ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DETERMINATION OF SOME MAJOR AND TRACE METALS LEVELS IN ONION (*ALLIUM CEPA* L.) AND IRRIGATION WATER AROUND MEKI TOWN AND LAKE ZIWAY, ETHIOPIA.

By

Reta Birhanu Kitata

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By Reta Birhanu Kitata

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LISTS OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
EPA	Environmental Protection Agency
EU	European Union
FAAS	Flame Atomic Absorption Spectrometry
FAO	Food and Agricultural Organization
LZW	Lake Ziway Water
MCL	Maximum Contaminant Level
ND	Not Detected
OL	Onion Irrigated with Lake Ziway Water
OW	Onion Irrigated with Well Water
RSD%	Percentage Relative Standard Deviations
SD	Standard Deviations
UAE	United Arab Emirates
USDA	United States Department of Agriculture
WHO	World Health Organization
WW	Well Water

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By: Reta Birhanu

Advisor: Prof. B.S. Chandravanshi

ABSTRACT

The levels of major (Ca and Mg) and trace (Cu, Co, Cr, Mn, Zn, Cd and Pb) metals were determined in onion (*Allium Cepa* L.) bulbS and well water and Lake Ziway water employed for irrigation were determined by flame atomic absorption spectroscopy(FAAS). Sample dissolution of onion was carried out by wet digestion with a total volume of 2 mL HNO_3 (69-72 %) and 2 mL $HClO_4$ (70 %) mixture in flask fitted with reflux condenser on Kjeldahl heating apparatus.

Higher levels of Ca (599 and 550 μg/g) than Mg (516 and 407 μg/g) in onion irrigated with well and Lake Ziway water, respectively were detected. Among trace metals, the highest level was that of Zn (18 and 14.1 μg/g) followed by Mn (8.8 and 13 μg/g) Cr (4.9 and 6.6), Cu (3.2 and 3.6 μg/g), Co (1.2 and 0.8 μg/g) and Cd (0.64 and 0.53 μg/g) in onion irrigated with well and Lake Ziway water, respectively all in dry weight basis. Lead was below detection limit of the analytical procedure in this study.

The levels of Ca and Mg detected in well water and the Lake Ziway water used for irrigation were (22.7, 9.9) and (30.2, 8.6 mg/L), respectively. Higher levels of Zn (0.08 mg/L) and Co (0.06 mg/L) were found in the lake water while higher Mn (1.1 mg/L) and Cr (0.44 mg/L) were determined in well water. Cu, Cd and Pb were below the method detection limit in both well and Lake Ziway water samples of the study area. Mn in well water and Cr in both water exceeded the FAO limit of maximum recommended level in irrigation water.

Key words: Onion (*Allium Cepa* L.), irrigation water, Lake Ziway, FAAS and wet digestion

INTRODUCTION

1.1. Background of the Study

Vegetables, along with fruits, are considered as "protective supplementary food," serving as the most rapid and low cost source of essential mineral elements and minerals for the majority of people in developing countries (Iyaka, 2007, Dosumu *et al*., 2003). The intake of 400–600 g/day of fruits and vegetables was reported to be associated with reduced incidence and risks of cancers, heart disease, and many chronic diseases of aging due to the health benefits of diverse phytochemicals abundant in these foods (Heber, 2004). The need for vegetables in human diet throughout the year and period of life is so found to be imperative since long. Indeed, global demand has been found to rise gradually which can be attributed to the increase in societal awareness on the food value of vegetables through proper education and exposure to other cultures (Taiga *et. al.,* 2008).

Supplying the rising need for vegetables throughout the year coupled with population rise has required major changes in agricultural practices such as introduction of mechanization of farming, use of various agrochemicals and variety of selected seed types, irrigation and postharvest processing processes (Plaster, 2003). On the other hand, environmental pollution has become major challenge causing contamination of agricultural soil, water and air which were then taken up by crop plants consumed by human (Awode, 2008, Kanakaraju *et. al.*, 2007). Therefore, the levels of various toxic organic and inorganic elements have generally increased; some of which were to the level harmful to man and other living things.

Plants, which are intermediate reservoirs of these metals, may take these metals from soil, water and air and accumulate them in or in their tissues due to their several biochemical mechanisms developed during their evolution and course of life which enables them adapt and tolerate new or chemically imbalanced environment (Adeyeye, 2005). Once trace metals enter food chain of the ecosystem, they are so non-biodegradables that they even get bio-accumulated and bio-magnified. In humans, they may get accumulated in vital organs for a prolonged period of time resulting in chronic diseases on set and finally reaching toxic level (Akinola *et. al.*, 2008,

Hashmi *et al*., 2007). Excess metal ions in the body may bind nonspecifically but with higher affinity to certain active sites of enzymes inhibiting normal process; form insoluble salts in biological fluids; participate in hydrolytic reactions that degrade biopolymers etc. (Lippard and Berg, 1994). On the contrary, essential trace metals are also vital part of healthy nutrition along with macronutrients and so their deficiencies contribute significantly for the common malnutrition problem in Africa (Deckelbaum *et al*., 2006), that there are attempt in developing "biofortified" staple foods to improve minerals like iron and zinc as well as vitamins status in low-income populations (Hawkes *et al*., 2007).

The assessment of metal contents (essential and non-essential) of vegetables is therefore necessary from the point of view of nutrition, toxicological, crop yield as well as many other applications (Nnorom *et al.*, 2007). As plants are so sensitive to their environment the levels of metals vary with the type of metals and plant species, growth media (soil, water, and air), season, means of cultivation, pollution incidence, dietary traditions of consumers and other post harvest treatments continuous determination and/or monitoring of the levels of metals in the crop plants is required (Iyaka, 2007).

As Nabrzyski (2006) and others verified, elements biochemistry is not entirely independent rather either synergetic effect or inhibiting effect on one another exists. Interaction between and among minerals, or minerals with some anionic species have important effect on mineral absorption as well as physiological actions. For example, absorption of calcium is enhanced by phosphorous; however aluminum may reduce absorption and utilization of P, Ca, Mg, Fe and Mn. Cadmium and lead also may hinder calcium and iron absorption. Therefore consideration of levels of different elements in food and biological samples is helpful.

A lot of researchers worldwide have indeed dealt with the issue and provided relevant information on metal contents of vegetables in general and some of them for onion varieties in particular along with the growth media mainly water, soil and air.

In Ethiopia, though we share the consequences of global ecological pollution, the issue has not been given concern and activities done to enhance societal awareness are far to sufficiency.

Studies conducted by Itanna and his co-workers were worth mentioning on this aspect, particularly on vegetables produced around the Capital. The work includes heavy metal contamination on vegetables (Swiss chard, potato, cabbage, red beet and onion) irrigated with industrial effluents at Akaki (Itanna, 1998) and bioavailabilty of trace metals in vegetable farms in Addis Ababa, Kera and Kolfe (Itanna, 2008). The work indicated pollution tendencies around the capital.

Study conducted on Tilapia fish (*Oreochromis Niloticus*) in Lake Ziway and Lake Awassa, (Kebede and Wondimu, 2004), has revealed an increasing trend of trace metals in the area probably from human activities in the surrounding. Nigussie (2004) has on the other hand, attributed some elevated trace metals levels observed in the sediments of the two lakes more of geological origin by continuous inputs from thermal springs as well as the diversified agricultural activities along the catchment of Lake Ziway. Others studies on metal concentration in fruits (Guta, 2005) are also considerable. Overall, the increasing concentration of metals from anthropogenic and natural source is obvious in the country.

Investigation of trace and major metals in onion (Allium cepa L., variety cepa) which is highly used for food seasoning allium across the globe was considered from nutritional as well as toxicological points of view. The roots of onion are so shallow that frequent and deep irrigation in the growing period, particularly in dry season that salt build-up in the soil may occur, which inturn required assessment of the irrigation water quality. This study dealt with assessment of level of trace metals in onion bulbs and irrigation waters of well and Lake Ziway from farm lands around Meki Town. The study area selection was due to modern agricultural practices like irrigation with water sources of well, rivers and Lake Ziway to supply a great share of vegetables to the surrounding community, the capital and other parts of the country, as well as its geographical location within the great East African Rift valley with low out late of and known of salt and minerals deposit.

1.2. Onion Cultivation, History, Composition and Use

1.2.1. Botany of Onion and Allium Plants

Allium vegetables are bulbous plants commonly identified by their characteristics onion or garlic taste and pungent odor, which are imparted to them by the flavonols and organosulfur compounds in them. Most commonly recognized members of the genus include onions (*A. cepa*), shallots (*A. oschaninii*), leeks (*A. ampeloprasum* var. *porrum*), and herbs like garlic (*A. sativum*) and chives (*A. schoenoprasum*) (Wellness Encyc., 1992).

Onion along with other related vegetables of genus Allium has been classified as a member of the *Lily* family (*Liliaceae),* though some botanists place them in a new family called *Aliaceae* (Ensminger *et al.*, 1995*)*. The common onion is classified as the variety *cepa* to distinguish it from other forms such as shallot, which is the aggregate variety. Therefore, the scientific name *Allium Cepa* L. refers to the common variety of onion. The plant is grouped taxonomically in phylum magnoliophyta, class liliopsida and order asparagales. Categories of onion like pearl onions, boiling onions, yellow globe onions, red globe onions, white globe onions and Spanish onions are distributed throughout the globe (Margen, 1992, WHO, 1999). In Ethiopia the onion, shallot, garlic and leek were the most available varieties of the alliums.

1.2.2. The Origin and Worldwide Distribution of Onion

Onions, one of the oldest vegetables in continuous cultivation since at least 4000 BC, have been likely originated in prehistoric times in the Central Asia and regions bordering Mediterranean Sea (Northwest India, Afghanistan, China and part of former USSR) (Margen, 1992). Onions has almost spread to everywhere long time ago and was widely consumed in ancient Egypt, Greek, and Rome and by now spins the totality of the world's cultures and human race (Ejaz,. *et al.*,2003).

The leading onion-producing nations include China, India, United States of America, former Russia, Turkey, Japan and Spain (Ensminger *et al.*, 1995). The annual worldwide production of

onion in 2004 was 55 million tons (Rafique *et al.*, 2008) and 50 million tons in 2002 (Bloem, 2004). Agricultural sample survey estimated that, in Ethiopia (CSA, 2008) about 18,013 ha of land was covered by allium plants and about 175, 606 tons were produced across the country in "Meher" season, September to December in 2007/08 cropping year. The figures had been 17, 980 ha of land and a total of 229, 678 tons of bulbs production in 2004/05 (Sendek *et al.*, 2008). The shallot variety, which is closely related to both onion and some even grouped under *Allium cepa* variety aggregatum, other species like *Allium oschaninii (Auger et al., 2005), Alluium ascalonicum (Nmeth and Piskula, 2007)* is one of the most important vegetable in Ethiopia, too (Jemal, 2006). However shallot is vegetative multiplied and bulbs are planted rather than seed (Auger *et al*., 2005) giving rise to a cluster of bulbs making it rather expensive and resulting in increasing shift of cultivation to onion.

1.2.3. Cultivation and Storage Conditions of Onion

Onions are a cool season crops adapted to a growing season of air temperature at 13 to 24 ^{o}C; with production of leaves and bulb enhanced by long days of light period. As onions have shallow and limited root systems which explore mainly the upper 30 cm of the soil, they are sensitive to small changes in the water supply.

Onions can be established in many ways: from seed, bare root transplants, multiple transplant plugs, sets started from seed previous year, and overwinter. In addition to the nitrogen-phosphorous and potassium fertilizers application of zinc was found to improve the yield as well as quality (total sugar and ash increase and but decrease in moisture) and storability of onion while sulfur decrease the yield and mentioned quality parameters. The active compound iso-alliin (systein-sulfoxides) content of onion was discovered to be improved by sulfur-fertilization was reported to be important tool to enhance the nutritional as well as phyto-pharmaceutical quality (Bloem *et al*., 2004).

Onions are affected by various diseases like purple blotch, botrytis neck rot and smudge, and white rot which occur by fungi: bacterial diseases smut, insect diseases including onion maggot and onion thrips. Onion yellow dwarf virus is one of the major viral pathogens of onion and garlic (Arya, 2006).

Onions are undercut or pulled and windrows, when at least 60% of top leaves are down. When cooled properly at 0^0C and 65% to 70% relative humidity, onions can be successfully stored for 6 to 8 months (Wikepidea, 2008). However, the alliin compound, characteristics component of allium plants, is so unstable being decomposed up to 93% by allinase enzyme within 2–3 min of bulb damage that harvesting technique, processing and freshness of the product are of great importance to minimize losses of the active compound (Bloem, 2004). The common onion processing techniques are canning, dehydration, freezing and pickling (Ensimenger *et al*., 1995).

Although onions have relatively longer storage time than other vegetables the non-structural carbohydrates, free amino acids are altered and dry matter contents are reduced (Hansen, 2001) when stored for long.

1.2.4. Chemical Composition of Onion

Among various compounds in onion and other allium plants are organo-sulfur compounds, flavonoids, non-structural carbohydrates, free amino acids, and some inorganic minerals are the most important class of compounds obtained in onion and other related allium vegetables. The chemical composition of allium species is of interest in phytochemistry, plant–insect relationships, chemotaxonomy, flavor industry, quality control of food and phytochemical preparations, pharmacognosy and medicine (Bloem, 2004).

i. Organosulfur Compounds: Organosulfur compounds are largely responsible for the characteristics taste and smell of allium vegetables. S-1-propenyl-L-cysteine sulfoxide (iso-alliin) is the main form in onions while S-2-propenyl-L-cysteine sulfoxide (alliin) is the predominant form in garlic and S-propyl analogue in chives (*Allium Schoenoprasum*) (Dewick, 2000). Many of the organo-sulfur compounds occur only as degradation products of the naturally present the cysteine sulfoxides upon damage of tissues (Figure 1) (WHO, 1999).

Crushing the bulb allows the contact between the cysteine sulfoxides located in the cytoplasm and the alliinase enzyme (carbon–sulfur bond lyase) localized in the vacuole. Subsequent α,β-elimination reactions of the sulfoxides, ultimately afford volatile and odorous low molecular-weight organo-sulfur compounds (Wellness Enc., 1992). In onion, *S*-propyl-L-cysteine sulfoxide degrade to thiosulfinates, thiosulfonates, cepaenes, *S*-oxides, *S*, *S*-dioxides, monosulfides,

disulfides, trisulfides, and zwiebelanes and (Z)-propanethial S-oxide (onion lachrymatory factor). Cooking further probes chemical changes; converting the odorous compounds into substances much sweeter than table sugars (Margen, 1992).

