ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

# STUDY OF FENNEL (*FOENICULUM VULGARI* MILL) FRUITS FOR MAJOR AND TRACE ELEMENTS CULTIVATED IN ETHIOPIA

BY

Feleke Demissie Endalamaw

**A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements fore the Degree of Masters Science in Analytical Chemistry.**

JULY - 2010

**Addis Ababa University**
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# ACKNOWLEDGEMENT

I am very grateful to my advisor Prof. B. S. Chandravanshi for his endless constructive comments, help in correcting, editing, criticizing and encouraging in accomplishing this work. I also like to appreciate his patience in following up this work from the very beginning to the end of the project. I have a special respect and appreciation to him, for fatherly advice in all aspect and achievements of today's success.

I would like to express my deepest gratitude to Wolaita Sodo University for allowing me to proceed my education and providing sponsorship. My appreciation also goes to Addis Ababa University for its provision of all resources necessary to accomplish my study from the very beginning to the end.

I am also thankful to the staff members of Chemistry Department, Dr. Yonas Chebude, Head of Department of Chemistry, Addis Ababa University for their kindly cooperation in facilitating conditions and resources throughout my study. In addition, I am thankful to Mrs Aster, department secretary for her kindly approach in all of my work. My at heart thanks should go to the technical assistant Mr. Henok Mekonen for his timely and heartful help in the sample analysis.

Finally, I would like thank miss Tigereda, Mesters Tigistu Bassie, Yazachew Alemu, Kebede Nigussie, Minaleshiwa Atilabachew, Mulunehe Worka, Motbinor Worka, Sunachew, Yalow and others who was supporting me in accomplishing this project.

Above all I thank the **Almighty God** who led me through all the rough and difficult times and gave me strength and encouragement to complete this study.

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# ABSTRACT

## STUDY OF FENNEL (*FOENICULUM VULGARI* MILL) FRUITS FOR MAJOR AND TRACE ELEMENTS CULTIVATED IN ETHIOPIA

**By: Feleke Demissie**

**Advisor: Prof. B.S. Chandravanshi**

Levels of selected metals (Ca, Mg, Fe, Mn, Cu, Cr, Co, Zn, Ni, Cd and Pb) were determined in fennel fruit and the soil from which it was grown. The samples were collected from the Addis Ababa and Gojam area (Northern Ethiopia). Oven dried fennel fruit was grinded and 0.5 g portion of the sample was digested by wet digestion method using 2.5 mL of $HNO_3$ and $HClO_4$ in quick-fit round bottom flasks (100 mL) fitted with reflux condenser by using Kjeldahl micro hot plate apparatus. Similar procedure was followed for soil digestion using 2 mL $HNO_3$ (69-72%), 4 mL 36% and 1.5 mL of $H_2O_2$ (36%). The levels of the above elements were determined using flame atomic absorption spectrometer. The highest concentration of Fe (28,000 µg/g) and Ca (23,000 µg/g) were obtained from the soil and fennel fruit, respectively. Moderate concentrations of Mg (3,400 µg/g and 3,300 µg/g) were obtained from the fruit and the soil, respectively. The level of Cd was lowest (1.91 µg/g and 2.86 µg/g) in the fruit and the soil, respectively. The concentration of Pb was below the methoddetection limit.

**Key words:** Fennel fruit, *Foeniculum vulgari* Mill, Major elements, Trace elements, Flame atomic absorption spectrometer.

# 1. INTRODUCTION

## 1.1 HISTORY, ORIGIN AND DISTRIBUTION OF FENNEL

Fennel is native to Southern Europe and the Mediterranean region and is cultivated mainly in India, Romania, Russia, Germany, France, Italy, Japan, Bulgaria, Greece, USA, Lebanon, Argentina, Madagascar, Indonesia, Vietnam, Brazil, Spain, Guatemala and Sri Lanka. Greek mythology claims Prometheus (a demigod in Greek mythology who stole fire from the gods and gave it to the human race) used the stalk of a fennel plant to steal fire from the gods. In medieval times, fennel was used in conjunction with St John's wort to keep away witchcraft and other evil things. This might have originated because fennel can be used as an insect repellent. Fennel is thought to be one of the nine herbs held sacred by the Anglo-Saxons [1].

Today, as throughout history, fennel (as herbs and spices) is used for their fragrance, flavour, colour, and medicinal properties. Five thousand years ago, the Sumerians (a member of an indigenous non-Semitic people of ancient Sumer in Babylonia) documented medicinal uses of a few herbs. Four thousand years ago, the Chinese used more than 300 herbs for their healing properties. Three thousand years ago, the Egyptians expanded use from the merely pharmaceutical (including mummification) to culinary and cosmetic use. The Greeks used herbs to adorn the heads of their heroes, and the ancient Romans used herbs for magic and sorcery. The middle ages saw herb cultivation, with studies revolving around the monasteries. Superstitions surrounded herbs well into the 18th and 19th centuries, until modern chemistry and the study of the physical sciences began to advance [2].

In the ancient world, herbs were largely medicinal and spiritual and were grown or gathered easily. Spices were a luxury item and, consequently, one of the world's most valuable trade goods. They were light and did not require special preservation like other foods. That, combined with their multiple uses, made spices a hot commodity. While we still use spices for dyes, medicines, and cosmetics, their most beloved characteristic is their flavour. Moreover, spices and herbs were reasons for the discovery of new places and transfer of knowledge worldwide [1, 3].

## 1.2 ETYMOLOGY

Fennel (*Foeniculum vulgare* Mill) is one of the precious spices. It belongs to the family Apiaceae (formerly the Umbelliferae) [4]. Etymologically, the word fennel developed from Middle English *fenel*, *feny*; from Latin *feniculum*, *foeniculum*, diminutive of *fenum, foenum*, meaning ‘hay’. In Ethiopia the plant is called Ensilal. Fennel plant is perennial, erect, glaucous green and grows to 2 m tall. The leaves grow up to 40 cm long; they are finely dissected, with the ultimate segments filiform, about 0.5 mm wide. The flowers are produced in terminal compound umbels 5–15 cm wide, each umbel section with 20–50 tiny yellow flowers on short pedicels. The fruit is a dry seed from 4–9 mm long, half as wide or less, and grooved [1, 5].

## 1.3 WORLD SPICE TRADE

Globally about 661.16 thousand-hectare area are cultivated with the fennel and 467.86 million tons of fennel are produced. Fennel is the 3rd form common spices produced in the world following chilli peppers (dry) and chilli peppers (green) 662.73 million tons and 5,424,803.01 million tons, respectively [6]. The major markets in the global spice trade are the USA, the European Union, Japan, Singapore, Saudi Arabia and Malaysia. The principal supplying countries are China, India, Madagascar, Indonesia, Vietnam, Brazil, Spain, Guatemala and Sri Lanka. From 2000 to 2004, the value of spice imports increased by an average of 1.9% per year and the volume increased by 5.9%. World trade in spices in 2004 consisted of 1.547 million tons, valued at USD 2.97 billion. An annual average rate of 7% was seen in the global import volume of spices in the period 2000–2002 [1].

## 1.4 USED PART OF FENNEL PLANT

**Fruits:** other than most of their relatives, they retain a green colour after drying. As a rule of thumb, a bright green colour indicates a good quality (figure 1 b.).

**Fennel pollen:** it is also known as spice of the angels, has a subtle fennel flavour, lacking some of the sweetness but with a distinct note of pine needles (though others might disagree with this association of mine), figure 1 a.

**Leaves and stalks:** of fennel can be eaten as a vegetable (figure 1 c.) [4].

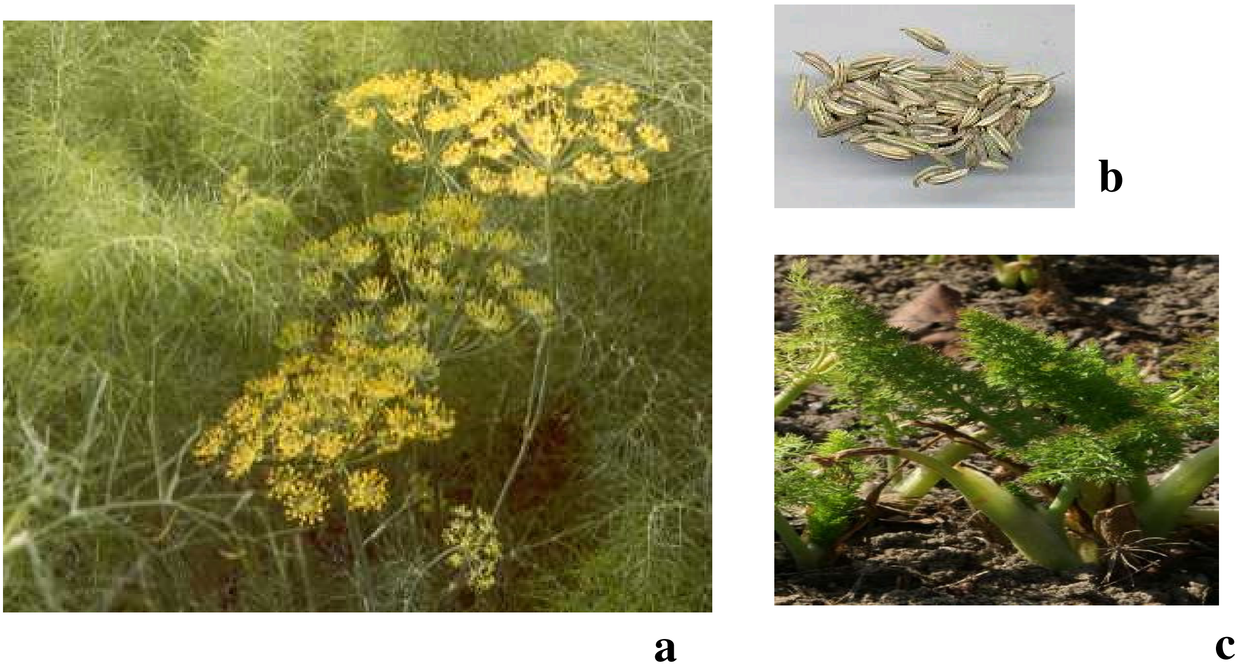


**Figure 1** (a - c**).** parts of fennel plant (fruit, pollen, leaf, and stalk)

Along with providing aromatic and medicinal value fennel fruits said to offer the most rapid and low cost source of trace elements for the majority of people in developing nations [7]. Since people throughout the world consume fennel in different ways and it can be source of micronutrients in addition to the flavour. They are considered as “protective supplementary food" as they contain significant quantities of minerals, vitamins, carbohydrates, essential oils and fixed oils that are required for normal functioning of human metabolic processes [1].

### 1.5 USES

Fennel is used almost in every country. For instance, in India, it is an essential ingredient in the spice mixture called *panch phoron* and in Chinese five-spice powders. In the west, fennel fruits are a very common ingredient, for instance in Italian sausages and Northern European rye breads. Much egg, fish and other dishes employ fresh or dried fennel leaves. Florence fennel is a key ingredient in some Italian and German salads, often tossed with chicory and avocado, or it can be braised and served as a warm side dish. One may also blanch and/or marinate the leaves, or cook them in risotto. In all cases, the leaves lend their characteristically mild, anise-like flavour.

It is one of the most important medicinal and aromatic plants due to its uses as a carminative, diuretic, anti-inflammatory, antimicrobial, Antioxidants act as radical scavengers, inhibit lipid peroxidation and other free radical-mediated processes and are able to protect the human body as well as processed foods from oxidative damage attributed to the reaction of free radicals. An estrogenic activity of fennel reduces pains of urination and menstruation and galactogogue present in fennel increase the production of milk in humans and other animals. Also, it is given to infants in the treatment of flatulence. The volatile oils of fennel are used to control flatulent dyspepsia and colic in children [5, 8, 9]. Moreover results of some study indicate that fennel oil-derived products could be useful for protecting humans from vector-borne diseases and nuisance insects, like mosquito and flies, although their inherent repellence can be affected by various factors such as the frequency and formulation of application, local conditions, and numerical density of mosquitoes or flies [10].

Fennel use is not bounded only in human beings but it extends to animals too. Extracts of fennel can be used to inhibit rumen methanogenic archaea without adversely affecting fermentation of feeds in the rumen at low dose levels as these extracts did not affect digestibility and protozoal numbers in the animal study [11]. In rats, fennel seed showed a significant enhancing effect on intestinal enzymes and provoked a higher rate of bile acids' secretion. Essential oil from fennel inhibits the opening of calcium channels and stimulates that of the potassium channels in smooth muscles, which increases motility of the small intestine and produces a significant shortening of the food transit time [12, 13].

Here in Ethiopia it is also used in different ways traditionally. In Gojam it is added in the local alcoholic drink '*Areki*' as a flavour. They are consumed directly the row seed to improve urination and menstrual problem. Traditionally fennel is used as powder seed together with garlic to resolve stomach-ache. The other use is to give flavour for the food they usually include it as spice directly to the '*wot*' or during preparation of '*Berbere*' and '*Shiro'*.

