ASSESSMENT OF THE LEVELS OF ESSENTIAL AND NON-ESSENTIAL ELEMENTS AND MAJOR QUALITY INDICATORS OF DIFFERENT VARIETIES OF BARLEY GROWN IN WOLISO DISTRICT SOUTH WEST SHOWA ZONE OF OROMIA REGIONAL STATE, ETHIOPIA

KUMSA GONFA DERA

A THESIS SUBMITTED TO

THE DEPARTMENT OF APPLIED CHEMISTRY

SCHOOL OF APPLIED NATURAL SCIENCE

PRESENTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENT FOR THE DEGREE OF MASTER'S IN CHEMISTRY

OFFICE OF GRADUATE STUDIES

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

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APPROVAL OF BOARD OF EXAMINERS

We, the undersigned, members of the Board of Examiners of the final open defence by Kumsa Gonfa have read and evaluated his thesis entitled "Assessment of the Levels of Essential and Non-Essential Elements and Major Quality Indicators of Different Varieties of Barley Grown in Woliso District South West Showa Zone of Oromia Regional State, Ethiopia" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in the partial fulfillment of the Degree of Master's in Chemistry.

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DECLARATION

I hereby declare that this MSc Thesis is my work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

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Table of Contents

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF APPENDICES

Appendix **Page**

ABBREVIATONS

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of variance
ATSDR	Agency of Toxic Substances and Disease Registry
CACC	Central Agricultural Census Commission
CSA	Central Statistical Agency
DCAP-OES	Direct Current Argon Plasma Optical Emission Spectrophotometer
ETAAS	Electro-thermal Atomic Absorption Spectrophotometer
FAAS	Flame Atomic Absorption Spectrophotometer
FAO	Food and Agriculture Organization
GFAA	Graphite Furnace Atomic Absorption
HGAAS	Hydride Gas Atomic Absorption Spectrophotometer
ICAP-OES	Inductively Coupled Argon Plasma Optical Emission Spectrophotometer
ICP-MS	Inductively Coupled Plasma Mass Spectrophotometer
WHO	World Health Organization
AD	Abba Dulla
BB	Black Barley
WK	Waketena
Abs	Absorbance
MDL	Method Detection Limit
IDL	Instrument Detection Limit
OECD	Organization for Economic Co-operation and Development
DI	De-ionized water
BDL	Below Detection Limit

ABSTRACT

Barley is one of the oldest cultivated cereal grains and grows almost in all agro-climatic conditions. Ethiopia is among the areas where barley is widely cultivated and consumed. In the present study three barley varieties (Abba dulla, Black Barley and Waketena), were collected from South West Showa Zone Woliso Distrct, Abadho Jawe Locality. The collected samples were treated and grounded to fine powder followed by digestion so as to prepare sample solutions for metals analysis. The levels of metals in the prepared solutions were analyzed using two different models of FAAS. The Shimadzu AA-6800 model was used for the analysis of Na, K, Mg and Ca metals where as the Varian spectra AA-20plus model was used for the analysis of Fe, Zn, Cu, Mn, Pb and Cd metals. Efficiency of the method was checked by standard addition (spiking) method. The average concentrations (mg/kg) of the analyzed metals in each variety were as follow: in AD Na (18.96), K (196.93), Mg (2.90), Ca (2.01), Fe (6.81), Zn (1.55), Cu (0.21), Mn (0.84), Pb (0.23) and Cd (BDL) ; in BB Na (25.77), K (241.90), Mg (11.95), Ca (2.24), Fe (8.82), Cu (0.15), Pb (0.14) and Cd (BDL) ; in WK Na (26.20), K (200), Mg (4.46), Ca (1.61), Fe (7.42), Cu (0.25), Zn (1.34), Mn (1.22), Pb (0.17) and Cd (BDL) respectively. The concentration of K was highest followed by Na and Fe in all samples though Black Barley is the most in K and Fe levels. The other barley variety, Waketena has got higher concentrations of Na, Mg as well as Cu and also K and Fe next to Black barley. This could be the reason why Waketena is known as a source of important diet to repair broken bone and as important feed for mothers and babies. The result of this study revealed that there were significant variations in the levels of Na, K, Mg, Fe, Zn, Cu and Mn among Abba dulla, Black Barley and Waketena Barley varieties. However, there was no significant variation in the levels of Ca, Pb and Cd among the varieties, except between Abba dulla and Waketena as well as Abba dulla and Black Barley for lead concentration. Zinc was found to be higher from trace metals next to Fe in the varieties under study. The significant levels of Pb metal obtained in Abba dulla barley variety needs further detailed investigation with more sampling areas.

Key words: *Barley Variety, Mineral Content, Assessment, Woliso District*

1. INTRODUCTION

1.1 Background

Barley is one of the oldest cultivated cereal grains. It's believed to be originated in the Fertile Crescent in the near East currently known as Israel, Northern Syria, Southern Turkey, Eastern Iraq, and Western Iran. There are many cultural evidences in Asia, Africa and Europe that barley was an important dietary. It played an important role as a sustaining food source in the evolution of humans (Newman and Newman, 2006). Moreover, different kinds of alcoholic beverages, fermented and unfermented foods prepared from barley in ancient time.

Barley grows best on well-drained soils and can tolerate higher levels of soil salinity than most other crops. Food barley is commonly cultivated in stressed areas where soil erosion, occasional drought or frost limits the ability to grow other crops (Mulatu and Grando, 2011). Barley grows during cold season and well adapted at altitude of 3000 m and more above sea level which may make it the only crop grown at such high altitude. However, it can be grown in almost all climatic regions in several production systems.

Ethiopia is among the areas where barley was grown in ancient times. Barley holds a unique place in farming and various sources agree that it has been in cultivation for at least the past 5000 years in the country. The first Ethiopians to have ever cultivated barley are believed to be the Agew people, in about 3000 BC (Zemede, 1996). At present, Ethiopia is the second largest barley producer in Africa, next to Morocco, accounting for about 25 percent of the total barley production in the continent (FAO, 2014).

Barley is the fifth important cereal crop after teff, maize, wheat and sorghum with annual production of about 1.2 million tons cultivated on an area of about 1.0 million hectares and it’s the most desirable crop in the highlands where there is a limited alternative crop (CSA, 2005).

Cereals are staple foods, providing a major source of carbohydrate, protein, B vitamins and minerals for the world's population. However, barley is nutritionally superior to other cereals in providing essential nutrients in biologically available forms (Goldberg G, 2003). Whole barley grain consists of about 65-68% starch, 10-17% protein, 2-3% free lipids, 4-9% β-glucans and 1.5-2.5% minerals. Barley is rich in fat-soluble vitamin E (tocotrienols) and contains varying amounts of vitamin B complex except vitamin B12. The major elements in barley grain are phosphorus, potassium, calcium, magnesium, sulfur, selenium, and sodium, the first two being the most abundant (Kaur, 2016).

Barley varies greatly in chemical composition, nutritive value and bioavailable energy content, due to genetic and environmental factors and interactions between the two. The diversity in soils, climate, altitude and topography, together with geographical isolation for long periods, are considered the main factors influencing the large diversity in Ethiopian barley (Newman and Newman, 2006).

As far as nutritional values of barley is concerned, in Ethiopia, there is a custom of using barley varieties for different purposes in various forms including porridge, gruel, popped (roasted), fermented, unfermented and thin bread. Some are consumed believing that it can repair broken (fractured) bone due to injury; others are assumed as good nutritional food for children and pregnant mothers. In most of developing countries like Ethiopia, such trends are not based on scientific evidences but on common practices. This may lead to high intake of some minerals or nutrients which can harm human health when consumed in excess amount. Elements such as Fe, Cu, Zn, Ni and Mn are considered as essential metals. However, if the concentrations of these metals are higher than their permissible limits they may create toxic effects in human. Heavy metal accumulation may pose a direct threat to human health (Turkdogan et al, 2003). In other way, using barley in high extent may not mean getting enough amount of the intended nutrient or mineral value. Thus, a complete profile of mineral composition and heavy metals in barley must be available for the users accordingly.

1.2 Statement of the problem

Woliso District is one of the areas where barley varieties are widely cultivated and locally used for different purposes. Barley is one of the cereal grains that is consumed alone or mixed with other grains or *Enset (Kocho*) as important dietary in most of the local areas of the district. As a matter of fact, all barley varieties may not provide the same mineral value for the consumers as required as possible. There is a natural fluctuation depending on the variety and conditions before and after harvest (Ehrenbergerova, 2008). However, there was no research conducted on assessing the mineral contents of barley varieties grown in this particular area, though there were some reports on nutritive values of barley as a whole. Therefore, it was very important to work on evaluating the mineral composition and heavy metal content of some barley types consumed widely in the area. This study was thus intended to fill the gap by evaluating the elemental composition of some common varieties of barley grown in Woliso District, Ethiopia. The vernacular names of varieties that evaluated were known as Waketena, Abba dulla and Black Barley. These varieties were targeted, because there is a common practice of using Waketena and Abbadulla species during injury to repair a broken bone, as essential diet for pregnant and birth gave mothers and for babies. On the other hand, the use of Black Barley is mostly restricted as a raw material, malt (*bikil*) in making home-made alcoholic drinks like *tella* and araki *(katikala*).

1.3 Significance of the study

Ethiopia is one of the most barley producer countries in Africa. There are different varieties grown in almost all parts of the country. These varieties are known with various common (local) names and widely used being as good sources of carbohydrate, fiber and minerals. However, there is a lack of research that conducted on the levels of essential and non-essential metal contents of barley varieties grown in the same area. Hence, this research work is significant:

- To provide baseline information for the consumers about the levels of essential and non essential metals in these varieties.
- To aware Woliso District Agricultural Office to have available data for other useful varieties as well.
- To initiate any interested body to conduct further detailed and comprehensive investigation.

1.4 Objectives of the study

1.4.1 General Objective

The main objective of this study is to assess the mineral and proximate composition of some common varieties of barley (Waketana, Abba dulla and Black Barley) grown in Woliso district of Abadho Jawe locality.

1.4.2 Specific Objectives

1. To determine the levels of Na, K, Mg, Ca, Fe, Mn, Cu, Zn, Pb and Cd concentration in three barley varieties.
2. To evaluate the moisture, volatile, ash and fixed carbon contents of these varieties.
3. To assess variability of these essential and non essential mineral contents among these barley varieties.
4. To compare the results with international standards.

2. LITRETURE REVIEW

2.1 Cereals

Cereals are the edible seeds or grains of the grass family, Gramineae. A number of cereals are grown in different countries, including rye, oats, barley, maize, teff, millet and sorghum. Cereals have a long history of use by humans. Cereals are staple foods, and are important sources of nutrients in both developed and developing countries. Cereals and cereal products are an important source of energy, carbohydrate, protein and fiber, as well as containing a range of micronutrients such as vitamin E, some of the B vitamins, magnesium and zinc. Cereals also contribute significant amounts of calcium and iron. Cereals and cereal products may also contain a range of bioactive substances and there is growing interest in the potential health benefits these substances may provide. There is evidence to suggest that regular consumption of cereals, specifically whole grains, may have a role in the prevention of chronic diseases such as coronary heart disease, diabetes and colorectal cancer. The exact mechanisms by which cereals convey beneficial effects on health are not clear. As there may be a number of positive health effects associated with eating whole grain cereals, encouraging their consumption seems a prudent public health approach (Kevith, 2004).

