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DETERMINATION OF Fe, Zn, Cr and Pb CONTENT IN SELECTED SPICES AVAILABLE FROM SELECTED MARKETS IN WESTERN AMAHARA REGION, ETHIOPIA

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Wisdom at the source of the Blue Nile

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DETERMINATION OF Fe, Zn, Cr and Pb CONTENT IN SELECTED SPICES AVAILABLE FROM SELECTED MARKETS IN WESTERN AMAHARA REGION, ETHIOPIA

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Thesis Approval Sheet

The thesis titled "DETERMINATION OF Fe, Zn, Cr and Pb CONTENT IN SELECTED SPICES AVAILABLE FROM SELECTED MARKETS IN WESTERN AMAHARA REGION OF ETHIOPIA" by Yeshiwork Yigzaw is approved for the degree of "Master of science in analytical chemistry."

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Declaration

I, undersigned, declare that the work described in this thesis is original work undertaken by myself for the Master of Science in analytical chemistry, at the college of science, department of chemistry BahirDar University. All sources of materials used for the thesis have been referenced and duly acknowledged.

Yeshiwork yigzaw

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Date of submission:__________________

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ABSTRACT

Many different spices make up Ethiopian cuisine, the main ingredient that is a must and need to be mastered and understood is Mixed berbere that contains multiple spices like coriander, sacred basil, ginger, and other spices that each brings their own complex flavor and fragments to the mix and may also contain essential and toxic metals. Although spices are widely used in Ethiopia, there is still paucity of information about their mineral contents. Some essential metals and toxic metal such as Fe, Zn, Cr and Pb were determined in selected spices (demblal/coriander seeds, besobela/sacred basil and senafich/ mustard seed) collected from three local markets of western Amhara region, Ethiopia by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Hot plate digestion method was applied for the digestion of the samples. Spike recoveries are 85.7% and 97.4% for Fe and Zn metals. The calibration curve regression coefficient value (R^2) for Fe, Zn, Cr and pb was 0.9989, 0.9997, 0.9995 and 0.9997 respectively. The concentration level of metals in selected spices were ranged from 20.2mg/kg to 1908.5mg/kg for Fe, 6.1mg/kg to 37.1mg/kg for Zn, 0.2mg/kg to 5.3mg/kg for Cr and ND (below detection limit) for Pb. The concentration of Fe, Zn and Cr in all the samples under the study were above WHO/FAO 2009 permissible limit of recommended (for Fe, Zn, Cr and Pb were 20mg/kg, 5.0mg/kg, 0.3mg/kg and 0.3mg/kg) that indicates the intake of such foods can cause the accumulation of these metals in various parts of the body. But the concentrations of Pb in all sample is below detection limit indicates these selected spice samples are free from toxicity of Pb metal.

Key words: Spices, essential metals, toxic metals, ICP-OES**.**

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1. INTRODUCTION

1.1. Background of the Study

In the last three decades, mainly because of their medicinal values, the use of spices and other herbs has increased markedly in most regions of the world, including Europe and North America. For instance, during this period, herbal medication in the USA has grown into an industry worth an average of $1.5 billion per year, with projected annual growth of 15 %(Abebe, 2006). Many common spices have outstanding antimicrobial effects. On the other hand, the process of preparation and handling can make them a source of food poisoning (Billing and Sherman, 1998). With the current emphasis on eating more healthy diets that are low in fat and salt, people 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, stir-fry, and pasta dishes can be derived from the bark, buds, flowers, leaves, fruit, seeds, rhizome, or roots of a plant (Mesfien et al, 2009). The presence of essential metals like iron, copper, nickel and zinc are very useful for the healthy growth of the body though very high levels are intolerable, metals like mercury, lead, cadmium etc are toxic at very low concentrations. The addition of spices that may be contaminated with trace and heavy metals to food as a habit may result in accumulation of these metals in human organs and lead to different health troubles. These metals may reach and contaminate plants, vegetables, fruits and canned foods through air, water and soil during industrial processing and packaging (Ozores et al, 1997). The study of these heavy metals is crucial because they have potential hazardous effect, not only on compounds but human health as well. This is due to their cumulative behavior and toxicity although they are generally present in agricultural soils at low levels (Ozkutlu et al, 2006).

The human body requires trace elements for healthy growth, development, and proper body functioning (Soomro et al 2008). Studying their contribution to the human diet becomes very important because excessive or insufficient intake may cause various nutritional diseases. Chromium potentiates insulin action, influencing carbohydrate, lipid, and protein metabolism. Chromium (III) is an essential element with low toxicity and rare deleterious effects of excessive intake (WHO 2010). Chromium (VI) is toxic and has respiratory, cardio vascular, gastrointestinal, hematological, hepatic, and neurological effects (Tchounwou et al 2012). Food consumption is the main source of daily chromium exposure for most people. Chromium is usually accumulated in liver, kidney, skin and lung. Small amount of Cr (III) is essential in controlling insulin, cholesterol and lipid biosynthesis (Adams and Happiness 2010).

Hexavalent chromium, Cr (VI) is known to be responsible for causing cancer in human body which is yet to be explored fully. However, it has been reported that Cr (VI) binds to double stranded deoxyribonucleic acid (DNA), therefore altering gene replication, repair and duplication. Severe and often deadly pathological changes (asthma, bronchitis, dermatitis, renal failure etc.) are occurred in cases of excessive intake of Cr, especially Cr (VI) (Goettsch 1991).

Iron is an essential element in haemoglobin, myoglobin, ferredoxins, and several enzymes active in porphyrin synthesis, transport of oxygen, and normal functioning of the immune system (WHO 1996). Deficiency in Fe causes anaemia. Infants, children, and pregnant mothers are the most susceptible to anaemia, an Fe deficiency (Goettsch 1991). Lead is a xenobiotic toxicant which can accumulate in body organs (gastrointestinal tract, and kidneys) and the central nervous system leading to poisoning (Wuana and Okieimen 2011). Children are most vulnerable to Pb poisoning due toneurological,neurobehavioural,anddevelopmentaleffects (Ahamed and Siddiqui 2007). Lead has been classified as a category 2B carcinogen (possible carcinogenicity to humans) by the IARC 2016. Airborne lead can be deposited on soil, water and plants thus reaching human body via the food chain. Lead is preferred to be accumulated in the skeleton and cause renal tubular damage and kidney damage (Lead, W.I., 1995). International Agency for Research on Cancer (IARC) classified cadmium and lead as human carcinogen (IARC, 1991).

Spices are essential foodstuffs in a country like Ethiopians. Many spices have been documented to possess anti-diabetic, anti-inflammatory, and anti-hypertensive potential (Jarup et al 1998). Only a small fraction of the many bio chemical's found in plants are relevant for the quality of spiciness; many classes are hardly ever found in spices, as their taste in unpleasant or they are not safe at all. Cadmium is present in most foodstuffs with varied concentrations. Cadmium exposure may cause kidney damage and/or skeletal damage (Satarug and Moore 2004).

