ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CHEMISTRY

Determination of Some Essential and Non-Essential Metals in Fenugreek Seed (*Trigonella Foenum-Graecum* L.) Cultivated in Different Parts of Ethiopia

BY

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ABSTRACT

Determination of Some Essential and Non-Essential Metals in Fenugreek Seed (*Trigonella Foenum-Graecum* L.) Cultivated in Different Parts of Ethiopia

By: Mebrahtu Hagos

Advisor: Prof. B. S. Chandravanshi

Fenugreek (*Trigonella foenum-graecum* L.) is native to *Ethiopia*. It is a plant mostly used as a spice and herbal medicine in most countries of the world. Fenugreek has many dietary and health benefits. It is used as anti-diabetes, hypercholesterolemia, anti-cancer, anti-microbial, against breast cancer, hypoglycemia, antioxidation, laxation, apetite stimulation, antiulcer, and immunomodulatory, spice in food, etc. In this study, the levels of major (Ca, K, Na, Mg), trace (Fe, Cr, Ni, Zn, Mn, Cu, Co), and toxic (Pb, Cd) metals in seed of fenugreek grown in different regions of Ethiopia were determined by flame atomic absorption spectrophotometry (FAAS). Wet ashing was used to digest 0.5 g fenugreek seed flour and was optimized using 1.5 mL of HNO_3 and $HClO_4$ acid mixtures (5:1 ratio), 30 min pre-digestion time, 45 min total digestion time and 150 ^{0}C temperature. Thirteen elements were analyzes and their concentrations are listed in the following ranges and order; Ca (15353–36771 mg/kg) > Fe (6041–18584 mg/kg) > K (6789–11517 mg/kg), > Pb (615–2624 mg/kg) > Na (201–1559 mg/kg), > Cd (285–464 mg/kg) > Cr (3–552 mg/kg) > Ni (31–108 mg/kg) > Mg (31–102 mg/kg) > Zn (15–33 mg/kg) > Mn(16–28 mg/kg) > Cu (N.D–35 mg/kg) > Co (4–15 mg/kg). A statistical analysis of variance (ANOVA) at 95% confidence level was used to test whether the variation between metal content of fourteen sample means were significant or not. In addition Pearson correlation coefficient was used to predict the dependence of metal levels of one with another.

Key words: Fenugreek seed (*Trigonella foenum-graecum* L.), Major elements, Trace elements, Toxic elements, Flame atomic absorption spectrophotometry

1. INTRODUCTION

1.1. Origin of fenugreek

Trigonella foenum-graecum (fenugreek) is native to *Ethiopia* and the area from the Eastern Mediterranean to central Asia and much cultivated in Pakistan, India and China [1-3]. This crop is native to an area extending from Iran to northern India and widely cultivated in China, India, Egypt, Ethiopia, Morocco, Ukraine, Greece, Turkey, etc [4]. The species *Trigonella foenum-graecum*, wild or cultivated, is widely distributed throughout the world. It has been reported as a cultivated crop in Portugal, Spain, United Kingdom, Germany, Australia, Switzerland, Greece, Turkey, Egypt, Sudan, Ethiopia, Kenya, Tanzania, Lebanon, Morocco, Tunisia, India, Pakistan, China, Japan, Russia, Argentina and USA [5]. The word "foenum-graecum" means in Latin "hay from Greece". A Latin substantive for hay, dried grass or fodder from meadows, and "graecum", "Greek". Common English name is fenugreek and vernacular names are Abake (Tigrigna), Abeshe (Guraginga), Abish (Amharic and Kore), Abishi or Sunqoo (Afan Oromo), Sets (Bas), Shiko (Sid), Shuko (Gam) and Shuqwa (Konta, wel) [6].

1.2. Botany of fenugreek

The genus *Trigonella* is one of the largest genera of the tribe Trifoliatae in the family Fabaceae and sub-family Papilionaceae. Among *Trigonella* species, *Trigonella foenum-graecum* (commonly known as fenugreek) is a flowering annual, with autogamous flowers occasionally visited by insects [4]. *Trigonella foenum-graecum* L. is erect aromatic annual to 50 cm tall, sparsely pubescent. Leaflets obovate, 20–50 x 8–17 mm, denticulate. Flowers are 1–2 in leaf–axils, corolla yellow or white, 12–18 mm long. In Ethiopia it grows at an altitude between 1600–2300 m [7]. Fenugreek *(Trigonella foenum-graecum* L.) is an annual dicotyledonous plant and it has two types of fenugreek flowers, i.e., cleistogamous (closed) and aneictgamous (open) flowers. However, the majority of fenugreek flowers are cleistogamous. The high frequency of cleistogamous flowers each with a protective papilionaceous corolla may have lead to development of fenugreek as a predominantly self-pollinated plant [8].

1.3. Chemical composition of fenugreek

Whole fenugreek seeds are about 50% fiber, with 20% of that mucilage. In addition to mucilage, fenugreek also contains protein, saponins, and the hypoglycemic phytochemicals coumarin, fenugreekine, nicotinic acid, phytic acid, scopoletin, and trigonelline [9-11] and 4-hydroxyisoleucine [10]. Fenugreek seeds have high contents of iron (21 mg/100 g), calcium (182 mg/100 g), zinc (4.9 mg/100 g) [12], lysins (5.7 g/16 gN) and beta-carotene [13]. Fenugreek seeds are a rich source of flavonoid compounds such as quercetin, luteolin, kaempferol, tricin, gallic acid, etc. Many workers have reported the antioxidant potential of fenugreek [14].

1.4. Major fenugreek growing areas in Ethiopia

In Ethiopia fenugreek is cultivated between altitudes of 1600 and 2300 m all over the country [6]. In Amhara regional state it is cultivated in the following zones of the state, namely North Gonder, South Gonder, East Gojam, West Gojam, North Shewa, South Wollo and North Wollo. In Tigray regional state it is cultivated in Central Tigray, Eastern Tigray and Southern Tigray zones. In Oromia regional state it is cultivated in the following zones: West Welega, Illubabor, East Shewa, Arsi, West Harerge, East Harerge, Kelem and Horoguduru. And in S.N.N.P. regional state it is cultivated in the following areas: Yem special wereda, Burji special wereda, Silitie and Alaba special wereda.

1.5. Production of fenugreek in Ethiopia

Even though, the production of fenugreek in Ethiopia is very low it has been used in many different types of foods and traditional medicines for a very long time. According to the central statistical agency (CSA) of Ethiopia the production of fenugreek seed in Ethiopia in 2008/09 was 376588 quintals in 33773 ha land coverage.

1.6. Dietary use and health benefits of fenugreek

Seeds of fenugreek have been reported for their pharmaceutical properties in treating such human diseases as diabetes and hypercholesterolemia [1]. Fenugreek leaves and seeds are consumed in different countries around the world for different purposes such as medicinal uses (anti-diabetic, lowering blood sugar and cholesterol level, anti-cancer, anti-microbial, against breast cancer [15], avoids blood poison of wounds [16], etc.), making food (stew with rice in Iran, flavor cheese in Switzerland, syrup and bitter run in Germany, mixed seed powder with flour for making flat bread in Egypt, curries, dyes, young seedlings eaten as a vegetable, etc.), roasted grain as coffee-substitute (in Africa), controlling insects in grain storages, perfume industries, etc. Fenugreek can be a very useful legume crop for incorporation into short-term rotation and for hay and silage for livestock feed, for fixation of nitrogen in soil and its fertility, etc [4, 17]. *Trigonella foenum-graecum* L. (Fenugreek) is a well-known leguminous herb grown extensively in Turkey and has been used as a cooking spice, for preparing "Çemen" and folk remedy for thousands of years. Çemen is composed of crushed classical fenugreek seeds, garlic and chilli pepper mixed to a paste with a little water. The çemen paste covering the slabs of pastýrma is both an important factor in the flavor and protects the meat from drying and spoiling by contact with the air, which would cause the fat in the pastýrma to oxidise and give a bitter flavor. The seeds are reported to have nutritive properties and to stimulate digestive process. *T. foenum-graecum* is known to have several pharmacological effects such as hypoglycemia, hypocholestrolemia, antioxidation, laxation, apetite stimulation, antiulcer, and immunomodulatory. The seeds of this ancient herb have been used as both a spice and an herbal remedy in the Middle East, India, and Egypt and slightly shorter time in Europe, China and other parts of the world. As a folk medicine fenugreek has been commonly used as a digestive aid and to treat intestinal disorders, diarrhea and other stomach upsets, chronic cough, bronchitis, tuberculosis, fever, sore throat and mouth ulcers, and diabetes. Poultices and other external formulations have been smoothed onto wounds, skin irritations, and areas afflicted by nerve pain. Some companies started to use the seeds or seed extract of *T. foenum-graceum* as an herbal supplement and nutrition in the last few years [18]. Fenugreek seeds and leaf infusion of *Phyllanthus emblica* Linn, together is given to treat chronic diarrhea [19]. Alcoholic extract of seeds of *T. foenum gracium* have a potent antihelminthic (antihelminthics are those agents that expel parasitic worms (helminthes) from the body, by either stunning or killing them) activity when compared with conventionally used drug. In addition to the above this plant is also used to treat fever, dysentery, heart diseases, aphrodisiac, diuretic, emmenagogue and tonic [3]. In traditional Indian dish, for pregnant and lactating women it is used to stimulate appetite and boost milk production [20]. In Ethiopia, fenugreek is used in preparing "Hilbet" a traditional white, soft and delicious food in Tigray regional state. It is also used in making tea, "Injera", flat bread mostly made of "teff" flour, spice in milk and in traditional medicine.

1.7. Minerals in foods

More than 60 elements may be present in foods. It is customary to divide the minerals into two groups, the major salt components and the trace elements. The major salt components include potassium, sodium, calcium, magnesium, chloride, sulfate, phosphate, and bicarbonate. Trace elements are all others and are usually present in amounts below 50 parts per million (ppm). The trace elements can be divided in to the following three groups: (i) essential nutritive elements, which include Fe, Cu, I, Co, Mn, Zn, Cr, Ni, Si, F, Mo, and Se; (ii) nonnutritive, nontoxic elements, including Al, B, and Sn; and (iii) nonnutritive, toxic elements, including Hg, Pb, As, Cd, and Sb. Metals are often present in the form of chelates. Chelates are metal complexes formed by coordinate covalent bonds between a ligand and a metal cation; the ligand in a chelate has two or more coordinate covalent bonds to the metal [21].

Calcium

All cells need calcium, but more than 99% of the calcium in the body is used to strengthen bones and teeth. This calcium represents 40% of all the minerals present in the body and equals about 1200 g. As calcium circulates in the blood stream, it supplies the calcium needs of body cells. Growth and bone development require an adequate calcium intake. Calcium requires an acid environment to be absorbed efficiently. Absorption occurs primarily in the upper part of the small intestine. Calcium is essential for blood clotting and for muscle contraction. If the blood calcium level falls below a critical point, muscles cannot relax after contraction; the body stiffens and shows signs of tetany. In nerve transmission, calcium works to release chemical messengers and permits the flow of ions in and out of nerve cells. Without sufficient calcium, nerve function fails, opening another path to tetany. Calcium helps regulate cellular metabolism by influencing the activities of various enzymes and hormonal responses. Dietary calcium may reduce risk of colon cancer, especially in people consuming a high–fat diet. Researchers suggest that a dietary calcium intake of 1500-2000 mg per day is needed to reduce colon cancer risk. The recommended dietary allowance (RDA) for calcium for adults is 800 mg per day. The current RDA extends the 1200 mg standard used for the teenage years to age 25, in order to contribute to building and maintaining a higher bone mass. The major risk from taking excess supplements is development of one form of kidney stones, as well as constipation, intestinal gas, and interference with absorption of other minerals. However, sticking to a daily limit of 2000 mg from diet plus any supplement use poses little risk for developing these problems [22].

Potassium

Potassium performs many of the same functions as sodium. Unlike sodium, which is found primarily outside cells as sodium ions (Na^+), potassium is found mostly inside cells as potassium ions (K^+). About 95% of the potassium in the body is in the intracellular fluid. Potassium participates in maintaining the body's fluid balance and works with sodium in conduction of nerve impulses. Unlike sodium, which tends to raise blood pressure in some people, potassium is more associated with reducing blood pressure. Although the body absorbs 90% of the K consumed, we rarely add potassium to our food. That is why; a potassium deficiency is more likely than sodium deficiency. Potassium stores may be depleted in some diseases states, such as long bouts of vomiting or diarrhea. Low blood potassium (hypokalemia) is a life-threatening condition. It often results in a loss of appetite, muscle cramps, confusion and a pathy, and constipation. Eventually, the heart will beat irregularly, decreasing its capacity to pump blood. The minimum potassium requirement for health for adults is 2000 mg per day. If the kidneys function normally, typical intakes of dietary potassium are not toxic. When the kidneys function poorly; potassium builds up in the blood, creating a condition called hyperkalemia. This inhibits heart function, causing slowed heart beats eventually stops beating. Consequently, in cases of reduced kidney function, close control of potassium intake becomes critical [23].