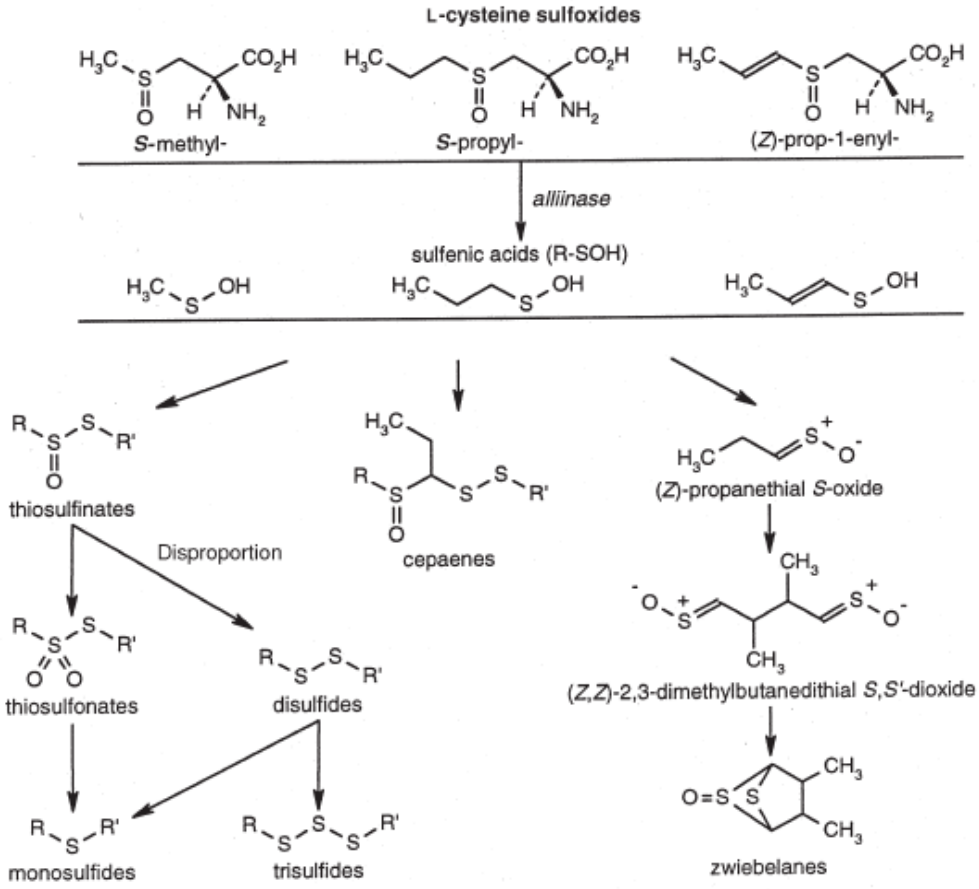


Figure 1. Degradation Products of Organo-sulfur Compounds upon Chopping Bulbs of Allium Vegetables. 7

ii. **Flavonoids** are also abundant in the allium genus of which the bulb onion is characterized by higher concentration of flavonoids compared to those of garlic and leek (Dosumu *et al.*, 2003). The extended conjugation across flavonoids structures and the number of hydroxyl groups enhance the antioxidant properties by reacting as reducing agents, hydrogen or electron donating agents or single oxygen scavengers and so prevent peroxidative damage of tissues (Saxena, *et al.*, 2007).

Flavonoids strongly absorb ultraviolet (UV) radiation (Malesev and Kuntic, 2007). As in other vegetables and fruits, the skin- non-edible dry peel, is richer in total flavonoids compared to the edible flesh and so peeling indeed results in their greater loss. Quercetin glycosides, group of flavonol were found to be not degraded when onion was cooked but transferred into the cooking water, turning the onion soup into a good source of flavonoids, with the total flavonoids content remaining unaltered during frying with oil and butter for 40 min in certain study (Nemeth and Piskula, 2007).

iii. Non-structural carbohydrates: non-structural carbohydrates in onion include the reducing glucose and fructose, the non-reducing sucrose and a series of oligosaccharides, the fructans. These carbohydrates account for approximately 60-80% of the dry matter content, while starch and rafinose are said to be absent (Benkeblia *et. al.,* 2004).

iv. Free Amino Acids and Dietary Fibers: are also very important as taste substances of onion of which glutamate is considered the most important taste sensation of onions. Arginine and glutamine are the most abundant, 60% of total free amino acids, amino acids in onion (Hansen, 2001). During maturation, free amino acids as well as carbohydrates are trans-located from senescing leaves (foliage) to the bulb. Dietary fibers play an important role in preventive nutrition and onion was found to have significant amounts of fibers, approximated to 3.86 % (Gyurova *et al.*, 2007).

1.2.5. Dietary Use and Health Benefits of Onion

Onions are one of the common vegetables used as seasonings in tremendous range of dishes, imparting characteristic flavor, aroma, or piquancy to food. Being among the several spices known of which the sensory quality of foods, the quantity and variety consumed.

Besides being one of the main condiments, onions are used as a household remedy for a long time as it promotes digestion, cure mild burns and asthma, lowering of blood cholesterol, and antidibetic (Srinivasan, 2005). Onion bulb was also reported to have, bacteriostatic and antibiotic and diuretic effects (Boulos, 1983).

Phytochemicals, biologically active plant chemicals other than traditional nutrients, such as flavonoids and other phenolics present in onion may contribute to the protective effects. Many of these phenolic phytochemicals have antioxidant capacity and may protect cells against the oxidative damage caused by free radicals. Oxidative stress can cause oxidative damage to large bio-molecules such as proteins, DNA and lipids, resulting in an increased risk of cancer and cardiovascular disease (Nemeth and Piskula, 2007)

1.3. Agricultural Soil and Water Pollution

The impact of modern technology upon agriculture has been as great as other industries, leading to increase in range of new agricultural practices involving modern farm equipments, arable land acreage increase, irrigation with various source of water from fresh water to sewage and sludge as well as intense use of agrochemicals to support the ever-growing world population and parallel rise in human aspiration and demand 'per capita'.

However, agrochemicals like chemical fertilizers, insecticides, fungicides, herbicides, plant-growth regulators, soil-and post-harvest fumigants etc increases soil and water contamination with heavy metals and other organic pollutants. Fertilizers frequently contain trace amounts of arsenic and heavy metals so; their repeated use may cause toxicity of soils (Chamley, 2003). P-fertilizers are among the sources of heavy metal input into agricultural systems. On average, phosphate rock, Malak *et al.*, (2007), contains 11, 25, 188, 32, 10, and 239 mg kg^{-1} of As, Cd, Cr, Cu, Pb and Zn, respectively.

Soil is an environmental, biochemical reaction system with three important phases: solid (i.e. mineral particles, organic debris, plant roots), solution (i.e. groundwater, rain water, biological excreta, products of biochemical reactions), and gas (i.e. atmospheric, products of biochemical reactions) which move towards equilibrium with one another. Agricultural soil is mentioned to be the most important sink for heavy metals due to soils' high metal retention capacities (Tokaliog *et al.*, 2006).

Although almost two-third of the 5,000 km^3 water annually used by people at the dawn of third millennium is found to be employed for farming (Chamley, 2003), water is so vulnerable to

pollution at all points of hydrological cycle that its quality is decreasing continuously (Dima *et al.*, 2006). Almost all forms of water on the earth's crust surface or sub-surface (sea, lake, rivers, rain etc. has been employed by human being for agricultural practice.

Surface waters, those collected in lakes, ponds, and rivers, etc either flowing or still are also widely used for agricultural production. Among these still or stationery water is more susceptible to pollutants than flowing water because of lack of self purification and processes like eutrophication (Tomar, 1999).

The lakes which are also used for irrigation farming in various parts of the globe, have a complex and fragile ecosystem, as they do not have self-cleaning ability and therefore readily accumulate pollutants (Lokeshwari and Chandrappa, 2006). Comprehensive studies related to the analyses of water, soil and vegetation around a particular lake are therefore crucial.

Groundwater has been found to be the main source of water supply for agriculture and other purposes due to several advantages it offers. The farmers could use the groundwater when and where they needed it. The improved prosperity enabled them to use higher yielding crops, fertilizers, and pest control, making the use of groundwater far more productive, and thereby resulting increasing dependence on groundwater. The over-exploitation of groundwater, however, has led to declining water levels, drying of shallow aquifers, and saline water intrusion. Excessive withdrawals of ground water in some India parts, for example has found to increase iron, fluoride and arsenic contamination (Singh, 2007).

Groundwater is susceptible to pollution, Bell (1999), from sources such as farm animals, man, sewers, and polluted streams, refuse disposal sites and so on. Besides, salt intrusion from coast that can extend several kilometers inland is another factor determining suitability of groundwater for irrigation as excess salt interferes with osmotic process and metabolisms (Dima *et. al.*, 2006).

Large and small industrial enterprises, the water industry, the urban infrastructure, agriculture, horticulture, transport, discharges from abandoned mines, and deliberate or accidental pollution incidents all affect water quality.

1.4. Metals Accumulation in Plants

Living organisms (plants, animals and microorganisms), store and transport metallic elements, as both to provide appropriate concentrations of them for latter use in metallo-proteins or cofactors and to protect themselves against the toxic effects of metal excess. Growth media including soil, nutrient solution, water and air are main sources of heavy metals to vegetables and other crops, which enter by roots or foliages through two main bio-sorption mechanisms: adsorption and/or absorption and accumulated in their tissues (Adeyeye, 2005, Abdullahi *et al.*, 2008).

Different vegetable species accumulate different metals depending on environmental conditions, metal species and plant, available forms of the heavy metals (Lokeshwari and Chandrappa, 2006). Many plants are found to be in a position to take up large quantities of certain elements from the environment and said hyper-accumulators of heavy metals (Olajire and Ayodele, 2003). These plants have been on use even as phytoremediation, removing or transforming contaminants through metabolic processes (Chamley, 2003).

Metal uptake by plants can be affected by several factors including metal concentrations in soils, soil pH, cation exchange capacity, organic matter content, types and varieties of plants, and age of the plant (Jung, 2008). The chemical forms of metals in which they enter the ecosystems and their final forms of existence greatly affect mobility, bio-availability, storage, retention and toxicity of the metals in living organisms, food and the environment depend on (Mocko and Wacllawek, 2004).

1.5. Physiological Role of Metals in Human Being

Mineral elements have a role in the body generally in building body tissue and regulating numerous processes. They are thus essential constituents of enzymes and hormones; regulate a variety of physiological processes (e.g. osmotic pressure maintenance, oxygen transport, muscle contraction, and central nervous system integrity), and are required for the growth and maintenance of tissues and bones. They are so potent and so important that without them the organism would not be able to use the other remaining constituents of food (Nabrzyski, 2006).

Minerals are usually classified into two main groups on the basis of their relative amounts in the body. One of the groups are macro-elements or macro-minerals occurring in relatively large amounts and needed in quantities of 100 mg or more per day which include calcium, magnesium, sodium and potassium. Minerals occurring small amounts and needed in quantities of a few milligrams or less per day are called microelements or trace elements, which includes iron, zinc, copper, manganese, cobalt, nickel, chromium and boron. Other trace metals like aluminum, lead, cadmium, mercury and arsenic are till now recognized as potentially harmful though further study is required for their benefit.

Trace elements are also classified in other ways into three groups: essential, probably essential and potentially toxic elements. Generally, an element is considered essential if it is necessary to support adequate growth, reproduction, and health through the life cycle when all other nutrients are eaten daily at optimal and safe levels (Nabrzyski, 2006). Kumar and Pastore (2007) however, emphasized that, what constituted 'safe' yesterday is no longer 'safe' today, and what is 'safe' today may not be 'safe' tomorrow exemplifying that the present 'safe' limit of 100 μg/L of lead in blood was actually 600 μg/L in 1960s and then it was brought to 300 μg/L in 1970s, which was then revised in 1985 to 250 μg/Land in 1991 to 100 μg/L.

Moreover, trace metals tend to bio-accumulate in plants and animals thereby causing deleterious effects, bio-concentrated in the food chain or attack specific organs in the body (Akinola *et. al.*, 2008). Therefore it is always recommended to be on the safe side by monitoring their level in the food and the environment. Some specific roles of selected metals are presented below.

Calcium

Calciumis also a macroelement required in larger quantity in bone and tooth formation, blood clotting, nerve stimulation and muscle contraction. Its deficiency is found to result in stunted growth, rickets, osteoporosis and tetany (Nabrzyski, 2006).

Magnesium

Magnesium is also macroelement required as component of bones and teeth, nerve stimulation, muscle contraction and activation of many enzymes like calcium. Its deficiency has been observed in alcoholism or renal disease.

Copper

Copper is an essential trace metal for animals and man and have diverse functions in plants. It is required in the formation of erythrocytes and hemoglobin as well as some enzymes like tyrosinase. Tyrosinase is required for catalytic conversion of tyrosine to melanin, the vital pigment located beneath the skin, which protects the skin from dangerous radiations. Copper is also involved in bone and elastic tissue development. The use of stored iron is reduced by copper deficiency suggesting that iron metabolism may depend on copper proteins (Bertini *et al*., 1998).

A daily dietary intake of 2 to 3 mg of copper is recommended for human adults (Hashmi *et. al.*, 2007). It is toxic to man when ingested at concentration of 250 mg/day (Acar *et. al.*, 2008). In chronic exposure, brain, liver, kidney and spleen may be injured and causes anemia.

Cobalt

The only apparent biological site of cobalt is vitamin B_{12} which is a cyno-complex. The deficiency of vitamin B_{12} is rarely observed but if exists, is pernicious anemia in humans due to vitamin B_{12} deficiency (Bertini *et al.*, 1998).

Chromium

Trivalent chromium is an essential microelement with a broad safety range and low toxicity that forms part of the glucose tolerance factor and helps to maintain normal glucose and lipid metabolism (Bertini *et al*., 1998). Its deficiency results in impaired growth, glucose intolerance and elevated blood cholesterol (Nabrzyski, 2006).

The higher oxidation state chromium (VI) ion has been found to be toxic with health effects like allergenic dermatitis, lesions of the skin and nose wall and a higher lung cancer incidence. As absorption of Cr^{+3} is so poor that oral intake has to be very high to reach damaging levels. The fraction of the Cr^{+3} species absorbed in the bowel from dietary intake, mainly is very low and usually ranges between 0.5 and 2%, however, the absorption of Cr^{+6} species is nearly six times higher (Velasco-Reynold *et. al.*, 2008).

Manganese

Manganese is a cofactor of a large number of enzymes; has a role in aging process as an antioxidant (Mn-superoxide dismutase); important for normal brain function, reproduction, and bone structure (Nabrzyski, 2006). Manganese plays critical role in oxygen evolution catalyzed by proteins of photosynthetic reactions (Bertini *et al.,* 1998). A daily intake of 2.5 to 5 mg of manganese by human assists in normal well being of cells (Hashmi *et. al.*, 2007).

Zinc

Zinc is an essential trace mineral and the second most abundant trace mineral in the body next to iron. Zinc though also almost hundred times as abundant as copper it is scarce (Nicholas and Egan, 1975). Zinc is stored primarily in muscle but is also found in red and white blood cells, the retina of the eye, bones, skin, kidneys, liver, and pancreas (Sarica *et. al.*, 2002). In men, the prostate gland contains more zinc than any other organ.

Among the well-characterised zinc proteins are carbonic anhydrase (assist blood pH maintenance), alcohol dehydrogenase, and a variety of hydrolases involved in metabolism of sugars, proteins, and nucleic acids (Bertini *et al.*, 1998). Recommended daily intake of zinc is about 15 mg which function as a co-factor for enzymes such as arginase and diaminase; in synthesis of DNA, proteins and insulin (Hashmi *et. al*., 2007).

Cadmium

Cadmium has been considered an extremely significant pollutant affecting all life forms because of its high toxicity and great solubility in soil and water and easy accumulation in roots of most plant tissues (Unyayar *et al*., 2005; Liu and Kottke, 2004). Cadmium is found to be a widespread heavy metal and released into the environment by power stations, heating systems, metal-working industries, waste incinerators, urban traffic, cement factories and as a by-product of phosphate fertilizers.

Most of the cadmium that enters the body is concentrated in the kidneys and liver with biological half-life of 10-35 years interfering with filtration leading to execretion of essential proteins and sugar from the body (Akinola *et. al*., 2008). Toxic effect of cadmium arises from its binding with the active -SH group in enzymes even replacing zinc in the enzymes preventing the normal physiological functions of enzymes. The principal long-term effects of low-level exposure to cadmium are chronic obstructive pulmonary disease, emphysema and chronic renal tubular disease, depressed immune system response and joint pain and loss of sense of smell, etc. There might also be effects on the cardiovascular and skeletal systems (Mor and Ceylan, 2008; Kocak *et al*., 2005).