## 1.6 ACCUMULATION OF METALS IN FENNEL FRUIT

Fennel plants can accumulate trace heavy metals in or on their tissue, thus this plant is intermediate reservoir, through which trace elements from soils, and partly from water and air, move to man and animals [14]. Growth media including soil, nutrient solution, water and air are main sources of heavy metals to plants, which enter by roots or on the surface of the plant thorough adsorption or absorption [15, 16].

Different fruit species accumulate different metals depending on environmental conditions, metal and plant species, available forms of the heavy metals [17]. Many plants are found to be in a position to take up large quantities of certain elements from the environment and said hyper-accumulators of heavy metals [18]. Fennel has this kind of nature.

The fennel fruit chemical composition on average, per 100 g edible portion: 8.8 g water; 15.8 g protein; 14.9 g fat; 36.6 g carbohydrates; 15.7 g fibre; and 8.2 g ash (containing 1.2 g Ca, 19 mg Fe, 1.7 g of K, 385 mg of Mg, 88 mg Na, 487 mg P and 28 mg Zn). The contents of vitamin A were: 6.75 mg; niacin 6 mg; thiamine 0.41 mg; riboflavin 0.35 mg; and energy value about 1440 kJ per 100 g. The fruit contains mucilage, sugars, starch, tannin, non-volatile oil and essential oil. The main components of the non-volatile oil are petroselenic, oleic, linoleic and palmitic acids. The fruit contains a fixed oil from 15 to 30% and a volatile essential oil up to 12%. The fruit also contains flavonoids, iodine, kaempferols, umbelliferone and stigmasterol and ascorbic acid; traces of aluminium, barium, lithium, copper, manganese, silicon and titanium [1].

Trace elements play an important role in chemical, biological, biochemical, metabolic, catabolic and enzymatic reactions in the living cells of plants, animals and human beings. More than sixty (60) elements are found in human body in various forms among which twenty-five (25) are considered essential to human health out of which fourteen (14) exist usually less than 1 $\mu g\ g^{-1}$ of tissue and so termed trace [19]. Though required in very small amount, deficiency of trace elements cause diseases, whereas their presence in excess may result in toxicity to human life disturbing normal functioning of organs and central nervous system. Anaemia from iron deficiency affects more than half of pregnant women and at least one-third of children under five years [20]. Cobalt is essential component of vitamin $B_{12}$; zinc is found in several enzymes and genetic material transcription; copper is key component of redox enzymes and choursomium has a role in glucose metabolism, iron in oxygen transport and so enables metabolism [21]. On the other hand, trace metals like lead, cadmium and mercury are known of their detrimental health effect. Cadmium, for example, has been considered as an extremely significant pollutant even in small amount, affecting all forms of life because of its high toxicity and great solubility in soil and water [22]. No level of lead in blood as well should be considered safe for children due to its neurotoxicity [23, 24].

As food is the major intake source of toxic trace metals by human beings and animals, contamination of food has become a burning issue in recent years particularly in most metropolitan cities [25]. This is attributed mainly to the problem of environmental pollution due to rapid urbanization and industrialization with improper environmental planning leading to discharge of industrial, agricultural and swage effluents into water bodies, lands and air.

Added to the heavy release of trace metals, their geo-accumulation, bioaccumulation, bio-magnification in bio-system and non-biodegradability enhances exponentially their concentration across food chain and the effect on human being [17, 18]. Once they enter the body, they may alter their oxidation state, may form complexes with other biological molecules [26].

Determination of inorganic elements in agricultural products is also attracting considerable attention for a new application, which is to identify the geographic origin of the agricultural products to provide necessary information to the consumers, agricultural farmers, retailers, and administrative authorities [27]. Another reason for analysis of metals in plants is for diagnosis of deficiency of essential metal nutrients so that appropriate minerals can be supplied for the proper growth of crops. High concentrations of heavy metals, metabolic process of the plants can be interfered, resulting in poor growth and sometimes-even death [28].

Although spices are consumed in small amount analysis of spics for trace metals is essential because they accumulate many trace metals at higher concentration [29-31] that are essential and toxic metals. Thus, analysis helps the community to be warned from risk arising from deficiency of essential metals or accumulation of trace metals. Especially same spices and herbs are used extensively in developing countries like Ethiopia for their medicinal, spiritual and flavour as row or prepared ways. Therefore, the determination of metal content of agricultural products across different parts of the globe were conducted from health risk assessment, nutrient content analysis for consumers, tracing of geographic origin of food products, nutritional status assessment of growing plants and assay of suitability of soil and water for farming viewpoints.

Trace and major metal concentration of fennel are done in some part of the world. The mean levels of minerals from different parts of the world are given in the Table 1 and Table 2 given below.

Table 1.Summary of major metal levels reported fennel fruit worldwide (all values are in μg/g).

| Country | Ca | Mg | Fe | Mn | Ref. |
|---|---|---|---|---|---|
| India | **23,900 ± 1300** | 5,110 ± 420 | **744 ± 20** | **72.3 ± 3.0** | 32 |
| Turkey | 10,780 ± 221 | 2774 ± 39 | 224.8 ± 9.2 | 27.8 ± 0.4 | 13 |
| Turkey | 6746 ± 1022 | 3400 ± 382 | 316 ± 35 | 33.4 ± 5.3 | 33 |

Bold fonts indicate the highest concentration of the metals
The * indicate lowest concentrations of the metals

Table 2. Summary of trace metal levels in fennel fruit worldwide, (all values in μg/g)

| Country | Cu | Cr | Co | Zn | Ni | Cd | Pb | Ref. |
|---|---|---|---|---|---|---|---|---|
| India | 10.9 ± 0.8 | 1.50 ± 0.35 | **0.54 ± 0.10** | 34.5 ± 4.4 | 1.46 ± 3.43 | 0.0861 ± 0.034 | **0.95 ± 0.83** | 32 |
| Turkey | **16.2 ± 0.4** | 1.04 ± 0.20 | 0.40 ± 0.02 | 37.0 ± 2.4 | 5.40 ± 0.14 | 0.004 ± 0.001 | 0.48 ± 0.074 | 13 |
| Turkey | 8.28 ± 0.85 | **35.93 ± 9.12** | | 20.8 ± 4.78 | **28.66 ± 1.38** | **0.50 ± 0.1** | 0.35 ± 0.11 | 33 |

Bold fonts indicate the highest concentration of the metals
The * indicate lowest concentrations of the metals

Trace metal assessment, particularly in Ethiopian fennel fruits is currently not available although fennel is consumed in the country in various ways. Moreover studies abroad indicates this plant accumulate some major and trace metals [1,13,32,33]. Therefore, this study aims to fill the gap at least partially in the area and initiate others on other parts (leaf, stalk, and bulb) of this plant.

In this study, the levels of trace metals, Ca, Mg, Fe, Mn, Cu, Cr, Co, Zn, Ni, Cd, and Pb in fennel fruits' samples and with soil samples in which fennel is grown from some area of Ethiopia have been determined using flame atomic absorption spectroscopy.

## 1.7 BIOAVAILABILITY OF HEAVY METALS IN CROPS

### 1.7.1 Bioavailability

Bioavailability is the proportions of total metals that are available for incorporation into biota (bioaccumulation). Total metal concentrations do not necessarily correspond with metal bioavailability. For example, sulfide minerals may be encapsulated in quartz or other chemically inert minerals, and despite high total concentrations of metals in sediment and soil containing these minerals, metals are not readily available for incorporation in the biota; associated environmental effects may be low [27].

### 1.7.2 Factors Affecting Heavy Metals Mobility and Bioavailability in Plants

Plant uptake of trace elements is generally the first step of their entry into the agricultural food chain. Plant uptake is dependent on (1) movement of elements from the soil to the plant root, (2) elements crossing the membrane of epidermal cells of the root, (3) transport of elements from the epidermal cells to the xylem, in which a solution of elements is transported from roots to shoots, and (4) possible mobilization, from leaves to storage tissues used as food (seeds, tubers, and fruit), in the phloem (the vascular tissue in plants which conducts sugars and other metabolic products downwards from the leaves) transport system. After plant uptake, metals are available to herbivores and humans both directly and through the food chain. The limiting step for elemental entry to the food chain is usually from the soil to the root [34].

Plant species and relative abundance and availability of necessary elements also control metal uptake rates. Abundant bio-available amounts of essential nutrients, including phosphorous and calcium, can decrease plant uptake of non-essential but chemically similar elements, including arsenic and cadmium, respectively. Bioavailability may also be related to the availability of other elements. For example, copper toxicity is related to low abundances of zinc, iron, molybdenum and (or) sulfate [32].

The bioavailability of elements to plants is also controlled by many factors associated with soil and climatic conditions, plant genotype and agronomic management, including: total concentration and speciation (physical-chemical forms) of metals, mineralogy, pH, redox potential, temperature, total organic content, and suspended particulate content, the type of plant root system and the response of plants to elements in relation to seasonal cycles [27].

Many of these factors vary seasonally and temporally, and most factors are interrelated. Consequently, changing one factor may affect several others. In addition, other poorly understood biological factors seem to strongly influence bioaccumulation of metals and severely inhibit prediction of metal bioavailability [34].

This variables are complex and varies from place to place so study from abroad only my not be sufficient to conclude. Moreover the agricultural practice, physico-chemical property of the soil as well as the climate of Ethiopia is different from the rest of the world. Thus conducting this study is mandatory.

## 1.8 OBJECTIVES OF THE STUDY

### 1.8.1 General Objective

The aim of the present study was to provide a reliable estimate for major, minor and trace elements in fennel (*Foeniculum Vulgari* Mill) fruits cultivated in Ethiopia.

### 1.8.2 Specific Objectives

- To determine selected major and trace elements in fennel fruit by flame atomic absorption spectrometer (FAAS).
- To determine selected major and trace elements in soil sample in which fennel was grown by flame atomic absorption spectrometer (FAAS).
- To compare the levels of the metals in fennel fruit and soil in which fennel plant was grown.

- To compare the level of metals in the fennel cultivated in Ethiopia with those of other countries.

## 2. EXPERIMENTAL

### 2.1 APPARATUS AND GLASSWARES

Stainless steel knife, less than 0.2 mm diameter sieve, blending device (Moulinex, France), pestle and mortar (Haldenwanger, Germany) apparatus and Digital Analytical Balance (Adam, Model WL 3000, Switzerland), Kjeldahl (Gallenhamp, England) apparatus hot plate, air-circulating oven (Digitheat, J.P. Selecta, Spain) and Flame Atomic Absorption Spectrophotometer (Buck Scientific, Model 210VGP AAS, East Norwalk, USA) instruments were used. Quick-fit round bottom flasks (100 mL), reflux condenser and Borosilicate volumetric flasks (25, 50 and 100 mL sizes), glasswares and 5-50 mL measuring cylinders (Duran, Germany), 1-5 mL pipettes (Pyrex, USA), 1-10 μL, and 100-1000 μL micropipettes (Dragonmed, Shangai, China) measuring devices were used.

### 2.2 CHEMICALS, REAGENTS AND STANDARD SOLUTIONS

Chemicals and reagents that were used in the analysis are all analytical grades, except the acids. 96-98% sulfuric acid (Riedel-de Haen, Germany) was used to soak quick-fit round bottom flasks. 69-72% $HNO_3$, (Supreme Enterprises Cantt, India) and 70% $HClO_4$ (A.C.S. Reagent, Aldrich, UK) were used for digestion of powdered fennel fruit samples. Aqua-regia (1:3 of 70% $HNO_3$, Supreme Enterprises Cantt, India and 36% HCl) and 36% $H_2O_2$ (Scharlau Chemie, European Union, UN2014) were used for digestion of powdered soil samples. Lanthanum nitrate hydrate (99.9%, Aldrich, Muwaukee, USA) was used to prevent the chemical interference of phosphate ion on Ca and Mg during the analysis of the samples. Stock standard solution of concentration 1000 mg/L in 2% $HNO_3$ of the metals Ca, Mg, Mn, Fe, Cu, Zn, Ni, Co, Cr, Pb and Cd (Buck Scientific Puro-Graphic) standard solutions were used to prepare intermediate standard solutions to obtain calibration curves for the determination of metals in the samples and recovery study. De-ionized water was used for dilution of sample and intermediate metal standard solutions and rinsing glassware and sample bottles prior to analysis.

## 2.3 PROCEDURES

### 2.3.1 Description of Sample Collection Sites

The study locations were Addis Ababa, Bichena, Debre Markos and Finote Selam. Bichena and Debre Markos are located in East Gojam-Ethiopia; they are 299 km and 265 km from Addis Ababa, respectively. Finote Selam is found in waste Gojam, which is 380 km away from the capital city. Gojam was selected because fennel is consumed in these zones every day in different ways, as spice, flavouring agent in local '*Areki*' and used as medicinal plant. Addis Ababa was selected because it is expected to be relatively contaminated area and to see the accumulation effect of the plant. Latitude, longitude, altitude, annual average temperature and rainfall and misture content of fennel fruit are given in table 3.

