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SCHOOL OF GRADUATE STUDIES

DETERMINATION OF THE LEVELS OF ESSENTIAL AND NON-ESSENTIAL METALS IN COMMERCIALY AVAILABLE ETHIOPIAN RED PEPPER (*Capsicum Annuum*)

BY

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DETERMINATION OF ESSENTIAL AND NON-ESSENTIAL METALS IN ETHIOPIAN RED PEPPERS

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To

My Wife and Son

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ABSTRACT

The concentrations of essential (Ca, Co, Cr, Cu, Fe, Mn, Ni, Zn) and non-essential metals (Cd and Pb) in Ethiopian red pepper samples were determined because such studies have not been reported in the literature. The pepper samples were collected from Alaba, Mareko and Addis Ababa open market (Merkato) and analyzed for metals using flame atomic absorption spectroscopy after wet digestion. The metal concentrations in the samples were found to be 161-222 μg/g, 1.02-2.24 μg/g, 27.5-73.6 μg/g, 2.12-3.71 μg/g, 99.5-157 μg/g, 9.72-18.95 μg/g, 2.71-6.68 μg/g and 20.8-58.4 μg/g for calcium, cobalt, chromium, copper, iron, manganese, nickel and zinc respectively. The mean levels of metals (Fe and Ca) in the red pepper samples from Alaba were found to be highest Co, Cu, Ni and Zn were found in the highest level in Mareko than with in Alaba and Merkato peppers. While chromium and manganese were available with maximum levels in Merkato peppers.

The concentrations of metals in Ethiopia pepper were comparable to values reported in literature from different parts of the world.

The trace metals levels in either sample were found to be lower than the WHO allowed limit. However Cr is found above the limit.

Keywords: Macro and micro (trace) metals, Pepper, pungency, AAS (Atominc absorption sepectomety)

1. INTRODUCTION

1.1. Origin and Distribution of Capsicum

The genus *Capsicum* was originated in the American tropics [1], with the majority of species having some range in Brazil and Bolivia. The primary centre of origin for domesticated *C.* annuum is in semitropical Mexico. Amazonia (in the northern area) is considered the centre for *C. chinense* and western Amazonia is perhaps the centre for C. *frutescens*, which is more domesticatedly variable in Central America. Bolivia is considered the centre of domestication for *C. baccatum* (in the subtropical east) and *C. pubescens* (in the mid-elevation Andes, where known only in cultivation) [2].

One of the greatest treasures of Columbus discovery of the new world was red pepper (chilies), Capsicum species. Columbus was given credit for introducing pepper to Europe and subsequently to Africa and Asia. In Asia, it was quickly incorporated in to native cuisines. It grows in the tropical, subtropical as well as temperate regions. From these centers of origin, the Chile pepper was brought to Central and North America, Asia, Africa and Europe through several avenues such as discovery travels by explorers and traders. In Europe, the chili was enthusiastically and rapidly incorporated into many cultures. Within 50 years, chilies spread from Spain to England and as far east as India [3].

Capsicum has been known since the beginning of civilization in the new world and is historically associated with the discovery of the new world. It has been a part of the human diet since 7500 B.C. Today the major producers of chile include Asia, Latin America, Africa, Europe and North America. The Capsicum genus represents a diverse plant group and produces the most consumed spice in the world [4, 5].

1.2. Classification of Capsicum

The genus capsicum is a member of the Solanaceae family that includes tomato, potato, tobacco and petunia. Capsicum is a small shrub that is perennial in suitable climatic condition.

It may live a decade or more in tropical Central America, but is more often cultivated as annual [6]. *Capsicum* contains approximately 2027 species, five of which are domesticated and cultivated in different parts of the world are *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens*. Among the five species of cultivated *Capsicum*, *C. annuum* is a dicotyledonous flowering plant which is the most well-known variety, has the highest economic importance of the cultivated chiles, and it is most commonly cultivated crops worldwide followed by *C. frutescens* [5]. This species includes the vast majority of the cultivated pungent and non-pungent (sweet) Capsicum peppers in temperate as well as some tropical areas. In the species *C. annuum*, throughout the world there is phenotypic diversity in plant habit and especially in shapes, sizes, colours, pungency and other qualities of the fruits [2].

Chile types are usually classified by fruit characteristics, i.e. pungency, color, shape, flavor, size and their use [6]. The fruit of Capsicum has a variety of names, such as 'chilli', 'chilli pepper' or 'pepper' depending on place (i.e. differences between the English-speaking countries) and type of fruits [1]. The two features commonly used in the classification are pod types (shape, size, color, and texture) and pungency [5]. The word 'chile' is the common name for any Capsicum species in Mexico, Central America and the Southwestern USA. In Asia, the spelling 'chilli' is more common and is always associated with highly pungent varieties of *C. annuum* and *C. frutescens*, while the non-pungent sweet bell peppers are referred to as 'Capsicums'. Pungent fruits of all cultivated Capsicum species as a collective class are called 'chillies' [7].

1.3. Red Pepper

Red pepper is made from the ground fruit of a plant in the Capsicum family. The fruits, commonly known as "chilies" or "chili peppers," are fiery red or orange pods which rarely grow to more than 4 inches in length. The ground product ranges from orange red to deep, dark red. The fruit is generally handpicked as it ripens and then allowed to dry in the sun, although

artificial drying is often employed in Europe and the United States. The fruit may be ground intact or after the removal of seeds, placenta parts, and stalks, increasing the fruit color and lowering the pungency [8].

The red pepper (*Capsicum annuum*) has been used since ancient times as a source of pigment to add or change the colour of foodstuffs, making them more attractive and acceptable to consumers. As a food colourant, red pepper has traditionally been in the form of paprika (ground powder), although today oleoresins are widely used. The red pepper is a vegetable known for its high antioxidant capacity. Their attractive red colour is due to a variety of carotenoid pigments, which include β-carotene with pro-vitamin A activity and oxygenated carotenoids, such as capsanthin, capsorubin and cryptocapsin, which are distinct to this genus and are shown to be effective free radical scavengers. Red peppers also contain high levels of flavonoids and natural phenolics, more specifically, quercetin, luteolin and capsaicinoids [9].

1.4. Red Pepper Production

Chili peppers are grown worldwide, either outside in fields or in greenhouses. The ability to produce a quality crop in such a wide range of climates and conditions has helped to make chili pepper a globally common crop. Because of the extensive cultivation, adaptation and variability of *C. annuum*, it is difficult to generalize to what is typical, and there is no single method for production. It is a warm-season crop, and highly susceptible to frost. For a high yield of good quality fruit mean air temperatures of 21-23°C were optimal during vegetative growth. The minimum temperature for growth and development is 18°C, below which growth is trivial, with plants in the 5-15°C range growing poorly. The most growth in the vegetative stage occurs at 25-27°C day temperature and 18-20°C night temperature.

The ideal soil for producing chili pepper is deep, well-drained, medium-textured sandy loam or loam that holds moisture and has some organic matter. Chili peppers require adequate amounts of most major and minor nutrients; the most utilized are normally nitrogen and phosphorus.

Chili pepper is sensitive to excessive water. Irrigation is not necessary in areas with regular and ample rain, although it generally is essential in arid and semi-arid regions.

Chili pepper can be harvested at different fruit stages, depending on the final use. Fresh chili pods often are harvested at a physiologically immature stage. The dehydrated and mash industries use physiologically mature fruits, generally showing red colour. The production of chili pepper for spice, vegetable, and other uses increases every year. It is estimated that it is annually cultivated on more than 1.5 million hectares, in numerous countries. Forty-six percent of production is in Asia (with China the principal producing country). Southern Europe is the second most important producing region, with 24% of world production. The countries with harvest area of more than 70,000 hectares are China, India, Indonesia, Mexico, Korea, Nigeria, Ghana and Tureky [2].

1.5. Peppers in Ethiopia

Ethiopia has a variety of fruits, leafy vegetables, roots and tubers adaptable to specific locations and altitudes (Table 1). The major producers of horticultural crops are small-scale farmers, production being mainly rain fed and few under irrigation. Shallot, garlic, potatoes and chillies are mainly produced under rain fed conditions. Tomatoes, carrots, lettuce, beetroot, cabbage and spinach are usually restricted to areas where irrigation water is available [10]. The pepper species which are grown extensively in Ethiopia are *C. frutescens* and *C. annuum* (Fig. 1). The *C. frutescens* mainly cultivated in Tigray, Gondar, Wollega, Illubabor, Keffa, Gamogofa, Bale, Harerege and probably also elsewhere in the Flora area. Cultivars of this species are the hot chilli peppers, an essential part of traditional cooking in most parts of the Flora area, and cultivated throughout Ethiopia. The ripe fruits, which are always hot or very hot, are dried and powdered. The powder is used in the hot, mixed spice *Mitmita* and in the spicy sauce *Awazi*, both very frequently used in cooking.

C. annuum cultivated in Tigray, Shewa, Illubabor, Keffa, Sidamo, Bale, Harerege (and

probably elsewhere in the Flora area); which is indigenous in Mexico and South America, but now cultivated and naturalized in almost all tropical countries. Numerous cultivars of this species exist, with every thing from sweet to pungent fruits. The entire and cut fruits are used green as ingrediences in cooked dishes and raw as a component in the fasting food *Beyayenetu*. The ripe fruits are dried and powdered and used as a component of the mixed spice *Berbere* [11].

(a) (b)

Fig. 1**.** The two common pepper species (a) *Capsicum frutescens* and (b) *Capsicum Annuum.*

Table 1. Recommended root crops and vegetables variety in different production areas 1983 – 2002.

Crop type	Varieties	Yield (t/ha)	Altitude (m.a.s.l.)
Onion	Red creole	19.8	1700-2200
	Bombay red	14.0	1700-2200
Tomato	Marglobe	27.7	500-2200
	Mney maker	24.6	1700-2200
	Roma VF	31.4	500-2200
Cabbage	Copenhagen	27.5	500-3000
	Early drum head	15.8	500-1700
Cauliflower	Glacier	21.6	1700-3000
Swiss chard	Long white ribbed	31.0	1700-3000
	Ford hook grant	15.9	500-1700
Carrot	Nates	21.0	500-3000
	Chantey	24.7	500-3000
Pepper	Bakolocal	20	1400-1900
	Marekofana	30	1400-2200

t/ha –tonnes per hectar.

m. a. s. l.- meters above sea level.

1.6. Contribution of Vegetables to Ethiopia Economy

In fact, though hot pepper has been cultivated for centuries in typical tropical climate within Ethiopia but the yield has remained very low due to limited improvement work on the crop. However, in the past three decades, diverse genotypes (more than 300) of the crop have been introduced from different regions of the world and local collections have also been made in the country. More than 100,000 tones (annual average) of dry fruit of hot pepper are produced in the country and used for export for worldwide market but substantial amount are consumed

locally as spice which exceeds the volume of all other spices put together in the country [12]. Vegetable crops are also important for food security in times of drought, famine and food shortage. It is an important cash crop and a crop of high value in both domestic and export markets, which provides a source of income for the farmers (producers); create employment opportunity and contribution to the national economy as export commodities (Table 2) [13].

Oleoresin (coloring) and capsaicin (hot) are extracted from red pepper (capsicum) for export purpose. The deep red colored and large podded cultivars (sweet/hot) have a very high processing demand in the country. The main processed product, oleoresin, is exported to different countries and the spiced ground is supplied to local market. From 1992/93 to 2003/04, a total of 616.16 tones of oleoresin, which worth 106.6 million Birr, was exported to different countries by Ethiopian Spices Extracting Factory [14].

Table 2. Estimate of area and yield of major vegetable crops for 2004 and 2005 in Ethiopia.

Crop	Area (ha)		Production (tones)		Yield (tones ha^{-1})	
	2004	**2005**	**2004**	**2005**	**2004**	**2005**
Head cabbage	1843	2120	8577	15208	4.7	7.2
Ethiopian cabbage	22377	27143	213592	265455	9.6	9.7
Tomatoes	3761	2919	54871	31655	14.6	12.4
Hot peppers (for green pod)	4471	4783	43187	44273	9.3	9.3
Hot peppers (for dry pod)	49611	59991	66908	72466	1.4	1.3
Swiss-chard	140	142	1075	680	7.6	4.8
Beetroot	1216	1486	140956	16480	11.6	11.1
Carrot	1808	1741	10023	17895	9.1	10.3
Onion	17017	17980	216689	229678	12.7	12.8
Garlic	9465	13657	135394	196741	14.3	14.4

1.7. Chemical Composition of Red Pepper

Capsicum comprises numerous chemicals including steam-volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fibre and mineral elements. Many chilli constituents are important for nutritional value, flavor, aroma, texture and color. Chillies are

low in sodium and cholesterol free, rich in vitamins A and C which are important anti-oxidant, and are a good source of potassium, folic acid and vitamin E. Green Capsicum has the highest amount of vitamin C, which decreases with maturity. Fresh fruits may contain up to 340 mg/100 g of vitamin C. The amount of vitamin C obtained from fresh green chilli peppers higher than that of citrus fruits and fresh red chilli has more vitamin A than carrots. Two chemical groups of greatest interest are capsaicinoids and carotenoids [4, 15, 16]. They are produced only in the fruit and are concentrated around the seeds and in the pericarp. Early taxonomies used the presence of capsaicinoids as a defining trait to characterize the genus Capsicum, but not all Capsicum species produce capsaicinoids [17].

