LEVELS OF SOME TOXIC HEAVY METALS IN SELECTED VEGETABLES, SOIL AND WASTEWATER AROUND EASTERN INDUSTRY ZONE, CENTRAL ETHIOPIA

MSc THESIS

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I hereby certify that we have read and evaluated this Thesis entitled "Levels of Some Toxic Heavy Metals in Selected Vegetables, Soil and Wastewater around Eastern Industry Zone, Central Ethiopia" prepared under our guidance by Dagne Bekele. We recommend that it be submitted as fulfilling the Thesis requirement.

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DEDICATION

This research thesis work is dedicated to my mother Beletu Damtew and to my father Bekele Bahiru who selflessly dedicated their whole life to the betterment of my education.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is my own work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and compilation of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

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ACRONYMS AND ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
ANRL	Agricultural and Nutritional Research Laboratory
APHA	American Public Health Association
ATSDR	Agency for Toxic Substances and Disease Registry
CV	Coefficient of Variation
DZARC	Debre Zeit Agricultural Research Center
EC	Electrical Conductivity
EDXRF	Energy dispersive X-ray fluorescence
EIAR	Ethiopian Institute of Agricultural Research
EIZ	Eastern Industry Zone
EPA	Environmental Protection Agency
FAAS	Flame Atomic Absorption Spectrophotometer
FAO	Food and Agricultural Organization
HASCL	Holeta Agricultural and Soil Chemistry Laboratory
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-OPS	Inductively Coupled Plasma-optical Emission Spectrometry
IDL	Instrumental Detection Limit
IS	Indian Standard
LSD	Least Significance Difference
MDL	Method Detection Limit
NAS	National Academy of Sciences
NRC	National Research Council
RSD	Relative Standard Deviation
SD	Standard Deviation
TDS	Total Dissolved Solids
USEPA	United State Environmental Protection Agency
USPHS	United States Public Health Service
WHO	World Health Organization

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LEVELS OF SOME TOXIC HEAVY METALS IN SELECTED VEGETABLES, SOIL AND WASTEWATER AROUND EASTERN INDUSTRY ZONE, CENTRAL ETHIOPIA

ABSTRACT

The purpose of this study was to determine the concentration of heavy metals in effluents coming out of EIZ and used as irrigation water source, heavy metals (Cr, Cd Zn, Fe Pb and Cu) in vegetables produced using the same effluent, and the soil contaminating levels as a result of irrigation using Flame Atomic Absorption Spectrophotometer (FAAS). The wet digestion and sequential fractionation extraction procedures were employed to solubilize the metals from the collected samples. The validation these procedures was performed by spiking the samples with a standard solution of each metal having a known concentration and percentage recovery values in the range of 91.0– 98.3% for the soil, 92.0–102% for the effluent, and 89.0–101% for the vegetable samples were obtained. The results obtained from this study showed overall concentration of heavy metals (Cr, Cd, Zn, Fe, Pb, and Cu) respectively, in the range of (2.90-3.77), (2.20-3.68), (45.65-62.46), (358.17-571.33), (4.60-5.50) and (10.20-15.07) (mg /Kg) in the edible parts of sampled vegetables. Whereas, concentration of these metals in the effluent samples (mg/L) were found to be Cr (0.20-1.04), Cd (0.04-0.08), Zn (0.07-0.21), Fe (2.89-5.15), Pb (3.11-45) and Cu (0.30-0.99). Similarly, concentrations (mg/Kg) of the metals in the soil samples were found to be in the ranges of 22.37-66.30, 27.93-45.33, 69.37-123.77, 7140.00-20065.00,18.82-64.87 and 68.47-146.10 for Cr, Cd, Zn, Fe, Pb and Cu, respectively. The modified Tessier sequential extraction procedure was used to fractionate the above six metals from the soil samples into five fractions. In this study the heavy metals were predominantly concentrated in residual fraction (F5); since lead was mainly associated with the organic matter bounded fraction (F4) (34.33-43.45%), it was found to be more bioavailable and mobile than the other investigated heavy metals. The mobility factors of Cr, Cd, Zn, Fe, Pb and Cu were 1.881-3.404, 0.908-1.938, 0.908-3.044, 0.216-0.443, 11.297-33.508 and 0.314-1.968, respectively, samples of soils collected from lands irrigated with wastewater around the EIZ. The concentrations of heavy metals (Cr, Cd, Zn, Fe, Pb and Cu) in the wastewater, soil and vegetable samples were above the recommended limit of both WHO and FAO. But Zn and Pb for the soil samples were smaller than WHO and FAO recommended limit. Based on facts obtained from this study we suggests concerned official body (ies) to take the necessary precaution measures for cleaning the polluted factory effluents.

Keywords: Heavy Metals, Sequential Fractionation, Mobility Factors, Effluent, Eastern Industry Zone

1. INTRODUCTION

Metals are elements, present in chemical compounds as positive ions, or in the form of cations (+ ions) in solution. Heavy metals are among the most serious environmental pollutants due to their high toxicity, abundance and ease of accumulation by various plant and animal organisms. Increasing of heavy metals in soil can be attributed to the contribution of effluent from waste water treatment plants, industries, mining, power stations and agriculture (Guevara-Riba *et al*., 2004). Heavy metals are extremely persistent in the environment. They are non-biodegradable and non-thermo degradable and therefore readily accumulate to toxic levels (Akguc *et al*., 2008).

Vegetables are rich sources of vitamins, minerals, and fibers and also have beneficial antioxidative effects. However, the intake of heavy metal contaminated fruits and vegetables may pose a risk to human health; hence the heavy metal contamination of food is one of the most important aspects of food quality assurance (Radwan and Salama, 2006; Khan *et al*., 2008). Plants take up heavy metals by absorbing them from airborne deposits on the parts of the plants exposed to the air from the polluted environments as well as from contaminated soils through root systems. Also, the heavy metal contamination of fruits and vegetables may occur due to their irrigation with contaminated water (Al Jassir *et al*., 2005).

Increasing industrialization has been accompanied throughout the world by the extraction and distribution of mineral substances from their natural deposits. Unlike many other pollutants associated with the environments, metals are nonbiodegradable and can undergo biomagnifications in living tissues. Uptake and accumulation of heavy metals by plants is either via the roots and foliar surfaces (Sawidis *et al*., 2001). Some factors which affect metal uptake include soil pH, metal solubility, soil conductivity nature, stages of plant growth and plant species type (Ismail *et al*., 2005; Sharma *et al*., 2006).

Industrial wastewater contains high levels of heavy metals that may pollute the environment once it is discharged to the nature. These metals include arsenic, chromium, copper, zinc, aluminum, cadmium, lead, iron, nickel, mercury, and silver. They are some of the most toxic types of water pollutants. According to Khaled *et al*. (2008), at least 20 metals are considered

to be toxic, and approximately half of these metals are emitted to the environment in quantities that are hazardous to the environment, in addition to the human health. Many people could be at risk of adverse health effects from consuming vegetables cultivated in contaminated soil. Many researchers have shown that some vegetables are capable of accumulating high levels of metals from the soil or water (Garcia *et al*., 1981). Heavy metals are one of a range of important types of contaminants that can be found on the surface and in the tissue of fresh vegetables. Heavy metals, such as cadmium, copper, lead, chromium and mercury, are environmental pollutants, particularly in areas under irrigation with wastewater (Garcia *et al*., 1981).

Soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, sewage sludge pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition (Khan *et al*., 2008). Heavy metal contamination in agricultural soils may lead to the disorder of soil functionality and retardation of plant growth, and influence human health through a contaminated food chain ((Khan *et al*., 2008).

Dry-ashing and wet-digestion are the common methods of soil, plant and water sample digestion for elemental analysis. Dry-ashing methods are comparatively simpler and safe than wet-digestion methods but may introduce error due to volatilization, especially for arsenic (As), selenium (Se), cadmium (Cd) and mercury (Hg). In addition, dry ashing may be problematic with pyrolytic organic materials as they may resist thermal decomposition at temperatures of about 550 ^{0}C and analyte reactions with the crucible material and sample contamination from combustion residues (Hoeing *et al*., 1998).

Wet-digestion methods are preferable because of the speed with which sample is processed. These techniques utilize strong inorganic acids ($HClO_4$, NHO_3, H_2SO_4 and HCl) and in some cases hydrogen peroxide (H_2O_2) to decompose the samples. Perchloric acid ($HClO_4$) was commonly used some time ago because of its strongly oxidizing ability but has largely been avoided because of handling issues, its capacity to react violently with organic compounds, and the possibility of explosion when dry. Additionally, the use of $HClO_4$ requires special

ventilation equipment. Nitric acid (HNO_3) in combination with hydrogen peroxide (H_2O_2) is an effective substitute for $HClO_4$, with the benefit of increased safety (Enders and Lehmann, 2012).

Sequential selective extraction techniques are commonly used to fractionate the solid-phase forms of metals in soils. Many sequential extraction procedures have been developed, particularly for sediments or agricultural soils, and despite numerous criticisms, they remain very useful (Christian *et al*., 2002). The mobility and bioavailability of heavy metal depend absolutely on their speciation or chemical forms. These forms are determined by sequential extraction technique, this method gives vivid information about metal affinity to the soil components together with the strength to which they are bound to the soil matrix. Also heavy metal fractions can give detail about soil origin, biological and physicochemical availability, and their mode of occurrence, mobility and transportation of trace metals (Kotoky *et al.*, 2003). Extraction procedures of soil are used both for the single-stage leaching and the sequential extraction. Among various methods of the sequential extraction of soil, Tessier's method is most often used both in case of soil samples, as well as sediments (Yoseph, 2015)

Some methods used in heavy metal analysis are AAS, EDXRF and ICP (Abolino *et al*., 2002). For analysis of various fractions obtained by sequential extraction, AAS, ICP-MS and ICP-AES and ICP-OES are used (Iwegbue, 2007). Also Milkessa (2013) used FAAS. ICP-MS and AAS are most preferred because they are not prone to polyatomic interferences and are less affected by matrix suppression (Harrison *et al*., 1981).The method used in the present study for analysis was AAS due to its availability. AAS is simple, sensitive and selective and has the advantage of being a fast method of analysis (Katz, 1984).

The aim of this study was to detect and determine the concentrations of beneficial as well as toxic metals viz. Cr, Cd, Fe, Pb, Cu and Zn in samples of industrial effluents, soils and selected vegetables from irrigation farms around Eastern Industry Zone, in which pesticide, fertilizer, municipal and industrial sewage effluents are known to be discharged into surrounding irrigation farms. Cabbage, lettuce, and tomato were selected and most commonly-consumed edible vegetables which are cultivated by using effluent wastewater, due to lack of clean irrigation water. The study is necessary, as a large number of people consume the

vegetables grown in this area. To date there is no research report on the levels of heavy metal concentrations in effluents, soils and vegetables, to elucidate the extent of the problems posted by this industry zone on the environment.

Objective of study

General Objective

To study levels of heavy metals (Chromium, Cadmium, Zinc, Iron Lead, and Copper) in samples of soil, wastewater and selected vegetables grown in the wastewater irrigated farmlands around Eastern Industry Zone, Dukem, Ethiopia.

Specific Objectives

i. To determine the total concentrations of heavy metals (Cr, Cd, Zn Fe, Pb, and Cu) in soils samples/farmers ‘fields irrigated with the Eastern Industry Zone effluents by using sequential fractionation and wet-digestion method.
ii. To determine the concentrations of heavy these metals in the effluent discharges coming from Eastern Industry Zone.
iii. To determine the concentration of these heavy metals in vegetable crops (cabbage, Lettuce and Tomato) grown around Eastern Industry Zone using the factory effluents as irrigation water source.

2. LITERATURE REVIEW

2.1. Soil

Soil is an essential natural resource for support of human life; but with time, its degradation has been constantly increasing due to the deposition of pollutants. The background concentration of metals in virgin soil depends primarily on the bedrock type from which the soil parent material was derived (Maldonado *et al*., 2008). In addition, anthropogenic inputs may increase metal concentrations, especially in highly industrialized parts of the world producing rare and heavy metals (Maldonado *et al*., 2008).

Soil acts as a thin layer of earth's crust which serves as a natural medium for the growth of plants and it is the unconsolidated mineral matter influenced by genetic and environmental factors. Soil is a very important natural resource to man as it is a source of his life on this planet. Without soil the earth would be as barren as the moon hence lifeless (Misra and Mani, 2009). According to Dukem town development plan team report the soil of the town is vertisol. This type of soil forms deep cracks during the dry season but logs water during the rainy season. The cracking of the soil during the dry season facilitates soil erosion. As a result, there are deep gorges in different parts of the town, especially along the courses of seasonal streams and Dukem river valley because of the very nature of the soil. Activities like quarrying and extraction of soil and also sand from the valleys of streams and river play their contribution for the formation of deep river gorges in different parts of the town (Abebe, 2012).

2.1.1. Soil Pollution

Soil is often contaminated by human activities and this is reflected in the high horizontal and vertical variability brought about by the anthropogenic influence on soil formation and development (Fong *et al*., 2008). A variety of human activities including municipal waste disposal, industrial emissions, military testing and agricultural practices have left their impacts on soils in the form of elevated and high level of toxicants. When plants decay, heavy metals that had been taken into the plants are redistributed so the soil is then again enriched with the pollutants (Sawidis *et al*., 2001).

2.1.2. Soil Pollution by Heavy Metals

Heavy metal pollution in soils refers to cases where the quantities of the elements in soils are higher than maximum allowable concentrations and this is potentially harmful to biological life at such locations (Gazso, 2001). Heavy metals occur at typical background in all ecosystems, however, anthropogenic releases can result in higher concentrations of these metals relative to their normal background values hence the pollution (Gazso, 2001). Heavy metals released from vehicular emission can accumulate in surface soils and their deposition over time can lead to abnormal enrichment, thus causing metal contamination of the surface soils (Fong *et al*., 2008).

To effectively remediate heavy metal contaminated soil, it is necessary to know the amount of toxic elements in the soil. However, only determining the total concentration of heavy metals in soil is because mobility and bioavailability are strongly dependent on the chemical phase of heavy metals in soil (Anrich *et al*., 2003; Brown *et al*., 2004).

Soil pH significantly influences heavy metal concentrations in both soil and plant tissues. The effect of soil pH on mobility of heavy metals is a well-researched topic (Li and Wu, 1999). As the soil pH decreases, metals are desorbed from organic and clay particles, enter the soil solution and, become more mobile (Li and Wu, 1999).

2.2. Vegetables

Vegetables are important ingredients in the human diet and contain essential nutrients and trace elements that have potential health benefits. Environmental pollution has caused the contamination of soil; on the other hand, wastewater irrigation resulted in significant infusion of non-essential elements in agricultural lands (Deribachew *et al*., 2015). Vegetables refer to the fresh edible portion of herbaceous plant roots, stems, leaves or fruits. They play an essential role in human diet. They constitute an important part of the human diet since they contain carbohydrates, proteins, as well as vitamins, minerals and trace elements. In addition to their high concentration of micronutrients, vegetables provide little dietary energy, making them valuable in energy limited diets. The fiber content has been reported to have beneficial effects on blood cholesterol and aids in the prevention of large bowel diseases, while in

diabetic subjects, they improve glucose tolerance. So increasing consumption of vegetables is fundamental goal of awareness among populations. Although an increase in the consumption of fresh produce contributes to the improvement of public health, it may also contribute to an increase in product related food borne illnesses (Yuk *et al*., 2006).

2.2.1. Heavy Metals in Vegetables

There is an inherent tendency of plants to take up toxic substances including heavy metals that are subsequently transferred along the food chain (Singh *et al*., 2010). Contamination of foods by heavy metals has become a challenge for producers and consumers. The main sources of heavy metals to vegetable crops are their growth media (soil, air, nutrient solutions) from which these heavy metals are taken up by the roots or foliage (Lokeshwari and Chandrappa, 2006). Vegetables can take up and accumulate heavy metals in quantities high enough to cause clinical problems to humans (Alam *et al*., 2003). Leafy vegetables grown on heavy metal contaminated soils accumulate higher amounts of metals than those grown in uncontaminated soils because of the fact that they absorb these metals through their roots (Alam *et al*., 2003).

2.2.2. Sources of Heavy Metal Pollution in Vegetables

Water contamination by heavy metals in some areas is practically inevitable due to natural process (weathering of rocks) and anthropogenic activities (industrial, agricultural and domestic effluents) (Sugiyama, 1994). In turn, industrial or municipal wastewater is mostly used for irrigation of crops mainly in periurban ecosystem. This is because wastewater is easily available coupled with disposal problems and scarcity of fresh water (Arora *et al*., 2008). The wastewater from the industries of mining, electroplating, and paint or chemical laboratories often contains high concentrations of heavy metals, including cadmium, copper and lead. These elements, at concentrations exceeding the physiological demand of the plants, not only could administer toxic effect in them but also could enter food chains, get biomagnified and pose a potential threat to human health (Sawidis *et al*., 2001; Mapanda *et al*., 2005).

Wastewater is known to contribute significantly to the heavy metal contents of soils; hence disposal of sewage and industrial waste into agricultural lands leads to contamination of crops

including vegetables grown on that land. This is because these effluents that are considered a rich source of organic matter and other nutrients also have high levels of heavy metals such as iron, manganese, copper, zinc, lead, cadmium, nickel and cobalt. Most of the heavy metals are extremely toxic because of their solubility in water (Arora *et al*., 2008).

Vegetables can absorb metals from soil as well as from deposits on the parts of the vegetables exposed to the air from polluted environments (Haiyan and Stuanes, 2003). Emission of heavy metals from the industries and vehicles may be deposited on the vegetable surfaces during their production, transport and marketing. Similarly, atmospheric deposition can significantly elevate the levels of heavy metals contamination in vegetables commonly sold in the markets of Varanasi, India (Sharma *et al*., 2008).

The uptake and bioaccumulation of heavy metals in vegetables is influenced by many factors such as climate, atmospheric depositions, the concentrations of heavy metals in soils, the nature of soil and the degree of maturity of the plants at the time of the harvest (Voutsa *et al*., 1996; Scott *et al*., 1996).

2.3. Wastewater and Irrigation

Wastewater is not just sewage. All the water used in the home that goes down the drains or into the sewage collection system is wastewater. This includes water from baths, showers, sinks, dishwashers, washing machines, and toilets. Small businesses and industries often contribute large amounts of wastewater to sewage collection systems (Lokeshwari, *et al*., 2006).

Irrigation is a supply of water to agricultural crops by artificial means, designed to permit farming in arid regions and to offset drought in semi-arid regions. The wastewater irrigation practices give very good crop yields because wastewater contains large amounts of organic material and some inorganic elements essential for plant growth. But it may also contain large amounts of non-essential heavy metals which can be transferred to animal and human beings through food chain (Murtaza *et al*., 2010).

The main sources of pollution that enter surface water bodies are industries, municipal solid waste and oily wastes from garages and fuel stations. Most of the water resources are gradually becoming contaminated due to the addition of foreign materials from the surroundings. These include organic matter of plant and animal origin, land surface washing and industrial and sewage effluents. Rapid urbanization and industrialization with improper environmental planning often lead to discharge of industrial and sewage effluents into rivers (Lokeshwari, *et al*., 2006).

Worldwide, 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. Waste water has deleterious effects on soil and it cannot be properly used for agricultural practices due to salinity and sodicity problems which impose harmful effects on seedlings of plants. Most of the leafy vegetables which were grown in contaminated soil accumulate higher amount of heavy metals in their leaves (Ansari and Malik, 2007).

Wastewater irrigation may lead to transport of heavy metals to soils and may cause crop contamination affecting soil flora and fauna. Some of these heavy metals may bio-accumulate in the soil while others, e.g., Cd may be redistributed by soil fauna such as earthworms (Kruse and Barrett, 1985).

In Ethiopia, from the increasing human population, uncontrolled urbanization and inadequate sanitation infrastructure cause serious quality degradation of surface waters. Now a day's water pollution from disposal of industrial wastewater is becoming an environmental concern in Addis Ababa city and its vicinity areas, where most (More than 40% of large and medium scale manufacturing industries are located (Mulu *et al*. 2013).

2.4. Selected Toxic Heavy Metals under Study

Heavy Metals are defined as those elements with a specific density at least five times the specific gravity of water, Heavy Metals include Cadmium (Cd), Copper (Cu), Lead (Pb), Zinc (Zn), Mercury (Hg), Arsenic (As), Silver (Ag), Chromium (Cr), Iron (Fe) and Platinum group elements, Copper and Zinc are essential trace elements for living organisms at low

concentration (<10 mg/L). However, they become toxic at high concentration (>10 mg/L). Most of these metal ion (Cd, Cu, Zn, Hg, As, Ag, Cr and Fe) can be released from the industries are in simple cationic forms (Volesky, 1995). Heavy Metals cannot be degraded including bio treatment and are very toxic even at low concentration (1.0-10.0 mg/L) (wang, 2006). Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs (WHO, 1984).