Table 3. Latitude, longitude, altitude, annual average temperature and rainfall and Moisture contents of fennel fruit and annual rain fall of the sites

| Name of sites | Latitude (N) | Longitude (E) | Altitude (m) | Annual average Temperature (°C) | Annual average rain fall (mm) | Moisture (%) | Ref. |
|---|---|---|---|---|---|---|---|
| A,A | 38° 44′ | 9°1′ | 2,300 | 22.8-10.6 | 1,180.4 | 10.13 | 35 |
| B | 10°27′ | 38°12′ | 2541 | 22-9.83 | 1048 | 9.75 | 36,37 |
| D.M | 10°20′ | 37°43′ | 2,515 | 14.2 – 18.6 | 1,278 | 10.21 | 36,38 |
| F.S | 10°42′ | 37°16′ | 1917 | | | 8.98 | 39 |

A.A = Addis Ababa, B = Bichena, D.D = Debre Markos and F.S = Finote Selam

### 2.3.2 Collection of Samples

Fennel fruit samples were collected from March 21 to April 15, 2010. A total of 5 sub-sites were selected for each site and 100 g soil and fennel fruit were collected from each sub-site. The sub-sites are selected randomly. The samples collected in each sub-sites were pooled and 0.5 kg sample were collected for each of the sites. The soil samples were collected at a depth of 20 cm from the surface of the top soil.

### 2.3.3 Sample Preservation, and Handling

Samples were sealed in washed and rinsed with de-ionized water and dried polyethylene bags and transported to the laboratory where sample pre-treatments were made. All the samples were dried in the oven for 48 hours at 80 $^{o}$C for fennel fruit and 72 hours for soil samples at which the mass of the samples became constant.

### 2.3.4 Apparatus Clean-up

All glassware, plastic containers and polyethylene bags were filled with aqueous detergent solution; inner and outer walls wiped briskly with foam and brushes; sufficient amount of water was used to remove the detergent followed by de-ionized water rinsing. Quick-fit round bottom flasks (100 mL) were soaked with concentrated sulphuric acid in the presence of catalytic amount (1 g/1000 mL of concentrated sulphuric acid) of potassium dichromate for 48 hour. Borosilicate volumetric flasks (25, 50 and 100 mL sizes) were soaked with about 10% (v/v) nitric acid for 24 hours followed by rinsing with de-ionized water. The glassware were then dried in hot air oven and stored in clean dry places free of contamination till use.

### 2.3.5 Optimization of Digestion Procedure

Optimum procedure of sample dissolution is required to give result, with minimum reagents, time temperature and simple procedure. Nitric acid alone or as a mixture with perchloric, sulfuric or hydrochloric acids is the most popular reagent used for sample decomposition, as it is a strong oxidizing agent and forms soluble nitrates with metals, sometimes strong oxidizing agents like hydrogen peroxide added. Sulfuric acid cannot be used during determination of elements like Ba, Ca, Pb, and Sr which form sulfates with low water solubility. Therefore, in this study nitric and perchloric acids were used during optimization procedure for fennel fruit and a total volume of acid was taken to be 4 mL [34]. The procedures followed by analyst were tested for their application and an optimum procedure, (Table 4), was attained for the digestion of samples for metal content determination using FAAS.

Table 4. Reagent types and volumes, temperature and time attempted during optimization of digestion of fennel fruit.

| No | Reagent volumes (mL) | | | Maximum temperature ($^0$C) | Time, (min) | Results |
|---|---|---|---|---|---|---|
| | $HNO_3$ | $HClO_4$ | Total | | | |
| 1 | 1 | 3 | 4 | 270 | 180 | Yellow |
| 2 | 1.5 | 2.5 | 4 | 270 | 180 | Lightly yellow |
| 3 | 2 | 2 | 4 | 270 | 180 | Almost clear |
| **4** | **2.5** | **1.5** | **4** | **270** | **180** | **Clear solution** |
| 5 | 3 | 1 | 4 | 270 | 180 | Clear solution |
| 6 | 3.5 | 0.5 | 4 | 270 | 180 | Almost clear |
| 7 | 2.5 | 1.5 | 4 | 120 | 180 | Yellow |
| 8 | 2.5 | 1.5 | 4 | 150 | 180 | Lightly yellow |
| 9 | 2.5 | 1.5 | 4 | 180 | 180 | Almost clear |
| **10** | **2.5** | **1.5** | **4** | **210** | **180** | **Clear solution** |
| 11 | 2.5 | 1.5 | 4 | 240 | 180 | Clear solution |
| 12 | 2.5 | 1.5 | 4 | 270 | 180 | Clear solution |
| 13 | 2.5 | 1.5 | 4 | 270 | 105 | Light brown with suspension |
| 14 | 2.5 | 1.5 | 4 | 270 | 120 | Yellow |
| 15 | 2.5 | 1.5 | 4 | 270 | 135 | Lightly yellow |
| **16** | **2.5** | **1.5** | **4** | **270** | **150** | **Clear solution** |
| 17 | 2.5 | 1.5 | 4 | 270 | 165 | Clear solution |

Therefore the procedure with total of 4 mL reagents volume (2.5 mL $HNO_3$ and 1.5 mL $HClO_4$), heating at 210 $^{o}$C and 150 min digestion time was selected for this study.

### 2.3.6 Digestion of Samples

**a.** Soil sample analysis: Applying the optimized procedure, 0.5 g of well-powdered soil sample was added into a round bottom flask (100 mL). To this flask 2 mL $HNO_3$ (69-72%), 4 mL 36% and 1.5 mL of $H_2O_2$ (36%) were added and the mixtures were digested on a micro Kjeldahl digestion apparatus by setting the temperature at 270 $^{0}$C for 3:0 hour. The residue of the digest was allowed to cool by leaving it for about 20 minutes at room temperature. After cooling, about 15 mL of de-ionized water was added to dissolve the precipitates formed on cooling and to dilute left over acid so that to minimize the dissolution of a filter paper by the digest residue while filtrating with Whatman, (110 mm, dia), filter paper. The round bottom flask was rinsed by using another 8 mL de-ionized water two times and filtered. The filtrate was filled to 50 mL with de-ionized water. Triplicate digestions were carried out for each sample. The digested samples were kept in the refrigerator, until the level of all the metals in the sample solutions were determined by FAAS. The blank solutions were prepared by digesting the mixture of reagents following the same digestion procedure and diluted to 50 mL with de-ionized water.

**b.** Fennel fruit sample analysis: Applying the optimized procedure, 0.5 g of well-powdered fennel fruit sample was added into a round bottom flask (100 mL). To this flask 2.5 mL $HNO_3$ (69-72%) and 1.5 mL $HClO_4$ (70%) were added and the mixtures were digested on a micro Kjeldahl digestion apparatus by setting the temperature at 210 $^{0}$C for 3:0 hours. The residue of the digest was allowed to cool by leaving it for about 20 minutes at room temperature. After cooling, about 15 mL of de-ionized water was added to dissolve the precipitates formed on cooling and to dilute left over acid so that to minimize the dissolution of a filter paper by the digest residue while filtrating with Whatman, (110 mm, dia), filter paper. The round bottom flask was rinsed by using another 8 mL de-ionized water two times and filtered. The filtrate was filled to 50 mL with de-ionized water. Triplicate digestions were carried out for each sample. The digested samples were kept in the refrigerator, until the level of all the metals in the sample solutions were determined by FAAS. The blank solutions were prepared by digesting the mixture of reagents following the same digestion procedure and diluted to 50 mL with de-ionized water.

### 2.3.7 Instrument Operating Conditions

Atomic absorption spectroscopic standard solutions containing 1000 mg/L of the particular metal were used for preparing intermediate standards (10 mg/L) and working standards by using de-ionized water. Working standards of metals solutions were prepared by diluting the intermediate standards solutions of the metal with de-ionized water. Three points of calibration curve were established by running the prepared working standard solutions in flame atomic absorption spectrometer (Buck Scientific Model 210GP). Immediately after calibration, the sample solutions were aspirated into the AAS instrument and direct readings of the metal concentrations was recorded. Three replicate determinations were carried out on each sample. The same analytical procedure was employed in the determination of elements in the blank. The operating conditions of AAS employed for each analyte are given in Table 5.

Table 5. Instrument operating conditions for determination of metals in the samples using flame atomic absorption spectrometer.

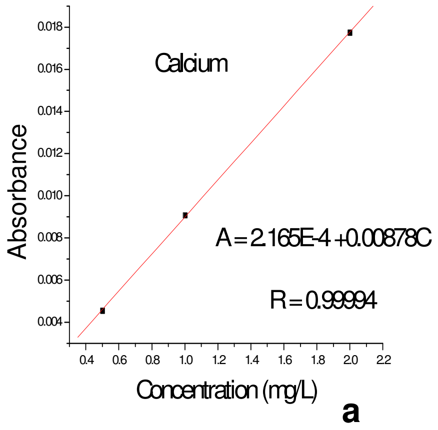
| Element | Detection limit (mg/L) | Energy | Current (mA) | Wave length (nm) | Slit width (nm) | Linear range (mg/L) |
|---|---|---|---|---|---|---|
| Ca | 0.010 | 3.606 | 2.0 | 422.7 | 0.7 | 3.000 |
| Mg | 0.001 | 3.951 | 1.0 | 285.2 | 0 .7 | 0.023 |
| Mn | 0.010 | 3.971 | 3.0 | 279.5 | 0.7 | 1.875 |
| Fe | 0.030 | 3.256 | 7.0 | 248.3 | 0.2 | 3.750 |
| Zn | 0.005 | 3.047 | 2.0 | 213.9 | 0.7 | 1.120 |
| Cu | 0.020 | 3.327 | 1.5 | 324.8 | 0 .7 | 15.000 |
| Cr | 0.050 | 3.536 | 2.0 | 357.9 | 0.7 | 3.000 |
| Co | 0.050 | 2.746 | 4.5 | 240.7 | 0.2 | 5.250 |
| Ni | 0.040 | 2.928 | 7.0 | 232.0 | 0.2 | 5.250 |
| Cd | 0.005 | 3.070 | 2.0 | 228.9 | 0.7 | 1.120 |
| Pb | 0.100 | 3.493 | 3.0 | 217.0 | 0.7 | 15.000 |

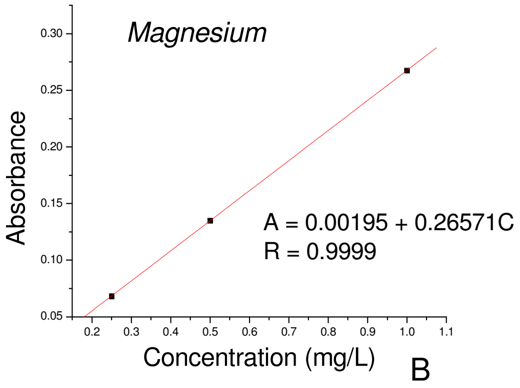
### 2.3.8 Instrument Calibrations

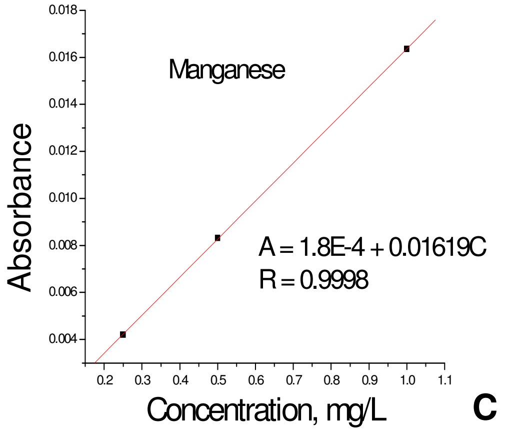
The instrument was calibrated using three series of working standards. The working standard solutions of each metal were prepared freshly by diluting the intermediated standard solutions. Concentrations of the intermediate standards, working standards, calibration curves and value of correlation coefficient of the calibration graph for each of the metals are provided (Table 6 and Figure 2 a - k).