Sodium

Almost all sodium dietary is absorbed. Its role within the body is primarily as the major positive ion (Na^+) in extracellular fluid. Sodium ions play a crucial role in conduction of nerve impulses. The concentration of sodium ions is much higher outside cells than inside cells, where as the concentration of potassium ions is much higher inside cells than outside cells. A low-sodium diet coupled with high perspiration losses or diarrhea depletes the body of sodium. This state can lead to muscle cramps, nausea, vomiting, and dizziness and eventually to shock and coma. The minimum sodium requirement for health is 500 mg/day for adults [23].

Magnesium

Magnesium is present in the plant pigment chlorophyll, making plant foods a rich source of this mineral. We absorb about 30%-40% of the magnesium in our diets, but absorption efficiently can increase to 80% when intakes of magnesium are low. Bone contains 60% of the body's magnesium. The rest circulates in the blood and operates inside cells in the form of magnesium as activating co-factor. Without magnesium, many enzymes would function less efficiently. Proper nerve, lung, and cardiac function require magnesium. Animals deficient in magnesium become very irritable. Eventually, if the deficiency becomes severe, the animals suffer convulsions and often die. In humans, a magnesium deficiency causes an irregular heartbeat, which is sometimes accompanied by weakness, muscle pain, disorientation, and seizes. However, a magnesium deficiency develops very slowly because we have large stores. A link between magnesium deficiency and sudden heart attacks has been observed, and its use in therapy for acute myocardial infarction is under investigation. Adequate magnesium status also contributes to bone health. The adults recommended dietary allowances (RDA) for magnesium is 350 mg/day for men and 280 mg/day for women. The daily value used for food labeling is 40 mg. High blood magnesium leads to weakness, nausea, and eventual malaise. Elderly people are at particular risk, as kidney function may be compromised [23].

Iron

Iron in foods occurs in several forms, which differ in their absorption by the body. Iron that is part of the hemoglobin and myoglobin molecules in animal flesh (about 40% of the total iron present), called heme iron, is absorbed more than twice as efficiently as simple elemental iron, known as non-heme iron. Non-heme iron is also present in animal flesh, eggs, and milk, as well as vegetables, grains, and other plant foods. The body uses several mechanisms to regulate iron absorption because the body cannot easily eliminate excess iron once absorbed. Eating meat with vegetables and grain products generally improves the absorption of non-heme iron present in the meal. Several dietary factors interfere with our ability to absorb iron. Phytic acid and other factors in grain fibers and oxalic acid in vegetables can all bind iron, reducing its absorption. Iron forms part of hemoglobin in red blood cells, myoglobin in muscle cells, and the cytochromes, which are components of the electron-transport chain in mitochondria. Iron also functions as a co-factor for some enzymes, including those involved in the synthesis of collagen and of various neurotransmitters (e.g., dopamine, epinephrine, norepinephrine, and serotonin). In addition iron is needed for proper immune function and plays a role in drug-detoxification pathways. Hemoglobin molecules in red blood cells transport oxygen from the lungs to all cells and assist in the transport of some carbon dioxide (CO_2) from cells to the lungs for excretion. Bone marrow cells synthesize red blood cells when stimulated by erythropoietin, a hormone synthesized mainly by kidneys. Erythropoietin is released in response to a decrease in oxygen concentration in the blood, blood loss, or binding of carbon monoxide (CO) to red blood cells. Erythropoietin targets the bone marrow to increase production of red blood cells. More than 90% of hemoglobin iron is recycled. Adult men lose about 0.9 to 1.2 mg iron per day from the GI (gastrointestinal) tract, urine, and skin. Women lose more iron because of menstrual blood loss. When averaged over the entire month, iron losses for women are about 1.4 to 2.2 mg/day, depending on the amount of menstrual blood loss. If the diet or the body cannot supply the needed amount of iron for hemoglobin synthesis, red blood cell synthesis is reduced. Eventually, the number of red blood cells falls so low that the amount of oxygen carried in the blood is decreased. Such a person exhibits anemia (characterized by a decreased oxygen-carrying capacity of the blood). The body needs to absorb about 0.9 to 1.2 mg of iron daily for men and 1.4 to 2.2 mg daily for women. The adult RDA for iron is 10 mg/day for men and 15 mg/day for women. Iron over load can be serious because it can easily lead to toxic symptoms. Even a large single dose of 60 mg can be life threatening to a 1-year-old. In addition, iron toxicity accompanies the genetic diseases called hereditary hemochromatosis. People with hemochromatosis over absorb iron. This disorder is characterized by increased absorption, saturation of iron-binding proteins, and deposition of hemosiderin in the liver tissue. Carriers of hemochromatosis may be prime candidates for heart diseases [23].

Chromium

Much is not known about chromium, but chromium deficiency may be related to diabetes in some individuals. The most-studied function of chromium is the maintenance of glucose uptake in to cells. Chromium enters the cell and likely increases the number of insulin receptors or enhances the transport of glucose across the cell membrane. In both animals and man, a chromium deficiency is characterized by impaired glucose tolerance and elevated serum cholesterol and triglycerides. The estimated safe and adequate daily dietary intake (ESADDI) of chromium is 50 to 200 µg/day. Marginal to low chromium intakes in the elderly may contribute to their increased risk for developing diabetes. Chromium intakes of less than 20 µg/day may be determined to a significant portion of the population that has marginally elevated blood glucose. High chromium intakes may result to liver damage and lung cancer [23].

Nickel

Plants and animals need nickel (Ni) for the activity of certain enzymes and perhaps for iron metabolism. Humans have never exhibited a deficiency of nickel when consuming a mixed diet. Researchers estimate that adults need about 100 to 300 µg of nickel per day [23].

Zinc

Although zinc has been recognized as essential nutrient in animals since the early 1900s, zinc deficiency was first recognized in humans in the early 1960s in Egypt and Iran. The deficiency was determined to be the cause of growth retardation and inadequate sexual development in humans. Over 300 enzymes require zinc as a co-factor for optimal activity. Adequate zinc intake is necessary to support many bodily functions, such as: nucleic acid synthesis and function (some factors that control expression of genes contain zinc-rich regions that bind DNA), Protein metabolism, wound healing and growth, immune function (intake the excess of the RDA do not provide any extra benefit to immune function), development of sexual organs and bone, storage, release and function of insulin, cell membrane structure and function, alcohol metabolism, and taste sensation. Zinc is important for the growth and behavioral developments of infants. Aside from inadequate growth, signs and symptoms of zinc deficiency include acne like rash, diarrhea, lack of appetite, fall in immune function, reduced sense of taste and smell, impaired appetite, hair loss, mental confusion, delivery of low birth weight infants by pregnant women and inadequate sexual development in children and adolescents. The adult RDA for zinc is 15 mg/day for men and 12 mg/day for women. The average adult intake of zinc is 15 mg and 9 mg daily for men and women, respectively. Although some women may have marginal intakes, there is no evidence of wide spread moderate or severe zinc deficiencies among otherwise healthy adult women and men. High zinc intakes, greater than about twice the RDA, inhibit copper absorption. Zinc does this by simulating synthesis of the mineral-binding protein metallothionein. One study has shown that zinc supplements at approximately three to five times the RDA can reduce HDL by about 15%, perhaps by interfering with copper metabolism. Low HDL is associated with an increased risk of developing heart diseases. Zinc intakes over 100 mg/day also result in diarrhea, cramps, nausea, vomiting and depressed immune system function, especially if intake exceeds 2 g/day [22, 23].

Manganese

It is easy to confuse the mineral manganese (Mn) with magnesium (Mg). Their names are similar, and in a few metabolic pathways they can substitute for each other. Manganese is a co-factor for certain enzymes, including pyruvate carboxylase (an enzyme in carbohydrate metabolism). Manganese is important in bone formation. No manganese deficiency symptoms have been observed in humans. Animals on manganese-deficient diets exhibit changes in brain function, bone formation, and reproduction. If human diets were low in manganese, the problems would probably appear in human as well. The estimated safe and adequate daily dietary intake of manganese is 2 to 5 mg/day. Average intakes fall within this range. Manganese toxicity has been seen in people working in manganese mines, and includes severe psychiatric abnormalities, hyper irritability, violence, hallucinations and impaired control of muscles [23].

Copper

Copper is a part of certain enzymes, contributes to the activity of other enzymes, and aids in iron metabolism. About 10% to 55% of dietary copper is absorbed, with higher intakes associated with lower absorption. Copper is excreted primarily via the bile in to the GI tract. Copper increases iron absorption by helping form a protein called ceruloplasmin (also called ferroxidase). This compound mobilizes iron by accepting an electron from Fe^{2+} to form Fe^{3+}, the form that can cross the cell membrane of intestinal and other body cells. Copper is part of an enzyme that forms cross-links in collagen and elastin connective tissue proteins. Copper is also part of enzymes that synthesize norepinephrine and dopamine, two neurotransmitters. Copper participates in the immune system function, blood clotting and cholesterol metabolism. Signs and symptoms of copper deficiency include anemia, decrease numbers of white blood cells (specifically, the neutrophils), bone loss, and inadequate growth. Copper deficiency has been linked to a form of heart disease in laboratory animals. Copper has an estimated safe and adequate daily dietary intake of 1.5 to 3 mg/day for adults. Our average intake is about 1 to 1.5 mg daily. Copper tends to cause vomiting at single doses greater than 10 to 15 mg. When copper is used to treat deficiency, it must be given in divided doses to limit this effect. An inherited condition called Wilson's disease results in accumulation of copper in the liver, brain, kidneys and cornea of the eye. If recognized early, treatment with agents that bind copper in blood stream and increase its excretion in the urine can prevent damage to these tissues and reduce the mental degeneration commonly seen in active cases [23].

Cobalt

Cobalt is an integral part of vitamin B_{12} (cobalamin), which is necessary for myelin formation and insulating layer found around nerves, to support red blood cell production, and it is also essential for the metabolism of fats, carbohydrates, the synthesis of proteins, and the conversion of folate to its active form. The average adult body contains 2 to 5 mg of vitamin B_{12}, of which most is stored in the liver. Vitamin B_{12} is available in several supplemental forms, of cyanocobalamin and hydroxycobalamin (hydroxycobalamin = injectable) are the main synthetic forms that have a cyanide molecule attached, while adenosylcobalamin and methylcobalamin occur as two coenzymatically active and more efficient forms. Cobalt deficiency is not a major problem though as long as one has adequate amount of vitamin B_{12}. Long term vitamin B_{12} deficiency can result in demyelination of large nerve trunks and the spinal cord, in reduced white blood cells, and in pernicious anemia with symptoms of severe fatigue, shortness of breath, dizziness and headaches. Overdosing on cobalt (> 5 mg/day) include abnormal thyroid functions, polycythemia and overproduction of red blood cells (erythropoiesis), with increased production of the hormone erythropoietin (EPO) from kidneys [24].

Lead

Ingested lead can cause anemia, kidney diseases and damage to the nervous system and can interfere with nerve impulse conduction. Lead toxicity is especially a problem for children because it is associated with IQ deficits, behavior disorders, slowed growth and impaired hearing, and possibly high blood pressure and kidney disease later in life. Exposed children who eat a high-fat diet low in calcium and low in iron absorb more lead. According to centers for Diseases Control and prevention (CFC) 10 µg/100 mL is a dangerous amount of lead toxicity [22, 23, 25].

Cadmium

Cadmium compounds are used as color pigment and in re-chargeable nickel-cadmium batteries. It is also present as a pollutant in phosphate fertilizers. Jarup *et al.* pointed out that cadmium is present in most food stuffs, but concentrations vary greatly. Cadmium exposure may cause kidney damage and/or skeletal damage [25].

1.8. Purpose, scope and limitations of the study

So many studies have been done on *Trigonella foenum-graecum* (fenugreek) globally specially in the areas of medicine and metal analysis. However, in Ethiopia none of these studies have been done. Therefore, the researcher would like to study the metal levels of essential and non-essential metals in *T. foenum-graecum* (fenugreek) cultivated in Ethiopia because this plant is used and cultivated though out of the country. The assessment of major metals (Ca, K, Na and Mg), trace metals (Fe, Cr, Ni, Zn, Mn, Cu, Co) and toxic metals (Pb, Cd) in *T. foenum-graecum* (fenugreek) samples collected from Amhara and Tigray regional states of Ethiopia was conducted to offer experimental data which can be used to identify the nutritional use as well as the toxicological status of the spice. It is also helpful to estimate the sources of metals and assess the pollution level. However, to obtain detailed information and come up with general conclusion on the metal levels of fenugreek cultivated in Ethiopia it may need collection of samples from the whole country, which was not possible in this study. Techniques other than FAAS could also be used to verify the accuracy and precision of the results.

1.9. General objectives

The main objective of this thesis is to determine the levels of essential and non-essential metals in *Trigonella foenum-graecum* L. (fenugreek) growing in Ethiopia.

1.10. Specific objectives

1. To develop an optimum working procedure for digestion of fenugreek seed samples.
2. To determine the concentrations of major (Ca, K, Na, Mg), trace (Fe, Cr, Ni, Zn, Mn, Cu, Co), and toxic (Pb, Cd) metals in seed of fenugreek by flame atomic absorption spectrophotometry (FAAS).
3. To compare the levels of the identified metals in fenugreek seed in different regions of Ethiopia with that of the data in the literature.
4. To compare the levels of the metals in fenugreek seed with that of the data on other spices available in the literature.