2.4.1. Sources of Heavy Metals

Toxic heavy metals have adverse effects on plants, animals and humans. Excess heavy metals in the soil originate from many sources, which include atmospheric deposition, sewage irrigation, improper stacking of the industrial solid waste, mining activities, the use of pesticides and fertilizers (Zhang *et al.*, 2011).

2.4.2. Chromium

Chromium is a naturally occurring element found in rocks, soil, plants, animals, and in volcanic dust and gases. Chromium is present in the soil as (Cr III) or chromate (Cr VI) ions Chromium (III) is an essential nutrient in the diet, but it is required in a very small amount (Cataldo *et al*., 1981). It is used for making steel and other alloys, bricks in furnaces, and dyes and pigments, and for chrome plating, leather tanning, and wood preserving. Chromium plating was once widely used to give steel a polished silvery mirror coating. Chromium is used in metallurgy to impart corrosion resistance and a shiny finish; as dyes and paints, its salts colour glass an emerald green and it is used to produce synthetic rubies; as a catalyst in dyeing and in the tanning of leather; to make moulds for the firing of bricks. Chromium (IV) oxide (CrO_2) is used to manufacture magnetic tape (Lee *et al*., 1997).

Most of the chromium in soil does not dissolve easily in water and can attach strongly to the soil. A very small amount of the chromium in soil, however, will dissolve in water and can move deeper in the soil to underground water. The movement of chromium in soil depends on the type and condition of the soil and other environmental factors (Rai *et al*., 1992).

A smaller percentage of total chromium in soil exists as soluble chromium (VI) and chromium (III), which are more mobile in soil. The mobility of soluble chromium in soil will depend on the sorption characteristics of the soil (Lokeshappa, 2012).

Chromium (VI) compounds are toxics and known human carcinogens. The toxicity of Cr (VI) derives from its ability to diffuse through cell membranes and oxidize biological molecules whereas chromium (III) is an essential element. Breathing high levels can cause irritation to the lining of the nose; nose ulcers; running nose; and breathing problems, such as asthma, cough, shortness of breath, or wheezing. Long term exposure can cause damage to liver, kidney, circulatory and nerve disorders, as well as skin irritation (Lokeshappa, 2012).

2.4.3. Zinc

Zinc 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. Zinc can be found in nearly all soils. It is present in most rocks and is weathered out and deposited into the soil. Zinc is also released by thermal outgassing and other volcanic events. Fallout from such events can be a significant source of zinc in soils and plants. Anthropogenic release is the primary source of zinc in the environment. Zinc is released from industrial and manufacturing facilities in wastewater effluent or from incinerators (ATSDR, 1994).

Zinc deficiency in the diet may be more detrimental to human health than too much zinc in the diet. Zinc is an essential element, present in the tissues of animals and plants even at normal, ambient concentrations. However if plants and animals are exposed to high concentrations of bioavailable zinc, significant bioaccumulation can results, with possible toxic effects (USPHS, 2000). Zn is an airborne pollutant, so in general it majorly accumulate to open and above-earth crops; however root crop plants also assimilate great proportion from Zn contaminated soils (Lokeshappa, 2012).

2.4.4. Cadmium

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 occurs naturally at low levels in the environment. Food, rather than air or water, represents the major source of cadmium exposure, although tobacco smoking adds significantly to the body‘s burden. Additional cadmium has been added to the environment through industrial processes such as cadmium metal production. Further cadmium has been added to agricultural soils through the use of phosphate fertilizers (WHO, 1989). Other sources include farmyard manure, sewage sludge, metal working industries, waste incinerators, urban traffic and atmospheric deposition; cement factories etc (Sanita and Gabbrielli, 1999).

Cadmium is very toxic, its long-term exposure to lower levels leads to a buildup in the kidneys and possible kidney disease, lung damage, and fragile bones. Hypertension, arthritis, diabetes, anaemia, cancer, cardiovascular disease, cirrhosis, reduced fertility; hypoglycemia, headaches, osteoporosis, kidney disease, and strokes are its some odd long term results (NAS/NRC, 1999).

2.4.5. Lead

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 (Wuana and Okieimen, 2011).

The major sources of lead in the environment are automobile exhaust, industrial wastewater, sludge and pesticides (Balba *et al*., 1991). Lead enters into the body system through air, water and food and cannot be removed by washing fruits and vegetables (Divrikli *et al*., 2003). It is a serious cumulative body poison, which can affect every organ and system in the body.

Exposure to its high levels can severely damage the brain, kidneys and ultimately cause death and long-term exposure result in decreased performance in some tests that measure the functions of the nervous system; weakness in fingers, wrists, or ankles; small increases in blood pressure; and anaemia. Others are abdominal pain, anaemia, arthritis, attention deficit, back problems, blindness, cancer, constipation, convulsions, depression, diabetes, migraine headaches, thyroid imbalances and tooth decay (NAS/NRC, 1999).

2.4.6. Iron

Iron is the second most abundant metal on the earth's crust. Iron occupies the 26^{th} elemental position in the periodic table. Iron is a most crucial element for growth and survival of almost all living organisms (Valko *et al*., 2005). It is one of the vital components of organisms like algae and of enzymes such as cytochromes and catalase, as well as of oxygen transporting proteins, such as hemoglobin and myoglobin (Vuori, 1995). An elevated dietary iron intake enhances the incidence of carcinogen-induced mammary tumors in rats and estrogen-induced kidney tumors in Syrian hamsters. Estrogen administration increases iron accumulation in hamsters and facilitates iron uptake by cells in culture. In humans, increased body stores of iron have been shown to increase the risk of several estrogen-induced cancers (Liehr and Jones, 2001).

Iron deficiency includes symptoms such as reduced resistance to infection, reduced work productivity, reduced physical fitness, weakness, fatigue, impaired cognitive function, and reduced learning ability, increased distractibility, impaired reactivity and coordination, itching, inability to regulate body temperature and eating pica (Beard, 2001)

2.4.7. Copper

Copper is one of the world's most widely used metals. According to Weiner (2008) the most common copper-bearing ores are sulfides, arsenates, chlorides, and carbonates. It reaches aquatic systems through anthropogenic sources such as industry, mining, plating operations, usage of copper salts to control aquatic vegetation or influxes of copper containing fertilizers (Nussery, 1998). Copper is an essential trace element to plants, animals and even humans, and although the concentration of copper is usually low in nature, it happens in adequate quantities

for growth in all aquatic environment. It is required for bone formation, maintenance of myelin within the nervous system, synthesis of haemoglobin, component of key metalloenzymes, plus it forms an important part of cytochrome oxidase, and assorted other enzymes involved in the redox reactions in the cells of animals (Nussery, 1998).

2.5. Sequential Extraction

Sequential extraction methods are based on the rational use of a series of more or less selective reagents chosen to solubilise successively the different mineralogical fractions thought to be responsible for retaining the larger part of metals (Gleyzes *et al*. 2002). Several sequential extraction procedures have been proposed for determining the speciation of particulate heavy metals. For example, Tessier *et al*. (1979) developed a five-stage extraction to evaluate the fractions of Cd, Cu, Fe, Pb, Mg, Ni and Zn in river sediments.

Three major experimental problems with sequential procedures have been recognized (1) the limited selectivity of extractants, (2) the redistribution of metals during extraction process, and (3) the deficiency of a reagent dose if metal content is too high (Tessier *et al*. 1979; Tipping *et al*., 1990; Kheboian and Bauer, 1987). These limitations mean that sequential extraction cannot be used to determine specific geochemical associations, but the approach is still of value in the assessment of soil contamination. Sequential extraction can be used to give an indication of the amounts of metals in various reservoirs which could be mobilized by changes in environmental chemistry (notably pH) (Kennedy *et al*., 1997).

The amount of inaccuracy introduced to sequential extraction process by resorption has been debated in the literature (Kim and Fergusson, 1991), but theoretically it may lead to a significant underestimation of bioavailability. Howard and Vandenbrink (1999) evaluated the utility of a sequential extraction process for counteracting resorption during sequential extraction analysis using sediments with a wider range in composition. They indicated that significant resorption may occur during sequential extraction analysis, thereby reducing the accuracy of the method. However, this problem may be important only at very high levels of contamination (Howard and Vandenbrink, 1999). Generally it is difficult to associated a given extractant reagent with a particular physicochemical phase. Instead the specificity of an

extractant is operationally defined according to what it extracts. Therefore, most validation studies have focused on the selectivity and completeness of extraction rather than the redistribution. Emphasis was also on major species rather than trace elements (Kheboian and Bauer, 1987). Keeping these limitations in mind, "operational speciation" results from sequential extractions still provide useful information on metal partitioning, beyond the simple elemental concentrations which are conventionally measured (Ho and Evans, 2000).

However, several researchers have addressed the limitations of sequential extraction. These limitations include the technical difficulties associated with achieving complete and selective dissolution and recovery of trace metals from those geochemical phases in soils and sediments. For example, the overlap of chemical reagents and readsorption of trace metals during extraction. Despite these inherent limitations, the sequential extraction scheme is still a very useful method for characterizing the trace metals in solid mattes (Li *et al*. 1995). A large number of sequential extraction procedures have been developed. Among them, the most widely used sequential extraction method was provided by Tessier *et al*. (1979) and was based on the fact that different forms of heavy metals retained in soils can be extracted selectively by a series of extracting reagents (Li *et al*., 1995; Ma and Rao, 1997; Lo and Yang, 1998; Ariza *et al*., 2000). Due to the above situations, this method was selected for this study. The sequential extraction procedure of Tessier, the chemical partitioning of heavy metals allows to five fractions representing the following chemical forms:

Exchangeable fraction: This fraction involves weakly adsorbed metals retained on the solid surface by relatively weak electrostatic interaction, metals that can be released by ion-exchangeable processes etc. Remobilisation of metals can occur in this fraction due to adsorption-desorption reactions and lowering of pH. Exchangeable metals are a measure of those traces metals which are released most readily to the environment. Corresponding metals in the exchangeable fraction represent a small fraction of the total metal content in soil, sewage. sludge and sediment and can be replaced by neutral salts. $MgCl_2$ is the most frequently used extraction agent for determination of ion-exchangeable amounts of different metals including Mn. The effect of this reagent combines two important properties, namely the high ion-exchange capacity of Mg (II) and the almost negligible complex formation ability of chloride ions. In addition, $MgCl_2$ do not attack organic matter, nor silicates and metallic

sulphides. Application of $MgCl_2$ may actually lead to dissolution of carbonates in some extent, but as it is in general assumed, this problem can be easily handled by reduction of contact times (Gleyzes *et al*., 2002; Krishnamurti *et al*., 2002).

The fraction bounded to carbonates: Carbonate tends to be a major adsorbent for many metals when there is reduction of Fe-Mn oxides and organic matter in the aquatic system. The most popular use reagent for the extraction of trace metals from carbonates phases in soils and sediments is 1 M sodium acetate adjusted to pH 5.0 with acetic acid. The carbonate fraction is a loosely bound phase and bound to changes with environmental factors such as pH. The time lag for the complete solubilisation of carbonates depends on some factors such as the type and amount of the carbonate in the sample, particle size of the solid (Tessier *et al*., 1979; Yoseph, 2015). In general, this fraction is sensitive to pH changes, and metal release is achieved through dissolution of a fraction of the solid material at pH close to 5.0 (Yoseph, 2015).

The fraction bounded to iron and manganese oxides: This is referred to as sink for heavy metals. Scavenging by these secondary oxides, present as coating on mineral surfaces or as fine discrete particles. This can occur as a combination of the precipitation, adsorption, surface complex formation and ion exchange (Yoseph, 2015).

The fraction bounded to organic matter: The bioaccumulation or complexation process being the primary source in which trace metal get associated with organic material such as living organisms, detritus etc. (Tessier *et al*., 1979). In organic phase, metallic pollutant bound to this phase are assumed to stay in the soil for longer periods but may be immobilized by decomposition process. Under oxidizing conditions, degradation of organic matter can lead to a release of soluble trace metals bound to this component. The extracts obtained during this step are metals bound to sulphides (Yoseph, 2015).

The organic fraction released in the oxidisable step is considered not to be bioavailable due to the fact that it is thought to be associated with stable high molecular weight humic substances that release small amount of metals in a slow manner. The most commonly used reagent for the extraction of metals in organic phases is hydrogen peroxide with ammonium acetate readsorption or precipitation of released metals. Other reagents such as H_2O_2/ ascorbic acid or

HNO_3 + HCl have been used which can dissolve sulphides with enhanced selectivity (Yoseph, 2015).

The residual fraction: Including mainly metals built in the crystal lattice of minerals in natural conditions they are practically inaccessible for living organisms and can be considered and permanently immobile (Zerbe *et al*., 1999; Yoseph, 2015).

2.6. Atomic Absorption Spectroscopy

The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires a standard with known analyte content to establish the relation between the measured and the analyte concentrations and relies on Beer Lambert's law (Skoog *et al*., 2005).

The sample is converted into atomic vapours by a process known as atomization. The precision and accuracy of this method depends on the atomization step and therefore a good choice of the atomization method is required. The two types of atomizers are continuous and discreet atomizers. In continuous atomizers the sample is fed into the atomizer continuously at a constant rate giving a spectral signal which is constant with time. Atomization methods that are of continuous type are flame, inductively coupled argon plasma and direct current argon plasma. With the discrete atomizers, a measured quantity of a sample is introduced as a plug of liquid or solid. The spectral signal in this case rises to a maximum and then decreases to zero. An electro thermal atomizer is one of the discrete types. The atoms then absorb radiations of characteristic wavelengths from an external source. This technique has been widely employed for elemental analysis in a number of matrices such as soils, water, nuts wine and wine products (Navin *et al*., 2000).

Figure 1 shows a schematic diagram for the components of AAS. The two sources of radiation are continuous source which makes use of deuterium and mercury lamps and a hollow lamp which consists of an anode made of either tungsten wire or wink and a hollow cathode made of either the element of interest or its own salt (Navin *et al*., 2000).

Flame atomization method consists mainly of a fuel and oxidant. Their temperatures are determined by flow rate and ratio of oxidant and fuel while the electro thermal atomizer is basically made of carbon rods. The free atoms are vaporized from the carbon atomizer into the optical light path to a monochromator which presents a monochromatic radiation to the detector. The radiations from the monochromator are received by detectors which converts them to electrical signals. Some commonly used detectors are photocells and photo multiplier tubes (Navin *et al*., 2000).

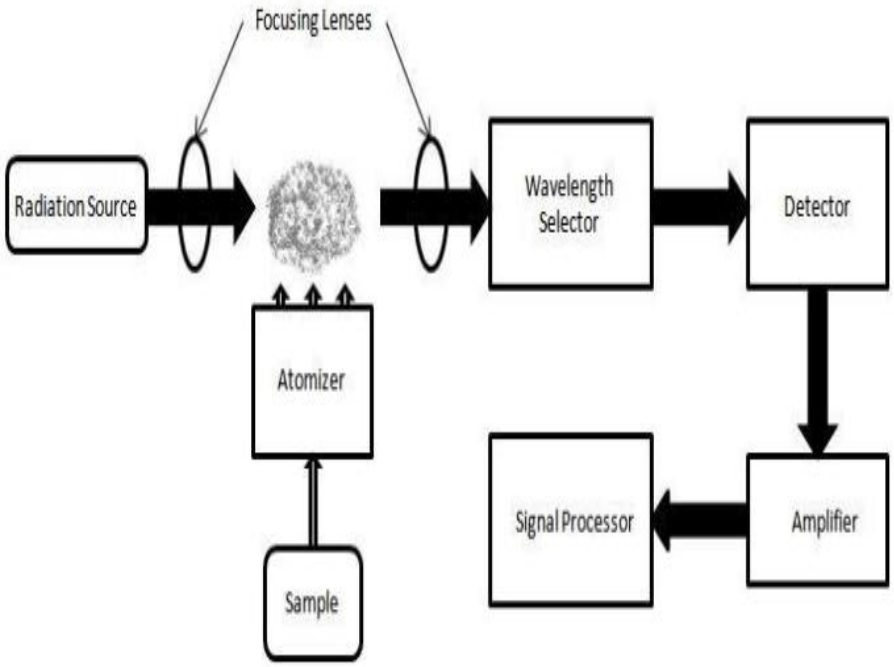

Figure 1. Schematic diagram of AAS equipment

3. MATERIALS AND METHODS

3.1. Description of the Study Area

This study was conducted around Eastern Industrial Zone in Dukem, Ethiopia. Dukem Town was founded in 1914 and is one of the 18 special zones of the Oromia Regional State of Akaki Woreda which is located at 37 Km distance from Addis Ababa City. It is a town in central Ethiopia, to the South of Addis Ababa and 10 Km to North West of Bishoftu Town. Its astronomical location is 08º45'25"-08º50'30" North Latitude and 38º51'55"-08º56'5" East Longitude (Abebe, 2012).

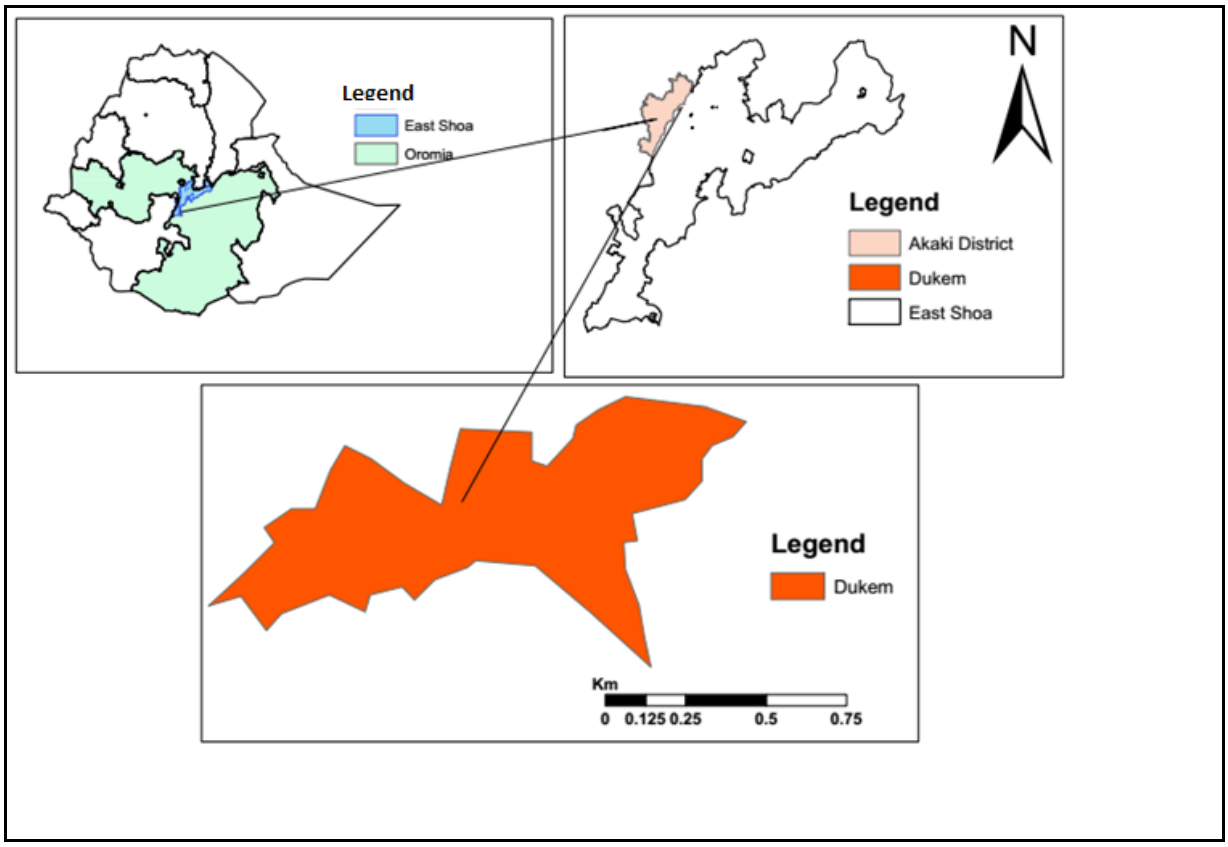

Figure 2. Location of study area

The Eastern Industrial Zone (EIZ) of Ethiopia is located at 35 km southeast of Addis Ababa, and 680 Km from the port of Djibouti with 200 hectares of land in Dukem. For Ethiopia, EIZ is the first and largest-scale industrial park. The Ministry of Industry of Ethiopia requires the

EIZ to focus on Chinese companies in the area of textile, apparel, building materials (including east steel, cement factory), mechanical manufacturing, and agricultural processing. Currently, 26 Chinese firms are operational and producing different products for export markets having agreement with EIZ in all targeted areas. In addition to the present 26 manufacturing industries, more than 20 other manufacturing industries are about to join the EIZ (Gebregeorgis, 2016). This implies that more wastewater from various industry of EIZ is discharged to the surrounding through one wastewater channel; this wastewater is used as source of irrigation water by the farmers around this industry area.

