ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

LEVELS OF ESSENTIAL AND NON-ESSENTIAL METALS IN GINGER (*Zingiber officinale*) CULTIVATED IN ETHIOPIA

BY

YOHANNES WAGESHO

JULY 2010

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Graduate Project (Chem. 774)

Submitted to the School of Graduate Studies of

Addis Ababa University

Department of Chemistry

In Partial Fulfilment of Requirements for

the Degree of Master of Science

in Chemistry

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AKNOWLEDGEMENTS

I praise the almighty God who gave me this opportunity and helped me to accomplish my study.

I would like to express my deepest gratitude to my advisor Prof. B. S. Chandravanshi, for his patience, friendly approach, sincere, and immense devotion to help me for the accomplishment of this work.

I would like to thank Dr. Yonas Chebude, Head of Department of Chemistry, Addis Ababa University for his cooperation in facilitating financial support provided for transport during sample collection, and chemicals and apparatus required in the study.

I would like to acknowledge Ato Minaleshewa Atlabachew for his positive approach in assisting during sample preparation and digestion procedure and Ato Henok Mekonnen for his cooperation in AAS analysis.

I would also like to express my gratitude to the Chemical Information Center staff members of AAU, Ms Birhane Tadesse and Ms Hasab Abera for their cooperation in printing and copying all necessary materials throughout my project work.

My appreciation is also extended to Arbaminch Teachers Education College for giving me the opportunity to join this postgraduate program and for sponsorship of my study.

My heart felt thanks go to my beloved father, mother, sisters and brothers who gave me moral support during my study.

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ABSTRACT

LEVELS OF ESSENTIAL AND NON-ESSENTIAL METALS IN GINGER (*Zingiber officinale*) CULTIVATED IN ETHIOPIA

By: Yohannes Wagesho

Advisor: Prof. B. S. Chandravanshi

Ginger (*Zingiber officinale* Roscoe) is one of the most widely used spices that contain several interesting bioactive constituents. Ginger has numerous health benefits and has been reported to possess antioxidant, antiseptic, anticarcinogenic, and antifungal, properties. In the present study, the level of essential (Ca, Mg, Fe, Zn, Cu, Co, Cr, Mn, and Ni) and non-essential elements (Cd and Pb) in ginger cultivated in Ethiopia (particularly in Tepi, Bombae, Hadaro and Ilubabur) and the soil where it has grown were determined by flame atomic absorption spectrometry. The optimized wet digestion procedure was evaluated using standard addition (spiking) method and an acceptable percentage recovery was obtained 93-106 and 93-107 for the metals in ginger and soil samples respectively except chromium in ginger. 0.5 g of oven dried ginger sample was digested using 3 mL of HNO_3 and 1 mL of $HClO_4$ at 210 ^oC for 3 h and 0.5 g dried soil sample was digested with reagent mixture of 6 mL aqua-regia and 1.5 mL H_2O_2 at 270 ^oC for 3 h. The mean metal concentration (μg/g dry weight basis) ranges in ginger and soil samples respectively are: Ca (2001-2543, 1773-3583), Mg (2700-4094, 1457-2442), Fe (41.8- 89.0, 21701-46950), Zn (38.5-55.2, 255-412), Cu (1.1-4.8, 3.8-33.9), Co (2.0-7.6, 48.5-159), Cr (6.0-10.8, 110-163), Mn (184-401, 1756-6465), Ni (5.6-8.4, 14.1-79.3) and Cd (0.38-0.97, 0.24-1.1). However, Pb was not detected in both ginger and soil samples. A statistical analysis of variance (ANOVA) at 95% confidence level was used to test whether the variation between the mineral content of four sample means were significant or not. The Pearson correlation was used to predict the dependence of metal levels on one another.

Key words: Ginger (*Zingiber officinale*), Essential elements, Non-essential elements, Flame Atomic absorption spectrophotometer.

1. INTRODUCTION

1.1. Background of the study

Spices are any of various aromatic vegetable productions such as pepper, cinnamon, nutmeg, mace, allspice, ginger, cloves, etc., used in cookery to season and to flavour sauces, pickles, etc [1].

The delightful flavour and pungency of spices make them indispensable in the preparation of edible dishes. Spices impart aroma, colour and taste to food preparations and sometimes mask undesirable odours. Volatile oils give the aroma, and oleoresins impart the taste. Aroma compounds play a significant role in the production of flavourants, which are used in the food industry to flavour, improve and increase the appeal of their products. They are classified by functional groups, e.g. alcohols, aldehydes, amines, esters, ethers, ketones, terpenes, thiols and other miscellaneous compounds. In spices, the volatile oils constitute these components. In addition, they are reputed to possess several medicinal and pharmacological properties and hence find position in the preparation of a number of medicines [2].

Ethiopia is among the largest consumer of spices in Africa. The major use of spices is in the preparation of a highly spiced stew known as 'Wot' which together with 'Injera' is consumed by a large proportion of the population everyday as their main food. In addition, spices are also used by the numerous ethnic groups in the country to flavor bread, meat, soups, different vegetables, and as medicines and perfumes [3].

The spice ginger is obtained from the underground stems or rhizomes of *Zingiber officinale* (Rosc.), a herbaceous tropical perennial belonging to the family Zingiberaceae. The whole plant is refreshingly aromatic, but it is the underground rhizome, raw or processed, that is valued as spice [1].

1.1.1. Origin and production of ginger (*Zingiber officinale)*

Ginger originated in South-East Asia, probably in India. Ginger is cultivated in several parts of the world, the most important producing regions being India, China, Nigeria,

Sierra Leone, Indonesia, Bangladesh, Australia, Fiji, Jamaica and Nepal. Among them India and China are the dominant suppliers to the world market [1].

1.1.2. Botany of ginger

Ginger is a monocotyledon belonging to the family Zingiberaceae and to the order Zingiberales. In the Zingiberaceae, it belongs to the subfamily Zingiberoideae, which are aromatic with unbranched aerial stems, distichous leaves, open sheaths and hypogeal germination, mainly confined to the old world tropics. Among them, ginger is a slender perennial herb, 30−100 cm tall with palmately branched rhizome bearing leafy shoots. The leafy shoot is the pseudostem formed by leaf sheath and bears 8−12 distichous leaves [1]. Figure 1 shows a) the whole plant and b) the under ground rhizome of ginger.

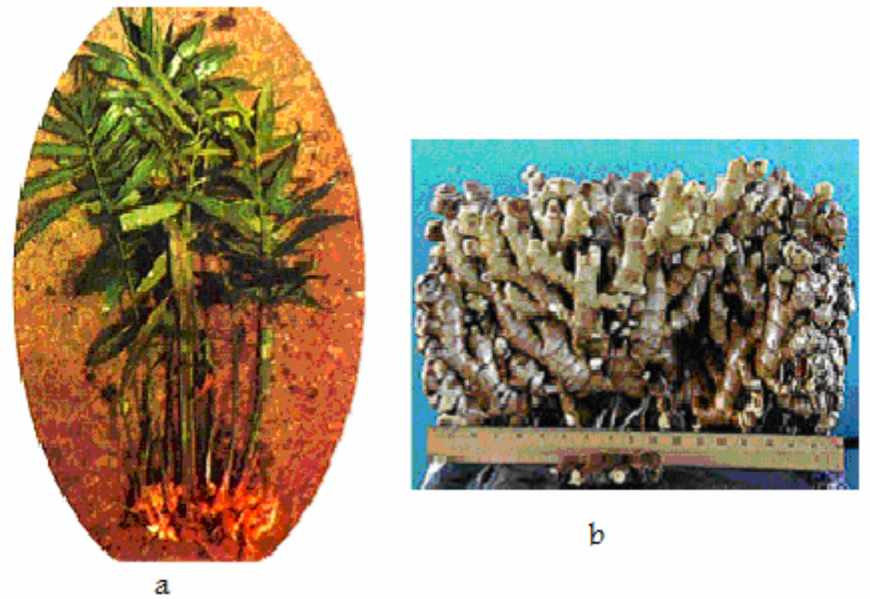


Figure 1. Ginger (*Zingiber officinale)*: a) flowering branch; b) fresh rhizome.

Ginger, the rhizome of *Zingiber officinale* Roscoe, one of the most widely used species of the family Zingiberaceae, is a common condiment for various foods and beverages in Ethiopia. Therefore, this study deals with assessment of level of metals (essential and non-essential) in ginger cultivated in Ethiopia and it aims to fill the gap at least partially in the area and initiate others for further study on ginger and closely related plants widely used throughout the country.

1.2. Major ginger growing areas in Ethiopia

Ethiopia is a land of diverse climate and soil type that enable prolific growth of several indigenous and exotic spices, herbs, medicinal and other essential oil bearing plants. Ginger is known in Ethiopia since the beginning of 13th century. The major ginger growing area in Ethiopia includes wetter regions at altitude bellow 2000 m in Kefa, Illubabur, Gamo Gofa, Sidama, and Wellega mostly in garden and around homesteads. Large scale production and marketing of ginger are also reported from Illubabur, Wolaita, Kembata-Tambaro, etc. Ginger is the cash crop for the Gumuz people in Benshangul Gumz region. Currently, it has become an important cash crop for farmers in southern and south-western parts of Ethiopia. There are also cultivations (though in small quantities compared to the wet parts of the country) in Gojam and Gonder regions to cover home consumption. The production of this spice has been expanding in most parts of the country, as it can be grown under varied climatic conditions that do not have frost problem. Ginger thrives well in areas with altitudes from sea level to 1500 m, mean annual temperature of 20–32 ^{o}C and with total rainfall greater than 1200 mm. The ideal soil type for the production of ginger is a well-drained, fertile and friable soil and with enough humus, neutral pH. Coffee soils or forest soils of south and south-western Ethiopia, having comparable varieties of soil with above ones, especially around Tepi and Bebeka are found suitable for ginger production [3, 4].

1.3. Dietary use and health benefits of ginger

Both fresh and dried ginger rhizomes are used worldwide as a spice, and ginger extracts are used extensively in the food, beverage, and confectionary industries in the production of products such as marmalade, pickles, chutney, ginger beer, ginger wine, liquors, biscuits, and other bakery products [5]. In Ethiopia it is among the important spices used in every kitchen to flavor stew, tea, bread and local alcoholic drinks [6].

Ginger is also widely used in both traditional and contemporary natural medicine. It has been used medicinally in India since ancient times. Ginger is included in the British, European, Chinese, and Japanese pharmacopoeias, as well as in many other national pharmacopoeias, and the World Health Organization has published a monograph for *Rhizoma zingiberis*. The medicinal uses of ginger are diverse and include for indigestion,

stomachache, malaria and fevers. It is chiefly used to cure diseases due to morbidity of Kapha and Vata. Ginger with lime juice and rock salt increases appetite and stimulates the secretion of gastric juices. It is said to be used for abdominal pain, anorexia, arthritis, atonic dyspepsia, bleeding, cancer, chest congestion, chicken pox, cholera, chronic bronchitis, cold extremities, colic, colitis, common cold, cough, cystic fibrosis, diarrhoea, difficulty in breathing, dropsy, flatulent, disorders of gallbladder, hyperacidity, hypercholesterolemia, hyperglycemia, morning sickness, prevention of motion sickness, nausea, rheumatism, sore throat, throat ache, stomach ache and vomiting in pregnancy [7].

1.4. Chemical composition of ginger

The unique flavor properties of ginger arise from the combination of pungency and aromatic essential oil. The main pungent compounds in fresh ginger are a series of homologous phenolic ketones known as gingerols. The gingerols are thermally unstable and are converted under high temperature to shogaol (after *shoga*, the Japanese word for ginger) [5]. Gingerols, a family of homologous compounds differentiated by the number of carbon atoms in their side chain, are the major pungent constituents, with [6]-gingerol [5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one] being the most abundant. Another homologous series which also accounts for the pungency of ginger is the shogaol family, the dehydrated form of the gingerols, resulting from the elimination of the OH group at C-5 with the formation of a double bond between C-4 and C-5 (Figure 2). Shogaols, which are more pungent than gingerols, are the major pungent compounds in dried ginger. The shogaols are known to occur naturally and also are formed chemically from the corresponding gingerols in pH 2.5–7.2 media and during thermal processing. The distinct aroma of fresh ginger comes from the volatile oils, the second major group of components of ginger [8].

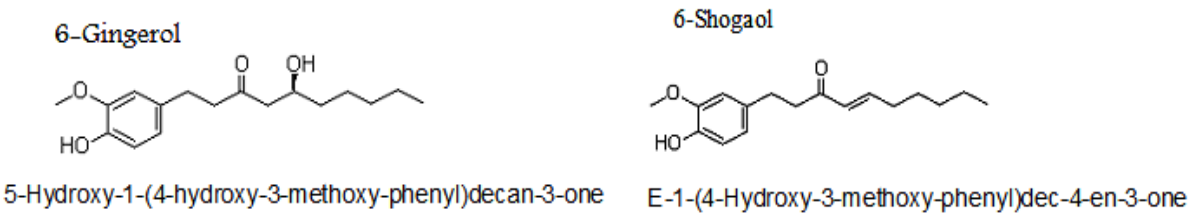


Figure 2. The structure of 6-gingerol and 6-shogaol.

The ginger rhizome contains a little steam volatile oil, fixed (fatty) oil, pungent compounds, resin, proteins, cellulose, pentosans, starch and mineral elements. Of these, starch is the most abundant and comprises 40–60% of the rhizome on a dry weight basis. The relative abundance of certain constituents can vary considerably between samples of ginger in both the fresh ('green') and the dried forms. The composition of the fresh rhizome is determined by the cultivar grown, the environmental conditions of growth and the stage of maturity at harvest. Further changes in the relative abundance of some constituents can also occur post harvest during the preparation and subsequent storage of dried ginger. A typical analysis of a market sample of green ginger gave the following values (as percentages): moisture, 80.9; protein, 2.3; fat, 0.9; carbohydrates, 12.3; fibre, 2.4; and minerals, 1.2. The principal minerals and vitamins in mg/100 g are Ca, 20; P, 60; and Fe, 2.6; the vitamins, thiamine, 0.06; riboflavin, 0.03; niacin, 0.6; and ascorbic acid, 6.0. In addition to starch, the dominant carbohydrate, the rhizome contains 7.6% pentoses on a dry weight basis and small quantities of the free sugars, glucose, fructose and sucrose. Ginger contains 1.6–2.4% nitrogen on a dry weight basis, of which non-protein nitrogen is roughly one third [2].

1.5. Mineral accumulation in ginger plants

Plants can manufacture vitamins, essential amino acids, and fatty acids but they can not manufacture minerals. Mineral uptake by plants can be affected by several factors including mineral concentrations in soils, soil pH, cation exchange capacity, organic matter content, types and varieties of plants, and age of the plant [9]. To get the minerals they need, plants rely on the soil in which they grow. Plants draw in minerals from the soil one tiny, some times microscopic, parcel at a time, through their vast, tentacle-like complex of roots and then put those minerals to work doing the job of sustaining life. During transport throughout a plant, minerals can exit in xylem and enter the cells that require them. Mineral ions cross plasma membranes by a chemiosmotic mechanism. Mineral nutrient concentration in roots may be 10,000 times more than in surrounding soil.