Approximately 90% of total intake of nickel comes from food. Excess amount of nickel can be stored in skin, lung, throat and kidney. Small amount of Ni (II) in human body play an important role in regulating prolactin and stabilization of RNA and DNA structures. Excess nickel in human body may lead to lung cancer, nose cancer, larynx cancer, prostate cancer, asthma and chronic bronchitis, heart disorders etc. Nickel in zero oxidation state is said to be more toxic than its other state (Khan and Qayyum, 2002, Mukherjee et al 2003). Contrary to the toxic metals, Iron, copper, magnesium, cobalt and zinc are known to be essential which are so significant for nutrition and other metabolic functions in human body. The presence of essential metals like iron, copper, nickel and zinc are very useful for the healthy growth of the body though very high levels are intolerable, metals like mercury, lead, cadmium etc. are toxic at very low concentrations. The addition of spices that may be contaminated with trace and heavy metals to food as a habit may result in accumulation of these metals in human organs and lead to different health troubles. These metals may reach and contaminate plants through air, water and soil during plants growth and food processing (Ozores Hampton et al 1997).

1.2. Statement of the Problem

Although many different herbs and spices make up Ethiopian cuisine, there is one main ingredient that is a must and need to be mastered and understood. The spice mixed berbere contains multiple spices (coriander, sacred basil, and black pepper etc.) that each brings their own complex flavor and fragments to the mix and May also contain essential and toxic metals (Gall and Shinkute 2009). Although spices are widely used in Ethiopia, there is still paucity of information about their mineral contents. It has to be noted that some minerals are essential to human at a certain concentration level and species are expected to be one contributor of the individuals daily need of minerals intake.

1.3. Objectives of the Study

1.3.1. General Objective

The main objective of this study was to determine the content of selected heavy metals (Fe, Zn, Cr and Pb) in selected spices (demblal/coriander seed, besobela/sacred basil and senafich/ mustard seed) available in three selected markets (DebreMarkose, FenoteSelam and BahirDar) from Amhara region of Ethiopia.

1.3.2. Specific Objective

- To determine the level of essential and toxic metal contents in coriander seeds, sacred basil and mustard seed**.**
- To compare level of Fe, Zn, Cr and Pb metals in coriander seeds, sacred basil and mustard seed spices with WHO limit**.**

1.4. Significance of the Study

In Ethiopia, spices have varied uses (Zuberi et al. 2014). However, there is little information available about the contribution of these heavy metals to the intake of mineral nutrients. Furthermore, safety of these spices with respect to heavy metal contamination have not been studied in depth. Monitoring the levels of heavy metal toxicity in spices would help ascertain the health impact of taking these spices, and provide relevant data on spices in the country. The information gained from this study is expected to be useful to the general people of this region either to select such kind of food on the basis of the abundances of the essential trace metals or to avoid/limit the food that exceeding the permissible concentration of the toxic metals.

2. LITERATURE REVIEW

Definition of spices: Spices are essential oils that give foods and beverages flavor, aroma and sometimes color. The term spice refers to any dried plant product used primarily for seasoning, be it the seed, leaves, bark or flowers. They can be marketed whole, ground to a powder or in the form of essential oils and oleoresins. Many spices are also used for other purposes. Plants such as turmeric (*Curcuma longa*) are increasingly in demand for natural therapies, while others such as peppers (*Capsicum spp.)* serve as substitutes for chemical dyes or pesticides. Another definition indicates: A spice is a dried seed, fruit, root, bark or vegetative substance used in nutritionally insignificant quantities as a food additive for the purpose of flavoring, and sometimes as a preservative by killing or preventing the growth of harmful bacteria. Many of these substances are also used for other purposes, such as medicine, religious rituals, cosmetics, perfumery or eating as vegetables. For example, turmeric is also used as a preservative; licorice as a medicine; garlic as a vegetable and nutmeg as a recreational drug (Goettsch 1991).

Figure 1: common spice samples

Definition of herbs: In general use, herbs are any plants used for flavoring, food, medicine, or perfume. Culinary use typically distinguishes herbs as referring to the leafy green parts of a plant (either fresh or dried), from a "spice", a product from another part of the plant (usually dried), including seeds, berries, bark, roots and fruits. In Ethiopia several culinary herbs are produced: chervil, chives, coriander, dill, green basil, lovage, mint, oregano, rocolla, thyme, vernonia, etc (Khan et al 2008).

Figure 2: selected spice samples

Heavy metals: Heavy metals are individual metals and metal compounds that can impact human health, "A metal of relatively high density (Specific gravity greater than about 5) or of high relative atomic weight (especially one i.e. Poisonous) one mercury or lead." It has been includes density, atomic weight, atomic number, or periodic table position. Density criteria range from above 3.5 g/cm^3 to above 7 g/cm^3. Atomic weight definitions start at greater than sodium (22.98) to greater than 40. Atomic numbers of heavy metals are generally given as greater than 20; sometimes this is capped at 92(uranium). Hawke' suggested referring to heavy metals as "all the metals in Groups 3 to 16 that are in periods 4 and greater. The term "heavy metals" was in use as far back as 1817, when Gmelin divided the elements into nonmetals, light metals and heavy metals. Light metals had densities of 0.860–5.0 gm/cm3; heavy metals 5.308–22.000. In 1868, Wanklyn and Chapman speculated on the adverse effects of the heavy metals "arsenic, lead, copper, zinc, iron and manganese" in drinking water (Prakash et al 2011).

Heavy metals play a very important role in human nutrition (Dospatliev and Katrandzhiev 2012). Trace metals are useful at very low concentrations, but these are toxic at high concentrations. Cadmium and lead are heavy metals which are widely distributed, naturally occurring, and potentially toxic elements. With increasing industrial use, environmental pollution and associated toxic exposure, concern has increased about their long term exposure and potentially toxic effects on human health specially infants and young children which are at the pick of growth (Divrikli and Soylak 2006). Heavy metals are mobile and easily taken up by the plants in the environment. Metals accumulation in herbs and spices may pose a direct threat to human health

(Ddepoju et al 2003). Different substances occur naturally in our environment as a consequence of natural events. Many diseases are caused by the inability of environment to support the mineral needs of human, plants and animals or man (Mobeen et al 2009). Untreated sewage and industrial effluent are commonly used for the cultivation of herbs and spices around farm lands (Ozcan 2004).

2.1. Importance of Spices

Herbs and spices grown in various regions of the world have been used for several purposes since ancient times. Several uses of these plants are for culinary purposes (Arora and Kaur 1999). Spices and herbs belong to condiments, substances which do not contain nutritive components. Although a few dozen different spice plants are of global importance, many more are used as condiments locally, in the regions of their natural occurrence. Some of these are traded in small quantities and used in ethnic restaurants. Spices have been recognized to have some medical properties due to antioxidant and antimicrobial action (Samotyja and Aurbanowicz 2005, Roussel and Verdeaux 2003). In the last three decades, mainly because of their medicinal values, the use of spices and other herbs has increased markedly in most regions of the world (WHO 2010). Many common spices have outstanding antimicrobial effects. On the other hand, the process of preparation and handling can make them a source of food poisoning. With the current emphasis on eating more healthy diets that are low in fat and salt, people 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, stir-fry, and pasta dishes can be derived from the bark, buds, flowers, leaves, fruit, seeds, rhizome, or roots of a plant (Sobukola and Kajihausa 2010).

