**DEBRE BIRHAN UNIVERSITY**

**COLLEGE OF NATURAL SCIENCE DEPARTMENT OF CHEMISTRY**

# INVESTIGATION OF CONCETRATION OF Fe, Mn, Cu AND Zn IN PEA IN WOGDIE WOREDA

**BY:**

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**ID PGS /375/09**

**FEBRUARY, 2021**

**Debre berhan**

**DEBRE BIRHAN UNIVERSITY**

**COLLEGE OF NATURAL SCIENCE DEPARTMENT OF CHEMISTRY**

# ASSESSMENT OF TRACE METAL (Fe, Cu, Zn AND Mn) IN SOIL AND PEA SAMPLE IN WOGDIE WOREDA THREE KEBELE SOUTH WOLLO ZONE AMHARA REGIONAL STATE

**BY:**

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**A PARTIAL FULFILMENT FOR THE REQUIREMENT OF MASTERS OF DEGREE IN CHEMISTRY**

**ADVISOR; TESFAYE DEMSSIE (Ph.D.)**

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**DEBRE BERHAN UNIVERSITY**

**POST GRAGUATE**

**COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCEs**

# APPROVAL SHEET I

This is to certify that the thesis entitled “ASSESSMENT OF TRACE METALS IN SOIL AND PEA SAMPLES IN WOGDIE WOREDA IN SOUTH WOLLO ZONE AMHARA REGIONAL STATE” submitted in partial fulfillment of requirements Degree of Master of Science in Chemistry, College of Natural and Computational Science, Debre Berhan University and is a faithful record of original research work carried out by Amlakie Girma under my guidance and supervision of Tesfaye Demssie (PhD). No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by him from various sources during the course of the investigation has been duly acknowledged. Therefore, I recommend that it be accepted as fulfilling the thesis requirements.

| | | |
|---|---|---|
| Tesfaye Demssie (Ph.D.) | ________________ | _________________ |
| Major Advisor | Signature | Date |

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# APPROVAL SHEET II

We, the undersigned members of the boarded of the examiners of the final open defense by Amlakie Girma have read and evaluated his thesis entitled "ASSESSMENT OF TRACE METALS IN SOIL AND PEA SAMPLES IN WOGDIE WOREDA THREE KEBELE IN SOUTH WOLLO ZONE AMHARA REGIONAL STATE" and examined the candidates. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree of Masters of Science in Chemistry.

| | | |
|---|---|---|
| TESFAYE DEMSSIE | ____________________ | ____________________ |
| Major Advisor | Signature | Date |
| ____________________ | ____________________ | ____________________ |
| Name of internal examiner | Signature | Date |
| ____________________ | ____________________ | ____________________ |
| Name of chairman | Signature | Date |

# STATEMENT OF THE AUTHOR

I declared and affirm that the work described in this thesis is my own original work and this thesis where works of any other investigator has been properly recognized through citation. This thesis has been submitted in partial fulfillment of the requirements for MSc. degree at Debre Berhan University. I also certainly declared that this thesis has neither been submitted elsewhere nor is being currently submitted; for the award of any academic degree, diploma, or certificate.

Name: Amlakie Grma

Date:________________________

Department:____________

Signature:____________________

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## Acronyms

| | |
|---|---|
| **AEZ:** | **Agro ecological zones** |
| **ANOVA:** | **Analysis of Variance** |
| **DA:** | **Development Agents** |
| **DNA:** | **Deoxyribonucleic Acid** |
| **EBI:** | **Ethiopian Biodiversity Institute** |
| **EIAR:** | **Ethiopian Institute of Agricultural Resea** |
| **FAO:** | **Food and Agricultural Organization of the United Nations** |
| **GP:** | **Germination Percentage** |
| **MW :** | **micro wave digestion** |
| **LW:** | **lomi wuhha** |
| **WHO:** | **World health organization** |
| **IDL** | **instrumental detection limit** |
| **LOD** | **limit of detection** |
| **LOQ** | **limit of quantification** |
| **OM** | **organic matter** |
| **ICP-OES** | **Inductively coupled plasma Emission spectroscopy** |

*ABSTRACT*

*The pea were cultivated at the high land areas that can be absorbed and collected important heavy metals which potentially important to human health. The current study determined the concentration of potentially important metals from soil and pea samples that grown in high land farmland and used to people. Essential elements required for nutrition of crop plants some of these trace elements are Fe, Mn, Zn,and Cu. A reduction in their concentration in soils results in unhealthy low intake by plants and consequently domestic animals and human beings.*

*From the analysis the range the concentration level of copper maximum mean concentration is 182.433 ± 5 and minimum mean concentration 4.977± 0.212, zinc maximum mean concentration is 1.97 ± 0.97 and minimum mean concentration 0.663± 0.0.04, Iron maximum mean concentration is 8.72 ± 0.30 and minimum mean concentration 2.802 ± 00.172, Manganes maximum mean concentration is 1.852 ± 0.079 and minimum mean concentration 0.526± 0.024 except manganes the other three are within the permissible limit of WHO .which implies that the concerned body should consider the amount since the accumulation of manganes causes poor bone* ***health*** *and symptoms resembling Parkinson disease.*

*Key words: concentration of copper, iron,zinc,and manganese in soil and pea*

# CHAPTER ONE

# 1. INTRODUCTION

## 1.1. Background of the study

In Ethiopia, agriculture is the mainstay of the majority of the population and major drivers of the national economy; agricultural production has been highly dependent on natural resources for centuries .However, increased human population has degraded vital natural resources in the country and became a serious threat to sustainable agriculture degradation of soil resources as a result of natural and anthropogenic factors is very common and low soil fertility is one of the bottlenecks to sustain agricultural production and productivity in Ethiopia. Hence, integrated soil nutrient management is an option as it utilizes available organic and in organic nutrients to build ecologically sound and economically a viable farming system [alem,2014]. pea (*Pisum sativum L.*) is a leguminous annual herbaceous plant a one-year lifecycle .Pea is considered a cool season crop with planting taking from winter to early depending on the location. Seed may be planted when the soil temperature reaches 10$^{o}$C, with plants ideally growing at temperature of 13-18$^{o}$C .

Pea (*Pisum sativum.*) is the most important cereal crop in Ethiopia and particularly in Wogdie woreda. However, the production of Pea in the region did not meet potential yield quantities enough secure a small land holders family consumption due to lack of proper soil fertility status, crop type selection and traditional farming practices. pea grains are often an important part of a healthy and nutritious diet for people. Whole pea grains specially provide many health benefits and are important in the prevention of chronic diseases. In addition to many protein and minerals essential to overall health. Pea and other grains are an excellent source of phytochemicals. In plants, these compounds have supporting functions, such as protecting the plant from external stresses like pests and weather extremes. In humans, many of these phytochemicals have antioxidant effects to prevent chronic diseases, including obesity, heart diseases and certain cancers [EL-MetwallyA.E,2010]. As grains are the largest single source of calories consumed worldwide, and they are arguably one of the most important sources of antioxidants and essential macro and micronutrients.

The group of essential elements for biological systems including plants, animals and humans includes both macro and trace elements. Out of these 17 elements, 9 essential elements have been classified as “macronutrients” as these are required in relatively large amount by the plants. These elements include C, H, O, N, P, K, Ca, Mg, and S. The remaining essential trace elements are often called “micronutrients” because they are required in small, but in critical concentrations by crops, livestock and human beings [AllowyB.J.,1990]. However, this does not mean they play a minor role in plant and animal nutrition.

and enzymatic processes [EL-MetwallyA.E,2010]. Soil supplies 14 out of 17 essential elements required for nutrition of crop plants and 8 of them are trace elements. These are Fe, B, Cl, Mn, Zn, Cu, Mo and Ni. A reduction in their concentration in soils results in unhealthy low intake by plants and consequently domestic animals and human beings. This in turn could result in an increased risk of mineral deficiency related symptoms, diseases and malnutrition thus worsening the current food and economic situation in Africa. Thus nutrients play a very important role in chemical, biochemical, physiological, metabolic, geochemical, biogeochemical,

Plants are considered as intermediate reservoirs through which heavy metals/ elements/ nutrients are transferred from soil to other organisms via a food chain. Several heavy metals are toxic to human beings. The metals are not toxic as the condensed free elements but are dangerous in the form of cat ions and when bonded to short chains of carbon atoms. Potential toxicity of trace metals result from the fact that they are transitional elements able to form stable coordinated compounds with a range of both organic and inorganic ligands. Many metals act as biological poisons even at parts of per billion (ppb) levels. The toxic elements accumulated in organic matter in soils are taken up by growing plants. The toxicity of these metals may result in blocking the essential biological functional groups of the biomolecules, displacing the essential metal ion present in biomolecules or and modifying the active confirmation of the biomolecules like polypeptides etc. The polypeptides store genetic information and their disruption can have serious results such as cancer or congenital deformation [AloowallyB.J.,1990].

Copper and Zinc are important component of proteins found in the enzymes that regulate many biochemical reactions in plants and animals. For example copper requires for the functioning of more than 30 enzymes, all of which are either redox catalysts (e.g., cytochrome oxidase and nitrate reductase) or Dioxygen carriers (e.g., hemocyanin) Copper and Zinc in cereals provide vital nutritional components for plants, animals and humans. A copper deficiency for example causes retardation of plant growth and in animals and man may cause many adverse effects on blood vessels, bone, central nervous system, kidney, liver and enzymes. High concentrations of copper in irrigated soils and ground water can also be a problem. Copper poses a threat to agricultural production and may impact the health of humans and animals [RowellD.L,1994].

