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ANALYTICAL CHEMISTRY STREAM

LEVELS OF ESSENTIAL AND NON-ESSENTIAL METALS IN KOCHO, ENSET (*ENSETE VENTRICOSUM*) AND ITS SUPORTING SOIL

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY IN PARTIALFULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ANALYTICAL CHEMISTRY.

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LIST OF ABBREVIATION

SNNPR	South Nation's Nationality People Region
AAS	Atomic Absorption Spectrophotometer
FAAS	Flame Atomic Absorption Spectrophotometer
MOD	Limit of Detection
SD	Standard Deviation

ABSTRACT

Enset (*Ensete vntricosum*) is a drought tolerant crop, grown in Ethiopia. It has many usages: food, fibers and traditional medicine. Being perennial, enset improves local climate and soil conditions. It could contribute to improve food security in several drought-prone parts of the world. The aim of this study was to determine the levels of essential and non-essential metal elements in Enset, Kocho and Soil.

This paper presents the concentration of K, Ca, Fe, Zn, Cr, Cu, Ni, Mn, Cd and Pb in Enset, Kocho and Soil were collected from Amogera water shade found in gurage zone Enemor Ena Ener Woreda (SNNPR).

A 4 mL 70% HNO_3 and 4 mL 70% $HClO_4$ for Enset and Kocho and 2.5 mL conc. HCl and 7.5 mL HNO_3 and 2 mL H_2O_2 for Soil was used for digestion method and determination was made by flame atomic absorption spectrometry. The percentage recoveries of the metals were in the range of 90 to 99%. The ranges of the concentrations (µg/g) of the metals on dry weight are: Fe 105-124; K 5250-5500; Ca 3250-3825; Zn 13.5-16.3; Cu 6.5-8.7; Ni 32.7-35.3; Cr 8.5-11.5; Mn 16.5-20.7; and Pb 0.8-1.1for Enset and Fe 172.5-182.5; K 5000-5025; Ca 10500-11250; Zn 6.7-9.5; Cu 3.7-8; Ni 33-34; Cr 8-9; Mn 10-13 and Pb 1.4-2.1 for Kocho and Fe 165: K 5750; Ca 1725; Zn 38.5; Cu 13.5; Ni 23.7; Cr 13.5; Mn 62.5 and Pb 18 for Soil, where as Cd was not detected in all samples. The concentration of K was highest followed by Ca in Enset, Kocho and Soil. Enset and Kocho also contain higher amount of Fe and Zn.

Key words: Enset, Kocho, Enemor Ena Ener, Amogera water shade, SNNPR, Soil, Gurage, Essential, Non- essential Metal Elements, FAAS

1. INTRODUCTION

1.1 Background and justification

Enset (*Enseteventricosum)* grows wild in a number of countries in central and eastern Africa including Congo, Mozambique, Uganda, Tanzania and Zambia [1]. Wild *E. ventricosum* grows on the highlands (1100—3100m) above sea level of Ethiopia, in small pockets aroundBonga town and the Omo River valley. In contrast, cultivated Enset grows in a wider area comprising the central, south and south—western parts of Ethiopia, mainly at higher altitudes (1500—3100 masl) [1,2].

Enset is a perennial root crop that is domesticated only in Ethiopia, grown mainly in the Southern Nations; Nationalities and people's Regional state (SNNPR) [3, 4] followed by Oromiya and Gambella Regional states [5]. It is an adaptable and drought resistant plant with nutritional and a variety of non-nutritional usages [6, 7].

Enset is an important staple or co-staple crop for more than 20% of the Ethiopian population [5], being a source of major foods such as Kocho, Bulla and Amicho. Medicinal uses of the different parts of Enset include curing fractured and broken bones, assisting with placenta discharge during childbirth, anti-diarrheal medication and as birth control method [7]. Parts of fresh and dry leaves of Ensetfind applications in packaging, storage, food serving, sanitation, separation and construction work. Starch extracted from Enset has proven and potential applications as binding, adhesive, and water absorbing agent in industrial production of textile, paper and pharmaceuticals [8].

Adaptability and resistance against drought are notable features of Enset; as a result the plant has been called "a tree against hunger" [7]. Together with its multiple usages, and these features of Enset increased recognition and interest to enhance its role aside from use as food crop and economic resource, as indicated in national workshop [9]. The increased interest in Enset has been challenged by a number of factors, most notably disease and environmental degradation [7, 10, 11]. However conservation and increasing yield of Enset get prior attention by a researcher to overcome problem related to environmental degradation and diseases.

Metallic composition of Enset thus appears an aspect that merits studying. Inadequate intake of mineral elements has been noted to be a major nutritional problem in human diet [12]. The nutritional composition of Enset would impact the health status of millions of people consuming it as a staple food. Relative to geographic distribution, cultivation and consumption rates, literature on essential and non-essential metals content of Enset, with suporting Soil is scarce [13, 14].

The mineral content of Enset varies widely due to numerous factors, such as disease, number of transplantation, sanitation, plant variety and soil contamination [2, 12, 13, 15, 16]. The general chemical composition of Enset is high in carbohydrates and low in proteins and fats as reported byAgren and Gibbson[17, 18].

The calcium and iron contents of Bulla and Kocho to vary as follow (mg element /100 g edible portion): 82 and 70 for Ca, 3.7 and 7.9 for Fe, respectively [17]. In a previous study, Ayalew and Minaleshewa [19, 20] have determined the levels of major, minor and trace elements in commercially available Enset and its food products from Wolkite and Wolliso towns, as widely known to consume Enset food products. The aim of this study is to analyze essential and non-essential metals nutrients in Enset, Kocho and Soil. So the above mentioned studies do not correlate their findings with the Soil. Therefore this study compares the metal nutrients of Enset with Kocho and Enset with Soil. And Enset plant covers large parts in the country of Ethiopia; the researchers do not cover all part of the Enset growing areas so this paper address the results which do not covered by the researcher.

The traditional methods of processing and storage of raw Enset as well as the resulting reprocessing activities may introduce extraneous materials and cause metallic compositional data unreliable. The aim of this study is to determine the levels of metallic contents in unprocessed Enset corm, Kocho and Soil. For this particular study, a plant from Amogera water shade in EnemorenaEnerWoreda was selected.

Ten frequently analyzed metallic elements in common food products (K, Ca, Fe, Zn, Mn, Cr, Cu, Ni, Cd and Pb) were determined in dried and digested Kocho, Enset, and Soil samples by flame atomic absorption spectrometry.

1.2 Significance of the study

Enset (*Ensetventricosum*) is one of the most important crop plants grown in Ethiopia, where it makes a major contribution to the food security of the country, feeding at least 15 million people in the highlands of South and South western Ethiopia. It buffers food shortage during dry spells and recurrent drought and has been dubbed as the “tree against hunger” [7]. Enset is a multi-purpose crop, with all parts of the plant being utilized for human food, animal feed, medicine, or ornamental uses [17]. Furthermore, it has the capacity for high yield, can be stored for long periods, can be harvested at any time of the year and at any stage over a period of several years [6], thereby offering advantages over seasonal crops, it contains large amount of carbohydrate and it used as energy food source for human being. Therefore studieson essential and non-essential metal elements are necessary to assess its role as source of essential nutrients.

The purpose of this study is to determine the essential and non-essential metal elements in Enset, Kocho and Soil, and the findings of this study is believed to provide adequate information on the distribution of essential and non-essential metal elements on Enset, Kocho, and Soil. Further more the information that was generated from this study useful for EnemorenaEner Woreda Agricultural Office, Water and Energy Office, Farmers that live in this Woreda, and researchers on Enset prospects. It also provides base line information for Gurage Zone Agricultural and mine and Energy Office.

1.3 Objectives of the study

1.3.1 General objective

The main objective of this study is to determine the levels of: essential and non-essential metal elements in Enset, Kocho and Soil.

1.3.2 Specific objectives

The specific objectives of this study are to:

1. Determine the level of essential and non-essential metal elements (K, Ca, Fe, Zn, Mn, Cu, Pb, Ni, Cd, and Cr) in the unprocessed edible part of Enset, and the processed part Kocho.

2. Determine essential and toxic metals in Enset and Kocho samples collected from Amogera water shade Enset farm in EnemorenaEnerWoreda using atomic absorption spectroscopy.

3. Determine the amount of these metals in the Soil of Amogera water shade farms where Enset is grown and

4. Compare the content of these metal elements in Enset and Kocho with the amount of the metals in the Soil.

5. Develop suitable digestion method for Enset and Kocho samples.

2 LITERATURE REVIEW

2.1 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 *Mussa*[7, 21, 22]. 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 [1, 22]. *E. superbum* and *E. glaucum* grow in Asia, where as *E. ventricosum*, *E. gilletti* and *E. hombleioccur* in sub-Saharan Africa and *E. perrieriin* Madagascar. *Enstventricosum* grows wild also in a number of countries in central and eastern Africa including Congo, Mozambique, Uganda, Tanzania and Zambia [1].

In Ethiopia, wild E. ventricosum occurs in the highlands of the county. The distribution is very restricted; the wild populations grow mainly around the city of Bonga (Kefficho administrative region) and in a smaller area 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 often not suitable for human settlements, human interference still prevails through the raising of domestic animals and cutting of trees and shrubs. Enset cultivation in Ethiopia may have started around the 15thcentury, South of Ghibe River. However, historical evidence regarding the origin and domestication of crops is seldom completely trustworthy [1]. In-spite of availability of different species and extensive distribution ofEnset, it is only in Ethiopia that the plant has been domesticated. Wild Enset propagates naturally by seed and domesticated Ensetvegitatively, and recognized more than 50 different varieties, colons, or landraces [1].

Domesticated Enset is also classified taxonomically as *Ensetventricosum* world have similar appearance and the domesticated plants subjected to similar processing for consumption in Ethiopia. Enset (*Ensetventricosum*) belongs to the order *sctaminae*, the family *musaceae*. The *Musaceae* family is subdivided into the genera Musa and Enset [23]. Enset system of cultivation is considered to be one of the last remaining sustainable, indigenous agricultural systems found in Africa [24]. Enset occurs in wild forms in East, Central and South Africa. Even though the banana fruit is known worldwide Enset (*Ensetventricosum*) is only cultivated as a crop in Ethiopia [25].

Outside Ethiopia, the use of Enset is reported from Vietnam, where it provided an emergency food during the Second World War. In parts of north and central Vietnam, the growing point was used as a vegetable [26]. The crop is grown in many regions but the dwellers of the central and southwestern parts

of Ethiopia are the only people who use Enset as a staple and co-staple crop [27]. These regions are among the most densely populated in the whole of Ethiopia and are inhabited by more than 11 ethnic groups, which show great variation in culture and agricultural practices [26].

There are many Enset clones in different agro ecologies. Farmers classify their landraces and give them different names based on several attributes that distinguish the landraces from one another. The names given by farmers to the different Enset clones separate the landraces linguistically, phenotypically and in terms of their utilization value [28]. This paper presents the results of investigation on the concentration of essential and non-essential metal elements in Enset (*Ensetventricosum*), Kocho and the Soil.

2.2 Enset and Its Part

Enset looks like a large, thick single stemmed banana plants. Bothe 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 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 underground 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 [7].

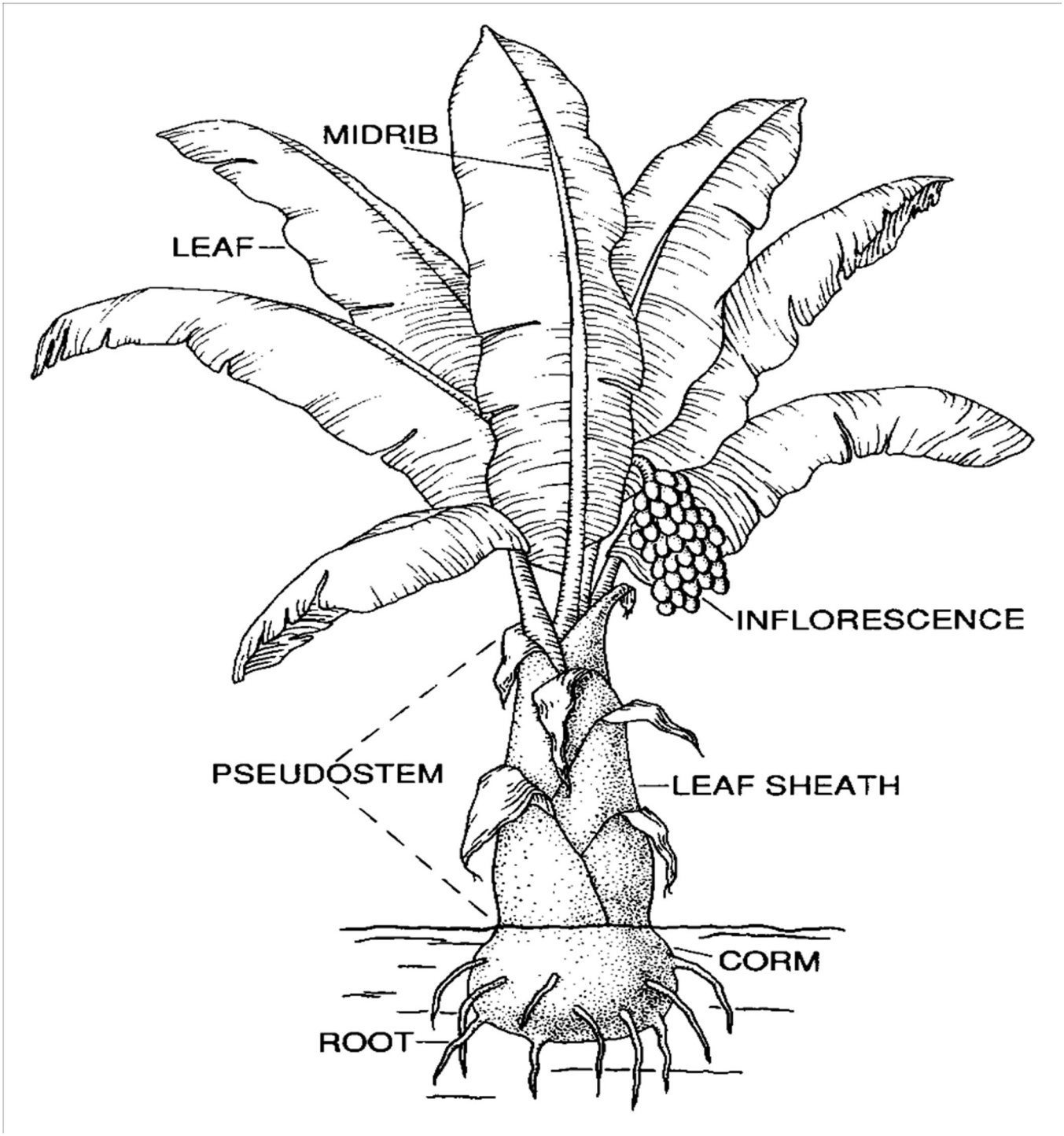

Figure 1 Parts of the Enset plant.