In Ethiopia, berbere is used to make a sauce called awaze, which is used much like roux but with berbere spice instead of flour. It is base for braised meats. Berbere spice is used to make Ethiopian favorites such as the chicken stew known as doro wot and the lentil stew, misr wot. The berbere spice blend contains various spices, each of which provides nutrition compounds. Chili pepper is the main components of berbere spice and they are rich in vitamins A and C. both vitamins are potent antioxidants that help to protect the body from damaging free radicals. Vitamin A is also necessary for vision support and for functioning immune system. Vitamin C is other benefits include the fact that body needs it to make serotonin as well as collagen, which the body uses to generate various tissues. The fenugreek in berbere spice is rich source of riboflavin, which is known as vitamin B2. The body uses riboflavin to maintain skin health and for increasing energy levels. Berbere seasoning also contains fenugreek, which is rich in iron copper and other minerals. The body uses iron and copper to make hemoglobin and copper may also be an antioxidant**.** Fenugreek seeds are an excellent source of fiber with a 100g serving able to provide more than half of daily requirement. Carom seeds and nigella seeds are both included in many berbere spice blends; these are also excellent source of fiber. Fiber is good for both cardiovascular and intestinal health. Chili pepper like those in berbere spice have been shown to reduce levels of cholesterol and triglycerides, thus reducing the like hood heart attack and stroke. Ginger is a popular ingredient in berbere spice and one of its oldest uses is as a remedy for gastro-intestinal aliments. Ginger can use to treat flatulence and nausea. The chemical that gives ginger its pungent flavor is zingerone, it is an effective treatment for certain types of diarrhea. Ginger can induce sweating, which may help with the treatment of cold and flu. In addition, the high vitamin A and C levels in chili peppers can also help to ward off infection (Salati 2009). Many informants believe that the predominant spice mix, which is the important component of many Ethiopian sauces, called locally berbere (composed of mainly C. annum powder mixed with other ground spices) is important to kill internal parasites. Berebere can be made in to paste known as dilik and eaten with injera. In Ethiopia, people who eat raw meat believe that Capsicum (hot pepper) kills dangerous micro-organisms in the meat and the stomach. It is used also against amoeba infection and intestinal worms (Jansen 1981). The medicinal use of C. annum as a treatment to ascariasis is also noted in the research work of Fisseha Mesfin (2007). Medicinal value of C. annum is again mentioned by Mathewos Agize (2008).

2.2. Spices in Ethiopia

Ethiopia has a long history of spice and herb production for the domestic market and has a unique, indigenous product, Korarima cardamom. Modest quantities of several spices have been exported for centuries to countries in the Middle East and exports to Europe have developed over the past twenty years. The history of spices in Ethiopia is an ancient one and spices remain as basic food items of the Ethiopian people. Ethiopia is the homeland for many spices, for example Korarima, long pepper, black cumin, Bishops weed and coriander. The average land covering by spices is approximately 222,700 ha and the production 244,000 ton/annum. At the moment, there are two spice extraction plants in Ethiopia, one public and the other under private ownership. The public spice extraction plant, the Ethiopian Spice Extraction Factory, has a processing capacity of 180 tons per year. The plant is capable of processing ginger from locally grown ginger root, capsium oleoresin from red pepper, and turmeric. Over 85% of its business is for paprika. The privately owned spice extraction plant in Ethiopia is Kassk Spices and Herbs Extraction PLC. This factory was built in Addis Ababa in 1997 and has a processing capacity of 120 tons per annum. Some of the extracted spice like black cumin seeds, ginger, cardamom, coriander, garlic, fenugreek and turmeric are exported overseas for food coloring, flavoring, etc. to Europe mainly Germany, Spain and Italy (Demirezen 2006).

2.3. Selected spices and their use

2.3.1. Sacred basil

Basil which is called besobla in Ethiopia is a popular herb that belongs to the family of Lamiaceae (mint). It is an annual spice herb permanently grown in warm conditions, but requires enough soil moisture throughout the growing season for high quality and yields. Basil is cultivated for the purpose of fresh market or for its aromatic leaves which are dried and used as a spice or flavoring agent. It may have originated in India and spread through Asia. Basil is now considered to be native to Africa, Asia, India, Middle East, Caribbean, and South America. It is produced commercially in Egypt, France, Hungary, Israel, Mexico, Indonesia, and United States. The quality of cultivated basil is determined by its appearance (color and absence of decay or insect damage), flavor, moisture content for the fresh market, and volatile oil content and total insoluble ash content for the processing market. Most commonly, basil leaves are used fresh or dried to add flavor to soups, tomato dishes, fish, poultry, vinegar, vegetables, salads, powdered beef, sausages, candy, gelatins, and ice cream (Hamasaki et al 1914). It is also used in most dairy products to flavor yoghurt and cheese. In addition to these, basil is used to flavor tea and to make berbere (Zuberi et al 2014). Basil is used medicinally to treat for coughs and fever in Africa. Basil was a traditional treatment for mild nervous diseases and taken as snuff in a dry powder form. Basil leaf tea alleviates vomiting, gas pains, and fevers while basil juice has been used to treat warts, worms, snake bites and insect bites (Sienkiewicz et al 2013). Commercially, the essential oil of basil is a component of mouth wash, medicine, soaps, shampoos, perfume, and liqueur. The tender stems, leaves and flowers are dried, ground and added to sauces either alone or blended with other spices to provide good flavor to the stew. It is an important ingredient of berbere and shiro powders. It is also one of the ingredients used in the preparation of clarified butter (spiced butter). The fresh leaves can be cooked or roasted in preparing roasted beef locally called tibsi, when fast preparation is required. It is explained that both dried and fresh inflorescences and leaves of basil are used as flavoring agent in the preparation of all kinds of wät. Dried ground basil is also used to flavor butter and is sometimes sprinkled in tea or coffee to add flavor. Basil oil obtained from Ocimum basilicum L. is a useful sources of compounds like methyl chavicol, eugenol, (E)-methyl cinnamate, thymol, linalool etc. (Jansen 1981). Herb complements meat, vegetables, cheese, and egg dishes. It is the presence of this essential oil that enables the plant to provide good flavor to the berbere, shiro, or butter (Coleman 1998).

Sacred basil (figure 3) is rich in antioxidant and renowned for its restorative powers, it has several benefits:

- Relieves stress / adaptogen
- Bolsters immunity
- Enhances stamina
- Provides support during cold season
- Promotes healthy metabolism
- A natural immuno-modulator

"Modern scientific research offers impressive evidence that sacred basil reduces stress, enhances stamina, relieves inflammation, lowers cholesterol, eliminates toxins, protects against radiation, prevents gastric ulcers, lowers fevers, and improves digestion (Kambel 2012, Maheshwari 2005).

