



ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
DEPARTMENT OF CHEMISTRY

**DETERMINATION OF THE LEVELS OF SELECTED HEAVY METALS
IN *LEPIDIUM SATIVUM* SEED USING FLAME ATOMIC ABSORPTION
SPECTROSCOPY (FAAS)**

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AUGUST 2024

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**A Thesis Submitted to the Department of Chemistry in Partial Fulfillment of the
Requirements for the Degree of Master of Science in Chemistry**

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This is to certify that the thesis prepared by Desalegn Abere Bantie entitled “Determination of the levels of Selected Heavy Metals in *Lepidium sativum* Seed by Using Flame Atomic absorption spectroscopy (FAAS)” submitted for the fulfillment of the requirements for Degree of Master of Chemistry complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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DECLARATION

I declare that this thesis entitled “Determination of levels of Selected Heavy Metals in *Lepidium sativum* Seed by Using Flame Atomic absorption spectroscopy (FAAS)” is my own work done under the supervision of Dr Negussie Negash, Department of Chemistry, and Addis Ababa University. It has not previously submitted in any form for obtaining any qualification at any university, all the references have been clearly recorded and acknowledged.

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

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Signature: _____

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
BDL	Below Detection Limit
cm	Centimeter
CS	Continuum Source
DL	Detection Limit
DNA	Deoxy-ribose Nucleic Acid
ELDL	Electrode less Discharge Lamps
ETAAS	Electro Thermal Atomic Absorption Spectroscopy
FAAS	Flame Atomic Absorption Spectroscopy
GC	Garden Cress
gcm^{-3}	Gram per Centi meter
GF	Graphite Furnance
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
gL^{-1}	Gram per Liter
gmol^{-1}	Gram per Molar
HCL	Hollow Cathode Lamp
IARC	International Agency for Research on Cancer
ICP	Inductively Coupled Plasma
IQ	Intelligent Quotient
LOD	Limit of Detection
LS	Line Source
MDL	Method Detection Method
mg	Milli gram

mg/kg	Milligram per Kilogram
MHz	Micro hearth
ml	Millimeter
mm	Millimeter
mm	Millimolar
MS	Mass Spectroscopy
MW	Microwave
nm	Nanometer
ND	Not detected
NTP	National Toxicology Program
ODS	Office of Dietary Supplements
PMT	Photo Multiplier Tube
ppm	Parts per Million
RSD	Relative Standard deviation
S	Slope
SD	Standard Deviation
UV	Ultraviolet
WHO	World Health Organization
μgL^{-1}	Micro gram per Liter
μL	Microliter

ABSTRACT

Traditional medicines have been used thousands of years with a great contribution made by human practice. One of the important traditional medicinal plant is *Lepidium sativum* or fetto in Amharic belonging to mustard family. The levels of heavy metals (Cr, Ni, Cu, Zn, Cd, and Pb) in *Lepidium sativum* were determined. The mean concentrations or levels of heavy and trace metals, Zn, Cd, and Pb in the seed of the selected medicinal plant were found to be (in mg/L) 0.6874 ± 0.0029 , 0.0094 ± 0.0028 , and 0.3695 ± 0.0013 respectively. The mean concentration of metals obtained in the sample were in the order of Zn>Pb> Cd. The concentrations of these trace metals were relatively higher than the maximum allowable concentration given by WHO. But the remaining three metals (Cr, Ni, and Cu) were below detection limit. Furthermore, monitoring such medicinal plants for heavy and trace metals concentrations is of great importance in protecting the community from the adverse effects of the heavy metals.

Key words: Adverse effects, FAAS, Heavy metals, *Lepidium sativum* and Medicinal plant

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the study

Traditional medicines have been used thousands of years with a great contribution made by human practice. They are used as primary health care providers within a community. Traditional medicines are important substances to health practices, approach, belief, and knowledge in incorporating plant, mineral and animal based medicines. Many medicinal plant species were leaves, seeds, and roots are the main parts of the plants used for the preparation of traditional medicines. Parts of medicinal plants are very vital, besides providing ecological, economic, and cultural services, in their uses for medication [1].

Lepidium sativum is an important herb that belonging to mustard family. It is also called garden cress and believed that the plant has its origin in Ethiopia but is now cultivated throughout the world. The botanical name of the medicinal plant is *Lepidium sativum*. It has a common name as common cress in English and other names are garden pepper cress, pepper cress, pepper wort, poor man's pepper. It is an edible herb that grows to the height of almost 50 centimeter [2]. It grows well in all types of soil and climate. Nowadays it is cultivated in the entire world like in Ethiopia, Egypt, in Asia countries, commercially grown in England, France, and Scandinavia etc. [3]. *Lepidium Sativum* is used in the world like added to salads for its tangy flavor, sandwiches, and soups [4]. In Ethiopia it is eaten as dried seed, spourts, and added to another food by its peppery flouring as spices. The plant has its own potential nutritional value like protein, carbohydrate, vitamins, fat, minerals, and amino acid [5].

Ethiopia is a country that located in the Horn of Africa between 3 and 15° north, latitude, and 33 and 48° east, longitude, and is also comprised of many regional states with varied agro ecological places. In Ethiopia many forms of medicinal plants including seeds, roots, trees, and leaves of those herbal medicinal plants used for many human and animal treatments [6]. Generally, traditional medicinal plants used for traditional medicine play a vital role in health care of the majority of the people in Ethiopia [7].

One of the important traditional medicinal plants is *Lepidium sativum* or fetto in Amharic. It is annual herb and originated primary in the highland region of Ethiopia and Eritrea. In Ethiopia the seed of the plant and its oil are used medicinally. The plant is used in treatment of cough, bleeding, asthma, constipation, diuretic, diabetic, microbial [7]. In the world it also used for treatment of hepatitis, menstrual cycle problems, arthritis, diarrhea, and vitamin C deficiency, migraine, cancer, fracture, cardiac disease. Thus, *Lepidium sativum* is a key plant that contributes a traditional medicine in the world [8].

Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. Their heaviness and toxicity are interrelated; heavy metals like mercury, cadmium, and lead also include metalloids such as arsenic that are able to induce toxicity even at low exposure. Heavy metals refer to any metallic chemical element that has atomic number greater than 20 and atomic weights in the range 63.5-200.6 g mol⁻¹ and atomic density above 5 g cm⁻³. They are dangerous because of they tend to bioaccumulate. This means an increase in the concentration of a chemical in biological organism over time, compared to the chemical's concentration in the environment. Thus, they are naturally existing elements having a high atomic mass [9]. They have a density of almost five times greater than water.

Heavy metals in high concentration can affect our body. They bind to parts of our cell and prevent our organs from doing their function. Problems related to heavy metals poisoning can be life threatening and they can cause irreversible damage. Heavy metal poisoning occurs when microscopic molecules of metals accumulate within our body after exposure. They attach to our cells and prevent them performing their functions. They get into our body by exposing into a large amount of metals with eating them with food, drinking, or working with metals. They can cause poisoning even in a microscopic form when they come to our body whether in skin contact or in breathing activity. The bodies of children are at a higher risk of heavy metal poisoning due to still developing and they are more sensitive to the harmful effect of heavy metals.

Heavy metals can pollute soil in multidirectional aspect. Their contaminations are the major causes of environmental pollutions. The polluting agents may be natural or anthropogenic. The major sources of heavy metal contamination into soil are river sediments, air pollution, sludge

waste, composts, agricultural chemicals like fertilizers and pesticides. Soils in agricultural activity may generally be contaminated by heavy metals toxicity such as Hg, Pd, Cd, Cr, As, Co, Ni, Cu, Zn, Fe, Sb, etc. They can be generated from long term uses of fertilizers excessively [10]. Heavy metals also are taken up by plants. So, one has to take care when using traditional medicines that are free from contamination of toxic heavy metals to save life. In the studied area the seed of a plant commonly used by the community as a medicine. Yet no determinations of heavy metals in the study area took place. So the purpose of this study is to determine the risk of heavy and toxic metals in the area.

Heavy metals can be detected by flame atomic absorption spectroscopy (FAAS), inductively coupled plasma (ICP), mass spectroscopy (MS), X-ray fluorescence and spectroscopy [11]. From these instruments flame atomic absorption spectroscopy (FAAS) is the best because it can give precise quantitative data. But the others are time, cost, chemical and energy consuming. Moreover, most of these procedures are not efficient for heavy metal determination. So they have limitations in the case of giving specific data [11].

1.2 Statement of the Problem

Heavy metals are the most serious environmental contaminants in agricultural activity. Our environment includes soil, water, and air. Soil is the main source of heavy metal entry into plants and consequently directly into the food chain. Fertilizers as well as any agricultural chemicals used by farmers and enter into the part of the plant. If the soil contains heavy metals, these are absorbed by the plant and eventually will come to the human being. The use of traditional medicines continues to expand across the studied area. Many people now take traditional medicines and their products for their health in different national health care settings.

However, research reports of adverse events tend to be sensational and give a negative impact regarding the use of traditional medicines. Traditional drug treatments had been additionally determined to include heavy metals. A number of diseases have been reported due to consumption of traditional medicines contaminated with heavy metals [12]. Currently, the majority of adverse events related to the use of traditional medicines either to poor product quality or to improper use. In order to expand knowledge about authentic adverse reactions to

traditional medicines, and to keep away from losing scarce sources for figuring out and studying destructive activities, activities as a result of such conditions will want to be decreased or eliminated. *Lepidium sativum* among vital medicinal plants is used to treat various ailments such as cough, bleeding, asthma, constipation, diabetic, and microbial. Thus, it is used by most people in Bahir Dar zuria woreda for the treatment of mentioned diseases. The level of heavy metals in the plant above the maximum allowable concentration given by WHO is a matter of great concern to public safety [13]. The problem is more pronounced in the case of the studied area because the traditional medicines used by the society without realizing the concentration of heavy metals.

