LEVELS OF SELECTED HEAVY METALS IN SOIL AND LEAFY VEGETABLES FROM HIRNA AREAS, ETHIOPIA

MSc THESIS

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DEDICATION

This thesis manuscript is dedicated to all my family.

STATEMENT OF THE AUTHOR

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BIOGRAPHICAL SKETCH

The author, Mr. Dejenu Teklewold, was born from his father Teklewold Haileslassie and mother Gebeyehu Werkeneh in West Hararghe Zone, Oromia Regional State, in 1978. He attended his primary school (grade 1-6) at Korke Primary School and junior and senior secondary school (grade 7-12) at Hirna.

After completing the Ethiopian Schools Leaving Certificate Examination (ESLCE) in 1993/1994, he attended teachers training institute (TTI) for primary school at Harar in 1994/95, following his graduation he was employed by the Ministry of Education as primary school teacher in West Hararghe Zone Tulo district in 1995. After serving for three years as primary school teacher, he attended diploma in Chemistry at the Alemaya University (presently Haramaya University) from 1999-2003 under the summer in-service program, than he attended BEd degree in chemistry at Haramaya University from 2004-2007 under the summer in service program. After serving for six years at Hirna senior school as chemistry teacher, he joined Haramaya University for his Postgraduate Studies in July 2013.

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ACRONYMS AND ABBREVIATIONS

FAAS	Flame Atomic Absorption Spectrophotometer
MDL	Method Detection Limit
SD	Standard Deviation
ND	Not detected
RSD	Relative Standard Deviation
TF	Transfer Factor
IDL	Instrumental Detection Limit

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LEVELS OF SELECTED HEAVY METAL IN SOIL AND LEAFY VEGETABLES FROM HIRNA AREAS, ETHIOPIA

ABSTRACT

Leafy Vegetables widely consumed in Hirna, Western Hararghe, Ethiopia such as cabbage (Brassica oleracea var), lettuce (Lectuca sativa L.) and Swiss chard (Beta vulgaris L.var .cicla) are cultivated on the main roadside soils. For this study, leafy vegetables and soil samples up on which they grow were collected at distances of 5,6 and 7m from the main road and the digestion procedures were optimized with a mixture of conc. 69-72 % HNO_3 and 70 % $HClO_4$ for the vegetables samples and a mixture of conc. 69 - 72 % HNO_3, 36-38 % HCl and 30 % H_2O_2 for soil samples and wet digestion procedure was employed to solubilized the metals from the samples. The validation was performed by spiking the samples with a standard solution of each metal having a known concentration and the percentage recovery values in the ranges of 88.6–108.4 % for soil and 87.9 - 98.67 % for vegetable samples. The samples were analyzed with the Flame Atomic Absorption Spectrophotometer (FAAS) to determine level of selected heavy metal (Pb, Cd, Cu, Zn, Ni, Co and Fe) in leafy vegetables and main roadside soils. The results showed that the levels of heavy metal in vegetable samples the ranges of 0.106 - 0.130 mg/L in lead, 1.856- 3.335 mg/L in zinc, 0.902- 1.13 mg/L in copper and 3.409- 5.929 mg/L of iron. The content heavy metal in soil samples also in the ranges of 0.137-0.179 mg/L in lead, 5.61-7.104 mg/L in zinc, 2.360-4.557 mg/L in copper and 7.778- 10.038 mg/L of iron. The levels of cadmium, cobalt and nickel were not detected (ND) in all samples. The heavy metal contents at the same distance from the road were found in leafy vegetables and soil samples in the following order: Fe > Zn > Cu > Pb. All these values were small when compared to the WHO/FAO safe limit, which is an indicated that the leafy vegetables samples in Hirna town area are safe for consumption without the risk of environmental toxicants.

Keywords: Leafy Vegetables, Heavy Metals, Roadside Soils

1. INTRODUCTION

1.1. Background

Vegetables are vital to the human diet, and in particular provide the well-known trace elements and heavy metals. Minor or trace elements are essential for good health if they come from an organic or plant source. In contrast, if they come from an inorganic or metallic source, they become toxic. The processes of plant growth depend on the cycle of nutrients including trace elements, from soil to plant (Mohamed *et al.,* 2003). Vegetables, especially leafy vegetables, accumulate higher amounts of heavy metals because they absorb these metals in their leaves.

Vegetables, especially those of leafy vegetables grown in heavy metals contaminated soils, accumulate higher amounts of metals than those grown in uncontaminated soils because of the fact that they absorb these metals through their leaves (Muhammad *et al*., 2008). It should be realized that most of these cultivated lands are contaminated with heavy metals contributed mainly through vehicular emissions, pesticides and fertilizers, industrial effluents and other anthropogenic activities (Halweil and Nierenberg, 2007). Environmental contaminants are widely distributed in air, water, soils and sediment and among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bio-accumulate in ecosystems (Censi *et al.,* 2006).

Fresh vegetables are important to our daily diet because they contain essential nutrients such as carbohydrates, proteins, vitamins, minerals and trace elements (Amin *et al*., 2013; Gebrekidan *et al*., 2013; Liu *et al*., 2013). There is a gradual increase consumption of vegetables due to increased awareness of food value of the vegetables and education exposure (Itanna, 2002; Chary *et al*., 2008; Bo *et al*., 2009). Heavy metals uptake also occurs as a result of deposits on different parts of the vegetables exposed to air from polluted environments. However, not all the traces of heavy metals in plants and animals are the results of human activity. Some arise through the absorption processes of naturally occurring soil components. Even foodstuffs grown in completely unpolluted areas are not entirely free of heavy metals; the absorption of very small amounts is therefore unavoidable in principle and has always occurred (Demirezen and Aksoy, (2006).

Heavy metals cannot be underestimated as these food stuffs are important components of human diet, they are very rich and comparatively cheaper sources of vitamin. The release of heavy metals is one of the most significant environmental problems caused by anthropogenic activities such as urban road construction, quarrying, agriculture, waste incinerations, sewage disposal, bush burning vehicle exhausts, industrial discharges, oil lubricants, automobile parts (Dolan *et al.*, 2006). Lead, cadmium, copper and zinc are the major metal pollutants of the roadside environments and released from fuel burning, wear out of tires leakage of oils, and corrosion of batteries and metallic parts such as radiators etc. The increased uptake of these metals by plants influence the natural contents of vegetables and thus poses serious health impacts. Most heavy metals are extremely toxic, and because of their solubility in water, contamination may readily reach toxic levels (Arora *et al*., 2008). Heavy metals are natural components of the earth's crust and cannot be degraded nor destroyed. They enter the human body through food, water and air.

Excessive intake of heavy metal-rich vegetables causes a series of health crisis viz reduction in immunological resistances, intra-uterine growth retardation, weakness in psycho-social behavior, malnutrition disabilities, upper gastrointestinal cancer and cardiovascular, kidney, nervous as well as bone diseases (Tongbin *et al*., 2009; Chang *et al.*,2013; Gebrekidan *et al*., 2013). These serious health problems happened due to intake of vegetables as a diet (Tongbin *et al*., 2009). The determination of heavy metal bioavailability is a direct method for assessing their toxicity and speciation analysis is the theoretic basis for evaluating metal bioavailability (Wang *et al*., 2008; Massas *et al*., 2013). Heavy metals are known as accumulative, refractory inorganic pollutants which widely exist in the environment. Their pollution effects will display through metal migration, enrichment and transformation (Wang, 2012). Heavy metal concentrations vary among different vegetables, which may be attributed to differential absorption capacity of vegetables for different heavy metals (Singh *et al*., 2010). Assessment of some heavy metals concentration in selected leafy vegetables collected in developing countries like Ethiopia, limited data are available on heavy metals in food products.

Due to the potential toxicity, persistent nature and cumulative behaviour of heavy metals in vegetables and fruits, there is an urgency to assess the food products to certify that the levels of such pollutants meet the approved international norms (Gebrekidan *et al*., 2013). A complete profile of mineral and heavy metals must be available for the nutritionist and consumers. Quality of vegetables affects consumers. Excessive levels could imply a risk as vegetables represent a high percentage of Ethiopians' diet.

A lot of work has been done on metal determination in vegetables in Addis Ababa (Yirgalem *et al*., 2012) and Tigray (Abraha Gebrekidan *et al*, 2013) and some data have been reported for leafy vegetables (Itanna, 2002. In line with this, there are no published literature reports on the concentrations of heavy metals in the vegetables available for the consumers in Hirna Town, Ethiopia with the view of knowing the health effect associated with the consumption of these leafy vegetables.

1.2. Objectives of the Study

1.2.1. General Objective

- To assess the levels of selected heavy metals (Cd, Pb. Fe, Ni, Co, Cu and Zn) in both soil and leafy vegetables (cabbage, lettuce and Swiss chard) from road side of Hirna, Western Hararghe, Ethiopia.

1.2.2. Specific Objectives

- To optimize the digestion procedure for both vegetable and soil samples.
- To validates the optimized method using recovery test.
- To determine the concentrations of Lead, Cadmium, Copper, Iron, Nickel, Cobalt and zinc in vegetables and soils sample using FAAS.

2. LITERATURE REVIEW

2.1. Heavy Metal

Heavy metals are currently of much environmental concern. Heavy metals are naturally occurring elements, and are present in varying concentrations in all ecosystems. There is a huge number of heavy metals. They are found in elemental form and in a variety of other chemical compounds. These metals are dangerous because they tend to bio accumulated in the food chain and they are harmful to humans and animals. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Several meanings have been assigned to heavy metals. Heavy metals can also be loosely defined as a subset of elements that exhibit metallic properties. It comprises the transition metals, some metalloids, lanthanides, and actinides. Using density as a defining factor (Järup, 2003) also defined heavy metals as those having a specific density of more than 5 g/cm^3 (Suciu *et al.,* 2008) They can also be chemical elements with the density greater than 4 g/cm^3 found in all kinds of soils, rocks and water in terrestrial and freshwater ecosystem (Adelekan and Abegunde, 2011).

Heavy metals can be said to be referred to as any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2010; Obodai *et al.*, 2011; Yahaya *et al.,* 2012). Therefore, heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4 °C (39 °F). Another school of thought also put heavy metals as having a density of 6.0 g/cm^3 or more (much higher than the average particle density of soils which is 2.65 g/cm^3) and occur naturally in rocks but concentrations are frequently elevated as a result of contamination (Asio, 2009). They can also be said to be intrinsic, natural constituents of our environments (Aderinola *et al.,* 2009). Therefore, they can be said to be a group of metals and metalloids with atomic density greater than 4 g/cm^3 or 5 times or greater than water (Obodai *et al*., 2011; Yahaya *et al.,* 2012). Heavy metals are members of a loosely defined subset of elements that exhibit metallic properties. It mainly includes the transition metals, some metalloids, lanthanides, and actinide. Heavy metals occur naturally in the ecosystem with large variations in concentration (Mohsen and Salisu, 2008) they are metallic elements that are toxic and has high density, specific gravity or atomic weight, metals with a potential negative health effect or environmental impact may be termed heavy metals (Kabata and Pendias,1999). There are

over 50 elements that can be classified as heavy metals, but only 17 that are considered to be both very toxic and relatively accessible. Lead, zinc, cadmium, magnesium and cobalt should be given particular attention, in terms of water pollution. Toxicity levels depend on the type of metal, its biological role, and types of organism that are exposed to it, toxic metals are often added to the streams as salt (sulfides, phosphate and carbonates), are very insoluble in hard water and usually travel with sediment. The transformation into readily accessible material is a complex process and depends on many factors such as pH, sediment presence and hardness.

The availability of these metals is determined by precipitation-dissolution reactions which are strongly affected by pH. Therefore at lower pH, heavy metals are more available and more reactive. Many of these metals then undergo Methylation as a result of bioaccumulation where bacteria absorb these elements and convert them from a metallic state into a toxic organometallic state. By becoming incorporated with an organic component, these metals become readily available to the first tropic level of the food chain and eventually lead to biological magnification throughout the system (Laura and Susan, 2009).