Figure 3: Sacred basil (besobela)

2.3.2. Coriander

Coriander seed (Coriandrum sativum L.) Coriander, commonly called dimbilal in Ethiopia, (Figure 4) is an annual spice and aromatic herb that belongs to the family of Umbelliferae/Apiaceae. Due to its wide range of climatic, ecological and topographic conditions, Ethiopia has long been known as a center of origin and diversity for coriander. It is mainly cultivated in Jima, Wellega, Sidamo, South and North Omo, Illubabour, and East and West Gojam (Ethiopian Investment Agency 2010).

Coriander is also cultivated in Bale and Gonder and used for flavoring soups and other foods. Since ancient time, the coriander seed are used to flavor foods and beverages, especially gin and are also used medically to treat various diseases, particularly used as carminative. It is also used in perfumes, soaps, detergents, and pharmaceutical preparations (Mengesha et al 2011). Coriander seeds are used locally in Ethiopia for flavoring purposes in the preparation of red pepper powder, bread, and sauces. It is boiled in water and drunk on an empty stomach to treat stomach-ache (Asfaw and Abegaz 2014).

Figure 4:* *Coriander (demblal)

2.3.3. Mustard Seed

Mustard seed used in the preparation of a slurry condiment called Awaze/senafiche which is added to germinated beans and eaten usually during fasting days. People drinking tella in tella bets (local beer houses) also use the condiment while eating germinated bean seeds. The condiment is also served with dulet (This dish is made from chopped liver, kidney, heart, gastro-intestinal parts mixed with bile (hamot), garlic, black pepper, celery, and sautéed in vegetable oil or butter). The slurry condiment known as (Awaze/senafiche) which is made of largely ground seeds of B. nigra (it also contains other spices), is eaten with injera or germinated beans in the morning before breakfast as a treatment for intestinal parasites (amoeba). It is also reported to be a treatment for malaria (Lev 2006) also noted the medicinal use of Brassica nigra among the

Ethiopian Jews in Israel Mathewos Agize (2008) also noted the medicinal use of B. nigra. People who eat raw meat also use the awaze as a seasoning. To prepare awaze/senafiche, the seeds of B. nigra are ground and blended with chopped bulbs of A. sativum and chopped fruits of fresh green C. annum and allowed to ferment for almost 3-5 days or more. Or alternatively it can be prepared from chopped bulbs of A. sativum, ground seeds of B. nigra and berbere which is allowed to be fermented for 3-5 days or up to a week. Then finally the slurry condiment is made. The leaves and tender stems of B. nigra are cooked and eaten like collards especially during rainy summer season. About the lant, it is similarly reported in (Anonymous 2006) the young tender leaves and stem tips of the plant may be eaten raw in salads and older leaves and stouter stems cooked and eaten like collards (leafy vegetables). The seeds are also used for mustard preparation. The young shoots and leave (Figure 5) are eaten cooked and the seeds used as a spice (Paulos Abebe 2008, and Mathewos Agize 2008).

Figure 5: ***Mustard seed (snafich)***

2.4. Method of Determination of Metals in Spices

2.4.1. Inductively coupled plasma optical emission spectrometry (ICP-OES)

The analytical technique applied in this research for extraction of metals from spices is hot plate digestion method and ICP-OES. The used methodologies had been either time consuming and costly, or importantly did not allow simultaneous estimation of the micro minerals concerned. Inductively coupled plasma optical emission spectrometry (ICP-OES) is well established as a method for multi elemental analysis and the determination of isotope ratios and overcomes many of these problems. This methodology allows simultaneous analysis of a wide range of trace elements. There are various reported methods used for the determination of heavy metals in digested spice samples. The most commonly reported analytical techniques are: Flame and graphite furnace atomic Absorption spectroscopy (FAAS and GFAAS), Inductively Coupled Plasma optical emission spectroscopy (ICP-OES), inductively coupled plasma mass spectroscopy and laser ablation inductively coupled plasma mass spectroscopy (ICPMS; LAICP-MS) and others (Demirezen and Aksoy 2006).

2.4.2. Digestion Methods

Wet acid digestion and dry ashing procedures reported in the literature are tedious and time-consuming, require the use of hazardous reagents and often lead to systematic errors and failures (Welna et al. 2011). Therefore, an interest in improving and simplifying sample preparation procedures prior to the elemental analysis has lately been observed. The most common approach to achieve this is to reduce the processing time and amounts of reagents used or completely eliminating the sample matrix destruction step. So far, only few studies have been carried out to compare the different procedures for the sample preparation of spice before their elemental analysis by petrochemical methods (Narin et al. 2004a).

Examined a microwave-assisted digestion procedure in a mixture of concentrated HNO_3 and H_2O_2 solutions (3+1 ml) and a hot-plate heating digestion procedure in a mixture of concentrated HNO_3 and $HClO_4$ solutions (7+8 ml) for the determination of Cu, Fe, K, Mn, Ni and Zn in black, green, white and herbal tea samples by FAAS. In both cited reports, it was shown that there were no significant differences in results obtained using compared procedures. (Mierzwa et al. 1998).

3. RESEARCH METHODOLOGY

3.1. Sampling

In this study three types of spices (besobela/sacred basil, demblal/coriander & senafich/mustard seed) were selected for this study.

Selected Spices; coriander seeds, sacred basil and mustard seed, 100g of each spices were collected from three selected markets (DebreMarkose, FenoteSelam and BahirDar) from spice sellers in Amhara Region of Ethiopia (Table 1).

Table 1: selected spice samples and their production areas

Types of selected spics	Amount collected	Selected Central markets
Coriander	100g	BahirDar
	100g	FenoteSlam
	100g	DebreMarkosse
sacred basil	100g	BahirDar
	100g	FenoteSlam
	100g	DebreMarkosse
mustard seed	100g	BahirDar
	100g	FenoteSlam
	100g	DebreMarkosse

3.2. Apparatus, Chemicals, Reagents and Instruments

All reagents and chemicals used in the study were being analytical grade. 35.4%, concentrated hydrochloric acid (LOBA chemie), 69.7% concentrated nitric acid (RANCHEM) and 30% H_2O_2 were used for digestion of powdered spice sample. Stock standard solutions of concentration of the metals Pb, Cr, Fe and Zn standard solutions were used to prepare intermediate standard solutions. Metal analysis experiments were carried out using inductively coupled plasma optical emission spectroscopy ICP-OES (PerkinElmer ICP OES optima 8000). Analysis were conduct at Analytical Chemistry laboratory of the department of chemistry at BahirDar University, Ethiopia**.**

3.3. Sample preparation for metal analysis

3.3.1. Powdering of the sample

All the samples were ground to fine powder using an electrical blender (FW100 high speed Universal Disintegrator) then sieved by 500µm mesh size and each powdered spice samples (Coriander seeds, Sacred basil and Mustard seed) were stored using sample holder (1273 Pcs cup) **(**Figure :5).