2.1.1 Nutritional and Compositional Facts of Cereals

All cereal grains in general have high energy values mainly from the starch portion, but, also from the fat and protein portions. Apart from moisture content and inedible substances such as cellulose, cereal grains contain carbohydrates mainly starches (comprising 65 to 75% of their total weight), as well as proteins (6 to 12%) and fat (1 to 5%) along with traces of minerals and vitamins. (Muhammad Haroon Sarwar, 2013)

Barley

Barley is one of a cereal grain with a height not exceeding 1m, producing four or six ears. Worldwide barley is mainly produced for feeding and malting. In Ethiopia however it's the main staple crop for human consumption.

2.2 Nutritional Composition of Barley

2.2.1 Carbohydrates

Carbohydrates are the chief sources of energy by providing 70 – 80% of the calories in the human diet and constitute the bulk of the total dry matter of the barley grain. Whole barley grain consists of about 65-68% starch which serves as energy source during germination (Kevith, 2004). Mono- and di-saccharides (sucrose, glucose, fructose and maltose) are present in lesser amounts, but their concentration is twice as high as in other cereals. Total dietary fiber ranges from 11-34% containing soluble dietary fiber within 3-20%. The non-starch polysaccharides in barley are β-glucans, arabinoxylans, and cellulose, the major one being β-glucans; these modify the energy value of barley. Significant differences in β-glucans content have been reported among barley types with various starch amylose contents, the average amount being 7.5% in high amylose, 6.9% in waxy, 6.3% in zero amylose waxy and 4.4% in normal starch types. The β-glucans content of barley grains is mainly determined by genetic factors and less by environmental factors. The low-digestible carbohydrates especially β-glucan and resistant starch have a positive impact on human health due to their role in lowering post-prandial blood glucose levels and in reducing the blood cholesterol level (Kaur, 2016).

2.2.2 Proteins

Barley grain consists of about 10-17% proteins. The proteins of barley can be divided into four solubility groups: albumins (water-soluble); globulins (soluble in dilute saline); prolamins (soluble in alcohol / water mixtures); and glutelins (soluble only in dilute acid or alkali). Prolamins, called hordeins in barley, are the major storage proteins and account for 35 to 50% of the total nitrogen in the grain. The albumins, globulins, glutelins consist predominantly of structural and metabolic proteins. Barley grains considerably vary in their protein content. The precise composition depends on the growth conditions and on the rate and timing of nitrogen fertilization (OECD, 2004).

2.2.3 Lipids

Lipids are present only in a small extent in cereals but they have a significant effect on the quality and the texture of foods because of their ability to associate with proteins due their amphipatic nature and with starch, forming inclusion complexes. Lipid levels in barley are considerably low about 2-3%. The major fatty acids in barley triacylglycerol are palmitic acid, oleic acid, linoleic acid, and linolenic acid. Fatty acids in barley are similar to those in wheat except that barley tends to have more linolenic acid. Barley is rich in fat-soluble vitamin E (tocotrienols) and contains varying amounts of vitamin B complex except vitamin B12 (OECD, 2004).

2.2.4 Minerals

Minerals are present in foods at low but variable concentrations and in multiple chemical forms. The role of minerals in food is to provide a reliable source of essential nutrients in a balanced and bioavailable form. They are most important factors in maintaining all physiological processes being as the constituents of the teeth, bones, tissues, blood, muscle and nerve cells (Hopman et al, 2008). The minerals considered to be essential for normal body functions include the major elements Sodium, Potassium, Calcium, Magnesium, Phosphorus, and Chloride ; trace elements Cobalt, Chromium, Copper, Iron,

Manganese, Molybdenum, Selenium and Zinc. The major constituents of the mineral fraction of barley are magnesium, phosphorus, potassium, calcium and sodium. The average mineral content varies significantly, and this appears to be due to a number of factors, such as variation types, growing soil, and fertilizer application (OECD, 2004). For instance the concentration of metals such as Fe, Zn, Mn, Cu, Pb and Cd in eight barley genotypes grown in the same soil was evaluated up on their variation on accumulation of these metals. The analysis revealed that there were differences between the targeted metals concentration in the barley samples due to variation in genotype (Ghulam et al, 2013).

2.3 Barley Crop in Ethiopia

Barley (*Hordeum vulgare* L.) is a major crop for large numbers of people living in the cooler, semi-arid areas of the world. In tropical Africa, Ethiopia is the only country where barley is a major crop, being the fifth most important crop both in area under cultivation and in production after teff, maize, sorghum and wheat (FAO, 2004). It is grown mainly in the highlands of the country and represents approximately an 11% share of the total area where grain is cropped (CACC, 2003). It is predominantly grown at altitudes ranging from 2000 to 3000 meters above sea level (Gebre and J.Van Leur, 1996) in various regions of the country. Barley is produced in both the main rainy season and the short rainy season. It is preferred by subsistence farmers because of its ability to grow on short rainy season. It is preferred by subsistence farmers because of its ability to grow on marginal farms, unlike other cereals. Traditionally, barley is cultivated under no or little external inputs such as fertilizer or chemicals to control the major pests. Barley has a wide range of uses. Its grain is used as a staple food, for malting and for making local drinks, and is sold for cash. Its straw and stem stubs are used for animal feed and thatching.

2.4 Consumption of barley and barley products

Ethiopia is not only the largest producer but also the biggest consumer of barley and various barley products in sub-Saharan of Africa (SSA). Barley is a main ingredient in staple foods (e.g., injera, porridge, and bread) and local drinks (e.g., Tella, Basso and Borde) in addition to its use for malting and animal feed. In 2013/14, household consumption accounted for 64 percent of the total barley production in the country (CSA, 2014). Barley serves as food, beverage, and feed for many highlanders in the country and as a substitute for other cereals. At the national level, it accounts for about 6 percent of the per capita calorie consumption (Berhane et al, 2011). Ethiopia's per capita food barley was 14 kilograms, which is more than three times the average for Eastern African countries four times that of Africa, and fourteen times the world average of consumption. However, food barley and barley products' contribution to the Ethiopian diets is small compared to other stable foods. In fact, it is the least important staple in both quantity and share of calories in total consumption (Berhane et al, 2011).

2.5 Facts about Ethiopian Barley Varieties and Their Dietary Contribution

Ethiopia is a well known country being as a source of numerous barley varieties. Even, West Showa and South West Showa Zones alone, comprises large number of barley varieties. For instance, Balame (white and black), Abbabadhas (white colored), Garbuguracha (black barley), Kitankite (white in color), Luka'a (Senefgebs), Abba dulla (purple) and Waketena (white) are among the most known barley varieties in the mentioned two zones (Firdissa et al, 2010). The seed color of barley species is dependent on different pigments which are located in different seed layers. A great diversity from yellowish-white to blue, purple and black is reported in the literature. There are evidences that Ethiopia is the home of colored barley varieties. For instance, (Susanne et al, 2011) well explained that most of black and blue genotypes came from Ethiopia, a country rich in colored barley varieties.The interest in introducing colored varieties is closely associated with their possible uses and health promoting effects. As already mentioned, Ethiopians are accustomed to use these varieties in many ways for different occations.

For instance, a variety called as *Abbabadhas* is used for preparation of delicious local foods like *Kinche, Chiko* and *Injera*. Balame for making of *injera, roasted, kinche, chiko, besso*, porridge and beverage. The other variety, *Garbuguracha* (black barley) is used as a raw material (malt) to make beverages. A variety named as *Samareta* (white or purple in color) is chosen in making *Besso, Injera, Shameta (Borde*) and roasted (*Kolo*) (Firdissa et al, 2010). From nutritional point of view, barley contains many nutrients including dietary fiber, antioxidants, vitamins, minerals, sphingolipids and unsaturated fatty acids (Sakhawat et al, 2014).

2.6 Importance and classification of minerals in food

Foods and beverages ingested by human represent a potentially proficient pathway of exposure to toxic and nutritionally important major, minor and trace elements. Many mineral elements occur in living tissues, foods and diets in such small amounts that they are frequently described as traces (Hunt, 2003). Vitamins and minerals play essential catalytic roles in metabolism and have other functional roles as well. Although needed in minute amounts, most vitamins are not synthesized in the human body in sufficient quantity, and therefore, together with essential minerals must be obtained from food or other exogenous sources. Minerals are inorganic substances found in the body that function in conjunctions with enzymes, hormones, vitamins and other compounds. They play important roles in nerve transmission, muscle contraction, blood formation and metabolism of macronutrients and energy production. Some minerals can either block or enhance absorption of other nutrients, including other minerals and some vitamins. They are present in the skeletal system and other hard tissues and constitute approximately 4% of the body's weight (Fraga, 2005).

The bulk of human body is composed of six major elements; oxygen, carbon, hydrogen, nitrogen, calcium and phosphorous and the six minor elements; sulfur, potassium, sodium, chlorine, magnesium, and silicon. If noble gases are excluded as unlikely to have a physiological function, seventy one elements of the periodic system remains and because of their low concentration in living mater, are termed the trace elements. The concentration of major and minor elements in living tissues can be expressed in grams

per kilograms. On the other hand, the concentration of trace elements in living tissues varies between 0.01 and 100 mg kg-1. It may not be appropriate to classify them as essential or toxic elements. It is logically wrong to establish a category of "toxic" elements, because any element may be potentially toxic and this property is but a function of concentrations to which humans are exposed. Essentiality of the trace elements is established when a further reduction below the range of tolerable levels, better known as "range of safe and adequate intakes", results in a consistent and reproducible impairment of a physiological function (Sanger and Hoesch, 2005).

2.6.1 Classification of trace elements

All major and minor elements are important; besides that, some of the trace elements, e.g. Cr, Fe, Co, Cu, Zn, Se, Mo and I are essential trace elements; and some of them; Mn, Si, Ni, B, V, and Sn are probably essential trace elements; and further some of them As, Cd, Pb, Al and Hg are considered potentially toxic, some possibly essential elements for animal and human life. Actually all essential elements may also be toxic in animals and humans if ingested at sufficiently high levels and for a long enough period (Sanger and Hoesch, 2005). Essential trace elements are required by man in amounts ranging from 50μg day-1 to 20 mg day-1. The organism can neither grow nor complete its life cycle without the element in question. The element should have a direct influence on the organism and be involved in its metabolism. The effect of the essential elements cannot be wholly replaced by any other elements (Windisch, 2002).

The bioavailability of essential elements depends on their chemical form, the composition of the diet and health situation of the individuals. Thus, establishment of the optimum daily requirements and determinations of actual daily intake of essential elements are important problems of trace elements in nutrition (Hunt, 2003). The physiological role of K, Ca, Fe, Zn, Cu, Na, Pb, Mg, Cd & Mn are briefly described below.

Potassium (K)

Potassium is the most abundant mineral found in the human body next to P and Ca. It is a major electrolyte of intracellular fluid. It helps to regulate the acid base balance osmotic pressure and water balance (Emmanuel, 2013). It is of great physiological importance, contributing to the transmission of nerve impulses, the control of skeletal muscle contractility, and the maintenance of normal blood pressure. It is obviously essential for plants and cannot been tirely replaced by any other elements. Symptoms of potassium deficiency are associated with disturbed cell membrane function, loss of appetite, disturbances in heart function, increase in blood pressure, mental disturbance, (e,g. depression)and muscle weakness (Minaleshewa, 2007).

