

**HAWASSA UNIVERSITY**  
**COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE**  
**DEPARTMENT OF CHEMISTRY**



**MSC THESIS**

**LEVELS OF SELECTED ESSENTIAL AND NON-ESSENTIAL METALS  
IN THE SOIL AND GINGER (ZINGIBER OFFICINALE) CULTIVATED  
IN KAMBATA ZONE, HADERO TUNTO WOREDA CENTRAL  
ETHIOPIA**

**BY**

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**ADVISOR: ADELEW ESTIFANOS (PhD)**

**OCTOBER, 2024**  
**HAWASA, ETHIOPIA**

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**A MASTER THESIS SUBMITTED TO THE SCHOOL OF GRADUATE  
STUDIES OF HAWASSA UNIVERSITY, COLLEGE OF NATURAL AND  
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PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE  
DEGREE OF MASTERS OF SCIENCE IN CHEMISTRY**

**OCTOBER, 2024  
HAWASA, ETHIOPIA**

## **DECLARATION**

This Thesis entitled “levels of selected essential and non-essential metals in the soil and ginger (*zingiber officinale*) cultivated in kambata zone, hadero tunto woreda Central Ethiopia is my original work has not been presented for a degree in any other university and that all sources or materials used for the thesis have been accordingly acknowledged.

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This thesis has been submitted for examination with my approval as university Adviser.

Advisor Name: Adelew Estifanos (PhD)

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**SCHOOL OF GRADUATE STUDIES**

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**APPROVAL SHEET-1**

This is to certify that the thesis entitled “levels of selected essential and non-essential metals in the soil and ginger (*zingiber officinale*) cultivated in kambata zone, hadero tunto woreda Central Ethiopia is submitted in partial fulfillment of the requirements for the degree of Master of science in chemistry the graduate program he Department of Chemistry, Hawassa University, and is a record of original research carried out by Beyene Bedebo ( PG ChemK/054/ 09 ), under my supervision, and no part of the thesis has been submitted for any other degree or diploma. The assistance and help received during the course of this investigation have been accordingly acknowledged. Therefore, I recommend that it is accepted as fulfilling the thesis requirements.

Dr. Adelew Estifanos

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Name of advisor

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**APPROVAL SHEET-2**

We, the undersigned, members of the board of Examiners of the final defense by Beyene Badebo have read and evaluated this thesis entitled “levels of selected essential and non-essential metals in the soil and ginger (*zingiber officinale*) cultivated in kambata zone, hadero tunto woreda Central Ethiopia” examined the candidate. This is therefore to certify that, the thesis has been accepted partial fulfillment of the requirements for the degree of Master of Science in Chemistry.

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## **ABSTRACT**

*Ginger is a common spice and condiment for many foods and beverage. Its extracts are widely utilized in the culinary, beverage, and confectionary industries for making product like liquors, biscuits , pickles, chutney, marmalade and ginger beer. The levels of essential (Ca, Zn, Cu, Co, Cr, Mn, and Ni) and non-essential (Pb) metals in ginger cultivated in four different sites of kembata zone Ethiopia and the soil where it was grown were determined by flame atomic absorption spectrometry. 0.5 g of oven dried ginger and soil samples were digested using 3 mL of HNO<sub>3</sub> and 2 mL of HClO<sub>4</sub> at 230°C for 2:30 h and a mixture of 6 mL aqua-regia and 2 mL H<sub>2</sub>O<sub>2</sub> at 250°C for 3 h, respectively. The mean metal concentration (mg/Kg dry weight basis) ranged in the ginger and soil samples, respectively, were: Ca (874–1253, 801–1550), Zn (4.16–7.1, 48–110), Cu (3.3–5.4, 8.4–13.3), and Mn (231–321, 527– 673), the other metals like Pb, Cr, Ni and Co were not detected in both the ginger and soil samples. There was good correlation between some metals in ginger and soil samples. This study revealed that gingers samples taken from sample site are good source of essential metals and free from toxic metal Pb, Cr, Co and Ni.*

**Keywords:** *Ginger;; Essential metals; Non-essential metals; Soil , Zingiber officinale*

# CHAPTER ONE

## 1. INTRODUCTION

### 1.1 Background of the Study

Ginger (*Zingiber officinale*) is a herbaceous tropical perennial belonging to the family Zingiberaceae. The Rhizome of ginger has been used as a drug in herbal traditions since antiquity as carminative or ant flatulent, diaphoretic, antispasmodic, expectorant, peripheral circulatory stimulant, astringent, appetite stimulant, anti-inflammatory agent, diuretic, and digestive aid. Moreover, it also imparts flavor and pungency to food and beverages and is particularly consumed as fresh paste, dried powder, slices preserved in syrup, candy, or flavoring tea [1] [2]. Ginger is homegrown to tropical India, South East Asia, Australia, and Japan, with the main center of diversity in Indo-Malaysia [3]. The cultivation of ginger was started in Ethiopia, during the 13th century when Arabs introduced it from India to East Africa [4]. It's the second most generally cultivated spice in Ethiopia, next to chilies. It's limited mostly within the wetter regions of Southern Nations, Nationalities and Peoples Regional State (SNNPRS) and a few parts of western Oromia [5]. Large-scale commercial production of ginger by farmers in SNNPRS is practiced especially in Boloso Bombe, at Wolaita Zone [6]. Plants store and transport metallic elements, both to supply appropriate concentrations of them for later use in metalloproteins or co-factors, and to guard themselves against the toxic effects of excess metals. Growth media including soil, nutrient solution, water, and air are the key sources of metals to vegetables and other crops, which enter by roots or foliage through two main bio-sorption mechanisms: adsorption and/or absorption and accumulated in their tissues [7] [8]. Metal uptake by plants is often suffering from several factors including metal concentrations in soils, soil pH, cation exchange capacity, organic matter content, types and diversities of plants, and age of the plant [9]. Major and minor elements are considered essential nutrients in food. The routine monitoring of the amount of these elements in crops is a common quality control process [10]. However, both deficiency and excesses of those essential trace elements give very serious problems in the human and animal body. Since ginger is a cash crop and one of the widely consumed spice in the study area and all over the world, the assessment of the levels of essential and heavy toxic metals have a particular interest with respect to human health and the quality of its products. Many kinds of researches were conducted on the determination of essential and

non-essential metal levels of ginger in Nigeria, India, Pakistan, and Romania [11] [12] [13] [14]. However, research related to the levels of essential and non-essential metals in ginger cultivated in Wolaita Zone, Southern Ethiopia was scarce. Therefore, this study will be focused on the determination of levels of essential (K, Na, Ca, Mg, Mn, Fe, Zn, Cu, Co) and non-essential (Cd and Pb) metals in ginger and its correlation with the concentration of metals in the supporting soil. It also aimed to assess some physico-chemical properties of the soil. Furthermore, the results of this study may help to identify the difference in mineral content of ginger with the area of cultivation and provide baseline information for researchers who need further investigations in this area.

## **1.2. Statement of the problem**

Ginger (*Zingiber officinale*) is an important tropical herbaceous perennial plant which has been used to treat many diseases, including gastrointestinal ulcers, cancer, arthritis, vomiting peptic duodenal; improve blood circulation, lowers blood glucose and cholesterol level [15]. Ginger is used as a spice around the world in both fresh and dried forms [16]. Chemical analysis indicates that ginger contains many active ingredients including terpenes, oleoresin, gingerols, shogaols, paradols, gingerone, vitamins and minerals [17]. Ethiopia is the homeland for many spices, including chiles, black cumin, ginger, fenugreek and coriander. Ginger is the second most widely cultivated spice next to chilies in Ethiopia [18]. It is known to have been cultivated in Ethiopia since 13<sup>th</sup> century [19]. In Ethiopia, ginger is the common spice added in many food stuffs [20]. In recent years, the intake of metals from food and water has become the potential health risk for the users [21]. Though metals are essential to humans and other living beings, they become noxious when their concentration higher than the thresholds [22]. Trace metals such as Cu, Zn, Fe, Mn and Cr are important for enzyme structuring; and synthesis hemoglobin and vitamin ; whereas metals such as Cd and Pb are toxic even at low concentration [23]. Heavy metals can be released into the environment through both natural and anthropogenic sources and transferred into human body through soil, dermal contact, breathing and food chain [24]. Prolonged use of unsafe heavy metals through food consumption can lead to chronic accumulation in the human kidneys and liver and cause toxicity [25]. Limited studies are available on the levels and health risks of metals in ginger growing regions of Ethiopia, especially Kambata Zone Hadero Tunto Woreda Central Ethiopia. There is no report so far about the levels of essential and non-essential metals for human in ginger residing Kambata Tambaro Zone, of Hadero Tunto Woreda Ethiopia.

