Addis Ababa University

College of Natural and Computational Sciences

Department of Chemistry

Analytical Chemistry Stream

MSc Thesis

Determination of metal contents in eggplants and their corresponding soil of the plant cultivated in Ethiopia

By

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Advisor: Prof: B. S. Chandravanshi

July, 2020

Declareation

I the undersigned, hereby declare that this research study is my own orgininal work and that all references have been correctly recorded and acknowldeged, and that it has not previously been submitted in full or in part to any university for academic qualification. The thesis "Determination of metal contents in eggplants and their corresponding soil of the plant cultivated in Ethiopia" is conducted under the supervision of Prof. B. S. Chandravanshi, Department of Chemistry, and Addis Ababa University, Ethiopia.

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

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Approved by the examining board

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List of abbreviations

AAS	Atomic absorption spectrometry
ACS	American Chemical Society
AES	Atomic emission spectrometry
ANOVA	Analysis of variance
BDL	Below detection limit
CV	Coefficient of variance
EC	Electrical conductivity
FAAS	Flame atomic absorption spectrometry
IDL	Instrument detection limit
ICP-MS	Inductively coupled plasma-mass spectrometry
LOD	Limit of detection
MDL	Method detection limit
MP-AES	Micro plasma-atomic emission spectrometry
ND	Not detected
NR	No result
R	Correlation coefficient
R^2	Regression correlation coefficient
RSD	Relative standard deviation
SD	Standard deviation
TDS	Total dissolved solid
µs	Microsiemens
WHO	World Health Organization

Abstract

Eggplant is one of an edible vegetable in the world including Ethiopia. It is among the widely produce vegetable in the world. The aim of this study is to determine the metal contents of eggplants and their corresponding soil sample collected from different areas in Ethiopia (Bishoftu, Koka, Alemtena, Meki and Zuway). Levels of selected metals (Na, Ca, Mn, Fe, Cu, Ni, Zn, Pb and Cd) were determined using flame atomic absorption spectroscopy. After proper sample pretreatment, known weight of eggplant sample was wet digested using 4 mL of HNO_3, 1 mL of $HClO_4$ for 2:30 h at 270 ^oC and for soil sample 3 mL of HNO_3, 1 mL of HCl and 1 mL of $HClO_4$ for 2:45 h at 270 ^oC. Then using the optimized conditions sample preparation was made and levels of metals were determined by FAAS.

The mean concentration range (mg/kg) of each metal in eggplant were Na (1,384 - 1,917) > Ca (110 - 158) > Mn (34.5 - 44.2) > Fe (55.9 - 94.8) > Cu (4.3 - 10.0), Zn (17.9 - 29.2) > Pb (1.8 - 4.5) and in soil samples Fe (4,714 - 5,508) > Na (843 - 1,120) > Ca (237 - 788), Mn (617 -763) > Zn (50.7 - 99.4) > Cu (44.8 - 74.0) > Pb (13.8 - 15.8), respectively. Ni and Cd were below the detection limit. The result indicates that eggplant is a good source of essential macro and micro metals and free from toxic metal Cd but not free from the toxic element lead, the concentration of Pb are higher than the WHO guidelines 0.2 mg/kg. ANOVA indicated that there is no significant different between the mean concentration of Fe and Cu, and Na and Pb among eggplant and soil samples at 95% confidence level, respectively. The bioavailability of the metals in eggplant was investigated by analysis of soil pH, electric conductivity, total dissolved solid and salinity. Bioaccumulation factors exhibited a significantly higher accumulation of Na from the soil to the eggplant but for the other element it is less than one.

Keywords: eggplant, heavy metals, minerals, soil properties, flame atomic absorption spectrometry

1. Introduction

1. 1. Background of the study

Vegetable is any edible part of a plant or fungus (Alexis, Sahoré, François, 2014). Vegetables belonging to the *Solanaceae* family have great importance because they have around 90 genera and (3000–4000) species. *Solanaceae* families are widely used by humans as drugs and mainly for food. Such as potato, tomato, eggplant and bell pepper are an example of *Solanaceae* family. Eggplant has bitterness test because it contains glycol alkaloids. According to the most accepted hypotheses, eggplants were first domesticated before 4000 years ago in south East Asia (Knapp, Bohs, Nee, Spooner, 2004).

Solanum melangema belongs to Solanaceae family. The name of eggplant differs from country to country. The name of eggplant in different countries are brinjal in England baigan in India, and many more names all over the world. Eggplant is commonly known as “Deberjan” in Ethiopia. Eggplant is member of the *Solanaceae* plant which includes many species and varieties. Eggplant has different shapes, sizes and colors. The skin of the eggplant has many colors but the purple, green and white are more common (Persid, Verma, 2014). Different sizes, shapes and colors of eggplant fruits are shown in Figure 1 and 2.

Figure 1. Different color of eggplant.

Figure 2. Different shape of eggplant.

Some of the shapes of eggplant are oblong, ovoid, or long cylindrical. The presence of glycol alkaloids which are of wide occurrence in the plants of Solanaceae family gives bitter taste. (Khemnani *et al.*, 2012).

Solanum melongena L. a member of Solanaceae is generally cultivated as fruit vegetable in sub-tropical and tropical regions of the world. Eggplant has been cultivated for centuries in Asia, Africa and Europe. Vegetables are appreciated because they contribute with many nutrients to the human diet, such as macro minerals K, Mg, Na, P, and also contain trace minerals, such as Cu, Cr, Fe, Mn, Ni, Zn (Xiao, Ping, Xian, 2016).

Eggplant is a good source of minerals and vitamins. The nutritional value of eggplant and tomato are almost the same. Eggplant is a popular vegetable which is rich in dietary fiber, phenolic constituents, water soluble sugars and free reducing sugars. Eggplant contains vitamins such as B6, K and C. Eggplant is a good source of essential minerals such as K, Mg, Na, P, Cu, Cr, Fe, Mn, Ni and Zn (Yamaguchi *et al.*, 2019). Eggplant has high ability of absorbing large amounts of cooking fats (Jenkins *et al.*, 2003).

1. 2. Statement of the problem

According to FAO (2000), food security is achieved when it is ensured that all people, all the time, have physical, social and economic access to sufficient and safe food which meets their dietary needs for healthy life (Rattan *et al.*, 2009).

According to Lenntech (1998-2014) heavy metals have effects on human health. High level of lead causes low intelligence quotient in children, nervous system and brain and fetus damages. Excess zinc levels in humans causes several physiological disorders in body systems. High doses cadmium causes bone defects, renal dysfunction and lung cancer (Lenntech, 1998-2014). According to Khair (2009), heavy metals are the major contaminants of food. Because of their level of toxicity (Khair, 2009).

Plants accumulate heavy metals like Cd and Pb from contaminated soil and water. Humans and animals get affected by toxic heavy metals through the consumption of contaminated vegetables cultivated in contaminated soils and water (Kumar, Agrawal, Marshall, 2007).

Environmental factors like temperature, soil, water and genetic factors have their own effects in the chemical composition as well as the physical characteristics of eggplant. The heavy metals are widely distributed in environment, in soil, in plants and animal tissues. Due to this heavy metals have a great impact for the contamination of soil and water so heavy metals are also affects vegetables, plant and human health (Khairiah, Zalifah, Aminah, 2004).

It is important to determine the metal content of eggplant because its mineral contents depend on the mineral contents of soils. The aim of this study is to investigate macronutrients (sodium and calcium), micronutrients (manganese, iron, copper, zinc and nickel) and toxic status (lead and cadmium) in eggplants and their corresponding soil. Ethiopia is an agricultural country so studying soil quality is very important. Flame atomic absorption spectrometry (FAAS) technique has been employed for the determination of metal contents in eggplants and corresponding soils, which are collected from different places in Ethiopia. Eggplant is used as vegetable, now a time eggplant is one of the edible vegetable in Ethiopia. Many study have been conducted in a different country but not in Ethiopia. Heavy metals are the major contaminants of food so the mineral content of food should be determine. Determining the metal content of eggplant is conducted by this study.

1. 3. Significance of the study

The output of this research will have a multiple benefits when viewed from various perspectives. Since eggplant is one of edible vegetable in Ethiopia, identifying the metal contents of it is very important. Soil is essential for growth of vegetables so it is important to determine the metal contents as well as the pH, salinity, total dissolved solid and electric conductivity.

Mostly the metal contents of the vegetable depend on the metal contents of soil due to this, knowing the quality of soil is very important. The generated analytical data and information on the chemistry of the eggplant could be used as a baseline information for future researches in various fields related to Ethiopian eggplant productive areas.

This study creates awareness to the people to cultivate and use eggplant and introduce the importance of it for human health. This study was conducted to investigate the concentration of heavy metals in eggplants and their corresponding soil.

1. 4. Objectives of the study

1. 4. 1. General objective

The main objective of this study is to determine the metal contents in eggplants and their corresponding soil samples collected from the selected areas of Ethiopia by using flame atomic absorption spectrometry (FAAS).

1. 4. 2. Specific objectives

- To determine metal contents of eggplants and corresponding soil samples by using flame atomic absorption spectrometry (FAAS).
- To compare the results of this study with reported in literature.
- To study the correlation of the analyzed metals in the eggplants and their corresponding soil.
- To determine electrical conductivity, pH, salinity and total dissolved solid content of soil to check the bioavailability of metals in vegetables.

2. Literature review

2. 1. Eggplant

Eggplant is well adapted to high rainfall and temperatures. Eggplant is a high-yielding crop and it has an adaptation for both hot and wet environments. Eggplant is an especially important source of nutrients in the diets of low income consumers (Hanson *et al.*, 2006). Eggplant contains nutrients such as dietary fiber, folate, ascorbic acid, vitamin K, niacin, vitamin B6, pantothenic acid, potassium, iron, magnesium, manganese, phosphorus, and copper (USDA, 2009).

Vegetables (potato, tomato, eggplant and bell pepper etc.,) constitute an important part of the human's diet. They are a good source of important nutrients like carbohydrates, proteins, minerals and vitamins (Arai, 2002). Eggplant is a good source of antioxidants, anthocyanins and phenolic acids which are beneficial to human health (Gajewski, Katarzyna, Bajer, 2009).

2. 2. The importance of eggplant for human health

Healthy and unhealthy people must eat fruits and vegetables daily. Fruits and vegetables are valuable sources of vitamins and minerals so peoples should be eat fruits and vegetables daily (Milton, 2003).

Eggplant contains many bioactive constituents that have healthy effects, for example minerals, vitamins, and other products which have several medicinal properties (Nisha, Nazar, Jayamurthy, 2009). Eggplant contains phenolic compounds which, used to reduce risk of developing chronic diseases such as cardiovascular disease, cancer, diabetes and disorders with age (Yeh, Yen, 2005).

A vegetables that belongs to the Solanaceae family such as potato, tomato, eggplant and bell pepper are important for health, because of their nutritional value. (Helmja *et al.*, 2007). White eggplant used to treat type-2 diabetes because of its high-insoluble fiber and low soluble carbohydrate contents (Kwon, Apostolidis, Shetty, 2008).

Eggplant has been used in traditional medicine to treat many diseases. For example in Asia, it used for the treatment of skin problems. Eggplant used to ease urination and increase sex initiative as well as it used as a purgative (Meyer *et al.*, 2014). Eggplant is a good source of antioxidants like phenolic acids, which are important to human health. Eggplant used for the treatment of arthritis, asthma, cancer, diabetes and bronchitis. The skin of eggplant contains antioxidant which used as free radical scavenger. Eggplant contains measurable amount of oxalates which develops kidney and gallbladder problems (Noda, Kneyuki, Igarashi, 2000). Based on its free radical scavenging activity eggplant is ranked among the top ten vegetables (Nisha, Nazar, Jayamurthy, 2009).

2. 3. Heavy metals

The definitions of heavy metals are based on its density, weight and toxicity. Any metallic elements that have a density greater than 4 g/cm^3 are called heavy metals. Some heavy metals are toxic at low concentration (Lenntech, 2004).