2.2. Sources of Heavy Metals in Contaminated Soils and Water

The presence of heavy metals in aqueous streams, air, soil and food has become a problem due to their harmful effects on human health even at low concentration in the environment. Heavy metal pollutants in wastewater are one of the problems facing human beings; heavy metal can be toxic to the life. Heavy metal contamination refers to the excessive deposition of toxic heavy metals in the soil caused by human activities. Heavy metals in the soil include some significant metals of biological toxicity, such as mercury (Hg), cadmium (Cd), lead (Pb), chromium (Cr) and arsenic (As) etc. Generally, metals enter the aquatic environment through atmospheric deposition, erosion of geological milieu or due to anthropogenic activities caused by industrial effluents, domestic sewage and mining waste (Aderinola *et al.,* 2009; Ene *et al.,* 2009; Bhagure and Mirgane, 2010; Adelekan and Abegunde, 2011; Obodai *et al.,* 2011), then also through urban storm, water runoff, land fill, mining of coal and ore. But naturally metals get to waters by chemical weathering of minerals and soil leaching (El Bouraie *et al.,* 2010). Heavy metals contamination can result from various sources such as purification of metals. For instance, the smelting of copper the preparation of nuclear fuels and electroplating, this produces chromium and cadmium (Ene *et al.,* 2009). Other sources of

heavy metals can be from dead and decomposing vegetation, animal matter, wet and dry fallouts of atmospheric particulate matters and from man's activities (Wufem, 2009; Yildiz *et al.,* 2010; Obodai *et al.,* 2011) reported on anthropogenic sources which lead to accumulation of heavy metals such as lead (Pb), zinc (Zn), copper (Cu) and nickel (Ni) in the environment (Nassef *et al.,* 2006). Such activities which give cement industry and mining operations (Suciu *et al.,* 2008; Obodai *et al.,* 2011).

Heavy metals are currently of much environmental concern. These metals are dangerous because they tend to bioaccumulation in the food chain and they are harmful to humans and animals. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment .Heavy metal contamination of arable soils is primarily caused by wastewater from mines being used to irrigate paddy fields (Adelekan and Abegunde, 2011), by emissions from nonferrous metal refineries (Ene *et al.,* 2009) land application of fertilizers animal manures, sewage sludge (Adelekan and Abegunde, 2011), pesticides and coal combustion residues (El Bouraie *et al.,* 2010). If there are no proper treatments or disposal of mine tailings and mine drainage then agricultural fields can be contaminated (Makino *et al.,* 2010; Wuana and Okieimen, 2011). Also manufacturing and the use of synthetic products (e.g. pesticides, paints, batteries, industrial waste and land application of industrial or domestic sludge) can result in heavy metal contamination of urban and agricultural soils (USDA and NRCS, 2000; Wei and Yang, 2010). Another important source of heavy metal contamination is human transport either by land, air, inland water or sea. The heavy metals in the aquatic environment can be found in sediments and suspended particulates (Aderinola *et al.,* 2009; Wufem, 2009).

Anthropogenic sources such as mining and metallurgy manufacturing, agriculture, industrial waste water discharges, sewage wastewater, fossil fuel combustion and atmospheric deposition and transport sectors can also introduce heavy metals into water bodies such as rivers and lagoons thereby contaminating them(Ene *et al.,* 2009; Varalakshmi and Ganeshamurthy, 2010). The metals dissolve and move downstream to lower reaches of the water bodies while others settle into the sediments (Kumar *et al.,* 2010; Oluyemi *et al.,* 2010; Obodai *et al.,* 2011; Rajaganapathy, 2011). The range of contaminant concentrations and the physical and chemical forms of contaminants will also depend on activities and disposal patterns for contaminated waste on the site. Excess heavy metals in the soil originate from many sources, which include sewage to soils pathway, solid wastes to soils pathway and agricultural supplies to soil path (Zhang *et al.,* 2011).

2.2.1. Agricultural Supplies to Soils

Fertilizers, pesticides and mulch are important agricultural inputs for agricultural production (Zhang and Zhang, 2007; Zhang *et al.,* 2011). Nevertheless, the long-term excessive application has resulted in the heavy metal contamination of soils. The vast majority of pesticides are organic compounds, and a few are organic - inorganic compound or pure mineral, and some pesticides contain Hg, As, Cu, Zn and other heavy metals (Arao *et al*., 2010). Heavy metals are the most reported pollutants in fertilizers. Heavy metal content is relatively low in nitrogen and potash fertilizers, while phosphoric fertilizers usually contain considerable toxic heavy metals. Heavy metals in the compound fertilizers are mainly from master materials and manufacturing processes.

The content of heavy metals in fertilizers is generally as follows: phosphoric fertilizer > compound fertilizer > potash fertilizer > nitrogen fertilizer (Boyd, 2010). Cd is an important heavy metal contaminant in the soil. Cd is brought to soils with the application of phosphoric fertilizers. Many studies showed that, with the application of a large amount of phosphate fertilizers and compound fertilizers, the available content of Cd in soils increases constantly, and Cd taken by plants increases accordingly. In recent years, the mulch has been promoted and used in large areas, which results in white pollution of soils, because the heat stabilizers, which contain Cd and Pb are always added in the production process of mulch. This increases heavy metal contamination of soils (Satarug *et al.,* 2003).

Table 1. Different source of heavy metals contaminating soils annually in the world

	Cd	Ni	Cu	Pb	Zn
Agriculture and food waste	0~0.3	6~45	3~38	1.5~27	12~150
Farmyard manure	0.2~1.2	3~36	14~80	3.2~20	150~320
Logging and timber Industry wastes	0~2.2	2.2~23	3.3~52	6.6~8.2	13~65
Municipal wastes	0.88~7.5	2.2~10	13~40	18~62	22~97
Municipal sludge	0.02~0.34	5~22	4.9~21	2.8~9.7	18~57
Organic wastes	0~0.01	0.17~3.2	0.04~0.61	0.02~1.6	0.13~2.1
Metal processing solid wastes	0~0.08	0.84~2.5	0.95~7.6	4.1~11	2.7~19
Fertilizer	0.03~0.25	56~279	93 ~335	0.42~2.3	0.25~1.1
Coal ash	1.5~13	0.2~3.5	0.05~0.58	45~242	112~484
Marl	0~0.11	0.22~3.5	0.15~20	0.45~2.6	0.15~3.5
Commodity	0.78~1.6	6.5~32	395~790	195~390	310~620
Impurities Atmospheric	2.2~8.4	11~37	14~36	202~263	49~135
Deposition Total	5.6~38	106~544	541~1367	479~1113	689~2054

Source Qin *et al.,* 2008

2.2.2. Waste Water

The application of municipal and industrial wastewater and related effluents to land dates back 400 years and now is a common practice in many parts of the world, it is estimated that 20 million hectares of arable land are irrigated with waste water. In several Asian and African cities studies suggest that agriculture based on wastewater irrigation accounts for 50 percent of the vegetable supply to urban areas (Bjuhr, 2007), farmers generally are not bothered about environmental benefits or hazards and are primarily interested in maximizing their yields and profits. Although the metal concentrations in wastewater effluents are usually relatively low, long-term irrigation of land with such can eventually result in heavy metal accumulation in the soil. Wastewater can be divided into several categories, sanitary sewage, chemical wastewater, industrial mining wastewater and urban mining mixed sewage etc. Heavy metals are brought to the soil by irrigative sewage and are fixed in the soil in different ways.

It causes heavy metals (Hg, Cd, Pb and Cr etc.) to continually accumulate in the soil year by year. Sewage irrigation is a feasible way to solve the problem of crop irrigation in the arid area. However heavy metal contamination caused by sewage irrigation must be paid enough attention. Quality of irrigative sewage must be strictly controlled within the national quality standard for irrigation water.

2.2.3. Solid Wastes to Soils

There are a variety of solid wastes which have complex composition of which mining and industrial solid waste contamination is the most serious. When these wastes are in the process of being piled or governed, heavy metals move easily due to the facilitation of sunlight, raining, washing and they spread to the surrounding water and soils at the shape of funnel and radiation. With the development of industry and the acceleration of urban environmental construction, sewage treatment is continuing to be strengthened. China now has more than 80 sewage treatment plants, with the estimated 400 million tons of sludge production. Due to the high content of organic matter, nitrogen and phosphorus in the sludge, soils become the main places for soil sludge treatment. In general, Pb, Cu, and Zn in the sludge will exceed the control standards easily (Ding, 2000). Solid wastes can expand contamination scope easily with the help of wind and water.

2.3. Heavy Metal Transport and Its Uses

Generally, heavy metals enter into the body system through air, food and water and bioaccumulation over a period of time (Lenntech, 2010; Obodai *et al.,* 2011). Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment (Lenntech, 2010). Emission of heavy metals to the environment occur via a wide range of processes and pathways, including to the air (e.g. during combustion, extraction and processing), to surface waters (via runoff and releases from storage and transport) and to the soil (and hence into ground waters and crops) (Järup, 2003). Certain contaminants move through the air and deposited as dust or by precipitation. Heavy metals enter the aquatic environment through atmospheric deposition. They usually remain either in soluble or suspension form and finally tend to settle down to the bottom or are taken up by organisms (Obodai *et al.*, 2011). Sediments are hosts of toxic metals and can therefore allow for the detection of heavy metals that may be either absent or in low concentration in the water column (Aderinola *et al.,* 2009).

The accumulated heavy metals in the sediments can remain present for many years also chemicals may be carried by winds and deposited on the surface of soils (Shayley *et al.,* 2009). Dietary intake of food may constitute a major source of long-term low-level body accumulation of heavy metals (Doherty *et al.,* 2011). Heavy metals have varied uses, even though in certain concentrations they tend to be dangerous. Some of its uses are as follows:

- In small amounts nickel can be used by the body to produce red blood cells and then as an ingredient of steel and other metal products (Asio, 2009; Lenntech, 2010).
- Cadmium compounds are used in re-chargeable nickel-cadmium batteries, pigments, stabilizers for polyvinyl chloride (PVC), alloys and electronic compounds (Järup, 2003; Wuana and Okieimen, 2011)
- Lead is also used in the manufacture of lead storage batteries, solders, bearings, cable covers, ammunition, plumbing, pigments and caulking (Wuana and Okieimen, 2011).
- Copper is also used in the production of blood hemoglobin, seed dressing, disease resistance, and regulation of water (Wuana and Okieimen, 2011).

2.4. Properties and Effects of selected Heavy Metals

Heavy metals have the ability to enter the human body through inhalation, ingestion and dermal contact absorption (Wei and Yang, 2010; Adelekan and Abegunde, 2011; Rajaganapathy, 2011).They also accumulate in soils, plants and in aquatic biota (Suciu *et al.,* 2008; Obodai *et al.,* 2011; Wuana and Okieimen, 2011). Heavy metals can persist for a long time within different organic and inorganic colloids before becoming available to living organisms (Fried ova, 2010; Adelekan and Abegunde, 2011). They are non-degradable and therefore do not decay with time. Heavy metals can be biomagnified if an organism excretes it slower than it takes in. They can therefore become dangerous to human beings and wild life (Kumar *et al.,* 2010; Adelekan and Abegunde, 2011).

Heavy metals can be described as contaminants in the soil environment because (i) through man-made cycles their rates of generation are more rapid relative to natural ones, (ii) they become transferred from mines to random environmental locations where higher potentials of direct exposure occur, (iii) compared to those in the receiving environment the concentrations of the metals in discarded products are relatively high, and (iv) the chemical form (species) in which a metal is found

in the receiving environmental system may render it more bio available (Wuana and Okieimen, 2011). Occurring as natural constituents of the earth's crust, heavy metals are by nature non - biodegradable and tend to be contaminants to living things in the environment (Aderinola *et al.*,2009; Bhagure and Mirgane, 2010; Obodai *et al.*, 2011). Therefore, the biota that inhabits contaminated sites is exposed to very high amounts of the heavy metals (Aderinola *et al.*, 2009).

Environmental problems can also result from irrigation with sewage effluent which introduces heavy metals though can help alleviate water shortages (Makin *et al.*, 2010). Heavy metals contamination threatens agriculture and other food sources for human population as well as poor vegetation growth and lower plant resistance against forests pests. There by having impact on the quality of food, groundwater, microorganisms and plant growth (Ene *et al.*, 2009). Their effect on microorganisms can give rise to decrease in litter decomposition and nitrogen fixation, less efficient nutrient cycling and impair enzyme synthesis (Adelekan and Abegunde, 2011). Heavy metals persist for a long time in the environment being non degradable and are trans- located to different components affecting the biota (Kumar *et al.*, 2010; Obodai *et al.*, 2011; Rajaganapathy, 2011). This situation poses a different kind of challenge for remediation.

The persistence of heavy metals can result in bioaccumulation and bio magnifications causing heavier exposure for some organisms than is present in the environment alone. For instance, mercury and selenium can be transformed and volatilized by microorganisms (USDA and NRCS, 2000). According to (Yildiz *et al.*, 2010) increasing exposure to toxic elements in marine and terrestrial organisms can have adverse toxicological effects. For instance, Coastal fish (such as the smooth toadfish) and sea birds (such as the Atlantic Puffin) are often monitored for the presence of such contaminants. Mention can also be made of Minamata disease and itai-itai disease from mercury and cadmium poisonings respectively (Azimi *et al.*, 2006). Heavy metal exposure is normally chronic (exposure over a longer period of time), due to food chain transfer. The heavy metals to be determined in both the soil and leaf vegetables samples are zinc (Zn), cadmium (Cd), lead (Pb), cobalt (Co), nickel (Ni) and copper (Cu).