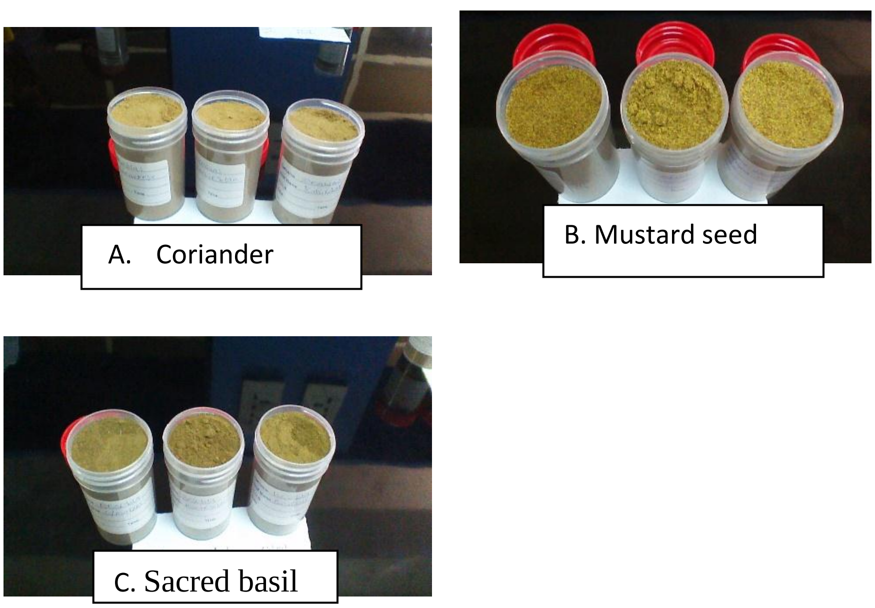


Figure 6: Powders of selected spice samples

3.3.2. Sample digestion procedure

Exactly 0.5g of powdered spice samples was weighted by using analytical balance and transferred in to 50 mL beakers. Same ratio of 35.4%, concentrated hydrochloric acid, 69.7% concentrated Nitric Acid and 30% H_2O_2 were added and heated at 80-250ºc temperature for time to get the optimum condition. Based on the result observed from the experiment 1:1:1 ratios of HNO_3, HCl and H_2O_2 at a temperature range of 80-250 ℃ for 3:00 hr. The samples were cooled and filtered by using 0.45 µm watman filter paper. Finally the digested sample was diluted by distilled water in to 50 mL volumetric flask and submitted for ICP- OES analysis (Table 2).

Table 2: Sample digestion optimization

No of trial	HNO_3	HCl	H_2O_2	Temperature	Time	Color
1	5ml	5ml	5ml	80℃-200℃	50min	Orange
2	5ml	5ml	5ml	100℃-200℃	1:20	Orange
3	5ml	5ml	5ml	150℃-250℃	1:00	Orange
4	5ml	4ml	4ml	150℃	1:50	Yellow
5	6ml	4ml	3ml	200℃	2:00	Yellowish
6	5ml	5ml	5ml	80℃-250℃	3:00	colorless

3.3.3. Standard solution preparation

Standard solutions of Fe, Zn, Cr and Pb were taken from 1000 ppm stock solution in order to prepare 10 ppm intermediate solution in 100 mL volumetric flask. Then 6 serial dilution with (0.002 ppm, 0.02 ppm, 0.2 ppm, 1 ppm, 2 ppm and 4 ppm) concentration were prepared in 50 mL volumetric flask from intermediate solution for calibration curve.

3.3.4. Spiking solution preparation

0.5 gram of coriander spice sample from BahirDar market was weighed and transferred in to 50 mL beaker this is labeled as spike. 150 µl Fe and 18 µl Zn were measured by using micro pupate from 1000 ppm stock solutions of (Zn and Fe) and added into beaker, then 5ml of HNO_3, 5mL of HCl acids were added on it and settled for 50 minutes. After 50 minutes the samples were heated for 2:30 hrs.' at 80-250℃ on a hot plate which is adjusted in the hood. The sample was cooled for 10 min and then 5mL of H_2O_2 was added and reheated for 30 min at 250℃, after that the colorless liquid was cooled for 10 min and diluted with 45 ml distilled water and filtered in to 50 ml volumetric flask using 4.5 µm watman filter paper. Finally the volume of digested samples were adjusted to 50 ml and submitted to ICP- OES analysis.

The levels of the spiked and un spiked spice samples were analyzed in duplicate and percent recovery (%R) was obtained by comparing the results between the level of concentration of metals in un spiked spice samples and that of in spiked spice samples as follow

$$\%\mathbf{R} = (\frac{\mathbf{Sp-Spu}}{\mathbf{S}}) \times \mathbf{100)} \text{ -------------------------- } \mathbf{(1)}$$

Where: **Sp**=Concentration of metal in spiked

Spu=Concentration of metal in unspiked

S=amount of spiked added concentration

3.4. Method Validation

3.4.1 Limit of Detection (LOD)

The efficiency of the experimental procedure proved by some parameters such as Method detection limit, spike recovery test and calibration of the instrument. Method detection limit is the lowest concentration of the analyte that can be detected. This value was obtained by multiplying the standard deviation of the blank by three. A general accepted definition of detection Limit is the concentration that gives a signal three times the standard deviation of the blank or background signal (Rhoades et al. 1992).

LOD = 3×Standard Deviation of Blank ---------------------------- (2)

3.4.2 Limits of quantization (LOQ)

The limit of quantitation (LOQ) is the lowest concentration of an analyte in a sample that can be quantitatively determined with acceptable precision and accuracy under the stated conditions of test. 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 (Welch et al. 2000, Ness and Cope 2016).

LOQ is calculated as:

LOQ = 10× Standard Deviation of Blank ----------------------- (3)

Table 3: Calculated limits values for the analytes

Element	Fe	Zn	Cr	Pb
LOD in mg/L	0.0042	0.001	0.0027	0.0024
LOQin mg/L	0.04	0.004	0.009	0.008
IDL in mg/L	0.0046	0.001	0.0023	0.0021
Standard divation of Blank in mg/L	0.0014	0.0004	0.0009	0.0008

4. RESULT AND DISCUSSION

4.1. Metal Analysis

4.1.1. Instrument calibration curve

The qualities of result obtained from ICP for metal analysis are highly affected by Calibration and standard solution preparation procedures. The Calibration curve was performed by using six series of diluted standard solutions (0.002, 0.02, 0.2, 1, 2, and 4 mg/L) were prepared by diluting 10mg/L the intermediate standard solution.

