DEBRE BIRHAN UNIVERSITY

SCHOOL OF POST GRADUATE STUDIES

CHEMISTRY DEPARTMENT

DETERMINATION OF ESSENTIAL NUTRIENTS (Zn, Fe, Cu, Cr) AND TOXIC HEAVY METALS (Cd, Hg, Pb) IN LENTIL SEED USING INDUCTIVE COUPLED-OPTICAL EMMISSION SPECTROPHOTOMETER IN NORTH SHOA ZONE ANGOLELA AND TERA DESTRICT

By:

SHEWAKENA MAMO

SUBMITTED IN ACCORDANCE WITH THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN THE SUBJECT CHEMISTRY

Advisor: Balkew Zewge (PhD)

February-2021

DEBRE BIRHAN, ETHIOPIA

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Advisor Approval sheet

This is to certify that the thesis entitled with **Determination of essential nutrients (Zn, Fe, Cu, Cr) and toxic heavy metals (Cd, Hg, Pb) in lentil seed using inductive coupled-optical emission spectrophotometer in north shoa zone Angolela district** submitted in partial fulfillment of the requirements for the Degree of Master of Science in Chemistry, in the graduate program of the Department of Chemistry which has been conducted by **Shewakena Mamo** were done under our supervision. Therefore, I recommend that the student has fulfilled the requirements and hence here can submit the thesis to the department.

Balkew Zewge (PhD)	__________________	________________
Name of Advisor	Signature	Date

DEBREBERHAN UNIVERSITY

School of Graduate Studies

APROVAL SHEET

We undersigned members of the Board of Examiners of the final open defense by **Shewakena Mamo** have read and evaluated his thesis entitled **Determination of essential nutrients (Zn, Cu, Cr, Fe) and toxic heavy metals(Cd, Hg, Pb) in lentil seed using inductive coupled-optical emission spectrophotometer in north shoa zone Angolela district** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the Degree of Master of Science in Chemistry

Name of Advisor Signature Date

______________________ ____________________ _______________

Name of Internal Examiner Signature Date

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Name of External Examiner Signature Date

________________________ ____________________ _______________

DSGS Approval Signature Date

Declaration

I the under signed, declare that this thesis is my original work and has not been presented for degree in any other university and that all source of materials used for the thesis have been duly acknowledged.

Name ________________________

Signature_____________________

Department___________________

Date ________________________

Acknowledgment

Behind my every success and achievement is the almighty God, whose kindness and help are invaluable forever.

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Table of contents

LIST OF FIGURES

LIST OF TABLE

LIST OF ABBREVIATIONS

ICP-OES	Inductively coupled optical emission spectrometer
SHA	Self Help Africa.
EDA	Emmanuel development Association
SPSS	Statistical package for social science.
ppm	Parts per million
WHO	World Health Organization
FAO	Food & Agricultural Organization
SD	Standard deviation.
PLC	Private Limited Company.
EC	Electrical conductivity.
pH	Power of hydrogen.
UK	United Kingdom
USA	United states of America.
ATSDR	Agency for Toxic Substance and Disease Registry.
DNA	Dioxy Nucleic Acid.
Cps	counts Per second
μS	Micro-Siemens.
FEPA	Federal Environmental Protection Agency
EU	European Union

ABSTRACT

The lentil seeds (Lens Culinaris Medik) of samples were collected from the farm land of Angowelela and tera district Chacha and Chefanen kebele. After the pre-treatment, of the digestion and ignition temperature and volume of reagents used the concentration of selected metals was determined by inductively coupled plasma optical emission spectrometry. The concentration of essential metals nutrients in the lentil seed samples were in the following ranges Zn(4.5739-4.439mg/kg), Cu(0.6137-0.662mg/kg), Fe(4.8313-2.622mg/kg), Cr(0.2882-0.295mg/kg) the concentration of toxic, heavy metals were Cd(0.1515-0.1367mg/kg), Hg(0.5945-0.7493mg/kg) and Pb(1.1348-1.009mg/kg). All the metal determined were found below the levels allowed for legumes crop by WHO/FAO except mercury and cadmium metal. T-test at 95% confidence interval indicated that there is positive and highly significant correlation ($r = 0.99^{*}$), between heavy metal lead with cadmium and iron with copper respectively for Chacha. Similarily positive significant correlation($r=0.99^{*}$) between heavy metal copper with zinc for Chefanen. However, the degree to which two securities are negatively or positively correlated might vary over time and are almost never exactly correlated, all the time. This condition may happen sometimes because one physico-chemical property may affect others and it may don't have consistent.

Key words: *Lens Culinaris Medik, ICP-OES, legumes*

CHAPTER ONE

1. Introduction

1.1. Background of the study

Lentil (Lens Culinaris Medik.) is a brushy annual plant of the legume family, grown for its lens-shaped seeds. It is about 15 inches tall and the seeds grow in pods, usually with two seeds in each. Its stem is thin, square and generally herbaceous and weak(Mulugeta,2009).Lentil being one of the first crops to be domesticated by man and continue to be an important food source for over 8000 years through subsequent cultivation. It is a diploid self-pollinating crop. It is the principal cool season legumes. Lentil is grown as a winter crop in Ethiopia and particularly important in Oromiya, Amhara and Tigray region. Lentil is one of the less selective legumes in terms of climate and soil features. It usually well adapted to various soil types ranging from clay to loam. Lentil is considered as drought resistant crop that can tolerate low annual rainfall distribution even in the range of 280-300mm in regard to temperature ,lentil can grow in different environments from cool temperature to subtropical dry zone. A soil pH of six to eight is conducive for lentil production, but it can also tolerate a moderate alkalinity. Lentil is mainly grown in the high lands of Ethiopia where rainfall is usually high. It provides affordable source of dietary proteins (22-35%), minerals, fibres, and carbohydrates to people and play a vital role in alleviating malnutrition and micronutrient deficiencies in developing countries. When lentils are planted in a field for the first time the necessary nitrogen fixing bacteria must be provide (UOM, 1990).Lentil is an annual crop cultivated mostly in rotation with cereals. Lentil plants are typically short, but can range from 20 cm to 75 cm in height depend on growing condition.

The most important product of the lentil plant is its seed. Lentil is used in several food products, both as a whole seed and in processed form (Myers, 2000). Like most legumes they are relatively high in protein about 14 g of protein per cooked cup (250 mL). The amino acid profile of lentil is similar to that of other beans and is complementary to cereal grains. They also contain thiamine, iron, magnesium and other nutrients. Lentil is rich in vitamins, minerals and proteins and the ideal to substitute peas and beans which are all high land crops. (SHA & EDA, 2005).Lentil is a warm season crop requiring 90 to 120 days of frost free conditions from planting to maturity (depending on the variety).The optimum temperature range for growth is between 27 and 30 ^{0}C.

Lentil is considered to be heat and drought tolerant (department of agriculture, forestry and fishery, 2010).

As mentioned in SHA and EDA newspaper the metals (nutrients) are useful for the different functioning of human body parts, for example, copper is used to change protein in suitable forms to our body. Metals are also essential for the normal growth and metabolism of plants and play very important role in the biosynthesis of some enzymes and growth hormones (Nanson and McElroy, 1963). The growth and metabolism of many plant species were reported to be affected adversely by excess supply of heavy metals (Foy et al, 1978).Sammantary and das (1997) studied the accumulation of heavy metals in lentil grown on metalliferous mine spoil also found that Cr, Ni and Fe were more easily transported to shoots and roots as compared to seeds and their presence in traces in the fruits. Here in this experimental research attempts will take to study the accumulation of heavy metals in seeds of lentil grown in north shoa zone, Angolela and Tera district two different kebele (Chacha, Chefana). The determination of heavy metals in lentil is selected as this experimental research title, because at the research area farmers use different insecticides fertilizers and herbicides which can pollute the soil on which lentil grows and many cause for the uptake of metals by lentil seeds. In addition to this farmers use flowing river water for irrigation to grow lentil but different waste materials including by-products of metal work and wood work were wasted to the river which may cause in the increment of heavy metals concentration in lentil seeds. Heavy metals are elements of high molecular masses, most of which belong to the transition elements (Silberberg, 2000). Studies have shown that soils of refuse dumpsite contain different kinds and concentrations of heavy metals (Odukoya, et al, 2000). In recent times, it has been reported that these elements accumulate and persist in soils at an environmentally hazardous levels (Alloway, 1996).

1.2. Statement of the problem

Higher concentration of heavy metals in lentil seeds causes for the health effect on human being and other living organisms. The effects of heavy metals toxicity on the lentil seeds were used as a model in this study. If the heavy metals are ingested beyond the permitted concentration, they could cause serious health disorders (Marakat, 2011). The rise in population in Angolela and Tera district has increased the number of dumpsite due to poor waste management schemes. It was a common practice to burn dumpsite wastes; this burning gets rid of organic matter and became ashes which were richer in heavy metal contents. These ashes were either dissolved in rain water and leached into the soil contaminating the underground water, or washed away by runoff into streams and rivers, thereby contaminating the environment; it was based on these facts that the study was aimed at determining the total concentration of metals in lentil seed using scientific method.This study also compare the obtained concentration of metals found in lentil seed with toxic levels provided in literature.

1.3. Objectives

1.3.1. General objective

To determine the concentration of heavy metals (Pb, Cd, Cu, Zn, Cr, Hg, Fe) in lentil seeds using inductively coupled plasma optical emission spectrometer.

1.3.2. Specific objectives

- ✓ To determine the concentrations of heavy metals(Pb ,Cr, Cd, Fe, Zn, Hg, Cu) in lentil seeds.
- ✓ To compare the concentration of the heavy metals with the standard limits of WHO/FAO.
- ✓ To explain the physico-chemical properties of soil (pH and electrical conductivity) on which lentil grows.

1.3.3. Significance of the study

The main significances of the study were to determine the heavy metals concentration present in lentil seed . High levels of heavy metals in lentil pose a health risk to human. Therefore, the determination of heavy metals would be important,

- ✓ To provide techniques on how to determine heavy metals concentration in lentil seeds,
- ✓ To provide background information to other researchers, who will be interested in similar research work . Since, the finding of this study directly concerned all the Kebele of Angolela and Tera administration, they were primarily benefiters together with Woreda agriculture office and region 3 agriculture bureau. Moreover, the stated problem concerting this farm land was a problem identified at a national level. So the finding, the data presented and the recommendation could be used for further investigation of the problem at a regional or national level.

The essential metals Cu, Fe, Cr, Zn were selected because of their important biological roles in the human body whereas Cd, Pb and Hg being non-essential were included due to their toxic nature

1.3.4. Limitation of the study

During conducting of the study the determining factor was:

- Unavailability of equipped laboratory with sufficient equipment.
- Time to do the experiment due to covide-19

CHAPTER TWO

2. Review of literature

2.1. Description of lentil crop

Food legumes are grain legumes or pulses, and are species of the plant family leguminose their seeds are consumed directly by human. They occupy an important place in global food and nutrition especially, in the dietary pattern of low-income group of people in developing countries. They can also establish a symbiosis with nitrogen-fixing soil bacteria. Turning atmospheric nitrogen in to a biologically usable form(Odogola,1994).Food legumes are grown throughout Ethiopia and account for 13% of cropped land that is concentrated in Amhara and Oromia regions(Rashid et al.,2010).

AS well-known, legumes crop plays an important role in agriculture. Lentil (LENS CUlinaris Medikus), a member of the legume family, were grown globally as seeds for human diet and straw for animal feed. Lentils were probably one of the oldest grain legume crops domesticated in the old world (Sandhu and Singh, 2007).They are a cool season crop with a restricted root system which is only moderately resistant to high temperature and drought (Agriculture and Agri-Food Canada, 2010),therefore, they are mainly grown in the cooler temperature zones of the world or in the winter season in the area such as India and Australia which have warm winter and hot summer(Yadav,2007).

2.2. Importance of lentils

Lentil is annual bushy herb plants with slender stem and many branches ,erect semi-erect or with a spreading growth habit (Sandhu andSingh,2007).The plant height in the range of 15 to 75 cm and there are 10 to 16 leaflets subtended on the rachis: upper leaves have simple tendrils while lower leaves are mucronate ;all leaves are alternate ,compound and pinnate(Muehlbauer et al.,1985).Flowers are self-pollinating and flower stalk produce 1-3 flowers that develop pods.

There are many different types of lentils, concerning seed color, shape or size. The most common types used in cooking are brown, red and green lentils. Brown lentils are mild in flavor and the least expensive generally red lentils have slighter sweeter taste than brown ones and are better for soups and stews. Green lentil are the finest and richest tasting but most expensive. Agriculture and Agri-Food Canada (2010) estimated that about 70% of the world lentil production was the red types, 25% green type and 5% brown and other types. Canada and USA mainly produce the green type, whereas the rest of the world produces the red type lentils.

As a kind of legumes plant crops, lentil contribute to the nitrogen in put on the farm due to the biological nitrogen fixation. The nitrogen fixed by lentils may be used by the following crop which is important especially in organic farming. Prakash et al.(2002) reported that there was a 23.4% increase in rice yields following lentil compared to wheat in India. Based on the result from (Campbell et al.(1992).

