ADDIS ABABA UNIVERSITY
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Quantitative Determination of the Accumulation Level of Selected Essential and Toxic Heavy Metals in Korarima (*Aframomum corrorima*), Ginger (*Zingiber officinale*), Turmeric (*Curcuma longa*) and Black pepper (*Piper nigrum*) Spice Plants and Their Corresponding Soils Cultivated in Jima Zone, South West Ethiopia.

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By

Tura Gemechu

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Tura Gemechu

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This is to certify that the thesis prepared by Tura Gemechu, Quantitative Determination of the Accumulation Level of Selected Essential and Toxic Heavy Metals in Korarima (Aframomum corrorima), Ginger (Zingiber officinale), Turmeric (Curcuma longa) and Black pepper (Piper nigrum) Spice Plants and Their Corresponding Soils Cultivated in Jima Zone, South West Ethiopia and submitted in partial fulfillment of the requirements for Degree of Masters of Science in Chemistry complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Dr. Negussie Megersa ______________ ______________________
Advisor Signature Date

Dr. Merid Tessema ______________ ______________________
Examiner Signature Date

Prof. B. S. Chandravanshi ______________ ______________________
Examiner Signature Date

____
Chair of Department or Graduate Program coordinator

Abstract

The level of accumulation of selected essential and non-essential metals, namely; Ca, Cu, Zn, Ni, Cd, Pb, and Cr have been investigated in the seeds, fruits, and rhizomes, which is the edible part of some spice plants used for food flavoring in Ethiopia and their respective soil samples. These include seed of Aframomum corrorima (korarima), fruit of Piper nigrum (black pepper), rhizomes of Zingiber officenale (ginger), and rhizomes of Curcuma longa (turmeric) and their respective soil samples. All dried plant and soil samples were digested by wet digestion using appropriate concentrated acids such as HNO_3, $HClO_4$, HCl, and H_2O_2. The contents of the metals in the digests were analysed using flame atomic absorption spectrometer. From analyzed essential elements: Ca (429.01 – 5369.67 mg kg^{-1}) was the predominant metal followed by Zn (31.88 – 67.70 mg kg^{-1}), and Cu (7.62 – 10.67 mg kg^{-1}) in all the spice plant. Whereas the non-essential or toxic metals like Cr, Cd, Ni and Pb were not detected in all spice plants. Acidity, organic matter content and electrical conductivity of soil samples were found in the range of 5.86 – 6.57, 13.93 - 20.59 % and 0.28 - 0.49 mS/m respectively. In the soil samples, Ca (1195.67 – 4147.17 mg kg^{-1}) was the most abundant metal followed by Zn (112.90 – 120.25 mg kg^{-1}), Cr (21.92 – 45.76 mg kg^{-1}), Ni (21.40 – 56.93 mg kg^{-1}) and Cu (13.03 – 28.67 mg kg^{-1}). While Pb and Cd were not detected in all soil samples. The levels of all metals determined in the spice plants and the respective soil samples are below the standard set by WHO/FAO and in good agreement with those reported in the literature and the standards set for the soil by various legislative authorities.

Keywords: Spice, Korarima, Ginger, Turmeric, Black pepper, Heavy Metal, Wet digestion, FAAS

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List of abbreviation

Abbreviations	Description
FAO	Food Agricultural Organization
WHO	World Health Organization
ADI	Acceptable Daily Intake
TDI	Tolerable Daily Intake
PTWI	Provisional Tolerable Weekly Intakes
AAS	Atomic Absorbance Spectroscope
ICP	Inductively Coupled Plasma
ICP-MS	Inductively Coupled Plasma Mass spectrometry
FAAS	Flame Atomic Absorbance Spectrometry
GFAAS	Graphite Furnace Atomic Absorption Spectroscope
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
IC	Ion Chromatography
XRF	X-ray Fluorescence
LOQ	Limit of Quantification
SD	Standard Deviation
RSD	Relative Standard Deviation
EC	Electrical Conductivity
OM	Organic Matter
TF	Transfer Factory
MDL	Method Detection Limit

1. INTORODUCTION

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 (*Elettaria cardamomum*) [19], Large Cardamom (*Amomum subulatum*) [20] and related spice family like Ginger (*Zingiber officinale*) [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 (*Aframomum corrorima*), ginger (*Zingiber officinale)*, turmeric (*Curcuma longa*) and black pepper (*Piper nigrum)* are the most common ones.

1.1. 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.

1.1.1. 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, Gamo Gofa, Debub Omo, Sidamo, Jima, Illubabor and Wollega). Outside of these areas, it is cultivated in the vicinity of Lake Tana and Gelemso and in Eritrea [31].

Figure 1: Matured red korarima capsual

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].

Species of korarima

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 *Elettaria cardamomum* 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].

Elettaria cardamomum 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 α-terpineol and limonene [31].

1.1.2. Ginger

Ginger (*Zingiber officinale*) 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 B_3 and B_6, 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].

(a) (b)

Figure 2: Ginger (*Zingiber officinale)*: a) ginger plant; b) fresh rhizome of ginger

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, Gamo Gofa, Sidama, Jima and Wollega. Large scale production and marketing of ginger are also reported from Wolaita, Kembata and Tambaro [28].

1.1.3. 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].

(a) (b)

Figure 3: Turmeric (Curcuma longa) (a) turmeric plant; (b) fresh rhizome of turmeric plant

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.

1.1.4. 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 carminitive, (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].

(a) (b)

Figure 4: Piper nigrum (a) Black pepper plant; (b) Matured fruit of black pepper

Table 1: Scientific and common names of studied spice plants

English name	Local name (Amharic)	Scientific name	Family	Used part
False cardamom	Korarima	Aframomum corrorima	Zingiberaceae	Seed
Ginger	Zinjible	Zingiber officenale	Zingiberaceae	Rhizomes
Turmeric	Irid	Curcuma longa	Zingiberaceae	Rhizomes
Black pepper	K.B*	Piper nigrum	Piperaceae	Fruit

Note: K.B: Kondo berbere

1.2. 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].

1.3. Heavy metals

Heavy metals are defined as metals having a density higher than 5 g/cm^3 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].

1.3.1. 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.

1.3.2. Factors affecting the bioavailability of heavy metal to plants

1.3.2.1. 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 pH < 7 than at pH > 7 [51].

1.3.2.2. 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].

1.3.3. 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].

1.3.4. 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 (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 haematotiopic 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].

1.3.5. 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 ($mg.kg^{-1}$ 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].

1.4. Sample preparation techniques for metal analysis in the plant materials

1.4.1. 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.

1.5. 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 non-essential metals can be determined by various spectroscopic or chromatographic methods, such as: Flame Atomic Absorbance Spectrometry (FAAS) or Graphite Furnace Atomic Absorption Spectroscope (GFAAS), Atomic Emission Spectrometry (AES), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Inductively Coupled Plasma Mass spectrometry (ICP-MS), X-ray fluorescence (XRF), and Ion chromatography (IC) [67].

1.6. Statement of the problem

It is well known that plant species accumulate considerable quantities of toxic heavy metals up taken from the soils where the plants grow. As a result, large number of the society who use these spices to flavor their food, may accumulate a significant quantities of these metals in the various parts of their tissue systems, since the dosage is not precisely regulated. Therefore, in addition to the possible toxicity encountered from the various constituents of the plant materials, consumers may also suffer from trace level metal toxicity and hence their normal body function can also be affected. Therefore, it is very timely and important to experimentally determine the extent to which these toxic metals are found in the plant species and the respective soil samples.

Hence, the current study is conducted to answer the following research questions.

- Is the concentration of heavy metals in the spice plants were below or beyond the permissible limit recommended by WHO?
- What are the most toxic heavy metals found in spices plant and their relative distribution in the plants and the respective soil samples?
- What is the contamination level of the Ethiopian spices relative to the other countries like Indian, Ghana and Nigeria?
- Is it harmful the concentration of heavy metals in spices of Ethiopia?

1.7. 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 consummations.
- To initiate further studies on nutritional, medicinal and toxicological effects that can be caused due to the use of spices through diet.

1.9. Objectives

1.9.1. General objective

The main objective of this study is to quantitatively determine the levels of selected essential and non-essential metals in korarima seeds, ginger, turmeric and black pepper cultivated in Ethiopia, and their relative distribution in the plant materials and the respective soils where each plant grow.