## **1.3. Research question**

1. How is the optimum digestion procedure for the determination of selected metals in the soil and ginger (*zingiberofficinale*) cultivated in the study area by FAAS?
2. What is the magnitude of the level of essential and non-essential metals (Ca, Zn, Cu, Cr, Ni, Cd and Pb) in the soil and ginger (*zingiberofficinale*) cultivated in the study area?

3. What is the level of metals in soil samples where ginger (*zingiberofficinale*) cultivated at the study area?
4. Are the levels of metals in the spices with that of soil in which they have been cultivated in the study area correlated?
5. Is the concentration of heavy metals in the spice plants were below or beyond the permissible limit recommended by WHO?

## **1.4. Objective of the study**

### **1.4.1. General objective**

The main objective of this study is to quantitatively determine the levels of selected essential and non-essential metals in the soil and ginger (*zingiber officinale*) cultivated in Kambatas zone, Hadero Tunto Woreda Central Ethiopia..

### **1.4.2. Specific objectives**

- ❖ To develop an optimum digestion procedure for the determination of selected metals in the soil and ginger (*zingiberofficinale*) cultivated in the study area by FAAS.
- ❖ To determine quantitatively the levels of essential and non-essential metals (Ca, Zn, Cu, Cr, Ni, and Pb) in the soil and ginger (*zingiberofficinale*) cultivated in the study area
- ❖ To investigate the level of metals in soil samples where ginger (*zingiberofficinale*) cultivated. in the study area
- ❖ To correlate the levels of metals in the spices with that of soil in which they have been cultivated in the study area..
- ❖ To compare the concentration of metals determined with the acceptable limits proposed by WHO.

## **1.5. Significance of the study**

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 or monitoring of the levels of metals in the plants. Therefore, the outcome of this research work

is ultimately help to ensure the dietary safety and nutritional use of spice for society. It is also helpful to estimate the sources of metals and assess the pollution level or toxicological status of the spices cultivated in Ethiopia. It helps as a baseline data for the health policy makers in Ethiopian on the daily intake of the heavy metals consumptions. To initiate further studies on nutritional, medicinal and toxicological effects that can be caused due to the use of spices through diet.

### **1.6. Scope of the study**

The study is delimited to Kambata zone because the cultivation of Spice is paramount significant at the study area. Limited studies are available on the levels and health risks of metals in ginger growing regions of Ethiopia, especially Kambata Zone Hadero Tunto Woreda Central Ethiopia. There is no report so far about the levels of essential and non-essential metals for human in ginger residing Kambatas Zone, of Hadero Tunto Woreda Ethiopia.

## CHAPTER TWO

### 2. REVIEW OF RELATED LITERATURE

#### 2.1. Over view of spices

Spices are dried parts of plants, which are commonly used as diet components often to improve color, aroma, palatability and acceptability of food; has a hot taste and used to enhance taste of foods [1]. They are used as a food additive for the purpose of food flavorings and sometimes as a preservative by killing or preventing the growth of harmful bacteria [2]. Spices are also used as medicines and religious rituals in Asia and in the other part of the world [3].

Many common spices have also outstanding antimicrobial effects. On the other hand, the process of preparation and handling can make them the source of food poisoning [4]. With the current emphasis on eating more healthy diets that are low in fat and salt, people's attentions towards eating habits are turning to various herbs and spices to flavor their food. The culinary herbs and spices that are used to enhance the flavor of vegetables, soups and pasta dishes can be derived from the bark (cinnamon), fruits (black pepper), flowers (sattfron), seeds (korarima), rhizome or roots (ginger, turmeric) of a plant [5, 6].

There are many reasons for which people use spices, though taste probably tops the list. There are several spices that simply smell good and those smells can be alternately soothing or exciting. Some of the common types of spices include ginger, garlic, cloves, cinnamon, rosemary, thyme, marjoram, black pepper, anise, nutmeg, turmeric, peppermint, dill, cardamom and coriander. Besides adding to the taste, spices have multifarious functions that include combating food borne microorganisms, reducing food poisoning [7] antioxidant function [8] and antimicrobial activity [9-11], fight against cancer causing cells [12], reduction of cholesterol level in the blood and prevention of several skin diseases [13].

Plants need proper quantities of mineral elements for growth and these minerals are found in the soil. Some of these mineral elements are essential metals while others are non-essential metals for the plant growth [14]. On the other hand, it is a well-known fact that soil pollution is a global issue since the beginning of this century. The use of fertilizers, herbicides, pesticides, selected hybrid seeds, widespread irrigation, traffic pollution and industrial activities are some of the sources for trace metals to contaminate the soil [15]. In this respect, contamination of agricultural

soils and of the environment with heavy metals in the past few years has attracted public and scientific interest due to their dangerous effects on human health [16, 17]. Heavy metals may be present in agricultural soils at low levels but can be accumulated in plant through time. This has led researchers to deal with the pollution of air, water, soil and food with heavy metals and to avoid their harmful effects [18] and to determine their suitability for human consumption.

Researchers now days are greatly concerned with the determination of essential and non-essential metal levels on the medicinal value and chemical composition of essential oils of spices in country like Nigeria, India, Saudi Arabia, Poland, etc. There is no report on the assessment of metal contents of spices cultivated in Ethiopia. An investigation of plants for their metal contents is indispensable because a survey of literature indicates that such study is scarce in Ethiopia. However, several works have been conducted in different countries on the analysis of metal contents of Cardamom (*Elettariacardamomum*) [19], Large Cardamom (*Amomumsubulatum*) [20] and related spice family like Ginger (*Zingiberofficinale*) [21]. Essential trace metal (Zn, Mn, Cu and Fe) levels in plants of medicinal importance [22], determination of heavy metals in common spices [23], evaluation of the heavy metals contents in spices and herbs available on the Polish market [24], levels of selected heavy metals in some Nigerian vegetables [25], monitoring of cadmium and micronutrients in spices commonly consumed in Turkey [26], assessment of the level of trace metals in commonly edible vegetables locally available in the markets of Karachi city [27]. 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.

Ethiopia is among the largest consumer of spices in Africa [28]. The major use of spices is in the preparation of a highly spiced stew known as 'Wot' which together with 'Injera' is consumed by a large proportion of the population everyday as their main food. In addition, spices are also used by numerous ethnic groups in the country to flavor bread, meat, soups, different vegetables, and as medicines and perfumes [28]. Among the spices used in Ethiopia korarima (*Aframomumcorrorima*), ginger (*Zingiberofficinale*), turmeric (*Curcuma longa*) and black pepper (*Piper nigrum*) are the most common ones.

## **2.2 Origin, distribution and uses of korarima, ginger, turmeric and black pepper**

The wide range of climatic, ecological and topographic conditions makes, Ethiopia to be the origin and diversity of several plants like coffee and several grain species [29]. However, there

are also several plants that may have potential to be used as food, spice or medicine, but are yet to be known outside the local usage. Korarima is an important spice and medicinal plant in Ethiopia, but little is known outside the country. It is an indigenous spice of Ethiopia. Ginger, turmeric and black pepper are also widely used as spices in the country.

### **2.2.1. Korarima**

Ethiopian cardamom, or false cardamom, is obtained from the plant's seeds (usually dried) and is extensively used in Ethiopia and Eritrean cuisine [30]. 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, GamoGofa, DebuOmo, Sidamo, Jima, Illubabor and Wollega). Outside of these areas, it is cultivated in the vicinity of Lake Tana and Gelemso and in Eritrea [31].