Heavy metals are also known as trace elements, micronutrients, microelements and minor elements. Some heavy metals such as cobalt, copper, iron, manganese, molybdenum and zinc are essential in trace amounts for plants and animals. Chromium, nickel and tin are essential for animals but asenic, cadmium, mercury and lead have not been shown to be important for either or animals plants (Mitchell, Bear, 1964).

Eggplant grown on a soil absorbs and accumulates heavy metals by its roots, shoots and fruits (Marshall *et al.*, 2007). Heavy metals cause varies diseases to human being for example Pb causes neurological damage, blood disorder and hypertension (Mapanda *et al*., 2005). Increasing the heavy metal content in the soil also increases the heavy metals uptake by plants. The heavy metals uptake by plants depending upon the soil type, plant growth stage and plant species (Farooq, Anwar, Rashid, 2008).

2. 4. Minerals

Minerals are inorganic substances. Minerals present in all body tissues and fluids. They are necessary for the maintenance of certain physicochemical processes which are essential to life. Minerals are chemical constituents used by the body in many ways (Soetan, Olaiya, Oyewole, 2010).

Based on their relative amount in human body mineral are classified in to two main groups. Those are macro minerals and micro minerals. Macro minerals are occurring in relative large amounts in human body whereas micro minerals are occurring in small amounts. Macro minerals needed in quantities of 100 mg or more per day but micro minerals needed less than 100 mg per day. Calcium, magnesium, sodium and potassium are macro minerals. Fe, Cu, Mn, Co and Zn are micro minerals. If the amount of essential heavy metals are above the recommended value they became toxic. Cadmium and lead are toxic elements. (WHO, 1996; Sager, Hoesch, 2005). Minerals are involved in numerous biochemical processes. An acceptable intake of essential minerals is necessary for good health. The recommended daily intake of Ca, Cu, Fe, Mg, Mn, K, Zn, P and Na are 1000, 2, 18, 400, 2, 1000, 15, 4000 and 2400 mg per day, respectively (FNB, 2001). The physiological role of Na, Ca, Mn, Fe, Cu, Zn, Ni, Pb and Cd are briefly described below.

Sodium (Na)

Sodium is a relatively abundant vital mineral in the human body. Sodium is not synthesized by the body. Both plant and animals are the source of sodium. A minimum intake of 2 g/day would be required to ensure the organization in its functions and a maximum of 8 g/day may also be established. Higher intake of Na causes cardiovascular disease (Alexis, Sahoré, François, 2014).

Calcium (Ca)

The most abundant mineral in our body is calcium. Ca does not make metabolism. 99% of calcium contributes to the formation of strong bones and teeth. 1% of calcium is used for blood clotting, muscle contraction, neural conduction and hormone release. Milk, dark green leafy vegetables, and fish are the major source of calcium. Calcium makes stronger and more resistant vital organs. Ca is excreted in the feces and urine. The recommended daily intake of calcium is 0.8 to 1 gram per adult. But, the amount of Ca intake depends on the age and the activity of individual. Excess calcium increases the risk of kidney stones, cause problems of a psychological or nervous, loss of appetite, nausea and vomiting, and muscle pain (Alexis, Sahoré, François, 2014).

Manganese (Mn)

Mn plays an essential role in the activation of certain enzymes. It participates in the metabolism of sugars and in the synthesis of lipids, particularly cholesterol. It is indirectly involved in the synthesis of sex hormones. It also participates in the formation of the skeleton, connective tissue and the elimination of free radicals from the body. The body absorbs manganese inhalation and from the gastrointestinal tract. The manganese intake recommended for an adult is 1 to 2.5 mg /day. Vegetables contain more than animal products. It is found in tea, cereals, nuts and legumes. Deficiency of Mn causes blood cholesterol or altered reproductive functions. Over dose of Mn cause neurological headache and drowsiness. The daily intake of Mn should be less than 10 mg (Alexis, Sahoré, François, 2014).

Iron (Fe)

It is essential to the production of hemoglobin in red blood cells and smooth muscle function. Iron deficiency causes anemia. Anemia is usually accompanied by a reduction in physical and mental abilities, weakening of the immune system. An adult male should consume 16 mg of iron per day and a woman 9 mg. There are two types of iron: Heme iron of animal origin is present in meat (especially red meat), fish and animal products. It is well absorbed (about 25%). The non-heme iron content in products of plant origin, eggs and dairy products and is less well absorbed (only 5%). Some substances help iron absorption such as vitamin C, others hinder such as tea, coffee and fibers (Alexis, Sahoré, François, 2014).

Copper (Cu)

Copper is an essential trace element and human must have a diet that gives Cu to the body. The body contains less than one gram of copper. Cu is involved in the formation of hemoglobin in the maintenance of cartilage, bones, in the fight against infections and proper functioning of the heart. Copper is used in osteoarthritis. It regulates thyroid, helps the absorption of fat and strengthens the immune system. Copper deficiency leads to increased risk of cardiovascular disease, anemia and osteoporosis. Deficiency of Cu causes nausea, vomiting and liver impairment. The daily intake of Cu is 2 μg. Cu is found in liver, shellfish, nuts, chocolate, potatoes and beans. (Alexis, Sahoré, François, 2014).

Zinc (Zn)

Zn is an essential trace element which is used for synthesis of enzymes. Zn enzymes are used for the metabolism processes of carbohydrate, lipid, and protein synthesis or degradation. The large concentrations of zinc found in our red blood cells, eyes, skin, hair and liver. Deficiency of Zn causes growth retardation, digestive disorders, dry skin and hair loss. Zinc is a vital element that acts as an antioxidant and prevents the harmful effects of free radicals. The recommended dietary allowance for zinc is 15 mg per day. Fish, meat, grains, soy, eggs, wheat bread, wheat germ are rich in Zn. (Alexis, Sahoré, François, 2014).

Nickel (Ni)

Nickel is believed to play a role in physiological processes as a co-factor in the absorption of iron from the intestine. Nickel increased the absorption of iron from the diet in iron deficiency but only when dietary iron was in the unavailable ferric form (Das *et al.*, 2008). Contact with nickel compounds can cause a variety of adverse effects on human health, such as nickel allergy in the form of contact dermatitis, lung fibrosis, cardiovascular and kidney diseases and cancer of the respiratory tract. Acute health effects of Ni manifest as a variety of clinical symptoms (nausea, vomiting, abdominal discomfort, diarrhea, visual disturbance, headache, giddiness, and cough). The most common type of reaction to nickel exposure is a skin rash at the site of contact (Duda-Chodak, Blaszczyk, 2008).

Lead (Pb)

Lead serves no useful purpose in the human body, and its presence in the body can lead to toxic effects, regardless of exposure pathway. Lead toxicity influences brain, heart, kidneys, liver, nervous system, and pancreas. It may cause many signs and symptoms such as abdominal pain, anemia, anorexia, anxiety, bone pain, brain damage, confusion, constipation, convulsions, dizziness, drowsiness, fatigue, headaches and hypertension. It also diminishes IQ in children (Ali, 2003).

Cadmium (Cd)

Cadmium has no known nutritional value, and it has been considered an extremely significant pollutant affecting all life forms because of its high toxicity and high solubility in soil and water and easy accumulation in roots of most plant tissues. Excessive Cd exposure may give rise to renal, pulmonary, hepatic, skeletal, reproductive effects, and cancer (Nordberg, 2003). Intake of cadmium-contaminated food causes acute gastrointestinal effects, such as vomiting and diarrhea (Godt *et al.*, 2006).

2. 5. Soils

Soil serves as a natural medium for growth of plants (Addis, Abebaw, Pashikanti, 2017). Soil is composed from minerals, organic matter, water, and air. Soil is composed of minerals (45%), organic matter (5%), water (30%), and air (20 %). Soil organic carbon, N, P and K are the major indicators of soil quality and fertility. It used for growth of plants (Mccauley, Jones, Jacobsen, 2005).

Plants get nutrient from environmental compartment. Metals present in the soil fractions vary in degree of mobility or solubility (Boke, Megersa, Teju, 2015).

Soil is one of the most important resources of the nature. All living things depend on plants. Plants grow in soil for day to day need. Soils are medium in which crops grow to food and cloth so soil is important for living organisms directly or indirectly (Nwachokor, Uzu, Molindo, 2009).

Heavy metals occupy a special position in soil chemistry because they play very important physiological roles in nature. The contaminant concentration in soil mainly depends on the adsorption properties of soil matter. Conductivity, pH, moisture content are affect the solubility of heavy metal ions in the soil. Heavy metals may enter the food chain as a result of their uptake by edible plants (Addis, Abebaw, Pashikanti, 2017).

Soil is the material that is found on the earth's surface and is made of organic and inorganic material. Soil contains various dead organic matter and various types of living organisms. The soil interfaces with the lithosphere, the hydrosphere, the atmosphere and the biosphere. Soil is a major component of the Earth's ecosystem (Maurya, Keshar, Mishra, 2018). Heavy metals are non-degradable so they accumulate in the soil at toxic levels. Plants grown in these soils also accumulate metals in their tissues (Weldegebriel, Chandravanshi, Wondimu, 2012). Measuring the pH and electrical conductivity (EC) parameters provides valuable information for assessing soil condition for growth of plant, nutrient cycling and biological activity (Kadam, 2016).

2. 6. Physico-chemical properties and soil quality

2. 6. 1. pH

Soil pH refers to the acidity or alkalinity and it measures hydrogen ion in soil. The most significant property of soil is its pH level. Its effects on all other parameters of soil. Therefore, pH is considered while analyzing any kind of soil. In mineral soils, pH is a general indicator of soil nutrient availability, presence of free lime (calcium carbonate), presence of excess sodium, and excess hydrogen. If the pH is less than 6 then it is said to be an acidic soil, the pH range from 6-8.5, it is a neutral soil and greater than 8.5, it is said to be alkaline soil. (Kekane *et al*., 2015)

2. 6. 2. Electrical conductivity

Electrical conductivity is very important property of the soil. It is used to check the quality of the soil. It is a measure of ions present in solution (Tale, Ingole, 2015). The electrical conductivity of a soil solution increases with the increased concentration of ions. Electrical conductivity is a very quick, simple and inexpensive method to check health of soils. EC indicates how much dissolved salt is in a given sample. The quality of soil is controlled by physical, chemical and biological components of a soil and their interactions (Kadam, 2016).

2. 6. 3. Salinity

Soil salinity refers to the total salt concentration in the soil solution (i.e., the aqueous liquid phase of the soil and its solutes) consisting of soluble and readily dissolvable salts including charged species (e.g., Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Cl^{-}, HCO_3^{-}, NO_3^{-}, SO_4^{-2} and CO_3^{-2}), non-ionic solutes, and ions that combine to form ion pairs (Corwin, 2003).

2. 6. 4. Total dissolved solids (TDS)

Total dissolved solids (TDS) is a measure of the dissolved combined content of all inorganic and organic substances present in a liquid in molecular, ionized, or micro-granular (colloidal sol) suspended form.

2. 7. Analytical methods for determination of metals

Metals may be determined satisfactorily by a variety of methods, with the choice often depending on the precision and sensitivity required. These methods are gravimeter, electrochemistry, spectrometry and chromatographic method. Among those analytical methods, spectroscopic such as flame atomic absorption spectrometry, graphite furnace atomic absorption spectrometry, atomic emission spectrometry, inductively coupled plasma atomic emission spectrometry, microwave plasma atomic emission spectrometry and inductively coupled plasma spectrometry. Atomic spectroscopy is used to quantify the elemental concentration based on the amount of energy absorbed or emitted and sometimes to qualify (determine) which element is present. Flame emission atomic spectrometry and plasma atomic emission are the two atomic emission techniques which used for the determination of metal contents in vegetables or any other things.

3. Experimental

3. 1. Equipment and apparatus

Polyethylene bags were used to pack the eggplant and soil sample from the sample site. Plastic knife was used to separate the skin of the eggplant from the fruit. Electronic series balance (OPTECH, A205EC, Italy) with precision of ±0.0001 g was used for weighing both eggplant and soil sample. Ceramic mortar and pestle was used to grind eggplant and soil samples. 250 mL round bottom flask fitted with reflux condenser were used with Kjeldahl (United Kingdom) apparatus to digest the dried and powdered eggplant and soil sample.