2.3. Nutritional use of lentil

The lentil seeds are rich in protein content, carbohydrates and calories (Muehlbauer et al,. 1985) Its seeds are also a good source of several essential minerals such as K, P, Fe, Zn and vitamin B for human nutrition (Bhatty,1988).Lentil seeds are most common used as main dishes, side dishes or as sprouted grain in salads with rice or rotis (Sandhu and Singh,2007).Its flour mixed with cereal crops for making breads and cakes(Williams and Singh,1988).Lentil plant residues such as leaves ,stems, husk and podwall left after threshing are also a good source of livestock feed.

2.4. Definition of metals and their classification

Metals are defined as elements that have a high electrical conductivity and luster which lose their electrons to form cations. metals are found naturally in the earth's crust and their composition vary among different localities, resulting in spatial variations of the surrounding concentration ,the metal distribution in the atmosphere is mentored by the properties of the given metal and by the various environmental factors (Khlifi and Hamza-Chaffai, 2010). Although metals are naturally occurring elements that are found throughout the earth's crust, most environmental contamination and human exposure result from anthropogenic activities such as mining and smelting operations, industrial production and use, domestic and agricultural use of metals and metal containing compounds (Shallari S, Schewartz C, Hsko A, Morel JL, 1998).

Roane and Pepper (2000) have classified metals into three categories on the basis of their biological functions and effects: -

1. The essential metals with known biological functions [Na, K, Mg, Ca , V, Mn, Fe, Co, Ni, Cu, Zn, Mo, and W]

2. The toxic metals [viz., Ag, Cd, Sn, Hg, Ti, Pb, Al] and metalloids [Ge, As, and Se] and

3. The non-essential, non-toxic metal with no known biological functions [viz., Rb, Cs, Sr and Ti]. These metals are sometimes found in accumulated forms in the cells as a result of non-specific sequestration and transport (Gadd, 1988).

2.5. Occurrence of heavy metals

In most terrestrial ecosystems, there are two main natural sources of heavy metals; the underlying parent material and atmosphere. The concentration of metals in soils depends on the weathering of the bed rock and on the atmospheric input of heavy metals. However, in recent times, the atmospheric activities, like mining, ore refining, combustion of fossil fuels, metal working industries, battery manufacturing, paints, preservatives, insecticides and phosphate fertilizers. etc. have led to the emission of heavy metals and the accumulation of these compounds in ecosystems (Weast, 1984; Gold bold and Muttermann, 1985; Nis, 1999) causing serious threat to the environment. The main sources of essential and nonessential metals to vegetable crops are their growth media (soil, air, nutrient solutions) from which these are taken up by the roots or foliage. Vegetables take up metals by absorbing them from contaminated soils, as well as from deposits on different parts of the vegetables exposed to the air from polluted environment (Umoru PE2013). Mineral elements play critical role in building body tissue and regulating numerous physiological processes. They are thus essential constituents of enzymes and hormones; regulate a variety of physiological processes (e.g., osmotic pressure maintenance, oxygen transport, muscle contraction and central nervous system integrity) and are required for the growth and maintenance of tissues and bone (RetaBKandChandravanshiBS2012).

2.6. Heavy metal concentration in plants

Due to modern agricultural practices and expansion of urbanization using insecticides, fertilizers and herbicide chemicals like heavy metals and essential metal may enter in the water supply, soil from the consumer wastes as the results of this lentil seed will absorb water from the soil that contaminated with heavy metals also contaminate the seed.

The heavy metals may be introduced in to soil and contaminated agricultural resources and deliberate addition of metal producing fertilizers (Street 2012). Several researches shown that heavy metal such as Cd, Hg and Pb affect human health chronic exposure to heavy metals like lead retard growth in children and dead to a series of kidney malfunction, anaemia, haematological and brain damage (schcwartz et al., 1986). Essential trace elements play a great role to prevent and fighting disease

Living organisms (plants, animals and microorganisms), store and transport metallic elements, as both to provide appropriate concentrations of them for later use in metallo-proteins or cofactors and to protect themselves against the toxic effects of metal excess. Growth media including soil, nutrient solution, water and air are main sources of heavy metals to vegetables and other crops, which enter by roots or foliages through two main bio-sorption mechanisms: adsorption and/or absorption and accumulated in their tissues (Adeyeye, 2005, Abdullahi et al., 2008).

Different vegetable species accumulate different metals depending on environmental conditions, metal species and plant, available forms of the heavy metals. Many plants are found to be in a position to take up large quantities of certain elements from the environment and said hyper-accumulators of heavy metals (Olajire and Ayodele, 2003). These plants have been on use even as phytoremediation, removing or transforming contaminants through metabolic processes (Chamley, 2003). Metal uptake by plants can be affected by several factors including metal concentrations in soils, soil pH, cation exchange capacity, organic matter content, types and varieties of plants, and age of the plant (Jung, 2008). The chemical forms of metals in which they enter the ecosystems and their final forms of existence greatly affect mobility, bio-availability, storage, retention and toxicity of the metals in living organisms, food and the environment depend on (Mocko and Wacllawek, 2004).

2.7. Effect of heavy metals on soil and lentil seed germination

Pollution of the biosphere by metals due to industrial, agricultural and domestic activities has created a serious problem for the safe and rational utilization of soils (srivastava et al, 2005; Avery, 2001; Igwe et al., 2005; kandoriet al., 1993; volesky, 1990). Industrial imputes and the agronomic applications of fertilizers, pesticides and heavy metal contaminated sewage continue to contribute the heavy metal accumulation in the soil (Herald et al, 2000). The pollution of the ecosystem by metals is real threat to the environment because heavy metals can’t be naturally degraded like organic pollutants and persist in the ecosystem having accumulated in different parts of the food chain (Igwe, et al., 2005; smejkalova et al., 2003).

Germination of seeds was favoured with increasing concentrations of Zinc (50 mM), which indicates the essentiality of Zinc to plants. More deleterious effects on the germination of lentil were observed in case of silver while moderate toxicity was observed in case of lead (Pb).Materials that find their entry in to the soil system persist and accumulate in toxic levels, hence becoming sources of pollution in the soil (Misra and Mani, 2009).The concentration of metals in soil and their impact on ecosystems can be influenced by many factors such as the parent rock, climate and anthropogenic activities (Jail, *et al.*, 2.4.5).

The impact of modern technology upon agriculture has been as great as other industries, leading to increase in range of new agricultural practices involving modern farm equipments, arable land acreage increase, irrigation with various source of water from fresh water to sewage and sludge as well as intense use of agrochemicals to support the ever-growing world population and parallel rise in human aspiration and demand ‘per capita’.

However, agrochemicals like chemical fertilizers, insecticides, fungicides, herbicides, plant growth regulators, soil-and post-harvest fumigants etc increases soil and water contamination with heavy metals and other organic pollutants. Fertilizers frequently contain trace amounts of arsenic and heavy metals so; their repeated use may cause toxicity of soils (Chamley, 2003). Fertilizers are among the sources of heavy metal input into agricultural systems. On average, phosphate rock, Malak et al., (2007), contains 11, 25, 188, 32, 10, and 239 mg kg-1 of As, Cd, Cr, Cu, Pb and Zn, respectively.

Soil is an environmental, biochemical reaction system with three important phases: solid (i.e. mineral particles, organic debris, plant roots), solution (i.e. groundwater, rain water, biological excreta, products of biochemical reactions), and gas (i.e. atmospheric, products of biochemical reactions) which move towards equilibrium with one another. Agricultural soil is mentioned to be the most important sink for heavy metals due to soils’ high metal retention capacities (Tokaliog et al., 2006).

2.8. Mineral Nutrients in the Soil

Soil is a heterogeneous material which may be considered as consisting of three major components: a solid phase, a liquid phase and a gaseous phase. All three phases specifically influence the supply of plant roots with nutrients. The solid phase may be regarded as the main nutrient reservoir. The inorganic particles of the solid phase contain cationic nutrients such as K, Na, Ca, Mg, Fe, Mn, Zn, and Co whilst the organic particles of this phase provide the main reserve of N and to a lesser extent also P and S. Colloidal soil particles are mostly negatively charged. The negative charge on the clay mineral surfaces arises largely because of isomorphous replacement of cations in the crystalline lattices where trivalent cations are substituted by divalent cations. The negatively charged surfaces of these various soil particles attract cations such as Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} as well as $Al^{3+}$and Mn^{2+} [Mengel, K.and Kirkby, E. A. 1978].

Plant growth involves the interaction of soil and plant properties. Soil is the normal medium for plant root growth. The plant's roots absorb nutrients and water from the soil and are an anchor to support the shoot. Maximum plant growth depends on the soil having the biological, chemical and physical conditions necessary for the root system to maximize the plant's required absorption of nutrients and water and to enable the biochemical reactions that occur in the root. The plants rate of absorption of nutrients involves processes going on in both the plant's root and the soil. Each of these processes is important in providing nutrients for use by the shoot [Barber, S. A. 1984].

Soil properties are greatly influenced by its pH. Soil pH values can differ widely from values of about 3 to as high as 10, being very low in in acid sulphate and podzolic soils and being rather high in calcareous and alkali soils. In alkali soils in particular very high pH values may occur as the soil solution contains weak acids (HCO_3^-) and strong bases (Na^+ or K^+). The H^+ concentration of the soil solution has a pronounced effect on a number of soil constituents and especially on the soil minerals, soil microorganisms and plant roots. High H^+ concentration favour the weathering of minerals resulting in a release of various ions such as K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Cu^{2+}, and Al^{3+} (Mengel, K.and Kirkby, E. A. 1978).

The soil organic matter, another important soil property, is the organic fraction derived from living organisms. It includes the living organisms, partly decomposed and decomposed plant and animal residue. The decomposed organic fraction is usually called humus (Tan, K. H. 1996).

Organic matter binds mineral particles in to a granular soil structure that is largely responsible for the loose, easily managed condition of productive soils. It is a major source of plant nutrients. As soil organic matter decays, the nutrient elements, which are present in organic combinations, are released as soluble ions that can be taken up by plant roots (Brady, N. C.; Weil, R. R. 2002).

Nutrients in the soil can be transported by two different mechanisms: by mass flow and by diffusion. Mass flow occurs when solutes are transported with the convective flow of water from the soil to plant roots. The amount of nutrients reaching the root is thus dependent on the rate of water flow or the water consumption of the plant and the average nutrient concentration of the water. The level of a particular nutrient around the root may be increased, decreased or remain the same depending on the balance between the rate of its supply to the root by mass flow and the rate of uptake by the root. Diffusion occurs when an ion is transported from a higher to a lower concentration by random thermal motion. Diffusion comes into effect when the concentration at the root surface is either higher or lower than that of the surrounding solution. It is directed towards the root when the concentration at the root surface is decreased and away from the root when it is increased (Mengel, K.and Kirkby, E. A. 1978).

Lead

Lead (Pb) is a well-known neurotoxin. Impairment of neurodevelopment in children is the most critical effect of lead poisoning. Exposure in the uterus, during breastfeeding and in early childhood may all be responsible for the effect. Lead accumulates in the skeleton and its transfer from bones during pregnancy and lactation causes exposure to feta’s and breast fed infants (ATSDR, 2007). Chronic exposure to Pb can affect physical growth and cause anemia, kidney damage, headache, hearing problems, speaking problems, fatigue or irritable mood (Simeonov *et al.*, 2010). The toxicity by Pb has multiple biochemical effects. It has the ability to inactivate enzymes, compete with calcium for incorporation into bones and interfere with nerve transmission and brain development (Ediin et al., 2000). It has been suggested that lead on a cellular and molecular level may permit or enhance carcinogenic events involved in DNA damage, DNA repair and regulation of tumor suppressor and promoter genes (Silbergeld, 2003). The common sources of lead are car batteries, tyre materials, coals, plastics and insecticides. The high level of Pb in soil could be attributed to Pb from car exhaust fumes, derived from leaded petrol (Alloway, 1996). The main sources of Pb in the environment include, dust from leaded paints of older houses, leaded gasoline and tap water from soldered pipes (Ediin, et al., 2000). The maximum allowable limits of lead in the soil in UK and USA are 100 mg/kg and 200mg/kg respectively (Mamtaz and Chowdhury, 2006), while it is 0.05 mg/kg in Nigeria. Lead is a naturally occurring heavy metal. It is seldom found in its elemental form; however, it is part of several ores including its own (galena, PbS). Pb is also a product of the radioactive decay of uranium206, thorium208, and actinium207. Pb has many industrial and commercial uses. It is used in the production of ammunition, as solder, in ceramic glass, and the production of batteries (ATSDR, 1999b).

Other sources of Pb in the environment include automobile exhaust, industrial wastewater, wastewater sludge, and pesticides (Balba et al., 1991). Because of its high toxicity, the use of lead in some products has been discontinued. Lead is no longer used in house paint because of the concern about the toxic effects of the accidental ingestion of paint chips or the inhalation of aerosolized lead from decaying paint. Most of the environmental lead contamination comes either from landfill leachate or from airborne lead particles deposited onto the soil (ATSDR, 1999b).

Pb behavior in soil is similar to Cd behavior in soil. However, (Khan and Frankland, 1983) showed that Pb was less mobile in soil than Cd. Very little of either Pb or Cd was leached through the soil profile. In fact, more Pb and Cd were removed from the soil by plants than was leached through the profile (Khan and Frankland, 1983). Several factors may influence the content and distribution of heavy metals in soil. Some of these factors are parent material, organic matter, particle size distribution, drainage, pH, type of vegetation, amount of vegetation, and aerosol deposition (Siegel, 2002).