The seed of korarima is mainly used as sources of spices in traditional Ethiopian dishes. It is the source of income for growers as its seeds fetches high prices in local and export markets. Parts of korarima are used in traditional medicine for humans and cattle. In addition, korarima is important plant for soil conservation as the rhizomes and leaves spread on the ground covering and protecting the soil from erosion in hilly areas the year around [32]. Occasionally they are also used to flavor coffee, tea, bread and butter. In Ethiopia, the seeds are used medicinally as a tonic, carminative and purgative. The arilloid flesh around the seed is edible [31]. The use of korarima is only known in Ethiopia and Eritrea [31].

Korarima is herbaceous, perennial and aromatic species classified in the monocotyledonous family Zingiberaceae, native to Ethiopia. The plant consists of an underground rhizome, a pseudostem and several broad leaves and resembles *Elettariacardamomum* species morphologically. Mature korarima can reach a height of 1-2 m. It sets seed after 3-5 years of planting depending on the planting materials used and it continue to bear seeds for a number of decades [33].

*Elettariacardamomum* is an important member of the family Zingiberaceae [33]. It is perennial herb with hairless leaves. The seeds have been widely used as a spice since the ancient times but are also an important Ayurvedic aphrodisiac and remedy in case of digestive problems, asthma, bronchitis and urinary complaints. It is used against bad breath, cough and nausea and may be

combined with laxative to treat indigestion, stomach pain, griping and flatulence. The seeds contain essential oil in concentration of about 4% of dry weight. The main compound is 1,8-cineole (representing 50% or more), with smaller amounts of  $\alpha$ -terpineol and limonene [31].

### **2.2.2. Ginger**

Ginger (*Zingiberofficinale*) is a herbaceous tropical perennial, the rhizomes of which is used as spices and it belongs to the family Zingiberaceae [34]. For long period of time, ginger has been widely used as a dietary supplement spice and a medicinal herb in traditional medicine [35]. Ginger is originated in the Southeast Asia [36] and then used in many countries as a spice and condiment to add flavor to food.

It contains a host of compounds, which include acid resins, vitamin C compounds (folic acid, inositol, choline and panthothenic acid), gingerol, sesquiterpene, vitamins B3 and B6, volatile oils, and bio-trace elements (Ca, Mg, P and K). The pungency of Ginger is due to gingerol, while its aroma is due to the volatile oils, which are bisaboline, zingiberene and zingiberol [37].

Ginger has been used in treating diarrhea, nausea, asthma and respiratory disorders. In addition to their medicinal activities, Zingiberaceae plants extracts also serve as a natural larvicidal agent [38].

The rhizome of ginger is one of the most widely used species of the family Zingiberaceae, is a common condiment for various foods and beverages in Ethiopia. The major ginger growing area in Ethiopia includes wetter regions of altitude bellow 2000 m in Kefa, Illubabur, GamoGofa, Sidama, Jima and Wollega. Large scale production and marketing of ginger are also reported from Wolaita, Kembata and Tambaro [28].

### **2.2.3. Turmeric**

Turmeric (*Curcuma longa*) is a perennial herb widely cultivated in tropical regions of Asia and Central America. It belongs to the family of Zingiberaceae. Turmeric has been used as coloring and flavoring agent and spice in many foods [39].

Turmeric is widely consumed in different countries for a different purposes, including a dietary spice, a dietary pigment, pharmaceutical industries and a folk medicine for the treatment of

various illnesses such as biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis [40].

There are different varieties of turmeric species growing in different countries all over the world. The most common turmeric species are *Curcuma aromatica*, *Curcuma caesia*, *Curcuma longa*, *Curcuma amada* and *Curcuma zedoary* [41].

*Curcuma longa* is the most chemically investigated species of Turmeric. To date, at least 235 compounds, primarily phenolic compounds and terpenoids have been identified, including diarylheptanoids (commonly known as curcuminoids), diarylpentanoids, monoterpenes, sesquiterpenes, diterpenes, triterpenoids, alkaloid, and sterols, etc. [42]. It is also cultivated by farmers in Ethiopia.

#### **2.2.4. Black pepper**

Black pepper (*Piper nigrum*) is a flowering vine in the family Piperaceae, cultivated for its fruit, is used as a spicy condiment in foods throughout the world and has high economic value. It is cultivated in southern parts of India and in Indonesia. It is known for its pungent taste, aroma and flavor and is used in Ayurvedic, herbal and folklore medicines [43]. It is an aromatic carminative stimulant. It contains antioxidant constituents and possesses anti-inflammatory and antimicrobial properties [44]. The spiciness of black pepper is due to the chemical piperine. Black pepper has long been recognized as a carminative, (a substance that helps prevent the formation of intestinal gas), a property likely due to its beneficial effect of stimulating hydrochloric acid production. In addition, black pepper has diaphoretic (promotes sweating), and diuretic (promotes urination) properties. Black pepper is an excellent source of manganese, a very good source of iron, calcium and vitamin K, and a good source of dietary fiber [45].

**Table 2.1: Scientific and common names of studied spice plants**

English name	Local name (Amharic)	Scientific name	Family	Used part
False cardamom	Korarima	Aframomumcorrorima	Zingiberaceae	Seed
Ginger	Zinjible	Zingiberofficenale	Zingiberaceae	Rhizomes
Turmeric	Irid	Curcuma longa	Zingiberaceae	Rhizomes
Black pepper	Kondo berbere	Piper nigrum	Piperaceae	Fruit

### 2.3 Chemical nature of spices

Spices are storehouses of many chemically active compounds that impart flavor, fragrance and piquancy [46]. Most spices owe their flavoring properties to volatile oils and in some cases, to fixed oils and small amount of resin, which are known as oleoresins. Most probably, no single compound is responsible for flavors; but a blend of different compounds such as alcohols, phenols, esters, terpenes, organic acids, resins, alkaloids, and sulphur containing compounds in various proportions produce the flavors. Beside these flavoring components, every spice contains the usual components such as proteins, carbohydrates, fiber, metals, tannins or polyphenols. Essential oils or extracts are also derived from these plant sources either as a primary processing or a secondary opportunity [47].

### 2.4. Heavy metals

Heavy metals are defined as metals having a density higher than 5 g/cm<sup>3</sup> of the total 90 naturally occurring elements, 53 are considered heavy metals and few are of biological importance. Based on their solubility under physiological conditions, 17 heavy metals may be available to living cells and have significance for the plant and animal communities within various ecosystems [48].

#### 2.4.1. Accumulation of heavy metals in plants

Soil contamination by heavy metals as a result of human activities is a serious environmental issue all over the world. Mining, smelting of metalliferous ores and metal scraps, electroplating, application of fertilizer and pesticides, sludge dumping and generation of municipal waste have been identified as the principal sources of soil contamination by heavy metals. Failure to mitigate

high heavy metal concentrations in soils may result in mobilization of heavy metal contaminants into the flora and fauna and subsequently into man with consequent deleterious health effects [49].

Accumulation of essential and non-essential metals in soil-plant system has become a global concern, as deficiency or excess of these metals poses a health threat to humans and other organisms when accumulated within the biological system. Edible crop plants grown on soils enriched with metals may be accumulating these metals to levels that may be deleterious to human health when consumed over time [50].

In general, most plants grow by absorbing nutrients from the soil. Their ability to do this depends on the nature of the soil. A soil contains some combination of sand, silt, clay, and organic matter. This combination depends on its location. Soil texture and its pH determine the extent to which nutrients are available to plants. The path taken by metal to transport into the plant is: soil > roots > stems > leaves.

## **2.4.2. Factors affecting the bioavailability of heavy metal to plants**

### **2.4.2.1. pH**

Soil pH is one of the factors which influence the bioavailability and the transport of heavy metals in the soil and it is accepted that as pH decreases, the solubility of cationic forms of metals in the soil solution increases and, therefore, they become more readily available to plants. According to Smith and Giller (1992) heavy metal mobility decreases with increasing soil pH due to precipitation of hydroxides, carbonates or formation of insoluble organic complexes, which are the main mechanisms of metal retention to soils. The amount of heavy metals mobilized in soil environment is a function of pH, properties of metals, redox conditions, soil chemistry, organic matter content, clay content, cation exchange capacity and other soil properties. Heavy metals are generally more mobile at  $\text{pH} < 7$  than at  $\text{pH} > 7$  [51].