2. EXPERIMENTAL

2.1. Instruments and apparatus

Digital heat oven (P Selecta J.P. Selecta, Spain) was used to dry the fenugreek seed samples at 80 ^{0}C for 24 h. Blender (Moulindex, France) was used to ground and homogenize the fenugreek seed samples and then the samples were allowed to pass through 1.4 mm sieve. Analytical balance (Adam equipment, Model WL 3000, Lion brand, U.K.) was used to weigh 0.5 g of each sample. Pippetes of 1 mL, 2 mL, 5 mL and 10 mL were used to measure different volumes of acids and/or oxidizing agents. The weighed sample and the needed amount of acids were poured in to 19/26 and 24/26 digestion flasks for the first 30 min pre-digestion time and then fixed with Gallenhamp Kjeldahl apparatus (U.K.) for the next 45 min full digestion time. For preparation of standard solutions and dilution of sample solutions 50 and 100 mL volumetric flasks were used. The sample solution was filtered with Whatman filter paper in a 50 mL volumetric flask. Then the flask was filled with deionized water up to the mark and was ready for farther analysis. Flame atomic absorption spectrophotometer (Buck Scientific Model 210 VGP AAS, East Norwalk, USA) using deuterium background corrector with air-acetylene flame was used for the determination of the concentration of the metals Ca, K, Na, Mg, Fe, Cr, Ni, Zn, Mn, Cu, Co, Pb, and Cd. Micropipettes (Dragonmed, Shangai, China, 100-1000 µL) were used for spiking of known concentrations of analyte during the recovery test.

2.2. Chemicals and reagents

All the chemicals and reagents used were analytical grade. Sulfuric acid 98%, H_2SO_4 (Fine Chem, Mumbai, India), perchloric acid 70%, $HClO_4$ (Fine Chem, Mumbai, India), hydrogen peroxide, 30%, w/w, extra pure (Spain), nitric acid, HNO_3 (pure 69-72%) (Spectrol, BDH, England) were used for the digestion of fenugreek seed samples. For preparation of calibration curves standard solutions (1000 mg/L in 2% HNO_3) for Ca, K, Na, Mg, Fe, Cr, Ni, Zn, Mn, Cu, Co, Pb, and Cd (Buck Scientific Puro-Graphic[tm]) were used. $LaNO_3.6H_2O$ (98%, Aldrich, USA) solution was used to prevent chemical interference from phosphates and other anions in the measurements of Ca, Mg, Fe, Zn, Mn and Cu [34]. Chemically pure deionized water (less than 2 µs/cm) was used for cleaning digestion flasks and volumetric flasks and dilution of samples. Chemical interference is caused by any component of the sample that decreases the extent of atomization of analyte. For example, SO_4^{2-} and PO_4^{3-} hinder the atomization of Ca^{2+}, perhaps by forming non-volatile salts. Releasing agents such as EDTA and 8-hydroxyquinoline are added to a sample to protect chemical interference. EDTA and 8-hydroxyquinoline protect Ca^{2+} from the interfering effects of SO_4^{2-} and PO_4^{3-}. La^{3+} can also be used as a releasing agent, apparently because it preferentially reacts with PO_4^{3-} and frees the Ca^{2+}. A fuel rich is recommended to reduce certain oxidized analyte species that would otherwise hinder atomization. Higher flame temperatures eliminate many kinds of chemical interference [26].

2.3. Sample collection

Fenugreek seed samples were bought from villagers from local markets in different regions of Ethiopia. And they were handled by polyethylene plastic bags, tied with rubber bands and finally transported safely to the Science Faculty of Addis Ababa University.

Table 1. Description of sampling areas.

No	Sample site	Zone	Region
1	Denboskie	North Gonder	Amhara
2	Denbia	North Gonder	Amhara
3	Ambagiorgis	North Gonder	Amhara
4	Dabat	Northern Gonder	Amhara
5	Jehaniagiorgis	West Gojam	Amhara
6	Keteteekli	Central Tigray	Tigray
7	Adet	East Gojam	Amhara
8	Bichena	East Gojam	Amhara
9	Meseretgamra	Southern Tigray	Tigray
10	Mayadrasha	Central Tigray	Tigray
11	Eidagaarbi	Central Tigray	Tigray
12	Hatsebo	Central Tigray	Tigray
13	Debrebirhan	North Shewa	Amhara
14	Korem	Southern Tigray	Tigray

Sample sites were selected both from Amhara and Tigray regional states based on the wider cultivation of the plant in these regions of the country. Fourteen sample sites were used in this study and these are Donbeskie, Koladiba, Ambagiorgis, Dabat, Johaniagiorgis, Keteteekli, Adet, Bichena, Meseretgamra, Mayadrasha, Edagaarbi, Hatsebo, Debrebirhan and Korem (Table 1).

2.4. Sample preparation

Fenugreek seeds were collected from different parts of Amhara and Tigray regional states of Ethiopia. They were bought from their corresponding local markets from peasants of that area. Half a kilogram was bought at most sample places and were placed in polyethylene plastic bags and finally transported to Science Faculty of Addis Ababa University for farther study. Each sample was washed with deionized water and then allowed to sun dry in an open air. After that each sample was oven dried at 80 ^{0}C for 24 h. Moreover, each sample was grounded with a stainless steel blender (Moulindex, France) and the flour was allowed to pass through 1.4 mm mesh and placed in clean labeled plastic bags.

2.5. Cleaning apparatus

The pipettes and volumetric flasks were first washed with water and detergent and then immersed in 10% HNO_3 solution for 24 h and finally rinsed with deionized water. 100 mL digestion flasks were first washed with 1% (w/v) $K_2Cr_2O_7$ in 98% (v/v) H_2SO_4 solution followed by 10% HNO_3 solution and finally rinsed by deionized water.

2.6. Optimization of the digestion procedure

There are different methods in which organic samples can be decomposed by inorganic acids in an open vessel and one of them is *wet ashing*. Wet ashing is the process of oxidation decomposition of organic samples by liquid oxidizing reagents, such as HNO_3, H_2SO_4, $HClO_4$, or mixture of these acids. This decomposition process converts the organic sample to carbon dioxide and water. Non-metallic elements such as halogens, sulfur, and nitrogen, are wholly or partially lost by volatilization in an open vessel [27]. There are also other digestion-procedures, i.e. dry and microwave digestion procedures. In terms of digestion efficiency, dry and wet digestion methods are more time consuming and complicated than the microwave digestion. However, the use of microwave digestion system in spice samples provides a better, safer and cleaner method of sample preparation [28] and is very expensive compared to the above two.

2.6.1. Digestion procedures of this study

To optimize the digestion of fenugreek seed sample different oxidizing agent and/or acid combinations were tried. For instance; mixture of conc. H_2SO_4/30% H_2O_2, conc. H_2SO_4/HNO_3/30% H_2O_2 and HNO_3/$HClO_4$ were tried. The first and second combinations were not good and only the last combination could give best result. Therefore, to optimize the fenugreek seed different ratios of HNO_3/$HClO_4$ were used and the best result was obtained at 5:1 ratio. The digestion for this study was optimized at 1.5 mL total volume of acid mixtures, 150 ^{0}C temperature, 30 min pre-digestion time and 45 min total digestion time. A mixture of HNO_3 and $HClO_4$ is used in this study because the same plant material was digested by these acids in other literatures. The digestion procedures used in this study is given in Table 2. The optimization of the digestion procedures for this study was done based on the parameters of time, volume, temperatures and reagent compositions. And the optimized condition was selected based on the criteria that could give minimum cost of the reagents, time and clear solution.

Table 2. Optimization of the parameters during wet ashing of fenugreek seed samples.

Ratio of HNO_3 to $HClO_4$	Total volume (mL)	Temperature of digestion (^0C)	Pre-digestion time (min)	Time of digestion (h)	Color of solution
I. Optimization of $HNO_3/HClO_4$ ratio					
1:1	6	270	15	3	Colorless solution
2:1	6	270	15	3	Clear pale yellowish solution
5:1	**6**	**270**	**15**	**3**	***Clear colorless solution**
1:2	6	270	15	3	Clear colorless solution
3:2	6	270	15	3	Clear very pale yellowish
II. Optimization of volume					
5:1	6	270	15	3	Clear colorless
5:1	5	270	15	3	Clear colorless
5:1	4	270	15	3	Clear colorless
5:1	3	270	15	3	Clear colorless
5:1	2	270	15	3	Clear colorless
5:1	**1.5**	**270**	**15**	**3**	***Very clear colorless**
III. Optimization of temperature					
5:1	1.5	30	15	3	Yellowish
5:1	1.5	60	15	3	Very pale yellowish
5:1	1.5	90	15	3	Very pale yellowish
5:1	1.5	120	15	3	Very pale yellowish
5:1	1.5	150	15	3	Very pale yellowish
5:1	**1.5**	**180**	**15**	**3**	***Very clear colorless**
5:1	1.5	210	15	3	Very clear colorless
5:1	1.5	240	15	3	Very pale yellowish
5:1	1.5	270	15	3	Very pale yellowish
5:1	1.5	300	15	3	Colorless solution
IV. Optimization of time					
5:1	1.5	180	15	1/4	yellowish
5:1	1.5	180	15	1/2	Clear yellowish
5:1	**1.5**	**180**	**15**	**3/4**	***Very clear colorless**
5:1	1.5	180	15	1	Very clear colorless
5:1	1.5	180	15	5/4	Very clear colorless
5:1	1.5	180	15	3/2	Very clear colorless
V. Optimization of pre-digestion time					
5:1	1.5	180	15	3/4	Very clear colorless
5:1	**1.5**	**150**	**30**	**3/4**	***Clear colorless**

* Indicates optimized condition.

2.7. Digestion of fenugreek seed samples

0.5 g of powdered fenugreek seed was transferred in to 100 mL digestion flask followed by 1.5 mL total volume of $HNO_3/HClO_4$ acid mixture, i.e. 5:1 ratio. The mixture was allowed to stay in an open air for 30 min pre-digestion time. In addition to the above, the sample mixture was digested at 150 ^{0}C for 45 min using Gallenkamp Kjeldahl apparatus. The sample in the digestion flask was allowed to cool for about 40 min and then filtered with 70 mm Whatman filter paper in a 50 mL volumetric flask. 1 mL lanthanum nitrate solution and deionized water were poured to the filtrate up to the mark. The solution in the volumetric flask was ready for further analysis using flame atomic absorption spectrophotometer.

2.8. Digestion of blank

To determine the concentration of metals in the acid mixtures, blank samples were digested in the same procedure as real fenugreek seed samples. Therefore, a total of 10 blanks were prepared.

2.9. Determination of essential and non-essential metals

An intermediate standard solution of concentration 10 mg/L for each metal was prepared by dilution of about 1000 mg/L stock standard solution. The prepared solution was used to calibrate the instrument before analyzing the concentration of the samples. A total of thirteen elements were analyzed using flame atomic absorption spectrophotometer in this study. All the twelve metals (Ca, K, Mg, Fe, Cr, Ni, Zn, Mn, Cu, Co, Pb, Cd) were analyzed by absorption mode of the instrument while Na was analyzed in the emission mode of the instrument to avoid ionization interference. Data was taken in triplicate readings.

Table 3. Instrument operating conditions for the determination of metals in fenugreek seed samples using flame atomic absorption spectrophotometer.

Element	Wavelength (nm)	Slit width (nm)	Detection limit (mg/L)	Lamp current (mA)	Energy
Na	589.0	0.2	0.002	2.0	2.010
Mg	285.2	0.7	0.001	1.0	3.970
K	766.5	0.7	0.01	2.0	3.410
Ca	422.7	0.7	0.01	2.0	3.604
Cr	357.9	0.7	0.05	2.0	3.698
Mn	279.5	0.7	0.01	3.0	3.943
Fe	248.3	0.2	0.03	7.0	3.598
Co	240.7	0.2	0.05	4.5	2.831
Ni	232.0	0.2	0.04	7.0	2.935
Cu	324.8	0.7	0.02	1.5	3.771
Zn	213.9	0.7	0.005	2.0	3.137
Cd	228.9	0.7	0.005	2.0	3.110
Pb	283.3	0.7	0.1	2.0	3.160

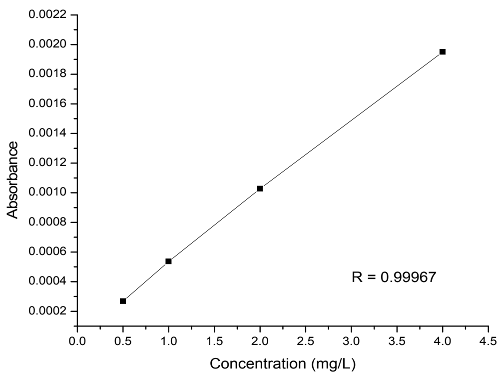
3. RESULTS AND DISCUSSIONS

3.1. Instrument calibration

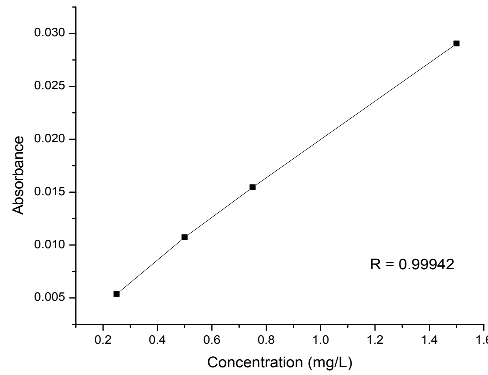
The atomic absorption spectrometer is sensitive to errors that come during calibration of the instrument and working standard solution preparations. The instrument was calibrated using four series of working standards. The working standard solutions of each metal were prepared freshly by diluting the intermediated standard solutions. Concentrations of the working standards and values of correlation coefficient of the calibration curve for each of the metals are listed in Table 4. The calibration graph of each of the metal of interest is shown in Figure 1.

