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MSc THESIS

Comparative Determination of Heavy Metals in Flower Farm and Road side Soils Collected from Holeta by using Flame Atomic Absorption Spectrophotometry

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Declaration

I certify that the research for this thesis, Comparative Determination of Heavy Metals in Flower Farm and Roadside Soils Collected from Holeta by Using Flame Atomic Absorption Spectroscopy, was done entirely by me under the guidance of Dr. Merid Tessema at the Chemistry Department of Addis Ababa University. All sources of information used in this thesis have been properly cited.

I solemnly declare that this thesis is not being submitted to any institution, anywhere, for the award of any academic degree, diploma, or certificate.

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This thesis has been submitted in partial fulfillment of the criteria for a master's degree in science at Addis Ababa University and has been placed in the department of chemistry to be available to the borrower under the library rule.

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List of abbreviations

ANOVA	Analysis of Variance
CVD	Cardiovascular Disease
DW	Dry Weight
EDC	Endocrine Disruptors
FAAS	Flame Atomic Absorption Spectrophotometry
GF-AAS	Graphite Furnace Atomic Absorption Spectrophotometry
HMI	Heavy Metal Ions
ICP-MS	Inductively Coupled Plasma- Mass Spectrophotometry
LOD	Limit of Detection
LOQ	Limit of Quantification
RDA	Recommended Dietary Allowance
ROS	Reactive Oxygen Species
RSD	Relative Standard Deviation
UIL	Upper Intake Limit
WHO	World Health Organization

Abstract

In this study the concentration of heavy metals: Mn, Cu, Zn, Ni, Cd, Pb, Fe, Mg and Ca were determined in soil by flame atomic absorption spectrophotometry (FAAS). The soil samples were collected from a flower farm (Holeta) and from the roadside around the flower farm. After proper sample preparation, a 0.5 g of dried powdered soil sample was digested with 3ml of HNO_3, 1ml of HCl at 240 ^{0}C for 2:45 hours and the concentration of the selected heavy metals were determined by flame atomic absorption spectrophotometry. The results showed that the soil samples in the two sampling areas contain high amount of iron and magnesium. As exception zinc being which is higher in the flower farm than on the road side, the mean metal concentration (mg/kg dry weight) in both soil samples are as follows: Fe (8807-9128) > Mg (1023-1119) > Mn (399-964) > Ca (60-118) > Zn (96.1-150.5) > Cr (34-48.54) > Cu (30.5-35.6) > Ni (21-33.43). A small amount of nickel was observed in both sample sites, while cadmium and lead were below the limit of detection. The detected metals in the present study were also compared with WHO recommended maximum permissible limit. Fe, Mg, Mn, Ca and Zn were above the WHO standard limit, but Cu, Ni and Cr were below the recommended permissible limit.At 95% confidence level between the two samples collected from the two sample sites the value obtained from ANOVA indicates that there is a significant difference among the mean concentrations of all the selected metals ($P<0.05$).

Key words*:* Soil, Heavy metal, Atomic Absorption Spectrometry (AAS) Flower farm, Environmental pollution, Pesticides, Fertilizer.

1. INTRODUCTION

1.1 Background of the study

Metals are natural constituents in nature. Metals are substances that readily donate electrons to form cations. Metals occur spontaneously in the earth's crust, and their composition varies around the world, which causes geographical changes in surrounding concentrations. Heavy metals are trace elements that are about five times denser than water and have a higher atomic weight that occur naturally in the earth's crust as oxides, carbonates, and sulphides [1]. The characteristics of each metal and numerous environmental conditions are used to monitor the distribution of metals in the atmosphere [2]. Most HMIs are dangerous environmental hazards and harmful to people [3]. With increased industrialization and its reliance on fossil fuels, urban development, and growth in agriculture, remediation of the environment contaminated with HMIs is becoming an increasingly relevant issue in many countries [4, 5].

Even at extremely low concentrations, metals are essential for the maintenance of a variety of physiological and biochemical functions in humans and animals. They become hazardous, however, when concentrations reach specified threshold. Heavy metals are known to have various adverse effects on health which might persist for complicated periods of times. Heavy metal exposure is growing as a result of environmental, dietary, and ecological problems [6, 7].

Some metals serve as essential micronutrients that maintain life, while others have far-reaching adverse effects on survival and fitness. Since essential metals are essential for metabolic processes, living things cannot survive if they are not consumed. Essential metals are involved in the production of electrochemical potentials at membranes, biological signaling, enzyme catalysis, protein structure, and up to 30% of proteins are thought to be metal-proteins [8]. The quality of food, the quality of groundwater, the activity of microbes, plant growth, etc. can all be impacted by the presence of heavy metals in soil [9]. The determination of heavy metal in soil samples is very important in monitoring environmental pollution[10].

Methods for the determination of heavy metals in soil mainly include flame atomic absorption spectrometry (FASS), graphite furnace atomic absorption spectrometry (GF-

AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES) [11]. These methods have accurate detection results and high reproductively [12, 13]. FAAS is widely accepted because the technique is element selective and it provides analytical sensitivities at the parts-per-million level and less. Example application areas that use FAAS as a routine method of metal analysis include various forms of industrial manufacturing, geology, medicine, and agriculture[14].

1.2. Statement of the problem

Floriculture, which includes the flowery business, is a branch of horticulture that focuses on the cultivation of flowering and attractive plants for use in gardens and floristry. In the Ethiopian economy the fastest-growing sector is the floriculture industry. The export sector in the country now has an alternate export product to the traditionally important export of coffee through the floriculture industry. To keep the development of the sector at its current high rate, however, a number of issues must be handled. One of the difficulties is the large consumption of various chemicals in the industry, which, when released into the environment, can cause environmental damage. More than 300 chemicals, including insecticides and growth regulators, are used in the cultivation of flowers, which can harm beneficial soil creatures and disrupt the biodiversity around the flower farms. It is well recognized that if harmful chemicals leach into groundwater or if contaminated runoff enters streams, lakes, or seas, soil pollution can result in water pollution. One source of heavy metal inputs is phosphorus fertilizers, as well superphosphate fertilizers also contain trace metal contaminants including cadmium and lead in addition to nutrients. Environmental effects of floriculture include waste disposal, toxic chemical use, and excessive water and soil use. In addition, they use a lot of fertiliser and insecticides in order to improve productivity.

1.3. Significance of the study

This study attempts to show the effects of industrial effluents and municipal solid wastes on soil quality by determining the content of heavy metals in soils from flower farms and roadside areas. Thus, this research aids in the start of monitoring in the area and offers recommendations for further research.

1.4. Objectives of the study

1.4.1. General objective

The main objective of this study is the comparative determination of the concentration of heavy metals in the soils of the selected flower farm and the roadside soil using Flam atomic absorption spectrometry (FAAS).

1.4.2 Specific objectives

- ✓ To assess the level of accumulation of selected heavy metals in soil samples
- ✓ To compare the concentration of heavy metals in soils from the flower farm and roadside
- ✓ To compare the results of this study with literature values

2. LITERATURE REVIEW

2.1. Heavy metals in the environment

Heavy metals are the most typical environmental pollutants in the world. Heavy metals are well-known environmental pollutants because of their toxicity and capacity to bioaccumulate in the human body. Heavy metal ecosystem contamination is a serious environmental problem that affects people's health. Heavy metals have the ability to mix with other elements in the environment, such as water, soil, and air, to form potentially toxic compounds. As a result of untreated waste water and fertilizer, heavy metals can build up in the soil to dangerous levels. Since heavy metals are poisonous and have negative impacts on both human and environmental health, the degree of their soil contamination is quite of concern [15].

Heavy metals are considered environmental pollutants since they are harmful to both plants and people. These heavy metals are persistent, build up, are not metabolised into other intermediate chemicals, and are difficult for the environment to break down. Heavy metals are naturally occurring substances that exist in different environments at variable concentrations. Toxins include heavy metals such as arsenic, cadmium, lead, and mercury. The International Agency for Research on Cancer classifies as carcinogenic category compounds arsenic, cadmium, chromium, and nickel. There is a link between the geological environment and chronic diseases. In reality, the geochemical environment is a significant contributor to serious health issues. International organisations like the WHO constantly assess these impact of metals on human health as a result of the substantial research that has been done on them [16. Due to their inherent persistence, inability to biodegrade and capacity to accumulate and enter an open system, heavy metals are thought to be carcinogenic to the ecosystem [17, 18]. Due to their bioaccumulation and bio magnification in the food chain, heavy metals pose a major threat to public health in addition to endangering ecosystems.