1.9.2. Specific objectives

- To develop an optimum digestion procedure for the determination of selected metals in korarima, ginger, turmeric and black pepper plant samples by FAAS.
- To determine quantitatively the levels of major, minor and trace metals (Ca, Zn, Cu, Cr, Ni, Cd and Pb) in korarima, ginger, turmeric and black pepper samples cultivated in Ethiopia.
- To investigate the level of metals in soil samples where the korarima, ginger, turmeric and black pepper are grown, and their relative distribution in the plants and respective soils.
- To correlate the levels of metals in the spices with that of soil in which they have been cultivated.
- To compare the concentration of metals determined with the acceptable limits proposed by WHO.

2. EXPERIMENTAL

2.1. Instrumentation and apparatus

Air circulating oven (PLT-125) was used for air drying of samples, ceramic mortar and pestle (Haldenwanger, Germany) were used for grinding and homogenizing the ground samples, digital electronic balance (PW-254, UK) was used for weighing the samples. Kjeldahl digestion apparatus (Gallenkamp, England), pH meter (AD-1020, Romania) 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) (Zeenit 700P, Germany) by using hollow cathode lamps with air-acetylene flame.

2.2. Chemicals and reagents

Chemicals and regents used in this study were all of analytical grades:, 69-72 % $HNO_{3;}$ sigma Aldrich (steintein, Germany), 70 % $HClO_{4;}$ Research Laboratory Fine Chemical Industry (Mumbai, India), 30 % H_2O_2, BDH Chemicals Ltd (Poole, England) and 36-38 % HCl Sigma Aldrich (Steinheim, Germany), were used for the digestion of target analytes in different matrix. Lanthanum nitrate hydrate 99.9 %, Hopkin and Williams Ltd (Essex, England) solution was used to prevent the chemical interference on calcium determination during analysis of the plant and soil sample. Stock standard solution (Buck Scientific Puro-Graphic, USA) having concentrations of 1000 mg L^{-1} 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 rising the apparatus prior to analysis.

2.3. 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.

2.4. Sampling

2.4.1. Description of sampling site

Edible part of the plant spices and their respective soil samples were collected from Jima Zone of Oromia Regional State, Southwest of Ethiopia. The distance of Jima Zone from Addis Ababa, the capital city of Ethiopia is 336 km with the altitude $7^0$40'42" N and longitude $36^0$49'24" E. It has an elevation of 1,780 m from sea level. The selection of the sampling site was based on the major spice growing area of Ethiopia.

2.4.2. Collection and preparation of samples

Matured fresh red korarima capsule, ginger, turmeric and black pepper 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. After being authenticated by Addis Ababa University’s herbaria, samples were washed several times with tap water to eliminate dirty and possible parasites and then rinsed with distilled deionized water and air dried to get a constant weight mass of the sample. 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. The dried, sieved and homogenized samples were stored in clean and dry polyethylene bags till digestion.

2.5. 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 HNO_3, $HClO_4$ and H_2O_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.

Table 2: Optimization of digestion procedure for 0.5 g of korarima ginger, turmeric and black pepper samples.

S.No.	Reagents used	Reagent volume (mL)	Temp. (^{0}C)	Digestion time (hr)	Observation
I. Optimization for reagent volume					
1	HNO_3:HClO4: H_2O_2	3:1:0	270	3	Colorless solution with some residue
2	HNO_3:$HClO_4$: H_2O_2	2:1:0	270	3	Slightly clear solution with some residue
3	HNO_3:$HClO_4$: H_2O_2	2:2:0	270	3	Almost colorless solution
4	HNO_3:$HClO_4$: H_2O_2	2:0:1	270	3	Yellowish solution with residue
5	HNO_3:$HClO_4$: H_2O_2	2:1:1*****	270	3	Clear and colorless solution
6	HNO_3:$HClO_4$: H_2O_2	3:2:1	270	3	Clear and yellowish solution
II. Optimization for temperature					
1	HNO_3:$HClO_4$: H_2O_2	2:1:1	120	3	Deep yellow
2	HNO_3:$HClO_4$: H_2O_2	2:1:1	150	3	Yellowish
3	HNO_3:$HClO_4$: H_2O_2	2:1:1	180	3	Yellowish
4	HNO_3:$HClO_4$: H_2O_2	2:1:1	210	3	Light yellowish color
5	HNO_3:$HClO_4$: H_2O_2	2:1:1	240	3	Almost clear solution
6	HNO_3:$HClO_4$: H_2O_2	2:1:1	270*****	3	Clear and colorless solution
III. Optimization for digestion time					
1	HNO_3:$HClO_4$: H_2O_2	2:1:1	270	1:45	Deep yellow
2	HNO_3:$HClO_4$: H_2O_2	2:1:1	270	2:00	yellowish
3	HNO_3:$HClO_4$: H_2O_2	2:1:1	270	2:15	yellowish
4	HNO_3:$HClO_4$: H_2O_2	2:1:1	270	2:30	Light yellowish color
5	HNO_3:$HClO_4$: H_2O_2	2:1:1	270	2:45	Almost clear solution
6	HNO_3:$HClO_4$: H_2O_2	2:1:1	270	3:00*****	Clear and colorless solution

Note: * Indicates the optimized volume, temperature and time of plant sample digestion

Table 3 : Optimization of digestion procedure for 0.5 g of korarima, ginger, turmeric and black pepper soil samples**.**

S.No.	Reagents used	Reagent volume (mL)	Temp. (^{0} C)	Digestion time (h)	Observation
I. Optimization for reagent volume					
1	HNO_3:$HClO_4$: HCl	3:1:0	240	2	Deep yellow with suspension
2	HNO_3:$HClO_4$: HCl	3:1:0	240	2	Slightly clear solution with some residue
3	HNO_3:$HClO_4$: HCl	3:2:0	240	2	Almost colorless solution
4	HNO_3:$HClO_4$: HCl	3:0:1	240	2	Yellowish solution with residue
5	HNO_3:$HClO_4$: HCl	3:1:1*****	240	2	Clear and colorless solution
6	HNO_3:$HClO_4$: HCl	3:2:1	240	2	Clear and yellowish solution
II. Optimization for temperature					
1	HNO_3:$HClO_4$: HCl	3:1:1	120	2	Deep yellow with suspension
2	HNO_3:$HClO_4$: HCl	3:1:1	150	2	Slightly clear solution with some residue
3	HNO_3:$HClO_4$: HCl	3:1:1	180	2	Almost colorless solution
4	HNO_3:$HClO_4$: HCl	3:1:1	210	2	Almost colorless solution
5	HNO_3:$HClO_4$: HCl	3:1:1	240*****	2	Clear and colorless solution
6	HNO_3:$HClO_4$: HCl	3:1:1	270	2	Clear and colorless solution
III. Optimization for digestion time					
1	HNO_3:$HClO_4$: HCl	3:1:1	240	1:00	Deep yellow with suspension
2	HNO_3:$HClO_4$: HCl	3:1:1	240	1:15	Slightly clear solution with some residue
3	HNO_3:$HClO_4$: HCl	3:1:1	240	1:30	Almost colorless solution
4	HNO_3:$HClO_4$: HCl	3:1:1	240	1:45	Almost colorless solution
5	HNO_3:$HClO_4$: HCl	3:1:1	240	2:00*****	Clear and colorless solution
6	HNO_3:$HClO_4$: HCl	3:1:1	240	2:15	Clear and colorless solution

Note: * Indicates the optimized volume, temperature and time of plant sample digestion

2.5.1. Wet digestion of plant samples

For digestion purpose, 0.5 g of powdered, sieved and homogenized plant samples were weighed and transferred into a 100 mL round bottom flask. To this, a freshly prepared 2 mL concentrated HNO_3 (69-72 %), 1 mL of H_2O_2 (30 %) and 1 mL of $HClO_4$ (70 %) were added based on the optimized procedure. 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 9 (270 ^{0}C) for 150 min until a clear and colorless solution was obtained following the optimized digestion procedure given in Table 2. After a total of 3:00 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 pepper with distilled-deionized water was carried out for diluting the sample to the final volume of 50 mL. To each sample 1 % w/v ‘matrix modifier’ lanthanum nitrate hydrate ($La_3(NO_3).3H_2O$) were added to the filtered solutions, so that lanthanum may bind the phosphate and liberate calcium in case if large phosphate exist in the sample. 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.