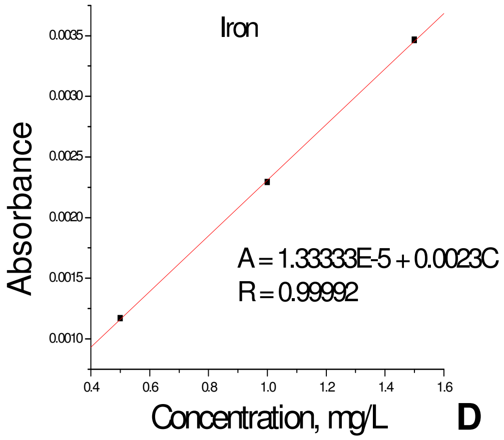
Table 6. Intermediate standards, working standards and correlation coefficients of the calibration curves for determinations of the metals using flame atomic absorption spectrophotometer

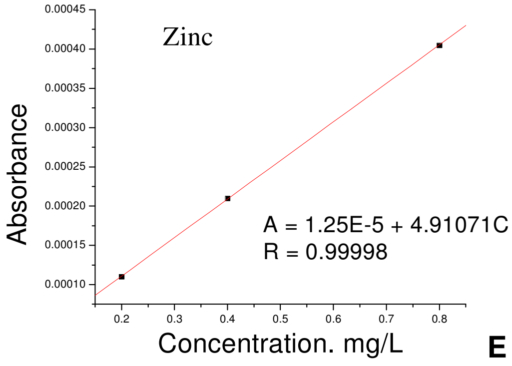
| Metal | Intermediate standard (mg/L) | Working standards (mg/L) | Correlation coefficient of calibration curve |
|---|---|---|---|
| Ca | 10 | 0.5, 1.0, 1.5 | 0.9994 |
| Mg | 10 | 0.25, 0.5, 1 | 0.9999 |
| Mn | 10 | 0.25, 0.5, 1 | 0.9998 |
| Fe | 10 | 0.5, 1.0, 1.5 | 0.9992 |
| Zn | 10 | 0.2, 0.4, 0.8 | 0.9998 |
| Cu | 10 | 0.5, 1.0, 1.5 | 0.9999 |
| Cr | 10 | 0.5, 1.0, 1.5 | 0.9992 |
| Co | 10 | 0.25, 0.5, 1 | 0.9995 |
| Ni | 10 | 0.25, 0.5, 1 | 0.9999 |
| Cd | 10 | 0.25, 0.5, 1 | 0.9999 |
| Pb | 10 | 0.1, 0.2, 0.4 | 0.9998 |

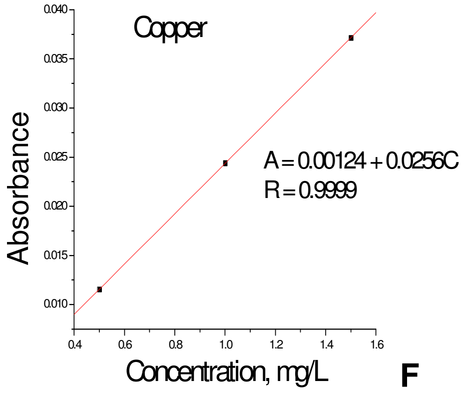

Calcium
Absorbance
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
A=2.165E-4+0.00878C
R=0.99994
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Concentration (mg/L)
a


Magnesium
Absorbance
0.30
0.25
0.20
0.15
0.10
0.05
A = 0.00195 + 0.26571C
R = 0.9999
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
Concentration (mg/L)
B


Manganese
Absorbance
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
A = 1.8E-4 + 0.01619C
R = 0.9998
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
Concentration, mg/L
C


Iron
Absorbance
0.0035
0.0030
0.0025
0.0020
0.0015
0.0010
A=1.33333E-5+0.0023C
R=0.99992
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Concentration, mg/L
D


Zinc
Absorbance
0.00045
0.00040
0.00035
0.00030
0.00025
0.00020
0.00015
0.00010
A = 1.25E-5 + 4.91071C
R = 0.99998
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Concentration. mg/L
E


Copper
Absorbance
0.040
0.035
0.030
0.025
0.020
0.015
0.010
A=0.00124+0.0256C
R=0.9999
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Concentration, mg/L
F

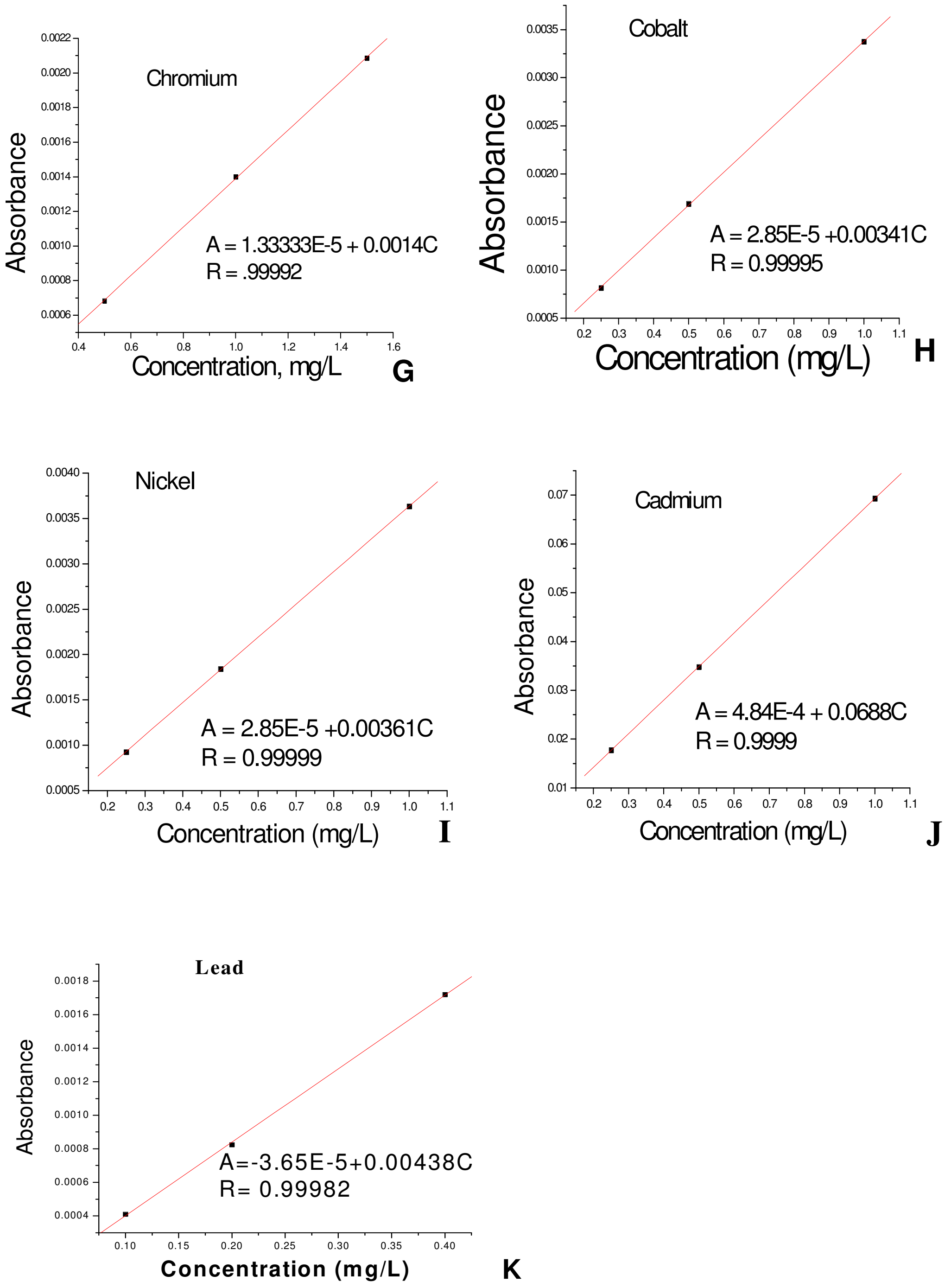


Figure 2 (a- k). Calibration curves of metals standard solutions.

### 2.3.9 Method Detection Limits

Detection limit is the lowest concentration level that can be determined to be statistically different from an analyte blank or the minimum concentration that can be detected by the analytical method with a given confidence limit [34]. The generally accepted and common definition of method detection limit is the concentration that gives a signal three times the standard deviation of the blank or background signal [33]. In this work, after digestion of three blank solutions, triplicate reading was obtained for each sample. Then the method detection limit of each element was calculated as tree times the standard deviation of the blank ($3\sigma_{blank}$, n = 3). Instrumental detection limit and method detection limit for fennel fruit and soil samples are presented in Table 7.

Table 7. Instrument and method detection limits for metals (for all metals, n = 3, fennel fruit and soil samples).

| Element | Instrument detection limit (mg/L) | Method detection limit for fennel fruit (mg/kg) | Method detection limit soil (mg/kg) |
|---|---|---|---|
| Ca | 0.010 | 3 | 5 |
| Mg | 0.001 | 0.4 | 0.5 |
| Mn | 0.010 | 0.5 | 1.5 |
| Fe | 0.030 | 0.2 | 0.4 |
| Zn | 0.005 | 0.6 | 2 |
| Cu | 0.020 | 0.5 | 2 |
| Cr | 0.050 | 3 | 3 |
| Co | 0.050 | 4 | 3 |
| Ni | 0.040 | 1 | 3 |
| Cd | 0.005 | 0.1 | 1 |
| Pb | 0.100 | 5 | 5 |

The method detection limits estimated were greater than the instrument detection limit for all metals (Ca, Mg, Fe, Mn, Cu, Cr, Co, Zn, Ni, Pb and Cd) in the fennel and soil. But the method detection limits are low enough to determine the metals at trace levels in the fennel and soil.

## 2.4 METHOD VALIDATION

To check accuracy of digestion procedure and efficiency of the FAAS instrument various methods can be followed. These are certified standard reference material analysing, spiking sample with known concentration of the analyte, and comparing the values obtained by different method, laboratory or analyst. In this work, spiked samples were prepared by adding a small known quantity of metal standard solutions to both the soil and fennel fruit by applying similar digestion procedure, analysing for the levels of metals and calculating the recovery percent.

### 2.4.1 Analytical Precision

The reproducibility of the analytical procedure was checked by carrying out a triplicate analysis and calculating the coefficient of variation of the mean for each metal. In almost all cases, triplicate results did not differ by more than 10% of the mean.

### 2.4.2 Study of Recovery (Procedure of Spiking)

#### a. Spiking for Fennel fruit sample

To confirm the efficiency of developed optimized procedures, spiking experiments in which known volume and concentration of standard solutions, were employed. From the stock solution (1000 mg/L) 200 µL for Ca, Mg and Fe and 0.50 g of Fennel fruits powder were added to the first batch round bottom flask. From 100 mg/L intimidate solution of the metals prepared from the stock solution and 200 µL Cu and Cr and 180 µL Co, and 0.50 g of fennel fruits powder were added to the second batch round bottom flask. From 100 mg/L intimidate solution of the metals and 160 µL Mn and Zn; 140 µL Ni, and 100 µL Cd from 10 mg/L and 0.50 g of fennel fruits powder were added to the third batch round bottom flask. Then samples were digested with the optimized procedures for fennel fruits sample in triplicates. After diluting the digested samples to 50 mL with distilled de-ionized water, they were analyzed with the same procedure followed for the analysis of fennel fruits samples (Table 8).

### b. Spiking for Fennel fruit sample

From the stock solution (1000 mg/L) 270 μL for Fe, from 100 mg/L 270 μL for Cu and from 10 mg/L 270 μL for Cd and 0.50 g of soil powder were added to the first batch round bottom flask. For Mg, Ca and Mn 330 μL of 1000 mg/L stock solution of the metals and 0.50 g of soil powder were added to the second batch round bottom flask. From 100 mg/L intimidate solution of the metals 212 μL Zn, Cr, Co and Ni and 0.50 g of soil powder were added to the third batch round bottom flask. Then samples were digested with the optimized procedures for soil sample in triplicates. After diluting the digested samples to 50 mL with de-ionized water, they were analyzed with the same procedure followed for the analysis of soil samples (Table 7).

Table 8. Recovery test results of fennel fruit sample.

| Metal | Amount added to fennel | Amount | Mean recovery |
|---|---|---|---|
| Ca | 411 | 384 | 93.4 ± 2.5 |
| Mg | 478 | 505 | 108 ± 4.5 |
| Fe | 455 | 463 | 101.9 ± 5.8 |
| Mn | 32.7 | 31.4 | 96.2 ± 3.7 |
| Cu | 41.3 | 40.6 | 98.2 ± 5.7 |
| Co | 37.6 | 36.6 | 97.4 ± 4.2 |
| Cr | 37.9 | 37.5 | 99 ± 3.5 |
| Zn | 29.7 | 29.7 | 100.1 ± 2.5 |
| Ni | 29.1 | 29.0 | 99.7 ± 5.1 |
| Cd | 1.91 | 2.07 | 93 ± 2.9 |

The obtained percentage recovery for fennel fruit varied from 93% for Cd to 108% for Mg which are in the acceptable range (Table 8). Thus the optimized procedure for the metal analysis in the fennel fruit was validated.

Table 9. Recovery test results of soil sample.

| Metal | Amount added to soil sample (mg/L) | Amount found (mg/L) | Mean recovery (%) |
|---|---|---|---|
| Ca | 660 | 611 | 92.6 ± 6.7 |
| Mg | 661 | 633 | 95.8 ± 6.5 |
| Mn | 657 | 685 | 104.2 ± 3.2 |
| Fe | 553 | 568 | 102.9 ± 7.3 |
| Zn | 42.8 | 47.2 | 110 ± 5.5 |
| Cu | 54.2 | 53.3 | 98.4 ± 2.8 |
| Cr | 42.2 | 39.9 | 94.5 ± 3.9 |
| Co | 42.4 | 41.1 | 97 ± 5.3 |
| Ni | 42.6 | 40.6 | 95.3 ± 6.9 |
| Cd | 1.35 | 1.34 | 99.2 ± 3.1 |

The obtained percentage recovery for the soil varied from 92.6% for Ca to 110% for Zn (Table 9). Thus the optimized procedure for the metal analysis in the soil was validated

## 3. RESULT AND DISCUSSIONS

### 3.1 DETERMINATION OF METALS IN FENNEL FRUIT AND SOIL SAMPLES

The fennel fruit and the soil samples on which the plant was grown were analysed for major (Ca, Mg, Fe, and Mn), trace essential (Cu, Zn, Cr and Co), and trace non-essential metals (Ni, Cd and Pb) with FAAS. The instrument was operated as per the instrument's manual. Each of the sets of successive samples were aspirated one after another in to the FAAS instrument set in concentration mode using the hallow cathode lamp of the respective metal. The values along with standard deviation of triplicate analysis are given from Table 10 to Table 13 for the fennel fruit and soil samples.