Zinc plays a part in the basic roles of cellular functions in all living organisms and is also involved in improving the human immune system. The optimum dietary intake for human adults is 15 mg Zn per day. Zinc acts as a catalytic or structural component in various body enzymes. Zinc deficiency is common in humans, animals and plants. More than 30% world's population suffers from Zn deficiency. Unsatisfactory intake and improper absorption of Zn in the body may cause deficiency of Zn such as; the human body will suffer from hair and memory loss, skin problems, weakness in body muscles and during pregnancy alsocauses stunted brain development of the fetus. Generally, Zn deficiency is expected in calcareous soils, sandy soils, peat soils, and soils with high phosphorus and silicon [SleemM.,2010].

## 1.2. Statement of problem

Currently the researcher identifies pea grains are among the major crop production in that research area. However the grains yields are not enough due to inadequate amount of micronutrients like copper, iron, manganese and zinc in that soil. Wogdie is one of the focus areas in the south Wollo zone where soil is unfertile and food production particularly pea grain has been on the decline for the last years. Which concentrations level of copper ,Iron, manganese and zinc are available for pea .Therefore the main issue of this experimental research will look to analyze the concentrations of copper, iron manganese and zinc in soil and its availability for maximizing the yield of pea grains.

## 1.7. Research questions

The research is focused towards answering the following main questions:

What is the level of the concentration of Zn, Mn, Cu and Fe in pea those are cultivated by farmers in wogdie.

1. Which essential elements are important for people?
2. There any difference concentration of Fe Cu ,Mn and Zn in pea three kebele?
3. Which element is high content in pea from that kebele?

### 1.3. Objective study

#### 1.3.1. General objective

The main objective of this study is to assess total concentration of copper, zinc iron and manganese pea grain samples from the wereda using instruments like Inductively Coupled Plasma Optical Emission Spectroscopy.

#### 1.3.2. Specific objective

1 Compare the concentration of copper, iron, Manganese and zinc in pea sample.

2 Develop pea suitable method for pea digestion.

3 Compare the level of this metal in pea with other similar legume plant.

### 1.4. Purpose of the study

Pea from the selected kebeles of the Wogdie woreda and finally give suggestion for farmers those were cultivating and using pea. The purpose of this study is to determine the available concentration of copper ,iron ,manganese and zinc from different soil samples using laboratory techniques which are important for the growth and yield of pea .

### 1.5. Research scopes

It is impossible to cover the whole aspects of the study area with the available time, covid-19 and resources, because the district wereda covers around thirty kebeles. It is advisable to limit the sample size and the scope of the problem is to a manageable kebeles in wogdie wereda, in South wollo of Amhara Region. Then the samples will investigate using Inductively Coupled Plasma Optical Emission spectroscopy (ICP - OES). Cu,Fe,Mn and Zn will extract using these instruments and chemicals which are suitable for the extraction process.

### **1.6**. Significances of study

The study was finding out the concentration of iron, zinc, manganese and copper from pea grains and process the extraction methods of these metals were using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) methods from different pea samples. This study was also attempt to assess the effectiveness of extracting metals from pea and it provides a chance to raise awareness on the use of micro nutrient especially to those who uses the pea grains from agricultural product. Therefore, the outcome of this research will give information about copper iron manganese and zinc micro nutrients for consumers, food chemists, researchers and food manufacturers.

## 1.8. Limitation of the study

From the beginning of the appropriate title selection to final draft of this study, I am faced in to different problems through process. Among such problems the major are the following: absence of internet access, shortage of budget and time, lack of sufficient and recent reference

# CHAPTER TWO

# REVIEW OF LITERATURE

## 2.1. Characteristics of pea

Pea (Pisum sativum L.), belongs to the Legumes which has an important ecological advantage because it contributes to the development of low-input farming systems by fixing atmospheric nitrogen and it serves as a break crop which further minimizes the need for external inputs(Gaba et al., 2015; Sallaku, Nasto, & Balliu, 2014).

Dry pea currently ranks second only to common bean as the most widely grown grain legume in the world with primary production in temperate regions and global production of 10.4 M tones in 2009. Pea seeds are rich in protein (23–25%), slowly digestible starch (50%), soluble sugars (5%), fiber, minerals and vitamins(Dahl, Foster, & Tyler, 2012). Pea has also been a model system in plant biology since the work of Gregor Mendel. The fundamental discoveries of Mendel and Darwin established the scientific basis of modern plant breeding in the beginning of the 20th century. Similarly, current progress in molecular biology, genetic and biotechnology has revolutionized plant breeding, allowing a shift toward molecular plant breeding and adding to its interdisciplinary nature. However, although the methods have been available for over a decade, there is still a large gap between plant biologists engaged in basic research and plant breeders(Foyer et al., 2016).

Pea (Pisum sativum L.) is one of the world's oldest domesticated crops. Its area of origin and initial domestication lies in the Mediterranean, primarily in the Middle East. Prior to cultivation, pea together with vetches, vetch lings and chickpeas was part of the everyday diet of hunter-gatherers at the end of the last Ice Age in the Middle East and Europe. Remains of these legumes occur at high frequencies in sites dating from the 10th and 9th millennia BC suggesting that domestication of grain legumes could even predate that of cereals. Thus, grain legumes were fundamental crops at the start of the 'agricultural revolution' which facilitated the establishment of permanent settlements(Ahmadi, Targhi, & Seyfi). Subsequently, during centuries of selection and breeding thousands of pea varieties were developed and these are maintained in numerous germplasm collections worldwide(Smýkal et al., 2012). Plant growth is often limited by the amount of available nitrogen when other soil nutrient deficiencies have been corrected by amendments or fertilizations. The rhizoid are able to supply available nitrogen to the soil by fixing the atmospheric nitrogen gas into organic compounds(Holdsworth et al., 2017; Plucknett & Smith, 2014). The members of the genus, Rhizobium are non-spore forming Gram negative rods, usually containing poly-hydroxybutyrate granules observable under phase contrast microscopy. These organisms occur as free-living microorganisms in soil or as micro-symbiosis in root nodules of leguminous plants(Puławska, Willems, De Meyer, & Süle, 2012; Puławska, Willems, & Sobiczewski, 2012). Rhizoid in root nodules are estimated to carry out between 50 to 70% of the world's biological nitrogen fixation, and the estimated annual biological fixation of atmospheric nitrogen varies between 100 x 106 and 180 x 106 metric tons per year (Huergo et al., 2012). Biological nitrogen fixation has of particular importance in agriculture. Leguminous plants that fix nitrogen well may grow on soils that are poor in available nitrogen, reducing the amendments with expensive nitrogen fertilizers (Huergo et al., 2012). Leguminous plants are also of crucial importance as animal feed present in the soil. Unfortunately, improvement in legume crop yields has not kept pace with those of cereals(Jacoby, Peukert, Succurro, Koprivova, & Kopriva, 2017).

## 2.2. Benefits from legumes

The ability of legumes to fix atmospheric $N_2$ and thereby add external N to the crop-soil ecosystem is a distinct benefit of legume culture. When fertilizer-N is expensive or unavailable, crop production systems depend on the N fixed by legumes to maintain the N cycle at a sustained productive lever. Such limitations of fertilizer-N availability and cost are not uncommon in many developing countries(Oke, 2014).The quantity of N biologically fixed each year by legumes varies greatly from zero to several hundred kg N per ha (Babiker, 2014). Many grain legumes are efficient at N fixation. Variables affecting quantity of nitrogen fixed include not only legume species and cultivar, but also such factors as soil type and texture, pH, soil nitrate-N level, temperature and water regimes, availability of other nutrients, and crop (especially harvest) management. .

## 2.3. Composition of pea

Pea (*pisum sativum*) glucose, sucrose , raff nose in the were present highest concentration in the protein concentration (7.1to11.1%) the pea protein concentrate contained 8.7% sugar .

## 2.4. Nutrient value of pea

All pea are lower in calcium and phosphorus than bean,but provide similar level of protein, carbohydrate fat. That are a good source of protein vitamin and a variety of minerals ,including phosphorous managanese ,magnesium potassium and iron. In addition, dried peas are an excellent source of dietary fiber and green pea s are a good source of vitamin C Vitamin K and carotenes.

## 2.5. Importance of pea

Peas are good source of vitamin C and E ,Zinc and other antioxidant that strengthen our immunity system that nutrients such as vitamin A and B and coumestrol help reduce inflammation and lower risk to chronic condition including diabetes heart diseases.

## 2.6. Heavy metals

### 2.6.1. The importance toxicity and source of iron

Iron has other important functions, too. "Iron is also necessary to maintain healthy cells, skin, hair, and nails," says Elaine Chottiner, MD, clinical assistant professor and director of General Hematology Clinics at the University of Michigan Medical Center said in an email interview.