Magnesium (Mg)

Magnesium represents about 0.05% of the weight of human body (the 4^{th} most abundant cation in the mammalian body) and the 2nd most abundant cation in intracellular fluid. It is essential to maintain both the acid-alkaline balance in the body and healthy functioning of nerves and muscles (including the heart), as well as to activate enzymes to metabolize blood sugars, proteins and carbohydrates .Magnesium composed 53% in bone, 46% in muscles and <1% in serum (one third bound to protein and two third exist in free or ionized state) (Emmanuel, 2013). It plays an important role in protein and nucleic acid synthesis and has a stabilizing and protecting effect on membranes. Indications of a magnesium deficiency may be muscle twitches, nervousness, abnormal heart beat and disorientation. Excess intake of magnesium causes weakness in people with kidney failure (Wardlaw and Insel, 1996).

Calcium (Ca)

Calcium is the fifth most abundant element in the human body. The calcium content of the human body is 25 to 30 g at birth (0.8% of the body weight) and between 900 and 1300 g in adult men (up to 1.7% of body weight). Over 99% of the total calcium of the

body is located in the bones, where it accounts for 39% of the total body bone mineral content, and in the teeth, mostly as hydroxyapatite. It also participates in glycogen metabolism (Tortora, 1997). Inadequate intake of calcium increases the risk of osteoporosis (boneless with no apparent cause). Excess intake of calcium may cause kidney stones and reduces mineral absorption in general (Wardlaw and Insel, 1996).

Iron (Fe)

Iron is an essential element in man and plays a vital role in the formation of haemoglobin and electron transport in human body (Kalagbor and Diri, 2014). It carries oxygen to the cells and is necessary for the production of energy, the synthesis of collagen, and the functioning of the immune system.

Effects of Iron Deficiency in Human health

- Cause anemia that can be detected by its symptoms such as low blood iron level, small red blood cells and low blood hemoglobin values (Tortora, 1997).
- Cause high risk of tissue hypoxia and heart failure, which can lead to death in young children and pregnant women (Viteri, 1998).
- Children suffering from Fe deficiency have poor attention spans, impaired fine motor skills and less capacity for memory (Walter et al, 1997).
- Iron deficiency in pregnant women may cause irreversible damage to fetal brain development leading to irreversible damage to intellectual development in their babies (Gordon, 1997).

In other way, great care must be taken not to take too much iron, as excess amounts are stored in the body's tissues and adversely affect the body's immune function, cell growth and heart health (Tozonou, 1998)

Sodium (Na)

Sodium is an essential nutrient involved in fluid and electrolyte balance and is required for normal cellular function. It is an essential mineral or micronutrient which along with potassium helps to regulate the body's fluid balance. Dietary deficiency of sodium is not

common due its widespread occurrence in foods. Sodium is present in foods as a normal constituent at a low level. It is also added to foods as NaCl (table salt) while processing, cooking and immediately prior to consumption. However, excess sodium intake is linked with high blood pressure and heart disease. The current recommendation is to consume less than 2,400 milligrams of sodium a day. This is about 1 teaspoon of table salt per day. It includes all salt and sodium consumed, including sodium used in cooking and at the table (J. Anderson et al, 2010).

Manganese (Mn)

Manganese is an essential element to both plants and animals. It is necessary for normal bone metabolism and important enzyme reactions. It also helps to maintain normal nerve, brain and thyroid function. While a deficiency of this mineral is uncommon, it is often lost in processed foods. A deficiency of manganese may affect brain health, glucose tolerance, normal reproduction, and skeletal and cartilage formation. Grains and cereal products are the best food sources of manganese, while animal products are the poorest. Toxicity from manganese is uncommon (Minaleshewa, 2007). Exposure to high level of Mn can cause both mental and emotional disturbance, along with increased slowness and clumsiness of the body movements. This disease is called manganism. Any brain injury due to the accumulation of Mn in the brain is permanent (ATSDR, 2001).

Copper (Cu)

Copper is the third most abundant essential trace element in the body after iron and zinc (Sarkari, 1994). Copper in living organisms, including humans, forms an essential component of many enzymes (cuproenzymes) and proteins. The biochemical role for copper is primarily catalytic, with many copper metalloenzymes acting as oxidases to achieve the reduction of molecular oxygen, for example cytochrome-C-oxidase and superoxide dismutase. Studies have also shown that copper is required for infant growth, host defense mechanisms, bone strength, red and white cell maturation, iron transport, cholesterol and glucose metabolism. Deficiency of copper causes low white blood cell

count and poor growth. Excess intake of copper can cause vomiting, nervous system disorder and Wilson's diseases (Wardlaw and Insel, 1996).

Zinc (Zn)

Zinc is an essential element found in the tissue of animals and plants even at normal ambient concentrations. However, if plants and animals are exposed to large concentrations of bioavailable Zn, significant bioaccumulations can result, with possible toxic effects. Zinc is the most ubiquitous of all trace elements involved in human metabolism. More than one hundred specific enzymes require zinc for their catalytic function. If zinc is removed from the catalytic site, activity is lost; replacement of zinc restores activity. Zinc participates in all major biochemical pathways and plays multiple roles in the perpetuation of genetic material, including transcription of DNA, translation of RNA, and ultimately cell division. When the supply of dietary zinc is insufficient to support these functions, biochemical abnormalities and clinical signs may develop (Minaleshewa, 2007).

Among micronutrients, Zn deficiency is occurring in both crops and humans (Welch and Graham, 2004). Zinc deficiency is currently listed as a major risk factor for human health and cause of death globally. According to a WHO report on the risk factors responsible for development of illnesses and diseases, Zn deficiency ranks 11th among the 20 most important factors in the world and 5^{th} among the 10 most important factors in developing countries. Zinc deficiency is responsible for many severe health complications, including impairments of physical growth, immune system and learning ability, combined with increased risk of infections, DNA damage and cancer development (Hotz and Brown 2004).

Lead (Pb)

Lead is a highly toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. 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 can affect every organ system. On a molecular level, proposed mechanisms for toxicity involve fundamental biochemical processes. These include lead's ability to inhibit or mimic the actions of calcium (which can affect calcium dependent or related processes) and to interact with proteins (including those with sulfhydryl, amine, phosphate, and carboxyl groups). Acute high lead exposure can cause serious physiologic effects, including death or long-term damage to brain function and organ systems. Effects of lead exposure vary according to exposure timing and levels, and other factors, and some effects may be latent (ATSDR, 2002).

Cadmium (Cd)

Cadmium is highly toxic metal that has no role in biological processes in living things. Even in low concentration, it can be toxic for living organisms (Wodaje, 2015). Cadmium can cause both acute and chronic intoxications. Its dose (10-30 mg/kg/day) can cause severe gastrointestinal irritation, vomiting, diarrhea and excessive salivation, and doses of 25 mg of cadmium per kg body weight can cause death. Low level chronic exposure to cadmium can cause adverse health effects including gastrointestinal, hematological, musculoskeletal, renal, neurological and reproductive effects. The main target organ for cadmium following chronic oral exposure is the kidney (Nordberg, 1999). Intake of cadmium can double if one smokes cigarettes because each cigarette contains about 0.2 mg/100 g cadmium, on the average (Harun and Ali, 2007). Cadmium can cause bone mineralization either through bone damage or by renal dysfunction. Studies on humans and animals have revealed that osteoporosis (skeletal damage) is a critical effect of cadmium.

2.7 Proximate Composition

The moisture, volatile matter, and ash results are typically among the primary parameters used for assessing the quality of a solid material. The procedures for the chemical analysis for the moisture, crude protein, ash, crude fiber, carbohydrate and fat contents were as outlined by the Association of official analytical chemists (AOAC, 1984).

2.7.1 Fiber content

Dietary fiber is defined as lignin plus the polysaccharide components of plants which are indigestible by enzymes in the human gastrointestinal tract. It is important for the removal of waste from the body thereby preventing constipation and many health disorders. Consumption of vegetable fiber has been shown reduce the cholesterol level, risk of coronary heart diseases, colon and blood cancers and hypertension, enhance glucose tolerance and increase insulin sensitivity. Common food sources of fiber are fruits, vegetables and whole grains. Barley is important cereal grain from a nutritional point of view due to its high dietary fiber contents particularly beta glucans which are beneficial to human health (Kaur, 2016).

2.7.2 Moisture content

The moisture content is considered as one of the most important quality criteria of barley to it for different purposes. Wet barley respires more rapidly than dry barley, which may lead to a rise in temperature. High temperature and humidity may then activate the growth of bacteria and fungi which can affect the storage and shelf life of barley. To avoid spoilage, immediate drying of barley after harvesting is needed. The low moisture content barley grains could have an extended shelf life in their raw form. The moisture content of a grain affects its stability and overall quality. Moisture content of a feed usually is calculated as the weight lost by material during application of heat to a sample (Thiex and Richardson, 2003).

Determination of Moisture

Methods which have commonly been used for this determination may be subdivided according as to whether their approach is physical or chemical. Physical methods include:
(a) Oven-drying, in which the weighed material is heated to a suitable temperature and the loss in weight determined
(b) Determination of loss in weight of the sample after drying in vacuo at room or elevated temperature with or without the use of desiccants
(c) Direct water determination by measuring after distillation with an immiscible Liquid
(d) other methods depending on the existence of a constant relation between water content and physical properties, such as electrical conductivity and detectable alteration of any significance other than the reversible loss of moisture. An oven temperature of 104-105 °C is desirable for the satisfactory determination of moisture in barley.

2.7.3 Volatile matter

Volatile matter in coal and coke is defined by the American Society for Testing Materials (ASTM, 1954) as "those products, exclusive of moisture, given off by a material as gas or vapor, determined by definite prescribed methods which may vary according to the nature of the material". It is one of the most important determinations in the proximate analysis.

2.7.4 Ash content

Ash, a residue that remains after ignition of organic matter, is used as starting point for the determination of elemental composition of food material. It is required by the human body for good health and growth (Husle, 1990).

2.7.5 Fixed carbon

Fixed carbon is a calculated value of the difference between 100 and the sum of the moisture, ash, and volatile matter where all values are on the same moisture reference base.

2.8 Analysis of metals

Metals may be determined satisfactorily by a variety of methods, with the choice often depending on the precision and sensitivity required. Several spectrometry techniques have been used for macro and trace elements determination in plants or biological materials. Flame Atomic Absorption Spectrophotometer (FAAS), hydride graphite atomic absorption spectrophotometer (HGAAS) or electrotermal atomization atomic absorption spectrophotometer (ETAAS) ,graphite furnace atomic absorption (GFAA), Inductively Coupled Argon Plasma Optical Emission Spectrometry (ICAP-OES), direct current argon plasma optical emission spectroscopy (DCAP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are most commonly used for the determination of metals in plant materials because of their inherent selectivity, sensitivity, precision and accuracy (Jacobs, 2007).

2.9 Sample decomposition

The various techniques for elemental determination do not all requires the same degree of sample matrix break down. The choice of a digestion technique should take into account the objective of the final determination and factors such as the matrix composition, the element contents, the possible interferences, the risk of loses and contaminations, the practicality and possible safety hazards in the laboratory (Ebdon et al, 1998). The purposes of sample decomposition are (Quevauviller et al, 1995).