World Health Organization (WHO) recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, further it regulates maximum permissible limits of toxic metals like lead, cadmium and arsenic which amounts to 10 ppm, 0.3 ppm and 1.0 ppm, respectively [14]. The widespread conception among the population that natural means safe and that drugs of natural origin are harmless and have no risk associated with their use, does not match reality. Some medicinal plants have inherent toxicity like any medicine; have side effects that can cause many diseases [15].

The analysis of heavy and trace metals like Zn, Cu, Cr, Pb, and Cd were determined by using FAAS in the medicinal plant, *Ocimum Lamiifolium* grown in Duguna Fango District, Wolaita zone, Southern Ethiopia [16]. The determination of the essential and non-essential metal levels of garden cress seed grown in Jage, Wandara, Taba, Siyara, and Hilena in Southern Ethiopia were also investigated by FAAS [17]. But the studies did not indicate full and sufficient information about the horizontal risk of the heavy and trace metals. This is one gap and initiative issue of this study. So, based on the above-presented consideration, no study has been done to determine heavy and toxic metals in the studied area. Thus, the present study focuses on the determination of the levels of heavy and toxic metals in the seed of *Lepidium Sativum* that is grown in Bahir Dar zuria woreda Meshenti kebele in order to protect the individuals from their adverse effects when used above the permissible limits.

1.3 Objective of the study

1.3.1 General Objective

The general objective is;

- ❖ To determine the concentration of selected heavy metals (Cr, Ni, Cu, Zn, Cd, and Pb) in *Lepidium sativum*.

1.3.2 Specific Objectives

The specific Objectives are the following.

- ❖ To determine the concentration of selected heavy metals (Cr, Ni, Cu, Zn, Cd, and Pb).
- ❖ To Compare and contrast their concentrations with WHO guidelines.
- ❖ To compare and contrast the concentration of heavy metals in this study with those reported in the literature.

1.4 Significance of the study

In this study the determination of selected heavy metals in *Lepidium sativum* from Meshenti were investigated and compared with those recommended by WHO. This was found necessary since the area exposed by agricultural and mini industrial residential sites. So, the risk of heavy metal levels superseded the recommended limit by WHO.

1.5 Limitation of the study

This study was conducted in short period of time and lack of modern instrumental apparatus. But it needs deep investigation about soil examination like pH, soil acidity, and degradation of the land that *Lepidium sativum* grows by running water and so on. Therefore, the earlier reasons are the limitations of the study.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Traditional Medicine of *Lepidium sativum*

Lepidium sativum is a shrub is cultivated as a medicine and culinary herb and belonging to the family Cruciferae (mustard family) [18]. It is a fast growing annual herb and is widely used in a traditional medicine for the treatment of various diseases. It is a traditionally well-known and widely used in Africa, China, and other far Eastern countries [18]. *Lepidium sativum* also called Garden cress (GC) is important herb and documented in the Ayurvedic Pharmacopoeia of India [19]. Its seed has been recommended as a very essential medicinal part and it is used as a medicine in Ayurvedic System of Medicine starting from Vedic era [19]. The seeds in addition to traditional medicine are regularly consumed as food and are considered to be an effective in different pathological activities. *Lepidium sativum* is mostly used as food and culinary practice for its spicy aroma, pungent taste, and tangy flavor. The parts of *Lepidium sativum* including leaf, seed, and stem have been studied to reveal interesting results for general and clinical applications in both current therapeutic and alternative medicine fields [20]. It has been used by many people of various cultures and societies throughout the world.



Figure 1 The leaf and seed of *Lepidium sativum* (Adapted from Wikipedia)

In Ethiopia, the *Lepidium sativum* seed traditionally used for treating skin problems, intestinal , eye disease, gastritis, liver diseases, amoebic dysentery, asthma, ring worm, malaria, stomach ache, tonsillitis, etc. [20]. In addition to this the seed is one of the potential types of food containing ingredient such as alkaloids, saponin, flavonoids, terpenoids and steroids, which have antioxidant, ant atherosclerotic, and hepatoprotective capacity as supported by various

literature [21, 22]. Generally, *Lepidium sativum* has a considerable importance in traditional medicine worldwide because its antioxidant and anti-inflammatory activities [23].

2.2 Heavy Metals

Heavy metals are naturally existing chemical elements having high atomic weight and density greater than 5 g cm^{-3} . They are less reactive than light metals like sodium and potassium. They have a density five times greater than water. Almost they are found through the crust of earth. They are also named as trace elements because of their presence in trace concentrations [24]. Heavy metals are denser and have higher atomic numbers.

Heavy metals may be essential for plants. The essentiality exerts biochemical and physiological functions in plants [25]. But their heaviness and their toxicity even in low level are inter-related. Nowadays, due to increasing ecological and global public health inter-related with environmental contamination by these heavy metal activities. Using these metals in industrial, agricultural, domestic and technological applications is one of the methods to increase the risk exposing with toxicity [26]. Their various domestic, agricultural, industrial, medical and technological applications have led to their wide distribution in the environment raising concerns over their potential effects on human health and the environment. The toxicity of them depends on various factors including the dose, route of exposure, and chemical species, as well as the age, genetics, gender, and multi nutritional status of exposed individuals. The most toxic heavy metals are cadmium, mercury, lead, and arsenic. They are included in the World Health Organization's list danger chemical of public concern. Because of absorbing into our skin, eating or drinking the metal from food or water, breathing in or inhaling tiny metals, heavy metals can enter into our body. So, they are a significant health hazards because of the characteristic of their bio-accumulate.

Heavy metal contaminations are main causes of environment deteriorations. The basic reasons are generally contaminated by heavy metal toxicity such as Cd, Pb, Hg, Cr, Zn, Cu, As, Co, Mn by using long term use of inorganic fertilizers. They mortify air, soil, and water quality and consequently cause health issues in plants, animals, and people. But they require only in small amount for human health.

2.2.1 Chromium (Cr)

Chromium is the 17th most abundant metal in the Earth's mantle [27]. It has density of 7.2 g/cm³. It occurs naturally as chromite (FeCr₂O₄) in ultramafic and serpentine rocks or complexed with other metals like crocoite (PbCrO₄), bentorite Ca₆(Cr,Al)₂(SO₄)₃ and tarapacaite (K₂CrO₄), vauquelinite (CuPb₂CrO₄PO₄OH), in the Earth's crust [28]. Chromium is widely used in industry as plating, alloying, textile dyes and mordents, pigments, ceramic glazes, refractory bricks. It exists in several oxidation states, like Cr (0), the trivalent Cr (III), and the hexavalent Cr (VI) species.

The levels of chromium in the soil increase mainly through anthropogenic deposition. Cr (VI) has higher solubility and thus bio-availability is more toxic at lower concentrations than Cr (III), which tends to form stable complexes in the soil [29]. When the amount of chromium in the soil rises, this can cause high concentration. Soil acidity is also one impact of chromium uptake by crops.

Chromium toxicity in vegetation is particularly depending on the chemical species of the element. It should be advisable that plants can take up soluble metal ions from water and soil during growth, especially during photosynthesis. In addition to the uptake of metal ions, they can detoxify metals into less harmful forms by chelating heavy metals or changing their oxidation state. But Cr accumulated in plants can affect nutrient uptake and physiological balance, there by disrupting development and growth. Metal uptake in plants is concentrated in the roots, but some species also redistribute a greater proportion of metals to above ground tissues, especially leaves. In animals, its toxicity increases the risk of developing cancer. The toxicity and carcinogenic properties of chromium (VI) have been known for a long period of time [30]. Because of the specific transport mechanisms, only limited amounts of chromium (III) enter the cells.

The carcinogenetic property of chromate dust has been known for a long time [31]. Allergic reactions in few people are also caused by of chromium. Chromates are often used to manufacture, leather products, paints, cement, mortar and anti-corrosives. Since the solubility and toxicity of chromium (VI) are greater than those of chromium (III), the oxidation reduction conversions between the two oxidation states have implications for movement and bioavailability of chromium in soils, groundwater, and plants.

Chromium (III) may be necessary for humans and shortages may cause disruptions of metabolisms. Although chromium (III) has been reported to be an essential nutrient but the uptake of too much can cause health effects as well, for instance skin rashes. Chromium (VI) is known to cause various health effects for people who work in the steel and textile industry. After breathing it in chromium (VI) can cause nose irritations and nosebleeds, respiratory problems, weakened immune systems, kidney and liver damage, alteration of genetic material, lung most cancers and death.

According to NTP, there is sufficient evidence for carcinogenicity in the hexavalent chromium compounds like calcium chromate, chromium trioxide, lead chromate, strontium chromate, and zinc chromate. International Agency for Research on Cancer (IARC) has listed chromium metal and its compounds as to its carcinogenicity [32]. Chromium has been reported to vary from the recommended level of 0.05 mg/L for domestic water [33]. The mean concentration of Cr in physico-chemical parameters of soil and water collected from Tanda Dam kohat has been reported as 1.313 ± 0.141 mg/L [34].

2.2.2 Nickel (Ni)

Nickel is a silver-white metal with atomic number of 28, atomic weight of 58.71, and density of 8.9 g/cm^3 . It has five naturally occurring stable isotopes, with mass numbers 58 (68.07%), 60 (26.23%), 61 (1.14%), 62 (3.63%) and 64 (0.93%). Nickel has the oxidation state of +2, +3 and +4, the most common valence state in the environment is the divalent oxidation state (Ni^{2+} or Ni (II)) [35]. On Earth, it occurs mostly in combination with iron and sulfur in pentlandite, with sulfur in millerite, with arsenic in the mineral nickeline, and with arsenic and sulfur in nickel galena [36]. It is also found in iron meteorites as the alloys kamacite and taenite.

