ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DETERMINATION OF SOME MAJOR AND TRACE METALS LEVELS IN KORARIMA (*AFRAMOMUM CORRORIMA*) CULTIVATED IN SOUTHERN AND SOUTHWESTERN ETHIOPIA

BY

BIRHANU MEKASSA

JULY 2010

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BIRHANU MEKASSA

ADVISOR: PROF. B. S. CHANDRAVANSHI

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Dedication

To my parents . . .

Halafi Mekassa and Gadissie Terefe

I dedicate this project work to my Dad and Mom who planted the seed of Wisdom within me, from which my thirst for knowledge grew. It was this seed that enabled me to truly appreciate and understand my self.

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LIST OF ACRONYMS AND ABBREVIATIONS

FAAS	Flame Atomic Absorption Spectrometry
FAO	Food and Agricultural Organization
WHO	World Health Organization
EPA	Environmental Protection Agency
RDA	Recommended Dietary Allowances
ANOVA	Analysis of Variance
ND	Not Detected
RSD	Relative Standard Deviation
SNNPRS	South Nations Nationalities and Peoples Regional State

DETERMINATION OF SOME MAJOR AND TRACE METALS LEVELS IN KORARIMA (*AFRAMOMUM CORRORIMA*) CULTIVATED IN THE SOUTHERN AND SOUTHWESTERN ETHIOPIA

By: Birhanu Mekassa

Advisor: Prof. B. S. Chandravanshi

ABSTRACT

In this study, the concentration levels of major (Ca and Mg), trace (Fe, Zn, Cu, Co, Cr, Mn, and Ni), and toxic (Cd and Pb) metals were determined in korarima (*Aframomum corrorima*) samples collected from southern and southwestern Ethiopia. The results were compared with the metal levels in the soil collected from the same area as korarima sample. A wet digestion procedure involving the use of 3 mL of HNO_3 (69-72%) and 1 mL of $HClO_4$ (70%) and modified aqua regia (HCl:HNO_3, 3:1) with hydrogen peroxide (6 mL of aqua regia and 1.5 mL of H_2O_2) were used to solubilize metals from the korarima and soil samples, respectively. Flame atomic absorption spectrometry was used to quantify the metals levels. The validity of the procedure was checked by spiking experiments and the recovery value ranges from 88 to 109% for korarima and from 89 to 104% for soil samples. Calcium and iron were found to be the highest metals determined in korarima and soil samples, respectively. Manganese is the most accumulated trace metal in korarima seed samples analyzed and the second most accumulated trace metal in the soil next to iron. The concentration of cadmium was within the range 0.9-1 μg/g dry wt in korarima samples. The level of lead in the korarima samples was below the method detection limit.

Key words: Korarima (*Aframomum corrorima*), Soil, Wet digestion, FAAS, Ethiopia.

1. INTRODUCTION

1.1. Background

Spices and herbs are used throughout the world to season food products and create the unique characteristic flavors of different cuisines. They have played a dramatic role in civilization and in the history of nations. The delightful flavor and pungency of spices make them indispensable in the preparation of palatable dishes. In addition, they are reputed to possess several medicinal and pharmacological properties and hence find position in the preparation of a number of medicines [1, 2].

1.1.1. Global spice trade

The most important spices traditionally traded throughout the world are products of tropical environments. The characteristics and environmental needs of the crops dominate the global spice trade [3]. The major markets in the global spice trade are the USA, the European Union, Japan, Singapore, Saudi Arabia and Malaysia. The principal supplying countries are China, India, Madagascar, Indonesia, Vietnam, Brazil, Spain, Guatemala and Sri Lanka. During the period from 2000 to 2004, the value of spice imports increased by an average of 1.9% per year and the volume increased by 5.9%. World trade in spices in 2004 consisted of 1.547 million tons, valued at US $ 2.97 billion. An annual average rate of 7% increase was seen in the global import volume of spices in the period 2000–2002 [4-6].

1.1.2. Major compounds in spices

Spices are widely used as flavoring ingredients in foods. They impart aroma, color and taste to food preparations and sometimes mask undesirable odors. Their aroma results from complex mixtures of volatile compounds, e.g. monoterpenes, sesquiterpenes, and their oxygenated derivatives which usually occur at low concentrations [7]. 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.

Spices produce a vast and diverse assortment of organic compounds, the great majority of which do not appear to participate directly in growth and development. These substances, traditionally referred to as secondary metabolites, assume great significance. Although noted for the complexity of chemical structures and biosynthetic pathways, the volatile and non-volatile natural products are perceived generally as biologically insignificant.

Secondary metabolites in spices have been a fertile area for chemical investigation for many years, driving the development of both analytical chemistry and of new synthetic reactions and methodologies. In recent years, there has been an emphasis on secondary metabolites in relation to dietary components, which may have a considerable impact on human health. The majority of herbs and spices constitute important bioactive secondary metabolites, which possess versatile pharmacological and medicinal properties. The structure–activity relationship of these compounds is an exciting field, where molecular biology and nanotechnology can definitely play a symbiotic role [2].

1.1.3. Research achievements on Ethiopian spices

Ethiopia is a land of diverse climate and soil types that enable prolific growth of several indigenous and exotic spices, herbs, medicinal and other essential oil bearing plants. Despite availability of the diverse agro-ecologies of the country to produce these huge plant species and as they are playing a significant economic role of the commodity on the national economy through generating considerable export earnings or import substitution, the research conducted on them is very limited due to various reasons.

Research on spices, herbs and medicinal plants has been running since the inception of coffee research as coffee diversification. However, for some reasons, research on spices

have been limited to the low land spices such as black pepper, cardamom, turmeric, ginger, cinnamon, and korarima.

Currently, the national spice herbs research project set a research priority for spice crops of korarima (*Aframomum corrorima*), black pepper (*piper nigrum L.*), ginger (*Zingibere officinale* Rosc.), cardamom (*Ellettaria cardamomum*), tumeric (*Curcuma domestica*), cinnamon (*Cinnamomum verum*), vanilla (*Vannila fragrance*), black cumin (*Nigella sativa*), coriander (*Coriandrum sativum*), fenugreek (*Trigonellafoenum-graecum* L.), etc [8].

1.2. Korarima (*Aframomum corrorima*)

1.2.1. Origin, distribution and uses of korarima (*Aframomum corrorima*)

Figure 1. Dried korarima fruits.

Figure 2. Matured red korarima fruit.

Several important crop plants have their origin in Ethiopia, among them coffee and several grain species. However, there are also several plants that may have a potential as food, spice or medicine, but are yet to be known outside the local usage. Korarima is an important spice and medicinal plant in large areas of Ethiopia, but little known outside the country. It is an indigenous spice of Ethiopia. The spice, known as korarima,

Ethiopian cardamom, or false cardamom, is obtained from the plant's seeds (usually dried) (Figure 1 and 2), and is extensively used in Ethiopia and Eritrean cuisine. The plant is native to western Ethiopia, southwestern Sudan, western Uganda, and Tanzania. It is widely distributed in southern and western Ethiopia (Provinces of Kefa, Gamo Gofa, Debub Omo, Sidamo, Illubabor and Wollega). Outside of these areas, it is cultivated in the vicinity of Lake Tana and Gelemso and in Eritrea [9-11].

Spices and herbs are the rich storehouses of different bioactive compounds and are well known for their beneficial effects on health [12]. The use of korarima is only known from Ethiopia and Eritrea. It occurs as a cultivated crop only in Ethiopia. The seeds (usually dried, sometimes fresh) are used to flavour all kinds of sauces locally called 'wot', for which they are ground and usually mixed with other spices. Korarima is sold in all the markets in Ethiopia, and is daily used by most families in rural areas. Korarima is used for adding flavour to local food, bread and butter. It is an ingredient in berbere, mitmita, awaze, and other spice mixtures, and is also used to flavor coffee. Additionally, the korarima seeds are widely used medicinally as a tonic, laxative, carminative and purgative drug, and is added to food for preserving purposes. The consumption of korarima as a spice may be used as source of antioxidants. The arilloid flesh around the seed is edible. Strings of fruits are sometimes used as an ornament, or as rosaries (by the Arabs), and in the past the fruits have been used as money in Ethiopia. In addition, korarima is an important plant for soil conservation as the rhizomes and leaves spread on the ground covering and protecting the soil from erosion in hilly areas. It is primarily the red fruits that are being used, but also other parts of the plant. The taste of korarima is similar to Indian cardamom, and has been used as a substitute for this [13-15].

1.2.2. Species of korarima

Korarima (*Aframomum corrorima*) or so-called Ethiopian cardamom is herbaceous, perennial and aromatic spice and medicinal crop of the species in the monocotyledonous ginger family, Zingiberaceae native to Ethiopia. It is a shade plant that grows wild in moist and open woodlands, in the same climate areas as wild coffee, but may also be

planted and cultivated. The plant consists of an underground rhizome, a pseudostem, and several broad leaves and resembles *Elettaria* species morphologically. The genus *Aframomum* comprises about 50 species and is widely distributed in the wetter parts of tropical Africa. It is closely related to *Amomum* from tropical Asia and was formerly included in it. *Aframomum zambesiacum* occurs in similar habitats as *Aframomum corrorima*. The seeds of the former species, however, are not used, and in Ethiopia it is called 'monkey's korarima'. Two major differences with the real korarima are that its leaves are less aromatic upon crushing, and its inflorescences bear 25–50 flowers (korarima only up to 5) [16, 17].

1.2.3. Ecological requirement

Korarima grows naturally at an altitude of 1700–2000 m altitude in slightly shaded, more or less open sites in higher altitude rain forest. Annual rainfall varies from 1300 mm to more than 2000 mm. There is no distinct dry season but usually most rain falls in June–August (50–60%). The annual average temperature is about 20°C. In Ethiopia, korarima grows in almost the same habitats as wild coffee species (*Coffea*). As discussed above korarima is a shade loving plant like cardamom (*Ellattaria cardamomum*) under natural forest condition. Shade level management is one of the key agronomic practices in korarima production. It was reported that shade level was 55-63%, which is suitable for korarima production. Shade is very important both for korarima and cardamom production since it creates suitable microclimate and regulates moisture and temperature, which facilitates optimum growth and root development particularly when korarima rhizomes produce very shallow roots at each nodes.

Korarima can be propagated by seed but planting rhizome parts is probably easier and quicker. Fruits mature about 2–3 months after flowering. Most probably, the flowers are open for only one day. Perhaps people influence the wild population of korarima by some kind of protection and aid wider dispersal by planting [9].

1.2.4. Harvest and processing of korarima

Korarima (*Aframomum corrorima*) as it is indigenous spice crops of Ethiopia, hardly done any work in the processing and market preparation methods and techniques. Korarima flowers and red ripe fruits can be found at the same time in the field due to the irregularity nature of flowering like that of cardamom. Maturity and harvesting time of korarima varies in different areas of Ethiopia but generally the plant flowers in May-August and harvesting is done in August-September. At the early stage, the color of capsules is green but when it matures and ready for harvest it turns to deep red color.

To get quality product of korarima the capsules should be red ripe and the seeds when removed from the capsule should be dark brown that have pungent and appreciable taste when crushed by teeth. There should be great care while harvesting the capsules of korarima not to create any opening on the capsules since through this opening important quality components (aroma and flavor) will be lost and it will serve as entrance for microorganisms.

Korarima capsules harvested from natural forests in the south and southwestern parts of the country are processed or dried in traditional ways. According to a survey conducted around Bonga the series of activities carried out by farmers in the preparation of korarima for market passes the following steps: (i) pre-drying: harvested capsules are stored in a warm place covered with straws, enset leaf or other materials for 10 to 15 days and (ii) drying: performed in two ways (a) sun drying (b) drying with smoke [8].

1.2.5. Chemical composition of korarima oil

Korarima oil has a similar chemical composition to that of the Indian cardamom (*Elettaria cardamomum*), except for its reduced content of α-terpinyl acetate, which is the major component in the latter. The major components of dried seed and pod oils of korarima were found to be 1,8-cineole (44.3%) and (E)-nerolidol (17.2%), respectively, while 32.6% of 1,8-cineole was recorded from dried seed. The major constituents in oil

from fresh pods were found to be α-terpinene (27.1%), β-pinene (15.4%), CC-phellandrene (8.5%), 1,8-cineole (6.7%) and *p*-cymene (6.4%), whereas the seed essential oil contained 1,8-cineole (39.3%) as the most abundant constituent followed by sabinene (10.4%) and geraniol (6.8%). High levels of 1,8-cineole found in seeds are in agreement with the dried seeds oil composition range (32-44%) of previous reports. It was observed that no significant differences of major constituents analyzed from fresh and dried plant samples of korarima and only minor differences of less abundant compounds. Generally, the seeds contained higher levels of monoterpenes, e.g. 1,8-cineole, sabinene, β-pinene and geraniol, accounting for 94% of the total identified compounds, in contrast to the pods with 84% of monoterpenes. (E)-nerolidol was obtained from oils of both pods (3.8%) and seeds (4.5%) [17].

1.2.6. Market conditions of korarima

Previously, Ethiopia was well known for its considerable exports of korarima capsules to the world market, mainly as a substitute for the Indian cardamom. However, the supply has greatly fluctuated during the past few decades, and the total annual korarima export has decreased to less than 60 metric tons in the years 1994-1998, fetching only some 2.1 million USD. This condition is mainly due to reduction of production as a result of destruction of the natural habitat of the plant. Compared to cardamom, korarima has a relatively wider adaptation and higher yield. Besides the large domestic consumption of korarima, Ethiopia exported it to Sweden, Finland, Sudan, India, Egypt and Saudi Arabia. Ethiopian cardamom had notably penetrated the Scandinavian market and was priced at 9 USD per kilogram in early 1978 as a substitute for Indian cardamom [17].