The capsaicinoids (CAPS) are alkaloids that make hot chilli pungent. A large number of carotenoids provide high nutritional value and the color to chilli. The rich supply of carotenoids contributes to chili peppers' nutritional value and colour [1, 2, 4, 15].

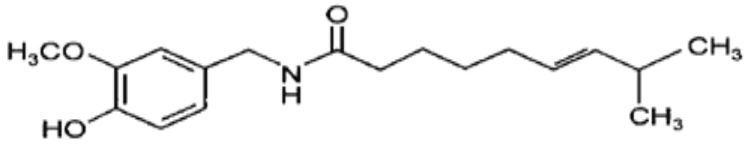
Some varieties of chilli are hot, while others are less hot. The main reason for this variation is the content of capsaicin, its analogues and derivatives. Capsaicin is the most abundant capsaicinoid followed by dihydrocapsaicin, with minor amounts of nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin and others.

Capsaicin is a white crystalline, fat-soluble compound formed from homovanillic acid that is insoluble in water, odourless, and tasteless (Fig. 2). Capsaicin is present in large quantities in the placental tissue which holds the seeds, the internal membranes and to a lesser extent in the other fleshy parts of the fruits of plants in the genus Capsicum. The secretion of capsaicin is an adaptation to protect the fruit from consumption by mammals while the bright colors attract birds that will spread the seeds.

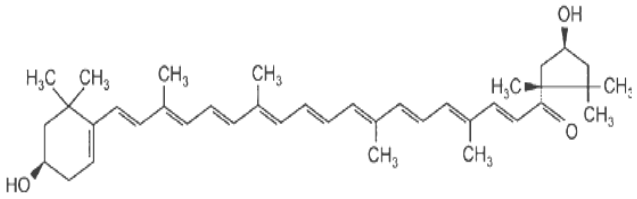
Varieties of chilli differ widely in CAPS content. The amount of CAPS in a given variety can vary depending on the light intensity and temperature at which the plant is grown, the age of the fruit and the position of the fruit on the plant. The red colour of mature pepper fruits is due

to several related carotenoid pigments, including capsanthin (Fig. 2), capsorubin, cryptoxanthin, and zeaxanthin, which are present as fatty acid esters. The most important

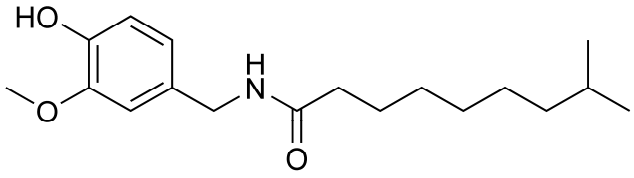
pigments are capsanthin and its isomer capsorubin, which make up 30–60% and 6–18%, respectively, of the total carotenoids in the fruit. The intensity of the red colour is primarily a function of the amount of these two pigments [7].



(a)



(b)



(c)

Fig. 2. The chemical structure of (a) Capsaicin, (b) Capsanthin and (c) dihydrocapsaicin.

1.8. Main Uses in Food Processing

Due to various chemical composition of pepper, it variously used for different purposes because of their nutritional value, flavour, aroma, texture, pungency and colour in a wide

assortment of foods, drugs, and cosmetics, while some are cultivated ornamentally, especially for their brightly glossy fruits with a wide range of colours, shape and sizes. It is also used as a source of pain relief for pharmaceutical use, and as a repellent. In many cases two or more of these properties are included in the same product; for example, paprika may be a source of colour, pungency, and flavour, but colour extracts have a very low content of capsaicin in contrast to the extracts used as flavouring agents [7, 18, 19].

1.8.1. Colour

People whose diets are largely colourless starches, such as rice or maize, use peppers to add colour to their bland, achromatic diets. Fruits of *Capsicum annuum*, paprika, paprika oleoresin, red pepper oleoresin, and dried chilli may all serve as a source of red colour in various processed products, but the primary sources of red colour are paprika and paprika oleoresin. Paprika is used in many products where no pungency is desired, but the colour, flavour, and texture of a finely ground powder is desired. These include processed lunchmeats, sausages, cheeses and other dairy products, soups, sauces, and snacks such as potato chips. Paprika oleoresin is used as a colour and flavour additive in many products where the texture is important and small particles of paprika powder would be undesirable. Capsanthin is a colour pigment which gives the characteristic colour to pepper [7, 15, 20].

1.8.2. Pungency

Capsicum frutescens is much more pungent than *Capsicum annuum*. Capsaicinoids are responsible for the heat, or pungency in chili pepper fruit. Among the capsaicinoids, capsaicin and dihydrocapsaicin together account for about 90% of pungency. Capsaicin is a potent chemical that can survive both cooking and freezing but apart from burning sensation; it also triggers the brain to provide endorphins, natural pain-killer that promotes a sense of well being. An increase in the level of capsaicin leads to a rise in the level of pungency of peppers, which plays an important role on preference of consumer. Typical aroma of red pepper comes from

many specific esters and pyrasine, thiazole and alcohol compounds [3, 15, 17]. Chili pungency is measured by determining the capsaicinoids content of the fruit, which can be accomplished by several industrial (laboratory) procedures, and as well by a subjective dilution and detection test ("taste test") scored as Scoville Heat Units (Table 3) [2].

The concentration of capsaicin varies according to the variety of pepper; the geographical origin and the climatic conditions. Capsaicin causes inflammation of the mucous membranes. Green and red peppers are valued for their sensatory attributes of colour, pungency and aroma and capsaicin content of red pepper is one of the main parameter that determines its commercial quality [21].

1.8.3. Flavour

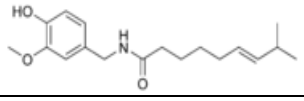
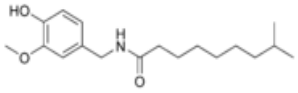
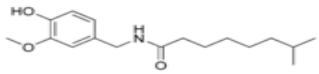
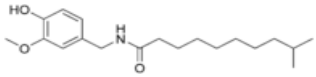
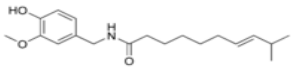
Paprika is valued for its flavour in many products in addition to its colour. Dried chilli is also valued for its contribution to flavour in chilli sauces and chilli powders. The flavouring principle is associated with volatile aromatic compounds and colour. As a general rule, when the colour of paprika or chilli powder fades, the flavour also disappears [7].

1.8.4. Pharmaceutical

Capsaicin, a major alkaloid among capsaicinoids produced only in Capsicum fruits has wide applications in the food, medicine, and pharmaceutical industries. As a medicine, it is most widely used and reliable as a salve to relieve muscle, joint, and toothache pain, to treat cough, asthma, and sore throat, as a stimulant, and to treat stomach ache, seasickness, and flatulence. It is also known to kill some types of cancer cells and provide relief in arthritis and respiratory ailments. Pharmaceutical application of capsaicinoids is attributed to its antioxidant, anticancer, antiarthritic, and analgesic properties. Coatings and powders are used with varying degrees of success to deter browsing animals and insects, and break children of thumb sucking and nail biting. Pepper spray, whose active ingredient is capsaicin, is used widely for personal

protection, law enforcement, and defense. However, prolonged contact with the skin may cause dermatitis and blisters, while excessive consumption can cause gastroenteritis and kidney damage. High levels of ground hot pepper have induced stomach ulcers and cirrhosis of the liver in laboratory animals. Body temperature, flow of saliva, and gastric juices may be stimulated by capsicum peppers [19].

Table 3. Types of capsaicinoid, their relative amount, heat content and chemical structure.

Capsaicinoid name	Abbrevi-ation	Typical relative amount	Scoville heat units	Chemical structure
Capsaicin	C	69%	16,000,000	
Dihydrocapsaicin	DHC	22%	15,000,000	
Nordihydrocapsaicin	NDHC	7%	9,100,000	
Homodihydro-capsaicin	HDHC	1%	8,600,000	
Homocapsaicin	HC	1%	8,600,000	

1.9. Quality of Dried Chilli

Like the other forms of peppers used in food processing, colour and pungency are the major quality factors, as well as aroma. Factors that affect the colour of dried chillies include the cultivar, the stage of maturity at harvest and subsequent curing, fruit drying conditions and the final moisture content. At less than 10% moisture, the colour appears bleached, while at levels greater than 10% there is darkening, possibly caused by non-enzymatic browning. The colour of crushed or ground chilli powder deteriorates faster than whole chillies, due to the auto-

catalyzed degradation of carotenoids. The major factor influencing colour retention during storage is the temperature, followed by the moisture content. The effect of air, light, and type of container is minimal [7].

1.10. Plant Nutrients

Vegetables are vital to human diet as they contain essential components needed by the human body such as carbohydrates, proteins, vitamins, minerals and also trace elements. Vegetables are considered as "Protective supplementary food" because they are highly beneficial for the maintenance of health and prevention of diseases and also important to neutralize the acid produced during digestion, besides being useful "roughage" according to food experts [22-24].

In recent years their consumption is increasing gradually, particularly among the urban community. This is due to increased awareness on the food value of vegetables, as a result of exposure to other cultures and acquiring proper education [25]. There are different kinds of vegetables; they may be edible roots, stems, leaves, fruits or seeds. Each group contributes to diet in its own way. Vegetables contribute minerals, vitamins and fiber to the diet. Minerals are very important and essential ingredients of diet required for normal metabolic activities of body tissues. Out of 92 naturally occurring minerals 25 are present in living organisms. They are constituent of bones, teeth, blood, muscles, hair and nerve cells. Vitamins cannot be properly assimilated without the correct balance of minerals [26, 27].

An element, which is required in amounts smaller than 0.01% of the mass of the organism, is called a trace element (for example Fe, Cu, Mn, Zn and Cr). Trace metals function mostly as catalysts for enzymatic activity in human bodies. However, all essential trace metals become toxic when their concentration becomes excessive [28]. The presence of trace elements in fruits and vegetables has been ascribed to their absorption from the soil and sources such as type of fertilizer, agricultural chemicals and contaminating dirts. Other sources of heavy metals

contamination of most foodstuffs may also include adaptation of mechanized farming, sprays, seed preservatives and components from global pollution [29-30].

1.11. Essential and Non-Essential Elements

Metals can be classified as either essential elements (important for life) or nonessential elements. Non-essential metals (with no known physiological functions to humans) are priority pollutants that pose potential risks to human health and the environment [31]. An element is essential when it is consistently determined to be present in all healthy living tissues and when its deficiency symptoms are noted, with depletion or removal, which disappears when the elements are provided to the tissues. There are 20 mineral elements essential for plant growth. Carbon (C), hydrogen (H) and oxygen (O) are supplied by air and water. The six macronutrients, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S), are required by plants in large amounts. The rest of the elements are required in trace amounts (micronutrients). Essential trace elements include boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), sodium (Na), zinc (Zn), molybdenum (Mo), and nickel (Ni). Studies have also shown that a number of other mineral elements are beneficial to the growth of plants and are required for some plants [32].

Heavy metals are metals having a density of 5 g/cc. These metals occur in all foods as natural or inherent components of plant and animal tissues and fluid and also may be present as a result of contamination or deliberate addition [33]. They have important positive and negative roles in human life. Some of heavy metals are considered essential including iron, zinc and copper, while some metal ions like cadmium, lead and mercury are non-essential metals which have toxic roles in biochemical reactions in our body [34].

Although only required in small amounts, trace elements (or micronutrients), are essential for plant growth. These nutrients often act as catalysts in chemical reactions. Trace element plays an important role in chemical, biological, biochemical, metabolic, catabolic and enzymatic

reactions in the living cells of plants, animals and human beings. Trace elements have great significance due to their tendency to accumulate in the vital human organs over prolong period of time. It is possible to have toxicities of trace elements, as well as deficiencies. In fact some of the trace element deficiencies in plants can cause nutrient deficiencies in the animals that graze those plants [35].

Metal accumulation in plants depends on the plant species, types of soil, environment and agricultural practice. Some plants may accumulate toxic metals at levels which may be harmless to the plant but could be harmful to humans if ingested. The total metal content in soil is the result of parent materials, fertilizers, atmospheric deposition, agrichemicals, and organic wastes [23]. In addition, they could be contaminated from various species including trace metals as farmers wash them with waste water before bringing them to market [36].

Keeping in mind the potential toxicity and persistent nature of heavy metals, and the frequent consumption of vegetables and fruits, it is necessary to analyze these food items to ensure the levels of these contaminants meet agreed international requirements.