### 2.6.2.Toxicity of iron

Iron is found in many over-the-counter (OTC) multivitamins. Iron toxicity from intentional or accidental ingestion is a common poisoning. Iron is an important component of hemoglobin, the substance in red blood The acute ingestion of iron is especially Iron is an essential mineral. "The major reason we need it is that it helps to transport oxygen throughout the body," says Paul Thomas, EdD, RD, a scientific consultant to the National Institutes of Health, Office of Dietary Supplements.cells that carries oxygen from your lungs to transport it throughout your body. Hemoglobin represents about two-thirds of the body’s iron. If you don't have enough iron, your body can't make enough healthy oxygen-carrying red blood cells. A lack of red blood cells is called iron deficiency anemia. Without healthy red blood cells, your body can't get enough oxygen. "If you're not getting sufficient oxygen in the body, you're going to become fatigued," Thomas says. That exhaustion can affect everything from your brain function to your immune system's ability to fight off infections. If you're pregnant, severe iron deficiency may increase your baby's risk of being born too early, or smaller than hazardous to children. Life-threatening toxicity is associated with pediatric ingestion of potent adult preparations, such as prenatal vitamins. Serious iron ingestion in adults is usually associated with suicide attempts. Accidental ingestions are more common in children less than 6 years. In addition, iron toxicity may also develop after multiple blood transfusions for a chronic disorder like thalassemia, sickle cell, and hematological cancers(H.-W. Yuen & Becker, 2019).

Iron toxicity is classified as corrosive or cellular. Ingested iron can cause direct caustic injury to the gastrointestinal mucosa, resulting in nausea, vomiting, abdominal pain, and diarrhea. Significant fluid and blood loss can lead to hypovolemia. Hemorrhagic necrosis of gastrointestinal mucosa can lead to hematemesis, perforation, and peritonitis. At the cellular level, iron impairs cellular metabolism in the heart, liver, and central nervous system. Free iron enters cells and concentrates in the mitochondria. This disrupts oxidative phosphorylation, catalyzes lipid peroxidation, forms free radicals, and ultimately leads to cell death(Jaiswal et al., 2019; H. Yuen & Gossman, 2017).

**2.6.3. Sources of iron**

- , for example spinach, silverbeet and broccoli
- lentils and beans
- nuts and seeds

grains, for example w

The best source of iron is animal-based foods, especially red meat and offal (such as liver).

Chicken, duck, pork, turkey, eggs and fish also have iron.

Iron is also found in many plant-based foods such as:

- green vegetables hole wheat, brown rice and fortified breakfast cereals
- dried fruit

The iron in animal-based foods is easier to absorb than the iron in plant-based foods. If you are a vegetarian or vegan, you need to take extra care with your diet to get enough iron.

## 2.7. Role of cu and zinc in human health

Copper is a catalyst for respiration and an activator of several enzymes. It is important for carbohydrate a protein synthesis. It may also play a role in carotene production. Copper proteins have diverse roles in biological electron transport and oxygen transportation; processes that exploit the easy inter conversion of Cu (I) and Cu (II). Copper is also a component of other proteins associated with the processing of oxygen. In cytochrome c oxidase, which is required for aerobic respiration, copper and iron cooperate in the reduction of oxygen. Copper is also found in many superoxide dismutases, proteins that catalyze the decomposition of superoxide, by converting it (by disproportionation) to oxygen and hydrogen Peroxide. Several copper proteins, such as the "blue copper proteins", do not interact directly with substrates, hence they are not enzymes. These proteins relay electrons by the process called electron transfer.

The mineral elements like Zn and Cu are as crucial for human health as organic compounds such as carbohydrates, fats, protein and vitamins. The daily dietary intake of young adult ranges from 2-3 mg for Cu and 15 mg for Zn. Intake less than these values can cause slow physiological processes. These micronutrients deficiencies in soil are not only hampering the crop productivity but also are deteriorating produce quality. High consumption of cereal based foods with low contents of micronutrients is causing health hazards in humans. The contents of micronutrients in food can be elevated either by supplementation, fortification or by agricultural strategies i.e., bio fortification and application of micronutrients containing fertilizers.SinceZinc and copper are essential elements they also serve as plant nutrients; they may be used as components of paint pigments. Consequently, their undue presence in the environment through industrial discharge can also be hazardous to man. Heavy metal absorption is governed by soil characteristics such as pH and organic matter content. Thus, high levels of heavy metals in the soil do not always indicate similar high concentrations in plants. The extent of accumulation will depend on the plant and heavy metal species under consideration. Copper is an essential element, with both deficiencies and excesses associated with impaired health. Its deficiency is known to cause various physiologic disorders such as anemia and bone abnormalities resulting from decreased activity of the copper requiring enzymes. Copper excess can cause hepatic and kid damage hemolytic anemia, and methaemoglobinemia. Copper is a moderately toxic element as compared to other transition materials. However, the toxic does of copper and its compounds can lead to serious problems. Severe oral intoxication will affect seriously the blood and kidneys. Cu is a nutritionally essential metal and is widely distributed in nature. At low concentrations it plays an important role in carbohydrates and lipid metabolism. Above trace levels, however, copper has many biological effects both as an essential and toxic element.

## 2.8. Soil physical properties

### 2.8.1. Morphological properties

Morphology of soil is the most important tool than physical and chemical properties of the soil in soil classification because it is observed under natural undistributed condition.

### 2.8. 2. Soil Structure And Constituent

Soil structure (aggregation)is affected by cation effect, clay particles interaction, and organic matter and soil moisture conditions. Soil structure has a major influence on the ability of soil to support plant growth, nutrient, receive and store water and to resist soil erosion, and the dispersal of chemical anthropogenic origin. Soil structural property variations could be related to organic matter and textural characteristics. Research studs reported that soil structure is strongly affected by change in climate, biological activities, soil management practices and physio chemical nature of the soil. Soil constituent refers to the manifestation of the physical force of cohesion and adhesion acting within the soil at a range of soil moisture contents. Most of the time consistence is described for three moisture levels: wet, moist and dry. It is a term used to describe the action of physical force of cohesion and adhesion on the attributes of soil material at these moisture contents that determines the resistance of soil material to crushing or rupture and its ability to charge the shapes or to be molded.

### 2.8.3. Soil Water Characteristics

Soil water characteristics is the basic parameter required to answer the wetness, quantity of water held in the soil the amount of water absorbed before surface runoff started, and the amount of water a particular soil supply to maintain optimum growth. Soil water is, therefore, the most critical limiting resources and will continue to be most critical crop production factor affecting production and sustainability in the land areas.

### 2.8.4. Soil Type Characteristics

Copper deficient soils have several characteristics that indicate where a deficiency will likely occur.

**Texture:** deep sandy and light loamy soils are much more likely to be copper deficient than medium or heavy textured soils. The present material forming these soils may contain low copper concentration.

**Organic matter:** copper is strongly bound to organic matter. As organic matter increase, the probability of copper deficiency increases, whereas solubility and mobility of zinc in decrease.

**Soil pH:** Copper availability is reduced as pH increases. And zinc solubility and mobility is soil decrease at high pH. A pH increase of 1 unit (between pH 7 and 8) means a 100-fold reduction in copper availability to the crop. Thus, for similar soils, as pH increases, so does the probability of copper deficiency.

## 2.9. Soil chemical properties

Soil chemical properties are those soil properties which are responsible and take part in the chemical reactions and processes of the soil and results of weathering of their mineral components, decomposition of organic materials and the activity of plants and animals pertaining to plant and animal growth and human development.

### 2.9.1 Soil Reactions

A soil reaction (pH) is a measure of the concentration of $H^+$ in the soil or in other words a measure of acidity or alkalinity of a soil. It is mostly related to the nature of the parent material, climate, organic matter, and topographic situations. This soil property can be referred to as a" master variable" because it regulates almost all biological and chemical reactions in soils. Soil pH indicates the state of weathering of a given soil and in slightly weathered soil the surface soil pH is neutral to slightly alkaline.

Most plants and soil organisms prefer pH range between 6.0 and 7.5.soil pH is the first parameter to be considered in soil fertility evaluation. Research reported that the PH of soil was moderately acidic with values ranging between 6.0 and 6.62 and this value indicates that there is no toxicity of Aluminum, Manganese and Hydrogen. The authors stated that pH value increased with soil depth because less $H^+$ ions are released from decreased organic matter decomposition, which is caused by decreased organic matter content with depth. Soil pH is most useful in soil sustainability evaluation and management as it provides information about then solubility and thus potential availability or phyto-toxicity of elements for crops.

### 2.9.2. Soil organic matter

Soil organic matter (OM) content has many benefits such as reservoir of plant nutrients especially Nitrogen, Phosphorus and maintaining micronutrient cations in available form, complexion Aluminum in less phytotoxic form and minimizing the effect of moisture stress. Soil OM increases the water holding capacity of soils improves aggregate stability and structure of soils and is a source of several essential plant nutrients. The positive effect of OM on structural stability is more pronounced on sandy than on more finally textured soils.

## 2.10. Copper and zinc in soils

An estimated 97 % of copper released from all sources in the environment is primary released to land. These include primary tailings and over burdens from copper mines and tailing from mills. The copper in tailings represents the portion of copper that could not be recovered from the ore and is generally in the form of insoluble sulfides or silicates. These wastes from electroplating, iron and steel producer, and discharged copper products (e.g. plumbing, wiring) that are not recycled. The copper content of municipal solid waste is approximately 0.16% much of this waste is land field directly or in the form residues following incineration. Emission factors in milligrams of copper released per grams of solid waste have been established for various industries. The factors would enable estimation of an industry's copper releases in terms of total quantity of solid waste discharged. Agricultural products are believed to constituent 2 % of the copper released to soil. However, even though the largest releases of copper are to land, uptake of copper in humans populations through ingestion of copper in soils are expected to be minimal in in comparison to the primary route of exposure through the ingestion of drinking water.