### **2.4.2.2. Organic matter**

Organic matter accumulates at the soil surface, mainly as a result of decomposing plant material. Whilst the organic matter content of soils is often small compared to that of clay, the organic fraction has a significant influence on metal binding [52]. Metal ions can be complexed by organic matter altering their availability to plants. The COO

groups in both solid and dissolved organic matter form stable complexes with metals. Hence, as the amount of organic matter present in soil increases the opportunity for forming stable metal-organic matter complexes increases. In general, plants are unable to absorb the large metal-complexes and so the bioavailability of metals decreases [52].

#### **2.4.2.3. Toxicity of heavy metals on human health**

Heavy metals have positive and negative roles on human health. Some of the heavy metals are considered essential including calcium, zinc and copper, while metals like cadmium, lead and chromium have toxic roles in biochemical reactions on our body [53].

Heavy metal-polluted food can severely reduce some vital nutrients in the body that are accountable for declining immunological defenses, growth delay, reduced psychosocial abilities, incapacities related with malnutrition and greater occurrence of upper gastrointestinal cancer degrees, encourage tumor and mutations at greater amounts in animals, harm to germ cells of both male and female animals, relentlessly infuriates the stomach which results in diarrhea and vomiting. Metals are non-decomposable and are recognized as main environmental contaminants causing cytotoxic, mutagenic and cancerous (carcinogenic) effects in animals [54]. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Chronic level ingestion of toxic metals has undesirable impacts on humans and the associated harmful impacts become perceptible only after several years of exposure [55].

Exposure to trace and heavy metals above the permissible level affects human health and may result in illness to human fetus, abortion and preterm labor, as well as mental retardation to children, among others. Adults also may experience high blood pressure, fatigue kidney and neurological disorders. The study of trace metals in the food chain is crucial because they have potential hazardous effects. Spices and food condiments are readily consumed in large amounts in different countries. Trace element food composition data are important for both consumers and health professionals [47].

#### **2.4.2.4. Classification of minerals**

Minerals are usually classified into two main groups on the basis of their relative amounts in the body. One of the groups is macro elements or macro minerals occurring in relatively large amounts and needed in quantities of 100 mg or more per day which include calcium

magnesium, potassium and sodium. Minerals occurring in small amounts and needed in quantities of a few milligrams or less per day are called microelements or trace elements, which includes zinc, copper, nickel and chromium. Other trace metals like aluminium, lead, cadmium, mercury and arsenic are till now recognized as potentially harmful. Actually all essential elements may also be toxic in animals and humans if ingested at sufficiently high levels and for a long enough period [56]. The physiological role of Ca, Cu, Zn, Cr, Ni, Cd, and Pb are briefly described below.

Calcium is required for the normal development and maintenance of the skeleton. It is stored in the teeth and bones, where it provides structure and strength [57]. Calcium is also important as a positive ion ( $\text{Ca}^{2+}$ ) in blood clotting, muscle contraction, and nerve impulse transmission. Excessive intakes of Ca induce constipation and place up to half of otherwise healthy hypercalciuric males at increased risk of urinary stone formation. A high calcium intake may inhibit the intestinal absorption of iron, zinc, and other essential minerals [58]. Its deficiency is found to result in stunted growth, rickets, and osteoporosis Cadmium is a cumulative toxin. Its levels in the body increase over time because of its slow elimination. It accumulates chiefly in the liver and kidneys, causing mutations, DNA strand breaks, chromosomal damage, cell transformation and impaired DNA repair in cultured mammalian cells. Cadmium is known to modulate gene expression and signal transduction and is also a known testicular and prostate carcinogen [59].

Chromium is one of the known toxic pollutants in the world. At an elevated concentration it is toxic for both plant and animals. The problems that are associated with chromium involve skin rashes, stomach ulcer, lungs cancer and ultimate death [60]. The acute toxicity of chromium (VI) is due to its strong oxidation properties. After it reaches the blood stream, it damages the kidneys, the liver and blood cells through oxidation reactions, resulting into haemolysis, renal and liver failure. Aggressive dialysis can improve the situation [59].

Copper is an essential trace metal for animals and human beings and have diverse functions in plants. It is required in the formation of erythrocytes and hemoglobin as well as some enzymes like tyrosinase. Copper is also involved in bone and elastic tissue development [61]. The deficiency of copper results anemia related to defective iron metabolism, skeletal defects, affect the central nervous system and the immune and cardiovascular systems notably in infants,

defects in pigmentation and structure of hair or wool, reproductive failure, and decreased arterial elasticity [61]. Excess intake of copper can cause vomiting, nervous system disorder and Wilson's diseases.

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 [53].

Nickel also plays some roles in body functions including enzyme functions. Nickel occurs naturally more in plants than in animal flesh. In very trace amounts, it may be beneficial to activate some enzyme systems, but its toxicity at higher levels is more prominent. However, nickel toxicity in humans is not a very common occurrence because the absorption of nickel is very low [53]. Low concentrations of Ni are beneficial for plant growth and development, respiration intensity and photosynthesis, as well as for the activity of antioxidant enzymes [60].

Lead has been shown to have toxic impact on a variety of metabolic processes essential to plant growth and development, including photosynthesis, transpiration, DNA synthesis, and mitotic activity. Lead, on the other hand, is known to exert its most significant effect on the nervous system, including motor disturbances, sensory disturbances, the haematotopic system and the kidney and ultimately, can cause major brain damage. Lead ingestion has been associated with deleterious health effect, including disorder of the central nervous system [59]. Sources of lead include metal smelting, pigments, lead battery manufacturing and lead contaminated petrol. In soil lead tightly binds itself to organic soil particles which may decrease the mobility of lead in most soils and may reduce uptake by plants. It has been suggested that the mobility of lead and copper is greater in sandy soils, which tend to lack organic matter, than in organic soils [60].

#### **2.4.2.5. The tolerable daily intake approach of heavy metal**

In view of avoiding undesirable health hazards consequent of excessive intake of toxicants like heavy metals or toxic metals, international and national scientific organisms such as FAO/WHO, European Union, etc have used the safety factor approach for establishing acceptable or tolerable

intakes of substances that exhibit threshold toxicity. The acceptable daily intake (ADI) or tolerable daily intake (TDI) or provisional tolerable weekly intakes (PTWI) are used to describe safe levels of intake for several toxicants including toxic metals. For chemicals that give rise to such toxic effects, a tolerable daily intake (TDI), i.e. an estimate of the amount of a substance in food, expressed on a body weight basis (mg.kg<sup>-1</sup> of body weight) that can be ingested over a lifetime without appreciable health risk. Exposure exceeding the TDI value for short periods should not have deleterious effects upon health. However, acute effects may occur if the TDI is substantially exceeded even for short periods of time. Besides, contaminants possessing very long half-lives can be accumulated in the body and chronic effects are most often observed when critical concentrations are reached in target tissues [62].

## **2.5 Sample preparation techniques for metal analysis in the plant materials**

### **2.5.1. Methods of sample decompositions**

For solid samples such as plant samples, the solid form must be transformed to liquid phase. The process known as digestion is required for the spectroscopic analysis of metals. The principle is the releasing of metals from the solid matrix to the acid solution during the extraction process. Conventional acid leaching procedures and microwave assisted acid digestion systems are used to prepare the sample for further spectroscopic analysis. The conventional procedures are open systems in which the solid material is extracted on a heating source in the presence of acid and/or acid mixtures [63]. The purpose of sample preparation is for converting all the species in which a given metal is present in such a way that it becomes present in one defined form, eliminating interfering substances from the matrix, and obtaining the metal in a homogeneous and easily accessible matrix [64].

#### **A. Wet digestion**

Wet digestion involves the utilization of mineral or oxidizing acids and an external heat source to decompose the plant samples matrix in open or closed vessels. The choice of the acid or the combination of the acids depends upon the nature of the matrix to be decomposed. Open systems are useful for samples such as food or agricultural products, but generally unsuitable for some samples that require lengthy dissolution times. Nitric acid is used as oxidant alone or in combination with other acids or sometimes with hydrogen peroxide, hydrochloric acid and

sulfuric acids, perchloric acids. Nitric acid is popular because of its chemical compatibility, oxidizing ability, availability, purity and low cost [65]. This yields a clear solution containing the metals for analysis by techniques such as AAS, ICP, ICP-MS or FAAS. The procedure could take place either in open system or closed system (bomb decomposing with conventional or microwave heating). Wet digestion has the advantage of being effective on both inorganic and organic materials, thus it often removes or minimizes the sample matrix, thus helping to reduce or eliminate some of the interferences [66]. In addition the method is simple, fast and inexpensive compared to other techniques [64].