Plants can uptake nickel from soil and results in another source in the diet. Sediments are also important sources in water. Generally, the concentrations detected in sediments show similarities with those detected in topsoil. Migration from contact with food and water could represent an additional source for the presence of nickel.

Nickel is released to soils from smelting and refining operations, disposal of sewage sludge, or use of sludge as a fertilizer and anthropogenic sources. The large a part of all nickel compounds which are dispensed to the surroundings will adsorb to sediment or soil debris and turn out to be motionless as a result. In acidic floor however, nickel is certain to grow to be extra cellular and it will regularly rinse out to the groundwater. Weathering and erosion of geological substances are herbal reasserts of nickel to soils. Typical average background concentrations of nickel in topsoil are in the order of few tens of mg/kg (< 50 mg/kg).

Nickel is one of the heavy metal that exists only at very low levels in the environment. Humans use nickel for many different applications. The maximum not unusual place utility of nickel is the use as a component of alloy and different steel products. It may be determined in metallic merchandise consisting of jewelry. Plants are known to accumulate nickel and as a result the nickel uptake from vegetables will be eminent [37].

The toxicity of nickel is generally low, but high amount have been reported to cause sub-lethal effects [38]. An uptake of too large quantities of nickel has health-related effects of lung and nose cancer, respiratory failure, birth defects, asthma and, allergic reactions such as skin rashes [38]. Generally, reports have been suggesting that nickel compounds are classified as human carcinogens [39]. Nickel and certain nickel compounds have been listed by the National Toxicology Program (NTP) as being reasonably anticipated to be carcinogens [40, 41]. The mean values of Ni metal levels in Tyume River water was recorded that ranged from 0.201 to 1.777 mg/L [42]. The mean concentration of Ni has been reported that was 15 ± 0.7 mg/kg from linseed sample in East Gojjam [43]. The mean concentrations of Ni have been reported from Garden cress in Pakistan Peshawar spot 1 and 2 were 0.15 ± 0.08 mg/L, and 0.08 ± 0.01 mg/L respectively [44].

2.2.3 Copper (Cu)

Copper is a chemical element that has atomic number 29. It is a soft and a reddish-gold colored metal. Copper changed into the primary metallic to be labored with the aid of using human beings and is a few of the maximum broadly used metals. Copper is used as a building material, and a constituent of various metal alloys. It is one of the few metals which could arise in nature in a without delay usable steel form (local metals). Pure copper is orange-purple and acquires a

reddish tarnish usable steel form (local metals). Pure copper is orange-purple and acquires a reddish tarnish whilst uncovered to air. Copper is the 25th most abundant element in earth's crust, representing 50 ppm compared with 75 ppm for zinc, and 14 ppm for lead [45]. The concentration of copper in ores averages only 0.6%, and most commercial ores are sulfides [46]. The common awareness of copper in poly metallic nodules is anticipated at 1.3%. The methods of extracting copper as well as other metals found in these nodules include sulphuric leaching, smelting and an application of the Cuprion process [47].

The high concentration of copper in animals causes to hepatic and occasionally renal changes are the most common effects. However, sensitivity to copper toxicities is highly species dependent. Copper does not now no longer spoil down with inside the surroundings and due to that it may acquire in plant life and animals while it is far discovered in soils. On copper-wealthy soil most effective a confined quantity of flora have a risk of survival. That is why there is not always lots plant range close to copper-disposing factories. Due to the consequences upon flowers copper is a critical hazard to the productions of farmlands. Copper can critically have an effect on the lawsuits of sure farmlands, relying upon the acidity of the soil and the presence of natural matter. Despite of this, copper-containing manures are nevertheless applied.

The bioavailability of copper from the food plan is set 65-70% relying on a selection of things inclusive of chemical form, interplay with different metals, and nutritional components. The serum copper awareness degrees as much as about 1.5 five mg/L in healthful persons. Gastrointestinal symptoms occur at whole blood concentrations near 3 mg/L [48].

Copper is important to good health. It is a critical nutrient in our each daily diet. Just as some copper is essential for good health, but too much can be harmful. Reports have been suggested that at high level of consuming copper as food ingestion can damage the liver and kidneys [49]. Inhalation of copper dirt and fumes (from copper generating and processing facilities) can have an effect on the respiration tract inflicting coughing, sneezing, and ache with inside the chest. Ingesting big quantities of copper compounds (including copper sulfate) can purpose toxic even to people with a wholesome liver and kidney failure. According to reports have been mentioned as people with Menkes disease, hereditary aceruloplasminemia, and Wilson's disease are

particularly sensitive to copper toxicity [50]. The mean concentration of Cu has been reported that was $25 \pm 0.8 \text{ mg kg}^{-1}$ from linseed in East Gojjam [43]. The mean concentrations of Cu have been reported from Garden cress in Pakistan Peshawar spot 1 and 2 were $0.37 \pm 0.00 \text{ mg/L}$, and $0.270 \pm 0.1 \text{ mg/L}$ respectively [44].

2.2.4 Zinc (Zn)

Zinc is one of the 24th most abundant elements in the Earth's crust. It has atomic weight 65.37; atomic number 30; specific gravity 7.14 at 20°C ; and its density is 7.14 g/cm^3 . Zinc is discovered with inside the air, soil, and water and is found in all foods. Soil contains zinc in the range 5-770 ppm with an average 64 ppm. Metallic zinc is also mixed with other metals to form alloys such as brass and bronze and has been used in construction of roofing, buildings and cladding [51]. Metallic zinc has many uses in industry. A common use for zinc is galvanization and to make dry car batteries. Zinc is important for many of the body's functions in small quantity.

Zinc is found in cells throughout the body. It enables the cells to develop and divide and is important for the pastime of enzymes, proteins and DNA. The frame cannot make zinc so it has to be acquired from food. It is specifically saved with inside the muscle and bone. We are uncovered to zinc compounds in food. The common each day zinc consumption through the diet ranges from 5.2 to 16.2 milligrams. Food may contain levels of zinc ranging from approximately 2 parts of zinc per million (2 ppm) parts of foods (leafy vegetables) to 29 ppm (meats, fish, and poultry).

Zinc enters the air, water, and soil because of both natural processes and human activities. Most zinc enters the surroundings because the end results of mining, purifying of zinc, lead, and cadmium ores. The activity of human for his necessity can increase zinc levels in the atmosphere. The degree of zinc in soil will increase specifically from disposal of zinc wastes from steel production industries and coal ash from electric powered utilities. Artificial fertilizers also contribute to increased levels of zinc in the soil.

Compared to numerous different steel ions with comparable chemical properties, zinc is highly

harmless. Zinc is an essential nutritional element that performs essential roles in the course of the whole body. Taking in an excessive amount of zinc may be harmful. According to the Office of Dietary Supplements Trusted Source (ODS), an excessive intake of zinc can cause zinc toxicity. This toxicity can cause gastrointestinal discomfort and, when chronic, may also disrupt nausea, diarrhea, and headaches. Only exposure to high doses has toxic effects, making acute zinc intoxication a rare event [52]. The salts of zinc are intestinal irritants and cause nausea and abdominal pain [53]. The high dosage of zinc has been reported that is able to damage nerve receptors in the nose, which can cause anosmia [54]. The higher mean value of Zn metal from Forcados River was recorded as 76.25 mg/L while lower mean value of Zn as 0.085 mg/L [55]. The mean concentration of Zn has been reported that was 29 ± 0.8 mg kg⁻¹ from linseed in Shoa [43]. The mean concentration of Zn has been reported from garden cress in Jage 0.0656 ± 0.033 mg/L, Taba 0.46 ± 0.045 mg/L, Hilena 0.057 ± 0.026 mg/L, and Siyara 0.058 ± 0.017 mg/L respectively [17].

2.2.5 Cadmium (Cd)

Cadmium is a soft, malleable, ductile, silvery-white divalent metal and its atomic number is 48. It is found in soils under agriculture from insecticides, fungicides, sludge and commercial fertilizers. Elevated concentrations in air, water, and soil might also additionally arise from industrial areas. Cadmium compounds are utilized in batteries and as pigments. Nanoparticles derived from cadmium compounds are used in solar panels and electronic applications [56]. Cadmium makes up approximately 0.1 ppm of Earth's crust. It is highly rarer than zinc, which makes up approximately sixty five ppm [57]. Cadmium is produced in particular as a byproduct of mining, smelting, and refining sulfide ores of zinc, and, to a lesser degree, lead and copper. Small quantities of cadmium, approximately 10% of consumption, are created from secondary sources, especially from dirt generated with the aid of using recycling iron and metal scrap. Sources of cadmium such as agricultural runoff, wastes from industry and atmospheric inflow of dust could have resulted in these levels [58].

Cd is a naturally occurring environmental toxicant, which is easily absorbed and accumulated by plants, and has strong teratogenic and mutagenic effects on organisms. Animal exposure to Cd

for a long time can easily cause diseases such as osteoporosis and kidney for a long time can easily cause diseases such as osteoporosis and kidney damage. A huge quantity of statistics indicates that the consumption of Cd with inside the human frame is especially from the diet. Therefore, it is miles very critical to recognize the inflow and delivery mechanism of Cd in plants.