1.2.7. Cardamom (*Ellettaria cardamomum*)

Small cardamom, known as the ‘queen of spices’, which belongs to the family of Zingiberaceae, is a rich spice obtained from the seeds of a perennial plant, *Elettaria cardamomum* Maton. It is one of the highly prized spices of the world and is the third most expensive spice after saffron and vanilla. Cardamom is one of those spices that

cross the sweet/savoury boundary between desserts and main dishes. The original home of this precious spice is the mountain of the southwestern parts of the Indian Peninsula. India had a virtual monopoly of cardamom until recently [6, 18].

As cardamom is exotic spice its introduction and adaptation, in Ethiopia, were not more than two and half decades. After introduction, multiplication of the planting materials was made at Tepi, Bebeka and Jimma. Adaptability and evaluation studies continued at Tepi, Bebeka, Jimma, Metu, Mugi, and Wonago. The utilization of this spice in Ethiopia is very high. Irrespective of the quality, the imported cardamom is found in every market in the country indicating its wide utilization. Cardamom is used as an aromatic, carminative and stimulant. The seeds have a warm, slightly pungent aromatic flavor. It is used mainly as a flavoring agent in tea and food preparations. Cardamom oil is a precious ingredient in food preparations, perfumery, health foods, medicine and beverages. Cardamom is also used internally for indigestion, nausea, vomiting and pulmonary disease with copious phlegm and also as a laxative to prevent stomach pain and griping, as well as flatulence. Cardamom plays an important role in a variety of special foods, vegetables, and meat dishes, for flavoring tea, butter, coffee, bread, and cakes in ground or whole forms as a sole or mixed with other spices [2, 8].

The chemical composition of cardamom differs considerably with variety, region and age of the product. Cardamom is rich in calcium, potassium, phosphorus and magnesium. Good amount of iron, sodium and manganese is present in it. Small amount of zinc and copper is found in it. The content of volatile oil in the seeds is strongly dependent on storage conditions, with an average yield from 2 to 5%. The oil is described as sweet, spicy, warm, lightly camphorated and citrusy. The volatile oil contains about 1.5% α-pinene, 0.2% β-pinene, 2.8% sabinene, 1.6% myrcene, 0.2% α-phellandrene, 11.6% limonene, 36.3% 1,8-cineole, 0.7% γ-terpinene, 0.5% terpinolene, 3% linalool, 2.5% linalyl acetate, 0.9% terpinen 4-ol, 2.6% α-terpineol, 31.3% α-terpinyl acetate, 0.3% citronellol, 0.5% nerd, 0.5% geraniol, 0.2% methyl eugenol and 2.7% *trans*-nerolidol. The basic cardamom aroma is produced by a combination of the major components, 1,8-cineole and α-terpinyl acetate [6, 8].

1.3. Agricultural practices and metals accumulation

1.3.1. Metals accumulation in plants

The widespread contamination with heavy metals in the last decades has raised public and scientific interest due to their dangerous effects on human health. This has led researchers all over the world to study the pollution with heavy metals in air, water, and foods to avoid their harmful effects and to determine their permissibility for human consumption. Food is the major intake source of toxic metals by human beings. The main sources of trace elements in foods include soil, agricultural practices, manufacturing processes and environmental sources such as traffic pollution and industrial activities. [19-21].

The use of fertilizers, herbicides, pesticides, selected hybrid seeds, widespread irrigation and modern farm equipments has in recent years increased the yield of crop production to support the increasing population and rural-urban migration [22]. Such progress nevertheless is often associated with various forms of environmental hazards. Regular soil spraying with pesticides increase abundance of harmful chemicals that is subsequently stored in ground minerals and water. Fertilizers frequently contain trace amounts of arsenic and heavy metals; so their repeated use may cause toxicity of soils [23].

Crops can accumulate these trace heavy metals in or on their tissue, thus they are intermediate reservoirs, through which trace elements from soils, and partly from water and air, transfer to man and animals [24]. Growth media including soil, nutrient solution, water and air are main sources of heavy metals to crops, which enter by roots or foilages through adsorption or absorption [22, 25].

Different crop species accumulate different metals depending on environmental conditions, metal species, plant species and available forms of heavy metals [26]. Many

plants are found to be in a position to take up large quantities of certain elements from the environment and said hyper-accumulators of heavy metals [27].

1.3.2. The role of metals in human beings

Although heavy metals are present in food in very minute quantities, the very human existence is due to their role in body metabolism. It has been established that whatever is taken as food might cause metabolic disturbance if it does not contain the permissible upper and lower limits of heavy metals. Trace elements play an important role in chemical, biological, biochemical, metabolic, catabolic and enzymatic reactions in the living cells of plants, animals and human beings. Thus, both deficiency and excess of essential micro-nutrients may produce undesirable effects.

Effects of toxic metals (cadmium, lead, etc) on human health and their interactions with essential heavy metals (trace metals) may produce serious consequences. From this viewpoint, metals such as iron, arsenic, lead, mercury, cadmium and nickel are considered suitable for studying the impact of various foods on human health. Cobalt is essential component of vitamin B_{12}; zinc is found in several enzymes and genetic material transcription; copper is key component of redox enzymes and chromium has a role in glucose metabolism and its deficiency is characterized by disturbance in glucose, lipid and protein metabolism, iron in oxygen transport and so enables metabolism. Lately, it has been reported that in patients with acute myocardial infarction, the mean concentration of serum nickel were significantly increased through the period of 1-36 h after the onset of symptoms. Cadmium primarily targets the renal and gastrointestinal systems. Renal failure and gastrointestinal irritation are the usual symptoms following a high oral dose intake of cadmium. Acute high dose lead poisoning has been known to cause haemolytic anemia and renal tubular dysfunction as well as subtle behavioural, constitutional and neurocognitive impairments [28-30].

Though required in very small amount, deficiency of trace elements cause diseases, whereas their presence in excess may result in toxicity to human life disturbing normal

functioning of organs and central nervous system. Anemia from iron deficiency affects more than half of pregnant women and at least one-third of children under five years [31]. In Ethiopia, the Food and Agricultural Organization estimate indicated prevalence of iron deficiency is 85% among children and 58% among pregnant women while in Kenya similar survey estimated 60% and 70%, respectively [32]. On the other hand, trace metals like lead, cadmium and mercury are known of their detrimental health effect. Cadmium, for example, has been considered as an extremely significant pollutant even in small amount, affecting all forms of life because of its high toxicity and great solubility in soil and water [33]. No level of lead in blood as well should be considered safe for children due to its neurotoxicity [34, 35].

As food is the major intake source of toxic trace metals by human beings, contamination of food has become a burning issue in recent years particularly in most metropolitan cities [36]. This is attributed mainly to the problem of environmental pollution due to rapid urbanization and industrialization with improper environmental planning leading to discharge of industrial, agricultural and swage effluents into water bodies, lands and air. Added to the heavy release of trace metals, their geo-accumulation, bioaccumulation, bio-magnification in bio-system and non-biodegradability enhances exponentially their concentration across food chain and the effect on human being [26, 27]. Once they enter the body, they may alter their oxidation state, may form complexes with other biological molecules but their essential integrity remains constant [37].

Spices and herbs grown in various regions of the world have been used for several purposes since ancient times. They are the sources of many bioactive compounds that can improve the taste of food as well as influence digestion and metabolism processes. Spices have been recognized to have some medicinal properties due to their antioxidant and anti-microbial action. However, they can also contain some undesirable components that can be harmful, e.g. micotoxins, pesticides, polycyclic carbohydrates and heavy metals residues [38].

1.3.3. Evaluation of metals contents

Determination of inorganic elements in agricultural products is attracting considerable attention for a new application, which is to identify the geographic origin of the agricultural products to provide necessary information for consumers, agricultural farmers, retailers, and administrative authority. The technique was based on the observation that difference in the composition of inorganic elements is based on the difference in cultivation conditions: features of the soil, such as its composition, pH, soil type, moisture and organic constituents, temperature and humidity [39]. The study of inorganic elements in biological samples is now well expanding into a field called bioinorganic chemistry, concerned with the study of elemental uptake, physiological action, storage, excretion and introduction as probes or drugs when necessary [40, 41].

An investigation of plants for their metal concentration is indispensable because a survey of literature indicates that such study is scarce in Ethiopia. The determination of metal content of spices and other vegetables across different parts of the globe can be conducted from the viewpoints of: health risk assessment, nutrient content analysis for consumers, to trace geographic origin of food products, nutritional status assessment of growing plants and assay of suitability of soil and water for farming. Another reason for analysis of metals in plants is for diagnosis of deficiency of essential metal nutrients so that appropriate minerals can be supplied for the proper growth of crops [42]. At high concentrations of heavy metals, metabolic process of the plants can be interfered, resulting in poor growth and sometimes-even death [43].

The assessment of metal contents (essential and non-essential) is necessary from the point of view of nutrition, toxicological, crop yield as well as many other applications. As plants are so sensitive to their environment, the levels of metals vary with the type of metals and plant species, growth media (soil, water, and air), season, means of cultivation, pollution incidence, dietary traditions of consumers and other post harvest treatments. This needs continuous determination and/or monitoring of the levels of metals in the plants [44].

Even though many research works have been carried out on the medicinal value and chemical composition of essential oils [10, 13-17] there is no report on the assessment of metal contents of korarima (*Aframomum corrorima)*. However, several works have been conducted on the analysis of metal contents of cardamom (*Elettaria cardamomum*), large cardamom (*Amomum subulatum*) and related spice family like ginger (*Zingiber officinale*). The work includes essential trace metal (Zn, Mn, Cu and Fe) levels in plants of medicinal importance [12], determination of heavy metals in common spices [19], evaluation of heavy metals contents in spices and herbs available on the polish market [38], levels of selected heavy metals in some Nigerian vegetables [28], monitoring of cadmium and micronutrients in spices commonly consumed in Turkey [45], assessment of trace metals in commonly edible vegetables locally available in Pakistan [46], microbial and heavy metals contamination of herbal medicines [47]. All these studies have shown the accumulation of metals in various amounts in different parts of the world depending on the type of spice and metals.

Metals (major, trace and toxic) assessment, particularly in korarima (*Aframomum corrorima)* is currently not available although exposure of the plants to the metals is inevitable. Therefore, this study aims to fill the gap at least partially in the area and initiate others on closely related spices widely used throughout the country. In this study, selected major and trace metals' levels in korarima samples collected from selected highlands of southern and southwestern Ethiopia (Kaffa, Gamo Gofa, and Illubabor**)** were determined using flame atomic absorption spectrometry.

1.4. Purpose, scope and limitations of the study

The assessment of major metals (Ca and Mg), trace metals (Cu, Cr, Mn, Zn, Co, Ni) and toxic metals (Cd, Pb) in korarima (*Aframomum corrorima)* samples collected from southern and southwestern Ethiopia was conducted to offer experimental data which can be used to identify the nutritional use as well as the toxicological status of the spice. It is also helpful to estimate the sources of metals and assess the pollution level. However, to obtain detailed information and come up with general conclusion on the metal levels of

korarima cultivated in Ethiopia it may need collection of samples from the whole country, which was not possible in this study. The use of other techniques than FAAS could also be used to verify the accuracy and precision of the results.

1.5. OBJECTIVES

A. General Objective

The main objective of this project was to determine the level of major and trace metals in korarima (*Aframomum corrorima*) cultivated in southern and southwestern Ethiopia.

B. Specific Objectives

i. To develop an optimum working procedure for digestion of korarima (*Aframomum corrorima)* and soil samples to determine major and trace metal contents by FAAS.

ii. To determine levels of selected metals (Ca, Mg, Fe, Zn, Cu, Co, Cr, Mn, Ni, Pb, Cd) in korarima (*Aframomum corrorima)* cultivated in Ethiopia.

iii. To compare the levels of major and trace metals in korarima (*Aframomum corrorima)* cultivated in different parts of southern and southwestern Ethiopia.

iv. To compare the levels of the metals in korarima (*Aframomum corrorima)* with Indian cardamom (*Elettaria cardamomum*).

v. To correlate the levels of metals in korarima (*Aframomum corrorima)* with that of soil.

vi. To compare the levels of metals found with data in literature.

2. EXPERIMENTAL

2.1. Apparatuses, instruments, chemicals and reagents

2.1.1. Apparatuses and instrument

Polyethylene plastic bags were used for collecting the korarima sample. A hot plate was used for drying the sample after removing the outer cover. Blender (Moulinex, France), porcelain mortar, pestles and crucibles (Haldenwanger, Germany) were used during pounding of the korarima sample. Analytical balance (Larko, LA114, 110 g/0.1 g) with precision of ± 0.0001 was used to weigh the korarima sample. Round bottom flasks with grounded glass (100 mL) fitted with reflux condenser were employed in digesting the sample on Kjeldahl heating apparatus (Gallenhamp, England). Borosilicate volumetric flasks (25, 50 and 100 mL) sizes 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 made use during measuring different quantities of volumes of sample solution, acid reagents and metal standard solutions. Metals' concentration determination was done by flame atomic absorption spectrophotometer (Buck Scientific, Model 210VGP AAS, East Norwalk, USA) equipped with deuterium background corrector and hollow cathode lamps with air-acetylene flame.

2.1.2. Chemicals and reagents

All reagents and chemicals used in the study were analytical grade. Nitric acid, 69-72% HNO_3 and perchloric acid, 70% $HClO_4$ both from Research-lab Fine Chem Industries Mumbai, India, were used for digestion of powdered korarima samples. Extra pure hydrogen peroxide, 30% H_2O_2, (Scharlau, European Union), and hydrochloric acid, 37% HCl were used during optimization procedure of the soil sample. Lanthanum chloride hydrate, 99.9% (Aldrich, USA) was used to prevent the chemical interference on Ca and Mg during the analysis of korarima sample. Stock standard solution of concentration 1000 mg/L in 2% HNO_3 of the metals Ca, Mg, Fe, Mn, Cu, Co, Zn, Cr, Ni, Pb and Cd (Buck Scientific Puro-Graphic) standard solutions were used to prepare intermediate

standard solutions. Distilled deionized water was used for dilution of sample and intermediate metal standard solutions and rinsing glassware and sample bottles.