2.4.1. Zinc (Zn)

Zinc (Zn) is a hexagonal crystal, bluish-white metal and a d-block metal. It is a transition metal located in period 4 and group 12. It also has atomic number 30, atomic mass 65.4, density

7.15 g/cm^3, melting point 693 K and a boiling point of 1180 K. Zinc (Zn) is actually a common element found in air, soil, water and all foods (Hardy *et al.,* 2008). It occurs naturally in soil but the concentrations are rising due to anthropogenic additions. Zinc can be found in nearly all soils. It is present in most rocks and is weathered out and deposited into the soil. Zinc is also released by thermal outgassing and other volcanic events. Fallout from such events can be a significant source of zinc in soils and plants. Anthropogenic release is the primary source of zinc in the environment Most additions are from industrial activities such as mining, coal, waste combustion and steel processing (Wuana and Okieimen, 2011), then also from the use of liquid manure, composted materials, fertilizers and pesticides in agriculture (Bhagure and Mirgane, 2010).

It is used in industry to make paint, dye, rubber, wood preservatives and as well as ointments (Hardy *et al.,* 2008). Zinc (Zn) pollutes water due to the large quantities present in the wastewater of industrial plants and the water- soluble forms present in the soil can contaminate ground water. It may increase the acidity of waters. Then can negatively influence the activity of microorganisms and earthworms thereby retarding the breakdown of organic matter (Wuana and Okieimen, 2011). The primary source of zinc in the area could be the attrition of motor vehicle brake linings and tire wear have been identified as possible sources of Zn (Bai *et al.,* 2008). In addition vehicular traffic may be major source of Zn and Cu (Rajaram *et al.*, 2014).

2.4.2. Cadmium (Cd)

Cadmium (Cd) is also a hexagonal crystal, silver white malleable and a d-block metal. This is a transition metal belonging to period 5 and group 12. It has atomic number 48, atomic mass 112.2, density 8.65 g/cm^3, melting point 594 K and boiling point of 1038 K. It is an essential micronutrient for plants and animals but may cause malfunctioning of metabolic processes (Wuana and Okieimen, 2011). Cadmium (Cd) enters the environment through the uncontrolled burning of coal and garbage and through the human's food chain directly or indirectly from plants or animals (Azimi *et al.,* 2006).

The application of agricultural inputs such as fertilizers, pesticides, bio solids (sewage sludge), and the disposal of industrial waste and the deposition of atmospheric contaminants increases the total concentration of Cd (Järup, 2003; Asio, 2009; Wuana and Okieimen, 2011). It can also result from burning of fossil fuels, sewage sludge, plastics waste, by product of Zn and lead refining, insecticides and motor oil. Cadmium (Cd) uses include Ni/Cd batteries, pigments, stabilizers for

polyvinyl chloride (PVC), in alloys, electronic compounds, barriers to control nuclear fission, phosphors in the production of televisions, anticorrosive coatings for metals, amalgam in dentistry and worm treatments for swine and poultry (Hardy *et al* ., 2008).

Cadmium (Cd) causes bone diseases (itai- itai), cardiovascular diseases, renal problems, severe pains in the joints, kidney and lung problems and also anemia due to decrease of iron adsorption by intestines (Azimi *et al.,* 2006; Hardy *et al.,* 2008). The first indicator of cadmium poison is the malfunctioning of the kidneys (Azimi *et al.,* 2006). Cd may damage the metabolism of calcium, which will cause calcium deficiency and result in cartilage disease and bone fractures, etc. Agency for Toxic Substances Management Committee has listed Cd as the sixth most toxic substance that damages human health. It affects sperm, reduces birth weight and a causal factor in cardiovascular diseases and hypertension (Asio, 2009: Adelekan and Abegunde, 2011), also Cd exposure can lead to situations such as neurotoxin, hypertension, carcinogenic, teratogenic, liver dysfunction, nausea, vomiting, respiratory difficulties, cramps and loss of consciousness (Bhagure and Mirgane, 2010; Adelekan and Abegunde, 2011).

2.4.3. Lead (Pb)

Lead (Pb) is cubic crystal, silver blue-white, soft and a p-block metal. It is located in period 6 and group 14. Lead has atomic number 82, atomic mass 207.2, density 11.4 g/cm^{3,} melting point 601 K and boiling point 2013 k. Lead exists as Pb^{+2} ion during the chemical reaction. Lead is a well-known highly toxic metal and a cumulative poison. The common industries that deal with lead are the battery manufacturing, motor vehicle repair, cable making and metal grinding industries and it is also, used in piping conducting materials, accumulators, lead chambers, printing characters, soldering, antiknock substances, colored pigments, radiation shielding, wrappings for food, tobacco and as an additive in gasoline.

A major use is the production of anti-knock compounds particularly tetraethyl lead, Pb $(C_2H_5)_4$ for addition to petrol. The exhausts from vehicles are a major source of the environmental contamination by lead. Lead is present in exhaust gases mainly as lead halides and oxides, but incomplete combustion result in about 10 % of alkyl lead compound also being present.

Other source of lead emissions are copper and nickel smelters, iron and steel production. Estimates vary as to the importance of vehicle emission as the source of the lead impurity. Lead exists in the oxidation states Pb^{2+} and P^{4+} with the divalent form being the more stable in most aquatic environments. Lead (Pb) accumulation in the body organs (i.e.,brain) may lead to poisoning (plumbism) or even death. Pb mainly enters human body through the digestive tract and respiratory tract, and then goes into the blood circulation in the form of soluble salts, protein complexes or ions, etc. 95% of the insoluble phosphate lead accumulates in bones. It affects and damages many of the body organs and systems, such as kidney, liver, reproductive system, nervous system, urinary system, immune system and the basic physiological processes of cells and gene expression. The presence of lead (Pb) may also affect the gastrointestinal tract, kidneys, and the central nervous system. For instance, children exposed to lead (Pb) suffer from impaired development, lower IQ, shortened attention span, hyperactivity and mental deterioration. Those at substantial risk are the children under the age of six (Hardy *et al.,* 2008; Asio, 2009; Wuana and Okieimen, 2011). Lead is a serious cumulative body poison which enters into the body system through air, water and food and cannot be removed by washing fruits and vegetables (Itanna, 2002).

2.4.4. Copper (Cu)

Copper (Cu) is a cubic crystal, reddish and a d-block metal. It is also a transition metal located in period 4 and group 11. Copper (Cu) has atomic number 29, atomic mass 63.5, density 8.96 g/cm^3, melting point 1357 K and boiling point 2840 K. It occurs in rocks, soil, water, air, plants and animals (Hardy *et al.,* 2008). It is also an essential micronutrient required in the growth of both plants and animals. Concerning humans copper (Cu) helps in the production of blood hemoglobin whiles in plants it is used in seed production, disease resistance and regulation of water (Wuana and Okieimen, 2011).

It is also used as a component in metal alloys, electrical wiring, preservatives for wood, leather and fabrics (Hardy *et al.,* 2008). Copper (Cu) is not magnified in the body or bio accumulate in the food chain. Cu, Zn and Ni are essential trace metals in the human body, but if the body takes excessive Cu, Zn and Ni from the outside environment, they will damage human health. Ni and Cu are tumor promoting factors, whose carcinogenesis effect has attracted global concerns. Workers who are in close contact with the nickel powder are more likely to suffer from respiratory cancer, and the

content of Ni in the environment is positively correlated with nasopharyngeal carcinoma (Chen, 2011). High doses of copper causes anemia, damages liver, kidney, stomach, intestinal irritation, neurological complications, hypertension and liver and kidney dysfunctions (Bhagure and Mirgane, 2010; Lenntech, 2010; Wuana and Okieimen, 2011).

2.4.5. Cobalt (Co)

Cobalt (Co) is cubic crystal, silver gray and a d-block metal. It is also a transition metal located on the periodic table in period 4 and group 9. It has atomic number 27, atomic mass 58.9, and density 8.9 g/cm ^{3} melting point 1768 K and boiling point 3143 K. There are three valence states of cobalt namely 0, +2 and +3 (Kim *et al.*, 2006). In nature, it is frequently associated with nickel, and both are characteristic minor components of meteoric iron. Cobalt exposure can be both natural and anthropogenic. The natural sources result from wind-blown dust, seawater spray, volcanoes, forest fires, and continental and marine biogenic emissions. Also, the anthropogenic sources can be from burning of fossil fuels, sewage sludge, phosphate fertilizers, mining and smelting of cobalt ores, processing of cobalt alloys, and industries that use or process cobalt compounds (Kim *et al.*, 2006). Having released into the atmosphere cobalt is deposited on soil, but in water may sorbs to particles and settle into sediment or sorbs directly to sediment.

Cobalt (Co) can be used in electroplating, as a ground coats for porcelain enamels, magnetic steels, some types of stainless steels and alloys for jet engines and gas turbines. Concerning humans, cobalt is important because it is part of vitamin B_{12}, which is essential component for human health (Bhagure and Mirgane, 2010). It is also used to treat anemia with pregnant women because it stimulates the production of red blood cells (Lenntech, 2012). Among individuals who are iron deficient there is an observable increase in cobalt absorption (Kim *et al.*, 2006). Inhalation and dermal exposure to cobalt in humans can result in bronchial asthma, interstitial lung disease, lung cancer, pneumonia, heart problems, thyroid damage, nausea, vomiting and diarrhea (Bhagure and Mirgane, 2010; Kim *et al.*, 2006; Lenntech, 2012). Then in animals exposure to cobalt could cause reproductive and developmental effects (Kim *et al.*, 2006).

2.4.6. Nickel (Ni)

Nickel (Ni) is also cubic crystal, silvery and a d-block metal. This is a transition metal belonging to period 4 and group 10. It has atomic number 28, atomic mass 58.7, density 8.9 g/cm ^{3}, melting point 1726 K and boiling point of 3005 K. It is an element that occurs in the environment only at very low levels and is essential in small doses, but it can be dangerous when the maximum tolerable amounts are exceeded (Asio, 2009; Wuana and Okieimen, 2011). Nickel contaminations in the soil are metal plating industries, combustion of fossil fuels, nickel mining and electroplating (Bhagure and Mirgane, 2010). Humans may also be exposed to nickel (Ni) by inhalation, drinking water, and eating contaminated food (Asio, 2009). It is used in the manufacture of stainless steel, coins, nickel for armor plates, burglarproof vaults, vegetable oils, ceramics and Ni-Cd batteries (Asio, 2009; Hardy *et al.*, 2008). Nickel (Ni) can result in lung, liver and kidney damage. In high quantities Ni can also cause cancer, respiratory failure, birth defects, allergies, dermatitis, eczema, nervous system and heart failure (Adelekan and Abegunde, 2011; Asio, 2009; Lenntech, 2010).

Table 2. Estimate of relative lead exposure in selected African countries (Adapted from 2002 / as cited in ESMAP (2003).

Estimate of relative Lead Exposure in Selected Countries (Adapted from WRI 2002 / as cited in ESMAP (2003)	Market Share of Leaded Gasoline (%) 2002	Motor gasoline consumed (million liters)	% gasoline consumed in urban areas	Maximum Lead concentration in Gasoline (g/L)	Average Actual Lead Conc. Gasoline (g/L)	Actual Leaded gas Emission (metric tons)	Total Urban Population in thousands (1995)	Exposure to leaded gasoline (tons per Million urban population (1995)
Ethiopia	100	188	70	0.6	0.06	11	8,695	0.9
Ghana	100	806	80	0.6	0.1	81	6,222	10.4
Kenya	100	458	70	0.4	0.2	92	7,763	8.3
Senegal	100	242	80	0.8	0.2	48	3,629	10.7
Tanzania	100	165	70	0.4	0.2	33	7,279	3.2

3. MATERIALS AND METHODS

3.1. Study Area

The study area, Hirna Town is, located in Eastern part of Ethiopia in Oromia Region 360 km far from Addis Ababa and 42 km from Asebe Teferi (Chiro), West Hararghe. It is found between $9^0$10'16.16" North latitude and 41^0 02'48.58" East longitudes with elevation 2260.70 m. The major economic activities of the town are: services (hotels and restaurants, traditional beverages and groceries), urban agriculture, trades, wood works, and metal works. In the urban areas of the town vegetables are highly cultivated. The study area, in which vegetables are largely cultivated, is located on the sides of the main road to and from Addis Ababa which crosses the town.

3.2. Chemicals and Reagents

Chemicals and reagents used in the analysis were all of analytical grade. 69-72 % HNO_3 (Fine Chemical Industries, Mumbai, India) and 70 % $HClO_4$ (Aldrich, UK) were used for the digestion of the vegetable samples (cabbage, lettuce and Swiss chard). Concentrated 36-38 % HCl (Hopkins and Williams, UK), 69-72 % HNO_3 (Fine Chemical Industries, Mumbai, India), and 30 % H_2O_2 (Scharlaw Chemie S.A) were used for digestion of soil samples. The working standard solutions of the selected metals were prepared freshly from the intermediated standard solution 100 mg/L which was obtained by diluting stock standard solutions. Stock standard solutions (Buck Scientific Puro-Graphic) of 1000 mg/L concentration of the heavy metals from which 100 mg/L intermediate standards obtained were used for the preparation of the calibration standards of each metal. Deionized water was used for sample preparation, dilution, and rinsing apparatus prior to analysis.