## **B. Dry ashing**

For plant samples that contain organic matter, which are being analyzed for nonvolatile metals, dry ashing is a relatively simple method for removing the organic matter that can be used for relatively large samples and requires shorter time of the entire analysts time. In the open vessel method, the sample is placed in a suitable crucible and is ignited in a muffle furnace. Crucibles used for ashing are usually made of silica, porcelain, platinum or Pyrex glass. After decomposition the residue is dissolved in acid and transferred to a volumetric flask. Typical ashing temperatures are 450 to 550 °C. Dry ashing is also conducted at 50-100 °C under reduced pressure in an oxygen plasma discharge. The major drawbacks of the method are the possible loss of some elements by volatilization, contamination of the sample by airborne dust, as it must be left open to the atmosphere and irreversible sorption of analyte into the walls of the vessel [67]. The most important advantages of the ashing are the ability to decompose large sample sizes and dissolving the resulting ash in a small volume of acid, and the need for little or no reagents.

## **2.6. Determination of metal contents within plant materials**

Metals contained in samples are determined by a wide variety of analytical methods, with the choice often depending on the precision and sensitivity of the instruments. Both essential and nonessential metals can be determined by various spectroscopic or chromatographic methods, such as: Flame Atomic Absorbance Spectrometry (FAAS) or Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), Atomic Emission Spectrometry (AES), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Inductively Coupled Plasma Mass spectrometry (ICPMS), X-ray fluorescence (XRF), and Ion chromatography (IC) [67].

## **CHAPTER THREE**

### **3. METHOD AND MATERIAL**

#### **3.1. Chemicals and reagents**

Chemicals and reagents used in this study were all of analytical grades: 69-72 % HNO<sub>3</sub>; sigma Aldrich (Steinheim, Germany), 70 % HClO<sub>4</sub>; Research Laboratory Fine Chemical Industry (Mumbai, India), 30 % H<sub>2</sub>O<sub>2</sub>, BDH Chemicals Ltd (Poole, England) and 36-38 % HCl Sigma Aldrich (Steinheim, Germany), were used for the digestion of target analytes in different matrix. Stock standard solution (Buck Scientific Puro-Graphic, USA) having concentrations of 1000 mg/L were used for preparation of serially diluted working standard solutions of each metals for calibration. Deionized water (chemical pure below 1.5 µS/cm) was used for sample preparation, dilution and rinsing the apparatus prior to analysis.

#### **3.2. Instrumentation and apparatus**

Air circulating oven was used for air drying of samples, ceramic mortar and pestle were used for grinding and homogenizing the ground samples, digital electronic balance (PW-254, UK) was used for weighing the samples. pH meter (pH 211 HANNA instrument) was used during pH measurement of the soil samples. Metal content of the plant and soil samples were determined by flame atomic absorption spectrophotometer (FAAS) (BUCK Scientific 210 VGP Atomic Absorption Spectrophotometer) by using hollow cathode lamps with air-acetylene flame.

#### **3.3. Cleaning of apparatus**

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. Then, the apparatuses were soaked with about 10 % (v/v) nitric acid for 24 hours followed by rinsing with distilled deionized water. Lastly, the glassware were then dried in hot air oven and stored in clean dry places free of contamination till use.



### **3.4.2. Collection and preparation of samples**

Matured fresh ginger samples were collected by hands protected with vinyl gloves. About 1 kg samples were bought and collected from farmers land. From a particular main site, four sub-sites were taken for the purpose of random sampling. About 250 g of the sample was taken from each sub-sites and then mixed in to a single polyethylene plastic bags to get 1 kg of one bulk sample. Then the collected samples were carefully packed in polyethylene bags, labeled and transported to laboratory for further treatment. Only the edible parts of each plant were used for analytical processes. All plant samples were grounded sieved (0.425 mm) and homogenized by mortar and pestle. Dried, sieved and homogenized samples were kept in clean polyethylene bags and stored in a desiccators till digestion. Soil samples were collected from 0 - 15 cm depth, from the spices plants origin with an auger (made of stainless steel). The collected soil samples were air dried followed by oven drying to obtain constant weight, grounded and sieved (0.425 mm) into coarse and fine fractions: the fine fractions were well mixed and homogenized by mortar and pestle.

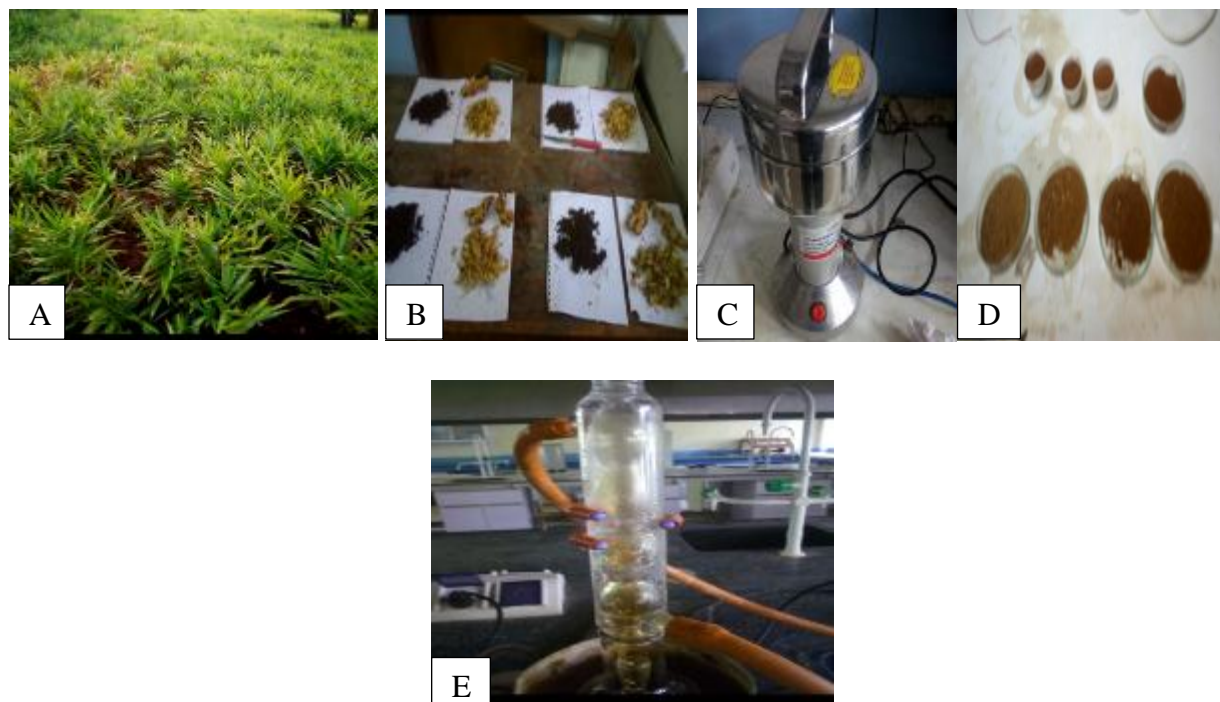
### **3.5. Optimization of the digestion procedure of plant material and soil samples**

Different digestion procedures for the digestion of the spice plant samples were carried out using  $\text{HNO}_3$ ,  $\text{HClO}_4$  and  $\text{H}_2\text{O}_2$  acid mixtures by varying parameters such as volume of the acid mixture, digestion time and digestion temperature as well as in environmental friendly method. Optimized procedures were selected based on the usage of lesser reagent volume, shorter digestion time and reasonably mild temperature for obtaining clear solutions of the resulting digests.