Heavy metals, including Pb, tend to accumulate in the clay fraction of the soil profile (Boon and Soltanpour, (Lee et al., 1997). Strong ionic bonds are formed between the cation and the clay particle. Acidic conditions will cause desorption of these cations into solution making them available for uptake by plants. Desorption to the soil solution also increase cation mobility through the profile (John and VanLaerhoven, 1972; Peles et al., Siegel, 2002). Decreased growth and yield have been observed in plants grown in Pb contaminated soils and plant. Balba et al., (1991) showed a significant decrease in plant biomass yield with increasing Pb treatments that varied with soil type. The highest adverse effects were on those plants grown in soils with high clay content.

Toxicity of Pb: Ninety-nine percent (99%) of the lead that enters the adult human body and 33% that enters a child's body is excreted in about 2 weeks (ATSDR, 1999b). Because of this, lead poisoning is a greater concern in children. Most of the accumulated lead is sequestered in the bones and teeth. This causes brittle bones and weakness in the wrists and fingers. Lead that is stored in bones can reenter the blood stream during periods of increased bone mineral recycling (i.e., pregnancy, lactation, menopause, advancing age, etc.). Mobilized lead can be deposited in the soft tissues of the body and can cause musculoskeletal, renal, ocular, immunological, neurological, reproductive, and developmental effects (Todd et al., 1996; ATSDR, 1999b).

Renal toxicity is now used as a biochemical and physiologic marker of chronic subclinical lead toxicity (Todd et al., 1996). Lead can also be transmitted through breast milk. Anemia, colic, impaired vitamin D metabolism, and growth retardation result from lead exposure during infancy or early childhood.

Lead exposure is also associated with several neurological effects, such as delayed neurological development, cognitive impairment, and effects on general brain function and it is carcinogenic. Some of these effects are irreversible and continue into adulthood (ATSDR, 1999b).

Lead is known as toxic element. The toxicity effect includes impairment of mental activity, reproductive and development problems, disorder in bone formation and renal function.

Zinc

Zinc can be found in nearly all soils. It is present in most rocks and is weathered out and deposited into the soil. Zinc is also released by thermal outgassing and other volcanic events. Fallout from such events can be a significant source of zinc in soils and plants. Anthropogenic release is the primary source of zinc in the environment.

Zinc is released from industrial and manufacturing facilities in wastewater effluent or from incinerators. Zinc is used as a constituent in several alloys, including brass, bronze, die-cast metals, and is combined with copper for the production of US pennies. Zinc is also used in electroplating, smelting, and ore processing (ATSDR, 1994). Mine tailings and drainage from mines can contain high concentrations of zinc.

Toxicity of Zn: zinc is the less toxic and an essential element in the human diet because it is required to maintain the proper functions of the immune system. It is also important for normal brain activity and is fundamental in the growth and development of the fetus. Zinc deficiency in the diet may be more detrimental to human health than too much zinc in the diet (ATSDR, 1994).

Although the average daily intake of zinc in the United States is 7-16.3 mg Zn/day, the Recommended Daily Allowance (RDA) for zinc is 15 mg Zn/day for men and 12 mg Zn/day for women (ATSDR, 1994). To compensate for Zinc deficiency some people use Zinc supplements.

If doses 10-15 times higher than the RDA are taken over a long period, anemia and damage to the pancreas and kidney can develop. Vomiting, diarrhea, abdominal cramping, and, in some cases, intestinal hemorrhage can occur from long-term exposure to high (i.e., >85 mg/kg/day) doses of zinc. Excessive dietary exposure of zinc can cause gastrointestinal distress, pancreatic damage and anemia , reduction of copper absorption and depressed immune function

Copper

Copper is the third most used metal in the world. Copper is an essential micronutrient required in the growth of both plants and animals. In humans, it helps in the production of blood hemoglobin. In plants, Cu is especially important in seed production, disease resistance and regulation of water. Copper is indeed essential, but in high doses it can cause anemia, liver, kidney damage and stomach and intestinal irritation. Copper normally occurs in drinking water from Cu pipes, as well as from additives designed to control algal growth. While Cu's interaction with the environment is complex, research shows that most Cu introduced into the environment is, or rapidly becomes, stable and results in a form which does not pose a risk to the environment. In fact, unlike some man-made materials, Cu is not magnified in the body or bio accumulated in the food chain.

In the soil, Cu strongly complexes to the organic implying that only a small fraction of copper will be found in solution as ionic copper, Cu(II). The solubility of Cu is drastically increased at pH 5.5 (Ewers.1991), which is rather close to the ideal farmland pH of 6.0– 6.5.

Copper and Zn are two important essential elements for plants, microorganisms, animals and humans. The connection between soil and water contamination and metal uptake by plants is determined by many chemical and physical soil factors as well as the physiological properties of the crops. Soils contaminated with trace metals may pose both direct and indirect threats: direct, through negative effects of metals on crop growth and yield and indirect, by entering the human food chain with a potentially negative impact on human health. Even a reduction of crop yield by a few percent could lead to a significant long-term loss in production and income. Some food importers are now specifying acceptable maximum contents of metals in food, which might limit the possibility for the farmers to export their contaminated crops (EPA, 2009).

Copper is required with iron for synthesis of hemoglobin it works with many enzymes such as those involved in protein metabolism and hormone synthesis deficiency of copper cause anemia, low white blood cell count and poor growth. excess intake of copper can cause vomiting, nervous system disorder and Wilson disease.

Cadmium

Cadmium is highly toxic metal and present in low concentration in nature. The toxicity of cadmium results lung damage, hypertension, hepatic, injury reproductive toxicity and bone effect in human body (Robards and Wars Fold, 1991).The level of cadmium in environment increased by human activity like urban and industrial waste disposals to soil and water. The permissible limit of Cd set by FAO (1984) IS 0.3mg/kg.

Exposures potentially of particular concern for children include disposal and recycling of electronic and electrical waste, as well as toys, jewelry and plastic containing cadmium. Cadmium can travel long distances from the source of emission by atmospheric transfer. It is readily accumulated in many organisms, notably mollusks and crustaceans. Lower concentrations are found in vegetables, cereals and starchy roots. Human exposure occurs mainly from consumption of contaminated food, active and passive inhalation of tobacco smoke, and inhalation by workers in a range of industries. Cadmium is a naturally occurring element, it is rarely found as a pure metal in nature. It is generally associated with oxygen, chlorides, sulfates, and sulfides. Cadmium is often a byproduct of the extraction of Pb, Zn, and Cu from their respective ores (ATSDR, 1999a). Carbonaceous shale, coal, and other fossil fuels are also sources of Cd. Volcanism is the largest natural source of Cd (ATSDR, 1999a). Anthropogenic sources of Cd in the soil and groundwater include the use of commercially available fertilizers and the disposal of sewage sludge as soil amendments (Peles et al., 1998; Gallardo-Lara et al., 1999).

Cadmium can accumulate in high concentrations in soils. John et al., (1972) report a Cd concentration of 95 ppm in a sample collected near a battery smelter near Vancouver, BC, Canada. Cadmium is recalcitrant in the soil profile, particularly in the surface horizons (John et al., 1972; Khan and Frankland, 1983). Most soil profiles have a A horizon, which is primarily topsoil composed of decaying organic matter such as leaves and grass, and a B horizon, which is composed of smaller clay-sized particles. In general, heavy metal concentrations are higher in the B horizons than in the A horizons (Lee et al., 1997).

Heavy metals tend to accumulate in the clay fraction of most soil profiles (Boon et al., 1992; Lee et al., 1997). Boon et al., (1992) concluded that the concentration of heavy metals in soil is dependent on clay content because clay-sized particles have a large number of ionic binding sites due to the higher amount of surface area. These results in the immobilization of heavy metals, and there is very little leaching through the soil profile (Khan and Frankland, 1983). Immobilization can increase the Cd concentration of the soil and ultimately lead to the increased toxicity of the contaminated soil. Higher soil Cd concentrations can result in higher levels of uptake by plants (John et al., 1972). However, specific soil properties can have a significant effect on the amount of heavy metal assimilated by the plant (John and VanLaerhoven, 1972; Peles et al., 1998).

Increased levels of Ca^{2+} can decrease the amount of Cd that is assimilated by plants (Larlson et al., 2000). Because of their similar size, Ca (II) is almost indistinguishable from Cd (II) A higher affinity for the essential trace metal Ca results in the decreased uptake of Cd into the plant. A similar relationship exists between P and Cd. John et al., (1972) showed that the addition of 1000 ppm of phosphorus to a Cd contaminated soil decreased the concentration of Cd 43% in the roots of oats. Trace metal deficiencies in plants have been associated with increases in heavy metal uptake.

Soil pH significantly influences heavy metal concentrations in both soil and plant tissues. The effect of soil pH on mobility of heavy metals is a well-researched topic (Peles et al., 1998). As the soil pH decreases, metals are desorbed from organic and clay particles enter the soil solution and, become more. When the pH is higher (i.e., >7), metals remain adsorbed and what metals in solution precipitate out in the form of salts. Variability in pH also affects the amount of Cd assimilated by the plant. John and VanLaerhoven, (1972), showed that higher pH resulted in lower Cd uptake. During the addition of lime to contaminated soils (essentially increasing the pH) decreased the uptake of heavy metals.

Khan and Frankland, (1983) reported that extremely high concentrations (180 µg g-1) of Cd in soil adversely affected plant development. In their research, radish plants were grown on soils contaminated with Cd and Pb. Within 3 weeks of planting, all plants that were grown in soil contaminated with 1000 µg Cd g-1 were dead. The concentrations of Cd in the soil that produced a 50% inhibition in growth were higher at the seedling stage than at the edible stage.

John et al., (1972), also reported that plant size and yield were reduced when 50 mg Cd (dosed as CdCl2) was added to 500g of soil. In both studies, chlorosis of the leaves was reported. Khan and Frankland, 1983), suggest additive effects from the application of Cd and Pb at the same time.

They document a considerable reduction in growth when Cd was added at 50 µg g-1 and Pb was added at 1000 µg g-1 (Khan and Frankland, 1983).

Toxicity of Cd: The Agency for Toxic Substances and Disease Registry (ATSDR) reports that the average American ingests about 30 µg Cd/day (ATSDR, 1999a). However, only about one tenth of this amount is actually absorbed into the tissues. Intake of Cd can double if one smokes cigarettes because each cigarette contains about 2 µg Cd. Acute doses (10-30 mg/kg-day) of cadmium can cause severe gastrointestinal irritation, vomiting, diarrhea, and excessive salivation, and doses of 25 mg CdI_2/kg body weight can cause death.

Low-level chronic exposure to Cd can cause adverse health effects including gastrointestinal, haematological, musculoskeletal, renal, neurological, and reproductive effects. The main target organ for Cd following chronic oral exposure is the kidney (ATSDR, 1999a). Because cadmium tends to accumulate in the kidneys. The highest Cd level in the renal cortex that does not cause significant proteinuria is 200 µg Cd/g (EPA, 1994; ATSDR, 1999a). A toxic kinetic model was used to determine the no-observable-adverse-effect-level (NOAEL) dose that would result in a renal cortex concentration of 200 µg Cd/g. To use the model, it was assumed that 0.01% of the daily Cd body burden is excreted in the urine or faces and that 2.5% of the Cd in food and 5% of the Cd in water are actually absorbed into the body tissues. The ATSDR concludes that there is insufficient evidence to determine whether oral exposure to Cd increases the risk for cancer. However, the United States Department of Health and Human Services (DHHS) has stated that cadmium compounds may be carcinogenic (ATSDR, 1999a).

The International Agency for the Research on Cancer (IARC) has classified Cd and Cd salts as possible human carcinogens. This classification is based on human lung cancer data from occupational inhalation.

Cadmium has no known nutritional function, and is highly toxic to humans. The biochemical effects of cadmium include interference with enzymatic activity, damaging kidney ,hypertension and anosmia(absence of smell).

Iron.

Iron is essential in oxygen supply as a component of hemoglobin and for oxygen storage as a component of myoglobin cytochromes it is a cofactor for some enzymes and used for immune function iron deficiency can also decrease learning ability of children iron toxicity usually result from ageneric disorder called hemochromatosis. this disease causes over absorption and accumulation of iron which can result in severe liver and heart damage.. Iron is also a component of enzymes with a functions in the metabolism of energy and protein and in the synthesis of proteins, tissues, hormones and neurotransmitters. Because iron easily react with oxygen, mechanism have involved that tightly limit the uptake of iron and control the reactivity of iron in the body (Domellof M.(2011). Iron requirements in infancy. Ann Nutr Metab.; 59(1):59-63.Iron absorption depends on total iron intake, dietary factors and the iron status of the individual. Bioavailability of iron differs between different types of foods, and is assumed to be about 10% from a mixed diet**.**

Iron is essential for the synthesis of chlorophyll and activates a number of respiratory enzymes in plants. The deficiency of iron results in severe chlorosis of leaves in plants. High level of exposure to iron dust may cause respiratory diseases such as chronic bronchitis and ventilation difficulty. Iron maintains a healthy central nervous system, prevents anemia and interrelated with the function of zinc and iron in the body(Akinyele and Osibanjo,1982).

Mercury

A particular characteristic of mercury is that is exist in the environment in a number of different chemical and physical forms. Each with different behaviour in terms of transport and environmental effects (Schroeder and Munthe, 1998).

Mercury combines with other elements to form organic and inorganic mercury compounds Metallic mercury is used to produced chlorine gas and caustic soda and also used in thermometer.