Even extremely trace concentrations of heavy metals can be considered a threat to the overall health of living organisms as well as their ecosystems because the intensity of their effects is correlated with their toxicity. Additionally, heavy metals like Cd, Pb, Ni, Mn, and Zn have been labelled as endocrine disruptors (EDCs) or estrogenic metals, which may affect gene expression due to hormonal effects.

2.2. Source of heavy metals

The most prevalent heavy metals found in contaminated soils are, in descending order of frequency: Pb, Cr, As, Zn, Cd, Cu, and Hg. Heavy metals are natural components of the earth's crust and persistent environmental contaminants; they are not degradable, enter the soil in various ways, and bioaccumulate over time [19]. Natural processes and human activity are two sources of heavy metal pollution of the soil. Sources of heavy metals pollution in soil include natural processes and anthropogenic activities [20].

In fact, during the past few decades, urban and industrial activities have contributed to the rise in metal contamination. Heavy metals come from both anthropogenic (man-made) and geogenic (natural) sources [1]. Although most heavy metals are formed naturally, some are also a result of human activity. The main human activities that release heavy metals into the environment are mining, smelting processes, the steel and iron industries, the chemical industry, traffic, and agricultural activities [12].

Natural sources

Different concentrations of heavy metals can be found naturally in a variety of soils. The metals in naturally existing, unpolluted soils originate in the lithosphere, the mineral portion of the soil that forms the rocks and minerals that make up the Earth's crust [21]. Volcanic eruptions, sea-salt sprays, forest fires, biogenic sources, and wind-borne soil particles have all been reported to significantly contribute to heavy metal pollution. Naturally occurring elements reach the soil from the parent substrate due to pedogenetic processes of weathering of parent materials at levels that are regarded as trace and rarely toxic [22].

Anthropogenic sources

The natural amounts of heavy metals in the soil may be exceeded as a result of various human activities. Agricultural practices, application of fertilizers and animal manures, sewage sludge, pesticides, wastewater irrigation, residues from coal combustion, runoff from terrestrial systems, industrial and domestic effluents, spillage of petrochemicals, emissions from rapidly expanding industrial areas, mine tailings, dumping wastes, disposal of high metal wastes, leaded gasoline, and paints are main source of heavy metals [23]. Sometimes,

even after these processes have stopped, the metals they release continue to accumulate in the soil and other areas of the environment. Because of their instability and solubility, which result in high bioavailability, heavy metals originating from anthropogenic sources are more harmful significantly contribute to soil pollution [24]. Heavy metals from the soil may accumulate in the bodies of humans and livestock through the food chain, harming human health either directly or indirectly.

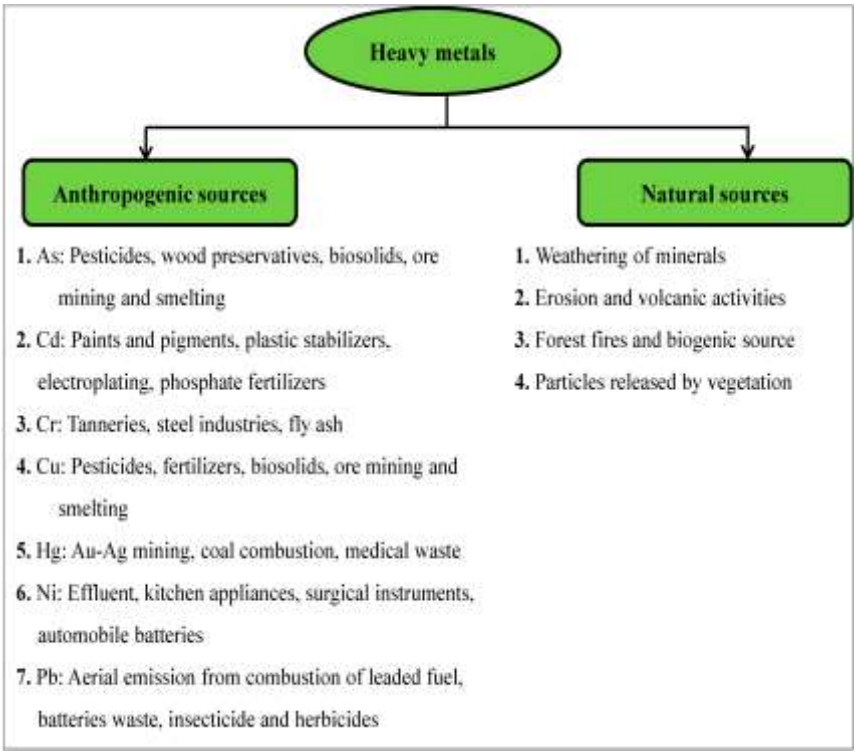

Figure 1. Sources of heavy metals

Heavy metal pollution is a significant factor in soil pollution. In contrast to other pollutants, heavy metals are dangerous since they do not degrade and build up on the earth's surface.

2.3. Occurrence of heavy metals

Natural occurrences like volcanic eruptions, rock weathering, and many others release heavy metals into the environment. Human activities such as industrial discharge, excessive vehicle use, resource extraction, anthropogenic activities, metallurgy, and so forth are equally

involved in contaminating the environment [25]. Almost all metals, whether dangerous or not, are present in nature and can be refined using a variety of techniques to produce the most pure and useful form. Metals likely escape the earth in the form of mixed ores and are gathered throughout the mining process. And after that, they pass through a number of procedures to get the purest form possible for use in subsequent processing.

Calcium

Calcium, the fifth-most prevalent element and most abundant metal in the human body, was not isolated until electricity was controlled. It combines with magnesium, aluminium, copper, and lead to form alloys [26]. Calcium is a crucial lithophile element, with a crustal abundance of 41,500 ppm. Consuming above 2500 mg daily may cause hypercalcemia and calcium-alkali syndrome [27]. Calcium intake below 2500 mg/day may contribute to arterial calcification and cardiovascular diseases, with supplement loading potentially more likely in older individuals [28].

Cadmium

Cadmium is a significant environmental pollutant, with occupational exposure from smelters and electronic waste [29]. Chronic exposure to Cd can cause kidney disorders, anemia, anosmia, cardiovascular diseases, renal problems, and hypertension, causing painful bone loss [30]. Cadmium is carcinogenic for number of tissue [31] and is classified as a human Carcinogen. Cadmium enters the brain parenchyma, causing neurological alterations, attention deficits, olfactory dysfunction, and memory deficits.

Chromium

Chromium, a naturally occurring element, is found in various forms, with stable Cr^{6+} and Cr^{3+} forms being the most stable [32]. The permissible value established by the U.S. EPA is 0.05 mg l^{-1}. However, in trace quantities, chromium is an essential nutrient for humans, setting such permissible limits is essential, because at elevated levels Cr is toxic [33]. Chromium (VI) is an important environmental contaminant [34]. Cr^{6+} is highly mobile and water-soluble, whereas Cr^{3+} is chemically more stable but less bioavailable due to its low permeability to biomembranes [35]. Cr^{6+} is about 100 times as hazardous as Cr^{3+} and 1000

times more mutagenic [36]. Chromium, a naturally occurring element, is found in various forms, with stable Cr^{6+} and Cr^{3+} forms being the most stable.

Nickel

Nickel, the 28th element, is a hard, ductile transition metal with a common +2 oxidation state [37]. Nickel, a ferromagnetic element, naturally occurs in the Earth's crust as oxides and sulphides and is found in soil, meteorites, and volcanoes [38]. Nickel poisoning risks include fibrosis, chronic bronchitis, reduced lung function, and allergic dermatitis.

Lead

Lead is a naturally occurring bluish-grey metal in Period 6, with atomic number 82, mass 207.2, density 11.4 g/cm3, melting point 327.4 °C, and boiling point 1725 °C. It is found in minerals with elements like sulphur or oxygen [49]. The most common sources of Pb in the environment include dust from leaded paintings in older dwellings, as well as leaded and tap water from soldered pipes [40]. Indoor pollutants are also a source, as is indoor smoking [41]. Lead, a low-mobility heavy metal, negatively impacts children's development, intelligence, and pregnant women's health due to its long soil residence.

Zinc

Zinc is one of the most abundant transition elements in the Earth's crust, and it is also a trace element required by all living organisms [42]. It is an important component of many proteins, acting as a cofactor or coenzyme in over 300 enzymes [43] , and enzyme regulation, protein stability, DNA binding, immunity, cell metabolism, and ROS production.