Table 4. Instrument detection limit, concentration of standard solutions and correlation coefficient of calibration curves.

Element	Wavelength (nm)	Instrument detection limit (mg/L)	3 x σ(blank) (mg/kg)	Concentration of calibration standard (mg/L)	R	Equation for calibration curve
Ca	422.7	0.01	1.5	0.5, 1, 2, 4	0.99967	$Y = 4.778x10^{-4}X + 4.9\ x10^{-5}$
K	766.5	0.01	0.01	0.25, 0.5, 0.75, 1.5	0.99942	$Y = 0.01876X + 0.00107$
Na	589	0.002	0.08	0.25, 0.5, 1,2	0.99996	$Y = 57.8X + 0.37$
Mg	285.2	0.001	0.004	0.25, 0.5, 1,2	0.99964	$Y = 0.14X + 1.79\ x\ 10^{-4}$
Fe	248.3	0.03	1.6	0.5, 1, 1.5, 3	0.99648	$Y = 0.00224X – 1.19\ x\ 10^{-4}$
Cr	357.9	0.05	0.08	0.5, 1, 1.5 ,3	0.99945	$Y = 0.02X – 1.48\ x10^{-4}$
Ni	232.0	0.04	0.07	0.25, 0.5, 1,2	0.99875	$Y = 0.003X – 2.19\ x\ 10^{-5}$
Zn	213.9	0.005	0.02	0.1, 0.2, 0.4, 0.8	0.99985	$Y = 0.17X + 1.71\ x\ 10^{-4}$
Mn	279.5	0.01	0.06	0.25, 0.5, 1, 2	0.99925	$Y = 0.02X + 1.49\ x\ 10^{-4}$
Cu	324.7	0.02	0.08	0.5, 1, 1.5, 3	0.9975	$Y = 0.01X – 2.81\ x\ 10^{-4}$
Co	240.7	0.05	0.06	0.25, 0.5, 1, 2	0.99957	$Y = 0.02X – 7.2\ x\ 10^{-5}$
Pb	283.3	0.1	0.1	1, 2, 3, 4	0.99962	$Y = 0.003X + 8.75\ x\ 10^{-5}$
Cd	228.9	0.005	0.02	0.25, 0.5, 1, 2	0.99919	$Y = 0.07X + 1.33\ x\ 10^{-5}$


Absorbance
Concentration (mg/L)
R = 0.99967
0.0002
0.0004
0.0006
0.0008
0.0010
0.0012
0.0014
0.0016
0.0018
0.0020
0.0022
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5

(a)


Absorbance
Concentration (mg/L)
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0.010
0.015
0.020
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0.030
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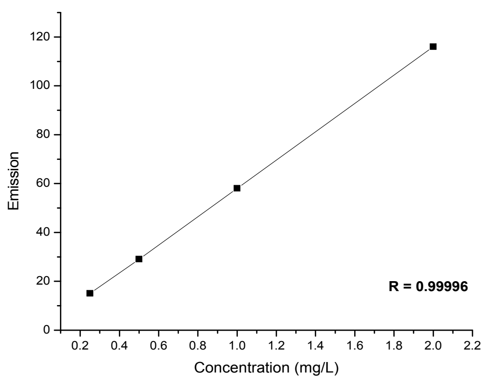
0.4
0.6
0.8
1.0
1.2
1.4
1.6

(b)


Emission
Concentration (mg/L)
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20
40
60
80
100
120
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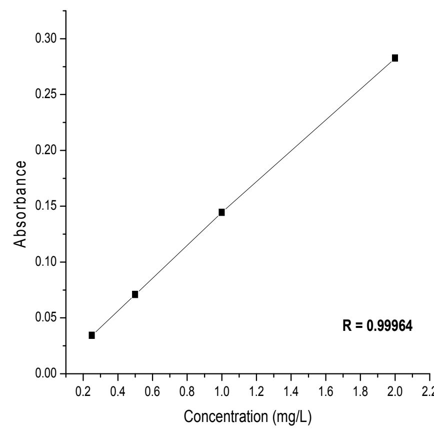
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2

(c)


Absorbance
Concentration (mg/L)
R = 0.99964
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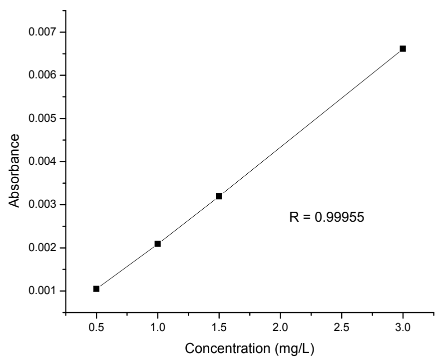
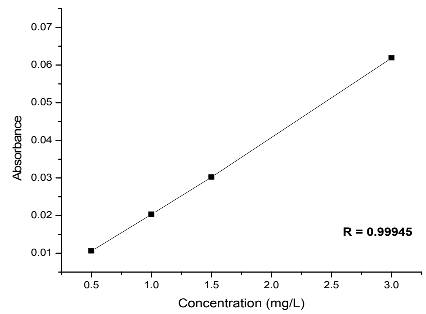
0.05
0.10
0.15
0.20
0.25
0.30
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2

(d)


Absorbance
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.5
1.0
1.5
2.0
2.5
3.0
Concentration (mg/L)
R = 0.99955

(e)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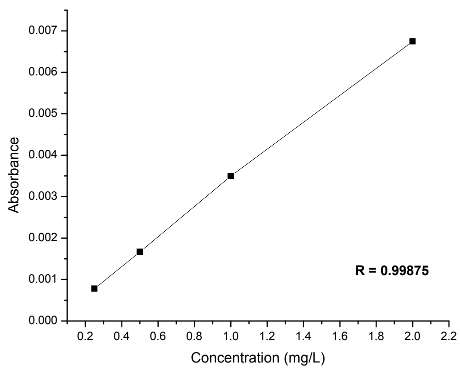
0.06
0.05
0.04
0.03
0.02
0.01
0.5
1.0
1.5
2.0
2.5
3.0
Concentration (mg/L)
R = 0.99945

(f)


Absorbance
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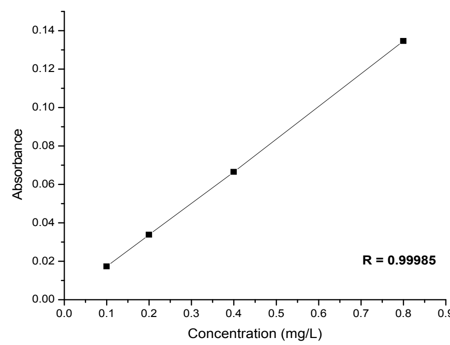
0.006
0.005
0.004
0.003
0.002
0.001
0.000
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Concentration (mg/L)
R = 0.99875

(g)


Absorbance
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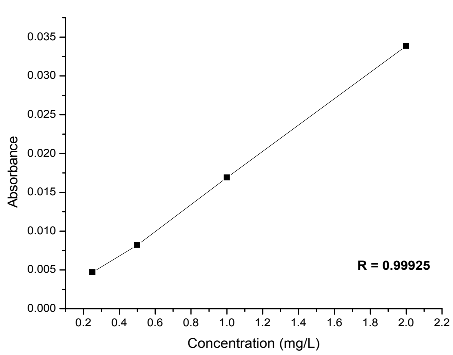
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Concentration (mg/L)
R = 0.99985

(h)


Absorbance
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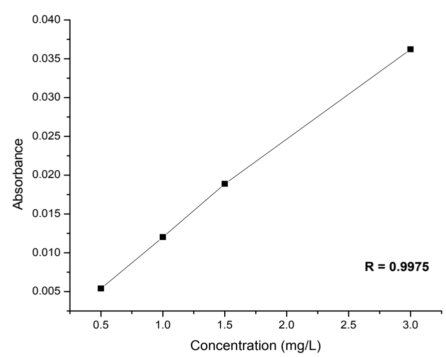
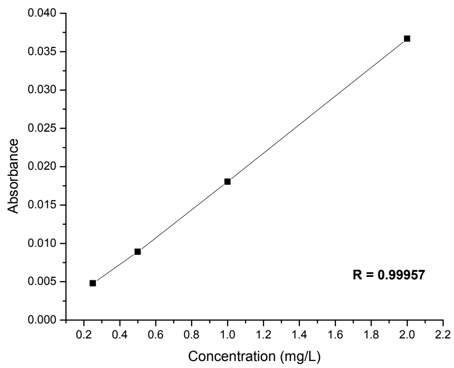
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Concentration (mg/L)
R = 0.99925

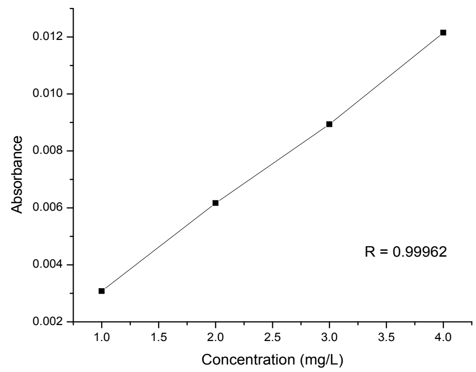
(i)


Absorbance
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.5
1.0
1.5
2.0
2.5
3.0
Concentration (mg/L)
R = 0.9975

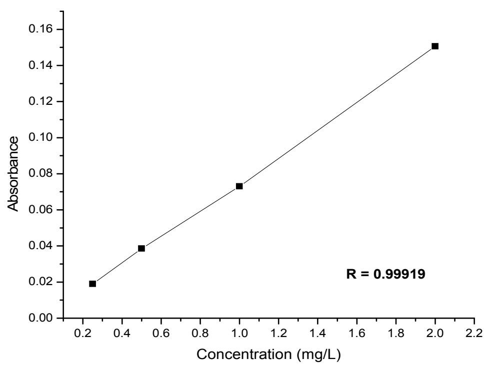
(j)



(k)



(l)



(m)

Figure1. Calibration curves of; (a) calcium, (b) potassium, (c) sodium, (d) magnesium (e) iron (f) chromium, (g) nickel, (h) zinc, (i) manganese, (j) copper, (k) cobalt, (l) lead, (m) cadmium.

3.2. Precision and accuracy

The errors in analytical results can be expressed in terms of accuracy and precision. Precision describes the reproducibility of measurements, i.e. the closeness of results that have been obtained in exactly the same way. And accuracy is the closeness of the measurement to its true or accepted value and is expressed by error [27]. In this study the precision of the results were evaluated by standard deviation of the triplicate samples with triplicate measurements of each sample (n = 9) while the accuracy and the validity of the analytical method were determined by spiking the samples with known concentrations of standard solution.

3.3. Method detection limit

A very important quantity in chemical analysis is the limit of detection. The LOD is the smallest concentration or amount that can be detected with reasonable certainty for a given analytical procedure [29]. In this study method of detection limit of each element was calculated as three times the standard deviation of blank (n = 10) and is given in Table 4. The method detection limits were slightly greater than that of the instrument detection limit.

3.4. Method validation

The efficiency of the optimized procedure was checked by spiking a known concentration of analyte to the sample. In this study 20% of the determined concentration of the metal was spiked first to the sample and then digested in the same way as usual. The spiked concentrations of these metals were 0.307, 13.6, 0.906, 30.7, 23.1, 0.35, 0.13, 0.06, 0.056, 0.05, 0.03, 1.8 and 0.6 mg/L for Ca, K, Na, Mg, Fe, Cr, Ni, Zn, Mn, Cu, Co, Pb and Cd, respectively. 50 µL each of Na, Fe, Ni, Mn, Cu, Co and Pb were spiked in to 100 mL digestion flask containing 0.5 g of fenugreek seed sample and in another flask 50 µL each of Ca, K, Mg, Cr, Zn and Cd were spiked and digested at the optimized digestion procedure. Both spiked and non-spiked samples were digested and analyzed in similar condition. Finally the percent recovery (% R) of the analyte was calculated and the results were between 94-109%.

Table 5. Recovery test of metals for analyzed fenugreek seed samples.