Cadmium (Cd)

Cd is a well-known environmental hazard, exerts a number of toxic effects in humans and animals [30, 37]. Cadmium is water soluble and can be transferred efficiently from soil to plants, which may affect human health if there is excessive intake from a contaminated food source. Increased concentrations of Cd in agricultural soils are known to come from human activities such as the application of phosphate fertilizer, sewage sludge, wastewater, and pesticides, mining and smelting of metalliferous ores with high Cd content and traffic [38, 39]. It accumulates mainly in the kidney and liver and high concentrations have been found to lead to chronic kidney dysfunction [40, 41]. The basis of Cd toxicity is its negative influence on enzymatic systems of cells, resulting from substitution of other metal ions in metalloenzymes and its very strong affinity to biological structures containing –SH groups, such as proteins,

enzymes, and nucleic acids. Many effects of Cd^{2+} action result from interactions with necessary micro- and macro elements [30, 37].

Lead (Pb)

Lead is a toxic, bio-accumulative heavy metal with no known biological function. Its sources may be natural, as it is found in the earth's crust and thus enters the food and water supply. It is absorbed by food stuffs (particularly green leafy vegetables) growing on soil where lead is present. Lead is absorbed by plants to varying degrees depending on the form of the metal present, and the pH and the temperature of the soil. It can adversely affect many organs, systems and numerous conditions such as high pressure, anemia, kidney damage, impaired wearing and mental retardation elevated levels in women may result in a shortened gestation period, while young children are considered at great risk because of their ability to effectively absorbed lead and thereby suffer mental and physical development retardation [30, 42].

Zinc (Zn)

Zinc is the fourth important micronutrient after vitamin A, iron and iodine, and is now receiving increasing global attention and is particularly necessary in cellular replication, the development of the immune response and as a cofactor for many enzymes of the body [43]. Although Zn has been found to have low toxicity to man, prolonged consumption of large doses can result in some health complications such as fatigue, dizziness and neutropenia. Some literature sources have also revealed that Zn could be toxic to some aquatic organisms such as fish. Copper, zinc and cobalt form the essential group of metals required for some metabolic activities in organisms [41].

Nickel (Ni)

Nickel is a naturally occurring element found in a number of mineral ores including sulfides,

oxides and silicates. It is present in the enzyme urease and as such is considered to be essential to plants and some domestic animals. Nickel related health effects such as renal, cardiovascular reproductive and immunological effects have been reported in animals. Its toxic effects in man are related to dermal, lung and nasal sinus cancers [41].

Copper (**Cu**)

Copper naturally is one of the significant essential elements for plant growth, and is usually mobile within plants, but at elevated contents of bioavailability copper; bioaccumulation can result with possible toxic effects on plants and animals through the food chain. One can get exposed to copper by breathing air, drinking water, eating food and by skin contact with soil, water and other copper containing substances. Most copper compounds found in air, water, sediment, soil and rock are so strongly attached to dust and dirt or imbedded in minerals that they cannot easily affect your health. Copper is essential in humans for bone formation, elastin synthesis, energy metabolism, nerve transmission, normal hair growth, pigmentation of the skin and red blood production and also serves as an antioxidant and helps the body to remove free radicals and prevent cell structure damage. It is regulated in the body via absorption, excretion and a combination of processes. However, very large single or daily intakes of copper can harm your health. Long term exposure to copper dust can irritate your nose, mouth and eyes, and cause headaches, dizziness, nausea and diarrhea. Higher level of copper in water may experience vomiting, diarrhea, stomach cramps and nausea [44, 45].

Manganese (Mn)

Manganese is found widely in nature, but occurs only in trace amounts in human tissues. In plants it is involved in chlorophyll formation, nitrate assimilation, enzyme systems and iron metabolism. Manganese deficiency is generally caused by a high soil pH, but can also be induced by an imbalance with other elements such as calcium, magnesium and iron. Manganese availability in limed soils is decreased with increasing levels of organic matter. The

human body contains a total of 15-20 milligrams of manganese, most of which is located in the bones, with the remainder found in the kidneys, liver, pancreas, pituitary glands and adrenal glands. In the human body, manganese functions as an enzyme activator and as a component of metalloenzymes. Because manganese plays a role in a variety of enzyme systems, dietary deficiency of manganese can impact many physiological processes. In experimental animals, manganese deficiency causes impaired growth, skeletal abnormalities and defects in carbohydrate and fat metabolism [46]. Excess manganese also causes irritation of the respiratory passages and impairment of movement [47].

Chromium (Cr)

Chromium is an essential element which is involved in the metabolism of carbohydrates, and which forms part of the glucose tolerance factor (GTF). As well as being associated with prevention of diabetes and cardiovascular disease, chromium seems to be influential in metabolism of cholesterol and various proteins [48].

Chromium can exist in various chemical valence states ranging from Cr^{2+} to Cr^{6+}, among which more stable chemical forms are Cr^{3+} and Cr^{6+} [49]. Cr^{3+} is considered to be the trace element essential for proper functioning of living organisms. Cr^{6+} was reported to exert toxic effects on biological systems, where as Cr^{3+} is generally not transported over great distances because of its low solubility and tendency to be absorbed in the pH range typical for natural soils and water. Hexavalent anionic form is more available for living organisms than Cr^{3+}, and plays a main role in removing this metal from water and soil systems [50]. Its deficiency is characterized by disturbance in glucose, lipids and protein metabolism [51].

Calcium (Ca)

Calcium in human body needs for numerous functions, such as building and maintaining the bones and teeth, blood clotting, transmitting of the nerve impulses and regulating heart's

rhythm. Ninety nine percent of calcium in a human body is stored in bones and teeth. The remaining one percent is found in the blood and other tissues. The body gets calcium by pulling it from the bones when blood levels of calcium drop too low, usually when quite a long time passes since having taken calcium with meal. Calcium can also be found in dark green leafy vegetables, dried beans and legumes, calcium fortified juice in addition to milk powder and dairy products are a convenient source of calcium for many people [52].

Cobalt (Co)

Cobalt is important in the plant world. In higher plants cobalt is an essential element for legumes because of its use by microorganisms in fixing atmospheric nitrogen. Cobalt is an essential element for the synthesis of vitamin B_{12}, which is required for human and animal nutrition. Cobalt can be taken into the body by eating food, drinking water, or breathing air. Gastrointestinal absorption from food or water is the principal source of internally deposited cobalt in the general population. Cobalt is an essential element found in most body tissues, with the highest concentration in the liver. Unlike other heavy metals, cobalt is safe for human consumption and up to 8 mg can be consumed on a daily basis without health hazard. Cobalt is usually considered non-toxic, however severe cardiac and some deaths in man resulted from consumption of large amounts of beer containing 1.2-1.5 mg/L cobalt. Inhalation and dermal exposure to cobalt in humans can result in sensitization. Bronchial asthma has been described in workers exposed to various forms of cobalt [53, 54].

Iron (Fe)

Iron is one of the most abundant metals in the universe. It is a mineral necessitates the red blood cell formation and also one of the most useful in biochemical functions. In the human body, iron is present in all cells and has several vital functions as a carrier of oxygen to the tissues from the lungs in the form of hemoglobin (Hb), as a facilitator of oxygen use and storage in the muscles as myoglobin, as a transport medium for electrons within the cells in the

of cytochromes, and as an integral part of enzyme reactions in various tissues. Iron in general has poor availability from foods derived from plant sources compared to foods from animal sources. Iron deficiency can be a very difficult diagnosis, since the symptoms such as lethargy, tiredness and dizziness, are non-specific can be found in a variety of ailments [55, 56].

1.12. Methods of Determination of Metals in Capsicum

Determination of elements at trace levels continues to be one of the most important issues of analytical chemistry. Accurate and sensitive techniques are needed to solve problems related to low concentration levels of trace elements regarding many controversial issues in fields of environment, health and technology. The most often employed tools in this area are atomic absorption spectrometry (AAS), inductively coupled plasma, optical emission and mass spectrometry (ICP-OES and ICP-MS) and atomic fluorescence spectrometry (AFS) that is used less often than the others [57, 58].

Flame atomic absorption spectrometry (FAAS) is one of the most extensively used techniques for the determination of various micro elements industrial fertilizers or some environmental samples with significant precision and accuracy, due to being simple; it provides analytical sensitivities at the parts-per-million level and less, speed, inexpensive, selective, considered as a specific technique with little interference as compared to other techniques and applicable to wide range of samples. The technique of AAS involves the conversion of an analyte molecule in to its constituent gas-phase atoms (atomization), and the subsequent absorption of radiation by these free atoms [59-62].

However, in some cases in determining trace metals by flame atomic absorption spectrometry can be amenable to spectral and chemical interferences. For FAAS the problem of direct spectral interference by elements is not very severe. Chemical interference is however the biggest source of interference in FAAS. For instance, elements that forms oxoanions such as

P, B, Si, and Cr can sometimes indirectly interfere by complexing the element of interest. Several approaches can be used to minimize such interference [63]. One method to reduce the effects of chemical interferences is to adjust the flame-gas stoichiometry. A fuel-rich flame produces a reducing environment, with correspondingly fewer of the reactive oxide species. An alternate approach is to use a hotter flame, such as the nitrous oxide-acetylene flame, to vaporize and dissociate any refractory metal oxides. A third commonly employed technique is to use a releasing agent that can compete with the metal-oxide formation equilibrium. An example often cited is the addition of lanthanum when analyzing for calcium in the presence of high concentrations of phosphate ions. In this example, the lanthanum competes with the calcium for complex formation with the phosphate ions in the flame. The lanthanum phosphate complex is more stable than the calcium phosphate complex and predominates, resulting in a greater number of free calcium atoms in the flame providing a higher sensitivity for the calcium [64].

1.13. **Purpose and scope of the study**

The levels of metals in red peppes were reported from different countries**.** Report from Nigeria indicates that the amount of cadmium, zinc, nickel, chromium, lead and copper were determined. U. A. Awode *et al.* found that the amount of cadmium, chromium and lead were higher than recommended by WHO, while others metals are below the limit.

Similarily in Canary Islands and Holland the levels of maco metals (Na, K, Ca and Mg) and micro metals (Fe, Cu, Zn, Mn and B) were determined. The concentration of metals studied in Canary Islands by R. Carmen *et al*. were also compared with certified concentration values and obtained comparable results.

The study of M. Bigdeli and M. Seilseour from Iran in the levels of trace metals reveled that, they are found below the permissible levels for consumption. Furthermore the presences of trace metals in red peppers were also dermined in Korea and Spain. However, there were no

studies carried out in determining the levels of metals in Ethiopian pepper. Therefore, this study focuses in determination of the major and minor elements in red pepper samples collected from markets of production sites (Alaba and Mareko) and to compare the result with commercially available samples sold on Merkato (Addis Ababa).

The finding of this study will

- Provide the levels of metals loaded and the consumer will have information while using the pepper.
- Help to develop the awareness of the user in the levels of metals in the pepper.
- The consumer will identify the type of pepper which is essential for health purpose in terms of metallic content and to provide away for researchers to find means of improving the metals content in pepper to be suitable for consumption.
- Provide a base line for researchers for further investigation.

1.14. Objective of the Study

1.14.1. General objective.

The objective of this project was to determine the concentrations of metals in pepper grown in different parts of the country.

1.14.2. Specific objective

- To develop a working procedure for the digestion and analysis of red pepper powder for their essential and non-essential metal contents by flame atomic absorption spectrometer.
- To determine the levels of Ca, Mn, Cu, Fe, Zn, Cr, Co, Pb, Cd and Ni metals in the Ethiopian (Alaba, Mareko and Merkato) red pepper using flame atomic absorption spectrophotometer (FAAS).

- To compare the levels of metals in red peppers sold in the markets of Alaba, Mareko and Merkato.
- To compare the levels of Ca, Mn, Cu, Fe, Zn, Cr, Co, Pb, Cd and Ni metals in the Ethiopian (Alaba, Mareko and Merkato) red peppers with the levels of metals in red peppers from the other part of the word.

2. EXPERIMENTAL

2.1. Description of the Study Areas

2.1.1. Alaba

Alaba Special Woreda is one of the special woredas in the Southern Nation, Nationalities and People Regional State and is part of the Southern Rift Valley of Ethiopia. Alaba is surrounded by Siltie zone in the north, Badawacho in the south, Oromia in east and Shashogo in the west (Fig. 3). Alaba Kulito is the main town of Alaba, which is located at a latitude and longitude of 7^0 19' N and 38^0 04' E and 315 kms away from Addis Ababa.

The woreda is found in Woina-Dega climatic zone with the mean annual temperature ranging between 23-25 ^oC and the mean annual rainfall between 700 - 900 mm. The woreda located at 1554 to 2149 m.a.s.l., but most of the woreda is found at about 1800 m.a.s.l. The woreda is knowned for the high quality red pepper production which, is mainly supplied to Addis Ababa and other parts of the country.

2.1.2. Mareko

Mareko is one of the woredas in the Southern Nations, Nationalities and Peoples' Regional State of Ethiopia. Part of the Gurage Zone, Mareko is bordered on the south by Lanfero, on the southwest by Seri, on the west by Meskan, on the north by Sodo, and on the east by the Oromia Region (Fig. 3). Koesh is the main town of the woreda which about 250 km from the capital city, Addis Ababa. It is located at 8^0 0' N and 38^0 31' E. The climate is subtropical and the

altitude of the area is 1700 m above sea level and pepper is one of the main crops in the woreda.