2.5.2. Wet digestion of soil samples.

Applying the optimized condition (Table 3), 0.5 g of dried, sieved and homogenized soil samples were transferred into a 100 mL round bottomed flask. To this 3 mL HNO_3 (69 - 72 %), 1 mL HCl (35 %) and 1 mL of $HClO_4$ (70 %) were added and the mixture was digested on a Kjeldahl digestion apparatus fitting the flask to a reflux condenser by setting the temperature first to dial at 4 (120 ^{0}C) for the first 30 min and then raised to dial 8 (240 ^{0}C) for the remaining 90 min until colorless solution samples obtained. The rest steps for the soil sample digestion were similar to that of plant sample digestion procedure.

2.6. 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^{-1}) which were prepared by diluting stock solutions (1000 mg L^{-1}) of the analyte metals. Working standards of metal solutions were prepared in 50 mL volumetric flasks by diluting with distilled deionized water. For all the metals investigation in spice plants and soil samples, deutrium background correction has been performed. The operating conditions for FAAS employed for each metal are given in Table 4.

Table 4: Instrumental operating conditions for determination of metals using flame atomic absorption spectrophotometer

Metals	Wave length (nm)	Acetylene Flow (mL/min)	Sample Uptake (mL)	Read time (sec.)	Slit width (nm)	PMT (V)	Lamp current (A)	Energy (KJ/mol)
Ca	422	60	1.4	3	1.2	300	2.0	73.1
Cd	228	40	1.4	3	1.2	300	2.0	89.9
Cr	335	115	1.4	3	0.2	300	2.0	71.3
Cu	324	40	1.4	3	1.2	300	1.5	71.4
Ni	232	55	1.4	3	0.2	300	0.7	72.3
Zn	213	40	1.4	3	0.5	300	2.0	71.6
Pb	283	65	1.4	3	1.2	300	2.0	79.3

Table 5: Concentration of standard solutions for FAAS instrument calibration and correlation coefficient of calibration curves.

Element	Concentration (ppm)	Absorbance	r^2	Linear Regression
Cd	0.25	0.11177	0.99982	Y = 0.41291*x + 0.01017
	0.5	0.21850		
	0.75	0.32099		
	1	0.42170		
Cr	1	0.08231	0.9992	Y= 0.08697*x - 0.00167
	2	0.17596		
	3	0.26086		
	4	0.34392		
Cu	0.25	0.05133	0.99998	Y= 0.00454 +0.19063*x
	0.5	0.10078		
	1	0.19528		
	2	0.38561		

Pb	1	0.03238	0.99281	Y= 2.8E^{-4} + 0.0335*x
	2	0.06676		
	3	0.10601		
	4	0.13096		
Ni	1	0.13617	0.99982	Y= 0.00811 + 0.12996*x
	2	0.27129		
	3	0.39718		
	4	0.52741		
Zn	0.25	0.16265	0.99967	Y= 0.00162 + 0.63974*x
	0.5	0.32370		
	0.75	0.47372		
	1	0.64576		
Ca	0.25	0.01455	0.99856	Y= 0.0569*x - 0.00108
	0.5	0.02727		
	1	0.05350		
	2	0.11373		

2.7. 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 have been utilized for plant and soil sample material digestion. Each of the black samples was analyzed for their metal content namely: Ca, Cu, Zn, Cd, Pb, Cr 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**.**

$$\mathrm{MDL} = \mathrm{Y_B} + 3\mathrm{SD_B}$$

Where Y_B is the concentration of analyte in the digestion blank solution and SD_B the standard deviation of the replicate measurements of the analytes in the blank. The value of the MDL obtained were compared with the instrument detection limit and found to have greater values, for both plant and soil samples **(**Table 6**).**

Table 6: MDLs (mg L^{-1}) and instrumental detection limit (mg L^{-1}) for investigated metals in plants and respective soil samples.

Metal	MDL for plant materials				MDL for soil samples				
	Korarima	Ginger	Turmeric	B.P *	Korarima	Ginger	Turmeric	B.P*	IDL*
Ca	0.11	0.13	0.10	0.12	0.08	0.11	0.09	0.10	0.025
Cd	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.012
Cr	0.06	0.06	0.06	0.06	0.08	0.09	0.09	0.09	0.05
Cu	0.05	0.05	0.07	0.08	0.04	0.12	0.14	0.13	0.035
Ni	0.08	0.08	0.09	0.08	0.12	0.13	0.11	0.10	0.07
Zn	0.06	0.06	0.04	0.07	0.09	0.12	0.12	0.12	0.012
Pb	0.43	0.43	0.43	0.43	0.44	0.44	0.44	0.44	0.3

Note: B.P*: Black pepper, IDL*: instrumental detection limit

2.8. Determination of limits of quantitation

Limit of quantitation (or limit of determination) is the lowest concentration of the analyte that can be measured in the sample matrix at an acceptable level of precision and accuracy. An acceptable level of precision is typically 10 to 20 % of relative standard deviation depending upon the concentration level measured. However, in the absence of specified precision, the limit of quantification is the same as the concentration that gives a signal 10 times the standard deviation of the blank [68]. Limit of quantitation is the lowest limit for precise quantitative measurements [69]. The limit of quantitation for each element was calculated by the formula given below.

$$LOQ = Y_B + 10SD_B$$

where LOQ limit of quantitation, Y_B is the concentration of analyte in the digestion blank solution and SD_B the standard deviation of the replicate measurements of the analytes in the blank.

Table 7: LOQ (mg L^{-1}) and instrumental detection limit (mg L^{-1}) for investigated metals in plants and respective soil samples.

Metal	LOQ for plant materials				LOQ for soil samples				
	Korarima	Ginger	Turmeric	B.P *	Korarima	Ginger	Turmeric	B.P*	IDL*
Ca	0.20	0.24	0.23	0.26	0.12	0.24	0.16	0.20	0.025
Cd	0.06	0.06	0.06	006	0.07	0.07	0.07	0.07	0.012
Cr	0.07	0.07	0.07	0.07	0.26	0.30	0.27	0.32	0.05
Cu	0.07	0.08	0.18	0.10	0.08	0.24	0.27	0.23	0.035
Ni	0.15	0.12	0.17	0.15	0.15	0.24	0.14	0.13	0.07
Zn	0.09	0.07	0.07	0.10	0.19	0.21	0.18	0.22	0.012
Pb	0.45	0.45	0.45	0.45	0.46	0.46	0.46	0.46	0.3

Note: B.P*: Black pepper, IDL*: instrumental detection limit

2. 9. 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 8 and 9) respectively, using the following equation:

$$\%\ \text{R} = \frac{\text{Amount of the analyte recoverde}}{\text{Amount of the analyte added}}\ \text{x}100$$

2.10. Analysis of the soil pH

Soil pH was measured by potentiometrical 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 and the mean values have been reported (Table 8).

2.11. Analysis of soil organic matter

Soil OM was measured by taking dried known mass of soils (5 g) in to crucible rack (stainless steel) by measuring to 0.0001 g accurately on electronic balance. A sample of soil is dried at 105° C before determination of OM to remove moisture and get constant weight. The sample is weighed, heated at 400° C for 4 hours in furnace and weighed again after the temperature drops below 150° C. Organic matter determination of the soil samples was performed in triplicates and the mean values have been reported (Table 8). It was calculated using the following formula [70].

$$\text{OM \%} = \frac{(Wt.at\ 105\ {}^{o}C) - (\text{Wt.at } 400\ {}^{o}\text{C})}{(Wt.at\ 105\ {}^{o}C)} \text{ x } 100$$

where OM, is organic matter, Wt. is weight of the soil samples at specified temperature.

2.12. 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 (Table 8).

3. RESULTS AND DISCUSSION

3.1. Physicochemical characteristic of soil samples

The soil pH was measured and found to be within the range of 5.86 ± 0.05 - 6.57 ± 0.08 (Table 8), which categorizes the soil under weakly acidic conditions and this probably is the favorable pH range for spice crops like cardamom and black pepper [71]. Soil pH is one of the most influencing parameters controlling the conversion of metals from the immobile solid phase forms to more mobile forms. The solubility of heavy metals is generally greater as pH decreases within the pH range of normal agricultural soils (approximately 5.0 - 7.0) [71]. It can further be inferred that the relatively higher pH values of the soils might have contributed for the low transfer of metals from the soils to plant parts. The soil pH analyzed is in normal range of agricultural soil.