3.3. Apparatus and Instruments

Polyethylene plastic bags were used to pack the collected samples, air-circulating oven (Genlab Limited, UK) was used for drying the samples, ceramic mortar and pestle Halden wanger, Germany) were used for homogenizing the ground afore mentioned plant samples, digital analytical balance (Mettler Tolendo, Model AG 204, Switzerland) with ± 0.0001 g precision was used to weigh samples, and digestion tubes were employed for digesting sample (electrical hot plate for digestion

Of samples, volumetric flasks of differing volumes (Pyrex, USA) were used during dilution and preparation of metal standards. Measuring cylinders, pipettes and micropipettes, burettes (Pyrex, USA) were used to measure volumes of sample solution, acid reagents and metal standard solutions, pH meter, conductivity meter, metal beaker, magnetic stirrer, Flame Atomic Absorption Spectrophotometer (FAAS) (Buck Scientific Model 210 VGP AAS, East Norwalk, USA) by using hallow cathode lamps with air acetylene flame. The concentration of iron (Fe), cadmium (Cd), copper (Cu), lead (Pb), Cobalt (Co), nickel (Ni) and zinc (Zn) in the vegetables and soil samples was determined by Flame Atomic Absorption Spectrophotometer (BUCK Scientific, MODEL 210 VGB) by using hollow cathode lamps with air-acetylene flame.

3.4. Sample Collection and Preparation

Each three vegetable species totally (9) samples were randomly and triplet for each samples were selected from sampling sites at a distances of 5, 6, and 7 m on road side soil of Hirna, by hand using gloves and packed into polyethylene bags, then brought to Haramaya University Research Laboratory for purpose of the experiment to determine the levels of Cd, Pb, Cu, Ni, Co, Fe and Zn in leafy vegetables. These vegetables were swiss chard (Beta vulgaris L.var. cicla), Lettuce (Lectuca sativa L.) and cabbage (Brassica oleracea var.). The vegetable leaves used for the experiment were purchased directly from local farmers at the sites located on road side of Hirna. The samples were washed with double distilled water to remove dust particles. After washing and cutting, the vegetables leaves were dried in oven for 3 - 4 days at temperature of 70 ℃ to remove moisture. After drying, the samples were gently grinded with clean agate mortar and pestle to make fine vegetable leaves. Sample collections were carried out according to the methods described by Udosen *et al.*, (2006).

The soil samples also similar vegetables samples each three soil samples totally (9) samples randomly selected and for each triplet samples were collected from sampling sites at distances of 5, 6 and 7 m were taken at a depth of 0 to 15 cm with the help of garden shovel cleaned with concentrated nitric acid. The soil samples were collected into plastic containers which had been pre-cleaned with concentrated nitric acid. The reason is to remove any traces of heavy metal contaminant. In the laboratory, each mixed soil sample was dried using an oven at 107 ℃ for 24 hours. The dried samples

were grinded using a grinding disc mill and used for metal analysis (Brigden *et al*., 2008; Vanloon, 1985; Vandecasteele and Block, 1993).

3. 5. Determination of pH and Electrical Conductivity of Soil

The bioavailability of heavy metals in the plant parts depends on a number of physical and chemical factors in the soils. These include: pH and electrical conductivity. A 10 g laboratory soil sample was weighed into five 50 mL beakers and 25 mL distilled deionized water was added to form 1:2.5 soil/water mixtures. Then, the beakers containing the mixture were placed on an automatic stirrer and stirred for 30 minutes; the samples then kept for about 5 minutes for the soil particles to settle. Finally, the pH meter electrodes were immersed into the soil/water mixture and the pH was measured on the upper part of the suspension (Sertse and Taye, 2000).

A 10 g laboratory soil sample was weighed into four 100 mL beaker then, 50 mL of distilled deionized water was added and the mixture was stirred using an automatic stirrer for 30 minutes. Finally, the conductivity of each sample was measured, from the upper part of the mixture, after the suspensions settled. The temperature during this measurement was displayed by the instrument automatically (Sertse and Taye, 2000).

3.6. Optimization of the Digestion Procedure

It is important to develop an optimum working procedure in order to get a reliable result from an analytical experiment. Thus, to prepare a clear and colorless sample solution that is suitable for the analysis using FAAS, different working procedures for the digestion were assessed using of HNO_3 and $HClO_4$ acids for leafy vegetable and of HNO_3, HCl and H_2O_2 for soils in varying quantities such as volume of the acids mixture, digestion time and digestion temperature. By examining the nature of the final digests obtained by varying the above parameters, the optimized procedure was selected depending up on the clearness of the digests, less digestion time, less reagent volume consumption and simplicity (Amde *et al.,* 2013). Accordingly to the digestion procedure was optimized in terms of the usage of lesser reagent volume, shorter digestion time and reasonable mild temperature for obtaining clear and colorless solutions of the resulting digests.

3.6.1. Optimization of Reagent Volume

To obtain a clear and colorless sample solution that is suitable for the analysis using flame atomic absorption spectrometry, different digestion procedures for the plant samples 0.50 g of (cabbage, lettuce, and swiss chard) were tried using 8 mL of varying volume of (v/v) the acid of concentrated HNO_3 and $HClO_4$ by keeping the digestion time and temperature of the method constant. To optimize the selected reagent volume digestion procedure of soil samples also carried out by 0.50 g of soil sample with 13 mL of varying volume the acid mixture of concentrated HNO_3 and HCl and different volume H_2O_2 by fixed digestion temperature and time were used. According to the digestion procedure was optimized in terms of the usage of lesser reagent volume for obtaining clear and colorless solution the resulting digests selected.

3.6.2. Optimization of Digestion Temperature

In order to optimize temperature, 0.50 g of vegetable samples mixed with the values of optimized reagent volume of acidic mixtures (HNO_3 and $HClO_4$) and fixed digestion time with varying temperature were used in order to obtain reasonably mild temperature for obtaining clear solutions of the resulting digests. The optimization of digestion temperature for soil sample was similar to the vegetable sample.

3.6.3. Optimization of Time

0.50 g of vegetable sample was mixed with the optimized reagent volume and temperature of digestion procedure with varying time in order to obtain less digestion time for clear and colorless solution. A similar approach was used to optimize the digestion time for the soil sample. The choice was made by observing that the final solution to be clear and colorless without any suspended matter. The procedures that required higher reagent volume longer digestion time and colored digest solution were rejected.

After optimization of experimental parameter, digestion of plants and soil based on selection optimization principles less reagent volume, less digestion time and mild temperature selected. For each of the leafy vegetables plant (9) samples, 0.5 g of powdered and homogenized samples were weighed and transferred to 250 mL of Erlenmeyer flasks in triplicate. To this, 4 mL of 1:1 (v/v) of HNO_3 and $HClO_4$ were added and digested in hot plate at 150 $°C$ for 2:00 hr. The digested solutions

were allowed to cool and 5 mL of distilled-deionized water was added to dissolve the precipitate formed on cooling and gently swirled and filtered into 50 mL volumetric flask through filter paper and was filled to the mark and allowed to settle (Vanloon, 1985; Vandecasteele and Block, 1993). The solution was kept in refrigerator for analyzed for the concentration of Cd, Cu, Ni, Co, Fe Zn and Pb by flame atomic absorption spectroscopy.

On the other hand, 0.50 g each samples were collected from a distance of 5, 6 and 7 m from main road soils of each vegetables samples of laboratory soil sample (9) were weighed into 100 mL three Erlenmeyer flasks. Then, soil sample was wetted by 5 mL of distilled-deionized water. Next 3 mL HNO_3 and 10 mL HCl were added into digestion flask. The samples were mixed with the acid mixture and digested over boiling water bath, at 95 $°C$ for two hours. After cooling, the digestion mixture was treated with 2 mL of H_2O_2 and filtered through filter paper. The filtrate was transferred in to a 100 mL volumetric flask and was diluted to the mark (Vanloon, 1985; Vandecasteele and Block, 1993) .Finally all the solutions were prepared in this way and kept in the refrigerator for subsequent metal determination.

Figure 1.The digestion set up and digested samples of plants and soils

3.7. Validation of the Analytical Procedure

In order to validate the analytical method, the following method validation parameters such as recovery of analyte, method limit of detection and limit of quantification studies were carried out (Prichard *et al*., 2007).

3.7. 1. Recovery Test

Recovery is one of the most commonly used techniques utilized for validation of the analytical procedure to evaluate how far the method is acceptable for its intended purpose. Because of the absence of certified reference material for the leafy vegetables and the corresponding soil samples: validity of the digestion procedures was assured by spiking the samples with a standard solution of known concentration of the target analytes. The spiked leafy vegetable plant and their soil samples were digested in triplicate following the same procedure used for digestion of the plant parts and the soil samples. The resulting digests of the spiked samples then were analyzed for their respective metal contents using FAAS and percent recoveries were calculated both for the plant parts and the soil samples using the following formula (Harvey, 2000).

$$R\ (\%) = \frac{\text{Metal Concentration in spiked--Metal Concentration in un spiked}}{\text{Concentration of metal in spiked}} \times 100$$

3.7.2. Method Detection Limit (MDL)

Detection limit is the lowest concentration level that can be determined to be statistically different from an analyte blank or the minimum concentration that can be detected by the analytical method with a given certainty. A general accepted definition of detection limit is the concentration that gives a signal three times the standard deviation of the blank or background signal. In this study, the detection limit of each metal was calculated as three times the standard deviation of the blank or 3 σ blank (Dean, 2003).

3.7.3. Determination of Limits of Quantification

The limit of quantification is the same as the concentration that gives a signal 10 times the standard deviation of the blank. Limit of quantification is the lowest limit for precise quantitative measurements. The quantification limit of each element was calculated as ten times the standard deviation of the blank solution or 10 σ blank (Dean, 2003).

3.8. Determination of Heavy Metal in the Samples

The samples were analyzed for each heavy metal of Zinc (Zn), Cadmium (Cd), Copper (Cu), Nickel (Ni), Iron (Fe) Cobalt (Co) and lead (Pb) in plants and soil sample using Flame Atomic Absorption

Spectrophotometer (FAAS). The method was used by direct aspiration of sample digest, using air acetylene flame.

3.9. Data Analysis

All the sample analyses in this study were carried out in triplicate and results were reported as mean ± SD. The significance of variation in metals concentration between samples was analyzed by one way ANOVA. The statistical analysis was conducted using Microsoft Office Excel 2007 and statistical method Statistical analyses were carried out for comparing mean concentration of heavy metals between the leafy vegetables in selected samples and respective soil samples of vegetables.

4. RESULTS AND DISCUSSION

4.1. Instrument Calibration and Operating Conditions

The data qualities obtained from FAAS for metal analyses are highly affected by the calibration curve. The calibration curves were prepared from a standards of known concentration; covering the concentration range expected in the sample and to determine the concentration of each metal in the sample solutions. The instrument was calibrated using series of working standards. The working standard solutions of each metal were prepared from intermediate standard solutions of the respective metals.

Different parameters such as wave length, the coefficients regression and method of detection limit of the calibration curves of each metal in samples are presented in the Table 9. The calibration curves of the metals showed good linearity with regression coefficients (R^2). Thus, the obtained calibration curves were linear, which assured that linearity of instrumental response for individual analyte.

4.2. Optimization of Experimental Parameter

For the evaluation of the total concentration of metals using FAAS, samples of leafy vegetables and soils require to be solubilized to reduce matrix effects originating from organic compounds and releasing elements in the form of their simple ions. The optimum digestion procedures selected for complete digestion of 0.50 g homogenized samples of leafy vegetables were 4 mL HNO_3 and 4 mL of $HClO_4$, temperature 150 ℃ and time of 2 hour. For soils samples also 0.5 g soil samples with the optimized selected reagent volumes were 3 mL HNO_3, 10 mL of HCl and 2 mL of H_2O_2 temperature of 95 ℃ and time 2 hour. The selected optimized method depending upon: minimum values for the reagent volume, digestion temperature and digestion time to give a clear digest from are presented in Tables (3-5) for vegetables samples and Tables (6-8) for soils samples.

Table 3. Optimization of digestion temperature for vegetable samples

No	Amount of Sample	Reagent added	Temp. (℃)	Digestion time (h)	Nature of the digest after filtration
1.	0.50 g	4 mL HNO_3 4 mL $HClO_4$	100	2:00	Clear and yellowish
2.	0.50 g	4 mL HNO_3 4 mL $HClO_4$	150	2:00	Clear and colorless (Optimum)
3.	0.50 g	4 mL HNO_3 4 mL $HClO_4$	200	2:00	Clear and colorless
4.	0.50g	4 mL HNO_3 4 mL $HClO_4$	250	2:00	Clear and colorless

As can be seen in the above Table, the digestion temperature (150 ℃) was selected for the next experiment.