-The EPA has determined that mercuric chloride and methyl mercury are possible human carcinogen.,2007).

Chromium

The most common forms of chromium are chromium (VI) and chromium (III) (Hilgenkamp, 2006).Although chromium toxicity in the environment is rare. It still presents some risks to human health since chromium can be accumulated on skin, lungs, muscle, fat, in liver, dorsal spin, hair, nails and placenta where it is traceable to various health conditions (Adeleken and Abegunde, 2011). The health effects brought about by the exposure to chromium (VI) include lung cancer, malignant neoplasia, chromium dermatitis and skin ulcers (Sarkar, 2005). Perforations and ulcerations of the nasal septum and bronchial asthma have also been reported. In one of the studies, a four-fold increase in childhood leukemia was attributed to possible consumption of water with chromium (VI) levels above standard recommended value of 300 mg/kg (Sarkar, 2005). The sources of chromium in the environment include, cement, leather, plastics, dyes, textiles, paints, printing ink, cutting oils, photographic materials, detergents, wood preservatives among others (Hilgenkamp, 2006). Other sources of chromium are power plants, liquid fuels, brown and hard coal and industrial and 17 municipal wastes. Non biodegradability of chromium is responsible for its persistence in the environment and on mixed with soil; it undergoes transformation into various mobile forms before ending into environmental sink (Adeleken and Abegunde, 2011). The maximum allowable limit of chromium in the soil set by United Kingdom is 300 mg/kg (EU, 1986) while the limit set by FEPA (1991) is 0.03 mg/kg in Nigeria.

Cr (III) is an essential element required for normal sugar and fat metabolism. It is effective to the management of diabetes and it is a cofactor with insulin. Cr (III) and its compounds are not considered a health hazard, while the toxicity and carcinogenic properties of Cr (VI) have been known for a long time (Kalagbor et al., 2014)

2.9. Heavy metal and contamination pattern

Heavy metal(Pb, Hg and Cd) are metallic element of high molecular masses, most of which belong to the transition element (Silberberg, 2000).There are different types of pollution, but pollution caused by toxic level of heavy metal pollutants is called heavy metal (Bose and Hemantaranjan, 2005). Heavy metal pollution has received the attention of researchers all over the world, mainly due to their harmful effects on living beings (Misra and Mani, 2009). Heavy metals can directly affect the growth of plants; an excess dietary intake of contaminated plants could also be dangerous to the health of humans and animals, the chemical composition of plants reflects almost the elemental composition of the soil.

Adeleken and Abegunde (2011) noted that heavy metals have low environmental mobility as a result of this; a single contamination could set a stage for a long term exposure of heavy metals to human, microbial, fauna, flora and other edaphic communities. The problem of atmospheric heavy metal pollution is not going to disappear overnight. Heavy metal pollution not only affects the production and quality of crops, but is environmentally problematic due their high persistence and toxic effects (Esakku, *et al.*, 2003). Toxic metals comprise a group of harmful minerals that have no known function in the body. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Toxic heavy metals include; lead cadmium, arsenic, mercury, aluminium, antimony, bismuth, barium and uranium. Heavy metals needed in lesser quantities are usually toxic in greater amounts. Toxic heavy metals have the ability to replace vital minerals, for instance Cadmium, which is located just below zinc in the periodic table of the elements and has an atomic structure very similar to that of zinc almost fits perfectly in the zinc binding sites of critical enzymes such as RNA transferase, carboxypeptidase and alcohol dehydrogenase in the body (Wilson, 2011). Again, in diagnostic medical applications, direct injection of gallium during radiological procedures, dosing with chromium in parenteral nutrition mixtures, and the use of lead as a radiation shield around x-ray equipment (Roberts, 1999) proves that toxic metals are not completely harmful as they can extend life by keeping bodies function.

2.10. Physico–chemical Parameters of Soils and Environmental Implications

2.10.1. Soil Quality

Soil is a crucial component of rural and urban environments and in both places land management is the key to soil quality (Chukwulobe and Saheed, 2014). The quality of soil depends on both its physical properties which include color, texture, moisture content, pH, electrical conductivity and chemical properties which include cation exchange capacity, phosphate-phosphorous, sulphate-sulphur, nitrate and nitrite-nitrogen. The physical properties and chemical properties largely determine the suitability of a soil for its planned use and the management requirements to keep it most productive. The behavior and fate of metals are governed by a range of different physico-chemical processes, which dictate their availability and mobility in the soil or sediment system. In the water phase, the chemical form of a metal determines the biological availability and chemical reactivity (sorption/desorption, precipitation/dissolution) towards other components of the system (Osazee *et al*, 2013). The binding form in the solid phase is related to the kinetics and equilibria of metal release to the liquid phase and hence the likelihood of remobilization and bioavailability (Tack *et al*, 1996). Metals and metalloids can be involved in a series of complex chemical and biological interactions. The most important factors which affect their mobility are pH, sorbent nature, presence and concentration of organic and inorganic ligands, including humic and fulvic acids, root exudates and nutrients. Furthermore, redox reactions, both biotic and abiotic, are of great importance in controlling the oxidation state and thus, the mobility and the toxicity of many elements, such as chromium, selenium, cobalt, lead, arsenic, nickel and copper (Tack *et al.*, 1996).

2.11. Method of metal analysis

Several techniques for the determination of heavy metal elements are currently in use. These include flame atomic absorption spectroscopy, inductively coupled plasma atomic emission Spectroscopy (ICP-OES) (Sonayei et al, 2009). Atomic absorption spectroscopy is used in many studies because of its simplicity, reliability and sensitivity (sarkar, 2005). Researchers who have employed atomic absorption spectroscopy in the analysis of metals include: Mamtaz and Chowdhury (2006) in the study of iron, copper, Manganese, lead, cadmium, magnesium, calcium and Zinc levels in urban solid waste. Mico *et al.* (2006) employed atomic absorption spectroscopy method in analysis of Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb and Zn in the agricultural soils.

2.12. Instrumentation

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES):

This technique is used for multi-element analysis of aqueous samples. Quantization is accomplished using multi-element standard solutions from which calibration curves were prepared. These instruments have a wide linear dynamic range (typically 3 to 5 orders of magnitude) that permits the analyst to measure both major and trace elements in the same solution without dilution. The technique has one major disadvantage as it yields very complex spectra with many overlapping lines, which may introduce a bias or increase the uncertainty in the final results. Detection limits range from 10-8 to 10-10 mg/L for approximately 70 elements (Theodore D. M. Method 200.5, 2003**).**Inductively coupled plasma optical emission spectrometer was the instrument used in this study for the determination of heavy metals composition of the sample (lentil seed). ICP-OES is a technique used to identify the presence and concentration of substances.

CHAPTER THREE

3. Method and Materials

3.1. Study Area

The study was conducted at two kebele in Angowelela and Tera district which is located 120 km north of Addis Ababa. The selection of the study area was based on the availability of sample, proximity to the study area, sampling cost. For this research work, raw lentil seeds were collected from the agricultural lands of Angolela and Tera district. Lentil seeds were collected from the field after crop harvested in order to investigate the distribution and concentration of heavy metals (Pb, Cd, Cu, Fe, Hg and Zn, Cr) in the produced lentil seeds . Whole lentil seeds were collected from the farmland of farmers of two different kebeles (Chacha kebele, Chefana kebele) administration representing the food as normally human. The lentil seeds were separated from different unwanted materials (matrices). The seeds had the same size. The representative sample of lentil seed was grounded to fine powder using porcelain mortar and pestle store in plastic bag (zip lock) at room temperature for further experimental use. Sample preparation was carried out in the laboratory of chemistry, Debreberhan University. The concentrations of metal ions were determined by ICP-OES in Debrezeyt, Horticoop Ethiopia PLC Soil and Water Analysis Laboratory.

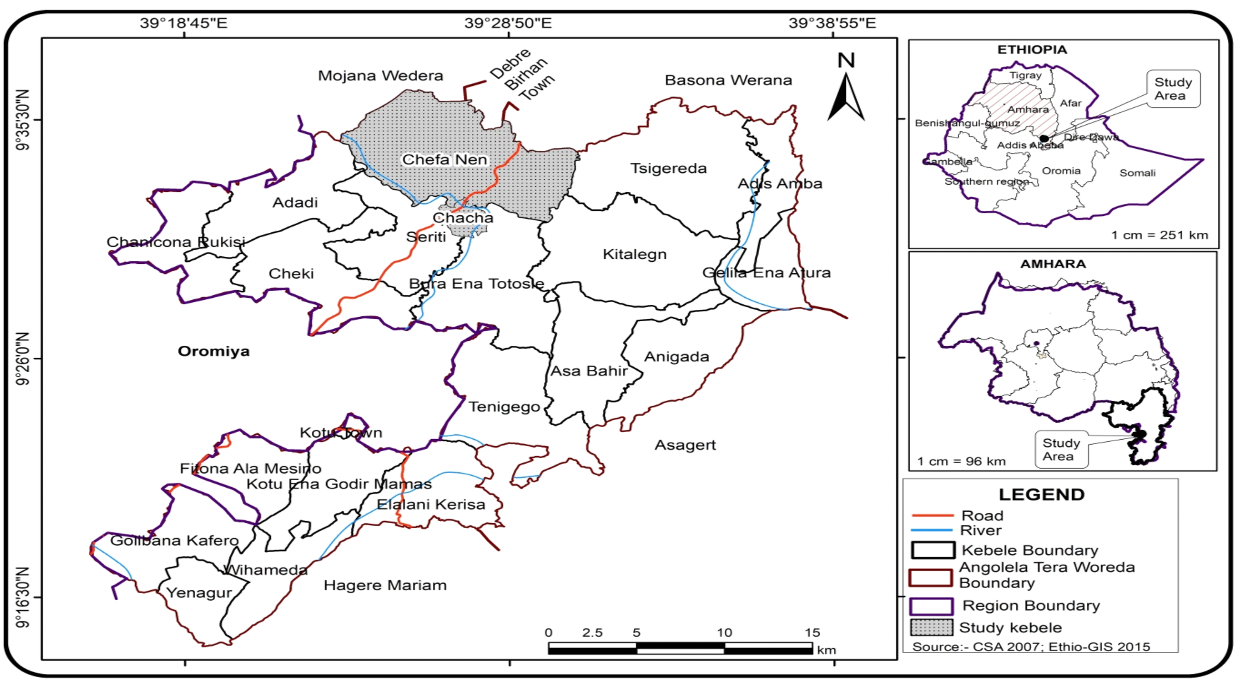

Figure 1 Map of the study area

3.2. Instrument and apparatus

Digital balance, muffle furnace, pH meter, conductivity meter, ICP-OES, air circulating oven and digestion tubes, crucible ,mortar, measuring cylinder ,conical flask, beaker, volumetric flask were used in this study.

3.3. Chemicals and reagents

All the reagents used in the analysis were analytical grade. Distilled water, 69% to 72% HNO_3 or 70% $HClO_4$, HCl (RESEARCH-LAB FINE CHEM INDUSTRIES MUMBAI, INDIA) for the digestion of the selected lentil seeds grains that had taken from the laboratory of chemistry, Debreberhan University.

3.4. Sample preparation

All glass wares, plastics, containers, crucibles, mortars and pestles were washed thoroughly. Glasses were washed with liquid soap, rinsed with distilled water and then soaked in 10% HNO_3 solution for 24 hours (Todorovi *et al.,* 2001). They were washed with distilled water and dried in drying oven at 105 ^{0}C for 5 hours (DIGI THEAT, J.P.SELECTA,S.A.SPAIN). All stock solution was prepared freshly, autoclaved and stored at recommended temperature till further use. During sample preparation, dilution and rinsing apparatus prior to analysis. The lentil seeds were first washed with distilled water several times to remove all the dirty particles. And then the dried lentil sample were crushed and homogenized in to fine powder with a grinding device (MOULINEX,FRANCE) and rinsed in dilute HNO_3.Finally, it stayed in refrigerator at 4 ^{0}C.

3.4.1. Procedures

5g of lentil seeds flour were weighed and transferred to a clean crucible, which was labelled according to the sample and dry- ashing process would be carried out in muffle furnace by stepwise increase of the temperature up to 550 ^{0}C and left to ash at this temperature for 6 hrs. The sample was removed from the furnace and allowed to cool and transferred in to a conical flask. The ash was wetted with water and 2.5 mL of concentrated HNO_3 was added. The conical flask with a glass cover still intact then placed on a warm hot plate to commence wet ashing. The digestion had been performed at a temperature of 90 to 95 ^{0}C for 1hr.The ash had been dissolved in 5 mL of 9.25% HCl and digested again on a hot plate until the white fumes ceased to exist and sample reached to 2 mL. Then 2 mL 70% $HClO_4$ was added to the cooled solution and heating resumed until clear solution was appeared. As all HNO_3 eventually evaporated, fumes of $HClO_4$ appeared; heating maintained until ashing was completed. The $HClO_4$ then removed by evaporation. The residue had been treated with 5 mL of concentrated HCl and the acid was refluxed in the beaker; an equal volume of water was then added with subsequent evaporation to dryness. This refluxing process with concentrated HCl followed by evaporation to dryness repeated. Finally, 1.0 mL concentrated HCl added and the mixture was warmed briefly; then 15 mL of water was added and the solution has been heated for about 15 min. After it was cooled 20 mL of distilled water were added and filtered using Whitman's filter. The filtered sample was diluted up to the mark of 50 mL standard volumetric flask, and transferred to a 50 mL polyethylene storage bottle until analysis. A sample was prepared identically in triplicate. ICP-OES measurement was then made for each individual element along with the appropriate standard solution. The sample was digested in triplicate. (Adapted by Wodaje Addis Tegegne, 2015).