3.2. Apparatus and Equipment

The instruments used for this study was FAAS, Agilent technology with model no. 210 for toxic heavy metal determination of wastewater, vegetable and soil samples and a Mcroprossecer based PH-EC-TDS Meter; Model 1615 was used for the determination of soil pH and conductivity.

The common laboratory apparatus which were used during the study include; different sized beakers, erlenmeyer flasks, funnels, volumetric flasks, block digester, fume hood, centrifuge, hydrometer, shaker, droppers, glass pipettes, spatula, measuring cylinders, plastic knife, vinyl gloves, steel less steel auger, stirrer, polyethylene bags, analytical balance, conical flasks and oven.

3.3. Chemicals, Reagents and Standard Solutions

All the chemicals used were analytical reagent grade. Deionized water and distilled water were used for all preparation and dilution purposes throughout the study. Nitric acid, HNO_3 (69%), ammonium acetate (NH_4Ac) Sodium acetate (NaAc), potassium chloride (KCl), acetic acid (HAc), magnesium chloride ($MgCl_{2),}$ hydroxide hydrochloride ($NH_2OH.HCl$), sulphuric acid, H_2SO_4 (98%) and hydrogen peroxide, H_2O_2 (30%) and hydrochloric acid (HCl) were used for digestion. Stock standard solutions of 1000 ppm were prepared for the selected heavy metals (Cr, Cd, Zn, Fe, Pb, and Cu). pH 4 and 7 Buffers were used for pH meter calibration and potassium chloride was used for conductivity meter calibration.

3.4. Sample Collection and Preparation

3.4.1. Cleaning of Glassware and Sample Containers

All sample containers and glassware used in the present study were washed in detergent and soaked in 30% nitric acid for 2 h to leach out adsorbed metal ion. They were then rinsed in tap water followed by deionized water before drying in dust free area (APHA, 1999); (Yoseph, 2015).

3.4.2. Vegetable Sample Collection and Preparation

The vegetable samples were collected in February, 2017 about 1 Kg edible part of cabbage (*Brassica oleracea*), lettuce (*Lactuca sativa*) and tomato (*Lycopersicon esculentum Miller*). To this effect, three farmer farmlands were selected and three subsamples were taken for collecting representative edible parts of the vegetables. The collection was done manually. The representative reputable samples were thoroughly mixed to give a composite sample as representative fraction of the vegetables (Deribachew *et al*., 2015). The bruised or rotten portions were removed and the remaining samples were packed in polyethylene bags for transporting to the DZARC ANRL. In the laboratory, the collected plant samples were washed with tap water and then with distilled water to eliminate adsorbed dust and particulate matters. The vegetable samples were cut and chopped into small pieces using plastic knife in order to facilitate drying. Accordingly, the samples were air-dried for six days and further dried in hot air oven at 50-60°C for 24 h, to remove moisture and maintain constant mass. The dried samples were ground into powder using acid washed commercial mortar and pestle and then sieved to 2 mm mesh size. The sieved samples were finally stored in polyethylene bags and kept in desiccators until the time of digestion.

3.4.3. Soil Sampling and Preparation

Soil samples (about 1 Kg) were collected from 0-20 cm depth from the site where the vegetables were grown (for each vegetable type) with an auger (Poggio *et al*., 2008) and the control soil sample was collected 2 km away from the study area (Milkessa, 2012). Then the samples were placed in clean polyethylene bags and transported to the DZARC ANRL for

pretreatment and analysis. Larger particles and other debris were removed from the soil and then soil samples were air dried in a dry and dust free place at room temperature (25 ^{0}C) for 5 days, followed by oven drying until getting constant weights. The samples were then ground with a mortar and pestle to pass through a 2 mm sieve and homogenized. The dried, sieved, and homogenized soil samples were placed in polyethylene bags until the time of digestion.

3.4.4. Wastewater Sampling and Preparation

The industrial wastewater (effluent) samples were collected from Eastern Industry Zone. Samples were collected during February 2017. A total of three samples were collected; measurement points for the sampling were designated as N_1 to N_3. Wastewater samples were collected at the discharge point designated as N_1, 200 meters away from the discharge point designated as N_2 and at 500 meters away from the discharge point designated as N_3 (Singh *et al*., 2012). Samples were collected in clean and dry polyethylene bottles. These three bottles immediately acidified with 1 mL nitric acid, for later analysis of metal concentrations. The purpose of the acid is to keep the metals in solution and to avoid adsorption to the container walls (APHA, 1999).

3.5. Digestion of Soil, Wastewater and Vegetable Samples

3.5.1. Digestion of Wastewater Samples

The water samples from each sampling bottle were mixed thoroughly by shaking. A 50 mL filtered aliquot of water sample was pipetted into a digestion flask. The metal percentage found in the water was determined by digestion in 3 mL concentrated HNO_3 and 3 mL H_2O_2 below 80 ^{0}C for 1 h until a clear solution was observed. The clear solution was diluted to 100 mL volumetric flask with distilled water and blank digestion was also carried out in the same way (Birtukan and Gebregziabher, 2014). The blank solution contained all reagents except wastewater. All samples were digested in triplicates. The digests were analyzed for the toxic heavy metals by using FAAS in Holeta Agricultural Research Center Chemistry Lab. The concentration of each metal was calculated using the formula below (Birtukan and Gebregziabher, 2014):

$$\textbf{Final concentration (mg/L)} = \frac{CM \times DF \times NV}{SV} \quad (1)$$

Where: CM = Concentration of metal, DF = Dilution factor, NV = Nominal volume, SV= Sample volume (mL).

3.5.2. Digestion of Vegetable Samples

A 0.5 g of homogenized powdered vegetables sample was placed in borosilicate digestion flask to which 10 mL of acid mixture containing HNO_3- HCl-H_2O_2 (8:1:1, v/v/v) ratio were added. The mixture was heated at 120 ^{0}C over 3 h on block digester. After digestion was completed, the clear and colorless solution was filtered out into 100 mL volumetric flask. Each digestion tube were rinsed with distilled water to collect any possible residue, and added to the volumetric flask and finally made up to volume with distilled water. All the dilute samples were stored in 100 mL plastic bottles (high density polyethylene) until analysis. Each vegetable sample was digested and analyzed in triplicate to confirm precision of the result. The blank solution was prepared by taking a mixture of 8 mL HNO_3, 1 mL HCl and 1 mL H_2O_2 and treating similarly as that of the sample (Street, 2008). The heavy metal concentrations were analyzed by FAAS in Holeta Agricultural Research Center Chemistry Lab.

3.5.3. Wet Digestion of Soil Samples

The 0.5 g dried and homogenized soil samples were transferred in to 100 mL digestion flask in triplicate. In each of these flasks, 5 mL of deionized water and 30 mL of a mixture HNO_3 (69%) and 37% HCl with volume ratio of 5:1 were added. The sample dissolved in the acid mixture was digested in digestion hood (at 200 ^{0}C) for 1 h and kept to cool. After adding 2 mL of H_2O_2 to the cold digestion mixture, the final, the mixture was filtered out through Whatman No. 42 filter paper to a 100 mL volumetric flask and finally diluted to the mark with distilled water (Loon, 1985; Hizkeal, 2012; Kedir, 2015). The varying filtrates obtained above were analyzed for the total content of each heavy metal by FAAS in Holeta Agricultural Research Center Chemistry Lab. The blank reagent was also digested following the same procedure as the soil sample.

3.6. Sequential Extraction Procedure (SEP) for Soil Samples

The modified Tessier's procedure, Ma and Rao (1997); Yoseph (2015) was used to determine operationally defined chemical species of the metals from soil. Five operationally defined fractions of the metals were removed by these sequential extractions. The SEP operationally groups heavy metals into the following five fractions:

3.6.1. Soluble and Exchangeable Fraction

The soluble and exchangeable metals from each of 2.5 g soil samples were extracted, into wide mouthed polypropylene bottle; 20 mL of 1 M $MgCl_2$ solution adjusted to pH of 7.0 were added. The bottles were shaken for 1 h at room temperature by an end-over end mechanical shaker. The extracts were separated from the solid residue by centrifugation (5000 rpm) for 15 min and filtered through Whatman No. 42 filter paper into 100 mL volumetric flask and kept for metal analysis.

3.6.2. The Fraction Bound to Carbonates

The carbonate bound metals in the residue left from the previous step were extracted with 20 mL of 1.0 M NaAc (CH_3COONa) solution adjusted to pH of 5.0 with HAc (CH_3COOH) by continuously shaking for 4 h at room temperature. It was then centrifuged for 15 min at 5000 rpm and filtered into 100 mL volumetric flask through Whatman No. 42 filter paper and kept for metal analysis.

3.6.3. The Fraction Bound to Iron and Manganese Oxides

Metals bound to iron and manganese oxides were extracted from the residue of the second extraction by shaking with 50 mL of 0.04 M $NH_2OH.HCl$/25 % HAc solution and placed in to a water bath for 5.5 h at 96 ^0C, then centrifuged for 15 min at 5000 rpm and filtered through Whatman No. 42 filter paper into 100 mL volumetric flask and stored for metal analysis.

3.6.4. The Fraction Bound to Organic Matter

Metals bound to organic matter were extracted by pouring 7.5 mL of a 0.02 M HNO_3 solution and 12.5 mL of a 30 % H_2O_2 solution adjusted to a pH of 2.0 onto the residue from 3.6.3. and heated for 2 h in water bath at 85 ^{0}C. After cooling, additional volume of 7.5 mL of 30 % H_2O_2 solution adjusted to pH of 2.0 was added while maintaining continuous agitation and at a temperature of 85 ^{0}C for another 3 h. These solutions were then cooled to room temperature. Then aliquot of 12.5 mL of 3.2 M NH_4Ac/ 20 % HNO_3 solution was added and shaken for 30 min, then centrifuged for 15 min at 5000 rpm and filtered through Whatman No. 42 filter paper into 100 mL volumetric flask and stored for metal analysis.

3.6.5. The Fraction Bound to Soil Matrix (Residual Fraction)

The residues from 3.6.4., were quantitatively transferred into a digestion vessel and treated with aqua regia (7 mL of 10 M HCl and 2.3 mL of 15.8 M HNO_3). The temperature of the reaction mixture was slowly raised until reflux conditions and maintained for 2 h, centrifuged, at 5000 rpm for 15 min and then filtered through Whatman No. 42 filter paper into 100 mL volumetric flask. All dilutions were made to 100 mL with 2 % (v/v) HNO_3. For each fraction a blank was subjected to the same procedure.

3.7. Heavy Metals Analysis

3.7.1. Instrument's Operating Conditions

Concentrations of Cr, Cd, Zn, Fe, Pb, and Cu in the extracted soil, wastewater and different kind of vegetables samples were determined by using FAAS in Holeta Agricultural Research Center Chemistry Laboratory. The instrument was calibrated using 1000 ppm standard solution of respective heavy metals as well as drift blanks. Calibration curves for each heavy metal was set to ensure the accuracy of the instrument and to confirm that the results of determination were true and reliable. Standard stock solutions of 1000 ppm for all the metals were obtained. These solutions were diluted to the desired concentrations to calibrate the instrument (A multi-element solution containing Cr, Cd, Zn, Fe, Pb, and Cu 100 ppm was

utilized to prepare elemental calibration solutions. This multi-element solution was diluted with 2% nitric acid to obtain working standards for each metal of interest).

Parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument based on the instrument instruction. Three replicate determinations were carried out on each soil, vegetable and wastewater samples. Hallow cathode lamp for each metal operated at the manufacturer's recommended conditions were used at its respective primary line source. The acetylene and air flow rates were managed to ensure suitable flame conditions. All the six metals (Cr, Cd, Zn, Fe, Pb, and Cu) were analyzed by the absorption mode of the instrument. Three readings were recorded for each digest by different FAAS conditions shown in Table 1 to give the maximum signal intensity.

Table 1. Instrumental operating condition for the analysis of metal in sample of wastewater, vegetable and their respective soil vegetable growing.

Element	Wavelength (nm)	Slit width (nm)	Lap current (Am)
Cr	357.9	0.7	7.00
Cd	228.9	0.7	4.00
Zn	213.9	0.7	5.00
Fe	248.3	0.2	5.00
Pb	283.2	0.7	5.00
Cu	324.8	0.5	4.00
As	193.7	0.5	10.00

3.7.2. Instrument Calibration

Calibration curves for Cr, Cd, Zn, Pb, Fe and Cu were obtained by using suitable standard solutions prepared from stock solutions. The quality of results obtained for heavy metal analysis using FAAS are seriously affected by the calibration and standard solution preparation procedures. Calibration standards for the elements analyzed were prepared in concentration range expected for the analytes in the samples analyzed. In addition, the calibration standards were prepared by taking into consideration the optimum working

ranges of the elements. The correlation coefficient (R^2) values that are closer to the absolute value of 1 indicate that there is a strong relationship between the variables being correlated whereas values closer to 0 indicate that there is no linear relationship (Gezahegn, 2013). As shown in Tables 2, 3 and 4 the correlation coefficients of metals were found to be from 0.993-0.999, which indicate strong relationship. The correlation coefficients of the elements were determined using prepared standards versus their corresponding absorbance. The prepared standard concentration and the corresponding correlation coefficients of the calibration curve for each metal in soil, vegetable and wastewater are presented in Table 2, 3 and 4, respectively. Also the calibration graph of each of the metal of interest in soil, vegetable and wastewater are shown in the Appendix Figure 1, 2 and 3, respectively.

Table 2. Concentrations of the working standard solutions and Coefficient of determinations of the calibration curve for analysis of soil samples.

Element	**Concentration (mg/L)**	**Coefficient of determination (R^2)**
Cr	0.25,0.5,1,2 and 2.5	0.997
Cd	0.25,0.5,1,2 and 2.5	0.999
Zn	0.5,1,2,4 and 8	0.998
Fe	0.5,1,2,4 and 8	0.999
Pb	0.25,0.5,1,2 and 2.5	0.995
Cu	0.5,1,2,4 and 8	0.996

Table 3. Concentrations of the working standard solutions and Coefficient of determinations of the calibration curve for analysis of vegetable samples.

Element	**Concentration (mg/L)**	**Coefficient of determination (R^2)**
Cr	0.01,0.02,0.04,0.08 and 0.16	0.996
Cd	0.01,0.02,0.04,0.08 and 0.16	0.997
Zn	0.01,0.02,0.04,0.08 and 0.16	0.996
Fe	0.25,0.5,0.72,1.0 and 1.25	0.997
Pb	0.01,0.02,0.04,0.08 and 0.16	0.994
Cu	0.01,0.02,0.04,0.08 and 0.16	0.995

Table 4. Concentrations of the working standard solutions and Coefficient of determination of the calibration curve for analysis of wastewater samples.

Element	Concentration (mg/L)	Coefficient of determination (R^2)
Cr	0.25,0.5,0.72,1.0 and 1.25	0.997
Cd	0.25,0.5,0.72,1.0 and 1.25	0.996
Zn	0.25,0.5,0.72,1.0 and 1.25	0.995
Fe	0.25, 0.50, 0.1, 2.0 and 2.5	0.993
Pb	0.25, 0.50, 0.1, 2.0 and 2.5	0.997
Cu	0.25,0.5,0.72,1.0 and 1.25	0.995

3.7.3. Method Detection Limit

Method detection limit is defined as the minimum concentration of analyte that can be measured. In other words, it is the lowest analyte concentration that can be distinguished from statistical fluctuations in a blank (Gezahegn, 2013). Three replicate blank samples were digested following the same procedures utilized for digesting the soil, vegetable and effluent samples. Each blank were assayed for its metal contents (Cr, Cd, Zn, Pb, Fe and Cu) by FAAS. The SD of the three replicate blanks was calculated to determine the MDL (David and Terry, 2008). Method detection limit (MDL) was then calculated according to equation indicated below (Meseret *et al*., 2013).

$$\mathbf{MDL = YB + 3SD} \qquad \mathbf{(2)}$$

Where: YB = Blank mean

Three blank samples were digested following the same procedure as the samples and each of the blank samples were analyzed for metal concentrations of Cr, Cd, Zn, Pb, Fe and Cu by FAAS. The standard deviations for each element were calculated from the three blank measurements to determine method detection limit. The values of method detection limit obtained in this study are given in Appendix Table 1 for vegetable, soil in wet digestion and wastewater samples and Appendix Table 2 for soil fractionation.

3.8. Procedure for Physico-chemical Analysis of Some Parameters

3.8.1. Soil pH Analysis

Soil pH was determined using portable pH meter (mcroprossecer based PH-EC-TDS meter, model 1615) in a 1:2.5 soils-water suspension in triplicate (Anderson and Ingram, 1996).

3.8.2. Electrical Conductivity (EC)

The EC of collected soil samples were determined electrometrically (soil water ratio was 1:5) by a conductivity meter (Mcroprossecer Based PH-EC-TDS Meter, Model 1615) (Anderson and Ingram, 1996).

3.8.3. Organic Carbon, Texture and Cation Exchangeable Capacity (CEC) of Soil Samples

The Walkley and Black (1934) wet digestion method was used to determine soil carbon content and percent soil OM obtained by multiplying percent soil OC by a factor of 1.724 following the assumptions that OM is composed of 58% carbon (Gemechu *et al*., 2015). Soil texture was determined following the method of Bouyoucos (1962) using a hydrometer. Each determination was made in triplicates. Cation exchangeable capacity (CEC) was determined from extracts which were obtained from residual exchangeable cations after removing unbounded ammonium salt with 95% ethanol and then extracting with 10% sodium chloride. The distillation of the extract was conducted using Kjeldahl distillation apparatus and the concentration determined by titration method (Jackson, 1967).

3.8.4. Moisture Content of Soil Samples

Soil moisture contents were determined by oven drying method. 10 g of composite soil samples were taken in to evaporating dish. The samples were oven dried at 105 ^{0}C for 24 hrs. Dry weights of the sample were taken till it showed its constant weight. The loss in weight corresponds to the amount of water present in the soil sample. The formula below was used to calculate the percentage of moisture content in each of the soil samples (Joel and Amajuoyi, 2009).

$$\textbf{Moisture content percent (MC \%)} = \frac{\text{Loss in weight on drying (g)}}{\text{Initial sample weight (g)}} \times 100 \qquad (3)$$

3.9. Method Validation

In present study due to the absence of certified reference materials for soil, vegetable and effluent samples in our laboratory, the validity of the digestion procedure, precision and accuracy of FAAS were assured by spiking soil, vegetable and effluent samples with standard of known concentration. The spiked and non-spiked vegetables, soil and effluent samples were digested following the same procedure employed in the digestion of the respective samples and analyzed in similar condition. Then the percentage recoveries of the analytes were calculated by: (Deribachew *et al*., 2015; Kedir, 2015).

$$\textbf{Recovery} = \left(\frac{\text{CM in the spik samples} - \text{CM in the non spik sample}}{\text{Amount added}}\right) x\ 100\ \% \qquad (4)$$

Where, CM = concentration of metal of interest

3.10. Statistical Analysis

The analyses of variance ANOVA were performed to examine the significance level of all parameters measured. Least Significant Difference (LSD) test was used for means comparison. The level of significance for the t-test and means comparison was $p<0.05$. Methodological precision was therefore evaluated with standard deviation (SD).

4. RESULTS AND DISCUSSION

4.1. Physico-Chemical Analysis

4.1.1. Physico-Chemical Analysis of Soil samples

Conductivity is a measure of the ability of aqueous solution to carry an electric current that depends on the presence and total concentrations of ions, their mobility and valance and on the temperature (Mulugeta, 2014). In this work, conductivities of the soil samples collected from EIZ irrigation farmlands were determined at 25 ^{0}C. In the collected soil samples growing tomato, cabbage and lettuce the conductivities were found to be 0.78±0.08, 0.50±0.01, and 0.43±0.04 mS/cm, respectively, and the control soil showed 0.84 ±0.01 mS/cm, which is significantly higher than cabbage and lettuce grown soil (Table 5).

Table 5. Selected physico-chemical properties of soils samples from lands irrigated with wastewater around the Eastern Industry Zone

Parameter		**Soil sample type**				**LSD**
		ST	**SC**	**SL**	**C**	**(0.05)**
pH (1:25)		7.90±0.02^b	7.05±0.03^c	7.13 ±0.02^c	8.30±0.10^a	0.11
EC in mS/cm		0.78±0.08^a	0.50±0.01^b	0.43±0.04^b	0.84 ±0.01^a	0.10
%OM		3.15±0.14^a	3.25±0.02^a	3.05±0.25^a	1.65 ±0.03^b	0.30
%MC		1.88±0.14^b	2.10±0.17^{ab}	2.18 ±0.13^a	0.96±0.03^c	0.28
CEC in (cmol(+)/Kg soil		46.70±2.49^a	42.45±1.56^b	38.99 ±0.93^c	32.26±0.53^d	3.36
Texture	%clay	46.67±0.42^b	48.27±0.61^b	53.47±0.61^a	53.07±2.57^a	2.75
	%silt	34.73±0.31^b	39.73±1.10^a	32.67±0.31^b	34.07±3.21^b	2.90
	%sand	18.60±0.40^a	12.00±0.53^c	13.87±0.81^b	12.87±1.10^{bc}	1.27
Class		Clay	Clay	Clay	Clay	

N.B: Where ST, SC and SL refer to soil sample taken from tomato, cabbage, lettuce growing farm land, respectively and C is control (reference soil) sample. Values are given as means of triplicates ± SD. The mean values in the same row having different superscript letters are significantly different from each other at 5% confidence interval.