The availability of zinc to plants depends on several soil factors such as, the concentration of zinc in solution, ion speciation, and the interaction of zinc with other macronutrient and micronutrient elements. The behavior of zinc ions in soils and their uptake by plants cannot be explained the total concentration of zinc in the soil. For example, the concentration of $ZnOH^+$ explained zinc adsorption on soil surfaces better than the total zinc concentration. Total Zn concentration in soils is largely dependent on the composition of the parent rock material.

## 2.11. Copper reaction in soils

The amount of copper available to plants varies widely by soils. Available copper can vary from 1 to 200 ppm in both mineral and organic soils. Copper is naturally present in soil in several soluble (hydroxy and carbonate) and insoluble (oxide and sulphide) forms and with the soluble form differing in its availability to plants depending on soil pH, clay content and the presence of organic matter. Available copper in soil is held mainly as cation ($Cu^{++}$) on surfaces of clay minerals or in association with organic matter. Copper present as impurity in silicate minerals or carbonates is largely available. Organic matter, soil texture and soil pH are the predominant factors influencing copper availability.

**Organic Matter:** Copper availability decrease as OM increases. OM binds copper more lightly than any other micro nutrient. This is not only reduces fixation by soil minerals and leaching, but also reduces availability to crops. Organic soils, therefore, are likely to be deficient in crops than mineral soils. Plants grown on newly reclaimed acidic organic soils occasionally exhibit copper deficiency symptoms in the first few years. After the organic matter begins to decompose when the soils are drained, sufficient copper is released to support normal crop growth.

## 2.12. Diagnosis of micronutrients deficiencies

**Plant deficiency symptoms**: Deficiency of micronutrient in soil and plants is a global nutritional problem and is prevalent in many countries with different magnitude of severity.)As the micronutrient deficient plants may exhibit characteristic symptoms, plant symptoms can be useful indicator of micronutrient deficiencies.

**Soil testing**: It is practical and most widely used technique for predicting micronutrients deficiencies in crops. An ideal soil test is one which is rapid, reproducible and correlates reliably with responses in plant yield, plant specific nutrient concentration or uptake of that nutrient. However, soil test levels at which micronutrient deficiency in plant can occur may vary to some extent according to soil type and crop species. Soil samples for the analysis can be taken at any time of the year but care is needed to ensure that a representative sample has been taken over the full area of the field. It is also important to avoid contamination of the soil samples by contact with metal equipment.

**Plant analysis:** An alternative to soil testing is to analyses samples of leaves or grain to determine the micronutrient status of both crop and soil on which it is growing. However, it is not often possible to rectify the problem to prevent the losses in the existing crop, but once diagnosed, the deficiency can be treated for future crops in time to prevent further losses of yield.

**Factors affecting micronutrient bioavailability:** Bioavailability of all four metallic micronutrients is significantly affected by soil pH, decreasing with increasing soil pH. The activity (consequent bioavailability) of Cu and Zn decreases 100-fold for each unit increase in soil pH. Amounts of exchangeable metals in soil are related to their concentrations in soil solutions, so soil pH affects exchangeable Cu and Zn similarly.

## 2. 12.1. Deficiency symptoms of copper

Copper containing enzymes play important roles in photosynthesis, respiration, and formation of lignin. Inadequate copper levels can lead to reduced starch production, reduced nodulation and nitrogen fixation in legumes, delayed flowering, maturity, and pollen sterility. Deficiency symptoms of Cu are dieback of stems and twigs, yellowing of leaves, stunted growth and pale green leaves that wither easily, poor pigmentation wilting and eventual death of leaf tips, and formation of gun pockets around central pith in oranges. Copper deficiency reduces plant vingor, and until the deficiency becomes serving, the symptoms are not well defined. In in small grains, grain yield decreases more than straw yield and increased lodging may occur. The tips of older leaves become necrotic (brown, dead tissues, and younger) leaves may remain unrolled. With severe deficiencies, the growing point of cereals may die, resulting in increased tille ring. In broad leaf plants, the upper portion wilts, the growing point may die, and the top leaves turn bluish green. If copper deficiency is severe enough, growth of small grains cereals and plants die after reaching the tile ring growth stage. Pea will not have grain in the head. Deficiency symptoms have only been observed when small grains are grown on peat soils. Whereas, in animals it is used for helping the body utilize iron, reduce tissue damage caused by free radicals, maintain the health of bones and connective tissues, produce the pigment called melanin, keep the thyroid gland to function normally, and preserve the myelin sheath that surrounds and protects the nerve.

## 2.12.2 Deficiency Symptoms of Zinc

The most common symptoms of Zn deficiency include stunted growth, shortened internodes and petioles and small malformed leaves (little leaf) which result in “rosette” symptom in young growth of cotyledons and “fan shaped” stem in monocotyledons. All crops are susceptible to zinc deficiency, but species differ considerably in their ability to tolerate low levels of zinc supply. Dry matter and grain yield of crop are determined by the ability of the roots of plants to extract nutrients from the soil at absorption rates that are non- limiting for growth.

## 2. 13.Fertilizer sources and management for copper

Copper fertilizers are available in both in inorganic and organic forms (Table 1). Follow recommended rates of copper fertilization closely. When 30lb/area of actual copper has been applied, discontinue application to avoid the development of copper toxicity. Copper can be broadcast or banded in soils or applied as a foliar spray. Broadcasting with nitrogen, phosphorous or potassium is the most common method of application. Applications of recommended amounts are good for 5-8 years depending on the soil and crop.

Table 1 Fertilizer sources of copper

| **Source** | **Formula** | **Percent of cu(% of Cu)** |
|---|---|---|
| Copper chelate | $Na_2CuEDTA$ | 13 |
| Copper sulphate | $CuSO_4$ | 25 |
| Cupric oxide | CuO | 75 |
| Cuprus oxide | $Cu_2O$ | 89 |

## **2.14**. Digestion method

### **2.14.1**. dry ashing

Dry ashing or dry oxidation is a process of minimizing the effect of organic materials in metal determination .Dry a shing is a sample preparation method that was described by several authors .During this process ,there is ignition of organic compound by air at atmospheric pressure and high temperature 450-550$^{o}$C in muffle furnace.

### **2.15.2. Wet Ashing**

Compared to dry ashing ,wet digestion shows a wide range of varieties ,concerning the choice of reagent or device used. The wet digestion system uses concentrated acid like $HNO_3$, $H_2SO_4$ , $HClO_4$ However conc.$HNO_3$ is the most selective oxidant for the destruction of the organic matter, Decompose fat, protein require the use of $H_2SO_4$ OR $HClO_4$.

### 2.15.3 Micro wave Assisted Digestion

MW assisted sample preparation by using $HNO_3$ or combination of HCl or $H_2SO_4$ in the presence or absence of $H_2O_2$ in order to destroy organic matter The advantage of MW is in interaction with samples and gives in fast heating of reaction mixture . The advantage of MW over dry or wet procedure is smaller reaction time, decreased contamination, and loss of volatile elements.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1. Description of the study area

### 3.1.1. Location

Wogdie is one of the woreda that are found in South Wollo Administrative zone of Amhara Regional State. It is far 583km from the capital city of Ethiopia (Addis Ababa) and 182km from Dessie, the center of South Wollo Administrative Zone. The woreda is located at $10^0$ 21' 26"- $10^0$ 46'24" north and $38^0$ 27' 30"- $38^0$ 56' 21" east with the total area of 1185km$^2$. Wogidi Wereda is boarded on north by Mekaneselam, on South by Weleka river it separates from Oromia Region, on west by Abay River which separates from east Gojam zone, on northeast by Legambo woreda and on east by Kelela Woreda (Wogidi woreda Communication Affairs Office, 2013). The study sites cover three central Kebeles around Wogidie town which are lomiwuha Abbey and Tungi kebele.

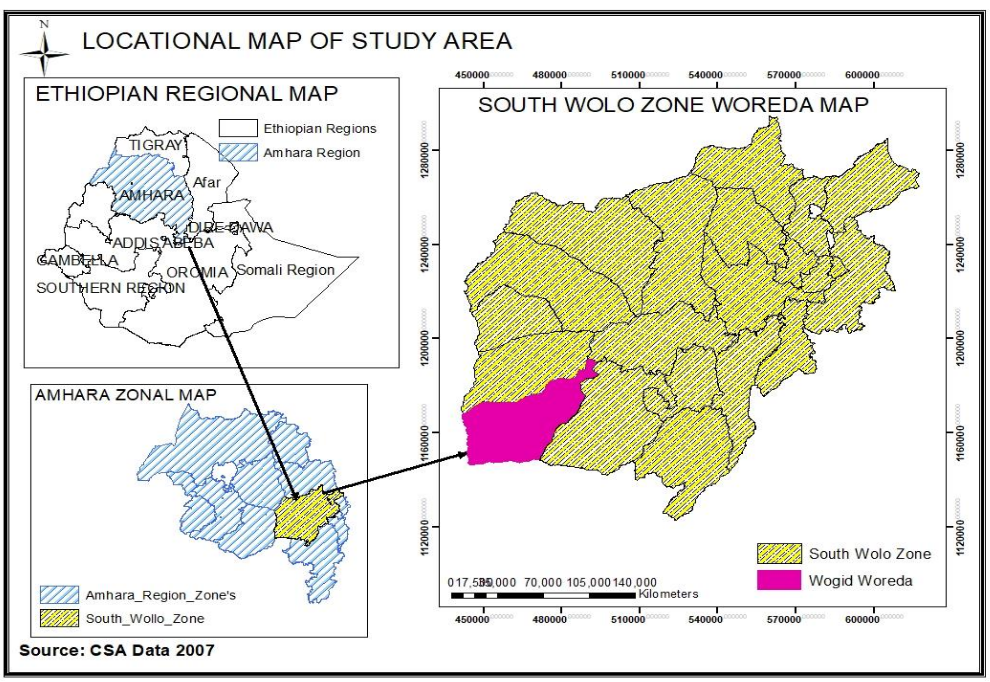


Figure 1 Study area

### 3.1.2. Population

The district has a total population of150, 914.Of these75, 843 are males and75, 071 are females. From the total population there are male households and female households. Among these 85 % lived in the rural which are 72,621 males and 71,910 and the rest 15 % were lived in the urban which are 3222 males and 3161 are females. From these total population 53.4% are workable (from 16-40 ages). The average family size is about six. The average land holding is about one hectare (Agriculture, 2008).