Lead

Most lead compounds are significantly toxic contaminants and impose significant health hazards in urban environments. Lead toxicity influences many bones, brain, heart, kidneys, liver, nervous system, and pancreas causing symptoms like abdominal pain, anxiety, brain damage, dizziness, hypertension, diminish in IQ in children, etc. Average Lead intake is approximated to be 0.03 mg/day as mean and 0.050 mg/day as high level for human body (Kocak *et. al*., 2005). Physicians and scientists agree that no level of lead in blood should be considered safe for children, as it was found that their digestive system can absorb up to 50% of lead they ingest (Kumar and Pastore, 2007; Mor and Ceylan, 2008).

1.6. Metals Analysis in Food Crops

Metals analysis in the environmental samples has been a major preoccupation for many years because of their toxicity towards terrestrials and aquatic plants and animals, human beings and the environment. The determination of metal content of onion and other vegetables across different parts of the globe were conducted from viewpoints: health risk assessment, nutrient content analysis for consumers, to trace geographic origin of food products, nutritional status assessment of growing plants and assay of suitability of soil and water for farming and as bio-indication for monitoring of environmental pollution.

Significant studies have been conducted to assess the levels of trace metals in onion and other vegetables from toxicological as well as nutritional aspects in Nigeria particularly with respect to Challawa River surrounded by industries (Akan *et al.*, 2009; Abdullahi *et al*., 2008; Iyaka, 2007, Abdullahi *et al*., 2008); China (Zhou *et al.*, 2000).; Chile (Badilla-ohlbaum, *et al.,* 2001); Pakistan (Hashmi *et al.*, 2007), UAE (Khan *et al*., 2006), Korea (Jung, 2008), Spain (Mendez *et al*., 2007) and other parts of the world.

Plants' high sensitivity to their environmental conditions has found new application in identifying geographic origin of vegetables accessed in markets to provide information for consumers as well as suppliers (Ariyam and Yasui, 2006). Another reason for analysis of metals in plants is for diagnosis of deficiency of essential metal nutrients so that appropriate minerals can be supplied for the proper growth of crops (Rafique, 2008). High concentrations of heavy metals, metabolic process of the plants can be interfered, resulting in poor growth and sometimes-even death (Tokalioglu *et al.*, 2006).

The study of inorganic elements in biological samples has recently been well expanding into a field called bioinorganic chemistry, concerned with the study of elemental uptake, physiological action, storage, excretion and introduction as probes or drugs when necessary (Lippard and Berg, 1994; Mann, 2001).

Although investigation of vegetables for their metal concentration is indispensable, a survey of literature indicates that such studies are scarce in Ethiopia. Studies conducted by Fisseha Itanna and were worth mentioning to exemplify for his work on heavy metal contamination on vegetables(swiss chard, potato, cabbage, red beet and onion leaves) irrigated with industrial effluents at Akaki (Itanna,, 1998), leafy vegetables of Addis Ababa (Itanna, 2002) and others published as well as unpublished. His work proved pollution tendencies in the various parts of the country. Others studies on metal concentration in fruits (Guta, 2005), are also to be mentioned.

The literature survey revealed that no study has been conducted on the determination of metals in the onion bulbs grown in Ethiopia. Hence it is worthwhile to determine the levels of essential and non-essential metals in onion bulbs cultivated in Ethiopia.

In this study, the levels of some macroelements (Ca and Mg,) and micro/trace metals (Fe, Cu, Zn, Mn, Ni, Co, Cr, Pb, Cd) in onion samples freshly collected from some irrigated farms around Lake Ziway and Meki Town in Ethiopia, will be determined using flame atomic absorption spectroscopic technique. The water samples used for irrigation will also be investigated for the respective metals and then compared with that of onion bulb.

1.7. Purpose, Scope and Limitations of the Study

The assessment of metals major (Ca and Mg) and trace (Cu, Cr, Mn, Zn, Co, Cd and Pb) in the onion bulbs samples collected from the farm fields nearby Lake Ziway was conducted to offer scientific data which can be used to asses the nutritional use as well as the toxicological status of onion. Besides, the analyzed irrigation water samples in the area can also be helpful to estimate the sources of metals to onion and investigate the pollution level and tendencies of the ground water and Lake Ziway. To obtain detailed information, comprehensive analysis of soil, water and the plant is important, which is not possible in this study. The dry season cultivation was the focus of this study though information on seasonal variations of metal levels is also considerable.

1.8. Objectives

A. General Objective

The main objective of this project is to determine the level of major, minor and trace metals in onion bulbs and irrigation water of well and Lake Ziway used around Meki Town, Ethiopia and trace the sources to one of the growth media, irrigation water, and finally assess the dietary uses and toxicity levels of the metals in onion from the area.

B. Specific Objectives

i. To determine levels of essential major and minor as well as toxic metals (Ca, Mg, Fe, Zn, Cu, Ni, Cr, Mn, Co Pb, Cd) in onion bulb samples and assess metal level statuses .

ii. To compare the levels of metals in irrigation waters with that of literatures values and water quality guidelines and assess suitability of the waters for irrigation.

2. EXPERIMENTAL

2.1. Materials

2.1.1. Apparatuses and Instrument

Chopping board and Teflon knife (PTFE) were used to cut onion bulbs in to pieces while air-circulating oven (DIGITHEAT, J.P., SELECTA, S.A., Spain) was for drying the bulb. Porcelain mortar, pestle and crucibles (HALDENWANGER, Germany) were used during pounding of the onion bulb. Analytical balance (LARKO, LA114, 110 g/0.1 g) with precision of ± 0.0001 were used to weigh the onion bulb samples. Round bottom flasks with grounded glass (100 mL) fitted with reflux condenser were employed in digesting the bulb sample on Kjeldahl heating apparatus (Gallenhamp, England). Borosilicate volumetric flasks (25, 50 and 100 mL sizes) were used for dilution of samples and preparation of metal standard solutions. Measuring cylinders (DURAN, Germany), pipettes (PYREX, USA), micro-liter pipettes (1-10 µL and 100-1000 µL size, DRAGONMED, Shangai, China) were employed for measuring different quantities of volumes of sample solution, acid reagents and metal standard solutions.

Concentrations metals were determined by flame atomic absorption spectrophotometer (BUCK SCIENTIFIC, Model 210VGP AAS, USA) equipped with deuterium background corrector and hollow cathode lamps with flame produced from air-acetylene as oxidant-fuel. Beside, spatulas, bottles and sample holders as well as funnels and wash bottles all polyethylene were used in the study.

2.1.2. Chemicals and Reagents

All reagents and chemicals used in the study were analytical grade. Nitric acid, 69-72 % HNO_3 and perchloric acid, 70 % $HClO_4$ both from Research-lab FINE CHEM Industries Mumbai, India were used for digestion of onion bulb powder samples. Extra pure hydrogen peroxide 30 % H_2O_2, (Scharlau, European Union), was used during attempt in optimization of digestion procedure. Lanthanum chloride hydrate, 99.9 % (Aldrich, USA) was used as a releasing agent for Ca and Mg determinations.

Stock standard solution of concentration 1000 mg/L in 2% HNO_3 of the metals, Ca, Mg, Mn, Cu, Zn, Co, Cr, Pb and Cd (BUCK SCIENTIFIC, PURO-GRAPHIC) as nitrate salt standard solutions were used to prepare intermediate standard solutions. Demineralized water (chemically pure with specific resistance of 18.0 MΩ/cm at the working environment) was used for dilution of sample and intermediate metal standard solutions and rinsing glassware and sample bottles.

2.2. Procedures

2.2.1. Apparatuses Cleaning

All glassware, plastic containers and polyethylene bags were filled with aqueous detergent solution for 24 h; inner and outer walls well wiped with foam and brushes; copious amount of water used to remove the detergent followed by rinsing with deionized water. The glassware were then soaked with about 10 % (v/v) nitric acid for 24 hrs while the polyethylene bags and bottles were soaked in the acid for 2 h; followed by rinsing with deionized water. The glassware were then dried in hot air circulating oven and stored in clean dry places free of contamination till use. Prior to each use the apparatus were soaked in diluted nitric acid and rinsed in deionized water.

2.2.2. Description of the Study Area

The study area was located in between Meki Town and northern part Lake Ziway (about 8^0 09' N latitude and 38^0 45' E longitudes and about 140 km south from the Capital, Addis Ababa) which is one of the Ethiopian Rift valley lakes (Figur 2a). The study area is located in the region of the Great Rift Valley known to encompass various lakes and water bodies. One of the lakes, Ziway Lake has two main tributaries rivers: Meki River which crosses Meki Town and Katar River from South East direction. Other river in the vicinity of the study area includes, Awash River which highly employed for irrigation and dammed for electrical power supply and irrigation at Koka.

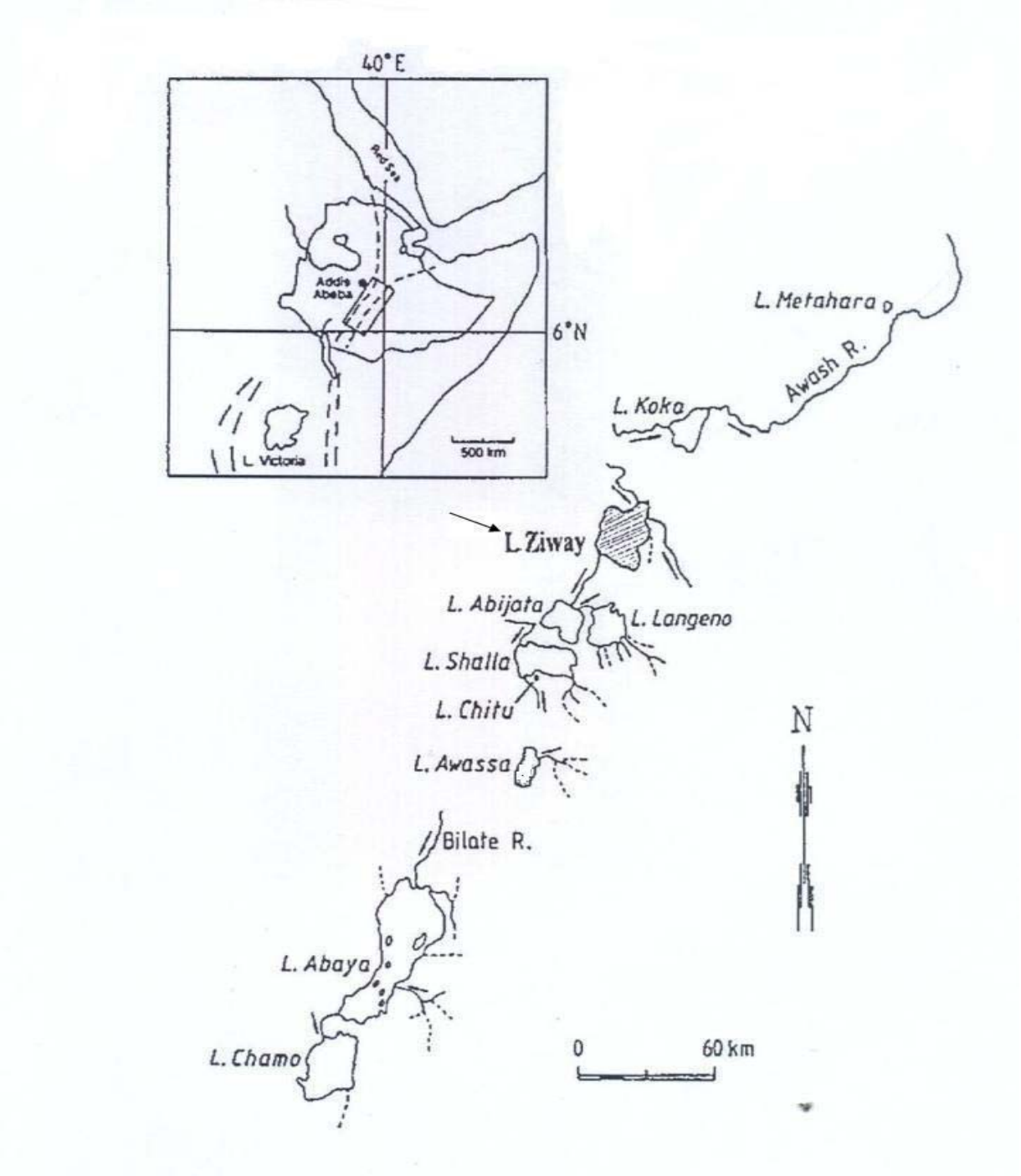


Figure 2 (a). Map of Ethiopian Rift Valley Lakes with their Drainage Pattern

(Lake Ziway- highlighted)

(Source: Adapted from Kebede, A. and Wondimu, T. *Bulletin of Chemical society of Ethiopia*, **2004**, 18(2), 119-130.)

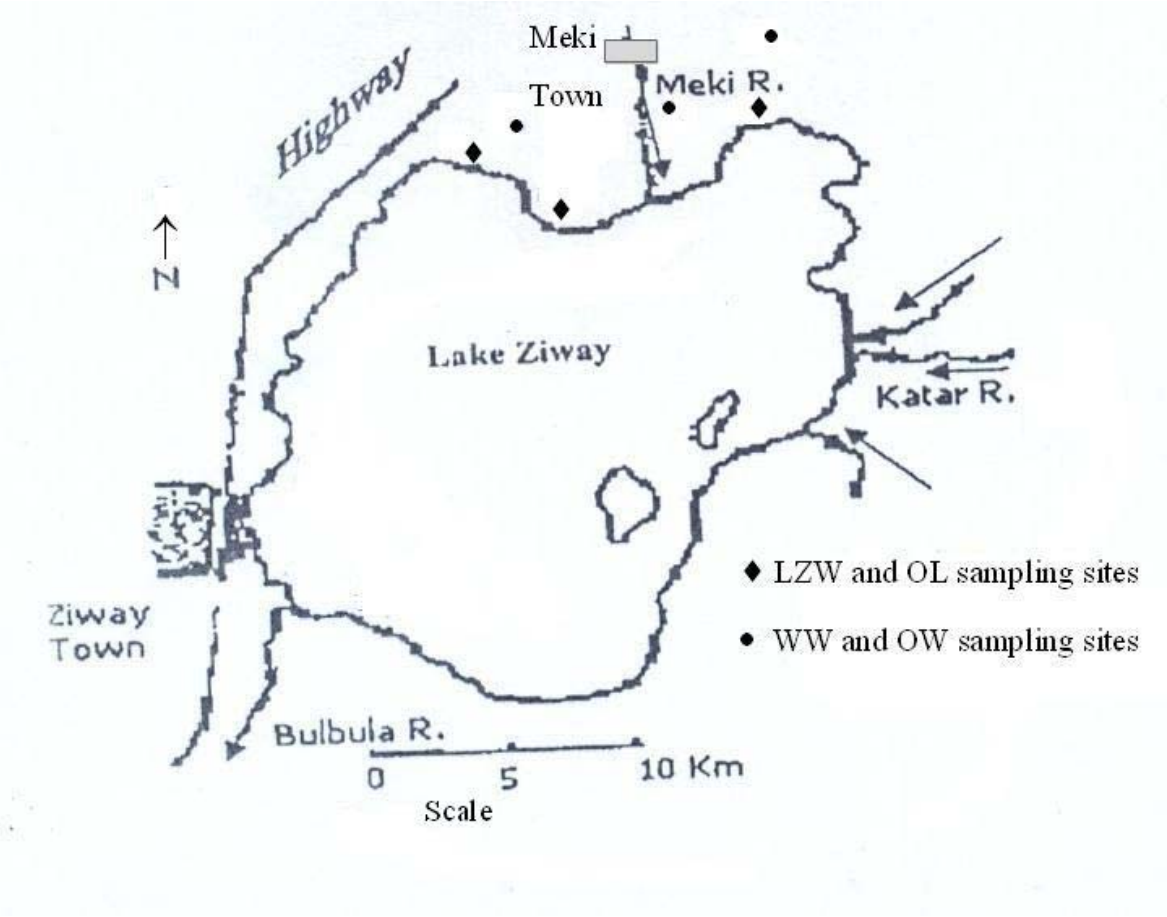


Figure 2 (b). Lake Ziway and the Sampling Sites of Irrigation Waters and Associated Onions

(Source: Adapted from page 21 of Nigussie, K. *Master of Science Thesis, unpublished*, Addis Ababa University, Ethiopia, **2004**.)