The relatively low electrical conductivity was observed in lettuce soil and relatively highest electrical conductivity was observed in tomato soil. Therefore, lettuce growing soils are able to give a toxic amount of metal from a small amount of soil. In line with this, Murray and McBride (1994) indicated that soils with low EC are able to give a toxic amount of metal from a small amount of soil (Hizkeal, 2012).

The pH value of the soils ranged from 7.13±0.02 to 8.30±0.10 (Table 5). According to Hizkeal (2012) soils with pH range of 5.6- 6.0, 6.1-6.5, 6.6-7.4, 7.4-7.8 and 7.8-8.4 are moderately acidic, slightly acidic, neutral or nearly neutral, slightly alkaline and moderately basic respectively, similarly soil with pH above 8.5 are strongly alkaline. Based on this, soil samples collected from tomato growing areas were moderately basic and soil samples collected from cabbage growing areas were nearly neutral whereas soil samples collected from lettuce growing areas were slightly acidic. Therefore, it indicates that the alkaline ranges of soils are known to limit the mobilization of heavy metals and thus minimize the uptake of heavy metals by plants (Sharma *et al*., 2007). Generally, most of the heavy metals are less available to plants under alkaline conditions than under acid conditions. pH is one of the factors which influence the bioavailability and the transport of heavy metal in the soil and heavy metal mobility decreases with increasing soil pH due to precipitation of hydroxides, carbonates or formation of insoluble organic complexes (Uduma, 2013). Heavy metals are generally more mobile at pH <7 than at pH >7. The amount of heavy metals mobilized in soil is a function of pH, properties of metals, redox conditions, soil chemistry, organic matter content, clay content, cation exchange capacity and other soil properties (Uduma, 2013).

Soil organic matter is a principal variable that affects the spatial distribution of heavy metals in soil (Afshin and Farid, 2007). Increase in soil organic matter content leads to elevation of soil adsorption capacity hence enhancing the accumulation of trace metals. Organic matters can therefore, be considered as an important medium through which heavy metals are incorporated into the soil (Afshin and Farid, 2007). Soil found in all type of samples investigated generally contained very high organic matter content with the highest for cabbage soil (3.25±0.02%). The organic matter content of the soil in this study area was generally higher when compared to that obtained by Inobeme *et al*. (2014); Gilbert and Osibanjo (2009) in a similar study, also for the control soil sample which was 1.65±0.03%.

CEC is a measure of the quantity of cations that can be adsorbed and held by a soil. CEC is used as a measure of fertility, nutrient retention capacity, and the capacity to protect groundwater from cation contamination. CEC is dependent on the organic carbon and clay in soil. In general, the higher the organic carbon and clay content, the higher the CEC. Cation exchange capacity (CEC) is an important parameter of soil, because it gives an indication of the type of clay mineral present in the soil and its capacity to retain nutrients against leaching (Landon, 1991). The vegetable growing soil samples were obtained very high CEC in range 32.26±0.53-46.70±2.49 cmol (+)/kg soil indicating its very high capacity to retain the cation. According to Metson (1961), the CEC were under very high (> 40 cmol (+)/ Kg, high (25-40 cmol (+)/ Kg), moderate and (12-25 cmol (+) /Kg) ranges Generally, CEC is derived from the clay and OM fractions (Landon, 1991) and can be affected by the different soil management practices such as cultivation, pesticide, fertilization and irrigation (Gao and Chang, 1996).

The texture class was also determined using the ‘textured triangular diagram. Soil suspension at a given depth becomes less as the particle settles. Its value at different time is related empirically to particle size, so that, by selection of times, a density can be a measure of sand, clay and silt. As indicated in Table 5, soil texture was similar for all samples. The particle size distribution of the soil showed that the soil contained higher composition of clay than silt and sand in all soil samples. Trace metals have preferential accumulation in the clay and silt fractions of soil. Generally, the concentrations of heavy metal in soil increase with decrease in the sizes of the soil particles (Inobeme *et al*., 2014).

4.1.2. Physico-Chemical Analysis of Wastewater Samples

Assessment and control of irrigation water quality depend upon physico-chemical properties like pH and electrical conductivity (EC) these are used to identify and quantify toxicants and to provide data that, for regulatory purposes, could be compared to allowable concentrations for particular recipient water (Pascoe, 1987). The concepts of water quality criteria are the basis for any kind of water pollution control for the characterization of water samples. The results of the physicochemical parameters of the wastewater samples investigated in the present study are presented in Table 6. The pH values of the wastewater samples from three sampling points (N1, N2 and N3) were 6.83±0.02, 6.95±0.03 and 7.04±0.02, respectively. The

pH of the wastewater samples were found to be in the recommended limits set by FAO (1985) and WWF (2007). The electrical conductivities (EC) of wastewater samples from the three sampling points were 675.33±9.02, 713.00±1.00 and 757.33±2.08 µS/cm, respectively. Electrical conductivities of wastewaters values collected from EIZ in N2 and N3 were above the recommended limit set by AFO (1985), but below the value set by critical limits as described by WWF (2007) for Pakistan. Water with high EC affects the soil structure, permeability and irrigation quality. Conductivity is measured to establish the pollution zone around an effluent discharge (Tessema *et al*., 2015). The minimum electrical conductivity of an effluent recorded in a similar study was found to be 3101 µS/cm and the maximum was 4630 µS/cm (Tessema *et al*., 2015).

Table 6. Selected physico-chemical property of wastewater samples from Eastern Industry Zone effluent (mean ± SD).

Parameter	**Wastewater type**					
	N1	**N2**	**N3**	**LSD (0.05)**	**FAO (1985)**	**WWF (2007)**
pH	6.83 ± 0.02^{c}	6.95 ± 0.03^{b}	7.04 ± 0.02^{a}	0.06	6.5-8.4	6.5-8.4
EC µS/cm	675.33 ± 9.02^{c}	713.00 ± 1.00^{b}	757.33 ± 2.08^{a}	11.66	700	1500

N.B: Where: N1, N2 and N3 refer to three sampling point 0, 200 and 500 m away from the discharge point, respectively. Values are given as means of triplicates ± SD. The means in the same row having different superscript letters are significantly different from each other at 5% confidence interval.

4.2. Method Validation

The procedures used in the current study were validated by spiking method and the percent recovery values are given in Table 7 for soil samples in wet-digestion method Tables 8, 9 and 10 for tomato, cabbage and lettuce samples, respectively and Table 11 for wastewater sample. Because of the absence of reference materials in the laboratory to check the efficiency of the analytical procedure percent recovery was calculated after small and known amount of the heavy metals from the stock solution were added or spiked in to the soil, vegetable and wastewater samples to be digested and concentration of both spiked and non-spiked samples were read. Results were obtained through calculation. Method validation is the process of

providing that a given analytical method is acceptable for its intended purpose (Duan *et al*., 2003). As shown in Tables 7, the percentage recovery for the soil samples lie in the range 93.33-120.83% which are within the acceptable range for all metals. As shown in Tables 8, 9 and 10, the percentage recoveries of vegetable samples lie in the range of 86.67-105 %, which is within acceptable range for all metals. Finally, for wastewater samples, the recoveries obtained varied from 90.67-105.70% as shown in Table 11. The obtained results are in acceptable range which is not less than 70% and no greater than 125% according to Duan *et al*. (2003) and which revealed that the digestion method and the FAAS analysis utilized in the present study were reliable.

Table 7. Values of the recovery analysis (X ± SD, n = 3) for soil sample

Heavy Metal	**Concentration before spiking (M± SDs) (ppm)**	**Concentration after spiking (M± SDs) (ppm)**	**Amount added (ppm)**	**%Recovery**
Cr	21.35±0.31	23.40±0.46	2.00	101.67
Cd	25.93±0.61	27.80±0.78	2.00	93.33
Zn	67.60±0.70	69.47±0.49	2.00	93.33
Fe	6965.00±31.74	6967.42±30.13	2.00	120.83
Pb	18.50±0.17	20.53±0.25	2.00	101.67
Cu	66.87±2.20	69.03±2.22	2.00	108.33

Table 8. Values of the recovery analysis (% R ± SD, n = 3) for tomato sample

Heavy Metal	**Concentration before spiking (M± SDs) (ppm)**	**Concentration after spiking (M± SDs) (ppm)**	**Amount added (ppm)**	**%Recovery**
Cr	2.97±0.21	3.16±0.23	0.2	96.67
Cd	2.21±0.12	2.39±0.60	0.2	93.33
Zn	45.63±4.37	45.81±4.38	0.2	90.00
Fe	361.50±13.70	361.67±13.71	0.2	86.67
Pb	4.70±0.16	8.89±0.15	0.2	95.00
Cu	10.14±0.33	10.35±0.35	0.2	105.00

Table 9. Values of the recovery analysis (% R ± SD, n = 3) for cabbage sample

Heavy Metal	Concentration before spiking (M± SDs) (ppm)	Concentration after spiking (M± SDs) (ppm)	Amount added (ppm)	%Recovery
Cr	2.90±0.10	3.10±0.12	0.20	100.00
Cd	3.20±0.10	3.40±0.10	0.20	98.00
Zn	51.53±0.60	51.73±0.62	0.20	100.00
Fe	593.33±5.86	593.53±5.87	0.20	100.17
Pb	5.26±0.21	5.45±0.20	0.20	98.33
Cu	11.72±0.24	11.90±0.24	0.20	93.67

Table 10. Values of the recovery analysis (% R ± SD, n = 3) for lettuce sample

Heavy Metal	Concentration before spiking (M± SDs) (ppm)	Concentration after spiking (M± SDs) (ppm)	Amount added (ppm)	%Recovery
Cr	3.43±0.64	3.64±0.62	0.20	100.00
Cd	3.69±0.02	3.90±0.02	0.20	96.67
Zn	62.46±1.43	62.64±1.43	0.20	90.00
Fe	557.33±8.62	557.54±8.60	0.20	105.00
Pb	5.54±0.45	15.72±0.47	0.20	91.67
Cu	15.11±0.18	15.29±0.20	0.20	90.00

Table 11. Values of the recovery analysis (% R ± SD, n = 3) for wastewater sample

Heavy Metal	Concentration before spiking (M± SDs) (ppm)	Concentration after spiking (M± SDs) (ppm)	Amount added (ppm)	%Recovery
Cr	0.80±0.00	1.01±0.01	0.20	104.67
Cd	0.07±0.00	0.28±0.02	0.20	102.30
Zn	0.10±0.00	0.30±0.01	0.20	93.67
Fe	3.50±0.01	3.69±0.01	0.20	96.17
Pb	2.20±0.05	2.41±0.06	0.20	105.70
Cu	0.56±0.02	0.74±0.02	0.20	90.67

4.3. Evaluation of Analytical Data

Errors in analytical results are most often expressed using accuracy and precision. The precision of an analytical procedure expresses the closeness or agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions whereas the accuracy of an analytical procedure expresses the closeness of measurements to the true value. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements (Miller and Miller, 2005; Meseret, 2013).

In the current study the precision of the results were evaluated by the standard deviation, and relative standard deviation of the results of three replicate measurements (n = 3). These parameters are useful in estimating and reporting the probable size of indeterminate error. The results of the present analysis are reported with the corresponding standard deviation. Tables 12 for soil wet digestion results, Table 13 for vegetable results, Table 15 for wastewater results and from Tables 16, 17, 18, 19, 20 and 21 for soil sequential extraction results show standard deviation (SD) of each metal for each vegetable, wastewater and soil samples.

4.4. Levels of Heavy Metals in Soil Samples

The concentrations of Cr, Cd, Zn, Pb, Fe and Cu in the digested samples of soil were determined by FAAS. The concentrations of these metals are presented with their respective SD in Table 12 in all types of soil samples iron were much higher than other.

As shown in Table 12, the recorded results of accumulated heavy metals in soil showed that zinc, iron, lead and copper showed relatively higher values for lands irrigated with wastewater around the Eastern Industry Zone compared to other heavy metals. This indicates that the wastewater might contain more sources of these metals.

In generally, heavy metals like, Lead (Pb), and cadmium (Cd) have no beneficial effects in humans, and there is no known homeostasis mechanism for them (Vieira *et al*., 2011). They are generally considered the most toxic to humans and animals; the adverse human health

effects associated with exposure to them, even at low concentrations, are diverse and include, but are not limited to, neurotoxic and carcinogenic actions (Jomova and Valko, 2010).

The metals considered in this study include the metals which are micro-nutrient such as iron, zinc and copper and the non-essential/toxic heavy metal which are toxic to plant when present in the soil at concentrations above tolerance levels for the non-essential metals, Cr, Pb and Cd are recognized as health hazardous and all have caused major health problems as a result of environmental pollution (Vieira *et al*., 2011).

Table 12. Mean concentration of Cr, Cd, Zn, Fe, Pb and Cu of soil sample in wet digestion method (n = 3, ± SD mg/kg)

Sample	**Heavy metals**					
Code	**Cr**	**Cd**	**Zn**	**Fe**	**Pb**	**Cu**
ST	50.50±0.53^{c}	45.33±1.53^{a}	114.86±10.33^{ab}	20065±149.64^{a}	63.00±2.26^{a}	146.10±3.08^{a}
SC	66.30±2.46^{a}	42.33±0.58^{b}	108.44±8.52^{b}	18318±60.39^{b}	64.87±0.45^{a}	142.77±3.23^{ab}
SL	62.23±2.35^{b}	45.00±1.00^{a}	123.77±7.71^{a}	12051±4.65^{c}	63.33±3.58^{a}	140.33±2.01^{b}
C	22.37±0.31^{d}	27.93±0.61^{c}	69.37±2.00^{c}	7140.00±133.32^{d}	18.82±0.08^{b}	68.47±1.10^{c}
LSD	3.16	1.83	12.23	212.03	4.07	4.37
FAO/WHO	50	3	300	5000	100	100
USEPA, 2002	150	3	300	-	300	140
EU, 2002	-	3	200	-	300	50

FAO/WHO (2001) values are given as means of triplicates ± SD. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

4.4.1. Chromium in Soil Samples

Chromium plays a vital role in the metabolism of cholesterol, fat, and glucose. Its deficiency causes hyperglycemia, elevated body fat, and decreased sperm count, while at high concentration it is toxic and carcinogenic (Chishti *et al*., 2011). As shown in Table 12 above, the Cr contents in the soil samples were found to be within the range of 50.50±0.53 and 66.30±2.46 mg/kg. The highest and lowest contents of Cr occurred in the soils of cabbage and tomato, respectively. The WHO/FAO (2001) permissible limit of chromium in soil is 50 mg/kg. So, the concentration of chromium found in the three soil samples from lands irrigated with wastewater around the Eastern Industry Zone might be harmful for human health. Major sources of Cr contamination include releases from electroplating processes and the disposal of Cr containing wastes (Smith *et al*., 1995).

Comparison of chromium level in the soil samples with that of the control soil sample (22.37±0.31 mg/kg) indicates that the higher levels found in all samples could possibly be attributed to the wastewater discharged from the industry zone. In similar study, Meseret (2013) reported that Cr concentration in soil 20.83-104.83 mg/kg. Also, Deribachew *et al*. (2015) reported 20.71-41.45 mg/kg. The results obtained in all vegetable soil samples indicate that CEC and organic carbon recorded high values, indicating high metal retention capabilities and this could account for the high heavy metal concentration in the soil samples (Divine, 2014).

4.4.2. Cadmium in Soil Samples

Cadmium is also a non-essential heavy metal. It is extremely toxic even at low concentration. It causes learning disabilities and hyperactivity in children (Hunt, 2003). As shown in Table 12, the experimental results showed that Cd concentration in soil samples occurred in the range of 42.33±0.58 and 45.33±1.53 mg/kg. The tomato soil observed to have the highest level (45.33±1.53 mg/kg) of Cd, while the cabbage originated soil had the smaller level (42.33±0.58 mg/kg) of Cd. Being a non-essential metal, it can be considered very toxic. In similar study, Meseret (2013) reported that the range of Cd concentration (mg/kg) in soil samples as 2.82-4.77 mg/kg, Deribachew *et al*. (2015) reported for the same as 0.79- 412.16 mg/kg. WHO/FAO (2001) permissible limit of cadmium in soil is 3 mg/kg. So, the concentration of

cadmium found in the three vegetables growing soil samples from lands irrigated with wastewater around the Eastern Industry might be harmful for human health. Comparison of cadmium level in the soil samples with that of the control soil sample (27.93±0.61 mg/kg) indicates that the higher levels found in all samples could possibly be attributed to the waste water discharged from the industry zone.

4.4.3. Zinc in Soil Samples

The natural range of zinc in soils is 10 – 300 mg/kg (Eddy *et al*., 2006). It is the basic component of a large number of different enzymes and plays structural, regulatory, and catalytic functions. It also has very important role in DNA synthesis, normal growth, brain development, bone formation, and wound healing. At high level, Zinc is neurotoxin (Adelekan and Abegunde, 2011). As shown in Table 12 above, the soil concentration of zinc in this study was within these natural ranges with values ranging between 108.44±8.52 mg/kg to 123.77±7.71 mg/kg. In the similar, Milkessa (2013) reported the concentration of zinc in soil samples range between 60.09-414.12 mg/kg, with those for uncontaminated soil earlier similar works by McGrath *et al*. (2001) and Kimani (2007) as 200 mg/kg and 133 mg/kg, respectively. Awokunmi *et al*. (2010) reported zinc levels in soils higher than those obtained in this study ranging between 350-3052 mg/Kg. The soil of lettuce had the highest contents (123.77±7.71 mg/kg) of Zn, while the soil of cabbage had the smaller concentration (108.44 mg/kg) of Zn. The WHO/FAO permissible limit of zinc in soil is 300 mg/kg. So, the concentration of zinc we obtained now is found to be below the permissible limit set by WHO/FAO (2001).

Comparison of zinc levels in the soil samples with that of the control soil sample (69.37±2.00 mg/kg) indicates that the higher levels found in all samples could possibly be attributed to the wastewater discharged from the industry zone. This implies that all the soil samples have zinc comfortably below the acceptable range. According to Odukoya *et al*. (2000) zinc is required in human nutrient for normal functioning of the body. The deficiency of zinc in man can lead to impaired growth, low energy balance and low protein intake, while excessive intake of zinc from plants can lead to vomiting, dehydration, electrolyte imbalance, abdominal pain, and lack of muscular co-ordination.

4.4.4. Iron in Soil Samples

Iron is the most abundant and most essential constituent for all plants and animals. On the other hand, at high concentration, it causes tissues damage and some other diseases in humans. It is also responsible for anemia and neurodegenerative conditions in human being (Fuortes and Schenck, 2000). As shown in Table 12 above, the results indicate that soil samples contained Fe in the concentration range of 12051±4.65 and 20065±149.64 mg/kg. This is lower than the value of iron the content reported by McGrath *et al*. (2001) as 80000 mg/Kg for certain contaminated soil. But other studies indicated lower values of iron as compared to what we obtained this study. Akubugwo *et al*. (2012) who recorded an iron metal content in the soils ranging between 73.62 mg/kg to 226.39 mg/Kg. Similarly Tsafe *et al*. (2012) reported a value of 195.25 mg/kg in the soils studied.

The tomato soil is said to have the highest contents of iron (20065±149.64 mg/kg), while the soil under lettuce is seen to have contained the least level (12051±4.65 mg/kg) of Fe. The WHO/FAO (2001) permissible limit of iron in soil is 5000 mg/kg. So, the concentration of iron found in the three soil samples from lands irrigated with wastewater around the Eastern Industry Zone might be harmful for human health. Comparison of iron level in the soil samples with that of the control soil sample (7140.00±133.32 mg/kg) indicates that the higher levels obtained from all samples could possibly be attributed to the high levels of iron in the waste water discharged from the industry zone.

4.4.5. Lead in Soil Samples

Lead is one of the more persistent metals and is estimated to have a soil retention time of 150 to 5000 years (Sobolev and Begonia, 2008). It is a non-essential heavy metal. Pb causes oxidative stress and contributes to the pathogenesis of lead poisoning by disrupting the delicate antioxidant balance of the mammalian cells. High level accumulation of Pb in body causes anemia, colic, headache, brain damage, and central nervous system disorder (Rehman *et al*., 2013). As shown in Table 12, the soil samples contained Pb concentrations in the range of 63.00±2.26–64.87±0.45 mg/kg. The WHO/FAO (2001) permissible limit of lead in soil is 100 mg/kg. This is within ranges of soils studies by Premarathna *et al*. (2011) who reported a range of 15 to 311 mg/kg. However, Awokunmi *et al*. (2010) reported very high levels of lead

in soils collected from various dumpsites ranging between 3500-6860 mg/kg. Aluko *et al*. (2003) also reported high values of lead in soil ranging from 1340 - 1693 mg/kg.