### 3.1.3. Land Use

It has an area of 112188 hectares of land. Of these, 40,964 hectares cultivated land; 2315 hectares grazing land; 50,544 hectares forest and bush/shrub land; and the rest 18,365 hectares are allotted for other activities (Agriculture, 2008).

### 3.1.4. Topography

The district has an altitude that ends from 1100 m to 2437 m. It generally covers the following physiographic areas defined by flat (50%), undulated slope (27%), mountainous slope (12 %), and dale or valley (11%).Topography diversification throughout the area is the reflection of geological processes that are responsible for the formation of the district (Agriculture, 2008).

### 3.1.5 Agro-Climate

According to the Ethiopian agro Ecological Zonation, Wogdie district is categorized under Woyin Dega (59.5%), Kola (39.7%), and Dega (0.85%).The mean annual rain fall for kola ranges from 500 to 900 mm and for dega ranges from 950 to 1100 mm. The average temperature of the area is from 24 to 27 $^{0}$C.The dominant soil types are loam (35.7%), brown (22.3%), red soil (22.15%), grey (10.05%) and 9.8% is for others (Agriculture, 2008).

### 3.1.6 Crop and Livestock Production System

The major economic activity for the population is agriculture. The agricultural production system is mixed crop-livestock farming. The major food crops cultivated are cereals (mainly teff,pea, wheat, maize, and etc.), pulses, fruits and vegetables through traditional farming practice. The major livestock species are cattle, goat and sheep, equines, hens and honey bee (Agriculture, 2008).

## 3.2. Experimental

### 3.2.1. Collection of sample

Recently harvested pea samples were collected from three kebele 03,05 and 06 efforts were made to record necessary information about the sample for later consideration .The samples were packed in to polyethylene plastic container bags for digestion.

### 3.2. 2. Procedure

Apparatus such as volumetric flasks ,measuring cylinder and digestion flasks and all necessary materials used for the experiment each of the pea samples are thoroughly washed with tap water and there after rinsed in distilled water so as to remove surface contaminants like soil ,dust and spray residues. The sample were then placed in acid washed clean crucible labeled according to the sample and oven dried at 85ºC for 24 hrs in drying oven .The dried pea sample ground and homogenized in to fine powder with a grinding device were washed with detergent and tap water rinsed with di ionized water.

### 3.2.3 Apparatus and chemical

Apparatus such as Borosilicate volumetric flask (100ml, 200ml, and 250ml), measuring cylinder, pipette, electronic beam balance (digital analytical balance), muffle furnace, reflux condenser or Gerhardt hot plate and refrigerator used for the measurement of pea of sample. Metals' concentration determination were performed on Model ARCOSFHS12 using the Inductively coupled plasma optical emission spectrometry (ICP-OES) used for the determination of heavy metal analysis and plastic bags (ice box) used for storing that pea samples until to move laboratory of the analysis take place.

The digested sample put within Pyrex flask and where heat in an electric hot plate. A drying oven(DIGITHEAT,J.P.SELECTA,S.a,Spain) was used to dry the washed pea seed samples .Mortar and pestle was used to grind weigh the pea sample.100ml round bottom flask used to digest pea sample .furnace used to ignite the pea sample .A refrigerator and powder the dried pea samples .Digital analytical balance with ±0.0001g precision was used to was used to keep the pea sample until determination.

Chemicals that were used for analysis of selected heavy metals (Mn,Fe,Zn and Cu) are analytical grade of HNO3 (69%) and HClO4 (70%) were used for digestion of pea powder. Stock standard solutions of having concentration 1000ppm in 3%HNO3 Zn, Fe, Mn Cu. Working standards solution used for the construction of calibration curves were prepared by appropriate dilution with deionized water of intermediate standard solution of 1000mg/L with 0.5%HNO3 for respective metals Zn, Fe ,Mn and Cu. All glass wares and other apparatus are thoroughly washing with distilled water, and then soaked in 10 %( v/v) HNO3 solution for 24hour followed by rinsing several times with distilled water. They were dried in hot air oven and kept in dust free space to avoid contamination until analysis began.

That the selective heavy metals were copper, manganese, iron, and zinc. Calibration curves for each metal were prepared using eight standard solutions. The usual procedure in the quantitative analysis method was to prepared series of standard solutions over a concentration range suitable for the sample being analyzed such that the expected sample concentrations are within the range established by the standard. These standard solution prepared by dilution from 1000ppm stock solution were as 0.056ppm,0.112ppm,0.168ppm,0.56ppm,1.12ppmand1.68ppm,2.24ppm,and2.8ppm for all elements Fe Zn Mn and Cu.

Calibration curves were drawn for copper Iron manganese and zinc by plotting metal ion concentration versus intensity

**3.2.4. Digestion of samples**

Concerning 5g of pea powdered with 5ml HNO3 will take in triplicate in flasks to digestion and then heated at120$^{o}$C.after cooling the mixture,3ml HClO4 acid (70%) was added and heated again at 120$^{O}$C until white fumes cease (or clear) to evolve. the solution was cooled in 50ml final volume measuring flask and a specified amount(10ml) of distilled water was added in the digested residue and filtered through what man filter paper No 1. then the volume of the filtrate was made up to mark with de ionized waters. .

Table 2 Instrument operating  parameter of determination of metals analysis by ICP-OES.

| Metals | Plasma power(watt) | Optical temperature($^{O}$c) | Nebulizer pressure(bar) | Argon pressure(bar) |
|---|---|---|---|---|
| Cu | 1400 | 15.05 | 1.96 | 6.75 |
| Fe | 1400 | 15.05 | 1.96 | 6.75 |
| Zn | 1400 | 15.05 | 1.96 | 6.75 |
| Mn | 1400 | 15.05 | 1.96 | 6.75 |

## 3.3. Method validation

### 3.3.1 Precision

In this study, the precision of an analytical procedure is usually expressed as the variance, relative, standard deviation and percentage relative standard deviation of a series of measurements (Mitra, 2004). The precision of the results was examined percentage relative standard deviation of the results three-sample (N=3) and triplicate reading for each sample giving nine measurements for a given bulk sample.

### 3.3.2 Method and instrument detection limit

The method detection limit is the lowest analytic concentration that can be distinguished from fluctuation in the blank, which usually corresponds to an average of blank signal plus three times the standard deviation of blank (Miller & Miller, 2018). The blank samples were digested following the same procedure with the samples and each of the samples was determined for the elements of interest (Cu, Mn,Fe and Zn) by using inductively coupled plasma-optical emission spectroscopy. The standard deviation for each element was calculated from the three blank measurements to determine the method detection limit of the instrument. Instrument detection limits are directly obtained from the instrument manual for all the elements under study. In this study, after digestion of three blank solutions containing $HNO_3$ and $HClO_4$ three readings were taken for each blank and the standard deviation of these was calculated. The method detection of each element was obtained by multiplying the standard deviation of the reagent blank by three(Miller & Miller, 2018).

In short **MDL= 3x∂blank**

Where**- ∂blank** is the standard deviation of the blank reading.

**3.4. Recovery Test (validation)**

In recovery test should be single randomly in a series of analyzed samples. The level of spiked examined can be equal to an expected value of analyzed samples were recognized by adding different volume of standard solution which analytical results must be evaluated to decide on the best values to report and establish the probable limit of errors of these values (Impellizzeri&Marcora, 2009). To determine the percentage of recovery of a spike, the sample is split into a known amount of standard solution and unspike portion. So, recovery is calculated as;

$$R=\frac{A-B}{Z}x100\%$$

Where A= metal content of the spiked sample

B=metal content of a non-spiked sample

Z=concentration of analyte added to the sample.

## 3.5. Data Analysis

Data will be analyzed using Microsoft Office Excel. The data will be expressed in term of descriptive statistics while the figures were presented with Mean values as (Mean ±SD). A Pea value less than 0.05 will be considered as Significant.

Determination of heavy metals and preparation of standard solution

Determination of heavy metal from the filter of pea grains and soil sample after digestion were carried out using the ICP-OES for Cu Zn, Fe, Mn .Working standard solution of Cu, Zn, Fe and Mn were prepared the stock solution containing 1000ppm of the element from highly purified compound of dust in distilled water .the ml/l (Intermediate standard solution) in 100ml volumetric flask was prepared from by dilution of 1000mg/l stock solution the 10mg/l solution five working standard solution were prepared at different concentration for Zn metal six standard working solution for Cu metal.