2.3 Food and Non-food Uses of Enset

Enset is an important co-staple food 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 [5]. 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) [7]. Bulla is the smallest water soluble starchy product that may be separated from Kocho during processing by squeezing and decanting the liquid. Amicho is the fleshly part of Enset plants and have medicinal value for humans and livestock to cure bone fractures, broken bone, childbirth problems (i.e. assisting to discharge the placenta), diarrhea and birth control inner portion of the Enset corm, which may be cooked and eaten separately, tasting similar to potato [29, 30].

Enset provides fiber as abyproduct of decorticating the leaf-sheaths. In addition (as an abottifacieant), fresh Enset leaves are used as bread food wrappers, serving plants, 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, are used as cleaning rags and brushes, baby cushions/diapers, and cooking pot stands [7, 31].

2.4 Mineral Nutrients in the Soil

Soil is a heterogeneous material which may be considered as consisting of three major components: a solid phase, a liquid phase and a gaseous phase. All three phases specifically influence the supply of plant roots with nutrients. The solid phase may be regarded as the main nutrient reservoir. The inorganic particles of the solid phase contain cationic nutrients such as K, Na, Ca, Mg, Fe, Mn, Zn, and Co whilst the organic particles of this phase provide the reserve of N and to a lesser extent also P and S. Colloidal soil particles are mostly negatively charged. The negative charge on the clay mineral surfaces arises largely because of isomorphous replacement of cations in the crystalline lattice where trivalent cations are substituted by divalent cations. The negatively charged surfaces of these various soil particles attract cat ions such as Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} as well as Al^{3+} and Mn^{2+} [32].

Plant growth involves the interaction of soil and plant properties. Soil is the normal medium for plant root growth. The plant's roots absorb nutrients and water from the soil and are an anchor to support the

shoot. Maximum plant growth depends on the soil having the biological, chemical and physical conditions necessary for the root system to maximize the plant's required absorption of nutrients and water and to enable the biochemical reactions that occur in the root. The plants rate of absorption of nutrients involves processes going on in both the plant's root and the soil. Each of these processes is important inproviding nutrients for use by the shoot [33].

Soil properties are greatly influenced by its pH values can differ widely from values of about 3 to as high as 10, being very low in acid, sulphate and podzolic soils and being rather high in calcareous and alkali soils. In alkali soils in particular very high pH values may occur as the soil solution contains weak acids (HCO_3^-) and strong bases (Na^+ or K^+). The H^+ concentration of the soil solution has a pronounced effect on a number of soil constituents and especially on the soil minerals, soil microorganisms and plant roots. High H^+ concentration favor the weathering of minerals resulting in a release of various ions such as K^+, Mg^{2+}, Ca^{2+}, Mg^{2+}, Cu^{2+} and Al^{3+} [32].

2.5 Mineral Contents of Plant Materials

The material of living plants consists of organic matter, water and minerals. The relative amounts of these three compounds may vary, but for green plant material always present in the highest proportions. The percentage distribution of these three components is in the following order of magnitude: 70% water, 27% organic material and 3% minerals. The minerals make up only a comparatively small proportion of dry matter. They are nevertheless of extreme importance because they enable the plant to build up organic material (photosynthesis). The mineral content of plants and plant organs is therefore of physiological and practical significance [32].

The main factor controlling the mineral content of plant material is the specific, genetically fixed nutrient uptake potential of the plant for the different mineral nutrients. This accounts for the fact that the N and K content of green plant material is about 10 times higher than that of P and Mg which in turn is about 100-1000 times higher than the content of the micronutrients. This general pattern occurs in all species of higher plants. The second factor controlling the mineral content of plant material is the availability of plant nutrients in the nutrient medium. The concentration of a particular mineral or plant nutrient in the plant increases in the form of a saturation curve as its availability in the nutrient medium increases [32].

Mineral contents differ considerably between plant organs. Generally the vegetative parts of the plant such as leaves, stems and roots vary to a higher extent in their mineral composition than fruits, tubers and seeds. The mineral content of plants is also very much dependent on age. Young plants and young

plant tissues have higher contents of N, K and P, where as in older plants and more mature plant parts, higher contents of Ca, Mn, Fe and B are often observed, provided the mineral content is expressed on a dry matter basis [32].

The analysis of plant material presents a type of approach in determining the nutrient availability of a soil. It is based on the concept that the content of a particular nutrient in the plant is greater the higher its availability in the soil. In principle, plant nutrients present in the plant must originally have been available in the soil. Unfortunately, however, it has drawbacks, as the mineral content in the plant not only depends on nutrient availability in the soil, but it is also affected by other factors such as the kind of plant organ or tissue, the age of the plant and the supply of the plant with other plant nutrients [32].

In most cases analytical data obtained from the analysis of leaf or tissue correlate fairly well with soil tests, however, it also reflects conditions of uptake. Leaf or tissue analysis provides a particularly useful means of assessing nutritional status of perennial plants such as trees, vines, Enset, forest trees various plantation crops [32].

2.5.1. Potassium (K)

The average K content of the earth's crust is in the order of about 2.3%. By far the greatest part of this K is bound in primary minerals or is present in the secondary clay minerals which largely make up the clay fraction of the soil. For this reason soils rich in clay are also generally rich in K and clay soils may often have excess of 4% total K [32]. Potassium is an essential element for all living organisms. In plant physiology it is the most important cation not only in regard to its content in plant tissues but also with respect to its physiological and biochemical functions [32].

Plants absorb large amounts of potassium, all of it in the form of the K^+ ion. The positive charges of the potassium cations help to maintain electrical neutrality in both soil and plants by balancing the negative charges of nitrates, phosphates, and other anions. Plants require relatively large amounts of potassium and often can use more than the soil can supply. Potassium is the third most likely element to limit plant growth [36].

Potassium is present in plants in the form of organic and inorganic salts. It does not form an integrated part of the structure of any known organic compound in plants. Nearly all potassium salts are soluble and highly ionized in solution. The K^+ions are mobile within the plant but will not leach out of healthy living plant tissue. They are readily leached from dead plant tissue [36].

A deficiency in potassium affects processed such as respiration, photosynthesis, chlorophyll development, and water content of leaves. The best known function of K is its role in stomata opening and closing. The highest concentrations of potassium are found in the meristematic regions of the plant. Potassium is essential as an activator for enzymes involved in the synthesis of certain peptide bonds [37]. It exerts a specific role in the conformation of the enzyme protein, thus enabling the alignment of appropriate active cites [32]. Potassium also can act as an activator for several enzymes involved in carbohydrate metabolism [37]. Potassium aids in the uptake of other nutrients and in their movement within the plant. The presence of potassium and other ions in the solution helps to maintain the osmotic concentration necessary to keep the cells turgid [36].

2.5.2. Sodium (Na)

The total sodium content of normal soils is approximately 0.63%.High soluble Na contents are very harmful to the physical condition of soils. Sodium tends to disperse the soil colloids. A dispersed soil is puddle when wet and hard when dry. Sodium is not considered an essential nutrient for plant growth. Sodium compounds are widely distributed in nature. The major mineral source is sodium aluminum silicates, also called sodic feldspars or plagioclase, e.g., albeit, $NaAlSiO_3O_8$. Sodium can also occur in nature as chloride, sulfate and borate compounds. The dissolution of sodium from albiteis attributed to hydrolysis reactions, which is a weathering process [34].

The chemistry of sodium is quite similar to that of potassium, but its behavior in the soil is somewhat different [36]. Sodium is not required by plants but can replace part of the K^+ requirement of some species. It is toxic to some plants at high concentrations. High soil pH usually accompanies Na+ accumulations in soil [38].

Sodium occurs in feldspars but not in micas. The sodium feldspars weather a little more rapidly than the potassium feldspars. Sodium ions that have been released to the soil solution are not subject to fixation and are less tightly held to cation exchange sites than potassium, magnesium or calcium. Sodium is therefore the easiest basic cation to leach from the soil. This accounts for the sodium content of soils gradually decreasing with time while the potassium content remains nearly constant. This can lead to the formation of sodic soil [36].

2.5.3. Calcium (Ca)

Calcium is a divalent alkaline earth cation. It is the fifth most plentiful element in the earth's crust, which has an average calcium concentration of 3.6%. Calcium is the dominant exchangeable cation in many soils. In general, only alkaline soils containing sodium, acid soils containing large amounts of hydrogen and aluminum, and serpentine derived soils high in magnesium have cations other than calcium as the dominant exchangeable cation. Exchangeable calcium is in equilibrium with soil solution calcium. The relative strength of bonding controls the equilibrium between soluble and exchangeable calcium. Bonding depends on the nature of the cation exchange site, degree of calcium saturation of soil exchange sites, complementary cations present, and the anion content of the soil solution [33].

The exchangeable calcium in a soil has an important relation to the soil pH and to the availability of several nutrient elements. The amounts of calcium and other basic cations present in a soil decline as a soil becomes more acid and increase as it becomes more alkaline. An excess of calcium causes calcium carbonate to precipitate and buffer the pH to a value near 8. Excess calcium usually results in the solubility of phosphorus, iron, manganese, boron and zinc and sometimes causes deficiencies of one or more of these essential plant nutrients [36].

Calcium dissolved in the soil solution can move by mass flow and by diffusion, but exchangeable calcium has very low mobility. It is reported that plant roots was not entered soil layers that are devoid of calcium even though other conditions are favorable for growth and calcium is available in other layers. Monovalent ions such as Na^+ and K^+ are more mobile because they are less strongly attracted to cation exchange sites than Ca^{2+} ions [36]. Calcium occurs in plant tissues as free Ca^{2+}, as Ca^{2+} adsorbed to in diffusible ions such as carboxylic, phosphorylic and phenolic hydroxyl groups. It is also present in Ca oxalates, carbonates and phosphates [32].

Calcium is a structural component of cell walls and is therefore vital in the formation of new cells. Furthermore, calcium is so integrated into cell walls that it cannot be removed from old cells to form new cells. Plants deficient in calcium are stunted because they produce fewer and smaller cells. They have weak stems because their cell walls are less than normal thickness [36].

A calcium shortage restricts the growth of roots as well as stems, leaves, etc. the inability of calcium deficient roots to elongate rapidly handicaps the plant for exploiting new portions of the soil volume to obtain water and nutrients. Restricted root growth could produce or aggravate other nutrient deficiencies as well [35].

Calcium is normally abundant in plant leaves. A calcium deficiency prevents the growth and unfolding of new leaves. It also prevents the growth of the margins of existent leaves and therefore results in curled leaves [36].

2.5.4. Magnesium (Mg)

The Mg content of most soils generally lies in the range of between 0.05% for sandy soils and 0.5% for clay soils. Magnesium in the soil is a constituent of many soil minerals; it present as exchangeable magnesium on the cation exchange complex and the soil solution as the soluble magnesium ion. Small amounts of magnesium may also be combined in the soil's organic fraction [33].

Plant roots absorb soluble and ultimately exchangeable magnesium, which is assumed to go into solution before absorption by the root. Exchangeable calcium plus magnesium usually accounts for more than 60% of the exchangeable cations on soils with pH 5.5 or higher. Exchangeable hydrogen, aluminum, potassium and sodium occupy the majority of remaining exchange sites. Exchangeable magnesium is almost always present in smaller quantities than calcium, though on serpentine derived soils, magnesium may be the dominant exchangeable cation [33].

Magnesium in the soil that can move rapidly to the root and be absorbed includes both exchangeable magnesium on the cation exchange sites and the smaller quantity in solution. Exchangeable magnesium is assumed to go into solution before movement to, and absorption by, the root. The quantity in solution is usually from 1 to 10% of the amount of exchangeable magnesium. The measure of exchangeable magnesium plus that in solution reflects the total available for movement to the root [33].

Magnesium is generally taken up by plants in lower quantities that Ca^{2+} or K^{+}. The content of Mg in plant tissues is usually in the order of 0.5% of the dry matter. In plant tissues a high proportion of the total Mg, often over 70%, is diffusible and associated with inorganic anions and organic acid anions such as malate and citrate. Magnesium is also associated with in diffusible anions including oxalate and pectate. The most well known role of Mg is its occurrence at the center of the chlorophyll molecule. Besides its function in the chlorophyll molecule Mg^{2+} is required in other physiological processes. One major role of Mg^{2+} is as a cofactor in almost all enzymes activating phosphorylation processes. Magnesium is therefore important throughout the metabolism [32].

2.6. The Micronutrients

A micronutrient is an element that plants must have to complete their life cycles but need only in a small amount. These elements have often been called trace elements or minor elements, but micronutrient is the preferable term [36]. The terms micronutrient and trace element must not be construed to imply that these nutrients are somehow less important than the macronutrients. To the contrary, the effects of micronutrient deficiency can be very severe in terms of stunted growth, low yield dieback, and even plant death. By the same token, where they are needed, very small applications of micronutrients may produce dramatic results [35].

Each plant requires only a tiny amount of each micronutrient, yet it must have that tiny amount. Clearly they cannot serve as building blocks of major plant compositions; the amounts involved are inadequate for such a role. Some micronutrients function in the enzyme systems of plants. A very small amount of such an element is all that is required to make enough enzymes to catalyze an essential plant process. Cation forming elements such as copper are more likely to serve as coenzymes that activate an enzyme but are not an integral part of the molecule [36].

Some micronutrients function in oxidation-reduction processes of plant metabolism. Elements such as iron, copper and manganese can change valences and thus enter intooxidation-reduction reactions. Several of the micronutrients are involved in the production of chlorophyll. Deficiencies of these elements can appear very similar to the pale green condition characteristic of nitrogen deficiency [36].

2.6.1. Iron (Fe)

Iron is present in soils in higher concentrations than any other nutrient. The lithosphere contains 5.1% iron, which forms compounds with sulfur and oxygen [34]. The greatest part of soil Fe usually occurs in the crystal lattices of numerous minerals. Iron minerals commonly found in soils include goethite, (Fe_2O_3), lepidocrocite, (FeOOH), and magnetite (fe_3O_4). Hematite gives a red color to soils, while goethite imparts a yellow color. Amorphous iron as $Fe(OH)_3$ is probably the most significant form in supplying iron for uptake by the plant [33].

In spite of the large amount of iron in the soil and the low quantities needed for plant growth, iron deficiencies occur because so little of the element is in an available form. Soils vary in total iron content from 0.02 to 10%, depending on their origin [33]. The content of soluble iron in soils is extremely low in comparison with the total Fe content. Soluble inorganic forms include Fe^{3+}, $Fe(OH)^{2+}$, $FeOH^{2+}$ and

Fe^{2+}. In well-aerated soils, however, Fe^{2+} contributes little to the total soluble in organic Fe except under high soil pH conditions [32].