2.2. Procedures

2.2.1. Apparatuses cleaning

All glassware, plastic containers and polyethylene bags were filled with aqueous detergent solution; inner and outer walls wiped briskly with foam and brushes; copious amount of water was used to remove the detergent followed by distilled deionized water rinsing. The apparatuses were then soaked with about 10% (v/v) nitric acid for 24 followed by rinsing with distilled deionized water. The glassware were then dried in hot air oven and stored in clean dry places free of contamination till use. Prior to each use the apparatus were soaked in diluted nitric acid and rinsed in distilled deionized water.

2.2.2. Description of the study area

The samples were collected from three study areas, Bonga located in Kaffa zone of South Nations Nationalities and Peoples Regional state, Kemba in Gamo Gofa zone of South Nations Nationalities and Peoples Regional state, and Metu, Illubabor zone, Oromia. The choices of the study sites were made on the bases of major korarima producing regions. Bonga is situated at a distance of 449 km, southwest of Addis Ababa. Topographically it lies at an altitude of 1650 meters above sea level and has a 'woyena dega' type of climate. The annual temperature of the zone ranges from 10.1 to 27.5 ^{0}C and the average annual rainfall is 1750 mm. Kemba is one of the woredas in Gamo Gofa zone of SNNPR state which is located at 680 km south from Addis Ababa. It is situated at 6^{0}4'N 37^{0}0'E and has an altitude that varies from 900-1400 m above sea level. The mean annual rainfall ranges from 400-200 mm and while the temperature ranges from 15-30 ^{0}C. Metu is located at 550 km from Addis Ababa in the Illubabor zone of the Oromia region. It has latitude and longitude of 8^{0}18'N 35^{0}35'E and an altitude of 1605 meters.

2.2.3. Sampling and sample pretreatment

2.2.3.1. Korarima capsule sampling and pretreatment

Recently harvested matured red korarima capsule samples were collected from three sites. The three sites are Bonga (Kafa, SNNPR state), Kamba (Gamo Gofa, SNNPR state), and Metu (Illubabor, Oromiya). About 1.5 kg of red korarima capsules were bought from farmers from each site. Efforts were made to record necessary information about the sample for the later consideration. From a particular main site, three sub-sites were taken for the purpose of random sampling. About 500 g of the sample was taken from each sub-sites and then mixed in to a single polyethylene plastic bags to get 1.5 kg of one bulk sample. Then the collected samples were packaged into polyethylene plastic bags, labeled and transported to laboratory for further treatment.

From each of the korarima capsule samples the outer cover were carefully removed and clean brown colored seeds were collected. The seed samples were then placed in acid washed clean porcelain crucibles labeled according to the sample and dried over hot plate and sun-dried until they become brittle. The dried korarima seed samples were pound into fine particles, homogenized with blending device (Moulinex, France), and stored in polyethylene bags for digestion.

2.2.3.2. Soil sampling and pretreatment

The soil samples (1.5 kg) were collected from the same site as that of the korarima capsules by digging the soil from 10 to 15 cm depth. Three sub-sites were taken for the purpose of random sampling for each main site. About 500 g of the soil sample was taken from a particular sub-site and then mixed in to a single clean plastic bag to obtain one bulk sample. The samples were then put in clean polyethylene plastic bags and brought to the lab for further pretreatment. After sun drying, the soil samples were pound into fine powder with porcelain mortar and pestle and then sieved with 500-μm size sieve. The

powdered sample was then kept in pre-cleaned screw capped polyethylene container till digestion.

2.2.4. Optimization of digestion procedure

Optimum procedure of sample dissolution is required to give at most result with minimum reagents, time and temperature, simple procedure and in an environmentally friendly manner. A mixture of nitric and perchloric acids was used for korarima sample decomposition where as hydrogen peroxide was added to aqua-regia solution (3 HCl: 1 HNO_3) in the case of soil sample as it is a strong oxidizing agent. The optimum procedures followed for the digestion of korarima and soil samples for metal content determination using FAAS were provided in Table 1 and 2, respectively.

2.2.4.1. Wet digestion of korarima seed

For digestion purpose, 0.5 g of powdered and homogenized korarima seed samples were weighed and transferred into a 100 mL round bottom flask. To this, 3 mL concentrated HNO_3 (69-72%) and 1 mL of $HClO_4$ (70%) were added. The mixture was then digested on Kjeldahl digestion apparatus (Gallenkamp, England) fitting the flask to a reflux condenser by setting the temperature to dial at 4 (120 ^{0}C) for 30 min followed by dialing at 7 (210 ^{0}C) for 120 min until a clear solution was obtained following the optimized digestion procedure given in Table 1.

After a total of 2:30 h, the digested solutions were allowed to cool for 10 min without dismantling the condenser from the flask and for 10 min after removing the condenser. To the cooled solution, two 5 mL portions of distilled-deionized water were added to dissolve the precipitate formed on cooling and gently swirled to reduce dissolution of the filter paper by digest residue. The cooled digested samples were filtered into a 50 mL standard flask with a Whatman filter paper (110 mm) to remove any suspended or turbid matter. Subsequent rinsing of the filtrate with 5 mL distilled-deionized water was

followed until the volume reached the mark. At this point, the solution was clear and colorless.

To each sample 1% 'matrix modifier' lanthanum nitrate hydrate were added so that lanthanum may bind the phosphate and liberate calcium and magnesium in case large phosphate exist in the sample [48]. For each korarima samples, triplicate digestions were carried out. Blank solutions were also digested accordingly in triplicate. The digested and diluted sample solutions were then kept in refrigerator until analysis time.

Table 1. Optimization of parameters for wet digestion of korarima samples
(Reagent types and volumes, temperature and time attempted during optimization).

No	Reagent volumes (mL)			Temperature (^{0}C)	Time (min)	Result
	HNO_3	$HClO_4$	Total			
1	2	2	4	270	180	Almost clear
2	3	1	4	270	180	Clear solution
3	3	2	5	270	180	Lightly yellow
4	3	3	6	270	180	Clear light yellow
5	4	1	5	270	180	Almost clear
6	4	2	6	270	180	Yellow with suspension
7	3	1	4	150	180	Clear light yellow
8	3	1	4	180	180	Almost clear
9	3	1	4	210	180	**Clear solution**
10	3	1	4	240	180	Clear solution
11	3	1	4	300	180	Yellowish
12	3	1	4	210	90	Yellowish
13	3	1	4	210	105	Yellowish
14	3	1	4	210	120	Lightly yellow
15	3	1	4	210	135	Almost clear
16	3	1	4	210	150	Clear solution
17	3	1	4	210	165	Clear solution

Table 2. Optimization of parameters for wet digestion of soil samples
(Reagent types and volumes, temperature and time attempted during optimization).

No	Reagent volumes (mL)			Maximum temperature (^{0}C)	Time (min)	Result
	Aqua-regia (3 HCl: 1 HNO_3)	Hydrogen peroxide	Total			
1	4	1.5	5.5	270	180	Light yellow
2	6	1.5	7.5	270	180	**Clear solution**
3	8	1.5	9.5	270	180	Yellowish
4	6	1.0	7.0	270	180	Light yellow
5	6	2.0	8.0	270	180	Yellowish
6	6	2.5	8.5	270	180	Yellowish
7	6	1.5	7.5	240	180	Yellow with suspension
8	6	1.5	7.5	300	180	Light Yellow
9	6	1.5	7.5	270	120	Yellow with suspension
10	6	1.5	7.5	270	150	Very light yellow
11	6	1.5	7.5	270	210	Yellow with suspension
12	6	1.5	7.0	270	240	Very light yellow

2.2.4.2. Wet digestion of soil sample

Soils and sediments are known to be accumulation pools for metals. For the analysis of metals in the soil, sample preparation is the critical step which involves steps from simple dilution to partial or total digestion. Wet digestion with oxidizing acids is the most common sample preparation procedure. Wet decomposition or acid digestion involves the

use of oxidizing agents like potassium dichromate in concentrated sulfuric acid, hydrogen peroxide, nitric acid, nitric acid with sulfuric or perchloric acid, aqua-regia, aqua-regia modified with hydrogen peroxide and hydrogen fluoride, etc [49-51]. For our purpose, a modified aqua-regia with hydrogen peroxide, H_2O_2, were used and optimized.

Applying the optimized procedure Table 2, 0.5 g each of the well-powdered soil samples was taken into a round bottom flask. To this flask 6 mL of aqua-regia, a mixture of hydrochloric and nitric acid (3:1) was added. After addition of 1.5 mL of hydrogen peroxide, H_2O_2, the mixtures were digested on a micro Kjeldahl digestion apparatus by setting the temperature first at 120 ^{0}C for 30 min and then increased to 240 ^{0}C for the next 30 min followed by 270 ^{0}C for the remaining 120 min. After 3:00 h, the digest was allowed to cool for a total of 20 min (10 min without dismantling the condenser and 10 min after removing the condenser). Distilled-deionized water was added to dissolve the precipitate formed on cooling. The cooled digested samples were filtered into a 50 mL standard flask with a Whatman filter paper using distilled-deionized water until the volume reached the mark. Lanthanum nitrate hydrate (1%) was added to liberate calcium and magnesium.

2.2.5. Analytical procedures for metal analysis and instrument calibrations

In this study, the concentrations of essential, non-essential and toxic metal levels in korarima (*Aframomum corrorima)* samples and soil samples were quantitatively determined using FAAS. The digested and diluted sample solutions were run to FAAS (Buck Scientific Model 210VGP AAS, East Norwalk, USA) for the trace metals analysis. Of course, GFAAS, ICP-AES, ICP-MS and AFS are also most widely used for trace determination but most of them are expensive and need greater operator experience than FAAS [48]. The metal standards were used for external calibration running through FAAS equipped with deuterium arc background corrector, air-acetylene flame and instrument parameter optimized according to the manufacturer guide. The operating conditions for FAAS employed for each metal are given in Table 3.

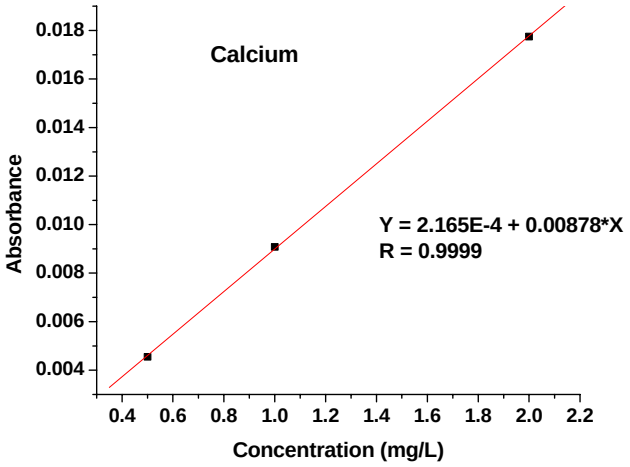
Table 3. Instrumental operating conditions for determination of metals using flame atomic absorption spectrophotometer.

Metals	Detection limit (mg/L)	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Energy (eV)
Ca	0.010	422.7	0.7	2.0	3.606
Mg	0.001	285.2	0.7	1.0	3.953
Fe	0.030	248.3	0.2	7.0	3.735
Zn	0.005	213.9	0.7	2.0	3.047
Cu	0.020	324.8	0.7	1.5	3.800
Co	0.050	240.7	0.2	4.5	2.746
Cr	0.050	357.9	0.7	2.0	3.586
Mn	0.010	279.5	0.7	3.0	3.882
Ni	0.040	232.0	0.7	7.0	2.928
Cd	0.005	228.9	0.7	2.0	3.094
Pb	0.100	283.2	0.7	2.0	3.131

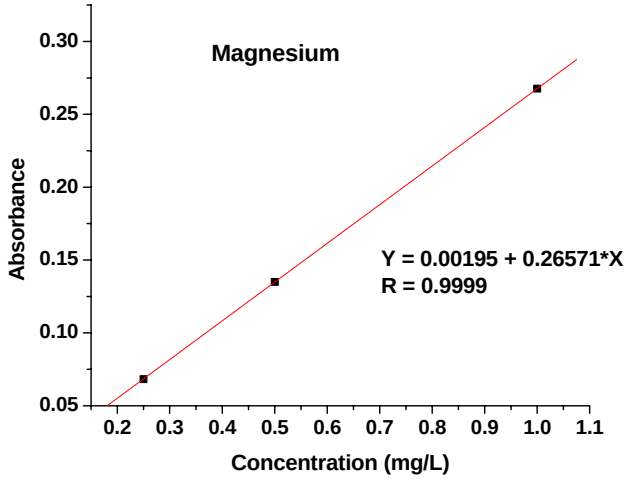
Calibration metal standard solutions were prepared for each of the metals from an intermediate standard solution of 10 mg/L made by diluting 1000 mg/L of stock metal standard solutions of atomic absorption spectrophotometer. Working standards of metal solutions were prepared in 50 mL volumetric flasks by diluting with distilled deionized water. Three appropriate working standard solutions of each of the metals with a concentration of which was close to that estimated from literatures were prepared from the intermediate standard solution. Concentrations of the intermediate standards, working standards, calibration curves and value of correlation coefficient of the calibration graph for each of the metals are given in Table 4 and Figure 3.

Table 4. Concentration of standard solutions for FAAS instrument Calibration and correlation coefficient of calibration curves.