Table 4. Optimization of reagent volume for digestion of vegetables samples

No	Amount of sample	Reagent added	Temp (℃)	Digestion time (h)	Nature of the digest after filtration
1	0.50 g	7 mL HNO_3 1 mL $HClO_4$	150	2:00	Brown yellowish color
2	0.50 g	4 mL HNO_3 4 mL $HClO_4$	150	2:00	Clear and Colorless (Optimum)
3	0.50 g	6 mL HNO_3 2 mL $HClO_4$	150	2:00	Clear and yellowish
4	0.50 g	5 mL HNO_3 3 mL $HClO_4$	150	2:00	Colorless but turbid

As can be seen in the above Table the smallest reagent volume of 4 mL HNO_3, 4 mL $HClO_4$ were selected for the next experiment to optimize digestion time.

Table 5. Digestion time optimization for vegetable samples

No	Amount of sample	Reagent added	Temp (℃)	Digestion time (h)	Nature of the digest after filtration
1	0.50 g	4 mL HNO_3 4 mL $HClO_4$	150	1:00	Milky and turbid
2	0.50 g	4 mL HNO_3 4 mL $HClO_4$	150	1:30	Clear but turbid
3	0.50 g	4 mL HNO_3 4 mL $HClO_4$	150	2:00	Clear and colorless (Optimum)
4	0.50 g	4 mL HNO_3 4 mL $HClO_4$	150	3:00	Clear and colorless

As can be seen in the above Table, the digestion time (2 h) was selected for the next experimental procedure.

Table 6. Optimization of reagent volume for digestion of soils samples

No	Amount of sample	Reagent added	Temp. (℃)	Digestion time (h)	Nature of the digest after filtrations
1	0.50 g	6 mL HNO_3 6 mL HCl 3 mL H_2O_2	100	2:00	Clear but pale yellowish color
2	0.50 g	3 mL HNO_3 10 mL HCl 2 mL H_2O_2	100	2:00	Clear and Colorless (Optimum)
3	0.50 g	5 mL HNO_3 5 mL HCl 5 mL H_2O_2	100	2:00	Clear but turbid
4	0.50 g	8 mL HNO_3 3 mL HCl 2 mL H_2O_2	100	2:00	Brown yellowish color

As can be seen in the above Table, the reagent volume of 3 mL HNO_3, 10 mL HCl and 2 mL of H_2O_2 were selected for the next experiment to optimize temperature.

Table 7. Optimization of temperature for digestion soil samples

No	Amount of sample	Amount of Reagent added	Digestion time (h)	Temp. (℃)	Nature the digest after filtration
1	0.50 g	3 mL HNO_3 10 mL HCl 2 mL H_2O_2	2:00	85	Milky turbid
2	0.50 g	3 mL HNO_3 10 mL HCl 2 mL H_2O_2	2:00	95	Clear and colorless solution (Optimum)
3	0.50 g	3 mL HNO_3 10 mL HCl 2 mL H_2O_2	2:00	105	Clear and colorless
4	0.50 g	3 mL HNO_3 10 mL HCl 2 mL H_2O_2	2:00	125	Clear but turbid

As can be seen in the above table, the digestion temperature (95 ℃) was selected for the next experiment to optimize the digestion time.

Table 8 Optimization of time for digestion soil samples for the selected reagent.

No	Amount sample	Reagent added	Temp. ℃	Digestion Time (h)	Nature of the digest after filtration
1	0.5 g	3 mL HNO_3 10 mL HCl 2 mL H_2O_2	95	1:00	Clear and yellowish
2	0,5 g	3 mL HNO_3 10 mL HCl 2 mL H_2O_2	95	2:00	Clear and colorless (optimum)
3	0.5 g	3 mL HNO_3 10 mL HCl 2 mL H_2O_2	95	2:30	Clear and colorless
4	0.5 g	3 mL HNO_3 10 mL HCl 2 mL H_2O_2	95	3:00	Clear and colorless

As can be seen in the above Table optimization time of 2 (h) was selected optimum.

4.3. Method of Validation

In order to validate the analytical method, the following method validation parameters such as, limit of detection and limit of quantification studies were carried out. The selected heavy metals elements in each digested blank were analyzed with FAAS. Then the limits of detection and quantification were calculated as three and ten times the SD of the blank (3 σ blank and 10 σ blank), respectively.

The values of limits of detection and quantification for leafy vegetables and soil samples of each selected heavy metals are summarized in Table 9 and 10, respectively. The values of the method detection limit of vegetable and soil samples the method obtained were compared with the instrument detection limit and found to have greater values in all case. This confirms that the method was good and acceptable. The method of detection limits vegetable samples and soil samples value lied in the ranges of 0.056 – 0.175 mg/L and 0.075 - 0.210 mg/L, respectively. The method quantification limit of vegetables and soil samples (MQL) value lied in the range of 0.76 - 0.95 mg/ L and 0.096 - 0.70 mg/ L, respectively.

In general the instrumental detection limits ranged from 0.005 to 0.1 mg/L which were below the method detection limits of both samples, indicating good sensitivity of the measuring instrument for analysis. The result shows both the MDL and MQL values were greater than the IDL; hence, the results of the analysis could be reliable.

Table 9. Instrumental parameter, coefficient of determination of the calibration and methods of detection limit

Metal	Wave length	Correlation Coefficient	IDL (mg/L)	MDL for Vegetable (mg/L)	MDL for soil samples
Cd	228.7	0.9994	0.005	0.056	0.090
Pb	283.3	0.9928	0.1	0.85	0.180
Zn	213.9	0.9929	0.005	0.068	0.075
Fe	248.3	0.9992	0.005	0.058	0.210
Co	240.7	0.9995	0.007	0.082	0.096
Ni	232	0.9993	0.008	0.175	0.087
Cu	324.7	0.9988	0.020	0.093	0.150

IDL - Instrumental Detection Limit, MDL- Method of Detection Limit.

Table 10. Limit detection quantification limits, (n = 6, MQL = 10 σ blank, in mg/L), for leafy vegetables and soils.

Metals	Cd	Pb	Zn	Fe	Co	Ni	Cu
MQL for vegetables	0.087	0.76	0.086	0.114	0.089	0.95	0.31
MQL for soils	0.096	0.6	0.25	0.7	0.13	0.29	0.501

MQL - Method Quantification Limit

4.3.1. Recovery of Spiking Samples

Percent recovery test was used for method validation which is the process used for evaluating if analytical method is acceptable for its intended purpose. The accuracy and precision of the method used for the study were tested by spiking the samples with a standard of known concentration of the analyte metals. The results indicated that the concentrations of the selected heavy metals determined in the present study using Flame Atomic Absorption Spectrophotometer were in agreement within the acceptable range for all metals. The procedures used in the current study were validated by spiking

method and the percent recovery values were given in (Tables 11) for heavy metal in soil samples and (Tables 12) for vegetable samples. The percentage recoveries for selected metals lie in the ranges of 86.25 – 103.48 % in cabbages, 85.87– 98.80 % in lettuces and 90.0– 102.05 % in Swiss chard samples. The percentage recovery for metals in the soils samples in the range of 89.625 – 107.50 %, which were within the acceptable range for metals. The precision of the method was expressed as RSD of the three replicate readings. Percentage of relative standard deviation of recovery spiking can be calculated in SD divided by mean of percent spiking. The RSD values obtained ranged from 0.26 to 1.67 % (Table 11) and 0.028 to 1.33 (Table 12) for leafy vegetable and soil samples respectively, which were under the required control limits ≤ 15 % (Ararso *et al.,* 2013). All the recovery values were within the acceptable range of 80–120 % for metal analysis (Harvey, 2000). The recovery analysis in soil sample of lettuce on distances of 5 m from main road in (Table 11) was given as a sample. Generally, good recoveries were obtained for metals which were detected in the present study. Thus the optimized procedure was good.

Table 11. The recovery analysis (% R ± SD, n = 3) of metals in soil samples.

Metals	Conc. in sample (mg/L)	Amount added (mg/L)	Conc. in spiked sample (mg/L)	Recovery (%)	% RSD
Co	ND	4	3.585 ± 0.06	89.625 ± 1.5	1.67
Cd	ND	6	5.586 ± 0.04	93.10 ± 0.67	0.72
Fe	10.035 ± 0.03	6	16.485 ± 0.03	107.50 ± 0.5	0.47
Ni	ND	4	3.84 ± 0.01	96.0 ± 0.25	0.26
Cu	4.557 ± 0.063	4	8.650 ± 0.025	102.33 ± 0.63	0.62
Pb	0.179 ± 0.001	0.5	0.673 ± 0.008	98.8 ± 1.60	1.62
Zn	7.131 ± 0.04	4	11.185 ± 0.053	101.35 ± 1.33	1.31

ND = Not Detected

Table 12. The recovery analysis (% R ± SD, n = 3) of metals in leafy vegetables samples

Vegetables	Metals	Conc. in sample	AmountAdded (mg/L)	Conc. in spiked Samples (mg/L)	Recovery %	% RSD
	Cd	ND	6	5.32 ± 0.002	88.67 ± 0.033	0.037
Cabbage	Fe	3.616 ± 0.051	6	9.825 ± 0.050	103.48 ± 0.83	0.80
	Ni	ND	4	3.45 ± 0.003	86.25 ± 0.075	0.087
	Cu	0.93 ± 0.032	4	4.852 ± 0.046	98.05 ± 1.15	1.17
	Co	ND	4	3.55 ± 0.012	88.75 ± 0.300	0.34
	Zn	1.936 ± 0.023	4	5.750 ± 0.042	95.35 ± 0.075	0.079
	Pb	0.130 ± 0.001	0.5	0.624 ± 0.003	98.80 ± 0.60	0.61
	Cd	ND	6	5.152 ± 0.012	85.87 ± 0.20	0.23
Lettuce	Fe	5.929 ± 0.029	6	11.85 ± 0.030	98.68 ± 0.50	0.51
	Ni	ND	4	3.58 ± 0.001	89.50 ± 0.025	0.028
	Cu	1.073 ± 0.063	4	4.825 ± 0.050	93.80 ± 1.25	1.33
	Co	ND	4	3.62 ± 0.003	90.5 ± 0.075	0.083
	Zn	3.087 ± 0.04	4	6.995 ± 0.031	97.7 ± 0.775	0.79
	Pb	0.114 ± 0.001	0.5	0.567 ± 0.007	90.60 ± 1.400	1.55
	Cd	ND	6	5.632 ± 0.004	93.87 ± 0.067	0.07
	Fe	4.133 ± 0.062	6	9.953 ± 0.040	97.0 ± 0.670	0.69
Swiss chard	Ni	ND	4	3.655 ± 0.002	91.38 ± 0.05	0.055
	Cu	1.183 ± 0.052	4	5.265 ± 0.020	102.05 ± 0.50	0.49
	Co	ND	4	3.68 ± 0.015	92.0 ± 0.375	0.41
	Zn	3.335 ± 0.058	4	6.935 ± 0.021	90.0 ± 0.525	0.58

SD - Standard Deviation, ND - Not Detected

4.4. Levels of Heavy Metals in the Leafy Vegetables Plants

The accuracy and precision of the method used for the study were tested by spiking the samples with a standard of known concentration of the analyte metals. Thus, in the present work, the concentration of the seven selected heavy metals (Fe, Cu, Zn, Co, Ni, Pb and Cd) in the three leafy vegetable samples were determined by FAAS using calibration curve. The determination was under taken at the three different distances from the main road (5, 6 and 7 m). The concentrations of cobalt, nickel and cadmium in samples of leafy vegetables were not detected.

To determine the level of Fe, Cu, Zn and Pb in vegetable samples, each sample was prepared in triplicate under the same experimental condition and analyzed. Then, the results of obtained expressed as mean ± SD and relative standard deviations (% RSD) are shown in Table 13. (% RSD can be calculated as SD of samples divided by mean of each samples than multiplied by 100). As can be seen, in the table the results obtained were with acceptable precision (% RSD less than 15). It can be also be noted that the concentration of heavy metals in the leafy vegetable samples varied from one to the other.

The level of lead was in the ranges of 0.101- 0.111 in cabbage, 0.116 - 0.130 in lettuce and 0.106 – 0.114 mg/L in swiss chard samples at different distance from the main road. All these values were less than the WHO/FAO safe limit, 0.3 mg/L. As can be seen from the Table 13 the concentrations of lead in different leafy vegetables were no significant different in the concentration of among the vegetable samples (cabbages, lettuce and swiss chard) and at different distance of sampling point at p less than 0.05.