#### **3.5.1 Digestion of ginger samples**

For digestion purpose, 0.5 g of powdered, sieved and homogenized ginger samples were weighed and transferred into a 100 mL round bottom flask figure 2. To this, a freshly prepared 3 mL concentrated  $\text{HNO}_3$  (69-72 %), 2 mL of  $\text{H}_2\text{O}_2$  (30 %) and 2 mL of  $\text{HClO}_4$  (70 %) were added based on the optimized procedure. The mixture was then digested on heating mantle using reflux apparatus fitting the flask to a reflux condenser by setting the temperature to (120 OC) for 30 min followed by dialing at (250 OC) for 150 min until a clear and colorless solution was obtained following the optimized digestion procedure . After a total of 02:30 hr, 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, 10 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 volumetric flask with a Whatman Number 41 filter paper to remove any suspended or turbid matter. Subsequent rinsing of the filter paper with distilled-deionized water was carried out for diluting the sample to the final volume of 50 mL. For all plant 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.



**Figure 2. Experimental procedure for digestion of ginger and soil sample**

(A) ginger sample of experimental site (B) drying of ginger sample (C) grinding of ginger sample (D) powder of ginger sample (E) digestion of ginger sample

### **3.5.2. Digestion of soil samples.**

In this procedure, 0.5 g of the soil sample was placed in flask and 6 ml of aqua-regia (3:1 ratio of HCl to HNO<sub>3</sub>) was added first before adding 2 ml of concentrated H<sub>2</sub>O<sub>2</sub>. The sample was swirled gently to homogenize then fitted to a reflux condenser and digested continuously for 3 hours on a reflux digestion block. The temperature was adjusted within certain time intervals to give the maximum temperature of 250°C. The digestion produces a clear colorless solution. The

digest was then cooled and a few drops of water were added before filtering using a Whatman No 42 filter paper. The filtrate was then diluted with de-ionized water to 100 ml and stored for further analysis [18].

### **3.6. Instrument calibration**

The data qualities obtained from FAAS for metal analyzes are highly affected by the calibration and standard solution preparation procedures. The instrument was calibrated using four series of working standard solutions. The working standard solutions of each metal were prepared freshly from the intermediate standard solutions (10 mg/L) which were prepared by diluting stock solutions (1000 mg/L) of the analyte metals. Working standards of metal solutions were prepared in 50 mL volumetric flasks by diluting with distilled deionized water.

### **3.7. Method detection limit**

Detection limit is the lowest concentration level that can be determined to be statistically different from an analyte blank [68] or the minimum concentration that can be detected by the analytical method with a given certainty [68]. For a measurement, detection limit can be properly estimated from the standard deviation of several blank determinations [69].

Blank plant and soil samples were digested in triplicate following the same procedure that has been utilized for plant and soil sample material digestion. Each of the blank samples was analyzed for their metal content namely: Ca, Co, Cu, Zn, Pb, Cr, Mn and Ni by FAAS. The pooled standard deviation of the replicate blanks was calculated from the three blank measurements to determine the method detection limit (MDL). It was calculated according to the equation indicated below.

$$\text{MDL} = 3\text{SDB}$$

Where SDB the standard deviation of the replicate measurements of the analytes in the blank.

### **3.8 Evaluation of analytical performance**

Recovery of the analytical method is one of the most commonly used techniques for validation of the analytical results. It is the technique used for evaluating how far the analytical method is acceptable for its intended purpose. Because of the absence of certified reference material for the spice plants and soil samples in the laboratory, the analyses were carried out, validity of the

digestion procedures, employed for the investigation, were assured by spiking the samples with a standard solution of known concentration of the analyte metals.

The spiking of plant and soil samples digested separately in triplicate following the same procedure used for digestion of the plant and soil samples. The resulting digest of the spiked samples were then analyzed for their respective metal contents using FAAS. From the recovered quantity of the analyte, percent recoveries were calculated both for the plant and soil samples (Table 2 and 3) respectively, using the following equation:

$$\% \text{ Recovery} = \frac{\text{Amount of the analyte recovered}}{\text{Amount of the analyte added}} \times 100$$

### **3.9 Analysis of the soil pH**

Soil pH was measured by potentiometric by a pH meter, in a suspension (1:2.5, w/v) of the soil and distilled-deionized water. Five gram of air dried soil (<0.425 mm) was weighed and transferred into 100 mL beaker and 12.5 mL distilled deionized water was added. The mixture was stirred by magnetic stirrer and the pH was measured after allowing the suspension to stand for a while at room temperature [70]. pH measurement of the soil samples was performed in triplicates.

### **3.10 Analysis of electrical conductivity of soil**

The EC of the soil was measured by conductometry in suspension of (1:5) soil: double distilled water. Five gram (5 g) of soil sample was measured and placed in a beaker, to this soil sample 25 ml of double distilled water was added and stirred by magnetic stirrer to make the soil sample solution uniform and homogenizes. EC was measured after allowing the suspension to stand for five minute at room temperature [70]. EC measurement of the soil samples was performed in triplicate and the mean values have been reported

## CHAPTER FOUR

### 4. RESULTS AND DISCUSSION

#### 4.1. Investigative Method Detection Limits

The detection limit for the developed methods was calculated by multiplying the standard deviation of six blank signals each determined in triplicate by three. The calculated Method Detection Limit (MDL) for ginger and soil samples is given in Table 4.1.

**Table 4.1: Method detection limit for ginger and soil**

Metal	Instrumental detection limit(mg/L)	Method detection limit for ginger(mg/L)	Method detection limit for soil (mg/L)
Pb	0.040	0.133	0.212
Co	0.050	0.091	0.090
Ni	0.020	0.113	0.064
Ca	0.010	0.072	0.166
Zn	0.005	0.036	0.045
Cr	0.040	0.051	0.112
Mn	0.030	0.045	0.066
Cu	0.005	0.052	0.052

#### 4.2. Evaluation of Analytical Method

As presented in Table 4.2 and Table 3, the percentage recovery for ginger and soil samples found in the range of 96.6 % - 103.5 %, which are within the acceptable range for metals [71]. The results confirm that the method is of good exactness and accuracy.

**Table 4.2. Recovery test for the optimized procedure of ginger sample**

Metal	Concentrations in the sample (mg/kg)	Amount added (mg/kg)	Conc. in spiked sample (mg/kg)	% Recovery	RSD
Cu	4.16 ± 0.057	10	14.53 ± 0.15	103.5 ± 1.15	1.11
Zn	7.1 ± 0.1	10	16.93 ± 0.15	96.66 ± 5.13	5.3
Ca	874 ± 0.577	50	924.3 ± 0.2	100 ± 0.4	0.4
Mn	232.33 ± 1.52	50	282.3 ± 0.2	100.2 ± 0.52	0.52

**Table 4.3: Recovery test for the optimized procedure of soil sample**

Metal	Concentrations in the sample (mg/kg)	Amount added (mg/kg)	Conc. in spiked	% Recovery	RSD
Cu	12.33 ± 0.57	10	22.2 ± 0.1	99.33 ± 1.52	1.53
Zn	82.33 ± 0.57	100	182.2 ± 0.15	100.3 ± 0.52	0.15
Ca	874.66 ± 1.15	100	901.3 ± 0.15	100.6 ± 0.15	0.15
Mn	673 ± 1.73	100	772.5 ± 0.36	100.2 ± 0.40	0.40

### 4.3. pH and Electrical conductivity of Soil

Soil pH and its carbon-based matter content are amongst those main factors that significantly affect the availability of macro- and micronutrients and yet toxic elements in the soils and their uptake by plant roots [72]. The pH and Electrical conductivity of soils that affect nutrient buildup in the soil and their accessibility to the plants were given in Table 4.4. The pH of studied soil samples was found to be within the range of 5.06 to 6.53 in which higher value observed in hadero lower value and a higher value was observed in Lalo . [73]. The higher acidity of the soils was mainly owing to the leaching of some basic cations [74] and probably due to poorly managed cultivation, inappropriate use of ammonium- based fertilizers, and accelerated erosions that implied the deterioration of soil quality [75]. The electrical conductivity of the soil was ranged from 0.03 to 0.08 dS/m. The low EC value was a result of the high rainfall of the area

leaching much of the salts in the sites. the low EC value was mainly because of the acidic nature of the soil under investigation [76].