The amount of iron present as Fe^{2+} and Fe^{3+}, in the soil solution depends on the hydroxide forms present in the soil, which in turn, depends on pH and a parameter related to the red ox potential. Iron solubility is largely controlled by the solubility of the hydrous Fe (III) oxides, which give rise to Fe^{3+} and its hydrolysis species [Fe $(OH)_3$]. The equilibrium is very much in favor of Fe $(OH)_3$ precipitation and is highly pH dependent, the activity of Fe^{3+} falling with increasing pH. At higher pH levels Fe3+ activity in solution decreases 1000 fold for each pH unit rise. The soluble Fe level reaches a minimum in the pH range of 6.5-8.0 acid soils are thus relatively higher in soluble inorganic Fe than calcareous soils where levels can be extremely low [32].

Iron forms stable complexes with organic compounds that occur in both the soil's solid phase and soluble organic compounds. Simpler organic compounds are citrate and oxalate. Iron compounds are more stable than combinations with most other nutrients, so that iron can replace them in the chelate except where mass-action displacement occurs due to highconcentrations of other ions present. Iron chelates have high stability constants, so that at pH levels below 7.0, iron is frequently the dominant cation in the chelate [33].

Iron may be supplied to plant roots as Fe^{2+}, Fe^{3+} or as Fe chelates. Absorption appears to be dependent on the ability of the roots to reduce Fe^{3+} to Fe^{2+}. The uptake of iron is considerably influenced by other cations. Competitive effects on iron uptake have been observed with Mn^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, K^{+} and Zn^{2+}. Heavy metals, in particular Cu and Zn are also known to displace Fe from chelate complexes forming corresponding heavy metal chelates. Iron uptake is particularly depressed by high pH, high phosphate and Ca^{2+} concentration in the nutrient medium. High pH levels as well as good aeration conditions favorthe oxidation of Fe^{2+} to Fe^{3+} and thus the precipitation of Fe (III) salts occurs [32].

The tendency for Fe to form chelate complexes and its ability to undergo a valency change are the two important characteristics which underlie its numerous physiological effects [32]. Iron has a number of important functions in the overall metabolism of the plant. Although iron is frequently taken up in the ferric state (Fe^{3+}), the ferrous state (Fe^{2+}) is generally accepted as the metabolically active form of iron in the plant [37].

The most well-known function of iron is in enzyme systems in which haem or haemin function as prosthetic groups [32]. Iron is necessary for the formation of chlorophyll and functions in some of the

enzymes of the respiratory system [36]. Iron is incorporated directly into the cytochromes, into compounds necessary to the electron transport system in mitochondria, and into ferredoxin [37].

An iron deficiency results in the younger leaves being small and pale green or yellow in color. This shortage of chlorophyll is called chlorosis. The younger leaves are more affected than the older leaves because iron is relatively immobile inside the plant. Often the veins remain green while the areas between veins turn yellow from iron chlorosis [36]. Primarily, this is because of the relative immobility of iron in the plant. Thus the younger leaves cannot withdraw iron from the older leaves [37].

2.6.2. Manganese (Mn)

Manganese is the eleventh most common element in the earth's crust, with an average concentration of 0.09% mg/kg. Manganese is present primarily as oxides and sulfides; it often occurs in association with iron. Soils have manganese concentrations that are usually in the range of 20 to 3000mg/kg, with an average of 600 mg/kg [33]. Soil manganese exists in three oxidation states Mn^{2+}, Mn^{3+} and Mn^{4+}. Manganese absorbed by plant roots is primarily as Mn^{2+}. Oxidation-reduction reactions in the soil influence the amount of each oxidation state present. The predominant oxidation states in most soils are Mn^{2+} and Mn^{4+}, with much more as Mn^{4+} than Mn^{2+} in aerated soils [33].

Total soil manganese may be divided into mineral manganese, organically complexed manganese, exchangeable manganese, and solution manganese. Manganese in solution may be either Mn^{2+} or manganese combined with soluble organic compounds. The equilibrium of manganese between these forms is influenced greatly by soil pH and red ox conditions [33]. Exchangeable manganese is held by the soil cation exchange sites and measured by displacement with ammonium acetate. It exists essentially as Mn^{2+}. A wide range of values can be obtained, with very acid soils having values above 1000mg/kg, while organic soils of high pH may have values less than 0.1 mg/kg [34]. Manganese availability is higher in acid soils due to the higher solubility of Mn compounds under low pH conditions. Soluble Mn^{2+} decreases 100 fold for each unit increase in pH. Under high soil pH conditions Mn availability can thus be inadequate to meet plant demand [32].

Manganese is an essential element in respiration and nitrogen metabolism; in both processes it functions as an enzyme activator. However, in many cases, especially with reactions in respiration, manganese can be replaced by other divalent cations, such as Mg^{2+}, Co^{2+}, Zn^{2+} and Fe^{2+} [37]. Manganese functions in chlorophyll development and in the enzyme systems of plants. Its various valences make it possible for manganese to be either a metallic coenzyme or a part of an organic molecule [36]. Manganese is also in

some way involved in the oxidation reduction processes in the photosynthetic electron transport system [32].

Manganese, like iron, is a relatively immobile in plants. Deficiency symptoms appear on the younger leaves of the plant first. The symptoms vary with the plant but include a pale color similar to iron chlorosis between the veins of broad-leaved plants. The similarity between manganese and iron causes a form of competition between the two elements. Symptoms of manganese toxicity correspond to those of iron deficiency [36]. Manganese deficiency also appears to have a marked effect on the chloroplast. The chloroplasts lose chlorophyll and starch grains, become yellow green in color, vacuolated, and granular and finally disintegrate [37].

2.6.3. Copper (Cu)

The average copper concentration in the earth's crust is approximately 70 mg/kg. Copper concentration varies with the type of rock; basalt may contain as much as 100mg/kg, while granite contains only approximately 10 mg/kg. Sedimentary rocks also vary in copper concentration. Limestone, sandstone and shale average approximately 4, 30 and 45 mg/kg of copper, respectively [33]. Copper occurs as Cu^{++} ions in most soils as Cu^{+} ions where the oxidation level is low. Copper ions are held so tightly by cation-exchange sites that they are even less mobile than Ca^{++}. The concentration in solution is only a few part per million. Copper is most soluble in acid soils, and its solubility decreases as the pH rises [36].

Copper can also be substituted isomorphously for manganese, iron and magnesium in various minerals. Copper oxides, carbonates and sulfates are not present in most soils, because the copper concentration found in soil solution is much lower than would be maintained by the solubility of these minerals. Copper in soil can occur in soil solution, both ionic and complexes, as an exchangeable cation on the exchange complex, as a specifically adsorbed non-exchangeable ion, in organic matter, included oxides, and in minerals [33].

Copper is taken up by the plant only very small quantities. It is thus about one-tenth of the Mn content. Copper uptake appears to be a metabolically mediated process and there is evidence that Cu strongly inhibits the uptake of Zn and vice versa [32]. There is little doubt as to the necessity of copper for normal plant metabolism. Copper acts as a component of phenolase, lactase and ascorbic acid oxidase, and its role as apart of these enzymes probably represent an important function of copper in plants [37].

Copper is important as a coenzyme that is needed to activate several plant enzymes. It is also involved in chlorophyll formation. Copper uptake seems to be inversely related to iron uptake. Too little copper

causes iron to accumulate in plants. Too much copper causes chlorotic symptoms similar to those of iron deficiency [36]. Copper is not readily mobile in the plant although it can be translocated from older to younger leaves [32]. Copper deficiency, symptoms occur mostly on new growth because copper is relatively immobile in plants [36]. Copper deficiency causes a necrosis of the tip of young leaves that proceeds along the margin of the leaf and gives it a withered appearance. Under more severe conditions, the leaves may be lost, and the whole plant may appear wilted [37].

2.6.4. Zinc (Zn)

The mean zinc concentration in the lithosphere is 80 mg/kg. Total soil zinc ranges from 10 to 300 mg/kg. Zinc is present in the soil in only the divalent form. Organic matter forms coordination complexes with zinc; they may be present in both the soil organic matter and soluble organic complexes in soil solution. Zinc that may become available for plant uptake is present as Zn^{2+} in the soil solution, exchangeable zinc on the cations-exchange sites, organically complexes zinc in solution and in the soil solid phase [33]. The levels of zinc in the plant materials are low, generally in the order of up to 100 ppm in the dry matter [32].

Zinc participates in the metabolism of plants as an activator of several enzymes [37]. In its function in some enzyme systems, Zn^{2+} resembles Mn^{2+} and Mg^{2+} in that it brings about the binding configuration between enzyme and substrate. Zn is required in the synthesis of tryptophan. As tryptophan is also a precursor of in dole acetic acid the formation of this growth substance is also directly influenced by Zn [32]. Zinc is needed for protein metabolism and appears to be involved somehow in the production of chlorophyll [37]. Plants suffering from zinc deficiency often show chlorosis in the interveinal areas of the leaf. These areas are pale green, yellow, or even white. Zinc deficiency is closely related to the inhibition of RNA synthesis. The deficiency prevents the normal development of chloroplast, grana and vacuoles are developed in them [32].

2.7. Further Elements of Importance

The elements K, Ca, Mg, Fe, Mn, Cu and Zn, with the possible exception of Na, are essential plant nutrients without which the plant would be unable to complete its life cycle. In addition to these nutrients are group of elements which can have a beneficial effect on the plant growth. Two well known elements of this type are Co and Si [32].

2.7.1. Cobalt (Co)

The Co concentration in the dry matter of plants grown in soil normally lies between 0.02 to 0.5 ppm. In soils the content is usually much higher and levels from 1 to 40 ppm are common although many values in excess of 40 ppm have been reported [32].

In the soil cobalt occurs primarily in the crystal lattices of ferromagnesian minerals and as such is unavailable to plants. After release from these minerals by weathering, Co^{2+} is held largely in exchangeable form or as oregano mineral complexes. Exchangeable Co^{2+} is very firmly bound and like Cu^{2+} the concentration in the soil solution is very low. Cobalt deficiency occurs in highly leached sandy soils, soils derived from acid igneous rocks or in highly calcareous or peaty soils. It is favored if the soil pH is neutral to alkaline [32].

It is now well established that Co is essential for microorganisms fixing molecular N_2 is still in question whether in addition to its requirement in symbiotic N_2 fixation, Co is essential for higher plants. It is clear, however, that low concentrations of Co can have a favorable effect on plant growth and there is some indication that there might be a Co requirement. Cobalt is also of importance in animal nutrition. It is well established that Co is a metal component of vitamin B-12 which is essential in N-metabolism [32].

2.8. Elements with More Toxic Effects

There is no clear division between elements which are toxic to plants and those which have a beneficial or even essential effect. The effect of any element on the plant depends not only on its chemical properties but also on its concentration and the presence and concentrations of other elements. Some elements such as Fe, Mn, Cu, Band Zn are essential at low concentrations but are toxic at higher levels. In the case of the heavy metals Pb and Cd, toxicity is induced by mimicking of lighter essential elements in uptake and biochemical behavior [32].

2.8.1. Cadmium (Cd)

Cadmium, a heavy metal, causes toxicity in humans in very small amounts when consumed. Cadmium occurs naturally in only trace concentrations in agricultural soils. Contamination of agricultural soils with Cd is derived from sources, such as phosphatic fertilizers manufactured from rock phosphates high in Cd and by the application of sewage sludge to a greater extent and by the pesticides and gypsum to lesser extent. Zinc smelters in the vicinity of agricultural soils can also be significant contributors to soil

contamination with Cd. Food crops grown on contaminated soils may take up substantial amounts of Cd and this could result in Cd entering the food chain of animals and humans when consumed [39].

There is now general concern that under certain conditions the cadmium content of plants may be raised and thus become hazardous to man. The sources of soil Cd are varied. Cadmium is added to soils in very small amounts in phosphate fertilizers. Along with other heavy metals, it is also present in sewage sludge. Levels from about 10 to as much as 1500 ppm Cd have been observed in the dry matter of sewage sludge, which is being used more and more on agricultural land [32].

There is considerable current interest in Cd in plant nutrition. Normal Cd levels in plant material are in the range of 0.1-1.0 ppm. Although the roots of several species can take up large quantities of Cd from solution, the movement of Cd through the plant is restricted. Cadmium appears to be held in the roots on exchange sites, and can be replaced by Ca^{2+}, Mn^{2+} and Zn^{2+}. As Ca^{2+} is normally the dominant cation in the soil solution it may substantially affect the uptake of Cd from the roots to the tops is particularly depressed by phosphate [32].

Cadmium and Zn are chemically very similar. Cadmium is thus able to mimic the behavior of the essential element Zn in its uptake and metabolic functions. Unlike Zn, however, Cd is toxic both to plants and animals. The basic cause of toxicity probably lies in the much higher affinity of Cd for thiol groupings (SH) in enzymes and other proteins. The presence of Cd therefore disturbs enzyme activity [32].

Most recently, interest in Cd has been directed at progressive accumulation in biological systems at low levels at which Cd generally occurs environmentally [40]. Toxic effects in man have been observed from the regular consumption of plants in excess of 3 ppm [32]. Continued exposure to small amounts of Cd leads to accumulation, in human and animal liver and kidney tissues resulting in damage and malfunction of these organs [40]. It disturbs the metabolism of Ca and P and cause bone disease, which is very painful, and causes excessive demineralization and embritlement of the skeleton [32].

2.8.2. Lead (Pb)

The total Pb content of agricultural soils lies between 2-200 ppm. Soils with levels in excess of this are limited to a relatively few regions where Pb mineral deposits occur. Lead airborne contamination in soils is usually restricted to the top few cm of the soil profile. These retention in the upper part of the soil profile probably relates the strong adsorption of Pb2+ to organic and clay colloids as well as to the formation of insoluble Pb chelates with organic matter. The availability of soil Pb is usually low. A high

soil pH may precipitate Pb as hydroxide, phosphate, or carbonate as well as possibly promoting the formation of Pb organic matter complexes [32].

Lead is a major chemical pollutant of the environment, and is highly toxic to man. No other pollutant than Pb has accumulated in man to average levels so close to those which are potentially clinically poisonous. Lead is toxic because it mimics many aspects of the metabolic behavior of Ca, and inhibits many enzyme systems. In animals, Pb toxicity interferes with Fe metabolism and the formation of heam [32].

Lead reaches soil and plant cover as an aerial deposit and in precipitation, irrigation water, mine drainage, leaf litter, or ground dust blown in from elsewhere. Pb is also added to soil as pesticide, such as Pb arsenate, or as an impurity in certain fertilizers such as limestone and superphosphates. Two pathways are available for Pb to enter plants: uptake by the roots and uptake by the foliage. Once inside the system, Pb seems to be retained by cell membranes, mitochondria, and chloroplasts [41].

Lead enters man by inhalation and ingestion. Absorbed and carried by the blood, it is accumulated in liver, kidney and bone up to about the fifth decade of life [41]. Pb causes brain damage particularly to the young. There is evidence that Pb pollution can induce aggressive behavior in animals which can also occur in humans [32].

2.9 Important Soil Properties

Plants normally derive their mineral components from the soil. The elements for plants can be subdivided into those required in relatively large amounts, the macronutrients and those required in small amounts, the micronutrients [46]. Soil pH and its organic matter content are among those major factors that greatly affect the availability of macro- and micronutrients and even toxic elements in soil and their uptake by plans [35].