3.5. Measuring of pH and electrical conductivity of soil

The soil pH and electrical conductivity test are best source available to know the nature of soil. The soil on which lentil grows was collected from the farm and taken in polythene bags. Sample was analyzed for pH and electrical conductivity following standard methods. De- ionized water was used for soil analysis. The pH of soil was measured using digital pH meter.

3.5.1. Procedures for measuring pH

The pH of the samples was determined with a handheld Hanna pH meter using the methods described in Kumar *et al*., 2008. Where a 20 g of the dried soil was weighed into a 50 cm^3 beaker, 20 cm^3 of distilled water was then added. The mixture was stirred with a glass rod and Allowed to stand for 30 minutes. A pre calibrated pH meter was inserted into the slurry and the pH was recorded.

3.5.2. Procedures for measuring electrical conductivity

The Electrical conductivity (EC) was determined using the modified method of (Kumar *et al*, 2008), and (Kalra and Maynard,1991); where 25 g of an air-dried soil sample placed in a 250cm^3 beaker, distilled water was added slowly drop by drop over the entire soil surface in a uniform pattern, until the soil appeared wet. A stainless steel spatula was used to form a homogeneous soil paste. The beaker was then covered with a Petri - dish, 50 cm^3 of distilled water was added and shaken for 1 hour. 40 cm^3 of the diluted extract was transferred into 100 cm^3 beaker and the electrode of the conductivity meter inserted to record the electrical conductivity of the soil recorded in µS/cm.

3.6. Preparation of standards and analysis of samples using ICP-OES

All standard stock solutions were 1000 ppm in nitric acid which was imported from inorganic ventures by dilution with 0.5% nitric acid. All standard solutions were prepared in 50 mL graduated tube by filling to their mark volume. So the linear graphs were drawn intensity against their concentrations. A mixture of working standard solution of heavy metals (Pb, Zn, Hg, Cu ,Fe, Cr, Cd) was prepared from the stock solutions containing 1000 ppm. Calibration curves were done for each element.

Figure-2 lentil plant showing the seeds.

Figure 3 Hot plate digestions.

3.7. Statistical (data) analysis

All statistical analyses were performed on hp pc computer using the Microsoft EXCEL(version 2010).Analysis of test($P<0.05$) were employed to examine statistical significance of difference in the mean concentration of heavy metals between lentil seed using statistical package for social sciences(SPSS) program. All treatments in the experiment were organized and analyzed in convenient way to make meaningful and readable.

Correlation can be also strongly correlated, if $r > 0.7$, whereas r values between 0.5 and 0.7 show moderate correlation between two different parameters (Sharma and Raju, 2013).

Positive and highly significant correlation ($r = 0.99^{*}$), ($r = 0.99^{*}$) between heavy metal lead with cadmium and iron with copper respectively for Chacha. Similarily positive significant correlation($r=0.99^{*}$) between heavy metal copper with zinc for Chefanen. However, the degree to which two securities are negatively or positively correlated might vary over time and are almost never exactly correlated, all the time. This condition may happen sometimes because one physicochemical property may affect other and it may make to don’t have consistent.

3.8. Method validation

Validation was concerned with assuring that a measurement process produced valid measurements. Results from method validation were used to judge the quality, reliability and consistency of analytical results. It was an integral part of any good analytical practice. The following typical validations characteristics were considered in the study accuracy, precision, detection limit, quantification limit, linearity and range.

Table 1 Instrumental operating condition for determination of heavy metals using ICP-OES from lentil seeds.

Heavy metals	Wave Length (nm)	IDL (mg/L)	Range (mg/L)	Lamp Current (mA)	Temperature (^{o} C)	Plasma Power (W)	Main Argon Pressure (bar)	Instrument model
Lead(Pb)	220.353	0.0007	0.0007-1.68	607	51.53	1400	6.75	ARCOS12
Copper	324.75	0.006	0.006-3.36	607	51.53	1400	6.75	ARCOS12
Zinc	213.856	0.008	0.008-3.36	607	51.53	1400	6.75	ARCOS12
Mercury	184.950	0.003	0.003-3.84	607	51.53	1400	6.75	ARCOS12
Cadmium	214.438	0.0001	0.0001-1.68	607	51.53	1400	6.75	ARCOS12
Iron	259.941	0.0018	0.0018-4.8	607	51.53	1400	6.75	ARCOS12
Chromium	267.71	0.001	0.001-1.68	607	51.53	1400	6.75	ARCOS12

CHAPTER FOUR

4.1. Result and Discussion

4.2. Instrument calibration

The qualities of results obtained for essential micronutrients (Cu, Zn, , Fe, Cr) and toxic heavy metals(Pb, Hg, and CD) by ICP-OES were seriously affected by calibration and standard solution preparation procedures.

Table-2 series of working standard and correlation coefficient of the calibration curves for the determination of heavy metals in lentil seeds using ICP-OES.

Heavy metals	Concentration Standards(mg/L)	Correlation coefficient (R) of calibration curves
Lead(Pb)	0,0.028,0.056,0.086,0.28, 0.56,0.84,1.12,1.4	0.9908
Zinc(Zn)	0,0.056,0.112,0.168,0.56, 1.12,1.68,2.24,2.8	0.9988
Cadmium (Cd)	0,0.028,0.056,0.084 0.28,0.56,0.84,1.12,1.4	0.9996
Mercury(Hg)	0,0.064,0.128,0.192,0.64 1.28,1.92,2.56,3.2	0.9993
Copper(Cu)	0,0.056,0.112,0.168,0.56 1.12,1.68,2.24,2.8	0.9998
Iron(Fe)	0,0.056,0.112,0.168,0.56 1.12,1.68,2.24,2.8	0.9998
Chromium(Cr)	0,0.028,0.056,0..084,0.28 0.56,0.84,1.12,1.4	0.9998