Iron

Iron is essential for plants, but excessive doses can cause oxidative stress and a pro-oxidant state in cells [44]. Soil iron concentrations range from 0.2% to 55%, with variations due to soil characteristics and other sources. Iron availability is influenced by anthropogenic activities, potentially impacting ecosystems [45], concentrations above 500 mg kg^{-1} dry weight (DW) are toxic to most plant species [46]. Iron poisoning affects 18% of soils worldwide, reducing pH, fertility, and agricultural yield, causing a 50% reduction and early crop loss [47].

Copper

Copper, a reddish metal, is a critical trace mineral found naturally in rock, soil, sediment, water, and air at a concentration of 50 mg/l on average [48]. Copper is naturally present in plants and animals, with RDA and UL values for adults and children, respectively, of 900 g/day and 10,000 g/day, according to the National Academies Institute of Medicine. It is essential for healthy development, lung elasticity, vascular function, neuroendocrine function, and iron metabolism. Consuming excessive copper can be hazardous and negatively impact bodily systems. It can be caused by illnesses and environmental factors like drinking water pollution, which can cause light blue or blue-green water with metallic tastes. Copper interferes with growth and photosynthesis at high concentrations; human activities cause dangerous levels [49].

Manganese

Manganese, abundant in rock, soil, and water, is sourced from natural sources like crystal rock, ocean spray, forest fires, vegetation, and acidic environments. Manganese in soils comes from atmospheric deposition, plant leaching, and anthropogenic sources like wastewater discharges and fossil fuel combustion [50]. Manganese is an essential micronutrient in most organisms that is required for the activity of a diverse set of enzymatic proteins (e.g., arginase and glutamine synthase) [51, 52]. It contributes to the structure of photosynthetic proteins and enzymes in plants. Thus, manganese plays two roles in plant metabolism: as an important micronutrient and, when present in excess, as a harmful element [53]. Manganese is essential for human health, supporting the brain and immune systems, enzyme cofactors, and regulating blood sugar levels [54].

Magnesium

Magnesium, the eighth element, is abundant in rocky minerals and seawater, ranking third in the Earth's crust. Magnesium is essential for metabolic activities in living organisms [55, 56]. Magnesium is a common mining-derived pollutant in freshwater habitats, coming from the leaching of minerals from waste rock [57] and controlled releases of mining wastewater [58]. Fertilisers, liming, and alloys for numerous industrial items such as automotive and aviation

parts, titanium, aluminium, and high-quality steel are also anthropogenic sources of Magnesium.

Soil pollution

Soil is the organic and inorganic material present on the earth's surface. Soil is an essential component of both rural and urban contexts, and land management is the key to soil quality in both [59]. Soils can become contaminated by heavy metals and metalloids accumulated from rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilisers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, petrochemical spillage, and atmospheric deposition.

Lead, chromium, zinc, cadmium, copper, iron, and nickel are the most commonly encountered heavy metals at contaminated sites. Although heavy metals are naturally present in soil, geological and anthropogenic activities increase trace element concentrations to levels that are toxic to plants and animals. Some heavy metals, such as Cu, Fe, Ni, and Zn, are required by life in trace amounts. Excessive concentrations of these elements, however, can be toxic to organisms [60]. Soil is a critical resource for meeting two human needs: a sufficient food supply and a healthy environment [61]. Heavy metal contamination in soils occurs when the amounts of elements in the soil exceed the maximum permitted concentrations, which can be hazardous to biological life in such areas [62]. Heavy metal concentrations in soil are linked to biological and geochemical cycles and are caused by anthropogenic activities such as agricultural practises, industrial operations, and waste disposal systems [63, 64]. Several studies on heavy metal contamination of soil and vegetables have been conducted [65].

Flowering farm

Floriculture is a horticultural discipline focusing on cultivating flowering and ornamental plants for gardens and floristry, including commercial production, marketing, and sale [66]. Ethiopia's floriculture industry emerged in 1980, exporting cut flowers to Europe and becoming an international player in Africa due to its high altitude and high-altitude cultivation [67. Roses and ornamental plants in greenhouses or fields are considered cut

flowers [68]. Environmentalists worry about excessive pesticide and chemical fertiliser use, contaminating water and soil, and harming ecosystems through waste disposal [69].

Flowers

Flowers are luxurious items with significant social value that are rarely used in food preparation. They are used as decorations, as part of celebrations, and as gifts. There are numerous flower kinds developed for floriculture. Roses, lilies, carnations, and daisies are among the most popular.

Ethiopian Rose

The Ethiopian rose is Africa's only native rose. The cream-colored rose can grow to be one of the largest in the world, with a diameter of 15cm. Soft, delicate layers of petals make a circular, symmetrical shape on top of a long stem in this beautiful flower. When grown at high altitudes, the rose provides rich, vitamin-rich fruit that is consumed on a regular basis amid food shortages. They are a popular love symbol in Ethiopia, and they are utilised on a variety of occasions [70].

Red-Hot Poker

Throughout Ethiopia, the Red-Hot poker flower blooms in the middle of summer. It is one of the Bale Mountains' most prominent tourist attractions. Hummingbirds are drawn to these remarkable rocket-shaped flowers because of their bright, eye-catching colours, such as red and orange. The flowers usually bloom from the bottom up on a shrub. Each stem has a single bloom. They grow in open, sunny areas with well-drained soil.

Hagenia Flower

The hagenia, also known as the kosso plant, is a native Ethiopian plant with a heavenly scented white, purple, orange, or green blossom. This plant, which is used as a common herbal remedy, thrives at high altitudes above 6,500 feet. The umbrella crown blossoms are

enormous and gorgeous, making them an excellent ornamental flower that adds colour to any garden.

Eucalyptus Flower

The Ethiopian eucalyptus flower is a branch of the world's tallest flower and is thought to be an old plant. Hundreds of fluffy stamens are produced by the tall eucalyptus tree, which ultimately produces colourful florets. These florets are available in pink, yellow, cream, red, and white.

Meskel Flowers

Meskel flowers look lovely in any garden. The little yellow blossoms are a type of flower found in the United States. They feature six to eight petals and a tiny brown centre. They are associated with pleasure and new beginnings in Ethiopia. Meskel flowers can be consumed once completely developed. They are typically used to embellish flower beds or as a decorative piece for a special occasion.

Aloe

Ethiopia has 45 native aloe species, with 66% being native. Aloes are essential for traditional medicine and soap production in Ethiopia. The plant's upright, succulent leaves form a rosette around the tubular blossoms, which are used in various colours. In Ethiopia, flowers are more than just decorative gifts; they are essential food sources during times of scarcity and treat medical disorders.

3. EXPERIMENTAL

3.1. Material and methods

3.1.1. Reagents and chemicals

All chemicals and reagents used in this study were of analytical grade chemicals. HNO_3 (60-70%), HCl (37%) were used for digestion. Working standard solutions for the selected heavy metals were prepared from stock standard solution of 1000 ppm.

3.1.2. Apparatus and equipment

Various apparatus and equipment, such as digital balance (Aczel, CY 224, England) for weighing soil samples, Kjeldahl apparatus for digesting samples, and flame atomic absorption spectrophotometer (FAAS) (Agilent 200 series AA), were used for determination of metals in this study.

3.2. Instrumentation

3.2.1. Flame atomic absorption spectrophotometry (FAAS)

Flame atomic absorption spectroscopy is very useful for the determination of a large number of elements, especially at trace levels. It is a widely used technique for the analysis of a wide variety of sample matrices including biota, soils, and water. Flame atomic absorption spectroscopy is a very reputable technique that is inexpensive and delivers accurate results even in a complex matrix [71].

In FAAS, samples are vaporized at very high temperatures and the concentrations of selected atoms are determined by measuring absorption at their characteristic wavelengths. This is typically accomplished by converting the sample into an atomic vapor by spraying a solution into a flame.

3.2.2. Wet digestion and instrumental analysis

In this study, the weighed samples were transferred to a 250 mL round-bottom flask for digestion, and 3 mL HNO_3 and 1 mL HCl were added [72] (). Then the mixture was heated to 240^0C for 3 hours on a Kjeldahl apparatus fitted with a reflux condenser. After the digestion is complete, the mixture was allowed to cool for 5–10 minutes. The clear solution was filtered into a 50 mL volumetric flask and finally made up to volume with distilled water. The entire diluted samples were kept in 50 mL plastic bottles until analysis. Each soil sample were prepared and analyzed in triplicate to confirm the accuracy of the results.

3.3. Description of study area

The study was carried out at flower farm and roadside around Holeta town in the special zone of Oromia Region, Ethiopia. Holeta has longitude of 9°3′N 38°30′E and an altitude of 2391 meters above sea level. Distance between cities Holeta, Oromia, Ethiopia and Addis Ababa, Addis Ababa is at 40km.