The digested samples were placed in a refrigerator (Hitachi, Tokyo, Japan) until the time of analysis. Filtration funnels (Kenutuf, England) and different sizes of filter papers such as Whatman^{TM} filter paper 110 mm were used for filtration of sample solution after digestion for both optimization and sample preparation processes. Volumetric flasks (50 mL) were used during dilution of samples and preparation of metals standard solutions. HTL pipettes with 0.01 mL division with pipette tips and micro pipettes (0 - 100 µL and 100 - 1000 µL) made of USA were used for measuring reagents volume, used during optimization, sample preparation, preparation of standard solutions and spike solutions. pH/ion meter (WTW Inolab pH/ION Level 2, Germany) using unfilled pH glass electrode was used to measure the pH of soil sample.

The electrical conductivity (EC), salinity and total dissolved solids (TDS) of soil and water samples were measured using Thermo Orion EC meter (USA). Magnetic stirrer was used to stir the mixture of soil sample with distilled water.

During the determination of the electrical conductivity (EC), salinity and total dissolved solids (TDS) of soil sample, glass beaker were used to place soil sample for the preparation of soil solution. ZEEnit 700p (Germany) flame atomic absorption spectrometry (FAAS) was used for analysis of the metals (Na, Ca, Mn, Fe, Cu, Zn, Ni, Pb and Cd).

3. 2. Chemicals and reagents

All chemicals and reagents used in the study were of analytical grade. 69.5% HNO_3 (69.5% reagent grade, ACS, ISO, European Union), 70% $HClO_4$ (Research-lab fine chem indusrties, India) and 37% HCl (Riedel-de Haen AG, Germany). A solution which has known electric conductivity (12.88 µs) was used for the calibration of the instrument which was used measure the electric conductivity, salinity and total dissolved solid of soil samples. A buffer solution which has known pH was used to calibrate the pH-meter which was used to measure soil samples were used for digestion of both eggplant and soil samples. Stock standard solution containing 1000 mg/L of the metal Na, Ca, Mn, Fe, Cu, Zn, Ni, Pb, and Cd were used for the preparation of calibration standard and for the spiking experiments

3. 3. Instrumentation

The analytical technique which was used in this research for determination of levels of selected heavy metals in eggplant part and their corresponding soil sample was flame atomic absorption spectrometery (FAAS) (ZEEnit 700p, Germany) equipped with deuterium ark background corrector, nebulizer, monochromator, detector, flame, data processor and hallow cathode lamps corresponding to metals of interest after destruction of organic matric. The operating parameters of FAAS for analysis of the metals are shown in Table 1.

Table 1. The operating parameters of FAAS for analysis of the metals.

Metals	Na	Ca	Mn	Fe	Ni	Cu	Zn	Pb	Cd
Wavelength (nm)	589	422	279	248	232	324	213	283	228
Lamp current (mA)	3	3	5	5	3	2	2	2	2
Slit width (nm)	0.8	0.2	0.2	0.3	0.2	1.2	0.5	1.2	1.2
Energy (kJ)	77.7	73.1	67	69	72.3	89.9	71.6	79.3	89.9
Oxidant	Air	Air	Air	Air	Air	Air	Air	Air	Air
Lamp	HCL	HCL	HCL	HCL	HCL	HCL	HCL	HCL	HCL
PMT(v)	256	300	369	464	300	300	300	300	300

PMT= photo multiplier tube

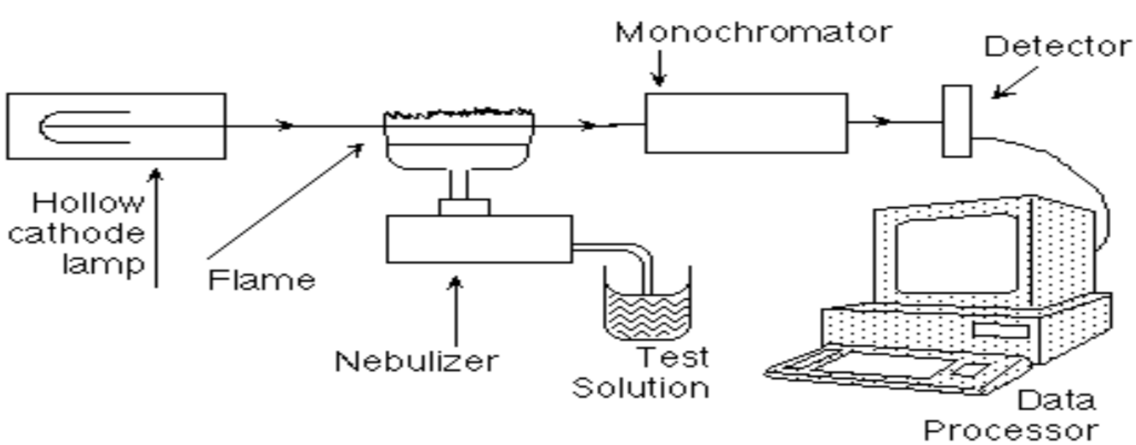


Figure 3. Schematic diagram of FAAS experimental setup.

3. 4. Description of sampling sites

The sample sites are located in Oromia region (Bishoftu, Koka, Meki, Ziway and Alemtena). These sample sites were selected due to their maximum production of eggplant in Ethiopia. In the area vegetables like eggplant, tomato, onion, cabbage, green pepper, potato were cultivated throughout the year. Modern farming practices like mechanized farming, application of agro-chemicals and selected seeds are significant agricultural inputs for getting better yield in the area. The geographical locations (latitude, longitude and elevation) of sampling sites are given in Table 2.

Table 2. The location of sampling site.

Sampling site	Latitude	Longitude	Elevation (m)	Distance from Addis Ababa (km)
Bishoftu	38°58'42.46" E	8°45'8.10"N	1,920	47.9
Koka	$39^0$1'54.45" E	$8^0$26'27.56" N	1603	90
Meki	38°49′E	8°9′N	1636	130
Zuway	38°43' E	7° 56'N	1,643	169
Alemtena	38.95°E	8.30°N	1,611	115 km

3. 5. Working procedures

3. 5. 1. Cleaning apparatus

All glass wares such as volumetric flask, round bottom flask, beaker, measuring cylinder, pipette, mortar and pestle were washed with detergents and tap water then soaked with dilute nitric acid for three days and then rinsed in distilled water. The glass wares were dried in hot air oven and kept in clean place till analysis begins.

3. 5. 2. Collection and preparation of eggplant and soil samples

About one kilogram eggplant were collected from five different sample sites using polyethylene bags. The eggplant samples were kept in polyethylene bags and transported to Analytical Chemistry laboratory of Addis Ababa University. In laboratory the skin of the eggplant was separated from the fruit and chopped the fruit into small size using plastic knife in order to facilitate drying. The samples were placed in laboratory till it dried. The dried samples were ground into powder using mortar and pestle and then sieved to 0.425 mm mesh size. The sieved samples were stored in polyethylene bags and placed in clean dry places free of contamination until the time of digestion.

The soil samples were collected from five sample sites. The soil samples were collected from the base of the uprooted of vegetable using auger and properly labeled and filled in polyethylene bag. Each soil sample was dried at ambient temperature for fifty days and crushed into powder using mortar and pestle and then sieved to 0.425 mm mesh size. The sieved samples stored in polyethylene bags and placed in clean dry places free of contamination until the time of digestion.

3. 6. Sample decomposition

The purpose of sample decomposition is for converting all the species in which a given element is present in such a way that it becomes present in one defined form, eliminating interfering substances from the matrix, and obtaining the element in a homogeneous and easily accessible matrix (Quevauviller, 1995). The digestion methods could be classified in to wet digestion, acid digestion, dry ashing, and microwave digestion. In this study wet digestion method was used.

3. 6. 1. Dry ashing

This method of sample preparation involves the incineration of samples in muffle furnace at a suitable temperature between 400 and 600 ^{o}c. The resulting ash from organic compound dissolve in dilute acids. Magnesium nitrate is commonly used as an ashing aid.

Dry ashing is a convenient and versatile method for preparing food samples for instrumental analysis. It allows use of relatively large sample sizes and can minimize contamination from reagents and needs minimal attention from the operator. Some disadvantages of dry ashing include time consuming operation, problems may be caused by incomplete combustion and absorption on the surface of the incineration crucibles and losses through volatilization.

3. 6. 2. Wet digestion

The decomposition of a sample by the addition of liquid reagents in order to solubilise it. Samples to be analysed for elemental metal content are usually prepared by digesting the matrix in a strong acid like $HClO_4$, HCl, H_2SO_4 and HNO_3. In the case of organic matrices, an oxidizing mixture is used to destroy the entire organic matrix and solubilize the sample. Wet acid digestion is one of the methods that are involved to get free metal ions in dissolved form from complex organic matrix based on changing different digestion parameters like volume ratio of reagents added, digestion temperature and digestion time.

Nitric acid is commonly used, because there is no chance of forming insoluble salts as might happen with $HClO_4$, HCl or H_2SO_4 as well as it is good oxidizing agent. Nitric acid is rarely used alone. It is best used in combination with sulfuric and/or perchloric acids for organic sample digestion. The only element that may be lost from a nitric/perchloric digestion is Hg. (Quevauviller, 1995).

3. 7. Optimization of digestion procedure

Optimization of digestion procedure is important to create optimum working conditions before starting analysis of real samples. Which means before preparing for analysis the volume of reagents, digestion time and temperature should be optimized. Wet acid digestion is one the method which is usually used for samples of organic matrix is based on varying different digestion parameters like volume ratio of reagents, digestion time and temperature. Kjeldhal apparatus is one of Wet acid digestion apparatus in which organic components are assumed to be decomposed in the form of different gaseous forms and other metallic elements are left in the solutions. The Optimization of digestion procedure was determined based on the usage of smaller reagent volume, shorter digestion time and reasonable mild temperature for obtaining clear and colorless solutions of the resulting digests.

3. 7. 1. Optimization of digestion procedure for eggplants

For each of the eggplant samples, 0.5 g of powder and homogenized was balanced and transferred to 250 ml of a round bottom flask. Then different volumes of HNO_3 and $HClO_4$ at quantified proportions (v/v) was added and digested at different temperatures (150, 180, 210, 240, 270 and 300 ^{o}C) for different period of time (105, 120, 135, 165 and 180 min). The optimized procedure was determined based on the formation of a clear colorless solution. The digested solution were allowed to cool and 5 mL of distilled water was added to dissolve residue formed on cooling and gradually swirled and filtered into 50 mL volumetric flask through Whatman 93 filter paper. Then the clear solution was diluted up to 50 mL volumetric flask distilled water. Finally, it was stored for analysis using Flame atomic absorption spectrometry.

Hence the optimization procedure for the sample preparation for the determination of macro, micro and toxic metal contents were made as shown in Table 3

Table 3. Different conditions tested for optimization of digestion procedure for 0.5 g of eggplant samples.

	Trial	Reagents used	Reagent volume ratio(mL)	Temperature (°C)	Digestion time(h)	Observation
Optimization for reagent volumes	1	HNO_3:$HClO_4$	3:1	300	3:00	Cloudy colorless
	2	HNO_3:$HClO_4$	2:2	300	3:00	Clear light yellow
	3	**HNO_3:$HClO_4$**	**4:1**	300	3:00	**Clear and colorless**
	4	HNO_3:$HClO_4$	3:2	300	3:00	Clear light yellow
	5	HNO_3:$HClO_4$	5:1	300	3:00	Clear and colorless
	6	HNO_3:$HClO_4$	4:2	300	3:00	Deep yellow
Optimization for time	1	HNO_3:$HClO_4$	4:1	300	1:45	Clear and light yellow
	2	HNO_3:$HClO_4$	4:1	300	2:00	Clear and light yellow
	3	HNO_3:$HClO_4$	4:1	300	2:15	Clear and light yellow
	4	**HNO_3:$HClO_4$**	**4:1**	300	**2:30**	**Clear and colorless**
	5	HNO_3:$HClO_4$	4:1	300	2:45	Clear and colorless
	6	HNO_3:$HClO_4$	4:1	300	3:00	Clear and colorless
Optimization for temperature	1	HNO_3:$HClO_4$	4:1	150	2:30	Yellow solution
	2	HNO_3:$HClO_4$	4:1	180	2:30	Yellow solution
	3	HNO_3:$HClO_4$	4:1	210	2:30	Clear and light yellow
	4	HNO_3:$HClO_4$	4:1	240	2:30	Cloudy and colorless
	5	**HNO_3:$HClO_4$**	**4:1**	**270**	**2:30**	**Clear and colorless**
	6	HNO_3:$HClO_4$	4:1	300	2:30	Clear and colorless

The bold font shows the optimized volume ratio, time and temperature.