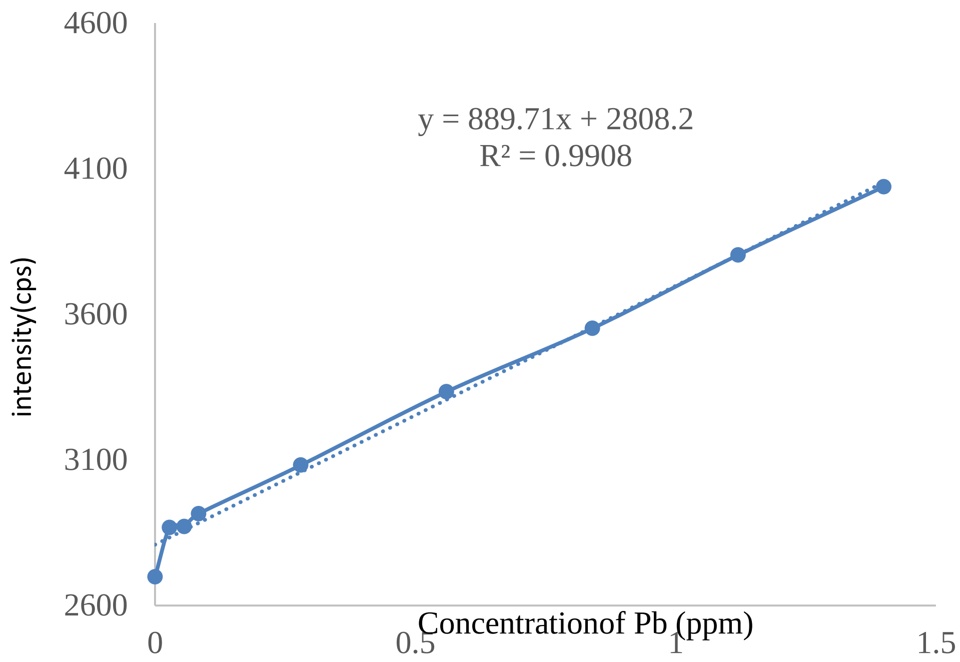

Figure-4 Calibration curve for lead

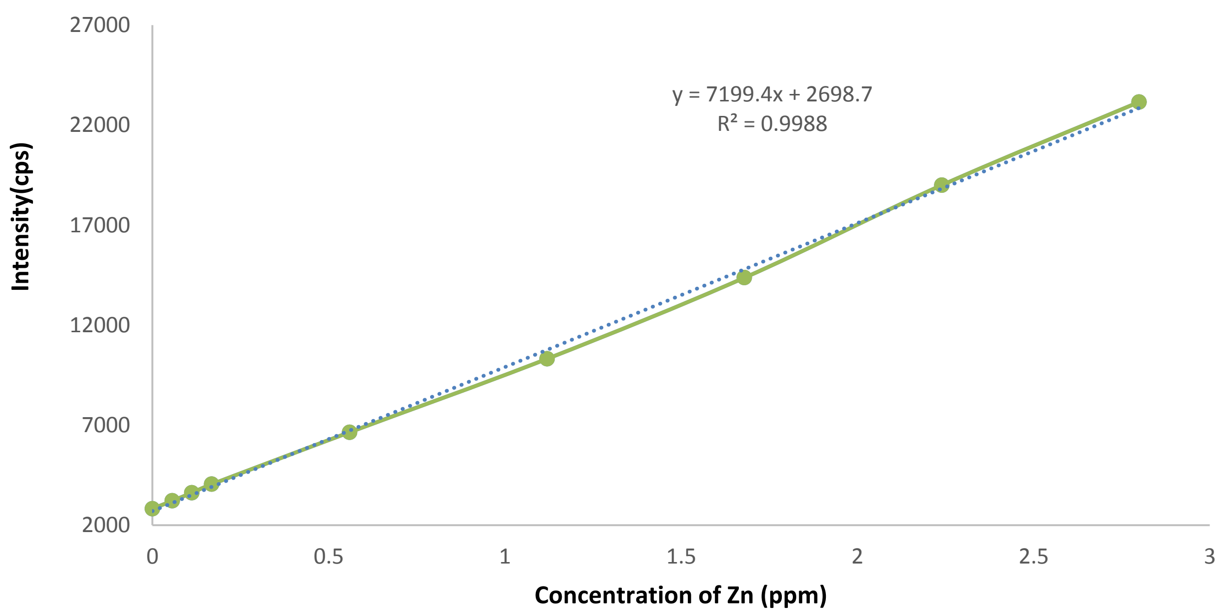

Figure-5 Calibration curve for zinc

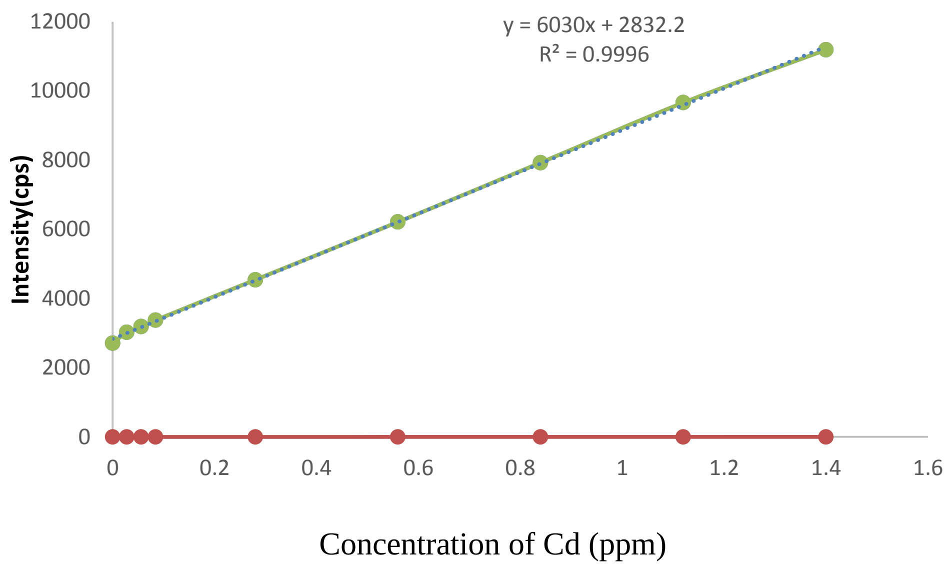

Figure-6 Calibration curve for cadmium

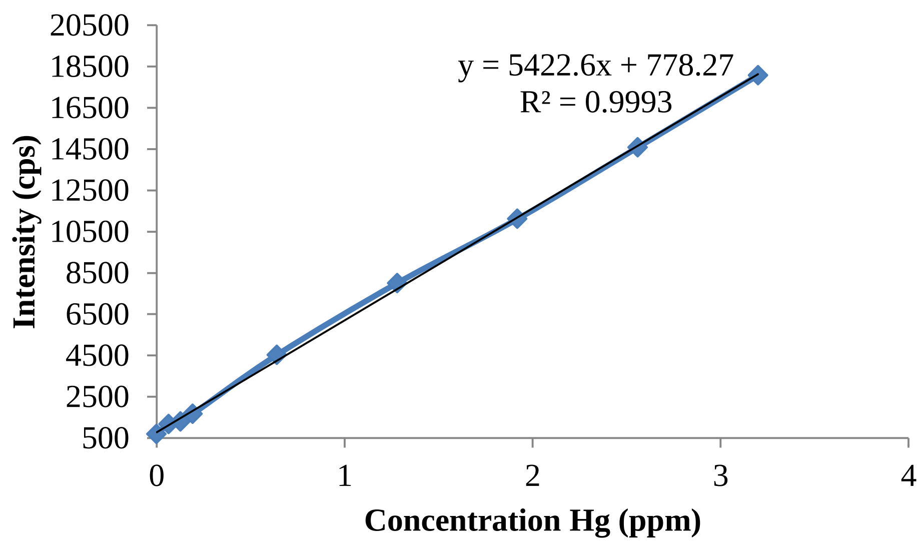

Figure-7 Calibration curve for mercury

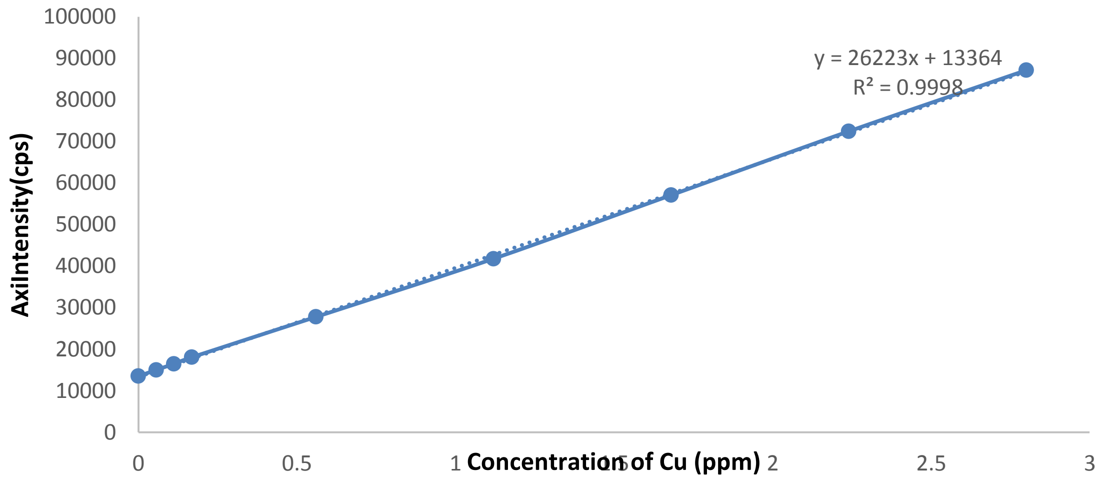

Figure-8 Calibration curve for copper

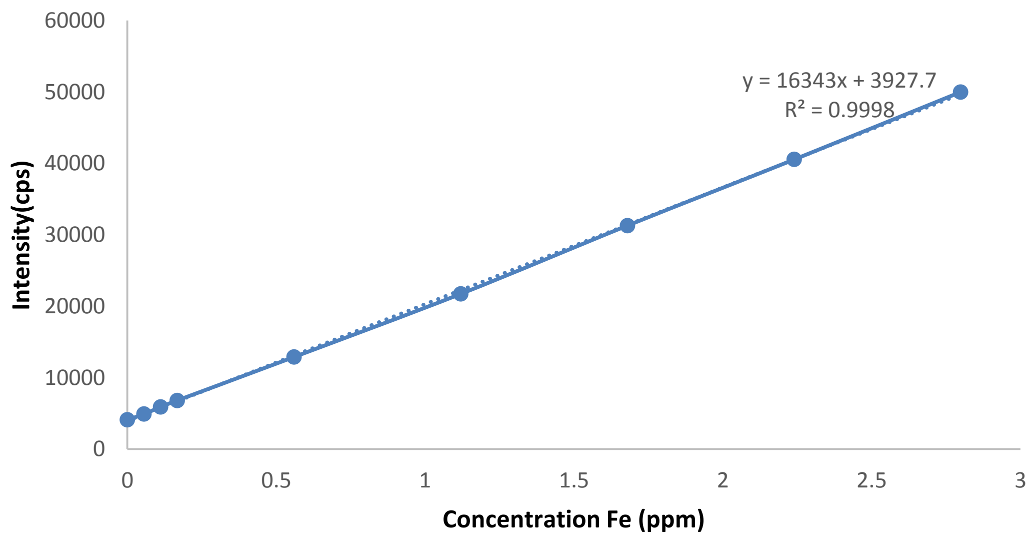

Figure-9 Calibration curve for iron

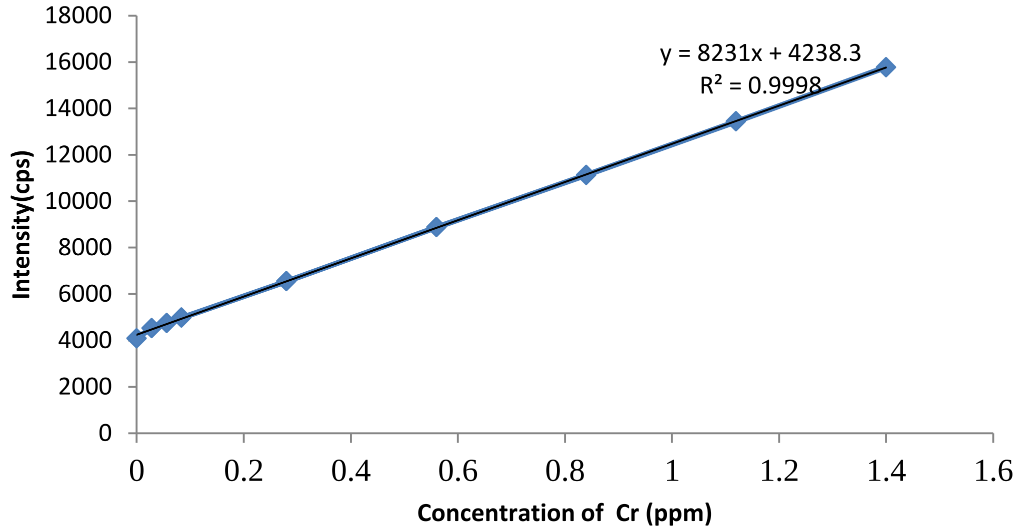

Figure-10 Calibration curve for chromium

The slope of the graphs in figure 4-10 showed that concentration and intensity have direct relationship.

The concentration of target analyte were measured in triplicate.

Table 3 ***the concentration of heavy metals in lentil seeds determined by ICP-OES***.

Study area	Description	Pb mg/kg	Zn mg/kg	Cd mg/kg	Hg mg/kg	Cu mg/kg	Fe mg/kg	Cr mg/kg
Chacha kebele	Mean ± SD	1.1348 ±0.2952	4.5793 ±2.2611	0.1515 ±0.0311	0.5945 ±0.0440	0.7615 ±0.3763	4.8313 ±3.4866	0.2882 ±0.1197
Chefanen kebele	Mean ± SD	1.009± 0.2246	4.439± 3.0499	0.1367 ±0.0369	0.7493 ±0.2896	0.662± 0.4809	2.622± 1.9242	0.295± 0.1600

As shown in the above table the concentration of Pb, Zn, Hg, Cu, Fe, Cr, Cd in each trial agree with each other. The standard deviations for each metal were low which indicates the precision is high. This confirmed that the precision is good because each measurement in each metal close to the average of the series. However, the precision in zinc and iron was highest compared with the precisions in other heavy metal determined. Therefore the measurement in iron and zinc was the most precise.

Table 4. Concentration of heavy metals compared with the recommended concentration by WHO/FAO in lentil seeds at Chacha kebele.

Heavy metals	Concentration determined Sample in mg/kg at Chacha kebele	Concentration Recommended by WHO/FAO in mg/kg
Lead(Pb)	1.1348	1.5
Zinc(Zn)	4.5793	100
Cadmium(Cd)	0.1515	0.1
Mercury(Hg)	0.5945	0.1
Copper(Cu)	0.7613	10
Iron(Fe)	4.8313	425
Chromium(Cr)	o.2882	1.3

Lead

Table 4 had been shown that the comparison between the experimental value and the standard value of WHO/FAO. The concentration of lead in lentil seed was 1.1348 mg/kg ±0.2952.

The permissible limit according to WHO/FAO standard (1996) is 1.5 mg/kg. the concentration of lead in the sample was slightly lowered compared with lead standard in WHO/FAO.

This confirmed that the amount of lead in lentil seed was in low. This experimental result showed that the concentration of lead in lentil seed was lower than the standard limit. The waste material and the fertilizers might not increase the concentration of lead in lentil seed as observed in this study.

Zinc

Table 4. had been shown that the comparison between the experimental value and the standard value of WHO/FAO. The concentration of Zinc in lentil seed was 4.5793 mg/kg ± 2.2611. The permissible limit according to WHO/FAO standard (1996) is 100mg/kg. The concentration of zinc in the sample was very lowered compared with zinc standard. This confirmed that the amount of zinc in lentil seed was in low. This experimental result showed that the concentration of zinc in lentil seed was lower than the standard limit. Zinc has no toxic effect if lentil seed is used as food in different forms because its concentration was lower than WHO limit. The waste material and the fertilizers might not increase the concentration of zinc in lentil seed as observed in this study.

Cadmium

Table 4. had been shown that the comparison between the experimental value and the standard value of WHO/FAO. The concentration of Cadmium in lentil seed was 0.1515 mg/kg ± 0.0311. The permissible limit according to WHO/FAO standard (2001) is 0.1 mg/kg. Cadmium was found to be more than the prescribed limits set by WHO/FAO i, e. 0.1mg/kg. The concentration of cadmium in the sample was higher compared with cadmium µexperimental result showed that the concentration of cadmium in lentil seed was higher than the standard limit. Cadmium fumes inhalation can cause acute pulmonary and kidney damage .It may cause acute and chronic poisoning causing adverse effects on immune and vascular-system(Maobe et al.,2012). The waste material and the fertilizers might increase the concentration of cadmium in lentil seed as observed in this study.

Mercury

Table 4.had been shown that the comparison between the experimental value and the standard value of WHO/FAO. The concentration of mercury in lentil seed was 0.5945 mg/kg ±0.0440. The permissible limit according to WHO/FAO standard is 0.1 µ/kg/day (US EPA), (Adopted CS 150-1985). The concentration of mercury in the sample was excessed compared with mercury standard. This confirmed that the amount of mercury in lentil seed was in excess. This experimental result showed that the concentration of mercury in lentil seed was higher than the standard limit. The waste material such as organic and inorganic mercury and the fertilizers might increase the concentration of mercury in lentil seed .

Copper

As the experimental analyses shown that in table 4 the concentration of copper in lentil seed was 0.7615 mg/kg ±0.3763.The permissible limit according to WHO (1996) standard is 10 mg/L ±0.3763. The concentration of copper in the sample was lowered compared with copper standard (10mg/L). Copper is a micro element which is essential in plant growth and occurs generally in the soil. Copper content has been reported to differ according to the soil type and pollution source (Onder S, Drsun S, Gezgin S, Demirbas (2007).

Iron

Table 4 had been shown that the comparison between the experimental value and the standard value of WHO/FAO. The concentration of iron in lentil seed was 4.8313mg/kg ±3.4866.The permissible limit according to WHO/FAO standard (1996) is 425mg/kg. The concentration of iron in the sample was lowered compared with iron standard in WHO/FAO. This confirmed that the amount of iron in lentil seed was in low. This experimental result showed that the concentration of iron in lentil seed was lower than the standard limit. The waste material and the fertilizers might not increase the concentration of iron in lentil seed as observed in this study.

Chromium

As the experimental analyses shown in table 4 the concentration of chromium in lentil seed was 0.2882mg/kg ±0.1197. The permissible limit according to WHO/FAO (1996) standard is 1.3 mg/L ±0.0202.Chromium is formed though natural processes and human activities. Chromium is not essential for plant growth, it was not detected in some plant sites due to the fact that uptake of chromium by plant shoot is generally low(Hoffman RD, Curnow RD(1973). This experimental result showed that the concentration of chromium in lentil seed was lower than the standard limit .The chromium concentration was lower than WHO limit. The waste materials and the fertilizers might not increase the concentration of chromium in lentil seed as observed in this study.

Table 5 Concentration of heavy metals compared with the recommended concentration by WHO/FAO in lentil seeds at Chefanen kebele.

Heavy metals	Concentration determined in mg/kg at Chefanen kebele.	Concentration Recommended by WHO/FAO in mg/kg
Lead(Pb)	1.009	1.5
Zinc(Zn)	4.439	100
Cadmium(Cd)	0.1367	0.1
Mercury(Hg)	0.7493	0.1
Copper(Cu)	0.662	10
Iron(Fe)	2.622	425
Chromium(Cr)	0.295	1.3

Lead

Table 5 had been shown that the comparison between the experimental value and the standard value of WHO/FAO. The concentration of lead in lentil seed was 1.009 mg/kg ±0.2246. The permissible limit according to WHO/FAO standard (1996) is 1.5 mg/kg. The concentration of lead in the sample was lowered compared with lead standard in WHO/FAO. This confirmed that the amount of lead in lentil seed was in low. This experimental result showed that the concentration of lead in lentil seed was lower than the standard limit. The waste material and the fertilizers might not increase the concentration of lead in lentil seed as observed in this study.