The area has been under continuous cultivation throughout the year and has been supplying significant portion of a wide variety of vegetables like tomato, onion, cabbage, green pepper, etc to the capital and local people consumption since long time. Modern farming practices such as mechanized farming, application of fertilizers, agrochemicals (pesticide, insecticides, fungicides, etc), and selected seeds are significant agricultural inputs used for getting better yield in the area. Artificial irrigation was highly practiced in the area, particularly, for dry season farming through the use of ground bore well waters, Lake Ziway and Meki River with engine pumps. Frequent and deep irrigation with water is applied for onion so as to keep the soil moisture level high and the shallow root of the plant can access.

2.2.3. Sampling and Sample Pretreatment

2.2.3.1. Irrigation Water Sampling and Pretreatments

Irrigation water samples (ground water and Ziway Lake water) were sampled in two rounds during the dry season farming (January to April) so as to make it composite sampling type. The first water sampling was done February 19-21, 2009 during early growth of the established onion plant while the second round sampling was carried out on March 29-30 during late bulb formation period. Plots of land (1-4 ha size), three irrigating with well and three Ziway Lake, were selected for the study on the bases of distance from the lake and Meki Town, size of the land and age of the plant during the study time.

The ground water samples were collected from pipes (PE or PVC) connected to water pumps discharging water just at the point where the water was delivered to the farm plots. In case of the lake, the irrigation water was diverted and travels certain distances along the channels after which it was driven by the pumps while the wells were much close to the farm fields usually.

During the first round sampling, pre-cleaned, and dried plastic bottles (LDPE, 2 L) with HDPE screw cups were used to collect the water samples. The bottles were filled and rinsed with the flowing irrigation water itself prior to sampling. From each six sites about half of the bottles (1000 mL) were filled the first day during 5 min flow of the water from the pipes while the rest was filled the next day in similar manner. The collected water samples were transported to laboratory, 500 mL of each well and lake waters filtered and preserved adding 2 % (v/v) nitric acid at about 4 ^{0}C in a refrigerator without freezing.

The second round sampling was carried out in similar manner except that 500 mL bottles were collected from each six sites for 3 min in each case for two days. The water samples from the second sampling were also mixed, filtered and 500 mL of both the lake and well taken. A total of 1000 mL stock sample volume of water was prepared for each well and lake water by combining three wells and three lake waters respectively and kept for analysis.

2.2.3.2. Onion Bulb Sampling and Pretreatment

Recently matured ten onion bulb samples which appear healthy were freshly collected from each of the six farm sites where the irrigation waters were collected, pulling out by hand. The sampling points selected were from different furrow irrigated spots on each plot of land. The bulb samples were put in clean plastic bags labeled in accordance with the water samples and brought to the lab for further pretreatment.

The onion bulbs, edible portion, was separated from the roots and remaining leaf parts with clean Teflon knives; washed with a running tap water so as to remove adsorbed soil particulates and then rinsed with deionized water, and air dried. Peeling the outer dry skin, the naked bulbs were chopped in to nearly uniform size to facilitate drying in similar rate. About 250 g of the sliced bulbs were put on acid-washed crucibles labeled according to the sample and dried in hot air oven at 80 ^{0}C for 48 h till it got brittle and crisp. Cooling to ambient temperature, the dried samples were pound in to fine powder with porcelain mortar and pestle and sieved. The powdered sample was then placed in pre-cleaned screw capped polyethylene container and stored in desiccators to keep to constant dry weight till digestion.

2.2.4. Optimization and Wet Digestion of Onion Bulbs

Elemental analysis of the majority of matrices for FAAS requires the sample solution to be in clear diluted usually in aqueous. Therefore complete or partial dissolution of the sample prior to instrumental analysis is applied. These can be done by dry ashing by mixing with a flux to get product which can be dissolved in water or dilute acid; or wet digestion with oxidizing agents and heat (Oliveira, 2003).

Wet digestion with acids is currently the most common technique used for decomposition of organic matter, especially food material (Biziuk and Kucznska, 2006). The technique may also involve conventional heating, microwave heating, UV and IR digestion in open or closed system. Adding the strong mineral acids or their combinations to the food in appropriate vessel (test tube, beaker, digestion flask, etc.), and heating with a Bunsen burner, hot plate, or aluminum block with programmed temperature is the most common procedure employed. Nitric acid alone or as a

mixture with perchloric or sulfuric acids is the most popular reagent used for sample decomposition, as it is a strong oxidizing agent and forms soluble nitrates with metals. Sometimes strong oxidizing agents like hydrogen peroxide are added. Sulfuric acid usage is not recommended during determination of elements like Ba, Ca, Pb, and Sr which form sulfates with low water solubility (Biziuk and Kucznska, 2006).

Various kinds of onion bulb sample digestion procedures were used by different analysts varying oxidizing acids and reagents strength and amount, time involved, simplicity of procedure and safety and other considerations (Abdullahi *et al.*, 2008, Hashmi, 2007, Iyaka, 2007). In this study digestion of onion bulb samples was performed by the optimized procedure (Table 1).

Table 1. Optimization of Parameters for Wet Digestion of Onion Bulb Samples. 24

(Reagent types and volumes, temperature, time and procedures)

No	Reagent volumes (mL)				Maximum temperature (^{0}C)	Time, (min)	Result
	HNO_3	$HCLO_4$	H_2O_2	Total volume			
1	6	-	-	6	150	150	Yellow
2	5	2	-	7	150	150	Lightly yellow
3	5	2	-	7	240	120	Almost clear
4	2	2	1	5	180	120	Yellow with suspension
5	3	3	-	6	180	120	Almost clear
6	2	2	-	4	240	150	Yellow solution
7*	**2**	**2**	**-**	**4**	**270**	**140**	**Clear solution**
8	2.5	2.5	-	5	210	75	Light brown
9**	2.5	2.5	-	5	210	190	Clear solution
10	3	2	-	5	210	150	Clear solution

* *Each added in two portions (1. + 0.5)* ** *Each added in two portions (1.5 + 1)*

Optimum procedure of sample dissolution is required to give at most result with minimum reagents, time and temperature, simple procedure and with small residue or waste generation, minimum contamination, as well as maintaining traceability of the result (Oliveira, 2003). Optimum digestion procedure was selected after varying different parameters.

Critical consideration was also given so that $HClO_4$ would not concentrate after evaporation of HNO_3 since $HClO_4$ combined with metals and undigested fats and oils can for spontaneously explosive compounds and also need special perchloric acid hood (Biziuk and Kucznska, 2006**).** The procedure with total of 4 mL reagents volume (2 mL HNO_3 and 2 mL $HClO_4$), heating at 270 ^{o}C and 140 min digestion time was selected for this study described in detail below.

Out of the dried onion bulb powder, 0.5 g was weighed and transferred to round bottom flask (100 mL) to which 1.5 mL HNO_3 (69-72 %) and 0.5 mL $HClO_4$ (70 %) were added. The mixture was then heated on Kjeldahl heating apparatus fitting the flask to a reflux condenser by setting the temperature to dial at 4 (120 ^{0}C) for 25 min and followed by dialing at 5 (150 ^{0}C) and 6(180 ^{0}C) each for 10 min. Cooling the mixture for 10 min, 0.5 mL of the HNO_3 (69-72 %) and 1.5 mL $HClO_4$ (70 %) were added and digestion was continued heating at 150 ^{0}C for 15 min, 180 ^{0}C for 30 min, 210 ^{0}C for 30 min and finally at 270 ^{0}C for 20 min till the solution became colorless (the solution was not allowed to dry).

The digest was allowed to cool to room temperature for 10 min without dismantling the condenser and further 10 min after removing the condenser. Then, 20 mL deionized water was added and filtered into 50 mL volumetric flask to remove any suspended or turbid matter. Three 5 mL portions of deionized water were used to rinse the flask for the remaining digest. About 1 % 'matrix modifier' Lanthanum chloride hydrate was added so that lanthanum may bind the phosphate and liberate calcium and magnesium in case large phosphate exist in the sample (Rouessac and Rouessac, 2000).The solution was then diluted to mark with deionized water and mixed thoroughly by shaking. Dilution is required to reduce the physical interferences caused, particularly variation in viscosity and surface tension which in turn affects sample aspiration rate (EPA, 1999). The digestion was carried out in triplicate for the samples (onion bulb cultivated with well and lake waters). Appropriate nine blank solutions were digested accordingly and stored in refrigerator at 4 ^{0}C without freezing till analysis time.

2.2.5. Analysis of Sample Solutions for Metal Levels

Biziuk and Kucznska (2006) phrased, "spectrophotometric methods AAS and AES using a flame, ICP, or an electrothermal device for atomization, are the most specific of all analytical methods, strictly connected with atomic structure, and can frequently be completed in a few minutes." Of this, atomic absorption spectroscopy can be used for quantification of more than 70 elements including alkali, alkaline earth, transition, and heavy metals in various samples with sensitivities from µg/g to ng/g or lower range (Rouessac and Rouessac, 2000).

In this study, FAAS were used for determination of metal concentration by using external calibration. The instrument parameters optimized according to the manufacturers was provided as Table 2. The instrument parameters were optimized according to the manufacturer guide.

Table 2. FAAS Instrumental Operating Conditions for Determination of Metals in Onion and Irrigation Water Samples.

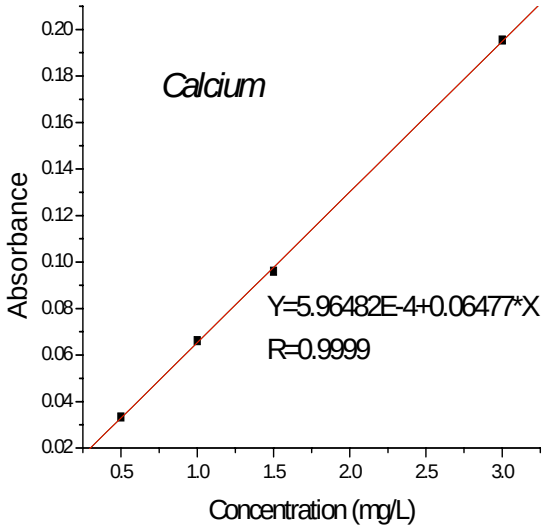
Element	Detection limit (mg/L)	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Energy
Ca	0.01	422.7	0.7	2	3.710
Mg	0.001	285.2	0.7	1	3.953
Cu	0.02	324.7	0.7	1.5	3.8
Co	0.05	240.7	0.2	4.5	3.453
Cr	0.05	357.9	0.7	2	3.586
Mn	0.01	279.5	0.7	3	3.886
Zn	0.005	213.9	0.7	2	3.017
Cd	0.005	228.9	0.7	2	3.094
Pb	0.1	283.2	0.7	2	3.475

Calibration metal standard solutions were prepared for each of the metals from an intermediate standard solution of 10 mg/L which was first prepared diluting the 1000 mg/L stock metal standard solutions. Four appropriate working standard solutions of each of the metals were finally prepared from the intermediate standard solution.

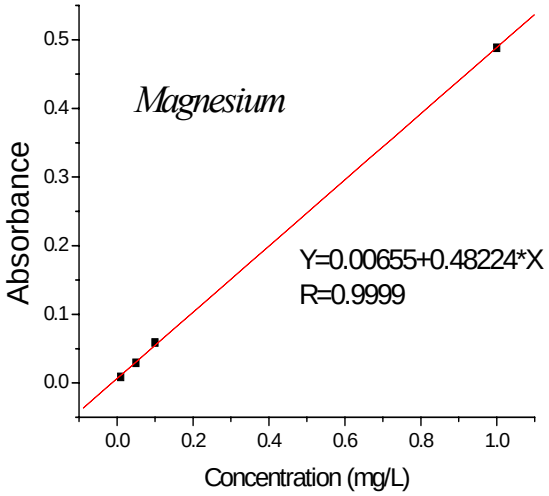
Concentrations of the intermediate standards, working standards, calibration curves and value of correlation coefficient of the calibration graph for each of the metals are provided (Table 3 and Figure 3). All correlation coefficients were higher than 0.999, indicating good relationship between concentration and absorbance in the range.

Table 3. Calibration Metal Standard Solutions and Correlation Coefficients of the Calibration Curves.

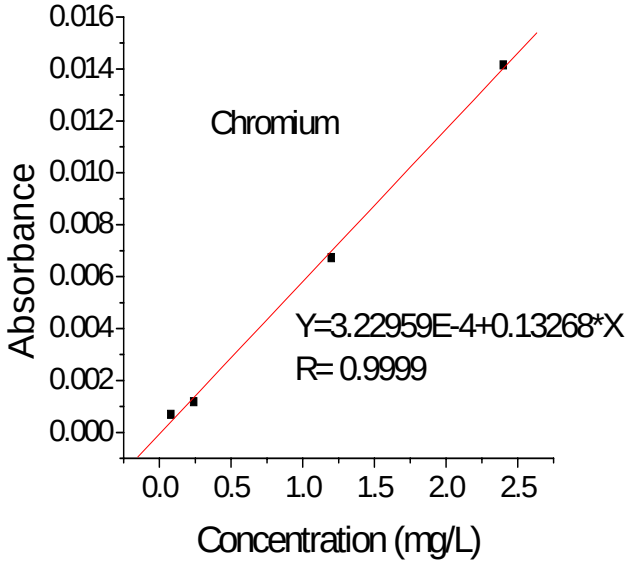
No.	Metal	Intermediate standard (mg/L)	Working standards (mg/L)	Correlation coefficient (*r*)
1	Ca	10	0.5, 1, 1.5, 3	*0.9999*
2	Mg	10	0.01, 0.05, 0.1, 1	*0.9999*
3	Cu	10	0.05,0.1, 0.2, 0.5	*0.9997*
4	Co	10	0.05, 0.1, 0.2, 0.4	*0.9997*
5	Cr	10	0.08, 0.24, 1.2, 2.4	*0.9999*
6	Mn	10	0.05, 0.2, 1.2, 1.8	*0.9999*
7	Zn	10	0.05, 0.2, 0.5, 0.7	*0.9993*
8	Cd	10	0.005, 0.3, 0.1, 0.15	*0.9999*
9	Pb	10	0.1, 0.3, 0.6, 1.2	*0.9993*