There is widespread contamination of soil from natural, geological, or industrial emissions. Cadmium is taken up from the soil via plants. Human exposure to cadmium in excessive way in long period of time may health impact. The vital impact has lengthy been taken into consideration to be the poisonous impact at the renal proximal tubules which can bring about scientific kidney disease. Measures of such tubular kidney effects, and of the urinary excretion of cadmium, were the premise for evaluation and control of risk, each with inside the occupational placing and management of risk, both in the occupational setting and for cadmium in food [59]. Reports have been shown that ingestion of cadmium in food causes vomiting, diarrhea, headache, attacking liver, kidneys, lung, brain, and bones [60].

The most dangerous form of occupational exposure to cadmium is ingestion of highly soluble cadmium compounds. Inhalation of cadmium fumes also can result initially in metal fume fever [61]. Cadmium in the human feeding system constitutes persistent threat to health. Adverse effects by cadmium compounds have been reported on a progression to pulmonary edema and chemical pneumonitis, sometimes culminating in death [62]. The mean concentrations of Cd from Tyume River water have been recorded from 0.030 ± 0.002 to 0.044 ± 0.003 mg/L [42]. The mean concentrations of Cd from frequently used herbal products in Pakistan were reported as (0.001 ppm to 0.006 ppm) [63]. The mean concentration of Cd also has been reported from Garden cress in Pakistan Peshawar spot 1 was 0.01 ± 0.00 mg/L [44].

2.2.6 Lead (Pb)

Lead is a soft, silvery white or grayish and one of the pollutant metals. Its density and atomic number is 11.3 g/cm³ and 82 respectively. Lead is a certainly taking place poisonous metallic exists in the Earth's crust. Its significant use has led to high environmental contamination, human

exposure and a significant public health problem in many parts of the world. It is one of the maximum pollutant metals. It dispersed while cars and factories burn fossil fuels at some stage in mining and smelting and as effluents from industries regarding the manufacturing of lead, battery, fertilizers, and pesticides [64].

Lead can exist in numerous oxidation states (0, +2, +4), with lead (II) on the whole related to inorganic lead compounds, and lead (IV) on the whole discovered in organolead compounds. Lead compounds have additionally been used considerably in pipes, pipe fittings, automobile batteries, ammunition, fishing gear, radiation shields, and weight belts, and environmental lead is basically the end result of these anthropogenic activities [65].

Lead can more widespread by human activity like mining, burning fossil fuels and manufacturing. Lead remains a part of our global today. It existed within the air, soil, dust, and the paint of a few homes. Lead is one of the most toxic and frequently encountered heavy metals in the environment [66]. Lead infected soil continues to be a primary hassle near highways and in a few city settings. It can also additionally mobilize from soil in lead bearing soil debris run off to floor waters for the duration of heavy rains.

Exposure to lead can cause serious health problems not humans, but also plants and animals. . Reports have been showed that lead causes to decreased fertility, causes cancer and minor effects like vomiting, nausea, and headache in humans [67]. Health threat of lead poisoning from soil children are particularly at risk [68]. Among these are reduced intelligent quotient (IQ) and attention span, hyperactivity, impaired growth, learning disabilities, hearing loss, and insomnia. Therefore, not only is prevention of lead poisoning the best cure, but it may be the only cure [69]. According to reports excessive exposure to lead may be the main environmental health threat to children [69, 70]. The mean concentration of Pb has been reported that was 28 ± 1 mg/kg from linseed in South Wollo [43]. The study has been recorded from Pakistan at Peshawar spot one Pb mean concentration was 0.23 ± 0.02 mg/L [44].

2.3 Flame Atomic Absorption Spectroscopy (FAAS)

Flame atomic absorption is a very common technique for detecting metal. It uses the principle

that atoms (ions) can absorb light as a specific, unique wavelength. When this specific wavelength of its light is provided, the energy (light) is absorbed by the atom. It makes use of absorption spectrometry to assess the concentration of an analyte in a sample. Electrons in the atom move from the ground state to an excited state by absorbing energy (light) at a specific wavelength. The amount of the light absorbed is measured and the concentration of the element in the sample can be calculated. FAAS is a very sensitive technique and it can be used to detect the concentration of different metals up to 1 ppm or even lower.

2.3.1 Instrumentation

In a typical FAAS instrument, the sample is converted into atomic vapors by a process of atomization. The analyte that should be investigated is atomized, thereby transiting from a ground to higher energy state. In the excited state, every element possesses a unique spectrum that distinguishes it from other elements. The spectra traces are very sharp such that there may be not often overlap, and FAAS takes gain of this phenomenon to become aware of and quantify those elements. For every test, a different light source (hallow- cathode lamp) is used. The analyte always present in solution first has to be atomized in a flame. The sample solution should be set through a small tube and transported to the nebulizer where it is broken up into a fine aerosol. After this the aerosol is carried to the flame, by a carrier gas and broken up into its individual atoms. It could be used for the analysis of large number of trace metals in a variety of sample materials. During analysis, the precise pattern practice and manage of chemical, ionization and spectral interferences are vital approach considerations. Flame atomic absorption spectrometry has the following steps.

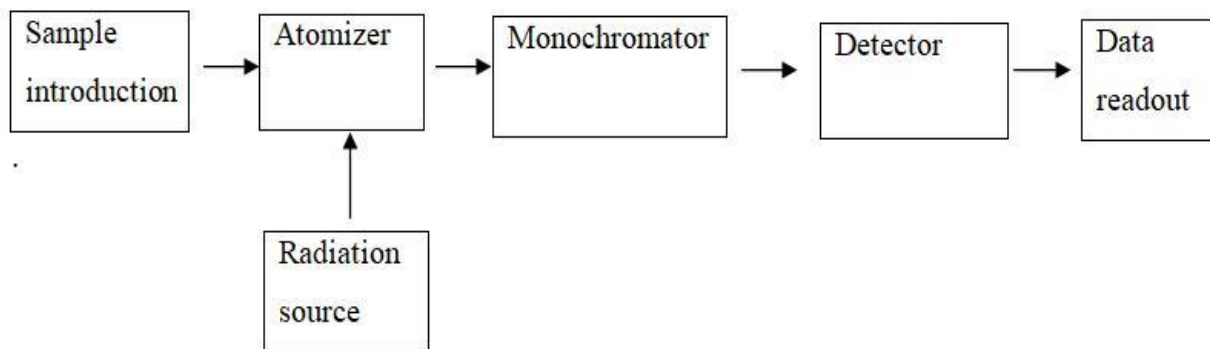


Figure 2 Schematic diagrams of FAAS

2.3.2 Radiation Source

Most instruments used the two sources of radiation namely line source (LS) and continuum source (CS). LS produce radiation at specific wave length while CS is typically produced by deuterium lamps [71].

2.3.3 Atomizer (Flame)

The part of the instrument that the sample is introduced into the atomizer. In FAAS the heat of the flame produces the free atoms. It has a function of produces a fine spray from a liquid and makes as homogeneous. It is the action of an atomization of the sample. It is also the source of thermal energy to excite the atoms. Samples are introduced via aspiration into the flame so must be liquid or gases.

2.3.4 Monochromator (Wave Length Selector)

Wave length selectors limit the radiation absorbed by a sample to a certain wave length or a narrow band of wave lengths. It isolates analytical line photons passing through the flame and remove scattered light of the other wavelength from the flame. This only impinges a narrow line on the photomultiplier tube.

2.3.5 Detector (Photomultiplier PMT)

A detector is a type of an instrument that converts radiation from a monochromator to a specified electrical signal. Photomultiplier tube is one of the types of detector in FAAS. Photomultiplier tube uses an external photoelectric signal. It determines the intensity of the analytical line exiting the monochromator.

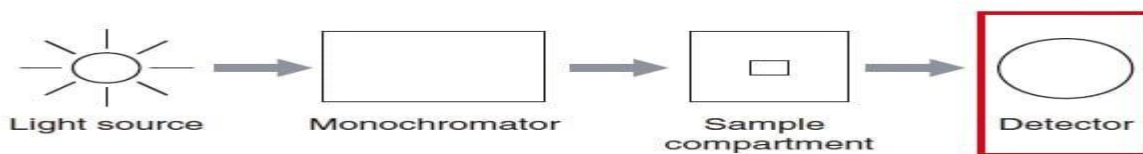


Figure 3 Diagram of detector

2.4 Method Validation

Method validation is the process of assessing analytical method. It was operated by adding the known concentration of the selected metal in 0.5 g of *Lepidium sativum* seeds. Therefore, adding or spiking was done in three triplicate groups. Then the percentage of recoveries was calculated. A spiking solution is a standard that is chosen for preparing a matrix spike; the concentration of the analyte in the spiking solution is usually much higher than the concentration found in the unspiked sample [72].

2.4.1 Precision

Precision is how close the measurements are to each other. The precision of an analytical method is the degree of agreement among individual test results when the method is repeated to multiple samplings of the same sample [73]. Precise measurements are enormously reproducible. It is an outline of random errors, a degree of statistical variability.

2.4.2 Accuracy

Accuracy is the degree to which the result of a measurement conforms to the correct value or a standard and essentially refers to how close a measurement is to its agreed value. The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value [74].

CHAPTER THREE

3 EXPERIMENTAL

3.1 Materials and Method

3.1.1 Description of the Study area

The study was conducted on the area of Amhara region North Gojjam zone in Bahir Dar zuria Woreda Meshenti kebele. The area is located in the North western Ethiopia about 547 kilometers of Addis Ababa, and 18 km / 11 mi away from Bahir Dar Town, at an elevation of 1,976 meters (6,483 feet) above sea level. The geological part of the place is Plateau basalt and its soil type is vertisoil that is low productivity [75]. This place has geographical coordinates are latitude 11.4739° or $11^{\circ} 28' 26''$ north longitude 37.28702° or $37^{\circ} 17' 13''$ east and its average temperature of and annual rainfall are 18°C and 528 mM respectively.