2.1.3. Merkato

Merkato is the name for the large open air market place in the Addis Ketema district of Addis Ababa, Ethiopia. It is also the largest open air market in Africa, covering several square miles and employing several people in different business entities. The primary merchandise passing through the Merkato is locally-grown agricultural products.

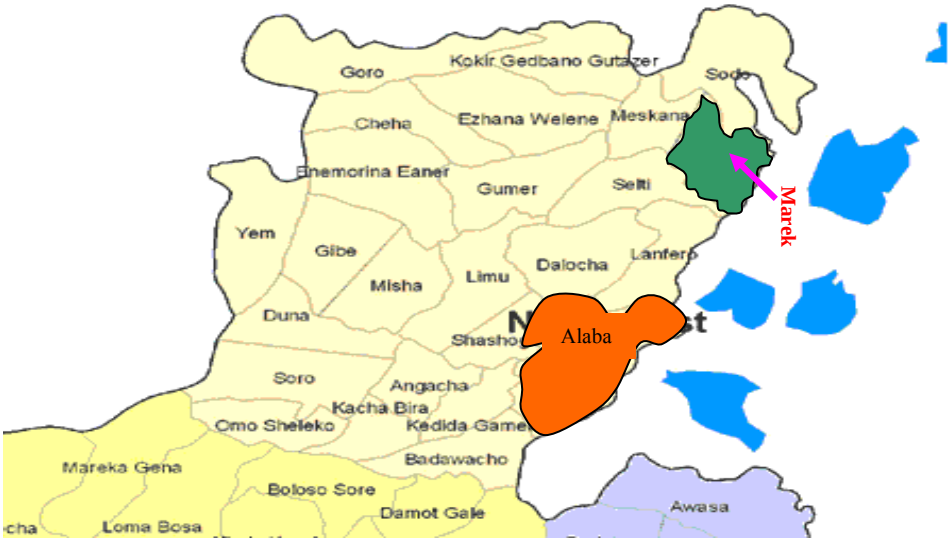


Fig. 3. Map of the sampling sites (Alaba and Mareko).
(Source**:** Microsoft ® Encarta® 2009, ©1993-2008 Microsoft Corporation)

2.2. Sample Collections

The most important single step in analysis is taking a sample of the material to be analyzed. If a non-representative sample is taken, no matter how excellent the analytical procedure may be or how expert the analyst, the result obtained will be incorrect. For correct sampling, first a

sample must be taken that is sufficient for all the analysis to be carried out comfortably with enough for at least duplicate analysis if necessary. Second, the sample must be representative of the material being sampled. If the sample doesn't represent the material, the analysis can't provide a reliable characterization of the material and the result is in error regardless of the accuracy of the analytical method [65].

For this study a total of three different pepper samples were taken from markets at Alaba Kulito (Alaba especial woreda administrative town), Koshe (Mareko woreda administrative town) in SNNPRS and Merkato at Addis Ababa, capital city of Ethiopia. The two areas in SNNPR were chosen for this study mainly because they are the main pepper producers, highly demanded and also mostly marketed to the market in Addis Ababa and most parts of the country for public consumption (Fig. 4). Merkato was chosen as study site, which is the place where red peppers grown in different parts of the country is easily available for marketing.

Fig. 4. Alaba-Mareko Lowland Pepper Livelihood Zone.
(Source: http://www.dpc. gov.et/gsdl/collect/)

2.3. Sample Handling

Half kilogram of the pepper sample was collected randomly from each bag with a total of 10 from different people. The collected sample was pooled together and mixed well to have a bulk sample. These were transported to the laboratory for processing and preservation. The sample size then reduced to about 100 g from the bulk. The pepper samples were thoroughly washed with tap water and there after with distilled water, sliced in nearly uniform size to facilitate drying of the pieces at the same rate and then dried in an oven at 105 ^{0}C for 24 hours, until they are brittle and crisp. At this stage, no microorganism can grow and care was taken to avoid any source of contamination. The dried samples were homogenized by grinded into fine particles using clean acid washed mortar and pestle until digestion [66, 67].

2.4. Analysis of Samples

2.4.1. Sample Digestion

When a sample is in solid form, the normal requirement is to convert the solid material (biological, environmental or geological) in to the liquid form. It is possible to analyze solids directly by using atomic spectroscopy, but this is not the preferred approach [68]. For trace element determination in plant materials require the decomposition of the sample before analysis to reduce organic matter interference and allow for the conversion of the metal into a form that can be analyzed by the Atomic Absorption Spectrophotometer (AAS). In this study, 0.5 g of sieved samples was then weighed in to 250 mL round bottom flask. The sample (red pepper) was digested on a micro Kjeldahl digestion apparatus with 2 mL of concentrated nitric acid (69-72%) for 1 h at 300^{o}C and then cooled to room temperature. The contents were further digested with a 3 mL mixture of nitric acid and perchloric acid (1:2) at the same temperature until a colorless solution was obtained. The sample solution was allowed to cool for 10 minutes at room temperature, and then the solution filtered with Whatman® (110 mm, dia) filter paper in to 50 mL volumetric flask. To use the portion of the samples remaining in the beaker, the

beaker was rinsed with small portion of deionized water and then filters in to the flask. Triplicate digestion of each sample was carried out together with blank digest with out the plant sample [69, 70]. The samples were stored in 50 mL volumetric flasks and placed in a freezer to avoid any decomposition until analysis.

2.4.2. Optimization of Working Procedure

In order to analyze metals present with pepper samples efficiently, different procedures for sample digestion were carried out and the one that consumed smaller reagent volume with smaller digestion time and produced clear solutions with no residue and suspended matter was selected for the routine digestion of the samples.

2.4.3. Accuracy and Precision

Precision is the extent of the consistency of results as they are obtained during repeated applications a specified determination method. As expression of precession, the standard deviation (relative standard deviation) of the measured values obtained under repeated conditions or the standard deviation of the measured values obtained under comparable conditions are generally used [71]. Accuracy describes how close the measured value is to the true value or accepted value of for the measurement. The accuracy of a data value may never, of course, be determined exactly since this would assume that the true value was already known with an absolute certainty [72].

2.4.4. Validation of Optimized Procedure (Recovery)

In order to ascertain the reliability of the method for the analysis of the samples for Ca, Fe, Cd, Cr, Zn, Cu, Cd, Ni, Mn and Pb, Certified Standard Reference Materials (CSRM) were not readily available for use, instead spiking method was adopted using digestion method. In spike a known amount of analyte is added (spiked) into the natural test sample matrix and its

response is measured (recovered) in the assay by comparison to an identical spike in the standard diluent. When samples were spiked with known amounts of the element of interest, the spike recovery may be used to check for losses of the element during sample digestion and subsequent processing, and to differentiate any matrix interferences in the elemental determinations. If RA (recovery of analyte A) is measured using a standard addition or spike procedure,

$$\mathrm{RA} = \frac{QA(O+S) - QA(O)}{QA(S)}$$

where QA(S) is the quantity of analyte A added (spike value) and QA(O+S) the quantity of A recovered from the spiked sample and QA(O) from the original sample. In all uses of spiked or standard addition procedures, it is essential to ensure that uniform chemical and/or isotopic distribution is achieved between the material added and that originally present in the sample. The concentration of analyte in the spike should be sufficiently high so as to minimize disturbance, by dilution, of the matrix. It should be noted that the addition of a spike only checks that part of a procedure after the addition of the spike. Recovery is also used to denote the ratio of observed value, X(obs), obtained from an analytical process via a calibration graph, divided by the reference value, X(ref). The reference value may be that of a certified reference material or the increased amount of analyte observed from the addition of a known amount of analyte to the test sample.

$$\mathrm{RA} = \frac{X(obs)}{X(ref)}$$

or

$$\mathrm{RA} = \frac{X(obs, O+S) - X(obs, O)}{X(S)}$$

where X(obs, O+S) is the observed value for the spiked sample, X(obs, O) the observed value for the original, unspiked sample, and X(S) the value for spike [73].

2.4.5. Method Detection Limit

Standard methods define the MDL as: “constituent concentration that, when processed through the complete method, produces a signal with a 99% probability that it is different from the blank”. The EPA defines it as: “the minimum concentration that can be determined, with 99% confidence, that the true concentration is greater than zero”. The MDL depends on the equipment, chemicals, methodology, materials, sample composition and even the analysts.

The MDL is different from the instrument detection limit (IDL). The instrument manufacturer’s manuals often contain numbers that can only be achieved under optimal conditions. But instruments often cannot be run under optimum conditions. For example in the case of ICP and ICP-MS, there may be matrix interferences; the sample introduction system may be contaminated by earlier samples, ICP-MS cones (for ICP-MS) may not be in perfect condition or the torch and injector tube may have deposits. Instrument detection limits are useful for comparison of different techniques (such as polarography, atomic absorption, ICP, ICP-MS) or different versions of the same type of instrument. Method detection limit is a useful parameter to determine the lowest concentration at which an analyte can be observed [74].

For determining the concentration of the metals Ca, Mn, Cu, Zn, Cr, Co, Pb, Cd, Ni and Fe in red pepper samples using FAAS six blank samples were digested. The standard deviations for each element were calculated from the six blank measurements to determine method detection limit of the procedure.

2.5. Instrumentation and Apparatus

A digital analytical balance (Mettler Toledo, Model AG 204, Swizerland) with a precision of ±0.0001 g was used to weigh peppers samples. A drying oven (DIGITHEAT, J. P. SELECTA, S. a, Spain) was used to dry the pepper samples in order to powdering red pepper samples. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the digested sample until analysis. A 250

mL round bottom flasks fitted with reflux condenser were used in Kjeldahl apparatus (Gallenkamp, England). Micropipettes (1-10 µL and 100-1000 µL) were used for measuring the volume of reagents and standards. BUCK SCIENTIFIC MODEL 210 VGP (East Norwalk USA) atomic absorption spectrophotometer equipped with deuterium are background corrector was used for analysis of the metals Ca, Mn, Cu, Zn, Cr, Co, Pb, Cd, Ni and Fe using air-acetylene flame.

2.6. Reagents and Chemicals

Analytical reagent (AnalaR) grade chemicals, reagents, distilled and deionized water were used throughout the study. All glasswares and plastic containers used will be washed with detergent solution followed by soaking in 10% (v/v) nitric acid and then rinsed with deionized water. HNO_3 (69-72%, Spectrosol, BDH, England) and $HClO_4$ (70%, Aldrich, Buck Scientific Model 210 VGP, East Norwalk, USA), were used to digest the matrix samples. Lanthanum chloride heptahydrated was used to avoid refractory interferences (to release calcium and magnesium from their common phosphates). The standard stock solutions containing 1000 mg/L, in 2 % HNO_3, of the metals Ca, Mn, Cu, Zn, Cr, Co, Pb, Cd, Ni and Fe (BUCK SCIENTIFIC GRAPHIC ^{tm}) were used for preparation of calibration standards in the analysis of the samples in non-spiking and in the spiking experiments.

3. RESULTS AND DISCUSSION

3.1. Optimization of Digestion Procedure

In optimizing the digestion procedure, different procedures for the samples were tried and as can be seen in Table 4 alternative 13, consumed smaller reagent (acid) volume, smaller digestion time and a colourless solution with no residue was obtained. On this basis total reagent volume 5 mL (3 mL nitric acid and 2 mL perchloric Acid), at a temperature of 300^{o}C for 2.5 h were chosen as the working conditions. The blank solutions were prepared by

digesting the mixture of reagents following the same digestion procedure as the samples and diluted to 50 mL with deionized water.

3.2. Calibration of instruments

Calibration of an instrument or a piece of equipment involves making of a comparison of a measured quantity against reference value. Instrument calibration is particularly important and must be checked on a day-to-day or run-to-run basis. For example, to calibrate a spectrophotometer response, select the appropriate reference material and measure the spectrometer response to it under the specified conditions and compare the measured value with the value quoted in the literature [75].

In this study, atomic absorption spectroscopic standard solutions containing 1000 mg/L were used for preparing intermediate standard solutions (10 mg/L) in 100 mL volumetric flask. As the values are given in Table 5 appropriate working standards were prepared for each of the metal solution by serial dilution of the intermediate solutions using deionized water. Each of the sets of serial dilutions was then aspirated one after the other into the atomic absorption spectrometry and their absorbance recorded. Calibration curves were plotted with four points for each of the trace metals standard using absorbance against concentrations (mg/L) and the calibration curve is given in Fig. 5. Immediately after calibration using the standard solutions, the sample solutions were aspirated into the AAS instrument and direct readings of the metal concentrations was recorded. Three replicate determinations were carried out on each sample. The same analytical procedure was employed in the determination of elements in each six digested blank. The operating conditions of AAS employed for each analyte are given in Table 6.

3.3. Analytical Results

3.3.1. Precision of Results

Precision is a measure of the spread or dispersion of a set of results. Precision applies to a set of replicate measurements and tells us how the individual members of that set are distributed about the mean value, regardless of where the mean value lies with respect to the true value. Precision indicates the repeatability and reproducibility of measurements. The first refers with-run and the latter reflects between-run precision of the method [75].