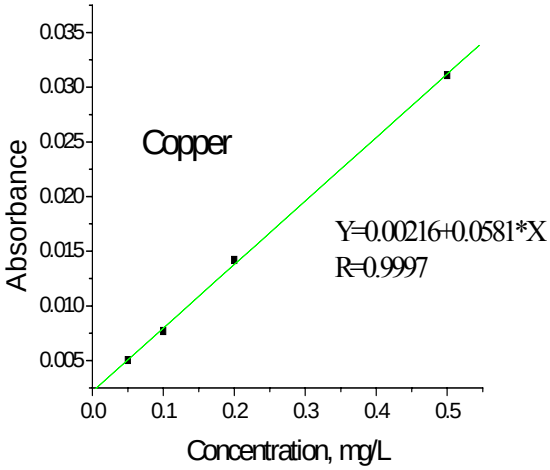
(a) Ca



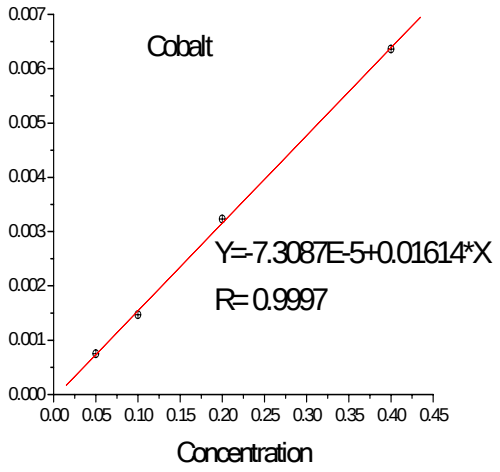
(b) Mg



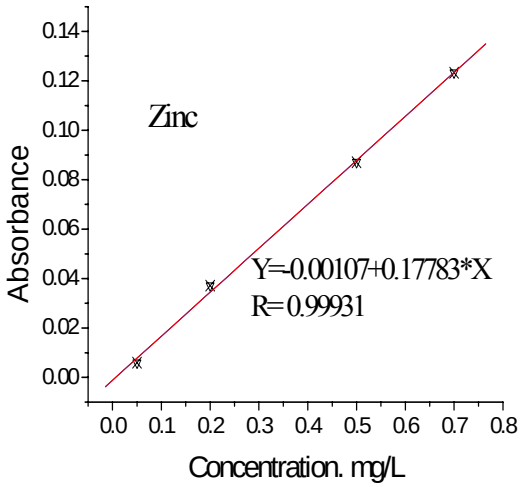
(c) Cr



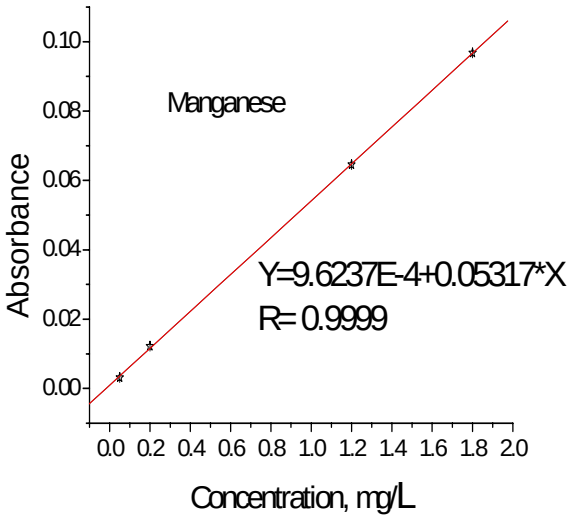
(d) Cu



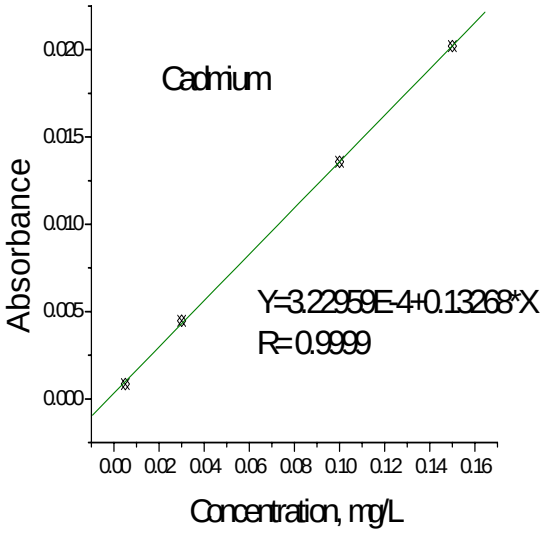
(e) Co



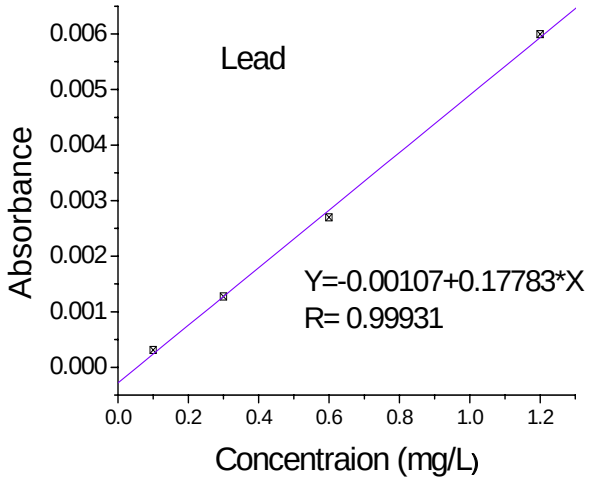
(f) Zn



(g) Mn



(h) Cd



(i) Pb

Figure 3 (a-i). Calibration Standard Curves of the Metals for FAAS Measurement.

2.2.6. Method Validation

Validation of a new analytical method or sample preparation technique need to be performed so as to asses its merits with respect to the purpose and type of analysis is a necessity (Mitra, 2003). This can be done through single operator figures of merits (accuracy, unknown sample analysis, precision, detection limit and linear dynamic range), equivalency testing with the existing method, and collaborative testing with other laboratories and others.

In this work, validation of the sample preparation method was done through recovery test, triplicate analysis of samples and detection limit establishing.

2.2.6.1 Spiking Experiment

In the spiking experiment, known quantities of the metals standard solutions were added to sub-samples of one sample for each (onion bulb cultivated with well water) and applying the whole procedure to the mixture (spiked sample) and the percent recoveries calculated.

For the i^{th} batch of analytes, the analytical results of concentrations for the 'spiked' and 'unspiked' portion of a samples (onion bulb or irrigation water) denoted by C_{sp} and C_{unsp}

respectively, percentage recovery is calculated as equation (1) where θ is the amount of analyte metal added to the spiked sample.

$$\%R = 100\left(\frac{C_{sp} - C_{unsp}}{\theta}\right)$$

2.2.6.1.1. Spiking of Onion Bulb Sample

Spiking of the onion bulb sample was carried out dividing the metals in two batches, Ca, Co and Cd & (Mg, Zn, Mn, Cu, Cr) so that acid reagents dilution could be minimized. Known concentrations of the metal standard solutions were added to each of the three flasks using micropipette. In the first batch, 100 µL of 1000 mg/L of Mg, 10 µL of 1000 mg/L Zn, 100 µL of 10 mg/L Mn, 100 µL of 10 mg/L Cr, and 120 µL of 5 mg/L Cu solutions were added to 0.5 g of onion bulb powder sample. In the second batch, 100 µL of 1000 mg/L Ca, 10µL of 10 mg/L Cd and 20 µL of 10 mg/L Co were spiked to the equal 0.5 g onion bulb sample in round bottom flasks (100 mL). The spiked samples were digested using the optimized procedures for the onion bulb sample and finally analyzed using FAAS so as to calculate the percent recoveries. Spiking experiment was carried out in triplicate for both batches. The percentage recoveries varied from 90 – 102.1 % (Table 4).

Table 4. Recoveries of Metals in Onion Bulb Samples.

Metal	Amount added to onion bulb sample (µg/g)	Amount found (mean value, µg/g)	Recovery (mean ± SD) %
Ca	200	204.1	102.1 ± 3.5
Mg	200	92.2	92.2 ± 5.9
Cu	1.2	1.12	93.5 ± 8.5
Cr	2	1.82	91.1 ± 6.0
Mn	2	1.86	92.8 ± 1.4
Zn	20	19.3	96.2 ± 4.9
Cd	0.2	0.18	90 ± 3.7

2.2.6.1.2. Spiking of Irrigation Water Sample

Three sub-samples of irrigation water from well each 40 mL were taken in volumetric flasks of size 50 mL to which known volume and concentrations of metal standards added. These were 275 µg of Ca, 375 µg of Mg, 0.75 µg of Zn, 12.5 µg of Mn, 6.88 µg of Cr quantities and the flask was filled to the mark by the water sample. Cobalt was spiked in Lake Water samples separately adding 2.5 µg in 50 mL volumetric flask water. The spiked samples were run on FAAS for metals content determinations to calculate the percent recoveries. The spiking experiment was done in triplicate.

The obtained percentage recovery ranges from 91.8 % to 104.2 % (Table 5), which was in the acceptable range. The optimized procedure for the metal analysis in the onion bulb was therefore suitable for analysis of the metals in the samples.

Table 5. Recoveries of Metals in Irrigation Water Samples.

Metal	Amount added to water sample (mg/L)	Amount found (mean value, mg/L)	Recovery (mean ± SD %)
Ca	5.5	5.28	96 ± 6.4
Mg	7.5	6.965	92.9 ± 2.8
Cr	0.1375	0.1433	104.2 ± 3.6
Mn	0.25	0.2436	97.4 ±5.6
Zn	0.015	0.0145	96.7 ± 3.9
Co	0.05	0.0459	91.8 ± 4.7

2.2.6.2. Method Detection Limit

Detection limit of an analyte is the smallest quantity (concentration) of analyte which can be detected but not quantified at a given confidence level. Detection limit of certain method may vary greatly often with matrix and experimental procedures (Ewing, 1985). The method detection limits estimated indicated that if the concentration of the respective element is at least equal to the detection limit, it can be detected but not necessarily quantified at the given confidence level.

In this study, method detection limit for each metals is estimated by digesting 9 analytical blanks with the optimized procedure for the onion bulb samples; while 6 blank solutions of 2 % (v/v) 69-72 % HNO_3 in deionized water (50 mL volumetric flasks) for irrigation water samples. Each blank solution was run with FAAS for the metals level in similar manner as the samples and standard deviations of the blanks' concentration were calculated. The method detection limit for the metal analyte is therefore here the concentration in the sample matrix at which analyte signal equals at least three times that of noise ($3\delta_{blank}$, where δ = SD of the blanks, n = 9 for onion bulb and n = 6 for irrigation water (Table 6).

Table 6. Method and Instrument Detection Limits for the Metals in the Onion and Water Samples. (n = 9 for onion bulb and n = 6 for irrigation water).

Metal	Instrument detection limit (mg/L)	Method detection limit for onion bulb (mg/g dry wt)	Method detection limit for irrigation water (mg/L)
Ca	0.01	0.002	0.18
Mg	0.001	0.001	0.03
Cu	0.02	0.0002	0.02
Cr	0.05	0.02	0.16
Mn	0.001	0.0004	0.002
Zn	0.005	0.001	0.01
Co	0.05	0.0002	0.03
Pb	0.1	0.002	0.10
Cd	0.005	0.00015	0.007

The method detection limits estimated were greater than the instrument detection limit for all of the metals under consideration (Ca, Mg Cu, Cr, Mn, Zn, Pb and Cd) in onion bulb and irrigation water samples except cobalt in water samples. The instrument detection limit was used for the analysis of cobalt in water since the method detection limits were found to be less than that of instrument.

2.2.6.3. Analytical Precision

The reproducibility of the analytical procedure was checked by carrying out a triplicate analysis and calculating the coefficient of variation of the mean for each metal. In most cases, triplicate results did not differ by more than 10 % of the mean.

3. RESULTS AND DISCUSSIONS

3.1. Distribution of Metals in Onion Bulb Samples

The levels of major and trace metals in the onion bulb samples irrigated with well and lake water determined with FAAS after sample dissolution with the optimized digestion procedure were expressed per dry weight as shown in Table 7. All analyses were done in triplicate.

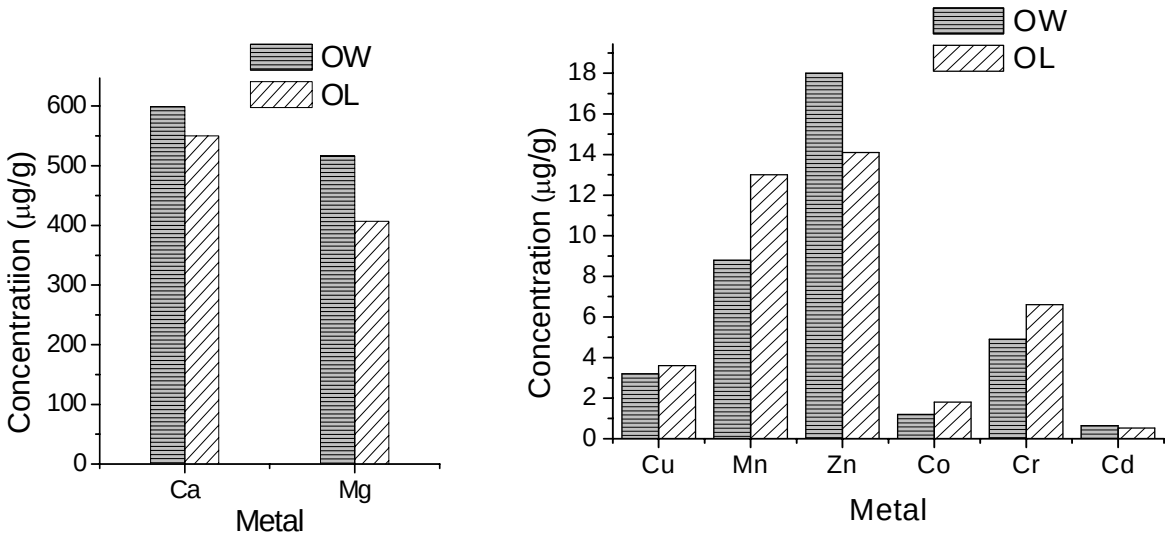
Table 7. Concentration (mean ± SD, n= 3 in μg/g dry wt) and RSD of metals in onion bulb samples.

Metal	Onion cultivated with well water (μg/g dry weight)	RSD (%)	Onion cultivated with Lake Ziway water (μg/g dry weight)	RSD (%)
Ca	599 ± 30	5.0	550 ± 20	3.7
Mg	516 ± 12	2.4	407 ± 30	7.4
Cu	3.2 ± 0.1	2.5	3.6 ± 0.2	4.3
Mn	8.8 ± 0.2	2.4	13 ± 0.8	6
Zn	18.0 ± 0.7	3.8	14.1 ± 0.4	3
Co	1.2 ± 0.5	4.3	1.8 ± 0.1	7.6
Cr	4.9 ± 0.2	3.7	6.6 ± 0.1	1.4
Cd	0.64 ± 0.07	6	0.53 ± 0.06	11
Pb	ND	-	ND	-

Zinc was the most accumulated trace metal in the onion bulb samples while Cd was the least with slightly higher amounts in the onion cultivated with well water. The levels of major metals were much higher than that of trace in the samples. The observed trend was Ca > Mg > Zn > Mn > Cr > Cu > Co > Cd in both onion samples. Lead was below method detection limit in the samples.

The two major metals (Ca and Mg) were detected in higher level in the onion bulb cultivated with well water than in lake water. On the contrary, the trace metals Cu, Mn, Co and Cr were higher in the onion bulbs irrigated with lake water except Zn and Cd which were higher in onion grown with well water (Figure 4).

Metal ion uptake into the roots of plants is extremely complex phenomenon occurring via both diffusion and mass flow of the soil solution (Bertini *et al*., 1998) and so great variation was observed in the metal levels in this study, too.



(a) Major metals (b) Trace metals

Figure 4 (a and b). Distribution of Metals in Onion Bulb (µg/g dry weight).