3. 7. 2. Optimization of digestion procedure for soil sample

For each of the soil samples, 0.5 g of powder and homogenized was balanced and transferred to 250 mL of a round bottom flask. Then different volumes of HNO_3, $HClO_4$ and HCl at quantified proportions (v/v) was added and digested at different temperatures (150, 180, 210, 240, 270 and 300 ^oC) for different period of time (105, 120, 135, 165 and 180 min). The optimized procedure was determined based on the formation of a clear colorless solution.

The digested solution were allowed to cool and 5 mL of distilled water was added to dissolve residue formed on cooling and gradually swirled and filtered into 50 mL volumetric flask through Whatman 93 filter paper. Then the clear solution was diluted up to 50 mL volumetric flask distilled water. Finally, it was stored for analysis using flame atomic absorption spectrometry (FAAS). Hence the optimization procedure for the sample preparation for the determination of macro, micro and toxic metal contents were made as shown in Table 4

Table 4. Different conditions tested for optimization of digestion procedure for 0.5 g of soil samples.

	Trial	Reagents used	Reagent volume ratio (mL)	Temperature (^oC)	Digestion time (h)	Observation
Optimization for reagent volumes	1	HNO_3:HCl:$HClO_4$	1:1:1	300	3:00	Deep yellow
	2	HNO_3:HCl: $HClO_4$	2:1:1	300	3:00	Deep yellow
	3	HNO_3:HCl:$HClO_4$	3:2:1	300	3:00	Light yellow
	4	**HNO_3:HCl:$HClO_4$**	**3:1:1**	300	3:00	**Clear and colorless**
	5	HNO_3:HCl:$HClO_4$	6:2:2	300	3:00	Clear and colorless
	6	HNO_3:HCl:$HClO_4$	6:1:1	300	3:00	Clear light yellow
Optimization for temperature	1	HNO_3:HCl:$HClO_4$	3:1:1	150	3:00	Deep yellow
	2	HNO_3:HCl:$HClO_4$	3:1:1	180	3:00	Clear light yellow
	3	HNO_3:HCl:$HClO_4$	3:1:1	210	3:00	Clear light yellow
	4	HNO_3:HCl:$HClO_4$	3:1:1	240	3:00	Clear and light yellow
	5	**HNO_3:HCl**:$HClO_4$	**3:1**:1	**270***	3:00	**Clear and colorless**
	6	HNO_3:HCl:$HClO_4$	3:1:1	300	3:00	Clear and colorless
Optimization for time	1	HNO_3:HCl:$HClO_4$	3:1:1	270	1:45	Deep yellow
	2	HNO_3:HCl:$HClO_4$	3:1:1	270	2:00	Clear and light yellow
	3	HNO_3:HCl:$HClO_4$	3:1:1	270	2:15	Clear and light yellow
	4	HNO_3:HCl:$HClO_4$	3:1:1	270	2:30	Clear and light yellow
	5	**HNO_3:HCl**	**3:1:1**	**270**	**2:45**	**Clear and colorless**
	6	HNO_3:HCl	3:1:1	270	3:00	Clear and colorless

The bold font shows the optimized volume ratio volume ratio, time and temperature.

3. 8. Digestion of eggplant samples

Using the optimized conditions (Table 3 and 4), 0.5 g eggplant powder sample were transferred to 250 mL round bottom flask and 4 mL 69.5% HNO_3 and 1 mL 70% $HClO_4$ was added. Then the solution was digested on a Kjeldhal digestion for the optimized time 2:30 h at the optimized temperature 270 ^oC. After 2:30 h of the digestion time the digested solution was permitted to cool to room temperature for about 30 min without disassembling the condenser.

Then at the time of disassembling the setup, 10 mL of distilled water was added to the solution. This was done by rinsing the neck of the round bottom flask and tip of condenser that was in contact to dissolve the residue formed to cooling and minimizing dilution of filter paper by digest residue while filtering with Whatman 110 mm filter paper into 50 mL volumetric flask.

Finally, the cooled solution was filled up to the mark with distilled water. This procedure was done three times for each eggplant samples. The digestion of the blank reagent was done in simultaneously with the samples. All the solutions were stored in refrigerator until the analysis was done. The solutions were used to determine the concentration of the target analyte of the sample.

3. 9. Digestion of soil samples

Using the optimized conditions (Table 3), 0.5 g soil powder samples were weighed and transferred to 250 mL round bottom flask and 5 mL of 3:1:1 ratio of HNO_3, HCl and $HClO_4$ were added. Then the solution was digested on a KJELDHAL digestion for the optimized time 2:30 h at the optimized temperature 270 ^oC. After 2:30 h of the digestion time the digested solution was permitted to cool to room temperature for about 30 min without disassembling the condenser.

Digested solutions were allowed to cool and 20 mL distilled water was added to dissolve the residue formed on cooling and vigorously swirled and then filtered into 50 mL volumetric flask through Whatman 110 mm filter paper. Finally the clear solution was diluted by distilled water. This procedure was done three times for each soil samples. The digestion of the blank reagent was done simultaneously with the samples. All the solutions were stored in refrigerator until the analysis was done. The solutions were used to determine the concentration of the target analyte of the sample.

3. 10. Determination of pH, conductivity, salinity and TDS in soil samples

Soil pH, conductivity, salinity and total dissolved solid (TDS) of prepared soil samples were determined by mixing 10 g of soil with 25 mL of distilled water in 50 mL beaker. The mixture was stirred by magnetic stirrer for 30 min. Then pH was measured after calibrating the pH meter with technical pH buffer of 4.01 and 10.00 pH value, the other three parameters (conductivity, salinity and TDS) were determined after direct calibration with standard solution.

3. 11. Analysis of eggplant and soil samples

For the analysis of all eggplant and soil samples calibration of the instrument with known concentration of standard solutions were done for each target analyte. Calibration metal standard solutions were prepared for each of the metals from an intermediate standard solution containing 10 mg/L which was prepared from the atomic absorption spectrometry standard stock solutions that contained 1000 mg/L. These standards were diluted with deionized water to obtain four working standards for each metal of interest.

The working standard solutions were prepared based on the sensitivity of the instrument for the particular metal. Three replicate determinations were carried out for each samples. All the metals which were determined by absorption and concentration mode and instrument read out was recorded for each samples of eggplant and soil and the blank solution of both eggplant and soil sample. The metal Na, K, Ca, Mn, Fe, Ni, Cu, Zn, Pb and Cd were analyzed by Flame atomic absorption spectrometry (FAAS) in both eggplant and soil samples.

3. 12. Method performance and validation

Based on the information from the correlation coefficients and their corresponding calibration curves of each metal it is possible to say the two variable concentration and intensity have a good positive correlation and linearity.

3. 12. 1. Precision and accuracy

Precision is the closeness or agreement between a replicate measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The common term used to measure variability is relative standard deviation (RSD. RSD is the parameter of choice for expressing precision in analytical sciences. In this study, the precision of the results were evaluated by the pooled standard deviation, and relative standard deviation of the results of fifteen measurements for a given sample (i.e. five samples (n = 5) and triplicate readings for each sample).

3. 12. 2. Method detection limit (MDL) and Method of quantification limit (MQL)

Method detection limit is the minimum concentration that can be detected by the analytical method with a given certainty. It is also the smallest concentration or amount of analyte that can be reliably shown to be present measured under specific confidence level. Limit of detection can calculated by multiplying the standard deviation of the blank (SD_b) three times ($LOD = 3SD_b$).

The detection limit (with all steps of the analysis included) is called the MDL. The practical method for determining the MDL is to analyze samples of concentration near the expected limit of detection. The limit of quantification (LOQ) is the minimum concentration of the that can be quantified and it is 10 time of the standard deviation (Boke *et al.*, 2015). The instrumental detection limit, method detection limit and method quantification are given in Table 5.

In this case, the value of method detection limit of each element's is more or less higher than that of the instrumental detection. This confirms that the method was good and acceptable. The limit of quantification also greater than the limit of detection. Both the limit of detection and quantification of the eggplant and were less than that of soil, except sodium. Based on the information in Table 5 instrumental detection limit is less than that of method detection limit in all metals.

Table 5.The instrumental detection limit, method detection limit and method of quantification.

Metals	IDL (mg/kg)	Eggplant sample		Soil sample	
		MDL (mg/kg)	MQL (mg/kg)	MDL (mg/kg)	MQL (mg/kg)
Na	0.02	1.7	5.7	1.1	3.7
Ca	0.025	0.14	0.46	0.48	1.6
Mn	0.02	0.16	0.50	0.52	1.7
Fe	0.04	0.47	1.6	0.52	5.2
Cu	0.035	0.14	0.47	0.43	1.43
Zn	0.012	0.38	1.3	0.83	2.8
Pb	0.30	0.32	1.1	0.48	1.6

Where IDL is instrumental detection limit, MDL is method detection limit and MQL is method of quantification limit.

3. 13. Validation of optimized procedure

The efficiency of the optimized procedure was verified by adding the known concentration of each meatal to 0.5 g sample of eggplant and soil. The experiment was done the validity of the optimized procedure for each eggplant and soil sample that were collected from the sample sites. The spiked and none spiked samples were digested and analyzed in the same way using optimized procedure of sample analysis. The percentage of the analytes were calculated by using the formula:

$$\text{Percent recovery} = \frac{\text{Spiked sample} - \text{Unspiked sample}}{\text{Amount added}} \times 100$$

The results obtained from recovery analysis are shown in Table 6 and the percentage of recovery lied between 93 up to 109% and 94 up to 103% for eggplant and soil samples respectively, which were within the acceptable range for all metals. Recovery test for the optimized procedure of eggplant and soil samples are given in Table 5.

Table 6. Recovery test results for the optimized procedure of eggplant and soil samples.

Metals	Eggplants				Soil			
	Na	Ca	Fe	Zn	Na	Ca	Fe	Zn
Unspiked sample (mg/kg)	1,451 ± 1	110 ± 1	94.8 ± 0.6	17.9 ± 0.3	1,016 ± 1	266 ± 1.	5,508 ± 1	44.8 ± 0.2
% spiking	20	50	50	50	20	50	20	50
Amount added (mg/kg)	290	55	47	9	203	133	1102	22
Spiked sample (mg/kg)	1721 ± 1	170 ± 1	140 ± 1	27 ± 0.1	1226 ± 1	391 ± 1	6,588 ± 1	70 ± 0.1
% Recovery	93	109	96	101	103	94	98	100

4. Results and discussion

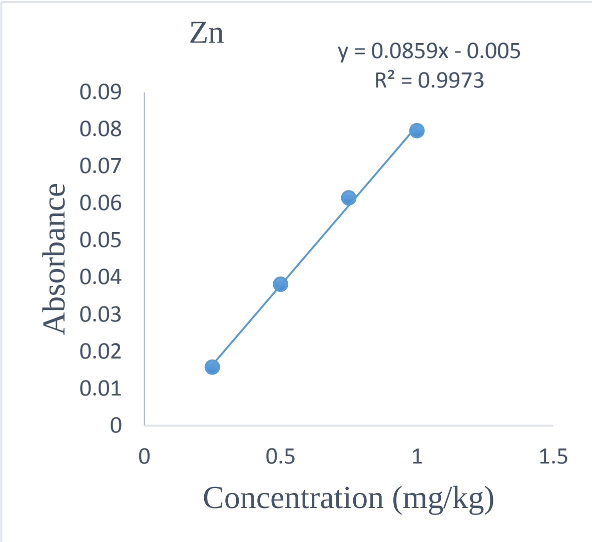
The concentration of metals in both eggplants and their corresponding soils samples were determined by using flame atomic absorbtion spectrometry and the precision of the result was evaluated as mean and standard deviation. Triplicate analysis were done to determine the mean values of each samples and triplicate samples were used for all sample sites.