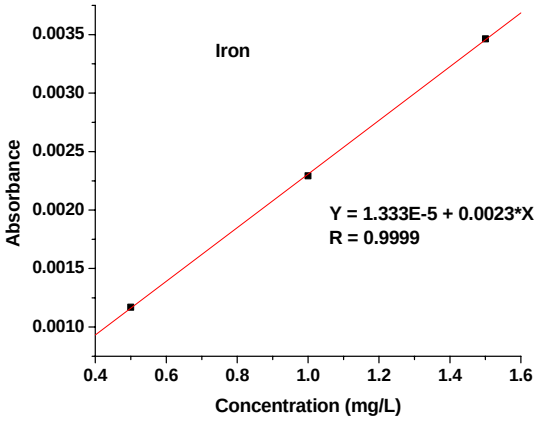
Metals	Concentration of intermediate standard (mg/L)	Concentration of the standards (mg/L)	Correlation coefficient (*r*)
Ca	10	0.5, 1.0, 2.0	0.9999
Mg	10	0.25, 0.5, 1.0	0.9999
Fe	10	0.5, 1.0, 1.5	0.9999
Zn	10	0.2, 0.4, 0.8	0.9977
Cu	10	0.5, 1.0, 1.5	0.9999
Co	10	0.25, 0.5, 1.0	0.9919
Cr	10	0.5, 1.0, 1.5	0.9989
Mn	10	0.25, 0.5, 1.0	0.9999
Ni	10	0.25, 0.5, 1.0	0.9999
Cd	10	0.25, 0.5, 1.0	0.9999
Pb	10	1.2, 2.4, 4.8	0.9999



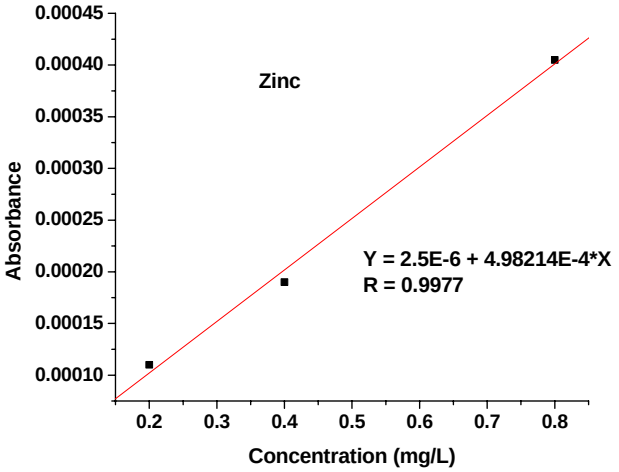
(a)



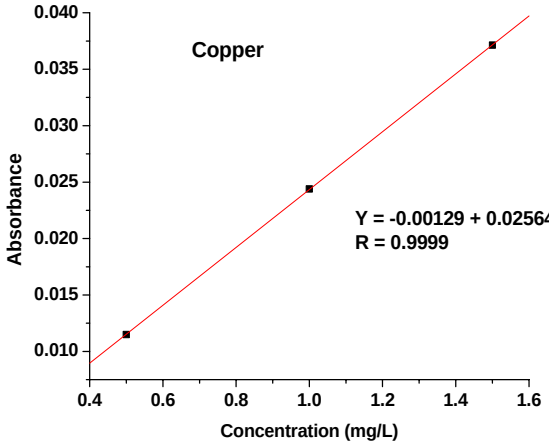
(b)


Iron
Absorbance
Concentration (mg/L)
Y = 1.333E-5 + 0.0023*X
R = 0.9999

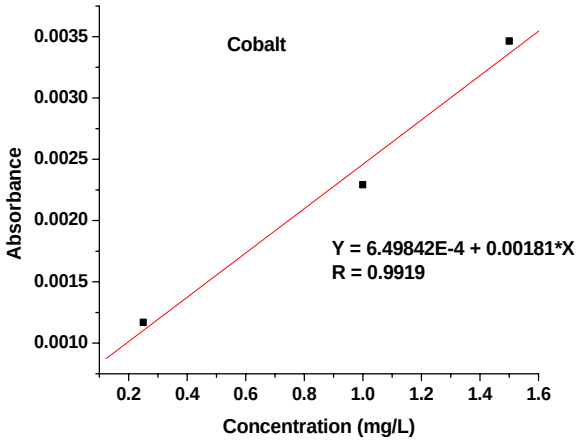
(c)


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

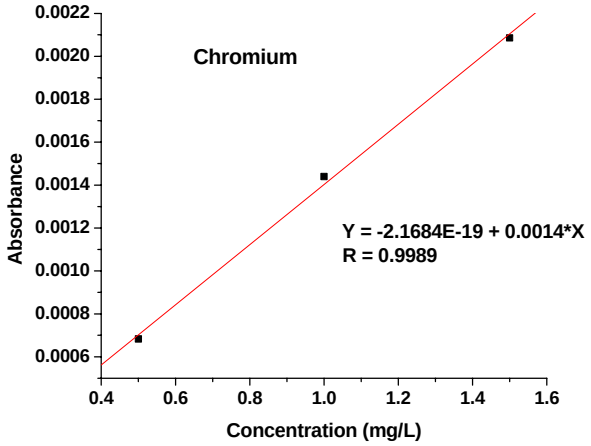
(d)


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

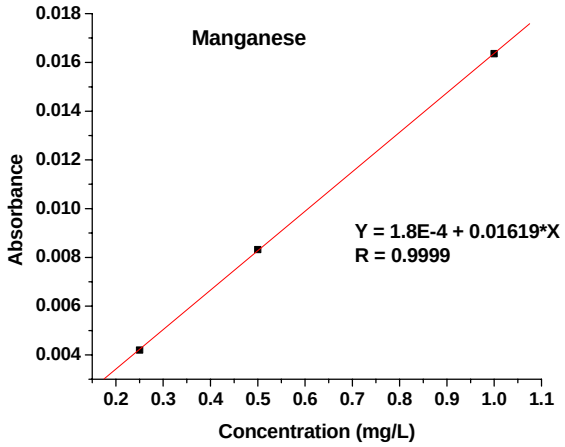
(e)


Cobalt
Absorbance
Concentration (mg/L)
Y = 6.49842E-4 + 0.00181*X
R = 0.9919

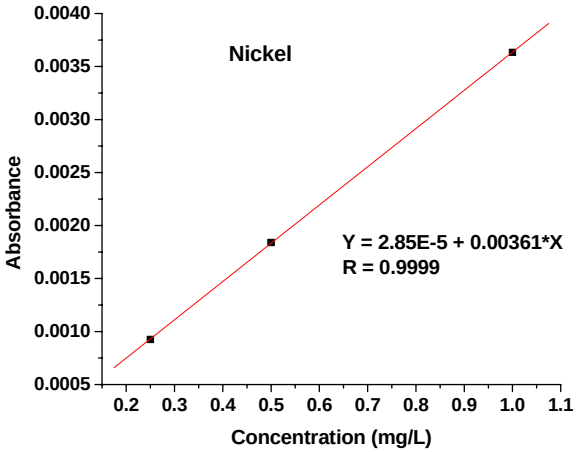
(f)


Chromium
Absorbance
Concentration (mg/L)
Y = -2.1684E-19 + 0.0014*X
R = 0.9989
0.0022
0.0020
0.0018
0.0016
0.0014
0.0012
0.0010
0.0008
0.0006
0.4
0.6
0.8
1.0
1.2
1.4
1.6

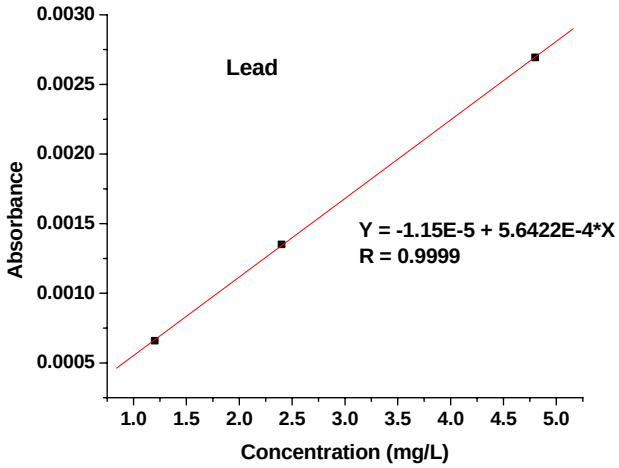
(g)


Manganese
Absorbance
Concentration (mg/L)
Y = 1.8E-4 + 0.01619*X
R = 0.9999
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1

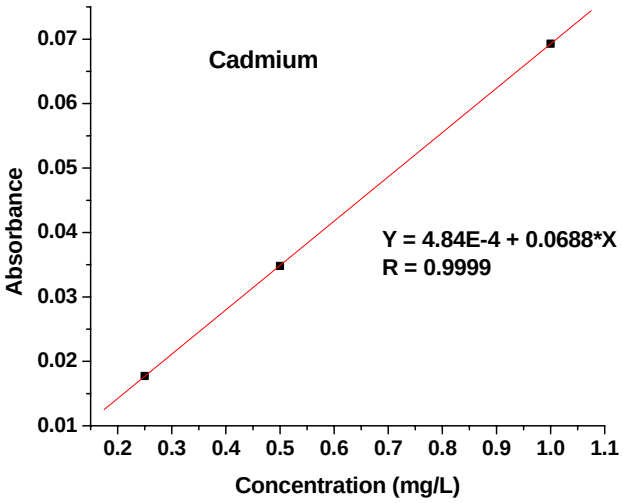
(h)


Nickel
Absorbance
Concentration (mg/L)
Y = 2.85E-5 + 0.00361*X
R = 0.9999
0.0040
0.0035
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1

(i)


Lead
Absorbance
Concentration (mg/L)
Y = -1.15E-5 + 5.6422E-4*X
R = 0.9999
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0

(j)



(k)

Figure 3. Calibration curves of metals standard solutions (a - k).

2.2.6. Method validation

In order to ascertain the reliability and efficiency of the developed optimized procedure, spiking experiments in which known volume and concentration of standard solutions added were employed. From the stock solution of (1000 mg/L) 196.0 µL of Ca, 162.5 µL of Mg, 10.5 µL of Fe, 36.0 µL of Mn and 4.25 µL of Zn solutions were added to 0.5 g of korarima sample. For the rest of the metals an intermediate standard solution (10 mg/L) was prepared and 200.0 µL of Cu, 75.0 µL of Co, 150.0 µL of Cr, 200.0 µL of Ni and 25.0 µL of Cd solutions were added to 0.5 g of korarima sample. Similarly, 100.0 µL of Ca, 181.5 µL of Mg, 130.0 µL of Fe, 97.0 µL of Mn were taken from 1000 mg/L stock solution. An intermediate standard solution of 10 mg/L was prepared from the stock solution of (1000 mg/L) and 400.0 µL of Zn, 275.0 µL of Cu, 80.0 µL of Co, 275.0 µL of Cr, 500.0 µL of Ni and 40.0 µL of Cd were added to the soil sample. Ca, Mg, Fe Mn and Ni were spiked in to one of the digestion sample in triplicate while Zn, Cu, Co, Cr and Cd in the second set of samples in triplicate. Then the samples were digested with the optimized procedures for both korarima and soil samples. After diluting the digested samples to 25 mL with distilled deionized water, they were analyzed by the same

procedure followed for the analysis of korarima and soil samples. As used for original samples triplicate spiked samples were prepared and triplicate readings were recorded. From these determinations, the precision and accuracy of the procedure were determined. The calculated percentage recovery varied from 89% for Co and Cd in korarima and soil samples respectively to 109% for Mn in korarima samples which were in the acceptable range as shown in Table 5 and 6. Thus, the optimized procedure for the metal analysis of both korarima soil samples was validated.

Table 5. Recovery experiment of korarima sample.

Metals	Amount added to korarima sample (µg/g)	Amount found (mean value, µg/g)	Mean recovery (%)
Ca	392.0	376.25	95.9 ± 2.7
Mg	325.0	329.48	101.4 ± 8.5
Fe	21.0	19.50	92.8 ± 4.3
Zn	8.5	7.76	91.3 ± 5.8
Cu	4.0	4.22	105.5 ± 1.9
Co	1.5	1.33	88.7 ± 4.5
Cr	3.0	2.89	96.3 ± 7.5
Mn	72.0	78.55	109.1 ± 10.3
Ni	4.0	3.78	94.5 ± 8.7
Cd	0.5	0.48	96.0 ± 8.4

Table 6. Recovery experiment of soil sample.

Metals	Amount added to soil sample (µg/g)	Amount found (µg/g)	Mean recovery (%)
Ca	200.0	179.21	89.6 ± 9.8
Mg	363.0	377.90	104.1 ± 7.8
Fe	260.0	253.52	97.5 ± 3.9
Zn	8.0	7.31	91.4 ± 1.7
Cu	5.5	5.06	92.0 ± 8.7
Co	1.6	1.50	93.7 ± 2.1
Cr	5.5	5.01	91.1 ± 5.9
Mn	194.0	175.02	90.2 ± 9.1
Ni	10.0	9.25	92.5 ± 3.5
Cd	0.8	0.71	88.7 ± 7.0

2.2.7. Method detection limit

Method detection limit is the lowest concentration level that can be determined to be statistically different from an analyte blank or is the minimum concentration that can be detected by the analytical method with a given confidence limit [52, 53]. There are numerous ways of determining detection limits of a given measurement. According to EPA (Environmental Protection Agency) of America, it is the minimum concentration of a substance that can be measured and reported with 99% confidence level that the analytical concentration is greater than zero. The generally accepted and common definition of method detection limit is the concentration that gives a signal three times the standard deviation of the blank or background signal [54, 55]. In this work, after digestion of 3 blank solutions (a mixture of 3 mL HNO_3 and 1 mL $HClO_4$ used for wet digestion of korarima samples) and 3 blank solutions (a mixture of aqua-regia and hydrogen peroxide for digestion of soil samples), triplicate reading was obtained for each sample. Then the method detection limit of each element was calculated as three times the standard deviation of the blank ($3\sigma_{blank,}$ n = 9) which is summarized in Table 7.