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. Organic matter contents for the soil investigated under this study ranges from 13.93 - 20.59 % which is high enough to form stable metal complexes and decreases the availability of metals to plant. In general, plants are unable to absorb the large metal-complexes and so the bioavailability of metals decreases. Copper ions forms strong coordination complexes with organic matter. Hence, Cu is often predominantly found bound to the organic matter fraction in the soil and soil organic matter can be the most important soil factor in determining Cu bioavailability [72].

Electrical conductivity is a numerical expression of the ability of a solution to carry an electric current. This ability depends on the presence of ions on their total concentration and on temperature of the measurement. It was founded that the EC of soil from study area farm ranges from 0.12 ± 0.01 to 0.49 ± 0.01 mS m^{-1} , which shows the EC of the soils are low. The results, therefore show that amount of ions in the soil are less. Electrical conductivity can also affect metal availability**.**

Table 8: Physicochemical characteristics of soil sample (pH, EC and OM) with (mean ± SD, n = 3) and percent relative standard deviations (% RSD)

	Korarima soil		Ginger soil		Turmeric soil		Black pepper soil	
	Mean ± SD	RSD	Mean ± SD	RSD	Mean±SD	RSD	Mean ± SD	RSD
PH	6.57 ± 0.08	1.30	5.91 ± 0.01	0.23	6.32 ±0.08	1.28	5.86 ± 0.05	0.87
E.C	0.28 ± 0.01	2.03	0.49 ± 0.01	2.34	0.12 ±0.01	9.89	0.12 ± 0.01	4.94
OM	20.59 ± 0.07	0.33	15.54 ± 0.04	0.26	15.38± 0.5	0.31	13.93 ± 0.06	4.31

Note: EC, electrical conductivity (mS/m), OM, organic matter (%), RSD (%)

3.2. Recovery of the analytical method

The accuracy of the optimized procedure was evaluated by analyzing the digests of spiked samples for plant and soil samples. As shown in Table 9, the present recovery for the plant samples lain in the range 84.0 - 98.25 % for korarima, 94.0 - 103.33 % for ginger, 88.0 - 110 % for turmeric and 92.5 - 106 % for black pepper spice plants which are within the acceptable range for all metals. Similarly, for soil samples 88.0 - 106.67 %, 87.0 - 113.3 %, 86.0 - 113.33 % and 93.33 - 107.5 % percent recovery range were obtained for korarima, ginger, turmeric and black pepper soil samples respectively as presented in Table 10, which are also within the acceptable range for all the investigated metals. Generally, good recoveries were obtained for all metals**,** thus the optimized procedure has good accuracy.

Table 9: Percent recover values (% R ± SD, n = 3) for plant samples.

Samples	Conc. (mg L^{-1})	Metals						
		Ca	Cd	Cr	Cu	Ni	Zn	Pb
Korarima	CIP[a]	1.72	BDL	BDL	0.37	BDL	0.67	BDL
	CSP[b]	2.00	-	-	0.94	-	0.95	-
	CA[c]	0.3	1	4	0.6	4	0.3	4
	AR[d]	0.28	0.84	3.91	0.57	3.93	0.28	3.87
	% R[e]	93.33 ± 2.3	84 ± 0.1	97.75 ± 0.7	95 ± 5.02	98.25 ± 2.50	93.33 ± 2.1	96.75 ± 1.98
Ginger	CIP[a]	1.63	BDL	BDL	0.27	BDL	0.76	BDL
	CSP[b]	1.93	-	-	0.95	-	1.07	-
	CA[c]	0.3	1	4	0.7	4	0.3	4
	AR[d]	0.31	0.94	3.87	0.68	3.96	0.31	3.94
	% R[e]	103.3 ± 1.0	94 ± 0.85	98.75 ± 0.1	97.14 ± 5.2	99 ± 3.3	103.33 ± 1.2	98.5 ± 1.98
Turmeric	CIP[a]	1.71	BDL	BDL	0.25	BDL	0.53	BDL
	CSP[b]	2.04	-	-	0.96	-	1	-
	CA[c]	0.3	1	4	0.7	4	0.5	4
	AR[d]	0.33	0.88	3.96	0.71	4.02	0.47	4.12
	% R[e]	110 ± 0.7	88±0.88	99 ± 0.3	101.42 ± 1.8	100.5 ± 0.32	94 ± 0.8	103 ± 1.6
Black pepper	CIP[a]	1.61	BDL	BDL	0.53	BDL	0.32	BDL
	CSP[b]	1.98	-	-	1.06	-	1.05	-
	CA[c]	0.3	1	4	0.5	4	0.7	4
	AR[d]	0.37	0.97	4.01	0.53	4.03	0.73	3.99
	% R[e]	92.5 ± 0.7	97 ± 0.69	100.25 ± 0.2	106 ±1.9	101± 0.94	104.28 ± 0.4	99.75 ± 1.3

Notes: [a] concentration in plant sample, [b] concentration in spiked plant sample, [c] amount added, [d] amount recovered, [e] percent recover

Table 10: Percent recover values (% R ± SD, n = 3) for soil samples**.**

Samples	Conc. (mg L^{-1})	Metals						
		Ca	Cd	Cr	Cu	Ni	Zn	Pb
Korarima	CIS[a]	1.76	BDL	1.09	0.97	1.32	0.73	BDL
	CSS[b]	2.07	-	2	1.29	2.21	1.04	-
	CA[c]	0.3	1	1	0.3	1	0.3	4
	AR[d]	0.31	0.88	0.91	0.32	0.89	0.31	3.67
	% R[e]	103.33 ± 1.0	88 ± 0.8	91 ± 0.29	106.67 ± 1.18	89 ± 0.66	103.33 ± 2.2	91.75 ± 2.0
Ginger	CIS[a]	1.73	BDL	2.28	0.55	1.05	0.64	BDL
	CSS[b]	2.07	-	3.15	1.03	1.98	0.97	-
	CA[c]	0.3	1	1	0.5	1	0.3	4
	AR[d]	0.34	0.87	0.87	0.48	0.93	0.33	3.73
	% R[e]	113.3 ± 0.4	87 ± 0.14	87 ± 0.8	96 ±1.6	93 ± 0.61	110 ± 0.9	93.25 ± 1.03
Turmeric	CIS[a]	1.65	BDL	2.13	0.50	1.07	0.68	BDL
	CSS[b]	1.92	-	3.09	0.93	2.08	1.02	-
	CA[c]	0.3	1	1	0.5	1	0.3	4
	AR[d]	0.27	0.91	0.96	0.43	1.01	0.34	4.02
	% R[e]	90 ± 0.6	91 ± 0.8	96 ± 0.6	86 ± 3.7	101±0.7	113.33 ± 0.3	100.5 ± 1.70
Black pepper	CIS[a]	1.19	BDL	2.13	0.28	1.13	0.71	BDL
	CSS[b]	1.47	-	3.14	1.01	2.17	1.03	-
	CA[a]	0.3	1	1	0.7	1	0.3	4
	AR[b]	0.28	0.98	1.01	0.73	1.04	0.32	4.3
	% R[c]	93.33 ± 0.4	98 ± 0.3	101 ± 0.57	104.28 ± 0.4	104±1.14	106 ±1.3	107.5 ± 0.3

Notes: [a] concentration in soil sample, [b] concentration in spiked soil sample, [c] amount added, [d] amount recovered, [e] percent recover

3.3. Determination of metals in spice plant and soil samples

The concentration of seven elements (Ca, Zn, Cu, Cr, Ni, Cd, and Pb) in the digested samples of spice plant and soils were analysed by FAAS. Among the analysed metals chromium, cadmium, nickel and lead were below the method detection limit in all spice plant samples. Lead and cadmium were also not detected in soil samples and the concentration value of the rest metals shown with their respective % RSD in Table 11 and 12. The most abundant metal among the investigated essential metals in spice plants was calcium followed by zinc whereas copper was the predominant among the tested micronutrient heavy metals. In soil sample the most abundant metal is calcium followed by zinc, nickel, chromium and copper respectively.

Table 11: Mean concentration (n = 3, x ± SD mg kg^{-1}) and percent relative standard deviations (% RSD) of metal in plant samples.