- 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

- Obtaining the element in a homogeneous and easily accessible matrix. The different decomposition methods could be classified in to three categories.

a) Wet digestion

The majority of wet digestion methods involve different combinations of five acids (HNO_3, H_2SO4, $HClO_4$, HCl, and HF) and H_2O_2. The choice of an individual acid or combinations of acids depends upon the nature of the matrix to be composed. The procedure could take place either in open system or closed system (bomb decomposing with conventional or microwave heating) (Santos et al, 2004).

b) Dry ashing

Dry ashing is usually performed by placing the sample in an open inert vessel and destroying the combustible (organic) portion of the sample by thermal decomposition using a muffle furnace. After decomposition the residue is dissolved in acid and transferred to a volumetric flask prior to analysis. Typical ashing temperatures are 450 to 550 °C. Magnesium nitrate is commonly used as an ashing aid. Dry ashing is also conducted at 50-100°C under reduced pressure in an oxygen plasma discharge. Keeping its advantage and simplicity, it has some limitation. These are (Santos et al, 2004):

- Losses due to retention to the ashing container and volatilization.
- Contamination from the muffle furnace and sample container
- Physical loss of 'low density' ashes when the muffle furnace door is opened.
- Formation of toxic gases in poorly ventilated area.

c) Fusion

Fusion is a powerful technique especially both for organic matrices and those with a high silica and alumina content. Since solid and aggressive fusion reagents are difficult to purify, fusion cannot be recommended as ultra-trace analysis. A second disadvantage is

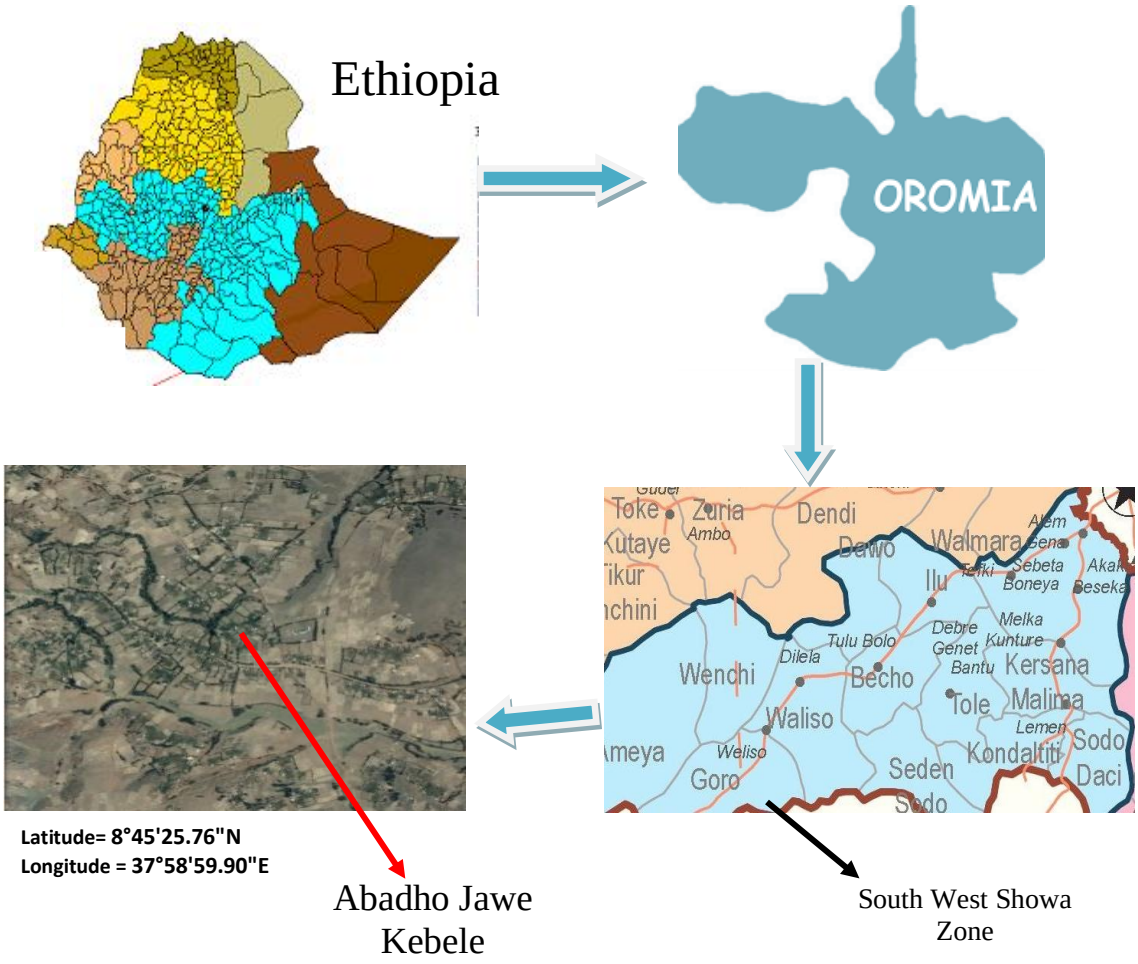
that the method is carried out in contact with ambient air. Risks of volatilizations are large (Negash et al, 2002).

3. MATERIALS AND METHOD

3.1 Description of the Study Area

Woliso district is one of the areas found in the South West Showa Zone of Oromia Regional State, Ethiopia. It is located along the main road from Addis Ababa to Jimma at a distance of 114 km from the capital city of the country Addis Ababa. Geographically, Dawo, Saden Sodo, Goro and Wenchi districts are the boundaries of this particular area. According to 2007 population census of Central Statistical Agency (CSA) report, the population size of Woliso district was 143,031 out of which 2,546 were urban whereas 140,485 were rural residents, and in the year 2014/2015 this number was projected to reach about 190,000. There are 37 rural localities and three small towns in the district. According to Woliso District administrative report 30 % of the area is highland and 70 % is with temperate ecological zone. The altitude of this area is from 1680-2883m above sea level. The annual rainfall is 1350-1500 mm with precipitation of 700-1200 mm annually. The average temperature of this district is maximum (25 ^{0}C) and minimum (10.3 ^{0}C). In most of the localities of this district, different varieties of barley are cultivated. Abadho Jawe is among the areas where barley is commonly cultivated and consumed in more extent.

Figure1: Map of the Study Area



Source: Google map & earth

3.2 Apparatus, chemicals, reagents and standard solutions

3.2.1 Apparatus

Analytical balance (OHAUS Switzerland) was used to weigh the barley samples, and air dry oven (Contherm 260M), was used to remove the moisture content of barley samples in the preparation of their powder form for metal analysis. Mortar and pestle was used to crush barley sample in to powder; 2.00 mm polyethylene sieve was used to remove large debris. Muffle furnace (BiBBY, Stuart) was used for fusion and ashing of barley samples. The digestion of barley samples were performed using hot plate. Borosilicate beakers (25, 50 and 100 mL) have used for the digestion of samples, and volumetric flasks (PYREX, UK) were used in holding the prepared sample solutions. Measuring cylinders and pipettes have been used during measuring different volumes of chemicals and reagents used. Flame atomic absorption spectrophotometers (Shimadzu AA-6800 and Varian spectraAA-20plus models) equipped with deuterium arc background correctors were used for analysis of the analyte metals using air-acetylene flame.

3.2.2 Chemicals, reagents and standard solutions

Chemicals and reagents used in the analysis were all analytical grade. Working standard solutions of Na, K, Mg, Ca, Fe, Zn, Mn, Pb and Cd were used for the calibration of instruments and spiking experiments. These standards were prepared in the laboratories of Ethiopian Geological Survey and Addis Ababa University where metal analysis and spiking processes have done. Double ionized water was used for dilution and preparation of reagents and standards. Concentrated nitric acid (HNO_3) 68% and hydrochloric acid (HCl) 35% were used for the digestion of the samples.

3.3 Procedures

3.3.1 Cleaning of apparatus

Apparatus such as volumetric flasks, measuring cylinders, beakers, porcelain crucibles, mortar, pestle, pipettes and polyethylene bottles were soaked with acid, washed with detergents and tap water, rinsed with de-ionized water, dried in oven and then kept in

dust free place until analysis of metal was performed. Prior to each use the apparatus were soaked and rinsed in de-ionized water.

3.3.2 Collection and Pretreatment of Samples

3.3.2.1 Sample Collection

In order to have representative and desired result, barley samples of three different varieties (Weketena, Abba Dulla and Black Barley) were collected from Abadho Jawe of Woliso District, South West Showa Zone. Sample collection was restricted only to this particular locality in order to reduce the effects of soil type, temperature, topography and other factors on mineral composition of these varieties. Abadho Jawe is one of the cold areas with highland ecological zone in the district. Crops like linseed, pea, beans, barley and wheat are cultivated widely in the area. Enset is the main food source in most of the local area of Woliso District. The three barley varieties were collected from randomly selected cultivator farmers in three sites of the locality. In one of the site, *Aruchu*, the three varieties (Abba dulla, Black Barley and Waketena) were collected from one cultivator farmer, where as in the other two sites *Tullu* and *Jawe* all varieties were collected from three different farmers respectively having nearby farmland for cultivation. This method of sample collection was applied to minimize more the effects mentioned above (soil type, topography of the landscape and fertility of the soil) on the variation of the levels of elements that analyzed. The samples that collected were those harvested in the same year (2016/2017). All the samples collected were homogenized according to their varieties (mixed 40 g each) and stored in polyethene bags.

3.3.2.2 Sample Treatment

The samples were placed in clean acid-washed porcelain crucibles and oven dried at 105^0C for 24h in a drying oven. The dried sample was then ground in to a fine powder form by using acid washed mortar and pestle, and was passed through a 2.0 mm sieve to remove large debris. The powdered samples were kept in polyethene bottles for further analysis.

3.4 Proximate Analysis

3.4.1 Determination of Moisture Content

The procedure for the determination of moisture content of the three barley varieties (Abba dulla, Black Barley and Waketena) was as outlined by the Association of Official Analytical Chemists (AOAC, 1990). This involves the drying of barley samples to a constant weight at 105 ^{0}C and calculating moisture content as the loss in weight of the dried samples. Accordingly, the crucible was thoroughly washed and dried in an oven at 100 ^{0}C for 30 minutes and allowed to cool in the desiccators. After cooling, the crucible was weighed and recorded. 2.03g of barley sample was placed in the crucible; weighted and registered. The crucible with sample then placed in air drying oven and allowed to dry for 4 hours at 105 ^{0}C; removed from oven, cooled and weighed. In 30 minutes interval this process continued until constant weight was obtained. Finally the moisture content of each barley sample (Abba dulla, Black Barley and Waketena) was calculated in triplicates as follows:

$$MC\% = \frac{W_2 - W_3}{W_2 - W_1}\ x\ 100 \qquad (1)$$

Where,

MC= moisture content

W_1 = weight of dry crucible

W_2= weight of moist sample + weight of dry crucible

W_3= weight of dry (sample + crucible)

$W_2 - W_3$= moisture mass

$W_2 - W_1$= initial mass

3.4.2 Determination of volatile matter

The oven dried sample of each barley variety was incinerated in muffle furnace at temperature of 950 ℃ for seven minutes (Roberto et al, 2013). The charred samples were removed from muffle furnace, allowed to cool and weighed for the determination of volatile matter in each barley varieties as follow:

$$VC\% = \frac{W_2 - W_3}{W_2 - W_1} x\ 100 \qquad (2)$$

Where,

VC = Volatile content

W_1 = weight of dry crucible

W_2 = weight of dry (sample + crucible)

W_3 = weight of charred sample

W_2-W_3 = volatile mass

W_2-W_1 = initial mass = mass of dry sample

3.4.3 Determination of Ash Content

Total ash content of the samples was determined using furnace incineration as described by (AOAC ,1990) based on the vaporization of water and volatiles with burning organic substances in the presence of oxygen in air to carbon dioxide at a temperature of 550 ^{0}C. After the determination of volatile content of barley varieties, the charred content was weighed and placed in muffle furnace at the above specified temperature for 6 hours until white ash formed. The resulting ash removed from the furnace; cooled in desiccators and weighed.

$$Ash\% = \frac{(Ash\ mass)}{(initial\ mass)}\ x\ 100 \qquad (3)$$

Where,

Initial mass= mass of dry sample

3.4.4 Determination of fixed carbon

Fixed Carbon is the barley residue besides ash, which remains after the removal of moisture and volatile contents (Roberto et al, 2013).