Table 4: Calibration curve and correlation coefficient value

Element	Equation	R^2 value	Standard solution in mg/L
Fe	Y=616967x-28678	0.9989	0.002, 0.02, 0.2, 1, 2, 4
Zn	Y=124083x+276.45	0.9997	
Cr	Y=780240x-17966	0.9995	
Pb	Y=19856x+732.58	0.9997	

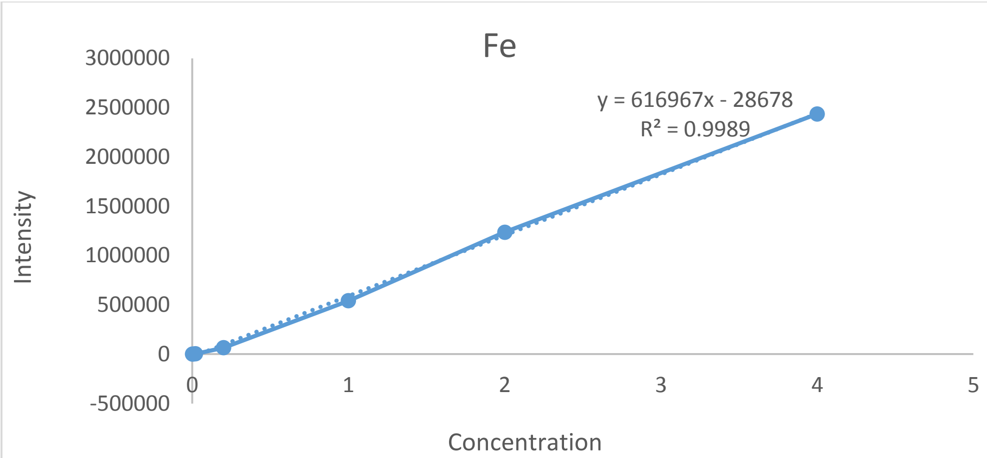
The correlation equation for Calibration curve of each metal standard using ICP-OES are represented by;-

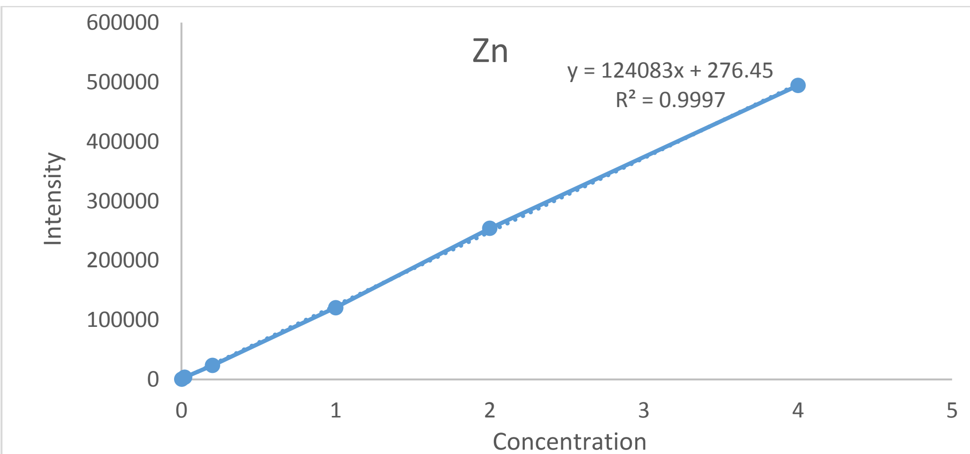
y= mx+b -------------------- (4)

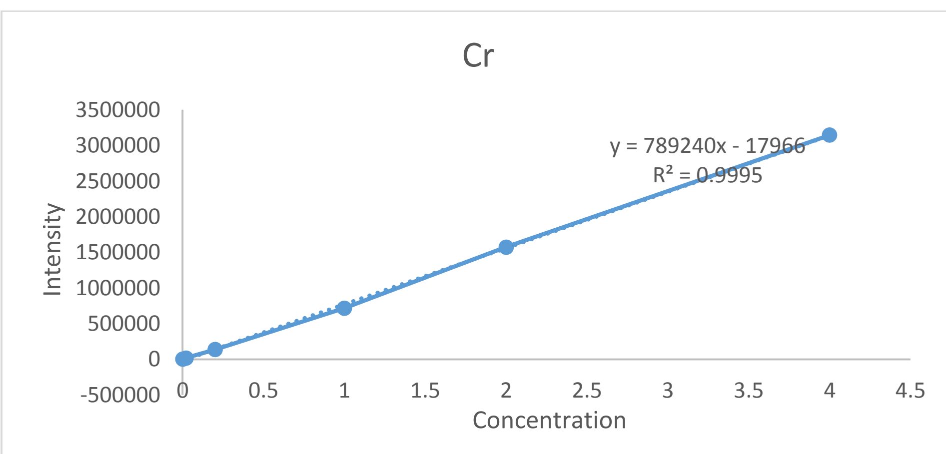
Where: y- intensity

X- Concentration

b- y-intercept


Fe
y = 616967x - 28678
R² = 0.9989
Intensity
3000000
2500000
2000000
1500000
1000000
500000
0
-500000
0
1
2
3
4
5
Concentration


Zn
y = 124083x + 276.45
R² = 0.9997
Intensity
600000
500000
400000
300000
200000
100000
0
0
1
2
3
4
5
Concentration


Cr
y = 789240x - 17966
R² = 0.9995
Intensity
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
-500000
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
Concentration

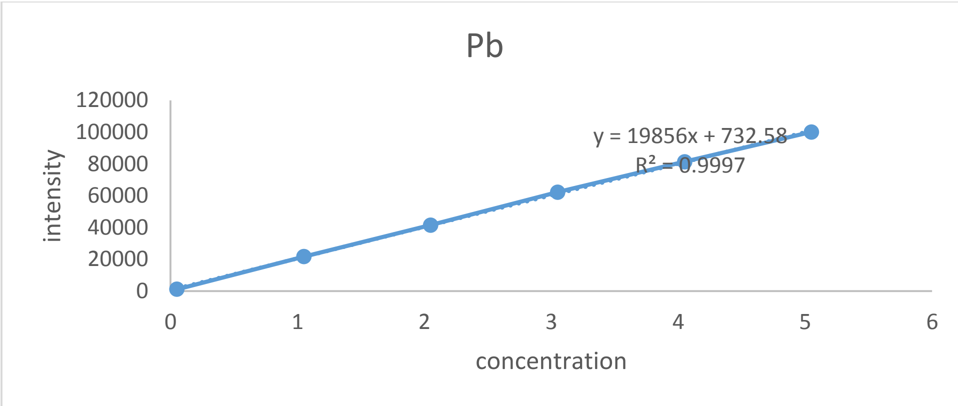


Figure 7: Calibration curve for Fe, Zn, Cr and Pb

4.2. The level of metal concentration in spice samples

The metal concentration in spice samples for this study was analyzed using ICP-OES, each sample have their own significance amount of metal concentration. The mean value of metal determined from doublet analysis for each sample and the results were recorded in terms of mean ± STD at 95% confidence interval. The analyses of selected spice samples (coriander, sacred basil and mustard seed) taken from three selected markets (debremarkos, fenoteSelam and BahrDar) spice markets were analyzed using ICP-OES instrument. The results of the analysis are summarized in table 4.