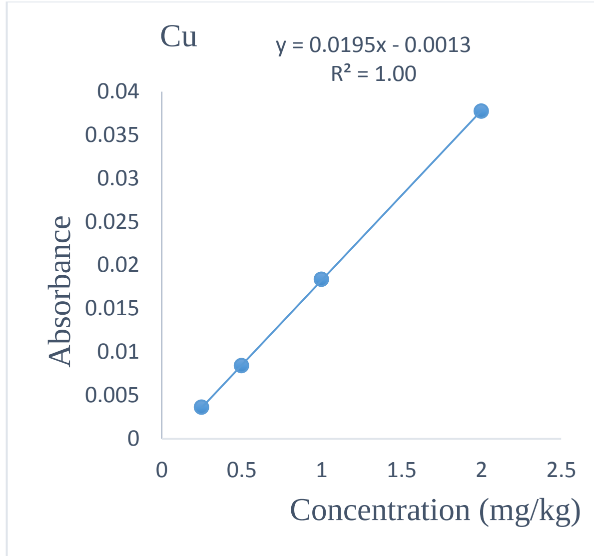
4. 1. Calibration of the instrument in the determination of selected metals by FAAS

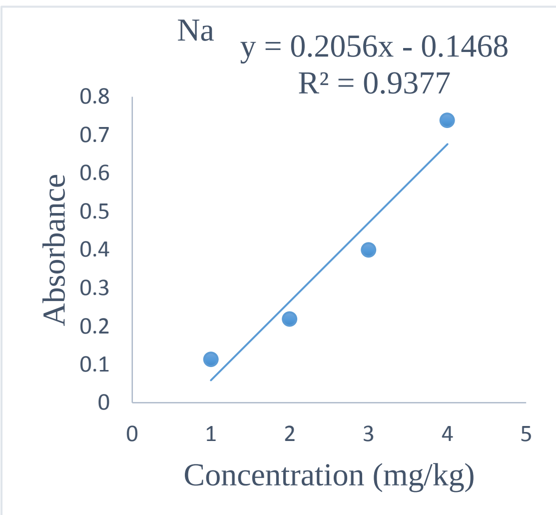
The metals (Na, Ca, Mn, Fe, Cu, Zn, Ni, Pb and Cd) in the digest of eggplant and soil samples from five different areas were determined three times by FAAS. Calibration curves were prepared to determine the concentration of metals in the sample solutions. Calibration curve exhibited good linearity with R^2 values ranged >0.99. Based on the information from the correlation coefficients and their corresponding calibration curves of each metal it is possible to say the two variable concentration and absorbance have a good positive correlation and linearity. Concentration of the working standards, calibration equation and correlation coefficient of the calibration curve for each metals are shown in Table 6 and Figure 7.

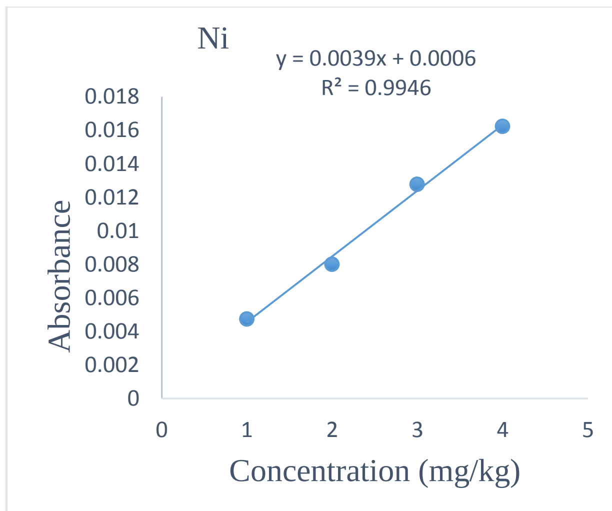
Table 7. Working standard concentration, calibration equation and correlation coefficient of each metals.

Metals	Concentration of standard(mg/kg)	Correlation coefficient	Calibration equation
Na	1, 2, 3, 4	0.9377	y = 0.2056x - 0.1468
Ca	0.25, 0.5, 1, 2	0.9998	y = 0.0141x - 0.006
Mn	0.5, 1, 2, 4	0.9973	y = 0.0182x + 0.0129
Fe	0.25, 0.5, 1, 2	0.9994	y = 0.007x - 0.0011
Cu	0.25, 0.5, 1, 2	1.000	y = 0.2056x - 0.0013
Zn	0.25, 0.5, 0.75, 1	0.9973	y = 0.0859x - 0.005
Pb	1, 2, 3, 4	0.9962	y = 0.0024x - 0.006
Ni	1, 2, 3, 4	0.9946	y = 0.0039x + 0.006
Cd	0.25, 0.5, 0.75, 1	0.9851	y = 0.0336x - 0.0013


Zn
y = 0.0859x - 0.005
R² = 0.9973
Absorbance
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
0.5
1
1.5
Concentration (mg/kg)


Cu
y = 0.0195x - 0.0013
R² = 1.00
Absorbance
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0
0.5
1
1.5
2
2.5
Concentration (mg/kg)


Na
y = 0.2056x - 0.1468
R² = 0.9377
Absorbance
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
Concentration (mg/kg)


Ni
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R² = 0.9946
Absorbance
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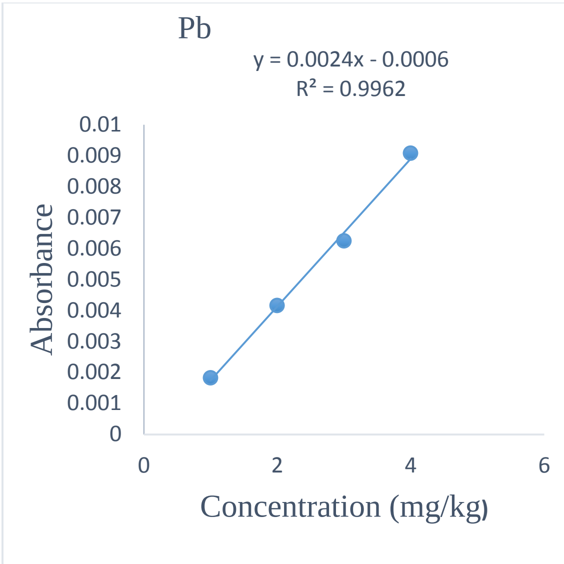
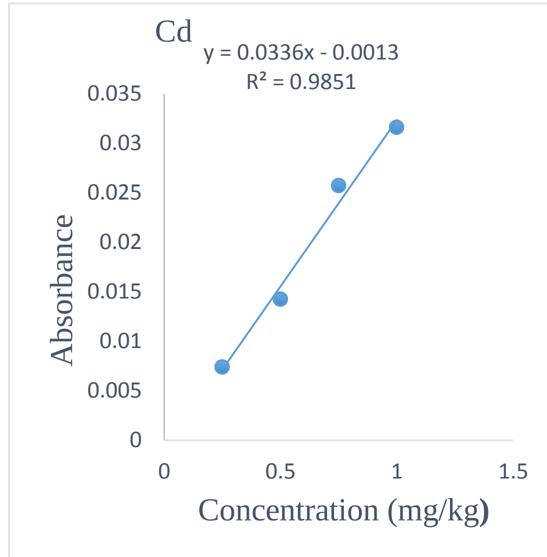
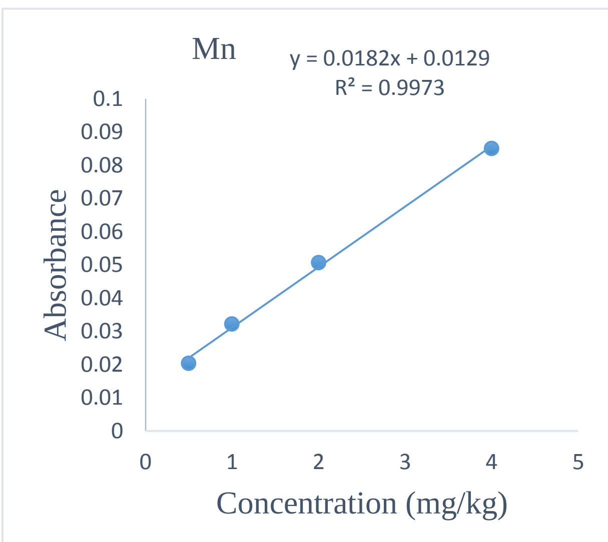
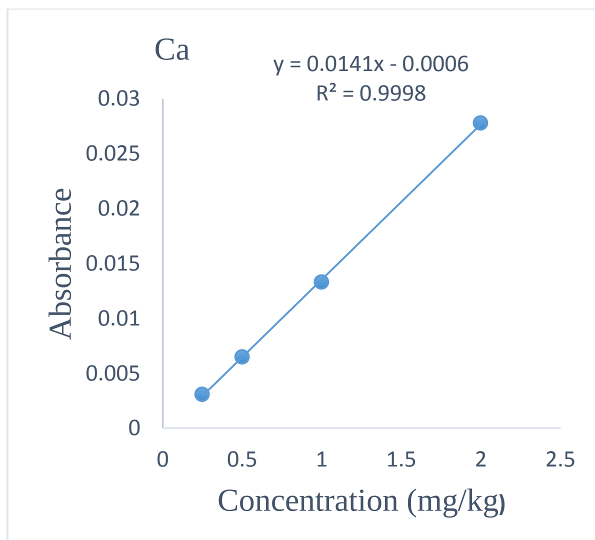
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
0
1
2
3
4
5
Concentration (mg/kg)


Pb
y = 0.0024x - 0.0006
R² = 0.9962
Absorbance
0.01
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
0
2
4
6
Concentration (mg/kg)


Cd
y = 0.0336x - 0.0013
R² = 0.9851
Absorbance
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0
0.5
1
1.5
Concentration (mg/kg)


Mn
y = 0.0182x + 0.0129
R² = 0.9973
Absorbance
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
1
2
3
4
5
Concentration (mg/kg)


Ca
y = 0.0141x - 0.0006
R² = 0.9998
Absorbance
0.03
0.025
0.02
0.015
0.01
0.005
0
0
0.5
1
1.5
2
2.5
Concentration (mg/kg)

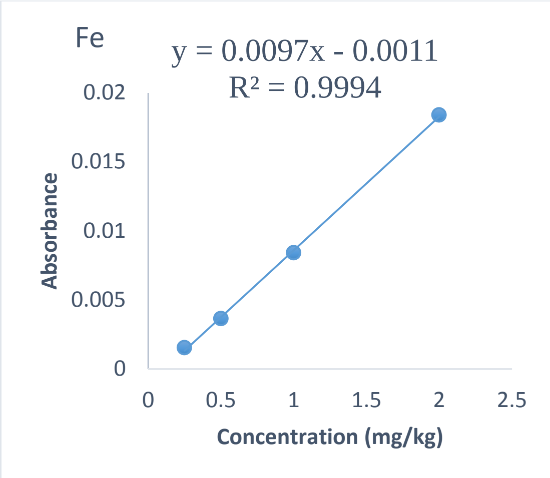


Figure 4. The calibration curves of the studied elements.

4. 2. The concentration of metals in eggplant samples

The result of the analysis showed that metal contents of eggplant samples were varied between the areas in which the plant was cultivated. This variation is attributed to the mineral composition of soil and water in which the plant has been grown and environmental factors influence the mineral content of the plant.