Plants absorb minerals in ionic form: for example, nitrate (NO_3^-), phosphates (HPO_4^{2-}), potassium ion (K^+), calcium (Ca^{2+}), etc; all have difficulty crossing a charged plasma membrane. In ideal word we would take in our daily requirement of minerals by eating plants that grow in mineral rich soils [10, 11].

1.5.1. Accumulation of heavy metals in plants due to soil contamination

Heavy metal contamination in ecosystems poses major environmental problems worldwide with substantial economic consequences. Soil pollution by metals differs from air or water pollution, because heavy metals persist in soil much longer than in other compartments of the biosphere. Heavy metals concentrations in soil are associated with biological and geochemical cycles and are influenced by anthropogenic activities such as metalliferous mining and smelting, metallurgical industries, sewage sludge treatment, warfare and military training, waste disposal sites, agricultural fertilizers and electronic industries [12]. Contamination and subsequent pollution of the environment by toxic heavy metals have become an issue of global concern due to their sources, widespread distribution and multiple effects on the ecosystem. Heavy metals are generally present in agricultural soils at low levels. Due to their cumulative behaviour and toxicity, however, they have a potential hazardous effect not only on crop plants but also on human health [13].

The contamination of soil by atmospheric deposition of toxic metals affects soil properties and further increases plant metal levels through root uptake [14]. Recent studies have also revealed that wastes dumpsites can transfer significant levels of these toxic and persistent metals into the soil environment. And eventually these metals are taken up by plants parts and transfer some into the food chain. Consequently, higher soil heavy metal concentration can result in higher levels of uptake by plants [15].

Heavy metals impact both the physiology and ecology of microorganisms and are known to inhibit a broad range of microbial processes including methane metabolism, growth, nitrogen and sulphur conversions. Metals generate many of their deleterious effects through the formation of free radicals, resulting in DNA damage, lipid peroxidation and depletion of protein sulfhydryls (for example, glutathione) [16].

Therefore, a comprehensive study related to the assessment of levels of essential and heavy toxic metals of plants and soil where the plant has grown is crucial with respect to human health and the quality of its products.

1.6. The role of metals in plants in human health

Humans require a suite of mineral elements in varying amounts for proper growth, health maintenance and general well being. Plant-derived foods have the potential to serve as dietary sources for all human-essential minerals, and with a well-balanced diet that includes mixed sources of grains, fruits, vegetables, roots and tuber crops, plant foods can make a significant contribution to daily mineral needs at all stages of the life cycle [17].

Minerals are essential components of our diet that serve as cofactors in the thousands of enzyme-controlled reactions that power the machinery of the cell. Throughout the body, minerals form critical structural elements, control the action of nerves and muscles, help maintain the body's water balance, and buffer the pH (acidity) of the cell and extracellular fluids. Although minerals only make up a small percentage (5%) of body weight, their role in the body is significant and life would not be possible without them [18].

Generally, too low or too high of a concentration of trace elements in human diet can affect the quality of human life. Equally, industrial-based metallic contamination of the air, soil and water supplies can have dramatic effects on human well-being. This is why water, air, soil and foods are tested for metals concentration to ensure that they meet acceptable standards and should not pose a health hazard.

1.6.1. Classification of minerals

Minerals are usually classified into two main groups on the basis of their relative amounts in the body. One of the groups is macroelements or macrominerals occurring in relatively large amounts and needed in quantities of 100 mg or more per day which include calcium, magnesium, sodium and potassium. Minerals occurring small amounts and needed in quantities of a few milligrams or less per day are called microelements or trace elements, which includes iron, zinc, copper, manganese, cobalt, nickel, chromium and boron. Other trace metals like aluminium, lead, cadmium, mercury and arsenic are till now recognized as potentially harmful. Actually all essential elements may also be toxic in animals and humans if ingested at sufficiently high levels and for a long enough period [19, 20].

The physiological role of Ca, Mg, Fe, Cu, Zn, Mn, Co, Cr, Ni, Cd, and Pb are briefly described below.

Calcium (Ca)

Calcium is required for the normal development and maintenance of the skeleton. It is stored in the teeth and bones, where it provides structure and strength [21, 22]. Calcium is also important as a positive ion (Ca^{2+}) in blood clotting, muscle contraction, and nerve impulse transmission.

Excessive intakes of Ca induce constipation and place up to half of otherwise healthy hypercalciuric males at increased risk of urinary stone formation. A high calcium intake may inhibit the intestinal absorption of iron, zinc, and other essential minerals [23]. Its deficiency is found to result in stunted growth, rickets, and osteoporosis.

Magnesium (Mg)

Soft tissue magnesium functions as a co-factor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes [24].

Excessive intakes of magnesium are harmful to people with impaired renal function resulting in magnesium retention is often associated with hypermagnesemia. Early symptoms of hypermagnesemia include nausea, vomiting**,** and hypotension. Its depletion results gastrointestinal tract abnormalities associated with malabsorption or excessive fluid and electrolyte losses; renal dysfunction with defects in cation reabsorption; and general malnutrition and alcoholism [23].

Iron (Fe)

Iron is a constituent of hemoglobin, myoglobin, and a number of enzymes and, therefore, is an essential nutrient for humans [23]. Iron has several vital functions in the body. It serves as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues [24].

Deficiency of iron results in anemia which is recognized by its symptom such as low blood iron level, small and red blood cells and low blood hemoglobin values. Functional indicators of iron deficiency may include reduced physical work capacity, delayed psychomotor development in infants, impaired cognitive function, impaired immunity and adverse pregnancy outcomes [21].

Iron toxicity usually results from a generic disorder called hemochromatosis. This disease causes over absorption and accumulations of iron, which can result in sever liver and heart damage [22].

Copper (Cu)

Copper is an essential trace metal for animals and human beings and have diverse functions in plants. It is required in the formation of erythrocytes and hemoglobin as well as some enzymes like tyrosinase. Copper is also involved in bone and elastic tissue development [25].

The deficiency of copper results anemia related to defective iron metabolism, skeletal defects, affect the central nervous system and the immune and cardiovascular systems – notably in infants, defects in pigmentation and structure of hair or wool, reproductive failure, and decreased arterial elasticity [21, 23]. Excess intake of copper can cause vomiting, nervous system disorder and Wilson's diseases [22].

Zinc (Zn)

Zinc is an essential component of a large number of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients [24].

Severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, eye and skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioural changes [21, 24].

The toxicity signs are nausea, vomiting, diarrhoea, fever, and lethargy and have been observed after ingestion of 4–8 g zinc. Long-term zinc intakes higher than the requirements could, however, interact with the metabolism of other trace elements. Copper seems to be especially sensitive to high zinc doses. A zinc intake of 50 mg/day affects copper status indexes, such as CuZn-superoxide dismutase in erythrocytes [23, 24].

Manganese (Mn)

Manganese is an essential element to both plants and animals. It is necessary for normal bone metabolism and important enzyme reactions. It also helps to maintain normal nerve, brain and thyroid function [26].

Signs of Mn deficiency include poor reproductive performance, growth retardation, congenital malformations in the offspring, abnormal formation of bone and cartilage, changes in carbohydrate and lipid metabolism, and impaired glucose tolerance. It also interferes with skeletal development [21, 23]. Exposure to high level of Mn can cause both mental and emotional disturbance, along with increased slowness and clumsiness of the body movements. This disease is called manganism. Any brain injury due to the accumulation of Mn in the brain is permanent [27].

Lead (Pb)

Lead serves no useful purpose in the human body, and its presence in the body can lead to toxic effects, regardless of exposure pathway. Lead toxicity influences brain, heart, kidneys, liver, nervous system, and pancreas. It may cause many signs and symptoms such as abdominal pain, anemia, anorexia, anxiety, bone pain, brain damage, confusion, constipation, convulsions, dizziness, drowsiness, fatigue, headaches and hypertension. It also diminishes IQ in children [28].

Cadmium (Cd)

Cadmium has no known nutritional value, and it has been considered an extremely significant pollutant affecting all life forms because of its high toxicity and great solubility in soil and water and easy accumulation in roots of most plant tissues. Excessive Cd exposure may give rise to renal, pulmonary, hepatic, skeletal, reproductive effects, and

cancer [29]. Intake of cadmium-contaminated food causes acute gastrointestinal effects, such as vomiting and diarrhoea [30].

Chromium (Cr)

Chromium(III) is an essential element in humans. Trivalent chromium is required for maintaining normal glucose metabolism; it acts as a cofactor for insulin. Chromium deficiency has been induced in several animal species, resulting in impaired glucose tolerance in the presence of normal concentrations of circulating insulin and, in severe cases, in a diabetes-like syndrome [23].

Chromium(III) is much less toxic than chromium(VI). The respiratory tract is the major target organ for chromium(VI) toxicity, for acute (short-term) and chronic (long-term) inhalation exposures. Shortness of breath, coughing, and wheezing were reported from a case of acute exposure to chromium(VI), while perforations and ulcerations of the septum, bronchitis, decreased pulmonary function, pneumonia, and other respiratory effects have been noted from chronic exposure. Inhaled chromium(VI) is a human carcinogen, resulting in an increased risk of lung cancer [31]**.**

Nickel (Ni)

Nickel is believed to play a role in physiological processes as a co-factor in the absorption of iron from the intestine. Nickel increased the absorption of iron from the diet in iron deficiency but only when dietary iron was in the unavailable ferric form [32].

Contact with nickel compounds can cause a variety of adverse effects on human health, such as nickel allergy in the form of contact dermatitis, lung fibrosis, cardiovascular and kidney diseases and cancer of the respiratory tract. Acute health effects of Ni manifest as a variety of clinical symptoms (nausea, vomiting, abdominal discomfort, diarrhea, visual disturbance, headache, giddiness, and cough). The most common type of reaction to nickel exposure is a skin rash at the site of contact [33].

Cobalt (Co)

The only known animal requirement for cobalt is as a constituent of vitamin B_{12}, which has 4% cobalt in its chemical structure. This means that a cobalt deficiency is really a vitamin

B_{12} deficiency. Cobalt deficiency symptoms include a loss of appetite, emaciation, weakness, anemia, and decreased production. Excessive amounts of cobalt produce cardiomyopathy with a high mortality risk [34].

1.7. Analysis of metals in plant materials

Metals contained in samples are determined by a wide variety of analytical methods, with the choice often depending on the precision and sensitivity required. Both macro and trace elements can be determined by various spectroscopic or chromatographic methods, such as atomic absorbance spectrometry using flame (FAAS) or graphite furnace (GFAAS) atomization, atomic emission spectrometry (AES), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), X-ray fluorescence (XRF), and ion chromatography (IC) [35]. Electroanalytical methods such as polarography and voltametry can also be used to determine the metal levels in various samples.

All these methods require pre treatment of samples to be analysed. One of these samples pre-treatment involve sample matrix break down or sample decomposition. In this project, FAAS was employed for determination of metal levels in ginger and soil samples where ginger has grown.

1.8. Sample decomposition

A common result of the sample preparation is the dissolution of the entire sample, producing a clear solution. The digestion method must be selected to suit the type of sample, the metals being determined, the analytical method and factors such as the matrix composition, the element contents, the possible interferences, the risk of loses and contaminations, the practicality and possible safety hazards in the laboratory [35, 36].

The purpose of sample decomposition is for converting all the species in which a given element is present in such a way that it becomes present in one defined form, eliminating interfering substances from the matrix, and obtaining the element in a homogeneous and easily accessible matrix [36].

The digestion methods could be classified in to wet digestion, acid digestion, dry ashing, and microwave digestion.

1.8.1. Wet digestion

Samples to be analyzed for elemental metal content are usually prepared by digesting the matrix in a strong acid. In the case of organic matrices, an oxidizing mixture is used to destroy the entire organic matrix and solubilize the sample. This yields a clear solution containing the metals for analysis by such techniques as AA, ICP, or ICP-MS. Nitric acid is commonly used, because there is no chance of forming insoluble salts as might happen with HCl or H_2SO_4. Hydrogen peroxide may be added to increase the oxidizing power of the digestion solution [35-37]. The procedure could take place either in open system or closed system (bomb decomposing with conventional or microwave heating).

1.8.2. Dry ashing

For samples that contain much organic matter, which are being analyzed for nonvolatile metals, dry ashing is a relatively simple method of removing the organic matter that can be used for relatively large samples and requires little of the analyst's time. In the open vessel method, the sample is placed in a suitable crucible and is ignited in a muffle furnace. Crucibles used for ashing are usually made of silica, porcelain, platinum, or Pyrex glass. After decomposition the residue is dissolved in acid and transferred to a volumetric flask prior to analysis. Typical ashing temperatures are 450 to 550 °C. Magnesium nitrate is commonly used as an ashing aid. Dry ashing is also conducted at 50-100°C under reduced pressure in an oxygen plasma discharge.

The major drawbacks of the method are the possible loss of some elements by volatilization, contamination of the sample by airborne dust, as it must be left open to the atmosphere and irreversible sorption of analyte into the walls of the vessel [35].

1.8.3. Microwave digestion

A microwave sample digestion system consists of a microwave oven, a rotating carousel holding several sample digestion bombs, and a system for venting these in a controlled fashion. It may also provide monitoring and recording of both temperature and pressure in the containers.

Digesting a sample in a closed container in a microwave oven has several advantages over open container dissolution methods. The containers are fabricated of high-temperature polymers, which are less likely to contain metal contaminants than are glass or ceramic beakers or crucibles. The sealed container eliminates the chance of airborne dust contamination. The sealed, pressurized containers reduce evaporation, so that less acid digestion solution is required, reducing blanks. The sealed container also eliminates losses of more volatile metal species, which can be a problem in open container sample decomposition, especially in dry ashing [35].

1.8.4. Fusion

Fusion is a powerful technique especially both for organic matrices and those with a high silica and alumina content. Since solid and aggressive fusion reagents are difficult to purify, fusion cannot be recommended as ultra trace analysis. A second disadvantage is that the method is carried out in contact with ambient air. Risks of volatilizations are large [38].

1.9. Significance of the study

As described in section 3.4., many researches were conducted on determination of essential and non-essential metal levels of ginger in Nigeria, India, Saudi Arabia, Poland, etc. In Ethiopia, the essential oil from ginger cultivated in Ethiopia was also investigated by Nigist Asfaw and Berhanu Abegaz [6]. The literature survey revealed that there was no study that has been conducted in the determination of metals in ginger cultivated in Ethiopia.

Since ginger is used as a spice for many peoples of Ethiopia and it is cash crop, the knowledge of their mineral level are of particular interest. Hence it is worthwhile to determine the levels of essential and non-essential metals in ginger cultivated in Ethiopia. Therefore, the out come of this research work will ultimately help to ensure the dietary safety of the society and improving the country's economy by increasing both quality and quantity of ginger cultivated in Ethiopia. Finally, the comparison was made on the mineral levels of ginger cultivated in Ethiopia with those of other countries [39-41].

1.10. Objectives

A. General objective

The main objective of this project is to determine the level of minerals in ginger (*Zingiber officinale)* cultivated in Ethiopia.