Table 7. Method and instrument detection limits for metals analysis in korarima and soil samples (for all metals, n = 9 for korarima and soil samples).

Metals	IDL (mg/L)	MDL for korarima (mg/kg)	MDL for soil (mg/kg)
Ca	0.01	1.5	0.8
Mg	0.001	0.8	0.5
Fe	0.03	0.5	0.5
Zn	0.005	0.9	1.0
Cu	0.02	0.3	0.5
Co	0.05	0.2	0.5
Cr	0.05	1.5	1.0
Mn	0.001	0.5	0.6
Ni	0.04	1.5	1.4
Pb	0.1	2.3	2.7
Cd	0.005	0.2	0. 7

The method detection limits estimated were greater than the instrument detection limit for all metals (Ca, Mg, Fe, Zn, Cu, Co, Cr, Mn, Ni, Pb and Cd) in korarima and soil samples. But the method detection limits are lower enough to detect the presence of levels of trace metals in the korarima and soil samples.

3. RESULTS AND DISCUSSION

3.1. Analytical precision

Accuracy and precision are probably the most often quoted terms to express the extent of errors in a given analytical results. Analytical results must be evaluated to decide on the best values to report and attempt to establish the probable limits of errors of the values. This can be done by determining the standard deviation, variance, coefficient of variance, relative standard deviation and range of series of measurements. In this study the precision of the results were evaluated by the pooled standard deviation and relative standard deviation of the triplicate samples with triplicate measurements of each sample (n = 9). It can be seen from Table 8 and 9 that the values of relative standard deviations

(%RSD) are less than 10% for all the mean concentrations except for Cd in Gamo Gofa, Cu in cardamom samples and Ca in Gamo Gofa soil samples which have slightly higher percentage relative standard deviation of 10.6, 11.2, and 11.0%, respectively. This shows the precision of the results obtained by this method is good.

3.2. Determination of metals in korarima and soil samples

The korarima and soil samples were analyzed for major (Ca, Mg), trace (Fe, Cu, Mn, Zn, Cr and Co), and toxic metals (Cd and Pb) with FAAS. The instrument was operated as per the instrument's manual. Each of the sets of series samples were aspirated one after another in to the FAAS instrument set in concentration mode using the hallow cathode lamp of the respective metal. The values along with standard deviation of triplicate analysis are given in Table 8 and 9 for korarima and soil samples, respectively.

Table 8. Average concentration (mean ± SD, n = 9 μg/g dry weight) and relative standard deviation (%RSD) of metals in korarima samples collected from different zones and cardamom samples.

Metals	Bonga	RSD %	Gamo Gofa	RSD %	Metu	RSD %	Cardamom	RSD %
Ca	1959 ± 23	1.2	1794 ± 43	2.4	2181 ± 59	2.7	2719 ± 35	1.3
Mg	1626 ± 34	2.1	1699 ± 29	1.7	2067 ± 61	2.9	2390 ± 41	1.7
Fe	43± 4	9.4	37 ± 1	2.5	46 ± 4	7.9	65 ± 2	3.3
Zn	17 ± 1	7.3	12 ± 1	7.0	18 ± 1	1.8	20 ± 1	4.7
Cu	8.3 ± 0.1	1.3	5.8 ± 0.4	7.1	7.1 ± 0.2	3.3	9.5 ± 1	11.2
Co	2.2 ± 0.2	6.9	2.0 ± 0.03	1.9	2.3 ± 0.2	7.9	2.6 ± 0.2	8.7
Cr	5.8 ± 0.4	6.9	3.8 ± 0.3	6.9	5.6 ± 0.4	6.3	8.3 ± 0.7	9.0
Mn	144 ± 5	3.5	141 ± 2	1.2	180 ± 4	2.2	355 ± 9.8	2.7
Ni	8.3 ± 0.7	9.4	6.6 ± 0.5	7.5	8.5 ± 0.2	2.1	11.7 ± 0.5	4.2
Pb	ND	-	ND	-	ND	-	ND	-
Cd	0.9 ± 0.05	6.3	1.0 ± 0.1	10.6	0.99 ± 0.04	3.8	0.87 ± 0.07	7.9

ND = Not detected (concentrations were below the method detection limit, 2.3 μg/g).

Table 9. Average concentration (mean ± SD, n = 9 μg/g dry weight) and relative standard deviation (%RSD) of metals in soil samples collected from different zones.

Metals	Bonga	RSD %	Gamo Gofa	RSD %	Metu	RSD %
Ca	1002 ± 24	2.4	665 ± 73	11.0	1048 ± 77	7.3
Mg	1818 ± 157	8.7	1170 ± 53	4.5	2469 ± 82	3.3
Fe	26357 ± 526	1.9	23570 ± 861	3.6	29301 ± 677	2.3
Zn	16.3 ± 0.2	1.3	16.9 ± 1.3	7.9	21.4 ± 1.2	5.9
Cu	12.6 ± 0.4	2.8	10.2 ± 0.9	8.6	12.9 ± 0.6	4.9
Co	2.7 ± 0.1	3.2	2.8 ± 0.2	7.8	2.9 ± 0.1	4.0
Cr	11.0 ± 0.8	7.3	10.9 ± 0.5	4.3	11.4 ± 0.4	3.1
Mn	971 ± 37	3.8	730 ± 8	1.1	947 ± 38	4.0
Ni	19.8 ± 0.3	1.5	16.3 ± 0.5	3.3	20.4 ± 0.6	2.9
Pb	ND	-	ND	-	ND	-
Cd	1.3 ± 0.02	1.9	2.0 ± 0.09	4.8	1.8 ± 0.04	2.1

ND = Not detected (concentrations were below the method detection limit, 2.7 μg/g).

3.3. Distribution patterns of metals in the samples

Korarima sample analyses for the metals indicate that except Pb, which was below the method detection limit, all the ten metals (Ca, Mg, Fe, Zn, Cu, Co, Cr, Mn, Ni, and Cd) were detected (Table 8). The levels of metals however differ significantly among each other and slightly between korarima samples cultivated in different zones. There is also a difference in metal concentration between korarima and Indian cardamom analyzed for comparison. The trends of variation of metals in korarima samples are Ca > Mg > Mn > Fe > Zn > Ni > Cu > Cr > Co > Cd while for soil samples it follows Fe >Mg >Ca >Mn Ni> Zn> Cu >Cr >Co> Cd.

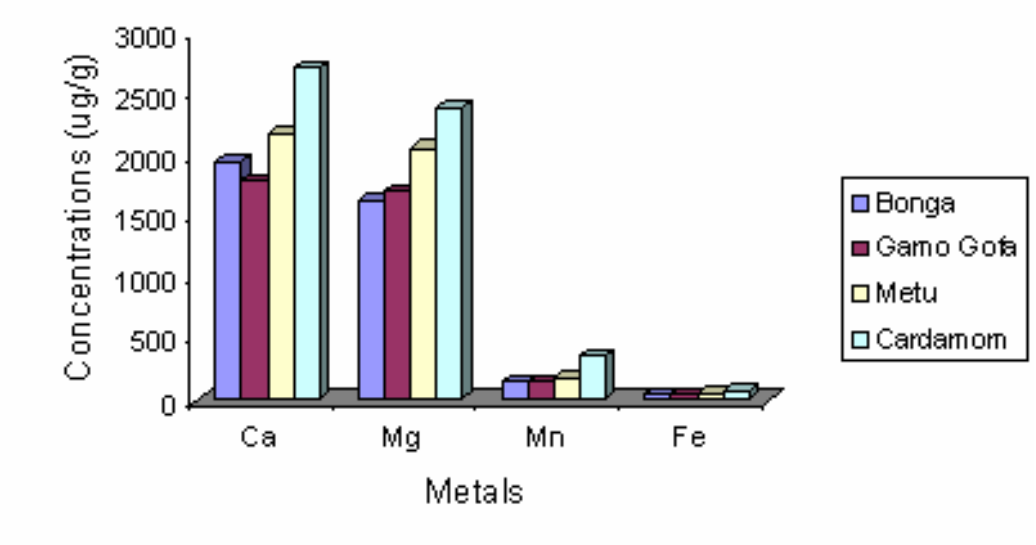
Out of the eleven metals studied in korarima and soil samples Pb were found to be below the method detection limits while the presence of the rest of metals, Ca, Mg, Fe, Zn, Cu,

Co, Cr, Mn, Ni and Cd in korarima and soil samples were observed. Metal ion uptake into the roots of plants is extremely complex phenomenon occurring via diffusion and mass flow of the soil solution. Chelation and surface adsorption, which are pH dependent, also affect availability of nutrient metal ions. In general, acidic soil retard the uptake of essential divalent metal ions but increase the availability of manganese, iron, and aluminum, all of which are normally of very limited availability because of hydrolysis of the trivalent ions.

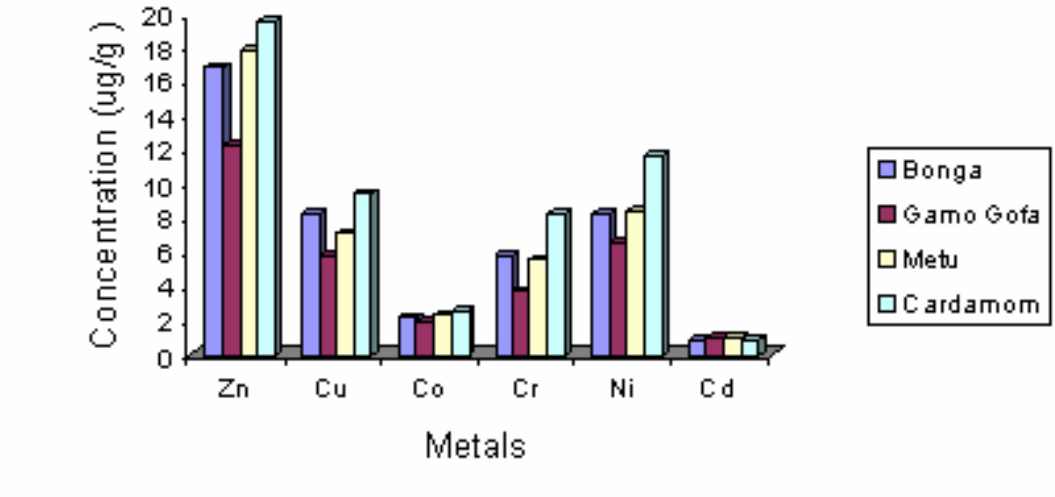
3.3.1. Distribution pattern of metals in korarima samples

In all the analyzed korarima and cardamom samples for major as well as trace metals, only lead was found to be below the method detection limit while others were detected. The concentration of metals determined in korarima samples indicate that calcium is highly absorbed by the plant while cadmium is the least determined in all the samples. As shown in Table 8, 1959 ± 23, 1794 ± 43, 2181 ± 59 and 2719 ± 35 μg/g dry weight of calcium was determined in Bonga, Gamo Gofa, Metu and Cardamom samples, respectively. For cadmium 0.9 ± 0.05, 1.0 ± 0.1, 0.99 ± 0.04 and 0.87 ± 0.07 μg/g dry wt was determined in the same order. Though the level of cadmium is the least among the metals, it deserves special concern due to its toxicity.

The levels of metals in both korarima and cardamom samples though comparable, higher concentration of major and trace metals were determined in cardamom samples in almost all the samples except for cadmium which is slightly higher in korarima samples. For korarima samples higher concentration of metals were determined for samples collected from Metu and the least for samples collected from Gamo Gofa except for cadmium (Figure 4 a & b).



(a)



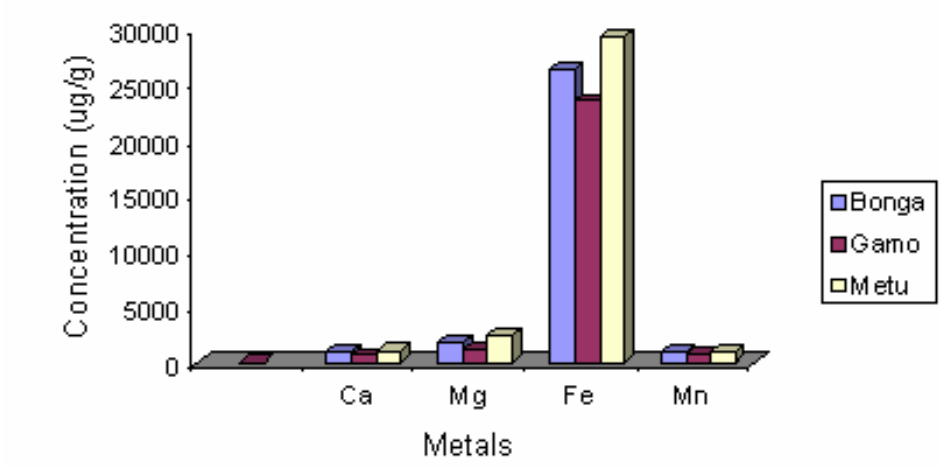
(b)

Figure 4. Concentration of (a) Ca, Mg, Fe and Mn and (b) Zn, Cu, Co, Cr, Ni and Cd in korarima and cardamom samples (µg/g dry weight).