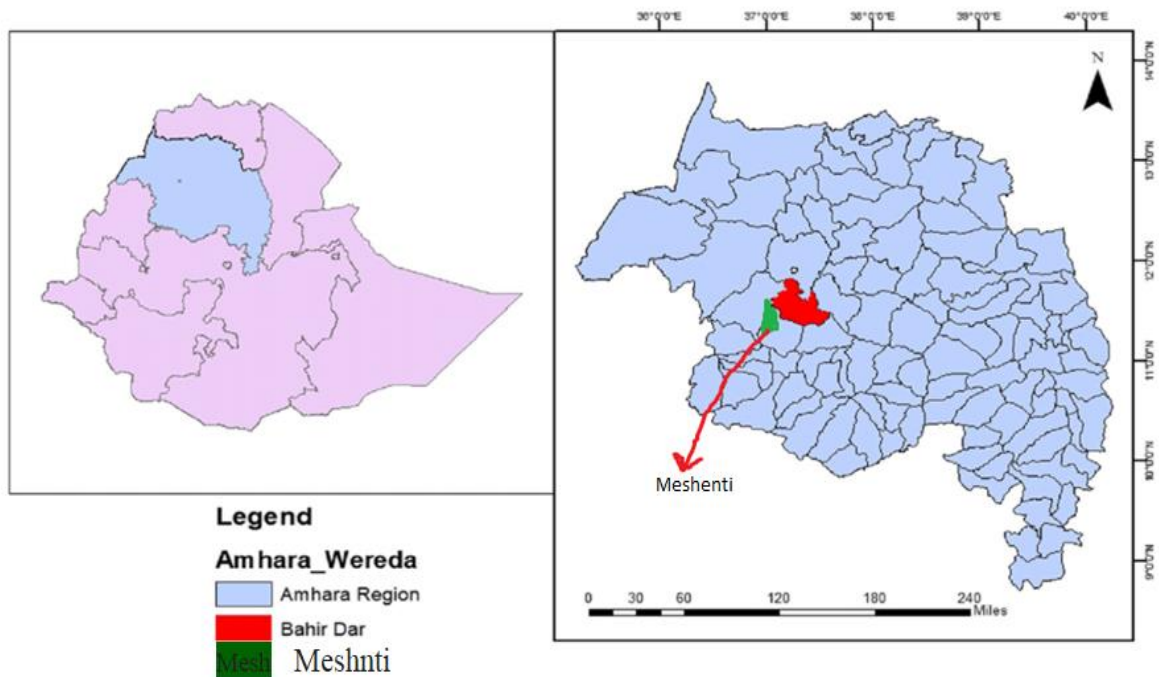


Figure 4 Sampling area of *Lepidium sativum* (Adapted from Wikipedia)

3.1.2 Apparatus and Instruments

In this work different types of apparatus and instruments were used. These are mortar and pestle, oven (J. P.SELECTA, s. a), analytical balance (SCIENTECH, ZSA, 120), plastic bags, spatula, crucible, Whatman filter paper no. 42, funnels, gloves, measuring cylinders, micro peppete different sized beakers and round bottomed flasks, digestion apparatus (Gollenhamp Kjeldahl apparatus), flame atomic absorption spectroscopy (Analitikyjena, Model nov AA, 400p), volumetric flasks and wash bottles.

3.1.3 Chemicals and Reagents

The chemicals and reagents in the project work were high purity of analytical grade. Chemicals nitric acid, (69% HNO₃), and per chloric acid, (70% HClO₄) were used for digestion of *Lepidium sativum* sample and blank. Standard solutions of the selected metals (Cr, Ni, Cu, Zn, Cd and Pb) were prepared from 1000 mg/L stock solution of Certified Reference Materials from Europe accredited laboratory. Distilled and deionized water were also used for rinsing of solution (samples).

3.1.4 Sample Collection and Preparation

The *Lepidium sativum* sample was collected from sampling area of Amhara region Bahir dar zuria worda in Meshenti kebele in plastic bag. The sample was transported to the laboratory and washed thoroughly using tap water and distilled water to remove soil and dirt particles from the grain of the samples. The samples were dried for five days at sun light and finally, they were dried in an oven. The samples were ground using pestle and mortar, mixed together to get a representative sample and stored in plastic bags for digestion. According to agricultural experts I have understood that figure 5 slightly color change of samples of *Lepidium sativum* comes from the maturity of the seed that collected from the farmers in the field of cultivation. The reddish color is higher matured than the yellowish.



Figure 5 Samples of *Lepidium sativum* seed

3.1.5 Digestion of *Lepidium sativum*

0.5 gram of triplicate samples were weighed using analytical balance (SCIENTEECH, ZSA, 120) and transferred in to 250 mL round bottomed flask and 2 mL of 69% of analytical grade nitric acid and 1 mL of 70% per chloric acid were added. The mixture was heated at a temperature of 210 °C for two hours on Gollenhamp Kjeldahl digestion apparatus. Using the same procedure this experiment was performed several times by varying the temperature at constant time, varying volume of acids at constant time and temperature, and varying the time at constant temperature and volume. The solutions were cooled 20 minute on the apparatus, and 5 minute at room temperature. Finally, the mixture was diluted with 10 mL distilled water then filtered using Whatman filter paper no. 42 in 50 mL volumetric flask, shaken and diluted to the mark with distilled water. The samples of *Lepidium sativum* were digested in triplicate then

labeled properly and kept for analysis. Three blanks were digested by the same procedure and each was analyzed for the required metals by FAAS.

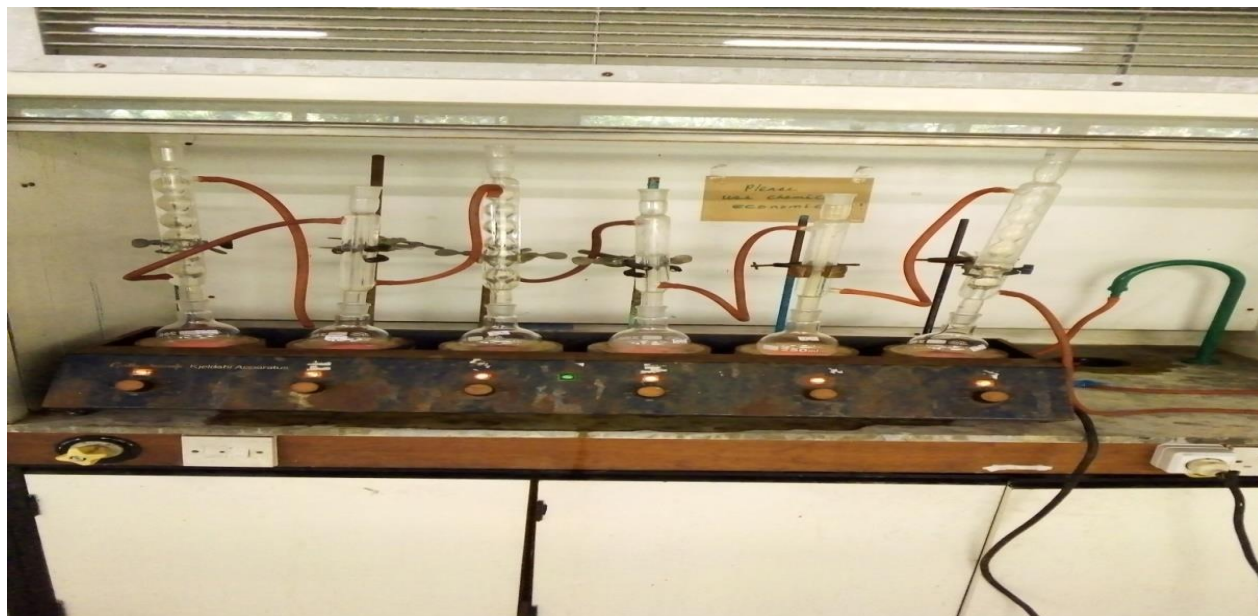


Figure 6 Digestion of *Lepidium sativum* samples



Figure 7 Digestion of blank solutions

3.1.6 Optimization of Working Procedures

As indicated in Table 1, 2, and 3 below, eight optimization procedures were used to get the optimum digestion conditions. The clear and highly colorless was selected. A temperature of 240 °C, 2:1 volume ratio of HNO₃ to HClO₄ acids respectively and a time of 2:40 hour was the optimized condition where clear colorless solutions were obtained. Therefore, procedure four was chosen as optimum condition for the digestion, because it took place at relatively minimum reagent volumes, low digestion time to give clear and colorless solution.

Table 1 Optimization procedure 1 was operated by varying time and volume of acids at constant temperature.

No	Weight of Sample(g)	Volume of reagents (mL)			Temp. (°C)	Time (min)	Results
		HNO ₃	HClO ₄	Total			
1	0.5	3	2	5	270	3:00	Yellowish
2	0.5	4	3	7	270	2:50	Clear pale yellow
3	0.5	5	2	7	270	2:10	Light yellowish
4	0.5	2	1	3	270	2:40	Clear and colorless (selected)
5	0.5	4	1	5	270	2:20	Clear and light yellow
6	0.5	6	1	7	270	2:10	Clear pale yellow
7	0.5	5	3	8	270	2:30	Slightly yellowish
8	0.5	3	1	4	270	2:40	Slightly colorless

Table 2 Optimization procedure 2 was operated by varying temperature and volume of acids at constant time.