3.4. Procedure

3.4.1. Sample collection

In this study, three soil samples were collected from the top at 30 cm depth of each sampling area using auger by applying random sampling technique. Then, after the three soil samples of each of the flower farm and roadside were mixed, homogenized, placed into clean polyethylene bags in triplicate, labeled and transported to Addis Ababa University, department of chemistry laboratory.

3.4.2. Sample preparation

In the laboratory, the samples were air dried and further dried in an oven at 60–70^0 C for four days. The soil sample were ground with a mortar and pestle and sieved through 2 mm sieve. A 0.5-g sieved soil sample was taken and digested by using Kjeldahl apparatus and finally the selected heavy metals were analyzed by Flame Atomic Absorption Spectrophotometer (FAAS).

Figure 2. Sample preparation

3.4.3. Sample decomposition

The purpose of sample decomposition is converting all the species present in a given sample to get one defined form, eliminating interfering substances from the sample matrix and obtaining the element in homogeneous and simple accessible matrix. Decomposition of solid samples is an important step in combined analytical methods. In most cases, when using highly sensitive measuring methods, such as flame atomic absorption spectrometry (FAAS), graphite furnace AAS, ICP-AES, ICP-MS, the sample is measured in an aqueous solution. The digestion methods could be classified in to wet digestion, dry ashing and microwave digestion. In this study wet digestion method was used for the analysis of soil samples.

3.5 Optimization of digestion procedure of soil samples

In this study, to prepare a clear colorless sample solution that is suitable for the analysis using FAAS, digestion procedures were optimized using the HNO_3 and HCl mixtures by varying parameters such as volume ratio of the acid mixture, digestion time and digestion temperature (Table 1). From the optimization procedures, 3 ml of the acid mixture of HNO_3 (69-70%) and 1 ml of HCl (37%) with the ratio of 3:1, digestion time of 2:45 hours and digestion temperature of 240^0C were the optimal condition for 0.5 g soil sample. These best settings

were chosen based on the clarity of the digests, the reagent volume consumed, the digestion duration, the simplicity, and the temperature employed to complete the digestion of the sample.

Figure 3. Digesting of soil samples

Table 1. Optimization for volume

Trial no	Volume ratio HNO_3:HCl	Digestion temp. (^oC)	Digestion time(hr.)	Observation
1	2:1	300	3:00	Clear and light orange
2	2:2	300	3:00	Clear and light yellowish
3	3:1	300	3:00	Clear and Colorless
4	3:2	300	3:00	Clear
5	3:3	300	3:00	Light yellowish with residue
6	4:1	300	3:00	Clear and light orange

Table 2. Optimization for temperature

Trial no	Volume ratio HNO_3:HCL	Digestion temp. (^oC)	Digestion time(hr.)	Observation
1	3:1	150	3:00	Slightly clear
2	3:1	180	3:00	Clear and Light yellowish
3	3:1	210	3:00	Clear and Light yellowish
4	3:1	240	3:00	Clear and Colorless
5	3:1	270	3:00	Almost clear
6	3:1	300	3:00	Slightly clear with some residue

Table 3. Optimization for time

Trial no	Volume ratio HNO_3:HCl	Digestion temp. (^oC)	Digestion time(hr)	Observation
1	3:1	240	1:45	Clear
2	3:1	240	2:00	Clear and light yellowish
3	3:1	240	2:15	Almost clear
4	3:1	240	2:30	Slightly clear
5	3:1	240	2:45	Clear and Colorless
6	3:1	240	3:00	Yellowish with some residue

3.6. Digestion of soil sample

Applying the optimized condition (Table 1, 2 and 3), 0.5 g of dried and homogenized soil samples were transferred into a 250 mL round bottomed flask. To this 4 ml of aqua-regia (3:1 ratio 69-70% of HNO_3 and 37% HCl, respectively) were added and the mixture was digested on a Kjeldahl digestion apparatus fitting the flask to a reflux condenser.

Figure 4. Optimized soil sample

4. RESULTS AND DISCUSSION

4.1. Calibration of the instrument

Calibration curves were constructed by first preparing a set of standard solutions with known concentrations of the selected heavy metals. The instrument response is measured for each, and plotted vs concentration of the standard solution.

Table 4.Concentration of working standard solutions, calibration equation and correlation coefficients of the calibration curves

Metals	Wave length	Concentration of standards (mg/l)	Calibration equation	Correlation coefficients (R^2)
Cr	357.9	2,4,6,8,10	Y= 0.011x + 0.001	0.9996
Cu	324.7	2,4,6,8,10	Y=0.0146x + 0.001	0.9993
Mn	279.5	2,4,6,8,10	Y=0.022x +0.0008	0.9996
Ni	332.0	2,4,6,8,10	Y=0.0119x +0.0618	0.9953
Cd	228.8	2,4,6,8,10	Y =0.0447x+0.0076	0.9999
Pb	217.0	2,4,6,8,10	Y =0.0447x+0.0076	0.9999
Zn	213.9	2,4,6,8,10	Y =0.0509x +0.0242	0.9993
Fe	248.3	1,3,5	Y =0.013x +0.0038	0.9973
Mg	285.2	1,3,5	Y = 0.0847x +0.0128	0.9994
Ca	422.7	1,3,5	Y=0.0135x + 0.0015	0.9994

Calibration curve for the selected metals

From the calibration curves shown below for each metal, it is possible to conclude that the relationship between absorbance and concentration is in good correlation and gives a linear graph due to its correlation coefficient range (0.9953 - 0.9999).