Fixed Carbon = 100- ([Moisture] + [Volatile] + [Ash]) (4)

3.5 Preparation of the Samples for Metals Analysis

3.5.1 Sample Digestion

A 5.0 g of each powdered barley variety was weighed and transferred in to a clean crucible labeled according to the sample type, and then dry-ashing process was carried out in a muffle furnace at 500 ℃ and left to ash at this temperature for 6 hours. The samples were removed from the furnace and allowed to cool, then carefully transferred to pre-cleaned borosilicate beakers (75 mL). The ash of each barley variety was wet using 10 mL de-ionized water followed by addition of 10 mL of concentrated nitric acid (HNO_3). The beakers then covered with watch glass; placed on hot plate and performed the digestion at a temperature of 90 ℃ until nitric acid and water evaporated totally. The resulting ash of each respective sample was placed again in muffle furnace adjusted to a temperature of 375 ℃ and digested for one hour to obtain the optimized white ash. The ashed powder then removed from the muffle furnace and allowed to cool. The resulting ash was dissolved in 25 mL de-ionized water and 5 mLof hydrochloric acid (35 %); placed on hot plate and boiled at 90 ℃ for 25 minutes until clear solution exist. After cooling, 20 mL of distilled water was added and filtered with Whatman filter paper (No. 541) in a pre-cleaned and rinsed 50 mL volumetric flask. This step eliminates some residues which would interfere the metals to be analyzed. The filtered solution was then diluted up to the mark of 50 mL standard volumetric flask; transferred to polyethylene bottle and stored in refrigerator until analysis. All samples were prepared identically in triplicates.

Blanks were prepared to check for background contamination by the reagents used. For preparation of six blank solutions, the same procedure of sample preparation was

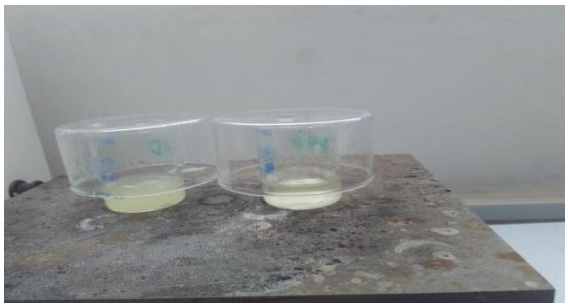
followed, that means 10 mL de-ionized water was added to pre-cleaned borosilicate beaker (50 mL) followed by addition of 10 mL concentrated nitric acid (68 %). The mixture containing beaker then placed on hot plate at temperature of 90 ℃ and left to heat until the acid and water evaporated totally. The resulting cooled beaker then placed in muffle furnace at temperature of 375 ℃ and allowed to digest for one hour. After the beaker was removed from furnace and cooled, 25 mL de-ionized water was added followed by 5mL concentrated HCl (35%) and placed on hot plate then boiled for 25 minutes. The beaker has removed from the furnace and 20 mL de-ionized water was added; filtered with whatman filter paper (No. 541) in to pre-cleaned volumetric flask (50 mL) and diluted up to the mark. The filtered blank solution then transferred to cleaned polyethylene bottle and stored in the refrigerator until the analysis.

Figure 2: Photos that show each stage of the barley samples in the processes of experimentation for the intended metal analysis

Black barley *Waketena* *Abba dulla*

Ash of each variety after removed from muffle furnace

While digesting on Hot plate with (DI water & HNO3)



While digesting on Hot Plate with (DI & HCl)

Final sample solutions prepared for metals analysis

3.5.2 Determinations of the essential and non-essential metals

Intermediate standard solutions containing 100 mg/kg were prepared in 1000 mL volumetric flask from the atomic absorption spectrophotometer standard stock solutions that contained 1000 mg/kg. Three or four working standards for each metal of interest were prepared from these intermediate standard solutions and used for calibration and spiking purposes. Then, Na, K, Mg and Ca were analyzed by flame atomic absorption spectrophotometer (SHIMADZU AA-6800) model and Fe, Zn, Cu, Mn, Pb and Cd were analyzed by flame atomic absorption spectrophotometer (Varian spectraAA.20plus) model equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the alignment of parameters (burner,

lamp current, slit width and wavelength) were optimized for maximum signal intensity of the instrument. For each element, respective hollow cathode lamp was used, and the solution was successively aspirated into the flame. Three replicate determinations were carried out for each sample at the laboratory of Ethiopian Geological Survey, Addis Ababa. The elements were determined by absorption/concentration mode and then the instrument readout was recorded for each solution. The same analytical procedure was employed for the determination of elements in the six digested blank solutions. The instrument operating conditions employed for each analyte are given in Table 1 below.

Table 1: Instrumental operating conditions for the determination of metals in Barley samples

Element	Wave length (nm)	Lamp current (mA)	Slit width (nm)	Instrument detection limit (mg/kg)	Flame type
Na	589.0	5	0.5	0.006	Air acetylene
K	766.5	5	1.0	0.006	"
Mg	285.2	4	0.5	0.006	"
Ca	422.7	10	0.5	0.005	"
Fe	248.3	5	0.2	0.006	"
Mn	279.5	5	0.2	0.006	"
Cu	324.8	4	0.5	0.007	"
Zn	213.9	5	5.0	0.002	"
Pb	217.0	5	5.0	0.060	"
Cd	228.8	4	4.0	0.002	"

Instrument detection limit (IDL) is the smallest signal above background noise that an instrument can detect reliably (Jeffrey, 1996). The value of instrumental detection limit given for each metal is that provided by the manufacturer of the instrument.

Figure 3: Flame Atomic Absorption Spectrophotometer (Simadzu AA-6800) model

Figure 4: Flame Atomic Absorption Spectrophotometer (varian spectraAA-20plus) model

3.6 Method Validation

3.6.1 Recovery Test (R %)

The effectiveness of analytical procedure can be checked by various methods. These are certified reference material analyzing and adding known concentration of analyte (spiking) in to the sample of interest. For this study, the effectiveness of analytical procedure was evaluated using the spiking method (recovery test). Spiking process was performed in the laboratory of Addis Ababa University. The resulting spiked samples were digested, diluted and analyzed for the essential and non-essential metals of interest. Levels of Ca, Zn, Cu, Pb and Cd metals in the spiked samples were also analyzed at Addis Ababa University and levels of the remaining metals (Na, K, Mg, Fe and Mn) in

the spiked samples were analyzed at the laboratory of Ethiopian Geological Survey, Addis Ababa. The spiking procedure was as below:

The 10 mg/kg standard of each Na and K were spiked at once in to a beaker containing 5 g of barley sample; 10 mg/kg of Mg, Ca, Fe and 5 mg/kg of Mn were spiked at once in the second beaker containing 5 g of the sample. Similarly, 2 mg/kg Cd and 1mg/kg of Zn, Cu & Pd were spiked at once in the third beaker containing a sample and the same digestion system was performed. The spiking levels were chosen based on the levels of standards used in the calibration process; which means, the amount that equal to one of the standard level used for the calibration was taken. Spiking process was performed in three categories to minimize the effect that might be arise due the concentration of one metal suppress the concentration of other metal. The spiked samples were determined for the respective metals of interest by FAAS. The recovery tests for the three samples were performed in triplicates. The percent recovery for each metal was calculated as below:

$$R\ \% = \frac{([\text{Concentration in Spiked Sample}]-[\text{Cononcentration in Unspiked Sample}])}{[\text{Total standard added}]} \times 100 \qquad (5)$$

3.6.2 Method detection limit

Method Detection Limit (MDL) is the minimum concentration of a substance that can be measured and reported with 99 % confidence that the analyte concentration is greater than zero, and is determined from analysis of a sample in a given matrix containing the analyte (Theodore, 2003). The limit of detection is most commonly defined as the amount of analyte that gives a signal equal to three times the standard deviation of a blank. In this study, after digestion of six blank solutions six readings were taken for each blank and the standard deviations of these were calculated. The calculated value in such away represent the measured minimum concentration of the analytes and the values reported with 99 % confidence that the analyte concentration greater than zero. The method detection limit of each element was obtained by multiplying the standard of the reagent blank by three (Jeffrey, 1996).

$$MDL=3XSD_{blank} \qquad (6)$$

SD_{blank} is the standard deviation of the blank readings

3.7 Data Analysis

The organized data were analyzed using SPSS software and Microsoft Excel 2007. The calibration curve of each element from its respective standards used was analyzed by Microsoft Excel 2007. Mean comparisons, variations in levels of essential and non-essential metals of interest and standard deviation of the triplicate measurement for each metal of interest in each barley varieties were analyzed using SPSS software. Descriptive data have generated and presented as means ± standard deviation for the results of proximate analysis and concentration of metals in each sample. The mean variations in data among the three barley varieties were analyzed using one-way ANOVA. The variation in mean values resulted are considered as significant if the calculated p-values < 0.05 at 95% confidence interval.

4. RESULTS AND DISCUSSION

4.1 Proximate composition of barley varieties

a) Moisture Content

The result of analysis carried out using equation (1) on moisture content of three barley varieties shows that Black Barley contains high moisture level 3.45 %, while both Abba Dulla and Waketena had equal moisture content of 2.97 % (Table 2). These values were lower when compared to the reported moisture content of barley and other cereals (maize and rice). For instance the moisture content of barley was reported as 9.65 % (Tefera et al, 2015). As reported by (Khader and Phani, 1997) the moisture content of maize was from 6.00 to 7.50 %. Similarly, (Oko et al, 2012) reported that different rice varieties have moisture content in the range of 3.67 to 8.0 %. This lower moisture content could be one of a factor that black barley has been used for malt in making homemade alcoholic beverages, as lower moisture content is required to inactivate enzymes that are responsible for unwanted earlier germination.

b) Volatile Matter Content

The present study indicated that (Table 2), WK variety has high volatile content (83.67 %) and AD with the minimum amount (81.32 %). The volatile content of the other variety (BB) was nearly between the two values (83.33 %).

c) Ash Content

As it can be observed from the result (Table 2), ash percent of Abba dulla was found to be maximum 2.88 % and Waketena contains the minimum amount 2.21 %. Generally, these values were in line with the literature values of ash content of barley and other cereals such as maize and rice. For instance 2.5 % ash content of barley whole grain was reported by (Sakhawat Ali, 2014). In similar manner, (Khader and Phani, 1997) reported the ash content of maize in the range of 1.96 to 2.95 % and, 0.5 to 2 % for different rice varieties.

Table 2: Average proximate composition of the three barley varieties under study (Mean ± STD)

Barley variety	Moisture%	Volatile%	Ash%	Fixed carbon%
AD	2.97 ±0.01	81.32 ± 0.27	2.88 ± 0.29	12.83 ± 0.04
BB	3.44 ± 0.01	83.33 ± 0.78	2.38 ± 0.29	10.84 ± 0.51
WK	2.97 ± 0.02	83.67 ± 0.51	2.21 ± 0.59	11.15 ± 0.78

4.2 Results of Method Validation

Instrument Calibration

The qualities of results obtained for essential and toxic metals analysis can be affected by the calibration and standard solution preparations procedures. The instrument was calibrated using three or four series of working standards. The working standard solutions of each metal were prepared freshly by diluting the intermediated standard solutions (Table 3).The calibration graph of each of metal of interest is shown in Appendix 2.