No	Weight of Sample(g)	Volume of reagents (mL)			Temp. (°C)	Time (min)	Results
		HNO ₃	HClO ₄	Total			
1	0.5	3	2	5	300	2:40	Slightly colorless
2	0.5	4	3	7	270	2:40	Clear pale yellow
3	0.5	5	2	7	250	2:40	Light yellowish
4	0.5	2	1	3	240	2:40	Clear and colorless (selected)
5	0.5	4	1	5	230	2:40	Clear and light yellow
6	0.5	6	1	7	220	2:40	Clear pale yellow
7	0.5	5	3	8	210	2:40	Slightly yellowish
8	0.5	3	1	4	200	2:40	Slightly colorless

Table 3 Optimization procedure 3 was operated by varying time and temperature at constant volume of acids.

No	Weight of Sample(g)	Volume of reagents (mL)			Temp. (°C)	Time (min)	Results
		HNO ₃	HClO ₄	Total			
1	0.5	2	1	3	300	2:40	Slightly colorless
2	0.5	2	1	3	270	3:00	Clear pale yellow
3	0.5	2	1	3	250	2:50	Slightly colorless
4	0.5	2	1	3	240	2:40	Clear and colorless (selected)
5	0.5	2	1	3	230	2:20	Clear and light yellow
6	0.5	2	1	3	220	2:10	Clear pale yellow
7	0.5	2	1	3	210	2:00	Slightly yellowish
8	0.5	2	1	3	200	2:30	Slightly colorless

3.1.7 AAS Operating Condition

In this study sample digestion apparatus (Gollenhamp Kjeldahl) and Flame Atomic Absorption Spectroscopy instruments were used. The digestion apparatus was optimized at a temperature of 240 °C for 2:40 hour and volume ratio of 2 mL HNO₃ and 1 mL HClO₄. The operating condition for Flame Atomic Absorption Spectroscopy is given in Table 2.

Table 4 Operating conditions

Operating Parameter	Cr	Ni	Cu	Zn	Cd	Pb
Wavelength (nm)	357	232	324	213	228	283
Flame type	Acetylene Gas	Acetylene Gas	Acetylene Gas	Acetylene Gas	Acetylene gas	Acetylene Gas
Photo multiplier tube	303	309	243	391	253	233

3.1.8 Stock Solution and Working Standards

For the metals analysis (Cr, Ni, Cu, Zn, Cd and Pb) the standard containing stock solution (1000 mg/L) for each metal was obtained from Europe accredited laboratory. These stock solutions were used to prepare the intermediate standards (10 mg/L) and different concentrations of working standards. The intermediate standards and working standards are prepared from the stock solution by serial dilution with deionized water. After the working standards were prepared the instrument was calibrated to obtain good correlation between absorbance and concentration which is used to determine the unknown concentration of the sample.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Instrument Calibration

Flame Atomic Absorption Spectroscopy (Analytikajena Model nova AA 400P) was used to determine the concentration of metals (Cr, Ni, Cu, Zn, Cd, and Pb) in *Lepidium sativum* sample. The qualities of the result obtained for analysis of heavy metals using FAAS are affected by calibration and standard solution preparation procedures. Calibration curves of the selected heavy metals were prepared to determine the concentration of metals in the sample solution. The instruments were calibrated using a series of working standards which were prepared from their intermediate solutions 10 mg/L. The stock solution, which is 1000 mg/L of each metal, was taken and 10 mg/L was prepared as an intermediate for preparing different concentration working standards. The working standards were prepared according to the sensitivity of each lamp in the AAS instrument. By using working standards the instrument was calibrated with good correlation coefficient.