As shown in Table 8 and Figure 5 the concentration of metals in eggplant samples collected from Bishoftu was decreased as Na > Ca > Fe > Mn > Zn > Cu > Pb. The pattern of concentration of the element in eggplant collected from Meki, Alemtena and Zuway are the same as that of Bishoftu. But the distribution of the concentration of metals in eggplant samples collected from Koka was decreased as Na > Ca > Fe > Mn > Zn > Pb > Cu.

The maximum overall mean levels in the eggplant samples of elements Na, Ca, Mn, Fe, Cu, Zn and Pb are 1,917, 158, 44.2, 94.8, 10.0, 29.2 and 4.5 mg/kg, respectively. The minimum overall mean concentration levels in eggplant samples of elements Na, Ca, Mn, Fe, Cu, Zn and Pb are 1,384, 110, 34.5, 55.9, 4.3, 17.9 and 1.8 mg/kg, respectively.

As shown in Table 8 the results of concentrations of metals showed that in eggplant samples, the higher amounts of Na and Pb are obtained from the sample that collected from Koka and as compared other sample site. The concentration of Ca, Cu and Fe are higher in Alemtena, Meki and Bishoftu sample sites, respectively as compared to other sample sites.

The concentration of Mn and Zn are higher in the sample site of Zuway as compared to other sample sites. From the selected metals mean concentrations in eggplant samples from five different sampling sites, the concentrations Na are highest than other metals mean concentrations in each sample sites and the mean concentrations of Pb are lowest than other metals mean concentrations in each sample sites except Koka sample site. The concentrations of Ni and Cd in eggplant samples collected from five different sample sites are below detection limit.

Table. 8. Concentration of metals (mg/kg, relative standard deviation, n = 3) in eggplant samples.

Metals	Concentration of metals (mean ± SD, mg/kg , n = 3)				
	Bishoftu	Koka	Alemtena	Meki	Zuway
Na	1,451 ± 1	1,917 ± 1	1,858 ±1	1,384 ± 1	1,600 ± 1
Ca	110 ± 1	114 ± 1	158 ± 1	120 ± 1	135 ± 1
Mn	39.5 ± 0.1	34.5 ± 0.2	41.8 ± 0.4	43.3 ± 0.2	44.2 ± 0.3
Fe	94.8 ± 0.6	85.3 ± 0.3	55.9 ± 0.3	72.2 ± 0.1	85.0 ± 0.2
Cu	6.40 ± 0.02	4.3 ± 0.02	6.5 ± 0.01	10.0 ± 0.3	6.7 ± 0.01
Zn	17.9 ± 0.1	18.2 ± 0.2	26.3 ± 0.5	21.6 ± 0.4	29.2 ± 0.2
Pb	4.20 ± 0.01	4.5 ± 0.01	1.8 ± 0.01	2.9 ± 0.01	3.6 ± 0.03
Ni	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND

Where, ND is not detected.

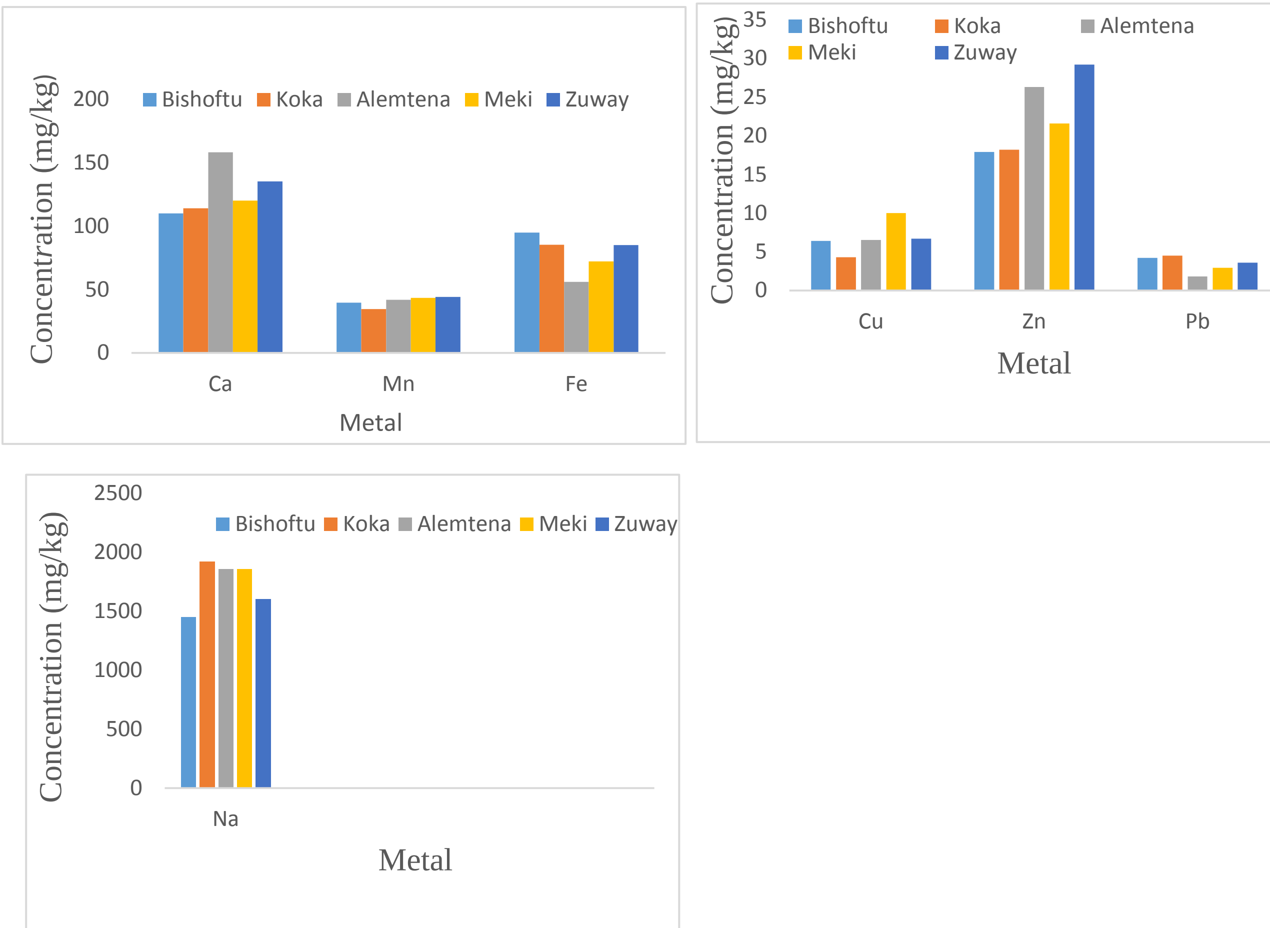


Figure 5. The comparison of the concentration of metal in eggplant samples

4. 3. The concentration of metals in soil samples

The result of the analysis showed that metal contents of soil samples were varied between the areas in which the plant was cultivated for some metals. This variation attributed to the mineral composition of soil and fertilizers in which the plant has been grown and environmental factors influence the mineral content of the plant.

As shown in Table 9 and Figure 7 the distribution of the concentration of metals in soil samples that collected from Koka, Alemtena and Meki were decreased as Fe > Na > Mn > Ca > Zn > Cu > Pb. The distribution of the concentration of metals in soil samples that collected from Bishoftu was decreased as Fe > Na > Mn > Ca > Cu > Zn > Pb. The distribution of the concentration of metals in eggplant samples that collected from Zuway was decreased as Fe > Na > Mn > Ca > Zn > Cu > Pb.

The maximum overall mean concentration levels in the soil samples of elements Na, Ca, Mn, Fe, Cu, Zn and Pb are 1,120, 788, 763, 5,508, 74.0, 99.4 and 15.8 mg/kg, respectively. The minimum overall mean concentration levels in soil samples of elements Na, Ca, Mn, Fe, Cu, Zn and Pb are 843, 237, 617, 4,714, 44.8,50.7and 13.8 mg/kg, respectively. As shown in Table 8 the results of concentrations of metals showed that the in soil samples the higher amounts of Na, Ca, Zn and Pb were obtained from the sample that collected from Zuway as compared to other sample site. The higher concentration of Mn, Cu and Fe were obtained from the sample that collected from Bishoftu as compared to other sample sites. From the selected metals mean concentrations in soil samples, the concentrations Fe are highest than other metals mean concentrations and the mean concentrations of Pb are lowest than other metals mean concentrations in each sample sites. The concentrations of Ni and Cd in soil samples are below detection limit.

Table 9. Concentration of metals (mg/kg, relative standard deviation, n = 3) in soil

Metals	Concentration of metals (mean ± SD, mg/kg , n = 3)				
	Bishoftu	Koka	Alemtena	Meki	Zuway
Na	1,016 ± 1	1,083 ± 1	1,320 ± 2	843 ± 1	1,120 ± 2
Ca	266 ± 1	248 ± 2	396 ± 1	237 ± 1	788 ± 1
Mn	763 ± 1	649 ± 2	653 ± 1	617 ±1	628 ± 1
Fe	5,508 ± 1	4,714 ± 1	5,010 ± 2	4,970 ± 1	5,355 ±1
Cu	74.0 ± 0.2	50.7 ± 0.2	58.3 ± 0.2	51.3 ± 0.2	74.0 ± 0.2
Zn	44.8 ± 0.2	55.5 ± 0.2	73.9 ± 0.4	51.5 ± 0.5	99.4 ± 0.2
Pb	15.0 ± 0.1	14.9 ± 0.1	13.8 ± 0.1	14.8 ± 0.2	15.8 ± 0.2
Ni	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND

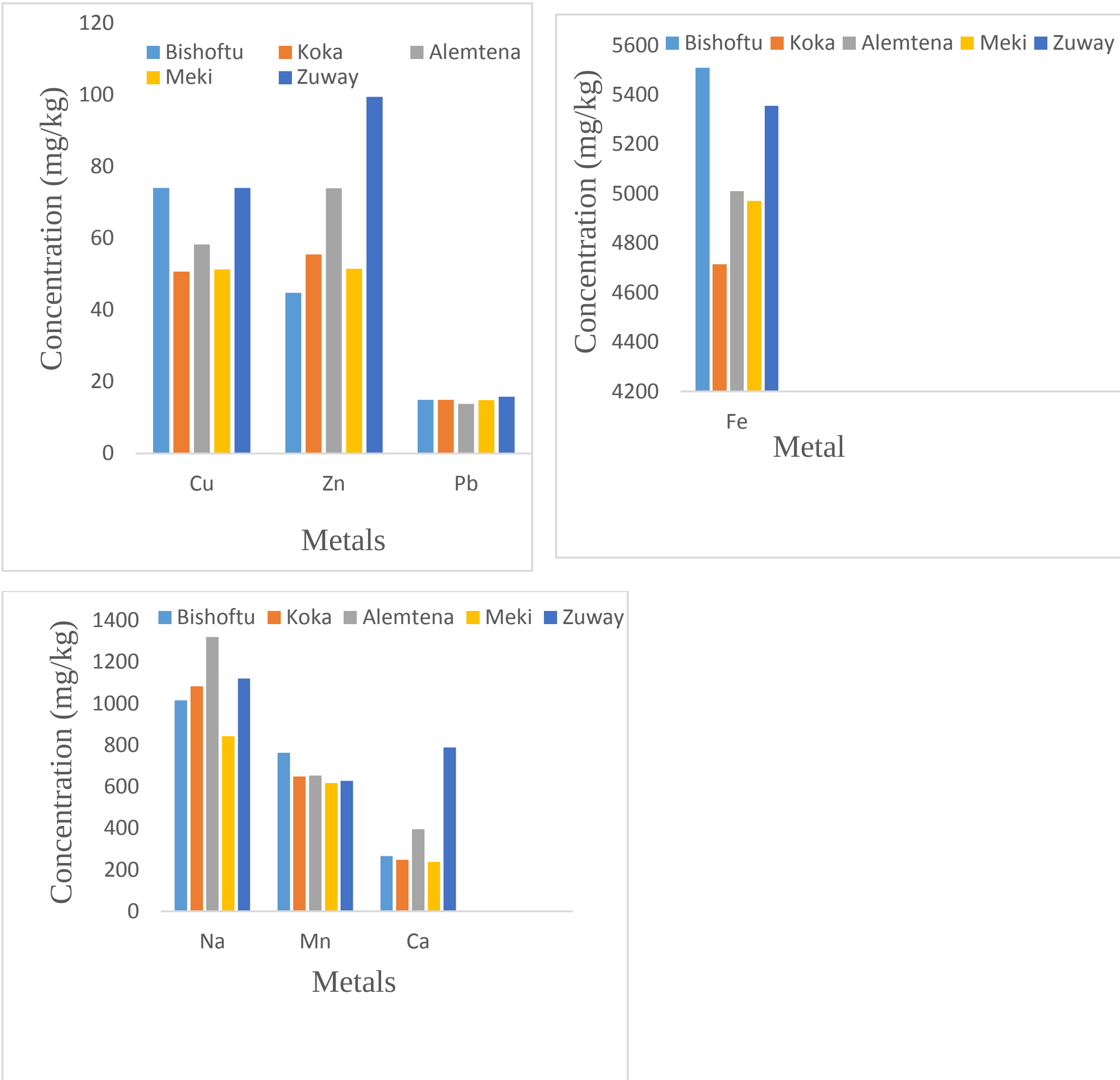


Figure 6. The comparison of the concentration of metal in soil samples.

