Determination of the Levels of Selected Heavy Metals and Health Risk Assessment in Lentil Crop (*Lens Culinaris Medik*) and Agricultural Soil at Dawunt Woreda, North Wollo, Ethiopia

By: Baynesagn Beyazn

A Thesis Submitted to the department of Chemistry,

Faculty of Natural and computational sciences

Presented in Partial ~~Ful~~fillment of the Requirement for the Degree of Master's in Chemistry (Analytical)

Office of Graduate Studies

Woldia University

Feb, 2023, Woldia, Ethiopia

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Advisor: Asamene Embiale (PhD)

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DECLARATION

I hereby declare that this Master Thesis entitled “Determination of the Levels of Selected Heavy Metals and Health Risk Assessment in Lentil Crop (*Lens Culinaris Medik*) and Agricultural Soil at Dawunt Woreda, North Wollo, Ethiopia is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation

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Name of the student	Signature	Date

RECOMMENDATION

I/we, the advisor(s) of this thesis, hereby certify that I/we have read the revised version of the thesis entitled "Determination of the Levels of Selected Heavy Metals and Health Risk Assessment in Lentil Crop (*Lens Culinaris Medik*) and Agricultural Soil at Dawunt Woreda, North Wollo, Ethiopia Prepared under my/our guidance by Baynesagn Beyazn submitted in partial fulfillment of the requirements for the degree of Master's of Science in chemistry (Analytical). Therefore, I/we recommend the submission of revised version of the thesis to the department following the applicable procedures.

_________________________ _________________________ _______________

Major Advisor Signature Date

_________________________ _________________________ _______________

Approval Sheet

I/we, the advisors of the thesis entitled "Determination of the Levels of Selected Heavy Metals and Health Risk Assessment in Lentil Crop (*Lens Culinaris Medik*) and Agricultural Soil at Dawunt Woreda, North Wollo, Ethiopia" and developed by Baynesagn Beyazn, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Major Advisor	Signature	Date
Co-advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Baynesagn Beyazn have read and evaluated the thesis entitled "Determination of the Levels of Selected Heavy Metals and Health Risk Assessment in Lentil Crop (*Lens Culinaris Medik*) and Agricultural Soil at Dawunt Woreda, North Wollo, Ethiopia and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Chemistry (Analytical).

Chairperson	Signature	Date
Internal Examiner	Signature	Date
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Final approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and Faculty Graduate Committee (SGC).

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Faculty Dean	Signature	Date
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LIST OF ABBREVIATION

ABS	Skin Absorption Factor
ADI	Average Daily Intake
AF	Adhesive Factor
AT	Average Time
BW	Body Weight
CF	Conversion Factor
C_l	Concentration of Heavy Metals in Lentil
ED	Exposure Duration
EF	Exposure Frequency
FAO	Food & Agricultural Organization
HMs	Heavy Metals
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
IngR	Ingestion Rate
InhR	Inhalation Rate
MDL	Method Detection Limit
MQL	Method Quantization Limit
PEF	Particular Emission Factor
SL	Skin Adherence Factor
SPSS	Statistical Package for Social Science.
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

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ABSTRACT

Health implications to the population due to the consumption of contaminated crops have been a great concern all over the world. In this study, the levels of heavy metals (Cr, Mn, Fe, Pb, Cd, Cu and Co) in lentil and its growing soil at Dawunt Woreda, Ethiopia have been determined using Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES) and possible health risks due various exposure routes have been also estimated. A wet digestion method was employed for the decomposition of soil and lentil samples. The levels of Cr, Mn, Fe, Pb, Cu and Co in agricultural soil were fund to be below the reference level set by (WHO) except Cd, which is found to be above the limit. Similarly, Cr, Cd, Mn, Fe, Pb, Cu and Co levels in lentil sample were also below the recommended values set by WHO. The following concentrations in dry weight (mg/kg) were obtained in lentil: 9.686±0.013 for Manganese, 60.4±0.09 for Iron, 0.75±0.005 for Cobalt, 5.70±0.04 for Copper, 0.25 ±0.004 for Cadmium, 0.35± 0.002 for Lead and 1.15±0.003 for Chromium. In soil, the concentrations of metals in dry weight (mg/kg) were: 19.85±0.35 for Manganese, 649.38±3.73 for Iron, 3.317±0.076 for Cobalt, 40.020±0.018for Copper, and 15.16 ±0.093 for Cadmium, 1.833± 0.029 for Lead and 69.063±1.931 for Chromium. The total health quotient (THQ) and the health index (HI) of the heavy metals were found < 1 for all metals through different exposure routes, suggesting no significant health risk for adult inhabitants. The total cancer risk (TCR) analysis have also revealed there is no potential adverse cancer risk as the TCR values were below the threshold level. Based on the results of this study, there would not be a significant health risk (both non-carcinogenic and carcinogenic) to the consumer.

Keywords: Lentil, Soil, ICP- OES, and Heavy Metals

CHAPTER ONE

1. INTRODUCTION

Lentil (*Lens culinaris Medik*) is one of the most ancient annual food crops that have been grown as an important food source for over 8,000 years [1]. Cultivated lentil is thought to have been originated and first domesticated in western Asia. Lentil has also been rapidly spread to Egypt, central and southern Europe, the Mediterranean basin, Ethiopia, Afghanistan, India, Pakistan, China and later to the new world including Latin America, Mexico, chili, Argentina, Colombia and more recently Canada [2, 3]

Lentil farming in Ethiopia is for own consumption and economic benefits. It is grown as a winter crop in Ethiopia and particularly important in Oromiya, Amhara and Tigray region. Lentil is one of the major highland pulses of Ethiopia that grows in rotation with Teff, wheat and barley particularly on the heavy black soils. The production of lentil mainly depends on soil type, altitude and agro ecologic conditions. In Ethiopia, its production is not mechanized and produced by smallholder farmers with fragmented plots of land mainly for household consumption [4].

The soil particles can bind various chemicals and, therefore, can be considered as an important sink for contaminants that may originate from different sources [5, 6]. The contaminations of soil quality by toxic metals have exerted long-term ecological and health risks. Agricultural crops which are being cultivated in the contaminated agricultural soils may cause serious carcinogenic and no carcinogenic risks to the human body. In the agricultural areas, toxic metals polluted soil can pose significant human health risks due to soil ingestion, inhalation, and dermal Contact Heavy metals can get into the soil through natural and anthropogenic sources. Anthropogenic sources of heavy metals include industrial effluents, domestic sewage, vehicle exhaust emissions, and excessive use of fertilizers, herbicides, and pesticides [7].

Heavy metal accumulation in soils can significantly affect human health through their environmental persistence, bioaccumulation, and transfer through food chains; consequently, control of pollution sources is required to minimize their impact [8]. Heavy metals in soil can be absorbed by plants and can cause reduction in their growth. HMs can enter in human bodies through all three medium - air, water and soil. Heavy metals (HMS), such as lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), and iron (Fe), contaminate agricultural soils and crops, such as garden vegetables, grains, and fruits, due to their concomitant and detrimental complications from their persistence and non-biodegradability [7, 9].

Some heavy metals are essential for biological systems in the human body, acting as both structural and catalytic components of proteins and enzymes, but may be toxic when safe concentrations are exceeded. These include Zn, Cu, and Ni. Other metals, such as Cd, Pb, and Cr, are harmful contaminants even at low concentrations. Soil heavy metals, especially Hg, Cr, Pb, Cd and Cu, tend to be accumulated in soils because of anthropogenic activities and their own easy migration properties as common industrial pollutants. Thus, it is crucial to closely monitor the concentration of heavy metals [10]. Dawunt woreda is the agricultural area in, North wollo Zone, Amhara region, Ethiopia, which has seen an upsurge in farming activities in recent years with extensive use of fertilizers and pesticides to increase productivity. This can pollute the soil on which lentil grows and many causes for the uptake of metals by lentil seeds. Here the main focus of this study is to investigate the levels of selected heavy metals (Fe ,Cu ,Mn ,Cd ,Cr ,Pb and Co) from soil and lentil corps grown in farm lands of Dawunt woreda, north wollo ,Ethiopia.

1.1 Statement of the problem

Population growth, urbanization, industrialization, and transportation are major contributors to the pollution of environments, which nowadays is considered a serious problem [11]. Environmental pollution by the heavy metals and associated food safety is a major global concern. These metals can pose a serious health implication to all living things in general and humans in particular if accumulated in elevated concentration above body requirements [12].
There is a growing awareness and concern that heavy metal contamination in agricultural soil and associated food crops can have an adverse effect on human health. Heavy metal pollution in farmland soil primarily originates from anthropogenic activities, such as fertilization, smelting, wastewater discharge and fossil fuel combustion [13].

Agricultural practices such application of phosphates fertilizers; pesticides and refuse derived composts can be important source of heavy metals in the soil. Continuous application of fertilizers to the soil may increase the heavy metal contents making it exceed the natural abundances in soils, and transfer of these metals into the human food chain despite the fact that these heavy metals may be present in minute quantities in fertilizers. Some of these metals such as Pb and Cd have no known use in the body and are toxic even at low levels. Excessive content of these metals in food is associated with a number of diseases, especially those of the cardiovascular, renal, nervous and skeletal systems [14].

In Dawunt farmers depend on rain-fed agriculture for their livelihoods. However, over time, continuous farming depletes nutrients in soil and as a result, fertilizers are used by farmers. The most type of commonly used fertilizer are nitrogenous and phosphate fertilizers. Its composition combines primary macronutrients that are important for the metabolic functions of a plant. In spite of this, improper use of fertilizers and pesticides can contaminate agricultural soil with potentially toxic heavy metals causing agricultural soil a source of pollution. This is because these fertilizers are containing toxic heavy metals. Since heavy metals do not undergo any ecological processes, these elements accumulate within the soil over time and can be transferred from plants to humans through the food chain. Consumption and exposure to these elements owing to polluted agricultural soil can cause potential harmful to humans' health. Humans include farmers who are in frequent contact with fertilizers and persons who consume these food products as they may be susceptible to acute or chronic heavy metal toxicity. Furthermore, the rise of population in Dawunt has increased the number of dumpsite due to poor waste management schemes. It was a usual 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. In addition to this, no work has been reported on the level of heavy metals in soils and crops in general of this area. It was based on these facts that the study was aimed at determining the total concentration of metals in soils and lentil crop, which is a common cereal and major food used by the local community.

1.2 Objectives of the study

1.2.1 General objective

The main objective of the study is to know the levels of Selected Heavy Metals and Health Risk Assessment in Lentil Crop (*Lens Culinaris Medik*) and Agricultural Soil at Dawunt Woreda, North Wollo, Ethiopia.

1.2.2 Specific objectives

- To determine the levels of Mn, Cu, Pb, Cd, Co, Fe and Cr in lentil and its growing soils.
- To compare the concentration of the heavy metals in lentil and soil sample with the standard limits of WHO/FAO.
- To estimate cancer and non-cancer health risk of inhabitants.
- To calculate the transfer factor of measured heavy metals.