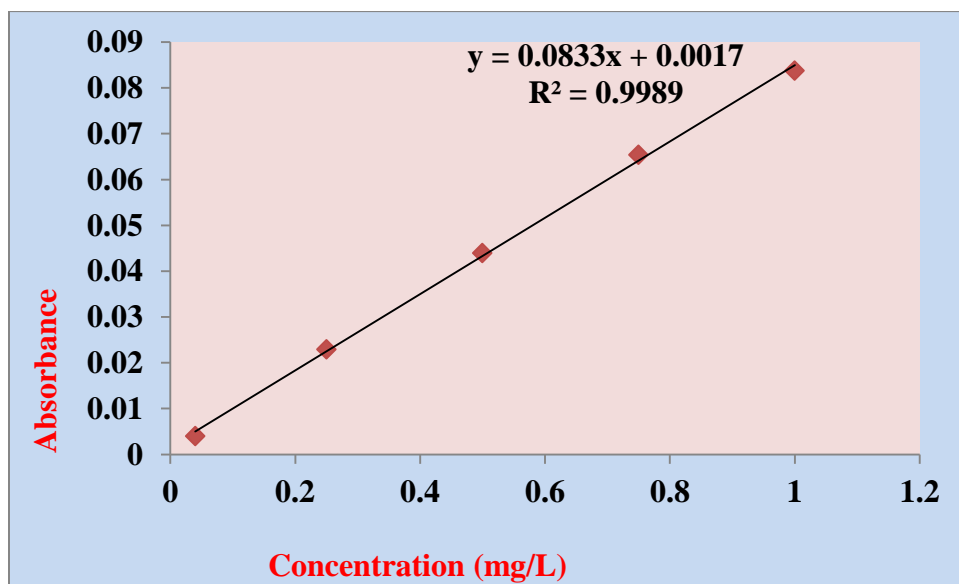


Figure 8 Calibration curve of Chromium

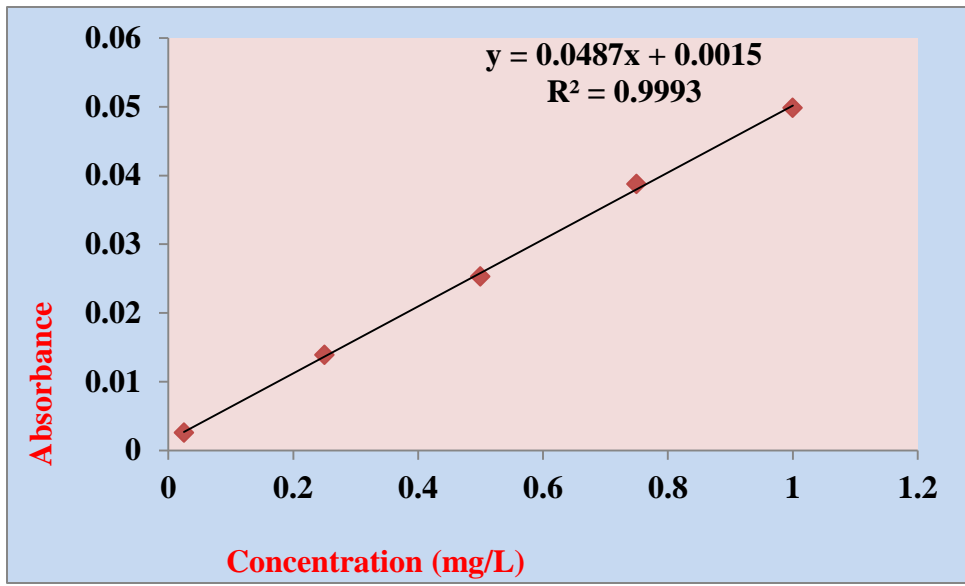


Figure 9 Calibration curve of Nickel

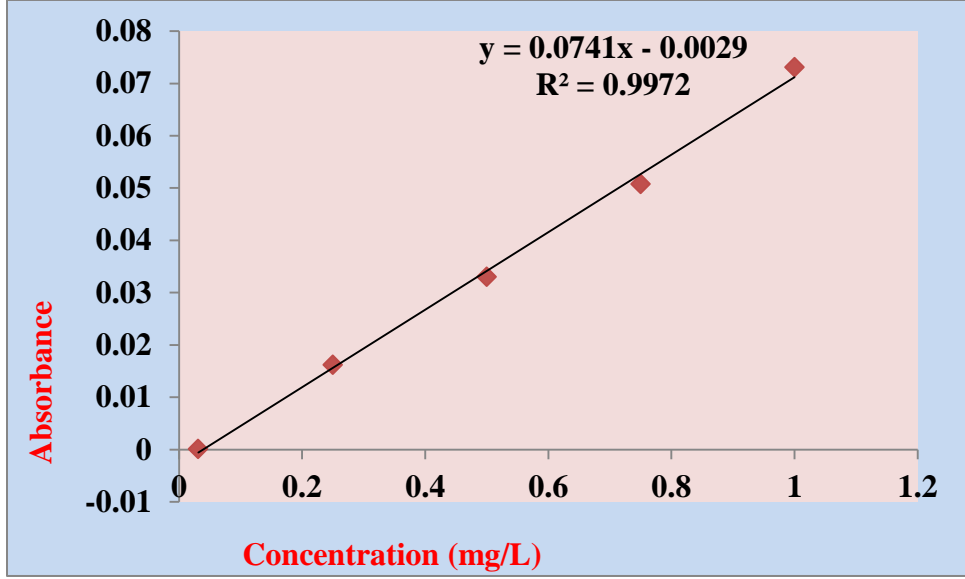


Figure 10 Calibration curve of Copper

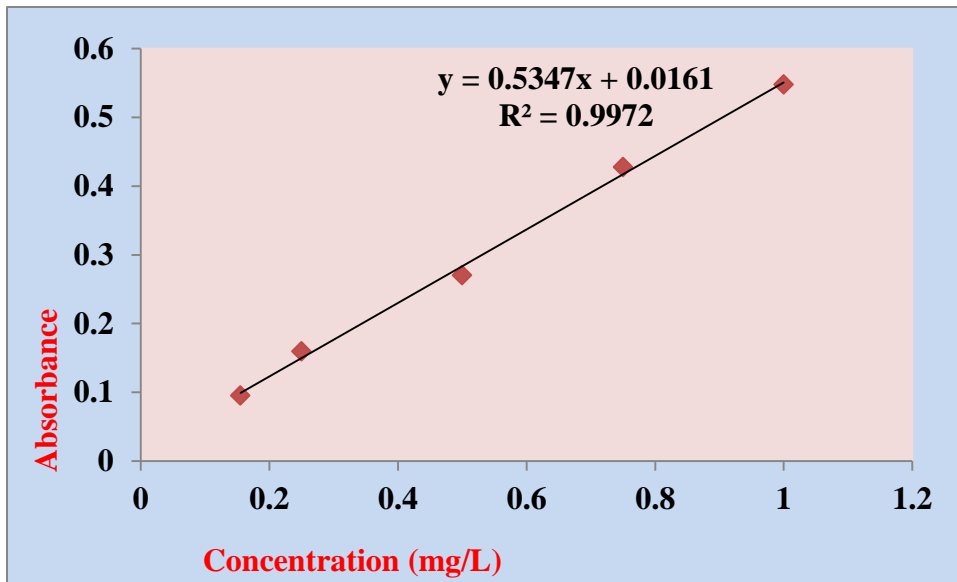


Figure 11 Calibration curve of Zinc

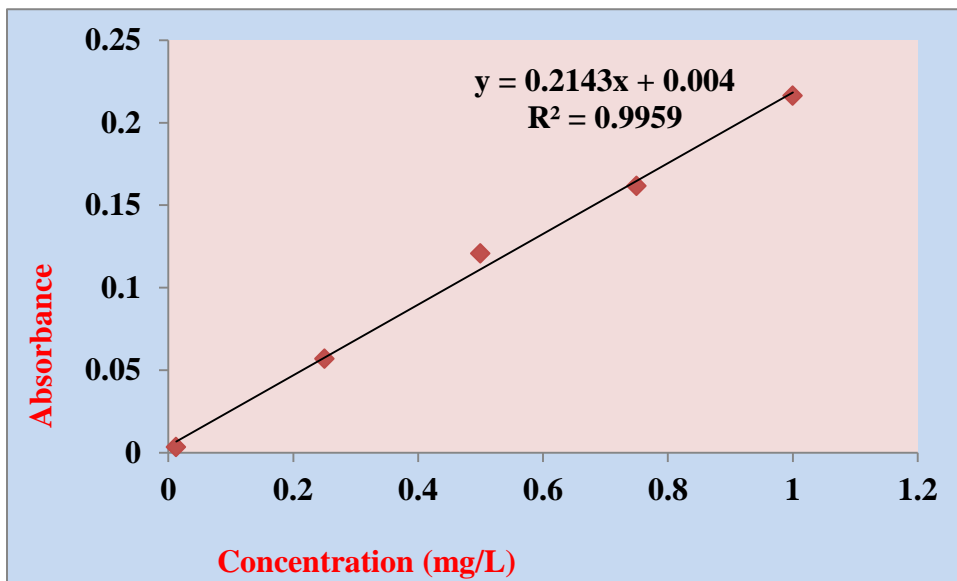


Figure 12 Calibration curve of Cadmium

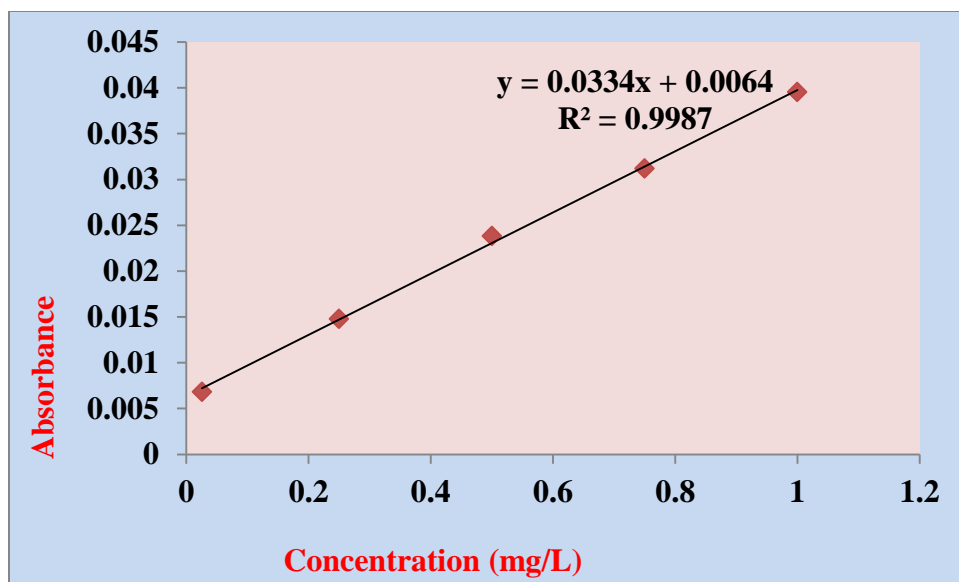


Figure 13 Calibration curve of Lead

Table 5 Concentration of standard solutions used to calibrate the instrument and their corresponding correlation coefficients for the determination of metals

Metals	Conc. of stock solution (mg/L)	Conc. Of Intermediate Solution (mg/L)	Conc. of standard series (mg/L)	Correlation coefficient
Cr	1000	10	0.04, 0.25, 0.5, 0.75, 1	0.9989
Ni	1000	10	0.025, 0.25, 0.5, 0.75, 1	0.9993
Cu	1000	10	0.0312, 0.25, 0.5, 0.75, 1	0.9972
Zn	1000	10	0.155, 0.25, 0.5, 0.75, 1	0.9972
Cd	1000	10	0.0125, 0.25, 0.5, 0.75, 1	0.9959
Pb	1000	10	0.0257, 0.25, 0.5, 0.75, 1	0.9987

4.2. Method Detection Limit (MDL)

MDL is the minimum concentration that can be detected by the analytical method. The method blank samples are used to calculate the MDL_b , which is a very similar calculation that also calculates the 99% confidence level that the result is derived from the sample rather from

contamination/noise [76]. The limit of detection (LOD) is the lowest concentration level that can be detected statistically [77]. In this study method detection limit of each metal was established by digesting three analytical blanks for *Lepidium sativum*. Each blank solution was also determined with FAAS as the same time and temperature as the *Lepidium sativum* sample. The standard deviations of the blank were determined to calculate MDL. $MDL = 3 \times SD/S$, where, SD is standard deviation of the blank and S is the slope.

Table 6 Method detection limit of metals

Metals	Cr	Ni	Cu	Zn	Cd	Pb
MDL (mg/L)	0.0048	0.0008	0.0011	0.0162	0.0039	0.0124

4.3 Recovery Test

Spike and recovery is used to determine whether analyte detection is affected by a difference between the diluent used to prepare the standard solution and the sample matrix. In spike-and-recovery, a known amount of standard is added (spiked) into the natural test sample matrix and its response is measured (recovered) in the assay by comparison to an identical spike in the standard diluent. The goal in assay development is to maximize signal-to-noise ratio while achieving identical responses for a given amount of analyte in standard diluent and sample matrix. The recovery of the selected metals in FAAS analytical method was investigated by calculating the percentage of metals. Then percentage recovery of the analyte was calculated by:

$$\text{Percentage Recovery} = \left(\frac{\text{Spike sample} - \text{unspike sample}}{\text{Spike amount}} \right) \times 100\%$$

In this experiment 10 ppm of standard solution was taken. In the first group 1375 μL of 10 ppm of Zn standard solution was spiked in a flask containing 0.5 g sample of *Lepidium sativum*. In the second group 19 μL of 10 ppm of Cd standard solution was spiked and in the third 735 μL of 10 ppm of Pb standard solution was spiked in the flask containing 0.5 g of the same sample. The calculated percentages were (91.66, 92.78 and 96.36) within the acceptable range of 80 to 120%

expected for the elements indicating good accuracy for the analysis procedure [78].

Table 7 Recovery values of metals for the analyzed *Lepidium Sativum*

Concentration (mg/L)				
Metals	Un-spiked Sample	Amount Spiked	Spiked Sample	Percent recovery (%)
Cr	BDL	NA	NR	---
Ni	BDL	NA	NR	---
Cu	BDL	NA	NR	---
Zn	0.6874	0.275	0.9524	96.36
Cd	0.0094	0.0036	0.0127	91.66
Pb	0.3695	0.147	0.5059	92.78

BDL= below detection limit, NA= not added and NR=not reported

4.4 Determination of Heavy Metals in *Lepidium sativum*

The concentration of six selective heavy metals (Cr, Ni, Cu, Zn, Cd, and Pb) in the digested *Lepidium sativum* sample were analyzed by using FAAS and the results are shown below the table.

Table 8 The mean Concentration of the analyzed metals (mean \pm SD)

Selected Metals	Cr	Ni	Cu	Zn	Cd	Pb
<i>Lepidium sativum</i> (mg/L)	BDL	BDL	BDL	0.6874 \pm 0.0029	0.0094 \pm 0.0028	0.3695 \pm 0.0013

Among the analyzed selected heavy metals given in Table 5 the level of chromium, nickel, and

copper were below the method detection limit (MDL) in *Lepidium sativum* samples taken, from the studied area while the other selected heavy metals zinc, cadmium, and lead were detected in *Lepidium sativum* sample.