Table 3: Series of working standards and correlation coefficients of the calibration curves for the determination of metals in the barley varieties using FAAS

Metal	Concentration of Standards	Absorbance	Regression Equation	Correlation Coefficient (R)
Na	5, 10, 20	0.1792, 0.4038, 0.8363	Y=0.042x-0.014	0.998
K	5, 10, 20	0.1813, 0.3556, 0.6974	Y=0.034x + 0.004	0.999
Mg	5, 10,20	0.1878, 0.3755, 0.7292	Y=0.036 + 0.004	0.999
Ca	5,10,20	0.2423, 0.4442, 0.8091	Y=0.04x + 0.023	0.995
Fe	5, 10, 20	0.062, 0.124, 0.246	Y=0.012x + 0.000	1.000
Mn	2.5, 5, 10	0.062, 0.127, 0.239	Y=0.023x + 0.002	0.998
Cu	0.5, 1, 2, 5	0.020, 0.043, 0.083, 0.210	Y=0.042x – 0.000	0.999
Zn	0.5, 1, 2, 5	0.055, 0.109, 0.197, 0.405	Y=0.079x + 0.018	0.990
Pb	0.5, 1, 2, 5	0.014, 0.030, 0.056, 0.141	Y=0.028x + 0.000	0.999
Cd	1, 2, 5	0.061, 0.116, 0.263	Y=0.052x + 0.006	0.997

Method Detection Limits

Six blank samples were digested following the same procedure as the samples and each of the samples were determined for the elements of interest (Na, K, Mg, Ca, Fe, Zn, Cu, Mn, Pb, and Cd) using flame atomic absorption spectrophotometers (Table 4). The standard deviation for each element was calculated based on equation (6) from six blank measurements for the determination of method detection limit (MDL) by SPSS Statistics 20.

Table 4: Method detection limit for metals analysis in AD, BB & WK barley samples (n=6)

Metal	MDL(mg/kg)	IDL (mg/kg)
Na	1.17	0.006
K	1.42	0.006
Mg	1.05	0.006
Ca	1.10	0.005
Fe	1.2	0.006
Mn	0.54	0.006
Zn	0.33	0.002
Cu	0.21	0.007
Pb	0.11	0.060
Cd	0.10	0.002

Recovery test (R %)

The results of recoveries tests of the metals levels in the spiked samples were between 80 % and 118.2 % (Tables 5-7). The mean percentage recoveries calculated according to equation (5) for all analytes were within an acceptable range, 80-120 % (Jeffrey, 1996) indicating the laboratory performance for each analyte is in control. Recovery values in the above range are acceptable for both major and trace analysis and the digestion procedure is believed to remove metal fractions that associated with organic matter.

Table 5: Recovery Test for Abba Dulla

Sample	Metal	Con in unspiked Sample (mg/kg)	Amount added (mg/kg)	Con in Spiked sample (mg/kg)	Recovery (%)
Abba Dulla (AD)	Na	24.0	10	33.50	95
	K	179.0	10	188.13	91.3
	Mg	19.0	10	27.10	81
	Ca	1.92	10	11.95	100.3
	Fe	9.63	10	18.54	89.1
	Cu	0.14	1	1.19	105
	Zn	1.36	1	2.28	92
	Mn	1.80	5	6.79	99.8
	Pb	0.28	1	1.15	87
	Cd				

Table 6: Recovery test for barley (Black Barley) sample

Sample	Metal	Con in unspiked Sample (mg/kg)	Amount added (mg/kg)	Con in Spiked sample (mg/kg)	Recovery (%)
Black Barley (BB)	Na	24.10	10	32.15	80.5
	K	296	10	305.20	92
	Mg	5.80	10	16.05	102.5
	Ca	2.59	10	12.65	100.6
	Fe	9.50	10	20.52	110.2
	Cu	0.10	1	1.22	112
	Zn	1.01	1	2.07	106
	Mn	1.51	5	6.76	105
	Pb	0.15	1	1.14	99
	Cd				

Table 7: Recovery test for barley (Waketena) sample

Sample	Metal	Con in unspiked Sample (mg/kg)	Amount added (mg/kg)	Con in Spiked sample(mg/kg)	Recovery (%)
Waketena (WK)	Na	23.30	10	34.12	118.2
	K	270	10	368.11	98.11
	Mg	14	10	22.75	87.5
	Ca	1.35	10	10.97	96.2
	Fe	8.25	10	19.0	107.5
	Cu	0.14	1	1.21	107
	Zn	1.63	1	2.71	108
	Mn	1.50	5	6.79	105.8
	Pb	0.35	1	1.15	80
	Cd				

4.4 Levels of metal contents in the analyzed barley samples

Iron (Fe)

Iron was found with its highest mean concentration in Black Barley (8.82 mg/kg) and lowest mean concentration (6.80 mg/kg) in Abba dulla analyzed samples (Table 8). The result was in line with the level reported, 8.4 mg/kg by (Abebe et al, 2007) for barley sample. The (FAO/WHO, 2001) maximum daily intake of iron concentration in food is 425 mg/kg and the result obtained in this study is below the recommended limit. All the three varieties contain the element (Fe) with level below the maximum tolerable limit, thus can be consumed safely as important source of it. One-way ANOVA shows that there was significant difference ($p<0.05$) in levels of Fe among the three barley varieties. Post hoc test also revealed that each variety significantly varied from one another by Fe level in them.

Zinc (Zn)

From the present investigation (Table 8) it can be seen that, the mean levels of Zn were 1.55 mg/kg in AD, 1.34 mg/kg in WK and 1.12 mg/kg in BB samples. The concentration of Zn obtained from this study is lower than (FAO/WHO, 2001) guidelines (99.4 mg/kg). One-way ANOVA revealed that there was a significant difference ($p<0.05$) in levels of Zn among the three samples. The result of post hoc test also revealed that all the varieties (Abba dull, Black Barley and Waketena) show significant variation from each other in Zn concentration that analyzed.

Table 8: Result of average concentrations of essential and non-essential metals in barley samples and statistical p-values (Average con. as mean ± Std, n=3)

	AD (mg/kg(	BB (mg/kg)	WK (mg/kg)	One-way ANOVA	
Metal	Mean ± Std	Mean ± Std	Mean ± Std	p-value	Significant
Na	18.96 ± 0.63	25.77 ± 1.57	26.20 ± 2.57	0.00	Significant
K	196.93 ±16.76	241.90± 2.70	200 ± 12.40	0.00	Significant
Mg	2.90 ± 0.19	11.95 ± 0.96	4.46 ±0.14	0.00	Significant
Ca	2.01± 0.16	2.24 ± 0.05	1.61± 0.07	0.55	Insignificant
Fe	6.80 ± 0.18	8.82± 0.72	7.42 ±0.17	0.00	Significant
Cu	0.21 ± 0.01	0.15 ± 0.02	0.25 ± 0.01	0.00	Significant
Zn	1.55 ± 0.04	1.12 ± 0.10	1.34 ± 0.08	0.00	Significant
Mn	0.84 ±0.01	1.38 ± 0.02	1.22± 0.03	0.00	Significant
Pb	0.23 ± 0.02	0.14 ± 0.01	0.17 ± 0.01	0.33	Insignificant
Cd	BDL	BDL	BDL		

BDL= Below Detection Limit

Manganese (Mn)

Manganese is essential element required for various biochemical processes. As can be seen from (Table 8), maximum mean concentration of manganese was obtained from Black Barley (1.38 mg/kg) and minimum value from Abba Dulla (0.84 mg/kg) varieties. The third variety, Waketena contains 1.22 mg/kg Mn. These levels further support the content of manganese analyzed as 1.67 mg/kg for barley sample (Wodaje, 2015). The analyzed concentrations were below FAO/WHO safe limit (500 mg/kg). One-way ANOVA and post hoc tests revealed that there was a significant difference ($p<0.05$) in the levels of manganese among and within the samples respectively.

Potassium (K)

The concentration of potassium in the barley types under study ranges from maximum content of 200 mg/kg in Waketena and minimum amount of 116.06 mg/kg in Black Barley. From the analyses carried out, it was quite evident that potassium is one of the dominant elements in barley. The estimated minimum requirement by adolescents and

adults are 2.0 g/day. However, many nutritionists now recommend higher dose of 3.5 g to reduce the risk of high blood pressure (FNB, 2004). Even though the concentration of potassium analyzed in the varieties seem low when compared to the recommended higher does, it is safe to use these varieties as a source of potassium. One-way ANOVA and post hoc test were revealed that there was significant differences ($p<0.05$) in the content of K among and within the samples under study respectively.

Magnesium (Mg)

In the present study the mean values of magnesium ranges from 2.90 to 11.95 mg/kg in the three barley samples. The maximum concentration (11.95 mg/kg) was recorded in Black Barley and minimum concentration (2.90 mg/kg) in Abba dulla sample. The third variety Waketena possessed 4.46 mg/kg magnesium concentration. The use of these varieties in diet is safe as the food and nutrition board of the institute of medicine (FNB, 1997) recommended up to 400 mg magnesium per day. Magnesium concentration was vary significantly among the three samples (one-way ANOVA, $P<0.05$). The post hoc analysis also implies that all the three barley varieties were varied in terms of Mg concentration.

Sodium (Na)

The content of sodium in the samples under study ranges from a maximum value of 26.20 mg/kg in Waketena to a minimum value of 18.96 mg/kg in Abba dulla variety (Table 8). The nutritional requirement for sodium is about 1100 – 3300 mg (FNB, 1989), however, much sodium is added to food in sodium chloride form and the contribution of unprocessed food source is only about 10 to 15%. Thus, the analyzed level of sodium could be safe to consume these barley varieties as good source of diet. Statistical one-way ANOVA ($P<0.05$) revealed that there is significant variation of sodium concentration between Abba dulla, Black Barley and Waketena barley varieties. All the three varieties under study show significant variation from each other in sodium level analyzed according to post hoc test.

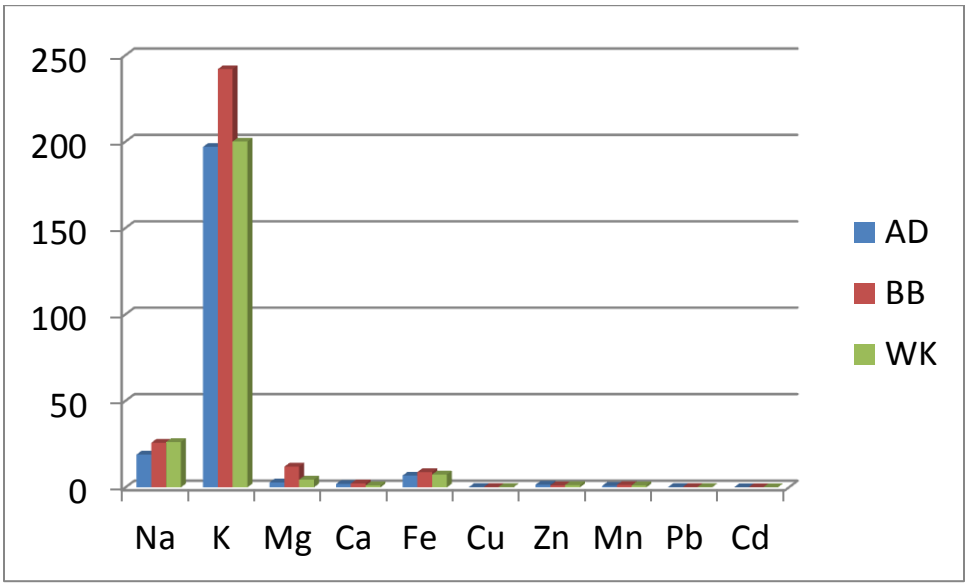


Fig 5: Levels of metals concentration (mg/kg) in three varieties of barley samples

Lead (Pb)

In the present study, the lead level ranges from maximum content of 0.23 mg/kg in Abba dulla to minimum value of 0.14 mg/kg in Black Barley which falls below the recommended safe limit (0.3 mg/kg) of FAO/WHO. Statistical One-way ANOVA shows that there was no significant variation in Pb concentration among Black Barley and waketena varieties under study. The result from post hoc test revealed that Abba dulla and Waketena varied from each other just as Abba dulla varied from Black Barley in the level of Pb concentration.