Metal	Concentration (mg/L)			Recovery (%)
	in un-spiked sample	Spiked amount	in spiked sample	
Calcium	152.9	30.7	184	101
Potassium	33.05	13.6	46.6	100
Sodium	5.635	0.906	6.621	109
Magnesium	0.55	0.088	0.64	102
Iron	102.4	23.1	126	102
Chromium	1.7	0.35	2.1	114
Nickel	1.068	0.13	1.196	99
Zinc	0.4156	0.06	0.4760	101
Manganese	0.306	0.056	0.36	96
Copper	0.339	0.05	0.86	94
Cobalt	0.168	0.03	0.2	107
Lead	12.6	1.8	14.3	94
Cadmium	3	0.6	3.58	97

3.5. Determination of metals in fenugreek seed samples

The concentrations of thirteen metals (Ca, K, Na, Mg, Fe, Cr, Ni, Zn, Mn, Cu, Co, Pb and Cd) were determined by digesting fenugreek seed flour by flame atomic absorption spectroscopy. As is shown in Table 6, the most abundant major element in fenugreek seed was calcium and from trace elements and toxic elements were iron and lead, respectively. The concentration of major elements, i.e. Ca, K, Na and Mg in fenugreek seed sample were found in the range of 1553–36771 mg/kg, 6789–11517 mg/kg, 201–1559 mg/kg and 31–102 mg/kg; respectively. In addition, the concentration of trace elements, i.e. Fe, Cr, Ni, Zn, Mn, Cu and Co were found in the range of 6041–18585 mg/kg, 3–552 mg/kg, 31–108 mg/kg, 15–33 mg/kg, 16–28 mg/kg, N.D–35 mg/kg and 4–15 mg/kg; respectively. Moreover, the concentration of toxic metals Pb and Cd were found in the range of 615–1814 mg/kg and 285–464 mg/kg; respectively. From the trace elements iron had the highest metal concentration due to the iron mineral, Fe_2O_3 rich contents of Ethiopian soils. In contrary to the above, among the plant major elements magnesium is the metal with least concentration. This is happened may be due to the plant has high affinity for the other metals than magnesium. However, unlike to the other spices studied in Ethiopia the concentrations of lead and cadmium in fenugreek seed were observed to be higher. The sources of these concentrations can be from the soil where this plant is cultivated or from the fertilizers that can be used by the farmers. Nevertheless, the metal content of the soils of the sample sites was not studied.

In Denboskie, the metal with highest concentration was calcium (36085 ± 1434 mg/kg) followed by Fe (17807 ± 1109 mg/kg), lead (1117 ± 12 mg/kg), K (7478 ± 83 mg/kg) and the least metal concentration was observed for cobalt (9 ± 1.1 mg/kg). Generally, the order of metals in decreasing order in fenugreek seed in Denboskie was; Ca > Fe > Pb > K > Na > Cd > Cr > Mg > Ni > Zn > Cu > Mn > Co. Fenugreek seed in Koladiba contained calcium with the highest concentration of all metals, i.e. 32612 ± 770 mg/kg followed by Fe (12854 ± 540 mg/kg), K (9618 ± 160 mg/kg), Pb (585 ± 75 mg/kg) and the least metal concentration was recorded by copper (5 ± 0.4 mg/kg). Generally, the order of metals by concentration in decreasing order in fenugreek seed in Koladiba sample site was represented as: Ca > Fe > K > Pb > Cr > Cd > Na > Ni > Mg > Zn > Mn > Co > Cu. Similar to Denboskie and Koladiba, Ambagiorgis fenugreek seed was very rich in calcium concentration (23374 ± 773 mg/kg) followed by Fe (8216 ± 1321 mg/kg), K (7788 ± 94 mg/kg) and the least metal concentration, i.e. 9 ± 0.5 mg/kg was recorded for copper. Generally, the order of metals by concentration in decreasing order in fenugreek seed in Ambagiorgis sample site was represented as: Ca > Fe > K > Pb > Cd > Na > Cr > Ni > Mg > Zn > Mn > Co > Cu. Dabat fenugreek seed contained calcium with highest concentration, i.e. 23350 ± 2400 mg/kg followed by iron and potassium with concentrations of 14669 ± 1395 mg/kg and 9845 ± 64 mg/kg, respectively. Copper was the metal with the least concentration, i.e. 7 ± 1 mg/kg in this site. Generally, the order of metals by concentration in decreasing order in fenugreek seed in Dabat sample site was represented as: Ca > Fe > K > Pb > Na > Cd > Cr > Ni > Mg > Zn > Mn > Co > Cu. In Jehaniagiorgis, the highest metal concentration in fenugreek seed was recorded by calcium, i.e. 32454 ± 3701 mg/kg followed by iron (15492 ± 1928 mg/kg) and potassium (8534 ± 67 mg/kg). However, copper metal was not detected in this sample site. Generally, the order of metals by concentration in decreasing order in fenugreek seed in Jehaniagiorgis sample site was represented as: Ca > Fe > K > Pb > Na > Cd > Cr > Ni > Mg > Mn > Zn > Co > Cu.

Highest metal concentration (26194 ± 1618 mg/kg) was recorded by calcium in Keteteekli sample site. Potassium and iron were the next metals with higher concentrations of 7582 ± 123 mg/kg and 6041 ± 745 mg/kg, respectively. Similar to Denboskie, cobalt was the metal with the least concentration (4 ± 0.3 mg/kg). Generally, the order of metals by concentration in decreasing order in fenugreek seed in Keteteekli sample site was represented as: Ca > K > Fe > Pb > Na > Cd > Cr > Mg > Ni > Zn > Mn > Cu > Co. In Adet, iron contained the highest concentration, i.e. 17318 ± 1049 mg/kg. Calcium and potassium follow iron with concentrations of 16443 ± 1521 mg/kg and 9354 ± 136 mg/kg, respectively. Cobalt and copper were the two metals with least concentrations, i.e. 7 ± 0.7 mg/kg and 7 ± 1 mg/kg, respectively. Generally, the order of metals by concentration in decreasing order in fenugreek seed in Adet sample site was represented as: Fe > Ca > K > Na > Pb > Cr > Cd > Ni > Mg > Mn > Zn > Co > Cu. In Bichena the three metals with higher metal concentrations were calcium, iron and potassium and their corresponding concentrations were 24114 ± 1154 mg/kg, 15690 ± 920 mg/kg and 9314 ± 78 mg/kg, respectively. In Bichena the metal with least concentration was copper, i.e. 5 ± 0.5 mg/kg. Generally, the order of metals by concentration in decreasing order in fenugreek seed in Bichena sample site was represented as: Ca > Fe > K > Cd > Na > Pb > Cr > Mg > Ni > Mn > Zn > Co > Cu.

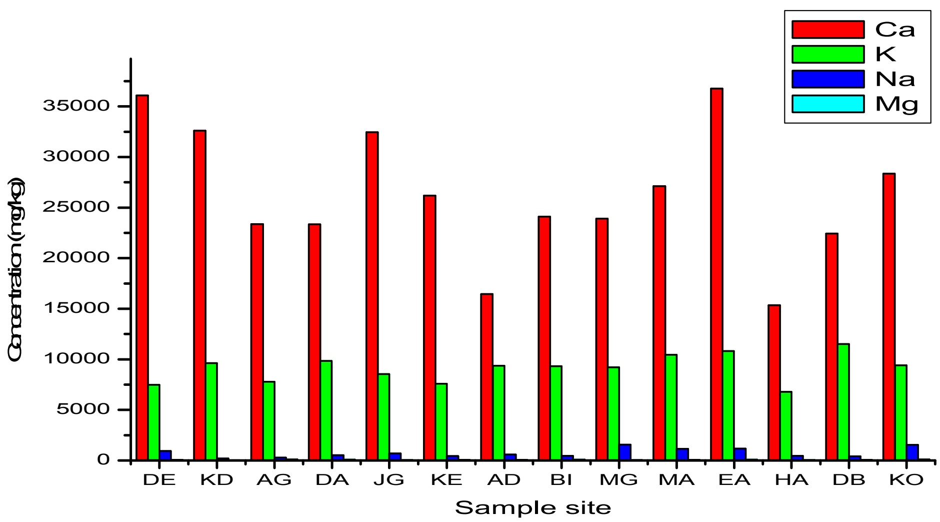
In Meseretgamra sample site, the three metals with higher concentrations were found to be calcium, sodium and potassium. The concentrations of these metals were 23900 ± 1326 mg/kg, 1559 ± 41 mg/kg and 9206 ± 211 mg/kg, respectively. Cobalt with 10 ± 0.6 mg/kg of dry mass was the least recorded metal concentration in Meseretgamra. Generally, the order of metals by concentration in decreasing order in fenugreek seed in Meseretgamra sample site was represented as: Ca > Na > Fe > K > Pb > Cd > Ni > Cr > Mg > Zn > Mn > Cu > Co. In Mayadrasha, the three metals with higher concentrations by dry mass in fenugreek seed were calcium, iron and sodium. Their corresponding concentrations were 27113 ± 2480 mg/kg, 18584 ± 1339 mg/kg and 1156 ± 5 mg/kg, respectively. However, the least metal concentration was obtained by cobalt, i.e. 11 ± 0.3 mg/kg by dry mass. Generally, the order of metals by concentration in decreasing order in fenugreek seed in Mayadrasha sample site is represented as: Ca > Fe > Na > K > Pb > Cd > Cr > Ni > Mg > Cu > Zn >Mn > Co. Similar to Mayadrasha, the three metals with higher concentration in Edagaarbi were calcium, iron and lead. Their concentrations were 36771 ± 1938 mg/kg, 15378 ± 945 mg/kg and 1776 ± 145 mg/kg, respectively. Cobalt was the metal with least concentration, i.e. 14 ± 0.9 mg/kg by dry mass in this site. Generally, the order of metals by concentration in decreasing order in fenugreek seed in Edagaarbi sample site was represented as: Ca > Fe > Na > K > Pb > Cd > Cr > Ni > Mg > Cu > Mn ≈ Zn > Co. Similar to Denboskie, the three metals with higher concentration in fenugreek seed in Hatsebo were calcium, iron and lead. Their concentrations were 15353 ± 1758 mg/kg, 11560 ± 1355 mg/kg and 1258 ± 12 mg/kg, respectively. Cobalt with 67 ±

0.7 mg/kg was the least metal concentration recorded in Hatsebo sample site. Generally, the order of metals by concentration in decreasing order in fenugreek seed in Hatsebo sample site was represented as: Ca > Fe > Pb > K > Na > Cd > Cr > Ni > Mg > Zn > Mn > Cu > Co. The three metals with higher concentration in Debrebirhan sample site were calcium, iron and potassium. The concentrations of these metals were 22433 ± 3115 mg/kg, 16104 ± 2307 mg/kg and 11517 ± 294 mg/kg, respectively. Cobalt with 13 ± 0.4 mg/kg by dry mass was the least concentration recorded among the analyzed metals. Generally, the order of metals by concentration in decreasing order in fenugreek seed in Debrebirhan sample site was represented as: Ca > Fe > K > Pb > Na > Cr > Cd > Mg > Ni > Cu > Zn > Mn > Co. Finally, in Korem the three metals with higher concentration were calcium, sodium and iron. The concentrations of these metals were 28903 ± 3138 mg/kg, 1532 ± 12 mg/kg and 14870 ± 1327 mg/kg, respectively. Differently from the entire above mentioned sample sites the metal with least concentration in Korem was chromium, i.e., 3 ± 0.5 mg/kg.

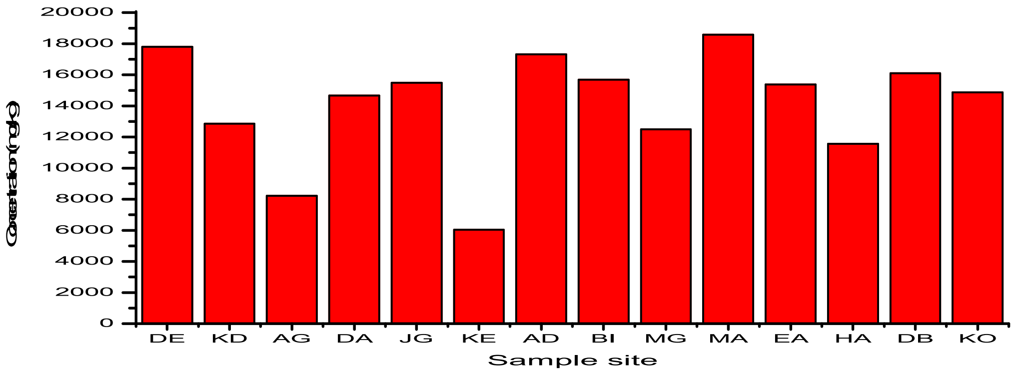
All in all, based on the mean results the analyzed metals in fenugreek seed were arranged in order of decreasing concentrations as follows: Ca > Fe > K > Pb > Na > Cd > Cr > Ni > Mg > Zn > Mn > Cu > Co. Mineral uptake in plants is a function of mineral concentrations in soils, soil pH, cation exchange capacity, organic matter content, types and varieties of plants and age of the plant [30]. The reason why the calcium metal concentration in fenugreek seed is very high in all sample sites is may be due to the presence of the thick mesozoic limestone and gypsum sequences in the blue nile river area in central Ethiopia and proterozoic marbles that occur in northern Ethiopia (Tigray) and west Ethiopia (Gojam). The marble and limestone could be the main sources of calcium in the soils of these sample areas. Moreover, calcium is among the major elements required by plants. In the above series the concentration of potassium is greater than sodium. This is happened because plants generally have a higher content of potassium than sodium [21]. Trace elements are ubiquitous in our environment, they are found in all of the foods we eat. In general, the abundance of trace elements in foods is related to their abundance in the environment. The most abundant trace element in the soil is iron. In this study iron was the trace element that was determined with highest concentration. Similarly, as many authors mention it the Ethiopian soil is rich of iron minerals, e.g. hematite (Fe_2O_3) and this may be the reason why Ethiopian fenugreek seeds are rich in iron contents. Moreover, trace elements get into foods by different pathways. The most important source is from the soil, by absorption of elements in aqueous solution through roots. Another, minor source is foliar penetration (industrial air pollution and vehicle emissions). Other possible sources are fertilizers, agricultural chemicals and sewage sludge [21].