Metals	Korarima		Ginger		Turmeric		Black pepper		WHO limits
	Mean ± SD	% RSD	Mean ± SD	% RSD	Mean ± SD	% RSD	Mean ± SD	% RSD	
Ca	627.15 ± 36.05	5.75	965.46 ±17.5	1.82	429.01 ± 1.89	0.442	5369.67 ±240.18	4.47	-
Cu	7.62 ± 0.27	3.54	8.02 ± 0.05	2.61	8.53 ± 0.02	0.66	10.67 ± 0.24	2.24	50 [4]
Cr	BDL	-	BDL	-	BDL	-	BDL	-	-
Ni	BDL	-	BDL	-	BDL	-	BDL	-	50 [4]
Zn	67.7 ± 2.8	4.14	67.02 ± 3.75	5.59	52.62 ± 3.27	6.22	31.88 ± 0.60	1.89	100 [4]
Cd	BDL	-	BDL	-	BDL	-	BDL	-	21[4]
Pb	BDL	-	BDL	-	BDL	-	BDL	-	100 [4]

Note: BDL: below detection limit

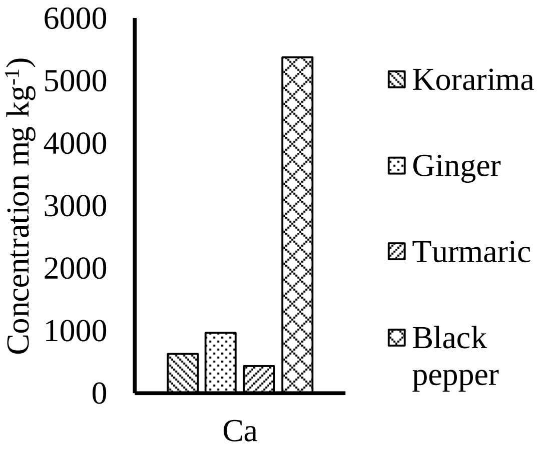
Table 12: Mean concentrations of metals (n = 3, x ± SD mg kg^{-1}), percent relative standard deviations (% RSD) of soil samples.

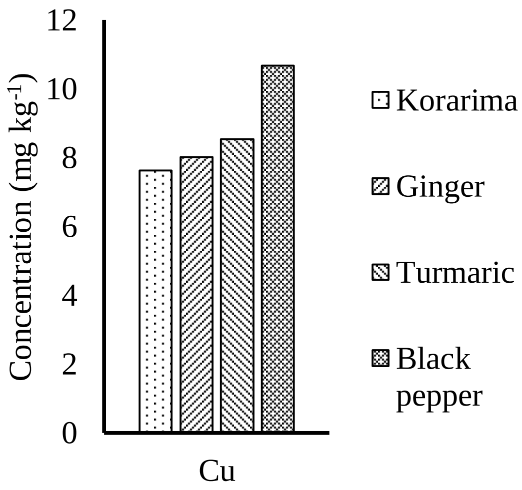
Metals	Korarima soil		Ginger soil		Turmeric soil		Black pepper soil	
	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)
Ca	3533.3 ± 176.54	4.99	2438.67 ± 41.04	1.68	4147.17 ± 329.25	7.94	1195.67 ± 35.85	2.99
Cd	BDL	-	BDL	-	BDL	-	BDL	BDL
Cr	21.92 ± 0.79	3.62	45.76 ± 0.8	1.95	42.74 ±1.18	2.76	42.78 ± 0.15	0.36
Cu	19.59 ± 0.58	2.96	13.03 ± 0.68	6.23	16.39 ± 0.29	2.80	28.67 ± 1.36	4.44
Ni	33.15 ± 1.55	4.69	26.27 ± 0.62	2.37	21.4 ± 0.13	0.58	56.93 ± 3.26	5.72
Zn	120.25 ± 9.53	7.92	112.9 ± 7.17	6.35	131.63 ± 7.98	6.06	113.8 ± 2.8	2.46
Pb	BDL	-	BDL	-	BDL	-	BDL	-

Note: BDL (below detection limit)

3.3.1. Concentration of metals in plant samples

In all the analyzed plant samples for major as well as trace metals, only calcium, copper and zinc were detected. Chromium, cadmium, nickel and lead was found to be below the method detection limit in all plant samples. The concentration of metals determined in plant samples indicate that calcium is more accumulated by the plant while copper is the least accumulated metal in all the plant samples. As shown in Table 11, 627.15 ± 36.05, 965.46 ± 17.54, 429.01 ± 1.89 and 5369.67 ± 240.18 mg kg^{-1}dry weight of calcium was determined in korarima, ginger, turmeric and black pepper respectively. For copper 7.62 ± 0.27, 8.02 ± 0.05, 8.53 ± 0.02 and10.67 ± 0.24 mg kg^{-1} dry weight was determined in the same order. The concentration of zinc found in korarima and ginger are comparable, 67.7 ± 2.8 and 67.02 ± 3.75 mg kg^{-1} respectively and turmeric contains 52.62 ± 3.27 mg kg^{-1}. The minimum concentration of zinc was obtained in black pepper 31.88 ± 0.60 mg kg^{-1}.





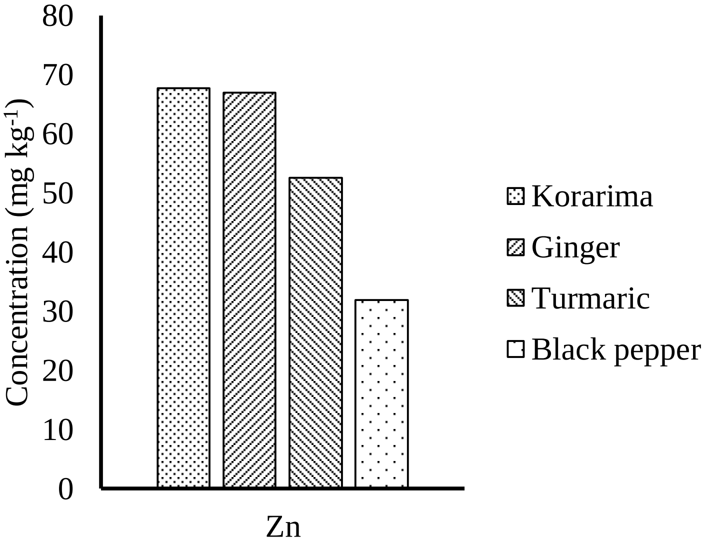
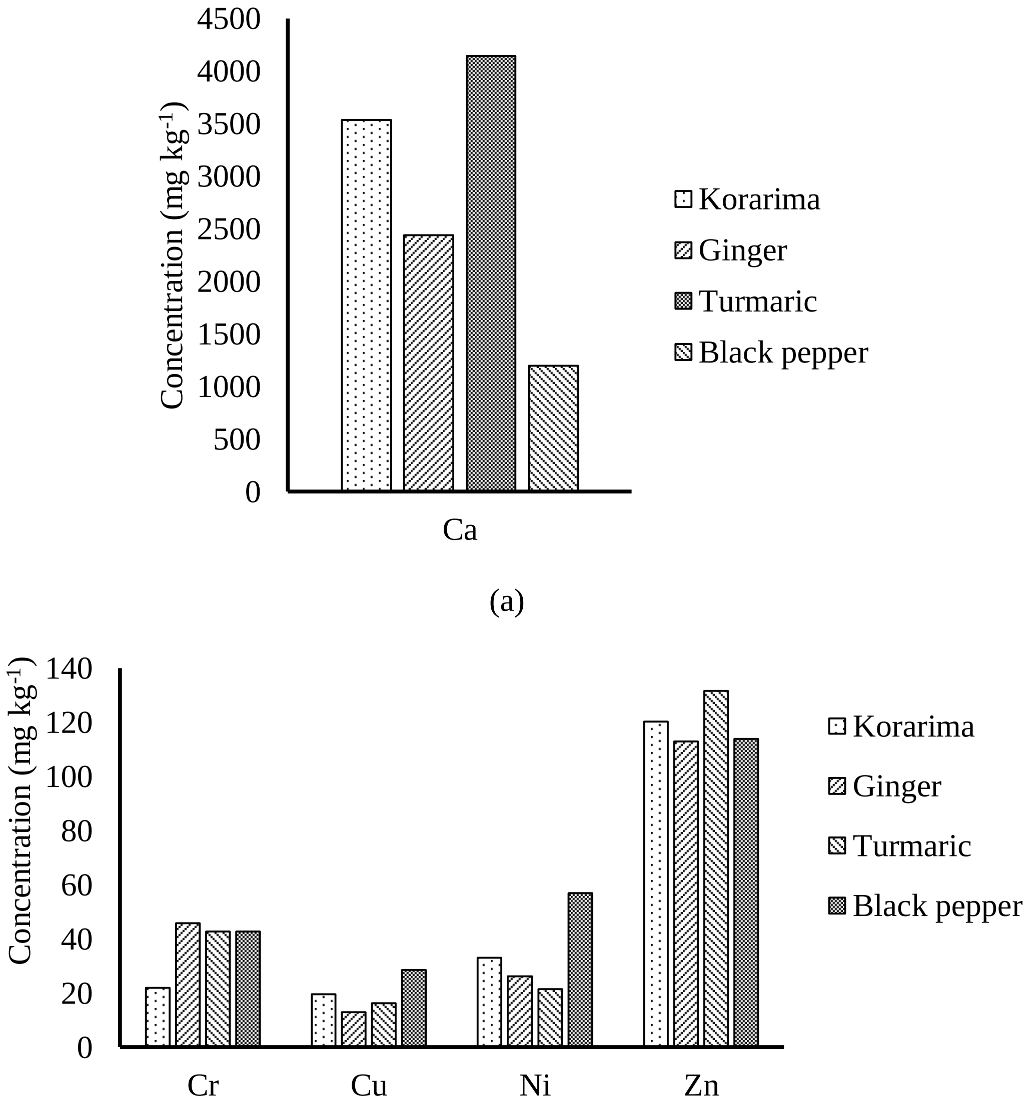