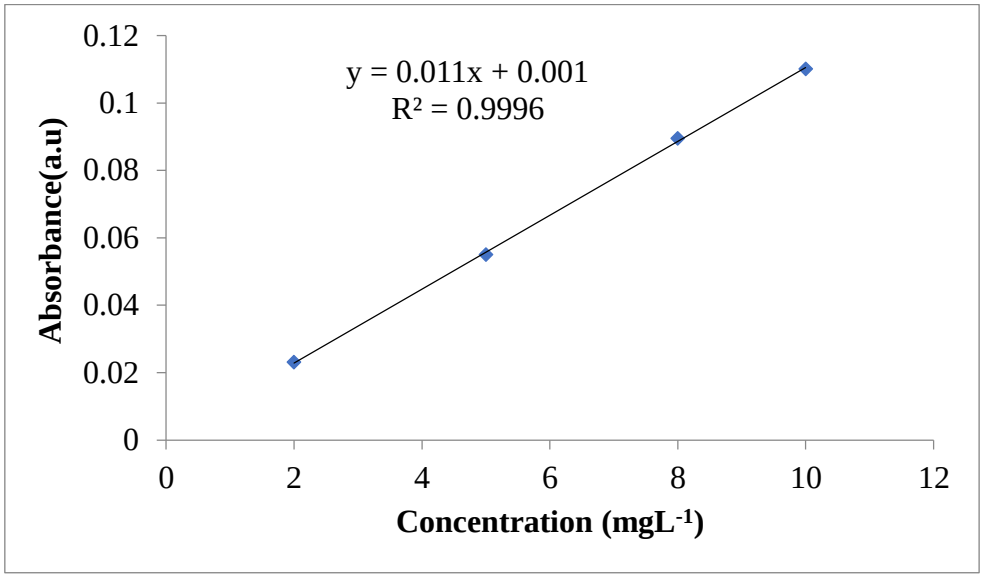

Figure 5. Calibration curve for chromium

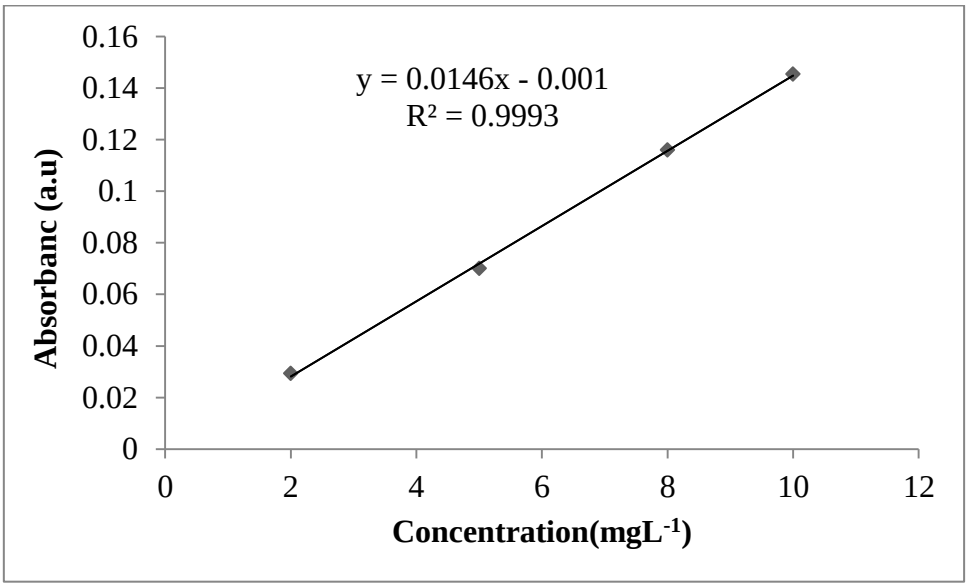

Figure 6. Calibration curve for copper

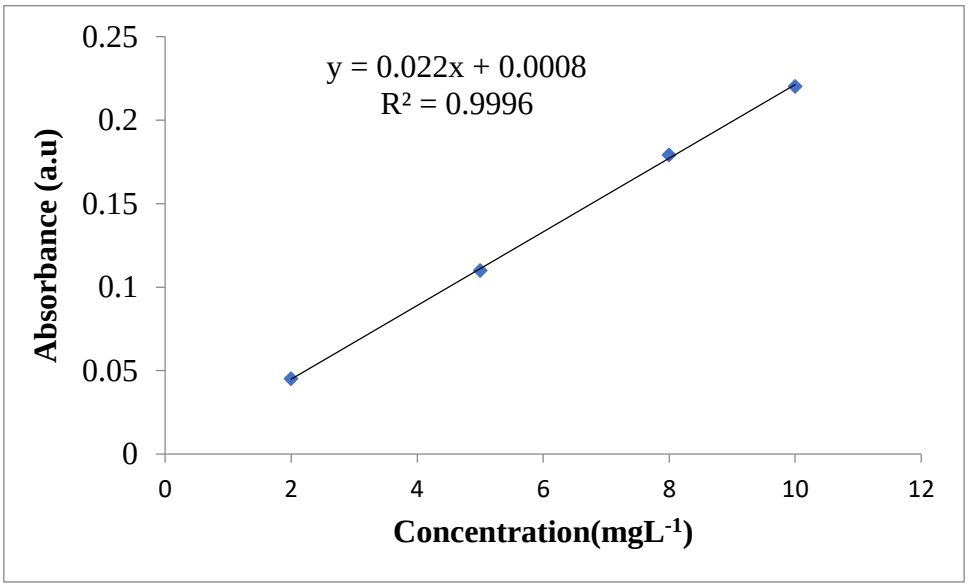

Figure 7. Calibration curve for manganese

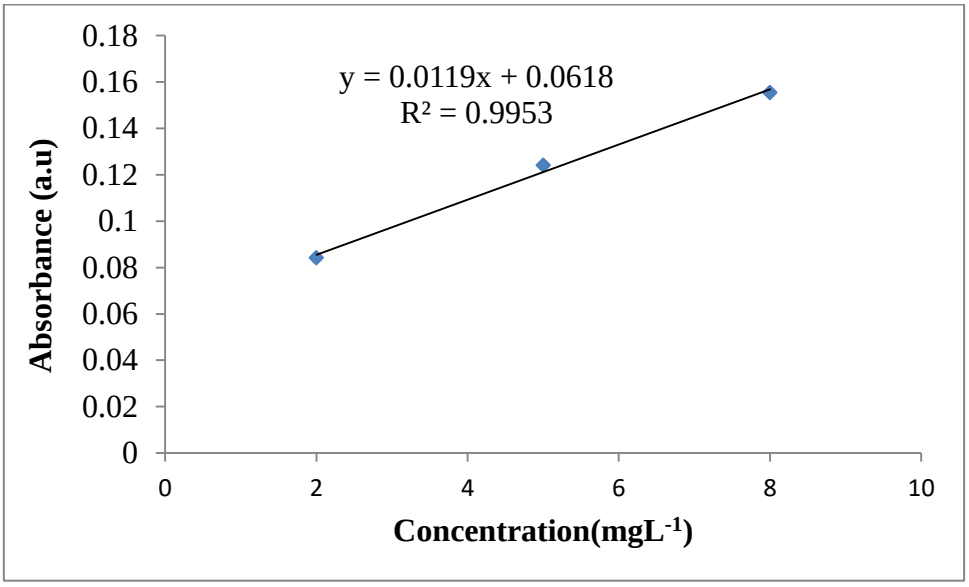

Figure 8. Calibration curve for nickel

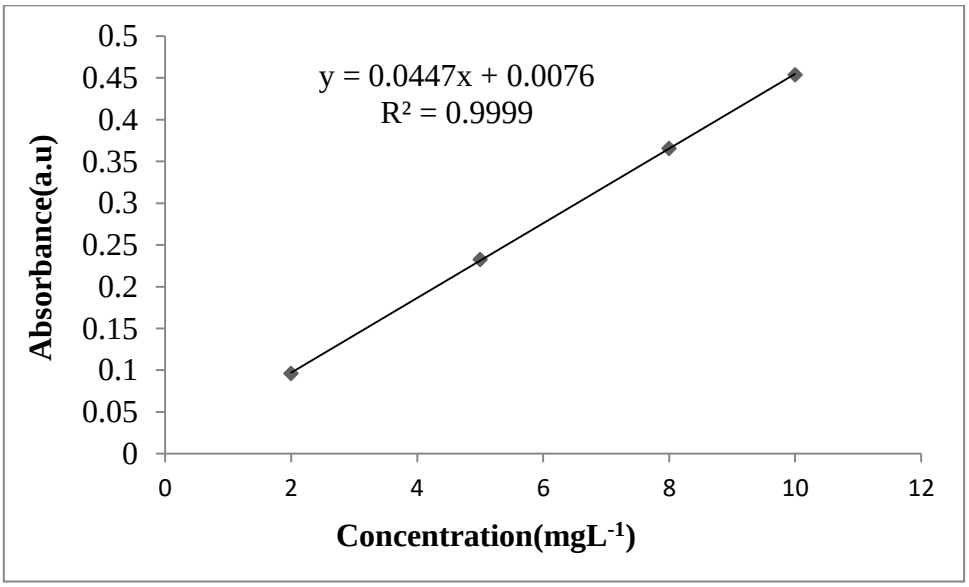

Figure 9. Calibration curve for cadmium

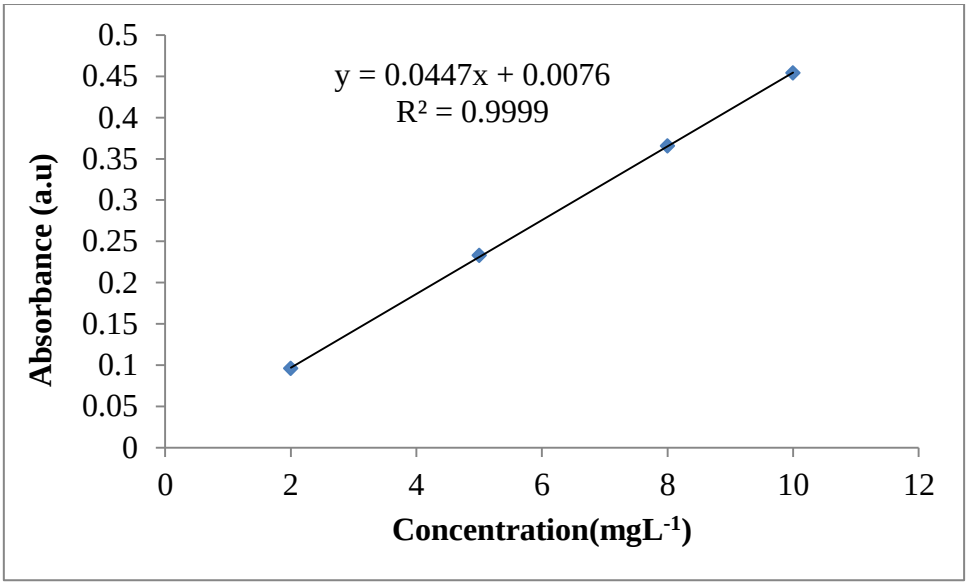

Figure 10. Calibration curve for lead

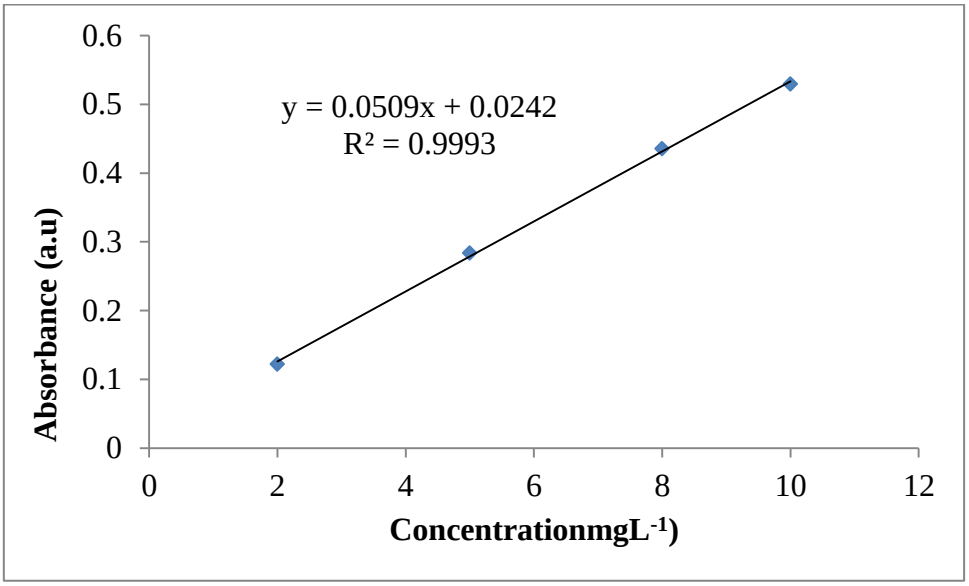

Figure 11. Calibration curve for zinc

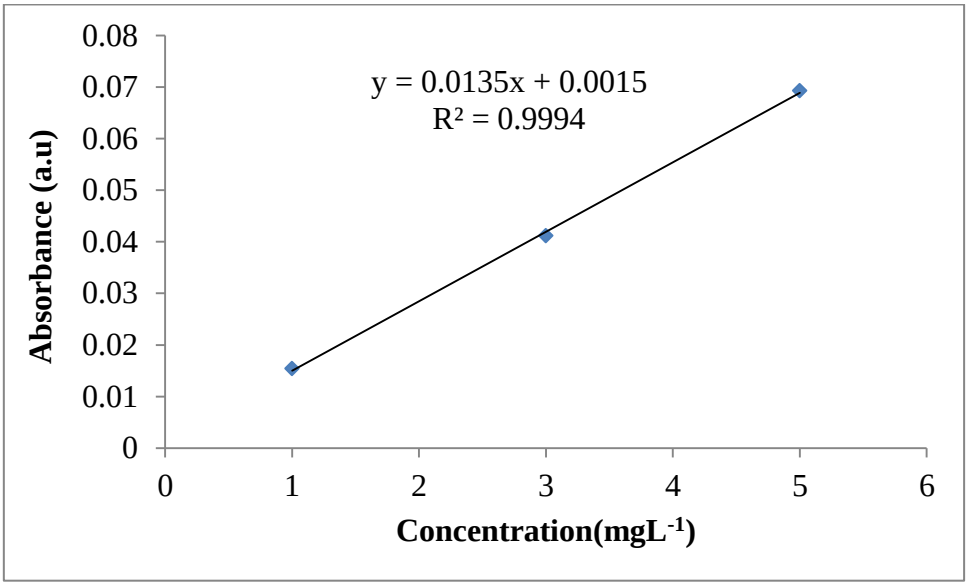

Figure 12. Calibration curve for calcium

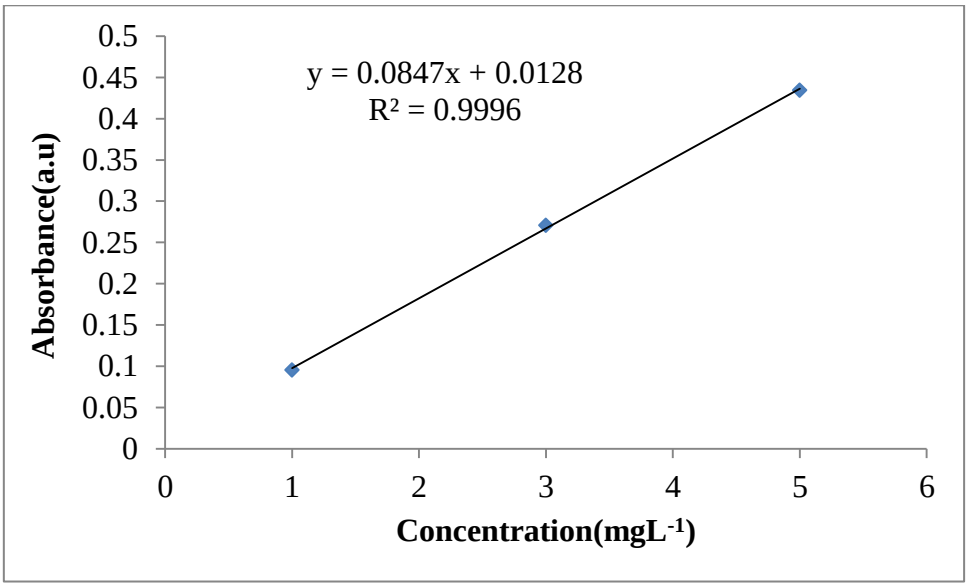

Figure 13. Calibration curve for magnesium

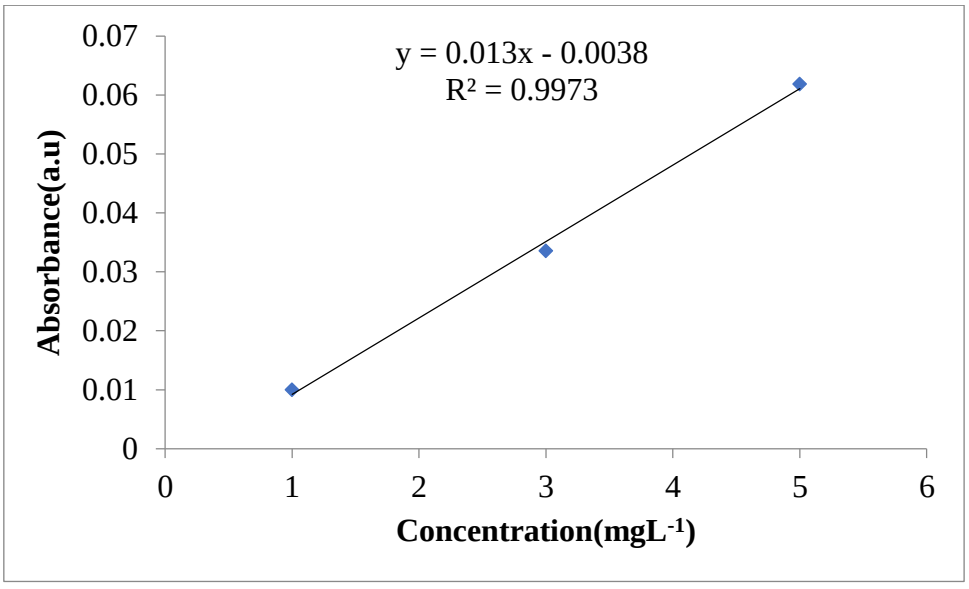

Figure 14. Calibration curve for iron

4.2. Determination of Metals in Soil Samples

The concentration of ten metals: Cr, Cu, Mn, Ni, Cd, Pb, Zn, Ca, Fe and Mg were analyzed in the digested soil samples by using Flame Atomic Absorption Spectroscopy. However, among the analyzed metals Cd and Pb were below the method detection limit. The levels of the detected metals are shown in Table 5 with their respective %RSD.

Table 5. Average concentration and relative standard deviation (%RSD) of heavy metals in each soil samples

Elements	Soil from flower farm	Soil from roadside
	Mean± SD(mg/kg)	Mean± SD(mg/kg)
Mn	399±9.847	964 ±41.623
Cu	30±0.651	36±1.301
Zn	150±8.238	96±5.824
Ni	21±0.451	33±2.854
Cr	34±1.389	48±1.106
Fe	8807±55.692	9128±166.106
Mg	1023±10.363	1119±11.609
Ca	60±2.837	118±5.462
Cd	BDL	BDL
Pb	BDL	BDL

BDL: Concentration of the tested heavy metal below the method of detection limit

The result shown in Table 5 indicates that iron has the highest concentration in the two sampling areas; however, similar to the other metals the concentration of iron in the road side was higher than the flower farm, and minimum amount of nickel was observed in both areas. Soil samples from the roadside contained higher amount of Mn, Cu, Ni, Cr, Mg and Ca than the flower farm, and a smaller amount of Zn were observed in road side. This may be due to irrigation from water source or as a result of metals mobility from a nearby waste depot to the roadside through leaching and run off.