In this study, to ensure the precision of the analysis, each sample was digested and analyzed in triplicate and the precision of the results were evaluated by the standard deviation and relative standard deviation of the results of samples with triplicate measurements of each sample were used for the analysis of metals in red pepper. The relative standard deviations of the measurements were found in the acceptable range.

Table 4. Different procedures tested during the optimization of red pepper samples.

No.	Weight of Sample (g)	Volume of HNO_3 (mL)	Volume $HClO_4$ (mL)	Total volume (mL)	Temp-erature (^oC)	Time (h)	Results
1	0.5	5	2	7	200	4:00	Clear and colorless
2	0.5	5	1	6	270	4:00	Clear and colourless
3	0.5	5	1	6	300	4:00	Clear and colourless
4	0.5	4	2	6	180	5:10	Clear and colourless
5	0.5	4	2	6	240	4:00	Clear and yellowish
6	0.5	4	2	6	270	3:45	Clear and yellowish
7	0.5	4	2	6	300	4:00	Clear and colourless
8	0.5	2	4	6	300	4:00	Clear and colourless
9	0.5	3	3	6	210	5:00	Clear and yellowish
10	0.5	3	3	6	240	4:30	Clear and colourless
11	0.5	3	3	6	300	4:00	Clear and colourless
12	0.5	5	0	5	240	3:50	Clear and yellow
13	0.5	3	2	5	300	2:30	Clear and colourless
14	0.5	3	2	5	240	2:40	Clear and colourless
15	0.5	4	1	5	300	2:45	Clear and colourless

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

Element	Conc. of standards in (mg/L)	Correlation coefficient
Ca	0.5, 1.0, 2.0, 2.5	0.99961
Cd	0.02, 0.08, 0.32, 0.72	0.99914
Co	0.5, 1.0, 1.5, 2.0	0.99964
Cr	0.5, 1.0, 2.0, 2.5	0.99937
Cu	0.5, 1.0, 1.5, 2.0	0.99975
Fe	0.5, 1.0, 2.0, 3.0	0.99967
Mn	0.025, 0.1, 0.4, 1.6	0.99985
Ni	0.05, 0.2, 0.8, 3.2	0.99984
Pb	0.1, 0.2, 0.5, 1.0	0.99942
Zn	0.1, 0.2, 0.4, 0.6	0.9995

3.3.2. Method Detection Limit

In this study method detection limit for the analysis of heavy metals using FAAS in red pepper samples were determined using reagent blank, i.e. 3 mL of 69-72% HNO_3 and 2 mL of 70% $HClO_4$ which was used for acidifying the sample solutions, was added to 50 mL of deionized water that was used for washing apparatus and for the dilution of standard solutions. Then the method detection limit of each element was determined as three times the standard deviation of the blank solution. As can be seen from Table 7, the method detection limit values of the investigated elements for AAS were found to be 0.0107 mg/g for Ca, 0.0058 µg/g for Fe, 0.0009 mg/g for Mn, 0.0017 mg/g for Cu, 0.0019 mg/g for Zn, 0.027 mg/g for Cr, 0.0005 mg/g for Co, 0.00167 mg/g for Pb, 0.003 mg/g for Cd and 0.0023 µg/g for Ni. These results indicate that the method can be used for the determination of metals at trace levels in the red pepper.

Table 6. Standard conditions of the Flame Atomic Absorption Spectrometer used in determination of different elements.

Metal	Wavelength (nm)	Instrument detection limit (mg/L)	Slit width (nm)	Lamp current (mA)
Ca	422.7	0.01	0.7	2.0
Mn	279.5	0.01	0.7	3.0
Cu	324.8	0.02	0.7	1.5
Zn	213.9	0.005	0.7	2.0
Cr	357.9	0.05	0.7	2.0
Co	240.7	0.05	0.2	4.5
Pb	283.3	0.1	0.7	2.0
Fe	248.3	0.03	0.2	7.0
Cd	228.9	0.005	0.7	2.0
Ni	232.0	0.04	0.2	7.0

Table 7**.** Instrument method detection limits for the analysis of red pepper samples by FAAS.

Metal	Ca	Cd	Co	Cr	Cu
IDL (mg/L)	0.01	0.005	0.05	0.05	0.02
[a]MDL (mg/g)	0.0107	0.0003	0.0005	0.027	0.0017

Metal	Mn	Fe	Ni	Pb	Zn
IDL (mg/L)	0.0102	0.03	0.04	0.1	0.005
[a]MDL (mg/g)	0.0009	0.0058	0.0023	0.00167	0.0019

[a]Values are three times mean of six blank determinations.

IDL = Instrument Detection Limit. MDL= Method Detection Limit.

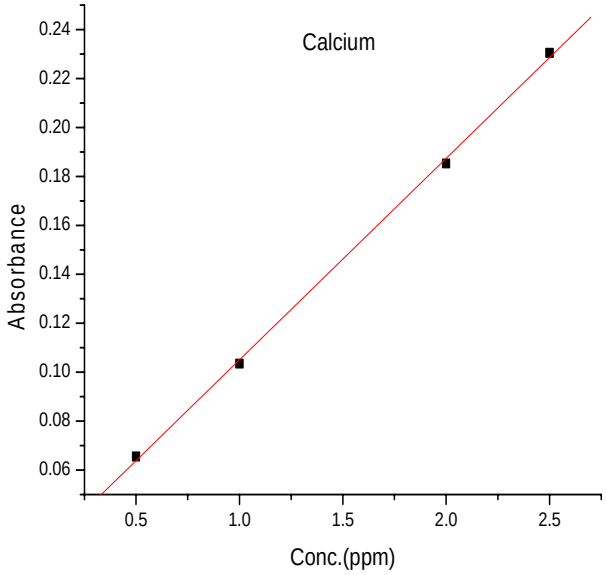


Fig. 5a. Calibration curve of calcium.

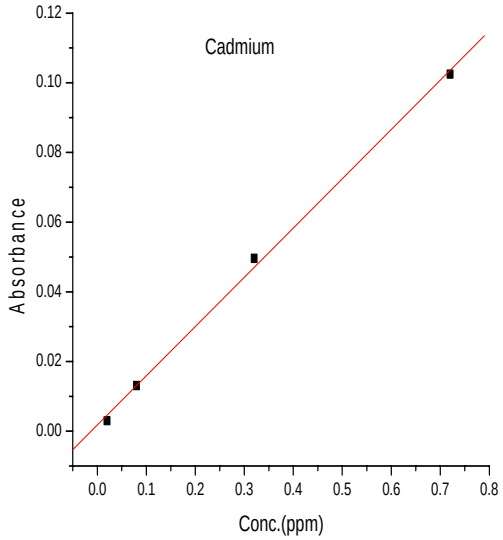


Fig. 5b. Calibration curve of cadmium

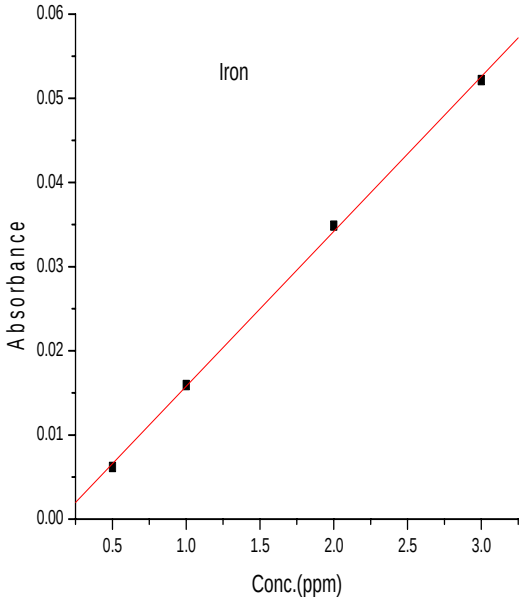


Fig. 5c. Calibration curve of iron

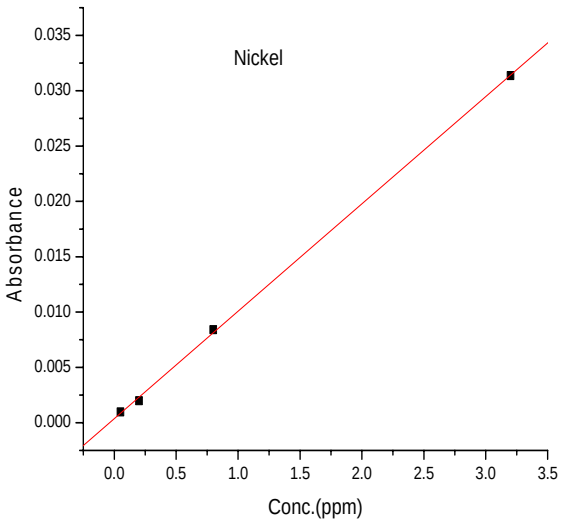


Fig. 5d. Calibration curve of nickel

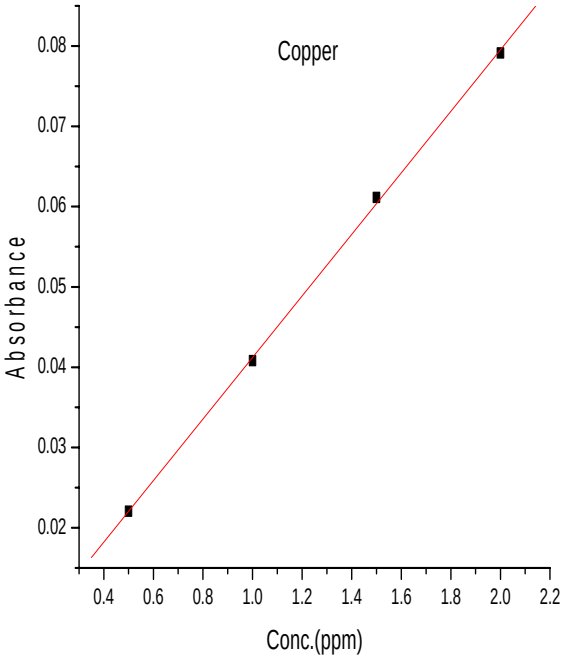


Fig. 5e. Calibration curve of copper

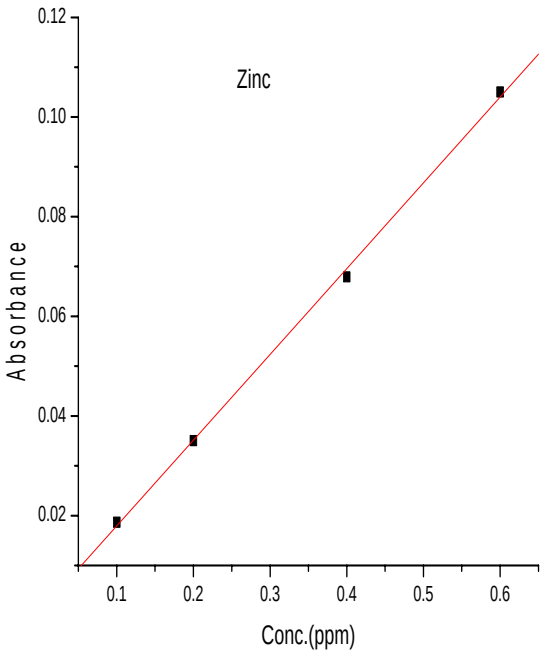


Fig. 5f. Calibration curve of zinc

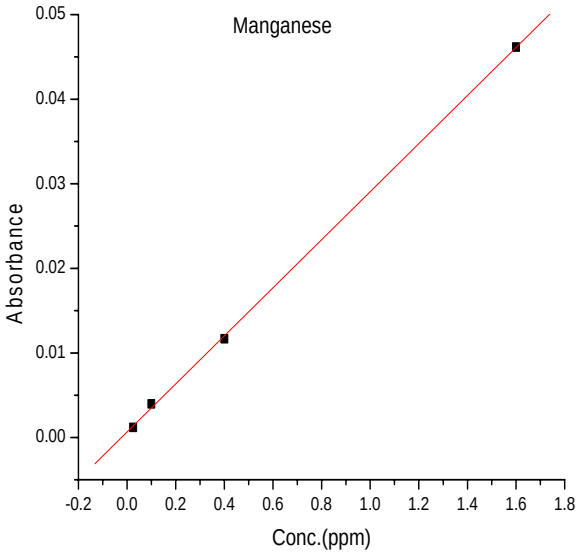


Fig. 5g. Calibration curve of manganese

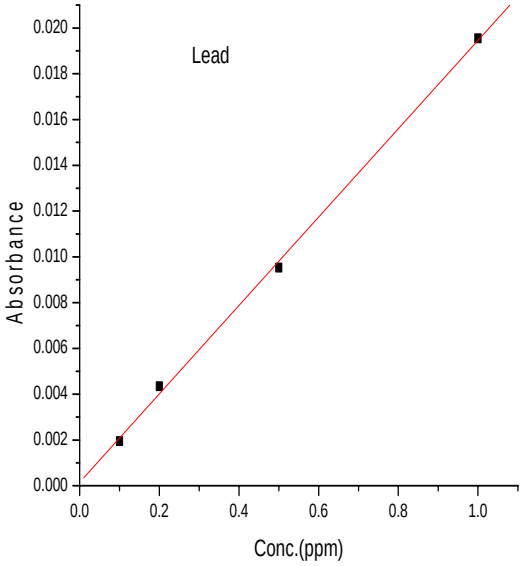
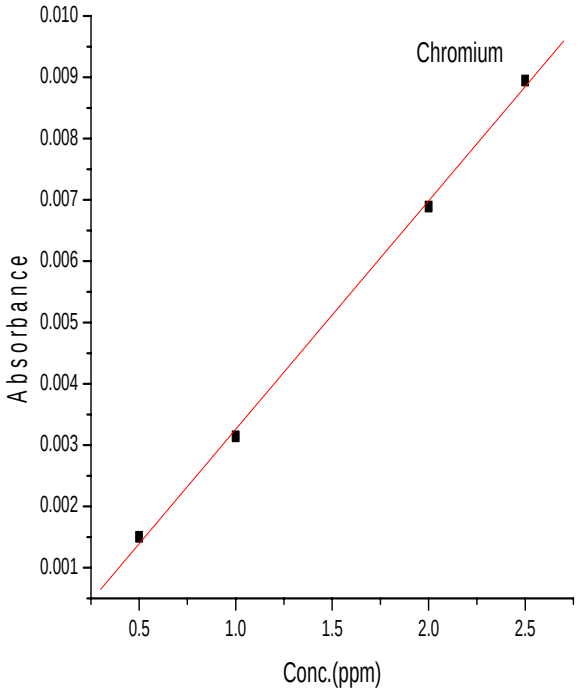