Vegetables grown at the roadside area contained higher Pb than that of grown far away from the road. Concentrations of analytes, especially lead, in the same vegetation at different sampling point were slightly different from each other. This may be explained by differences in traffic, soil property like pH, the solubility of heavy metals in soil or biological feature of vegetation in each sampling point. One likely source of the lead contamination in the vegetables could be as a result of acid-lead batteries (Astwan, 2008). However, (Pb) comes mainly from automobile exhaust and vehicular emissions for example tire wear, bearing wear, break lining wear (Poggio *et al.*, 2009).

The concentration of zinc in leafy vegetables was found in the ranges of 1.856 - 1.936 in cabbage, 2.904 - 3.087 in lettuce and 3.194 - 3.335 mg/L in swiss chard samples at different distances from main road. The zinc levels in leafy vegetables were lower than the WHO/FAO safe limit 99.40 mg/L. With regards to distance from the main road, Zn concentration was no significant among sampling point from the main road but, when compared with among vegetables samples, Zinc content was higher in the Swiss chard and lowest in the cabbage. This might be the zinc content of the plants varied with pH and contents of zinc naturally in the soil samples of the plants. However, ANOVA test for significant difference result showed that the difference concentration of zinc in different leafy vegetables were statistically significant at ($P < 0.05$). Among all heavy metals, zinc was the least toxic and an essential element in human diet as it is required to maintain the functioning of the immune system.

On the other hand concentration of copper was found ranges of 0.896-0.957 in cabbages, 1.060-1.073 in lettuce and 1.135 - 1.183 mg/L in swiss chard samples at different distance from main road. This value is less than the WHO/FAO safe limit of 73.00 mg/L. The concentrations copper in different vegetables were different. The ANOVA result showed that the contents of copper in different leafy vegetable were different and it is statistically significant, at ($P < 0.05$). Similarly, the level of iron was found in ranges 3.409-3.616 in cabbage, 5.790 - 5.929 in lettuce and 3.81- 4.133 mg/L in swiss chard at different distance from main roadside. These values were far lower than the WHO/FAO safe limit of 425.00 mg/L. The concentrations of iron were varied in the different leafy vegetables. Relatively, high concentration of iron was found in lettuce and the lowest concentration in cabbage (Table 13). Heavy metal contents in different plants at the same distance that means at 5, 6 and 7 m from the road was found in the following order: Fe > Zn > Cu > Pb. This may be related with abundance element the earth crust. The same order of heavy metal contents was found in vegetables at the same sampling points.

Table 13. Level of Heavy Metals (mg /L) in Leafy Vegetables (mean ± SD, n = 3).

Distance from road(m)	Vegetable	Pb	Fe	Cd	Zn	Ni	Cu	Co
5		0.111 ± 0.002^{J} RSD % 1.80	3.616 ± 0.051^{c} RSD % 1.410	ND	1.936 ± 0.023^{f} RSD % 1.188	ND	0.957 ± 0.012^{I} RSD % 1.254	ND
6	Cabbage	0.105 ± 0.001^{j} RSD % 0.95	3.409 ± 0.03^{c} RSD % 0.88	ND	1.902 ± 0.057^{f} RSD % 2.997	ND	0.923 ± 0.021^{I} RSD % 2.28	ND
7		0.10 1 ± 0.001^{j} RSD % 0.99	3.551 ± 0.04^{c} RSD % 1.126	ND	1.856 ± 0.041^{f} RSD % 2.209	ND	0.896 ± 0.024^{I} RSD % 2.68	ND
5		0.130 ± 0.001^{j} RSD % 0.77	5.929 ± 0.029^{a} RSD % 0.489	ND	3.087 ± 0.04^{e} RSD % 1.296	ND	1.073 ± 0.063^{h} RSD % 5.87	ND
6	Lettuce	0.121 ± 0.004^{j} RSD % 3.31	5.8 ± 0.03^{a} RSD % 0.517	ND	3.033 ± 0.037^{e} RSD % 1.22	ND	1.066 ± 0.054^{h} RSD % 5.07	ND
7		0.116 ± 0.001^{j} RSD % 0.86	5.79 ± 0.07^{a} RSD % 1.21	ND	2.904 ± 0.029^{e} RSD % 0.999	ND	1.060 ± 0.043^{h} RSD % 4.06	ND
5		0.114 ± 0.001j RSD % 0.88	4.133 ± 0.062^{b} RSD % 1.5	ND	3.335 ± 0.058^{d} RSD % 1.74	ND	1.183 ± 0.052^{g} RSD % 4.39	ND
6	S. chard	0.110 ± 0.002^{j} RSD % 1.82	3.81 ± 0.051^{b} RSD % 1.34	ND	3.278 ± 0.057^{d} RSD% 1.739	ND	1.155 ± 0.063^{g} RSD % 5.45	ND
7		0.106 ± 0.002^{j} RSD % 1.89	4.017 ± 0.049^{b} RSD % 1.22	ND	3.194 ± 0.029^{d} RSD % 0.908	ND	1.135 ± 0.031^{g} RSD % 2.73	ND

Note; Means with the same letter in given column are not significantly different.

4.5. Level of Heavy Metals in the Soil Samples

The concentration of heavy metals in soil depended mainly on the characteristics of the soil samples and distance from the source of contamination. Uptake and accumulation of heavy metals by shoots and roots varied with heavy metal type and plant species. The capacity of accumulating different heavy metals of different plant species is related to the heavy metal content in soil and the alternative absorptivity to heavy metals (Lu *et al.,* 2014). The bioavailability of heavy metals in the plant parts depends on a number of physical and chemical factors in the soils.

These include: PH and electrical conductivity. In the present study in order to see the bioavailability of the metals we have considered the pH and electrical conductivity of roadside soil samples. The pH values of the soils up on which the respective leafy vegetables grown were within the range of 6.10 -7.30 which can categorize the soils under weakly acidic to weakly basic category (Table 15). Reports indicated that increasing rates of nitrogen fertilizers generally increase soil acidity (Ishibashi *et al*., 2004).

Soil pH is one of the most influential parameter controlling the conversion of metals from the immobile solid-phase forms to more mobile and/or bioavailable solution-phase forms. The conductivities of the soil samples collected from all the sampling sites, in this study, were determined at 25 °C, which was done automatically by the conductivity meter (Table 15). The soil samples were collected from the areas from where the leafy vegetable plants were collected. In this study the concentration of heavy metals in the soils samples showed some sort variation. The concentrations of Cd, Ni and Co in the soil samples were not detected, whereas the other metals Pb, Cu, Fe and Zn were detected (Table 14).

The concentrations of lead were found in the ranges of 0.137- 0.150, 0.161-0.179 and 0.144-0.156 mg/L in the soil samples where cabbage, lettuce and swiss chard, respectively. The levels lead slightly higher in leafy vegetable of lettuce. The reason may be lettuce absorb heavy metals through high surface area their leaves. The availability of lead in soil is directly dependent upon the pH of the soil. The lead (II) ions become less soluble as the soil pH is raised. In near-neutral soils pH 6-8 lead is strongly bound to soil particles and may not be available for plant uptake and becomes more soluble in acidic soils (pH less than 5). Lead is the least mobile heavy metal in soils, especially under non acidic conditions (McBride, 1994). The concentrations of lead in the soils of leafy vegetables samples ANOVA test for significant difference showed no significance difference among soil of leafy vegetables and at different distances of sampling points at ($P < 0.05$). This might be an indication that the roadside soils are not significantly influenced by vehicular emission. All these values were small when compared to the maximum safe limits of Pb in the soils (Table 14).

The concentrations of zinc were found in the ranges of 5.61- 5.95, 7.012-7.131 and 6.366 - 6.756 mg/L in the soil samples where cabbage, lettuce and swiss chard collected, respectively. The concentrations of zinc different in soil respective of leafy vegetables were significant difference. This may be due to the natural contents of the metal in the soil. The concentrations of copper were found in the ranges of 3.291-3.531, 4.400 - 4.557 and 2.360 - 2.723 mg/L in the soil samples where cabbage, lettuce and swiss chard collected, respectively. The concentrations of Cu varied in different soil of leafy vegetables statically significant manner. This could be attributed due to the different nature of the vegetable species that accumulate different metals depending on their environmental conditions, metal species and forms of heavy metal (Jung, 2008). The concentrations of iron were found in the ranges of 7.778 - 8.435, 9.668 - 10.038 and 9.373 - 9.603 mg/L in the soil samples where cabbage, lettuce and swiss chard, respectively. In the concentrations of the heavy metals Fe, Zn, Cu and Pb among analyzed in the soils samples. In general, the mean concentration of heavy metals in soils were collected from all sampling site decreased in the order of: Fe > Zn > Cu > Pb.

When heavy metal concentrations in roadside soils with urban traffic in Beijing, China compared with the present study concentrations of Cd, Cu, Pb and Zn on west side decreased from 0.323, 29.1, 36.3 and 94.0 mg/kg (1 m from the road) to 0.125, 25.3, 23.4 and 67.7 mg/kg (30 m from the road), respectively; the concentrations of Cd, Cu, Pb and Zn on east respectively; the concentrations of Cd, Cu, Pb and Zn on east side decreased from 0.311, 43.1, 36.5 and 109.3 mg/kg (1m from the road) to 0.156, 28.5, 28.1 and 77.7 mg/kg (30 m from the road), respectively (Xi Chen *et al.*, 2010). The concentration Cd, Cu, Pb and Zn decrease with increasing the distance from the road due to the influence may be vehicular emission or others. The present study also the concentration of similar element decrease with increasing distance from the road except cadmium not detected.

The results of the study indicated that the level of heavy metals in the study areas showed that the concentrations were relatively higher in soil samples compared to vegetables samples. Demirezen and Aksoy, (2006) also reported that, the level of heavy metals in vegetables were generally lower than the soil samples. Such results might be attributed due to root activity, which seems to act as a barrier for translocation of metals (Yusuf *et al.*, 2003). In general, the results of the heavy metals analyzed in the study areas showed that their concentration level was below the standard guide lines

for maximum limit proposed for agricultural and the results also show that the levels of contamination of soils samples by the heavy metals were not polluted by toxic heavy metals.

Table 14. Level of Heavy Metals (mg /L), (mean ± SD, n = 3) in the respective Soil of Leafy Vegetables.

Distance From Road (m)	Soils of	Pb	Fe	Cd	Zn	Ni	Cu	Co
5		0.150 ± 0.004^{A} RSD % 2.67	8.435 ± 0.197^{c} RSD % 2.334	ND	5.954 ± 0.017^{f} RSD % 0.286	ND	3.531 ± 0.041^{h} RSD % 1.16	ND
6	Cabbage	0.142 ± 0.001^{A} RSD % 0.70	7.778 ± 0.145^{c} RSD % 1.864	ND	5.939 ± 0.093^{f} RSD 1.566	ND	3.434 ± 0.051^{h} RSD % 1.485	ND
7		0.137 ± 0.002^{A} RSD % 1.46	7.897 ± 0.183^{c} RSD % 2.317	ND	5.61 ± 0.201^{f} RSD % 3.583	ND	3.291 ± 0.052^{h} RSD % 1.58	ND
5		0.179 ± 0.001^{A} RSD % 0.56	10.035 ± 0.03^{a} RSD % 0.299	ND	7.131 ± 0.04^{d} RSD % 0.28	ND	4.557 ± 0.063^{g} RSD % 1.185	ND
6	Lettuce	0.168 ± 0.001^{A} RSD % 0.60	9.826 ± 0.172^{a} RSD % 1.75	ND	7.066 ± 0.007^{d} RSD % 0.099	ND	4.475 ± 0.02 ^{g} RSD % 0.447	ND
7		0.161 ± 0.003^{A} RSD % 1.86	9.668 ± 0.049^{a} RSD % 0.51	ND	7.012 ± 0.014^{d} RSD % 0.20	ND	4.40 ± 0.0479^{g} RSD % 1.089	ND
5	Swiss chard	0.156 ± 0.003^{A} RSD % 1.92	9.603 ± 0.0798^{b} RSD % 0.83	ND	6.756 ± 0.107^{e} RSD % 1.584	ND	2.723 ± 0.051^{i} RSD % 1.873	ND
6		0.150 ± 0.001^{A} RSD % 0.67	9.373 ± 0.135^{b} RSD % 1.44	ND	6.378 ± 0.04^{e} RSD % 0.63	ND	2.593 ± 0.063^{i} RSD % 2.43	ND
7		0.144 ± 0.001^{A} RSD % 0.69	9.465 ± 0.0997^{b} RSD % 1.053	ND	6.366 ± 0.078^{e} RSD % 1.225	ND	2.360 ± 0.084^{i} RSD % 3.56	ND
Max. Limit for soil (mg/kg)		100^{b}	5000^{c}	3.0^{b}	300^{b}	50^{b}	100^{b}	50^{b}

Note; Means with the same letter in a given column are not significantly different.

Source: Ewers^{b} Source: Kabata-Pendias and Pendias^{c}

The average mean of PH and electrical conductivity of different sampling points of soil in (5, 6 and 7 m) Corresponding vegetables samples were given in Table 15.