2.9.1 Soil pH

The degree of soil acidity or alkalinity, expressed as soil pH, is a most variable that affects a wide range of soil properties chemical, biological and indirectly even physical. This chemical variable greatly influences the availability for root uptake of many elements, including both nutrients and toxins [35].

Soil supply plants with the inorganic mineral nutrients in the form of dissolved ions. To be taken up by plant a nutrient element must be in a soluble form and must be located at the root surface which is in

such intimate contact with soil particles that a direct exchange may take place between nutrient ions absorbed on the surface of soil colloids and the H^+ ions from the surface of root cell walls [35].

Most plants grow best in slightly acidic soils (pH 6-7). In this pH range, nearly all plant nutrients are available in optimal amounts for plant growth. Soils with a pH below 6.0 are more likely to be deficient in some available nutrients. Ca, Mg and K are especially deficient in acid soils [34].

Soil pH, especially in well-aerated soils, has a decided influence on the availability of all the micronutrients except chlorine. Under acid conditions, most micronutrient cations are soluble and freely available some times at toxic levels [35]. In strongly (pH 4.0-5.0) and very strongly (pH 3.0-4.0) acid soils, Al, Fe and Mn may exist in very high amounts because of their increased solubility. If phosphates are present, these elements react with the phosphates to form insoluble phosphates. In alkaline soils (pH > 7), Fe, Mn, Zn and Cu become unavailable for plant growth [34].

Table 1: safe values for Pb, Cd, Cu, Zn and Cr in fruits and vegetables recommended by WHO/ FAO (2007)

Elements	Maximum allowable limits (mg / Kg dry weight)
Pb	5
Cd	0.2
Cu	40
Zn	60
Cr	2.3

2.10. 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 [12, 13, 28-31]. 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 Kocho 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 and edible as staple and co-staple food studies on essential and non-essential 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 [12]. Since Enset is the tree against hunger, the knowledge of its mineral concentration is of particular interest.

2.11. Distribution pattern of metals in processed Enset foods

Plants accumulate minerals essential for their growth from the environment and can also accumulate metals such as Cd, Co and Ag, Na, which have no known direct benefit to the plant [47]. Plants have developed several biochemical processes for the mobilization and uptake of minerals, to chelate and solubilize soil minerals, the roots secret metal-chelating molecules such as phytosiderophores. Metal-chelating proteins such as metallothioneins also function in plants to bind metals and enhance transport and metabolism. This biochemical complexation maintains the metal in a soluble form that is available for metabolism and may explain the relatively high bio-availability of minerals found in plants [47, 48] Enset plant is one of those plants which have long roots. As reported by Debebe [19], Enset plant is known to accumulate essential and non-essential plant nutrients and hence its processed foods.

The corn and the false stem of Enset after they have been grated or decorticated and mixed up, the watery part are allowed to squeezed out to get bulla and the solid material gives Kocho after fermentation. Then these Enset products allowed fermenting for a set of time, which depends upon the weather condition (i.e., the temperature and amount of fermented Kocho added as a yeast), harvesting season meaning that age of the plant and variety or clone of the species [7,49.50].

Therefore the essential and non-essential metals in Kocho and their quality will be affected by different factors, physical and chemical properties of the soil, application of natural (manure) and artificial fertilizers, storage and processing of Kocho, age of harvesting (processed) Enset plant, climatic condition of the region and other factors are the main contributors for the mineral contents of Kocho [53].

This paper compares the amounts of nutrient in Enset, Kocho and Soil, since nutrients obtained from the plant is come from the Soil and the environment. Enset plant covers large parts in the country of Ethiopia; the researchers do not cover all part of the Enset growing areas so this paper address the results which do not covered by the researcher.

2.12. Sample decomposition

The various techniques for elemental determination do not all requires the same degree of sample matrix break down. The choice of a digestion technique should take into account the objective of the final determination and factors such as the matrix composition, the element contents, the possible interferences, the risk of loses and contaminations, the practicality and possible safety hazards in the laboratory [58, 59, 69].

The purposes of sample decomposition are:

1. Converting all the species in which a given element is present in such a way that it becomes present in one defined form.
2. Eliminating interfering substances from the matrix.
3. Obtaining the element in a homogeneous and easily accessible matrix.

The different decomposition methods could be classified in to three categories.

I. Wet digestion

The majority of wet digestion methods involve different combinations of five acids (HNO_3, H_2SO_4, $HClO_4$, HCl, and HF) and H_2O_2. The choice of an individual acid or combinations of acids depends upon the nature of the matrix to be composed. The procedure could take place either in open system or closed system (bomb decomposing with conventional or microwave heating) [58, 60, 61, 66-67].

II. Dry ashing

Dry ashing is usually performed by placing the sample in an open inert vessel and destroying the combustible (organic) portion of the sample by thermal decomposition using a muffle furnace. After decomposition the residue is dissolved in acid and transferred to a volumetric flask prior to analysis. Typical ashing temperatures are 450 to 550 ^{u}C. Magnesium nitrate is commonly used as an ashing aid. Dry ashing is also conducted at 50- 100 ^{u}C under reduced pressure in an oxygen plasma discharge. Keeping its advantage and simplicity, it has some limitation [44, 58-59, 61-69]. These are:

- Losses due to retention to the ashing container.
- Losses due to volatilization.
- Contamination from the ashing container.
- Contamination from the muffle furnace.
- Physical loss of 'low density' ashes when the muffle door is opened (air currents).
- Difficulty in dissolving certain metal oxides.
- Formation of toxic gases in poorly ventilated area.

III. Fusion

Fusion is a powerful technique especially both for organic matrices and those with a high silica and alumina content. Since solid and aggressive fusion reagents are difficult to purify, fusion cannot be recommended as ultra trace analysis. A second disadvantage is that the method is carried out in contact with ambient air. Risks of volatilizations are large [44, 69].

3. MATERIALS AND METHODS

3.1. Apparatus, Instrument and Chemicals

3.1.1. Apparatus and Instrument

A digital analytical balance (Mettler Toledo, Model AG204, Switzerland) with ±0.0001 g precision was used to weight Kocho, Enset and Soil samples. A drying oven (Digit heat, J. P. Selecta, S. a, Spain) was used to dry Kocho sample. Mortar and Pestle was used to graound Enset, and Soil samples. A 250 ml round bottomed flasks fitted with reflux condensers were used in Kjeldahl apparatus. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the digested samples until analysis.ANALYTIK Gena ZEEnit700P (German) atomic absorption spectrophotometer equipped with deuterium ark back ground correctors was used for the analysis of the analyte metals using air-acetylene flame.

3.1.2. Reagents and Chemicals

Reagents used in the analysis were all analytical grade. 70% HNO_3 (spectrosol, BDH, England) and 70% $HClO_4$ (Aldrich, A. C.S. reagent Germany) 30% H_2O_2 and conc. HCl were used for digestion of Kocho, Enset and Soil samples. Lanthanum nitrate hydrated (98%, Aldrich, Muwaukee, USA) was used to avoid refractory interference (for releasing calcium from the phosphates). Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Zn, K, Ca, Mn, Ni, Pb, Fe, Cu, Cd and Cr (BUCK SCIENTFIC PURO-GRAPHIC ^{tm}) were used for preparation of calibration standards and in the spiking. Deionized water was used throughout the experiment for sample preparation, dilution and rinsing apparatus prior to analysis.

3.1.3. Cleaning apparatus

Apparatus such as volumetric flasks, measuring cylinder and digestion flasks were washed with detergents and tap water, rinsed with deionised water, soaked in nitric acid for one day, rinsed with deionised water three times, dried in oven and kept in contamination free place until analysis.

3.2. Description of the Study area

3.2.1. Location

EnemorenaEnerWoreda is situated in Gurage Zone of Southern Nations, Nationalities and People's Regional State (SNNPR), Ethiopia. Amogera water shade is found in Enemor ena Ener woreda, geographically it extends from 37^{o} 51'7.711'' North latitude and from 7^{o} 59'57.382'' East longitude. Mean annual temperature is 26.5 ^{0}C with an average annual rainfall of 1780 mm. The woreda is located 197 and 42 kilometers to south from Addis Ababa and Wolikite town respectively; and its administrative center is Gunchire, and its altitude is 2097 m above sea level. It is surrounded in North by ChehaWoreda, in South by Hadiya Zone and EndegagnWoreda, in the West Yem Special Woreda and Oromiya Region and in East by GetoWoreda. The altitude ranges between1001 and 3400 meters above sea level. The Woreda has a total land size of 107584 hectares[37].

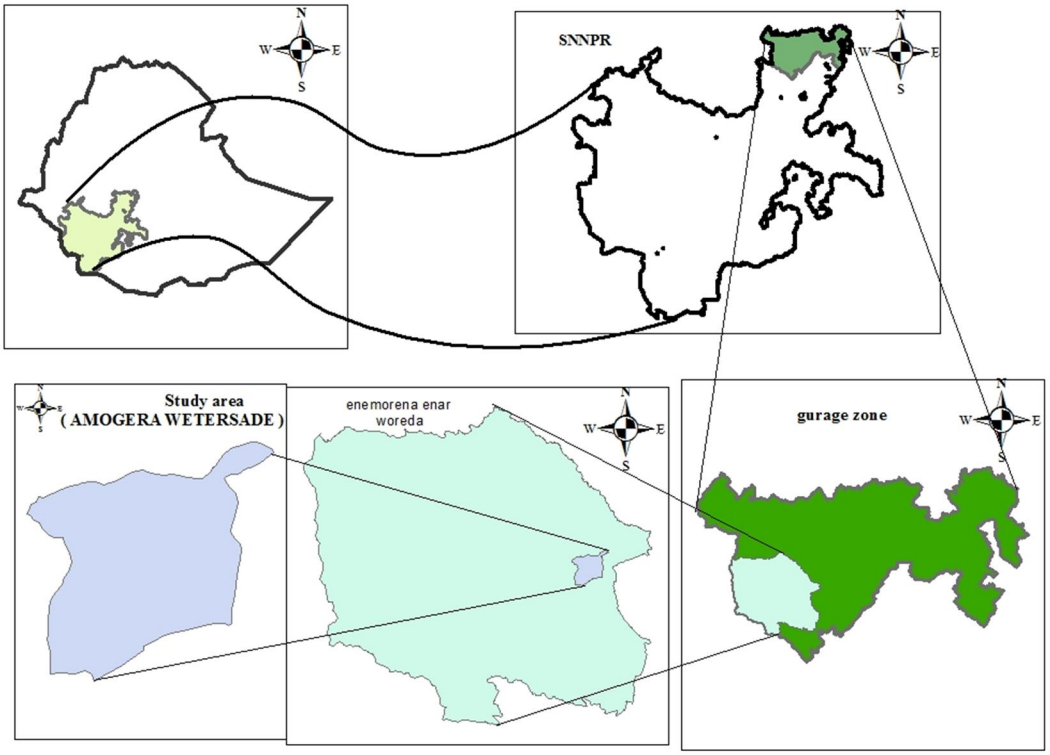

Figure: 2 Location Map of Study Area

3.3. Sampling and sample preparation

3.3.1. Collection and preparation of Enset sampling

An Enset farm was selected randomly from Amogerawater shade in EnemoreenaEnerWoreda. From this farmlands two unprocessed Enset samples were collected, that were 10 m separation between them. One kilogram of corm, edible part of these plantsweretaken for each sample from the farm. The soil was removed mechanically and then washed with distilled water, while the roots were removed from the plant with hands before the samples were cut in to pieces. The sample was packed in clean plastic bags, sealed and transported to the laboratory for processing and analysis. The Enset sample was sun dried for several weeks to reduce the moisture content and to get constant weight so that to express the result in dry mass basis. The sun dried samples were finely grounded in a mortar using a pestle and sieved through 0.5 mm sieve. The prepared samples were kept in dry clean plastic bags until digestion.

Table 2: Some characteristics of Enset samples collected from Amogera water shade.

Characteristics	Sample number	
	Enset 1	Enset 2
Height (m)	5	5.3
Age (years)	7	7

3.3.2. Collection and preparation of Kocho samples

The processed Kocho (5 Kg) was taken from the pit and put in to a plastic bucket and soaked in water, the fibers that were floated removed, transferred into other plastic bucket by sieving in order to removed unloaded fibers, it was transferred into a container, which has very small hole to remove water, to lich out the water from the Kocho after the water removed it transferred into plastic container and dried with sunthis process was performed by the people live the study area, the sun dried Kocho was collected from the study area. To be sure for complete removal of moisture it was allowed to dry in the oven for 24 hours at 60^0C temperature [20]. It was kept in plastic bag until digestion takes place.

3.3.3. Soil Sample Collection and preparation

At the spots where the Enset samples were plucked a total of 2(about 500g each) corresponding soil samples were taken at 75cm canopy radius of each Enset plant in 20cm depth using pickaxe and spade. The samples were collected in clean polyethylene bags. All soil samples were air dried ;friable fragments (>2mm) such as shells, organic debris, schist, weathered rock, calcareous, nodules and other concretions of pathogenic nature, gravel, stones etc were removed. The dried soils were grounded manually using ceramic mortar and pestle to pass a 2mm sieve and store in clean card boxes until analysis.

3.4. Digestion of Samples

3.4.1. Digestion of Enset Sample

By applying the optimized procedure, 2g of powdered Enset sample was placed in a 250 mL round bottomed flask and 4 mL 70% HNO_3 and 4ml 70% $HClO_4$, 2 mL 30% H_2O_2 were added to it. The mixture was heated under reflux for 3 hour on a kjeldahl digestion unit setting the temperature to 120 ^{0}C. At the end of heating period the mixture was cooled to room temperature for 25 min. The cooled digest and its washing were filtered into a 50mLvolumetric flask. Finally the digest was diluted to the mark with deionised water it was ready for the determinations by FAAS [38]. The digests were prepared in triplicate for each sample. A blank solution containing the same reagents (4 mL 70% HNO_3, 4 mL 70% $HClO_4$ and 2 mL 30% H_2O_2) and subjected to the same digestion procedure was prepared for correcting effect of the blank.

3.4.2. Digestion of Kocho Samples

By applying the optimized procedure, 2g dried Kocho sample was transferred in to a 250 mL round bottomed flask, to this 4 mL of 70% HNO_3, 4 mL of 70% $HClO_4$ and 2 mL of 30% H_2O_2 was added and the mixture was digested on a micro kjeldahl digestion apparatus by setting the temperature 120^{0}C for 3 hour then the digested solution was allowed to cool for 25 min without dismantling the condenser from the flask. To the cooled solution deionized water was added (5-8 drop) to dissolve the precipitate formed on cooling. The cooled digest and its washing was filtered in to a 50 mL volumetric flask, finally the digest was diluted to the mark (50mL) with deionised water and ready for the determinations by FAAS. The digests were

prepared in triplicate for each sample. The blank solution was prepared following the same digestion procedure as the sample.