B. Specific Objectives

(i) To develop an optimum working procedure for digestion of ginger samples to determine mineral contents by FAAS.

(ii) To determine the levels of major, minor and trace elements (Ca, Mg, Fe, Zn, Cu, Co, Cr, Mn, Ni, Cd, and Pb) in ginger samples cultivated in Ethiopia.

(iii) To assess the level of minerals in soil samples where the ginger has grown.

(iv) To correlate the levels of minerals in the ginger with that of soil in which it has been cultivated.

(v) To compare the levels of the minerals in the different soil samples from different sample sites.

(vi) To compare the levels of minerals found in ginger cultivated in Ethiopia with the data of mineral levels in literature from abroad.

2. EXPERIMENTAL

2.1. Equipment and reagents

2.1.1. Equipments

Chopping board (PTFE, China) and Teflon (PTFE) knife were used to cut ginger rhizome in to pieces. A drying oven (Digitheat, J.P. Selecta, Spain) was used to dry ginger sample. Electronic blending device (Moulinex, France) was used for grinding and homogenizing the sample to determine the total metal content of the ginger. Mortar and pestle was used to grind soil sample. Analytical balance (Larko, LA114, 110 g/0.1 g) with precision of ± 0.0001 g was used to weigh the ginger and soil sample. A 100 mL round bottomed flasks fitted with reflux condensers were used in Kjeldahl apparatus hot plate to digest the dried and powdered ginger and soil samples. Borosilicate volumetric flasks (25, 50 and 100 mL) were used during dilution of sample and preparation of metal standard solutions. Measuring cylinders (Duran, Germany), pipettes (Pyrex, USA), micropipettes (Dragonmed, 1-10 μL, 100-1000 μL, Shangai, China) were used during measuring different quantities of volumes of sample solution, acid reagents and metal standard solutions. Flame atomic absorption spectrophotometers (Buck Scientific Model 210VGP AAS, East Norwalk, USA) equipped with deuterium arc back ground correctors and hollow cathode lamps with air-acetylene flame was used for the analysis of the analyte metals (Ca, Mg, Cu, Zn, Mn, Ni, Fe, Co, Cr, Pb and Cd in the ginger and soil sample.

2.1.2. Reagents and chemicals

Reagents that were used in the analysis were all analytical grade. HNO_3 (69-72%) and $HClO_4$ (70%) [Research- Lab Fine Chem Industries Mumbai 400 002 (India)] were used for digestion of ginger samples. Aqua-regia prepared from 3:1 ratio of 37% HCl (Riedel-deHaën) and (69-72%) HNO_3, and extra pure hydrogen peroxide 30% H_2O_2, (Scharlau, European Union), were used for digestion of soil sample. Lanthanum nitrate hydrate (98%, Aldrich, Muwaukee, USA) was used to avoid refractory interference (for releasing calcium and magnesium from their phosphates). Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Ca, Mg, Cu, Zn, Mn, Ni, Fe, Co, Cr, Pb and Cd (Buck Scientific Puro-Graphic) were used for preparation of calibration standards and in the spiking experiments. Deionized water (chemically pure with conductivity 1.5 μs/cm and below)

was used for dilution of sample and intermediate metal standard solutions prior to analysis and rinsing glassware and sample bottles.

2.2. Procedure

2.2.1. Cleaning apparatus

Apparatus such as volumetric flasks, measuring cylinder and digestion flasks were washed with detergents and tap water, rinsed with deionised water. The digestion flasks were soaked with 1% (w/v) potassium dichromate in 98% (v/v) H_2SO_4 and the volumetric flasks were soaked in 10% (v/v) HNO_3 for 24 hours followed by rinsing with deionized water, dried in oven and kept in dust free place until analysis begins. Prior to each use the apparatus were soaked and rinsed in deionized water.

2.2.2. Description of sampling areas

Samples were collected from four major ginger production area in Ethiopia namly Tepi, Bombae, Hadaro and Ilubabur. Tepi is located in the Sheka zone, Southern Nations, Nationalities and Peoples' Region (SNNPR), 596 km southwest of Addis Ababa with an elevation of 1097 meters above sea level and lies between 7.12-7.89 latitude and 35.24 to 37.90 longitudes. The annual mean temperature of the area ranges between 15.1-27.5 ^{o}C and the annual mean rainfall ranges 1201-1800 mm. Bombae is located in Wolayita Zone, SNNPRS, 390 km south west of Addis Ababa, lies between 6º51" and 7º35" North Longitude; and 37º46" and 38º1" East Latitude. Hadaro is located in Kembata zone of SPNNR. It is 270 km far from the capital city of Ethiopia which is Addis Ababa. Illubabor is located in the Oromia Region. It has a latitude and longitude of 8°18′N 35°35′E and an altitude of 1605 meters.

2.2.3 Collection and preparation of ginger and soil samples

2.2.3.1 Collection of samples

Ginger sampling

Fresh ginger samples were collected from the farmlands of four areas in southern and south western Ethiopia particularly Illubabur (Oromia region), Tepi (Sheka, SNNPR), Hadaro (Kambata, SNNPR), and Bombae (Wolayita, SNNPR). These sampling sites are selected based on large scale production area of ginger in country so that the sample partly represents the whole ginger cultivated in Ethiopia. To draw the representative sample from each sampling sites, three sub samples (500 g each) were taken from farmlands which are roughly two km away from each other. These farmlands were randomly chosen from the three triangular corners of the area. Half kilogram of fresh ginger samples were collected from each farmland and put in clean polyethylene plastic bags labelled and brought to the laboratory for further pre-treatment. The three sub-samples were mixed together after grinding in a blender to homogenize and form bulk samples that represent each sampling areas. Finally four ginger bulk samples one from each stated areas were prepared for analysis. Twelve with 0.5 g aliquot (three from each bulk sample) were taken for final digestion.

Soil sampling

For comparative analysis of mineral levels in ginger and the soil where the ginger has cultivated, the soil samples were collected from the surface horizon (15-20 cm) depth of the same four sampling areas of ginger. Sampling was done similar to ginger. Half kilogram soil samples were collected from each farmland. The three sub-samples were mixed together after grinding using a mortar and pestle to homogenize and form bulk samples that represent each sampling areas. Finally four soil bulk samples one from each stated areas were prepared for analysis. Twelve with 0.5 g aliquot (three from each bulk sample) were taken for final digestion.

2.2.3.2. Sample Preparation

Ginger sample preparation

Fresh ginger collected from the sampling areas was kept in plastic bags. The rhizomes were washed with a running tap water so as to remove adsorbed soil particulates and then rinsed with deionized water. The thin outer skin of ginger was removed by plastic knife and chopped in to pieces nearly uniform size to facilitate drying uniformly. The sample was exposed to sun for two days to reduce the moisture content. Then, to have constant mass, the sample was oven dried at 80 ^{o}C for 24 hours so as to express the result in terms of dry mass basis. The dried ginger was powdered using electronic blender and sieved to prepare fine powder of ginger for digestion.

Soil sample preparation

The soil sample collected from the four sampling area were air dried to constant weight for three days and sieved through a 2-mm polyethylene sieve to remove large debris, stones, and pebbles. Then, the samples were ground using a mortar and pestle to pass a 500-μm sieve, homogenized, and ready for digestion.

2.2.3.3. Determination of moisture content of ginger

To determine the moisture content of fresh ginger, first it was carefully washed with tape water to remove adsorbed soil particulates and exposed to air to vaporize water on the surface of it. Then it was weighed with electronic balance to record the initial weight with its moisture content. After oven drying at 80 ^{o}C for two days it was re-weighed and re-dried until it gave constant mass. As can be seen from Table 1, the moisture content of four samples was between 75.0−84.9%. Therefore, fresh ginger cultivated in Ethiopia has comparable moisture content with the value reported by Govindarajan [42], which is 80.9% for typical analysis of market sample of ginger.

Table 1. Moisture content of ginger.

Sampling area	The mass before drying (g)	The mass after oven dried (g)	Moisture content (%)
Ilubabur	55.9	14.0	75.0
Hadaro	83.9	14.2	83.0
Bombae	98.1	14.9	84.9
Tepi	116.5	23.0	80.3

2.2.4. Optimization of digestion procedure and sample digestion

Wet digestion of plant material with acids in an electrical heating block, has been well established and widely used for the determination of nutrient concentrations in plant samples [43]. Adding the strong mineral acids or their combinations (commonly nitric or/and perchloric acid – as they are strong oxidizing agent and forms soluble salts with metals) to the plant material in appropriate vessel (test tube, beaker, digestion flask, etc.), and heating with a Bunsen burner, hot plate, or aluminum block with programmed temperature is the most common procedure employed.

2.2.4.1. Optimization of digestion procedure of ginger samples

The basic requirements for sample preparation for analysis are to get an optimum condition for digestion. The optimum condition is the one which required minimum reagent volume consumption, minimum reflux time, clarity of digests, and ease of simplicity.

In this study, to prepare a clear colorless sample solution that is suitable for the analysis using FAAS, different ginger digestion procedures were optimized using the HNO_3 and $HClO_4$ acid mixtures by varying parameters such as volume of the acid mixture, digestion time and digestion temperature (Table 2). From the optimization procudures, the acid mixture of 3 mL of HNO_3 (69-70%) and 1 mL of $HClO_4$ (70%), digestion time of 3 hours and digestion temperature of 210 ^oC were found the optimal condition for 0.5 g ginger sample. These optimum conditions were selected based on clarity of digests, minimum reagent volume consumption, minimum digestion time, simplicity and minimum temperature applied for complete digestion of sample.

Table 2. Different conditions tested for optimization of digestion procedure for 0.5 g ginger samples.

Trial No.	Reagent(s) used	Reagent volume (mL)	Temp. (^{0}C)	Digestion time (h)	Observation
I. Optimization for reagent volume					
1	HNO_3:$HClO_4$	3:3	270	3:00	Deep yellow
2	HNO_3:$HClO_4$	4:2	270	3:00	Yellow
3	HNO_3:$HClO_4$	3:2	270	3:00	Clear yellow
4	HNO_3:$HClO_4$	4:1	270	3:00	Almost clear
5	HNO_3:$HClO_4$	2:2	270	3:00	Clear light yellow
6	HNO_3:$HClO_4$	**3:1***	270	3:00	Clear and colourless
II. Optimization for temperature					
1	HNO_3:$HClO_4$	3:1	150	3:00	Deep yellow
2	HNO_3:$HClO_4$	3:1	180	3:00	Light yellow
3	HNO_3:$HClO_4$	3:1	**210***	3:00	Clear and colourless
4	HNO_3:$HClO_4$	3:1	240	3:00	Clear and colourless
5	HNO_3:$HClO_4$	3:1	270	3:00	Clear and colourless
6	HNO_3:$HClO_4$	3:1	300	3:00	Clear and colourless
III. Optimization for digestion time					
1	HNO_3:$HClO_4$	3:1	210	1:45	Deep yellow
2	HNO_3:$HClO_4$	3:1	210	2:00	Light yellow
3	HNO_3:$HClO_4$	3:1	210	2:15	Light yellow
4	HNO_3:$HClO_4$	3:1	210	2:30	Clear light yellow
5	HNO_3:$HClO_4$	3:1	210	2:45	Clear and colourless
6	HNO_3:$HClO_4$	3:1	210	**3:00***	Clear and colourless

* Indicate the optimal condition for the given parameter.

2.2.4.2. Optimization of digestion procedure of soil samples

The measurement of metal concentration in soil, sediment and waste, is generally a combination of a digestion procedure for dissolution of elements and a subsequent measurement of the dissolved elements [44].

The conventional aqua-regia digestion procedure consists of digesting soil samples is so widely used that the European Community Bureau of Reference has certified several soil and sludge samples based on it, in addition to the total elemental concentrations [45]. Wilson *et al.* reported that digesting soil sample with aqua-regia produced the most accurate, efficient and reproducible results [46]. Hur *et al.* evaluated 1:1 HNO_3:HCl, 1:3 HNO_3:HCl (aqua-regia), and 1:3:0.5 HNO_3:HCl:H_2O_2 (modified aqua-regia), and they reported that, the aqua-regia and modified aqua-regia were the most effective digestion reagents [47].

Considering the findings mentioned above, the modified aqua-regia (HNO_3 + HCl + H_2O_2) was selected as digestion reagent for soil sample digestion in this work and optimized as presented in Table 3. The optimum conditions for soil sample digestion were a reagent mixture of 6 mL aqua-regia (3:1 ratio of HCl to HNO_3) and 1.5 mL H_2O_2, digestion temperature of 270 ^oC and digestion time of 3 hours for 0.5 g soil sample.

Table 3. Different conditions tested for optimization of digestion procedure for 0.5 g soil samples.

Trial No.	Reagent(s) used	Reagent volume (mL)	Temp. (^{0}C)	Digestion time (h)	Observation
I. Optimization for reagent volume					
1	Aqua-regia:H_2O_2	6.5:1	300	3:00	Deep yellow with suspension
2	Aqua-regia:H_2O_2	**6:1.5***	300	3:00	Light yellow with no suspension
3	Aqua-regia:H_2O_2	5.5:2	300	3:00	Light yellow with no suspension
II. Optimization for temperature					
1	Aqua-regia:H_2O_2	6:1.5	240	3:00	Deep yellow with suspension
2	Aqua-regia:H_2O_2	6:1.5	**270***	3:00	Light yellow with no suspension
3	Aqua-regia:H_2O_2	6:1.5	300	3:00	Light yellow with no suspension
III. Optimization for digestion time					
1	Aqua-regia:H_2O_2	6:1.5	270	2:00	Deep Yellow with suspension
2	Aqua-regia:H_2O_2	6:1.5	270	2:30	Light yellow with suspension
3	Aqua-regia:H_2O_2	6:1.5	270	**3:00***	Light yellow with no suspension

* Indicate the optimal condition for the given parameter.

2.2.4.3. Digestion of ginger samples

Applying the optimized condition (Table 2), 0.5 g of dried and homogenized ginger samples were transferred into a 100 mL round bottomed flask. Then 4 mL of a mixture of HNO_3 (69-72%) and $HClO_4$ (70%) with a volume ratio of 3:1 (v/v) was added and the mixture was digested on a Kjeldahl digestion apparatus fitting the flask to a reflux condenser by setting the temperature first to dial at 4 (120 ^oC) for 30 min and then increased to dial 9 (210 ^oC) for the remaining 2 hour and 30 minutes. The digest was allowed to cool to room temperature for 10 min without dismantling the condenser from the flask and for 10 min after removing the condenser. To the cooled solution 15 mL of deionized water was added to dissolve the precipitate formed on cooling and to minimize dissolution of filter paper by the digest residue while filtering with Whatman, (110 mm, diameter), filter paper into 50 mL volumetric flask. The round bottom flask was rinsed subsequently with 5 mL deionized water until the total volume reached around 45 mL. To this final solution, 3 mL lanthanum nitrate solution (1% w/w) was added and the solution was filled to the mark (50 mL) with deionized water. The digestion was carried out in triplicate for each bulk sample. Digestion of a reagent blank was also performed in parallel with the ginger samples keeping all digestion parameters the same. The digested samples were kept in the refrigerator, until the level of all the metals in the sample solutions were determined by FAAS.