Fig. 5h. Calibration curve of lead



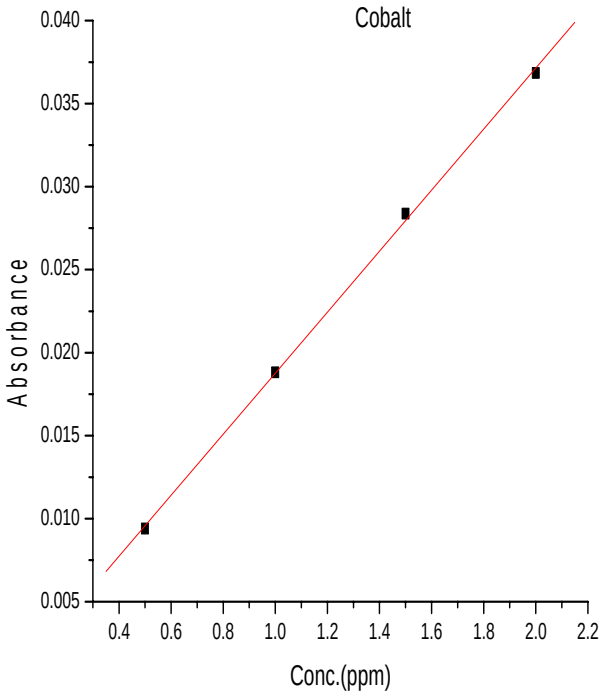


Fig. 5i. Calibration curve of chromium

Fig. 5i. Calibration curve of cobalt

3.3.3. Validation of Optimized Procedure (Recovery)

In order to ascertain the reliability of the method for the analysis of the samples for major and minor metals, Certified Standard Reference Materials (CSRM) were not available for use in our laboratory or around, instead the validity (accuracy) of the analytical procedures and efficiency of atomic absorption spectrophotometer used for sample treatment and analysis were tested by spiking experiment in a selected sample. From the stock solution (1000 mg/L) standard solutions the following concentration of metals were taken. A 30 µL of Co, 50 µL of Cu, 250 µL of Ca, 50 µL of Cr, 10 µL of Fe, 10 µL of Mn, 10 µL of Ni and 10 µL Zn from 1000 mg/L, solutions were added to 0.50 g red pepper samples and samples were spiked in triplicate as the procedures as were used for the original. As the values given in Table 8, the results of the

recoveries for all the metals in the analyzed pepper sample were all found within 100 ± 10 percent. This indicates that contamination was not a problem in the digestion procedure and the recovery results were in good agreement with expected values. Therefore the recovery test and reproducibility of the method were found satisfactory to validate the experimental protocol.

Table 8. Recovery results obtained for validation of the optimized procedure after spiking with standard solutions.

Metal	Amount added (µg/g)	[a]Amount found (µg/g)	[b]Recovery (%)
Ca	45	228 ± 7	90.5 ± 8.0
Co	0.09	2.32 ± 0.40	93.4 ± 0.5
Cr	5	32.3 ± 3.1	95.8 ± 4.1
Cu	0.5	4.16 ± 0.20	90.6 ± 0.3
Fe	10	109 ± 9	97.8 ± 11.0
Mn	2	11.60 ± 0.87	97.2 ± 0.9
Ni	1	7.76 ± 0.32	107.9 ± 0.5
Zn	10	67.7 ± 1.7	93.0 ± 1.7
Cd	-	[c]ND	-
Pb	-	[c]ND	-

[a] Concentration values are average of three analyzed samples ± standard deviation.

[b] Recovery values are mean ± standard deviation.

[c] Concentration values of the studied metal below method detection limit.

3.4. Distribution Pattern of Metals in Different red pepper Samples

The levels of essential (Ca, Co, Cr, Cu, Ni, Mn, Zn and Fe) and non essential metals (Cd and Pb) were determined by Flame Atomic Absorption Spectroscopy. All the pepper samples contain the metals mentioned above except cadmium and lead which were found below their method detection limit (0.0003 mg/g and 0.00167 mg/g respectively). In all the three pepper samples being analyzed, the Ca was found in appreciable amounts. This maximum availability compared with other metals indicated that it is the major components of plant nutrients. On the other hand, iron is the most abundant trace metal with in all the samples. The mean, standard deviation and relative standard deviation of the concentration of the metals in the pepper samples collected from three sites are presented Table 9.

Table 9. Average metals concentration and relative standard deviation of red pepper samples.

Metal	Alaba		Mareko		Merkato (Addis Ababa)	
	Conc.($\overline{X}$ ± SD)	RSD (%)	Conc.($\overline{X}$ ± SD)	RSD (%)	Conc.($\overline{X}$ ± SD)	RSD (%)
Ca	222 ± 4	1.9	187 ± 4	2.1	161 ± 7	4.3
Co	1.04 ± 0.1	9.1	2.24 ± 0.14	6.1	1.02 ± 0.10	10.2
Cr	46.6 ± 2.6	5.7	27.5 ± 2.6	9.5	73.6 ± 1.4	1.8
Cu	2.12 ± 0.19	9.2	3.71 ± 0.28	7.7	3.54 ± 0.16	4.6
Fe	157 ± 4	2.8	99.5 ± 7.3	7.4	103 ± 2	1.7
Mn	15.0 ± 0.2	1.6	9.72 ± 0.16	1.7	18.95 ± 0.63	3.3
Ni	2.71 ± 0.10	3.7	6.68 ± 0.30	4.6	2.81 ± 0.12	4.3
Zn	20.8 ± 1.2	5.5	58.4 ± 0.5	0.8	27.3 ± 0.7	2.4
Cd	[b]ND		ND		ND	
Pb	[b]ND		ND		ND	

[a]Data presented as mean ± standard deviation.

[b] concentration below method detection limit.

3.4.1. Levels of Metals in Alaba Pepper

The pepper from Alaba contains a more pronounced concentration of calcium than those metals being analyzed (Cu, Cd, Cr, Fe, Mn, Ni, Zn, Co). As placed in Fig. 6 and Table 9, the amount of calcium in Alaba pepper was found 222 μg/g. With in the essential trace elements iron was detected in a highest level (157 μg/g) followed by chromium (46.6 μg/g). The level of cobalt is generally low compared to any other metals studied in this sample. The concentration of Co examined was 1.04 μg/g. The levels of the two toxic metals, Cd and Pb were found below detection limit. This indicates that the Alaba pepper is safer for consumption in regarding with the amount of these metals. As can be seen in Table 10 and Fig. 6 and 7, the concentration pattern of metals in this sample follows the order Co < Cu < Ni < Mn < Zn < Cr < Fe < Ca.

Table 10. The levels of metals in Alaba pepper.

Metal	Ca	Fe	Co	Mn	Ni
Conc. (μg/g)	222 ± 4	157 ± 4	1.04 ± 0.09	15.00 ± 0.24	2.71 ± 0.01

Metal	Zn	Cr	Cu	Cd	Pb
Conc. (μg/g)	20.8 ± 1.2	46.6 ± 2.6	2.12 ± 0.19	ND	ND

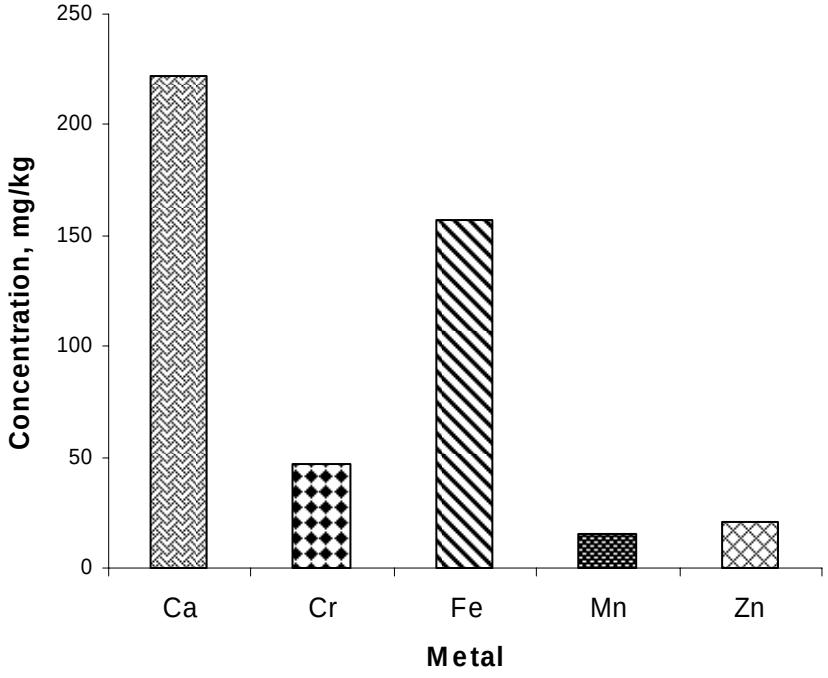


Fig. 6**.** The mean concentration of Ca, Cr, Fe, Mn and Zn Alaba pepper sample.

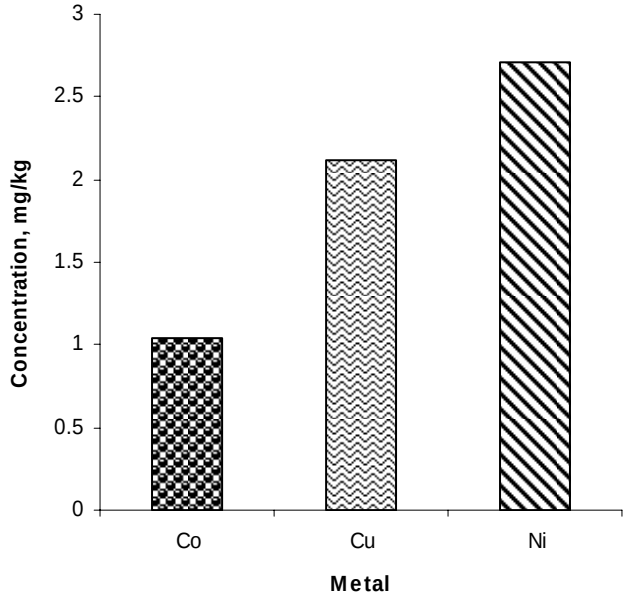


Fig. 7**.** The mean concentration of Co, Cu and Ni in Alaba pepper.

3.4.2. Levels of Metals in Mareko Pepper

The results of the analysis of red pepper in a sample form Mareko showed that the sample contains the highest value of Ca with identified concentration 187 μg/g followed by iron with a value of 99.5 μg/g. Of all the micro nutrients, zinc content was found in highest amount next to iron 58.4 μg/g. The trend of occurrence of the metals concentrations in red pepper samples from Mareko is in the order of Cd < Co < Cu < Ni < Mn < Cr < Zn < Fe < Ca (Table 11 and Fig. 8 and 9). This trend suggests that peppers of Mareko have least concentration of Co (2.24 μg/g).

Table 11. The levels of metals in Mareko pepper.