1.3 Significance of the study

The main intention of the study is to provide concrete information on the magnitude of the heavy metals in lentil crops and soil of cultivation at Dawunt farm farmland. It also pointed out the possible route of exposure pathway particularly the heavy metals in soil. The results of this study can also serve as baseline information to the policy makers, environmental monitoring agents, and governmental stakeholders and in designing appropriate preventive measures to ensure that the soil quality is improved. More over the finding also gives background information to other researchers, who will be interested in similar research work.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 The Lentil crop (*Lens Culinaris Medik*)

Lentil is one of the most widely grown legume crop in the world [15]. The enrichment of lentil with high protein makes it the prefer pulse crop to rural poor household in the world who are not afford expensive animal products. Because of this nature, lentil is called poor man's meat due to its low price compared to meat [16]. Taxonomically, lentil belongs to the kingdom-plantae, family-fabacea, subfamily-faboidea, genus-lens, and species-Lens culinaris Medik. Some vernacular names include Adas (Arabic), Mercimek (Turkey), Misser (Ethiopia), Masser (India), Lentille, Lentille Cultivée, Lentilles (French), Linse, Linsen (German), Lente, Lenticchia, Lenticchie (Italia) [17]. Lentil is mainly grown in the highlands of Ethiopia where rainfall is usually high. However, it is highly susceptible to excessive moisture stress and very sensitive to water logging and even with short period of exposure it can cause complete crop failure. It is well adapted to various soil types ranging from sand to clay loam when there is good drainage [18]. A soil pH of 6-8 is conducive for lentil production, but it can also tolerate a moderate alkalinity [1]. It is widely grown in areas having an altitude range of 1700-2400 meters above sea level with annual rainfall ranging from 700-2000 mm in Ethiopia. In addition, the crop is capable of germinating at a temperature above freezing point but optimum germination occurs at the range of 18-21°C. On the other hand, a temperature exceeding 27°C can harm the crop aggressively however the ideal temperature for optimum growth and yields of lentil is around 24°C [4].

2.1.1 Nutritional status and Lentil varieties

Lentils are becoming increasingly popular and important sources of vegetable protein. Lentil seed contains 1.1% fat, 59% carbohydrate, and is also rich in important vitamins, minerals, and soluble and insoluble dietary fiber. High protein content (22 to 34.6%) and 55% starch, low level of anti-nutrients, high fiber content and ability to grow in low water stress conditions are the main attributes that make lentils important legume crops. Lentils also contain significant amounts of mineral elements like Ca, Mg, Fe, Mn, Cu, Co, Ni, B and Se [19]. 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 lentils are the finest and richest tasting but most expensive [20].

2.1.2 Uses of lentils as food

Lentil is primarily grown for its mature seeds, which are consumed mainly in sauces and soups. In Ethiopia, it is used in 'kik wot' (sauce of split seeds), soup (from whole seeds or flour), 'nufro' (boiled and salted), 'azifa' (cooked and mashed) and 'elbet' (paste from flour). Many other dishes are prepared from lentil in different countries. Some of these include spicy lentil salad, lentil burgers with coriander-yoghurt sauce, lentil and mushroom cottage pie and lentil potatoes. In India, split seeds (dhal) are used in soups and the whole seed is eaten salted and fried. The seeds are ground into flour used for cakes and bread and for the preparation of special foods, e.g. for infants and invalids. Young pods, sprouted seeds and leaves are eaten as vegetable. Lentil seeds are occasionally fed to animals as a source of protein, particularly to poultry. The seeds are believed to remedy constipation and other intestinal problems. In India they are applied as a poultice to slow-healing sores. In Ethiopia, the seeds are credited with aphrodisiac properties [17].

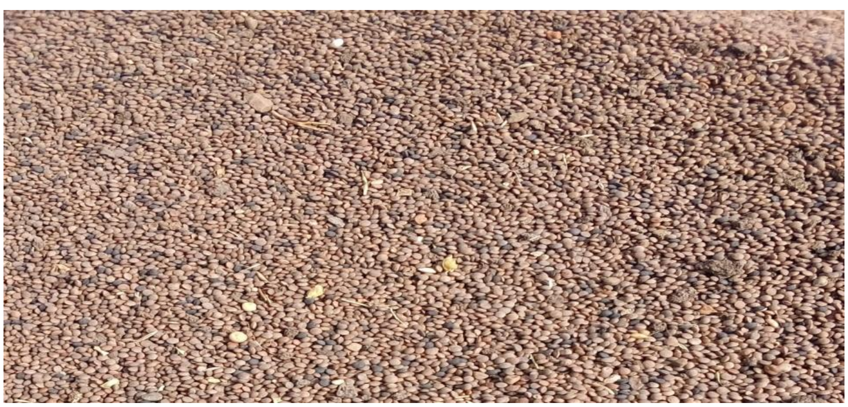

Figure 1 Lentil crop (10:00 am, November 12/2022, Guder)

2.2 Heavy metals

The term "heavy metal" can be defined as metals which have specific weights more than 5 g/cm^3 Metals, in terms of their role in the metabolism of the human body, are classified into two main groups: essential and non-essential [21]. The essential metals are those that play a key role in metabolic pathways. These include Fe, Cu, Zn, Se, Mn, Ni, and Co On the other hand, non-essential metals such as As, Pb, Cd, and Hg can be biologically accumulated in the tissues of microorganisms and human body to cause toxic effects even at low concentrations [22].

Heavy metals are natural constituents of the Earth's crust. Because they cannot be degraded or destroyed, heavy metals are persistent in all parts of the environment. In small amounts, they enter the human body via food, drinking water and air. Living organisms require varying amounts of "heavy metals". Iron, cobalt, copper, manganese, molybdenum, and zinc are required by humans [23, 24].

Human activities affect the natural geological and biological distribution of heavy metals through pollution of air, water, and soil. Humans are also responsible for altering the chemical forms of heavy metals released to the environment. Such alterations often affect a heavy metal's toxicity by allowing it to bio-accumulate in plants and animals, bio-concentrate in the food chain, or attack specific organs of the body. Bioaccumulation refers to an increase in the concentration of a metal in a biological organism over time, compared to the normal concentration in the environment Many metals and other chemicals accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) [25].

2.2.1. Occurrence of heavy metals in the environment

Metals in the environment may be present in the solid, liquid or gaseous state. They may be present as individual elements, and as organic and inorganic compounds. The movement of metals between environmental reservoirs may or may not involve changes of state. The geosphere is the original source of all metals (except those that enter the atmosphere from space in the form of meteorites and cosmic dust). Within the geosphere, metals may be present in minerals, glasses, and melts. In the hydrosphere, metals occur as dissolved ions and complexes, colloids, and suspended solids. In the atmosphere, metals may be present as gaseous elements and compounds and as particulates and aerosols. Gaseous and particulate metals may be inhaled and solid and liquid (aqueous-phase) metals may be ingested or absorbed, thereby entering the biosphere. In addition to being the original source of all terrestrial metals, the geosphere may represent a sink for metals. The atmosphere and hydrosphere also constitute sinks for metals;

however, from a geological perspective, they are more likely to be considered as agents of transport [26].

2.2.2. Behavior of heavy metals in the Environment

The main source for metal input to plants and soils is atmospheric deposition. Volatile metalloids such as As, Hg, Se, and Sb can be transported over long distances in gaseous forms or enriched in particles, while trace metals such as Cu, Pb, and Zn are transported in particulate phases. In terrestrial ecosystems, soils are the major recipient of metal contaminants, while in aquatic systems sediments are the major sink for metals, Freshwater systems are contaminated due to runoff and drainage via sediments or disposal, while groundwater is impacted through leaching or transport via mobile colloids. A number of biogeochemical processes take place at the heterogeneous interface between the rock, soil, water, air and living organisms. These processes or interactions in turn control the solubility, mobility, bioavailability and toxicity of metals. Metals are found in soil solution as free ions or complexes to inorganic or organic ligands. Both die free ions and the metal ligand complexes can be (i) taken up by plants, (ii) retained on mineral surfaces, natural organic matter, and microbes, (iii) transported through the soil profile into groundwater via leaching or by colloid-facilitated transport, (iv) Precipitated as solid phases, and (v) diffused in porous media such as soils [27-29]. Microorganisms can transform metals such as Hg, Se, Sn, As and Cr by means of oxidation reduction and methylation (the process of replacing an atom, usually a H atom, with a methyl group) mechanisms and di-methylation reactions. These processes affect transport or mobility and solubility or toxicity of metals. For example, methylated (organic) forms of Hg are more toxic than inorganic forms of the element and they bio-accumulate in organisms. Methylation is favored in environments characterized by low oxygen levels, low pH, and high soil organic matter (SOM) contents. Heavy metal contamination of soil is a far more serious problem than air or water pollution because heavy metals are usually tightly bound by the organic components in the surface layers of the soil. Consequently, the soil is an important geochemical sink which accumulates heavy metals quickly and usually depletes them very slowly by leaching into groundwater aquifers or bio-accumulating into plants [30, 31]. Heavy metals can also be very quickly trans-located through the environment by erosion of the soil particles to which they are adsorbed or bound and re-deposited elsewhere. The transport, cycling, fate, bioavailability and toxicity of heavy metals are markedly influenced by their physic-chemical forms in water, sediments and soil. Whenever a heavy metal or its compound is introduced into an aquatic environment, it is subjected to a wide variety of physical, chemical and biological processes. Often, heavy metals experience a change in the chemical form as a result of these processes and so their distribution, bioavailability and other interactions in the environment are also affected. They can leach into living systems from natural ore deposits and other sources such as waste disposal of heavy metal containing waste. In fact, waste disposal accounts for higher percentage of most heavy metals in the environment [32, 33].

2.2.3. Heavy metal toxicity

The toxicity of heavy metals is attributed basically to the role they metals play in biological processes as well as the way they interact with essential elements both at intestinal and organ level once absorbed in the body . They are able to remove electrons from the amino-acids or bases of DNA that cause a reaction which disrupts the ability of the cell to carry out their biological functions. These modified biological molecules lose their ability to function properly and result in malfunction or death of the affected cells [34]. In some cases toxic metals displace chemically related metal ions that are required for important biological functions. The alteration of these structures leads to toxic consequences that have an array of disorders. Further, heavy metals are non-biodegradable, thermo stable and bio accumulate [35]. The threat that heavy metals pose to human and animal health is aggravated by their long-term persistence in the environment [36].

2.3. Pollution Problems

Heavy metal pollution comes from the need to immobilize the metals released to the environment or mobilized by and partially lost through human technological activities. It has been established that dissolved metals (particularly heavy metals) escaping into the environment pose a serious health hazard. They accumulate in living tissues throughout the food chain, which has humans at its top, multiplying the danger. Thus, it is necessary to control emissions of heavy metals into the environment. Due to increase in the world population and development of industrial applications, environmental pollution problem has become a series problem [37].

2.4. Heavy metals in the soil

Soils are the main reservoirs for heavy metals generated by industrial activities e.g., metal finishing, paint pigment and battery manufacturing, leather tanning, mining activities, municipal wastewater sludge, urban composts, pesticides, phosphate fertilizers, or from atmospheric depositions. Manmade activities are primarily responsible for the increasing concentrations of heavy metals in agricultural land. Soil, especially those found in and around the industrial area are usually highly contaminated with the heavy metals, including, Zn, Ni, Cd, Cu, Pb, etc. [32]. In the soil that most of the foods we eat grow. The water we drink seeps through the soil in to the ground reservoirs or flows over it to the rivers. Mining, manufacturing and use of synthetic products such as pesticides, paints, industrial wastes, fertilizers and land application of industrial or domestic sludge result in heavy metal contamination of urban and agricultural soil. Although fertilizers are soil nutrient enhancements applied to the soil to promote plant growth they are however found to contain in minutes quantities other elements mostly heavy metals which either have no known use or may be toxic to man and plants are, most of which are responsible for many adverse health effects [38]. Inputs of heavy metals into environment include atmospheric deposition from industrial areas, dumping and treatment of wastes, commercial fertilizers, using sewage sludge, and other processes originated from degradation of various materials [39]. Land filling of municipal solid waste can lead to several metals including Cd, Cu, Pb, Sn, and Zn being dispersed in to the soil [40]. Higher soil heavy metal content can result in higher levels of uptake by plants. Soil acts as a long term sink for heavy metals such as Zn, Cu, Pb, Ni and Cd which have residence times that range from hundreds to thousands of years depending on the element and soil properties. Heavy metals, also occur naturally but rarely at toxic levels. Excess heavy metal accumulation in soils is toxic to human and other animals. Exposure to heavy metals is normally chronic due to food chain transfer. Metal contamination in agricultural soil is of increasing concern due to food safety issues and potential health risk associated with intake of contaminated plants [41].