Table 15. pH and Electrical Conductivity of Soil of Leafy Vegetables (Mean ± SD, n = 3).

Vegetables	pH	Electrical conductivity in (μS)
Cabbage	6.65 ± 0.02	5.85 ± 0.02
Lettuce	6.10 ± 0.05	7.75 ± 0.04
Swiss chard	7.30 ± 0.05	6.17 ± 0.05

4.6. Transfer Factor

The most important pathway through which human exposed to the toxic metals are soil- plant-human (food chain) and soil-human (incidental soil ingestion). Out of the two soil-to-plants transfer is the key components of human exposure to metals. The ability of a metal species in its different forms to migrate from the soil through the plant parts and makes itself available for consumption can be represented by the transfer factor. The translocation factor (TF) or mobilization ratio (Singh *et al*., 2010) is defined as follows:

$$TF = \frac{\text{Concentration of metals in plants parts}}{\text{Concentration of metals in soil}}$$

In the present study, the transfer factor of different heavy metal from soil to vegetable is presented in (Table 16). The value were found in the ranges of (0.72-0.76) for Pb, (0.24 -0.48) Cu, (0.330 - 0.46) Zn and (0.41- 0.60), Fe and the trend of transfer factor for heavy metals in vegetable samples studied were in order of Pb > Fe > Zn > Cu. Heavy metals in the study area were found to show low transfer factor values. The reason may be able to be attributed to the high retention rate of the metal in soil and therefore they are less mobile in the soil at pH of 6.1 - 7.30 of the studied area (Table 15).The solubility of heavy metals is generally greater as pH decreases within the pH range of normal agricultural soils (approximately 5.0 - 7.0) (Wang *et al.*, 2006). The high pH values of soils could have accounted for low transfer of metals from soil to plant. The present study of transfer

factor the concentration of heavy metals in lettuce on a distance of 5 m, cabbage on 6 m and swiss chard on a distance of 7 m with corresponding soil from the main road were considered as a samples. Transmission of metals from soil to plant tissues is studied using an index called Transfer Factor (TF). It is calculated as a ratio of concentration of a specific metal in plant tissue to the concentration of same metal in soil, both represented in same units. Higher TF values ($\geq$ 1) indicate higher absorption of metal from soil by the plant. On the contrary, lower values indicate poor response of plants towards metal absorption and the plant can be used for human consumption (Singh *et al*., 2010).The present study compared with other literature, different vegetables samples, different TF due to different centration of heavy metals in soils and plants.

Table 16 .Comparison of transfer factors (TF) for heavy metals from soil to vegetable with similar and other edible plants from the literature

Concentration metal in vegetable					
Name of plant	Cu	Zn	Fe	Pb	Reference
Cabbage	0.09	0.02	15.65	0.56	Abreha Gebrekidan
Lettuce	0.66	0.22	102.68	0.39	*et al.*, 2013
Swiss chard	0.59	0.15	103.93	0.51	
Cabbage	0.27	0.32	0.44	0.74	
Lettuce	0.24	0.43	0.59	0.72	Present study
Swiss chard	0.48	0.50	0.42	0.74	

4.7. Comparison of the Metal Contents in Soils of Leafy Vegetables and with Other Reported Values

In different studies the composition of heavy metals content in vegetables and soil samples have been analyzed at different distance from roadside. These studies include; the level of lead in the roadside soils of Addis Ababa, Ethiopia (Endale Teju *et al.*, 2012**)**; level of heavy metal pollution of soils and vegetables grown near roadside soils at Gazipur, Banglades (Habib *et al*., 2012); level of heavy metals contamination of roadside soils of North, England (Wiliam *et al*., 2006). Although various chemical analyses target to similar objective, there may also be a different in sampling,

sample preparation and analytical techniques. Considering all these, the results of the present study were compared to the findings of other authors. According to Endale *et al.,* **(**2012), the levels of lead in the roadside soil of Addis Ababa were generally higher than100 µg/g. Moreover, the contribution of vehicular emission for lead in roadside soils of Addis Ababa was indicated from the observed difference in lead levels between the sites that are exposed to vehicular emission. The average concentrations of lead in the roadside soils were found to be (418.6 ± 3.4) µg/g/.

Wiliam *et al.*, (2006), determined level Pb, Zn, Cu and Cd in contamination of roadside soils of North, England at a distance of 6, 10, 20 and 30 m from the road. The result indicated that the concentration of metals considered were found to in the ranges of (25.0 - 1198.0) µg/g Pb, (0.3 - 3.8) µg/g Cd, (15.5-240) µg/g Cu and (56.7-480) µg/g Zn at different distance from road. The highest concentrations these metals were detected in the samples collected from the border zone of the verges and there was a trend of gradual decrease in the metal content with the increasing distance from the paved road. All the four heavy metals exhibited a significant decrease in the roadside soils with the increasing distance from the road. The border zone had the highest mean concentration of the four metals whereas the ditch zone (30 m) exhibited the lowest mean concentration. In present study, the level of lead, copper and zinc was found not significantly decrease as the distance from road increase and cadmium which was not detected.

Habib *et al*., (2012), determined level of lead, cadmium, and nickel in vegetable and roadside soils along a major highway in Gazipur, Bangladesh. In this study, samples of soils were collected at distances of 0, 50, 100, and 1000 m from the main road. The result indicated in the ranges (13.0-20.8) µg/g in Pb, (0.23-0.29) µg/g in Cd and (24.1- 36.3) µg/g in Ni in soil from different sampling distance. Concentrations of lead (Pb) and nickel (Ni) in soil and vegetables decreased with distance from the road increased, indicating their relation to traffic and automotive emissions. The concentration of cadmium (Cd) was found to be independent of distance from road. When the result is compared with the present study, the level Pb not significantly decreased with distance from the road and Cd and Ni not detected.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The optimum conditions were validated through spiking experiment and good percentage recovery values in the ranges of 89.625–107.5 % for soil and 85.87 – 103.48 % for vegetable which were within the acceptable range for metals. The concentrations of heavy metals in the roadside soil samples and leafy vegetables in grown in the soil were determined. The study indicated that the soil in the study area was not contaminated and the concentration of the heavy metals in both soil and vegetables samples: Fe, Zn, Cu and Pb were far below the maximum tolerable levels set by FAO/WHO. The levels of Cd, Ni and Co in both samples were not detected. The concentrations of Fe, Cu and Zn vary in different leafy vegetable and soil samples were ANOVA test showed statically significance at $P < 0.05$. With regard to distance from the main road, the concentrations of lead, copper, iron and zinc not significance difference in any sampling point in both samples.

The heavy metals contents at the same distance from the road were found in leafy vegetables and soils sample in the following order: Fe > Zn > Cu > Pb. Regular monitoring of toxic heavy metals in vegetables by concerned bodies is vital to prevent disproportionate build up in the food chain. In general, the results showed that the levels of contamination of the soils by the heavy metals were not that much pronounced. Therefore, the soils studied were not harmful for cultivation of leafy vegetables and other agricultural purposes.

5.2. Recommendation

Based on the results of this study, the following points are forwarded.

1. Although this study showed the concentrations of the studied metals to be lower in both the soil and vegetable samples, it is better if further analysis on other essential and toxic metals is conducted to draw strong conclusion.

2. This study might be repeated with other instrument or other than (FAAS) to compare the metal contents of the selected leafy vegetables and soil.

3. The concentration of heavy metals in similar other vegetables samples that are not covered by this research should be determined and monitoring of the levels of heavy metals in commercially available vegetables should be encouraged.

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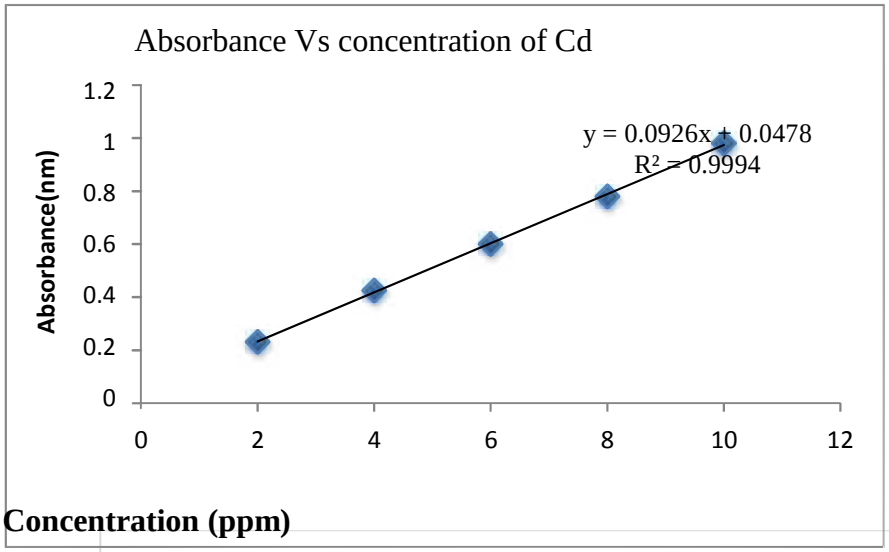
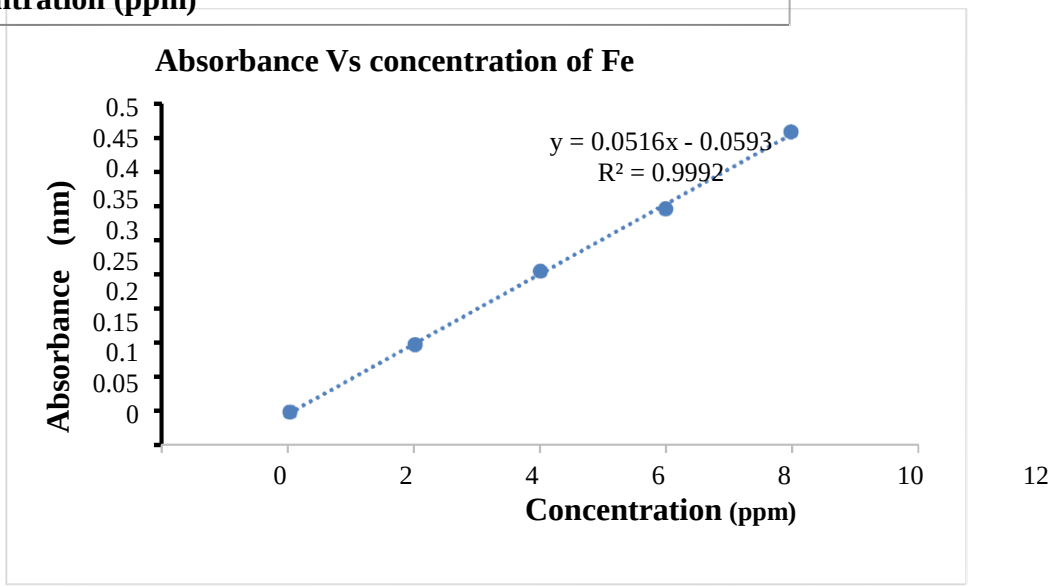
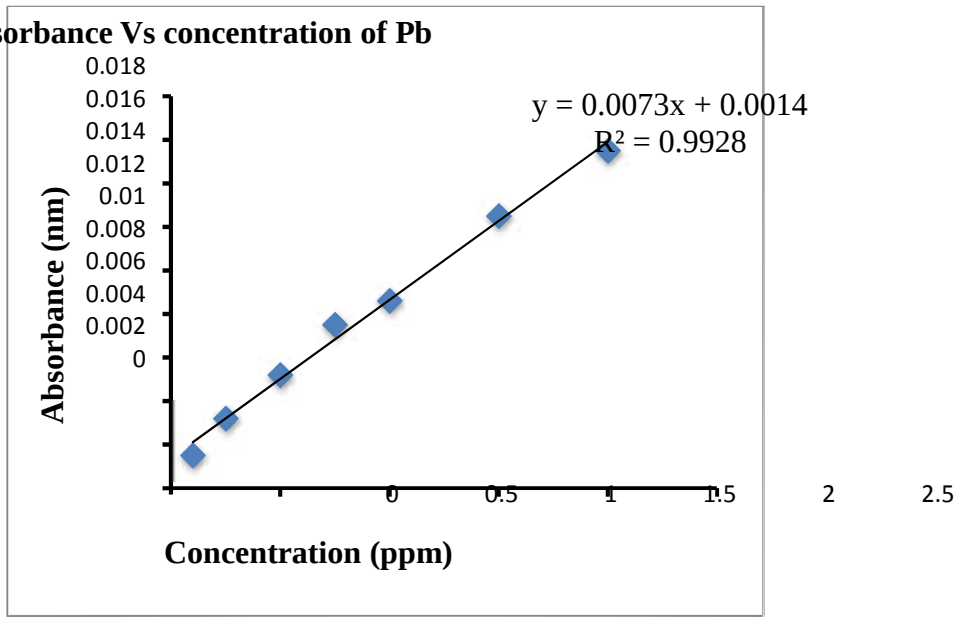
7. APPENDIX