(OW is onion irrigated with well water and OW is onion irrigated with Lake Ziway water)

3.2. Distribution of Metals in Irrigation Water Samples

The concentration of metals in irrigation water samples collected from the pipes at the points of entrance to the farm fields were analyzed after filtering and acidifying as expresses in mg/L of water solution for the detected metals (Table 8). Four trace and the two major metals were detected while Cu, Cd and Pb were below the method detection limits. Cobalt was detected in lake water but not in well water.

Table 8. Concentration (mean ± SD, in mg/L) and RSD of Metals in Irrigation Water Samples.

(n= 3 for all metals except Cu, Co and Cd for which n = 6)

Metal	Well water (mg/L)	RSD (%)	Lake water (mg/L)	RSD (%)
Ca	22.7 ± 0.5	2.2	9.9 ± 0.2	2
Mg	30.2 ± 1.0	3.3	8.6 ± 0.1	1.2
Mn	1.1 ± 0.01	0.9	0.050 ± 0.005	10
Zn	0.040 ± 0.004	10	0.080 ± 0.004	5
Cr	0.44 ± 0.01	2.3	0.42 ± 0.02	4.8
Co	ND	-	0.060 ± 0.002	3.3
Cu	ND	-	ND	-
Cd	ND	-	ND	-
Pb	ND	-	ND	-

The trends observed was Mg > Ca > Mn > Cr > Zn in well water and Ca > Mg > Cr > Zn > Co > Mn in lake water employed for irrigation in the study area. Among the trace metals, Mn was detected in highest quantity in well water samples. Comparing the water samples, the two major metals (Ca and Mg), Mn and Cr were found at higher level in well water than in the lake water samples while Zn and Co were higher in the lake water (Figure 5).

Tomar (1999) pointed out that unlike macromolecular organic pollutants, inorganic elements and gases like H_2S and CH_4, can get access easily through the soil, and, once introduced, their effect continue for long time because of slow natural dilution process and difficulty in artificial removal or treatment of ground water. Total hardness in water is contributed by metal cations like Ca^{2+} and Mg^{2+} primarily and also Fe^{2+}, and Mn^{2+}, St^{2+}, and Al^{3+} which might also be the case in the well water samples.

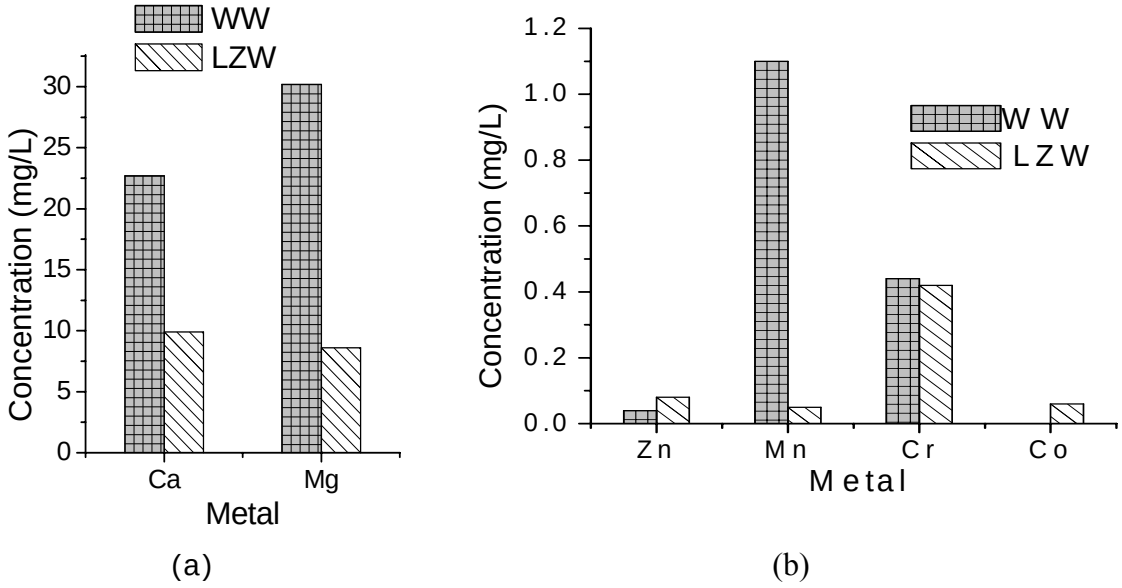


Figure 5. Distribution of Metals in Irrigation Water Samples: (a) Major and (b) Trace Metals (WW= Well water and LZW-Lake Ziway Water)

3.3. Comparisons of Metal Levels between Water and Onion Bulb Samples

Up on comparing the levels of metals in the plant (onion) and the growth media (irrigation water), Ca and Mg were higher in well water and onion cultivated with the well water. Therefore, these metals were taken up by the plants significantly from the irrigation waters applied.

Much higher concentration of Mn was observed in well than lake water which was not reflected in the associated onion plant. This variation might be because the Mn in the well water might not be bio-available for onion. Zn was detected at higher level in lake water than in well but the level

observed in the plant was the reverse that it was higher in onion cultivated with well water. The soil and other anthropogenic input might be principal source for the plant.

Cobalt levels were higher in Lake Ziway water samples and associated onion plant cultivated with it which might indicate significant contribution by the irrigation water applied. The levels of Cr were more comparable in the irrigation water samples than in the onion plant which was higher in onion grown with lake water.

Generally, much higher concentrations of the metals analyzed were detected in the plant than the growth media (irrigation water) indicating the bio-accumulation of the metals. Bioaccumulation is of course a normal and essential process enabling the organism to have reserve for latter use for metallo-proteins or cofactors or protect themselves against toxic effects. Kebede and Wondimu (2004) described bioaccumulation of chemicals in generals as governed by the net results of the interactions of uptake (by breathing, swallowing, or absorbing), storage (by binding to proteins, dissolving in fats, or displacing some elements from biological molecules), and elimination via metabolic processes.

In case of onion, the bioaccumulation of both trace and major metals were attempted to be verified by roughly comparing their concentration in one of the growth media, irrigation water and those in plants (Figures 5 and 6). The figures were drawn with logarithmic scale of the concentrations (mg/kg for onion bulb and mg/L for waters

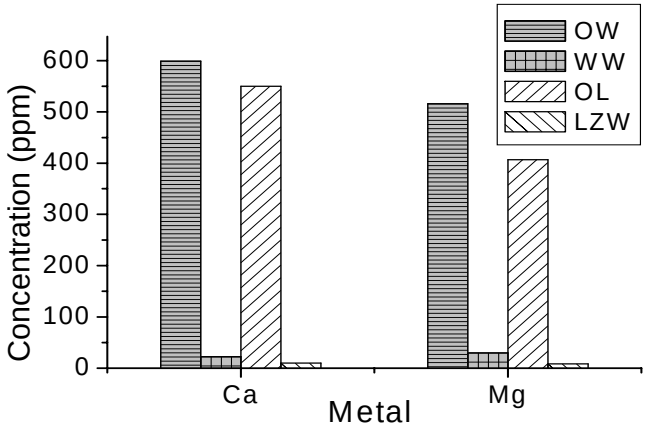
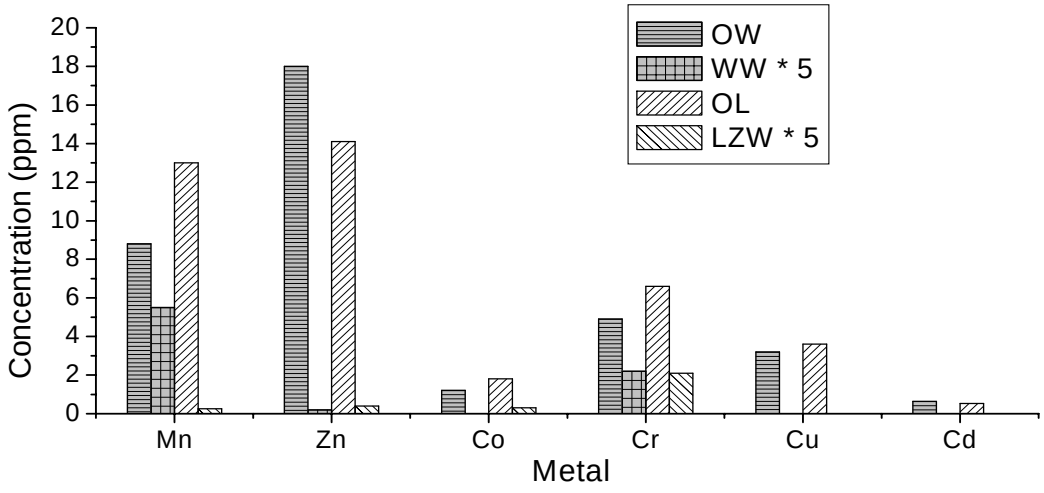


Figure 6. Comparison between Levels of Major Metals in Onion Plant and Water.



Figur

e 7. Comparison between Levels of Trace Metals in Onion Plant and Water.

(The levels of trace metals in WW and LZW in the graph were the actual concentration multiplied by 5).

However, metals are persistent in the environment and tend to biomagnified accross the food chain up to the top where human beings might be highly exposed (Biziuk and Kuczynska, 2006). As the immediate consumer of onions is human beings, there is a tendency of further accumulation of the metals in human tissues.

3.4. Metal Specific Distribution Patterns

Calcium

Calcium was the most accumulated metal in onion bulbs studied with mean concentrations and standard deviations 599 ± 30 and 550 ± 20 μg/g dry wt in onion bulbs fed with well and lake water respectively, 22.7 ± 0.5 and 9.9 ± 0.2 mg/L in the agricultural water pumped from well and lake respectively.

Calcium is essential macronutrient for both plants and animals. It is involved in cell division, bone and teeth building, and blood coagulation. The ion along with magnesium causes hardness

of water. There are broad range of Ca-bearing minerals in soil and water and usually abundant in ground and surface water with 0.1-1.2 % range in soil (Perk, 2006). Calcium in plants is a critical component of cell walls and membranes stabilizing and also assists in protein formation and carbohydrate transport (Plaster, 2003).

Magnesium

Magnesium concentration level in this study varies from 516 ± 12 and 407 ± 30 µg/g dry wt in onion bulbs irrigated with well and lake; 30.2 ± 1.0 and 8.6 ± 0.1 mg/L in corresponding well and lake water. The result reveals that high concentration of magnesium exists in well water and onion cultivated with it as in the case of calcium indicating that the irrigation water employed was good source of the metal.

In plants magnesium is constituent of every chlorophyll molecule of green plants. It is also associated with synthesis of plant protein, P-metabolism, and synthesis of plant oils along with sulfur (Adeyeye, 2005). In animals magnesium has a role in bone and tendons building with calcium. Minerals like dolomite are major sources of magnesium in soil and water while anthropological source are generally rare which might also describe the possible sources of Mg for the onion in the study area.

Copper

Copper concentration in onion bulbs watered with well and lake were 3.2 ± 0.1 and 3.6 ± 0.2 µg/g dry wt. respectively. However the levels in irrigation waters were below detection limit of the instrument. Therefore the principal source of copper for onion plant might be the soil or soil contaminants.

Copper is an essential micronutrient for many plants and animals. For human being 1-3 mg each day for normal body function. Human toxicity from copper is generally rare but prolonged exposure to children may damage liver and cause death. Copper deficiency in plants may cause depress in crop yield. Sources of copper in water and soil are Cu mining and smelting as well as agrochemicals. Copper as it is applied to treat plant disease like fungicides (e.g. Bordeaux mixture) it can accumulate on top soil. High level of copper in soil is phytotoxic and harmful to

soil microorganisms (Plaster, 2003). Copper content of normal plant tissues varies according to species but is usually within the range 1 – 25 mg/kg which inclusive of the level in onion samples. The accumulation of copper in plant roots is also found to decrease root length and suppress the formation of lateral root (Itanna, 1998).

Zinc

The concentration of zinc obtained in onion bulbs irrigated with well and lake water were 18.0 ± 0.7 and 14.1 ± 0.4 μg/g dry weight of the bulbs revealing higher accumulation by the former. The corresponding results for well and Lake Ziway samples applied to the onion farm were 0.040 ± 0.004 0 and 080 ± 0.004 mg/L Zn, respectively.

Zinc is an essential trace metal involved in growth and DNA synthesis in human with normal daily intake of 7-16 mg per day for adults. For plants n foods zinc concentration 6 mg/kg to 24 mg/kg were observed (Perk, 2006). Though not common, long term human exposure may result in Cu deficiency, reduced immune system and anemia. Phytotoxic effect of excess zinc for plants is more concern particularly when zinc level in soil is above 300 mg/kg. Mining and smelting activities and domestic waste products (galvanized steel, skin care cosmetic products and pigments) are principal causes which lead to local soil and ground water pollution by zinc. Zinc is most available in acidic soil (Plaster, 2003).

The levels of zinc obtained in edible portion of onion in this study are within the ranges estimated in plants. In the normal daily consumption of onion bulb as condiment, the level of zinc cannot have negative effect on human.

Manganese

Manganese was the second most accumulated trace metal next to zinc in onion bulb and even the one obtained in highest quantity in irrigation water from well water samples. The levels in onion bulbs fed with well and lake water were 13 ± 0.8 and 8.8 ± 0.2 μg/g dry wt, respectively and 1.1 ± 0.01 and 0.05 ± 0.005 mg/L for the respective well and Lake Ziway samples.

Perk (2006) explained that manganese oxide surface precipitated forms manganese nodules or coat on sediments or rock which when subsurface is in contact with the precipitate may possess

concentration up to about 1 mg/L which might also be the case in the study area which might be the case in well water. He further added acidic ground water may also contain high levels of dissolved Mn^{2+} ion up to 1 mg/L. Manganese absorbed by plants is stored mostly in leaves and so released to soil or run off as soon as the plant's leaves shade giving rise to temporary increase in manganese concentration in water bodies during autumn.

Chromium

The chromium content of onion bulbs samples in this study were 4.9 ± 0.2 and 6.6 ± 0.1 μg/g dry wt. for those irrigated with ground water (well) and surface water (lake); while the corresponding values were 0.44 ± 0.01 and 0.42 ± 0.02 mg/L for the waters, respectively. Compared to trace metals chromium ranks second in both plant and water samples. Higher levels were obtained in onion bulbs of lake water though the difference is very small for the water used during irrigation.

The trivalent chromium, Cr(III) is essential trace element for adult human being with safe and adequate daily requirement of 50-200 μg and toxic only at high level. However Cr(VI) is toxic, long time exposure resulting in kidney and liver damage and being carcinogenic. Plants absorb more quantities of Cr(VI) though Cr(VI) is more toxic than Cr(III) for them. The hexavalent chromium, Cr(VI) is more soluble in soil moisture and water while Cr(III) is largely present in soil as relatively unavailable, insoluble ion or hydroxides. This is because Cr(III) is readily hydrolyzed at neutral pH and extremely insoluble (Tomar, 1999). Soil and ground water pollution by Cr(VI) is primarily by leaching from wastes disposed through industrial processing of tannery, pigments, steel and others.

Cobalt

The cobalt concentrations in onion bulb samples were 1.2 ± 0.5 and 1.8 ± 0.1 μg/g dry wt for those irrigated by well and lake water, respectively. The level in corresponding well water was below instrument detection limit; however in the Lake Ziway analyzed 0.055 ± 0.002 mg/L Co obtained.

Though deficiency of cobalt is rare, the onion from the study area can be its good source in addition to animal products.

Cadmium

Cadmium concentration obtained by this study is one of concern 0.64 ± 0.07 and 0.53 ± 0.06 µg/g in onion bulb irrigated with well and Lake Ziway water while the level in water samples were found below detection limit.