The instrument was calibrated with working standard after the parameter were adjusted to give maximum signals intensity. All pea grains soil and blank sample were analyzed for all metal using ICP-OES.

## 3.6. Limit of Detection and limit of Quantification

Detection limit is defined as minimum concentration that can be detected by the analytical method with a given certainty .The limit of detection is often taken as three times standard deviation of the blank and limit of quantification is taken as ten times standard deviation of the blank.

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# CHAPTER FOUR

# 4. RESULT AND DISCUSSIONS

## 4 .1 Optimization of the digestion procedure

In this study the pea and soil sample were made ready for the analysis after wet digestion using the fume hood digester heating block .Hence different digestion procedure were tested by varying the volume of reagent digestion time was reagent composition and temperature .The nature of the final digest was examined, clear and colorless solution was selected and the procedure taken as an optimum.

Table 3 Analytical results for Recovery test of the optimized procedure for pea samples

| Metals | IDL(mg/L) | LOD(mg/L) | LOQ(mg/kg) | Coeeficient of determination($R^2$) |
|---|---|---|---|---|
| Cu | 0.005 | 0.082 | 0.287 | 0.9999 |
| Zn | 0.005 | 0.083 | 0.303 | 0.9998 |
| Fe | 0.005 | 0.203 | 0.771 | 0.9998 |
| Mn | 0.004 | 0.117 | 0.535 | 0.9999 |

## 4.2. Recovery test of the optimized procedure

Method validation is a way of testing a particular analytical method to see if it is suitable for its Intended purpose. The validation process begins in method development in that the documentation must include a record of the method development process giving details of the conditions explored by the rationale in the progress of the process. The efficiency of the optimized digestion procedure was checked by adding known concentrations of each metal in 0.5 g sample. For the recovery analysis, 50, 50, 50, and 100 μg of Cu, Zn, Fe, Mn, respectively were spiked to the samples all at once. Each recovery test for the sample was performed in triplicates. Standard metal solutions were used to fortify the sample to the specified metal given in Tableb3 and the percentage recovery was calculated using equation (1).

R = [(Amount after spike – amount before spike)/ Amount added] x100%------ (1)

Recoveries of the metals in the spiked pea sample are between 98% and 99.9 %. The results of this recovery test are indicated in Table 3. The mean percentage recoveries for all analytes were within an acceptable range (75-125%), indicating the laboratory performance for each analyte is in control. Recovery values in the above range are acceptable for both bulk and trace analysis and the digestion procedure is believed to remove metal fractions associated with organic matter. The lower recovery of copper may be due to incomplete digestion of the standard samples while the high recovery value of iron could be attributed to either contamination or incomplete digestion of the lentil samples.

Table 4 :Attempted digestion procedure for six sample selected kebele

| Trial | Volume ratio in(ml) | Weight of sample | Temperature | **Time** | Observation |
|---|---|---|---|---|---|
| 1 | $8HNO_3$:$6HClO_4$:$H_2O_2$ | 0.5g | 200 | **1:00** | Red residue with suspension |
| 2 | $6HNO_3$:$5HClO_4$:$2H_2O_2$ | 0.5g | 150 | **1:20** | Yellow residue |
| 3 | $5HNO_3$:$3HCl_4$:$2H_2O_2$ | 1g | 150 | **1:20** | Dark residue |
| 4 | $5HNO_3$ :$1HClO_4$ | 0.5g | 120 | **1:00** | Black residue |
| 5 | $5HNO_3$:$1H_2SO_4$:$1H_2O_2$ | 0.5g | 80 | **1:00** | Yellow solutionprecipitate |
| 6 | $5HNO_3$:$3HClO_4$:$1H_2O_2$ | 0.5g | 150 | **1:00** | Clear solution |

## 4.3. Soil reaction($P^H$)

pH of soils from the investigated location is reported in Table 4 the result show that one of the sample from sites were strongly The acidic with PH of 4.08 most of the sample were medium in acidic with ph range 5.39 to 6.70 one of the soil sample was alkaline with a PH of 7.22 and mean of PH 6.00 indicate the majority of the soil sample were acidic nature.

Attend in soil $P^H$ ofLlomiwuha(LW)<Abbey(A)<Tungi(TU)

## 4.4.Soil Electrical conductivity

Table 4 pH of soil and electrical conductivity was measured by taking 2g of soil in 10ml distilled water to prepare suspension (1:5w/v).The Electrical conductivity meter was calibrating using 0.01N KCl reference solution before taking electrical conductivity with conductivity of 1.2309 ms/cm .The conductivity was measured in micro siemenpercentimeter (µs/cm) and individual result in Table 4 .The electrical conductivity of all the selected soil solution obtained after measuring in micro siemencentimeterwas lower indicate there small amount soluble salt .The pH and ECof soil from Tungi was found to be higher whereas lower pH was recorded from soil of Lomiwuha having 4.08 and the conductivity was obtained from soil of tungi having 0.022µs/cm .The smaller the conductance the lower the soluble the sat is found in that soil.

Table 5 pH of soil and electric conductivity

| Soil sample site | $P^H$ | EC(µS/CM) |
|---|---|---|
| TU | 7.22 | 0.032 |
| A | 6.70 | 0.042 |
| LW | 4.08 | 0.037 |

Note EC=electrical conductivity

TU=tungi,A=Abbey LW=lomiwuha

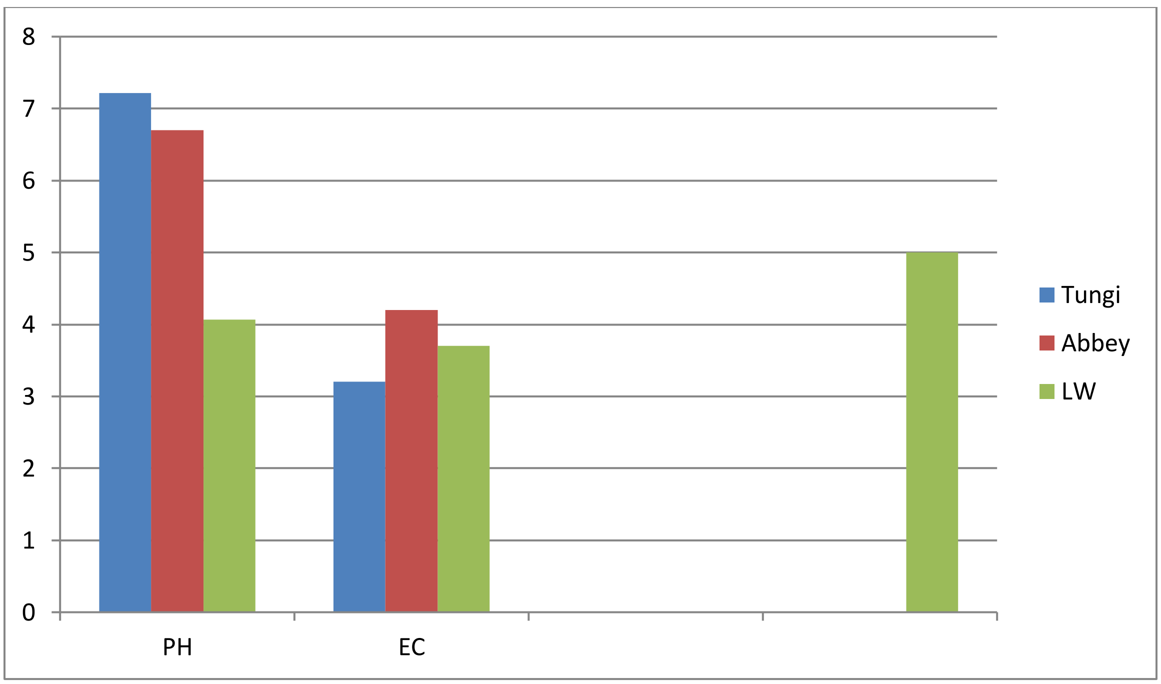


Figure 2 :The pH of study area pea soil and pea grains sample.

In this study the concentration of copper zinc, iron and manganese were determined using ICP-OES( ARCOS FHS12) spectrometer Since total heavy metal concentration analysis of soil and pea grains plays a vital play in prediction and diagnosis of deficiency related disease and environmental toxicity problems in living system .Trace metal like Cu Zn Mn and Fe content in soil and pea grains produced in the selected area in wogdie woreda .During experiment different concentration of standard solution and intensity were measured. The content of Cu Zn Mn and Fe in different soil and pea grains sample of wogdie woreda were determined using calibration curve of standard solutions.

The levels of Cu, Zn, Fe and Mn in pea samples obtained from farms in the selected area around Wogidie were determined in triplicates using computerized ICP-OES. The validity of the ICP-OES results were assessed by spiking of samples with standards of known levels and calculating percentage recoveries.

## 4.5. Results and Analysis of Cu, Zn, Fe and Mn

The results and analysis of each heavy metals based on laboratory findings using mean concentration of Cu, Zn, Fe and Mn are examined based on their mean concentration with in pea and in the soil as follow.

### 4.1.1. Copper (Cu)

The result of Cu concentration with in pea and in the soil at three concentration of laboratory finding analyzed as follow (Table 6).