Copper (Cu)

The present investigation revealed that the concentration of copper (Table 8) varies from maximum 0.25 mg/kg in Waketena to minimum content of 0.15 mg/kg in Black Barley. A daily intake of 0.9 mg/day of copper is recommended for adults (FNB, 2001). Copper concentrations in these samples fall below this recommended safe limit (0.9 mg/day) implying that, these barley varieties can be consumed safely as copper is an essential trace metal that is vital to health of humans and required by body for bone and connective tissue production (EFSA, 2006). Statistical result of ANOVA ($p<0.05$) revealed that there is significant variation between the mean concentration of copper metal in Abba dulla, Black Barley and Waketena barley varieties. Post hoc test also showed that all these varieties varied significantly by the copper level within them.

Cadmium (Cd)

As can be observed from the result of the present study (Table 8), Cd concentration is reported as below detection limit. That means 0.03 mg/kg level was recorded in both Waketena and Abba dulla and 0.02 was recorded in Black Barley in which both values were below detection limit (0.1 mg/kg). Even though these values were below the safe limit of FAO/WHO standard (0.2 mg/kg) care should be given since it can harm human health even at low concentration. Statistical result of ANOVA ($p >0.05$) shows that the mean concentration of cadmium among the three varieties was not significantly different. Post hoc test also showed that there was no significant variation between these verities in cadmium concentration within them.

Calcium

The concentration of calcium in the three barley varieties under study was lower when compared to that of K and Na. The mean concentration of Ca that recorded in this study was 2.01 mg/kg in Abba dulla, 2.24 mg/kg in Black Barley and 1.61 mg/kg in Waketena

barley variety. Calcium levels that were analyzed in the three barley varieties were lower when compared to the value reported value for barley (Table, 9). The mean concentration of calcium between Black Barley and Waketena varieties showed no significant variation (P >0.05). However, significant variation was observed among Abba dulla and Waketena varieties. Post hoc test further revealed that both Abba dulla and Waketena varied significantly from each other with their calcium concentration.

Table 9: Comparison of the analyzed concentration of major metals in barley samples with literature values of other cereals (mg/kg)

Cereal		Macro metals				Reference(s)
		Na	K	Mg	Ca	
Barley	Analyzed	18.96-26.20	116-200	2.90-11.95	1.61-2.24	This study
	Reported				45	Abebe et al, 2007
Wheat	Reported	30	1500	200	1400	Holland et al., 1988
		126.5-169.4	761.1-933.9	179.43-200.6	226.4-226.7	Raquel et al, 2013
Rice	Reported	40	3400	1200	1200	Holland et al., 1988

Table 10: Comparison of the analyzed concentration of trace metals in barley samples with literature values of other cereals (mg/kg)

Metal	Barley		Wheat	Maize	Sorghum Reported	Reference
	Analyzed	Reported	Reported	Reported		
Fe	6.8-8.82	31.85 8.4	10.64	0.40	36.45	(Wodaje, 2015) (Abebe et al, 2007)
Zn	1.12-1.55	3.85	8.54	0.66	5.40	(Wodaje, 2015)
Cu	0.14-0.21	0.15	1.72	0.13	1.25	"
Mn	0.84-1.38	1.67	7.67	0.42	2.34	"
Pb	0.14-0.23	0.03	0.05	-	0.08	"
Cd	BDL	-	-	-	-	"

5. CONCLUSSION AND RECOMMENDATION

In this study, assessment of the levels of Na, K, Mg, Ca, Fe, Cu, Zn, Pb and Cd metals in the three varieties of barley, Abba dulla (AD), Black Barley (BB) and Waketena (WK) was performed.

After digestion of samples carried out, analysis of each metal was done using FAAS of two models, Shimadzu AA-6800 for Na, K Mg, Ca metals and Varian Spectra AA-20plus for the remaining metals of interest. The variation of levels of these metals among the samples was analyzed using statistical one-way ANOVA, and the individual variation of the three barley varieties due to the levels of these analyzed metals was analyzed by post hoc test. As a result there were significant differences in the level of the majority of the metals (Na, K, Mg, Fe, Zn, Cu and Mn). However, there was no significant variation in the levels of Pb and Cd among the varieties, except between AD and WK as well as AD and BB for lead concentration according to post hoc test. In case of calcium level, there was significant variation between AD and WK as well as AD and BB barley varieties, but no significant variation was observed between BB and WK barley varieties.

Relatively, all the analyzed barley samples contain high K, Na and appreciable Fe concentrations. Waketena has got higher concentrations of Na, Mg, Cu and also more levels of K and Fe next to Black Barley (Fig. 5). This can be one of the factors that Waketena is commonly consumed to repair fractured bone as K, Fe and Cu metals are used for bone and connective tissue development (Johns and Duquette, 2006)). In addition, this particular variety (Waketena) is chosen as good source of nutrients for pregnant and birth gave mothers as well as for babies may be due to relatively more Fe levels. Iron carries oxygen to the cells and is necessary for the production of energy, the synthesis of collagen, and the functioning of the immune system. Babies born to mothers that are iron-deficient are commonly stunted and unhealthy (EFSA, 2006). The other

variety, Black Barley contains K, Mg, Ca, Fe and Mn metals in more extent. Thus it can be consumed as important dietary in addition to its use in making home-made alcoholic drinks because these elements are all essential nutrients. The third barley variety, Abba dulla contain more concentration levels of Zn and Pb metals.

The presence of Pb was detected in all the samples under study, though Abba dulla variety has got relatively more concentrations. Lead level that analyzed in the three varieties was below FAO/WHO safe limits. As the result of this study shows, moisture, volatile, ash and fixed carbon content of these varieties were almost in line with the literature values (Tefera et al, 2015; Sakhawat, 2014).

The level of metals (Na, K, Mg and Ca) analyzed in the samples under study were below the literature values (Table 9). This may be due to variation in instruments, persons who run the FAAS instruments while metals analysis, method used, topography and the whole situation in which the samples grown. However, the analyzed levels of Fe, Cu, Zn, Pb and Cd metals were almost in line with the reported literature values of barley and other cereals (Table 10).

Finally, even though the results of this research work alone are not enough to give general conclusion about the metal contents of barley varieties grown in Woliso District as a whole, the three barley varieties can be consumed as good sources of essential elements, but further investigation is recommended with more sampling area especially on Abba dulla variety as it contained more lead concentration.

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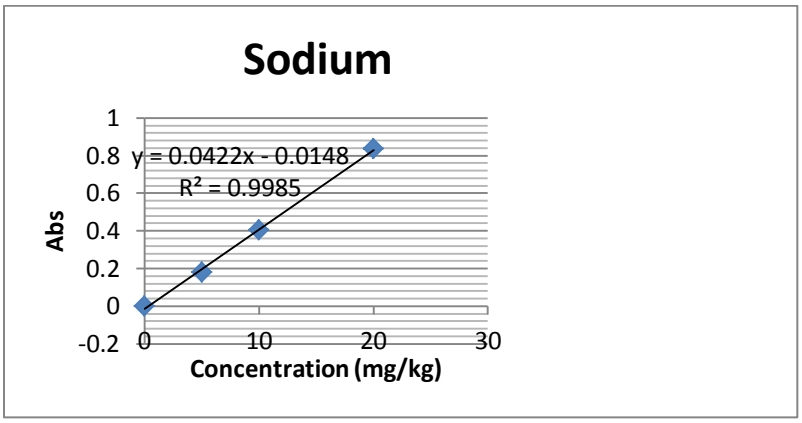
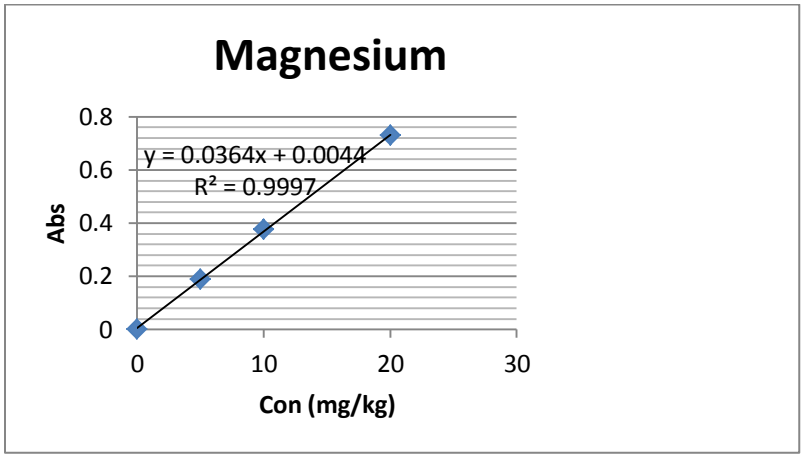
Appendix 1: Data of triplicate measurements of concentrations of metals in three barley samples by FAAS instruments in mg/kg.

Abba dulla (AD)										
	Na	K	Mg	Ca	Fe	Cu	Zn	Mn	Pb	Cd
1	18.46	199.60	3.01	1.92	6.97	0.22	1.60	0.84	0.25	BDL
2	18.75	212.19	2.67	2.19	6.81	0.21	1.52	0.85	0.22	“
3	19.67	179.00	3.02	1.92	6.62	0.20	1.53	0.83	0.22	“
mean	18.96	196.93	2.90	2.01	6.80	0.21	1.55	0.84	0.23	
std	0.63	16.76	0.19	0.16	0,18	0.01	0.04	0.01	0.02	
RSD	3.32	8.51	6.55	7.96	2.64	4.76	2.58	1.19	8.7	
Black barley (BB)										
1	24.10	245.00	11.45	2.30	9.23	0.13	1.01	1.36	0.15	BDL
2	26.00	240.00	11.78	2.20	7.99	0.16	1.22	1.39	0.14	“
3	27.21	240.70	12.62	2.22	9.24	0.16	1.13	1.39	0.13	“
mean	25.77	241.90	11.95	2.24	8.82	0.15	1.12	1.38	0.14	
std	1.57	2.70	0.60	0.05	0.72	0.02	0.10	0.02	0.01	
RSD	6.09	1.11	5.02	2.23	8.16	13.33	8.93	1.45	7.14	
Waketena (WK)										
1	25.00	187.60	4.45	1.35	7.49	0.25	1.43	1.23	0.18	BDL
2	24.45	200.00	4.32	1.55	7.23	0.24	1.31	1.24	0.17	“
3	29.15	212.40	4.61	1.93	7.54	0.26	1.28	1.19	0.16	“
mean	26.20	200.00	4.46	1.61	7.42	0.25	1.34	1.22	0.17	
std	2.57	12.40	0.14	0.07	0.17	0.01	0.08	0.03	0.01	
RSD	9.81	6.20	3.14	4.35	2.29	4.00	5.97	2.46	5.88	