Figure 5: Concentration of Ca, Cu and Zn in korarima, ginger, turmeric and black pepper plant samples (mg kg^{-1} dry weight).

3.3.2. Concentration of metals in soil samples

The soil samples were also found to contain detectable metals of Ca, Cu, Zn, Cr and Ni. Again, Pb and Cd were not detected in soil samples collected from the same origin of plant material sites. The concentration of metals in soil samples almost follows the same trend as that of plant samples. The concentration of Ca in soil samples is in decreasing order of : ginger soil > korarima soil > turmeric soil > black pepper soil , Cr investigated in ginger soil sample is greater than all of the soil samples and the concentration level of Cr obtained in turmeric soil and black pepper soil are comparable. Minimum amount of Cr was obtained in korarima soil samples. Copper was the least detected metal in all soil samples compared to other detected metals. Zinc trend of variation in soil sample is in decreasing order of: turmeric soil > korarima soil > black pepper > ginger soil. Concentration level of Ni is in decreasing order of: black pepper soil > korarima soil > ginger soil > turmeric soil.



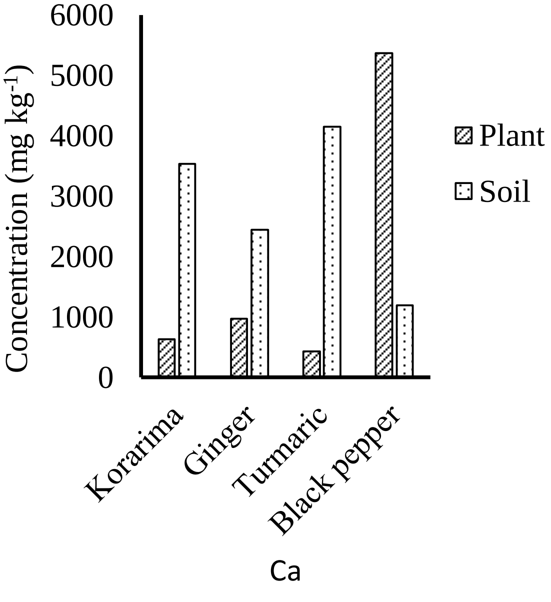
(a)

(b)

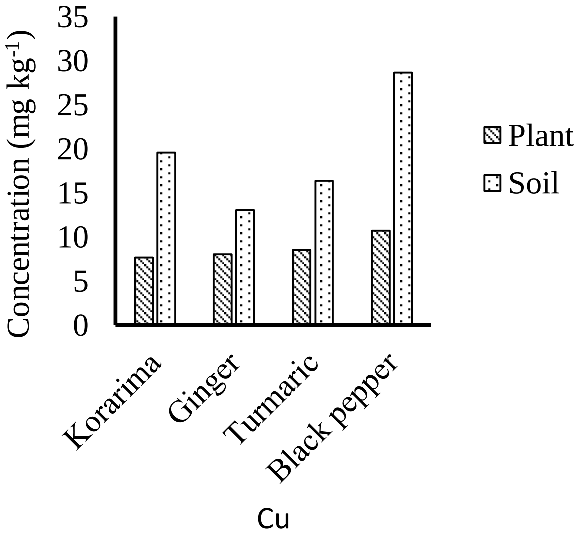
Figure 6: Concentration of (a) Ca and (b) Cu, Cr, Ni and Zn in korarima, ginger, turmeric and black pepper soil samples (mg kg^{-1} dry weight).

3.4. Comparison of metals in plant and soil samples.

The concentrations of metals determined are higher in soil samples than the plants grown on the same origin soils except for black pepper soil which was less than the black pepper plant for calcium metal. This indicates that only a small portion of the soil metals are transferred to the plant parts and the root acts as a barrier to the translocation of metals within the plants. The studied soil samples were found to contain higher concentrations of all metals than the spice plants except for black pepper soil for calcium metal Figure 7 (a - c) ; this is because of the sorption processes of metals from soil by plants is significantly affected by metal level in the soil, soil pH, the presence of competing ligands, the ionic strength of the soil solution, organic matter content of soil, types and varieties of plant, plant age and the simultaneous presence of competing metals [73]. The relationships between the metal contents of spice plant and soil samples in this study were also analyzed. As it can be seen from Figure 7 (a - c), 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 Ca where the level in the soil is extremely low as compared to that of the black pepper plant, which is may be due to the high uptake of the metal by plant, but for the rest plant the concentration of metals in plants is less than their corresponding soil. This unproportionality variation in level of metals in spice plant and soil may be resulted from the difference in availability of absorbable form of metals in soil due to difference in soil acidity or the presence of competing ligands and differences in their organic matter content (Table 8). It can further be inferred that the relatively higher pH (5.86 ± 0.05 - 6.57 ± 0.08) values of the soils might have contributed for the low transfer of metals from the soils to the plant parts.



(a)



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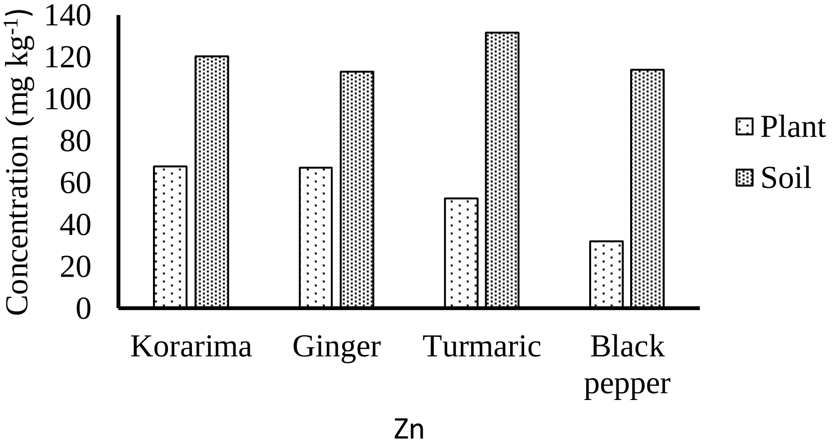

(c)

Figure 7: **(a - c)** Comparisons of metal levels in korarima, ginger, turmeric and black pepper plant with respective soil samples.

3.5. Translocation factor

Metals from soil are absorbed by plant roots and then distributed in various plant tissues. Such transmission of metals from soil to plant tissues is studied using an index called as Transfer Factor (TF). It is calculated as a ratio of concentration of a specific metal in plant tissue to the concentration of same metal in soil, both represented in same units. Higher TF values (>1) indicate higher absorption of metal from soil by the plant and higher suitability of the plant for phyto-extraction and phytoremediation. On the contrary, lower values indicate poor response of plants towards metal absorption and the plant can be used for consumption [74, 75].

$$\text{TF} = \frac{\text{concentration of metals in plant tissue}}{\text{concentration of metals in corresponding soil}}$$

Table 13: Gives a list of TF values for Ca, Cu and Zn in the four spice plants varieties. The current study showed that the TF values varied between 0.103 - 4.491 for Ca, 0.183 - 0.389 for Cu and 0.280 - 0.594 for Zn. All the values reported here were found to be less than 1 except for Ca in the black pepper plant, which is greater than one. For Zn, TF value was detected to be highest in all spice plant (except in black pepper), while the TF values of Cu is comparable with that of Ca (except in black pepper). Thus it can be concluded here that, the plant species that are nutritionally

insignificant to humans are absorbing higher concentration of Zn (except for black pepper). The TF values for Zn were found to be higher than Ca and Cu (except for black pepper) indicating that the plants show higher affinity towards Zn than Ca and Cu. Overall TF values are thus found to be significant.