Table 6 **Descriptive Statistics of Cu in the pea and in the soil**

| | N | Mean | | Std. Deviation |
|---|---|---|---|---|
| | Statistic | Statistic | Std. Error | Statistic |
| copper in the pea | 3 | 4.979000 | .1534938 | .2658590 |
| copper in the soil | 3 | 6.58 | .372 | .644 |
| Valid N (listwise) | 3 | | | |

As we see in this item in three concentrations (N=3) the heavy metal (Table 6) Cu with pea had mean concentration 4.97±0.266mg/L with standard deviation 0.266 while in the soil showed 6.58±0.644 standard deviation0.644. This shows that the mean concentration of Cu in the soil is higher than mean concentration of Cu in pea. Which tells us that some concentration copper is remain in the soil while it is absorbed by the roots of the pea which indicated that the heavy metal that are there in the soil has a direct effect on the plants which are growing on it and has a consequence accumulation in animal and human beings similar to the suggestion (Jacoby et al., 2017).

Table 7:The Permissble concentration of selected metal with WHO and FAO

| Metals | Permissible concentration(mg/L) WHO | Permissible conc(mg/L)FAO |
|---|---|---|
| Cu | 0.1 | 0.2 |
| Zn | 15 | 2 |
| Fe | 425 | 200 |
| Mn | 0.2 | 0.2 |

In the present study the level of Cu in pea seed sample found to be much higher than the maximum permissible level (0.1mg/L) set by WHO or 0.2mg/L of FAO (S. Khan, Cao, Zheng, Huang, & Zhu, 2008). The accumulation of elevated of Cu in Pea seed of wogdie farms might be attributed to the accumulation from different weed killer inputs of agriculture from the industries to the water and the gas emission from vehicles because this site is not much far from the main road.

Table 8:**Paired Samples Test of Cu from farm soil and from Pea**

| | | Paired Differences | | | T | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | | | |
| | | | | | | | |
| Pair 1 | copper in the pea - copper in the soil | -1.601 | 0.389847 | 0.225079 | -7.113 | 2 | 0.019 |

As shown in (Table 8) using paired sample T-test, the mean concentration difference of cu in pea and from the soil showed that there is statistically significant difference; since the Sig. value 0.019 is greater than the significance level 0.05. This mean concentration difference shows that the source of the metal is mainly the soil. Therefore, there should be care when we use weed killers fertilizers which are not free from such type of heavy metals.

### 4.1.2. Zinc (Zn)

The result of Zn concentration with fertilizer and without fertilizer at three concentrations of laboratory reports analyzed using paired descriptive statistics as follow (Table5)

Table 9:**Descriptive Statistics of Zn in the pea and in the soil**

| | N | Mean | | Std. Deviation |
|---|---|---|---|---|
| | Statistic | Statistic | Std. Error | Statistic |
| zinc in the pea | 3 | 1.158222 | .0291168 | .0504318 |
| zinc in the soil | 3 | 1.741667 | .4040967 | .6999159 |
| Valid N (listwise) | 3 | | | |

As can be seen in the table 5 above the three concentrations (N=3) the heavy metal (Table 9) Zn with pea had mean 1.158±0.050mg/L with standard deviation 0..0504318 while in the soil showed 1.742±0.699 standard deviation0.699. This shows that the mean concentration of Zn in the soil is higher than mean concentration of Zn in pea again. Shows that some concentration zinc is remain in the soil while it is absorbed by the roots of the pea which indicated that the heavy metal that are there in the soil has a direct effect on the plants which are growing on it and has a consequence accumulation in animal and human beings similar to the suggestion (Jacoby et al., 2017).

In the present study the level of Zn in pea seed sample(2.3mg/L) found to be much less than the maximum permissible level 15mg/l of WHO but a little bit greater than 2mg/L of FAO (Salah, Esmat, & Mohamed, 2013). The accumulation of elevated of Zn in pea seed of wogdie farms might be attributed to the accumulation from different weed killer inputs of agriculture from the industries to the water and the gas emission from vehicles because this site is not much far from the main road.

Table 10: **Paired Samples T-test of Zn from farm soil and from Pea**

| | | Paired Differences | | | T | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | | | |
| | | | | | | | |
| Pair 1 | zinc in the pea - zinc in the soil | -0.58344 | 0.749788 | 0.432891 | -1.348 | 2 | 0.31 |
| | | | | | | | |

As can be seen from Table 10:

when we compare the mean concentration of Zn in the soil and in the Pea, there is no statistically difference between them. Which illustrated that Zn present in the soil has a good permeability than copper, and absorbed by the root of pea plants. Which is in agreements with the suggestion of (A. Khan, Khan, Khan, Qamar, & Waqas, 2015).

**4.1.3. Iron (Fe)**

The result of Iron concentration with in pea and in the soil at three concentration of laboratory finding analyzed as follow (Table 11).

Table 11 **: Paired Samples descriptive Statistics of iron in the pea and in the soil**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | Iron in the pea | 4.799000 | 3 | .1127431 | .0650922 |
| | Iron in the soil | 5.486667 | 3 | .0981495 | .0566667 |

As shown in the above Table 11: the descriptive statistics, the mean concentration of Fe in the pea is 4.799 ± 0.1127 with standard deviation 0.11274, and in the farm soil in which the pea grow is 5.487 ±0.0982 with standard deviation 0.0982. Demonstrated that the concentration of the soil iron is slightly greater than the concentration of iron in the pea shows that some iron compound are there in the form of insoluble iron compounds.

In the present study the level of Fe in pea seed sample(5mg/L) found to be much less than the maximum permissible level (425/Kg) set by WHO (Guerra, Trevizam, Muraoka, Marcante, & Canniatti-Brazaca, 2012)The accumulation of elevated of Zn in pea seed of wogdie farms might be attributed to the accumulation from different wee dkiller inputs of agriculture from the industries.

Table 12:**Paired Samples T-test of iron from farm soil and from Pea**

| | | Paired Differences | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | | | |
| Pair 1 | Iron in the pea - Iron in the soil | -.68766 | .1306688 | .0754417 | -9.115 | 2 | .012 |

As shown in (Table 8) using paired sample T-test, the mean concentration difference of Fe in pea and from the soil showed that there is statistically significant difference; since the Sig. value 0.012 is less than the significance level 0.05. This this mean concentration difference shows that the source of the metal is mainly the soil. And, and there is some compound of iron which are not soluble and remain in the soil but exposed in the soil when it is digested.

As shown in the above Table11 there is huge amount of iron in the pea as well as in the soil which is accumulate through years and affect the tissue of plants and animals.

Table 13: **Descriptive Samples Statistics of Mn in the pea and in the soil**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | manganse in the pea | .999778 | 3 | .0232626 | .0134307 |
| | Manganese in the soil | 1.066633 | 3 | .1154989 | .0666833 |

As indicated in the above Table 13: the descriptive statistics, the mean concentration of Mn in the pea is 0.9998 ± 0.02326 with standard deviation 0.02326, and in the farm soil in which the pea grow is 1.06667 ±0.1155 with standard deviation 0.1155. Verified that the concentration of the soil Manganes in soi is slightly greater than the concentration of iron in the pea shows that some manganes compound is not easily absorbed by the root of the pea plan due to their insolubility which is similar to (Elnabris, Muzyed, & El-Ashgar, 2013).

Table 14:**Paired Samples T- test of Mn from farm soil and from Pea**

| | | Paired Differences | | | T | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | | | |
| | | | | | | | |
| Pair 1 | manganse in the pea - manganes in the soil | -0.06686 | 0.125855 | 0.072662 | -0.92 | 2 | 0.455 |

As shown in the above Table 14 using the paired sample T-test results, the mean concentration Difference of Mn in the pea and Mn in the soli showed that there is no statistically significant difference; since the Sig. value 0.0.455 is higher than the significance level 0.05. This statistical insignificant mean difference from Mn in the pea to Mn in the soil showed -0.06686mg/L with standard deviation 0.125855. shows that even though there is a slight difference between Mn in the pea and in the soil but there is no as such different between them similar to((Elnabris et al., 2013) ).