3.6. Distribution of pattern of the metals in different sample sites

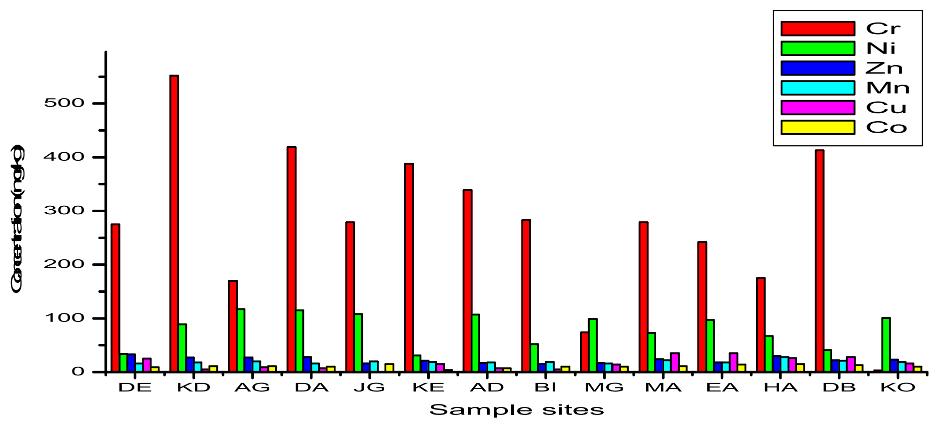
The distribution pattern of metals in the sample sites of this study are represented in Figure 2 (a–d) and Table 6.



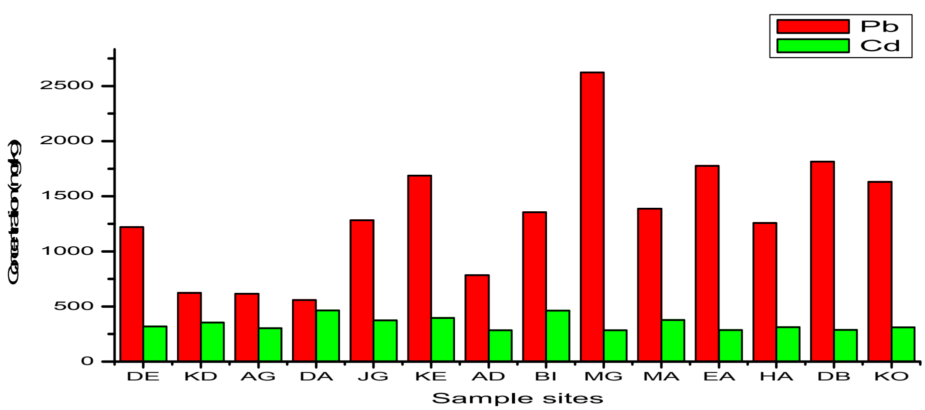
(a)



(b)



(c)



(d)

Figure 2. Average metal concentration of (a) major metals, (b) iron, (c) trace metals, (d) toxic metals in fenugreek seed in all sample sites.

Table 6. Concentration (mean ± SD, n = 3, mg/kg by dry mass) of major, minor and toxic metals in fenugreek seed in different sample sites of Ethiopia.

Sample site	Concentration in mg/kg											
	Ca	K	Na	Mg	Fe	Cr	Ni	Zn	Mn	Cu	Co	Pb	Cd
Denboskie	36085 ± 1434	7478 ± 83	942 ± 17	74 ± 4	17807 ± 1109	275 ± 16	34 ± 4	33.0 ± 0.3	16.0 ± 0.4	25 ± 0.8	9.0 ± 1.1	1220 ± 16	318 ± 12
Koladiba	32612 ± 770	9618 ± 160	201 ± 6	31 ± 5	12854 ± 540	552 ± 9	89 ± 9	27.0 ± 0.3	18.0 ±0.5	5.0 ± 0.4	11.0 ± 0.2	624 ± 75	355 ± 9
Ambagiorgis	23374 ± 773	7788 ± 94	286 ± 9	99 ± 2	8216 ± 1321	170 ± 13	117 ± 16	27 ± 0.5	20.0 ±0.8	9 ± 0.5	11 ± 0.5	615 ± 75	303 ± 11
Dabat	23350 ± 2400	9845 ± 64	529 ± 21	86 ± 4	14669 ± 1395	419 ± 42	115 ± 5	28 ± 0.4	16.0 ±0.3	7 ± 1	10.0 ± 0.4	559 ± 36	464 ± 14
Jehaniagiorgis	32454 ± 3701	8534 ± 67	699 ± 7	51 ± 5	15492 ± 1928	279 ± 17	108 ± 12	16.0 ± 0.6	20.0 ±0.6	N.D	15.0 ± 0.6	1282 ± 124	374 ± 10
Keteteekli	26194 ± 1618	7582 ± 123	442 ± 9	55 ± 4	6041 ± 745	388 ± 16	31 ± 5	21.0 ± 0.5	19.0 ±0.4	15 ± 0.2	4.0 ± 0.3	1687 ± 136	396 ± 10
Adet	16443 ± 1521	9354 ± 136	578 ± 4	67 ± 6	17318 ± 1049	339 ± 29	107 ± 5	17.0 ± 0.4	18.0 ±0.5	7 ± 1	7.0 ± 0.7	785 ± 42	285 ± 22
Bichena	24114 ± 1154	9314 ± 78	452 ± 14	96 ± 5	15690 ± 920	283 ± 24	52 ± 3	15.0 ± 0.7	19.0 ±0.6	5.0 ± 0.5	10.0 ± 0.3	1355 ± 42	463 ± 10
Meseretgamra	23900 ± 1326	9206 ± 211	1559 ± 41	42 ± 7	12493 ± 919	74 ± 10	99 ± 10	17.0 ± 0.2	16.0 ±0.5	14.0 ± 0.6	10.0 ± 0.6	2624 ± 101	285 ± 32
Mayadrasha	27113 ± 2480	10451 ± 220	1156 ± 5	57 ± 6	18584 ± 1339	279 ± 10	73 ± 2	24.0 ± 0.3	22 ± 0.8	35 ± 1	11.0 ± 0.3	1387 ± 91	378 ± 20
Edagaarbi	36771 ± 1938	10807 ± 375	1172 ± 6	96 ± 5	15378 ± 945	242 ± 14	97 ± 7	18 ± 1.1	18 ± 2	35 ± 1	14.0 ± 0.7	1776 ± 145	286 ± 11
Hatsebo	15353 ± 1896	6789 ± 88	453 ± 10	44 ± 3	11560 ± 1355	175 ± 13	67 ± 5	30 ± 0.2	28 ± 0.8	26.0 ± 0.3	15.0 ± 0.4	1258 ± 12	312 ± 12
Debrebirhan	22433 ± 3115	11517 ± 294	415 ± 10	69 ± 5	16104 ± 2307	413 ± 37	41 ± 3	22 ± 0.2	21.0 ± 0.7	28 ± 4	13.0 ± 0.7	1814 ± 67	287 ± 16
Korem	28903 ± 3138	9401 ± 155	1532 ± 12	102 ± 6	14870 ± 1327	3.0 ± 0.5	101 ± 9	23 ± 0.2	19.0 ± 0.7	16.0 ± 0.4	10.0 ± 0.2	1631 ± 54	311 ± 17
Mean	**26364**	**8448**	**744**	**69**	**14077**	**278**	**81**	**23**	**19**	**17**	**11**	**1330**	**344**
Range	**(15353-36771)**	**(6789-11517)**	**(201-1559)**	**(31-102)**	**(6041-18584)**	**(3-552)**	**(31-108)**	**(15-33)**	**(16-28)**	**(N.D-35)**	**(4-15)**	**(615-2624)**	**(285-464)**

3.7. Comparison of metal levels among sample sites

The comparative study of the metal concentrations of fenugreek seed in this study are presented in Table 7. Among all the thirteen elements analyzed in fenugreek seed in this study, calcium showed the highest metal concentration. The mean concentration of Ca in this study was 26364 mg/kg. In addition, the highest Ca concentration, i.e. 36771 ± 1938 mg/kg by dry mass of fenugreek seed was recorded by Edagaarbi sample site. Moreover, the three sample sites with higher calcium metal concentrations were Edagaarbi, Denboskie and Koladiba. Their corresponding concentrations are 36771 ± 1938 mg/kg, 36085 ± 1434 mg/kg and 32612 ± 770 mg/kg, respectively. And the sample site with least calcium metal concentration was Hatsebo with 15353 ± 1758 mg/kg. The RDA and RDI for people in the age range of 25–50 for calcium are 800 mg/day and 1000 mg/day, respectively [22, 31]. The first three sample sites with highest K metal concentrations by dry mass were Debrebirhan, Edagaarbi and Mayadrasha. And their corresponding concentrations were 11517 ± 294 mg/kg, 10807 ± 375 mg/kg and 10451 ± 220 mg/kg, respectively. The site with minimum potassium concentration in its fenugreek seed was Hatsebo (6789 ± 155 mg/kg). The estimated minimum potassium requirement for healthy person above 18 is 2000 mg/day [22]. In Canada, the median dietary intakes range is from 3.2 to 3.4 g/day for men and 2.4 to 2.6 g/day for women [32]. The three sample sites with higher Na metal concentration by dry mass were Mesretgamra, Korem and Edagaarbi with 1559 ± 41 mg/kg, 1532 ± 12 mg/kg and 1172 ± 6 mg/kg, respectively. However, the sample with the least Na concentration was Koladiba, i.e. 201 ± 6 mg/kg. The estimated minimum sodium requirement for healthy person above 18 is 500 mg/day [22]. The AI (Adequate Intake) for sodium for older adults and elderly is set at 1.3 g/day for men and women 50 through 70 years of age, and at 1.2 g/day for those 71 years of age and older [32]. The three sample sites with highest magnesium metal concentrations were Korem, Ambagiorgis and Bichena. The concentrations were 102 ± 6 mg/kg, 99 ± 2 mg/kg and 96 ± 5 mg/kg, respectively. The sample site with least magnesium concentration in its fenugreek seed was Koladiba, i.e. 31 ± 4 mg/kg. Dietary reference intakes (DRIs) for Mg in the ages 25–50 are 420 mg/day for male and 320 mg/day for women [31]. This indicates the amount of magnesium that can be obtained from fenugreek seed is very small, hence, people are recommended to eat other foods in order to attain the needed amount of magnesium per day. The three sample sites with greater Fe concentrations in fenugreek seed samples were Mayadrasha, Denboskie and Adet and their corresponding concentrations are 18584 ± 1339 mg/kg, 17807 ± 1109 mg/kg and 17318 ± 1049 mg/kg, respectively. Conversely, the sample site with minimum iron concentration was Keteteekli with 6041 ± 745 mg/kg. The recommended dietary allowance for iron for 23–50 years age is 10 mg/day for male and 18 mg/day for female [22, 33]. The determined concentration of iron in fenugreek is very rich compared to the above mentioned values, therefore, people are recommended not to take this seed regularly. Koladiba, Dabat and Debrebirhan were among the sample sites of this study with higher chromium concentrations. Their corresponding concentrations are 552 ± 9 mg/kg, 419 ± 42 mg/kg and 413 ± 37 mg/kg, respectively. 3 ± 0.5 mg/kg was the least chromium metal concentration by dry mass in fenugreek seed recorded in Korem. The AI for chromium is 35 µg/day and 25 µg/day for young men and women, respectively [32, 33]. In addition to the above, the ESADDIs for adults for chromium is 50-200 µg/day [22]. Fenugreek seed is rich in chromium, hence, it should not be taken daily in large quantities so as to minimize the risk from chromium contamination. The three sample sites with higher Ni concentrations were Ambagiorgis, Dabat and Jehaniagiorgis. Their corresponding concentrations are 117 ± 15 mg/kg, 115 ± 5 mg/kg and 108 ± 12 mg/kg, respectively. Nevertheless, the sample site with least Ni concentration was Keteteekli (31 ± 5 mg/kg). The adequate intake of nickel for people above the age of 19 is 2.3 mg/day for male and 1.8 mg/day for female [32]. The three sample sites with maximum Zn concentration were Denboskie, Hatsebo and Dabat. The above sample sites contain Zn with the following concentrations: 33 ± 0.3 mg/kg, 30 ± 0.2 mg/kg and 28 ± 0.4 mg/kg, respectively. On the other hand, the sample site with least Zn concentration was Bichena, i.e. 15 ± 0.7 mg/kg. The ESADDIs and RDA for zinc for people in the age range of 25–50 is 15 mg/day [22, 31]. The three sample sites with highest manganese concentrations by dry mass were Hatsebo, Mayadrasha and Debrebirhan. And their corresponding concentrations are 28 ± 0.8 mg/kg, 22 ± 0.8 mg/kg and 21 ± 0.7 mg/kg, respectively. The minimum manganese concentration is recorded by Meseretgamra, Dabat and Denboskie, i.e. 16 ± 0.5 mg/kg, 16 ± 0.3 mg/kg and 16 ± 0.4 mg/kg, respectively. For adults the estimated safe and adequate daily dietary intake (ESADDIS) of manganese is 2–5 mg/day [22]. The sample sites with higher copper concentrations were Mayadrasha (35 ± 1 mg/kg) and Edagaarbi (35 ± 1 mg/kg) followed by Debrebirhan with 28 ± 4 mg/kg of fenugreek seed by dry mass. But copper was not detected in Jehaniagiorgis sample site. The ESADDIs of copper for adults is 1.5–3 mg/day [22]. The three sample sites with greater cobalt concentrations were Hatsebo, Jehaniagiorgis and Edagaarbi with values of 15 ± 0.4 mg/kg, 15 ± 0.6 mg/kg and 14 ± 0.7 mg/kg, respectively. However, the sample site with least cobalt concentration was Keteteekli with 4 ± 0.3 mg/kg by dry mass. Meseretgamra, Debrebirhan and Edagaarbi recorded the higher lead concentration when compared to other sample sites. The above mentioned sample sites had 2624 ± 101 mg/kg, 1814 ± 67 mg/kg and 1776 ± 145 mg/kg lead concentrations by dry mass, respectively. Bichena was the sample site with the least lead concentration (422 ± 42 mg/kg). 464 ± 14 mg/kg, 463 ± 10 mg/kg and 396 ± 10 mg/kg were the three higher cadmium concentrations recorded by Dabat, Bichena and Keteteekli sample sites, respectively. While Adet and Meseretgamra recorded the least cadmium concentration, i.e. 285 ± 22 and 285 ± 32 mg/kg by dry mass, respectively. From all the metals analyzed in fenugreek seed in this study, cobalt showed the least metal concentration. The mean concentration of cobalt in fenugreek seed by dry mass in this study was 11 mg/kg.