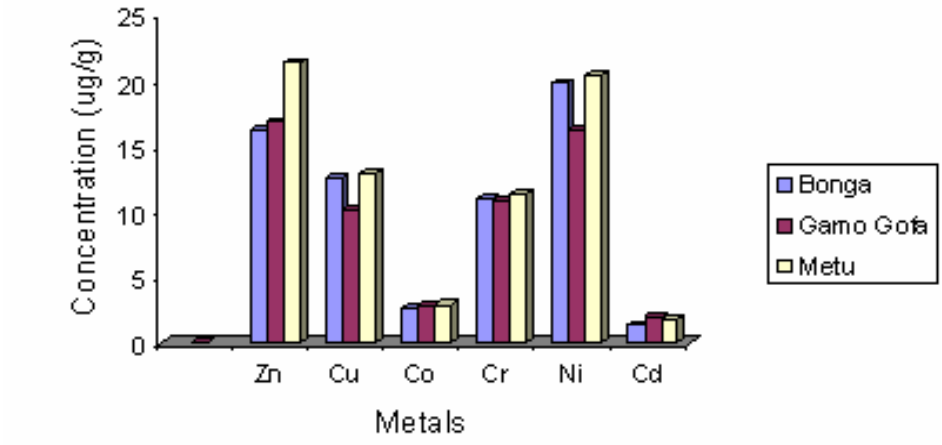
3.3.2. Distribution pattern of metals in soil samples

The soil samples were also found to contain detectable metals Ca, Mg, Fe, Zn, Cu, Co, Cr, Mn, Ni, and Cd. Again, lead was not detected in soil samples collected from any of the three sites. The concentration of metals in soil samples almost follows the same trend

as that of korarima samples brought from each zone, i.e. higher amounts of metals were determined in samples collected from Metu, Illubabor Zone and the least was in Kemba, Gamo Gofa Zone except for Mn and Cd in which the highest concentration were determined in Bonga and Gamo Gofa samples respectively. The highest metal concentration level obtained from this study was determined for Fe (29301 ± 677 µg/g dry weight) and the least for Cd (1.8 ± 0.04 µg/g dry weight) both brought from Metu as shown in Table 9 and Figure 5 (a). The level of metals in soil samples collected from the three zones generally follow the order Fe > Mg > Ca > Mn > Ni > Zn > Cu > Cr > Co > Cd.



(a)



(b)

Figure 5. Distribution of metals in soil samples: (a) Ca, Mg, Fe and Mn and (b) Zn, Cu, Co, Cr, Ni and Cd (µg/g dry weight).

3.3.3. Metal specific distribution patterns

Calcium

Calcium is a divalent alkaline earth cation. It is the fifth most plentiful element in the earth's crust. Calcium is the dominant exchangeable cation in many soils. It is an essential macronutrient for both plants and animals. The exchangeable calcium in the soil has an important relation to soil pH and to the availability of several nutrient elements. The amounts of calcium and other basic cations present in the soil decline as soil becomes more acidic and increase as it becomes more alkaline. An excess of calcium causes calcium carbonate to precipitate and buffer the pH to a value near 8. Excess calcium usually results in the solubility of phosphorus, iron, manganese, boron, and zinc and sometimes causes deficiencies of one or more of these essential plant nutrients.

Calcium is the most accumulated metal in korarima samples studied with mean concentrations and standard deviations of 1959 ± 23, 1794 ± 43, 2181 ± 59 and 2719 ± 35 μg/g dry weight in Bonga, Gamo Gofa, Metu and Cardamom samples, respectively. Higher level of calcium was observed in korarima and soil samples collected from Metu and the least in Gamo Gofa samples. High concentrations of Ca are important because of its role in bones, teeth, muscles system and heart functions and the study shows satisfactory level of Ca accumulation.

Magnesium

Magnesium in the soil is a constituent of many soil minerals; it is present as exchangeable magnesium on the cation-exchange complex and in soil solution as the soluble magnesium ion. Small amounts of magnesium may also be combined in the soil's organic fraction. Plant roots absorb soluble and ultimately exchangeable magnesium, which is assumed to go into solution before absorption by the root. Exchangeable calcium plus magnesium usually accounts for more than 60% of the exchangeable cations on soils with pH o f 5.5 or higher.

Magnesium is generally taken up by plants in lower quantities than Ca^{2+} or K^{+}. The content of Mg in plant tissues is usually in the order of 0.5% of the dry matter. The most well known role of Mg is its occurrence at the center of the chlorophyll molecule. Besides its function in the chlorophyll molecule Mg^{2+} is required in other physiological processes. One major role of Mg^{2+} is as a cofactor in almost all enzymes activating phosphorylation processes. Mg is therefore important throughout the metabolism [49].

Magnesium is the second most accumulated metal in both korarima and soil samples analyzed in this study. The concentration level of Mg varies from 1626 ± 34 to 2067 ± 61 μg/g dry wt in korarima samples; 1170 ± 53 to 2469 ± 82 μg/g dry wt in the corresponding soil. The result reveals that high concentrations of magnesium exist in korarima and soil samples collected from Metu as in the case of calcium.

Iron

Iron is an essential element for both plant productivity and nutritional quality. Iron is present in soils in higher concentrations than any other nutrient. In spite of the large amount of iron in the soil and the low quantities needed for plant growth, iron deficiencies occur because so little of the element is in an available form. The content of soluble iron in soils is extremely low in comparison with the total iron content. The amount of iron present as Fe^{2+} and Fe^{3+} in the soil solution depends on the hydroxide forms present in the soil, which in turn, depends on pH. Although iron is frequently taken up in the ferric state (Fe^{3+}), the ferrous state (Fe^{2+}) is generally accepted as the metabolically active form of iron in the plant [49].

Improving plant iron content was attempted through genetic engineering of plants overexpressing ferritins. However, both the roles of these proteins in plant physiology, and the mechanisms involved in the regulation of their expression are largely unknown [56]. Iron is essential for the synthesis of chlorophyll and heme or haemin which function as prosthetic groups [57]. High pH and high level of $CaCO_3$, low levels of organic matter and some other soil factors are predominantly responsible for low availability of Fe to

plants. Plant species and varieties show different response to Fe nutrition even they are grown in the same conditions [58].

Iron is by far the most accumulated metal determined in the soil samples studied in this work. The concentration varies from 23570 ± 861 µg/g dry wt in Gamo Gofa to 29301 ± 677 µg/g dry wt in Metu soil samples. The iron content of korarima sample ranges from 37 ± 1.0 to 46 ± 4.0 µg/g dry wt in Gamo Gofa and Metu sites respectively indicating that low availability of soluble iron in the soil. The permissible limit set by FAO/WHO (1984) in edible plants was 20 µg/g [59].

Zinc

Zinc is one of the important metals for normal growth and development in human beings. Deficiency of zinc can result from inadequate dietary intake, impaired absorption, excessive excretion or inherited defects in zinc metabolism. Zinc deficiency is of growing concern in the developing world because of the consumption of plant foods that have inhibitory components for zinc absorption. Especially, in these populations, zinc deficiency is related to the high consumption of bread made without yeast [60]. Zinc is an essential trace metal involved in growth and DNA synthesis in human with normal daily intake of 7-16 mg per day for adults.

The levels of Zn in plant materials are low, generally in the order of up to 100 µg/g in the dry matter. Zinc participates in the metabolism of plants as an activator of several enzymes. In its function, Zn^{2+} resembles Mn^{2+} and Mg^{2+} in that it brings about the binding configuration between enzyme and substrate. Zinc is needed for protein metabolism and appears to be involved somehow in the production of chlorophyll. Plants suffering from Zn-deficiency often show chlorosis in the interveinal areas of the leaf. These areas are pale green, yellow, or even white. Zn deficiency is closely related to the inhibition of RNA synthesis. The deficiency prevents the normal development of grana, vacuole and chloroplast.

The concentration of zinc determined in korarima and soil samples were from 12.3 ± 0.87 to 17.9 ± 0.33 and 16.3 ± 0.2 to 21.4 ± 1.2 μg/g dry weight respectively. While the highest level of zinc was observed in Metu samples, the lowest level of zinc was determined in Gamo Gofa and Bonga samples for korarima and soil samples respectively. The permissible limit set by FAO/WHO (1984) in edible plants was 27.4 μg/g, which is higher than the present study. According to Bowen and Allaway, the range of Zn in agricultural products should be between 15 to 200 μg/g [59].

Copper

Copper is an essential micronutrient for many plants and animals. For human being 1-3 mg each day for normal body function. Human toxicity from copper is generally rare but prolonged exposure to children may damage liver and cause death. Copper is taken up by the plant in only very small quantities. It is thus about one-tenth of the Mn content. Copper uptake appears to be a metabolically mediated process and there is evidence that Cu strongly inhibits the uptake of Zn and vice versa. Copper occurs as Cu^{++} ions in most soils and as Cu^{+} ions where the oxidation level is low. Copper is most soluble in acidic soils, and its solubility decreases as the pH rises [49].

Copper concentration in this study was from 5.8 ± 0.4 to 8.3 ± 0.1 μg/g dry wt in korarima samples and 10.2 ± 0.9 to 12.9 ± 0.6 μg/g dry wt in soil samples. The highest level of copper was found in Bonga korarima samples and Metu soil samples. Gamo Gofa samples contain the least level of copper in both korarima seed and soil samples analyzed in this study. The principal source of copper might be the soil or soil contaminants. The permissible limit set by FAO/WHO (1984) in edible plants was 3.00 ppm. According to Bowen and Allaway, the range of Cu in agricultural products should be between 4 to 15 ppm in agreement with the present study [59].

Cobalt

The Co concentration in the dry matter of plants grown in soil normally lies between 0.02 to 0.5 μg/g. In soils, the content is usually much higher and levels from 1 to 40 μg/g are common although many values in excess of 40 μg/g have been reported. Low concentrations of Co can have a favorable effect on plant growth. Cobalt is also of importance in animal nutrition. It is well established that Co is a metal component of vitamin $B_{12,}$ which is essential in N-metabolism [49].

The concentration levels of cobalt ranges from 2.0 ± 0.03 μg/g dry wt in Gamo Gofa to 2.3 ± 0.2 μg/g dry in Metu for korarima samples. The level in the corresponding soil sample lies between 2.7 ± 0.08 and 2.9 ± 0.1 μg/g dry wt. for Bonga and Metu sites respectively, indicating that the lowest concentration of cobalt was determined in Bonga for korarima samples and Gamo Gofa for soil samples. Cobalt is the second least traces metal determined next to cadmium in both korarima and soil samples. The safety limit for human consumption of Co is 0.05 to 1 mg/day in humans.

Chromium

The trivalent chromium, Cr(III) is essential trace element for adult human being with safe and adequate daily requirement of 50-200 μg and toxic only at high level. However Cr(VI) is toxic, long time exposure resulting in kidney and liver damage and being carcinogenic. Plants absorb more quantities of Cr(VI) though Cr(VI) is more toxic than Cr(III) for them. The hexavalent chromium, Cr(VI) is more soluble in soil moisture and water while Cr(III) is largely present in soil as relatively unavailable, insoluble ion or hydroxides. This is because Cr(III) is readily hydrolysed at neutral pH and extremely insoluble. Soil and ground water pollution by Cr(VI) is primarily by leaching from wastes disposed through industrial processing of tannery, pigments, steel and others.

The chromium content of korarima samples in this study were 5.8 ± 0.4, 3.8 ± 0.3, 5.6 ± 0.4 μg/g dry wt in Bonga, Gamo Gofa and Metu sites respectively. For soil samples the

results are 11.0 ± 0.8, 10.9 ± 0.5, 11.4 ± 0.4 μg/g dry wt in the same order. The result shows highest level of chromium in Bonga sites for korarima and Metu for soil samples though the difference is very small. Chronic exposure to Cr may result in liver, kidney and lung damage. The tolerable limit of Cr set by FAO/WHO (1984) in edible plants was 0.02 μg/g. This study shows korarima plant accumulate Cr above this limit [59].

Manganese

Manganese is the eleventh most common element in the earth's crust, with an average concentration of 0.09%, or 900 mg/kg. Soil manganese exists in three oxidation states – Mn^{2+}, Mn^{3+}, and Mn^{4+}. Manganese absorbed by plant roots is primarily as Mn^{2+}. Manganese availability is higher in acidic soils due to the higher solubility of Mn compounds under low pH conditions. Soluble Mn^{2+} decreases 100 fold for each unit increase in pH. Under high soil pH conditions, Mn availability can thus be inadequate to meet plant demand. Manganese, like iron, is a relatively immobile element in plants. Manganese absorbed by plants is stored mostly in leaves and so released to soil or run off as soon as the plant's leaves shade, giving rise to temporary increase in manganese concentration in water bodies during autumn.

Manganese is an essential element in respiration and nitrogen metabolism; in both processes, it functions as an enzyme activator. Manganese functions in chlorophyll development and in the enzyme systems of plants. Manganese is also in some way involved in the oxidation-reduction processes in the photosynthetic electron transport system [49].

Manganese is the third most accumulated metal next to calcium and magnesium in korarima samples and the fourth highest metal determined in soil samples analyzed in this study. The levels of manganese lies between 144 ± 5 and 180 ± 4μg/g dry wt for korarima samples and 730 ± 8 to 971 ± 37 μg/g dry wt for soil samples. Thus, the highest level was determined in Bonga sites for both samples and the least in Metu and

Gamo Gofa for korarima and soil samples respectively. The permissible limit set by FAO/WHO (1984) in edible plants was 2 μg/g.

Nickel

Nickel holds a special place among the heavy metals unlike cadmium, lead, mercury, arsenic and several other metals that are not the components of plant enzymes. Ni is a constituent of urease and small quantities of Ni (0.01 to 5 μg/g dry wt.) are essential for some plant species. On the other hand, Ni is not as import for plant metabolism as Zn and Cu. However, same as with other heavy metals, high Ni concentrations may turn toxic to plants [60].