**Table 4.4 pH and electrical conductivity of soil**

Sample site	pH	EC (dS/m)
Hader	5.06 ± 0.15	0.03 ± 0.01
Lalo	6.53 ± 0.11	0.05 ± 0.01
Checho	5.93 ± 0.05	0.08 ± 0.005
Mugunga	5.73 ± 0.05	0.043 ± 0.005

#### 4.4. Spreading of Metals in Ginger Samples

The levels of essential and non-essential metals in the ginger samples determined with FAAS after sample dissolution with the optimized digestion procedure were articulated per dry weight as shown in Table 4.5 and Figure 1. Ginger contains a higher amount of essential metal Ca (876.6 – 1253 mg/kg), followed by Zn (4.16 – 7.1 mg/kg). The higher level of Ca, and Zn in the ginger was probably due to the fact that nutrient elements such as N, P, S, and Ca, are highly mobile in the plant tissue [41]. The other probable reason for the higher concentrations Ca is due to the presence of fertilized soil with manure and organic residues which is rich with these metals [77]. The concentration of Mn (231.3 - 321 mg/kg) in the ginger was higher among determined essential trace metals. Higher levels of Mn in the ginger may be attributed to the availability of this micronutrient in relatively acidic soils of the farmland. The chemical forms of Mn existing in the soil depend on the soil pH. In acidic soil, an easily absorbed form,  $Mn^{2+}$  released from soil by  $H^+$ , which is produced from  $NH_4^+$  [78], The observed trend was  $Ca > Mn > Zn > Cu$  and  $Co$ , Cr, Pb and Ni were bellow detection limit in ginger samples.

**Table 4.5. Concentration (Mean  $\pm$  SD, n = 3 in mg/kg dry weight) of metals in ginger sample from four sample sites.**

Metals	Hadero	Lalo	Checho	Mugunga
Cu	4.16 $\pm$ 0.05	3.36 $\pm$ 0.208	5.4 $\pm$ 0.1	3.5 $\pm$ 0.173
Zn	7.1 $\pm$ 0.1	5.46 $\pm$ 0.152	6.43 $\pm$ 0.15	4.16 $\pm$ 0.057
Ca	874.6 $\pm$ 1.15	1148 $\pm$ 1.154	1253 $\pm$ 0.57	1181.3 $\pm$ 1.52
Mn	232.3 $\pm$ 1.15	231.3 $\pm$ 1.52	321 $\pm$ 1.15	253.3 $\pm$ 0.57
Pb	ND	ND	ND	ND
Ni	ND	ND	ND	ND
Cr	ND	ND	ND	ND
Co	ND	ND	ND	ND

**ND = not detected**

#### **4.4.1 Distribution of metals in ginger sample**

##### **4.4.1.1. Calcium**

The mean concentration of Calcium was ranged from 874 to 1253 mg/kg in ginger. The highest level of calcium in ginger was observed in Checho and the lowest concentration was found in Hadero. The pattern of concentration of calcium in ginger among different sampling sites was in the order of Checho > Mugunga > Lalo > Hadero Comparable levels of Ca (1031 mg/kg and 1161 mg/kg) in the ginger were reported from Pakistan and Nigeria respectively [79].

##### **4.4.1.2. Manganese**

The concentration of Manganese in ginger was ranged from 231 to 321 mg/kg. The highest level of Mn was observed in Checho and the lowest concentration was found in Lalo. The result obtained is comparable with the value obtained in the literature [80]. Higher Mn levels in the studied plant may be attributed to the availability of this metal in relatively acidic soils. The concentration of Mn in ginger obtained from checho was significantly higher than those sampled from the other sites. This may be due to the difference in the type of agricultural practice in Checho

#### 4.4.1.2. Zinc

As specified in Table 5, the level of Zn in ginger was ranged from 4.16 to 7.1 mg/kg. The highest mean concentration of Zn in ginger was found in Hadero and the lowest concentration was observed in Mugunga. Comparable levels of Zn in ginger 13.93 mg/kg have been reported by [81]. Zinc levels in this study were also below permissible values of 60 mg/kg in vegetables [82] implying that there was no Zn contamination in ginger.

#### 4.4.1.4 Copper

The concentration levels of Copper in the soil ranged from 3.36 to 5.4 mg/kg. The highest mean concentration values were found in Checho and low mean concentration were found in Lalo. The difference in Cu levels in the sampling sites could be attributed to the difference in their parent materials.

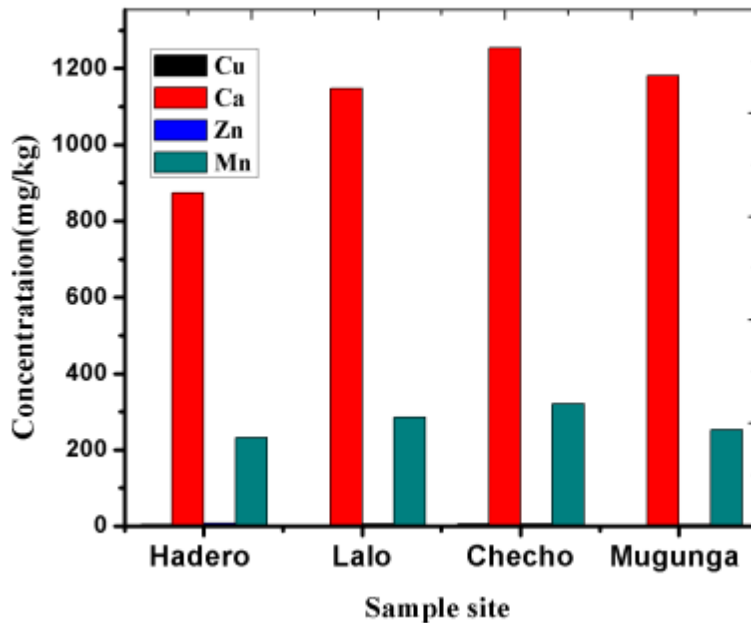


Figure 3: Level of Cu, Ca, Zn and Mn in ginger sample

#### 4.5. Spreading of Metals in Soil Samples

The levels of essential and nonessential metals in the soil samples determined with FAAS after sample dissolution with the optimized digestion procedure were expressed per dry weight as shown in Table 4.6 and Figure 2. The soil sample collected from four sampling areas was found

to contain measurable metal content of Ca, Mn, Cu, and Zn. There was a significant difference in the concentration of different metals within the soil samples and appreciable differences recognized between the same metals of from sample sites. Concentration of Ca (801 - 1550 mg/kg) in the soil was higher when compared to Zn (48.2 – 110.6 mg/kg) and Mn (527 - 573 mg/kg). In general, the concentration pattern of metals in soil was decreased as Ca > Mn > Zn > Cu. Among the analyzed metals, Co, Ni, Cr and Pb were found to be below the detection limit of the method used in this study.

**Table 4.6 Concentration (Mean  $\pm$  SD, n = 3 in mg/kg dry weight) of metals in soil samples**

Metals	Hadero	Lalo	Checho	Mugunga
Cu	12.3 $\pm$ 0.57	13.3 $\pm$ 1.15	8.4 $\pm$ 0.15	9.3 $\pm$ 0.2
Zn	82.2 $\pm$ 0.57	95.3 $\pm$ 1.52	48.2 $\pm$ 0.57	110.6 $\pm$ 0.57
Ca	801 $\pm$ 1.52	900 $\pm$ 0.57	949.6 $\pm$ 1.52	1550.6 $\pm$ 2.08
Mn	673 $\pm$ 1.73	619 $\pm$ 1.15	527 $\pm$ 1.52	577 $\pm$ 1.52
Pb	ND	ND	ND	ND
Ni	ND	ND	ND	ND
Cr	ND	ND	ND	ND
Co	ND	ND	ND	ND

ND = not detected

#### **4.5.1 Distribution pattern of metals in soil**

##### **4.5.1.1 Calcium**

The mean concentration of Ca was ranged from 801 to 1550 mg/kg in the soil sample. The highest content of calcium in the soil was observed in Mugunga and the lowest concentration was found in hadero. This lower concentration of Ca in the soil of hadero may be due to relatively higher rainfall, which causes the intensive leaching of Ca in the soils. [83]. One-way analysis of variance showed that the mean concentration of Ca in the soil was significantly different among the studied sites. The concentration of Ca in the soil (1435 - 1775 mg/kg) reported by Feleke (2010) was in the range with the present study [84].