2.5. Uptake of heavy metals by plants

Contamination by HMs in soils and their effects in agriculture have been associated with natural sources and anthropogenic factors [42]. In mining areas, contamination by HMs in rivers and soils has been attributed to the presence of mining tailings close to the watersheds [43]. Other origins of HM contamination of soils are atmospheric deposition, irrigation with polluted water, pesticides, herbicides, and fertilizers [44]. HMs in the environment can be transferred from soils to plants that are consumed by humans [45]. The incorporation of toxic HMs in crops and crop-based processed foods is a potential health risk to the global population with potential serious implications for food security in certain regions of the world [46]. The main sources of heavy metals to plants are the air or soil from which metals are taken up by the root or foliage. Some heavy metals are essential in plant nutrition, but plants growing in a polluted environment can accumulate these elements at high concentrations, causing a serious risk to human health. The uptake of metal concentration by roots depends on speciation of metal and soil characteristics and type of plant species etc. Consequently, metal mobility and plant availability are very important when assessing the effect of soil contamination on plant metal uptake, as well as translocation and toxicity or ultra- structural alterations [47]. The path taken by metal to transport into the plant is: soil roots stem leaves. The minerals, dissolved in the water, get transported often accompanied by various organic molecules supplied by root cells. Once in the xylem, water with the minerals that have been deposited in it move up in the vessels and tracheid's. Minerals enter the root by active transport into the symplast of epidermal cells and move toward and into the stele through the plasmodesmata connecting the cells. Usually metals preferentially concentrate in the roots, followed by the twigs and leaves. Additional sources of these metals for plants are rainfall, atmospheric dusts and plants protection agents, which could be adsorbed through the leaf blades. An important source of contamination, in vegetable crops, is considered to be foliar uptake of atmospheric heavy metals emissions [48]. 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. 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, anemia, hematological and brain damage. Essential trace elements play a great role to prevent and fighting diseases [20].

2.6. Sources and health effects of selected heavy metals

Cadmium

Cadmium is a non-essential trace element that is widely distributed in the environment. Both natural and anthropogenic sources can elevate Cd concentrations in soils and groundwater, which are important for maintaining healthy supplies of food and safe drinking water. Elevated Cd doses are carcinogenic to humans. Important anthropogenic Cd sources include mining, atmospheric deposition of combustion emissions and the use of Cd containing fertilizers. Besides anthropogenic Cd sources, Cd is also incorporated into sulfides, carbonates, and phosphorites resulting in elevated Cd concentrations in associated rock types. In aqueous solutions, Cd generally occurs as the divalent Cd^{2+} and it is mobilized mainly in toxic, acidic conditions [49, 50]. Cd is used widely in electroplating industries, solders, batteries, television sets, ceramics, photography, insecticides, electronics, metal-finishing industries, and metallurgical activities. It can be introduced into the environment by metal-ore refining, cadmium containing pigments, alloys, electronic compounds, cadmium containing phosphate fertilizers, detergents, and refined petroleum products. Rechargeable batteries with nickel– cadmium compounds are also sources of cadmium and excess in of cadmium causes renal dysfunction, bone degeneration, liver, and blood damage. It has been reported that there has been indicated evidence of the carcinogenicity resulting from cadmium intake [51, 52].Cd is one of the most phytotoxic heavy metals, which is easily absorbed by vegetables due to its lipid solubility. Therefore, closely monitoring the Cd concentrations in soil is crucial [53]. Cadmium is released mainly from Ni–Cd batteries, coatings and plating's, stabilizers for plastics, fossil fuel combustion, phosphate fertilizer, and waste incineration. Besides, Zn, Pb, and Cu mining; ferrous and non-ferrous metal production; and cement production also contribute to the concentration of Cd in the environment [54]. Cd is strongly adsorbed to organic matter in soils. When Cd is present in soils it can be extremely dangerous, as the uptake through food will increase. Soils that are acidified enhance the cadmium uptake by plants. Human uptake of Cd takes place mainly through food. Foodstuffs that are rich in Cd (liver, mushrooms, shellfish, mussels, cocoa powder and dried seaweed) can greatly increase the Cd concentration in human body. Tobacco smoke transports Cd into the lungs. Blood will transport it to the rest of the body. Other high exposures can occur with people who live near hazardous waste sites or factories that release Cd into the air and people that work in the metal refining industry. When people breathe in Cd, it can severely damage the lungs. This may even cause death [29, 52]. Cd is one of the most toxic and mobile elements in the environment it can replace calcium in minerals due to its similar ionic radius, identical charge and similar chemical behavior. Therefore, Cd can enter the human body and accumulate to a high level in several organs. Chronic Cd poisoning cause’s renal tubular. Cd exposure is also associated to glucose metabolism disorders, breast and lung cancer, cerebral infarction and cardiac failure [14].

Chromium

Chromium is a hard, steel-gray metal found naturally in earth's crust in form of chromite ore ($FeOCr_2O_3$). It is a transition metal and belongs to group VI in the periodic table as the first element of the group. It exists in various oxidation states ranging from Cr (II) to Cr (VI). Among these states, Cr (III) and Cr (VI) are most common and highly stable. Naturally, Cr is found in all sort of environmental components including air, water, and soil but in trace amount [55]. It is considered to be harmful to living organisms, although at low concentrations it is an essential micronutrient. In soils, its innate concentration varies within 10–50 mg/kg, depending on the mineralogy of the parent bedrock material [56]. Among heavy metals, Cr is widely utilized in multiple industrial activities. It is used mainly in industrial leather processing and finishing, in the production of refractory steel, drilling muds, electroplating cleaning agents, catalytic manufacture, and in the production of chromic acid and specialty chemicals. Cr compounds are also used in the metalworking industries, cooling tower water, and, until a short time ago, to preserve wood. All these activities have contributed to a significant increase in Cr concentration in the environment. The leather and tannery industries in particular, are the most responsible for the stream of this metal into the biosphere. It exist in all environmental compartments, including water, air, and soil, at different concentrations. Cr content in freshwater varies from 0.1 to 117 μg L^{-1} while in sea water, from 0.5 to 50 μg L^{-1}. Cr may enter the natural waters by weathering of Cr-containing rocks and direct discharge from leaching of soils. In the aquatic environment, Cr may undergo reduction, oxidation, precipitation, sorption, and desorption [57]. Cr, being necessary for the normal metabolism of sugar, lipid and proteins in mammals, becomes an essential micronutrient in the diet of animals and humans. There is no known requirement of Cr in metabolic pathways of plants and microorganisms. Though high levels of Cr are always toxic, the toxicity level depends on its oxidation state. Cr (VI) is considered highly toxic as it causes severe ill effects on human and animal health like diarrhea, ulcers, eye and skin irritations, and kidney dysfunction and lung carcinoma. It is not only highly carcinogenic and mutagenic but is also known to cause birth defects and decrease in reproductive health. The main mechanism of Cr (VI) toxicity in prokaryotes as well as eukaryotes is related to its easy diffusion across the cell membrane, followed by reduction of Cr (VI) in cells giving rise to free radicals that may directly cause DNA alterations and other toxic effects [58, 59].

Cobalt

Cobalt is abundantly present in sedimentary igneous rocks. Co being characterized with chalcophilic, siderophilic, lithospheric in nature [59], it occurs in earth crust as sulfides minerals i.e. carrollite [$Cu(Co,Ni)_2S_4$], linnaeite [Co_3S_4], pyrite [$(Fe,Co)S_2$] and carbonate minerals i.e. cobaltoan dolomite [$(Ca,Mg,Co)CO_3$] as well as other minor portions in erythrite [$Co_3(AsO_4)_2{\bullet}8H_2O$] and heterogenite [$CoO(OH)$] [60]. The concentration of magnesium bound Co significantly lowers down to 3 mg/kg [61]. Hard metal manufacturing units act as the most important man-made source of Co [62]. Cement industries, carbide tool grinding, e-waste processing, diamond polishing, and bottom ash from incinerators release substantial amount of Co in the environment. Cosmetic products paint industry battery; monitor or television display and biomedical wastes potentially increase Co concentration and ultimately contribute to Co abundance in soil-plant animal-human system. Co is a technologically important metal, with unique properties. Lithium cobalt oxide ($LiCoO_2$) and its derivatives are commonly used as the positive electrode in lithium-ion batteries. This has an ordered laterite structure, with ions situated in well-defined positions stabilized by Co. This results in longer and safe battery life. Co is also found in high performance metal alloys used in jet engines, and in cutting and grinding tools. It is used in desulphurization catalysts, in digital storage devices, semiconductors and in hydrogen production. It therefore has a key role in delivering the technologies required for a net zero transition and is essential to achieve several Sustainable Development Goals (SDGs). Its use in energy storage systems is important in stabilizing renewable energy grids, decarbonizing electric mobility systems, and in mitigating climate change [63]. Co has been recognized as a beneficial element for leguminous crops at very lower concentrations (0.1-10 $\mu g\ g^{-1}$ biomass), but not categorized as an essential element for all plants unlike the other macro and micronutrients [64]. Notably, Co has been found to be essential for biosynthesis of vitamin B12 in animals including human beings and microorganisms [65]. Ruminants may be subjected to Co deficiency if their feeds i.e., forages are produced on Co deficient soils containing less than 5 ppm of Co as a whole [66]. Co aids in production of red blood corpuscles, thus constitutes a great option to fight against anemia in human body [67]. Besides, promotional functions of Co for stimulation of growth, appetite and release of energy in human body have also been reported. Nevertheless, rise in Co concentrations in crop plants is of serious concern as it undoubtedly incorporates toxicity in the human food chain [68]. Evidently, intake of Co beyond optimum limit may hamper the development of foetus during pregnancy, causes hematological and endocrine malfunctions, damage the muscles of heart leading to cardiac failure and can escalate the haemoglobin concentration in blood [69]. Though rare, still Co is supposed to cause neurological damage and cardiomyopathy if its concentration in blood circulating system is higher [70].

Copper

Copper is the third most used metal in the world 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. Cu is indeed essential, but in high doses it can cause anemia, liver, kidney damage and stomach and intestinal irritation. Cu 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 [20, 71].

Cu is found naturally as sulphides, oxides and carbonates. Due to its chemical properties it is one of the few metals to be found naturally in inactive form and it is for this reason that it has been known and used by man for many years. The major sources of Cu in land are mining operations, agriculture, solid waste and sludge from treatment works. Agricultural use of Cu products accounts for 2% of Cu released to the soil. Most Cu deposited on soil is strongly adsorbed and remains in the upper few centimeters of soil [48]. Cu is an essential element for human life, but excessive intake results in its accumulation in the liver and produces gastrointestinal problems, anemia, liver and kidney damage. Continued inhalation of Cu-containing sprays is linked with an increase in lung cancer. People with Wilson's disease are at greater risk for health effects from overexposure to copper. Cu can be released into the environment by both natural sources (e.g. wind-blown dust, decaying vegetation, forest fires and sea spray) and human activities (mining, metal production, wood production and phosphate fertilizer production). Because Cu is released both naturally and through human activities, it is very widespread in the environment. Cu is often found near amines, industrial settings, landfills and waste disposals [29, 72].