2.2.4.4. Digestion of soil samples

Applying the optimized condition (Table 3), 0.5 g of dried and homogenized soil samples were transferred into a 100 mL round bottomed flask. To this 6 mL of aqua-regia (3:1 ratio of 37% HCl to (69-72%) HNO_3 respectively) and followed by 1.5 mL of 30% H_2O_2 were added and the mixture was digested on a Kjeldahl digestion apparatus fitting the flask to a reflux condenser by setting the temperature first to dial at 6 (180 ^oC) for the first 30 min and then raised to dial 8 (240 ^oC) for the next 30 min and finally raised to dial 9 (270 ^oC) for the remaining 2 hours. The rest steps were similar for both ginger and soil sample digestion procedure.

2.2.5. Analysis of ginger and soil samples for metal levels

Calibration metal standard solutions were prepared for each of the metals from an intermediate standard solution containing 10 mg/L which was prepared from the atomic absorption spectroscopy standard stock solutions that contained 1000 mg/L. These

secondary standards were diluted with deionized water to obtain three working standards for each metal of interest (Table 5). Then, Ca, Mg, Cu, Zn, Mn, Ni, Fe, Co, Cr, Pb and Cd were analyzed with FAAS (Buck Scientific Model 210GP) equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument. Three replicate determinations were carried out on each sample. Hallow cathode lamp for each metal operated at the manufacturer's recommended conditions were used at its respective primary source line. The acetylene and air flow rates were managed to ensure suitable flame conditions. All eleven metals were determined by absorption/concentration mode and the instrument readout was recorded for each solution manually. The same analytical procedure was employed for the determination of elements in digested blank solutions. The operating conditions for FAAS employed for each analyte are given in Table 4.

Table 4. Instrumental operating conditions for determination of metals in ginger and soil samples using FAAS.

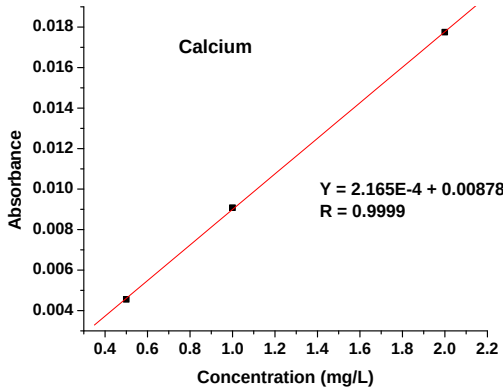
Element	Wavelength (nm)	Detection limit (mg/L)	Slit width (nm)	Lamp current (mA)	Energy (erg)
Ca	422.7	0.010	0.7	2.0	3.606
Mg	285.2	0.001	0.7	1.0	4.091
Cu	324.7	0.020	0.7	1.5	3.970
Zn	213.9	0.005	0.7	2.0	3.047
Mn	279.5	0.001	0.7	3.0	3.971
Ni	232	0.040	0.2	7.0	2.928
Fe	248.3	0.030	0.2	7.0	2.546
Co	240.7	0.050	0.2	4.5	2.746
Cr	357.9	0.050	0.7	2.0	3.750
Cd	228.9	0.005	0.7	2.0	3.07
Pb	217	0.100	0.7	3.0	3.16

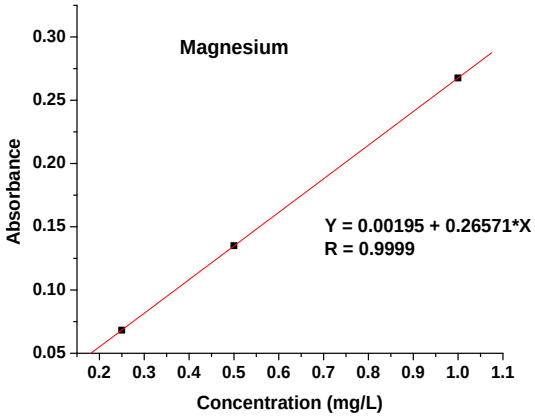
2.2.6. Instrument Calibration

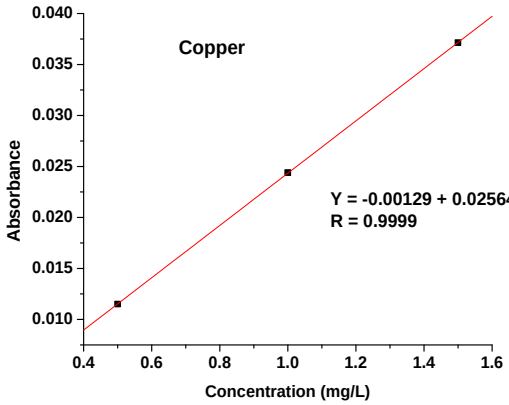
The data qualities obtained from FAAS for metals analyses are highly affected by the calibration and standard solution preparations procedures. The instrument was calibrated using three series of working standards. The working standard solutions of each metal were prepared freshly by diluting the intermediated standard solutions (10mg/L). Concentrations of the intermediate standards, working standards and value of correlation coefficient of the calibration graph for each metal are listed in Table 5. The calibration graph of each metals of interest is shown in Figure 3.

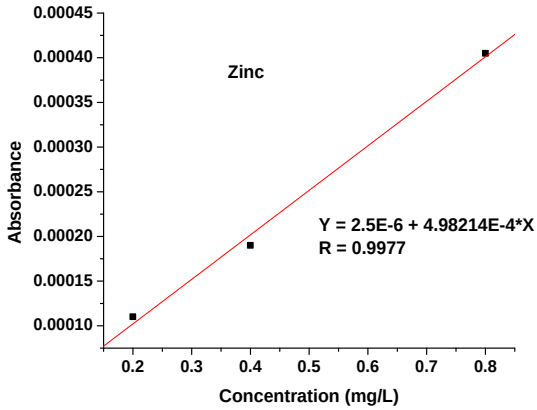
Table 5. Intermediate standards, working standards and correlation coefficients of the calibration curves for determinations of metals using FAAS.

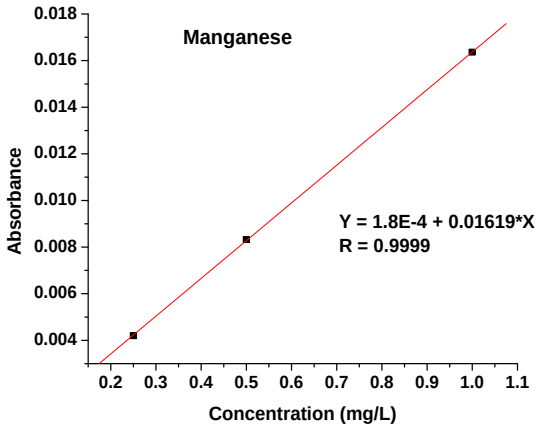
No.	Metal	Concentration of intermediate standard (mg/L)	Concentration of working standards (mg/L)	Correlation coefficient of calibration curves
1	Ca	10	0.5, 1.0, 2.0	0.9999
2	Mg	10	0.25, 0.5, 1.0	0.9999
3	Cu	10	0.5, 1.5, 1.5	0.9999
4	Zn	10	0.2, 0.4, 0.8	0.9977
5	Mn	10	0.25, 0.5, 1.0	0.9999
6	Ni	10	0.25, 0.5, 1.0	0.9999
7	Fe	10	0.5, 1.0, 1.5	0.9999
8	Co	10	0.25, 0.5, 1.0	0.9919
9	Cr	10	0.5, 1.0, 1.5	0.9989
10	Cd	10	0.25, 0.5, 1.0	0.9999
11	Pb	10	1.2, 2.4, 4.8	0.9999

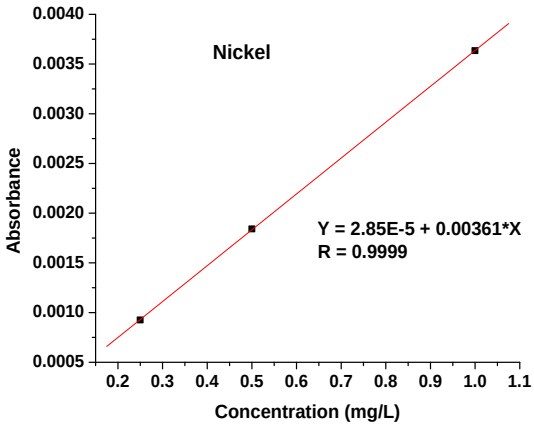

Calcium
Y = 2.165E-4 + 0.00878*X
R = 0.9999
Absorbance
Concentration (mg/L)


Magnesium
Y = 0.00195 + 0.26571*X
R = 0.9999
Absorbance
Concentration (mg/L)


Copper
Y = -0.00129 + 0.02564*X
R = 0.9999
Absorbance
Concentration (mg/L)


Zinc
Y = 2.5E-6 + 4.98214E-4*X
R = 0.9977
Absorbance
Concentration (mg/L)


Manganese
Y = 1.8E-4 + 0.01619*X
R = 0.9999
Absorbance
Concentration (mg/L)


Nickel
Y = 2.85E-5 + 0.00361*X
R = 0.9999
Absorbance
Concentration (mg/L)

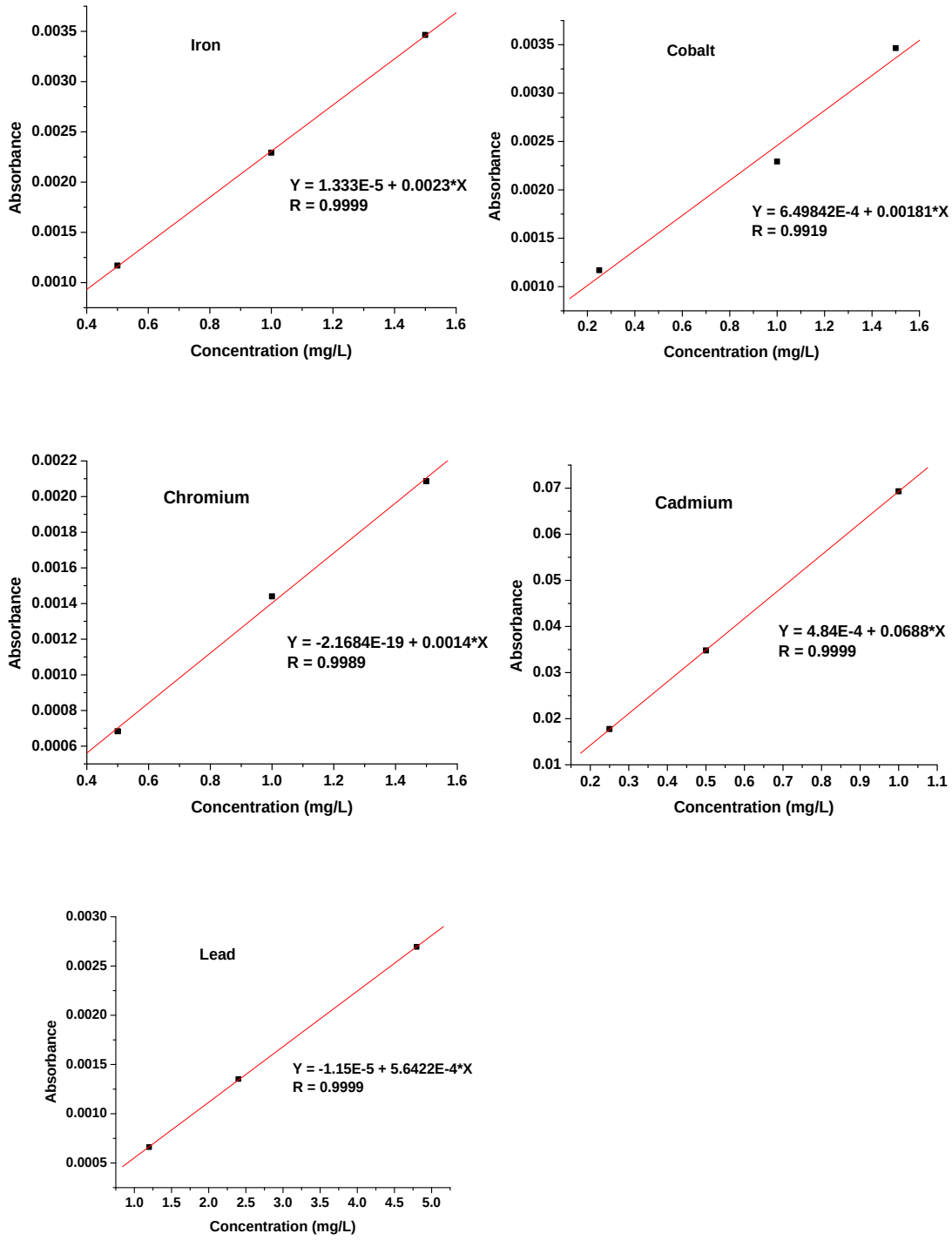


Figure 3. Calibration graphs of metal standard solution.

2.2.7. Method performance and method validation

The criteria used for evaluating analytical methods are called figures of merit. Based on these characteristics, one can predict whether a method meets the needs of intended purpose. These figures of merit are accuracy, precision, sensitivity, detection limits, and the quantitation limits [35].

2.2.7.1. Precision

The precision of an analytical procedure expresses the closeness or agreement between a replicate measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions (repeatability or reproducibility). The common term used to measure variability is the coefficient of variation (CV) or relative standard deviation (RSD), which may also be expressed as a percentage and it is the parameter of choice for expressing precision in analytical sciences [35].

In this study, the precision of the results were evaluated by the pooled standard deviation, and relative standard deviation of the results of nine measurements for a given bulk sample (i.e. three samples (n = 3) and triplicate readings for each sample).

2.2.7.2. Method detection limit

According to IUPAC, the limit of detection (LOD) is defined as “the smallest concentration or amount of an analyte that can be reliably shown to be present or measured under defined condition”, and the limit of detection is “the lowest concentration level that can be determined to be statistically different from a blank (99% confidence)”. The LOD is typically determined to be in the region where the signal-to-noise ratio is greater than 3 but not necessarily quantitated as an exact value [48].

In the present study, method detection limit for each metal was estimated by digesting six analytical blanks with the optimized procedure for both ginger and soil samples. Triplicate analyses of six blank samples for all elements were performed and the pooled standard deviation of the six blank reagents was calculated. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank (S_{blank}) by three (MDL = 3 x S_{blank}, n = 6). As shown in Table 6, the method detection limit of each element is above the instrument detection limit.

2.2.7.3. Method limit of quantitation (LOQ)

The lowest concentration level at which a measurement is quantitatively meaningful is called the limit of quantitation (LOQ). The LOQ is most often defined as 10 times the signal/noise ratio. If the noise is approximated as the standard deviation of the blank (s_b), the LOQ is 10 x S_b [35].

In this study, LOQ was obtained from triplicate analysis of six reagents blanks which was digested in the same digestion procedure as ginger and soil samples. The LOQ was calculated by multiplying pooled standard deviation of the reagent blank by ten (LOQ = 10 x S_{blank}, n = 6) and the value for each elements was listed in Table 6.

