2006.

27. http://www.whfoods.com/genpage.php?tname=nutrient&dbid=77, accessed 5 June 2006.

28. Agency for Toxic Substances and Disease Registry (ATSDR), 1993.Toxicological Profile for Cadmium, U.S. Department of Health and Human Services, Atlanta, GA.

29. Ranjhan, S.K.; Krishna Gopal, 1980. *Laboratory Manual for Nutrition Research*, Vikas Publishing House, New Delhi, pp 83-84.

30. Admasu, T.; Struik, P.C.; 2000. *Infulence of Repeatitive and Leaf Pruning on Dry Matter and Food Prodution* [*Enset Ventricosum*Wew.[Cheesman]]'American Association for the Advancement of Science, Field Crops Research, 68, pp 61-74.

31. http://www.fda.gov/ohrms/dockets/ac/02/slides/3841s1_07__lachman/tsld027.htm, accessed 7 June 2006.

32. http://72.14.207.104/search?q=cache:ml3PVvg9czEJ:www.apvma.gov.au/guidelines/analytical_methods.pdf, accessed 7 June 2006

33. Analytical methods committee (2001). What should be done with results below the detection limit? Mentioning the unmentionable, *amc technical brief*, 5.

34. http://s142412519.onlinehome.us/uw/pdfs/A2523.PDF, accessed 21 June 2006.

35. http://s142412519.onlinehome.us/uw/pdfs/A2521.PDF, accessed 21 June 2006.

36. http://s142412519.onlinehome.us/uw/pdfs/A2524.PDF, accessed 21 June 2006.

37. Murphy, H. F., 1968. A report on the fertility status and other data on some soil of Ethiopia, Experimental Station Bulletin, 44, pp 311.

38. Welz, B.; Sperling, M.1999. *Atomic Absorption Spectrometry*, 3rd ed., Wiley- vch Wenhiem, pp 335 – 769.

39. http://s142412519.onlinehome.us/uw/pdfs/A2528.PDF, accessed 21 June 2006

40. http://www.healthyeatingclub.com/info/booksphds/books/foodfacts/html/data/data5c.html, accessed 31 May 2006.

41. http: // www. Trace elements in food – how much you have to eat in order to get your RDA. htm, accessed 31 May 2006.

42. Holscher, D.; Schneider, B.; 1998. Phenylphenalenones from *Enset Ventricomsum*. Elsevier Science, *Phytochemistry* 49. (7), 2155-2157.

43. Dean, J. R., 1997. *Atomic Absorption and Plasma Spectroscopy*, John Wiley and Sons, Chichester, pp 1-72.

44. Van Loon. J. C., 1980. *Analytical Atomic Absorption Spectroscopy, Selected Methods*, Academic Press, New York, pp 1-76, 158-174.

45. Kulshrestha, S. K., 1994. *Food Preservation*, Vikas Publishing House, New Delhi, pp13-27.

46.World Health Organization; 2002. *Principles and Methods for the Assessment of Risk from Essential Trace Elements*; World Health Organization, Geneva.