Metal	Ca	Fe	Cu	Co	Cr	Zn
Conc. (μg/g)	187 ± 4	99.5 ± 7.3	3.71 ± 0.28	2.24 ± 0.14	27.5 ± 2.6	58.4 ± 0.5

Metal	Mn	Ni	Cd	Pb
Conc. (μg/g)	9.72 ± 0.16	6.68 ± 0.29	ND	ND

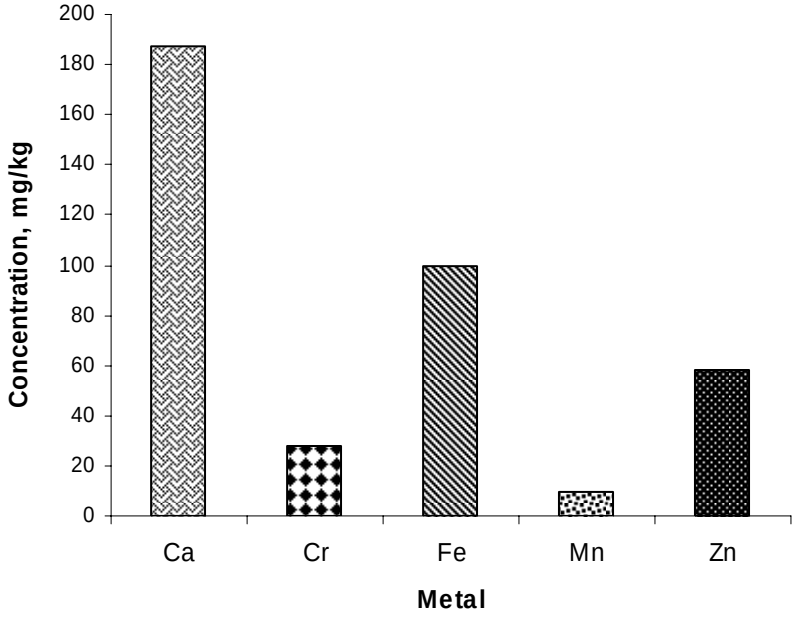


Fig. 8. The mean concentration of Ca, Cr, Fe, Mn and Zn in Mareko pepper sample

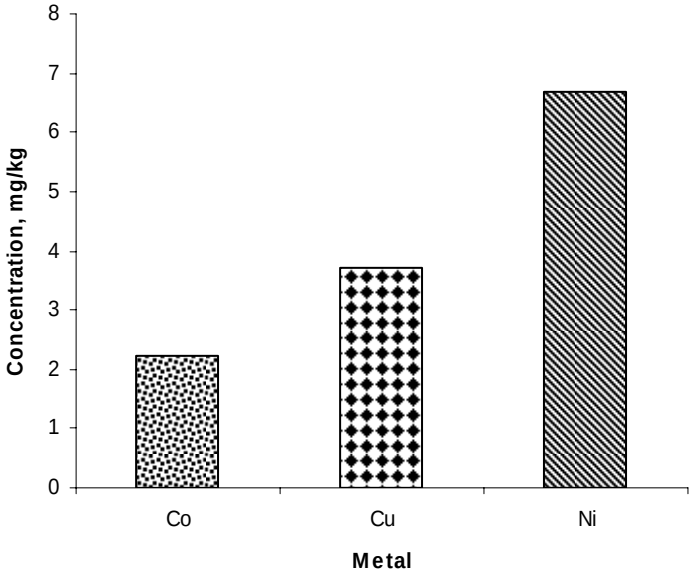


Fig. 9. The mean concentration of Co, Cu and Ni in Mareko pepper.

3.4.3. Levels of Metals in Merkato Pepper

As within the other pepper samples, the pepper from Merkato contains the highest value of calcium (161 μg/g). Iron had significantly higher concentration than the rest of trace metals 103 μg/g, followed by Cr (73.6 μg/g), while cobalt was found to occur in least concentration 1.02 μg/g. As indicated in Table 12 and Fig. 10 and 11, the concentration of the metals are ranked in the order Co < Ni < Cu < Mn < Zn < Cr < Fe < Ca.

Table 12. The levels of metals in Merkato pepper.

Metal	Ca	Fe	Cu	Co	Cr
Conc. (μg/g)	161 ± 7	103 ± 2	3.54 ± 0.16	1.020 ± 0.104	73.6 ± 1.4

Metal	Mn	Ni	Zn	Cd	Pb
Conc. (μg/g)	18.95 ± 0.63	2.81 ± 0.12	27.3 ± 0.7	ND	ND

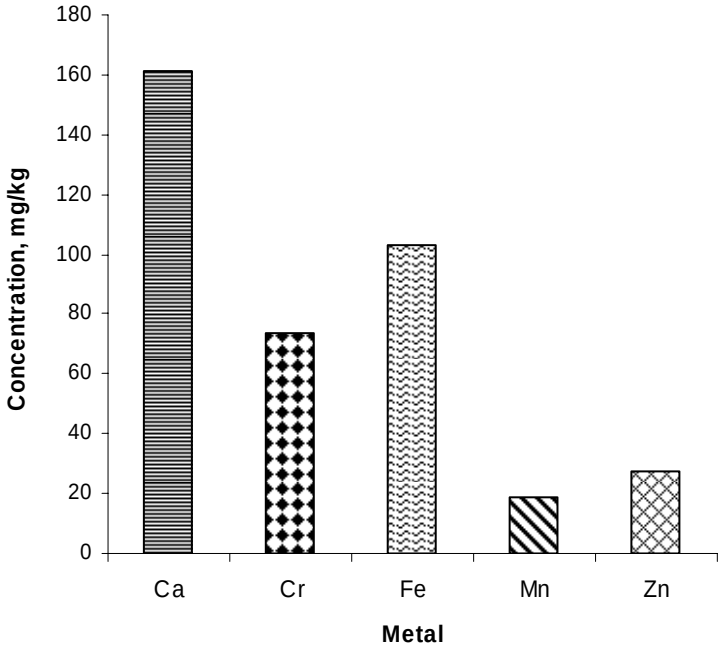


Fig. 10. The mean concentration of Ca, Cr, Fe, Mn and Zn in Merkato pepper sample.

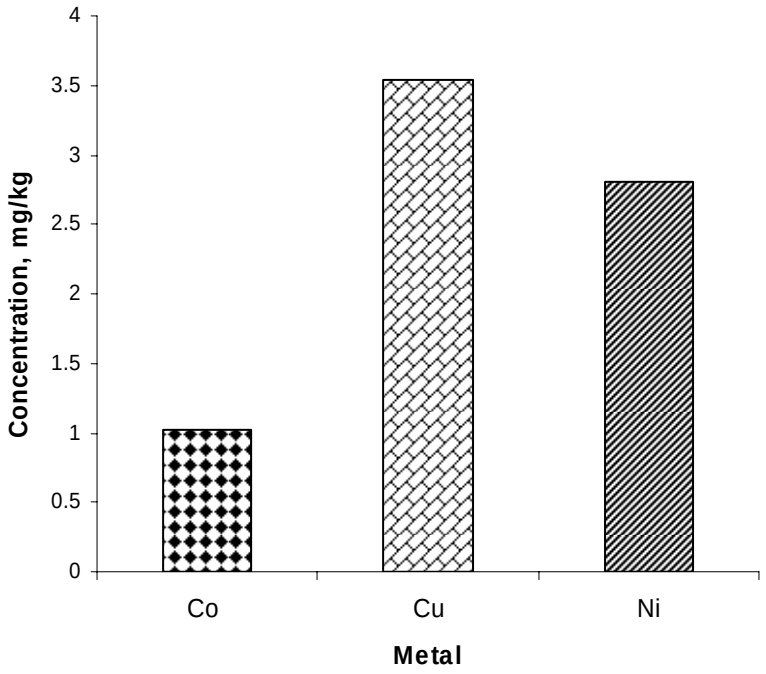


Fig. 11. The mean concentration of Co, Cu and Ni in Merkato sample.

3.5. Comparison of the Levels of Metals in the Three Pepper Samples

All pepper samples collected from the study areas showed the presence of Ca, Co, Cr, Cu, Fe, Mn, Ni and Zn, while cobalt and lead were found to be below their detection limits within the three pepper samples. The metal composition of pepper samples are given in Table 9, showing that the mineral concentration in all samples is comparatively varied and their concentration are more pronounced than those of the trace metals.

In all the analyzed metals calcium (Ca) content was high as compared to other metals (Fig. 12). Its concentration ranges from 161 to 222 μg/g. Alaba provided maximum of 222 μg/g while its concentration was found minimum in Merkato (161 μg/g). The high Ca content is a characteristic of plant food, the order of its concentration is Alaba > Mareko > Merkato. The second abundant metal found in pepper was iron. Its mean concentration was found between 99.5 μg/g and 157 μg/g. The maximum amount was found in Alaba pepper (157 μg/g), while

minimum amount was found in Mareko (99.5 μg/g). The total accumulation of iron was in the order of: Alaba > Merkato > Mareko.

Of the trace metals, in all pepper samples the concentration of Co was occured relatively least amount than any other trace element analyzed. But its concentration varies within the pepper samples analyzed. The Co contents in the study were in the range of 1.02 – 2.24 μg/g (Table 9). The highest and the least concentration were observed in Mareko (2.24 μg/g) and in Merkato (1.02 μg/g), respectively. The concentration pattern of cobalt follows the order Mareko > Alaba > Merkato. The nickel contents in the samples were 2.71 μg/g, 6.68 μg/g and 2.81 μg/g in Alaba, Mareko and Merkato, respectively. Moreover, the lowest level of Ni was accumulated in Alaba pepper and the highest was in Mareko.

Zinc was the second most accumulated trace metal next to iron in Mareko pepper, but it is the third abundant metal next to iron and chromium in Alaba and Merkato peppers. In this study, it was detected in the range of 20.81 - 58.4 μg/g, while the highest level of Zn was seen in peppers of Mareko and the lowest level in peppers from Alaba. The high concentration of Zn was probably because it is considered as essential micronutrients for plants growth and can easily taken up by plants. As can be seen in Table 9, the manganese contents in the samples were found 15.0 μg/g, 9.72 μg/g and 18.95 μg/g in peppers of Alaba, Mareko and Merkato, respectively. The order in the level of Mn follows the pattern Mareko < Alaba < Merkato.

Concerning to chromium in the study, the concentration with in the analyzed pepper samples was found in the range of 27.5 – 73.6 μg/g. These higher values find Cr the second most abundant trace metal in red pepeper samples following iron, with the exception in Mareko which is exceeded by Zn. The maximum chromium content was found in Merkato with concentration of 73.6 μg/g. The concentration of Cr metal determined from the samples of the study areas decreased in the order; Merkato > Alaba > Mareko.

In the case of copper, the mean concentrations were found in the range of 2.12 - 3.71 μg/g. The

highest was found in Mareko (3.71 μg/g) while the lowest concentration observed in Alaba pepper (2.12 μg/g). The daily in take of Cu by human being is 0.5-2 mg and the Ethiopian pepper is one of a good source of Cu.

The trend of occurrence of the metals concentration in red pepper samples in Alaba is in the order of Ca > Fe > Cr > Zn > Mn > Ni > Cu > Co. This trend suggests that calcium found in highest concentration and Co is in least. However, the trend of occurrence of metals concentration in Merkato follow different order of; Ca > Fe > Cr > Zn > Mn > Cu > Ni > Co. In the same manner, the metals accumulated in Mareko showed a different pattern where the highest metal accumulation was Ca, followed by Fe, Zn, Cr, Mn, Ni, Cu, Co.

In general, the results of this study revealed that the highest concentration of Ca and Fe were found in Alaba, Co, Cu, Ni and Zn were highest in Mareko pepper while Cr and Mn found maximum in Merkato (Fig. 13 and Table 13). The variations in concentration and order of accumulated metals were different in the samples probably influenced by the site locations (i.e, the mineral content of the soil on which the plants grow).

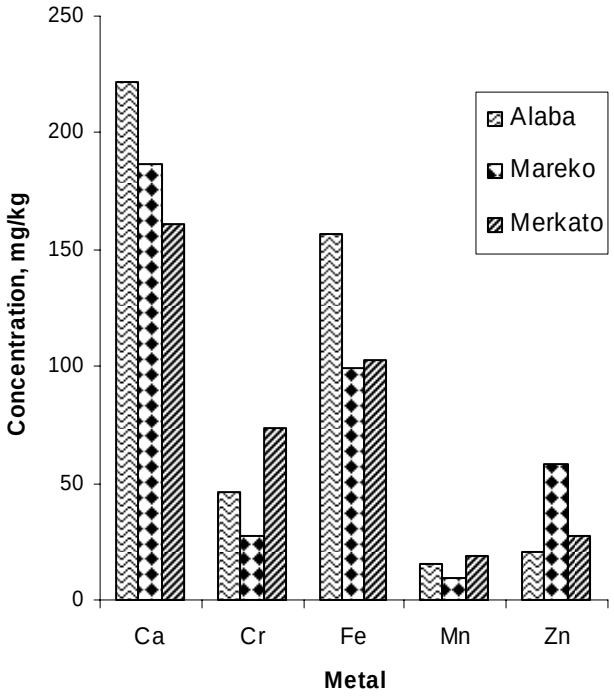


Fig. 12. The mean concentration of Ca, Cr, Fe, Mn and Zn in the three pepper samples.