Lead

The major sources of Pb contamination include its release from chemical, petrochemical and metallurgical industries, disposal of Pb-based paint, emissions from leaded petroleum, solid waste incinerators, and mining and smelting operations. Pb is one of the ubiquitously distributed most abundant toxic elements in the soil. It exerts adverse effect on morphology, growth, and photosynthetic processes of plants. A high lead level in soil induces abnormal morphology in many plant species [35]. Pb contamination is a serious environmental problem that poses a great risk to public health and the environment. As such, the remediation of Pb contaminated soils is an urgent priority for environmental professionals [73, 74], The toxic elements like Pb in soil can cause a great deal of health problem due to its high toxicity, and its accumulation in the human body can result in serious health issues like neurotoxicity, carcinogenicity, renal problems and diminished longevity in humans. Pb contaminated soil is very lethal for humans as well as animals since animals exposed to Pb were observed to develop severe health conditions like central disorders of nervous system, blindness, ataxia and convulsions. The maximum acceptable concentration for lead in food stuffs is around 1 mg/kg. Long-term exposure to lead can result in a buildup of Pb in the body and severe symptoms. These include anemia, pale skin, a decrease handgrip strength, abdominal pain, nausea, vomiting and paralysis of the wrist joint. Prolonged exposure may also result in kidney damage. If the nervous system is affected, usually due to very high exposure, the resulting effects include severe headache, coma, delirium and death. Continued exposure can lead to decreased fertility and/or increased chance of miscarriage or birth defects. Pb is present in exhaust gases mainly as lead halides and oxides, but incomplete combustion results in about 10% of alkyl Pb compounds also being present. Other sources of Pb emissions are Cu and Ni smelters, Fe and steel production. It exists in the oxidation states Pb^{+2} and Pb^{+4} with the divalent form being the more stable in most aquatic environments [24, 37]. In addition, the production of television picture tubes, pigments, petroleum fuels, printing, glass industries, photographic materials, etc., also adds pb^{2+} the environment. People living near hazardous waste sites may be exposed to Pb by breathing air, drinking water, eating foods or swallowing or touching dust or dirt that contains Pb. For others (people who do not live near hazardous waste sites), exposure to Pb may occur by eating foods or drinking water that contain Pb, by spending time in areas where leaded paints have been used, by working in jobs where Pb is used, by having hobbies in which lead may be used such as sculpturing (lead solder) and staining glass [75].

Iron

Iron is the fourth most abundant element in earth's crust (after oxygen, silicon, and aluminum). The mean iron content of soil, sediment, and rocks is about 5%. Most of the Fe in soils is present as iron oxides; in fact the typical soil colors (brown, red, yellow) are partly due to various iron oxides differing in their physical/chemical properties and their color. Fe, Al, and manganese oxide soil minerals are important sinks for heavy metals in soil and residual-amended soils [52, 76]. Fe can occur in either the divalent (Fe^{+2}) or trivalent (Fe^{+3}) states. Fe occurs predominantly as Fe^{+3} oxides in soils. The divalent state (or ferrous state) can be oxidized to the trivalent state (or ferric state), where it may form oxide or hydroxide precipitates, and become unavailable to plants as a micronutrient. Roots of some plants are able to reduce Fe from the ferric to the ferrous state, and allow iron uptake into the plant [43]. Fe has many important functions in plant growth and development, such as involvement in the biosynthesis of chlorophyll, respiration, chloroplast development and improves the performance of photosystems. It is an essential part of many enzymes. It also participates in the oxidation process that releases energy from sugars and starches and in response of that converting nitrate to ammonium in plant. It plays an essential role in nucleic acid metabolism .The availability of Fe in soils is affected by soil properties such as soil pH, calcium carbonate content, organic matter, accumulation of phosphorus, ion imbalance, soil texture, soil temperature, poor soil aeration, high humidity and soil compaction. Availability of Fe and most micronutrient is largely pH depended; availability decreases as pH increase. The lower the pH value of soil solution, causes the higher availability of soluble Fe [77, 78].Iron in drinking water is present as Fe^{2+} or Fe^{3+} in suspended form. It causes staining in clothes and imparts a bitter taste. It comes into water from natural geological sources, industrial wastes, and domestic discharge and also from byproducts. Excess amount of Fe (more than 10 mg/kg) causes rapid increase in pulse rate and coagulation of blood in blood vessels, hypertension and drowsiness [79]. Although most mineral soils are rich in Fe, the expression of Fe toxicity symptoms in leaf tissues occurs only under flooded conditions, which involves the micro-bail reduction of insoluble Fe^{+3} insoluble Fe^{+2}. Fe toxicity in tobacco, canola, soybean and Hydrilla verticillata are accompanied with reduction of plant photosynthesis and yield and the increase in oxidative stress and ascorbate peroxidase activity. The appearance of Fe toxicity in plants is related to high Fe^{+2} uptakes by roots and its transportation to leaves and via transpiration stream. The Fe^{+2} excess cause free radical production that impairs cellular structure irreversibly and damages membranes, DNA and proteins. Fe toxicity is not common, but some plants do secrete acids from the roots, which lowers soil pH. These plants can take up too much Fe, leading to toxicity [66].

Manganese

Manganese is a common trace element in the lithosphere with concentrations in rocks varying between 350–2000 mg/kg. Mn is naturally occurring in many surface and ground water sources and in soils that erode into these waters. Human activities are also responsible for much of this Mn contamination in water in some areas. It exists naturally in sediments and soils, primarily as a result of parent material weathering. The concentration of Mn in soils and sediments is affected by anthropogenic activities such as the use of chemical and organic (biosolids, animal manure, and similar organic waste) fertilizers in agricultural land, mining, and metal smelting[80]. Overexposure to Mn in the workplace and in food has been linked to toxic effects, including those on the heart, liver, lungs, reproductive system, and fetal development. Mn toxicity has also been linked to toxicity in other organ systems, including the central nervous system [81]. Mn is essential element required for various biochemical processes. The kidney and liver are the main storage place for the Mn in the body. Mn is essential for the normal bone structure. However, chronic exposure to higher doses is detrimental to human health .In higher doses Mn is toxic and its toxicity varies with route of exposure, chemical species, age, sex and animal species. The nervous system has been determined to be the primary target organ with neurological effects generally observed. Syndrome called Mn may result from chronic exposure to higher levels of

Mn. It is characterized among other symptoms, weakness, tremors, a masklike face and psychological disturbance [82].

2.7. Methods of analysis of heavy metal

Metals may be determined satisfactorily by a variety of methods; with the choice often depending on the precision and sensitivity required. Several spectrometric techniques have been used for macro and trace element determinations in plants or biological materials. The different techniques so far reported for the determination of metals in plant products are: Direct current argon plasma optical emission spectroscopy (DCP-OES), Flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption (GFAA), inductively coupled argon plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). These methods are most commonly used for the determination of materials because of their inherent selectivity, sensitivity, precision and accuracy [83]. For this study, the ICP-OES was used because of its availability, reproducibility and time efficiency.

2.8. 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 mgL^{-1} for approximately 70 elements [20]. 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) and soil.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Description of the study area

Dawunt Woreda is one of the 13 woreda of North Wollo Administrative Zone of Amhara National Regional State. The administrative center of the woreda is Kurba which is found 352 kms far from Bahirdar city and 192 kms from Woldia town (the main town of North Wollo administrative zone), and 593 km north west of Addis Ababa. The woreda is bordered on the south by, Koreb on the north by, Wadila, Tachgayint in the west and on the East by Delanta woreda. Astronomically the woreda is situated between 11°20'0''N-11°36'30''N latitude and 38^{0}33'30''E-39°0'30''E longitude. The average annual rainfall of the woreda is 600 mm with high variability between 250 mm (minimum) and 950 mm (maximum). Most of the rainfall falls during the ″summer″ season from June to September (it is most intense in July and august).The mean annual temperature is approximately 20 degree centigrade [84].

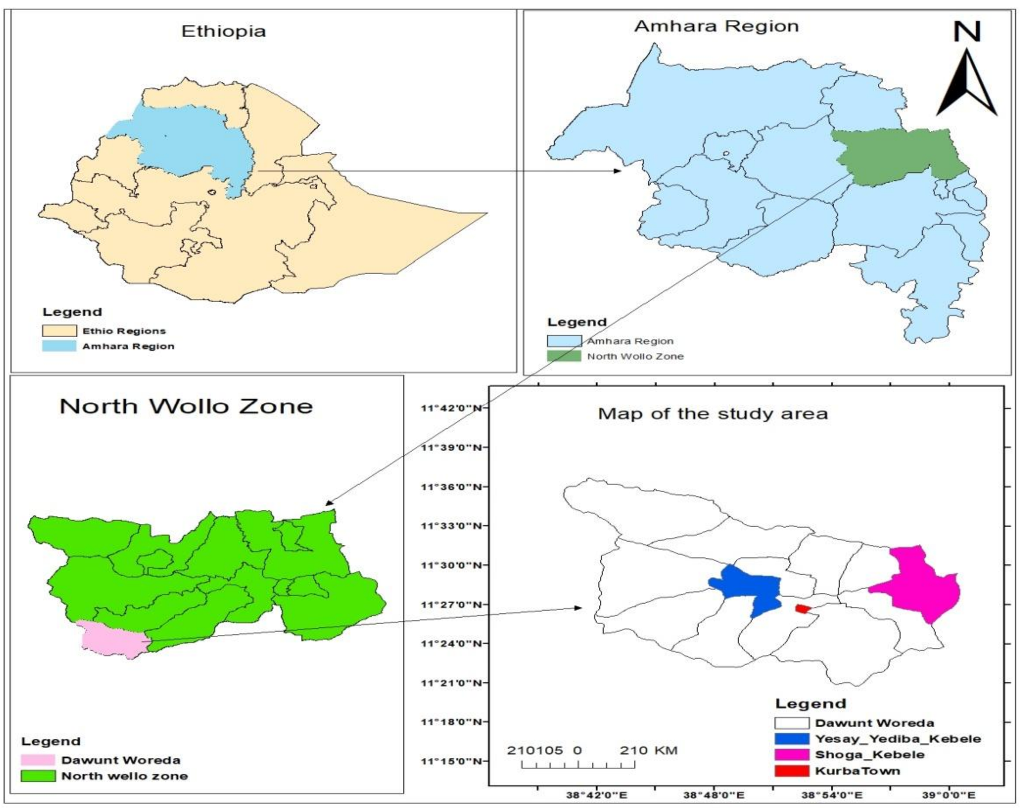

Figure 2: Map of the study area Source: Arc GIS (202

3.2 Research design

The experimental design which was used involved the determination of levels of selected heavy metals in both soil and lentil samples from three lentil growing kebeles namely Yesay-yediba, Shoga and Kurba. Samples were obtained from different farms randomly selected in each region, then dried, weighed and digested before the digests were analyzed for the chosen elements using ICP-OES. Sample preparation was carried out in the laboratory of chemistry, Woldia University. The concentrations of metal ions were determined by ICP-OES in Debrezeyt, Horticoop Ethiopia PLC Soil and Water Analysis Laboratory.