Table 10. Average concentration (mean ± SD, n = 3 in µg/g dry weight) and relative standard deviation of metals in the fennel and soil from Addis Ababa site.

| Metal | Fennel fruit (µg/g dry weight) | RSD (%) | Soil (µg/g dry weight) | RSD (%) |
|---|---|---|---|---|
| Ca | **20,544 ± 286** | 1.4 | 1,435 ± 37 | 2.6 |
| Mg | 2,392 ± 68 | 2.8 | 2,866 ± 59 | 1.8 |
| Fe | 1,136 ± 67 | 5.9 | **27,628 ± 574** | 2.1 |
| Mn | 45.8 ± 1.1 | 2.7 | 1,933 ± 131 | 6.8 |
| Cu | 103 ± 4 | 3.6 | 101 ± 6 | 5.6 |
| Cr | 97.7 ± 2.9 | 3.1 | 141 ± 5 | 3.6 |
| Co | 67.7 ± 3.7 | 5.8 | 143 ± 4 | 2.5 |
| Zn | 37.1 ± 2.0 | 5.3 | 101 ± 3 | 2.7 |
| Ni | 18.7 ± 0.5 | 2.1 | 97.7 ± 3.6 | 3.6 |
| Cd | 1.9 ± 0.1 | 4.0 | 2.1 ± 0.1 | 1.8 |
| Pb | ND | | ND | |

Table 11. Average concentration (mean ± SD, n = 3 in µg/g dry weight) and relative standard deviation of metals in the fennel and soil from Bichena site.

| Metal | Fennel Fruit (µg/g dry weight) | RSD (%) | soil (µg/g dry weight) | RSD (%) |
|---|---|---|---|---|
| Ca | 23,020 ± 242 | 1.1 | 1,775 ± 37 | 2.4 |
| Mg | 1,965 ± 83 | 4.2 | 2,257 ± 22 | 1.0 |
| Fe | 1,901 ± 81 | 4.3 | 27,195 ± 181 | 0.7 |
| Mn | 51.4 ± 1.3 | 4.1 | 1,979 ± 15 | 0.7 |
| Cu | 48.4 ± 0.6 | 0.8 | 51.0 ± 0.5 | 0.9 |
| Cr | 91.4 ± 2.4 | 4.2 | 127 ± 6 | 4.7 |
| Co | 26.2 ± 0.3 | 3.3 | 54.3 ± 0.7 | 1.4 |
| Zn | 37.8 ± 2.2 | 5.9 | 99.0 ± 2.2 | 2.3 |
| Ni | 24.2 ± 1.1 | 5.7 | 100 ± 1 | 0.8 |
| Cd | 1.67 ± 0.1 | 4.0 | 2.9 ± 0.03 | 1.2 |
| Pb | ND | | ND | |

Table 12. Average concentration (mean ± SD, n = 3 in µg/g dry weight) and relative standard deviation of metals in the fennel and soil from Debre Markos site.

| Metal | Fennel Fruit (µg/g dry weight) | RSD (%) | soil (µg/g dry weight) | RSD (%) |
|---|---|---|---|---|
| Ca | 21,373 ± 96 | 0.5 | 1,634 ± 76 | 4.7 |
| Mg | 3,456 ± 93 | 2.7 | 3,306 ± 194 | 6.7 |
| Fe | 1,484 ± 62 | 4.2 | 26,863 ± 174 | 0.6 |
| Mn | 48.1 ± 6.2 | 0.5 | 1,981 ± 23 | 1.1 |
| Cu | 62.5 ± 0.3 | 1.2 | 67.9 ± 2.1 | 3.2 |
| Cr | 94.0 ± 4.7 | 4.8 | 129 ± 6 | 4.5 |
| Co | 49.5 ± 0.9 | 6.9 | 115 ± 1 | 1.1 |
| Zn | 44.7 ± 2.5 | 5.6 | 104 ± 4 | 4.3 |
| Ni | 24.0 ± 0.7 | 2.7 | 130 ± 2 | 1.5 |
| Cd | 1.89 ± 0.08 | 4.4 | 2.41 ± 0.2 | 6.8 |
| Pb | ND |  | ND |  |

Table 13. Average concentration (mean ± SD, n = 3 in µg/g dry weight) and relative standard deviation of metals in the fennel and soil from Finote Selam.

| Metal | Fennel Fruit (µg/g dry weight) | RSD (%) | soil (µg/g dry weight) | RSD (%) |
|---|---|---|---|---|
| Ca | 22,860 ± 568 | 2.5 | 1,770 ± 41 | 2.3 |
| Mg | 1,309 ± 58 | 4.5 | 1,257 ± 50 | 3.9 |
| Fe | 1,467 ± 74 | 5.0 | 28,034 ± 75 | 0.3 |
| Mn | 30.6 ± 0.7 | 1.4 | 1,460 ± 36 | 2.4 |
| Cu | 23.9 ± 0.2 | 1.0 | 69.6 ± 0.6 | 0.8 |
| Cr | 90.9 ± 0.4 | 0.4 | 129 ± 6 | 2.5 |
| Co | 70.8 ± 0.6 | 0.9 | 133 ± 7 | 5.2 |
| Zn | 40.8 ± 1.3 | 3.3 | 103 ± 1.8 | 1.8 |
| Ni | 24.2 ± 0.6 | 2.6 | 161 ± 1 | 0.2 |
| Cd | 1.59 ± 0.1 | 6.4 | 1.66 ± 0.1 | 5.3 |
| Pb | ND |  | ND |  |

## 3.2 DISTRIBUTION PATTERNS OF METALS IN THE SAMPLES

Fennel fruit sample analyses for the metals indicate that except Pb which was below the method detection limit, all the ten metals (Ca, Mg, Fe, Mn, Cu, Cr, Co, Zn, Ni, and Cd) were detected (Table 8 to Table 11). The levels of metals however differ significantly among each other and to some extent between sampling sites. The general trends of variation in fennel fruit sample in Addis Ababa site is Ca > Mg > Fe > Cu > Cr > Co > Mn > Zn > Ni > Cd, in Bichena site is Ca > Mg > Fe > Cr > Mn> Cu > Zn > Co > Ni > Cd, in Debre Markos is Ca > Fe > Mg > Cr > Cu > Co > Mn > Zn > Ni > Cd and in Finote Selam is Ca > Fe > Mg > Cr > Co > Zn > Mn > Ni > Cu > Cd. In all the cases concentration of Ca is so high and Cd is so low.

The soil (from which the fennel was grown) sample analyses for the metals indicate that except Pb which was below the method detection limit, all the ten metals (Ca, Mg, Fe, Mn, Zn, Cu, Cr, Co, Ni, and Cd) were detected (Table 8 to Table 11). The levels of metals however differ significantly among each other and to some extent between sampling sites. The general trends of variation in soil sample in Addis Ababa site is Fe > Mg > Mn> Ca > Co > Cr > Cu > Zn > Ni > Cd, in Bichena site is Fe > Mg > Mn> Ca > Cr > Ni > Zn > Co > Cu > Cd, in Debre Markos is Fe > Mg > Mn > Ca > Ni > Cr > Co > Zn > Cu > Cd and in Finote Selam is Fe > Ca > Mn > Mg > Ni > Co > Cr > Zn > Cu > Cd. In all the cases concentration of Fe is so high and Cd is so low.

Metal ion uptake into the roots of plants is extremely complex phenomenon occurring via both diffusion and mass flow of the soil solution. Chelation and surface adsorption, which are pH dependent, also affect availability of nutrient metal ions. In general acid soil conditions retard uptake of essential divalent metal ions but increase the availability of manganese, iron, and aluminium, all of which are normally of very limited availability because of hydrolysis of the trivalent ions [34].

## 3.3 DISTRIBUTION PATTERN OF METALS IN FENNEL FRUIT IN THE FOUR SAMPLING SITES

Variation in average concentration of major metals in fennel fruit was grown from the four sits is discussed here; Ca ranged from 23,020 (Bichena site) to 20,544 (Addis Ababa site), Mg ranged from 3,456 (Debre Markos site) to 1,309 (Finote Selam site), Fe ranged from 1,901 (Bichena site) to 1,136 (Addis Ababa site) and Mn ranged from 51.4 (Bichena site) to 30.6 (Finote Selam site).

Trace metals ranged from for Cu 103 (Addis Ababa site) to 23.9 (Finote Selam site), for Cr 97.7 (Addis Ababa site) to 90.9 (Finote Selam site), for Co 71.8 (Finote Selam) to 26.2 (Bichena site), for Zn 44.7 (Debre Markos site) to 37.1 (Addis Ababa site), for Ni 24.2 (Bichena site) to 18.7 (Addis Ababa site), for Cd 1.91 (Addis Ababa site) to 1.59 (Finote Selam site).

In these metals there is no any logical sequence. Their concentration varies randomly. Generally, Cu, Cr, and Cd have highest value in Addis Ababa while Ca, Fe, Zn and Ni have lowest value. Ca, Fe Mn and Ni have highest concentration in Bichena site and only Co has lowest concentration. In Debre Markos site Mg and Zn have highest value and no lowest value. Finote Selam site has highest concentration of Co and lowest Mg, Mn, Cu and Cr concentrations.

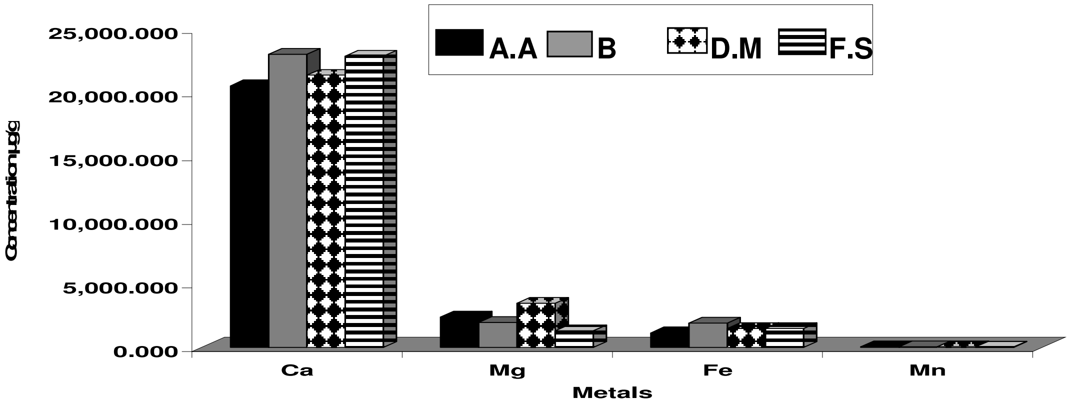
Table 13 and Figure 3 ( a - c) indicated that Cu and Cr were the most accumulated trace metal in the fennel fruit sample while the level of cadmium was the least among the metals; however due to its toxicity deserves special concern.

Table 14. Average concentration of metals in the fennel fruit samples from the four sites.

| Metals | Addis Ababa | Bichena | Debre Markos | Finote Selam |
|---|---|---|---|---|
| Ca | 20,544* | **23,020** | 21,373 | 22,860 |
| Mg | 2,392 | 1,965 | **3,456** | 1,309* |
| Fe | 1,136* | **1,901** | 1,484 | 1,467 |
| Mn | 45.8 | **51.4** | 48.1 | 30.6* |
| Cu | **103** | 48.4 | 62.5 | 23.9* |
| Cr | **97.7** | 91.4 | 94.0 | 90.9* |
| Co | 67.7 | 26.2* | 49.5 | **70.8** |
| Zn | 37.1* | 37.8 | **44.7** | 40.8 |
| Ni | 18.7* | **24.2** | 24.0 | 24.2 |
| Cd | **1.91** | 1.67 | 1.89 | 1.59* |
| Pb | **ND** | **ND** | **ND** | **ND** |

Bold fonts indicate the highest concentration of the metals in the sites.