Cadmium has not been known to have beneficial effect for plants and animals, indeed long term human exposure causes kidney damage and ultimately failure and being carcinogenic. Normal intake of cadmium is 1-3 µg per day (Perk, 2006). The divalent cadmium is found to be more labile in soil and sediments and so more bio-available since it is less strongly adsorbed than other divalent metals. The largest source of cadmium in human food chain is from application of phosphate fertilizer in which cadmium exist as impurity. In the environment it exists as a byproduct of Zn, Pb and Cu refining industries; wastes disposed from batteries, paints and plastics. During burning of fossil fuels at higher than 400 ^{0}C volatile cadmium can travel as aerosol several kilometers from the source and deposited.

Lead

Lead concentration in both onion and irrigation water samples were below the method detection limit. Lead in soil and water has low mobility because of low solubility of lead hydroxide, carbonate and phosphate. In natural water usually it exists at low level due to adsorption on mineral and organic sediments (Perk, 2006). Lead can cause brain and kidney damage, decrease in hemoglobin production and male fertility. Besides, lead is absorbed in to blood from their gastro-intestine that they are more susceptible to the toxicity. Therefore the results from obtained by the study concerning lead is in accordance with the requirement.

3.5. Comparison of Metal Levels in Onion of the Study with Literature Values

The major metals (Ca and Mg) and trace metals determined in different countries from different points of view (nutritional, health problems, crop yield etc) were presented in Table 9 along with the current study for comparison for comparison.

Table 9. Summary of Trace Metal Concentrations of Onion Reported in Literature.

Country/ Metal	Nigeria	Pakistan [a]	Ethiopia (PRESENT STUDY)		China, Shanghai[b]	Chile	UAE[c]	Ethiopia (onion leaves)	Korea	Poland	Turkey	Spain
			OW	OL								
Pb	2.00-9.5	-	ND	ND	0.040		0.24 (9.52)		4.23 ± 1.16	0.45 ± 0.69	13.1 ± 0.7	
Cd	0.22-0.89	-	0.6 ± 0.07	0.53 ± 0.06			0.06 (8.00)	0.018	1.88 ± 0.35	0.04 ± 0.02		
Co	0.03-0.22	-	1.2 ± 0.5	1.8 ± 0.1								
Cr	3.00-7.1	1.1 ± 0.00	4.9 ± 0.2	6.6 ± 0.1	0.070		4.2 (0.71)	2.81				
Cu	0.34-1.00	0.8 ± 0.00	3.2 ± 0.1	3.6 ± 0.2	0.900	7.7 ± 1	0.10-1.6	5.24	26.4 ± 4.58	3.47 ± 1.24		0.33 ± 0.01
Mn	1.00-6.6	1.9 ± 0.01	8.8 ± 0.2	13 ± 0.8			76 (2.58)					0.75 ± 0.09
Zn	2.00-4.55	3.4-8.33	18 ± 0.7	14.1 ± 0.4	3.280	31 ± 8		15.4	256 ± 79.5	10.94 ± 3.49		1.72 ± 0.13
Mg			516 ± 12	407 ± 30			241 (2.15)					92.8 ± 6.4
Ca			599 ± 30	550 ± 20			84 (4.59)					148 ± 8
Ref.	*	Hashmi *et al.*, 2007			Zhou *et al.*, 2000	**	Khan *et al.*, 2006	Itanna, 1998	Jung, 2008	Mocko, 2004	***	Mendez *et al.*, 2007

[a]- mean ± 2Stdev at 99% CL in onion bulb [c] value in parenthesis are relative standard deviation [b]Mean for the year 1992-1993

* (Akan *et al.*, 2009; Abdullahi *et al.*, 2008; Iyaka, 2007) ** (Badilla-Ohlbaum *et al.,* 2001) *** (Baytak and Turker, 2004)

The amounts of Ca and Mg in onion bulb of present study were higher than that obtained by Mendez *et al*., (2007) and Khan *et al*., (2006).

Copper concentration in onion bulbs determined in this study was less than 7.7 and 15 mg/kg dry wt. for onion and tomato respectively reported by Badilla-Ohlbaum et al. (2001). The level was also below the values reported for onion leaves samples in the range of 6.917-9.833 mg/kg by Abdullahi *et al*., (2008). Copper concentration as high as 26.4 mg/Kg was also found by Jung, 2008 in samples taken in the vicinity of Cu-W mine. The present level attained were however higher than 0.8 and 1.6 mg/kg dry wt. reported by Iyaka (2007), 0.8 mg/kg by Hashmi *et al*., (2007) and 0.10 Khan *et al*., 2006. The concentrations of this study were comparable with that of 3.47 mg/kg reported by Mocko (2004).

Furthermore the present result in onion bulb cultivated with well as well as lake water were below the standards of USDA nutrient data base mean content of edible onion part (Itanna, 1998), maximum tolerable content in vegetables, FAO/WHO joint limit (5.0 mg/kg dry wt.) and phytotoxic range of 20 - 100 (Abdullahi *et al*., 2008). The safe and adequate intake of copper for adult person is estimated to be 1.5-3.0 mg (Szefer and Nriagu, 2006). The onion bulb in the study area can therefore be a safe and good dietary source of copper for consumers.

The mean concentration of zinc in the onion bulb of the study area 18 mg/kg and 14.1 mg/kg for those irrigated with well and lake water were highest among trace metals but comparable with 18.3 mg/kg mean zinc content of edible part of onion set by USDA and 100 - 400 mg/kg phytotoxic range (Abdullahi *et al*., 2008). However, the concentrations were higher than FAO/WHO Limit of 0.3 mg/kg and even the more constraint WHO/EU Limit of 0.2 mg/kg in vegetables (Abdullahi *et al*., 2008). The levels were also higher than literature reports of Hashmi et al (2007), Akan et al (2009), Abdullahi *et al*. (2008), Iyaka (2007), Zhou *et al*. (2000) and Mendez *et al*. (2007). Much higher concentrations of 256 ± 79.5 mg/Kg were however reported by Jung (2008) and 31mg/kg by Badilla-Ohlbaum *et al.* (2001). In addition the levels were below the 20 mg/kg, and 100 - 400 mg/kg of MAFF, and phytotoxic range, respectively (Abdullahi *et al*., 2008).

"Zinc has now joined iodine and iron among trace elements of which worldwide and urgently need to be addressed," as emphasized by Reilly (2001). The deficiency of zinc is manifested by growth retardation, loss of appetite, reduced immunity, delayed sexual maturity in males, etc.

The recommended daily allowance of zinc is 12-15 mg for an adult person. Societies in which meat is not a major component of their foods are particularly found to practice as low intake as 5 mg/day. The onion bulb in the study area therefore can be used as a good dietary source of zinc. Ingestion of 75-300 mg/day in the form of dietary supplements has been shown to interfere with absorption of Cu, Fe and other trace metals while 1 g zinc salt ingestion causes irritation of intestine, nausea and abdominal pain (Nabrzyski, 2006).

The average manganese composition of onion bulbs in the study area 8.8 mg/kg and 13 mg/kg dry wt. for those watered with well and Lake Ziway were found to be above 0.2 WHO/EU Limit for vegetables (Abdullahi *et al*., 2008), and the 1.9 mg/kg dry wt. reported by Hashmi *et al*. (2007). Mnagaense concentration as high as 76 mg/kg dry wt. has been reported however by Khan *et al*. (2006). Manganese is so important in brain and bone development and safe and adequate daily intake of 2-3 mg for an adult is recommended (Nabrzyski, 2006).

Chromium level in edible part of onion in the study area, 4.9 and 6.6mg/Kg dry wt. in the vegetable irrigated with well and lake water were within the range of 3.00-7.1mg/Kg dry wt. determined in Nigeria (Akan *et al*., 2009; Abdullahi *et al.*, 2008; Iyaka, 2007) and 3.87-8.87 mg/kg dry wt. estimated by Audu and Balwant (2004) cited by Abdullahi *et al*. (2008). The level was however higher than reported values of 1.1 (Hashmi *et al*., 2007), 0.070 (Zhou *et al*., 2000) and 4.2 mg/kg dry wt. (Khan *et al*., 2006). In addition it exceeded the 2.30 mg/kg dry wt maximum load of vegetables (Codex, 2001) and 0.1 - 0.2 mg/kg FAO/WHO joint limit (Akan *et al*., 2009). The levels were below toxic stage for human, 200 mg/kg dry wt. (Abdullahi *et al*., 2008). Trivalent chromium is essential in glucose tolerance factor with safe and adequate daily intake of 0.05 - 0.02 mg/adult (Nabrzyski, 2006).

Trivalent chromium is known to exist in soil usually largely as insoluble and unavailable compounds (Tomar, 1999) which might indicate that the high level in the onion bulb might be from contamination of ground water and Lake Ziway with manucipal and industrial wastes of tannery, pigments, steel and others, disposed in to tributary rivers (Meki River and Katar River)

as well as Mojo River or to soils. The hexavalent chromium, which is toxic and carcinogenic, might also be in significant proportions that further speciation of the chromium in the onion bulb from the study area, is needed to assess hazardous effect for human consumption.

Cobalt levels obtained by this study in onion bulbs irrigated with well and lake water were 1.2 and 1.8 mg/kg dry wt respectively. Assessing literatures data on cobalt level of vegetables particularly onion is few, however one recent study conducted in Nigeria by Akan and his coworkers (2009) found cobalt in onion in the range of 0.03-0.22 mg/kg dry wt which was much lower than the values estimated in this study. Cobalt exists as a component of vitamin B_{12}, cobalamin whose deficiency if occurred causes pernicious anemia in human being. The recommended dietary allowance of cobalt is 0.002 mg/adult as vitamin B_{12} (Nabrzyski, 2006).

Cadmium toxicity danger to human health was brought to the world's attention by the itai-itai tragedy in post-World War II Japan, when rice fields were polluted with Cd contained in factory effluents. Cadmium triggers the need for concern in the study area which were mean 0.64 and 0.53 mg/Kg for the bulb grown with water from well and water diverted from Lake Ziway through furrow irrigation respectively. These levels were much higher than the 0.018 mg/kg dry wt. of onion leaf content estimated before 11 years by Itanna (1998) in farmlands fed with effluents from Akaki textile factory as well as 0.06 (Khan *et al*., 2006), and 0.04 mg/kg dry wt. (Mocko, 2004). However the values in this study were lower than the 0.8 - 1.00 (Abdullahi *et al*., 2008), and 1.8 mg/kg dry wt. (Jung, 2008) reported in the literature. Values in the range of 0.05 - 0.43 Davis and White, 1981 (as cited in Abdullahi *et al*., 2008) were also mentioned in the literature.

Generally, the levels determined in this study were above the WHO/FAO Limit (0.02 - 0.2) WHO/EU Limt (0.01), and MAFF (0-1 mg/kg) Abdullahi *et al*., 2008 but did not reach phytotoxic level estimated phototoxic range of 1-30 mg/kg Abdullahi *et al*.(2008).The principal sources of cadmium might be the use of Cd-contaminated phosphate fertilizers, wastes disposed from battery, pigments, plastics and burning of fossil fuels. Repeated use of P-fertilizers such as triple superphosphate may therefore result in accumulation of these elements and increase the contamination potential, especially of Cd, in the soil Malak *et al*. (2007).

Lead has got no nutritional benefits for human being with little doubt and can cause both chronic and acute poisoning affecting hemoglobin synthesis in bone marrow, the critical target organ. Lead is found however in every organ of the body, with a toatal of 100-400 mg in adult male. Absorption from food is 10% for adults, which may reach up to 50% for children and transported attached to blood cells. Overall levels of lead in foods throughout the world as Reilly (2006) stated being surprised ranges from 0.01 to 0.25 mg/kg in a uniform manner. In the literatures values as high as 4.23 Jung (2008), 0.24 Khan *et al*. (2006), 6-13.00 mg/kg dry wt. Abdullahi *et al*., 2008 were reported in onion vegetables. The results of this study shoed lead content of the onion bulb below the method detection limit that assures the low lead exposure of the farm fields as well as waters in the study area.

3.6. Comparison of Levels of Metals in Irrigation Water with Literature Values

The concentration of Co and Cd of Lake Ziway sample in this study was comparable with that of Kebede (2004). However, chromium was much higher while copper lower in this study (Table 11).

Compared with the Bellandur Lake in India, levels of Cu, Zn and Pb in Lake Ziway were lower while that of chromium and cadmium were higher in this study. The Cu and Zn determined in surface water of Nigeria seems to be much concentrated in the metals than that of the ground water and Lake in the present study.

Table 11. Summary of Metal Concentrations in Waters Reported in Literature.

Country	**Nigeria**	**India**	**Ethiopia**		**Ethiopia**	**Ethiopia (Present study)**	
	Borehole,hand dug, surface water	Bellandur lake (dry season)	Awash River	Channels, range	Lake Ziway water	Well water	Lake Ziway water
Pb		0.006	< 0.1*	<0.1*		ND	ND
Cd		0.0033	< 0.1*	< 0.0001-	0.009-0.01	ND	0.0062 ± 0.0004
Co				-	0.05-0.064	ND	0.055 ± 0.002
Mn			0.106 ± 0.002	0.053-0.125		1.06 ± 0.01	0.054 ± 0.005
Fe	0.32-0.67	0.777	0.142 ± 0.012	0.124-0.709		-	-
Ni	0.15-0.28	0.0065	-	-		-	-
Cr		0.005	-	-	0.06	0.44 ± 0.01	0.42 ± 0.02
Zn	0.12-0.16	0.113	0.092 ± 0.002	0.012-0.184		0.037 ± 0.004	0.084 ± 0.004
Cu	0.12-0.34	0.016	0.037 ± 0.002	0.011-0.066	0.03-0.04	ND	ND
Mg			6.67 ± 0.05	6.82 ± 0.23			
Ca			26.87 ± 0.11	28.57 ± 0.38			
Reference	Jatau *et al.*, 2008	Lokeshwari and Chandrapp	Guta, 2005 * MDL		Nigussie, 2004		

3.7. Comparison of Metals in the Irrigation from the Study Area with Water Quality Standards

Poor quality water may affect irrigated crops by causing accumulation of salts in the root zone, by causing loss of permeability of the soil due to excess sodium or calcium leaching, or by containing pathogens or contaminants which are directly toxic to plants or to those consuming them. Contaminants in irrigation water may accumulate in the soil and, after a period of years, render the soil unfit for agriculture (FAO, 1985).

Table 12. Recommended Maximum Concentrations of Trace Metals in Irrigation Water.

Metal	**Guideline value* (mg/L)**	**Toxicity remarks**
Mn	0.2	Toxic to a number of crops at few-tenths to a few mg/l, but usually only in acid soils.
Zn	2	Toxic to many plants at widely varying concentrations; reduced toxicity at pH > 6.0 and in fine textured or organic soils.
Cr	0.1	Not generally recognized as an essential growth element. Conservative limits recommended due to lack of knowledge on its toxicity to plants
Cu	0.2	Toxic to a number of plants at 0.1 to 1.0 mg/l in nutrient solutions.
Co	0.05	Toxic to tomato plants at 0.1 mg/l in nutrient solution. Tends to be inactivated by neutral and alkaline soils.
Fe	5	Not toxic to plants in aerated soils, but can contribute to soil acidification and loss of availability of essential phosphorus and molybdenum.
Ni	0.2	Toxic to a number of plants at 0.5 mg/l to 1.0 mg/l; reduced toxicity at neutral or alkaline pH.

Source: Table Adapted from Ayers, R.S and Westcot, D.W. FAO of the United Nation, Irrigation and Drainage Paper; Water Quality for Agriculture, 1985 of Table 21.

*The maximum concentration is based on a water application rate which is consistent with good irrigation practices (10 000 m^3 per hectare per year).