4.3 Comparison of Concentration of Metals in flower farm and road side soils

The soil samples which were collected from the two sampling areas were found to contain significant levels of Mn, Cu, Zn, Ni, Cr, Fe, Mg and Ca. The results showed that the soil samples in the two sampling areas contain high amount of iron and magnesium. As an exception, zinc is higher in the flower farm than on the roadside. The mean metal concentration (mg/kg) in both soil samples are as follows: Fe > Mg > Mn > Ca > Zn > Cr > Cu > Ni. A small amount of nickel was observed in both sample sites, while cadmium and lead were below the method detection limit.

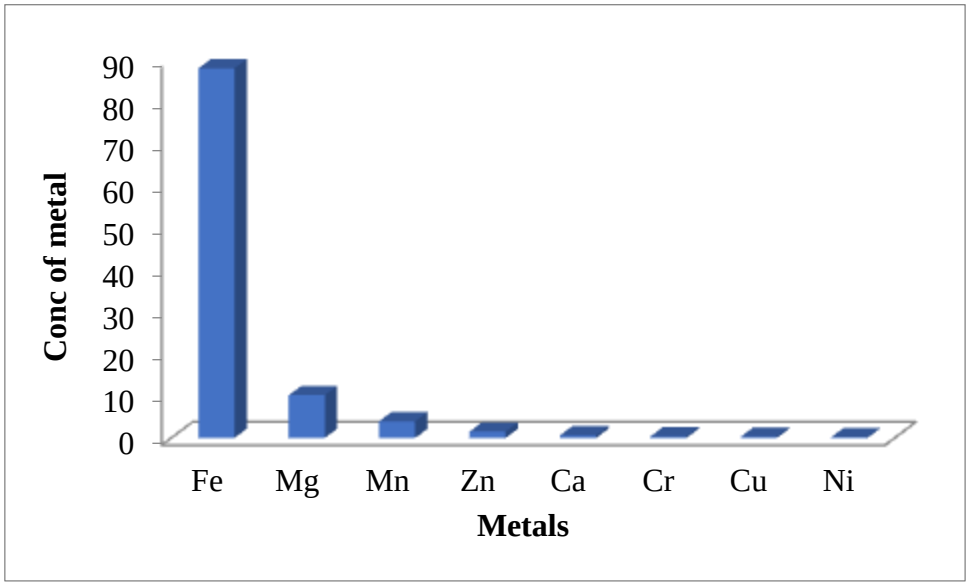

Figure 15.Comparison of metal to metal concentration in flower farm

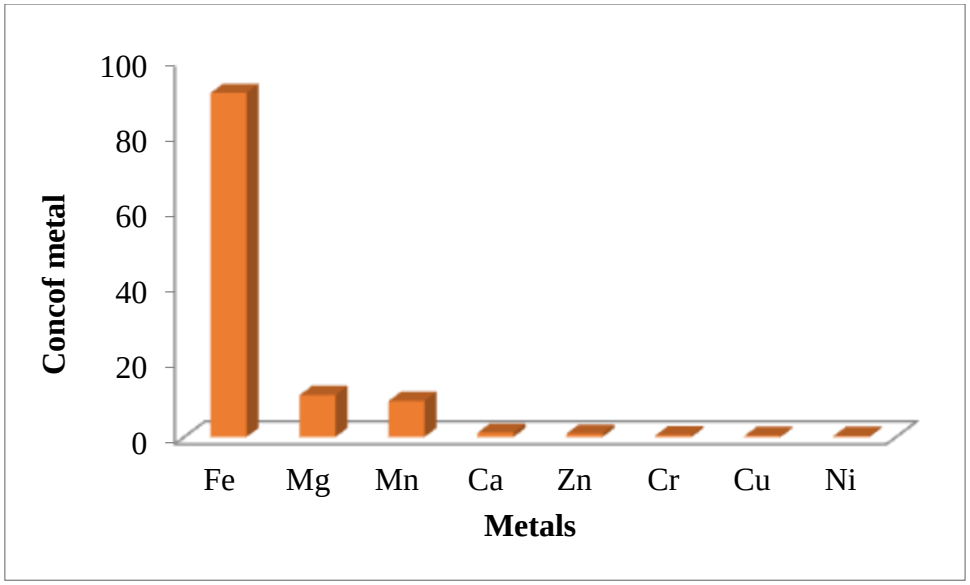

Figure 16.Comparison of metal to metal concentration in roadside soil

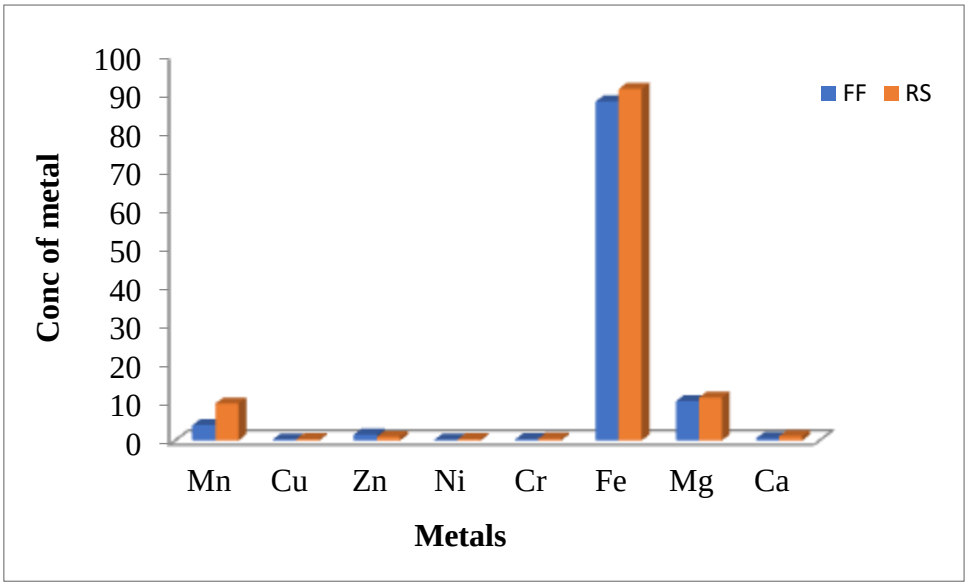

Figure 17.Comparison of metal to metal concentration for flower farm and roadside

4.4. Analytical methods detection limit

4.4.1. Limit of detection (LOD)

The limit of detection (LOD) is defined as the smallest concentration or amount of an analyte that can be reliably shown to be present or measured under defined condition, and the limit of detection is the lowest concentration level that can be determined to be statistically different from a blank (99% confidence). The LOD is typically determined to be in the region where the signal-to-noise ratio is greater than 3 but not necessarily quantitated as an exact value.

In this study, method detection limit for each metal was estimated by digesting six analytical blanks with the optimized procedure for soil samples. Six blank samples were analyzed in triplicate for each element, and the combined standard deviation of the six blank reagents was computed. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank by three. The method detection limit of each element is above the instrument detection limit.

4.4.2. Limit of quantification (LOQ)

The limit of quantification (LOQ), refers to the smallest analyte concentration or mass, which can be quantitatively analysed with a reasonable reliability by a given procedure [73].

In the present study, LOQ was obtained from triplicate analysis of six reagents blanks which were digested in the same digestion procedure as soil samples. The LOQ was calculated by multiplying the pooled standard deviation of the reagent blank by ten.

Table 6. LOD and LOQ

Metals	SD (mg/L)	LOD(mg/L)	LOQ(mg/L)
Mn	0.009	0.026	0.09
Cu	0.032	0.096	0.32
Zn	0.113	0.339	1.13
Ni	0.057	0.170	0.57
Cr	0.219	0.657	2.19
Fe	0.305	0.914	3.05
Ca	0.614	1.840	6.14

4.5. Validation of analytical method

4.5.1 Recovery

In this study the reliability of the optimized procedure was checked by adding known concentration (10mgL^{-1}) of standards to each metal. Thus, spiking was done in three triplicate groups. In the first group 1 mgL^{-1} of Mn, 0.061 mgL^{-1} of Cu, 0.075 mgL^{-1} of Zn and 0.064 mgL^{-1} of Ni added to 0.5g sample. The procedure was as follows: each of 1000 µL of Mn, 305 µL of Cu, 375 µL of Zn and 320 µL of Ni was added to 0.5g of soil samples in 250 ml round bottomed flask and digested in the same way as the soil samples. The final volume of the digest was diluted to 50 ml and the metal concentrations were determined from the calibration curve. The amount of spiked metals recovered after digestion of spiked samples was used to calculate percentage recovery.

$$\% \text{ Recovery} = \frac{(C_M \text{ in the spiked sample}) - (C_M \text{ in the non-spiked sample})}{C_M \text{ added for spiking}} X\ 100$$

Where: C_M = Concentration of metal

The recovery values for the soil samples are given in Table 7. The table indicates that all the recovery results are between 88 to 114.2% which is within the accepted range (80 -120%). Thus good recoveries were obtained for all metals in the soil samples.