The * indicate lowest concentrations of the metals in the sites.



a

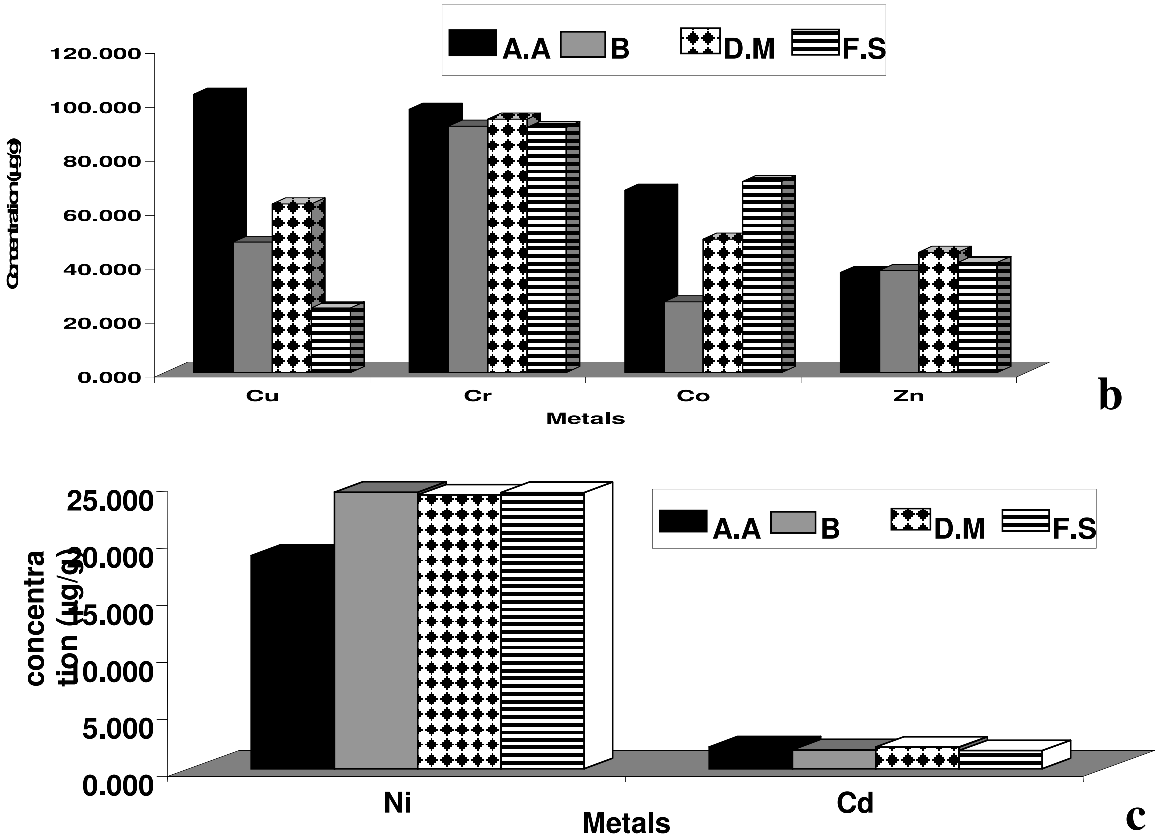


Figure 3 ( a - c). Concentration of major and trace metals in fennel fruit (µg/g dry weight). A.A = Addis Ababa, B = Bichena, D.D = Debre Markos and F.S = Finote Selam

### 3.4 DISTRIBUTION PATTERN OF METALS IN THE SOIL SAMPLES IN THE FOUR SITES

Variation in average concentration of major metals in the soil on which fennel was grown from the four sits is discussed here. Ca ranged from 1,775 (Bichena site) to 1,435 (Addis Ababa site), Mg ranged from 3,306 (Debre Markos site) to 1,257 (Finote Selam site), Fe ranged from 28,034 (Finote Selam site) to 2,6863 (Debre Markos site) and Mn ranged from 1,981 (Debre Markos site) to 1,460 (Finote Selam site). Mg and Mn have their highest value in Debre Markos site while Fe and Ca have highest concentration in Finote Selam, Bichena sites, respectively. It has been found that, Ca and Ni have lowest value in Addis Ababa; trace essential elements (Cu, Cr, Co and Zn) get their lowest value in Bichena site. In Debre Markos only Fe has very minimum value whereas Mg, Mn and Cd get their lowest value in Finote Selam.

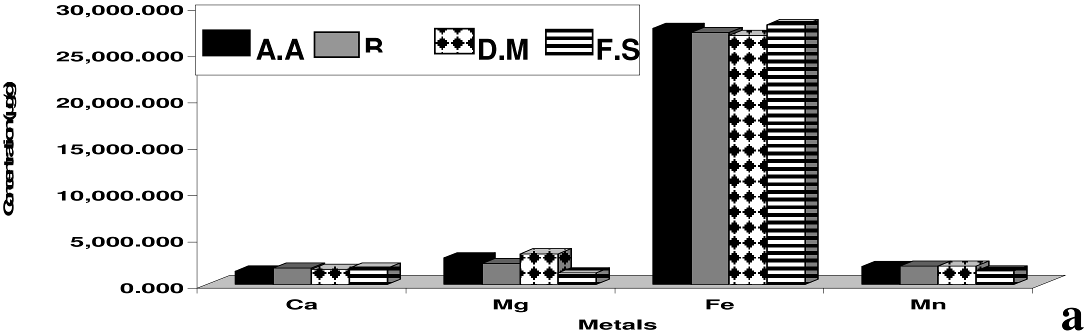
Trace metals ranged from for Cu 101 (Addis Ababa site) to 51.0 (Bichena site), for Cr 141 (Addis Ababa site) to 127 (Bichena site), for Co 143 (Addis Ababa site) to 54.4 (Bichena site), for Zn 104 (Debre Markos site) to 99.9 (Bichena site), for Ni 161 (Finote Selam site) to 97.7 (Addis Ababa site), for Cd 2.86 (Addis Ababa site) to 1.66 (Finote Selam site). Except Cd which has lowest concentration in Finote Selam and Ni in Addis Ababa all the other essential trace metals minimum values were found in Bichena site. All the trace metals accumulation found in Addis Ababa (Cu, Cr, Co and Cd) except Zn in Debre Markos and Ni in Finote Selam sites. Table 15 and Figure 4 (a - c) indicated that Co and Cr were the most accumulated trace metal in the soil sample while the level of cadmium was the least among the metals.

Table 15. Average concentration of metals in the soil of the four sites.

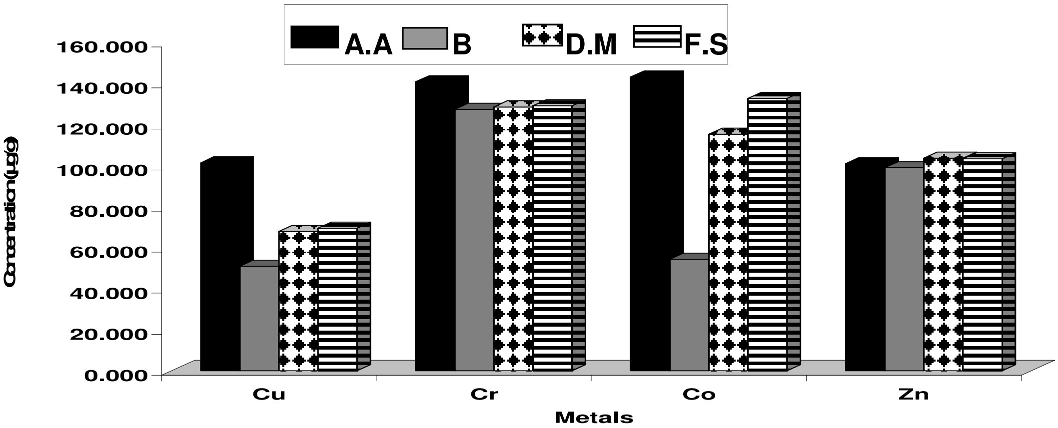
| Metals | Addis Ababa | Bichena | Debre Markos | Finote Selam |
|---|---|---|---|---|
| Ca | 1,435* | **1,775** | 1,634 | 1,770 |
| Mg | 2,866 | 2,257 | **3,306** | 1,257* |
| Fe | 27,628 | 27,195 | 26,863* | **28,034** |
| Mn | 1,933 | 1,979 | **1,981** | 1,460* |
| Cu | **101** | 51.0* | 67.9 | 69.6 |
| Cr | **141** | 127* | 128 | 129 |
| Co | **143** | 54.4* | 115 | 133 |
| Zn | 101 | 99.9* | **104** | 103 |
| Ni | 97.7* | 100 | 130 | **161** |
| Cd | **2.86** | 2.10 | 2.41 | 1.66* |
| Pb | **ND** | **ND** | **ND** | **ND** |

Bold fonts indicate the highest concentration of the metals in the sites
The * indicate lowest concentrations of the metals in the sites

In comparison between soil and fennel fruit sample except for Ca fennel fruit showed less or comparable accumulation of metals found in the soil. For Mg and Cu fennel showed comparable accumulation to that of the soil but for Fe, Mn, Cr, Co, Zn, Ni and Cd it showed low accumulation than available in the soil in all the sites. This may indicates that they are not found in bio-available form or fennel has poor accumulation for those metals.



a



b

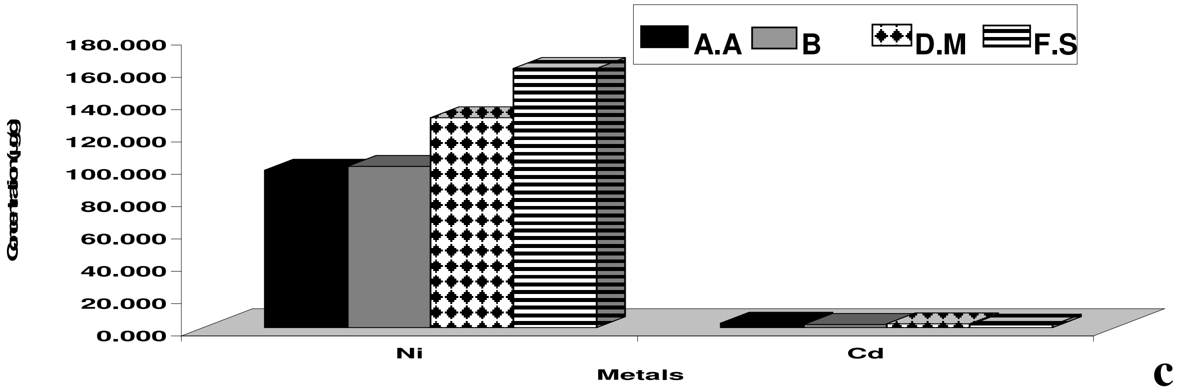


Figure 4 (a-c). Concentration of major and trace metals in soil (µg/g dry weight). A.A = Addis Ababa, B = Bichena, D.D = Debre Markos and F.S = Finote Selam

## 3.5 METAL SPECIFIC DISTRIBUTION PATTERNS

### Calcium

Calcium was the most accumulated metal in fennel fruit studied with mean concentrations varies between 23020 to 20544 µg/g dry wt of fennel fruit in the four sites. Ca was the fourth most accumulated metal in the soil of the three sites and the third in Finote Selam site with mean concentrations varied between 1775 to 1435 µg/g dry wt of soil. Higher level of calcium was observed in fennel fruit and soil sample of Bichena site.

Calcium is essential macronutrient for both plants and animals. It is involved in cell division, bone and teeth building, and blood coagulation. The ion along with magnesium causes hardness of water. There are broad range of Ca-bearing minerals in soil and water and usually abundant in ground and surface water and soil. Calcium in plants is a critical component of cell walls and membranes stabilizing to prevent leakiness and also assist in protein formation and carbohydrate transport [34].

### Magnesium

Magnesium was second most accumulated metal (except in Finote Selam in which it was the third) with concentration level in this study varied from 3,456 to 1,309 µg/g dry wt in fennel fruit. It was the second most accumulated metal in the soil of the three sites and the fourth in Finote Selam site with mean concentrations varied between 3306 to 1257 µg/g dry wt of soil. Higher level of Mg was observed in fennel fruit and soil sample of Debre Markos site. In both sample Mg concentration was comparable.

In plants magnesium is constituent of every chlorophyll molecule of green plants. It is also associated with synthesis of plant protein, P-metabolism, and synthesis of plant oils along with sulfur. In animals magnesium has a role in bone and tendons building with calcium. In water magnesium contributes to total water hardness. 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 [33].

### Manganese

Manganese was fourth most accumulated metal in all the four sites with concentration levels varied from 51.4 to 30.6 µg/g dry wt in fennel fruit. It was the third most accumulated metal in the soil of the three sites and except Finote Selam site in which it was the fourth with mean concentrations varied between 1981 to 1460 µg/g dry wt of soil. Higher level of Mg was observed in fennel fruit and soil sample of Debre Markos site.

Manganese oxide surface defragmentes form manganese nodules or coat on sediments or rock. Thus this rock or sediment withering and increase the bioavailability of it. Manganese absorbed by plants is stored mostly in leaves and so released to soil soon as the plant's leaves shade. This may be one reason for the decrease in concentration in the plant than in the soil. The other reason may be the fact that total soil manganese divided into mineral manganese, organically complexed manganese, exchangeable manganese, and solution manganese. Manganese in solution may be either $Mn^{2+}$ or manganese combined with soluble organic compounds. The equilibrium of manganese between these forms is influenced greatly by soil pH and redox conditions. Thus it may be available in a very small quantity in the last form.

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 $Fe^{2+}$. 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. Manganese is also in some way involved in the oxidation reduction processes in the photosynthetic electron transport system [33].

### Copper

Copper was second most accumulated trace essential metal (except in Addis Ababa in which it was the first trace essential metal) with concentration level in this study varied from 103 and 23.9 µg/g dry wt in fennel fruit. It is the list accumulated metal next to Cd in the soil of the three sites and the third in Addis Ababa site with mean concentrations varied between 101 to 51.0 µg/g dry wt of soil. Higher level of Cu was observed in fennel fruit and soil sample of Addis Ababa site.