3.3. Instruments and apparatus

Stainless steel auger, Mortar and pestle, polyethylene bags, Beaker, Electric thermostatic heated dry box oven (model: 202AB, Analytical balance (ADAM, 110 g/0.1 g), measuring cylinders, pipette, and micropipette (Merck KGaA, Darmstadt, Germany), Whatman No. 42 filter paper , ICP-OES Spectro-Arcos (Model: ARCOS FHS12, USA) were used for the determination of metals in lentil and soil samples considered in this study.

3.4. Chemicals and Reagents

All reagents and chemicals used in this study were analytical grade. Distilled, Nitric acid, (69%, Merck, France), 37% HCl (Fine Chem. Industries Mumbai, France), $HClO_4$ (Fine Chem. Industries Mumbai, France), and extra pure hydrogen peroxide 30% H_2O_2, (Scharlau, European Union) , Stock standard solutions containing 1000 ppm of the metals Cd, Co, Cu, Fe, Mn, Cr and Pb were used in this study.

3.5 Cleaning Apparatus

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. They were washed with distilled water and dried in Electric thermostatic heated dry box oven (202AB) at 105^0C for 5 hours.

3.6 Sample collection and digestion

3.6.1 Soil samples

A total of 15 soil samples 500 g from each farm were collected in June 2022 from fifteen different agricultural lands that are used for cultivation of lentil using soil sampler. The soil samples were collected from topsoil at the depths of 0-20 cm using soil auger which represents the plough layer and average root zone for nutrients uptake and heavy metals burden by lentil plants[50].The collected samples then mixed thoroughly into one composite sample to get a representative sample of 1 kg. The sample was then placed in a polythene bag, sealed and labeled appropriately then transported to the laboratory. In the laboratory, soil samples were dried in an oven at 80ºC to a constant weight, ground using mortar and pestle, then passed through a 1.0 mm sieve and stored in labeled containers awaiting further analysis.

3.6.2 Soil sample digestion

The soil samples were dried in open air for one week, crushing and sieved mechanically using a 1.0 mm sieve. After which, 1.25 g of sample was digested with 20 mL aqua regia (HCl/HNO_3 3:1) in a beaker (open-beaker digestion) on thermostatically controlled plate. The digest were heated to near dryness and cooled to ambient temperature .Then 5.0 mL of hydrogen peroxide was added in parts to complete the digestion and the resulting mixture heated again to dryness in a fume cupboard. The beaker walls were washed with 10 mL of distilled and 5 mL HCl were added, mixed and heated again. The resulting digest was allowed to cool and transferred in to a 5 mL standard flask and made up to the mark with distilled water. Pb, Cd, Cu, Co, Cr, Mn and Fe heavy metal elements were then analyzed by direct aspiration of the sample solution in to Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) [85].

3.6.3 Lentil samples

Lentil seeds were sampled from local farmers in household level. About 300 g of lentil sample from fifteen households from each farmer were collected, and then combined to form a representative sample. After the composite sample were made, about 500 g of the lentil sample was taken and transported to the laboratory, and then the sample were washed with distilled water and then rinsed several times with de-ionized water. It was then dried in an oven at 70ºC for 24 hours. After cooling the samples were ground to fine powder and packed in clean, labeled and decontaminated plastic containers for further analysis.

3.6.4. Lentil sample digestion

5g of lentil seeds flour were weighed and transferred to a clean crucible, and dry-ashing process would be carried out in muffle furnace by step wise increase of the temperature up to 550^0C 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 95^0C 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. All the sample and blank were digested in triplicate [20].

3.7. Method validation

In order to validate the analytical method, Method detection limit (MDL), instrumental detection limit (IDL), limit of quantification and Recovery test were carried out [86].

3.7.1. Method detection limit (MDL)

Method detection limit is defined as the minimum concentration of analyte that can be measured. For the present study, the detection limit was found by three times the standard deviation of the mean reagent blank signal. Analyses of blank of all metals of interest were performed and the standard deviation of the blank reagents was calculated [87].

$LOD = 3 \times SD_b$ ……………………………………………………………………………….(1)

Where SD_b is the standard deviation of the method blank

3.7.2 Limit of quantification

The limit of quantification (LOQ) is the lowest concentration of an analyte in a sample which can be quantitatively determined with acceptable uncertainty. LOQ was obtained from triplicate analysis of seven method blanks which were digested in the same digestion procedure as the actual samples. The LOQ was calculated as [88].

$LOQ = 10 \times SD_b$ ……………………………………………………………………………...(2)

Where SD_b is the standard deviation of the method blank

3.7.3 Recovery test

Recovery is one of the most used techniques utilized for validation of the analytical results and evaluating how far the method is acceptable for its intended purpose. The validity of the digestion procedures for the elements Fe, Co, Cr, Cu, Pb, Mn and Cd was assured by spiking the samples with a standard solution of known concentration of the target analytes. Recovery was then calculated as [86]:

$$\%Recovery = \frac{\text{conc.in spiked sample} - \text{conc.in unspiked sample}}{\text{Actual spike conc.}} \text{ X } 100 \ \ldots\ldots\ldots\ldots(3)$$

3.8 Transfer factor (TF)

The transfer factor is an indicator of the potential transfer of heavy metals from the soil to the edible part of crops. TF value that is significantly higher than one indicates the high capacity of the plant to absorb metals from the agricultural soil. The TF for each heavy metal were estimated on Eq. (4) [89].

$$TF = \frac{\mathbf{Clentil}}{\mathbf{Csoil}} \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(4)$$

where Clentil and Csoil represent the heavy metal concentration in lentil and in agricultural soil (mg/kg dry weight), respectively.

3.9 Health risk assessment method

In this study, four exposure pathways associated with soil HMs were explored: soil particle ingestion, dermal contact, soil particle inhalation, and dietary intake of lentil. The estimated average daily intake of HMs (ADI) via soil ingestion (ADI_{ing}), dermal contact (ADI_{dermal}), inhalation (ADI_{inh}) and food intake (ADI_{food}) was as follows [90, 91].

$$ADI_{ing} = \frac{C \, X \, R_{ing} \, X \, EF \, X \, ED}{BW \, X \, AT} X10^{-6} \ldots\ldots (5)$$

$$ADI_{inh} = \frac{C \, X \, R_{inh} \, X \, EF \, X \, ED}{PEF \, X \, BW \, X \, AT} \ldots\ldots (6)$$

$$ADI_{der} = \frac{C \, X \, SA \, X \, AF \, X \, ABS \, X \, EF \, X \, ED}{BW \, X \, AT} X10^{-6} \ldots\ldots (7)$$

$$ADI_{len} = \frac{C \, len \, X \, IngR \, X \, EF \, X \, ED}{BW \, X \, AT} X10^{-6} \ldots\ldots (8)$$

Where ADI_{ing} = average daily intake through ingestion, ADI_{inh} = average daily intake through inhalation, C = exposure-point concentration, mg/kg; IRS = ingestion rate, 100 EF = exposure frequency, 350 days/year ED = exposure duration, 30 years CF = units conversion factor, 10^{-6} kg mg^{-1}. SA = exposure skin area, 5700 cm^2. AF = adherence factor, 0.07 mg cm^{-2} ABS = dermal absorption fraction, 0.01 BW = body weight, 65 kg AT = averaging time for no carcinogens, 365 x ED InhR = inhalation rate 20 m^3 /day [21,92 , 93].

3.9.1 Hazard quotient (HQ)

Hazard quotient (HQ) was determined on the basis of ADI from ingestion (ADI_{ingest}), dermal (ADI_{dermal}), and inhalation (ADI_{inhal}), it was calculated by dividing the average daily dose to a specific reference dose (RfD) [43]. The equation used for estimating the HQ is as follows:

$$HQ = \frac{ADI}{RFD} \ldots\ldots (9)$$

Where: HQ is the target hazard quotient, ADI is the chronic daily intake of heavy metal (mg/kg), and RfD is the reference dose (mg/kg/day. If the target hazard quotient (HQ) is higher than (HQ

> 1), there will be a severe health hazard to human, whereas there will be no severe human health effects (HQ< 1) [94].

3.9.2 Hazard index (HI)

To assess the overall potential for no carcinogenic effects from more than one heavy metal, a HI has been formulated based on the guidelines for health risk assessment of chemical mixtures([95]. If the values of HI > 1, it means that there are possible adverse health effects; if HI < 1 1, it indicates the reverse [92].

$$HI = \sum_{K=1}^{n} HQ \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(10)$$

3.9.3 The total cancer risk (CR)

The cancer risk is assessed by the total cancer risk (CR) values of heavy metals, which is the product of the daily exposure intake (ADI) and its corresponding cancer slope factor (CSF). The CR is calculated as Eq. (11).

$$CR = ADI \times CSF \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(11)$$

Where: CR= Cancer risk over a lifetime by individual heavy metal ingestion, CSF = oral cancer slope factor in (mg/kg/day).

If the values of CR are greater than 10^{-4} , it indicates the cancer risk effects human health; if CR is lower than 10^{-6} , it means the reverse[43].

Table 1. RfD and CSF value of heavy metals [96].

Heavy metals	Cd	Fe	Co	Cu	Mn	Cr	Pb
RFD value in mg/kg/day	$5X10^{-4}$	$7X10^{-2}$	$3\ x10^{-4}$	$4X10^{-2}$	$4.1X10^{-1}$	$3X10^{-4}$	$3.5\ X10^{-3}$
CSF value in mg/kg/day	$3.8X10^{-1}$	-	-	-	-	$5X10^{-1}$	$8.5X10^{-3}$

3.10. Data Analysis

The data derived from various determinations was subjected to statistical analysis by using Pearson Correlation. It used to correlate the different metals within the same soil or lentil. Calculations (such as mean, standard deviation, and percent recovery) were done using SPSS software and scientific calculators.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Calibration Curves

Calibration curves for Fe, Cd, Mn, Pb, Cr, Co and Cu were obtained by using suitable standard solutions prepared from stock solutions. The serious of standard solutions, correlation coefficient and the calibrations equations for each metal were given in Table 2. Correlation coefficients of the elements were determined using prepared standards concentration versus their corresponding intensity. Thus, the correlation coefficients of all calibration curves were ≥ 0.995, which shows that there was a very high positive correlation between concentration and intensity, and therefore were employed in determining the unknown concentrations of the respective metals.

Table 2. Correlation coefficients and equations of the calibration curves.

Heavy metals	Concentration Standards (mg/L)	Correlation coefficient (R^2)	Calibration equation
Cu	0.5,1.0,1.5,2,2.5,3.0	0.9994	$y = 3946.2x - 174.25$
Mn	0.5,1.0,1.5,2,2.5,3.0	0.9995	$y = 37553x + 192.97$
Cr	0.2,0.4,0.6,0.8,1.0,1.2,1.4,1.6	0.9967	$y = 66517.88x - 111.34$
Fe	1,2,3,4,5,6	0.9967	$y = 10400x + 780.2$
Co	0.5,1.0,1.5,2,2.5,3.0	0.9945	$y = 9378.9x - 327.9$
Cd	0.2,0.4,0.6,0.8,1.0,1.2,1.4,1.6	0.9968	$Y=16819.45X-256.92$
Pb	0.2,0.4,0.6,0.8,1.0,1.2,1.4,1.6	0.9958	$Y=901.5X+3.09$

4.2 Recovery, MDL, IDL and LOQ

Table 3 showed that the experimental results of MDL and MQL. As shown in the table the MDL is greater than the detection limit of the instrument (ICP-OES). This is a clear indication that the instrument used was in good sensitivity for the analysis of the concentration of the heavy metals in the sample.