Zinc

Table 5 had been shown that the comparison between the experimental value and the standard value of WHO/FAO. The concentration of zinc in lentil seed was 4.439 mg/kg ±3.0499. The permissible limit according to WHO/FAO standard (1996) is 100mg/kg. The concentration of zinc in the sample was lowered compared with zinc standard in WHO/FAO. This confirmed that the amount of zinc in lentil seed was in low. This experimental result showed that the concentration of zinc in lentil seed was lower than the standard limit. The waste material and the fertilizers might not increase the concentration of zinc in lentil seed as observed in this study.

Cadmium

Table 5 had been shown that the comparison between the experimental value and the standard value of WHO/FAO. The concentration of Cadmium in lentil seed was 0.1367 mg/kg ± 0.0369. The permissible limit according to WHO/FAO standard is 0.1 mg/kg(2001). The concentration of cadmium in the sample was excessed compared with cadmium standard. This confirmed that the amount of cadmium in lentil seed was in excess. This experimental result showed that the concentration of cadmium in lentil seed was higher than the standard limit. Cadmium fumes inhalation can cause acute pulmonary and kidney damage. It may cause acute and chronic poisoning causing adverse effects on immune and vascular-system (Maobe et al., 2012).The waste material and the fertilizers might increase the concentration of cadmium in lentil seed as observed in this study.

Mercury

Table 5 had been shown that the comparison between the experimental value and the standard value of WHO/FAO. The concentration of mercury in lentil seed was 0.7493 mg/kg ±0.2896. The permissible limit according to WHO/FAO standard (Adopted CS 150-1985) is 0.1 mg/kg. The concentration of mercury in the sample was excessed compared with mercury standard. This confirmed that the amount of mercury in lentil seed was in excess. This experimental result showed that the concentration of mercury in lentil seed was higher than the standard limit. The waste material and the fertilizers might increase the concentration of mercury in lentil seed as observed in this study.

Copper

As the experimental analyses shown that in table 5 the concentration of copper in lentil seed was 0.662 mg/kg ±0.4809.The permissible limit according to WHO (1996) standard is 10 mg/L ±0.4809. The concentration of copper in the sample was lowered compared with copper standard (10mg/L). Copper is a micro element which is essential in plant growth and occurs generally in the soil. Copper content has been reported to differ according to the soil type and pollution source (Onder S, Drsun S, Gezgin S, Demirbas (2007). This confirmed that the different waste materials which entered in to the irrigation water and fertilizer that farmers used might not be the cause for the increment of the concentration of copper in lentil seed sample. But copper is an essential nutrient that is incorporated in to a number of metalloenzymes involved in haemoglobin formation ,drug/ xenobiotic metabolism, carbohydrate metabolism, catecholamine biosynthesis, the cross linking of collagen ,elastin and hair keratin, and the antioxidant defense mechanism. The lower concentration of copper in lentil seed might be decrease the function of copper.

Iron

Table 5 had been shown that the comparison between the experimental value and the standard value of WHO/FAO. The concentration of iron in lentil seed was 2.622mg/kg ±1.9242 . The permissible limit according to WHO/FAO standard (1996) is 425mg/kg. the concentration of iron in the sample was lowered compared with iron standard in WHO/FAO. This confirmed that the amount of iron in lentil seed was in low. This experimental result showed that the concentration of iron in lentil seed was lower than the standard limit. The waste material and the fertilizers might not increase the concentration of iron in lentil seed as in this observed study.

Chromium

As the experimental analyses shown in table 5 the concentration of chromium in lentil seed was 0.295mg/kg ±0.1600. The permissible limit according to WHO/FAO (1996) standard is 1.3 mg/L ±0.0202.Chromium is formed though natural processes and human activities. Chromium is not essential for plant growth, it was not detected in some plant sites due to the fact that uptake of chromium by plant shoot is generally low(Hoffman RD, Curnow RD(1973). This experimental result showed that the concentration of chromium in lentil seed was lower than the standard limit .The chromium concentration was lower than WHO limit. The waste materials and the fertilizers might not increase the concentration of chromium in lentil seed as observed in this study.

Table 6. Concentration of heavy metals compared with the sample site area

Heavy metal	Heavy metal concentration at Chacha kebele	Heavy metal concentration at Chefanen kebele	Range
Lead(Pb)	1.1348	1.009	1.1348-1.009
Zinc(Zn)	4.5793	4.439	4.5793-4.439
Cadmium(Cd)	0.1515	0.1367	0.1515-0.1367
Mercury(Hg)	0.5945	0.7493	0.5945-0.7493

Copper(Cu)	0.7613	0.662	0.7613-0.662
Iron(Fe)	4.8313	2.622	4.8313-2.622
Chromium(Cr)	o.2882	0.295	o.2882-0.295

Generally the concentration heavy metal lead at chacha kebele(1.1348mg/kg±0.2952 slightly greater than lead concentration at Chefanen kebele(1.009mg/kg±0.22465, the concentration heavy metal zinc at Chacha kebele(4.5793±2.261) greater than zinc concentration at Chefanen kebele(4.439±3.o499) the concentration heavy metal cadmium at chacha kebele (0.1515±0.0311) greater than cadmium concentration at Chefanen kebele(0.1367±0.0369,) the concentration heavy metal copper at Chacha kebele(0.7613±0.3763) greater than copper concentration at Chefanen kebele(0.662±0.4809), the concentration heavy metal iron at chacha kebele (4.8313±3.4866) greater than iron concentration at Chefanen kebele (2.622±1.9242),and mercury concentration at Chefanen(0.7493±0.2896) greater than mercury concentration at chacha and Chromium concentration at Chefanen kebele (0.295±0.1600) greater than Chromium concentration at chacha kebele(0.2882±0.1197) base on the data obtained from the experimental analysis. Zinc and iron metal concentration is obtained more than the other heavy metals.

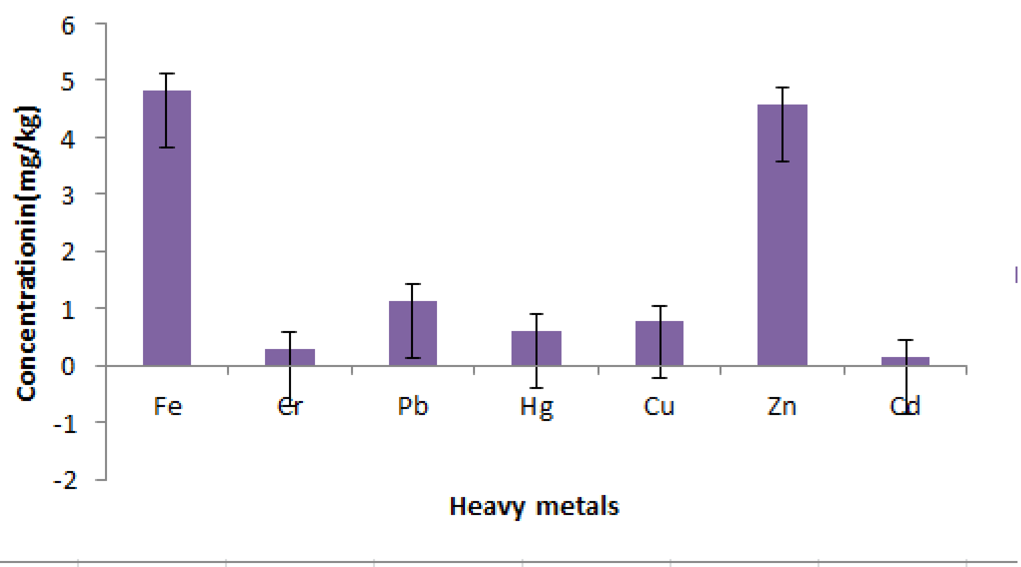

Figure 11. Mean concentration of Zn , Hg, Cr, Fe, Cu, Cd and Pb in (mg/kg) at Chacha.

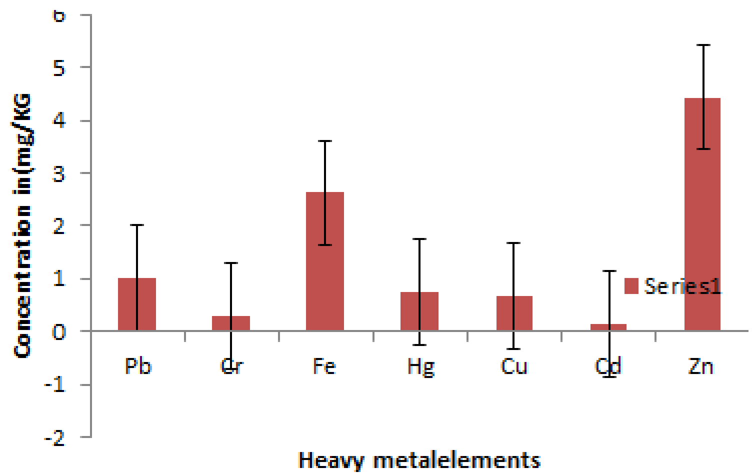

Figure 12. Mean concentration of Zn , Hg, Cr, Fe, Cu, Cd and Pb in (mg/kg) at Chefanen

4.3. Recovery test

Method validation is the way of testing a particular analytical method to check, if it is suitable for its intended purpose. The efficiency of the digestion procedure was checked by adding known concentration of each metal in a 5g of sample. For recovery analysis a mixture of 4mg/kg was spiked to all sample.

$$\textbf{Percentage recovery} = \frac{\textit{Concentration in spiked sample} - \textit{Concentration in unspiked sample}}{\textit{Amount spiked}} \times \mathbf{100}$$

<u>Percentage recovery of the sample at Chacha kebele</u>

Table 7 had been shown that the percentage recoveries of heavy metals (Pb ,Zn, Cu ,Cr, Cd ,Hg, Fe) value of the concentration of heavy metals obtained for this work. Percentage recoveries of Pb 115.6±0.2952 % ,Zn 115.59±2.2611 % Cd 117.96±0.0311 %, Hg 11.78±0.0440%, Cu 120.96±0.3763 %, Fe 104.83±3.4866 % and Cr 93.03±0.1197 % respectively. The percentage recovery values of Zn, Cd, Hg, Cr Cu, Pb, Fe were within the acceptable range of 80 to 120% expected for the elements indicating good accuracy for the analysis procedure (Duan, et al.,2003).These results therefore confirmed that the reliability of the method for the determination of the metals (Zn, Cd, Hg, and Cr, Pb, Cu, Fe).

Table 7. Percentage recovery of the sample at Chacha kebele

Heavy metals	Unspiked Amount(mg/kg)	Amount added in(mg/kg)	Amount after spiked(mg/kg)	Standard deviation	%Recovery± Standard deviation
Lead(Pb)	1.1348	4	5.759	0.2952	115.6±0.2952
Zinc(Zn)	4.5793	>>	9.203	2.2611	115.59±2.2611
Cadmium(Cd)	0.1515	>>	4.870	0.0311	117.96±0.0311
Mercury(Hg)	0.5945	>>	5.0657	0.0440	111.78±0.0440
Copper(Cu)	0.7615	>>	5.600	0.3763	120.96±0.3763
Iron(Fe)	4.8313	>>	9.0247	3.4866	104.83±3.4866
Chromium(Cr)	0.2882	>>	4.0096	0.1197	93.03±0.1197

Percentage recovery of the sample at Chefanen kebele

Table 8 had been shown that the percentage recoveries of heavy metals (Pb ,Zn, Cu ,Cr, Cd ,Hg, Fe) value of the concentration of heavy metals obtained for this work. Percentage recoveries of Pb 91.9±0.2246%, Zn 114.275±3.0479%, Cd 120.42±0.0369%, Hg 108.54±0.2896%, Cu119.62±0.4809%, Fe 117.77±1.9242% and Cr 118.01±0.1600% respectively. The percentage recovery values of Zn, Cd, Hg, Cr, Pb ,Cu, Fe were within the acceptable range of 80 to 120% expected for the elements indicating good accuracy for the analysis procedure (Duan, et al.,2003).These results therefore confirmed that the reliability of the method for the determination of the metals (Zn, Cd, Hg, and Cr Pb, Cu, Fe)

Table 8 Percentage recovery of the sample at Chefanen kebele

Heavy metals	Unspiked Amount(mg/kg)	Amount added in(mg/kg)	Amount after spiked(mg/kg)	Standard deviation	%Recovery± Standard deviation
Lead(Pb)	1.009	4	4.685	0.2246	91.9±0.2246
Zinc(Zn)	4.439	>>	9.010	3.0479	114.275±3.0479
Cadmium(Cd)	0.1367	>>	4.9538	0.0369	120.42±0.0369
Mercury(Hg)	0.7493	>>	5.091	0.2896	108.54±0.2896
Copper(Cu)	0.662	>>	5.4468	0.4809	119.62±0.4809
Iron(Fe)	2.622	>>	7.333	1.9242	117.77±1.9242
Chromium(Cr)	0.295	>>	5.0154	0.1600	118.01±0.1600

4.4. Physio-chemical properties of the soil

4.4.1. pH

In mineral soil pH is a general indicator of soil mineral availability presence of the lime (calcium carbonate).Soil pH is an indication of the acidity or the alkalinity of the soil. If the pH of the soil is 7 the soil is neutral. if the pH is less than 7 If it is acidic and if the pH is greater than 7 the soil is basic(alkali).pH can affect the activity of micro-organisms responsible for breaking down of organic matter and most chemical transformations in the soil.

4.4.2. Conductivity

Conductivity is a measure of the ability of aqueous solution to carry an electric current that depends on the presence and total concentration of ions, their mobility and valance and on the temperature (Belachew Tolla, 2006).An electrical conductivity would provide valuable information for assessing soil condition for plant growth, nutrient cycling and biological activity.