3.4.3. Soil Sample Digestion

0.5g of soil was accurately weighed and was transferred in to 250ml round bottom flask 7.5ml conc. HNO_3 and 2.5ml conc. HCl; 2ml of H_2O_2 were added to the flask and was digested on a kjeldahl digestion block under reflux condenser for 3h at 120^0c. The digest was left to stand for 25min to cool down to room temperature then to the cooled solution deionnized water was added to dissolve the precipitate formed on cooling. The cooled digest and its washing were filtered in to a 50 mL volumetric flask and were filled up to the mark with deionized water. The solutions were used for the analysis of the total metal concentrations by FAAS. The digests were prepared in triplicate for each sample. The blank solution was prepared following the same digestion procedure as the sample [39].

3.4.4 Soil pH

Soil pH was measured potentiometrically by a pH meter in a suspension of a 1:2.5 soil water mixture. About 10 g of air dried soil (<2 mm) was weighed and transferred in to 100 ml beaker and 25 ml of water was added. The sample was stirred by a magnetic stirrer and the pH was measured after allowing the suspension to stand for 1 hour at room temperature [43]

3.4.5 Optimization of the Working Procedure

The basic requirements of sample preparation for analysis are to get an optimum condition for digestion. In this study to prepare clear and colorless sample solution which is suitable for analysis using FAAS, different Enset and Kocho digestion procedures were optimized using HNO_3, $HClO_4$ and H_2O_2 acid mixtures by varying parameters reagent volume, temperature and time. The optimized procedures were selected based on; clarity of digests, minimal reagent volume consumption, simplicity and temperature applied for complete digestion of samples. Optimized digestion procedures for Enset and Kocho sample are shown in Table 3

Table 3: Different trial tested during the optimization procedures for Enset and Kocho

Method	Amount of sample(gm)	Reagent volume(ml)	Temperature(^{0}C)	Time (min)	Result before filtration and dilution
1	2 2	3ml 70% HNO_3 4ml 70% $HClO_4$ 2ml 30% H_2O_2	120	60	Yellowish solution with residue
2	2 2	4ml 70% HNO_3 3ml 70% $HClO_4$ 2ml 30% H_2O_2	120	120	Light yellow solution with suspension
3	2 2	4ml 70% HNO_3 4ml 70% $HClO_4$ 2ml 30% H_2O_2	120	180	Clear, colorless solution with small residue
4	2 2	4ml 70% HNO_3 4ml 70% $HClO_4$ 2ml 30% H_2O_2	120	150	Clear, red color solution with residue
5	2 2	3.5ml 70% HNO_3 2.5ml 70% $HClO_4$ 2ml 30% H_2O_2	120	160	Blood red color solution with residue
6	2 2	3ml 70% HNO_3 4.5ml 70% $HClO_4$ 2ml 30% H_2O_2	120	180	Clear, colorless solution with small residue

3.5. Determination of metals in the sample

In this study a total of ten metals for each of Enset, Kocho and soil samples were analyzed using flame atomic absorption spectrophotometer with external calibration curve. For each metal three replicate determinations was carried out for ten metals (K, Ca, Cr, Mn, Ni, Cu, Zn, Cd, Fe, and Pb) by absorption mode while K was determined by emission mode. The operating conditions of the instrument for FAAS employed for each analytes are shown in Table 4.

Table: 4 Instrumental operating conditions for determination of metals in Enset, Kocho and Soil samples using FAAS.

Elements	Parameters			
	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Instrumental detection limit (mg/l)
K	766.5	0.8	4	0.05
Ca	422.7	1.2	3	0.025
Cr	357.9	0.2	4	0.025
Ni	341.5	0.2	2	0.035
Cu	324.8	1.2	2	0.05
Zn	213.9	0.5	2	0.07
Mn	279.5	0.2	5	0.032
Pb	283.3	1.2	2	0.03
Cd	228.8	1.2	2	0.012
Fe	248.3	0.2	4	0.02

3.5.1. Instrument Calibration

The instrument was calibrated using standard working series of solutions of each of the metals. The standard working solutions of each metal were prepared freshly by appropriate dilution of the intermediate standards. Working standard solutions and values of correlation coefficients of the calibration graph for each of the metals are presented in Table 5. The calibration graph of each metals of interest is shown in Appendix.

Table 5 Concentration values of working standard solutions and correlation coefficients of calibration graph.

Metal	Wavelength (nm)	Conc. of standard series (mg/L)	Correlation coefficient of calibration graph
Ca	422.7	0.25, 0.5, 1, 2	0.998
K	766.5	2, 4, 6	0.995
Cr	357.9	0.25, 0.5, 0.75,1	0.993
Ni	357.9	1, 2, 3, 4	0.991
Cu	324.8	0.25, 0.5, 1, 2	0.996
Zn	213.9	0.25, 0.5, 0.75,1	0.992
Fe	248.3	2, 4, 6, 8	0.999
Mn	279.5	0.25, 0.5, 1, 2	0.998
Pb	283.3	0.25, 0.5, 1, 2	0.998
Cd	228.8	0.25, 0.5, 1, 2	0.993

3.5.2. Method Detection Limit

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

Seven blank samples (deionised water, nitric acid and perchloric acid) were digested and each of the samples were determined for the elements, metal concentrations for K, Ca, Ni, Fe, Cu, Zn, Mn, Cr, Pb and Cd by atomic absorption spectrophotometer (AAS). The standard deviations for each element were calculated from the seven blank measurements of the elements, to determine method detection limit of the instrument, taking the absorbance of 7

blank solution and calculated in Y= mx + b to obtain the concentration of the blank solution. Calculating the standard deviation of this concentration, finally the detection limits were obtained bymean concentration of the blank plus 3δ of the reagent blank.

3.5.3. Recovery Tests

Due to the absence of certified reference material for Enset, Kocho and Soil sample in the laboratory, the efficiency of the optimized procedure was checked by adding known concentration of ach metal in 2 g of Enset and 2 g of Kocho samples. The spiked and non-spiked samples were digested and analyzed in similar condition. Then the percentage recovery of the analyte calculated by:

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

As shown in Table 6, 7 and 8, the results of percentage recoveries for the studied metal nutrients in Enset and Kocho were all between 88 to 97% and 86 to 97% resepectively.Therefore, this verifies that the optimized digestion procedure was valid (good accuracy) for Enset and Kocho sample analysis.

Table 6 Recovery test results for Enset samples

Metal	Conc. in sample (ppm)	Amount added (ppm)	Conc. in spiked sample (ppm)	% Recovery
Fe	4.97	2	6.87 ± 0.51	95± 0.5
K	2.10	2	4.04 ± 0.004	97±0.0045
Mn	0.83	2	2.7 ±0.029	93±0.032
Ca	1.30	2	3.2 ± 0.004	95± 0.02
Zn	0.65	2	2.4 ± 0.005	88± 0.04
Cu	0.26	1	1.22± 0.002	97± 0.0074
Ni	1.31	1	2.22 ± 0.003	91±0.24
Cr	0.46	1	1.42 ± 0.001	96± 0.009
Pb	0.033	1	1.00 ± 0.0002	92± 0.006

Table 7 Recovery test results for Kocho samples

Metal	Conc. in sample (ppm)	Amount added (ppm)	Conc. in spiked sample (ppm)	% Recovery
Fe	7.3	2	9.2 ± 0.50	95± 0.46
K	2.00	2	3.95 ± 0.11	97± 0.07
Mn	0.55	2	2.45 ±0.01	95± 0.01
Ca	4.2	2	6.1 ± 0.001	95± 0.37
Zn	0.38	2	2.1 ± 0.001	86± 0.02
Cu	0.15	1	1.11 ± 0.004	96±0.01
Ni	1.34	1	2.29 ± 0.002	95± 0.005
Cr	0.33	1	1.29 ± 0.004	96± 0.003
Pb	0.087	1	1.05 ± 0.0001	90± 0.002

Table 8 Recovery test results for Soil samples

Metal	Conc. in sample (ppm)	Amount added (ppm)	Conc. in spiked sample (ppm)	% Recovery
Fe	6.61	2	8.5 ±0.05	94± 0.05
K	2.30	2	4.17± 0.1	93± 0.12
Mn	2.44	2	4.2 ± 0.01	88± 0.01
Ca	0.69	2	2.66 ± 0.01	99±0.05
Cu	0.51	1	1.45 ± 0.001	95± 0.004
Zn	1.54	2	3.5 ± 0.0003	98± 0.003
Ni	0.95	1	1.9± 0.01	97± 0.6
Cr	0.54	1	1.51 ± 0.001	97± 0.3
Pb	0.73	1	1.69 ± 0.005	96± 0.01

The values of percentage recoveries for the studied Essential, non-essential and toxic metals in soil samples were within the range 88 to 99.

3.5.4 Analytical Method Detection Limit

The calculated limit of detection for Enset, Kocho and Soil are given in Table 9. The method detection limits are generally comparable with that of instrument for Enset, Kocho and Soil samples.

Table 9 Method detection limits for Kocho, Enset and Soil samples

Metal	Instrument detection limit (mg/l)	Method detection limit for Enset (mg/g)	Method detection limit for Kocho (mg/g)	Method detection limit for Soil (mg/g)
Ca	0.025	0.110	0.032	0.109
K	0.05	0.252	0.07	0.07
Zn	0.07	0.14	0.072	0.08
Cr	0.025	0.046	0.029	0.052
Fe	0.02	0.037	0.05	0.035
Cu	0.05	0.07	0.092	0.07
Ni	0.035	0.051	0.042	0.037
Pb	0.03	0.05	0.07	0.06
Cd	0.012	0.022	0.03	0.021
Mn	0.032	0.042	0.034	0.037

3.5.5 Soil pH

The value of soil pH of the soil for the selected field of the farm is 6.1, which categorize the soil under moderately acidic soil. The moderate acidity of the soil is mainly attribute to the continuous use of natural fertilizer which is manure and organic rsidues.

3.6. Data analysis

Depending upon the type and nature of results at hand, there are different statistical methods used to check whether there is a difference in results of analysis or not; and if there is a difference, statistical analysis will tell us the difference is significant or not. For this study t-test is used to test hypothesis about differences between two or more means. The calculation can be made using detail calculations following the formula. It can also be made on a computer using excel. Therefore excel was used to calculate the presence or absence of significant difference in mean concentration of each metal between Enset and Kocho, soil and Enset as indicated in section 4.5

4. RESULTS AND DISCUSSION

4.1 Levels of metals

Analytical data for nutrient elements K, Ca, Mn, Fe, Cu, Zn, Cr, Ni and for the toxic metals Cd and Pb in soil, Enset and Kocho are given in Tables 10, 11 and 12 and Figures 3, 4 5 and 6.Among the identified elements except cadmium which is below the detection limit all have been identified. The most abundant metal among the macro-element isCa followed by K where as Fe and Zn content of Kocho, Enset and Soil are the predominant among the tested essential and non-essential nutrient. The remains metals vary from their respective samples.

Table 10 mean concentration (X ± SD, n=3, µg/g dry weight) of essential and non-essential metal in Enset samples from Amogera water shade.

Metal	Enset
Fe	124.3 ± 1.1
K	5250 ± 0.004
Mn	20.8 ± 0.003
Ca	3250 ± 0.04
Cu	6.5 ± 0.01
Zn	16.3 ± 0.1
Cr	11.5 ± 0.001
Ni	32.7± 0.5
Pb	0.83 ± 0.001
Cd	ND

ND =Concentration of the tested metal below the detection limit.

Table 11mean concentration (X ± SD, n=3, µg/g dry weight) of essential and non-essential metal in Kocho samples from Amogera water shade.

Metal	Kocho
Fe	182.5 ± 0.4
K	5000 ± 0.1
Mn	13.75 ± 0.05
Ca	10500 ± 0.7
Cu	6.5 ± 0.02
Zn	9.5 ± 0.001
Cr	8.3 ± 0.02
Ni	33.5 ± 0.01
Pb	2.2 ± 0.003
Cd	ND

ND =Concentration of the tested metal below the detection limit.

Table 12mean concentration (X ± SD, n=3, µg/g dry weight) of essential and non-essential metal in Soil samples from Amogera water shade.

Metals	Soil
Fe	165 ± 0.04
K	5750 ± 0.11
Mn	62.5 ± 0.01
Ca	1725 ± 0.1
Cu	13.5 ± 0.002
Zn	38.5 ± 0.01
Cr	13.5 ± 0.01
Ni	23.7 ± 1
Pb	18 ± 0.02
Cd	ND

ND =Concentration of the tested metal below the detection limit.

4.1.1 Concentration of Metals in Kocho

As it can be seen from Table 11 and Figure 3 and 4, there is a variation in concentration of essential and non-essential nutrients within the processed food (Kocho). Except for Cd, this is below detection limit in Kocho, Enset and Soil. The concentration of metals in µg/g in the samples are: Fe 182.5; K 5000; Ca 10500; Zn 9.5; Cu 6.5; Ni33; Cr 8.3; Mn 13.7 and Pb 2.17. The pattern of concentration of elements in Kocho collected from Amogera water shade was decreased as Ca> K> Fe > Zn >Mn> Cr > Cu > Ni >Pb. As can be seen from the trend, the table and the figure mentioned above Kocho can be good source of essential metals that are essential to human.

As can be seen from the Table 11 and Figure 3 and 4Kocho contain higher amount of Ca, K and Fe the higher levels of K in Kocho according to Mirschner [51] was due to the fact that nutrient elements such as N, P, K, S and Mg are highly mobile in the plant tissue and translocation from old plant tissue to new plant tissue.

Generally, since the soils, which have been used for cultivating the plant is highly fertilized with manure and organic residues therefore the Soil was high in available iron, potassium and calcium. Hence the plant has high amount of these metals [49].

It has been reported that Fe, Cu and Zn are the main elements that plant could accumulate and pass up the food chain. Therefore, the detection of Cu and the concentration of Zn from trace metals in Kocho may be because of the fact that these ions are readily transferred from the soil to plants, and accumulate in the root and tuber of the false stem of Enset plant hence in Kocho.

Since the soil types of Enset growing areas of Ethiopia are moderately acidic to slightly basic with the pH ranges from 5.6 to 7.3, the plant expected to have a better accumulation of micronutrients like iron and zinc [49, 53].

Table 13 Data from other source (literature)

Food	Element (µg/g)					
Kocho	K	Ca	Mn	Fe	Zn	Ref
	2753-4380	498-584	8.58-10.13	92.5-135	27.08-31	19
		1400-2260	36-101	3.2-7.2		56
		60070	-		17	
	32037	6	70			
	1200	53	-		71	
	5000	10500	13.75	182.5	9.5	PS
Cu	Cr	Ni	Pb	Cd		
3.4-4.3	5.96-6.42	5.61	Nd	Nd	19	
6.5	8.3	33.5	2.2	Nd	PS	

Note: **PS**= Present study

As can be seen from the above table, the reported concentration range in (µg/g) of K, Ca, Mn, Fe, Zn, Cu, Cr and Ni respectively were: 2753-4380, 320-2260, 8.58-10.13, 36-138, 3.2-31, 3.4-4.3, 5.46-6.42 and 5.6. Except Fe and Zn which are with in the range, the rest are different. The variation may be attributed from different reason, these can be age of harvested Enset plant, precaution taken during processing, physical and chemical nature of the soil and its mineral nutrients, either of the reasons may lead such variations. Therefore the mineral nutrients of the study Kocho is sufficient as compared with that of the literature.