Table 13: Transfer factor (TF) values of spice plants for metals Ca, Cu and Zn

Metal	Korarima	Ginger	Turmeric	Black pepper
Ca	0.177	0.395	0.103	4.491
Cu	0.389	0.183	0.243	0.372
Zn	0.563	0.594	0.400	0.280

A TF values of Ca in spice plant is in decreasing order of: black pepper > ginger > korarima > turmeric. For Cu: korarima > black pepper > turmeric > ginger and for Zn: ginger > korarima > turmeric > black pepper.

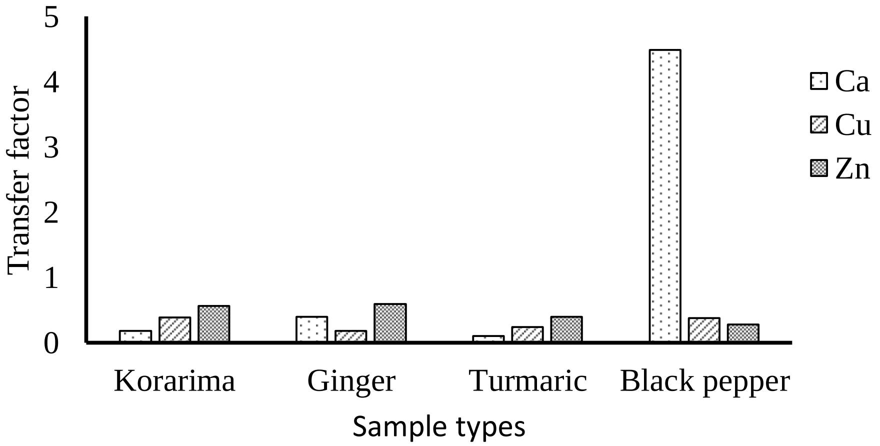

Figure 8: Comparison of TF values of metals in korarima, ginger, turmeric, black pepper spice

3.6. Trend of variation of metals in plants

Calcium concentration in fruits of black pepper, rhizomes of ginger, seeds of korarima and rhizomes of turmeric were found to be 5369.67 ± 240.18, 965.46 ± 17.54, 627.15 ± 36.05 and 429.01 ± 1.89 mg kg^{-1} respectively. The higher level of calcium in the studied spice plants is expected as the nutrient is highly mobile in the plant tissue and is translocated to different parts. Furthermore, broad concentration range of calcium bearing minerals in soil and water samples and also abundant in ground water and surface water can easily absorbed by the plants. Among the studied plant materials, black pepper fruits had the highest calcium contents and a turmeric rhizomes had the minimum concentration of calcium contents.

Cadmium was not detected in the samples of the present study (it is blew the detection limit). The uptake of cadmium is controlled by different mechanisms in different species. Physical conditions like salinity, temperature, pH of the medium, contents of organic matter and presence of other metals in the soil influence the cadmium uptake and accumulation in spice plants. WHO permissible limit for Cd is 21 mg kg^{-1} for edible plants [4].

Chromium was not been detected in any of the spice plant materials (below the detection limit of the instrument). This could be because Cr is immobilized in the vacuoles of the root cells of plants and showed less translocation, thus rendering it less toxic. This may be a neutral toxicity response of the plants. According to Macnicol and Bekett, the toxic levels of Cr in plants range from 1 to 10 mg kg^{-1} dry weight [76].

Copper is an essential microelement for plants. But high concentration of copper affects respiration, metabolism of carbohydrates, lipids and proteins. The concentration range of copper for the assayed spice plant materials were found to be (7.62 - 10.67 mg kg^{-1}) Table 10. The lowest concentration (7.62 ± 0.05) was observed in korarima plant and the highest concentration (10.67 ± 0.24) was observed in black pepper plant. The result obtained for all the plant materials are in the permissible limit of copper set by WHO (50 mg kg^{-1}) for edible plants [4]. The concentration of copper obtained in the plant materials of the present study is comparable with the reported values for other spice plants [60]. Cu availability for plant uptake is dependent upon number of plant factors such as root intrusion, water and ion fluxes and their relationship to the kinetics of metal solubilization in soils; biological parameters, including kinetic of membrane transport, ion interactions and metabolic fate of absorbed ions.

Zinc concentration range was found to be (31.88 - 67.7 mg kg^{-1}). Among the investigated plant materials, seeds of korarima accumulated higher zinc concentration (67.7 ± 2.8 mg kg^{-1}) which is almost comparable with the rhizomes of ginger (67.02 ± 3.75). The minimum concentration of zinc is obtained in fruit of black pepper (31.88 ± 0.60). Zinc contents of the studied plant materials has been compared with the limit proposed by FAO/WHO in the edible plants (100 mg kg^{-1}). It was found that all the plant materials accumulated zinc blow this limit. The obtained results of the present study are in good agreement with the concentrations of zinc reported for other spice plants of gingers and black peppers [4].

Nickel was also not detected in all spice plants. 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]. WHO permissible limit for nickel in edible plant is 50 mg kg^{-1} [4].

Lead: Lead was not detected in all plant materials. This is because 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 is greater in sandy soils, which tend to lack organic matter, than in organic soils [60], which is the organic matter content of soil range from (13.93 - 20.59 %) which forms a complex with the metal and reduces the uptake of lead by plants. WHO permissible limit for lead in edible plant is 100 mg kg^{-1} [4].

3.7. Trend of variation of metals in soil samples.

The metal contents of the soil samples are presented in Table 11 as indicated in the table Ca (1195.67 ± 35.85 - 4147.17 ± 329.25 mg kg^{-1}) is the predominant metals followed by zinc (112.9 ± 7.17 - 131.63 ± 7.98 mg kg^{-1}) in all soils. Chromium (21.92 ± 0.79 - 45.76 ± 0.89 mg kg^{-1}), nickel (21.4 ± 0.15 - 56.93 ± 3.28) and Copper (13.03 ± 0.29 - 28.67 ± 1.36 mg kg^{-1}) were the least concentration metals in the investigate soil samples.

The variation trend of metals in the korarima soil samples follows the decreasing order of: Ca > Zn > Ni > Cr > Cu, concentration level of the metals in ginger soil samples followed the decreasing order of: Ca > Zn > Cr > Ni > Cu. The level of metals in turmeric soil samples followed the same order as that of Ginger soil samples and black pepper soil samples also followed the same trend variation of metals as like that of korarima soil samples.

3.8. Comparison of metal levels of spice plants with literature values

Comparison of levels of metals in spice plants of Ethiopia with other spice plants reported in various parts of the world are summarized in Table 13. The results of present study are in good agreement with the most of reported values, especially for the essential metals investigated. The concentration level of copper in the spices used for comparison ranges from 2.11 to 48.2 mg kg^{-1}, while the value determined in this study lies from 7.62 to 10.67 mg kg^{-1} which is within the range. The mean concentration of Zn is from 11.40 to 134.49 mg kg^{-1} in the literature and from 31.80 to 67.7 mg kg^{-1} in this study. No literature report is found to compare calcium metal detected in Ethiopian spices with other country. The level of Cd in most literature is below the detection limit and Pb was also found to be below the detection limit in literature, which is in good agreement with our study values, that assures the low lead and cadmium exposure of the farmlands, however the report of Pb in ginger from Ghana was very high (115 mg kg^{-1}). The minimal risk levels for hazardous Pb, and Cd metal through oral route and its acute effect are 0.0002 and 0.0002 mg kg^{-1} per day, respectively [23]. Whereas the human need from spice plant is very few grams per day, hence there is no risk from used spices plant in the current investigation for food flavoring.

Chromium was not reported in some of the spices in the literatures considered. However, there are reported values of Cr with concentration range of 0.01 to 3.2 mg kg^{-1}, while in the results of the present study it is below the detection limit of the instrument was recorded. The nickel concentration of the present study is below the detection limit of the instrument. The concentration of Ni in literature ranges from 0.96 to 43 mg kg^{-1} which is less than the WHO permissible limit (50 mg kg^{-1}) and much higher than the concentration of spice plant of Ethiopia which is below the detection limit of the instrument. However, Ni toxicity is not a very common occurrence because its absorption by plant is very low [53].