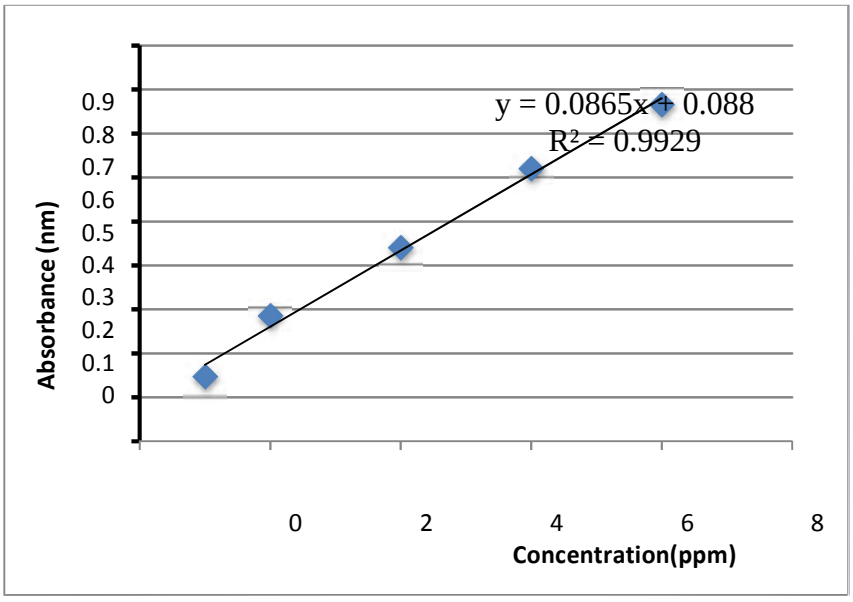
Appendix- I

1. Concentration versus Absorbance of Selected Heavy Metals

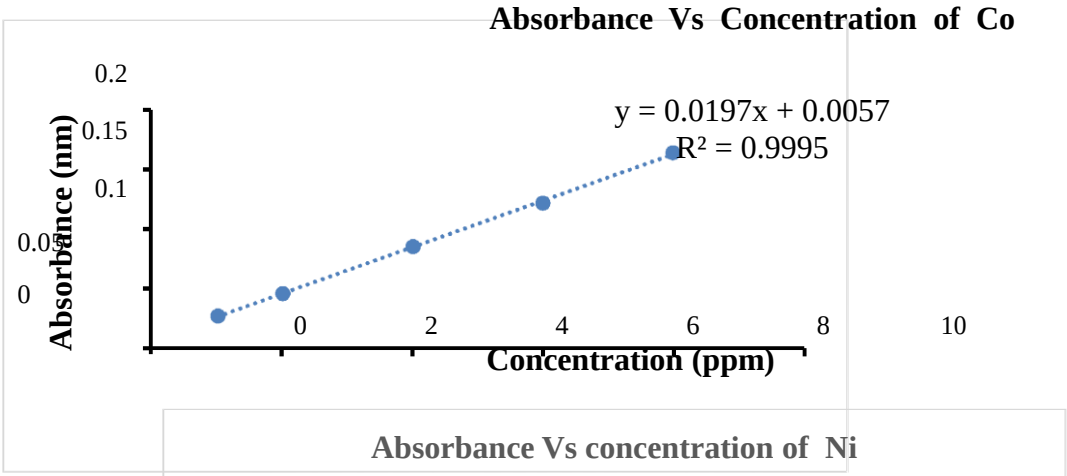


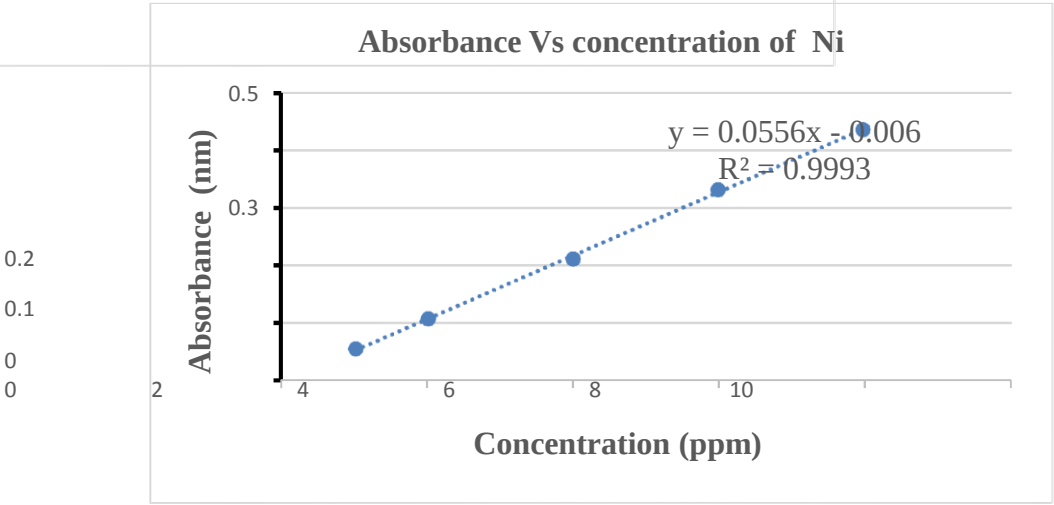


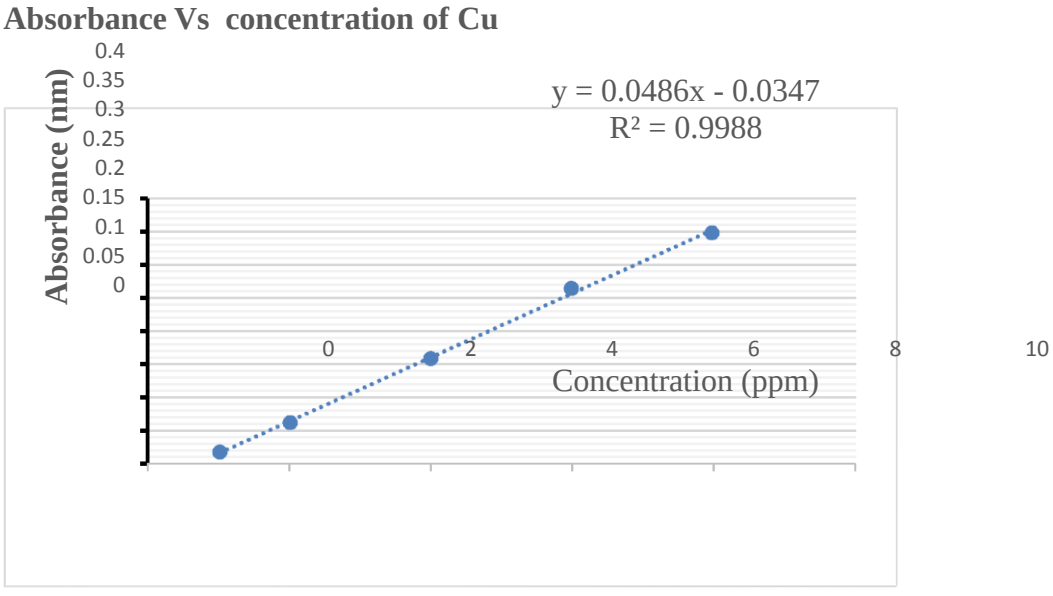




Absorbance Vs Concentration of Zn


Absorbance Vs Concentration of Co
y = 0.0197x + 0.0057
R² = 0.9995
Absorbance (nm)
Concentration (ppm)
0.2
0.15
0.1
0
0
2
4
6
8
10


Absorbance Vs concentration of Ni
y = 0.0556x - 0.006
R² = 0.9993
Absorbance (nm)
Concentration (ppm)
0.5
0.3
0.2
0.1
0
0
2
4
6
8
10


Absorbance Vs concentration of Cu
y = 0.0486x - 0.0347
R² = 0.9988
Absorbance (nm)
Concentration (ppm)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
2
4
6
8
10

The ANOVA Procedure for Plant Samples

T- Tests (LSD) for D5

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	0.00163
Critical Value of t	2.06390
Least Significant Difference	0.068

Means with the same letter are not significantly different.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Variety
A	5.92867	3	Lettuce Fe
B	4.13300	3	Swiss chard Fe
C	3.61600	3	Cabbage Fe
D	3.33533	3	Swiss chard Zn
E	3.08700	3	Lettuce Zn
F	1.93633	3	Cabbage Zn
G	1.18300	3	Swiss chard Cu
H	1.07333	3	Lettuce Cu
I	0.95700	3	Cabbage Cu
J	0.13052	3	Lettuce Pb
J J	0.11414	3	Swiss chard Pb
J J	0.11097	3	Cabbage Pb

The ANOVA Procedure

T- Tests (LSD) for D6

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha 0.05
Error Degrees of Freedom 24
Error Mean Square 0.001971
Critical Value of t 2.06390
Least Significant Difference 0 . 0 7 4 8

Means with the same letter are not significantly different.

t Grouping	Mean	N	Variety
A	5.78667	3	Lettuce Fe
B	3.80967	3	Swiss chard Fe
C	3.40933	3	Cabbage Fe
D	3.27800	3	Swiss chard Zn
E	3.03333	3	Lettuce Zn
F	1.90200	3	Cabbage Zn
G	1.15533	3	Swiss chard Cu
H	1.06633	3	Lettuce Cu
I	0.92267	3	Cabbage Cu
J	0.12097	3	Lettuce Pb
J			
J	0.10953	3	Swiss chard Pb
J			
J	0.10500	3	Cabbage Pb

The ANOVA Procedure

T- Tests (LSD) for D7

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	0.000965
Critical Value of t	2.06390
Least Significant Difference	0 . 0 5 2 3

Means with the same letter are not significantly different

T Grouping	Mean	N	Variety
A	5.79867	3	Lettuce Fe
B	4.01667	3	Swiss chard Fe
C	3.55100	3	Cabbage Fe
D	3.19400	3	Swiss chard Zn
E	2.90367	3	Lettuce Zn
F	1.85633	3	Cabbage Zn
G	1.13500	3	Swiss chard Cu
H	1.05967	3	Lettuce Cu
I	0.89567	3	Cabbage Cu
J	0.11637	3	Lettuce Pb
J			
J	0.10594	3	Swiss chard Pb
J			
J	0.10137	3	Cabbage Pb

The ANOVA Procedure for Soil Samples

T-Tests (LSD) for D5

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha 0.05
Error Degrees of Freedom 24
Error Mean Square 0.005432
Critical Value of t 2.06390
Least Significant Difference 0 . 1 2 4 2

Means with the same letter are not significantly different.

T Grouping	Mean	N	Variety
A	10.03533	3	Lettuce Fe
B	9.60267	3	Swiss chard Fe
C	8.43467	3	Cabbage Fe
D	7.13067	3	Lettuce Zn
E	6.75633	3	Swiss chard Zn
F	5.95433	3	Cabbage Zn
G	4.55733	3	Lettuce Cu
H	3.53100	3	Cabbage Cu
I	2.72267	3	Swiss chard Cu
J	0.17880	3	Lettuce Pb
J			
J	0.15563	3	Swiss chard Pb
J			
J	0.14650	3	Cabbage Pb

The ANOVA Procedure

T Tests (LSD) for D6

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	0.007168
Critical Value of t	2.06390
Least Significant Difference	0.1427

Means with the same letter are not significantly different.

T Grouping	Mean	N	Variety
A	9.82567	3	Lettuce Fe
B	9.37267	3	Swiss chard Fe
C	7.77833	3	Cabbage Fe
D	7.06600	3	Lettuce Zn
E	6.37800	3	Swiss chard Zn
F	5.93967	3	Cabbage Zn
G	4.47533	3	Lettuce Cu
I	2.59300	3	Swiss chard Cu
J	0.16833	3	Lettuce Pb
J	0.14967	3	Swiss chard Pb
J J	0.14213	3	Cabbage Pb

The ANOVA Procedure

T Tests (LSD) for D7

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	24
Error Mean Square	0.00901
Critical Value of t	2.06390
Least Significant Difference	0.16

Means with the same letter are not significantly different.

T Grouping	Mean	N	Variety
A	9.66833	3	Lettuce Fe
B	9.46467	3	Swiss chard Fe
C	7.89667	3	Cabbage Fe
D	7.01200	3	Lettuce Zn
E	6.36633	3	Swiss chard Zn
F	5.61000	3	Cabbage Zn
G	4.39967	3	Lettuce Cu
H	3.29100	3	Cabbage Cu
I	2.36000	3	Swiss chard Cu
J	0.16100	3	Lettuce Pb
J J	0.14423	3	Swiss chard Pb
J J	0.13690	3	Cabbage Pb