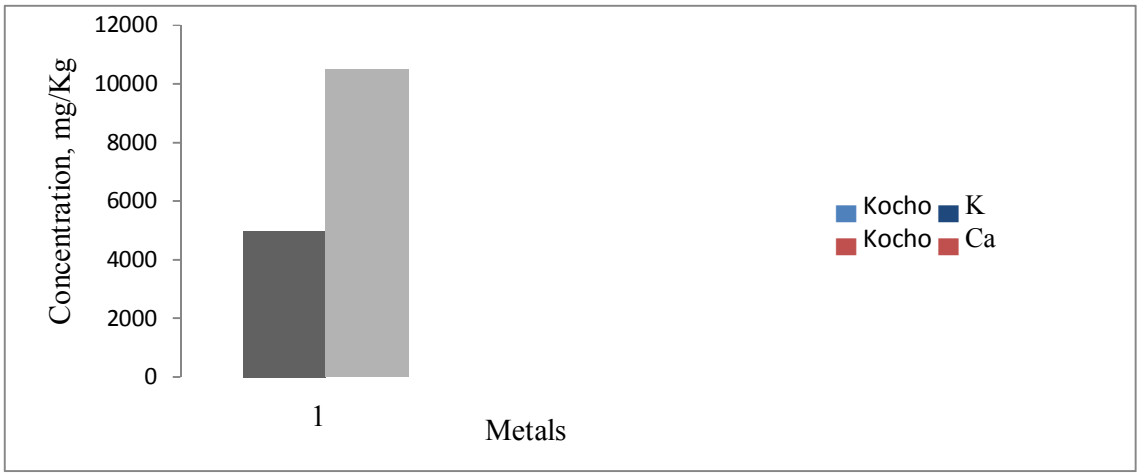

Figure 3 Metal concentrations of essential and non-essential metals in Kocho collected from Amogera water shade.

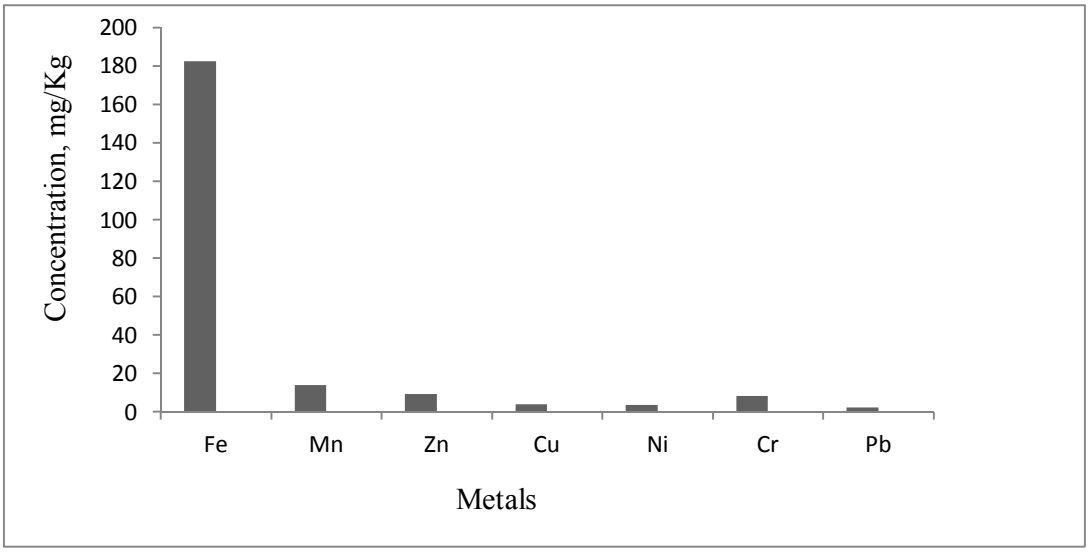

Figure 4 Metal concentrations of essential and non-essential metals in Kocho collected from Amogera water shade.

4.2 Concentration of metals in Enset

Food derived from Enset is protein poor but it contains large amounts of energy [55]. Enset is a major source of carbohydrate, calcium and iron [21-22, 17].

The concentration of selected metals in Enset samples are given in Table 10. 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 [52].

The concentration of selected metals was lowest for lead and highest for potassium. The concentration of the metals in µg/g in the samples are: Fe 124.3; K 5250; Ca 3250; Zn 16.3; Cu 6.5; Ni 32.7; Cr 11.5; Mn 20.7 and Pb 0.83.The trend is K >Ca> Fe >Mn> Zn > Cr >Cu > Ni >Pb. As shown in Table 10 and Figure 5 and 6. A high accumulation of potassium, calcium and iron relative to others is due to their higher nature of abundance in the soil [53]. Whereas, lead concentration was the lowest, this may be due low absorption constant and liable to form complexion with organic mater in the soil.

In relation to the micronutrients the concentration of iron and zinc are higher than the rest. This is as a result of plant requires high amount of abundance of iron in the soil and the pH of the soil ranges from 5.6 to 7.3 [22, 54]. For zinc the reason is due to acidic to slightly basic pH of the soil and high abundance of zinc in the region [22, 54].

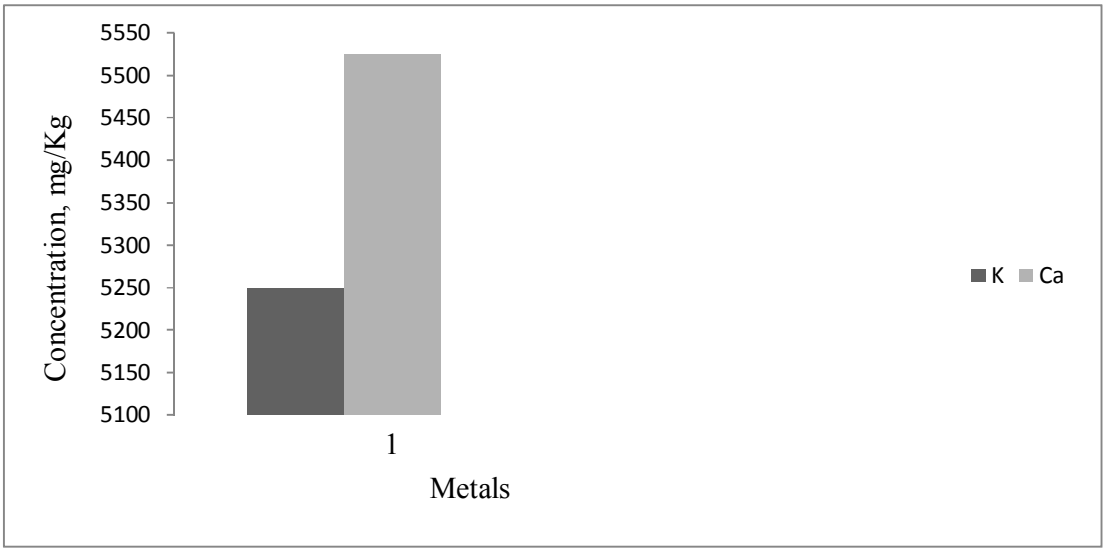

Figure 5 Metal concentrations of essential and non-essential metals in Enset collected from Amogera water shade.

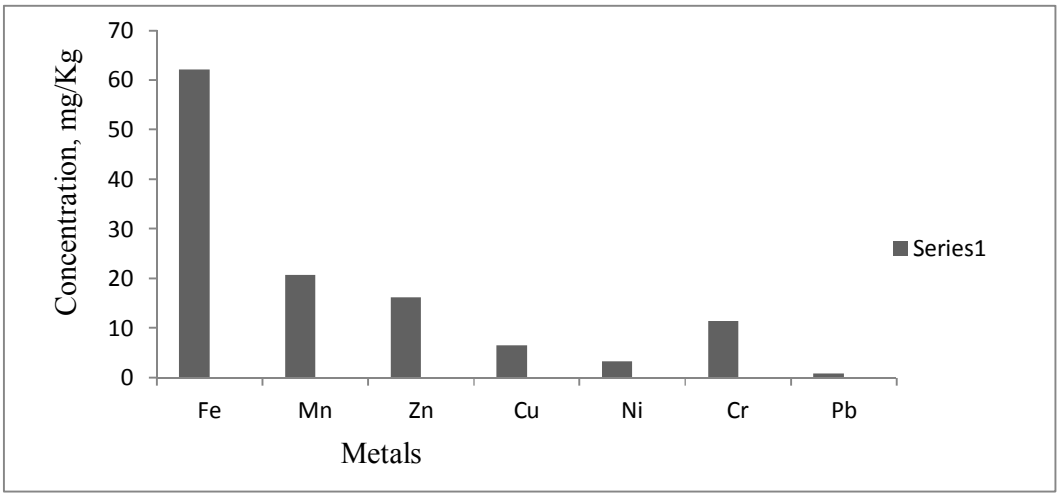

Figure 6 Metal concentrations of essential and non-essential metals in Enset collected from Amogera water shade.

4.3 Concentration of metals in Soil

The concentration of selected metals in soil samples are given in Table 12 and Figure 7 and 8. The trend is: K > Ca > Fe >Mn > Zn > Ni >Pb> Cr > Cu. Among the essential metals K, content of soil is higher within the amount of 5750 µg/g followed by Mn which is 62.5µg/g and Ca with 1725µg/g. The much higher K in soil may be due to the use of fertilizer compostsa the farmers told me. Fe is the predominant metal with the concentration amount 165µg/g. The concentration of Pb in this soil is higher when compared to Cu and Cr; Cd is below detection limit in this soil.

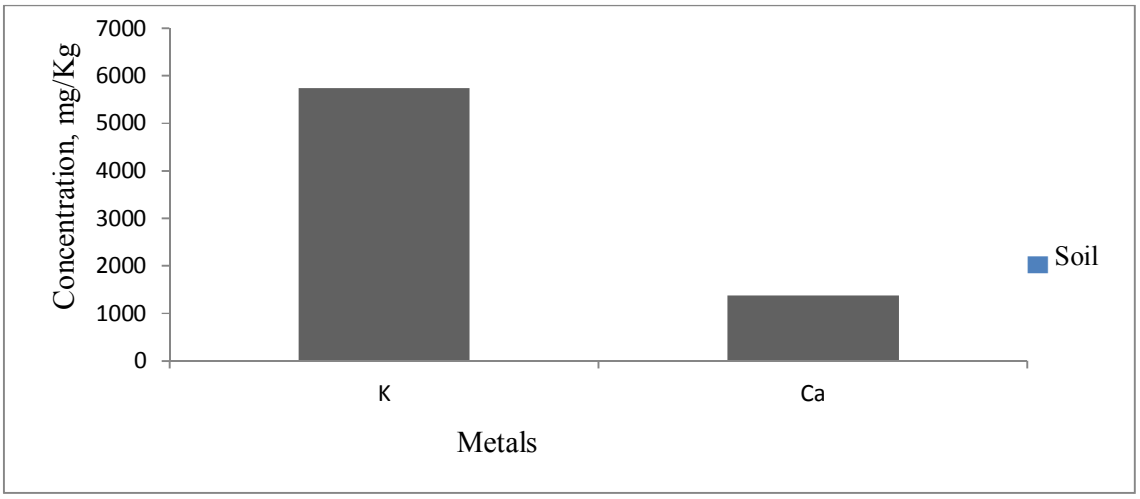

Figure 7 Metal concentrations of essential and non-essential metals in Soil collected from Amogera water shade.

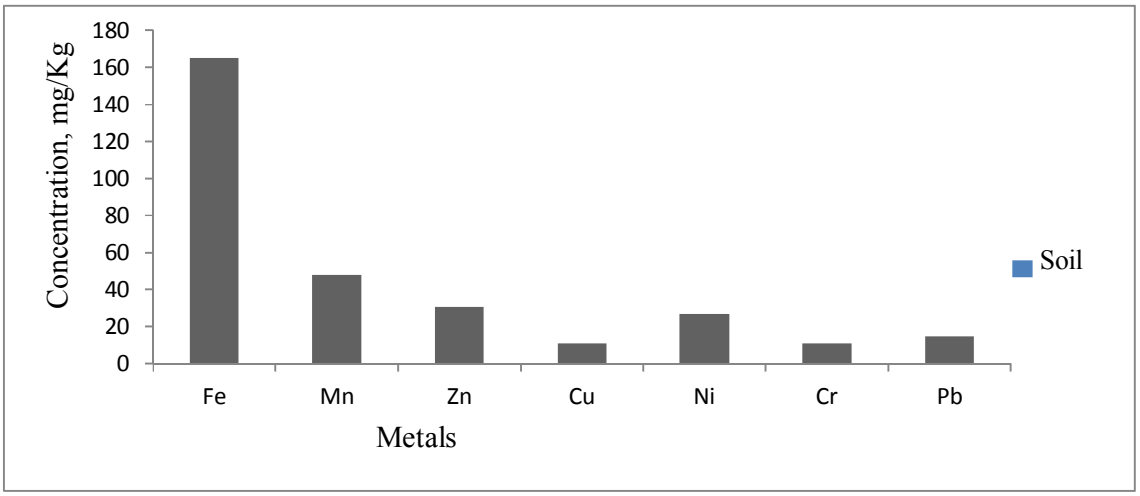

Figure 8 Metal concentrations of essential and non-essential metals in Soil collected from Amogera water shade.

4.4 Correlations of concentrations of metal nutrients in Enset, Kocho and Soil

Kocho is obtained by fermenting the scraped and decorticated edible part of Enset leaf sheath and the corn. During this processes a number of physicochemical processes will be taking place. As reported by many authurs [56, 57], food processing will enhance the nutritional quality of food, and some anti-nutritional elements will also be minimized during fermentation. For instance as reported by Abebe et al. [56], the phytate concentration fermented Enset dramatically decreased as compared to unfermented one. Therefore the bio-availabilities of mineral nutrients from processed Enset foods are greatly enhanced.

Food processing has inevitable consequences on the nutritional value of foods. Beside this enhancement in nutritional quality, there may be loss of some food nutrients and enhancements of certain nutrients through contamination.

Knowledge of the various food processes and the nutrient losses that occur during each process will allow improvements to be made and losses as well as contamination to be minimized. The essential and non-essential nutrients contained within foods show varying degrees of stability when foods are stored or processed.

The comparative study of the mineral contents of Kocho, Enset and Soil is explained as follow. The concentrations of essential and non-essential metal in Enset, Kocho and Soil, collected from Amogera water shade is given in Table 10, 11 and 12. The comparative results

of these nutrients in Enset, Kocho andSoil are also shown in Figure 9 and 10. From Figure 9 and 10, the concentration of metals except iron and calcium all others are higher in Soil, next Enset and lastly in Kocho, in the case of iron calcium higher in processed Kocho next in soil and lastly in Enset. Cadmium is below the detection limit in all samples. For the metals K, Mn, Cu, Zn and Cr the trend is Soil >Enset > Kocho, but for iron and calcium the trend is Kocho > Soil >Enset.