Copper is an essential micronutrient for many plants and animals. Sources of copper in water and soil are Cu mining and smelting as well as agrochemicals. Copper as it is applied to treat plant disease like fungicides (e.g. Bordeaux mixture) it can accumulate on top soil. High level of copper in soil is phytotoxic and harmful to soil microorganisms. The bio-availability of copper for plants decreases with increase in pH of soil. Copper content of normal plant tissues varies according to species but is usually within the range 1 – 25 mg/kg. The accumulation of copper in plant roots is also found to decrease root length and suppress the formation of lateral root [41]. Copper acts as a component of phenolase, lactase, and ascorbic acid oxidase, and its role as a part of these enzymes probably represent an important function of copper in plants [32]. Copper is important as a coenzyme that is needed to activate several plant enzymes. It is also involved in chlorophyll formation. Copper uptake seems to be inversely related to iron uptake. Too little copper causes iron to accumulate in plants [33].

### Chromium

Chromium was first most accumulated trace essential metal (except in Addis Ababa in which it was the second trace essential metal) with concentration level in this study varied from 97.7 and 90.9 µg/g dry wt in fennel fruit. It is the first in Bichena, second in Addis Ababa and Debre Markos and the third in Finote Selam in the concentration value of essential trace metals in the soil with mean concentrations varied between 141 to 127 µg/g dry wt of soil. Higher level of Cr was observed in fennel fruit and soil sample of Addis Ababa site. This result goes with previous study conducted in 2002 (81.0 mg/kg, 115 mg/kg, 283 mg/kg and 86 mg/kg for the farms peacock, Kera, Kolfe and Akaki respectively) [41].

Chromium occurs naturally in the Earth's crust. Continental dust is the main source of exposure to natural chromium present in the environment. As a result of human activities, however, chromium is released into the environment in larger amounts. The general population is exposed to chromium by eating food or food supplements, drinking water, and inhaling air that contain chromium [40].

### Cobalt

Cobalt was the second in Finote Selam, the third in Addis Ababa and Debre Markos and the fourth in Bichena in accumulation rank from trace essential metals with concentration level in this study varied from 70.8 to 26.2 µg/g dry wt in fennel fruit. It is the first in Addis Ababa and Finote Selam sites, second in Debre Markos and third in Bichena in the concentration value of essential trace metals in the soil with mean concentrations varied between 143 to 54.4 µg/g dry wt of soil. Higher level of Co was observed in fennel fruit and soil sample of Finote Selam and Addis Ababa sites, respectively.

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

It is now well established that Co is essential for micro-organisms fixing molecular $N_2$. It is still in question whether in addition to its requirement in symbiotic $N_2$ fixation, Co is essential for higher plants. It is clear, however, that low concentrations of Co can have a favourable 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 [33].

### Zinc

Zinc was the third in Bichena and Finote Selam, the fourth in Addis Ababa and Debre Markos in accumulation rank from trace essential metals with concentration level in this study varied from 44.7 to 37.1 µg/g dry wt in fennel fruit. It was the second in Bichena, the third Debre Markos and Finote Selam sites and fourth in Addis Ababa in the concentration value of essential trace metals in the soil with mean concentrations varied between 104 to 99.9 µg/g dry wt of soil. Higher level of Co was observed in Fennel fruit and soil sample of Addis Ababa site.

Zinc is an essential trace metal involved in growth and DNA synthesis in human. Though not common, long term human exposure may result in Cu deficiency, reduced immune system and anaemia. Phytotoxic effect of excess zinc for plants is more concern particularly when zinc level in soil is above 300 mg/kg. Mining and smelting activity and domestic waste products (galvanized steel, skin care cosmetic products and pigments) are principal causes which lead to local soil and ground water pollution by zinc. Zinc is most available in acidic soil [34].

### Nickel

Nickel was the first from trace non-essential metals in accumulation rank in all of the sites with mean concentration level which varied from 18.7 to 24.2 µg/g dry wt in fennel fruit. Similarly it was first none-essential trace metals in the soil of all the sites with mean concentrations varied between 97.7 to 161 µg/g dry wt of soil. Highest value of Ni is found in Finote Selam soil and fennel fruit.

### Cadmium

Cadmium was the least concentrated trace metal both samples and in all the sites with highest concentration in Addis Ababa and lowest in Finote Selam for both fennel samples and soil with mean concentration which ranged between 1.91 to 1.59 and 2.86 to 1.66, respectively.

Cadmium has not been known to have beneficial effect for plants and animals. It causes toxicity in humans even very small amounts is consumed and it has also accumulation effect. 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. Indeed long term human exposure causes kidney damage and ultimately failure and being carcinogenic [33].

The normal intake of cadmium is 1-3 µg per day. The divalent cadmium is found to be more labile in soil and sediments and so more bio-available since it is less strongly adsorbed than other divalent metals. The largest source of cadmium in human food chain is from application of phosphate fertilizer in which cadmium exist as impurity. In the environment it exists as a by-product of Zn, Pb and Cu refining industries; wastes disposed from batteries, paints and plastics. During burning of fossil fuels at higher than 400 $^{0}$C volatile cadmium can travel as aerosol several kilo-meters from the source and deposited [34].

**Lead**

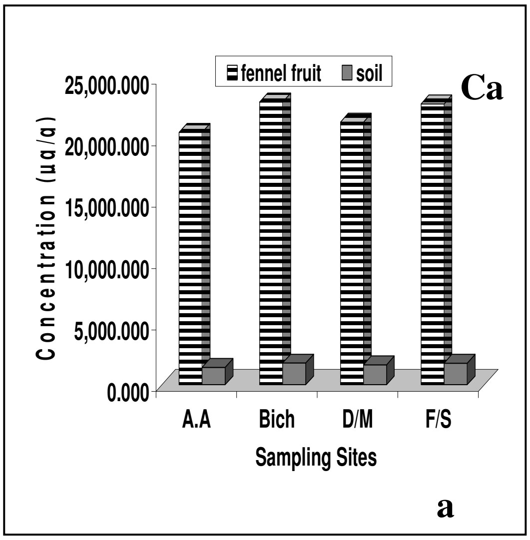
Lead concentration in both fennel fruit and the soil samples were below the method detection limit. Lead in soil and water has low mobility because of low solubility of lead hydroxide, chiloride, carbonate and phosphate. In natural water usually it exists at low level due to adsorption on mineral and organic sediments [34]. Lead can cause brain and kidney damage, decrease in hemoglobin production and male fertility. Besides, lead is absorbed in to blood from their gastro-intestine that they are more susceptible to the toxicity. Therefore the results obtained in this study concerning lead are in accordance with the requirement.

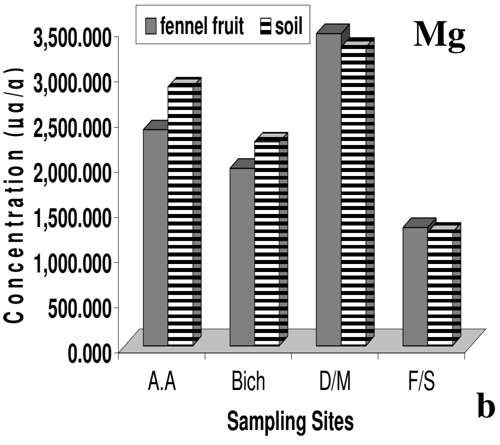
### 3.6 COMPARISONS OF METAL LEVELS BETWEEN FENNEL FRUIT AND SOIL SAMPLES

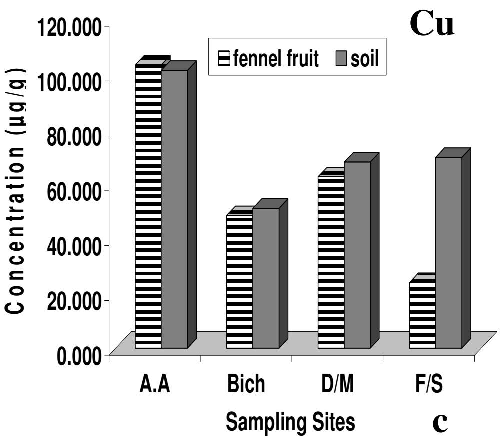
Besides metals are persistent in the environment and tend to bio-accumulate in plants and organisms, even becoming biomagnified in the food chain to the top where human beings are highly exposed. Bioaccumulation is of course a normal and essential process enabling the organism to have reserve for latter use for metalloproteins or cofactors or protect themselves against toxic effects [34].

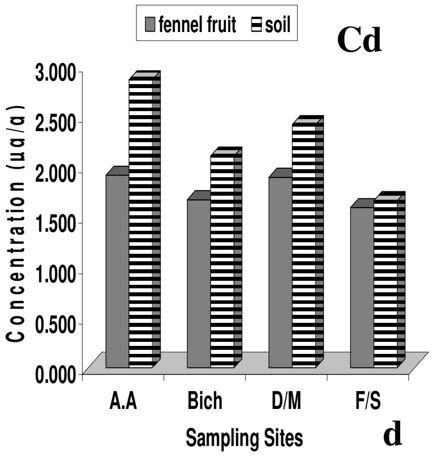
In case of fennel the bioaccumulation of both trace and major metals were attempted to be verified by roughly comparing their concentration in one of the growth media, soil and those in plants. Unlike most plants and living organisms, higher concentration was observed for calcium only (Figure 5 a). This may be because it is a seasonal plant.

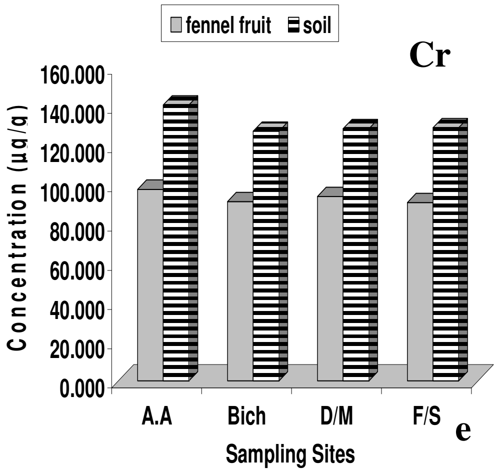
Metals like Mg, Cu and Cd have comparable concentration in the plant as that of the soil (Figure 5 b, d). In Cr moderate difference in concentration was observed (Figure 5 e). In the case of Zn and Co the concentration in the soil was higher than the fennel fruit at roughly by half (Figure 5 f, g). In other metals like Fe, Mn and Ni the concentration of the soil is very very high (Figure 5 h, j).

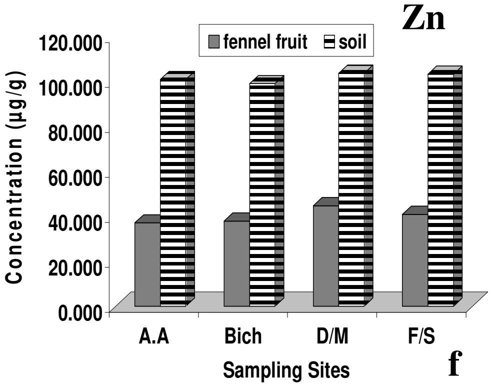

Ca
fennel fruit
soil
25,000.000
20,000.000
15,000.000
10,000.000
5,000.000
0.000
Concentration (µg/g)
A.A
Bich
D/M
F/S
Sampling Sites
a


Mg
fennel fruit
soil
3,500.000
3,000.000
2,500.000
2,000.000
1,500.000
1,000.000
500.000
0.000
Concentration (µg/g)
A.A
Bich
D/M
F/S
Sampling Sites
b


Cu
fennel fruit
soil
120.000
100.000
80.000
60.000
40.000
20.000
0.000
Concentration (µg/g)
A.A
Bich
D/M
F/S
Sampling Sites
c


Cd
fennel fruit
soil
3.000
2.500
2.000
1.500
1.000
0.500
0.000
Concentration (µg/g)
A.A
Bich
D/M
F/S
Sampling Sites
d


Cr
fennel fruit
soil
160.000
140.000
120.000
100.000
80.000
60.000
40.000
20.000
0.000
Concentration (µg/g)
A.A
Bich
D/M
F/S
Sampling Sites
e


Zn
fennel fruit
soil
120.000
100.000
80.000
60.000
40.000
20.000
0.000
Concentration (µg/g)
A.A
Bich
D/M
F/S
Sampling Sites
f