Table 7. Comparison of sample sites by metal concentrations.

Rank	Metals												
	Ca	K	Na	Mg	Fe	Cr	Ni	Zn	Mn	Cu	Co	Pb	Cd
1st	EA	DB	MG	KO	MA	KD	AG	DE	HA	MA	HA	MG	DA
2nd	DE	EA	KO	AG	DE	DA	DA	HA	MA	EA	JG	DE	BI
3rd	KD	MA	EA	BI	AD	DB	JG	DA	DB	DB	EA	MA	KE
4th	JG	DA	MA	EA	DB	KE	AD	AG	AG	HA	DB	DB	MA
5th	KO	KD	DE	DA	BI	AD	KO	KD	JG	DE	KD	JG	JG
6th	MA	KO	JG	DE	JG	BI	MG	MA	KE	KO	AG	HA	KD
7th	KE	AD	AD	DB	EA	JG	EA	KO	BI	KE	MA	EA	DE
8th	BI	BI	DA	AD	KO	MA	KD	DB	KO	MG	DA	KO	HA
9th	MG	MG	HA	MA	DA	DE	MA	KE	KD	AG	BI	KD	KO
10th	AG	JG	BI	KE	KD	EA	HA	EA	AD	DA	KO	DA	AG
11th	DA	AG	KE	JG	MG	HA	BI	MG	EA	AD	MG	KE	DB
12th	DB	KE	DB	HA	HA	AG	DB	AD	DE	KD	DE	AD	EA
13th	AD	DE	AG	MG	AG	MG	DE	JG	DA	BI	AD	AG	MG
14th	HA	HA	KD	KD	KE	KO	KE	BI	MG	JG	KE	BI	AD

Where: MG = Meseretgamra, KO = Korem, EA = Edagaarbi, MA = Mayadrasha, DE = Denboskie, JG = Jehaniagiorgis, AD = Adet, DA = Dabat, HA = Hatsebo, BI = Bichena, KE = Keteteekli, DB = Debrebirhan, AG = ambagiorgis, KD = Koladib

3.8. Comparison of metal concentrations of fenugreek seed in this study and the data in the literature

The concentration of calcium in fenugreek seed in Ethiopia was the highest, i.e. (26364 mg/kg) followed by Turkey (2341.2 ± 146.3 mg/kg), U.A.E. (84 mg/kg) and the least is recorded in Saudi Arabia (1.281 mg/kg). The concentration of potassium in Ethiopia was intermediate (9120 mg/kg) between the concentration of potassium in Saudi Arabia (271.6 mg/kg) and U.A.E. (18203 mg/kg). The concentration of sodium in fenugreek seeds in U.A.E. (37280 mg/kg) is quiet higher than the sodium concentration in Ethiopia (744 mg/kg) and Saudi Arabia (20.30 mg/kg). The magnesium concentration in Ethiopia was the least (69 mg/kg) compared to the concentration in U.A.E. (168 mg/kg) and Turkey (1372.7 ± 44.6 mg/kg).

Fenugreek seed from Ethiopia contained the highest (14077 mg/kg) iron concentration. This is may be due to the soils in Ethiopia are rich in iron minerals, e.g. Fe_2O_3. Next to Ethiopia, higher iron concentration in fenugreek seed is observed in Iran (702.3 ± 30.0), U.A.E. (588 mg/kg), Turkey (62.6 ± 5.4 mg/kg) and the least was found in Saudi Arabia with only (2.737 mg/kg). The concentration of chromium in India are quiet larger (56380-57310 mg/kg) followed by Ethiopia (278 mg/kg), Iran (23.0 ± 1.6 mg/kg), U.A.E. (1.8 mg/kg) and the least concentration is recorded in Turkey with (0.641 ± 0.292 mg/kg). Nickel concentration in Indian fenugreek seed is by far greater than the others, i.e. (31050-42250 mg/kg) followed by Ethiopia (18 mg/kg), U.A.E. (3.0 mg/kg) and the least is recorded in Turkey (2.085 ± 0.623 mg/kg). The highest zinc concentration is observed in India with a range of (47150–65480 mg/kg) followed by Iran (110.8 ± 13.0 mg/kg), Turkey (54.6 ± 5.2 mg/kg), Ethiopia (23 mg/kg), Egypt (14.605 ± 4.60 mg/kg) and the least is observed in Saudi Arabia (23 mg/kg). The concentration of manganese in Ethiopian fenugreek seed is intermediate (19 mg/kg) when compared to the others. The highest concentration is recorded in U.A.E. (48 mg/kg) followed by Iran (28.0 ± 1.3 mg/kg) and the least is observed in Turkey with concentration of (15.9 ± 1.4 mg/kg). Copper concentration in fenugreek seed in India are by far greater than the others with a range of (1790–4280 mg/kg) followed by Ethiopia (17 mg/kg), Turkey (9.4 ± 1.3 mg/kg), Egypt (7.25 ± 0.29 mg/kg), U.A.E. (0.14 mg/kg) and the least with (0.104 mg/kg) in Saudi Arabia. Cobalt concentration in Indian fenugreek seed is the highest in the range (5610–9180 mg/kg) compared to cobalt concentration in Ethiopia (11 mg/kg) and Turkey (0.413 ± 0.067 mg/kg). However, cobalt was not detected in Saudi Arabian fenugreek seed.

As is shown in Table 8, cadmium concentration is highest in India (1360–1770 mg/kg) followed by Ethiopia (344 mg/kg), Saudi Arabia (40 mg/kg), U.A.E. (0.30 mg/kg), Egypt (0.15 ± 0.11 mg/kg), Turkey (0.127 ± 0.068 mg/kg) and the least cadmium concentration is recorded in Saudi Arabia (0.017 mg/kg). Lead concentration in India is the highest (5620–4880 mg/kg) followed by Ethiopia (1330 mg/kg), Turkey (0.393 ± 0.310 mg/kg), Egypt (0.26 ± 0.12 mg/kg), U.A.E. (0.25 mg/kg) and Saudi Arabia (N.D –0.091 mg/kg). However, the Indian fenugreek was grown in an industrially polluted area with sludge water.

Therefore, the toxic metals cadmium and lead in Ethiopian fenugreek seed seem to be higher in concentration when compared to the fenugreek seeds from other countries except India. Hence, government or authorized bodies should make people concerned about the risk of these metals from regular intake of fenugreek seed in foods.

Table 8. Comparison of fenugreek seed metal concentrations in this study and results in the literature.

Sample origin	Concentration in mg/Kg													Ref.
	Ca	K	Na	Mg	Fe	Cr	Ni	Zn	Mn	Cu	Co	Pb	Cd	
*India	–	–	–	–	–	57310	31050	47150	–	1790	5610	4880	1770	[34]
*India	–	–	–	–	–	56380	42250	65480	–	4280	9180	5620	1360	[34]
Turkey	2341 ± 146	–	–	1373 ± 45	62.6 ± 5.4	0.6 ± 0.3	2.1 ± 0.6	54.6 ± 5.2	15.9 ± 1.4	9.4 ± 1.3	0.41 ± 0.07	0.4 ± 0.3	0.13 ±0.07	[18]
*Iran	–	–	–	–	702.3 ± 30.0	23.0 ± 1.6	–	110.8 ± 13.0	28.0 ± 1.3	–	–	–	–	[8]
Egypt	–	–	–	–	–	–	–	14.6 ± 4.6	–	7.3 ± 0.3	–	0.3 ± 0.1	0.2 ± 0.1	[35]
Saudi Arabia	–	–	–	–	–	–	–	–	–	–	Nill	Nill	40	[36]
U.A.E.	84	18203	37280	168	588	1.8	3.0	–	48	0.14	–	0.25	0.30	[37]
Saudi Arabia	1.3	276.1	20.3	–	2.7	–	–	0.375	–	0.104	–	0.091	0.017	[38]
Ethiopia	26364	8448	744	69	14077	278	81	23	19	17	11	1330	344	(Present study)

* Indicates mg/g units were changed in to mg/kg.

3.9. Comparison of metal concentrations of fenugreek seed and other spices and in the literature

The concentration of metals determined in *Trigonella foenum-graecum* L. (TFG) is compared with other thirteen spices studied in Ethiopia and abroad (Table 9). These spices were *Zingiber officinale* (ZO), *Aframomum corrorima* (AFC), *Elettaria cardamomum* (EC), *foeniculum vulgari* mill (FVM), *Coriandrum Sativum* (CS), *Allium cepa* (AC), *Anethum graveolus* (AG), *Mentha Viridis* (MV), *Spinacia oleraceae* (SO), *Arnica montana* (AM), *Carum petroselium* (CP), *Raphanus stivus* (RS), and *Thymus vulgari* (TV).

Calcium is studied in all spice types mentioned in Table 9 and the highest calcium metal concentration was recorded in TFG (15353–36771 mg/kg) and the least is observed in AC (84 mg/kg). The descending order in the concentration of calcium in these spices is as follows; TFG > FVM > EC > ZO > AFC > TV > AM > RS > CP ≈ CS > AG ≈ MV > SO > AC. The least potassium concentration was recorded in TFG (6789–11517 mg/kg) and the highest is recorded in AC (86422 mg/kg). The descending order in the concentration of potassium in these spices is as follows; AC > AG > MV > CP > TV > AM > SO > CS > RS > TFG. Among the spice mentioned in Table 9, SO is the richest in sodium concentration with (56433 mg/kg) followed by RS (46500 mg/kg), CS (40302 mg/kg), AG (22000 mg/kg), AC (15113 mg/kg), CP (15000 mg/kg), AM (12091 mg/kg), TV (11000 mg/kg), MV (4000 mg/kg) and the least was TFG (201–1559 mg/kg). The highest magnesium concentration is observed in ZO (2700-4094 mg/kg) and the least in TFG (31–102 mg/kg). The descending order in the concentration of magnesium in these spices is as follows; ZO > FVM > E C > AFC > TV > SO > AC > RS > CS > AM > MV > AG > CP > TFG.

Similar to calcium and chromium, highest iron concentration was recorded in TFG (6041–18584 mg/kg) and the least in AFC (37–46.5 mg/kg) both are from Ethiopia. The descending order in the concentration of iron in these spices is as follows; TFG > FVM > TV > AM > SO > RS > MV > AG > CS > CP > AC > ZO > EC > AFC. Chromium metal concentration was analyzed in all spice types and TFG was with the highest concentration (3–552 mg/kg) and the least is recorded in SO (1.4 mg/kg). The highest nickel concentration was recorded in TFG (31–108 mg/kg) and the least in SO (2.0 mg/kg). The descending order in the concentration of nickel in these spices is as follows; TFG > TV > FVM > CP > AM > AC > EC > AFC > ZO > MV > RS > AG > CS > SO. Zinc metal concentration was studied in only five spices. The highest zinc concentration is recorded by ZO (38.5–55.2 mg/kg) and the least by AFC (12.3 mg/kg). Concentration of zinc in TFG was in between the above concentrations. The least manganese concentration in those spices was recorded in TFG (196–28 mg/kg) and the highest by ZO (184–401 mg/kg). The descending order in the concentration of manganese in these spices is as follows; ZO > EC > AFC > RS > AG > TV > AM > CS > AC > CP > MV > FVM > SO > TFG. Highest copper concentration is recorded in FVM (23.9–103 mg/kg) and the least in RS and AG (0.05 mg/kg) each. And copper is not detected in CP. The descending order in the concentration of copper in these spices is as follows; FVM > TFG > EC > AFC > ZO > CS > SO > AC > TV > MV > AM > AG ≈ RS. Cobalt was studied only in five spices and the highest is recorded in FMV (26.2–70.8 mg/kg) and the least in AFC (2.0–2.3 mg/kg). The concentration of cobalt in TFG was in between the above concentrations. The descending order in the concentration of cobalt in these spices is as follows; FVM > TFG > ZO > EC > AFC.