From this trend it is possible to conclude that Kocho contains higher amounts of iron, calcium and potassium. Particularly the increment in Fe concentration in processed food is every processing material are made of iron and wood. In addition to this there may be contamination with dust particles during fermentation and storage. In condition of this some minerals may be released out from the matrix during fermentation.

Cu, Cr, Zn and Mn The concentration of all these metals in the processed food types are below the concentration range when compared with their respective Enset and Soil. This may be beside loss in concentration; there may be substantial contamination during processing. In addition to this, these metals may form water soluble complex in the plant material so that will not be easily leached out during squeezing.

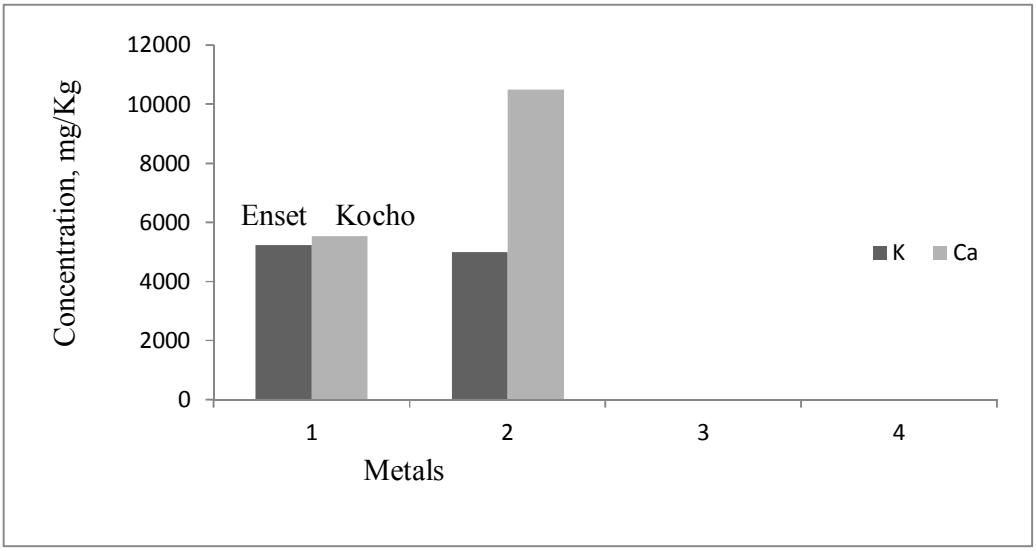

Figure 9 A comparative studies of metal concentrations of essential and non-essential metals in Soil, Koch and Enset collected from Amogera water shade.

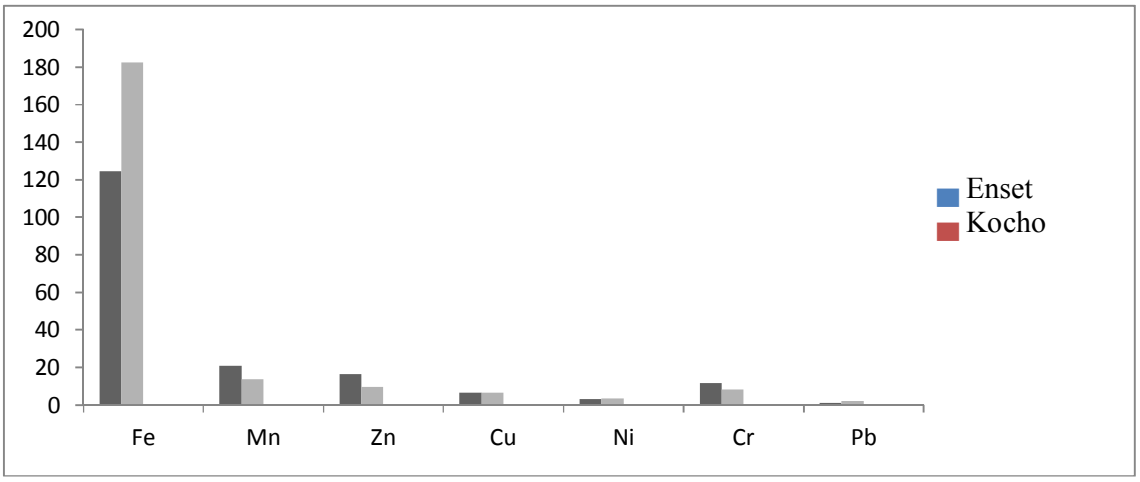

Figure 10 A comparative studies of metal concentrations of essential and non-essential metals in Soil, Koch and Enset collected from Amogera water shade.

Table 14 Metal content of Enset and Kocho (dry mass basis) in µg/g from Amogera water shade

Sample type	Elements									
	K	Ca	Mn	Fe	Cu	Zn	Cr	Ni	Pb	Cd
Enset	5250	3250	20.8	124.3	6.5	16.3	11.5	32.7	0.38	Nd
Kocho	5000	10500	13.75	182.5	6.5	9.5	8.3	33.5	2.2	Nd

As can be seen from the above table 14 there is a decrease in the concentration of these metals in Kocho. A number of processes followed during Enset food processing from decortications to fermentation and preparing for cooking. During these processes some metals are definitely lost from Kocho or added to Kocho, storage conditions and traditional processes can strongly affect different characteristics of Kocho, which determine their mineral content and nutritional value.

4.5 Statistical Analysis

Analysis of t-test for the comparison of Soil and Enset, Enset and Kocho samples

Table 15 Analysis of t-test between the Soil and Enset samples at 95% confidence level.

Metals	Mean concentration of Soil	Mean concentration of Enset	Sd	t-calculate	t-critical	Remark
K	1.73	1.70	0.084	0.305	2.78	Nosignificant difference between the mean
Fe	6.39	4.97	0.658	2.158	2.78	No significant difference between the mean
Mn	2.504	0.828	0.793	2.116	2.78	No significant difference between the mean
Ni	0.962	1.314	0.056	6.311	2.78	There is significant difference b/n the mean
Ca	0.695	1.296	0.258	2.328	2.78	No significant difference b/n the mean
Zn	1.544	0.137	0.094	14.98	2.78	There is significant difference b/n the mean
Cr	0.546	0.463	0.0049	16.85	2.78	There is significant difference b/n the mean
Cu	0.508	0.261	0.0123	19.72	2.78	There is significant difference b/n the mean
Pb	0.732	0.033	0.0087	80.51	2.78	There is significant difference b/n the mean

Sd = standard deviation of the difference between the means

Table 16 Analysis of t-test between the Enset and Kocho samples at 95% confidence level.

Metals	Mean concentration of Enset	Mean concentration of Kocho	Sd	t-calculate	t-critical	Remark
K	1.702	1.698	0.076	0.052	2.78	No significant difference between the mean
Fe	4.971	7.322	0.7	3.359	2.78	There is significant difference between the mean
Mn	0.827	0.548	0.017	16	2.78	There is significant difference between the mean
Ni	1.314	1.1.344	0.198	0.149	0.149	No significant difference b/n the mean
Ca	1.296	4.221	0.428	6.825	2.78	There is significant difference between the mean
Zn	0.653	0.312	0.044	7.749	2.78	There is significant difference b/n the mean
Cr	0.463	0.320	0.0094	14.56	2.78	There is significant difference b/n the mean
Cu	0.261	0.152	0.012	9.12	2.78	There is significant difference b/n the mean
Pb	0.0334	0.0867	0.0021	25.2	2.78	There is significant difference b/n the mean

For the present study the significance of variance between the samples has been studied using t-test. The test indicates the presence and absence of significance difference in mean concentration of each metal between Soil and Enset, Enset and Kocho. The following results were obtained.

From Table 15 and 16, seen that for K, Fe, Mn and Ca, there is no significant differences at 95% confidence interval (p=0.05) was observed in the mean concentrations between Soil and Enset samples. The mean concentration of K do not differ significantly for Enset and Kocho, while Ca, Fe and Mn differ significantly for Enset and Kocho samples when compared with Soil and Enset samples.

For Ni, Zn, Cr, Cu and Pb differ significantly for Soil and Enset samples. But Ni not differs significantly for Enset and Kocho samples. All others (i.e. Zn, Cr, Cu and Pb) differ significantly in Enset and Kocho samples.

5. CONCLUSIONS AND RECOMMEDATION

The study determined concentrations of Essential and non-essential nutrients in Enset, Kocho and Soil, which is collected in Amogera water shade. The results showed that the ability of Enset and Kocho to accumulate relatively high amount of K, Ca and Fe among the determined Essential nutrients.

The soils the study farms was found to contain high levels of K followed by Na, Fe, Mn, Zn, Ni, Pb, Cu and Cr, in this soil levels of Cd was below detection limit. In general, the levels of most of the metal, except Fe and Ca, in the studied soil were found to correlate positively with the levels found in Enset and Kocho.

Enset and its product Kocho is one of the most popular widely consumed in Southern part of Ethiopia. Thus, this study may have significance in delivering a preliminary data in levels of nutrient elements in Enset and Kocho. It might be useful in pointing directions for studies that will be conducted on the nutrient levels of Enset and Kocho with soil it growth in the country.

The data may also helpful for researches that may be conducted on agronomy and physiology of the plant, nutrient requirement and soil-plant nutrient balance of Enset.

Since the soil types of Enset growing areas of Ethiopia are moderately acidic to slightly basic with the pH ranges of 5.6 to 7.3 [22, 17], the plant expected to have a better accumulation of essential metals like iron and zinc, moreover, Enset from the investigated results of the concentration of metals, it is rich in calcium, iron and potassium. However due to the unfamiliarity and less advantageous of using artificial fertilizers as for the 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 optimized digestion method for Enset and Kocho analysis was effective for the majority of the minerals and a good percentage recovery was obtained.

Enset is the first plant which has been cultivated early and about around 20% of Ethiopian peoples are dependent on Enset as food. But, till now there was not enough information about the mineral content of Enset, therefore, this study is essential and crucial to give highlights on the mineral content of Enset and Kocho evenif the number of samples and sites are limited.

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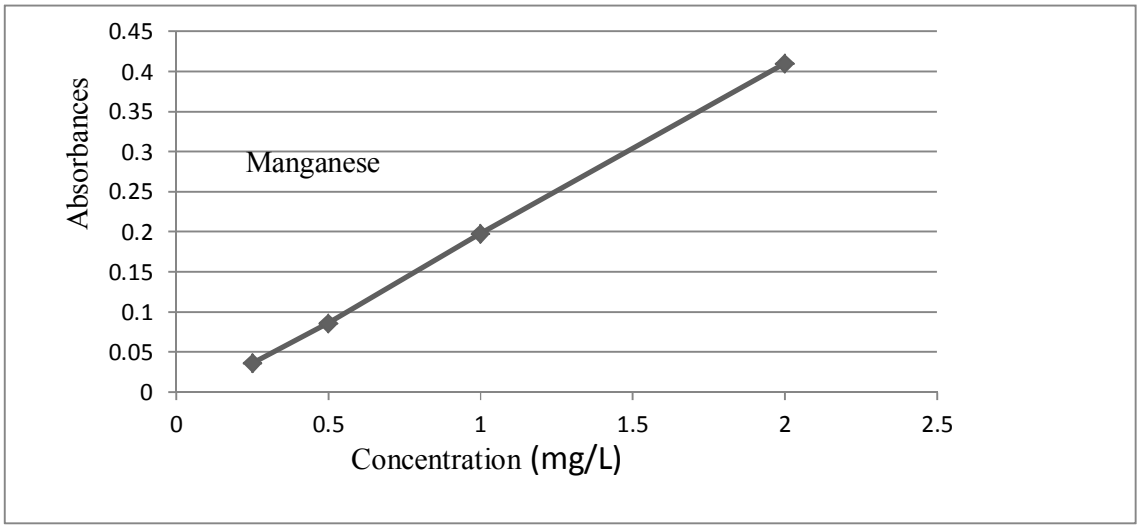
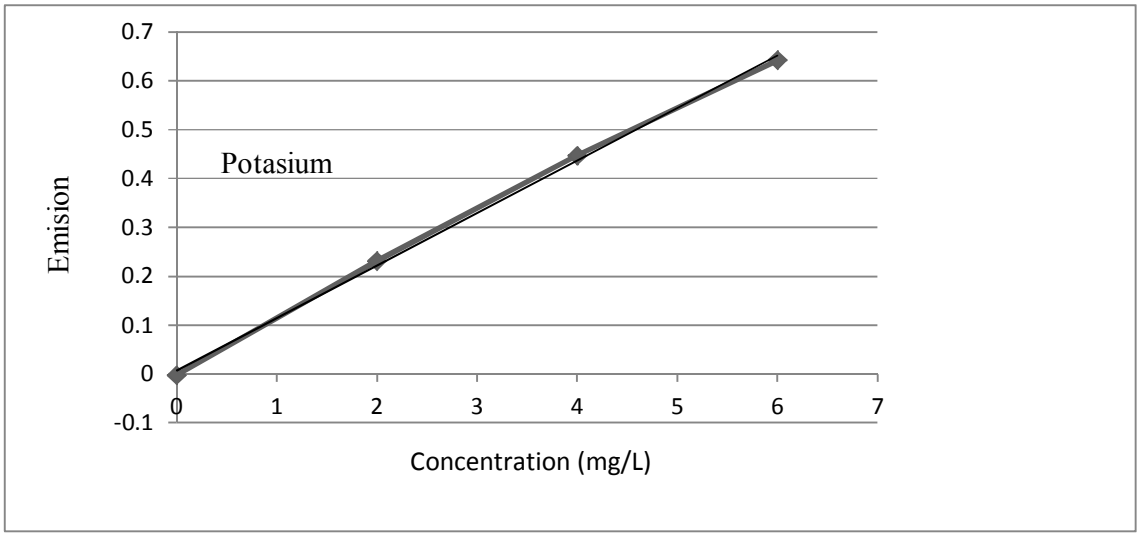
Appendix