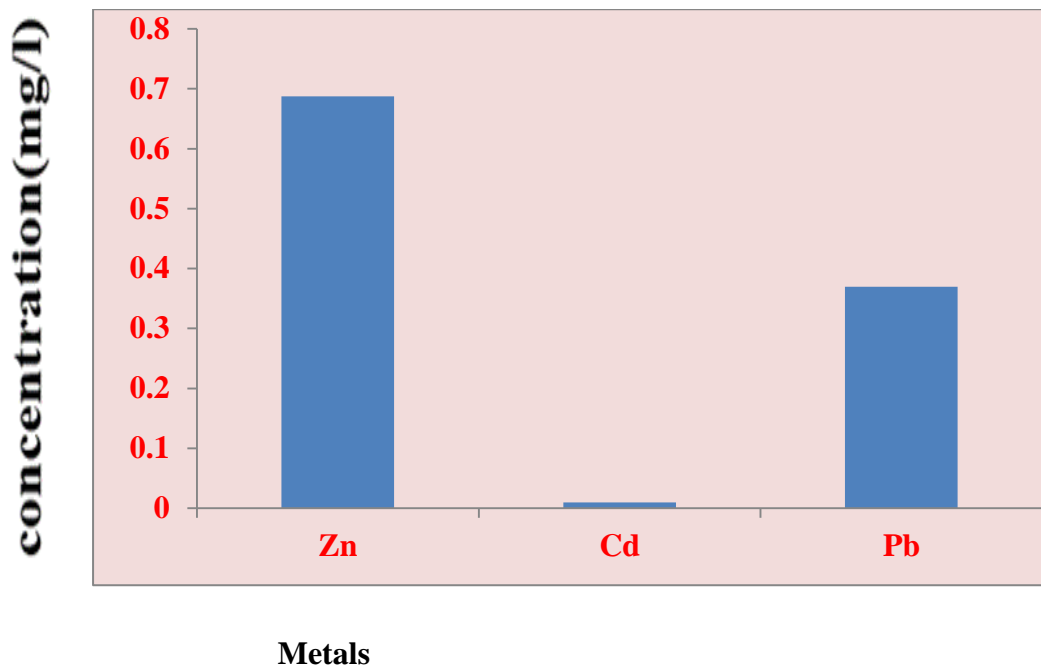


Figure 14 Graph of concentration of the selected heavy metal (Zn, Cd, and Pb) in *Lepidium sativum*

4.5 Comparison of metal concentrations with those reported in the literature in *Lepidium sativum* seed

The concentrations of Cr, Ni, and Cu in this study were below the detection limit (0.0048, 0.0008, and 0.0011) in mg/L respectively. But concentrations of Zn, Cd, and Pb have variations compared to other studied site that has been reported in literatures as explained by table 6 below, because of effect of fertilizers, manures, and water for irrigation used by farmers.

4.5.1 Zinc comparison with other reports

The mean concentration of zinc in the analyzed was 0.6874 ± 0.0029 mg/L. This value was higher than those reported in Jage 0.0656 ± 0.033 mg/L, Taba 0.46 ± 0.045 mg/L, Hilena 0.057 ± 0.026

mg/L, and Siyara 0.058 ± 0.017 mg/L [17]. Since the site is affected by artificial fertilizers and mini industries, zinc has higher concentration than the above report. The high level of Zn in the studied area may have health risk in the community as the result of high dosage of the metal in *Lepidium sativum* seed.

4.5.2 Cadmium comparison with other reports

The mean concentration of cadmium in the analyzed in *Lepidium sativum* in this study was 0.0094 ± 0.0028 mg/L. The result was lower than those reported in Pakistan at Peshwar spot1 0.01 ± 0.00 mg/L [44] and Romania at Iasi 0.148 ± 0.036 mg/L [79]. Since Cd is one of very toxic metal in human being, but it is relatively lower than in the above reports.

4.5.3 Lead comparison with other reports

The mean concentration of Pb in the analyzed in *Lepidium sativum* in this study was 0.3695 ± 0.0013 mg/L. But this result was higher than those reported in Pakistan at Peshawar spot one 0.023 ± 0.02 mg/L [44], Pakistan at Peshawar spot two 0.016 ± 0.006 mg/L [44], and Pakistan at Peshawar spot three 0.014 ± 0.008 mg/L [44]. Like Cd lead also toxic heavy metal and has health hazard to the people consuming the seed. Based on the above concentrations, recommendation is necessary to control and monitoring of the adverse effects the heavy metals.

Table 9 Comparison of metal concentrations with those reported in the literature in *Lepidium sativum* seed

Country	Place	Cr	Ni	Cu	Zn	Cd	Pb	Method	Reference
Pakistan	Peshawar (Spot one)	0.04± 0.00	0.15± 0.08	0.37± 0.00	NR	0.01± 0.00	0.23± 0.02	FAAS	[44]
Pakistan	Peshawar (Spot two)	0.03± 0.00	0.08± 0.01	0.07± 0.01	NR	ND	0.16± 0.06	FAAS	[44]
Pakistan	Peshawar (Spot three)	0.11± 0.03	ND	0.24± 0.01	NR	ND	0.14± 0.08	FAAS	[44]
Romania	Iasi	0.165± 0.035	NR	NR	NR	0.148± 0.036	NR	FAAS	[79]
Ethiopia	Jage	NR	NR	0.45± 0.05	0.0656± 0.033	NR	ND	FAAS	[17]
Ethiopia	Taba	NR	NR	0.381± 0.010	0.46± 0.045	NR	ND	FAAS	[17]
Ethiopia	Hilena	NR	NR	0.072± 0.020	0.057± 0.026	NR	ND	FAAS	[17]
Ethiopia	Siyara	NR	NR	0.039± 0.00	0.058± 0.017	NR	ND	FAAS	[17]
Ethiopia	Meshenti	BDL	BDL	BDL	0.6874± 0.0029	0.0094± 0.0028	0.3695± 0.0013	FAAS	This study

ND=not detected, BDL=bellow detection limit, NR=Not reported

4.6. Comparison of the concentration of selected heavy metals in *Lepidium sativum* sample with the guideline set by WHO

Metals obtained from the different sources are released in to the environment and tend to bio accumulate in plants, organisms and even biomagnified in the food chain where human beings are highly exposed. So, if *Lepidium sativum* is polluted with heavy metals, metals will be transferred to animals and humans immediately and it affects human health if the concentrations of heavy metals are above threshold limit. The concentrations of Zn, Pb, and Cd detected in *Lepidium sativum* are comparably higher than the WHO maximum allowable concentrations. The concentration of the selected heavy metals (Cr, Ni, Cu, Zn, Cd, and Pb) obtained in this study were, Cr(BDL), Ni(BDL), Cu(BDL), Zn(0.6874 ± 0.0029), Cd(0.0094 ± 0.0028), and Pb(0.3695 ± 0.0013) mg/L respectively. The concentration of heavy metals in *Lepidium sativum* sample vary in the order of Zn>Pb> Cd but the concentrations of Cr, Ni, and Cu are below the detection limit.

The concentration of zinc in the analyzed *Lepidium sativum* sample was (0.6874 ± 0.0029) mg/L. The maximum allowable concentration of zinc is 0.35 mg/L given by WHO [80]. As shown in Table 7, the concentration of zinc in *Lepidium sativum* was higher than the maximum allowable concentration of zinc given by WHO [80].

The concentration of cadmium in the analyzed *Lepidium sativum* sample was (0.0094 ± 0.0028) mg/L which is higher than the maximum allowable concentration (0.003) mg/L of cadmium given by WHO [80].

The concentration of lead in the analyzed *Lepidium sativum* sample was (0.3695 ± 0.0013) mg/L. Like cadmium the level of lead detected from *Lepidium sativum* is higher than the maximum allowable concentration (0.01 mg/L) of lead which is given by WHO [80].

Chromium, nickel, and copper were not analyzed by flame atomic absorption spectrometric method because the levels of them in *Lepidium sativum* sample were below the detection limit of the instrument. Generally, the detected metals meaning zinc, lead, and cadmium are higher than the maximum allowable concentration that is given by WHO, but the remaining three metals are below the detection limit.

The above three heavy and trace metals (Zn, Cd, and Pb) that have higher concentrations than internationally accepted permissible limits may have health risk to the consumer in the studied area.

Table 10 Maximum allowable concentration of selected heavy metals by WHO in seed.

Selected metals	Cr	Ni	Cu	Zn	Cd	Pb
WHO (mg/L)	0.05	0.02	0.55	0.35	0.003	0.01 [80]
For this Expt	BDL	BDL	BDL	0.6874	0.0094	0.3695

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The main purpose of this study was the determination of selected heavy metals (Cr, Ni, Cu, Zn, Cd, and Pb) in *Lepidium sativum* seed sample. The concentrations of these heavy metals were determined by using FAAS. The result showed that the concentrations of three heavy metals (Zn, Pb, and Cd) analyzed in *Lepidium sativum* seed sample were relatively higher than the maximum allowable concentration that is given by WHO. The remaining other three metals (Cr, Ni, and Cu) were below the detection limit. The contamination of traditional medicine is due to the accumulation of the heavy metals in different parts of the medicinal plants like the seed. Hence, the concentrations of these metals should be free of their toxic effects. The level of heavy and trace metals may pose health risk for people eating this *Lepidium sativum* seed as traditional medicine. Therefore, the concentrations of the metals analyzed in this traditional medicine taken from Meshenti is unsafe for consumption as food, traditional medicine, and spice in the case of the concentration of lead, zinc, and cadmium.

5.2. Recommendation

This work was conducted in a short period of time, limited amount of budget and lack of required instrument. Due to this it was difficult to cover more sample sites and collect larger sample size from all areas of Bahirdar zuria woreda and samples during all seasons to have more representative sample for the study. So it is recommended for other researchers to take more time, sufficient money and large number of samples to address of the limitation of this study.

This work also did not study the level of heavy metal in the soil, and chemicals like artificial fertilizers, insecticides, herbicides, source of water, compost, and weather condition of the growing area of *Lepidium sativum*. Therefore it is recommended for researchers to investigate soil and artificial chemicals that affect the concentration of heavy metal on *Lepidium sativum*.

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