Lead has been known to have harmful health effects even at lower levels and there is no known safe exposure level. It is appropriate to note that exposure to amount of lead above 0.01mg/kg is detrimental to health, as it may result in possible neurological damage to fetuses, abortion and other complications in children under three years (Asemave *et al*., 2012). So, the concentrations of lead found in all three soil samples collected from farmlands irrigated with wastewater around the Eastern Industry Zone might be harmful for human health. Comparison of lead levels in the soil samples with that of the control soil sample (18.82±0.08 mg/kg) indicates that the higher levels obtained from all samples could possibly be attributed to the high levels of lead in the wastewater discharged from the industry zone.

4.4.6. Copper in Soil Samples

Being an essential trace element, it is necessary for many enzymes. It is needed for the normal growth and development. High concentration of Cu causes metal fumes fever, hair and skin decolorations, dermatitis, respiratory tract diseases, and some other fatal diseases in human beings (Khan *et al*., 2008). Copper content was determined in three vegetable originated soil samples. All the tested samples contained the significant amount of Cu. As shown in Table 12 above, highest level (146.10 ±3.08 mg/kg) of Cu was found in tomato soil and the soil of lettuce had the smallest level (140.33±2.01 mg/Kg) of Cu. WHO/FAO (2001) permissible limit of lead in soil is 100 mg/kg. Also the concentration of copper was above the concentration permissible limit set by EU (2002); USEPA (2010) as shown in Table 12. So, the concentration of copper found in the three soil samples from farmlands irrigated with wastewater around the Eastern Industry Zone might be harmful for human health. Comparison of copper level in the soil samples with that of the control soil sample 68.47±1.10 mg/Kg) indicates that the higher levels obtained from all samples could possibly be attributed to the high levels of copper in the waste water discharged from the industry zone.

4.5. Heavy Metal Concentration in Vegetable Samples

Vegetables like cabbage (*Brassica oleracea* L.), lettuce (*Lactuca sativa* L.) and tomato (*Lycopersicon esculentum Miller*) were analyzed for total metals content. The level of heavy metals in vegetables varies by the ability of plants to selectively accumulate some of these elements. Bioavailability of the elements depends on the nature of their association with the constituents of a soil. Additional sources of these elements for plants are rainfall, atmospheric dusts, plant protection agents and fertilizers that can be absorbed through the leaf blades (Harris, 1982; Gezahegn, 2013). The concentrations of, Cr, Cd, Zn, Pb, Fe and Cu in sample of vegetables (cabbage, lettuce and tomato) that grown with wastewater discharges of factories around EIZ irrigation farm land were presented in Table 13. From the study, it is revealed that most of the metals were accumulated to greater or lesser extents in the vegetable samples with compared to WHO standard as shown below in Table 13. The vegetables are consumed by the urban population of the city of Dukem and cities present near Dukem like Addis Ababa, Debre Zeit, etc. thus exposing the population to dangerous levels of heavy metals. The results presented demonstrate that there is a risk associated with consumption of vegetables grown on these irrigation land farm, with the vegetable still looking apparently healthy and growing well despite accumulating heavy metals to concentrations which substantially exceed maximum values considered safe for human consumption.

Table 13. Mean concentration of Cr, Cd, Zn Fe, Pb and Cu of vegetable samples in wet digestion method (means ± SD mg/kg), n=3

Vegetable	**Heavy metals**					
Type	**Cr**	**Cd**	**Zn**	**Fe**	**Pb**	**Cu**
Tomato	2.97±0.21[b]	2.20±0.10[c]	45.63±4.37[b]	358.17±3.33[c]	4.60±0.10[b]	10.20±0.40[c]
Cabbage	2.90±0.10[b]	3.20±0.10[b]	51.53±0.60[b]	571.33±13.50[b]	5.47±0.35[b]	11.87±0.31[b]
Lettuce	3.77±0.12[a]	3.68±0.06[a]	62.46±1.43[a]	547.17±8.00[a]	5.50±0.40[a]	15.07±0.31[a]
LSD	0.20	0.22	6.73	12.03	0.87	0.92
WHO (1999)	1.2	0.2	1.5	150	0.5	2.0
CMH (2005)	0.5-1.0	0.05-0.2	-	-	0.1-0.3	-
FAO (1985)	-	0.01	2.00	-	5.00	0.20

The results of this study, heavy metal concentrations in vegetable samples were compared with WHO permissible values Source, WHO (1999), CMH: Chinese Ministry of Health. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval. The concentrations of heavy metals in the vegetables in the study area of the present research work were found to be much above the permissible limits given by WHO (1999); CMH (2005); FAO (1985). Food chain contamination by heavy metals has become a burning issue in past years because of their potential accumulation in biosystems through contaminated water, soil and air (Dogheim *et al*. 2004).

4.5.1. Distribution of Chromium in Vegetables

Exposure of human to chromium may occur through breathing, drinking, or eating food containing chromium or even through skin contact. Exposure to elevated levels chromium leads to skin irritation, ulceration, damage to circulatory and nerve tissues which cause health problems. However, daily uptake of it within a certain range of concentrations (up to 200 μg/day) by human beings and animals is considered to be essential for carbohydrate and lipid metabolism (Girmaye, 2012). In this study the chromium contents in vegetable samples were obtained to have ranged from 2.90±0.10-3.77±0.12 mg/kg and these, result were higher than permissibility level set by WHO (1999) is 1.2 mg/Kg as shown in Table 13. In the similar study Deribachew *et al*. (2015) reported cabbage had generally the highest concentrations of Cr (12-14 mg/kg) at Haramaya University vegetable farm when compared with the present study. In line to these results reported by Edema *et al*. (2009) stated that the highest concentration of chromium was found in leaves of vegetables. Banerjee *et al*. (2011) in his part stated that Cr content in brinjal (Solanum melongena) and Indian spinach was exceeding the safe value.

4.5.2. Distribution of Cadmium in Vegetables

The vegetable samples collected around EIZ irrigation farmlands contained Cd concentrations in the range of 2.20±0.10-3.68±0.12 mg/kg as shown in Table 13. The concentration of Cd was maximum (3.68±0.12 mg/kg) in lettuce sample and the minimum (2.20±0.10) was found in the tomato sample. According to WHO/FAO (1999) permissible level is 0.2 mg/kg. The results obtained from this study compared with studies done by Deribachew *et al*. (2015) the

amount of cadmium in cabbage samples was 1.2-2.5. In line with this result reported by Prabu (2009) found that the Cd accumulation was more in leafy vegetables such as lettuce, Swiss chard, spinach and radish (Raphanus sativus) when compared with the present study. Similarly Jamali *et al*. (2007) reported that the concentration of Cd in vegetables grown from domestic wastewater was ranging between 0.14 mg/kg spinach and 0.30 mg/ kg brinjal (Solanum melongena) on a dry weight basis.

The high concentration of Cd in the vegetables might be due to the use of untreated industrial effluent. Applications of untreated industrial effluent build up concentration of metal into the soil (Chary *et al*., 2008). From the soil, metals can transfer to the vegetables and accumulate in the tissues of vegetables. Several compounds of Cadmium are used in chemical industries and in the manufacture of pesticides, herbicides used in agriculture (Ogundele *et al*., 2015). Cd is more soluble as compared to other metals so, it can accumulate more into the vegetables tissues (Farid *et al*., 2015). The permissible limits of Cd is (0.20 mg/Kg) in the plant tissue proposed by WHO (1999). The efficiency of plants to absorb metals can be evaluated by their ability of metal uptake or soil to plant transfer factor (Farid *et al*., 2015).

4.5.3. Distribution of Zinc in Vegetables

In this study, results show that the levels of zinc in the vegetables studied had a range of 45.63±4.37-62.46±1.43 mg/kg and WHO (1999) permissible limit is 1.50 mg/kg (Table 13). All the ventures exhibited very high concentration compared to the permissible limit set by WHO (1999); CMH (2005); FAO (1985). The concentration of Zn in vegetables was found to be in the order of Lettuce > Cabbage > Tomato. The high concentration of Zn and other trace heavy metals present in the parts of the vegetables may be due to the absorption ability of the plants to get the trace heavy metals from the polluted soils.

A similar study that is conducted by Muhammad *et al*. (2008) reported that the zinc concentrations in lettuce was 1.893 mg/kg and in cabbage was 0.678 mg/Kg samples by which super passed the maximum permissible level of Zn set by WHO (1999). Also this study was higher than compared with studies reported by Akubugwo *et al*. (2012) on Amaranthus hybridus vegetables which reported values of zinc of 1.06 to 2.82 mg/kg. Zn is used in many factories because of their heat conducting properties and as such released during mechanical

abrasion of vehicles, and from engine oil combustion and tyres of motor vehicle (Ogundele *et al*., 2015). The concentration may be as a result of the number of factories emissions realized to the irrigation farm land.

4.5.4. Distribution of Iron in Vegetables

In this study, Fe concentration from the plants sites varied between 358.17±3.33-571.33±13.50 mg/kg and WHO (1999) permissible limit is 150 mg/kg (Table 13). Akubugwo *et al*. (2012) reported an even higher iron metal content of up to 147.41 mg/Kg in the Amaranthus hybridus vegetables. The concentration of Fe was almost all the ventures exhibited very high concentration compared to its permissible limit. By this way, the concentration of iron in vegetables was found to be in the order of Cabbage > Lettuce > Tomato. The high concentration of Fe and the other trace heavy metals present in the parts of the plants may be due to the absorption ability of the plants to get the trace heavy metals from the polluted soils. Iron as an essential element for all plants has many important biological roles in the processes as diverse as photosynthesis, chloroplast development and chlorophyll biosynthesis (Marschner 1995). In humans, increased body stores of iron have been shown to increase the risk of several estrogen-induced cancers (Liehr and Jones, 2001).

4.5.5. Distribution of Lead in Vegetables

Results show that the levels of lead in the vegetables studied had a range of 4.60±0.10 to 5.50±0.40 mg/kg as shown in Table 13. The results obtained from this study compared with studies done by Deribachew *et al*. (2015) the amount of lead in cabbage samples was 5.5-12. A similar study that is conducted by Girmaye (2012) reported that lead concentrations of lettuce ranged from 2.3-5.30 mg/kg sample by which super passed the maximum permissible level of Pb set by WHO (1999); CMH (2005); FAO (1985). In this study, the high level of Pb in lettuce suggests that the wastewater used for irrigation was not good for irrigation of crops in general and leafy vegetables in particular. Data showed that in all vegetables, lead concentration is more than permitted level, so they are not suitable for consumption.

Lead is a toxic element that can be harmful to plants, although plants usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield. In

many plants, Pb accumulation can exceed several hundred times the threshold of maximum level permissible for human (Bigdeli and Seilsepour, 2008). In leafy vegetables the accumulation of airborne lead largely exceeds the soil borne part taken up via roots. Air borne lead is mainly accumulated at the leaf surface and can be removed to a larger extent by washing of the vegetables (Tyagi, 2014).

4.5.6. Distribution of Copper in Vegetables

Copper is the third most used metal in the world. It is an essential micronutrient required in the growth of both plants and animals. In humans, it helps in the production of blood haemoglobin. In plants, Cu is especially important in seed production, disease resistance, and regulation of water. Copper is indeed essential, but in high doses it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation (Wuana and Okieimen, 2011).

In this study, Cu concentration from the vegetable sites varied between 10.20±0.40-15.07±0.31 mg/kg and WHO (1999) permissible limit is 2.0 mg/kg (Table 13). The concentration of Cu in the study vegetables were ventures exhibited high concentration compared to its permissible limit set by WHO (1999); CMH (2005); FAO (1985). The concentration of copper in vegetables was found to be in the order of Lettuce > Cabbage > Tomato. The high concentration of Cu present in the parts of the plants may be due to the absorption ability of the plants to get the trace heavy metals from the polluted soils. Cu is especially important in seed production, disease resistance, and regulation of water. Copper is indeed essential, but in high doses it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation (Martinez and Motto, 2000).

4.5.7. Comparison of Heavy Metals Concentration from the Current Study with those Reported on the Literature

The heavy metal (Cr, Cd, Zn Fe, Pb, and Cu) levels in vegetables samples (tomato, cabbage and lettuce) from‘fields irrigated with the Eastern Industry Zone were compared with different literature reported in table 14 below.

Table 14: Comparison of metal concentration in the vegetables with other reports in similar studies

Vegetable	Source of Heavy metals	Heavy metals in mg/kg						Reference
		Cr	Cd	Zn	Fe	Pb	Cu	
Tomato	Industrial effluents	2.97	2.20	45.63	358.17	4.60	10.20	resent study
	Agricultural activities	0.34	0.11	-	-	5.25	201.75	Liu *et al*., 2006
	Wastewater	-	0.20	3.80	-	5.50	0.05	Mohod, 2015
	Wastewater	0.33	0.03	4.97	118.40	4.40	3.68	Khan *et al*., 2011
	Swage water	2.12	13.56	-	-	6.80		Perveen *et al*., 2012
Cabbage	Industrial effluents	2.90	3.20	51.53	571.33	5.47	11.87	Present study
	Swage water	1.20	16.71	-	-	48.00	-	Perveen *et al*., 2012
	Wastewater	0.57	0.22	-	-	0.31		Girmaye, 2012
	Wastewater	0.38	0.26	1.38	12.84	2.24		Khan *et al*., 2015
	Transport & Market	16.28	6.17	-	310.50	22.76		Dingkwoet *et al*., 2013
	Tannery effluent	0.43	0.18	-	-	3.82		Gebrekidan *et al*., 2013
Lettuce	Industrial effluents	3.77	3.68	62.46	547.17	5.50	15.07	Present study
	Wastewater	1.86	0.36	-	-	0.53	-	Girmaye, 2012
	Swage water	2.20	15.25	-	-	2.20	-	Perveen *et al*., 2012
	Wastewater	0.41	0.51	0.84	13.20	1.52	-	Khan *et al*., 2015
	Transport & Market	11.07	-	-	584.90	37.81	-	Dingkwoet *et al*., 2013
	Tannery effluent	0.32	0.30	-	-	1.55	-	Gebrekidan *et al*., 2013

4.6. Heavy Metal Transfer Factor (TF) from Soil to Vegetables

The transfer coefficient quantifies the relative differences in bioavailability of metals to plants and is a function of both soil and plant properties. The coefficient is calculated by dividing the concentration of a given metal in a vegetable crop by the total metal concentration in the soil. Higher transfer coefficient represents relatively poor retention in soils or greater efficiency of plants to absorb metals. Low coefficient demonstrates the strong sorption of metals to the soil colloids (Coutate, 1992). Soil-to-plant transfer is one of the key components of human exposure to metals through food chain. Transfer Factor (TF) or Plant Concentration Factor

(PCF) is a parameter used to describe the transfer of trace elements from soil to plant body and it is also is a function of both soil and vegetables properties. The transfer coefficient is therefore calculated by dividing the concentration of heavy metals in vegetables by the total heavy metal concentration in the soil (Tasrina *et al*., 2015).

$$\mathbf{TF} = \frac{CMV}{CMS} \qquad (5)$$

Where, CMV = Concentration of metal in edible part of vegetable and CMS = Concentration of metal in soil.

In the present study, the TF of different heavy metal from soil to vegetable are presented in Table 15. Higher transfer factors reflect relatively poor retention in soils or greater efficiency of vegetables to absorbs metals. Low transfer factor reflects the strong sorption of metals to the soil colloids (Wierzbicka, 1995). The TF or PCF value ranges were: Cr (0.04 to 0.06), Cd (0.05 to 0.08), Zn (0.40 to 0.50), Fe (0.02 to 0.05) , Pb (0.07 to 0.09) and Cu (0.07 to 0.11) and the trend of TF for heavy metal in vegetable samples investigated are in order: Zn > Cu > Pb > Cd > Cr > Fe.

The mobility of metals from soil to plants is a function of the physical and chemical properties of the soil and of vegetable species, and is altered by innumerable environmental and human factors (Alloway and Ayres, 1997; Tasrina *et al*., 2015). The highest TF values were found to be 0.50 and 0.11 for Zn and Cu respectively. These might be due to higher mobility of these heavy metals with a natural occurrence in soil and the low retention of them in the soil than other toxic cations (Alloway and Ayres, 1997; Tasrina *et al*., 2015). According to the soil to plant transfer factor (TF) calculated for tested metals and leafy vegetables consumed by the local residents, it can be concluded that Cu and Zn are high accumulator among the investigated metals. However, the higher concentrations of these heavy metals are due to the waste water irrigation, solid waste combustion, agrochemicals and vehicular exhausts.

Table 15. Transfer factors (TF) for heavy metals from soil to vegetable

Vegetable Types	Heavy metals					
	Cr	Cd	Zn	Fe	Pb	Cu
Tomato	0.06	0.05	0.40	0.02	0.07	0.07
Cabbage	0.04	0.08	0.48	0.03	0.08	0.08
Lettuce	0.06	0.08	0.50	0.05	0.09	0.11

4.7. Comparison of Metals in the Plants and Soil Samples

Mostly, the concentrations of essential and non-essential metals are higher in soils than vegetables grown on the same soils. This indicates that only a small portion of soil metals is transferred to the vegetables and the root acts as a barrier to the translocation of heavy metals within plant (Davies and White, 1981). The concentrations of metals in the vegetables and their corresponding soil samples are given in Table 16 for the study sites. The concentrations of all essential and non-essential heavy metals were found to be higher in the soil samples than in the vegetables. This may reveal that the main source of metal contents of vegetables is from their corresponding soil content which might be affected by industrial effluent, the environmental interferences like pesticides, fertilizers and other additives that farmers use. Variations in transfer factor among the different vegetables may be attributed to differences in the concentration of metals in the soil and differences in element uptake by different vegetables (Deribachew *et al*., 2015).

Table 16. Heavy metals concentration comparison in the vegetables and their corresponding soil samples of the vegetables origin in mg/Kg

Code	Cr_v	Cr_s	Cd_v	Cd_s	Zn_v	Zn_s	Fe_v	Fe_s	Pb_v	Pb_s	Cu_v	Cu_s
T	2.97	50.50	2.20	45.33	45.63	114.86	358.17	20065	4.60	63.00	10.20	146.10
C	2.90	66.30	3.20	42.33	51.53	108.44	571.33	18318	5.47	64.87	11.87	142.77
L	3.77	62.23	3.68	45.00	62.46	123.77	547.17	12051	5.50	63.33	15.07	140.33

Where: V = Vegetable, S = Soil, T = Tomato, C= Cabbage and L = Lettuce

4.8. Level of Heavy Metals in Wastewater Samples

The distribution of heavy metals (Cr, Cd, Zn, Pb, Fe and Cu) in wastewater samples from the Eastern Industry Zone has been evaluated. Wastewater samples were collected from the discharge point, 200 and 500 m away from point of discharge and taken to the laboratory for chemical analysis. As shown in Table 17, the results obtained revealed that the concentration of the heavy metals were found to be in the order of Fe> Pb> Cr>Cu> Zn>Cd. Moreover the concentration of each heavy metal was higher than the permissible limit set by WHO (2006) which could pose a huge threat to human health and the natural environment.

Table 17. Mean concentration of Cr, Cd, Zn, Fe, Pb and Cu in mg/L of wastewater samples from Easter Industry Zone (n = 3, mean ± SD)

Wastewater	**Heavy metals in mg/L**					
Samples type	**Cr**	**Cd**	**Zn**	**Fe**	**Pb**	**Cu**
N1	1.04 ± 0.00^{a}	0.08 ± 0.00^{a}	0.21 ± 0.00^{a}	5.13 ± 0.04^{a}	3.11 ± 0.04^{a}	0.99 ± 0.06^{a}
N2	0.83 ± 0.00^{b}	0.07 ± 0.00^{b}	0.10 ± 0.00^{b}	3.62 ± 0.04^{b}	2.19 ± 0.05^{b}	0.56 ± 0.02^{b}
N3	0.2 ± 0.00^{c}	0.04 ± 0.00^{c}	0.07 ± 0.00^{b}	2.89 ± 0.06^{c}	1.98 ± 0.04^{c}	0.30 ± 0.00^{c}
LSD	0.01	0.01	0.03	0.12	0.11	0.07
WHO (2006)	0.10	0.003	0.03	0.30	0.05	0.01
USEPA(2010)	0.10	0.005	2.00	-	0.015	1.00

N.B: The mean values in the same column having different superscript letters are significantly different from each other at 5% confidence interval. The concentration of metals were determined at point of 0 m (N1), 200 m (N2) and 500 m (N3) from point of discharge.