Y=0.0008673 +0.05173X

R= 0.998

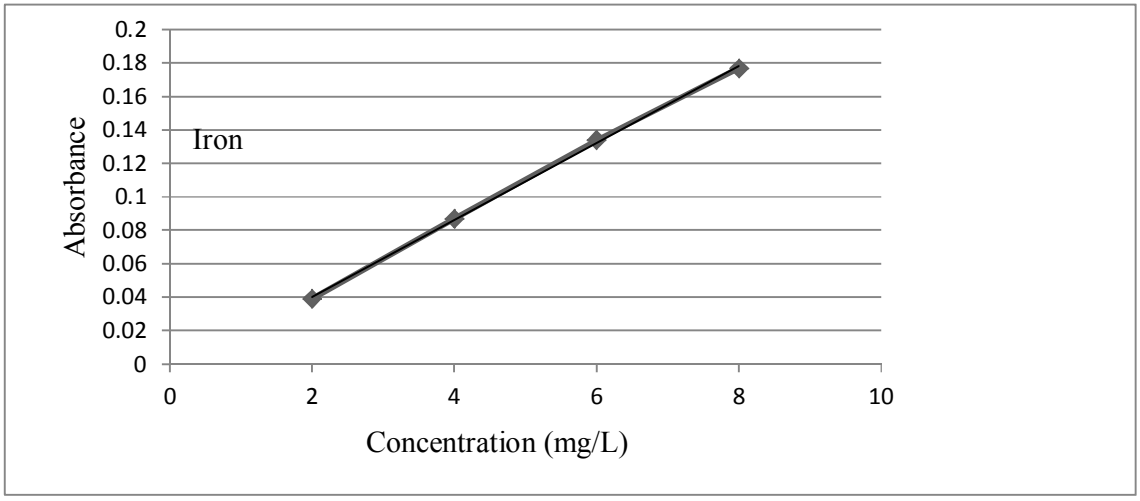
Appendix 1 Calibration graph of Mn standard solution



Y = 0.006596 + 0.10762X

R= 0.995

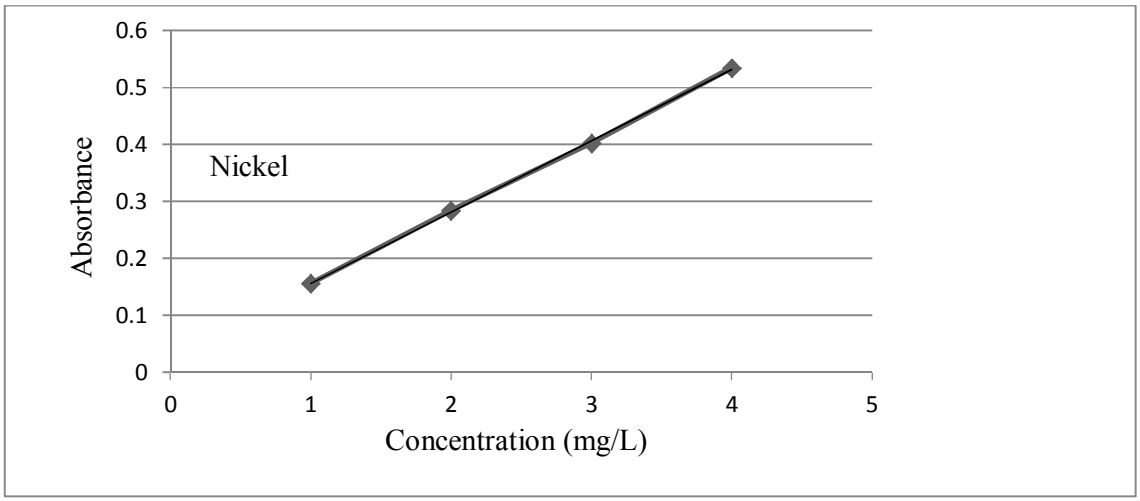
Appendix 2 Calibration graph of K standard solution.



Y = -0.006202 + 0.02305X

R = 0.999

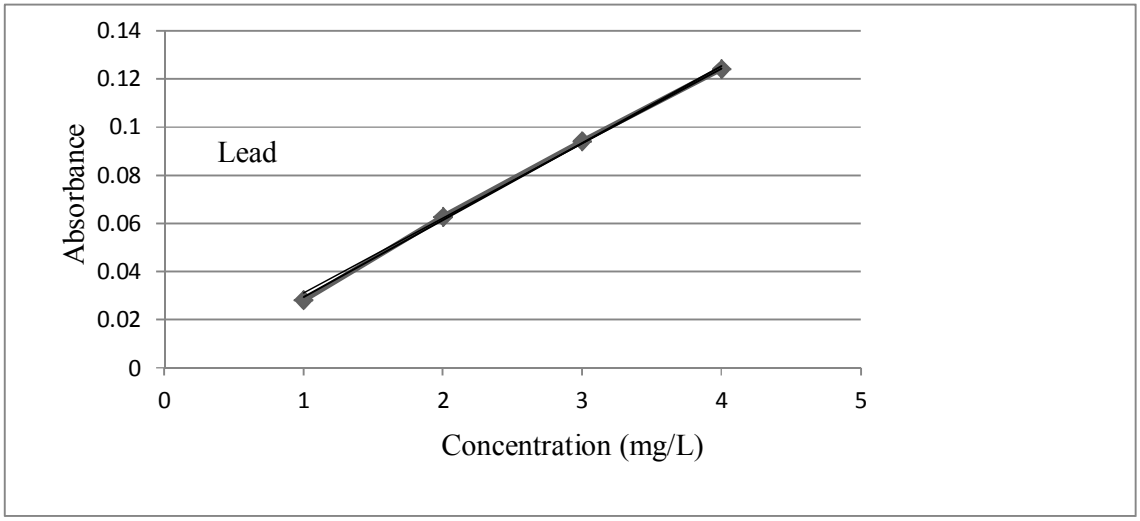
Appendix 3 Calibration graph of Fe standard solution.



Y =-0.005 + 0.135x

R = 0.991

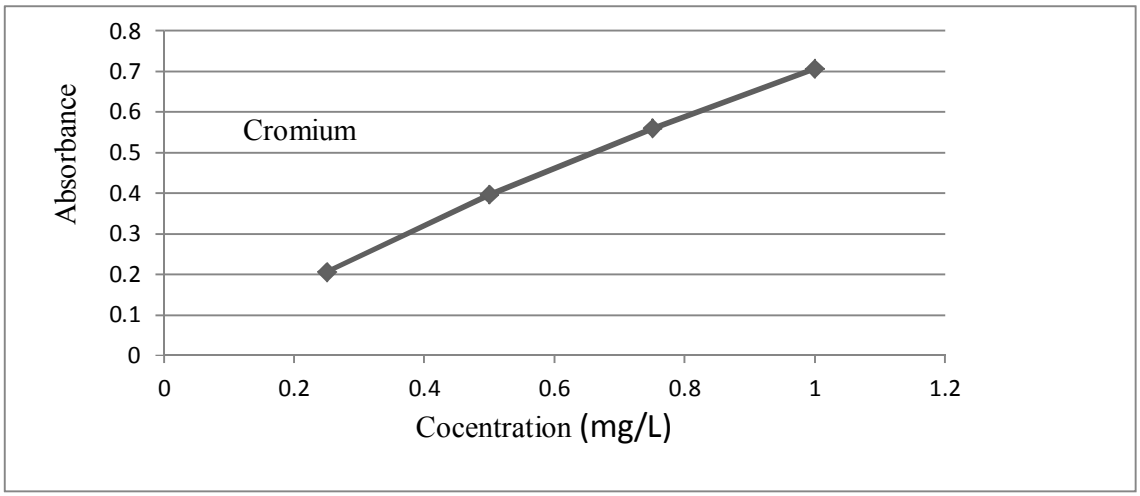
Appendix 4 Calibration graph of Ni standard solution



Y = 0.00782 + 0.03135X

R = 0.998

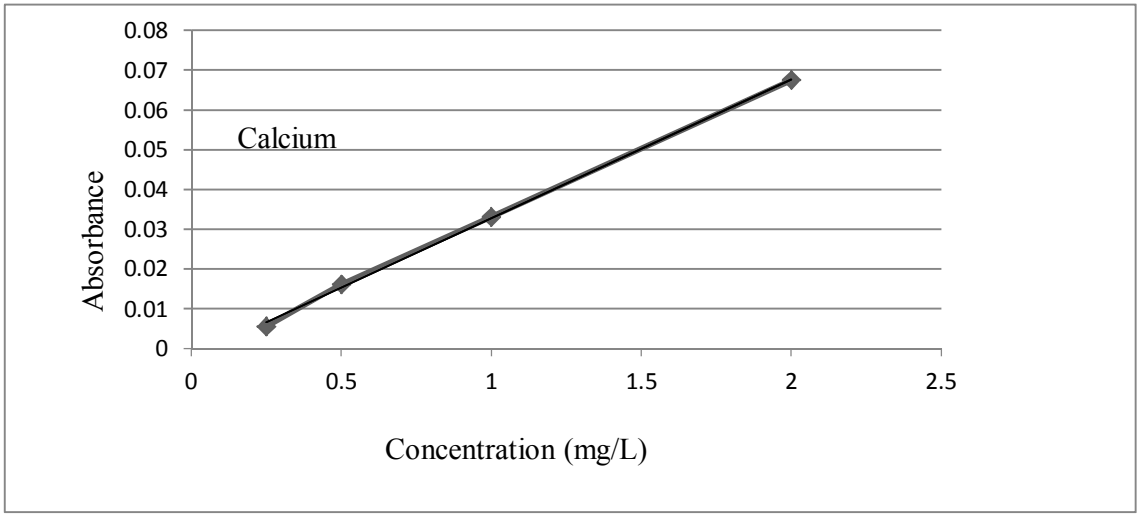
Appendix 5 Calibration graph of Pb standard solution



Y = 0.00718 + 0.07342X

R = 0.993

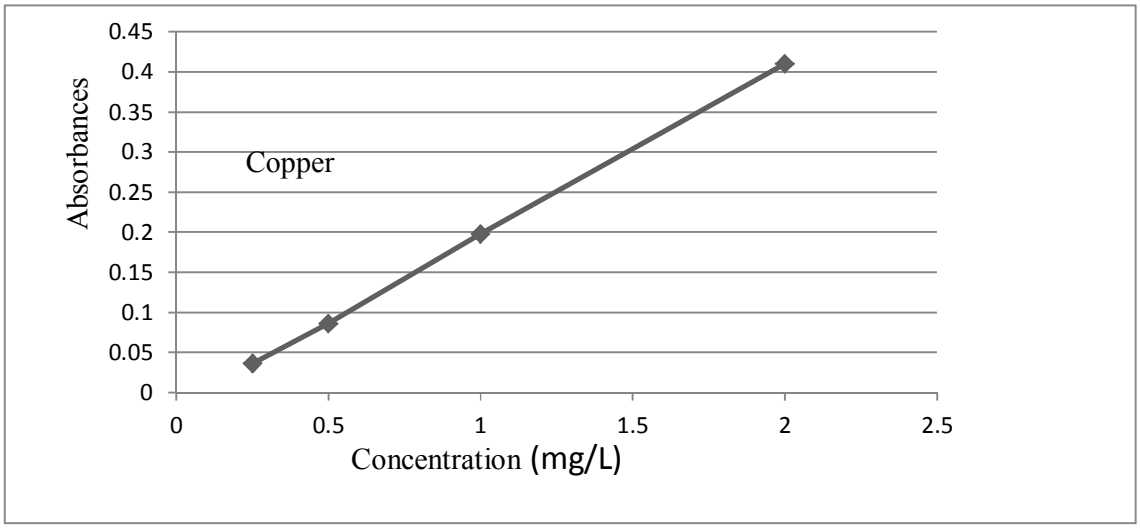
Appendix 6 Calibration graph of Cr standard solution



Y = 0.001824 + 0.03472X

R = 0.998

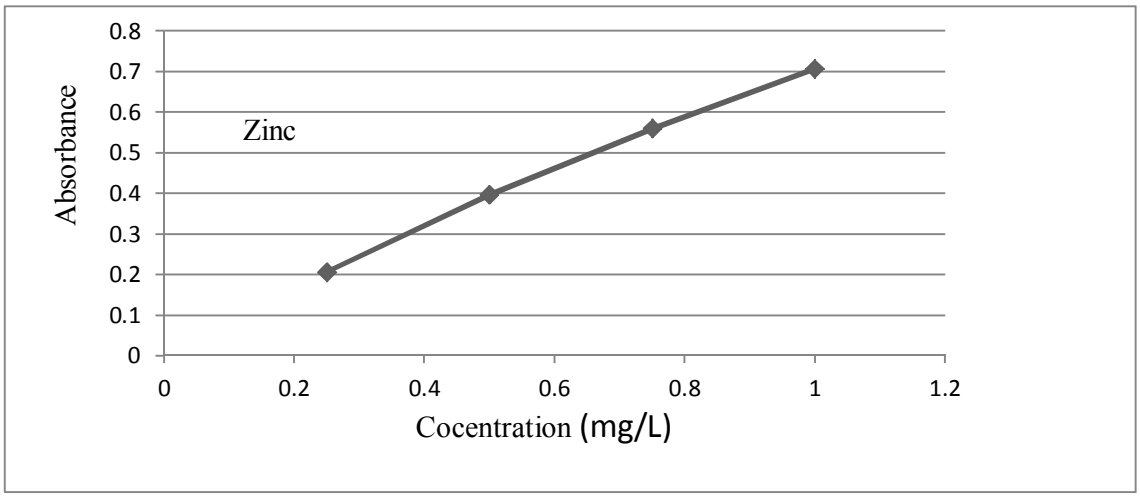
Appendix 7 Calibration graph of Ca standard solution



Y = 0.011134 + 0.20936X

R = 0.996

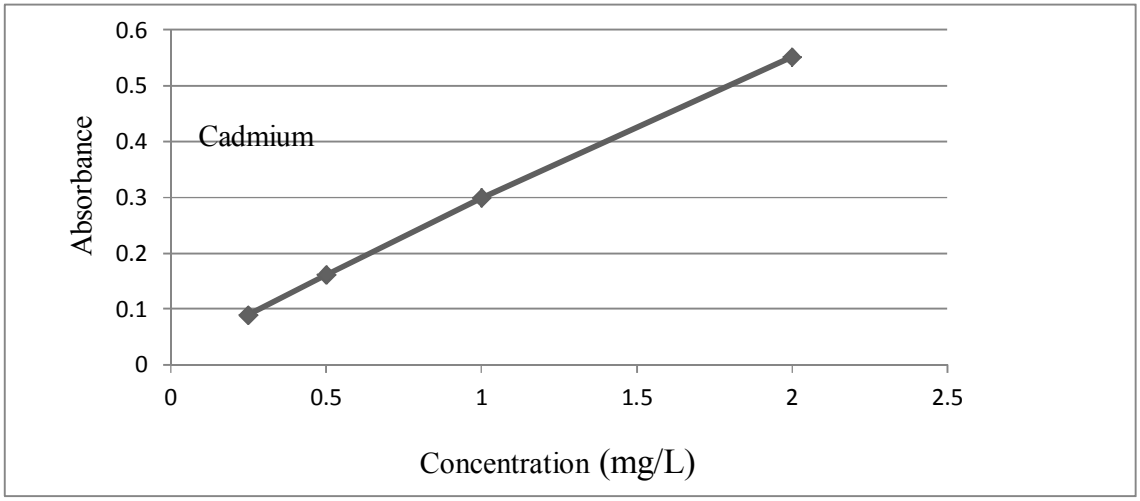
Appendix 8 Calibration graph of Cu standard solution



Y = 0.012467 + 0.70066X

R = 0.992

Appendix 9 Calibration graph of Zn standard solution



Y =-0.007078 + 0.27188X

R = 0.993

Appendix 10 Calibration graph of Cd standard solution