Table 3. The values of instrumental detection limit (IDL), method detection limit (MDL) and limit of quantification (LOQ)

Heavy metal	IDL	MDL	LOQ
Cu	0.001	0.003	0.01
Fe	0.002	0.171	0.57
Mn	0.002	0.009	0.03
Cd	0.001	0.003	0.01
Co	0.007	0.009	0.01
Cr	0.001	0.006	0.02
Pb	0.002	0.006	0.02

The percentage recoveries of heavy metals (Mn, Co, Cu, Cr, Cd, Pb, and Fe) obtained for soil and lentil samples in this work was given in Table 4 and Table 5, respectively. The results of percentage recoveries for the studied metal in soil and lentil samples were within the acceptable range (80 to 120%). The results for the elements indicating good accuracy for the analysis procedure[86].These results therefore confirmed that the reliability of the method for the determination of the metals (Mn, Cd, Co, and Cr Pb, Cu, and Fe).

Table 4. Recovery test of soil sample

Heavy metals	Unspiked Amount(mg/kg)	Amount added in(mg/kg)	Amount after spiked(mg/kg)	%Recovery
Mn	19.850±0.35	7.94	27.28±1.967	93
Fe	649.38±3.73	259.7	1864.9±6.10	83
Co	3.317±0.076	1.32	4.6±0.229	98
Cu	40.020±0.018	16.0	54.4±4.534	90
Cd	15.160±0.093	6.0	20.26±0.135	90
Pb	1.833±0.029	0.7	2.5±1.409	100
Cr	69.063±1.931	27.6	92.76±0.368	86

Table 5. Recovery test of lentil sample

Heavy metals	Unspiked Amount(mg/kg)	Amount added in(mg/kg)	Amount after spiked(mg/kg)	%Recovery
Mn	9.686±0.013	3.8	13.4±0.130	98
Fe	60.4±0.09	24.16	81.66±0.31	88
Co	0.75±0.005	2.0	19.84±0.016	95
Cu	5.70±0.004	4.0	40.62±0.053	87
Cd	0.25±0.004	2.0	19.17±0.008	94
Pb	0.35±0.002	2.0	19.48±0.027	95
Cr	1.15±0.003	1.5	15.12±0.012	93

4.3 Levels of selected heavy metals in soil and lentil samples

The mean levels of Cd, Cu, Co, Pb, Mn, Fe and Cr in soil and lentil samples from lentil growing Dawunt Woreda farm lands are given in Table 6.

Table 6. Concentration of heavy metals in soil and lentil samples (mg/kg ± SD) along with the recommended concentration by WHO/FAO (mg/kg)

Heavy metals	lentil sample	WHO/FAO	soil sample	WHO/FAO
Pb	0.35±0.002	1.5	1.833 ±0.029	100
Co	0.75±0.005	8	3.317±0.076	50
Cd	0.25±0.004	0.1	15.160±0.093	3
Cr	1.15±0.004	1.3	69.063	100
Cu	5.70±0.004	10	40.20±0.018	100
Mn	9.68±0.013	25	19.850±0.350	2,000
Fe	60.4±0.09	425	649.38±3.733601	50,000

4.3.1 Concentration of heavy metals in soil sample

The concentration of total heavy metals (Fe, Mn, Cu, Co, Cd, Cr and Pb) in soil samples are presented in (Table 6). The mean concentration of heavy metals decreased in the order of: Fe > Cr > Cu > Mn > Cd > Co > Pb. The mean concentration of these selected heavy metals in soils of this study was also compared to other study conducted in Ethiopia and other countries as follows: Lead concentration in this study was lower than 203 mg/kg in agricultural soil in china [97], 118.36 mg/kg road dust in Pakistan [91]. While, higher than 0.013 mg/kg farmlands in Egypt [80], lower than 46.47 mg/kg in agricultural soils of Central Ethiopia [41]. And also level of Co found in this study was lower than 22.20 mg/kg in agricultural soil in Iraq [98] and 106.6, 167.5, 177.6, and 140.7 mg/kg in sample sites of Debre Markos, Dejen, Bichena, and Debre Werk, respectively in soils used for cultivation of garlic in East Gojjam Zone [88].

The levels of Cd in this study was lower than 27.93 - 45.33 mg/kg reported in soil samples around Eastern Industrial Zone in Dukem, Ethiopia [87]. However, the levels of Cd in this study was higher than, 1.20 mg/kg in agricultural soils near the industrial areas of Bangladesh [71]. Cd concentration also higher than (2.5 mg/kg, 2.3 mg/kg, and 2.4 mg/kg) in soil samples of Debre Markos, Dejen , and Debre Werk, respectively [88]. The concentration Cr found in this study was higher than agricultural soils in Pakistan 1.222 to 1.310 mg kg^{-1} [59], (1.50 –2.86) mg/kg investigated in the city of Debre Markos [99] and 5.78 mg/kg in Bangladesh [71].

Cu concentration were lower than70 mg/kg agricultural soils in Italy [100], 62.4 mg/kg in Brazil [101], 106.5 mg/kg in the city of Debre Werk [88]. Likewise, concentration levels of Mn in this study was higher than the values (16.4 mg/kg) obtained farmlands of Indonesia [102] .but lower than agricultural soils in mojo area 1696.67 mg/kg [78]. As well as, the mean concentration of Fe levels was also compared to other study conducted in other countries and Ethiopia. The level of iron were lower than in Urban Soil of Western Iraq were 24718 mg/kg [103], (4642 mg/kg) reported in soil around mojo area [78]. However, lower values of iron (280 mg/kg) have also been reported in agricultural soils in Nigeria [104].

The concentration of Fe was highest among the heavy metals analyzed from the present investigation and the levels obtained were found to be 649.38 mg/kg. The high level of Fe might be due its abundance because iron is the fourth most abundant element in the earth's crust. Results from this study showed that concentration value of Cu, Cr, Pb, Fe, Co and Mn, in the samples were generally lower than the WHO/FAO maximum permissive limits. The low levels of heavy metals are indicative of low levels of contamination of farmlands were cultivated by these heavy metals in spite of increasing use of fertilizers and pesticides in modern agriculture, as well as emissions from other anthropogenic sources. Even though these heavy metal concentrations fell below the critical permissible concentration level in most heavy metals, it seems that their persistence in the soils of the study site may lead to increased uptake of these heavy metals by plants. However, the level of Cd in the studied soil samples exceeded the WHO target value [105] implying that there was Cd contamination in soil. This reason for this might be due to use of manure and phosphate fertilizer.

Generally all the levels of all analyzed elements in this investigation are different from other findings in the literature. The possible reason for this might be due to: soil type, climatically variation, types of fertilizers and pesticides used by farmers.

4.3.2 Concentration of heavy metals in lentil sample

The levels of Pb in this study were lower 1.1348 mg/kg than lentil samples in north shewa [20] and 0.142 to 0.176 mg/kg in lentil grain samples collected from (Dejen area (Gojjam), Welenchitti area (East Shewa) and Menz-mama Midir)Ethiopia [106]. While ,the level of Pb are higher than Pea sample which has a value of 0.13 mg/kg were obtained in Nigeria [47]. In addition to this the levels of Co in this study are higher than (0.3 mg/kg) in lentil grain samples collected from (Dejen area (Gojjam), Welenchitti area (East Shewa and Menz-mama Midir) Ethiopia [20]. The levels of Cadmium in this study were higher than 0.1515 mg/kg reported in lentil samples in north shewa [20] and lower than 0.31 mg/kg reported in lentil grain samples collected from (Dejen area (Gojjam), Welenchitti area (East Shewa and Menz-mama Midir) Ethiopia [106]. The level of Cd also lower than 0.75 mg/kg reported in pea samples in Nigeria [47] and higher than 0.004 mg/kg in bean sample in Nigeria [3].

In the present investigation the concentration of Cr were higher than those obtained from lentil sample in north shewa zone 0.288 mg/kg [20] and lower than 17.38 mg/kg have been reported in pea from Nigeria [3]. The levels of Cu in lentil obtained in the present investigation were higher than 0.7613 mg/kg reported in lentil samples in north shewa [20] and 0.27 mg/kg reported in lentil grain samples collected from (Dejen area (Gojjam), Welenchitti area (East Shewa and Menz-mama Midir) Ethiopia [106]. The levels of Cu obtained in this study are also higher than those obtained from pea in Nigeria which was 0.75 mg/kg [47] and 0.046 mg/kg recorded in bean samples in Nigeria [3]. The concentration of Mn in lentil sample were higher than the value 6.95 mg/kg reported in lentil grain samples collected from (Dejen area (Gojjam), Welenchitti area (East Shewa) and Menz-mama Midir)Ethiopia[106] and bean in Nigeria which was 0.0049 mg/kg [3]. The levels of Fe in this study were lower than 64.6 mg/kg reported in lentil grain samples collected from (Dejen area (Gojjam), Welenchitti area (East Shewa and Menz-mama Midir) Ethiopia [106] and 4.8313 mg/kg reported in lentil samples in north shewa [20]. The level of Fe also higher than 0.031 mg/kg in bean samples found in Nigeria [3].

Outcomes from this study revealed that concentration value of Cu, Cr, Pb, Fe, Co, Cd and Mn, in lentil sample were generally lower than the WHO/FAO maximum permissive limits [105]. The low levels of heavy metals are indicative of low levels of contamination by these heavy metals in spite of increasing use of fertilizers and pesticides in modern agriculture.

Generally all the levels of all analyzed elements in this investigation are different from other reports in the literature. The possible reason for this is probably due to differences in instrumentation and soil type. Furthermore, different crops vary in their ability to accumulate heavy metals in their tissues as well as cultivation of different lentil species and application of different methods of farming.

4.4. Transfer factor

All TF values of Mn, Fe, Co, Cu, Cr, Pb, and Cd were given in Table 7. Thus, TF values were ranged from 0.017 to 0.48, where all the values were below 1. This implies that these metals to transfer from the soils to the plant is low, that is metal bioavailability in the soil might be very low. The transfer factors of all elements are within normal range in plant [107].

Table 7. Transfer factors from soil to lentil

Element	Transfer factor
Mn	0.48
Fe	0.09
Co	0.23
Cu	0.14
Cd	0.017
Pb	0.19
Cr	0.017

4.3 Health risk analysis

The total daily intakes (total chronic daily intake [ADI]) and HI of agricultural soils through three exposure pathways are shown in Tables 1 and 2.

Table 8. ADI of heavy metals from soil and lentil crop via three exposure routes

Heavy metals	Fe	Mn	Cu	Co	Cd	Pb	Cr
Ingestion	$9x10^{-4}$	$2.9\ x10^{-5}$	$5.9\ x10^{-5}$	$4.8x10^{-6}$	$2.2x10^{-5}$	$2.6\ x10^{-6}$	$1\ x10^{-4}$
Dermal	$3.8x10^{-5}$	$1.1x10^{-6}$	$2.3\ x10^{-6}$	$1.9x10^{-7}$	$8.9x10^{-7}$	$1x10^{-7}$	$4\ x10^{-6}$
Inhalation	$1.9x10^{-7}$	$5.8\ x10^{-6}$	$1.1x10^{-8}$	$9.7x10^{-10}$	$4.4\ x10^{-9}$	$5.3x10^{-10}$	$2\ x10^{-8}$
Lentil ingestion	$5\ x10^{-6}$	$7.9\ x10^{-7}$	$4.6\ x10^{-7}$	$6.1\ x10^{-8}$	$2\ x10^{-9}$	$2.8\ x10^{-8}$	$9.4\ x10^{-9}$

The distribution of total ADI of heavy metals in soils was ranked as follows: Fe > Cr > Cu> Mn > Cd> Co > Pb through ingestion. Fe > Cu > Mn > Cr > Cd> Co > Pb Dermal contact and through inhalation Fe > Mn > Cu > Co > Pb> Cr > Cd. The ADI values were highest for oral ingestion in adult inhabitants. The ingestion of soil particles appeared to be the main exposure pathway for heavy metals in adults. The dermal contact and inhalation were the second and third mostly likely pathways.