The nickel content of korarima samples investigated in this study lies in the range of 6.6 ± 0.5 to 8.5 ± 0.2 μg/g dry wt for korarima samples and 16.3 ± 0.5 to 20.4 ± 0.6 μg/g dry wt for soil samples. The highest and the lowest level of nickel were determined in Metu and Gamo Gofa samples for both samples, respectively. Actually, there is no significance difference between the levels of nickel analyzed in each sites. The permissible limit set by FAO/WHO (1984) in edible plants was 1.63 μg/g higher than the present study [59].

Lead

The total Pb content of agricultural soils lies between 2 - 200 μg/g. Soils with levels in excess of this are limited to a relatively few regions where Pb mineral deposits occur. The availability of soil Pb is usually low. A high soil pH may precipitate Pb as hydroxide, phosphate, or carbonate as well as possibly promoting the formation of Pb organic matter complexes.

Lead is a major chemical pollutant of the environment, and is highly toxic to man. Lead can cause brain and kidney damage, decrease in hemoglobin production and male fertility. Lead enters man by inhalation and ingestion, absorbed and carried by the blood; it is accumulated in liver, kidney, and bone up to about the fifth decade of life. Lead

causes brain damage particularly to the young. There is evidence that Pb pollution can induce aggressive behavior in animals which can also occur in humans [49].

Lead concentration in both korarima and soil samples of the present study were below the method detection limit. However, values as high as 0.4, 0.6 and 0.78 µg/g dry wt was reported in spices of the same family [19, 38]. The permissible limit set by FAO/ WHO (1984) in edible plants was 0.43 µg/g [59]. Therefore, further investigation of lead with other alternative method and large sample size was recommended to come up with a firm conclusion.

Cadmium

Cadmium occurs naturally in only trace concentrations in agricultural soils. Contamination of agricultural soils with Cd is derived from sources, such as phosphate fertilizers manufactured from rock phosphates high in Cd and by the application of sewage sludge to a greater extent and by the pesticides and gypsum to lesser extent. Food crops grown on contaminated soils may take up substantial amounts of Cd and this could result in Cd entering the food chain of animals and humans when consumed.

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

Most recently, interest in Cd has been directed at progressive accumulation in biological systems at low levels at which Cd generally occurs environmentally. Toxic effects in man have been observed from the regular consumption of plants in excess of 3 µg/g. Continued exposure to small amounts of Cd leads to accumulation in human and animal liver and kidney tissues resulting in damage and malfunction of these organs. It disturbs

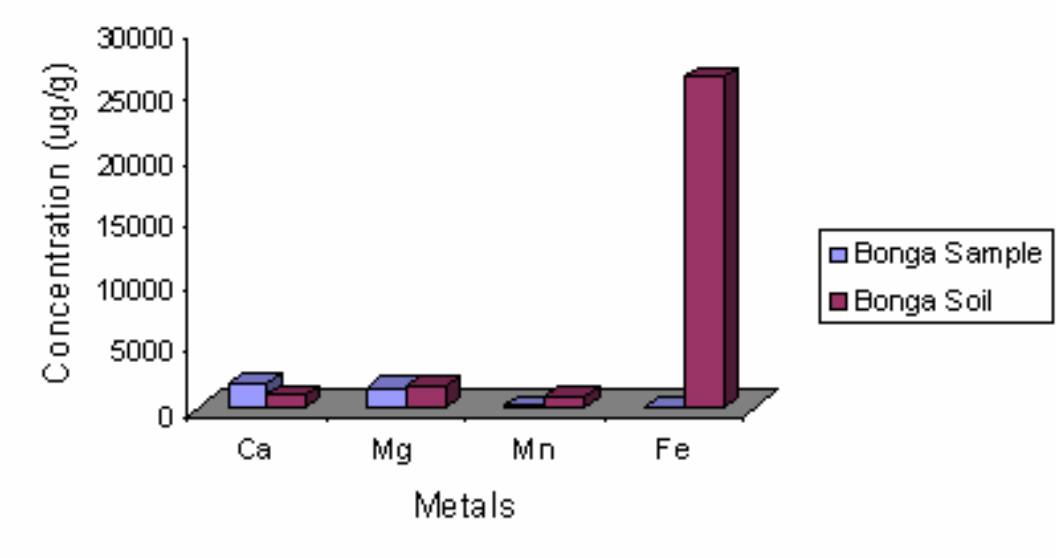
the metabolism of Ca and P and causes bone disease, which is very painful, and causes excessive demineralization and embrittlement of the skeleton [49].

Cadmium is the least of all the metals determined in this study. The concentration level of cadmium obtained by the present study is 0.9 ± 0.05, 1.0 ± 0.1, 0.99 ± 0.04 μg/g dry wt in Bonga, Gamo Gofa and Metu sites for korarima samples respectively. In the soil samples analyzed the Cd concentration is 1.3 ± 0.02, 2.0 ± 0.09, 1.8 ± 0.04 μg/g dry wt in the same order. The permissible limit set by FAO/WHO (1984) in edible plants is 0.21 μg/g. The present study shows Cd is accumulated slightly above this limit [59].

3.3.4. Comparisons of metal levels between korarima and soil samples

The relationships between the metal contents of korarima and soil samples in this study were also analyzed. As it can be seen from Figure 6 (a-f), there is direct correlation in the level of metals in the two samples, i.e. as the concentration of metal in the soil increases its accumulation in the plant also increases. However, there are some anomalies for certain metals investigated, like iron where the level in the soil is extremely high as compared to that of the korarima plant. Iron level in the soil obtained from this study is from 600 to 800 times greater than that determined in the korarima plant. The content of soluble iron in soils is extremely low in comparison with the total iron content. The amount of iron present as Fe^{2+} and Fe^{3+} in the soil solution further depends on the hydroxide forms present in the soil, which in turn, depends on pH.

Plants show greater tendency to absorb high amount of calcium from the soil, which is also shown in this study. Higher amount of calcium was determined in the korarima plant than the soil in all of the sites investigated in this study. Nevertheless, the reverse is true for all the other major and trace metals studied. The level of magnesium in the korarima seed and soil sample studied is comparable and relatively higher values were determined in the soil sample. For all the other trace metals, relatively greater concentration was found in the soil samples as compared to the korarima sample, Figure 6.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Concentration (ug/g)
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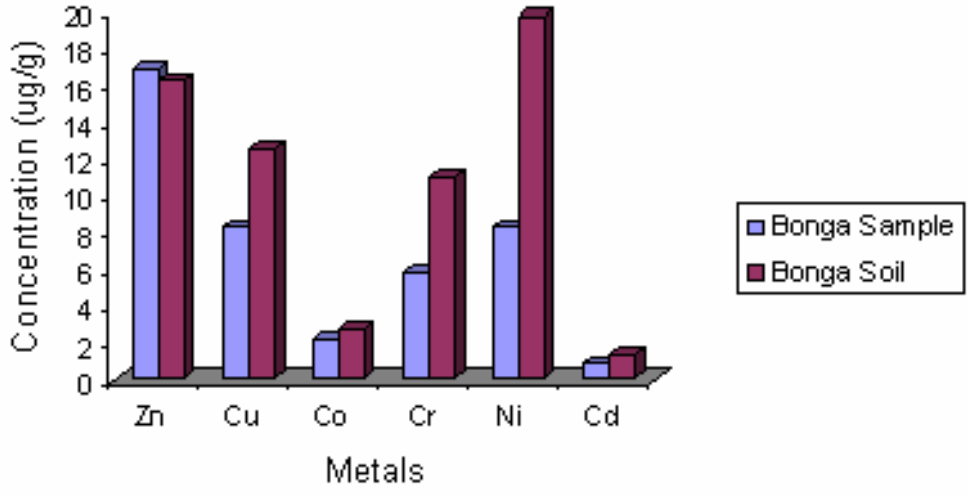
Metals
Bonga Sample
Bonga Soil

(a)


20
18
16
14
12
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8
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2
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Concentration (ug/g)
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Cu
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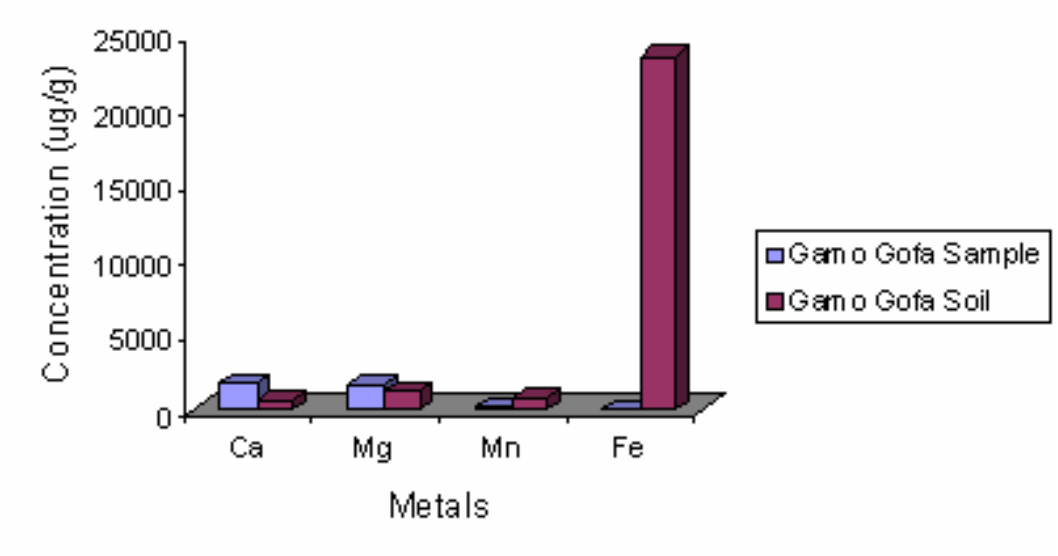
Cr
Ni
Cd
Metals
Bonga Sample
Bonga Soil

(b)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Concentration (ug/g)
Ca
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Mn
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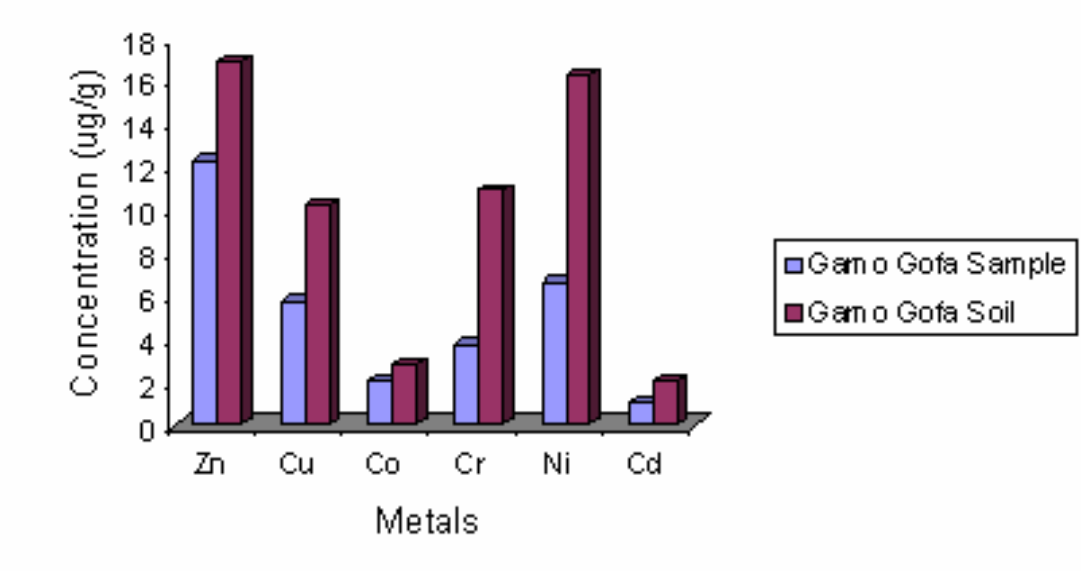
Metals
Gamo Gofa Sample
Gamo Gofa Soil

(c)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Concentration (ug/g)
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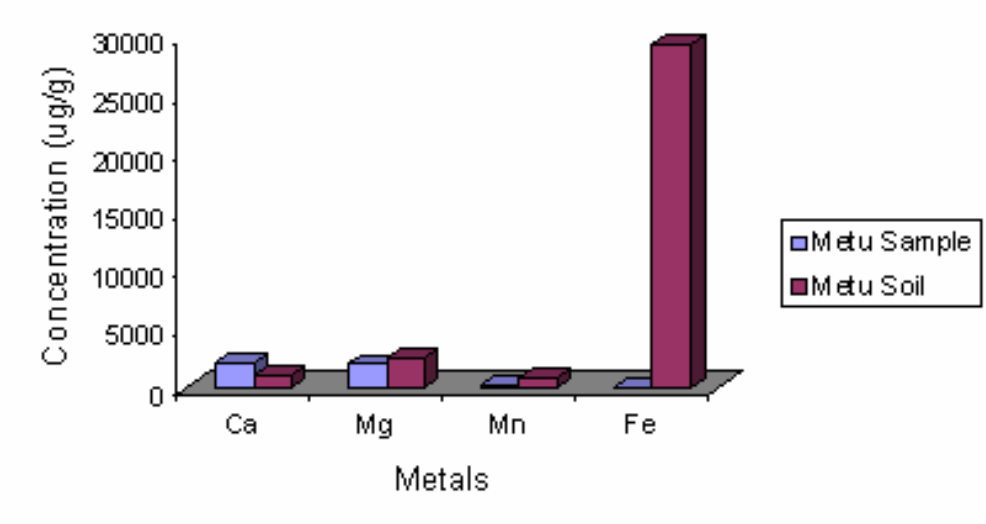
Metals
Gamo Gofa Sample
Gamo Gofa Soil

(d)



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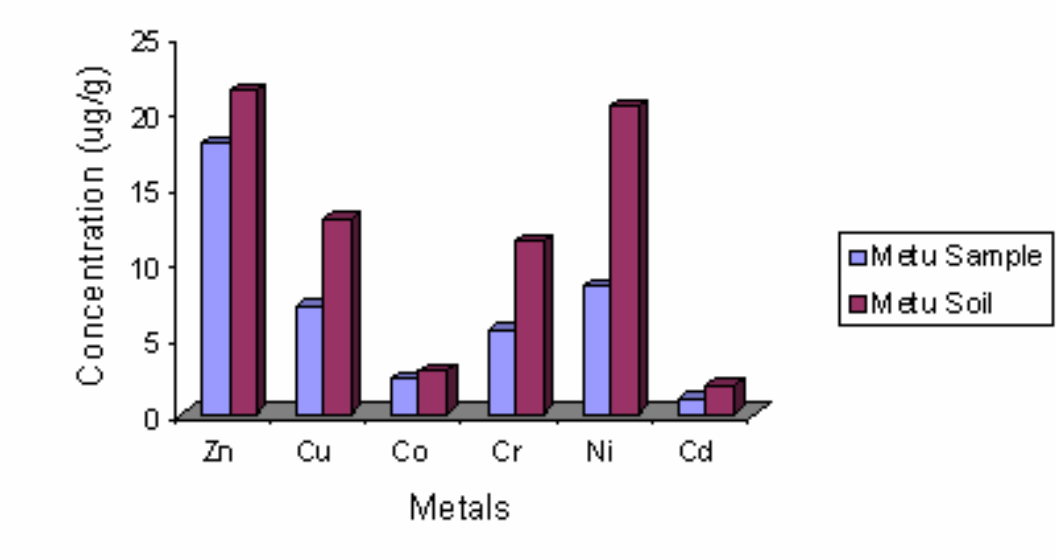

(f)

Figure 6. Comparisons of metal levels in korarima plant with soil.