Table 14: Comparison of metal concentrations (mg kg^{-1}) in spice plants with other spice plants from some other parts of the world.

Spice plants	Concentration (mg kg^{-1}) of metals in plants								Ref.
	Origin	Ca	Cu	Cr	Cd	Ni	Zn	Pb	
Elettaria cardamomum	Pakistan	-	48.2	-	-	-	50.6	-	77
Coriander	India		20.79	0.64	BDL	8.96	134.49	-	78
Marjoram	Turkey		11.3	3.2	BDL	3.2	83.7	BLD	53
Peppermint	Egypt	-	2.11	-	BDL	0.96	11.4		79
Rosemary	Ghana,	-	11.0	-	-	35	71	-	4 ,
	Turkey	-	-	-	BDL	-	-	BDL	53
Nutmeg	Nigeria	-	0.01	0.01	0.5	9.3	7.2	5.3	80
Ginger	Ghana	-	9.0	-	-	43	73	115	4
Korarima	Ethiopia	627.15	7.62	BDL	BDL	BDL	67.7	BDL	Present
Ginger	Ethiopia	965.46	8.02	BDL	BDL	BDL	67.02	BDL	work
Turmeric	Ethiopia	429.01	8.53	BDL	BDL	BDL	52.62	BDL	
Black pepper	Ethiopia	5369.67	10.67	BDL	BDL	BDL	31.80	BDL	

3.9. Comparison of metals in soil samples with different standards.

For all soil samples of the current study, the concentration ranges observed in the investigated essential and non-essential metals were Ca (1195.67 - 4147.16), Cu (13.03 - 28.67), Cr (21.92 - 45.76), Ni (21.40 - 56.93) and Zn (112.9 - 131.63). Compared to the established international regulatory standards, the concentration of Cu, Cr and Zn in the soil samples studied were found to be lower than maximum permissible limits set by Great Britain (100, 50 and 300 mg kg^{-1} respectively), while the concentration of Ni found in black pepper soil was a little bit higher than maximum permissible limit set by UK, which was 50 mg kg^{-1} [81], but lower than set by WHO which is 68 mg kg^{-1}[82]. The concentration range of the Cu, Cr, Ni and Zn are also found to be below the limit for all the investigated soil samples which also agree with the standard set by the USEPA [81]. However, the concentration of Zn in all soil sample is above the limits set by WHO, 50 mg kg^{-1} [82]. The relatively high concentration range of Ca investigated, in this study might be useful for growing plants since concentration of Ca in soil is tolerable. Concentration ranges of metals are within the acceptable range for soil samples compared to the other international standards.

3.9. Pearson correlation of metals in spice plant sample

Correlation is a statistical measurement of the relationship between two variables. Possible correlations range from +1 to -1. A zero correlation indicated that there is no relationship between the variables. A correlation of -1 indicates a perfect negative correlation, meaning that as one variable goes up, the other goes down. A correlation of +1 indicates a perfect positive correlation, meaning that both variables move in the same direction together.

Correlation between heavy metals in four spice plants as mean heavy metal contents are calculated for each plants separately. There are positive correlations between metals in ginger and turmeric plants and these relations are statically significant for both plants and all metals but are not significant for Cu and Zn in ginger plant (0.3043) which was weak positive correlation Table -15. A strong positive correlation was found between Zn and Ca while strong negative correlation was found between Cu - Ca and Zn - Cu for korarima plant. A weak positive correlation between Zn and Cu was observed for black pepper plant and negative correlation was found between Cu - Ca and Zn - Cu. Positive correlations for metal contents between metals in spice plants are found in expected results, because plants take nutritional elements from the environment through soil and leaves. The high negative correlation between Ca and Cu indicate the large absorption of Ca may affect the absorption of Cu in plant. The other metals have weak negative or positive correlation indicating that the presence or absence of one metal affect in lesser extent to the other.

Table 15: Pearson Correlation of metal in spice plants (a - d)

(a) korarima plant

	Ca	Cu	Zn
Ca	1		
Cu	- 0.99303	1	
Zn	0.9998	- 0.9904	1

(b) ginger plant

	Ca	Cu	Zn
Ca	1		
Cu	0.98576	1	
Zn	0.46016	0.3043	1

(c) turmeric plant

	Ca	Cu	Zn
Ca	1		
Cu	0.86903	1	
Zn	0.98702	0.77829	1

(d) black pepper plant

	Ca	Cu	Zn
Ca	1		
Cu	- 0.87369	1	
Zn	0.24518	- 0.68583	1

Table 16: Pearson Correlation of metal in soil (a - d)

(a) soil from which korarima was sampled

	Ca	Cr	Cu	Ni	Zn
Ca	1				
Cr	-0.9992	1			
Cu	0.95158	-0.96259	1		
Ni	-0.23671	0.2735	-0.52391	1	
Zn	-0.93228	0.94537	-0.99834	0.57214	1

(b) soil from which ginger was sampled

	Ca	Cr	Cu	Ni	Zn
Ca	1				
Cr	0.92039	1			
Cu	-0.98872	-0.85145	1		
Ni	0.77034	0.4597	-0.85715	1	
Zn	-0.7659	-0.96027	0.67127	-0.19361	1

(c) soil from which turmeric was sampled

	Ca	Cr	Cu	Ni	Zn
Ca	1				
Cr	-0.14615	1			
Cu	-0.93718	-0.20814	1		
Ni	0.82721	-0.67675	-0.57923	1	
Zn	-0.99521	0.24217	0.89858	-0.87819	1

(d) soil from which black pepper was sampled

	Ca	Cr	Cu	Ni	Zn
Ca	1				
Cr	-0.98323	1			
Cu	0.94905	-0.9906	1		
Ni	0.75776	-0.86407	0.92479	1	
Zn	0.88963	-0.958	0.98822	0.97213	1

4. CONCLUSION AND RECOMMENDATION

Study of metal contents of the spice plants (korarima, ginger, turmeric and black pepper) used for enhance taste of foods cultivated in Jima zone, south west of Ethiopia and the soil samples from their corresponding sampling sites have been carried out in this study for their contents of Ca, Cd, Cr, Cu, Ni, Zn and Pb. Concentration level of seven essential and non-essential selected metals have been analyzed by FAAS. It is founded that Ca, Cu and Zn metals were detected in spice plants and the rest metals are below the detection limit of the instrument. Black pepper contains the highest concentration of Ca, these element is very important for human being and required in much amount in daily diet. Therefore black pepper is a good source of calcium. Soil sample contains Ca, Cu, Cr, Ni and Zn metals while, Pb and Cd were below the detection limit of the instrument. The digestion procedure of both plant and soil samples followed the optimized procedure , which was optimized to suit the matrices under question, allows the use of acids with minimum volumes leading to reduced blank values, low detection and quantitation limit. The effectiveness of the chosen optimized digestion procedure were revealed by excellent recover obtained during the analysis for both plant and soil samples.

The present study revealed that the essential and the trace metal content of the selected spice plants of Ethiopia were within the safe limits, the concentration of all the determined metals were below the maximum limit sited by WHO/FAO. Therefore it is concluded that these levels may not contribute to metal toxicity and the human health is unlikely to be directly affected by these spice plants when consumed as part of diet. It is imperative to regulate the metal load of elements in our diets. The present study provides a base line data for health policy maker towards maintaining a healthy life style.

Since spices are widely consumed all over the world, the assessment of levels of essential and heavy toxic metals are particular interest with respect to human health, the quality of its products and environmental safety. Thus, the present study will give brief information about the metal contents of the spice plants cultivated in Ethiopia. It might be useful in pointing directions for studies that will be conducted on the nutrient levels in regard to the mineral status of the spice plants. Therefore, further study of the relationship between the metal levels of the spice 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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DECLARATION

“I hereby declare that the project submitted for the Degree of Master of Science (Analytical Chemistry), at Addis Ababa University, Addis Ababa, Ethiopia is my own original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references.”

Name: Tura Gemechu

Signature: ________________

Place of submission: Addis Ababa University, June 2014

This project has been submitted for examination with my approval as University advisor

Name: Negussie Megersa (PhD)

Signature _____________________

Addis Ababa University
June 2014