Table 6. Instrument detection limit, method detection limit and quantitation limit for metals of interest determined in ginger and soil samples.

Metal	Instrument detection limit (mg/L)	MDL for ginger (mg/g dry wt)	MDL for soil (mg/g)	MQL for ginger (mg/g)	MQL for soil (mg/g)
Ca	0.01	0.006	0.005	0.02	0.01
Mg	0.001	0.002	0.003	0.01	0.01
Cu	0.02	0.001	0.002	0.001	0.01
Zn	0.005	0.0006	0.003	0.002	0.01
Mn	0.01	0.0005	0.002	0.002	0.01
Ni	0.04	0.003	0.007	0.009	0.03
Fe	0.03	0.005	0.004	0.01	0.01
Co	0.05	0.002	0.003	0.006	0.01
Cr	0.05	0.006	0.006	0.01	0.01
Cd	0.005	0.0002	0.001	0.0003	0.01
Pb	0.1	0.002	0.004	0.007	0.01

2.3. Validation of optimized procedure

The efficiency of the optimized procedure is checked by various methods. These are certified standard reference material analyzing and spiking sample with known concentration of the analyte. In this work, the method validation was established by spiking experiments. The spiked samples were prepared by adding a small known quantity of metal standard solutions.

For spiking ginger sample, 200 μL of 1000 mg/L Ca, 300 μL of 1000 mg/L Mg, 12.5 μL of 1000 mg/L Zn, 75 μL of 1000 mg/L Mn, 31.5 μL of 40 mg/L Ni, 10 μL of 1000 mg/L Fe, 43.5 μL of 40 mg/L Co, 25 μL of 40 mg/L Cu, 62.5 μL of 40 mg/L Cr and 30μL of 10 mg/L Cd standard solutions were added to round bottomed flask (100 mL) containing 0.5 g ginger sample. For soil sample spiking, 350 μL of 1000 mg/L Ca, 250 μL of 1000 mg/L Mg, 235 μL of 1000 mg/L Fe, 65μL of 1000 mg/L Mn, 75 μL of 1000 mg/L Zn, 20 μL of 1000 mg/L Ni, 200 μL of 40 mg/L Cu, 40 μL of 1000 mg/L Co, 35 μL of 1000 mg/L Cr and 32 μL of 10 mg/L Cd standard solutions were added to round bottomed flask (100 mL) containing 0.5 g soil sample.

The spiked and non-spiked samples were digested and analysed in similar condition. Then the percentage recovery of the analyte was calculated by:

$$\%R = \frac{C_M \ \textit{in the spiked sample} - C_M \ \textit{in the non-spiked sample}}{C_M \ \textit{added for spiking}} x100\%$$

where, C_M = concentration of metal of interest.

As shown in Table 7 and 8, the results of percentage recoveries for the studied metal nutrients in both ginger and soil samples were within the acceptable range (93-106% in the ginger and (93-107%) in the soil samples except for chromium (Cr) in ginger sample. Therefore, this verifies that the optimized digestion procedure was valid for both ginger and soil sample analysis. The lower recovery of chromium may be attributed from the strong matrix analyte interaction.

Table 7. Recovery test for the optimized procedure of ginger sample.

Metal	Conc. in sample (μg/g)	Amount added (μg/g)	Conc. in spiked sample (μg/g)	Amount recovered (μg/g)	Recovery (%)
Ca	2254	400	2641 ± 39	386 ± 5	96.5 ± 6
Mg	2897	600	3462 ± 7	565 ± 4	94.2 ± 3.2
Cu	3.96	2	6.08 ± 0.34	2.12 ± 0.22	106 ± 3
Zn	52.6	25	75.9 ± 4.2	23.3 ± 1.2	93.2 ± 2.4
Mn	383	150	526 ± 15	144 ± 9.3	96.0 ± 4.2
Ni	5.34	2.5	7.94 ± 0.77	2.6 ± 0.37	104 ± 2.6
Fe	42.9	20	61.8 ± 5.3	18.8 ± 0.43	94.0 ± 3.4
Co	7.21	3.5	10.8 ± 0.87	3.63 ± 0.85	104 ± 5.2
Cr	8.79	5	13.2 ± 0.52	4.43 ± 0.26	88.6 ± 2.8
Cd	0.87	0.6	1.50 ± 0.03	0.63 ±0.04	105 ± 2.3

Table 8. Recovery test for the optimized procedure of soil sample.

Metal	Conc. in sample (μg/g)	Amount added (μg/g)	Conc. in spiked sample (μg/g)	Amount recovered (μg/g)	Recovery (%)
Ca	3521	700	4173 ± 53	652 ± 12	93.1 ± 2.6
Mg	2429	500	2912 ± 17	484 ± 17	96.8 ± 4.3
Cu	35.4	16	52.5 ± 3.4	17.1 ± 2.6	107 ± 2.8
Zn	382	150	530 ± 7	148 ± 7	98.7 ± 4.2
Mn	6423	130	6556 ± 48	133 ± 3	102 ± 6.4
Ni	72.8	40	112 ± 6	38.8 ± 4.7	97.0 ± 5.4
Fe	46813	470	47273 ± 214	461 ± 16	98.1 ± 4.4
Co	146	80	221 ± 8	74.5 ± 3.6	93.1 ± 3.2
Cr	128	70	203 ± 13	74.9 ± 4.7	107 ± 1.4
Cd	1.02	0.65	1.64 ± 0.08	0.62 ± 0.04	95.4 ± 5.2

3. RESULTS AND DISCUSSION

3.1 Determination of metals in ginger and soil samples

The concentration of eleven elements (Ca, Mg, Fe, Zn, Cu, Co, Cr, Mn, Ni, Cd, and Pb) in the digested samples of ginger and soil were analysed by flame AAS. Among the analysed metals lead was below the method detection limit (0.002 mg/g in the ginger and 0.004 mg/g in the soil) and the concentration value of the rest metals shown with their respective % RSD in Table 9 and 10. The most abundant metal among the macro-elements in ginger is Mg followed by Ca whereas Mn content was the predominant among the tested micronutrient heavy metals followed by Fe, Zn, Co and Cu. In soil sample the most abundant metal is Fe followed by Mn, Ca, Mg, Zn, Cr, Co, Cu, Ni, and Cd.

Table 9. Average concentration (mean ± SD, n = 3, μg/g dry weight basis) and relative standard deviation (% RSD) of major, minor and toxic metals in ginger samples from Tepi, Bombae, Hadaro and Ilubabur.

Metal	Tepi ginger sample		Bombae ginger sample		Hadaro ginger sample		Ilubabur ginger sample	
	mean ± SD	% RSD	mean ± SD	% RSD	mean ± SD	% RSD	mean ± SD	% RSD
Ca	2001 ± 47	2.4	2543 ± 93	3.6	2187 ± 24	1.1	2486 ± 41	1.7
Mg	2993 ± 9	0.3	2700 ± 57	2.1	2764 ± 11	0.4	4094 ± 105	2.6
Cu	4.78 ± 0.34	7.1	1.86 ± 0.18	9.5	2.53 ± 0.19	7.6	1.10 ± 0.05	4.6
Zn	55.2 ± 3.9	7.0	39.6 ± 0.5	1.2	38.5 ± 0.5	1.2	54.0 ± 2.7	4.9
Mn	385 ± 9	2.3	285 ± 4.3	1.5	184 ± 3.6	2.0	401 ± 12	3.1
Ni	5.61 ± 0.44	7.9	5.46 ± 0.48	8.9	6.78 ± 0.53	7.8	8.40 ± 0.32	3.8
Fe	44.2 ± 3.3	7.4	55.4 ± 5.0	9.0	41.8 ± 2.8	6.7	89.0 ± 6.1	6.8
Co	7.58 ± 0.46	6.0	5.68 ± 0.40	7.1	2.04 ± 0.14	6.8	2.18 ± 0.18	7.2
Cr	9.28 ± 0.61	6.6	6.02 ± 0.14	2.3	9.17 ± 0.62	6.7	10.8 ± 0.2	2.2
Cd	0.97 ± 0.08	7.9	0.38 ± 0.02	5.03	0.38 ± 0.02	5.04	0.70 ± 0.07	9.8
Pb	ND		ND		ND		ND	

ND: Concentration of the tested heavy metal below the method detection limit (< 0.002 mg/g).

Table 10. Average concentration (mean ± SD, n = 3, μg/g dry weight basis) and relative standard deviation (% RSD) of major, minor and trace elements in soil samples from Tepi, Bombe, Hadaro and Ilubabur.

Metal	Tepi soil sample		Bombae soil sample		Hadaro soil sample		Ilubabur soil sample	
	mean ± SD	% RSD	mean ± SD	% RSD	mean ± SD	% RSD	mean ± SD	% RSD
Ca	3583 ± 16	0.4	2056 ± 10	0.5	2040 ± 43	2.1	1773 ± 39	2.2
Mg	2432 ± 141	5.8	1457 ± 45	3.1	1657 ± 8	0.5	2442 ± 8	0.3
Cu	33.9 ± 0.5	1.5	3.76 ± 0.07	1.8	6.77 ± 0.17	2.5	33.7 ± 0.8	2.5
Zn	389 ± 36	9.4	344 ± 28	8.3	255 ± 14	5.3	413 ± 39	9.4
Mn	6465 ± 81	1.3	1756 ± 26	1.5	1919 ± 28	1.5	4675 ± 32	0.7
Ni	79.3 ± 1.2	1.5	14.1 ± 0.3	2.0	21.4 ± 1.0	4.7	73.1 ± 4.7	6.4
Fe	46950 ± 600	1.3	21768 ± 821	3.8	21701 ± 407	1.9	46172 ± 484	1.1
Co	159 ± 2.8	1.8	57.1 ± 2.1	3.6	48.5 ± 0.6	1.2	132 ± 1.9	1.5
Cr	139 ± 12	8.7	110 ± 7.6	6.9	114 ± 1.8	1.6	163 ± 2.5	1.6
Cd	1.08 ± 0.08	7.1	0.24± 0.02	7.9	0.73 ± 0.06	7.9	1.20 ± 0.07	5.6
Pb	ND		ND		ND		ND	

ND: Concentration of the tested heavy metal below the method detection limit (< 0.004 mg/g).

3.2. Distribution pattern of the metals in different samples

The levels of metals differ significantly among each other and there is slight difference between the same metals from different sampling area. The distribution pattern of the metals in both ginger and soil samples are discussed in the following subtopics.

3.2.1. Distribution pattern of the metals in ginger samples

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 [9].

As it can be seen from Table 9 and 11 and Figure 4, there is large difference in concentration of different metals within ginger sample and slight variation in metals of the same type along with geographical location. The range of concentration (in mg/kg) pattern of elements in ginger sample collected from four sampling area is given in Table 11.

Ginger contains higher amount of Mg (2700-4094 mg/kg), followed by Ca (2001-2543 mg/kg). The higher levels of Mg in the ginger is probably due to the fact that nutrient elements such as N, P, K, S, and Mg are highly mobile in the plant tissue and trans-located from old plant tissue to new plant tissue. The other probable reason for higher concentration of Mg and Ca is if the soil which have been used for cultivating the plant, are highly fertilized with manure and organic residues, they were high in available potassium, calcium and magnesium. Hence, the plant has high amount of these metals.

Mn (184-401 mg/kg) was the most accumulated trace metal followed by Fe (41.8- 89.0 mg/kg) and Zn (38.5-55.2 mg/kg) in ginger sample. Higher Mn levels in the ginger may be attributed to the availability of this micronutrient heavy metal in relatively acidic soils of the farmland. The chemical forms of Mn present in soil are known to depend on soil pH. In acidic soil, the easily absorbed form, Mn^{+2} released from soil by H^{+}, which is produced from NH_4^{+} [49], can be readily taken up and accumulated in the ginger.

It has been reported that Fe and Zn, are the main elements that plant could accumulate and pass up in the food chain. Therefore, the high concentration of Zn from trace metals next to Mn and Fe in ginger may be because of the fact that these ions are readily transferred from the soil to plants, and accumulate in plants.

The levels of other essential trace metals detected in ginger were Co (2.04-7.58 mg/kg), Ni (5.61-8.40 mg/kg), Cr (6.02-10.84 mg/kg) and non-essential heavy metals Cd (0.38-0.97 mg/kg). The level of Cd was the least among the metals; however due to its toxicity deserves special concern. The non-essential heavy metal, Pb, was found to be below the method detection limit. In general the concentration pattern of metals in ginger was decreased as Mg > Ca > Mn > Fe > Zn > Cr > Ni > Co > Cu > Cd.

As it can be seen from Table 9 and 11, ginger can be a good source of major, minor and trace metals that are essential to human in addition to its food flavouring purpose. The

small amount of cobalt and copper found in ginger does not contradict with the requirement of the metal for proper functioning of the body, because these metals are required in small amount (Co = 0.3 mg/day as a constituent of vitamin B_{12} and Cu = 3.5 mg/day).

Table 11. Range of metal concentration in ginger samples.

Metal	Conc. range (mg/kg)	Metal	Conc. range (mg/kg)
Ca	2001-2543	Ni	5.61-8.40
Mg	2700-4094	Fe	41.8- 89.0
Cu	1.10-4.78	Co	2.04-7.58
Zn	38.5-55.2	Cr	6.02-10.84
Mn	184-401	Cd	0.38-0.97

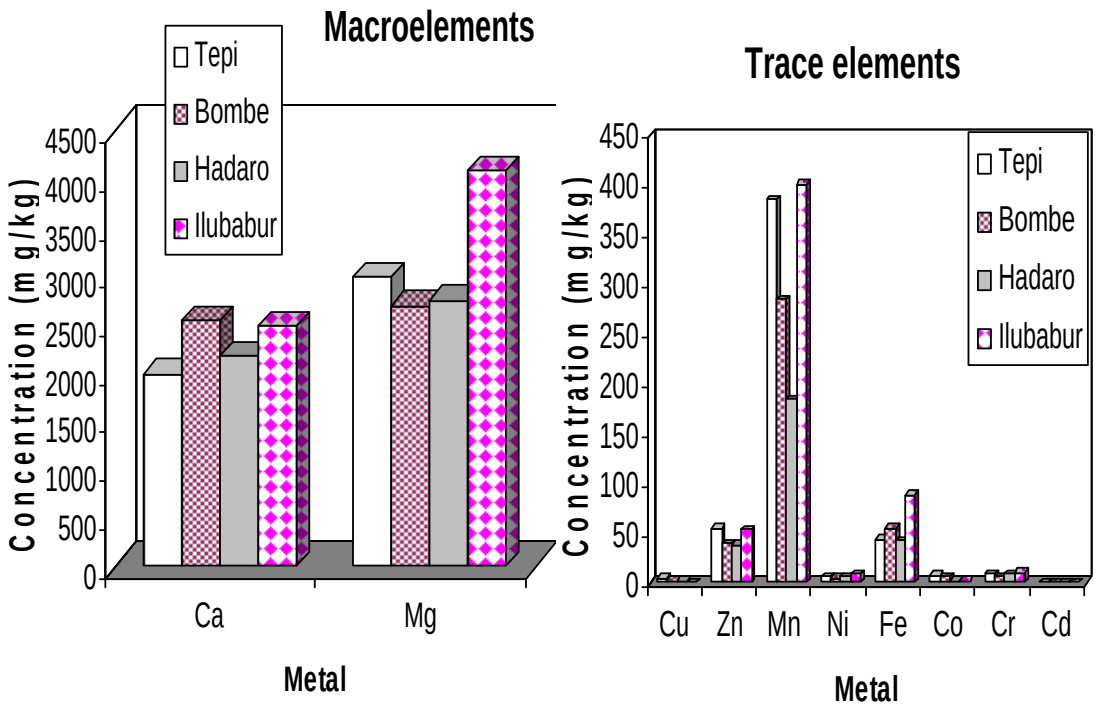


Figure 4. Distribution pattern of macro- and trace elements in ginger sample.