Electrical conductivity is one of the simplest and least expensive soil measurements available to check soil quality, Soil electrical conductivity is a measure that integrates many soil properties affecting crop productivity.

Table 9 . *Soil characteristics at Chacha kebele.*

Parameters	*Test value*	*Mean ± SD*	*Method of analysis*
pH	*6.6,6.8,6.7*	*6.7±0.1*	*Digital pH meter*
Electrical conductivity (µS/cm)	*136,139,140*	*138.33±2.082*	*Conductivity meter*

Table 9. *Showed that the pH and electrical conductivity value of soil measured on pH meter and conductivity meter respectively. Soil samples are categorized as acidic (low pH),<6.5,normal(medium pH): 6.5-7.8 and alkaline(high pH),>7.8 depending on the pH value. Depending on electrical conductivity soil samples are also categorized as normal:<0.8dS/m*

As the experimental analyses showed that the pH of the soil sample was 6.7±0.1.Which confirmed that the soil was normal(medium) which was weakly acidic and lentil can grow best at this pH of the soil The experimentally analyzed pH of the sample soil was in this range that was 6.7

The experimental analyses showed that the electrical conductivity of the soil sample was 138.33µS/cm ±2.082(0.1383 µS/cm±2.082).This confirmed that the soil sample was normal because its EC was 0.1383 µS/cm and which was in the range of normal electrical conductivity(<0.8dS/m).All this result suggested that good quality of soil around this soil sample was taken. Values might been vary significantly from season to season, and are influenced by lime, fertilizer added and climate conditions .By getting information about pH and EC of soil farmers can easily decide the amount of fertilizers to be added to soil and fertility value of their soil to make the production economic.

Table 10. *Soil characteristics at Chefanen kebele.*

Parameters	*Test value*	*Mean ± SD*	*Method of analysis*
pH	*6.8,6.9,6.7*	*6.8±0.1*	*Digital pH meter*
Electrical conductivity µS/cm)	*171,174,176*	*173.67±2.52*	*Conductivity meter*

Table 10. *Showed that the pH and electrical conductivity value of soil measured on pH meter and conductivity meter respectively. Soil samples are categorized as acidic (low pH),<6.5,normal(medium pH): 6.5-7.8 and alkaline(high pH),>7.8 depending on the pH value. Depending on electrical conductivity soil samples are also categorized as normal:<0.8dS/m*

As the experimental analyses showed that the pH of the soil sample was 6.8±0.1.Which confirmed that the soil was normal (medium) which was weakly acidic and lentil can grow best at this pH of the soil The experimentally analyzed pH of the sample soil was in this range that was 6.8.

The experimental analyses showed that the electrical conductivity of the soil sample was 173.67±2.52 (0.17367µS/cm±2.082).This confirmed that the soil sample was normal because its EC was 0.17367 µS/cm and which was in the range of normal electrical conductivity(<0.8dS/m).

All this result suggested that good quality of soil around this soil sample was taken. Values might been vary significantly from season to season, and are influenced by lime , fertilizer added and climate conditions. By getting information about pH and EC of soil farmers can easily decide the amount of fertilizers to be added to soil and fertility value of their soil to make the production economic.

Table 11. the mean concentration of heavy metals (Pb, Zn ,Cd ,Hg, Cu, Fe ,Cr) of the **blank** identified by ICP-OES.

Heavy metals	Mean	Mean ± SD
Lead(Pb)	0.399	0.399±0.012
Zinc(Zn)	0.043	0.043±0.004
Cadmium(Cd)	0.022	0.022±0.001
Mercury(Hg)	0.096	0.096±0.001
Copper(Cu)	0.016	0.016±0.001
Iron(Fe)	0.058	0.058±0.005
Chromium(Cr)	0.114	0.114±0.004

Table 11. Showed that the mean and standard deviation of the **blank**. As the experimental analysis signified that the mean was 0.399±0.012 for lead, 0.043±0.004 for zinc, 0.022±0.001 for cadmium, 0.096±0.001 for mercury, 0.016±0.001 for copper, 0.058±0.005 for iron and 0.114±0.004 for chromium. AS observed in table 11 the standard deviation of the blank of each metal was less than the least concentration of each metal. Therefore the concentration of the heavy metals in the blank was the least compared with their concentration in the sample. All the above information's had been confirmed that the method was more reliable for the determination of the heavy metal in the sample.

4.5. Method detection limit (MDL)

Detection limit is the lowest concentration of analyte that can be detected confidently by the analytical method with a give certainty and stated with 95% confidence that the analyte concentration is greater than zero, for the present study, the detection limit was found by three times the standard deviation of the mean reagent blank signal. Analysis of blank of all metals of interest were performed and the standard deviation of the blank reagents was calculated(R Caulcutt, R. Boddy,(1983).

The two important parameter in method validation are detection limit and quantification limit. To determine detection limit and quantification limit seven blank samples were digested following the same procedure as for the sample was digested and the concentration of each elements (Pb, Cu, Zn ,Cr, Hg, Cd, and Fe) determined by ICP-OES.

Method detection limit of each element is calculated as three times the standard deviation of blank

Detection limit= 3× Standard deviation of blank

4.6. Method quantification limit

Method quantification limit is the lowest concentration of analyte that can be measured in sample matrix at an acceptable level of precision and accuracy, it is the same as the concentration which gives a signal ten times the standard deviation of blank. It is calculated as,

Quantification limit= 10 × Standard deviation of blank

Table 12 Detection and quantification limit of heavy metals (Pb, Cu, Cr, Zn ,Fe, Cd and Hg) in lentil.

Heavy metals	MDL in mg/kg ±SD	MQL in mg/kg ±SD
Lead(Pb)	0.036 ±0.012	0.12±0.012
Zinc(Zn)	0.012±0.004	0.04±0.004
Cadmium(Cd)	0.003±0.001	0.01±0.001
Mercury(Hg)	0.003±0.001	0.01±0.001
Copper(Cu)	0.003±0.001	0.01±0.001
Iron(Fe)	0.015±0.005	0.05±0.005
Chromium(Cr)	0.012±0.004	0.04±0.004

Table 12 showed that the experimental analysis of MDL, and MQL. As shown in the table the

MDL is greater than the detection limit of the instrument (ICP-OES), which confirmed that the method was available and the instrument better detected the concentration of the heavy metals in the sample.

The MQL was the least which indicated that the accuracy is high. Therefore , the method (procedure) was more reliable

CHAPTER FIVE

5.1. Conclusion

The present study tried to examine the concentration of some essential metals nutrients (Cu, Fe, Cr and Zn) and toxic heavy metals (Pb, Cd and Hg) in lentil seed collected from two different area (chacha and chefanen kebele) in Angowelela and tera district north shoa zone Amhara region. The concentration of the metals (Pb, Cu, Cd, Cr, Hg, Fe, Zn) have been analyzed by inductively coupled plasma optical emission spectrometer. The concentration of essential metals nutrients in the lentil seed samples were in the following ranges Zn (4.5739-4.439mg/kg), Cu(0.6137-0.662mg/kg), Fe(4.8313-2.622mg/kg), Cr(0.2882-0.295mg/kg) the concentration of toxic, heavy metals Cd(0.1515-0.1367mg/kg), Hg(0.5945-0.7493mg/kg) and Pb(1.1348-1.009mg/kg).All the metal determined were found below the levels allowed for legumes crop by WHO/FAO except mercury and cadmium metal. T-test at 95% confidence interval indicated that there is positive and highly significant correlation ($r = 0.99^{*}$), between heavy metal lead with cadmium and iron with copper respectively for Chacha. Similarily positive significant correlation($r=0.99^{*}$) between heavy metal copper with zinc for Chefanen. The recoveries for the seven analyzed metals were between 90 and 120% which revealed that an acceptable digestion method for the analysis of lentil seed samples. The level of metals (Pb, Cu, Zn, Cr, Cd, Fe, and Hg) obtained shown that a comparable result with other reported values by WHO. The concentrations of copper, lead, chromium, zinc and iron were investigated in this research were lower than the values reported by WHO. The concentration of mercury and cadmium was slightly higher than the values reported by WHO. The concentration of mercury and cadmium might cause health problems since their concentration were above the recommended level, which were (o.5945mg/kg±0.0440 and 0.7493 mg/kg±0.2896) for mercury and (0.1515mg/kg±0.0311 and 0.1367mg/kg±0.0369) for cadmium Chacha kebele and Chefanen kebele respectively. This concentration was above the WHO acceptable level (maximum 0.1mg/kg) WHO. Cadmium fumes inhalation can cause acute pulmonary and kidney damage. It may cause acute and chronic poisoning causing adverse effects on immune and vascular-system (Maobe et al., 2012).The excess mercury concentration in lentil seed might result from house hold material like cosmetics containers, anthropogenic activities, inorganic and organic mercury compounds that entered to the soil and water which was lentil seed plant could grow. Therefore to use lentil seed for a day today consumption, it had to be taken care in cultivation processing, transports and sells of lentil seed to the consumer.

5.2. Recommendation

- This study will give brief information about the mineral (Pb, Cu, Zn, Cd, Hg, Fe, and Cr) content of lentil seed.
- The study could also be extended to other parts of lentil growing areas so that complete and general information can be obtained about the mineral composition of lentil in the country.
- The study further expanded on the minerals which were not dealt in this research. Moreover, the mineral content of the soil, where lentil in growing, should be studied so that the level of toxic metals can be monitored and fertilizers can be supplemented for deficient minerals
- Effective promotion of high-yielding improved lentil varieties to increase implementation by farmers due to advantageous of lentil over other leguminous crops.
- For improved nutritional value lentil compensates peas and beans.

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Appendix

Correlation coefficient between heavy metals in the lentil seed sample site

Correlation analysis is a bivariant method which was applied to describe the relation between two different parameters. The relationship between contents of heavy metals properties in the lentil seeds with heavy metals were analyzed by Pearson's correlation coefficient for each lentil seeds sample. The high correlation coefficient (near +1 or −1) means a good relation between two variables and correlation around zero means no relationship between them at a significant level of 0.05 and 0.01.

Correlation can be also strongly correlated, if $r > 0.7$, whereas r values between 0.5 and 0.7 show moderate correlation between two different parameters (Sharma and Raju, 2013).

Positive and highly significant correlation ($r = 0.99^{*}$), ($r = 0.99^{*}$) between heavy metal lead with cadmium and iron with copper respectively for Chacha. Similarly positive significant correlation($r=0.99^{*}$) between heavy metal copper with zinc for Chefanen. However, the degree to which two securities are negatively or positively correlated might vary over time and are almost never exactly correlated, all the time. This condition may happen sometimes because one physicochemical property may affect other and it may make to don't have consistent

Table 13. pearson's correlation Coefficient (r) of heavy metals in the lentil seed sample at chacha.

Heavy metals	Pb	Zn	Cd	Hg	Cu	Fe	Cr
Pb	1	0.09	0.99*	0.92	0.67	0.63	0.83
Zn	0.09	1	0.16	0-.32	0.80	0.83	0-.48
Cd	0.99*	0.16	1	0.89	0.71	0.68	0.79
Hg	0.92	-0.32	0.89	1	0.31	0.26	0.99
Cu	0.67	0.80	0.71	0.31	1	0.99*	0.14
Fe	0.63	0.83	0.68	0.26	0.99*	1	0.09
Cr	0.83	-0.48	0.79	0.99	0.14	0.09	1

* Correlation is significant at the 0.05 level (2-tailed).

	Pb	Zn	Cd	Hg	Cu	Fe	Cr
Pb	1.00						
Zn	0.09	1.00					
Cd	0.99*	0.32	1.00				
Hg	0.92	-0.32	0.89	1.00			
Cu	0.67	0.80	0.71	0.31	1.00		
Fe	0.63	0.83	0.68	0.26	0.99*	1.00	
Cr	0.83	0.48	0.79	0.99	0.14	0.09	1.0

* Correlation is significant at the 0.05 level (2-tailed)

Table 14.pearson’s correlation Coefficient (r) of heavy metals in the lentil seed sample at Chefanen.

Heavy metals	Pb	Zn	Cd	Hg	Cu	Fe	Cr
Pb	1	0-.97	0.58	0.89	0-.96	0.75	0.49
Zn	0-.97	1	0-.38	0-.76	0.99*	0-.58	0-.27
Cd	0.58	0-.38	1	0.89	0-.32	0.98	0.99
Hg	0.89	0-.76	0.89	1	0-.72	0.97	0.84
Cu	0-.96	0.99*	0-.32	0-.72	1	0-.53	0-.22
Fe	0.75	0-.58	0.98	0.97	0-.53	1	0.94
Cr	0.49	0-.27	0.99	0.84	0-.22	0.94	1

* Correlation is significant at the 0.05 level (2-tailed).

	Pb	Zn	Cd	Hg	Cu	Fe	Cr
Pb	1.00						
Zn	*- 0.97*	*1.00*					
Cd	0.58	-0.38	1.00				
Hg	0.89	-0.76	0.89	1.00			
Cu	-0.96	0.99*	-0.32	-0.72	1.00		
Fe	0.75	-0.58	0.98	0.97	-0.53	1.00	
Cr	0.49	-0.27	0.99	0.84	-0.22	0.9	1.00

* Correlation is significant at the 0.05 level (2-tailed)