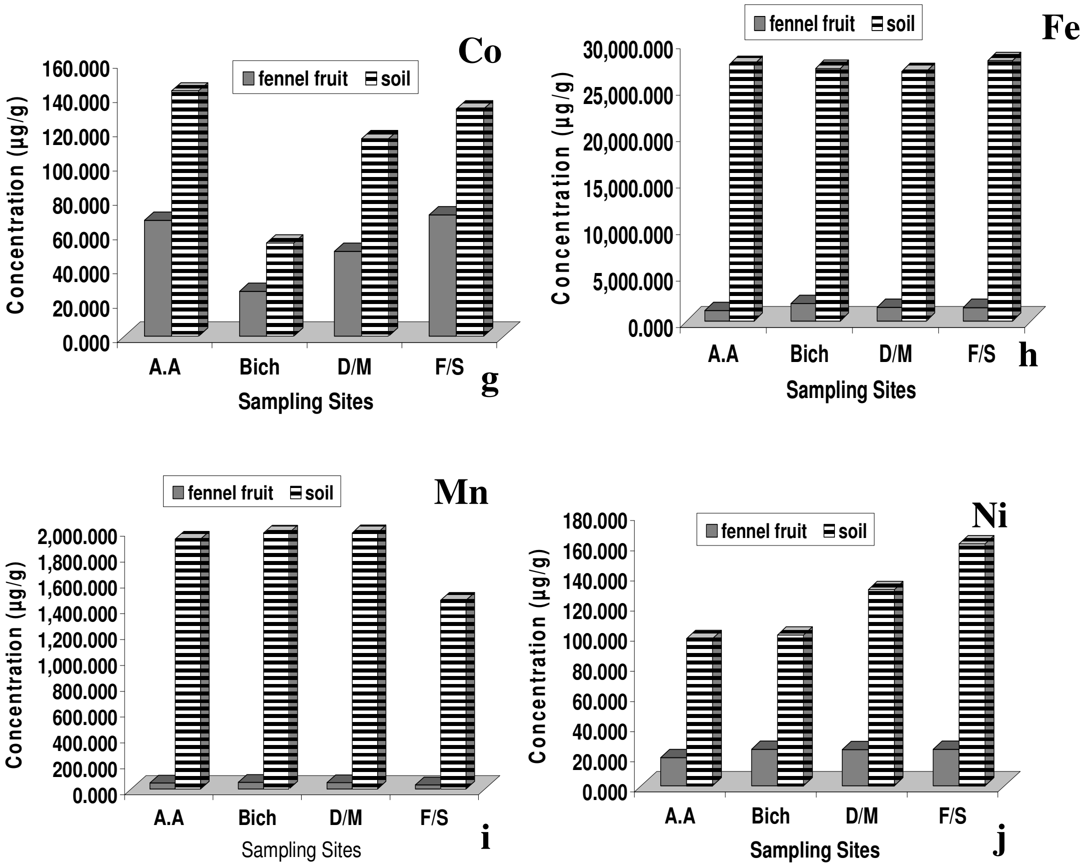


Figure 5 (a- j). Comparison of concentration of metals in the fennel fruit to metals in the soil

## 3.7 COMPARISON OF METAL LEVELS OF THE STUDY WITH LITERATURE VALUES

### 3.7.1 Comparison of Major Metals in Fennel Fruit Samples with Literature Values

The major metals (Ca, Mg, Fe and Mn) are determined in the fennel fruit in different countries from different points of view (nutritional, health problems, crop yield, etc). Some of the available studies were presented in Table 16 along with the current study for comparison.

Table 16. Summary of metal levels reported fennel fruit worldwide (all values are in µg/g).

| Country | | Ca | Mg | Fe | Mn | Ref. |
|---|---|---|---|---|---|---|
| India | | **23,900 ± 1300** | **5,110 ± 420** | **744 ± 20** | **72.3 ± 3.0** | 32 |
| Turkey | | 10,780 ± 221 | 2774 ± 39 | 225 ± 9 | 27.8 ± 0.4 | 13 |
| Turkey | | 6746 ± 1022 | 3400 ± 382 | 316 ± 35 | 33.4 ± 5.3 | 33 |
| **Ethiopia** | A.A | 20,544* | 2,392 | 1,136* | 45.8 | Present study |
| | B | **23,020** | 1,965 | **1,901** | **51.4** | |
| | D.M | 21,373 | **3,456** | 1,484 | 48.1 | |
| | F.S | 22,860 | 1,309* | 1,467 | 30.6* | |

A.A = Addis Ababa, B = Bichena, D.D = Debre Markos and F.S = Finote Selam
Bold fonts indicate the highest concentration of the metals.
The * indicate lowest concentrations of the metals.

The highest mean level of Ca, in this study was lower than study from India [32] but higher than from Turkey and from the undefined source [1, 13, 33]. The highest mean level of Mg in this study was lower than value obtained in the undefined source [1], slightly higher than from Turkey [23, 33], and higher than study from India [32]. Even the lowest mean level of Fe in this study was very high than studies from India, Turkey and undefined source value [1, 13, 32, 33]. The highest mean level of Mn in this study was lower than study from India but higher than others [1, 13, 32, 33]. Generally the values are acceptable except Fe which is very high than the literature value.

### 3.7.2 Comparison of Trace Metals Concentration of Fennel Fruit with Literature Values

Comparisons of levels of metals in fennel fruit reported in various parts of the world are summarized in Table 17.

Table 17. Summary of heavy metal levels in fennel fruit worldwide (all values in µg/g).

| Country | | Cu | Cr | Co | Zn | Ni | Cd | Pb | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| India | | 10.9 ± 0.8 | 1.50 ± | **0.54 ±** | 34.5 ± 4.4 | 1.46 ± | 0.0861 ± | **0.95 ±** | 32 |
| Turkey | | **16.2 ± 0.4** | 1.04 ± | 0.40 ± | 37.0 ± 2.4 | 5.40 ± | 0.004 ± | 0.48 ± | 13 |
| Turkey | | 8.28 ± 0.85 | **35.93 ±** | | 20.76 ± | **28.66 ±** | **0.50 ±** | 0.35 ± | 33 |
| **Ethiopia** | A.A | **103** | **97.7** | 67.7 | 37.1* | 18.7* | **1.91** | ND | **Present study** |
| | B | 48.4 | 91.4 | 26.2* | 37.8 | **24.2** | 1.67 | ND | |
| | D.M | 62.5 | 94.0 | 49.5 | **44.7** | 24.0 | 1.89 | ND | |
| | F.S | 23.9* | 90.9* | **70.8** | 40.8 | 24.2 | 1.59* | ND | |

A.A = Addis Ababa, B = Bichena, D.D = Debre Markos and F.S = Finote Selam
Bold fonts indicate the highest concentration of the metals.
The * indicate lowest concentrations of the metals.

Results of this study is very high than the literature values given in Table 17 for Cu, Cr, and Co. This variation may be due to difference in the geographical location, climate, agricultural chemicals like fertilizers or errors in sample preparation and analysis. Results of this study for Zn, Ni and Cd go with literature values.

### 3.7.3 Comparison of Major Metals in the Soil Samples with Literature Values

Table 18. Summary of metal levels reported soil worldwide (all values are in µg/g).

| Country | | Ca | Mg | Fe | Mn | Ref. |
|---|---|---|---|---|---|---|
| **Ethiopia** | J. | 490 ± 20 | 1,130 ± 10 | 26,200 ± 120 | 1,470 ± 30 | 33 |
| | A.A | | | 4,290 | 6,587 | 37 |
| | A.A | 1,435* | 2,866 | 27,628 | 1,933 | Present |
| | B | **1,775** | 2,259 | 27,195 | 1,979 | |
| | D.M | 1,634 | **3,306** | 26,863* | **1,981** | |
| | F.S | 1,770 | 1,257* | **28,034** | 1,460* | |

A.A = Addis Ababa, B = Bichena, D.D = Debre Markos, F.S = Finote Selam and J = Jima. Bold fonts indicate the highest concentration of the metals in the sites.

The * indicate lowest concentrations of the metals in the sites.

Table 19. Comparison of trace metal concentrations of the soil sample with values reported in literature.

| Country | | Cu | Cr | Co | Zn | Ni | Cd | Pb | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Ethiopia | Jim. | 25.6 ± 0.9 | | 19.9 ± 3.8 | 71.3 ± 0.5 | | **2.10 ± 0.25** | ND | 33 |
| | A.A | **55.0** | **115** | **43.0** | **2,986** | **115** | | | 37 |
| | A.A | | 47.8 ± 8.9 | | 113 ± 8 | | 0.25 | **6.02 ±0.34** | 27 |
| **Ethiopia** | A.A | **101** | **141** | **143** | 101 | 97.7* | **2.86** | ND | **Present study** |
| | Bich | 51.0* | 127* | 54.4* | 99.9* | 100 | 2.10 | ND | |
| | D/M | 67.9 | 129 | 115 | **104** | 130 | 2.41 | ND | |
| | F/S | 69.6 | 129 | 133 | 103 | **161** | 1.66* | ND | |

A.A = Addis Ababa, B = Bichena, D.D = Debre Markos, F.S = Finote Selam and J = Jima.
Bold fonts indicate the highest concentration of the metals in the sites.
The * indicate lowest concentrations of the metals in the sites.

The highest mean value of Cu in the literature is comparable to only Bichena site but lower than other sites. The mean value of Cr in this study was comparable to the highest literature value except for Addis Ababa site. Except for Bichena site the mean level of Co is higher than literature value. The highest literature value is extremely high than the current study but other values are comparable with mean Zn level of this study. Ni level of this study was comparable with literature value except for Finote Selam and Debre Markos sites. The highest literature value is comparable with mean value of Bichena and Debre Markos sites and it is lower than Addis Ababa site and higher than Finote Selam site.

## 3.8 STATISTICAL ANALYSIS

The comparison of variance in analysis and sampling, the equality of means and correlation between the metals in fennel fruit and soil samples were done separately using the Microsoft Excel (office 2007) and Microcal Origin 6.0 softwares.

### 3.8.1 Analysis of Variance

Variation in the mean levels of metals between the samples were tested whether it was from just a random error or treatment (i.e. difference in mineral contents of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides, etc or other variations in cultivation procedures)

Significant differences were obtained ($p < 0.05$) at 95% confidence levels for all metals in the soil and fennel fruit except Zn in the soil.

### 3.8.2 Pearson Correlation of Metals

The high association between metals, evidenced by high positive correlation coefficient, can arise from common anthropogenic or natural sources as well as from similarity in chemical properties [34, 42]. Cd, Ni and Mg was the least associated with metals in the soil and fruit which might imply different sources. The Pearson correlation matrices using correlation coefficient (r) for the samples were shown from Table 20 fennel fruit and soil samples of the four sits.

Table 20. Correlation of metals in the fennel fruit and soil samples in the four sits (n = 9).

| | Ca | Mg | Fe | Mn | Cu | Cr | Co | Zn | Ni | Cd |
|---|---|---|---|---|---|---|---|---|---|---|
| A.A | -0.842 | **0.425** | -0.663 | 0.985 | **-0.290** | **0.464** | **0.300** | -0.993 | 0.683 | 0.500 |
| B | **-0.395** | **0.000*** | **-0.092** | 0.947 | 0.615 | 0.957 | **-0.366** | 0.704 | **-0.211** | -0.500 |
| D.M | -0.613 | -0.707 | 0.979 | 0.756 | 0.936 | -0.699 | 0.977 | -0.998 | **0.219** | **-0.013** |
| F.S | -0.721 | -0.689 | 0.569 | -0.966 | -0.693 | -0.950 | -0.500 | 0.862 | -0.984 | -0.619 |

A.A = Addis Ababa, B = Bichena, D.D = Debre Markos, F.S = Finote Selam
Bold font indicates poor relation ($r <$ ± 0.500)
The * indicate no relation

There is poor relation for Mg, Cu, Cr, and Co in Addis Ababa site, Ca, Fe, Co and Ni in Bichena site and Ni and Cd in Debre Markos site between metals in the fennel fruit and soil. There is no relation between Mg in the soil and fennel fruit in Bichena site. The rest metals have good relation (at least greater than ± 0.5) between metals in the fennel fruit and soil.

## 4. CONCLUSION AND RECOMMENDATIONS

### 4.1 CONCLUSION

In this study fennel fruit and the soil in which the plant was grown was analysed for major and trace (Ca, Mg, Fe, Mn, Cu, Cr, Co, Zn, Ni, Cd and Pb) metals in the fruit. In fennel fruit it was found very high concentration of Ca, moderate Mg, and considerable concentration of Mn, Cu, Cr, and Co. Relatively low concentration of Ni and very low concentration of Cd was found in it.

Iron had the highest concentration of metals in soil followed by Ca and Mg. Cu, Cr, Co, Zn, and Ni concentration of the soil was considerable. The concentration of Cd was higher in the soil than in the fruit. Concentration of Pb in both the soil and the fruit was below the methoddetection limit. In all the metals except for Ca higher concentration were found in the soil than in the fruit.

The optimized wet digestion method for the fruit analysis was found efficient for all analysed metals as evaluated from good recovery values. The optimized method used minimum reagent, energy and time. Minimum consumption of reagent lead to reduced blank correction. Similarly optimized procedure was selected for wet digestion of the soil.

The presence of relatively high concentration of Ni and moderate concentration of Cd indicates that the areas are contaminated to some extent. For instance, in the case of Addis Ababa the concentration of Ni was 18.7 in the fruit and 97.7 in the soil. The concentration of this metal in the other sites is even higher in both the soil and the fruit. The reverse sequence was observed in the case of Ca.

### 4.2 RECOMMENDATIONS

It is recommended that continuous monitoring of the water, soil and plant for heavy metal accumulation is necessary because trace element are not bio-transformed to other formes and they have a tendency to accumulate on the soil and biota. Thus to reduce the bio-availability of trace metals in the soil should be monitored frequently. Similarly the plants should be examined frequently for trace metal. The use of organic manure instead of industrial fertilizers may minimize the accumulation of toxic metals in the fruit and the soil.

This study is limited to some part of the country due to economy, time, and labour shortage. Thus further country wide research should be conducted by me or other researchers.

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# DECLARATION

**I, the undersigned, declare that this is my original work and has not been submitted for a degree in any other University, and that all sources of material used for the thesis have been accordingly acknowledged.**

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**Place and Date of submission: School of Graduate Studies,**
**Addis Ababa University**
**July, 2010**