4.8.1. Concentration of Chromium (Cr) in Wastewater Samples

The concentrations of chromium in the wastewater samples at N1, N2 and N3 were 1.04±0.00, 0.83±0.00 and 0.2±0.00 mg/L, respectively. These values are above the permissible limit set by WHO (2006 and USEPA (2010) (Table 17) for crop irrigation. Chromium a toxic pollutant due to its harmful effects on human health, especially in its hexavalent form (Abagale *et al*.,

2013). A remedy must be sought for Cr levels in the effluent of industries where the metal is high in effluent.

4.8.2. Concentration of Cadmium (Cd) in the Wastewater Samples

The results show that the Cd concentrations in the wastewater samples at N1, N2 and N3 was 0.08±0.00, 0.07±0.00 and 0.04±0.00 mg/L, respectively. The sample at N1 recorded the highest concentration than that of samples at N2 and N3 (Table 17). The concentrations of Cd obtained in the present study are above the permissible limit set by WHO (2006); USEPA (2010) for crop irrigation. Cadmium is considered to be hazardous metal because of its toxicity and accumulation capacity in the living system (Singh *et al*., 2005). Cadmium used in industry finds its way into many water supplies. Old galvanized pipes and new plastic (PVC) pipes are sources of cadmium in water pump (Tyagi, 2014).

4.8.3. Concentration of Zinc (Zn) in the Wastewater Samples

The other heavy metal determined in the wastewater sample was Zn. Its concentrations in the samples at N1, N2 and N3 were 0.158±0.00, 0.132±0.00 and 0.249±0.00 mg/L, respectively. The sample at N1 recorded the highest concentration than that of samples at N2 and N3 (Table 17), and all the samples exceeded the recommended maximum concentration for crop irrigation (WHO, 2006). But the concentration of Zn was below the permissible limit set by (USEPA, 2010). Zn is the least toxic and is an essential element in the human diet as it is required to maintain the proper functioning of the immune system, normal brain activity and is fundamental in the growth and development of the foetus, but a very high concentration of zinc is very toxic, hence harmful to the human body (Helen and Othman, 2014).

4.8.4. Concentration of Iron (Fe) in Wastewater Samples

Concentrations of iron in the wastewater samples at N1, N2 and N3 were 2.89±0.04, 3.62±0.04 and 5.13±0.06 mg/L, respectively. Iron concentrations in three samples were above the recommended maximum concentration (RMC) for irrigation (WHO, 2006) and (USEPA (2010) (Table 17). The concentration of iron can therefore be said to be high for crop irrigation purposes in the area. The highest concentration was recorded in sample at the point of

discharge. According to Ayers and Westcot (1985), high concentration of iron in wastewater contributes to soil acidification and loss of availability of phosphorus and molybdenum when applied to the soil. Wastewater containing high concentration of Fe can increase soil acidity and diminish phosphorous in soil (Abagale *et al*., 2013).

4.8.5. Concentration of Lead (Pb) in Wastewater Samples

Lead has been used widely for application in metal products, cables and pipelines, as well as paints and pesticides. Lead is one of the metals that have the most damaging effects on human health (Bainies, 1999). Concentrations of lead in the wastewater samples at N1, N2 and N3 were 3.11±0.04, 2.19±0.05 and 1.98±0.04 mg/L, respectively. The lead value were of to be higher than recommended limit of Pb for irrigation water set by WHO (2006) and USEPA (2010) (Table 17). This makes the water unsuitable for human consumption as Pb is known to be toxic even at low levels with resultant ill-health effects as chronic exposure has been linked to growth retardation in children (Muiruri, 2009). Significant difference was evident from all the sampling point, with sampling point at the point of discharge recording the highest Pb mean value of 3.11±0.04 mg/L which can be attributed due to the untreated industrial effluent. A generally decrease in the mean concentration of Pb from point of discharge to 500 meter away from point of discharge may be due to settlement effect.

4.8.6. Concentration of Copper (Cu) in the Wastewater Samples

As shown in Table 17, the concentrations of Cu in wastewater samples were found in the range of 0.3±0.00-0.99±0.06 mg/L. these values were found to be higher than recommended limit of Cu for irrigation water set by WHO (2006) and lower than the recommended level set by USEPA (2010) (Table 17). This could be attributed to the reason of anthropogenic activities and industrial effluent released without treatment. In general, the concentration of copper was above the permissible level in three the wastewater samples of EIZ effluent. Significant difference was evident from all the sampling points. Generally a decrease in the mean concentration of Cu from the point of discharge to 500 meter away from point of discharge could be due to settlement effect.

4.9. Determination of the Concentrations of Selected Heavy Metals in the Five Chemical Fractions of Soils

Soil has long been regarded as a repository for society's wastes. Gradually mobilized by biogeochemical processes, soil contaminants can pollute water supplies and consequently enter the food chains. Heavy metals, such as Cr, Cd, Zn, Fe, Pb and Cu are all potential soil pollutants. Soils consist of heterogeneous mixtures of organic and inorganic solid components as well as a variety of soluble substances. Therefore, metal distribution among specific forms varies widely based on the metal's chemical properties and soil characteristics (Milkessa, 2012). Thus, it is important to evaluate the availability and mobility of heavy metals to establish environmental guidelines for potential toxic hazards and to understand chemical behavior and fate of heavy metal contaminants in soils (Milkessa, 2013).

The sequential extraction used in this study is useful to indirectly assess the potential mobility and bioavailability of heavy metals in the soils. The five chemical fractions are operationally defined by an extraction sequence that follows the order of decreasing solubility (Tessier *et al*., 1979).

Assuming that bioavailability is related to solubility, then metal bioavailability decreases in the order: exchangeable > carbonate > Fe-Mn Oxide > organic > residual. This order is just a generalization and offers only qualitative information about metal bioavailability. Based on the above information, one can further assume that metals in the nonresidual fractions are more bioavailable than metals associated with the residual fraction. The nonresidual fraction is the sum of all fractions except the residual fraction. The highest amounts of metal were concentrated in the residual fraction except for cadmium and lead (51.17-57.45%) and (79.62-83.78%) which were concentrated in the non-residual fraction respectively (Appendix Table 3). This indicates that metals were mostly associated with more stable soil fractions and should be less available to growing plants. The statistical analysis (ANOVA) performed on the results obtained from the sequential extraction procedure showed that metal concentrations in soil were significantly different ($P < 0.05$) from each other.

4.9.1. Chromium in Soil Fractionation

Table 18. Chemical fractionation of Cr (mg /kg) in soil samples from lands irrigated with wastewater around the EIZ (n = 3, ± SD mg/Kg)

Cr	F1	F2	F3	F4	F5	F1+F2+F3+F4+F5	Wet-digestion
ST	0.61±0.03[c]	0.26±0.02[c]	0.96±0.04[c]	2.81±0.03[b]	32.27±1.65[b]	36.86±1.	50.50±0.
	1.66%	0.71%	2.60%	7.60%	85.550%	73	53
SC	0.79±0.05[b]	0.44±0.02[a]	1.62±0.40[a]	4.23±0.23[a]	29.05±0.01[c]	36.13±0.	66.30±2.
	2.19%	1.22%	4.48%	11.71%	80.40%	71	46
SL	0.91±0.03[a]	0.37±0.03[b]	1.15±0.02[b]	4.46±0.05[a]	35.27±0.39[a]	42.52±0.	62.23±2.
	2.14%	0.87%	3.55%	10.49%	82.95%	52	35
C	0.19±0.01[d]	0.21±0.00[d]	0.21±0.00[d]	0.30±0.02[c]	20.35±0.09[d]	21.26±0.	22.37±0.
	0.89%	0.99%	0.99%	1.41%	95.72%	12	31
LSD	0.05	0.04	0.07	0.26	1.73		

N.B: Values are given as means of triplicates ± SD. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

The percentage distribution of chromium is in the range of 1.66 to 2.19 % in F1 (Soluble and exchangeable fraction), 0.71 to 1.22% in F2 (Carbonates bound fraction), 2.60 to 4.48 % in F3 (Fe-Mn Oxide bound), 7.60 to 11.71 % F4 (Organic matter bound fraction), 80.40 to 85.55% in the residual fraction (F5) (Table 18). The greater level of cadmium in the residual fraction probably indicates the greater tendency for chromium to become unavailable once it is in soils. Generally, this predominance of Cr in the last three fractions (Fe-Mn Oxide bound, organic matter and residual) suggests that it would be less available to plants. A metal in F1 and F2 (soluble and exchangeable and carbonate bound) fractions is the most mobile and is readily available for biological uptake by the plant (Gezahegn, 2013). The mobility and bioavailability of chromium in the samples is found to be in the order of control < tomato originated soil < cabbage originated soil < lettuce originated soil.

4.9.2. Cadmium in Soil Fractionation

Cadmium is very soluble and easily leached by rain water or swept by rain water especially when the soil pH is in the acidic range. Therefore its concentration was very low relative to concentration of Zn, Fe, Pb and Cu (Yoseph, 2015).

The percentage distribution of cadmium is in the range of 0.57 to 1.05 % in F1 (Soluble and exchangeable fraction), 0.34 to 0.89% in F2 (Carbonates bound fraction), 1.09 to 1.37 % in F3 (Fe-Mn Oxide bound), 48.79 to 48.88 % F4 (Organic matter bound fraction), 48.05 to 48.83% in the residual fraction (F5) (Table 19). Percentage of cadmium present in soil samples were F4 ~ F5 > F3 > F1 > F2 except for the control sample (F4 > F5 > F3 > F1 > F2). The greater level of cadmium in the organic matter bound fraction probably indicates the greater tendency for cadmium to become unavailable once it is in soils. The association of with organic fraction may be due to high formation of organic- complexes chiefly from agrochemicals (Olajire *et al*., 2003). The mobility and bioavailability of cadmium in the samples is found to be in the order of tomato originated soil > cabbage originated soil > lettuce originated soil > control soil. Association of Cd to the residual fraction does not generally constitute an environmental risk. This is due to the stable nature of Cd and the fact that the metals are bonded firmly within a mineral lattice that restricts the bioavailability of this metal (Milkessa, 2013).

Table 19. Chemical fractionation of Cd (mg /kg) in soil samples from lands irrigated with wastewater around the EIZ (n = 3, ± SD mg/Kg)

Cd	**F1**	**F2**	**F3**	**F4**	**F5**	**F1+F2+F3+ F4+F5**	**Wet-digestion**
ST	0.41 ± 0.01^{a}	0.35 ± 0.01^{a}	0.48 ± 0.02^{b}	19.13 ± 0.05^{a}	18.84 ± 0.02^{a}	39.21±0.11	45.33±1.53
	1.05%	0.89%	1.22%	48.79%	48.05%		
SC	0.35 ± 0.12^{b}	0.20 ± 0.02^{b}	0.42 ± 0.02^{c}	18.85 ± 0.02^{b}	18.81 ± 0.03^{a}	38.63±0.21	42.33±0.58
	0.91%	0.52%	1.09%	48.80%	48.69%		
SL	0.22 ± 0.02^{c}	0.13 ± 0.01^{c}	0.53 ± 0.03^{a}	18.85 ± 0.00^{b}	18.83 ± 0.10^{a}	38.56±0.16	45.00±1.00
	0.57%	0.34%	1.37%	48.88%	48.83%		
C	0.14 ± 0.01^{d}	0.11 ± 0.00^{c}	0.09 ± 0.00^{d}	8.34 ± 0.01^{c}	18.13 ± 0.14^{b}	26.81±0.16	27.93 ± 0.61^{c}
	0.52%	0.41%	0.34%	31.11%	67.62%		
LSD	0.03	0.03	0.03	0.06	0.19		1.83

Values are given as means of triplicates ± SD. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

4.8.3. Zinc in Soil Fractionation

The percentage distribution of zinc is in the range of 0.36 to 1.93 % in F1 (Soluble and exchangeable fraction), 0.61 to 0.1.25% in F2 (Carbonates bound fraction), 0.61 to 0.1.25% in F3 (Fe-Mn Oxide bound), 4.52 to 5.17 % F4 (Organic matter bound fraction), 91.05 to 93.25% in the residual fraction (F5) (Table 20). Percentage of zinc present in soil samples were F5 > F3 > F4 >F2 > F1. The greater level of zinc in the residual fraction probably indicates the greater tendency for zinc to become unavailable once it is in soils.

The mobility and bioavailability of zinc in the samples is found to be in the order of lettuce originated soil > tomato originated soil > cabbage originated soil > control sample. Zn was mostly associated with the residual fractions and the organic bound fraction. Zn has the lowest concentration in the exchangeable and carbonate fractions (Table 20). The strong association of Zn with residual and organic fraction was also reported by Fayun *et al*., (2008) in soil collected around industrial zone. Zn has the lowest concentration in the carbonate, exchangeable and Fe-Mn oxide fractions were reported by Adekola *et al*. (2012).

Table 20. Chemical fractionation of Zn (mg/kg) in soil samples from lands irrigated with wastewater around the EIZ (n = 3, ± SD mg/Kg)

Zn	**F1**	**F2**	**F3**	**F4**	**F5**	**F1+F2+F3+ F4+F5**	**Wet-digestion**
ST	0.36 ± 0.01^{b}	1.64 ± 0.03^{a}	5.08 ± 0.21^{c}	4.44 ± 0.12^{c}	89.56 ± 0.63^{c}	101.08±1.00	45.33±1.53
	0.36%	1.62%	5.03%	3.39%	88.60%		
SC	0.45 ± 0.03^{b}	0.75 ± 0.01^{c}	9.02 ± 0.10^{a}	6.41 ± 0.01^{a}	115.56 ± 0.41^{a}	132.19±0.56	42.33±0.58
	0.34%	0.57%	6.82%	4.85%	87.42%		
SL	2.04 ± 0.06^{a}	1.32 ± 0.12^{b}	6.07 ± 0.04^{b}	4.77 ± 0.30^{b}	96.18 ± 0.16^{b}	110.38±0.71	45.00±1.00
	1.85%	1.25%	5.50%	4.32%	87.14%		
C	0.09 ± 0.00^{d}	0.63 ± 0.01^{c}	0.09 ± 0.00^{d}	1.64 ± 0.03^{d}	65.55 ± 0.21^{d}	68.00±0.25	27.93±0.61
	0.13%	0.93%	0.13%	2.41%	96.40%		
SLD	0.08	0.13	0.18	0.31	0.75		

Values are given as means of triplicates ± SD. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

4.9.4. Iron in Soil Fractionation

The percentage distribution of iron is in the range of 0.11 to 0.13 % in F1 (Soluble and exchangeable fraction), 0.10 to 0.34% in F2 (Carbonates bound fraction), 9.52 to 15.14% in F3 (Fe-Mn Oxide bound), 5.06 to 21.28% F4 (Organic matter bound fraction), 73.88 to 84.98% in the residual fraction (F5) (Table 20). Percentage of iron present in soil samples were F5 > F3 > F4 > F2 > F1. The greater level of iron being in the residual fraction probably indicates the greater tendency for iron to become unavailable once it is in soils. A metal in F1 and F2 (soluble and exchangeable and carbonate bound) fraction is the most mobile and is readily available for biological uptake by the plant. The mobility and bioavailability of iron in the samples is found to be in the order of tomato originated soil > lettuce originated soil > cabbage originated soil > control sample. Adekola *et al*. (2012) reported Fe was found to be most concentrated in the residual fraction as well as in the organic and Fe-Mn oxide bound fractions to a lesser degree. However, Navas and Lindhorfer (2003) also reported Fe to be most concentrated in the residual fraction.

Table 21. Chemical fractionation of Fe (mg /Kg) in soil samples from lands irrigated with wastewater around the EIZ (n = 3, ± SD mg/kg)

Fe	F1	F2	F3	F4	F5	F1+F2+F3+F4+F5	Wet-digestion
ST	19.96± 0.14[c]	63.63±0.45[a]	1796.40±1.74[c]	954.50±10.05[c]	16040.74±9.91[a]	18875.23±22.29	20065±149.64
	0.11%	0.34%	9.52%	5.06%	84.98%		
SC	18.56±0.21[b]	15.96±0.12[b]	2487.87±10.52[a]	1657.20±16.38[a]	11820.73±113.89[b]	16000.32±141.12	18318±60.39
	0.12%	0.10%	15.14%	10.36%	73.88%		
SL	20.41±0.11[a]	20.33±0.15[c]	2107.87±5.03[b]	1170.75±21.28[b]	11830.67±115.54[b]	15150.03±142.11	12051±4.65
	0.13%	0.34%	13.91%	7.73%	78.09%		
C	10.71±0.53[d]	8.08±0.02[d]	466.53±22.76[d]	163.47±5.14[d]	6153.27±3.95[c]	6802.10±32.40	7140.00±133.32
	0.16%	0.12%	6.69%	2.40%	90.46%		
LSD	0.46	0.39	29.35	28.61	172.96		

Values are given as means of triplicates ± SD. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

4.9.5. Lead in Soil Fractionation

Table 22. Chemical fractionation of Pb (mg /Kg) in soil samples from lands irrigated with wastewater around the EIZ (n = 3, ± SD mg/kg)

Pb	**F1**	**F2**	**F3**	**F4**	**F5**	**F1+F2+F3+ F4+F5**	**Wet-digestion**
ST	12.27±0.42[a]	12.00±0.20[a]	7.87±0.31[c]	26.93±0.1[a]	13.36±0.04[a]	72.43±1.09	63.00±2.26
	16.94%	16.57%	10.87%	37.18%	18.45%		
SC	10.33±0.12[b]	10.40±0.60[b]	9.20±0.02[b]	24.87±0.1[b]	9.9±0.10[b]	64.70±0.96	64.87±0.45
	15.96%	16.07%	14.22%	38.44%	15.30%		
SL	5.12±0.11[c]	6.47±0.31[c]	10.57±0.04[a]	23.87±1.2[b]	8.91±0.01[c]	54.94±1.76	63.33±3.58
	9.31%	11.77%	19.24%	43.45%	16.22%		
C	0.32±0.02[d]	1.50±0.2[d]	0.70±0.30[d]	5.53±0.04[c]	8.06±0.04[d]	16.11±0.60	18.82±0.08
	1.99%	9.31%	4.35%	34.33%	50.15%		
LSD	0.46	0.63	0.36	1.34	0.10		

Values are given as means of triplicates ± SD. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

The percentage distribution of lead is in the range of 9.31 to 16.94 % in F1 (Soluble and exchangeable fraction), 11.77 to 16.57% in F2 (Carbonates bound fraction), 10.87 to 19.24% in F3 (Fe-Mn Oxide bound), 37.18 to 43.45% F4 (Organic matter bound fraction), 15.30 to 18.45% in the residual fraction (F5) (Table 22). Soil OM has a large surface negative charge/ cation exchange capacity and elements such as Pb are observed to accumulate in the organic-rich, surface horizons (Zimdahl and Skogerboe, 1977). A metal in F1 and F2 (soluble and exchangeable and carbonate bound) fraction is the most mobile and is readily available for biological uptake by the plant. The mobility and bioavailability of lead in the samples is found to be in the order of tomato soil > cabbage soil > lettuce soil > control sample. Pb has the lowest concentration in the exchangeable and carbonate bound fraction (Table 22). Many researchers have reported varying concentrations of Pb in different fractions, Chuckwujindu (2007) reported high percentage concentration of Pb in the residual fraction. However, Olajire (2003) reported Pb to be most concentrated in the non-residual fraction while other researchers

found Pb to be associated with organic matter fraction (Livette *et al*., 1979). The varying concentration of Pb in the different fractions could be attributed to varied nature of industrial activities.

4.9.6. Copper in Soil Fractionation

The percentage distribution of copper is in the range of 0.30 to 0.59 % in F1 (Soluble and exchangeable fraction), 0.19 to 1.38% in F2 (Carbonates bound fraction), 6.71 to 11.67 % in F3 (Fe-Mn Oxide bound), 11.26 to 25.04% F4 (Organic matter bound fraction), 62.07 to 80.83% in the residual fraction (F5) (Table 23). Percentage of copper present in soil samples were F5 > F4 > F3 > F2 > F1. The greater level of copper in the residual fraction probably indicates the greater tendency for copper to become unavailable once it is in soils. A metal in F1 and F2 (soluble and exchangeable and carbonate bound) fraction is the most mobile and is readily available for biological uptake by the plant. The mobility and bioavailability of copper in the samples is found to be in the order of tomato originated soil > cabbage originated soil >lettuce originated soil > control sample. Many researchers have reported varying concentrations of Cu in different fractions. Adekola *et al*. (2012) reported high percentage concentration of Cu in organic matter, Fe-Mn oxide and residual fraction. The dominance of Cu in the organic phase has also been reported by others (Chuckwujingu, 2007).