Table 7: Recovery test results for the optimized procedure of metals in soil samples

Metals	Conc. of metals in unspiked sample (mgL^{-1})	Amount Spiked (mgL^{-1})	Conc. of metals in spiked sample (mgL^{-1})	Amount recovered (mgL^{-1})	Recovery (%)
Mn	3.991	1	5.133	1.142	114.2
Cu	0.305	0.061	0.364	0.967	96.7
Zn	1.501	0.075	1.567	0.880	88
Ni	0.213	0.064	0.279	1.031	103.1

4.5.2. The correlation coefficient of metals

Correlation coefficient is a measure of association between two variables, and it ranges between –1 and 1. If the two variables are in perfect linear relationship, the correlation coefficient will be either 1 or –1. The sign depends on whether the variables are positively or negatively related. The correlation coefficient is 0 if there is no linear relationship between the variables [74]. Thus, in this study linear correlation tests were performed to investigate the correlations between metals concentration in the soil samples and are presented in Table 8 below.

Table 8. Metal to metal correlations

	Mn	*Cu*	*Zn*	*Ni*	*Cr*	*Fe*	*Mg*	*Ca*
Mn	1							
Cu	-0.856	1						
Zn	0.671	0.958	1					
Ni	0.089	-0.591	0.798	1				
Cr	0.936	0.620	-0.368	0.266	1			
Fe	0.491	-0.870	-0.975	0.912	0.154	1		
Mg	-0.913	0.571	0.311	0.325	-0.998	-0.093	1	
Ca	0.990	0.775	0.560	-0.052	0.976	0.363	-0.961	1

As shown Table 8 above between Mn-Ca(r=0.990), Mn-Cr (r=0.936), Cu-Zn(r=0.958), Ni-Fe(r=0.912) and Cr-Ca(r=0.976) was observed highest correlation. Higher correlation was observed between Mn-Zn(r=0.671), Cu-Ca(r= 0.775), Zn-Ni(r=0.798) and Zn-Ca(r=0.560). Medium correlation was observed between Mn-Fe (r=0.491) and Cu-Mg(r=0.571). Lower correlation was observed between Zn-Mg(r=0.311), Ni-Cr(r=0.266),Ni-Mg(r=0.325) and Fe-Ca(r=0.363). There is no correlation between Mn-Cu(r= -0.856), Mn-Ca(r=-0.913), Cu-Ni (r= -0.591), Cu-Cr (r= -0.620), Cu-Fe(r= -0.870), Zn-Cr(r= -0.368),Zn-Fe(r= -0.975), Ni-Ca(r= -0.052),Cr-Mg(r= -0.998), Fe-Mg(r= -0.093) and Mg-Ca(r= -0.961).

4.6. Statistical analysis of variance (ANOVA)

Analysis of variance (ANOVA) is a widely used statistical method to compare group means. One-way ANOVA can compare the mean of more than two groups of sample. ANOVA use the F statistic to compare whether the difference between samples means are significant or not. If the calculated value of F is greater than the value obtained from the table at specified confidence level and degree of freedom, the differences in sample means are significant. In this study, soil samples were collected from two different areas and the metal level of each sample was analyzed by FAAS. During the processes of sample preparation and analysis a number of random errors may be introduced in each aliquot and in each replicate measurement. The variation in sample mean of the analyte was tested by using ANOVA, whether the source for variation was from experimental procedure or heterogeneity among the samples. In the present study, data were analyzed by using Microsoft Excel 2010. Descriptive statistics were used to analyze the data obtained from heavy metal concentrations of the soil samples.

Table 9. Analysis of variance of soil samples

	Metals							
	Mn	Cu	Zn	Ni	Cr	Fe	Mg	Ca
F cal	522	37.83	87.16	52.62	194.74	10.075	114.15	271.4
P value	0.000022	0.0035	0.00073	0.00019	0.00015	0.0337	0.00044	0.00008
F crit	7.71	7.71	7.71	7.71	7.71	7.71	7.71	7.71

As shown in the table above, the statistical analysis of variance indicated that Fcal > Fcrit since; there is a significant difference among the mean concentration of all the selected metals ($P<0.05$) at 95% confidence level between the two samples collected from the two sample sites.

4.7. Comparison of heavy metals concentration in soil samples with other reported values

In Table 10 below the mean concentration of the metals determined by the FAAS was compared with other different countries. As shown in the table the concentration of manganese in this study ranges from 399 - 963.6 mg/kg, which is higher than its concentration in Nigeria. The amount of copper and zinc, which range from 30.5 -36.6 mg/kg and 96.1 - 105.5 mg/kg respectively, are higher than the concentration of copper and zinc in China and Nigeria, but they are lower than the other reports in Ethiopia. The concentration of nickel, which ranges from 21-33.4 mg/kg in this study, is comparable with concentration of nickel in China and Nigeria, but higher than in India. The level of chromium, which ranges from 34 - 48.54 mg/kg, is higher than the concentration of chromium in India and it is comparable with the concentration of chromium in China. The concentration of manganese, copper, zinc, nickel and chromium in this work is lower than the concentrations in other research in Ethiopia.

Table 10. Comparison of heavy metal concentration in soil samples with other reported Values (mg/kg)

Country	Methods	Mn	Cu	Zn	Ni	Cr	Pb	Cd	References
Ethiopia	ICP- MS	7746–13,093	201–442	749–1381	318–623	557–7022	81–472	1.0–2.0	[75]
India	AAS	NA	NA	NA	1.57-6.6	0.5-2.46	1.10-7.9	1.52-5.42	[76]
China	ICP-MS	NA	7.9-13.6	28-38	9.5-22.1	31-56	22-28	0.07-	[13]
Nigeria	AAS	7.65 - 207.25	2.0 - 20.50	8.70 - 20.50	2.60 - 37.15	NA	9.6 - 54.60	NA	[77]
Ethiopia	FAAS	399 - 963.6	30.5 - 35.6	96.1 - 150.5	21 - 33.4	34 - 48.54	ND	ND	This work

NA= not analysed

ND = not detected

— = between

4.8. Comparison of metals concentration with WHO standard value

Table 11. Comparison of heavy metals concentration in soil samples with WHO standard level (WHO 1996)

Metals	Conc. of metals ($mgkg^{-1}$)	WHO recommended limit of metal in soil ($mgkg^{-1}$)
Mn	399 - 963.6	12
Cu	30.5 - 35.6	36
Zn	96.1 - 150.5	50
Ni	21 -33.4	35
Cr	34 - 48.54	100
Fe	8807 – 9128	5000
Mg	1023 – 1119	60
Ca	60 – 118	100

As shown in Table 11 the concentration of Fe, Mg, Ca Mn and Zn in both the flower farm and roadside samples are above WHO recommended permissible level, whereas the concentration of Cu, Ni and Cr were found below WHO recommended permissible levels. This may result from use of various fertilisers, pesticides and wastes disposal practises in order to improve productivity in floricultures.

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

Thus, in the present work, the concentration of essential metals (Mn, Cu, Zn, Ni, Fe, Ca, Mg and Cr) and toxic metals (Cd and Pb), were determined by using FAAS in the soils of flower farm and roadside. The result indicates that the samples had a variable level of each analyzed metal. Based on the obtained results at 95% confidence level there is a significant difference among the mean concentrations of all the selected metals in the two sampling areas. Fe, Mg, Mn, Ca and Zn were above the WHO standard limit, but Cu, Ni and Cr were found to be within the recommended permissible limit. Soil samples from the roadside contained higher amount of Mn, Cu, Ni, Cr, Mg and Ca than the flower farm, and smaller amount of Zn were observed in road side. This may be due to irrigation from water source or as a result of metals mobility from a nearby waste depot around the flower farm to the roadside through leaching and run off.

5.2. Recommendations

It is sure that some pesticides from flower farms would get into our primary focus, human beings, and have detrimental impacts. As a result, flower farms should transition to organic farming, which relies on natural pest and disease control methods such as crop rotations, composting, encouraging natural predators of common pests, and developing healthy flowers with natural resistance to pests and diseases, in order to reduce the associated risk. In addition, wastewater must be appropriately treated before being discharged into the environment. Furthermore, attention and regular monitoring of the soil pollution status of Ethiopia's floriculture industry are essential.

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