Table 9. HQ of heavy metals via three exposure routes and lentil crop through ingestion

Heavy metals	Fe	Mn	Cu	Co	Cd	Pb	Cr
Ingestion	$1.2X10^{-2}$	$7X10^{-5}$	$4\ X10^{-3}$	$1.6\ X10^{-2}$	$1.7\ X10^{-3}$	$7.4\ X10^{-4}$	$3.3\ X10^{-1}$
Dermal	$5.4\ X10^{-4}$	$2.7\ X10^{-6}$	$5.7\ X10^{-6}$	$6\ X10^{-4}$	$1.8\ X10^{-4}$	$2.9\ X10^{-5}$	$1.3\ X10^{-2}$
Inhalation	$2.7\ X10^{-6}$	$1.5\ X10^{-7}$	$2.8\ X10^{-5}$	$3.2\ X10^{-7}$	$1.8\ X10^{-4}$	$2.9\ X10^{-4}$	$1.3\ X10^{-2}$
HI	$1.2X10^{-2}$	$7.3\ X10^{-5}$	$4X10^{-3}$	$1.6\ X10^{-2}$	$2.1\ X10^{-3}$	$7.7X10^{-3}$	$3.6\ X10^{-1}$
Lentil ingestion	$7.1\ x10^{-5}$	$1.9x10^{-7}$	1.210^{-5}	$2x10^{-5}$	$5.7\ x10^{-9}$	$8x10^{-6}$	$3.1\ x10^{-7}$

The potential non-carcinogenic toxic effects posed by toxic metals are usually characterized by calculating HQ. The HQs of individual metal are presented in Table 9. The noncancerous health risks related to individual element exposure through soil ingestion, dermal contact, and inhalation was low for all the investigated metals resulted in a HQ < 1, indicating low risk for adults. The combined effects of exposed metals and were calculated as HI and the data indicated that the HI values were also lower than one. Therefore, when considering the total exposure HI of ingestion, dermal contact, and inhalation, there was lower chance of having noncancerous risk for adult's health at the studied sites.

Table 10. Carcinogenic risk of adult via three exposure routes in soil and lentil crop through ingestion

Inhabitant	Exposure pathways	Cd	Cr	Pb
Adult	Ingestion	2.2×10^{-9}	5×10^{-7}	9.8×10^{-7}
	Dermal contact	8.9×10^{-11}	2×10^{-7}	3.8×10^{-8}
	Inhalation	4.4×10^{-11}	1×10^{-9}	2×10^{-11}
	Total risk	2.3×10^{-9}	7×10^{-7}	1×10^{-7}
	Lentil ingestion	2×10^{-11}	4.5×10^{-8}	1×10^{-8}

As presented in Table 10, the carcinogenic risks from Cd, Cr and Pb at all sites via ingestion, dermal contact, and inhalation were below acceptable ranges. The carcinogenic risks of Cd, Cr and Pb due to exposure from studied soil via ingestion, dermal contact, and inhalation pathways can be negligible at Dawunt farmland. Among the three exposure pathways, the ingestion of soil seems to be the major pathway of exposure to hazardous elements followed by dermal contact and inhalation. Hazardous elements could be accumulated in human for a long time and especially no cancer adverse effects of these toxic metals to the tissues of adult inhabitant can become more serious. According to the result of this study, health risk for adult due to toxic metal exposure through soil could not be observed.

4.4 Statistical analyses

4.4.1 Pearson correlation of metals

The correlation between two variables reflects the degree to which the variables are related. The most common measure of correlation is the Pearson Product Moment Correlation (called Pearson's correlation in short) has been used in this study. High correlation coefficient (near +1 or −1) means a good relation between two variables, and its concentration around zero means no relationship between them at a significant level of 0.05% level, it can be strongly correlated, if $r > 0.7$, whereas r values between 0.5 and 0.7 shows moderate correlation between two different parameters. [90].

Table 11. Pearson correlation of heavy metals in lentil

	Correlations						
	Fe	Cr	Co	Cu	Cd	Pb	Mn
Fe	1						
Cr	-0.982	1					
Co	-0.756	0.619	1				
Cu	0.786	-0.655	-0.999*	1			
Cd	0.000	-0.189	0.655	-0.619	1		
Pb	-0.918	0.826	0.954	-0.967	0.397	1	
Mn	0.997*	-0.993	-0.705	0.737	0.075	-0.885	1

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

As can be seen from Table 11, the results of the correlation coefficients showed strong positive correlation between Cu with Fe ($r = 0.786$), Pb with Cr ($r = 0.826$), Pb with Co ($r = 0.95$), Cu with Mn ($r = 0.737$), and Pb with Fe ($r = 0.736$). This strong positive correlation shows that the elements are closely associated, thus suggesting their common origin.

There were also moderate positive correlations Co with Cr ($r = 0.619$) and Co with Cd ($r = 0.655$). Strong negative correlations were found between Cr with Fe ($r =-0.982$), Pb with Fe ($r = -0.918$), Mn with Cr ($r = -0.993$), Cu with Co (r -0.999^*), and Mn with Co (r -0.705). Moderate negative correlations were found between Cd with Pb($r = -0.967$) and Mn with Pb($r = -0.885$). The other elements have weak negative or positive correlation indicating that the presence or absence of one element affect in lesser extent to the other. [24].

Table 12. Pearson correlation of heavy metals in soil

	Fe	Cr	Co	Cu	Cd	Pb	Mn
Fe	1						
Cr	0.659	1					
Co	-0.99*	-0.694	1				
Cu	-0.860	-0.182	0.834	1			
Cd	-0.034	0.730	-0.014	0.539	1		
Pb	0.960	0.420	-0.945	-0.969	-0.314	1	
Mn	0.972*	0.818	-0.982	-0.715	0.203	0.866	

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

From Table 12, the results of the correlation coefficients showed strong positive correlation between Pb with Fe (r =0.960), Mn with Fe (r = 0.972*), Mn with Cr (r = 0.818), Mn with Pb (r = 0.866), Cd with Cr (r = 0.730). This strong positive correlation shows that the elements are closely associated, thus suggesting their common origin. There were also moderate positive correlations Cr with Fe (r = 0.659) and Cd with Cu (r = 0.539). Strong negative correlations were found between Co with Fe (r =-0.999*), Cu with Fe (r = -0.860), Pb with Co (r = -0.945), Pb with Cu (r -0.969) and Mn with Co (r -0.982). Moderate negative correlations were found between Co with Cr (r = -0.694). The other elements have weak negative or positive correlation indicating that the presence or absence of one element affect in lesser extent to the other.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

In this study tried to examine the levels of seven selected heavy metals and health risk assessment in lentil crop and the soil where it has grown collected from Dawunt Woreda, north Wollo, Ethiopia. The concentration of metals (Fe, Mn, Cu, Co, Cr, Cd and Pb) have been analyzed by Using inductively coupled plasma optical emission spectrometer. The mean concentrations of these heavy metals in the digested lentil samples were in mg/kg; Mn (9.75-9.77), Fe (59.4-60.9), Co (0.70-0.79), Cu (5.66-5.74), Cd (0.24-0.25), Pb (0.84-0.94) and Cr (1.13-8). For soil sample were in mg/kg; Mn (19.50-20.20), Fe (645.2-652.50), Co (3.25-3.40), Cu (40.00-40.40), Cd (15.05-15.22), Pb (1.80-1.85) and Cr (67.75-71.28). The levels these heavy metals in lentil and the soil where it has grown were found below the standard limit value of WHO/FAO except Cd in soil exceeds the safe limit. The human health risk assessment revealed that ingestion was the main pathway of exposure to HMs. The non-carcinogenic and carcinogenic risks of the total analyzed metal were both lower than maximum threshold value.

The recoveries for the seven analyzed metals were between 80 and 120% which revealed that an acceptable digestion method for the analysis of lentil and soil samples. Pearson correlation indicated that there is positive and highly significant correlation (r = 0.972*), between heavy metal Mn with Fe in lentil and soil.

5.2 RECOMMENDATIONS

• The intakes of the selected heavy metals due to consumption of lentil pose no health risk.

• The levels of heavy metals in soil and lentil crop are below WHO standard limit except Cd in soil sample. Since, the level of Cd exceed the safe limit farmers should minimize the consumption of phosphate fertilizer in agricultural activities.

This study covered only seven elements. More heavy metals other than the one considered in this study as well as major elements should be analyzed in order to provide a comprehensive data on the metal profile of the lentil crop and soil. Further investigation should be carried out on physical and chemical composition of soils.

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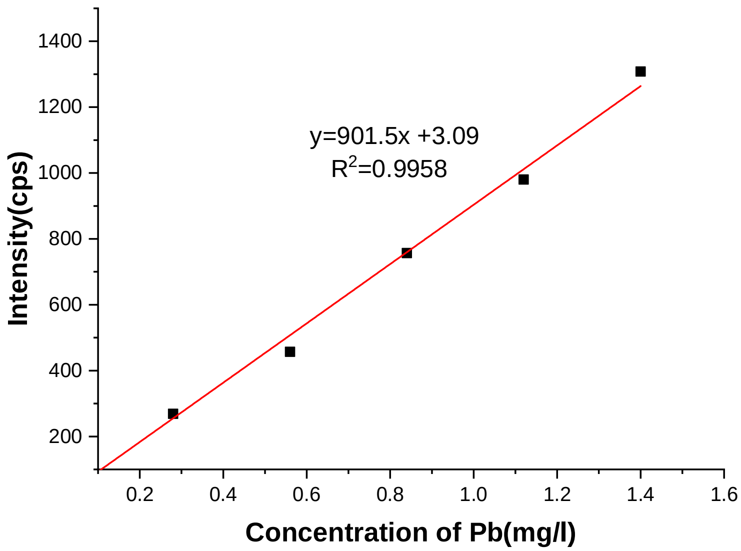
7. Appendix



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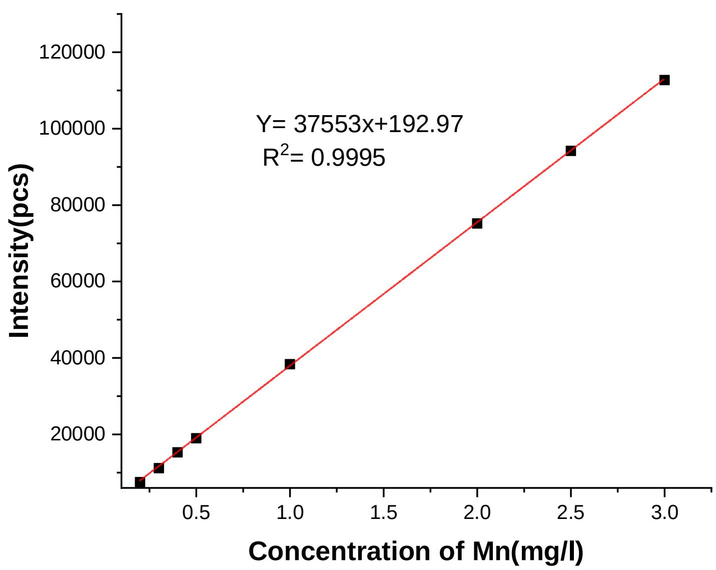
R^2=0.9958
Intensity(cps)
1400
1200
1000
800
600
400
200
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Concentration of Pb(mg/l)