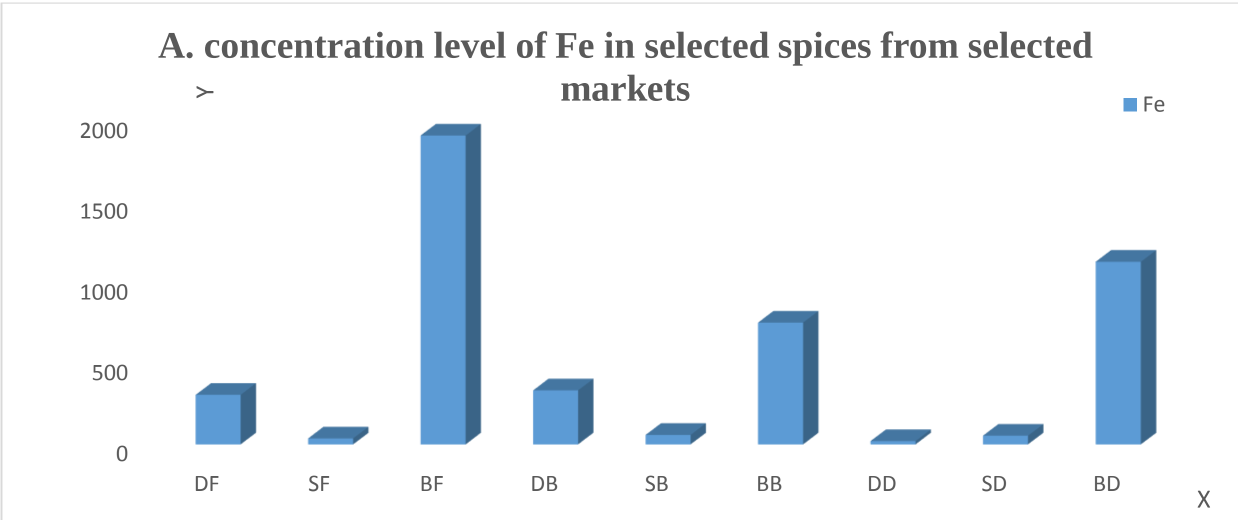
Table 5: Level of metal concentration (Mean ±STD) in mg/kg of spice samples

Sample name	Spice market	Sample code	Fe	Zn	Cr	Pb
Coriander	FenoteSelam	DF	306.8 ±6.73	11±0.05	1.3±0.03	ND
Coriander	BahirDar	DB	334.1±3.81	6.1±0.09	3.4±0.08	ND
Coriander	DebereMarkose	DD	20.2±6.34	10.6±0.13	0.8±0.03	ND
Mustard seed	FenoteSelam	SF	36.8±0.36	ND	0.6±0.01	ND
Mustard seed	BahirDar	SB	58.6±1.32	26.5±0.48	0.2±0.02	ND
Mustard seed	DebereMarkose	SD	53.7±6.46	13.2±0.12	1.2±0.01	ND
Sacred basil	FenoteSelam	BF	1908.5±4.93	30.1±0.06	5.3±0.02	ND
Sacred basil	BahirDar	BB	753.6±2.37	37.1±0.11	2.5±0.04	ND
Sacred basil	DebereMarkose	BD	1130±6.44	33.8±0.11	2.9±0.02	ND

As shown in Table 5 the concentration of metals (Fe, Zn, Cr and Pb) in mg/kg respectively in coriander sample collected from FenoteSelam market is (306.8, 11, 1.3 and ND), in mustard seed sample is (36.8, ND, 0.6 and ND) and in sacred basil sample is (1908.5, 30.1, 5.3 and ND). The concentration of the above listed metals in selected spices collected from BahirDar market in coriander sample is (334.1, 6.1, 3.4 and ND), in mustard seed sample is (58.6, 26.5, 0.2 and ND) and in sacred basil sample is (753.6, 37.1, 2.5 and ND). These selected metals concentration in selected spices collected from DebreMarkose market in coriander sample is (20.2, 10.6, 0.8 and ND), in mustard seed sample is (53.7, 13.2, 1.2 and ND) and sacred basil sample is (1130, 33.8, 2.9 and ND). As shown in table 4, the concentration of Fe, Zn and Cr in sacred basil showed the highest concentration as compared to in other seed samples collected from the different markets. Furthermore, these metals were found to be high in Coriander seed samples compared with Mustard seeds. However, this trend was reversed in Sample collected from FinotSelam market. Although Zn was abundantly quantified in Mustard seeds collected from BahirDar and DebreMarkos markets, it was not detected in sample from FinoteSelam. Whereas, Pb was not detected in all the studied samples and sampling sites. In all detected samples the concentration of Fe is highest as compared with Zn, Cr and Pb metals.