4. 4. Comparison of average metal concentrations (mg/kg) in eggplant and soil samples

The ranges of the concentrations of the metals determined by FAAS in eggplant and soil samples collected from five samples sites (Bishoftu, Koka, Alemtena, Meki and Zuway) are given in the Table 9. The overall mean concentrations determined (mg/kg) in the eggplant s samples were in the ranges of Na (1,384 - 1,917) > Ca (110 - 158) > Fe (55.9 - 94.8) > Mn (34.5 - 44.2) > Cu (4.3 - 10.0) Zn (17.9 - 29.2) > Pb (1.8 - 4.5) and in the soil samples Fe (4,714-5,508) > Na (843 - 1,120) > Ca (237 - 788 Mn (617 -763)) > Zn (50.7 - 99.4) > Cu (44.8 - 74.0) > Pb (13.8-15.8), respectively.

Table 10. Metals concentration (mg/kg) range in eggplants and their corresponding soil sample from the five study areas.

Metals	Range of the metal concentration (mg/kg)	
	Eggplant	Soil
Na	1,384-1,917	843-1,120
Ca	110-15 8	237-788
Mn	34.5-44.2	617-763
Fe	55.9-94.8	4,714-5,508
Cu	4.3-10.0	44.8-74.0
Zn	17.9-29.2	50.7-99.4
Pb	1.8- 4.5	13.8-15.8

4. 5. Comparison of average metal concentrations (mg/kg) of eggplant and soil sample

Based on the data obtained from the Table 10 and Figure 6, except the concentration of sodium the concentration of other metal in the soil is higher than that of the eggplant. This is due to the capability plant species to up take metal sodium. The concentration of Fe is higher with compared to the concentration of other metals in the soil, this may be due to the low capability of the plant that uptake Fe or the soil is rich in Fe by nature or different man made factories. The overall mean concentrations of metals that determined in five sample sites of eggplant were Na > Ca > Fe > Mn > Zn > Cu > Pb. The overall mean concentrations of metals that determined in five sample sites of soil were Fe > Na > Mn > Ca > Zn > Cu > Pb.

Table 11. Comparison of average metal concentrations (mg/kg) of eggplant and soil samples.

Metals	Eggplant	Soil
	Mean of the five sites	Mean of the five sites
Na	1,642	1076
Ca	127	387
Mn	40.7	662
Fe	78.6	4021
Cu	6.8	62.0
Zn	22.6	65.0
Pb	3.4	14.8

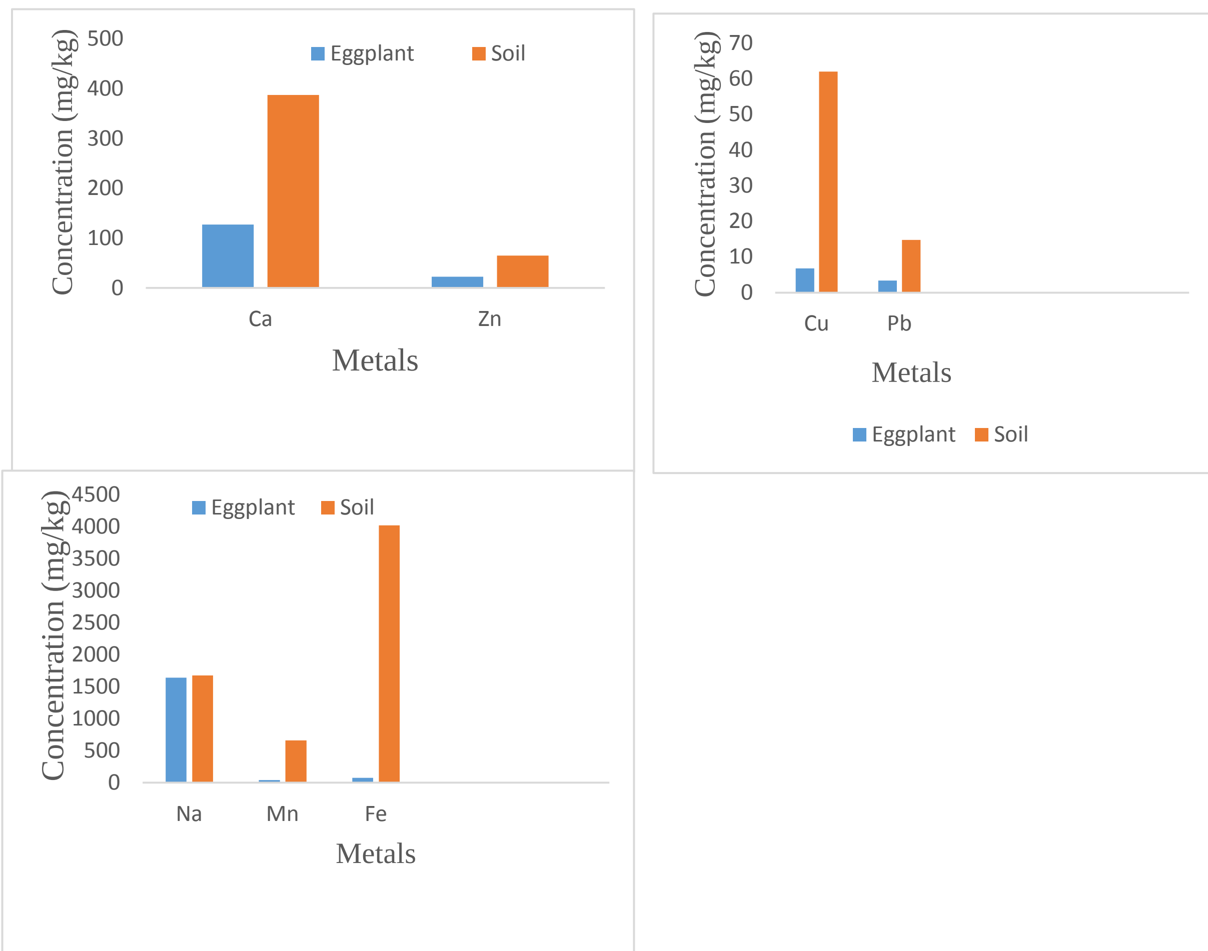


Figure 7. Comparison of average metal concentrations (mg/kg) of eggplant and soil samples.

4. 6. Accumulation factor of metals from soil to plants

Transmission of metals from soil to plant is studied using an index which is called transfer factor (TF). It is calculated as a ratio of the concentration of a specific metal in the plant to the concentration of same metal in soil both represented in the same units. If TF is greater or equals to one the absorption of metal from soil by the plant is higher. On the other hand, lower value TF indicates the poor response of plants towards metal absorption and the plant can be used for human consumption. Loading and accumulation of metals in the soil depend on different factors such as the chemical form of elements, pH, and organic matter content as well as electric conductivity (Mirecki, 2015).

TF is calculated by the formula: $TF = C_{plant} / C_{soil}$

Where, C_{plant} and $C_{soil\ are\ the\ concentration}$ of metals in the plant and the concentration of metals in the soil respectively.

Metal transfer factor from soil to plant is a key component of human exposure to metals via the food chain. Transfer factor of metals is essential to investigate the human health risk. Table 12 summarizes the TF values for were eggplants collected from five sample sites. The range of TF values of eggplant was 1.41–1.77, 0.171– 0.506, 0.046 – 0.070, 0.01 – 0.017, 0.086 – 0.111, 0.293 – 0.419 and 0.1 30 – 0.302 for Na, Ca, Mn, Fe, Cu, Zn and Pb, respectively. The distribution of metals in eggplant was Na > Ca > Zn > Pb > Cu > Mn > Fe. The TF value of Na is higher than the other metals in all sample sites and its TF value is greater than one, this indicates that the obtained sodium from other sources (water, fertilizers and air). The TF value of the metal Ca, Mn, Fe, Cu, Zn and Pb were less than one in all sample sites. This indicates high metal concentration in soil in relation to levels in the eggplant plant this reflect low uptake of metals by eggplant from the soil, therefore , lower values indicate the poor response of plants toward metal absorption and the plants can be used for human consumption.

Table 12. Transfer factor (TF) values of eggplant samples.

Metals	Sample sites				
	Bishoftu	Koka	Alemtena	Meki	Zuway
Na	1.43	1.77	1.41	1.64	1.43
Ca	0.486	0.460	0.399	0.506	0.171
Mn	0.052	0.053	0.046	0.071	0.070
Fe	0.017	0.018	0.011	0.015	0.016
Cu	0.086	0.085	0.111	0.195	0.091
Zn	0.399	0.328	0.356	0.419	0.293
Pb	0.282	0.302	0.130	0.196	0.229

As show in Figure 7 the transfer factor of sodium, iron and lead are higher in Koka sample site. The transfer factor of Ca, Cu, Mn and Zn are higher in Meki sample site. The transfer factor of Na, Mn, Fe and Pb are lower in Alemtena sample site. The transfer factor of Ca and Zn is lower in Zuway sample site where as the lower transfer factor of Cu was observed in Koka sample.

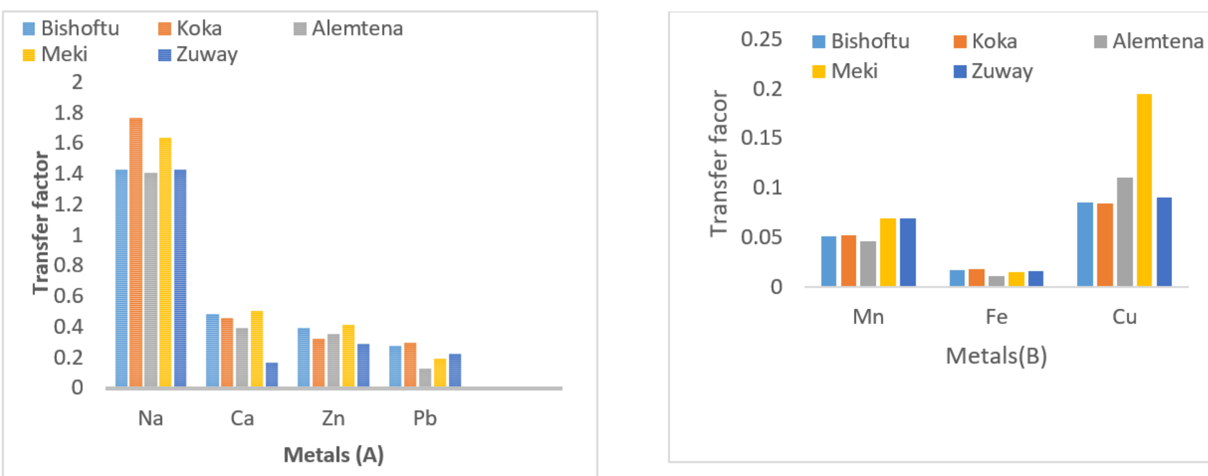


Figure 8. Transfer factor (TF) values of eggplant sample which is collected from five different sample site.