3.2.2. Distribution pattern of metals in soil sample

The soil sample collected from four sampling areas were found to contain detectable metal content of Ca, Mg, Fe, Zn, Cu, Co, Cr, Mn, Ni, and Cd in all the four soil samples and their values are given in Table 9. Among the analysed metals, Pb was found to be below the detection limit of the method used in this study. There is significant difference in concentration of different metals within soil sample and appreciable difference in the same metals of different sample. The determined concentration range of metals from four soil sampling area are given in Table 12.

As shown in Table 10 and 12 and Figure 5, the concentration of Fe (21701-46950 mg/kg) in soil exceeds much the concentration of macro-elements, Ca (1773-3583 mg/kg) and Mg (1457-2442 mg/kg), this is due to the presence of excess amount of hematite (Fe_2O_3) in the soil.

Concentration of Mn (1756-6465 mg/kg) an essential trace metal in these soils is higher when compared to the micronutrient heavy metals Zn (255-413 mg/kg), Cu (3.76-33.9 mg/kg), Cr (110-163 mg/kg), Co (48.5-159 mg/kg) and Ni (14.1-79.3 mg/kg). On the other hand, level of the toxic heavy metal Cd ranges from 0.24-1.08 mg/kg. The level of Pb, the other tested toxic metal, in soils of all the samples was found to be below the detection limit of the method used in this study. In general, the concentration pattern of metals in soil was decreased as Fe >> Mn > Ca > Mg > Zn > Cr > Co > Ni > Cu > Cd.

Table 12. Range of metal concentration in soil samples.

Metal	Conc. range (mg/kg)	Metal	Conc. range (mg/kg)
Ca	1773-3583	Ni	14.1-79.3
Mg	1457-2442	Fe	21701-46950
Cu	3.76-33.9	Co	48.5-159
Zn	255-413	Cr	110-163
Mn	1756-6465	Cd	0.24-1.08

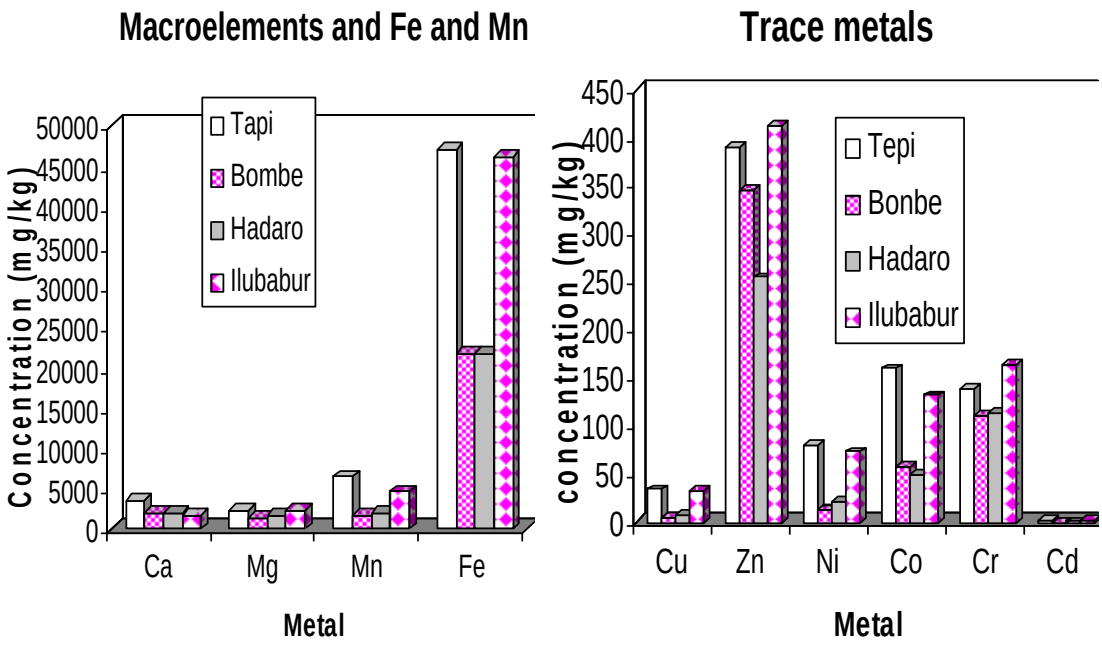


Figure 5. Distribution pattern of macro- and trace elements in soil sample.

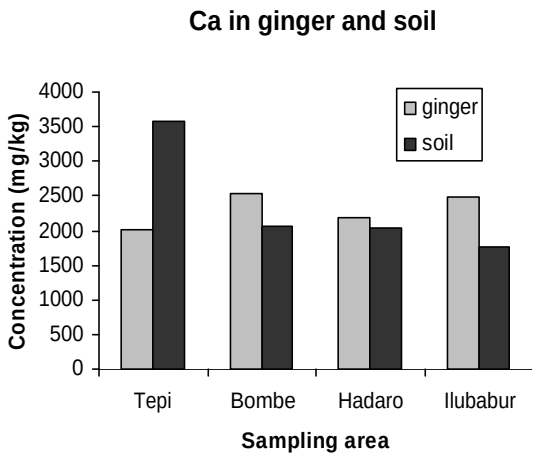
3.3. Comparisons of metal levels between ginger and soil sample

Plants absorb whatever is present in the soil medium and therefore the metals are also absorbed and become bioaccumulated in the roots, stems, fruits, grains and leaves of the plant, which may finally be transferred to man in the food chain. The sorption processes of metals by plants is significantly affected by metal level in the soil, soil pH, the presence of competing ligands, the ionic strength of the soil solution and the simultaneous presence of competing metals [50].

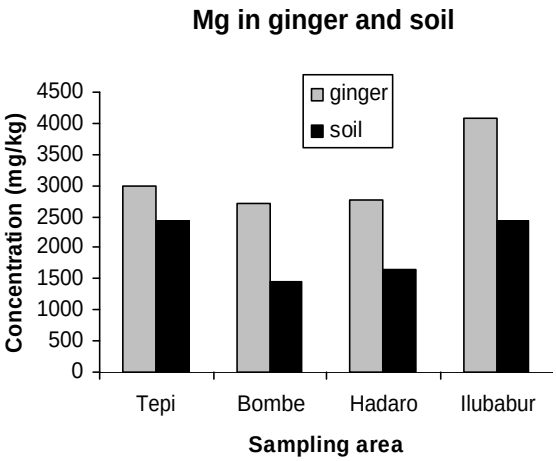
In this work, comparative study has been established to correlate the metal level of ginger with the soil where it has grown. As it can be seen from Table 9 and 10 and Figure 6 (a-k), for most elements (Mg, Mn, Zn, Fe (except in Tepi sample), Cu (except in Ilubabur sample), Cr, and Cd) the metal levels of ginger was directly proportional to the metal levels of soil where it has grown. Taking the level of Mg as counter example in both four ginger and soil samples, the Mg level of ginger sample from Ilubabur > Tepi > Hadaro > Bombae and the same order is true for Mg level of respective soil sample. This relation partly verifies that the metal content of the plant is a function of the metal level in the soil where it has grown. For the rest three metals (Ca, Ni and Co), the metal levels in some sampling area of ginger were varies unproportional to levels of metal in the corresponding

soil. This unproportional variation in level of metals in ginger and soil may be resulted from the difference in availability of absorbable form of metals in soil due to difference in soil acidity or the presence of competing ligands.

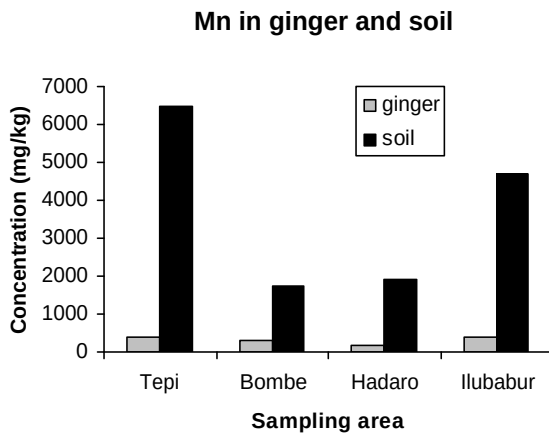
For most metals there was large difference in concentration of the same metals in different ginger sample (for example the concentration of Mg, Mn, Zn, Fe, and Cd of Illubabur and Tepi sample were higher than the other sample sites), this may be attributed to the difference in mineral concentration, the pH and organic matter content of the respective soil. The other probable reason is that the Tepi and Ilubabur sample sites are near to the city and may be such differences are observed as a result of higher population and industrial activities in cities and municipalities which lead to higher production of assorted waste than in the rural settlements of Bombae and Hadaro.



a)



b)



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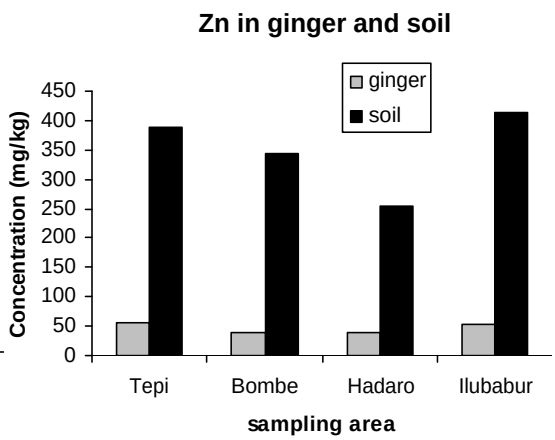
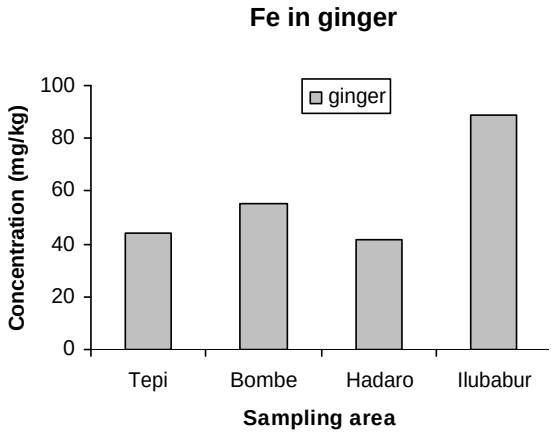
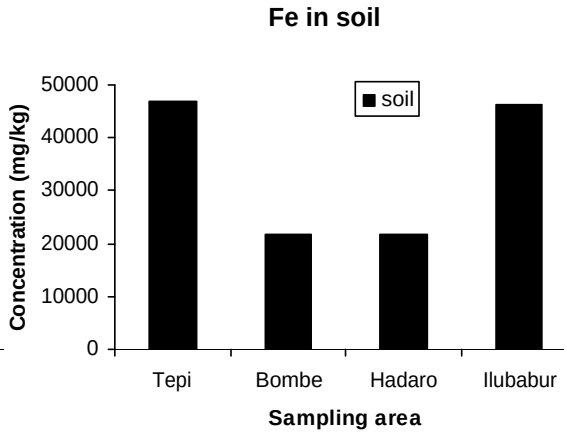


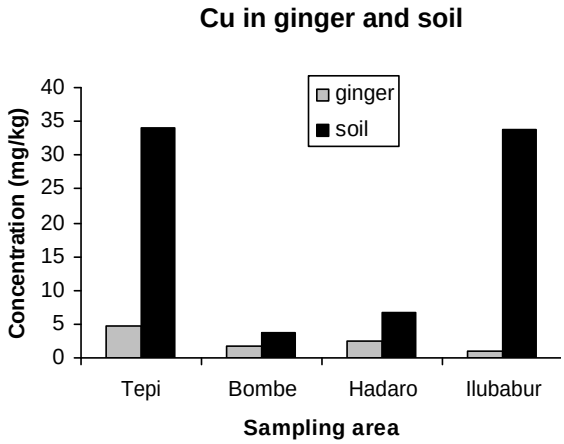
d)


Fe in ginger
Concentration (mg/kg)
100
80
60
40
20
0
ginger
Tepi
Bombe
Hadaro
Ilubabur
Sampling area

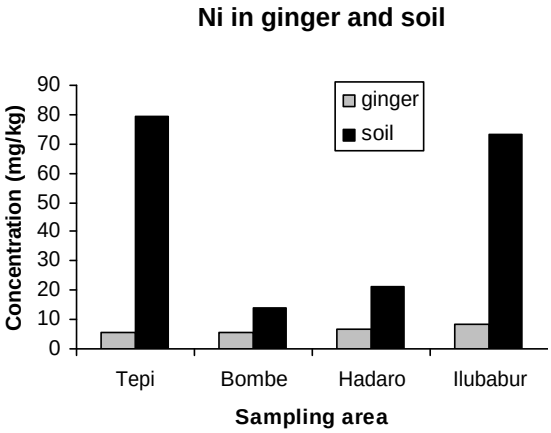
e)


Fe in soil
Concentration (mg/kg)
50000
40000
30000
20000
10000
0
soil
Tepi
Bombe
Hadaro
Ilubabur
Sampling area

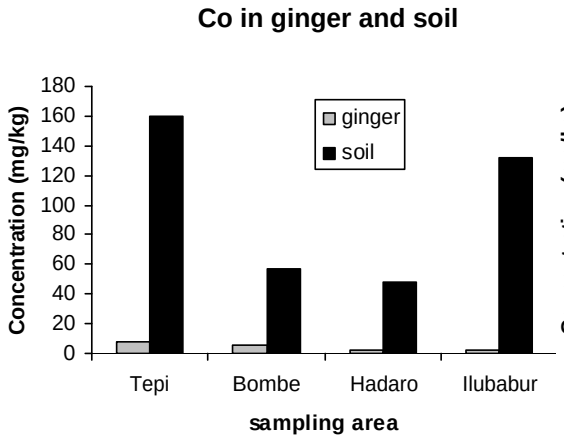
f)


Cu in ginger and soil
Concentration (mg/kg)
40
35
30
25
20
15
10
5
0
ginger
soil
Tepi
Bombe
Hadaro
Ilubabur
Sampling area

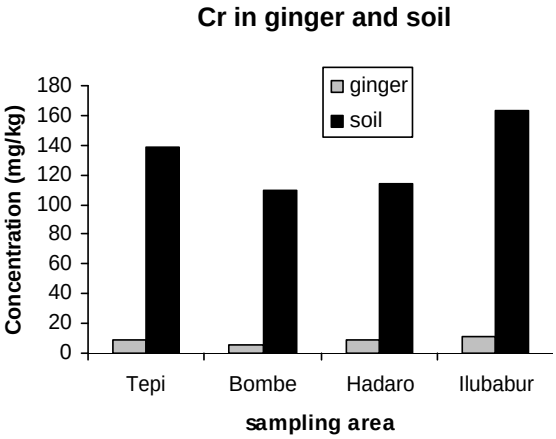
g)


Ni in ginger and soil
Concentration (mg/kg)
90
80
70
60
50
40
30
20
10
0
ginger
soil
Tepi
Bombe
Hadaro
Ilubabur
Sampling area

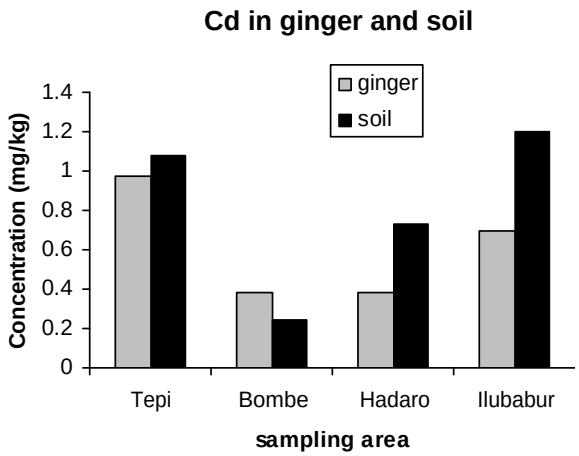
h)


Co in ginger and soil
Concentration (mg/kg)
180
160
140
120
100
80
60
40
20
0
ginger
soil
Tepi
Bombe
Hadaro
Ilubabur
sampling area

i)


Cr in ginger and soil
Concentration (mg/kg)
180
160
140
120
100
80
60
40
20
0
ginger
soil
Tepi
Bombe
Hadaro
Ilubabur
sampling area

j)



k)

Figure 6. Comparison of metal levels between ginger and soil from four sample area.