4.3. Comparison of results

4.3.1. Comparison the levels metal concentration of spice samples found in this study



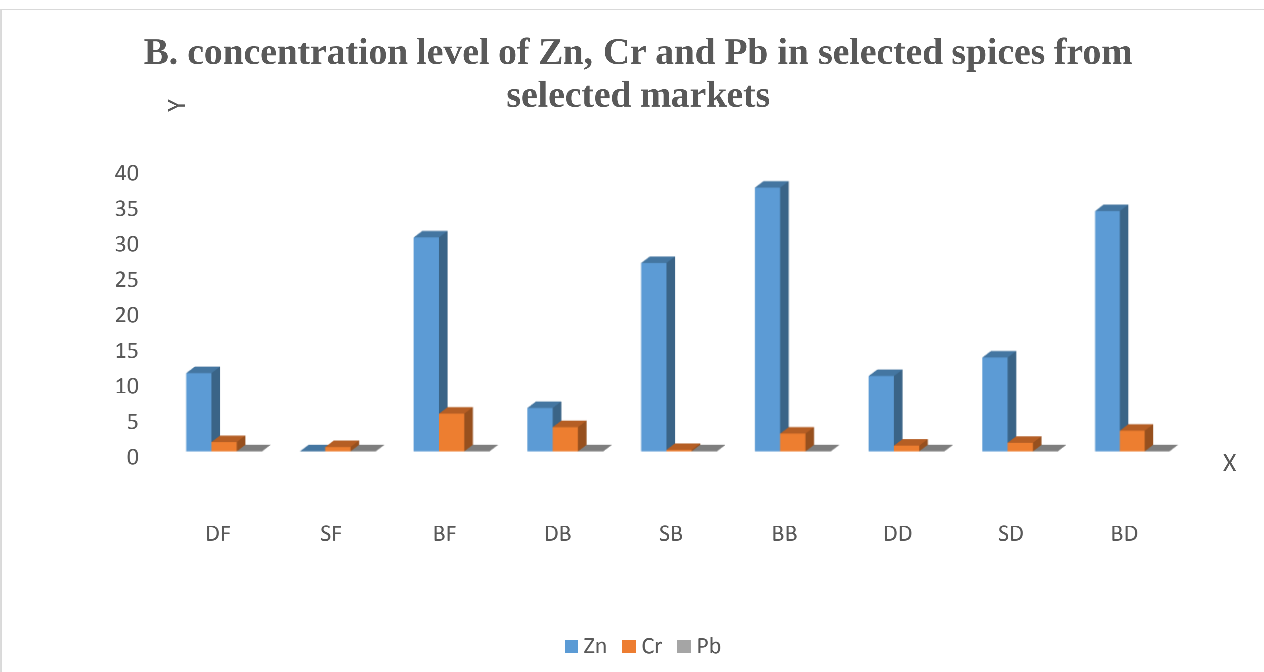


Figure 8: Concentration level of selected metals from selected markets

As shown from figure 8(A) the concentration of Fe is high in Besobela/secured basil sample in all selected markets. The highest concentration of Fe is showed in BF (Besobela/secured basil sample from fenoteSelam market) and lowest concentration of Fe is showed in DD (Demblal/Coriander sample from DebreMarkose market). The high concentration of Fe is showed in sacred basil and coriander samples from selected sites and the lower concentration of Fe is noticed in mustard seed sample.

As shown figure 8(B) the high concentration of Zn is showed in sacred basil sample from all selected sits and in mustard seed sample collected from BahirDar market. The lower concentration of Zn is noticed in coriander sample from selected sites and in mustard seed sample collected from DebreMarkose and FenoteSelam markets. The lowest concentration of Zn is showed in mustard seed sample it was not detected from FinoteSelam market. The high concentration of Cr is showed in sacred basil sample from selected sits and in coriander sample collected from BahirDar market. The lower concentration of Cr is noticed in mustard seed sample from selected sites and in coriander sample collected from DebreMarkose and FenoteSelam markets. The lowest concentration of Cr is showed in mustard seed sample from BahirDar market. The concentration of Pb is not detected in all selected samples.

4.3.2. Comparison metal analysis of this study with literature values

Table 6: Comparison of metals concentration in coriander sample with literature values and metals concentration in coriander, sacred basil and mustard seed samples with permissible limit

Type of sample	Place of sample and permissible limit source	Determination method	Level of metal concentration in mg/kg				Reference
			Fe	Zn	Cr	Pb	
Coriander, mustard seed and sacred basil	Ethiopia(Present study)	ICP-OES	20.2-1908.5	6.1-37.1	0.2-5.3	ND	Present study
coriander	Ethiopia(Present study)	ICP-OES	20.2-306.2	6.1-11	0.8-1.3	ND	
coriander	Nigeria	AAS	4.08-300.4	30-44.02	0.01	0.11-11.5	Iwegbue et al 2015
coriander	India	ICP-OES	1.699-5.475	0.009-2.575	0.114-0.156	0.18	Kumaravel and Alagusundaram 2014
Spice	permissible limit in mg/kg	-	20	5.0	0.3	0.3	WHO/FAO 2009

Table 6 shows level of metal concentrations of spice in Ethiopia 20.2-1908.5mg/kg for Fe, 6.1-37.1mg/kg for Zn, 0.2-5.3mg/kg for Cr and ND (below detection limit) for Pb. The concentration of Fe, Zn and Cr in this study is above WHO/FAO permissible limit, except Cr for mustard seed sample from BahirDar market is below WHO/FAO permissible limit but selected spices (coriander seeds, sacred basil and mustard seed) in this study were free from Pb metal contamination. As compared the concentration of metals in coriander sample with AAS determination method of Nigeria reported value the level of Zn and Pb (30-44.02 mg/kg and 0.11-11.5 mg/kg) is higher than the present study and the level of Fe and Cr (20.2-306.2 mg/kg and 0.8-1.3 mg/kg) is higher in this study ICP-OES determination value. The concentration of Fe, Zn and Cr in coriander sample in this study are higher than Indian ICP-OES determination value. But the concentration of Pb is higher in Indian reported value. This variation of level of metal concentration may be due to the variation of analytical methods, plant spices, soil type and agricultural inputs.

4.4. Recovery test results of metal determination

One sample is selected for recovery test since all the samples have similar matrix. Percentage recovery (% R) was calculated by spiking known amounts of metal concentration into 0.5 g spice sample DB (Demblal/Coriander sample collected from BahirDar spice market). The results of percentage recovery (Table 7) were 85.7% and 97.4% which confirms that the analytical method of metal concentration measurement used in this study is reliable (Table 7).

Table 7: Recovery test result for spice samples

Metal	Fe	Zn
Spiked amount of stock solution in mg/kg	300	35
Metal concentration in spiked sample in mg/kg	1153	53.1
Metal concentration in unspiked sample in mg/kg	895.5	19
% Recovery	85.7	97.4

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Essential metals like iron and zinc are very useful for the healthy growth of the body but though very high levels are intolerable, metals like mercury, lead, cadmium etc are toxic at very low concentrations. The addition of spices that may be contaminated with trace and heavy metals to food as a habit may result in accumulation of these metals in human organs and lead to different health troubles.

The main objective of this study was to determine the concentration of selected essential and toxic metal from selected spices (coriander, sacred basil and mustard seed) on selected spice markets of Amhara region of Ethiopia using ICP-OES. The result of the concentration of Fe, Zn and Cr was above WHO/FAO permissible limit but the concentration of Pb was below detection limit in all samples. The average mean concentration range of Fe, Zn, Cr and Pb were 20.2mg/kg-1908.5mg/kg for Fe, 6.1mg/kg-37.1mg/kg for Zn, 0.2mg/kg-5.3mg/kg for Cr and ND (below detection limit) for Pb.

The high concentration of Fe, Zn and Cr was found in sacred basil and coriander samples from selected sites and the lower concentration of Fe is noticed in mustard seed sample. Except the concentration of Pb the concentration of other metals in this study is higher than the reported values from Nigeria and India spices but the concentration level of Zn and Pb in the present study is lower than Nigeria reported value. As compared the concentration of metals in coriander sample with AAS determination method of Nigeria reported value the level of Zn and Pb (30-44.02mg/kg and 0.11-11.5mg/kg) is higher than the present study and the level of Fe and Cr (20.2-1908.5mg/kg and 0.2-5.3mg/kg) is higher in this study ICP-OES determination value(Table 6). This variation of level of metal concentration may be due to the variation of analytical methods, plant spices, soil type and agricultural inputs.

5.2. Recommendations

Determining the level of concentration of essential and toxic metals in spice samples is very important to check the level of toxic and essential metal permissible limit set by WHO/FAO. So stockholder government, educated people, Ethiopian food agency, farmers etc. participate on this issue to check the concentration limit and to decrease the metal accumulation in agricultural soil on the production areas. In addition to this further studies should be carried out on essential and toxic metals in the spice samples to check the level of metals permissible limit.

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