As shown in the above Table 13 the concentration of manganes is (1.02mg/L) which is greater than the maximum permissible limit of either WHO(0.2mg/L) or FAO(0.2mg/L) which tell us that there will be health risk around wogdie woreda concerning manganess concentration(Addis & A bebaw, 2017)

Table 15:**The concetration of four Metals in the three places**

| Place | Tripilicates | Cu | Zn | Fe | Mn |
|---|---|---|---|---|---|
| Place 1 | Concentration1 | 5.094 | 0.82 | 2.69 | 0.548 |
| | concentration2 | 5.168 | 0.82 | 2.956 | 0.527 |
| | concentration3 | 4.675 | 0.884 | 2.979 | 0.502 |
| | Mean | 4.979 | 0.841 | 2.875 | 0.526 |
| | Std | 0.266 | 0.037 | 0.161 | 0.024 |
| Place-2 | Concentration1 | 4.761 | 0.627 | 8.374 | 1.762 |
| | concentration2 | 5.184 | 0.656 | 8.916 | 1.904 |
| | concentration3 | 4.987 | 0.706 | 8.869 | 1.891 |
| | Mean | 4.977 | 0.663 | 8.72 | 1.852 |
| | Std | 0.212 | 0.04 | 0.3 | 0.079 |
| Place-3 | Concentration1 | 176.713 | 1.86 | 2.958 | 0.639 |
| | concentration2 | 185.003 | 2.04 | 2.618 | 0.648 |
| | concentration3 | 185.584 | 2.011 | 2.831 | 0.577 |
| | Mean | 182.433 | 1.97 | 2.802 | 0.621 |
| | Std | 4.962 | 0.097 | 0.172 | 0.039 |

As shown in the Table 15 :the concentration Copper, Zinc, Iron and Manganes when we compare the three places the third place is higher in average with all four metals with copper is the highest $182.433 \pm 4.962$ and manganes the list with the concetration $0.526 \pm 0.024$. Therefore, those people who are living in the third place should taker about this metals. Cu>Fe>Mn>Zn

**Table 16 concentration of standard solution and intensity**

| Copper standards | | Zinc standards | | Iron standards | | Manganese standards | |
|---|---|---|---|---|---|---|---|
| Concentration(mg/l) | intensity | Concentration | Intensity | concentration | Intensity | concentration | Intensity |
| 0.056 | 1502906 | 0.056 | 3211.79 | 0.056 | 4900.39 | 0.056 | 5225.8 |
| 0.112 | 16508.7 | 0.112 | 3619.41 | 0.112 | 5903.83 | 0.112 | 7954.64 |
| 0.168 | 18079.2 | 0.168 | 4043.38 | 0.168 | 6813.31 | 0.168 | 10851.3 |
| 0.56 | 27792.3 | 0.56 | 6647.12 | 0.56 | 12888.5 | 0.56 | 29106.6 |
| 1.12 | 41788.6 | 1.12 | 10315.4 | 1.12 | 21721..3 | 1.12 | 56798.8 |
| 1.68 | 57084.5 | 1.68 | 14370.2 | 1.68 | 31279 | 1.68 | 85914.1 |
| 2.24 | 72426.5 | 2.24 | 18997.2 | 2.24 | 40529 | 2.24 | 115795 |
| 2.8 | 8712.3 | 2.8 | 23153.7 | 2.8 | 49983.9 | 2.8 | 145107 |

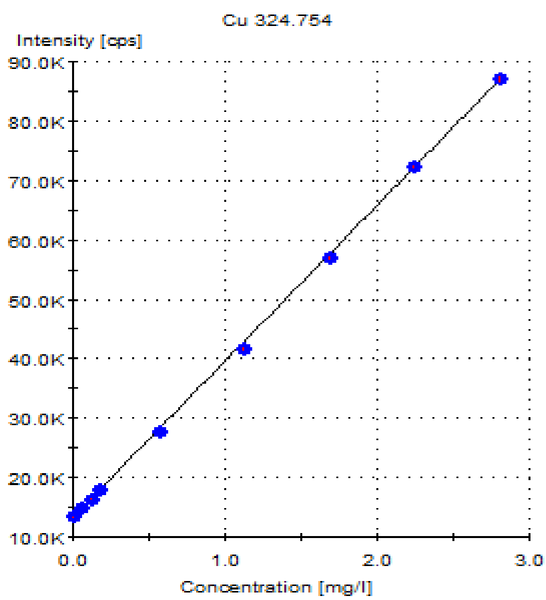


**Figure 3:Thecalibration curve of Cu**

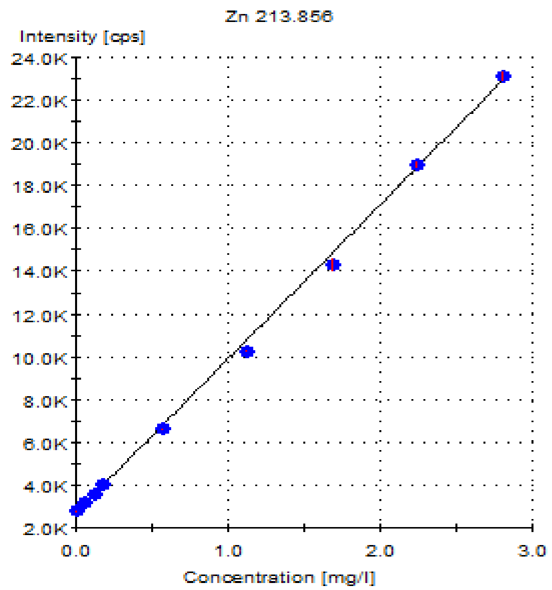


Figure 4: Thecalibration curve of Zn

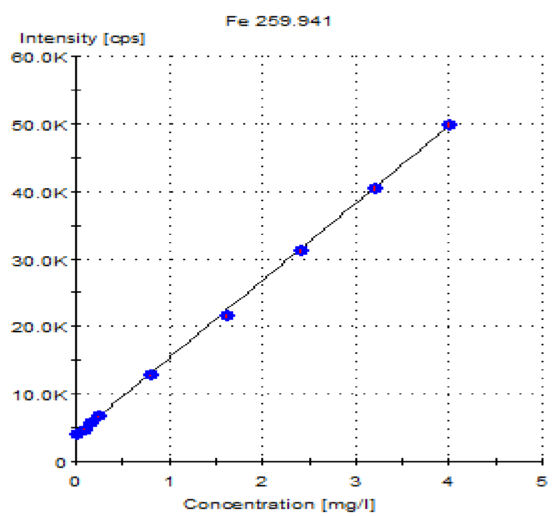


**Figure 5:The Calibration curve of iron**

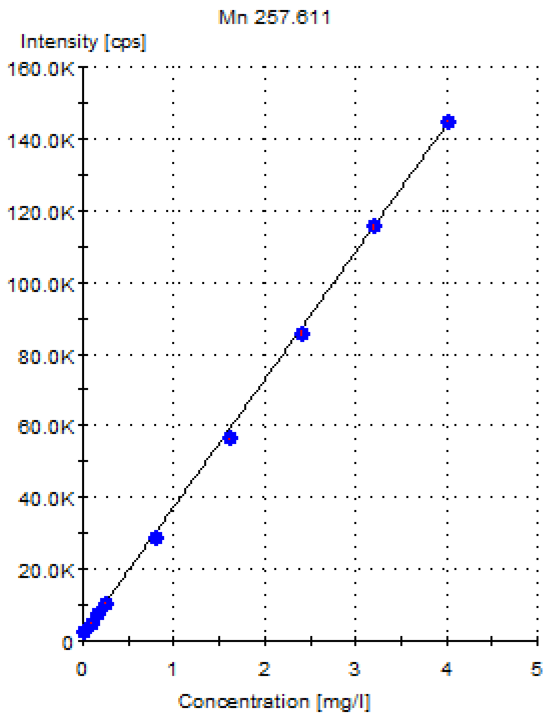


**Figure 6: the calivration Curve of manganes**

**Calculation:** The analysis of copper and zinc in soil pea grain samples were in mg/L , and it possible to express in mg/kg of the product is given by the formula:

$$C = \frac{C_s D_F}{m} \text{X}100\%$$

Where, C = the concentration in mg/kg

Cs = the concentration in mg/L of the soil and pea sample from instrumental reading

m = the mass in grams of the soil and pea sample prepared for analysis

DF= dilution factor (volume of solution in ml)

# CHAPTER FIVE

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusions

The chemical analysis of composite soil samples in wogdie woreda of different Kebeles in which pea is one of the livelihood indicated that Fe Mn Cu and Zn in the soil are sufficient to support good crop growth for now, however; the variations in the level of copper and zinc among the different pea cultivars should be significant in limiting high and quality yield for consumption than inherent zinc and copper deficiency in the soils.

From the analysis the range the concentration level of copper maximum mean concentration is 182.433 ± 5 and minimum mean concentration 4.977± 0.212, zinc maximum mean concentration is 1.97 ± 0.97 and minimum mean concentration 0.663± 0.0.04, Iron maximum mean concentration is 8.72 ± 0.30 and minimum mean concentration 2.802 ± 00.172, Manganes maximum mean concentration is 1.852 ± 0.079 and minimum mean concentration 0.526± 0.024 except manganes the other three are within the permissible limit of WHO .which implies that the concerned body should consider the amount since the accumulation of manganes causes poor bone **health** and symptoms resembling Parkinson disease, such as shaking (tremors).

## 5.2. Recommendations

This study revealed the yield of pea grain in the study area was different from the analysis result of copper Iron, manganese and zinc concentration in soil and pea grain samples of the selected kebeles. Therefore, the following recommendations are given to maximize the yield of pea grain in all cultivated areas of the selected kebeles.

- It is important to develop awareness for farmers to soil fertility management practices should be focus on the restoration and increasing soil water conservation, nitrogen content of the soils and improving traditional farming practices.
- Soil erosion induced problems facilitated by steep slope gradient found to be a great challenge in the study area similar to most Ethiopian highlands. Therefore,the

implementation and existed maintenance of soil and water conservation structures and practices should be increased and expanded across the terrains to tackle the problem.

Further studies are recommended in the study area samples including been white and soil with respect to heavy metals including Arsenic, Mercury, lead, were not addressed in the present study. The accumulation of metals in plants is also a factor of the plant type, growth media, applied agrochemicals, season of cultivation, global pollution status and local pollution incidence. Therefore, further assessment in other parts of the country and including other metals and nonmetallic constituents are possible area. Comprehensive study of relation between the soil, water and plant is the preferred optional method to trace the sources of the minerals that assessment of soil composition of the area was recommended particularly for the toxic chromium. Generally the high level of metals might be originated from weed killer and agriculture medicines indiscriminately released from this medicine.

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