3.4. Comparison of metal levels of ginger of this study with literature values

The comparative study of the metal concentration of ginger determined in this study and reported values of other researchers are presented in Table 13.

The Ca content of ginger determined in this study (Ethiopia) was a bit higher than that of reported by [51] and almost comparable to that of reported by [52]. The level of Mg of this study was comparable to those studied in Nigeria [52] but much lower than those in India [51].

Manganese concentration in ginger determined in this study (Ethiopia) was in the range of 184-401 mg/kg and its value reported by [51] and [53] lie in this range. The iron content reported in Saudi Arabia [54] is less than the iron content determined in this study. However, the iron content reported by [51] is higher than the result of the present study. The mean concentration of zinc determined in this study is a bit lower than the value determined in India [51]. The content of zinc in ginger sample of current study (Ethiopia) was above the permissible limit set by FAO/WHO in edible plants (27.4 mg/kg). However, according to Bowen and Allaway, the range of Zn in agricultural products should be between 15 to 200 mg/kg [55].

The nickel concentration of the present study is lower than the nickel content determined in Nigeria [53]. The nickel content determined in the present study (Ethiopia) is higher than the permissible limit set by FAO/WHO in edible plants (1.63 mg/kg). However, Ni toxicity

in human is not a very common occurrence because its absorption by the body is very low [55].

From Table 13, one can see that the concentration of copper determined in this study (Ethiopia) is almost the same as the value reported in India [51]. The chromium content of ginger reported by [56, 57] are less than the current result. The concentration level of Cr in ginger of this study (Ethiopia) is higher than the permissible limit set by FAO/WHO in edible plants (0.02 mg/kg). Chronic exposure to Cr may result in liver, kidney and lung damage [55].

The level of cobalt concentrations obtained in this study (Ethiopia) was 2.04-7.58 mg/kg and which is higher than the value reported in Saudi Arabia [58]. The content of cadmium in ginger determined in the present study (Ethiopia) was 0.38-0.97 mg/kg and which is higher than the content of Cd studied in Nigeria [59] and Saudi Arabia [58]. The level of cadmium in ginger from present study is above the permissible limit set by FAO/WHO in edible plants (0.21 mg/kg). The results of this study reveal that the content of lead in ginger was below the method detection limit and that assures the low lead exposure of the farmlands.

The minimal risk levels for hazardous Pb, and Cd metal through oral route and its acute effect are 0.0002 and 0.0002 mg/kg per day, respectively [58]. Whereas the human need from ginger is very few grams per day, hence there is no risk from used ginger in the food.

Table 13. Comparison of determined metals concentration, (mg/kg, dry mass basis) with reported values.

Metal	Concentration (mg/kg)	Country	Reference
Ca	1700	India	51
	2610	Nigeria	52
	2001-2543	Ethiopia	This study
Mg	4210	Nigeria	52
	9200	India	51
	2700-4094	Ethiopia	This study
Mn	367.9	Nigeria	53
	313.42	India	51
	184-401	Ethiopia	This study
Fe	216.64	India	51
	19.44	Saudi Arabia	54
	41.8- 89.0	Ethiopia	This study
Zn	72.53	India	51
	38.5-55.2	Ethiopia	This study
Ni	108.1	Nigeria	53
	5.61-8.40	Ethiopia	This study
Co	0.32	Saudi Arabia	58
	2.04-7.58	Ethiopia	This study
Cu	4.47	India	51
	1.10-4.78	Ethiopia	This study
Cr	0.47	Pakistan	56
	0.5	Pakistan	57
	6.02-10.8	Ethiopia	This study
Cd	0.12	Nigeria	59
	0.07	Saudi Arabia	58
	0.38-0.97	Ethiopia	This study
Pb	< 0.021	Nigeria	59
	ND	Ethiopia	This study

ND: Concentration of the tested heavy metal below the method detection limit (< 0.004 mg/g).

3.5. Comparison of metal level in soil with literature values

The natural content of metals in the soil is directly related to the mineralogic and granulometric composition and the origin of the matrix soil and its range is very wide [60].

The content of Ca determined in this study was a bit higher than the value determined by Micheal Yemane in Ethiopia [61] whereas the concentration of Mg was comparable.

The concentrations of Fe (21701-46950 mg/kg), Zn (255-413 mg/kg), Cr (110-163 mg/kg) and Co (48.5-159 mg/kg) in the soil in this study were comparable with the value of Fe (2000-48000 mg/kg), Zn (1.80-438 mg/kg), Cr (7-150 mg/kg) and Co (0.1-100 mg/kg) reported by Jankiewicz *et al.* in Poland [60].

Soils of the farmland with clay texture and dark reddish brown color, is an indicative of the presence of excess amount of hematite (Fe_2O_3). Soils with low pH contain high amounts of Fe and Al oxides. Thus, Fe is the predominant metal within the concentration range of 2000-48000 mg/kg in these soils whereas Mn content is in the range of 1756-6465 mg/kg.

The contents of Cu (3.76-33.9 mg/kg) and Cd (0.24-1.08 mg/kg) determined in this study are in good agreement with the value of Cu (0.18-68.75 mg/kg) and Cd (0.21-1.02 mg/kg) reported by Buszewski *et al.* in Poland [62]. The level of Pb, the other tested toxic metal, in soils of all soil samples was found to be below the method detection limit and which was in agreement with the reported value by Micheal Yemane [61].

3.6. Statistical analysis

3.6.1 Analysis of variance

T-tests and analysis of variance (ANOVA) are widely used statistical methods to compare group means. While the independent sample t-test is limited to comparing the means of two groups, while the one-way ANOVA can compare the mean of more than two groups of sample. ANOVA use the F statistic to compare whether the difference between sample means are significant or not [63]. If the calculated value of F (the ratio of SD between samples to SD within samples) is greater than $F_{critical}$ (the value obtained from the table at

specified confidence level and degree of freedom), the differences in sample means are significant.

In this study, ginger samples were collected from four different areas and the metal level of each sample was analysed by FAAS. During the processes of sample preparation and analysis a number of random errors may be introduced in each aliquot and in each replicate measurement. The variation in sample mean of the analyte was tested by using ANOVA, whether the source for variation was from experimental procedure or heterogeneity among the samples (i.e. difference in mineral contents of soil, pH of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides etc or other variations in cultivation procedures).

As it can be seen from the Table 14, there exist statistically significant differences at $F_{3,8}$ at 95 % confidence level in mean concentration of all the nine metals except Ni beyond what is expected from variation in experimental procedure. The source for this significant difference between sample means may be the difference in mineral contents of soil or pH of soil which predict the extent of mineral absorption by ginger.

For Ni, since the calculated value of F (3.14) is less than the critical value of F (4.07 for 3 degree of freedom between sample and 8 degree of freedom within samples), the difference between samples mean is not significant. The variance among results of Ni in four ginger samples should not be attributed to anything more than random error in the analytical procedure.

Table 14. Analysis of variance (ANOVA) between and within ginger samples at 95% confidence level.

Metal	Comparision	SD	df	$F_{calculated}$	$F_{critical}$	Remark
Ca	Between samples	255.38	3	4.96	4.07	Significant difference between sample means
	With in samples	51.44	8			
Mg	Between samples	650.05	3	14.24	4.07	Significant difference between sample means
	With in samples	45.64	8			
Cu	Between samples	1.58	3	8.32	4.07	Significant difference between sample means
	With in samples	0.19	8			
Zn	Between samples	9.96	3	5.89	4.07	Significant difference between sample means
	With in samples	1.69	8			
Mn	Between samples	100.50	3	13.82	4.07	Significant difference between sample means
	With in samples	7.27	8			
Ni	Between samples	1.38	3	3.14	4.07	No significant difference between sample means
	With in samples	0.44	8			
Fe	Between samples	21.78	3	5.09	4.07	Significant difference between sample means
	With in samples	4.28	8			
Co	Between samples	2.72	3	9.38	4.07	Significant difference between sample means
	With in samples	0.29	8			
Cr	Between samples	2.02	3	4.93	4.07	Significant difference between sample means
	With in samples	0.41	8			
Cd	Between samples	0.29	3	7.25	4.07	Significant difference between sample means
	With in samples	0.04	8			

where, SD - is standard deviation of between sample and within sample

df - is degree of freedom of between sample and within sample

3.6.2. Pearson correlation of metals

A correlation coefficient is a number between -1 and +1 that measures the degree of association between two variables (call them concentration of metal X and Y). A positive value for the correlation implies a positive association (large values of X tend to be associated with large values of Y and small values of X tend to be associated with small values of Y). A negative value for the correlation implies a negative or inverse association (large values of X tend to be associated with small values of Y and vice versa).

In this study, to correlate the effect of one metal concentration on the concentration of the other metal, the Pearson correlation matrices using correlation coefficient (r) for the samples were used and presented in Table 15, 16 and 17 for ginger, soil and ginger with soil samples, respectively.

3.6.2.1. Pearson correlation of metals within ginger sample

Table 15. Correlation matrices for metals in ginger sample (n = 4).

	Ca	Mg	Cu	Zn	Mn	Ni	Fe	Co	Cr	Cd
Ca	1									
Mg	0.315	1								
Cu	-0.907	-0.453	1							
Zn	-0.289	0.680	0.310	1						
Mn	0.032	0.683	0.120	0.924	1					
Ni	0.320	0.866	-0.623	0.311	0.232	1				
Fe	0.665	0.916	-0.711	0.443	0.592	0.783	1			
Co	-0.376	-0.426	0.731	0.286	0.370	-0.813	-0.438	1		
Cr	-0.312	0.751	0.002	0.655	0.399	0.781	0.439	-0.450	1	
Cd	-0.554	0.397	0.616	0.941	0.814	0.022	0.115	0.513	0.527	1

From the result depicted in Table 15, there is high positive correlation for Mg with (Ni, Zn, Mn, Fe and Cr), Cu with (Co and Cd), Zn with (Mn, Cr and Cd), Mn with Cd, Ni with Cr and moderate correlation for Cr with Cd; which may be arise from common anthropogenic or natural sources as well as from similarity in chemical properties. The high negative correlation between Ca and Cu indicate the large absorption of Ca may affect the

absorption of Cu in ginger plant. The other metals have weak negative or positive correlation indicating that the presence or absence of one metal affect in lesser extent to the other.

3.6.2.2. Pearson correlation of metals within soil sample

Table 16. Correlation matrices for metals in soil sample (n = 4).

	Ca	Mg	Cu	Zn	Mn	Ni	Fe	Co	Cr	Cd
Ca	1									
Mg	0.428	1								
Cu	0.445	0.996	1							
Zn	0.252	0.749	0.800	1						
Mn	0.706	0.937	0.948	0.737	1					
Ni	0.505	0.994	0.997	0.779	0.967	1				
Fe	0.461	0.987	0.997	0.839	0.953	0.995	1			
Co	0.403	0.313	0.393	0.806	0.477	0.395	0.464	1		
Cr	0.052	0.919	0.917	0.789	0.744	0.887	0.908	0.274	1	
Cd	0.282	0.942	0.910	0.512	0.808	0.905	0.875	-0.023	0.883	1

In soil samples, there is high positive correlation for all metals except Ca with (all metals except Mn), Co with (all metals except Zn). The low negative or positive correlation of Ca and Co with the other metals in the soil samples may be associated with chemical properties like insoluble carbonates.

3.6.2.3. Pearson correlation of metals between ginger and soil samples

Table 17. Pearson correlation coefficient for metals in ginger with soil sample (n = 4).

Metal	Ca	Mg	Cu	Zn	Mn	Ni	Fe	Co	Cr	Cd
r	-0.812	0.723	0.288	0.857	0.829	0.345	0.459	0.744	0.814	0.766

where, r is the Pearson correlation coefficient between metal level in ginger and metal level in soil.

As shown in Table 17 and Figure 6 (b, c, d, i, j and k), one can see that the more level of metals like Mg, Zn, Mn, Co, Cr and Cd in the soil, the more accumulation of corresponding metals in the ginger. This verifies that the dependence of metal concentration in the plant on metal concentration of respective soil.

4. CONCLUSION AND RECOMMENDATIONS

The level of essential (Ca, Mg, Fe, Zn, Cu, Co, Cr, Mn, and Ni) and non-essential elements (Cd and Pb) in ginger (*Zingiber officinale)* cultivated in Ethiopia and the soil where it has grown were determined by flame atomic absorption spectrometer.

An efficient digestion procedure for ginger and soil sample was developed and validated through standard addition (spiking) method and a good percentage recovery was obtained (100 ± 10) for all minerals identified except chromium in ginger.

The level of metals in ginger determined in this study could be put in the following order Mg (2700-4094 mg/kg) > Ca (2001-2543 mg/kg) > Mn (184-401 mg/kg) > Fe (41.8-89.0 mg/kg) > Zn (38.5-55.2 mg/kg) > Cr (6.02-10.8 mg/kg) > Ni (5.61-8.40 mg/kg) > Co (2.04-7.58 mg/kg) > Cd (0.38-0.97 mg/kg). The non-essential heavy metal, Pb, was found to be below the method detection limit. The results of this work confess that ginger accumulates relatively higher amounts of Mg and Mn among the determined macro- and micronutrients, respectively.