Table 23. Chemical fractionation of Cu (mg /Kg) in soil samples from lands irrigated with wastewater around the EIZ (n = 3, ± SD mg/kg)

Cu	**F1**	**F2**	**F3**	**F4**	**F5**	**F1+F2+F3+F4+F5**	**Wet-digestion**
ST	0.78 ± 0.02^{a}	1.83 ± 0.21^{a}	8.90 ± 0.20^{c}	21.40 ± 0.10^{b}	99.7 ± 0.70^{b}	132.61±1.	146.10±3
	0.59%	1.38%	6.71%	16.14%	75.18%	23	.08
SC	0.54 ± 0.01^{b}	0.97 ± 0.12^{b}	14.50 ± 0.10^{a}	31.10 ± 0.20^{a}	77.10 ± 2.00^{c}	124.21±2.	142.77±3
	0.43%	0.78%	11.67%	25.04%	62.07%	43	.23
SL	0.46 ± 0.01^{c}	0.29 ± 0.01^{c}	11.33 ± 0.15^{b}	17.20 ± 0.20^{c}	123.43 ± 1.60^{a}	152.71±1.	140.33±2
	0.30%	0.19%	7.42%	11.26%	80.83%	97	.01
C	0.11 ± 0.00^{d}	0.10 ± 0.00^{c}	0.21 ± 0.00^{d}	5.19 ± 0.02^{d}	61.19 ± 0.74^{d}	66.80±0.7	68.47±1.
	0.16%	0.15%	0.31%	7.77%	91.60%	6	10
LSD	0.03	0.27	0.20	0.27	2.59		

N.B: Values are given as means of triplicates ± SD. The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

4.10. Comparison and Recoveries of the Elements in Soil Samples

4.10.1. Comparison between Result of Fractional Extraction and Wet - Digestion Procedures

As depicted in Table 24, for all of the samples, the concentration of Cr and Cd determined in wet digestion method are found to be greater than the total concentration obtained from fractional analysis. The same is true for Zn, Fe, Pb and Cu except for the cabbage (CS), lettuce soil (LS), tomato soil and lettuce soil, respectively. In similar study Yoseph (2015) reported that concentration of lead and cadmium in wet digestion method are greater than total concentration obtained from fractional analysis.

Table 24. Comparison between fractional extraction and wet digestion results in mg/kg (mean ± SD) where n = 3

Element		**TS**	**CS**	**LS**	**C**	**WHO**
Cr	WD	50.50±0.53	66.30±2.46	62.23±2.35	21.37±0.31	50
	FE	36.86±1.73	36.13±0.71	42.52±0.52	21.26±0.12	
Cd	WD	45.33±1.53	42.33±0.58	45.00±1.00	27.93±0.61	3
	FE	39.21±0.11	38.63±0.21	38.56±0.16	26.81±0.16	
Zn	WD	114.86±10.33	108.44±8.52	123.77±7.71	69.37±2.00	300
	FE	101.08±1.00	132.19±0.56	110.38±0.71	68.00±0.25	
Fe	WD	20065±149.64	18318±60.39	12051±4.65	7140.00±133.32	5000
	FE	18875.23±22.29	16000.32±141.12	15150.03±142.11	6802.10±32.40	
Pb	WD	63.00±2.26	64.87±0.45	63.33±3.58	18.82±0.08	100
	FE	72.43±1.09	64.70±0.96	54.94±1.76	16.11±0.60	
Cu	WD	146.10±3.08	142.77±3.23	140.33±2.01	68.47±1.10	100
	FE	132.61±1.23	124.21±2.43	152.71±1.97	66.80±0.76	

N.B: Where: WD= Metal from wet digestion, FE= Metal from Fractional extraction

4.10.2. Element Recoveries

Validation of the analytical results was tested by recovery experiments because there was no standard reference material (SRM), which is more preferential or needed to control the accuracy of the method studied, in our laboratory. An important consideration in the reliability of a sequential extraction data is the percentage recovery relative to a single digestion using a mixture of strong mineral acids or generally a mixture of strong acids at the digestion of the residual phase of the sequential extraction protocol employed (Boch *et al*., 2002). Recovery is defined as follows:

$$\textbf{Recovery} = \left(\frac{\sum \textbf{n Sequential etraction procedure}}{\textbf{Single digesion with strong acids}}\right) \boldsymbol{x}\ \mathbf{100\ \%} \qquad \textbf{(6)}$$

Where n is the concentration of a given element and the single digestion with strong acids used for reference was a mixture of strong acids used in the residual fraction digestion (Boch *et al*., 2002). The analytical results acquired are depicted in Appendix Table 4. Recovery values of the tested elements for the scheme were calculated according to the equation above, and generally agreed with each other although some recoveries deviated from acceptable values. For example, low recoveries were obtained for Cr in three vegetable originated soil sample shown in table. Some recoveries, however, were higher than 100% for Zn, Fe and Pb in cabbage, tomato and lettuce originated soil respectively. Already, in the use of sequential extraction procedures for partitioning/speciation of metals, sample contamination or loss could occur during the extraction steps. This phenomenon may cause the observed experimental errors, i.e., obtaining low or high recovery. In a similar study low recoveries were obtained for Pb (60.58%), for Cu (60.57%), for Cr (63.18%), for Cd (66.23%), for Zn (61.04%), respectively. Some recoveries, however, were higher than 100% for Pb, Cu, Cd, Cr, Zn, (Uduma, 2013**)**.

4.10.3. Bioavailability and Mobility Factors of Heavy Metals

The sequential extraction procedures results provided information on the potential mobility and bioavailability of the elements investigated in this research. The distribution of heavy metals in the sample allows us to predict their mobility and bioavailability. The bioavailability factor was expressed as the ratio of the available concentration of a metal in soil to its total concentration. It shows the potentials of a particular metal from the soil matrix to enter the soil solution from which it can be absorbed by plants. Mobility factor was expressed as percentage of the Bioavailability factor (Kabata and Singh, 2001).

$$\mathbf{BF} = \frac{F1 + F2}{F1 + F2 + \cdots + F5} \qquad \mathbf{(7)}$$

$$\mathbf{MF} = \frac{F1 + F2}{F1 + F2 + \cdots + F5} \; \boldsymbol{x}\ \mathbf{100} \qquad \mathbf{(8)}$$

Table 25, shows the mobility, and bioavailability factors for all the sequential extractions steps. The high MF and BF values of soil Pb may be interpreted as symptoms of relatively high liability and biological availability of the metals in soil. Similar characteristics distribution patterns were observed for Cu Cd, Cr and Zn (Table 25). The average mobility of Cr, Cd, Zn Fe, Pb and Cu levels in all the five fraction fractions were in the order: Pb > Cr > Zn>Cd > Cu > Fe.

Table 25. The bioavailability and mobility Factor of Heavy Metals in soil sample fractionation (n = 3)

Elements	Sample code	F1	F2	Sum of F1 and F2	Sum of Fraction	Bioavailability Factor	Mobility Factor
Cr	ST	0.61	0.260	0.870	36.860	0.024	2.360
	SC	0.79	0.440	1.230	36.130	0.034	3.404
	SL	0.91	0.370	1.280	42.520	0.030	3.010
	C	0.19	0.210	0.400	7.260	0.019	1.881
Cd	ST	0.41	0.350	0.760	39.210	0.019	1.938
	SC	0.35	0.200	0.550	38.630	0.014	1.424
	SL	0.22	0.130	0.350	38.560	0.009	0.908
	C	0.14	0.140	0.280	15.110	0.010	1.040
Zn	ST	0.36	1.640	2.000	101.080	0.020	1.979
	SC	0.45	0.750	1.200	132.190	0.009	0.908
	SL	2.04	1.320	3.360	110.380	0.030	3.044
	C	0.09	0.630	0.720	22.870	0.011	1.059
Fe	ST	19.96	63.630	83.590	18875.230	0.004	0.443
	SC	18.56	15.960	34.520	16000.320	0.002	0.216
	SL	20.41	20.330	40.740	15150.030	0.003	0.269
	C	10.71	8.080	18.790	2802.060	0.003	0.276
Pb	ST	12.27	12.000	24.270	72.430	0.335	33.508
	SC	10.33	10.400	20.730	64.700	0.320	32.040
	SL	5.12	6.470	11.590	54.940	0.211	21.096
	C	0.32	1.500	1.820	8.380	0.113	11.297
Cu	ST	0.78	1.830	2.610	132.610	0.020	1.968
	SC	0.54	0.970	1.510	124.210	0.012	1.216
	SL	0.46	0.290	0.750	152.710	0.005	0.491
	C	0.11	0.100	0.210	26.800	0.003	0.314

4.11. Pearson's Correlation

4.11. 1. Pearson's Correlation between Heavy Metals in Soil and Vegetable

The relationship between contents of different elements in soil and vegetable were analyzed by Pearson's correlation coefficient. The correlation analysis is a bivariant method which is applied to describe the relation between two different parameters. The high correlation co-efficient (near +1 or −1) means a good relation between two variables, and its concentration around zero means no relationship between them at a significant level of 0.05% level, it is strongly correlated, if $r > 0.7$, whereas r values between 0.5 and 0.7 show moderate correlation between two different parameters (Sharma and Raju, 2013).

Simple statistical correlation was calculated for heavy metal concentration in soil and vegetable to identify the relationship that existed between the concentration of heavy metals in the soil and the vegetable. Pearson's correlation at 5% significance was calculated for finding relationship between content of elements in soil and vegetable. The results showed that the relationship among the elements Cr, Cd, Zn, Fe and Pb, were negative correlations for tomato, but Cu are shows positive correlations for tomato and cabbage as shown in Table 26. Also Zn and Fe show positive correlation for cabbage, but Cr and Pb show negative correlation. In the case of lettuce, Cr, Zn and Fe show positive correlation, which Cd, Pb and Cu are showing negative correlation as shown in Table 26. But there was no correlation observed with Cd in cabbage.

Negative correlation indicated that higher concentration of heavy metals present in soils but in comparison much lower concentration were found to be in vegetables of that soils. This was due to poor retention capabilities of different edible parts of vegetables (Marshall *et al*., 2008). TF values decreases with increasing respective metal concentration in soils, indicating an inverse relationship between transfer factor and metal concentration such inverse relationship were also reported by Wang *et al*. (2006) for vegetables. Correlation analysis between metal concentrations in different fractions of soil and vegetables was performed at 95% and 99% confidence levels. Positive and negative correlations were observed; positive values indicated the bioavailability of these metal fractions to vegetables, while negative values showed that

metal concentrations in particular fractions were not bioavailable to plants (Bashir *et al*., 2014).

Table 26. Correlation between metal contents of vegetable and soil samples of the plants origin

Vegetable	**Cr**	**Cd**	**Zn**	**Fe**	**Pb**	**Cu**
Tomato	-0.45392	-0.65465	-0.96532	-0.43355	-0.90687	0.17854
Cabbage	-0.59214	0.0000	0.482770	0.55092	-0.36836	0.53008
Lettuce	0.63872	-0.82199	0.98631	0.96862	-0.86603	-0.81935

4.11.2. Correlation of Heavy Metals in Soils

The Pearson correlation matrices using correlation coefficients (r) for the soil samples are shown in Appendix Table 5, Appendix Table 6, Appendix Table 7 and Appendix Table 8 for tomato soil, cabbage soil lettuce soil and control soil samples, respectively.

In tomato soil samples, Cr and negatively correlated with Zn, Pb, Cu, , EC,%OM %silt, %sand, but Cd, Fe, %MC, %clay and CEC show positive correlated with Cr. Whilst, the remaining tested metals correlate each other positively. The pH of the soils had no correlation with Cr. Cd was negative correlation with Pb, Cu, EC, %OM %silt, CEC, but with other heavy metal and physicochemical properties a positive correlation was recorded with Cd in tomato soil. Positive correlation between metal concentration and physic-chemical parameters could imply a significant effect on the amount of heavy metals in the soil, since the mobility and bioavailability of metals present in the soils depend on physic-chemical properties of both the metals and the soil (McEldowney *et al*., 1993). Zn had negative correlation with all metal except Cu and Cd in tomato soil, also negative correlation with all physicochemical properties except %OM and CEC. Fe had positive correlation with all physicochemical properties except %OM and %MC. Pb had negative correlation with all metals except Cu, but positive correlation with physicochemical properties except %MC, %clay and CEC. Cu had positive correlation with all physicochemical properties except %clay and %CEC. All correlation values were within the significance of $P \geq 0.05$ (Appendix Table 5).

In cabbage soil sample, %OM was positively correlated with all heavy metal except Fe and Cu. pH was negatively correlated with Cr, Cd and Pb, but positive correlation with Zn, Fe and Cu in cabbage soil. EC, %MC, %silt and CEC were negatively correlated with Cr, Cd and Pb, but had positive correlation with Fe. Heavy metals (Cr, Cd and Pb showed were positive correlation with %clay, but %clay negative correlation with Zn, Fe and Cu. All correlations were in the significance of $P \geq 0.05$ (Appendix Table 6).

In lettuce soil sample, Zn, Cu and %clay were positively correlated with all metals, also Cd and Fe were positively correlated in all metals except Cr. In addition to this, Fe was also negatively correlated to Pb. PH, EC and %sand were negatively correlated with all metals. %MC, %OM and % silt were negatively correlated with all metals except Cr. CEC was negative correlation with metals except Cd and Fe. All correlations were in the significance of $P \geq 0.05$ (Appendix Table 7).

In the control soil sample, Pb and pH were negatively correlated with all metals except Cu. Likewise, %OM and %clay were negatively correlated with all metals except Zn and %sand and CEC which were negatively correlated to all heavy metals except Pb and Cu. Cu and %silt were positively correlated with all metals except for Zn and Fe. Cd was positively correlated with all metals except Pb. All correlations were in the significance of $P \geq 0.05$ (Appendix Table 8).

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary and Conclusion

As stated earlier, the major purpose of this study was to find out the level of heavy metals in soil, from three farmer farm and three subsamples from each farm for each edible part of the vegetables (tomato, cabbage and lettuce) were collected manually and composited in to one for each vegetable. Similarly vegetable originated soil samples were collected at depth of 0-20 cm by steel less steel auger. The soil and vegetable samples were subjected to wet-digestion, sequential extraction and the concentration of heavy metals were determined via FAAS. Studied was carried out in the vegetables, soils and wastewater from irrigated farm around EIZ, to assess heavy metals contamination distribution due to industrialization, urbanization and agricultural activities. Overall concentrations of Cr, Cd, Zn, Fe and Pb and Cu in three sample type were determined. The relationship between different physico-chemical properties and heavy metal concentrations of soil samples and between soil samples and perspective vegetables heavy metal concentrations were analyzed by Pearson's correlation coefficient.

Our study showed that soil wet-digestion method 5:1 ratio of HNO_3 to H_2O_2 were used and the concentrations of Cr, Cd, Zn, Fe and Pb and Cu for soil samples were found to be ranged from 22.37-66.30, 27.93-45.33, 69.37-123.77, 7140.00-20065.00, 18.82-64.87 and 68.47-146.10 mg/kg respectively. The concentration of heavy metals in the soil display the following decreasing trend: Fe > Cu > Zn > Cr > Pb > Cd. These concentrations of heavy metals in soil samples were above the recommended level set by FAO/WHO (2001), EU (2002) and USEPA (2002) for irrigation soil.

Given the importance of vegetables in the food pyramid, their safety is very important from view point of public health. Vegetable contamination by heavy metals can lead bioaccumulation of these toxic and disease-causing elements in the body of consumers. Therefore, in this study, the concentrations of some heavy metals (Cr, Cd, Zn, Fe, Pb and Cu) in edible vegetables which are grown around Eastern Industry Zone irrigation farm were investigated. In vegetable samples overall concentrations of these heavy metals in acid mixture digestion method (8:1:1) ratio of HNO_3: HCl: H_2O_2) were found to be ranged from 2.90-3.77,

2.20-3.68, 45.65-62.46, 358.17-571.33, 4.60-5.50 and 10.20-15.07 mg/kg respectively. Vegetables were above the FAO/WHO (2006) permissible limits. The concentration of heavy metals in the vegetable samples display the following decreasing trend: Fe > Zn > Cu > Cr > Pb > Cd. The study revealed that the concentrations of all metals in the vegetables were found to be above the safe limits set by different international organizations for consumption, posing a serious health hazard to humans. Therefore, regular monitoring of effluents, soils, and vegetables are essential to prevent excessive build-up of the toxic heavy metals in food. Thus, the health risk and the extent of heavy metal contamination can be reduced. The soil–plant transfer factor (TF) decreased in the following order- TFZn > TFCu > TFPb > TFCd > TFCr > TFFe. Transfer factor 0.50 was obtained for Zn.

The analysis of wastewater for heavy metal contamination is an important step in ensuring human and environmental health. Excess levels of heavy metals might cause several short term and long term health effects to the human beings. Wastewater was collected from Eastern Industry Zone effluent in the point of 0, 200 and 500 m away from the discharge point. The wastewater samples were obtained and subjected to acid mixture digestion (3 mL HNO_3+ 3 mL H_2O_2) and the concentration of metals were determined via FAAS. Overall concentrations of Cr, Cd, Zn, Fe and Pb, Cu in the wastewater samples in acid mixture digestion method ranged from 0.20-1.04, 0.04-0.08, 0.07-0.21, 5.13-2.89, 1.98-3.11and 0.30-0.99 mg/kg respectively. The concentration of heavy metals in the wastewater samples display the following decreasing trend: Fe > Pb > Cr > Cu > Zn > Cd, these concentrations are above the recommended level set by WHO (2006) for irrigation water.

A sequential extraction procedure was used to fractionate Cr, Cd, Zn, Fe and Pb, Cu present in soils of tomato, cabbage and lettuce and reference (control) soils. Different geochemical fractions are operationally defined by an extraction sequence that generally follows the order of decreasing solubility. The highest amount of Cr, Zn and Fe metals in the studied soils was concentrated in the residual fractions, which is highly stable. However, in most of the soils, a significant percentage of Cd, Pb and Cu metals were associated with non-residual fractions. Therefore, they should be evaluated when studying the pollution levels of heavy metals in soils. Among the non-residual fractions, the organic fractions contained the greatest amount of

Pb in all soil samples. This metal can be released into the environment under extremely oxidized conditions.

High concentrations of Cr and Cu in lettuce soils, Pb, Fe and Cd in tomato soils and Zn in cabbage soils were found. The sequential extraction used in this study is useful to indirectly assess the potential mobility and bioavailability of heavy metals in the soils. The mobility and bioavailability of these metals were studied and a very high amount of these metals were concentrated at the residual, organic and Fe-Mn Oxide fractions. However, a very small concentration of these heavy metals was also found at the exchangeable and carbonate fractions. Mobility factor of, Cr, Cd, Zn, Fe, Pb and Cu in soil samples ranged from 1.881-3.404, 0.908-1.938, 0.908-3.044, 0.216-0.443, 11.297-33.508 and 0.314-1.968 respectively.

5.2. Recommendation

Based on the findings from this study, the following points are recommended.

- The effluent from EIZ has heavy metal contaminants that are beyond the recommended concentration limit. Therefore the industry zone effluent should not be used for irrigation purpose.
- The current farmers' irrigation practice using the EIZ effluent as irrigation source showed high concentration of toxic heavy metals in the vegetables as well as the irrigated top and sub-soils surrounding this industry zone. This concentration level of the heavy metals in all 3 vegetable types was detrimental that can cause health problem for human/consumers.
- The concerned Administrative bodies, health departments, district and regional level agricultural offices and of course, farmers in the area should be aware of the problem associated with the effluent coming out of EIZ.
- There should be an alternate study on how the farmers can use the effluent from EIZ safely for irrigation purpose.
- Management of EIZ must be aware the situation and they should install and/or establish an urgent mitigation/cleaning measures on the effluents coming out of their industries.

- Health science researchers are advised to launch additional assessment and supplementary information on consumers who obtain or buy vegetables harvested from EIZ.
- This study might be repeated with GFAAS and ICP-OES to compare the heavy metal contents of the selected sample types.