However, lead metal concentration in TFG was the highest (615–1814 mg/kg) and the least in AM (0.10 mg/kg). In AFC, EC and FVM, it was not detected and in ZO it was not studied. The descending order in the concentration of lead in these spices is as follows; TFG > TV > SO > RS > CS > AG ≈ CP > AC > MV > AM. Similar to lead, cadmium is studied in all spice types and the highest concentration was recorded in TFG (285–464 mg/kg) and the least in AC (0.06 mg/kg). The descending order in the concentration of cadmium in these spices is as follows; TFG > ZO > FVM > AFC > EC > MV > AM > CS > TV > AG > CP > RS ≈ SO > AC.

Table 9. Comparison of metal concentrations of fenugreek seed and other spices in the literature.

Botanical name	English name	Concentration in mg/Kg													Ref.
		Ca	K	Na	Mg	Fe	Cr	Ni	Zn	Mn	Cu	Co	Pb	Cd	
Trigonella foenum-graecum L.	Fenugreek	15353-36771	6789-11517	201-1559	31-102	6041-18584	3-552	31-108	15-33	16-28	N.D-35	4-15	615-1814	285-464	Present study
Zingiber officinale	Ginger	2001-2543	—	—	2700-4094	41.8-89.0	6.02-10.84	5.61-8.40	38.5-55.20	184-401	1.10-4.78	2.04-7.58	—	0.38-0.97	[30]
Aframomum corrorima	Corarima	1794-2181	_	_	1626-2067	37.0-46.5	3.8-5.8	6.6-8.5	12.3 ± 17.9	143.8-179.6	5.8 ± 8.3	2.0 -2.3	N.D	0.9-1.0	[39]
Elettaria cardamomum	Cardamom	2719 ± 35	_	_	2390 ± 41	64.8 ± 2.2	8.3 ± 0.7	11.7 ± 0.5	19.6 ± 0.9	355.4 ± 9.8	9.5 ± 1.0	2.6 ± 0.2	N.D	0.87 ± 0.07	[39]
foeniculum vulgari Mill	Fennel	20544-23020	_	_	1309-3456	1136-1901	90.9-97.7	18.7-24.2	37.1-44.7	30.6-51.4	23.9-103	26.2-70.8	N.D	1.59-1.91	[40]
Coriandrum Sativum	Coriander	210 (1.81)	27555 (2.19)	40302 (2.65)	212 (1.93)	348 (2.60)	1.8 (2.06)	3.44 (4.11)	—	77 (9.24)	0.08 (2.60)	_	0.37 (7.50)	0.28 (9.98)	[37]
Allium cepa	Onion	84 (4.59)	86422 (0.67)	15113 (2.23)	241 (2.15)	256 (3.88)	4.2 (0.71)	12.8 (9.81)	—	76 (2.58)	0.10 (9.97)	_	0.24 (9.52)	0.06 (8.00)	[37]
Anethum graveolus	Dill	168 (3.26)	73476 (3.21)	22000 (3.25)	141 (3.25)	432 (1.90)	2.2 (1.17)	4.0 (4.06)	—	160 (8.77)	0.05 (5.60)	_	0.26 (0.92)	0.15 (7.90)	[37]
Mentha Viridis	Spearmint	168 (3.85)	69138 (1.10)	4000 (4.91)	165 (1.83)	568 (0.84)	3.0 (0.94)	7.0 (10.05)	_	72 (3.90	0.08 (2.75)	_	0.18 (6.85)	0.34 (5.29)	[37]
Spinacia oleraceae	Spinach	126 (4.10)	46092 (1.50)	56433 (1.92)	312 (2.56)	692 (1.20)	1.4 (1.54)	2.0 (1.00)	_	44 (10.04)	0.14 (10.00)	_	0.45 (2.81)	0.4 (5.80)	[37]
Arnica montana	Mountain arnica	378 (2.32)	53000 (1.39)	12091 (2.86)	208 (2.20)	700 (2.67)	1.8 (2.24)	14.0 (10.00)	_	92 (4.43)	0.07 (10.03)	_	0.10 (5.29)	0.30 (6.23)	[37]
Carum petroselium	Parsely	210 (2.13)	63300 (0.87)	15000 (1.69)	115 (2.68)	332 (0.38)	3.7 (3.20)	15.0 (4.52)	_	72 (4.58)	N.D	_	0.26 (2.91)	0.13 (9.25)	[37]
Raphanus stivus	Radish	294 (2.36)	16779 (1.67)	46500 (0.95)	234 (2.15)	644 (1.51)	7.0 (2.6)	5.4 (7.87)	_	172 (4.42)	0.05 (4.90)	_	0.43 (5.93)	0.40 (6.20)	[37]
Thymus vulgaris	Thyme	463 (1.25)	54776 (1.01)	11000 (1.23)	375 (1.98)	1322 (0.95)	10.7 (5.30)	25.0 (2.90)	_	96 (9.64)	0.09 (5.04)	_	0.46 (6.20)	0.18 (9.45)	[37]

* The values in parenthesis are % RSD.

3.10. Statistical analysis

3.10.1. Analysis of variance

ANOVA is an extremely powerful statistical technique which can be used to separate and estimate the different causes of variation [41]. In this study a one way ANOVA with the help of SPSS software program was used to compare the mean of more than two groups of samples. ANOVA uses the F-test statistic to compare whether the difference between samples means are significant or not [30].

In this study *Trigonella foenum-graecum* L. (fenugreek seed) were collected from 14 different regions of Ethiopia and the metal levels of 13 metals were analyzed using FAAS. However, sample preparation and digestion process could result random errors during instrumental analysis. Therefore, one way ANOVA with the help of SPSS software program was used to analyze the variance that could result due to the difference in the metal content of the soils of the sample sites, acidity of the soil, atmospheric condition of the environment, water, fertilizers, etc.

Hence, Table 10 shows that all the sample means have significant differences at $F_{13, 28}$ at 95% confidence level. Nevertheless, no significant difference was observed between sample means in case of zinc metal. The reason why there is significant difference in the means of the samples is may be there is difference in the metal content of the soils of these sample sites, pH of the soils and the type of the fertilizers used by these environments. However, the difference between sample means of zinc is not significant at $F_{13, 28}$ at 95% confidence level most probably due to random errors in analytical procedures and instrumental errors.

Table 10. Analysis of variance between and within samples of fenugreek seed samples at 95% confidence level.

Metal	Comparison	Sum of squares	Df	Mean squares	$F_{calculated}$	$F_{critical}$	Remark
Calcium	Between Groups	165846.118	13	12757.394	14.124	1.75	Significant difference between sample means
	Within Groups	25291.588	28	903.271			
	Total	191137.706	41				
Potassium	Between Groups	7178.693	13	552.207	97.575	1.75	Significant difference between sample means
	Within Groups	158.460	28	5.659			
	Total	7337.153	41				
Sodium	Between Groups	730.544	13	56.196	8.869	1.75	Significant difference between sample means
	Within Groups	177.423	28	6.337			
	Total	907.967	41				
Magnesium	Between Groups	1.911	13	.147	39.474	1.75	Significant difference between sample means
	Within Groups	.104	28	.004			
	Total	2.015	41				
Iron	Between Groups	68970.497	13	5305.423	3.946	1.75	Significant difference between sample means
	Within Groups	37647.982	28	1344.571			
	Total	106618.479	41				
Chromium	Between Groups	57.718	13	4.440	12.487	1.75	Significant difference between sample means
	Within Groups	9.956	28	.356			
	Total	67.673	41				
Nickel	Between Groups	3.707	13	.285	18.896	1.75	Significant difference between sample means
	Within Groups	.422	28	.015			
	Total	4.129	41				
Zinc	Between Groups	.128	13	.010	1.567	1.75	No Significant difference between sample means
	Within Groups	.176	28	.006			
	Total	.304	41				
manganese	Between Groups	.038	13	.003	36.143	1.75	Significant difference between sample means
	Within Groups	.002	28	.000			
	Total	.041	41				

Copper	Between Groups	.531	13	.041	193.820	1.75	Significant difference between sample means
	Within Groups	.006	28	.000			
	Total	.537	41				
Cobalt	Between Groups	.034	13	.003	43.241	1.75	Significant difference between sample means
	Within Groups	.002	28	.000			
	Total	.036	41				
Lead	Between Groups	1271.601	13	97.815	602.529	1.75	Significant difference between sample means
	Within Groups	4.546	28	.162			
	Total	1276.147	41				
Cadmium	Between Groups	15.441	13	1.188	31.501	1.75	Significant difference between sample means
	Within Groups	1.056	28	.038			
	Total	16.497	41				

Where, Df is degree of freedom.

3.10.2. Pearson correlation of metals with in fenugreek seed sample

The product moment-correlation coefficient (r) has values that range $-1 \leq r \leq +1$. And r value of -1 describes perfect negative correlation, i.e. all the experimental points lie on a straight line of negative slope. Similarly, r value of +1 describes perfect positive correlation, all the points lying exactly on a straight line of positive slope. However, a zero r value does not mean that the two components (e.g. y and x in the formula) are entirely unrelated; it only means that they are not linearly related [41]. The values of correlation coefficients between metal concentrations in fenugreek seed are given in Table 10. The fenugreek seed metal vs. fenugreek seed metal system showed good positive correlations of sodium–lead, manganese–cobalt and iron–potassium with the corresponding r values of 0.659, 0.521 and 0.526, respectively. However, inverse relationship occurred between sodium and chromium (-0.698). All the other relationships had weak positive or negative linear relationship implies, the presence of one metal does not affect for the presence of the other within the plant.

Table 11. Pearson correlation of fenugreek seed metals vs. fenugreek seed metals system.

	Ca	K	Na	Mg	Fe	Cr	Ni	Zn	Mn	Cu	Co	Pb	Cd
Ca	1												
K	0.158	1											
Na	0.313	0.215	1										
Mg	0.109	0.182	0.140	1									
Fe	0.203	0.526	0.342	0.123	1								
Cr	0.080	0.252	-0.698	-0.334	0.043	1							
Ni	-0.062	0.146	0.179	0.153	0.021	-0.253	1						
Zn	0.047	-0.386	-0.237	-0.066	-0.108	0.133	-0.177	1					
Mn	-0.491	-0.252	-0.291	-0.208	-0.151	-0.128	-0.147	0.204	1				
Cu	0.292	0.220	0.405	-0.032	0.315	-0.192	-0.348	0.125	0.336	1			
Co	0.105	0.160	0.010	-0.072	0.230	-0.163	0.251	0.038	0.521	0.472	1		
Pb	0.106	0.191	0.659	-0.127	-0.011	-0.475	-0.299	-0.458	-0.086	0.448	0.035	1	
Cd	0.025	-0.026	-0.319	0.081	-0.021	0.391	-0.122	-0.040	-0.126	-0.376	-0.179	0.32	1

4. CONCLUSION AND RECOMMENDATION

The levels of major (Ca, K, Na, Mg), trace (Fe, Cr, Ni, Zn, Mn, Cu, Co), and toxic (Pb, Cd) metals in *Trigonella foenum-graecum* L. (fenugreek seed) grown in different parts of Ethiopia were determined by flame atomic absorption spectrometry.

The levels of these metals in fenugreek seed were in the following ranges and order; Ca (15353–36771 mg/kg) > Fe (6041–18584 mg/kg) > K (6789–11517 mg/kg), > Pb (615–2624 mg/kg) > Na (201–1559 mg/kg), > Cd (285–464 mg/kg) > Cr (3–552 mg/kg) > Ni (31–108 mg/kg) > Mg (31–102 mg/kg) > Zn (15–33 mg/kg) > Mn(16–28 mg/kg) > Cu (N.D–35 mg/kg) > Co (4–15 mg/kg). The result of this study shows that the fenugreek seed grown in Ethiopia are rich in all the above mentioned essential and non-essential metals. The one-way ANOVA results at 95% confidence level showed that there was significant difference among mean concentrations of all metals with the exception of zinc. This result can be attributed due to differences in pH of the soils, mineral composition of the soils and type of fertilizers. Furthermore, to correlate metal concentrations among each other metal level vs. metal level in fenugreek seed using Pearson's correlation coefficient (r) was determined and except for few relationships most relationships showed weak positive or negative linear relationships. From this relationship it can be understood that the presence of one metal in the plant does not influence the presence of the other.

Fenugreek seed has many benefits for both the producers and consumers in Ethiopia; however some of the very toxic elements were also analyzed with higher concentrations in this study, e.g. lead and cadmium. Prolonged accumulation of heavy metals through food stuff may lead to chronic effect in the kidney and liver of humans and causes disruption of numerous biochemical processes leading to cardiovascular, nervous, kidney and bone diseases [42]. Therefore, government or other concerned bodies are also supposed to strengthen the study of metal levels in most widely used spices and herbal medicines.

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Declaration

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

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