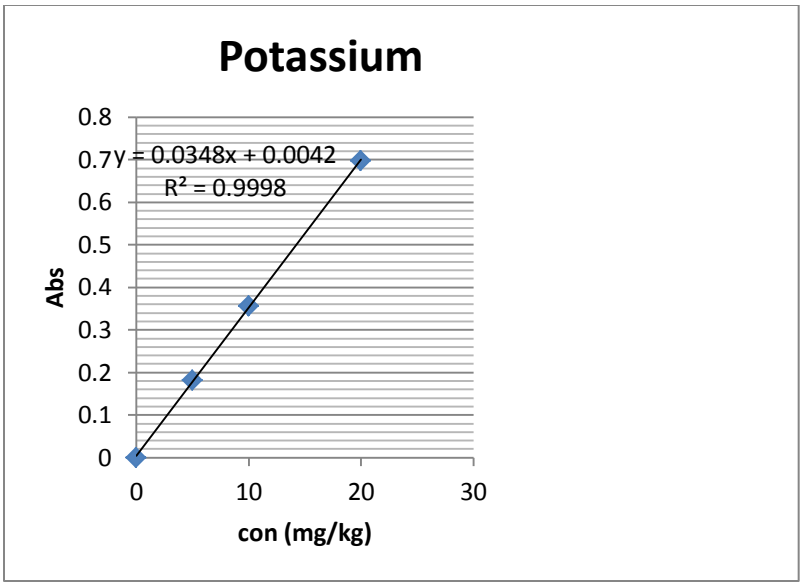
Appendix 2: Figure 4(a-j): The calibration graphs of ten metals of interest analyzed from barley varieties using Microsoft Excel 2007



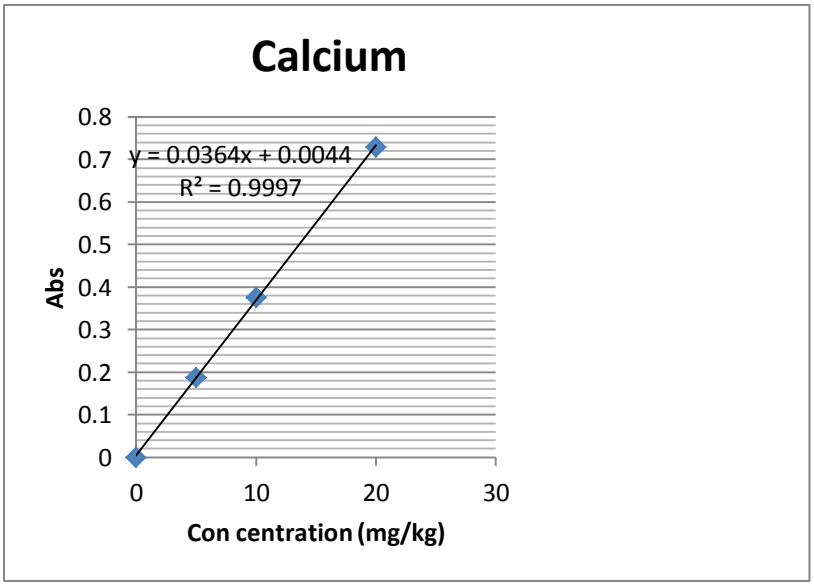
a) Calibration graph of sodium standards



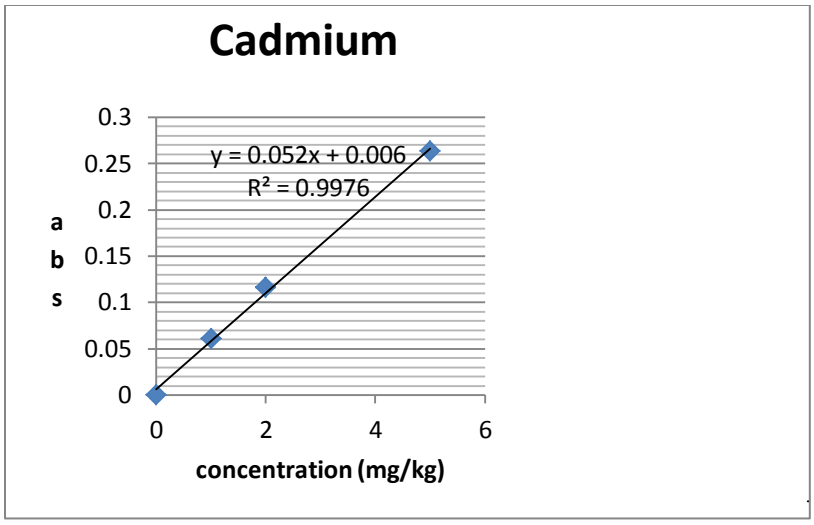
b) Calibration graph of magnesium standards



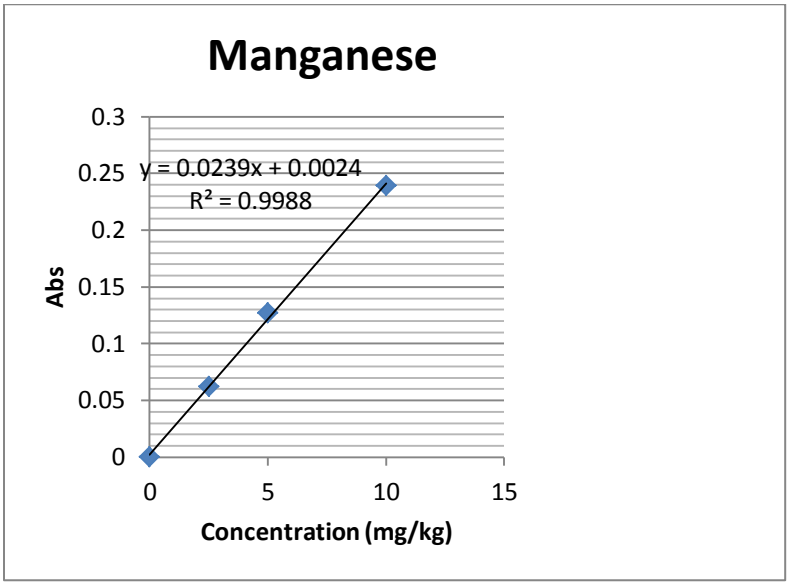
c) Calibration graph of potassium standards



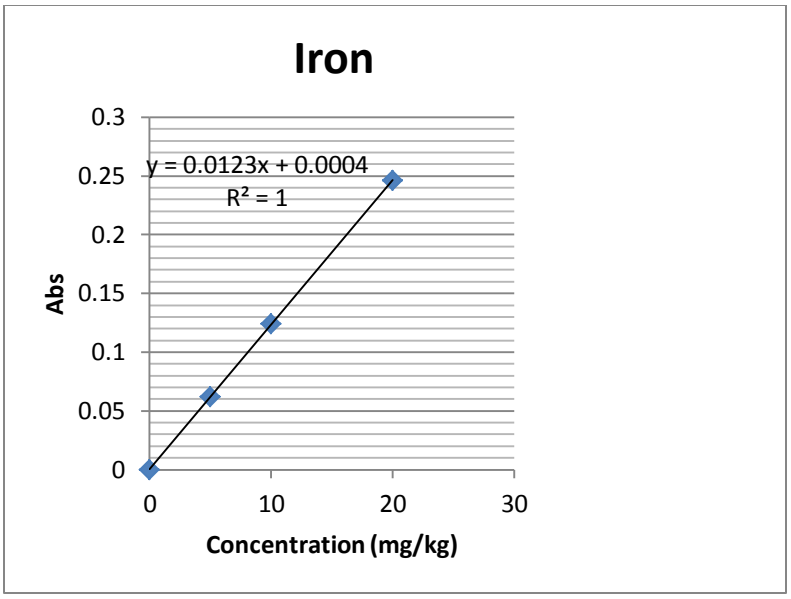
d) Calibration graph of calcium standards



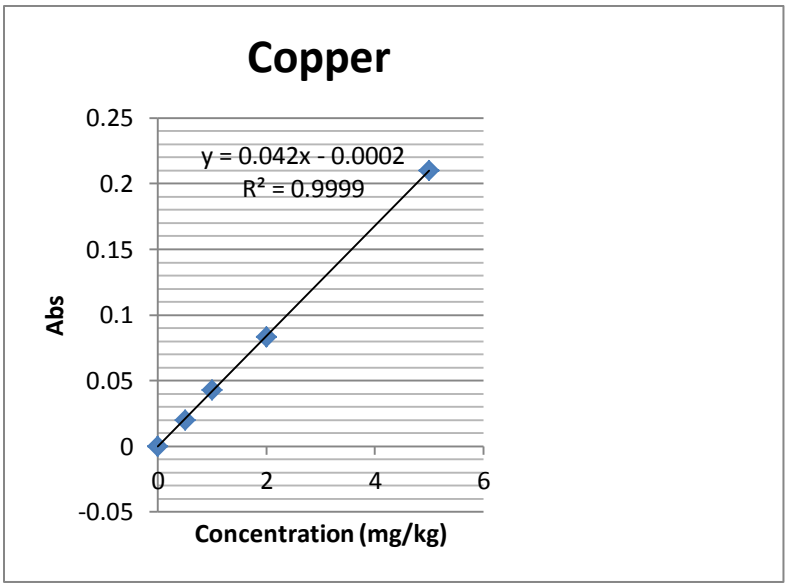
e) Calibration graph of cadmium standards



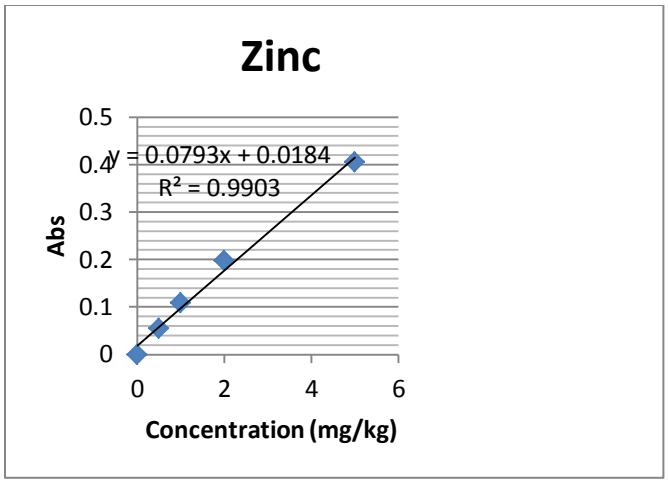
f) Calibration graph of manganese standards



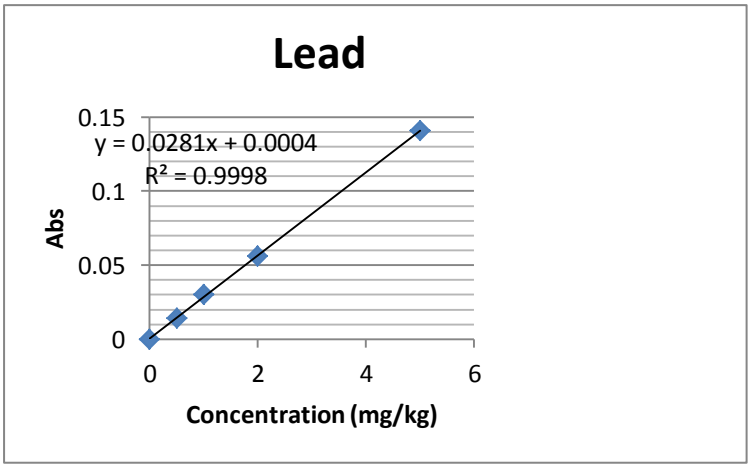
g) Calibration graph of iron standards



h) Calibration graph of copper standards



i) Calibration graph of zinc standards



J) Calibration graph of lead standards