The ANOVA results at 95% confidence level suggest that there were significant difference in the mean concentration of all metals except Ni between the four sampling areas which could be attributed to the difference in mineral contents of soil or pH of soil which predict the extent of mineral absorption by ginger.

The level of metals in the soil which was collected from ginger cultivated farmland was also determined for comparative study. The soils of the study farmland were found to contain high levels of Fe followed by Mn, Ca, Mg, Zn, Cr, Co, Ni, Cu, Cd. The level of Pb in soils of all samples was found to be below detection limit of the method used in this study.

To correlate the effect of level of metal in the soil with level of the same metal in ginger or any other metal, the Pearson correlation matrices using correlation coefficient (r) for the samples were used. The large positive value of r was obtained for most metals indicating that the more level of metals in the soil, the more accumulation of corresponding metals in

the ginger. In general, the levels of most of the metals in the studied soils were found to correlate positively with the levels found in the ginger.

Since ginger is a cash crop and one of widely consumed spice all over the world, the assessment of levels of essential and heavy toxic metals is particular interest with respect to human health and the quality of its products. Thus, the present study will give brief information about the mineral contents of it. It might be useful in pointing directions for studies that will be conducted on the nutrient levels in regard to the mineral status of the soil in which ginger is growing in relation to soil pH. Secondly, it is recommended to assess the correlation of essential and trace toxic metals concentration in ginger with that of the irrigation water (if any) using low detection limit instruments.

5. REFERENCES

1. K. V. Peter (Ed.), *Hand Books of Herbs and Spices*, Woodhead Publishing Ltd., Abington, England, **2001**, 211–222.
2. V. A. Parthasarathy, B. Chempakam and T. J. Zachariah, *Chemistry of Spices*, CAB International, Wallingford, UK, **2008**, 70–93.
3. Nigist Asfaw and Sebsebe Demissew, *Aromatic plants in Ethiopia*, Shoma books, Addis Ababa, **2009**, 27–185.
4. Girma H/micheal, Digafe Tilahun and Edossa Etissa, *Spices Research achivements*, Revised edn., Ethiopian Institute of Agricultural Research, Addis Ababa, **2008**, 12–23.
5. Parashuram Mishra, Isolation, spectroscopic characterization and molecular modeling studies of mixture of curcuma longa, ginger and seeds of fenugreek, *Int.J. PharmTech Res*.,1, **2009**, 79–95.
6. Nigist Asfaw and Berhanu Abegaz, Chemical constituents of the essential oils of *Zingiber officinale* Roscoe cultivated in Ethiopia, *SINET: Ethiop. J. Sci.*, 18, **1995**, 133–135.
7. Zingiber officinale, http://www.allianceingredients.com/pdfdocs/ZINGIBER_OFFICINALE.PDF, accessed on Feb. 25, 2010.
8. S. D. Jolad, R. C. Lantz, A. M. Solyom, G. J. Chen, R. B. Bates, and B. N. Timmermann, Fresh organically grown ginger (*Zingiber officinale*): composition and effects on LPS-induced PGE_2 production, *Phytochemistry*, 65, **2004**, 1937–1954.
9. M. C. Jung, Heavy metal concentrations in soils and factors affecting metal uptake by plants in the vicinity of a Korean Cu-W mine, *Sensors*, 8, **2008**, 2413–2423.
10. H. S. Harold, M. K. Leslie, History of Nutrition Symposium: Trace Element Nutrition and Human Health. *J. Nutritional Science*, 130, **2000**, 4835–4845.
11. H. Lokeshwari, G.T. Chandrappa, Impact of Heavy Metal Contamination of Bellandur Lake on Soil and Cultivated Vegetation, *Current Science*, 91, **2006,** 622–627.
12. P. K. Padmavathiamma and L. Y. Li, Phytoremediation Technology: Hyper-accumulation Metals in Plants; *Water Air Soil Pollut*, 184, **2007**, 105–126.

13. E. I. Uwah, N. P. Ndahi and V. O. Ogugbuaja, Study of the Levels of Some Agricultural Pollutants in Soils, and Water Leaf (Talinum triangulare) Obtained in Maiduguri, Nigeria, *Journal of Applied Sciences in Environmental Sanitation*, 4, **2009**, 71−78.
14. J. Pandey and U. Pandey, Accumulation of heavy metals in dietary vegetables and cultivated soil horizon in organic farming system in relation to atmospheric deposition in a seasonally dry tropical region of India, *Environ Monit Assess*, 148, **2009**, 61−74.
15. G. A. Ebong, M. M. Akpan and V. N. Mkpenie, Heavy Metal Contents of Municipal and Rural Dumpsite Soils and Rate of Accumulation by *Carica papaya* and *Talinum triangulare* in Uyo, Nigeria; *E-Journal of Chemistry*, 5, **2008**, 281−290.
16. G. E. Udofia, J. P. Essien, S. I. Eduok and B. P. Akpan, Bioaccumulation of heavy metals by yeasts from Qua Iboe estuary mangrove sediment ecosystem, Nigeria; *African Journal of Microbiology Research*, 3, **2009**, 862−869.
17. M. A. Grusak, Enhancing Mineral Content in Plant Food Products, *Journal of the American College of Nutrition*, 21, **2002**, 178−183.
18. Minerals-Learn the role of minerals in maintaining a healthy body; http://www.expert-nutrition.com/minerals.html, accessed on May 22, 2010.
19. M. Sanger, J. Hoesch, Macro- and Micro- Element Levels in Cereals Grown In Lower Austria. *Central Europian Agriculture*, 6, **2005**, 461−467.
20. World Health Organization (WHO). *Trace Elements in Human Nutrition and Health*, WHO, Geneva, **1996.**
21. Commonwealth Department of Health and Ageing, Australia Ministry of Health, New Zealand National Health and Medical Research Council; *Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes*; Commonwealth of Australia and New Zealand Government, **2005**, 243−248.
22. G. M. Wardlaw, P.M. Insel, *Perspective in Nutrition*, 3rd ed., Mosby- Year Book: Boston, **1996**, 524−562.
23. National Research Council (US), Food and Nutrition Board: *Recommended Dietary Allowances*, 10th ed. Washington, DC: National Academy Press, **1989**.

24. Food and Agriculture Organization of the United Nations and World Health Organization, *FAO/WHO expert consultation on human vitamin and mineral requirements*, Bangkok, Thailand, **2001**.
25. I. Bertini, H. B. Gray, S. J. Lippard, J. S. Valentine, *Bioinorganic Chemistry*, Viva Books, New Delhi, **1998**.
26. C.J. Rebouche, I. Carnitine, M.E. Shils, J.A. Olson, M. Shike, A. C. Ross, *Modern Nutrition in Health and Disease.* 9th ed. Williams and Wilkins, Philadelphia: Lippincott, **1999**, 505–512.
27. Agency of Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Manganese* CAS# 127-18-4, Atlanta, GA: U.S.; **2001.**
28. M. A. Taher, Flame atomic absorption spectrometric determination of trace lead after solid-liquid extraction and preconcentration using 1-(2-pyridylazo)-2-naphthol, *Croat. Chem. Acta*, 76, **2003**, 273–277.
29. G. Nordberg, the ChinaCad Group, Cadmium and human health: A perspective based on recent studies in China, *The Journal of Trace Elements in Experimental Medicine*, 16**, 2003,** 307–319**.**
30. J. Godt, F. Scheidig, C. Grosse-Siestrup, V. Esche, P. Brandenburg, A. Reich, and D. Groneberg, The toxicity of cadmium and resulting hazards for human health; *J. Occup Med Toxicol*., 1, **2006,** 1–22.
31. Chromim compounds/*Technology Transfer Network Air Toxics Web.* http://www.epa.gov/ttn/atw/hlthef/chromium.htm, accessed on May 24, 2010.
32. K. K. Das, S. N. Das and S. A. Dhundasi, Nickel, its adverse health effects and oxidative stress, *Indian J. Med. Res.*, 128, **2008**, 412–425.
33. A. Duda-Chodak, U. Baszczyk, The impact of nickel on human health, *J. Elementol*. 13, **2008**, 685–696.
34. F.H. Nielsen, W. Mertz, (Ed.) *Trace elements in Human and Animal Nutrition*. San Diego: Academic Press, **1987**, 245–273.
35. S. Mitra, *Sample Preparation Techniques in Analytical Chemistry*, John Wiley and sons, Inc., Hoboken, **2003**, 227–244.
36. P. Quevauviller, E.A. Maier, B. Griepink, (Eds.) *Quality Assurance for Environmental Analysis*, Elsevier: Amsterdam, 17, **1995,** 64–65.

37. G. Somer, A. N. Unlu, The Effect of Acid Digestion on the Recoveries of Trace Elements: Recommended Policies for the Elimination of Losses, *Turk J. Chem*. 30, **2006**, 745–753.
38. D.C. Harris, *Quantitative Chemical Analysis*, 4th ed., W.H. Freeman and Company, New York, **1982**, 84.
39. D. R. Hashmi, F. A. Khan, G. H. Shaiki, and T.H. Usman, Detrmination of trace metals in the vegetable procured from local markets of Karachi by AAS, *J. Chem. Soc. Pak*. 27, **2005**, 354–356.
40. Z. Krejpcio, E. Krol, and S. Sionkowski, Evaluation of Heavy Metals Contents in Spices and Herbs Available on the Polish Market, *Polish J. of Environ. Stud*., 16, **2007**, 98–99.
41. A. E. Aiwonegbe and E. U. Ikhuoria, Levels of selected heavy metals in some Nigerian vegetables, *Trends in Applied Science Research*, 2, **2007**, 77–78.
42. V. S. Govindarajan, Ginger – Chemistry, technology and quality evaluation: Part I. *CRC Critical Reviews*, 17, **1982**, 1–96.
43. L. Huang, R. W. Bell, B. Dell, and J. Woodward, Rapid nitric acid digestion of plant material with an open-vessel microwave system, *communications in soil science and plant analysis*, 35, **2004**, 427–440.
44. S. Gaudino, C. Galas, M. Belli, S. Barbizzi, P. Zorzi, R. Jacimovic, Z. Jeran, A. Pati and U. Sansone; The role of different soil sample digestion methods on trace elements analysis: a comparison of ICP-MS and INAA measurement results, *Journal for Quality, Comparability and Reliability in Chemical Measurement*, 12, **2007**, 84–93.
45. M. Chen and L. Q. Ma, Comparison of three aqua-regia digestion methods for twenty florida soils, *Soil Science Society of America Journal*, 65, **2001**, 491–499.
46. B. Wilson, A. Braithwaite and F. B. Pyatt, An evaluation of procedures for the digestion of soils and vegetation from areas with metalliferous pollution, *Toxicological and Environmental Chemistry*, 87, **2005**, 335–344.
47. J. Hur, S. Yimb and M. A. Schlautman, Copper leaching from brake wear debris in standard extraction solutions, *Journal of Environmental Monitoring*, 5, **2003**, 837–843.

48. H. Chen, Determination of 1-hydroxypyrene in human urine by acid hydrolysis coupled to solid-phase microextraction and semi-microcolumn liquid chromatography, *Analytical Sciences*, 23, **2007**, 1221–1225.
49. Y. Ishibashi, H. Matsuo, Y. Baba, Y. Nagafuchi, T. Imato, T. Hirata, Association of manganese effluent with the application of fertilizer and manure on tea field, *Water Research*, 38, **2004**, 2821–2826.
50. M. S. Zubillaga, E. Bressan and R. S. Lavad, Heavy metal mobility in polluted soils: effect of different treatments, *American Journal of Environmental Sciences*, 4, **2008**, 620–624.
51. K. N. Devi, H. N. Sarma and S. Kumar, Estimation of essential and trace elements in some medicinal plants by PIXE and PIGE techniques, *Nuclear Instruments and Methods in Physics Research B*, 266, **2008**, 1605–1610.
52. I. A. Ogunwandea and N. O. Olawore, Heavy trace metals and macronutrients status in herbal plants of Nigeria, *Food Chemistry*, 85, **2004**, 67–71.
53. E. I. Obiajunwa, A. C. Adebajo and O. R. Omobuwajo, Essential and trace element contents of some Nigerian medicinal plants, *Journal of Radioanalytical and Nuclear Chemistry*, 252, **2002**, 473–476.
54. S. S. Alwakeel, Microbial and heavy metals accumulation of herbal medicines, *Research Journal of Microbiology*, 3, **2008**, 683–691.
55. S. Jabeen1, M. T. Shah, S. Khan and M. Q. Hayat, Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan, *Journal of Medicinal Plants Research*, 4, **2010**, 559–566.
56. D. R. Hashmi, F.A. Khan, G.H. Shaikh and T.H. Usmani, Determination of trace metals in the vegetables procured from local markets of Karachi city by Atomic absorption spectrophotometery, *Journal of Chemical Society of Pakistan*, 27, **2005**, 353–357.
57. D. R. Hashmi, S. Ismail and G. H. Shaikh, Assessment of the level of trace metals in commonly edible vegetables locally available in the markets of Karachi city, *Pakistan Journal of Botany*, 39, **2007**, 747–751.
58. M. A. Al-Eed, F. N Assubaie, M. M. El-Garawany, H. EL-Hamshary and Z. M. Eltayeb, Determination of heavy metal levels in common spices, *Journal of Applied Science*, 17, **2002**, 87–98.
59. A. E. Aiwonegbe, E. U. Ikhuoria, Levels of selected heavy metals in some Nigerian vegetables, *Trends in Applied Sciences Reasearch*, 2, **2007**, 76–79.

60. B. Jankiewicz, D. Adamczyk, Assessing heavy metal content in soils surrounding the lodz ec4 power plant, Poland, *Polish Journal of Environmental Studies*, 16, **2007**, 933–938.
61. Micheal Yemane, Levels of essential and non-essential metals in leaves of the tea plant (*Camellia sinensis* L.) and soils of Wushwush farms, Ethiopia, Graduate Project, Addis Ababa University, **2006**.
62. B. Buszewski, A. Jastrzębska, T. Kowalkowski, A. Gorna-Binkul, Monitoring of selected heavy metals uptake by plants and soils in the area of Torun, Poland, *Polish Journal of Environmental Studies*, 9, **2000**, 511–515.
63. R. G. Bereton, *Chemometrics: Data analysis for the laboratory and chemical plant*, John Wiley and Sons, London, **2003**, 197–206.

DECLARATION

I, the undersigned, confirm that the results reported in this project work were my original work under the supervision of my advisor in Faculty of Chemical and Physical Sciences, Department of Chemistry, Addis Ababa University in the academic year 2009/2010 and all sources of materials used for the project work have been duly acknowledged.

Name : Yohannes Wagesho

Signature: _____

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

Advisor Name: _____

Signature :_____

Place and date of submission: School of Graduate Studies

Addis Ababa University

July 2010