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7. APPENDICES

Appendix Table 1. Method detection limits for vegetable, soil in wet digestion and wastewater samples

Element	IDL mg/L	MDL of vegetable sample in mg/L	MDL of soil sample in mg/L	MDL of wastewater sample in mg/L
Cr	0.006	0.007	0.011	0.006
Cd	0.002	0.002	0.003	0.003
Zn	0.001	0.001	0.004	0.002
Pb	0.01	0.016	0.054	0.010
Fe	0.006	0.006	0.009	0.007
Cu	0.003	0.004	0.005	0.004

Appendix Table 2. Method detection limits for sequential extraction of soil samples

Element	IDL	MDL				
		F1	F2	F3	F4	F5
Cr	0.006	0.008	0.008	0.008	0.105	0.012
Cd	0.002	0.004	0.003	0.003	0.006	0.005
Zn	0.001	0.002	0.003	0.003	0.005	0.004
Fe	0.01	0.008	0.007	0.010	0.012	0.011
Pb	0.006	0.013	0.024	0.022	0.035	0.044
Cu	0.006	0.005	0.005	0.006	0.006	0.007

Appendix Table 3. Chemical fractionation of heavy metals in (mg /Kg) in soil sample collected from lands irrigated with wastewater around the EIZ (n = 3)

Sample Code		Cr	Cd	Zn	Fe	Pb	Cu
	Residual	32.27	18.84	89.56	16040.74	13.36	99.70
ST	Non-residual	4.64	20.37	11.52	2834.49	59.07	32.91
	Sum	36.91	39.21	101.08	18875.23	72.43	132.61
	% Non-residual	12.57	51.95	11.40	15.02	81.55	24.82
	% Residual	87.43	48.05	88.60	84.98	18.45	75.18
	Residual	6.35	6.43	20.42	2153.27	2.06	21.19
SC	Non-residual	0.72	8.68	2.45	648.79	8.05	5.61
	Sum	7.07	15.11	22.87	2802.06	10.11	26.80
	% Non-residual	10.20	57.45	10.71	23.15	79.62	20.93
	% Residual	89.80	42.55	89.29	76.85	20.38	79.07
	Residual	35.27	18.83	96.18	11830.67	8.91	123.43
SL	Non-residual	7.25	19.73	14.20	3319.36	46.03	29.28
	Sum	42.52	38.56	110.38	15150.03	54.94	152.71
	% Non-residual	17.05	51.17	12.86	21.91	83.78	19.17
	% Residual	82.95	48.83	87.14	78.09	16.22	80.83
	Residual	6.35	6.43	20.42	2153.27	2.06	21.19
	Non-residual	0.72	8.68	2.45	648.79	8.05	5.61
C	Sum	7.07	15.11	22.87	2802.06	10.11	26.80
	% Non-residual	10.20	57.45	10.71	23.15	79.62	20.93
	% Residual	89.80	42.55	89.29	76.85	20.38	79.07

Appendix Table 4. The percentage recovery of sequential extraction of soil samples relative to a single digestion method

Element	sample code	sum of fraction	single acid digestion	% Recovery
Cr	ST	36.86	50.50	72.99
	SC	36.13	66.30	54.49
	SL	42.52	62.23	68.33
	C	21.26	21.37	99.49
Cd	ST	39.21	45.33	86.50
	SC	38.63	42.33	91.26
	SL	38.56	45.00	85.69
	C	26.81	27.93	104.18
Zn	ST	101.08	114.86	88.00
	SC	132.19	108.44	121.90
	SL	110.38	123.77	89.18
	C	68.00	69.37	98.03
Fe	ST	18875.23	20065	94.07
	SC	16000.32	18318	87.35
	SL	15150.03	12051	125.72
	C	6802.10	7140.00	90.46
Pb	ST	72.43	63.00	114.97
	SC	64.70	64.87	99.74
	SL	54.94	63.33	86.75
	C	16.11	18.82	85.60
Cu	ST	132.61	146.1	90.77
	SC	124.21	142.77	87.00
	SL	152.71	140.33	108.82
	C	66.80	68.47	97.56

Appendix Table 5. Correlation between pH, EC, %MC %OM, %Clay, %Silt, %Sand CEC and metals in tomato soil samples of the vegetable grown

	Cr	Cd	Zn	Fe	Pb	Cu	pH	EC	%MC	%OM	%clay	%silt	%sand	CEC
Cr	1.000													
Cd	0.866	1.000												
Zn	-0.776	0.987	1.000											
Fe	0.982	0.945**	-0.881	1.000										
Pb	-0.585	-0.101	-0.058	-0.042	1.000									
Cu	-0.779	-0.361	0.209	-0.647	0.964	1.000								
pH	0.000	0.500	-0.631	0.189	0.811	0.627	1.000							
EC	-0.965	-0.705	-0.898	0.583	0.777	0.916	0.262	1.000						
%MC	0.852	0.477	-0.331*	-0.923	-0.992	0.738	-0.523	-0.960	*1.000*					
%OM	-0.908	-0.996	0.969	-0.971	0.191	0.444	-0.419	0.766	-0.555	1.000				
%clay	0.817*	0.419	-0.270	0.693	-0.946	-0.998	-0.577	-0.940	0.998*	-0.500	1.000			
%silt	-0.866	-1.000**	-0.945	0.987	0.101	0.361	-0.500	0.705	-0.477	0.996	-0.419	1.000		
%sand	-0.763	0.327	-0.473	0.189	0.000	0.907	0.982	0.440	-0.675	-0.240	-0.721	-0.327	1.000	
CEC	0.329	-0.187	0.341	0.145	-0.958	-0.848	-0.944	-0.565	0.774	0.097	0.813	0.187	-0.989	1.000

**. Correlation is significant at the 0.01 level (2-tailed).

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

Appendix Table 6. Correlation between pH, EC, %MC %OM, %Clay, %Silt, %Sand, CEC and metals in cabbage soil samples of the vegetable grown

	Cr	Cd	Zn	Fe	Pb	Cu	pH	EC	%MC	%OM	%clay	%silt	%sand	CEC
Cr	1.000													
Cd	0.110	1.000												
Zn	0.470	-0.826	1.000											
Fe	-0.576	-0.876*	0.451*	1.000										
Pb	0.999	0.064	0.510	-0.537	1.000									
Cu	-0.944	0.223	-0.734	0.274	-0.958	1.000								
pH	-0.681	-0.803	0.326*	0.991	-0.646	0.402	1.000							
EC	-0.231	-0.992	0.750	0.929*	-0.186	-0.102	0.870	1.000						
%MC	-0.958	-0.391	-0.196	0.787	-0.943	0.810	0.863	0.501	*1.000*					
%OM	0.916	0.500	0.076	-0.856	0.896	-0.732	-0.918	-0.603	-0.993	1.000				
%clay	0.734	0.756*	-0.255	-0.978*	0.702	-0.469	-0.997*	-0.831	-0.898	0.945	1.000			
%silt	-0.549	-0.891*	0.479	0.999	-0.510	0.243*	0.986	0.940*	0.766	-0.839	-0.971	1.000		
%sand	0.296	0.982	-0.704	-0.951	0.251	0.035	-0.901	-0.998	-0.558	0.655	0.866	-0.961	1.000	
CEC	-0.420	-0.948	0.603	0.984	-0.378	0.098	0.951	0.980	0.663	0.749	0.925	0.989*	-0.991	1.000

*. Correlation is significant at the 0.01 level (2-tailed).

Appendix Table 7. Correlation between pH, EC, %MC %OM, %Clay, %Silt, %Sand, CEC and metals in lettuce soil samples of the vegetable grown

	Cr	Cd	Zn	Fe	Pb	Cu	pH	EC	%MC	%OM	%clay	%silt	%sand	CEC
Cr	1.000													
Cd	-0.661	1.000												
Zn	0.356	0.466	1.000											
Fe	-0.827	0.969	0.231	1.000										
Pb	0.751	0.000	0.885*	-0.249	1.000									
Cu	0.541	0.274	0.979	0.026	0.962	1.000								
pH	-0.320	-0.500	-0.999*	-0.269	-0.866	-0.970	1.000							
EC	-0.366	-0.457	-1.000	-0.221	-0.890	-0.981	0.999	1.000						
%MC	0.220	-0.878	-0.833	-0.731	-0.479	-0.701	0.854	0.827	*1.000*					
%OM	0.325	-0.924	-0.768	-0.801	-0.381	-0.620	0.792	0.761	0.994	1.000				
%clay	0.493	0.327	0.989	0.082	0.945	0.998	-0.982	-0.990	-0.740	-0.663	1.000			
%silt	-0.507	0.982	0.625	0.904	0.189	0.450	-0.655	-0.616	-0.952	-0.980	0.500	1.000		
%sand	-0.181	-0.619	-0.983*	-0.404	-0.786	-0.925*	0.990	0.981*	0.919	0.871	-0.945	-0.756	1.000	
CEC	-0.959	0.419	-0.608	0.632	-0.908	-0.758	0.576	0.616	0.067	-0.042	-0.720	0.240	0.454*	1.000

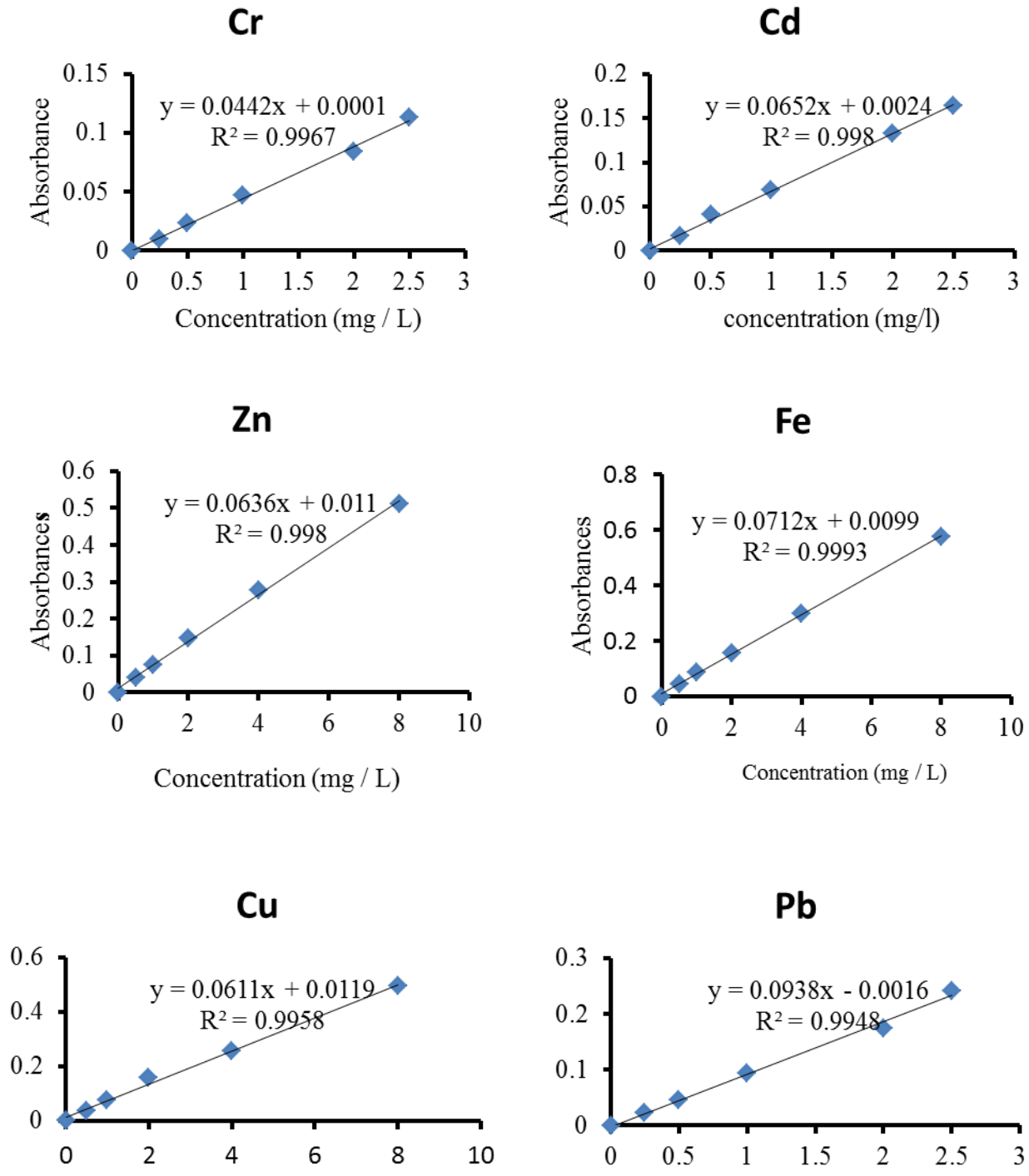
*. Correlation is significant at the 0.05 level (2-tailed).

Appendix Table 8. Correlation between pH, EC, %MC %OM, %Clay, %Silt, %Sand, CEC and metals in control soil sample

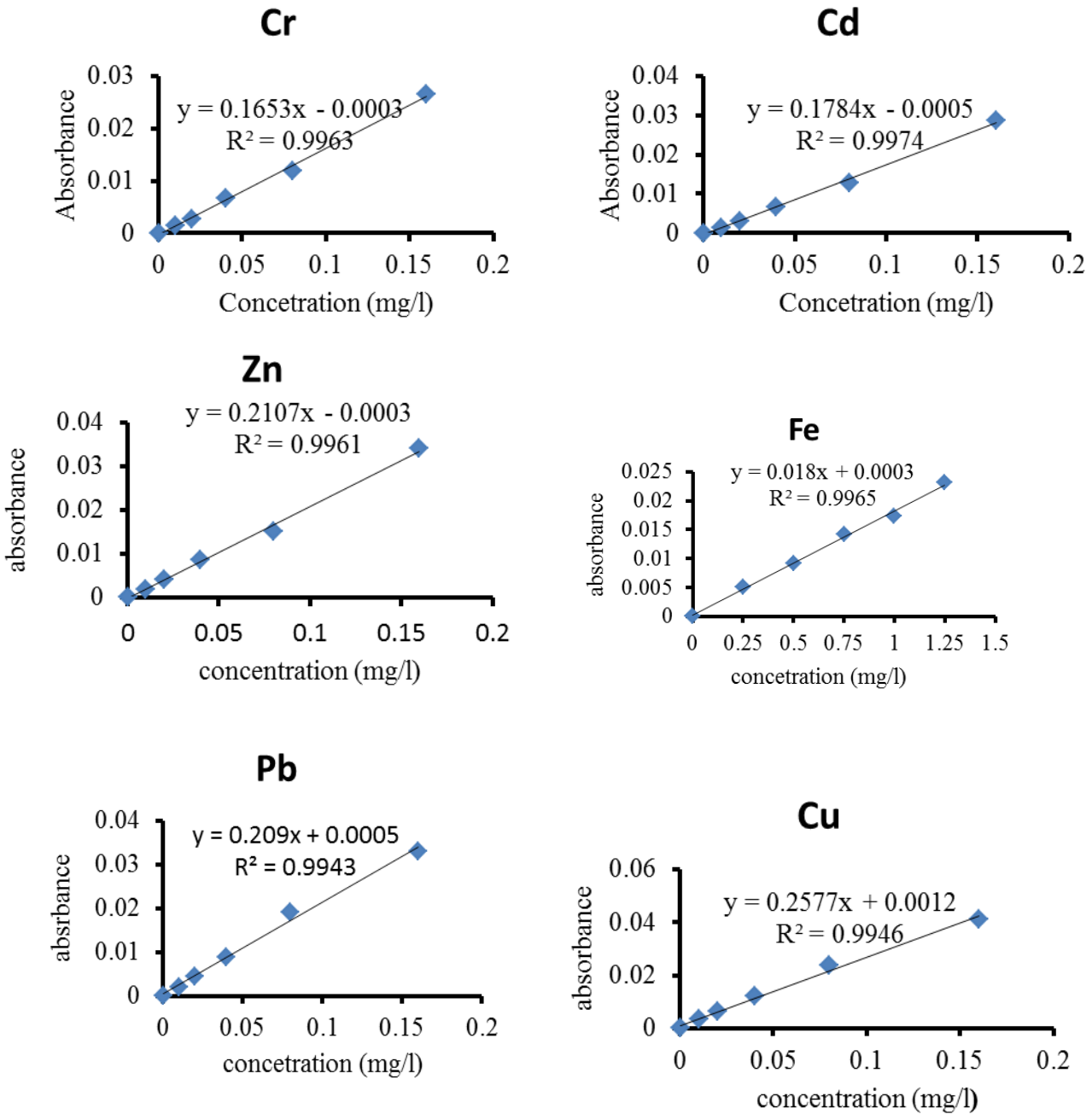
	Cr	Cd	Zn	Fe	Pb	Cu	pH	EC	%MC	%OM	%clay	%silt	%sand	CEC
Cr	1.000													
Cd	1.000	1.000												
Zn	0.381	0.381	1.000											
Fe	0.405	0.405	1.000	1.000										
Pb	-0.500	-0.500	-0.991	-0.994	1.000									
Cu	0.040*	0.040	-0.909	-0.897	0.845	1.000								
pH	-0.982	-0.982	-0.200	-0.225	0.327	-0.228	1.000							
EC	0.969**	0.969	0.143	0.168	-0.272	0.284	-0.998	1.000						
%MC	0.956	0.956	0.635	0.654	-0.731	-0.254	-0.884	0.856	*1.000*					
%OM	-0.327	-0.327	0.749	0.731	-0.655	-0.957	0.500	-0.549	-0.037	1.000				
%clay	-0.645	-0.645	0.461	0.438	-0.339	-0.789	0.778	-0.813	-0.394	0.933	1.000			
%silt	0.849	0.849	-0.166	-0.140	0.034	0.562	-0.933	0.952	0.657	-0.778	-0.952	1.000		
%sand	-0.971**	-0.971	-0.592	-0.613	0.693	0.201**	0.908**	-0.882	-0.999	0.091	0.442	-0.697	1.000	
CEC	-0.959	-0.959	-0.626	-0.646	0.724	0.243	0.889	-0.861	-1.000	0.048	0.403	-0.665	0.999	1.000

**. Correlation is significant at the 0.01 level (2-tailed).

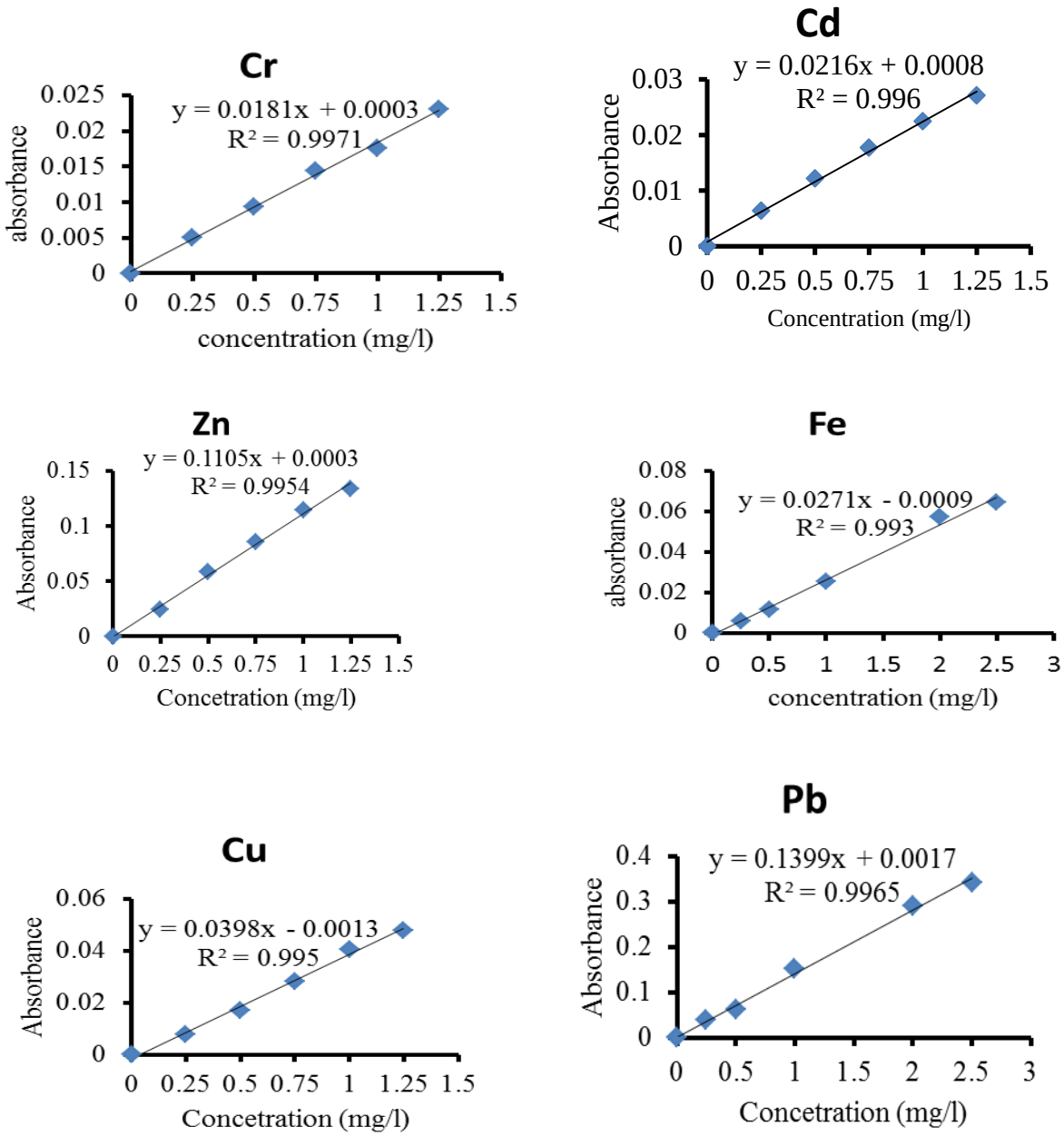
*. Correlation is significant at the 0.05 level (2-tailed).



Appendix Figure 1. Calibration Curves of heavy metals (Cr, Cd, Zn, Fe, Pb and Cu) for soil samples



Appendix Figure 2. Calibration Curves of heavy metals (Cr, Cd, Zn, Fe, Pb and Cu) for vegetable samples



Appendix Figure 3. Calibration Curves of heavy metals (Cr, Cd, Zn, Fe, Pb and Cu) for wastewater samples