3.3.5. Comparison of metal levels of the present study with literature values

The metal levels of korarima plant of the present study was compared with the literature values of similar plant species and spices as korarima plant is indigenous to Ethiopia and there is no study conducted on the metal content of this spice. Therefore, literature values of the metal levels of cardamom (*Elettaria cardamomum*), large cardamom (*Amomum*

subulatum) and ginger (*Zingiber officinale*) were used for comparison with the present study Table 10 and 11.

Table 10. Summary of the comparison of metals (Ca, Mg, Fe, Zn and Cu) levels reported in small cardamom, large cardamom and ginger with the present study.

Spices	Metals (μg/g dry wt)					Ref.
	Ca	Mg	Fe	Zn	Cu	
Cardamom (*Elettaria cardamomu*)	- -	- -	441 ± 61 73 ± 2.2	50.6 ± 2.6 25 ± 0.8	48.2 ± 20.6 5 ± 0.7	12 45
Large cardamom (*Amomum subulatum*)	-	-	285 ± 44	45.3 ± 5.2	14 ± 3.3	12
Ginger (*Zingiber officinale*)	- - - - - 2610 ± 10	- - - - - 4210±10	2475 ± 1110 - - 13.3 ± 0.01 - 144 ± 0.05	19.7 ± 1.9 5.0 ± 0.9 4.8 ± 0.05 3.4 ± 0.02 - 33.3± 0.01	49.4 ± 2.7 3.0 ± 0.8 - 1.7 ± 0.01 2.35-8.3 14.4 ± 0.01	12 45 28 46 38 61
Korarima (*Aframomum corrorima*)	1794 - 2181	1626 - 2067	37.0 - 46.3	12.3± 17.9	5.8 ± 8.3	**Presents tudy**
*Cardamom (*Elettaria* cardamomum) analyzed for comparison	2719 ± 35	2390 ± 41	64.8 ± 2.2	19.6 ± 0.9	9.5 ± 1.0	

Table 11. Summary of the comparison of metals (Co, Cr, Mn, Ni, Pb and Cd) levels reported in small *cardamom*, large cardamom and ginger with the present study.

Spices	Metals (μg/g dry wt)						Ref.
	Co	Cr	Mn	Ni	Pb	Cd	
Cardamom *(Elettaria cardamom-um)*	-	-	2840 ± 112	-	-	-	12
	-	-		-	-	0.15 ± 5.2	45
	ND	-	168 ± 2.5	-	0.4	0.14	19
Large card-amom *(Amomum subulatum)*	-	-	223 ± 18	-	-	-	12
Ginger *(Zingiber officinale)*	-	-	1014 ± 52	-	-	-	12
	-	-	73 ± 3.4	-	-	0.072 ± 4.1	45
	0.32	-	-	-	0.6	0.07	19
	-	0.04 ± 0.01	-	0.21 ± 0.03	0.01	0.012 ± 0.03	28
	-	-	-	-	0.21 - 0.78	0.02 - 0.04	38
	-	0.05 ± 0.01	1.6 ± 0.01	-	-	-	46
Korarima *(Aframom-um corrorima)*	2.0-2.3	3.8 - 5.8	143.8 - 179.6	6.6 - 8.5	ND	0.9 - 1.0	**Presents Study**
Cardamom(Elettaria cardamom-um)*	2.6 ± 0.2	8.3 ± 0.7	355.4 ± 9.8	11.7 ± 0.5	ND	0.87 ± 0.07	

* It was bought from the market for comparison.

The data presented in Table 10 and 11 clearly indicated that most of the values reported in the literature are in agreement with the present study, especially for the essential metals investigated. The concentration level of iron in the spices used for comparison ranges from 13.3 to 2475 μg/g dry wt while the value determined in this study lies from 37.0 to 46.3 μg/g dry wt, which is within the range. The mean concentration of Zn is from 3.4 to 50.6 in the literature and from 12.3 to 17.9 μg/g dry wt in this study. The level of copper in literature is in the order of 1.7 to 48.2 μg/g dry wt in the spices considered where as from 5.8 to 8.3 μg/g dry wt was observed in this study. Therefore, the concentration level of both zinc and copper is within the ranges of the literature values.

Cobalt was not reported in some of the spices in the literatures considered. However, there are reported values of cobalt with concentration of 0.32 μg/g dry wt while in the results of the present study from 2.0 to 2.3 μg/g of cobalt was recorded. The chromium concentration in literature is very small (0.04 - 0.05 μg/g dry wt) compared to the results of this study (3.8 - 5.8 μg/g dry wt). The level of manganese is in agreement with the literature values of the spices considered for comparison in this study. The literature value of Mn ranges from 1.6 to 2480 μg/g dry wt and the present study is from 144 - 180 μg/g dry wt, which lies within the range.

The nickel level of the present study is very high as compared to the literature values. Lead was not detected in the present study because the concentration was below the detection limit of the instrument. However, in the literature values as high as 0.01 to 0.78 μg/g dry wt of lead was detected in the spices used for comparison in different country. Cadmium is the least metal determined in the present study. The observed concentration of cadmium lies in the range of 0.9 - 1.0 μg/g dry wt. But, spices with cadmium concentration ranging from 0.012 to 0.151 μg/g dry wt were reported. The principal source of cadmium might be the use of cadmium containing phosphate fertilizers such as triple super phosphate.

In general, the mean concentrations of metals observed in the current study were more or less comparable with the reported values. However, relatively higher concentrations of Co, Cr, Ni and Cd were observed. Furthermore, lower concentrations of Fe, Zn, Cu and Mn were determined in this study than the reported values even though there are some results with lower concentrations. The concentration of lead was below the instrument detection limit in the current study.

3.4. Statistical analysis

The comparison of variance and the equality of means in analysis and sampling as well as the correlation between metals in korarima and soil samples were determined separately through one-way ANOVA using origin 6.0 and Microsoft Excel Software.

3.4.1. Analysis of variance

Variation in the mean levels of metals between the samples were tested whether it was from just a random error or treatment (i.e. difference in mineral composition of the soil, climatic condition, soil inputs like fertilizers, pesticides, herbicides or variations in soil parameters like pH).

The result indicated that significant differences were obtained ($p < 0.05$) at 95% confidence levels for Ca, Mg, Zn, Cu, Mn, and Cr in korarima samples collected from Bonga, Gamo Gofa and Metu sites. However, the variations for Fe, Ni, Co and Cd were not significant ($p > 0.05$). For the soil samples analyzed, except Zn, Cr and Co all the other metals (Ca, Mg, Fe, Cu, Mn, Ni and Cd) differ significantly.

3.4.2. Pearson correlation of metals

The results of the Pearson correlation matrices using correlation coefficient for korarima and soil samples were shown in table 12 and 13, respectively. The correlation between metals in the korarima seed and soil samples is presented in Table 14.

Table 12. Correlation matrices of metals in korarima samples.

	Ca	Mg	Mn	Fe	Zn	Cu	Ni	Cr	Co	Cd
Ca	1									
Mg	0.827	1								
Mn	0.932	0.974	1							
Fe	0.977	0.690	0.834	1						
Zn	0.905	0.511	0.689	0.974	1					
Cu	0.446	-0.131	0.092	0.625	0.784	1				
Ni	0.872	0.447	0.635	0.955	0.997	0.827	1			
Cr	0.766	0.273	0.481	0.884	0.966	0.917	0.982	1		
Co	0.962	0.645	0.799	0.998	0.986	0.671	0.971	0.911	1	
Cd	-0.007	0.554	0.355	-0.218	-0.431	-0.897	-0.495	-0.648	-0.277	1

Table 13. Correlation matrices of metals in soil samples.

	Ca	Mg	Mn	Fe	Zn	Cu	Ni	Cr	Co	Cd
Ca	1									
Mg	0.915	1								
Mn	0.980	0.817	1							
Fe	0.909	0.999	0.809	1						
Zn	0.502	0.808	0.321	0.816	1					
Cu	0.999	0.911	0.981	0.905	0.494	1				
Ni	0.999	0.925	0.974	0.919	0.524	0.999	1			
Cr	0.734	0.945	0.585	0.949	0.955	0.727	0.751	1		
Co	0.110	0.501	-0.088	0.513	0.914	0.101	0.135	0.755	1	
Cd	-0.639	-0.275	-0.779	-0.262	0.343	-0.646	-0.619	0.052	0.693	1

As shown in Table 12 and 13, for the majority of the metals analyzed except cadmium, magnesium and copper in the korarima seed and cadmium, cobalt, zinc and chromium in the soil samples the correlation is significant ($r > 0.8$) at 95% confidence level. Moreover, cadmium has shown a negative correlation with almost all the metals analyzed in the two samples, which shows weak association with other metals. A very high (near 1) positive correlation indicates high association of the metals, which may arise from natural source or environment or similarity in chemical properties. Magnesium with copper in the plant and manganese with cobalt in the soil also shows negative correlation.

Table 14. The correlation between metals in the korarima seed and soil samples.

Metals	Ca	Mg	Mn	Fe	Zn	Cu	Ni	Cr	Co	Cd
r	0.879	0.779	0.483	0.989	0.551	0.824	0.999	0.583	0.327	0.981

The correlation coefficient (r) between the metals in korarima and soil samples collected from each of the three sites was also investigated. The results shown in Table 14 indicate very high correlation for nickel and very weak correlation for cobalt between the two samples.

4. CONCLUSIONS AND RECOMMENDATIONS

Korarima (*Aframomum corrorima*) or the Ethiopian cardamom is a renowned spice and medicinal crop native to Ethiopia. The dried fruits are part and parcel of the daily dishes of the Ethiopians. The korarima capsules are used as a substitute for the Indian cardamom in the world market. Korarima occurs as a cultivated crop only in Ethiopia [15].

Although the data set is relatively small to draw authorative conclusions about the metal contents, the study indicated the presence of major metals (Ca and Mg), trace essential metals (Fe, Zn, Cu, Co, Cr, Mn, Ni) and trace toxic metal (Cd) in korarima seed and soil samples. Lead was found to be below the method detection limit in the samples analyzed in this study. The investigation of metal levels revealed that, there is a direct relationship between the korarima seed analyzed and the soil on which it is grown. Generally, lower

levels of the investigated metals were found in the korarima samples than the soil samples except for Ca because of the transportation of metals from the soil to the plant.

The recommended dietary allowances (RDA) as mg day^{-1} person $^{-1}$ for copper, iron, zinc and manganese are 2, 18, 15 and 5, respectively. The levels of copper, iron, and manganese in the samples were relatively higher than the RDA of Food and Nutrition Board of the National Academy of Sciences, United States. But, the level of zinc is in agreement with this RDA value. The safety limit for human consumption of Co is 0.05 to 1 mg/day in humans. Although there are no RDA value for cadmium, lead, nickel and chromium, some values are given by Food and Nutrition Board of the National Academy of Sciences, United States and other authorities. The recommended daily intake for chromium is 0.20 mg day^{-1}. Provisional tolerable intake for lead and cadmium is 0.21 and 0.06 mg day^{-1}, respectively. Average daily intake from food for nickel is 0.30 mg day^{-1}. The levels of Cd, Ni and Cr were also higher than the levels given above by World Health Organization and Food and Nutrition Board of the National Academy of Sciences-United States [62].

Generally, the consumption of spices in daily food is very negligible compared to other food items as they are added or used in very small quantities. Thus, the concentration level of metals is relatively small, particularly for these trace essential and trace toxic metals. This means that the level of these metal contents is in accordance with the RDA. However, special attention should be given to trace metals particularly to those trace toxic metals as they accumulate over time and cause serious health impacts. Moreover, there is a growing interest in the use of spices recently all over the world.

Therefore, further study of the relationship between the metal levels of the korarima plant and soil samples is recommended by taking large number of sites and different methods to come up with a comprehensive conclusion. Additional investigation on soil pH, nature of agricultural inputs like fertilizers, pesticides, herbicides are also recommended.

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