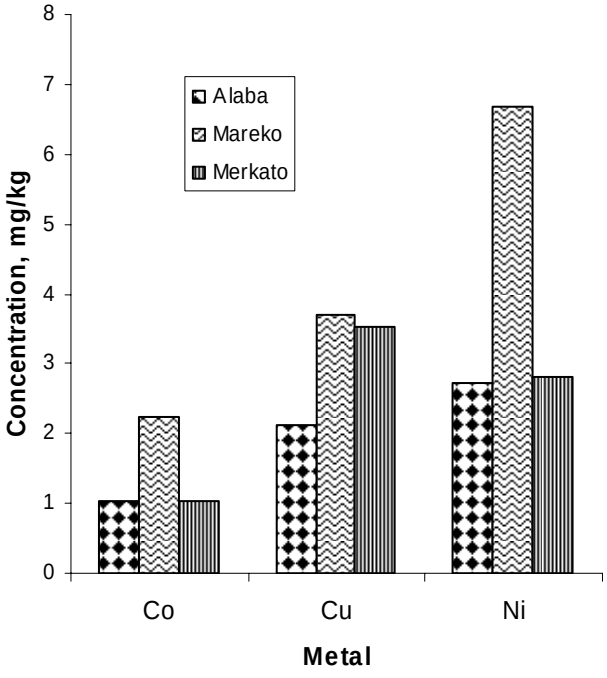


Fig. 13. The mean concentration of Co, Cu and Ni in pepper samples.

Table 13. Samples which contain the highest concentration of metals

Sample	Alaba	Mareko	Merkato
Metal	Ca, Fe	Co, Cu, Ni, Zn	Cr, Mn

3.6. Comparison of Current Study with Results from Other Countries

There are some reports from different countries on the analysis of the metal contents of red peppers. It is important to compare the results obtained from the analysis of red pepper in Ethiopia with the values sited in other countries. This comparision helps to identify the differences in composition and if there exits a deviation from certain guide lines.

The levels of calcium in the samples analyzed ranged from 161 - 222 μg/g. The Ca concentrations obtained from this study were higher than those recorded by Rubio et al. from Holland and Spain. But, it is comparable with the values of Canary Islands (123 μg/g - 231 μg/g) [76].

As can be seen in Table 14, iron was found to be the highest and cadmium occurred in least within the literatures cited. The amount of iron obtained in this study were lower than levels of iron reported by Bigdeli and Selsepour (413.7 μg/g) and Soyalk *et al.* (234.1 μg/g), but it was found in higher levels than given by Rubio *et al.* from Canary Islands and Spain [34, 76, 77].

Zinc is present in appreciable amount in these samples analyzed (20.8 - 58.4 μg/g). These concentrations were higher when in comparision to literature reports from Canary Islands, Spain and Tureky [76]. Rubio *et al.* reported the concentration range of as low as 1.0 - 2.3 μg/g in Canary Islands and 2.6 μg/g in Spain and Soyalk *et al.* reported as low as 7.84 μg/g of Zn. However, Zn concentrations in these samples analysed are shown to be in a good agreement

with the data obtained by Bigdeli and Selsepour (39.02 μg/g) [24], Jung (28.1 μg/g) [77] and with the results of Awode *et al.* with concentration range of Zn 10.40 - 35.10 μg/g from Nigeria [66].

When the levels of Mn obtained in this study (9.72 – 18.95 μg/g) compared with literature value, it is higher than with the results reported by Rubio *et al.* and lower than with the values obtained by Bigdeli and Selsepour. However, the values of Mn were agreeing with results given by Soyalk *et al.* which is 11.9 μg/g [34].

As given in Table 14, concentrations of Ni (2.71 - 6.68 μg/g) obtained in this study and results given by Soyalk *et al.* (2.92 μg/g) and Awode *et al.* (2.73 - 6.90 μg/g) are comparable. But, Co concentration found in this study is higher than data cited by Bigdeli and Selsepour (0.23 μg/g).

In comparing the results of this study with those cited in literature, the concentration of Cu found in this study was higher than value reported by Rubio *et al.*, 0.8 μg/g and 0.7 μg/g from Canary Islands and Spain, respectively. However, it is lower than with the values reported by Awode *et al.* (14.52 μg/g) [70], Bigdeli and Selsepour (24.1 μg/g) and Jung (8.45 μg/g), while more or less it agrees with the results of Soyalk *et al.* (4.2 μg/g).

As can be seen from Table 14, the chromium contents of some pepper samples around the world have been reported from Tureky as 7.15 μg/g [34] and from Nigeria as 10.40 - 35.10 μg/g [70]. It is found that the level of Cr in pepper samples from Turkey is much lower than that of these samples (27.5 - 78.6 μg/g). While the values for the Cr contents of the analyzed samples were generally are at the same level of the literature values from Nigeria [70].

Regarding to the non-essential trace elements (Cd and Pb) in most literature Cd and Pb were detected up to very low level. However, in the present study the levels of these two metals were found below their detection limit.

The results of the study show that the minimum and maximum values of cobalt are 1.02 and 2.24 μg/g. The value reported for Co by Bigdeli and Selsepour from their study in Iran is much lower than accumulated in Ethiopian peppers.

As mentioned above, the results obtained in this study were comparable favorably with the findings of other researchers from other parts of the world. However, results with those given by Rubio *et al.* are observed that there are great differences about the contents of macro and trace elements. This difference in metals content may be ascribed to variation in composition of soil types, sample sites, sample treatment, the presence of agents which either increases or decreases the metal content.

Table 14. Comparison of concentrations of metals in the Ethiopian red peppers with that reported in the rest of the world.

Origin	Metal (μg/g)								References
	Cd	Cr	Cu	Fe	Mn	Ni	Zn	Pb	
Canary Island	NG	NG	0.3─1.3	2.2─4.6	0.3─0.8	NG	1.0 ─ 2.3	NG	[76]
Iran	0.03	NG	24.10	413.70	28.58	NG	39.02	1.41	[24]
Turkey	0.65	7.15	4.2	234.1	11.9	2.92	7.84	0.79─	[34]
Nigeria	0.25─1.07	10.4─35.10	7.56─21.07	NG	NG	2.73─6.90	10.40─35.10	11.3─27.0	[66, 70]
Korea	0.27	NG	8.45	NG	NG	NG	2.81	0.38	[77]
Spain	NG	NG	0.8	5.5	1.0	NG	2.6	NG	[76]
This study	ND	27.5─78.6	2.22─3.71	99.5─157	9.72─18.95	2.71─6.68	28.8─58.4	ND	

ND- Not detected. NG- Not given.

Origin	Ca	Co	References
Iran	NG	0.01	[24]
Holland	130	NG	[76]
Spain	100	NG	[76]
Canary Island	123─231	NG	[76]
Present study	161─222	1.02─2.24	

Moreover, the levels of metals found in Ethiopian red peppers are compared with the maximum limit of metals in red pepper given by WHO guide lines. On this basis the levels of Co, Cu, Fe, Mn, Ni and Zn in the analyzed pepper samples were found below the guide lines, while the levels Cr which are occurred above the value given by WHO ML (Table 15). Chromium and its compounds are essential for human beings; the daily requirements are 0.05-0.5mg, and the body contains about 0.03mg per kg [47]. Evenif the levels of Cr in this study was found higher than recommended by WHO, the total Cr daily in take (0.05-0.5 mg) is too much higher than the amount available in pepper. Thus, the toxic effect of Cr from the studied peppers may be further prevented by minimizing the cromium contribution for the daily intake from different food sources.

Table 15**.** Recommended concentration of some traces metals in red pepper by WHO**.**

	Metal conc.(μg/g)									
	Zn	Fe	Mn	Cu	Ni	Cr	Co	Pb	Cd	Refere-nces
This study	20.8-58.4	99.5-157	9.72-18.95	2.12-3.71	2.71-6.68	27.5-73.6	1.02-2.24	ND	ND	
WHO ML	100	425	500	73	67	2.3	50	0.3		[24, 70].

WHO ML-World health Organization Maximum Limit.

3.7. Statistical Analysis of Variance (ANOVA)

In analytical work there are often more than two means to be compared. The possible sources of variation are due to the random error in measurement, which causes a different result to be obtained each time a measurement is repeated under the same conditions and the other source is due to controlled or fixed effect factor; for example the condition which the solution was stored, the method of analysis used and the analyst carrying out the experiment. ANOVA is a

powerful statistical technique which can be used to separate and estimate the different causes of variation. ANOVA can also be used in situations where there is more than one sources of random variation [78].

One-way ANOVA can be used when there is only one factor being considered and replicate data from changing the level of the factor are available. In the present study one-way ANOVA was used to compare the means between all the three pepper samples and the calculations were made using SPSS software.

In comparing the means of calcium in the three pepper samples at the 95% confident level, their means are significantly different. Of these samples, Alaba has the highest while Mareko has the lowest means. In similar manner, the results of one-way ANOVA also indicated that, there exist significant differences between the levels of chromium and manganese with in all the three pepper samples. The means of Cr and Mn were found maximum in Merkato pepper samples.

The analysis of the difference in the means of Co showed that significant differences were observed between peppers from Alaba and Mareko and between Mareko and Merkato. While within pair of means of Alaba and Merkato no significant differences were seen.

The ANOVA analysis of iron indicated the existence of significant differences ($p < 0.05$) between means of Merkato and Alaba and between Alaba and Mareko, but no significant differences between Merkato and Mareko peppers were observed for the elemental iron.

Concerning to the means of Ni and Zn, the means between Alaba and Mareko and between Merkato and Mareko have significant differences. But, no significant differences observed between the means of Ni and Zn in Alaba and Merkato samples ($p > 0.05$).

Statistically, there were significant differences ($p < 0.05$) in the means of copper in Alaba with other peppers. However, no significant differences were observed in the copper levels between Merkato and Mareko ($p > 0.05$).

To sum up, the results of one-way ANOVA revealed that means of Ca, Cr and Mn showed significant differences within all samples analyzed in this study. However, other metals show both significant and non-significant differences between their means.

5. CONCLUSION AND RECOMMENDATION

Red pepper is consumed with substantial amount locally as spice and also it is important cash crop which has high value in export markets. However, no literature values revealed the levels of metals in Ethiopian red peppers, only focused on the economic aspects. Owing to this, the present study tried to examine the distribution of metals (Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni, Zn and Pb) in pepper samples collected from Alaba, Mareko and Merkato using flame atomic absorption spectroscopy.

The study shows that the levels of Ca, Co, Cr, Cu, Fe, Mn, Ni and Zn in pepper samples were found in appreciable amount while lead is found below method detection limit. Of the leveled metals, calcium was observed in highest level. Similarly, iron accumulated in highest level among the micro metals. For the pepper samples from Alaba, Mareko and Merkato, the amount of the analyzed metals were found to follow decreasing order; Ca > Fe > Cr > Zn > Mn > Ni > Cu > Co, Ca > Fe > Zn > Cr > Mn > Ni> Cu > Co and Ca > Fe > Cr > Zn > Mn > Cu > Ni > Co, respectively.

A recovery study of the analytical procedure was carried out by spiking the analyzed samples with varied amounts of standard solutions of the metals. The average recoveries obtained were 100 ± 10 percent and are in a good agreement with expected values. This indicated that the analyzed samples contain appropriate levels of metals and procedures for determining the metals were reliable.

The levels of metals analyzed in Ethiopian peppers were compared with the levels of metals in peppers from other parts of the world. It was observed that the composition of red peppers in Ethiopia was comparable with the results reported from different countries with little exception.

Concentrations of metals between samples were also compared using one-way ANOVA. The results of ANOVA analysis indicated that there were significant differences in the levels of most metals between the three samples. For the metals Ca, Cr and Mn, the means are found significantly varied within the analyzed pepper samples, while other metals show significant and non-significant differences between their means. The variation in the levels of metals between samples certified the difference in the composition of soils from which the pepper grown.

The results obtained in the present study were compared with international guidelines for red pepper. All the metals determined were found below the levels allowed for vegetables by WHO, except chromium. The levels of Cr in all the three samples are slightly higher than 2.3 μg/g recommended by WHO.

At last, it is recommended that the findings of this study will provide preliminary information for researchers on the levels of metals in Ethiopia red peppers. The levels of chromium in the peppers analyzed were higher than the level recommended by WHO. Even though Cr is above WHO maximum limit, its toxicity depends on the forms of its availability. The toxic effects of Cr contributed from red pepper can be prevented by controlling the total chromium daily intake from different sources not to exceed the WHO level. Moreover, inorder to safer consumer from health risk further works should be carried out to monitor the sources of the metal more closely in areas where the peppers are grown in order to reduce the levels and to provide adequate protection for human health.

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Declaration

I the undersigned confirm that the results reported in this work were obtained by research carried by me under the supervision of my advisor in the Faculty of Science, Department of chemistry, Addis Ababa University in the academic year 2008/2009.

Name: Molla Tefera Signiture_____

This project has been submitted for examination with my approval as a university adivisor.

Advisor_____

Signiture _____

Place and date of submission: School of Graduate Studies
Addis Ababa University
June 30, 2009.