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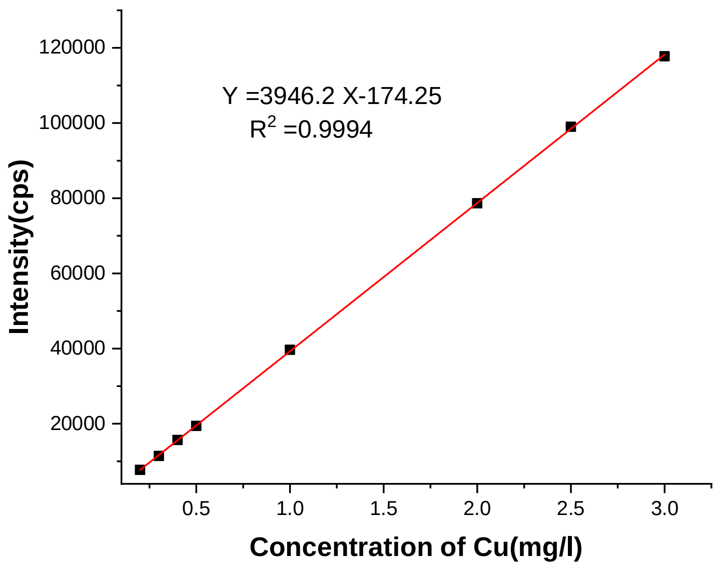
Intensity(pcs)
120000
100000
80000
60000
40000
20000
0.5
1.0
1.5
2.0
2.5
3.0
Concentration of Mn(mg/l)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R2 =0.9994
Intensity(cps)
Concentration of Cu(mg/l)
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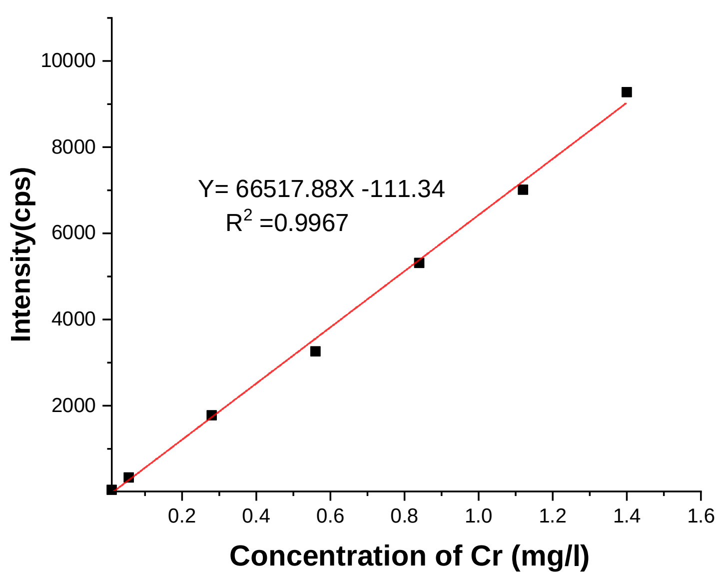
1.5
2.0
2.5
3.0
20000
40000
60000
80000
100000
120000


Y= 66517.88X -111.34
R2 =0.9967
Intensity(cps)
Concentration of Cr (mg/l)
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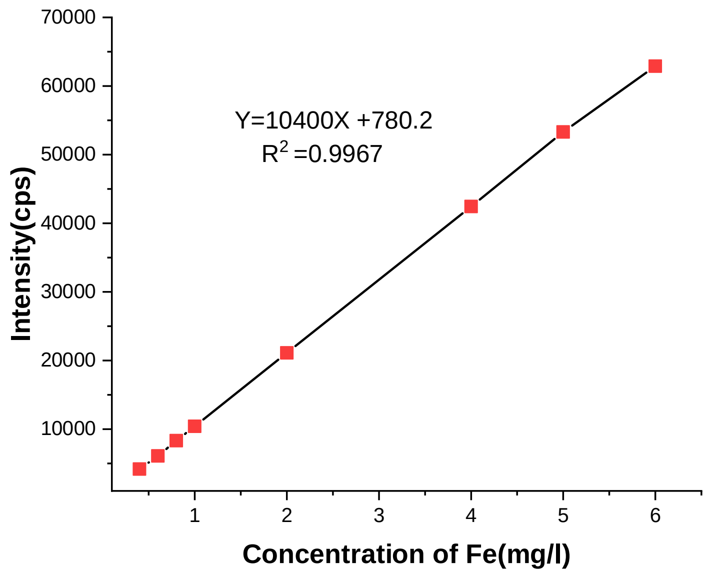
0.4
0.6
0.8
1.0
1.2
1.4
1.6
2000
4000
6000
8000
10000



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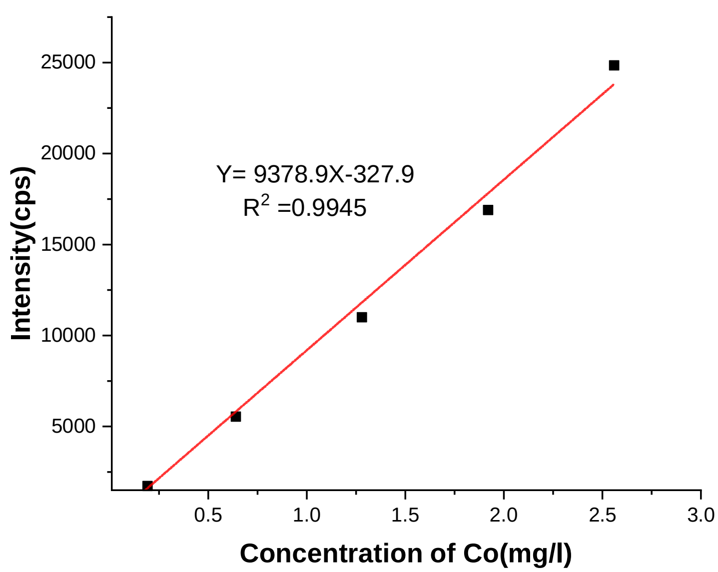
Intensity(cps)
Concentration of Fe(mg/l)



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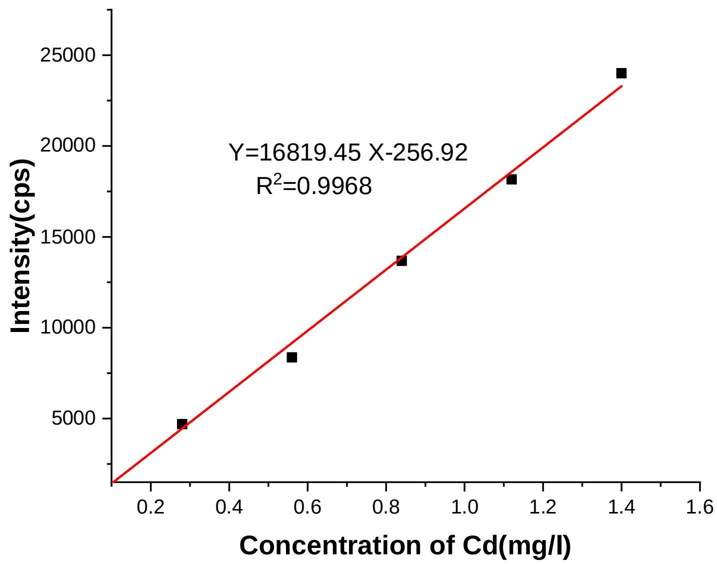
R^2 =0.9945
Intensity(cps)
Concentration of Co(mg/l)



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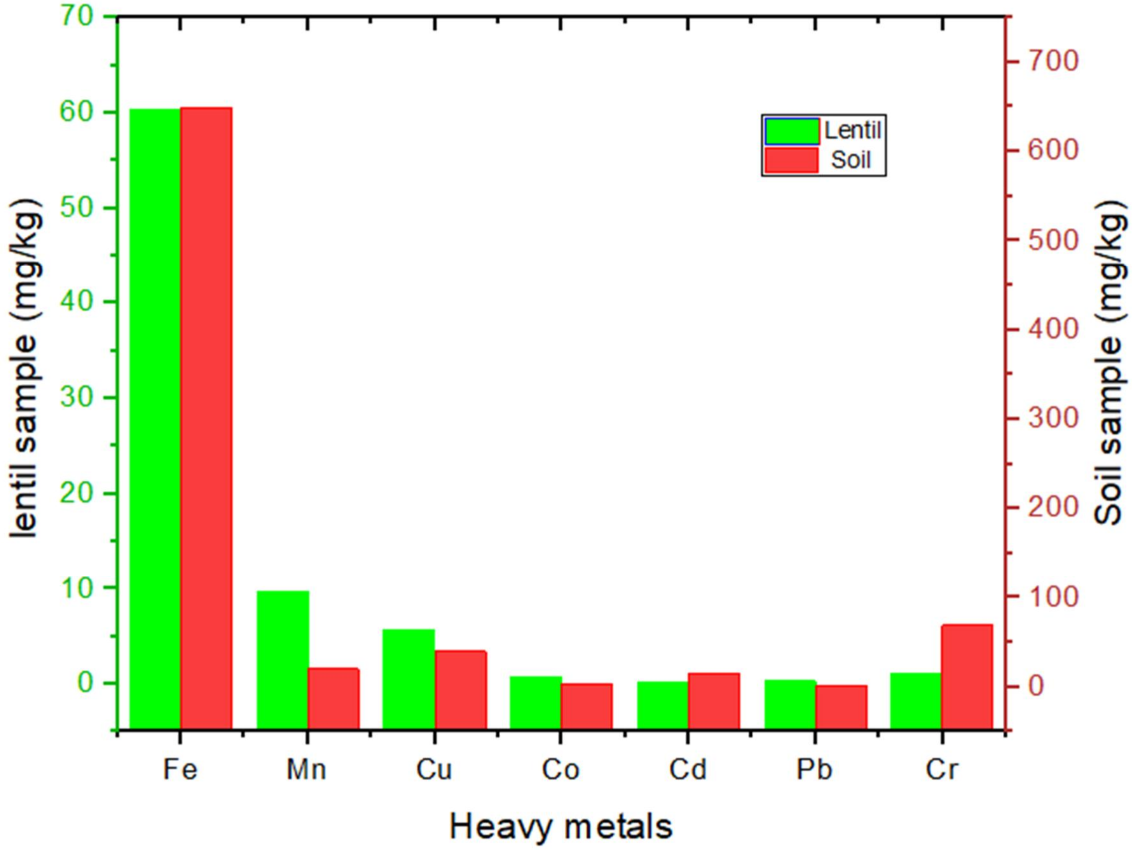
Intensity(cps)
Concentration of Cd(mg/l)



Lentil
Soil
lentil sample (mg/kg)
Soil sample (mg/kg)
Fe
Mn
Cu
Co
Cd
Pb
Cr
Heavy metals