4. 7. Comparison of the heavy metal contents in eggplant sample with other reported values

As shown in Table 13, the concentrations of Mn and Pb in this study are higher than the concentrations of Mn and Pb in other reported values. The concentration of Cd is determine by (Persid, Verma, 2014), (Uddin, Islam, Baten, 2016) and (Fevzi, 2005) but it is not detected in this study. The concentration of Cu is lower in the study of (Rashid et al., 2015) with compare to this study. The concentration of Fe is higher in the study of (Uddin, Islam, Baten, 2016) with compare to other study including this study. The concentration of Zn is higher in the study of (Demirezen, Akso, 2005) with compare to this study. The concentration of Ni is determine by (Demirezen, Akso, 2005) and (Fevzi, 2005) but it is below not detected in this study.

Table 13. Comparison of heavy metals mean concentration (mg/kg) of the eggplant with reported in the literature.

Zn	Cu	Mn	Fe	Ni	Cd	Pb	Country origin	Reference
21.4	8.4	13.4	63.4	NR	2.40	3.24	South America	Persid, Verma, (2004)
35.8	0.13	28.8	96.8	NR	NR	NR	Bangladesh	Rashid et al., 2015
53.9	22.2	NR	NR	2.18	0.30	3.3	Turkey	Demirezen, Akso, (2006)
16.4	11.7	NR	285	NR	NR	0.24	Bangladesh	Uddin et al., (2016)
39.7	14.4	8.2	NR	1.5	1.1	NR	Turkey	Fevzi, (2005)
22.6	6.8	40.7	78.6	ND	ND	3.4	Ethiopia	This study

NR and ND are no result and not detected, respectively.

4. 8. Analysis of variance (ANOVA)

Analysis of variance (ANOVA) is a statistical technique that used to compare data sets. Analysis of variance (ANOVA) is a statistical method that used to test differences between two or more variables. When we are comparing more than three groups based on one factor variable, then it said to be one way analysis of variance. ANOVA uses the F statistic to compare whether the difference between sample means is significant or not (Hepp *et al.*, 2014). If $F_{calculated}$ (the ratio of SD between samples to SD within samples) is greater than $F_{critical}$ (the value obtained from the table at specified confidence level and degree of freedom), in sample means are significant. If $F_{calculated}$ (the ratio of SD between samples to SD within samples) is less than $F_{critical}$ (the value obtained from the table at specified confidence level and degree of freedom), in sample means are not significant.

In this study, eggplant and soil samples were collected from five different areas and the metal levels of each sample was analyzed by FAAS. During the processes of sample preparation and analysis a number of random errors may be introduced in each aliquot and in each replicate measurement. The variation in sample mean of the analyte was tested by using ANOVA, whether the source for variation was from experimental procedure or heterogeneity among the samples (i.e. difference in mineral contents of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides etc or other variations in cultivation procedures).

If $F_{calculated}$ larger than $F_{critical}$ and $p < 0.05$, there is significant difference between the sample, so based on this idea results of variance analysis showed that there were significant differences in the mean values of Na, Ca, Mn, Zn and Pb between eggplant samples but there was no significant difference in the mean values of Fe and Cu between the samples.

Table 14. Analysis of variance (ANOVA) between and within eggplant samples at 95% confidence level.

Parameters	Na	Ca	Mn	Fe	Cu	Zn	Pb
$F_{Calculated}$	55.4	38.5	32.4	0.445	2.00	6.60	29.0
$F_{Critical}$	3.48	3.48	3.48	3.48	3.48	3.48	3.48
p–value	8.7×10^{-7}	4.7×10^{-7}	1.1×10^{-5}	0.774	0.171	0.007	1.8×10^{-5}

If $F_{calculated}$ higher than $F_{critical}$ and $p < 0.05$, there is significant difference between the sample, so based on this idea results of variance analysis showed that there were significant differences in the mean values of Na, Ca, Mn, Fe, Cu and Zn between eggplant samples but there was no significant difference in the mean values of Pb between the samples.

Table 15. Analysis of variance (ANOVA) between eggplant and within soil samples at 95% confidence level.

Parameters	Na	Ca	Mn	Fe	Cu	Zn	Pb
$F_{Calculated}$	4.74	12.8	9.40	13.3	21.4	9.41	0.548
$F_{Critical}$	3.48	3.48	3.48	3.48	3.48	3.48	3.48
p–value	7.6×10^{-7}	0.001	0.002	0.001	6.9×10^{-5}	0.002	0.705

4. 9. Pearson correlation of metals

The Pearson correlation coefficient (r) is used to measure the strength of linear association between two variables and attempts to draw a line of best fit through the data of two variables. The Pearson correlation coefficient (r) indicates that how far away all these data points are to the line best fit. A correlation coefficient of +1 indicates that there is a strong (perfect positive) correlation relationship between the two variables this means the one variable increases the other variables also increase and vise verse. A correlation coefficient of -1 indicates that there is a weak (negative) correlation relationship between the two variables this means the one variable increases the other variables decrease and Vis verse.

The correlation values are categorized as no correlation (r = 0.00-0.19), low correlation (r = 0.20-0.39), medium correlation (r = 0.40-0.59), higher correlation (r = 0.60-0.79) and highest correlation (r = 0.8-1.00) (Melina et al., 2016). Linear regression correlations test were performed to investigate the correlation between metal concentration in the eggplant and soil samples are summarized in Tables 16-18.

According to Table 16 the correlation coefficients between metals in eggplant shows highest correlation between Zn/Mn and Cu/Na. Medium positive correlation between Mn /Ca, Zn/Ca, Cu/Ca and Ca/Na. Pb/Fe and Cu/Mn have low positive correlation. Zn/Cu has no correlation coefficient between them. All the correlation between Ca/Na, Mn/Na, Fe/Na, Zn/Na, Pb/Na, Fe/Mn, Cu/Fe, Zn/Fe, Zn/Na, Pb/Ca, Pb/Cu, Fe/Ca and Pb/Zn show negative correlation.

Table 16. Pearson correlation coefficients between metals concentrations in eggplant samples.

	Na	Ca	Mn	Fe	Cu	Zn	Pb
Na	1.00						
Ca	0.5356	1.00					
Mn	-0.0484	0.5225	1.00				
Fe	-0.3520	-0.9238	-0.6668	1.00			
Cu	0.8538	0.4181	0.2790	-0.4043	1.00		
Zn	-0.2552	0.4518	0.9009	-0.4695	0.0340	1.00	
Pb	-0.6720	-0.5123	-0.2064	0.2047	-0.4754	-0.4011	1.00

According to Table 17 the correlation coefficients between metals in soil shows highest correlation between Cu/Fe, Pb/Na and Pb/Ca. Medium positive correlation between Zn/Na, Zn/Ca, Fe/Mn, Cu/Mn, Zn/Cu, Fe/Ca and Pb/Mn. The correlation coefficients between metals Ca/Na, Mn/Na, Cu/Na and Pb/Zn in the soil sample have low positive correlation. The correlation coefficients between metal Zn/Fe and Pb/Cu in the soil have no correlation coefficient between them. All the correlation between Fe/Na, Mn/Ca, Cu/Ca, Zn/Mn and Pb/Fe have negative correlation.

Table 17. Pearson correlation coefficients between metals concentrations in soil samples.

	Na	Ca	Mn	Fe	Cu	Zn	Pb
Na	1.00						
Ca	0.3791	1.00					
Mn	0.3718	-0.2579	1.00				
Fe	-0.0348	0.4217	0.4433	1.00			
Cu	0.3789	-0.2782	0.4849	0.9068	1.00		
Zn	0.5236	0.5642	-0.2782	0.1836	0.4519	1.00	
Pb	0.8202	0.8202	0.5642	-0.1386	0.1747	0.3213	1.00

According to Table 18 the correlation between Na with Ca, Mn, Fe, Cu, Zn and Pb have negative correlation, this indicates that when the concentration of Na increase in both eggplant and soil the concentration of Ca, Mn, Fe, Cu, Zn and Pb decreases and vice versa. Calcium has higher correlation with the Mn, Fe, Cu, and Pb as well as it has highest correlation with Zn. This shows the when the concentration of Ca increases/ decreases the concentration of Zn, Mn, Fe, Cu, and Pb also increases/decreases.

Cu has higher correlation with Mn and Fe this indicates that when the concentration of Cu increases the concentration of Mn and Fe, if its concentration decreases the concentration of Mn and Fe also decreases. Zn has strong positive correlation with Mn, Fe and Cu. Pb has strong positive correlation with Mn, Fe and Cu. The correlation between Fe and Mn is very strong, its value is 0.9903. This indicates that Fe/Mn has a good linearity in the eggplant and soil sample.

Table 18. Pearson correlation coefficients between metals concentrations in eggplant and soil samples.

	Na	Ca	Mn	Fe	Cu	Zn	Pb
Na	1.00						
Ca	-0.4408	1.00					
Mn	-0.8104	0.5930	1.00				
Fe	-0.8269	0.6503	0.9903	1.00			
Cu	-0.5553	0.6410	0.8359	0.8544	1.00		
Zn	-0.6089	0.92244	0.8022	0.8357	0.7391	1.00	
Pb	-0.7237	0.6161	0.9270	0.9019	0.6670	0.8071	1.00

4. 10. Important soil properties

4. 10. 1. Physicochemical properties of soil

The physicochemical properties of soil of the eggplants farmlands are given in Table 20. Based on the information given in Table 19, the pH lied between 6.81 and 8.13. The highest pH (8.13) was found in Koka farmland soil and the lowest pH (6.81) was found in Bishoftu farmland soil. Thus, the pH of all the farmland soil samples is near to neutral and in the range of the normal pH range of soil 6.5-8.4 which used for cultivation of vegetable. Based on this range all the farmland soil samples have excellent quality for cultivation purposes. The highest conductivity of 303 µs and total dissolved solid (TDS) of 176 mg/L was found in Zuway farmland soil, and the lowest conductivity of 208 µs and total dissolved solid (TDS) of 124 mg/L was found in Bishoftu farmland soil sample. Except Bishoftu farmland soil sample all the farmland soil samples have the same salinity value which is 0.2%.

Table 19. Mean concentration (mean ± SD), pH, conductivity, salinity and total dissolved solid (TDS) of soil sample.

Sample Site	pH mean ± SD	Conductivity (µs) mean ± SD	Salinity (%) mean ± SD	Total dissolved solid (TDS) (mg/L)
Bishoftu farmland	6.81 ± 0.02	208 ± 4	0.1 0.0	124 ± 3
Koka farmland	8.13 ± 0.03	282 ± 2	0.2 0.0	134 ± 2
Meki farmland	7.60 ± 0.04	293 ± 5	0.3 0.0	133 ± 3
Alemtena farmland	7.80 ± 0.03	287 ± 3	0.2 ± 0.0	143 ± 2
Zuway farmland	8.10 ± 0.02	303 ± 3	0.2 ± 0.0	1763

5. Conclusions and recommendation

The study determined levels of metals Na, Ca, Mn, Fe, Ni, Cu, Zn, Pb and Cd in eggplants and their corresponding soils which collected from different sample sites in Ethiopia. Metal were determined using FAAS. The concentration of Ni and Cd were below detection limit. The investigation of the metal levels also revealed that for most of the metals determined, there was a direct relationship between the levels in the eggplant and those in the soil in which the plant was grown. The study also showed that the metals were present at different concentrations in the samples from different sites.

Comparable results were found with some of the values reported in the literature and for Pb metals; the concentrations slightly exceeded the permissible levels by WHO, which could be attributed to the agricultural practices employed such as the use of fertilizer and herbicides. The bioavailability of in the plant was checked by analysis of soil some physicochemical properties of soil. The study showed the metal accumulation factor from the soil to the plant by calculating the transfer factor.

The metal contents of eggplant were not studied before so this study will give information for future researcher. The toxic metal Pb was detected. The source of lead in soil should be decreased as much as possible. The soil of Ethiopia is comfortable for the cultivation of eggplant so every farmer can be cultivated it in every agricultural season.

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