#### **4.5.1.2 Manganese**

The level of manganese in the soil ranged 527 to 673 mg/kg. The soil from hadero had the highest mean level of manganese and the lowest mean concentration was found in checho. One-way analysis of variance showed that the mean concentration of Mn in the soil was significantly different among the studied sites. The concentrations of manganese found in this study were lower than the FAO/WHO (2001) recommended limit in the agricultural soil indicating that there was no Mn toxicity.

#### **4.5.1.3 Zinc**

As shown in Table 6, Zn levels in the soil ranged from 48 mg/kg to 110 mg/kg. The mean concentration of Zn in the soil samples was highest in Mugunga which was significantly higher than other sampling sites. This is attributed to farming methods used by farmers in Mugunga such as the use of fertilizers and the application of compost manure. Use of agrochemicals such as fertilizers and pesticides has been reported as one source of Zn in the soil [85] while application of compost manure not only results in Zn accumulation in soil but also causes an increase in Zn mobility and enhances Zn leaching [86]. The concentration levels of Zn in this study were in the range with the value (25.50 - 440.30 mg/kg) obtained by [87]. The levels of Zn in this study showed that there was no Zn contamination in the soil since the maximum permissible limit of Zn concentrations in soil is 300 mg/kg [88]

#### **4.5.1.4 Copper**

The concentration levels of Cu in the soil ranged from 8.4 to 13.3 mg/kg. The highest mean concentration values were found in Lalo the difference in Cu levels in the sampling sites could be attributed to the difference in their parent materials. Copper concentration levels reported in this study were below the permissible limits for agricultural land use of 100 mg/kg [89] implying that there was no Cu contamination in the soil.

### **4.6. Correlation of Metal levels between Ginger and Soil Samples**

The higher concentration of metals in the soil than ginger indicates most metals in the soil were not in the bio-available form. To correlate the effect of one metal on the concentration of the other metal, the Pearson correlation matrices using correlation coefficient ( $r$ ) for the samples were used and presented in Table 7. As shown in Table 7, metals Zn, and Mn showed a positive correlation in soil and ginger indicating that the more level of each metal in the soil, the more the

accumulation of corresponding metals in the ginger. This verifies that the dependence of metal concentration in the plant on the metal concentration of respective soil. Mn in ginger in ginger showed a negative correlation Zn the soil. Zn in ginger showed a positive correlation with Cu whereas a negative correlation with Zn in the soil. Magnesium in soil showed a negative correlation with Cu in the soil and Zn in ginger.

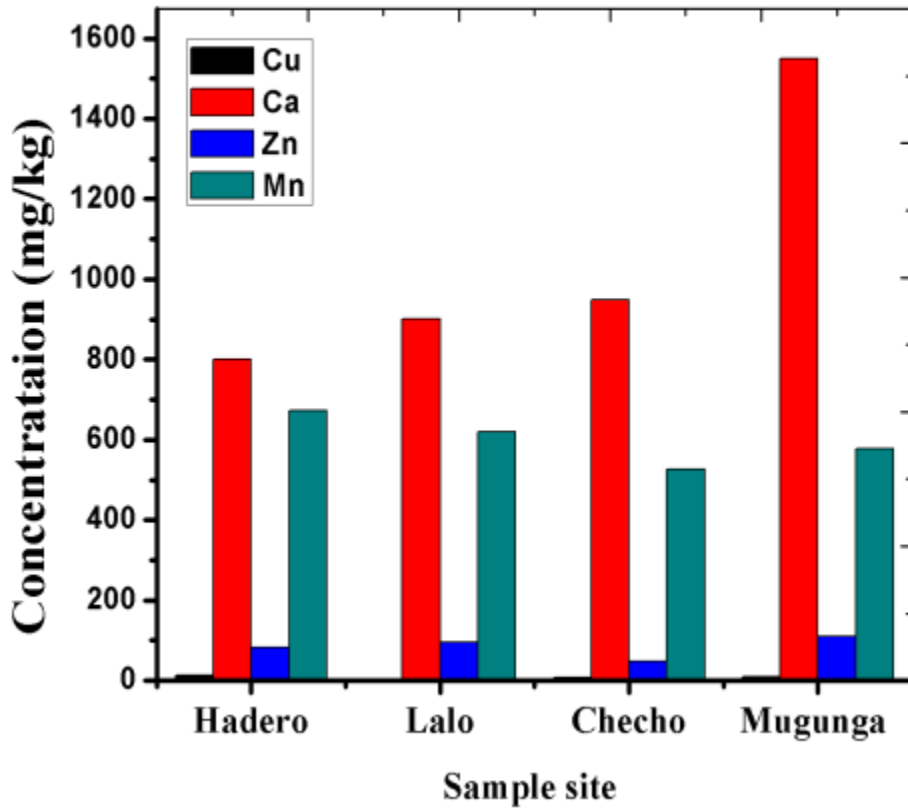


Figure 4: Level of Cu, Ca, Zn and Mn in soil sample

**Table4. 7 Correlation Coefficient Strength among ginger and Soil**

parameters	Cu in soil	Cu in ginger	Zn in soil	Zn in ginge	Ca in soil	Ca in ginge	Mn in soi	Mn in ginger
Cu in soil	1							
Cu in ginger	-0.607461	1						
Zn in soil	0.3825304	-0.955710854	1					
Zn in ginger	0.3195751	0.50574541	-0.62551	1				
Ca in soil	-0.625173	-0.227281919	0.4319434	-0.92840008	1			
Ca in ginger	-0.607388	0.217813179	-0.17972	-0.63070515	0.6474324	1		
Mn in soil	0.8402539	-0.523766604	0.4214932	0.442695429	-0.5983937	-0.92514506	1	
Mn in ginger	-0.838993	0.87215893	-0.782856	0.03061195	0.2227582	0.651339446	-0.8732446	1

#### 4.7. Correlation of Metals within Ginger Sample

As shown in Table 8, there was a negative correlation for Ca with Zn Positive correlation for Zn with (Cu), Mn with (Cu, Zn, and Ca) was observed. Metals interact with other metals in the uptake process by the plants. This is because some metals have similar chemical properties. For example, Mn shows properties of both the alkaline earth cation such Ca<sup>2+</sup> and the heavy metals (Zn ). This could be the probable reason for high positive correlation for Ca with Zn), (Mn) and Zn which may arise from common anthropogenic or natural sources as well as from similarity in chemical properties.

**Table 4.8. Pearson Correlation matrices for metals in ginger sample**

Parameters	Cu	Zn	Ca	Mn
Cu	1			
Zn	0.50575	1		
Ca	0.21781	-0.6307	1	
Mn	0.87216	0.03061	0.65134	1

## CHAPTER FIVE

### 5. CONCLUSION AND RECOMMENDATIONS

#### 5.1 Conclusion

The optimized wet digestion method was used and evaluated by spiking ginger and soil samples and their percentage recoveries were within the range of 96 % - 109 % which was within the acceptable range. The mean metal concentration (mg/Kg dry weight basis) ranged in the ginger and soil samples, respectively, were: Ca (874–1253, 801–1550), Zn (4.16–7.1, 48–110), Cu (3.3–5.4, 8.4–13.3), and Mn (231–321, 527– 673), the other metals like Pb, Cr, Ni and Co were not detected in both the ginger and soil samples. The result showed that ginger accumulates relatively higher amounts of Ca, and Mn among the determined essential metals. Non-essential metals analyzed in this study Ni, Cr, Pb and Co were below the detection limit. Hence, the plant is safe for dietary and medicinal uses with reference to analyzed metals. A positive correlation between metals in the ginger and soil was obtained for Zn and Mn indicating that the more level of metals in the soil, the more accumulation of corresponding metals in the ginger. The studied soil was found to be acidic in nature.

#### 5.2 Recommendations

Based on the results of the current study the following ideas were recommended

- More sensitive analytical instruments should be used to analyse small concentration of heavy metals in ginger and soil sample
- Determination of essential and non-essential metals in ginger sample of other site
- Assessment of proximate analysis of ginger sample

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