Comparison of the levels of trace metals in irrigation water samples from well and Lake Ziway, most of the metals, Cr, Zn, Co, Cu, Cd, and Pb were below the maximum concentration in irrigation water set by FAO (1985). The limit set by FAO for irrigation water quality for Cd and Pb were 0.01 and 5 mg/L, respectively. Chromium in both well and lake water (0.44 and 0.42 mg/L respectively) however, exceeds the set limit of 0.1 mg/L for total chromium. While Mn in lake water was below the limit, Mn detected in well water exceeded the maximum limit of 0.2 mg/L for irrigation water in general.

3.8. Statistical Analysis

Linear regression analysis of calibration curve was used to calculate unknown concentration, sensitivity, correlation coefficients and standard deviation. Average concentration and standard deviations of triplicate measurement for the sample were reported. Where necessary Q-test was used to decide whether to reject or accept outlying measurement value. Variance in sampling and analysis were determined by F-test through One-way ANOVA (Miller and Miller, 2005). Student t-test was calculated to identify whether the means of the concentration between the two onion bulbs and the two irrigation water samples vary significantly by Statistical Package for Social Science (SPSS statistic 15.0 Microsoft window) as well as excel work sheet (Microsoft Office Excel, 2007). Origin (origin 7.0, OriginLab Corporation) was employed to draw some of the curves.

Variation in the mean levels of metals between the samples were tested whether it was from just a random error or treatment (i.e. difference in mineral contents of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides etc. or other variations in cultivation procedures)

Significant differences were obtained in variance ($p < 0.05$) at 95 % confidence levels for Ca, Cu, Co and Cr in onion bulbs cultivated with well and Lake Ziway water. However, the variations for Mg, Zn and Cd were not significant ($p > 0.05$) in the two samples.

For the irrigation water samples, except Ca and Mg all other metals (Cr, Mn and Zn) do not differ significantly ($p > 0.05$) which might be due to similarity of source of the metals.

4. CONCLUSIONS AND RECOMMENDATIONS

The results in the study are comparable with the data reported in some countries. However, chromium and cadmium in onion exceeded the WHO/FAO allowable limits. Lead, one of the trace toxic metals was below method detection limit in both onion plant and irrigation water samples. The incidence of trace metals was higher in the lake water than the ground water on the contrary to the major metals which were higher in the ground water. This may be associated with the higher organic matter in the surface water which can form various complexes with the transition metals. Besides the rock to which the ground water and the sediment to which the lake water were in continuous contact also has great role in determing the metal levels The chromium level detected might be from wastes carried by the tributary rivers of Meki River which crosses Meki Town or industrial discharge from effluents of Mojo Tannery factory contaminating Mojo River.

Generally, concerning the metal levels, particularly the trace metals, both water samples were suitable for irrigation when discharged in a controlled manner. The onion bulb chopped daily in most kitchens and a condiment of variety of dishes can be a good dietary source of zinc and manganese trace essential metals.

The levels of metals in waters and the plants when compared was an indication of bioaccumulation of metals in the tissues of plant tissue from their growth media. Correlation test performed on the mean values of metals indicated significant association between the metals in the plant as well as those in the water among themselves which might be traced to common source or similarity in properties.

The benefits and toxicity analysis arising from metals in diet is a complex factor of dietary culture of the society, quantity of consumption, level of absorption from gastro-intestinal tract, the ways and time of intake, physical conditions of the person and level of other metals and food stuffs. 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 off further study.

Comprehensive study of relation between the soil, water and plant is the preferred recommended method to trace the sources of the minerals that assessment of soil composition of the area was recommended particularly for the toxic cadmium. The study area is also producer of various other vegetables and crops that an area of study can be advanced for detailed data. Closely related and highly consumed allium vegetables (shallot, garlic and chives), also needs critical consideration due to their frequency of consumption as condiments and therapeutics.

Irrigation water quality assessment also needs parameters like sodium adsorption ratio, salinity, p H, water infiltration rate, and nitrogen content that comprehensive analysis may be conducted in the study area.

REFERENCES

Abdullahi, M.S., Uzairu, A., Harrizon, G.F. and Balarabe, M.L. Trace Metals Screening of Tomatoes and Onions from Irrigated Farmlands on the Bank of River Challawa, Kano, Nigeria, *International Journal of Environmental Resourcse*, **2007**, 6(3), 1869-1878.

Abdullahi, M.S., Uzairu, A. and Okunola, O.J. Determination of Some Trace Metal Levels in Onion Leaves from Irrigated Farmlands on the Bank of River Challawa, Nigeria, *African Journal of Biotechnology*, **2008**, 7(10), 1526-1529.

Acar, O., Ilimb, M., Kilic, Z. and Turker, R. A. Cadmium, Lead, Copper and Manganese Determination in Human Deciduous Teeth by Electrothermal Atomic Absorption Spectrometry Using a Lanthanum, Palladium and Citric Acid Mixture as Chemical Modifier *International Journal of Environmental Analytical Chemistry,* **2008**, 88(12), 869–878.

Adeyeye, E.J. Trace Metals in Soils and Plants from Fadama Farms in Ekiti State, Nigeria, *Bulletin of Chemical Society of Ethiopia*, **2005**, 19(1), 23-34.

Adeyeye, E. I. Distribution of Major elements (Na. K, Ca, Mg) in the various anatomical parts of Fadama crops in Ekiti state, Nigeria, *Bulletin of Chemical Society of Ethiopia*, **2005**, 19(2), 175-183.

Akan, J.C., Abdulrahman, F.I., Ogugbuaja V.O., and Ayodele J.T. Heavy Metals and Anion Levels in Some Samples of Vegetable Grown Within the Vicinity of Challawa Industrial Area, Kano State, Nigeria, *American Journal of Applied Sciences* **2009**,6 (3): 534-542.

Akinola, M.O., Njoku, K.L. and Ekeifo, B.E. Determination of Lead, Cadmium and Chromium in the Tissue of an Economically Important Plant Grown Around a Textile Industry at Ibeshe, Ikorodu Area of Lagos State, Nigeria, *Advances in Environmental Biology*, **2008**, 2(1), 42-47.

Ariyam, K. and Yasui, A. The Determination Technique of the Geographic Origin of Welsh Onion by Mineral Composition and Perspectives for the Future, *Japan Agricultural Research Quarterly*, **2006**, 40(4), 333-339.

Awode, U.A., Uzairu, A., Balarabe, M.L., Harrisson, G.F.S. and Okunola, O.J. Assessment of Peppers and Soils for Some Heavy Metals from Irrigated Farmlands on the Bank of River Challawa, Northern Nigeria, *Pakistan Journal of Nutrition* **2008**, 7 (2): 244-248.

Auger, J., Arnault, I., Legin, A., Rudnitskaya, A., Seleznev, B. Sparfel, G. and Dore, C. Comparison of gas chromatography–mass spectrometry and electronic tongue analysis for the classification of onions and shallots, *International Journal of Environmental Analalytical Chemistry*, **2005**, 85(12–13), 971–980.

Badilla-Ohlbaum, R., Ginocchio, R., Rodriguez, P.H., Cespedes, A., Gonzalez, S., and Allen, H.E. Relationship Between Soil Copper Content and Copper Content of Selected Crop Plants in Central Chile, *Environmental Toxicological Chem*istry, **2001**, 20(12), 2749-2757.

Baytak, A.; Türker, R. Determination of Lead and Nickel in Various Samples by Using Atomic Absorption Spectrometry after Preconcentration on Ambersorb-572 Resin with Edta, *Adnan Menderes University, 4th Aacd Congress, , Kuşadası-Aydin/ Turkey Proceedings Book* 011.Doc Sıtk,.**2004**,

Benkeblia, N.; Onodera, S.; Yoshihira, T.; Kosaka, S.; Shiomi, N. Effect of Temperature on Soluble Invertase Activity, and Glucose, Fructose and Sucrose Status of OnionBulbs (*Allium Cepa*) in Store, *International Journal of Food Science and Nutrition*, **2004**, 55(4), 325-331.

Bertini, I., Gray, H. B., Lippard, S. J. and Valentine, J. S. *Bioinorganic Chemistry*, Viva Books, New Delhi, **1998**.

Biziuk, M. and Kuczynska, J. Mineral Components in Foods, Szefer, P.; Nriagu, J. O. (ed). (Chemical and Functional Properties of Food Components Sereies), CRC Press, Boca Raton, **2006**.

Bloem,E.; Haneklaus, H. and Schnug, E. Influence of Nitrogen and Sulfur Fertilization on the Alliin Content of Onions and Garlic, *Journal of Plant Nutrition*, **2004**, 27(10), 1827–1839.

Boulos, L. Medicinal Plants of North Africa, USA, Reference Publications, **1983**.

Central Statistical Agency (CSA). The Federal Democratic Republic of Ethiopia,. Agricultural Sample Survey: Area and Production Crops for 2007/2008. *Statistical Bulletin* Vol. I (417), **2008**.

Chamley, H. Fertlizers and Pesticides. *Geosciences, Environment and Management,* Elsevier Science, Netherlands, **2003**.

Deckelbaum, R.J., Palm, C., Mutno, P., Declerck, F. Econutrition: Implementation Models from the Millenium Villages Project in Africa, *Food and Nutrition Bulletin*, **2006**, 27(4), 335-342.

Dewick, P.M. *Medicinal Natural Products: A Biosynthetic Approach*, Wiley and Sons, Chichester, **2001**.

Dima, G., Popescu, I. V., Stihi, C., Oros, C., Dinu, S., Manea, L., and Vlacicu, G. Fe, Mn, and Zn Concentrations determinations from Iolomita River by Atomic Absorption Spectroscopy, *Romanian Journal of Physics*, 51 (5-6), 667-674, **2006**.

Dosumu, O. O., Salami, N. and Adekola, F. A. Comparative study of trace levels in some local vegetable varieties and irrigation water from different parts locations in Ilorin, Nigeria, *Bulletin of Chemical Society of Ethiopia*, **2003**, 17(1), 107-112.

Ejaz, S.; Woong, L.C.; Ejaz, A. Eztract of Garlic (Allium Sativum) in Cancer Chemoprevention, Experimental Oncology, **2003**, 93-97.

Elmaci, A., Teksoy, A., Topaç, F.M., Ozengin, N., Kurtolu, S. and Savaba_kaya, H. Assessment of heavy metals in Lake Uluabat, Turkey, *African Journal of Biotechnology*, **2007**, 6 (19), 2236-2244.

Ensminger, M.E., Ensminger, A.H., Kolande, J.E. and Robson, J.R. Onion, *The Concise Encyclopedia of Food and Nutrition,* CRC Press, London, **1995**.

Ewing, G.W. Atomic Spectroscopy, *Instrumental Methods of Chemical Analysis* (5th ed), McGraw-Hill, New York, **1985**.

Food and Agricultural Organization (FAO) of United Nation, Irrigation and Drainage Paper; Water Quality for Agriculture, Ayers, R.S and Westcot, D.W, Rome, 1985

Gedamu, F. The Effect of Alternative Sources of Organic Fertlizers in Increasing the Yield of Onions (*Allium Cepa L*), *Master of Science Thesis*, Addis Ababa University, Ethiopia, **1982**.

Gyurova, S, Atanassova, M. and Tzenkova,, R. Dietary Fiber Content of Thirteen Bulgarian Vegetables. *Journal of the University of Chemical Technology and Metallurgy*, **2007**, 42(1), 117-118.

Guta, S. Levels of Essential Nutrients and Toxic Metal in Fruits of Awara Melka and Nura Era Farms, Ethiopia, *Master of Science Thesis unpublished*, Addis Ababa University, Ethiopia, **2005**.

Han, J.; Jin, F. and Egashira, K. Environmental Impact Assessment of Vegetable Fields by the Heavy Metal Concentration in Yantai City Of Shandong Province, China, *Journal of Faculty of Agriculture*, **2007**, 52 (1), 129–134.

Hansen, S.L. Content of Free Amino Acids in Onion (*Allium Cepa* L.) as Influenced by the Stage of Development at Harvest and Long-term Storage, *Acta Agriculturae Scandinavica, Section B-Plant Soil Science*, **2001**, 51, 77-83.

Hashmi, D.R., Ismail, S. and Shaikh, G.H. Assessment of the Level of Trace Metals in Commonly Edible Vegetables Locally Available in the Markets of Karachi City, *Pakistan Journal of Bot*any, **2007**, 39(3), 747-751.

Hawkes, C.J., Ruel, M., Babu, S.J. From Harvst to Health: Challenges for Developing Biofortified Staple Foods and Determining their Impact on Micronutrient Status, *Food and Nutrition*, **2007**, 28(2).

Heber, D. Vegetables, Fruits and Phytoestrogens in the Prevention Vegetables, Fruits and Phytoestrogens in the Prevention Vegetables, Fruits and Phytoestrogens in the Prevention of Diseases, *Journal of Postgraduate Medicine*, **2004**;50:145-149.

Horsfall, M., Horsfall, M.N. and Spiff, A.I. Speciation of Heavy Metals in Inter-tidal Sediments of the Okrika River System, River State, Nigeria, *Bulletin of Chemical Society of Ethiopia*, **1999**, 13(1), 1-9.

Itanna, F., Brever, J. and Olsson, M. The Fate And Bioavailability of Trace Elements Applied to Two Vegetable Farms in Addis Ababa, *African Journal of Agricultural Research*, **2008**, 3(11), 797-807.

Itanna, F. Metals in Leafy Vegetables Grown in Addis Ababa and Toxicological Implications, *Ethiopian Journal of Health Development*, 2**002**, 16(3), 295-302.

Itanna, F. Meatal concentrations of some vegetables irrigated with industrial liwuid waste at Akaki, Ethiopia, *SINET: Ethiopian Journal of Science*, **1998**, 21(1), 133-144.

Iyaka, Y.A. Concentration of Cu and Zn in Some Fruits and Vegetables Commonly Available in North-central Zone of Nigeria, *Electronic Journal of Environmental Agricultural Food Chemistry*, **2007**, 6(6), 2150-2154.

Jatau, B.S., Bajeh, I. and Innocent, S. Trace Metals in Surface Water in Kaduna South Industrial Area North-Central; Nigeria, *Research Journal of Applied Sciences*, **2008**, 3(1), 81-87.

Jung, M. C. Heavy Metal Concentrations in Soils and Factors Affecting Metal Uptake by Plants in the Vicinity of a Korean Cu-W Mine, *Sensors,* **2008***, 8*, 2413-2423.

Kanakaraju, D., Mazura, N. S. and Khairulanwar, A. Relationship between Metals in Vegetables with Soils in Farmlands of Kuching, Sarawak, *Malaysian Journal of Soil Science,* **2007,** 11, 57-69.

Kebede, A. and Wondimu, T. Distribution of Trace Elements in muscle and organs of Tilapia, Oreochromis Niloticus, from Lakes Awassa and Ziway, Ethiopia, *Bulletin of Chemical society of Ethiopia*, **2004**, 18(2), 119-130.

Khan, S.A.; Ahmad, I.; Mohajir, M.S. Evaluation of Mineral Contents of Some Edible Medicinal Plants, *Pakistan. Journal of Pharmaceutical Science*, **2006**,19(2), 141-148.

Kocak, S.; Tokusoglu, O.; Aycan, S. Some Heavy Metals and Trace EssentialElements Detection in Canned Vegetable Foodstuffs by Differential Pulse Polarography, DPP, *Electronic Journal of Environmental Agricultural Food Chemistry*, **2005**, 4(2), 871-878.

Kumar, A.; and Pastore, P. Lead and Cadmium in Soft Toys. *Current Science*, **2007**, 93(6), 812-822.

Lippard, S. J. and Berg, M. *Bioinorganic Chemistry*, *Principles of Bioinorganic Chemistry*, University Science Books, California, **1994**.

Liu, D.; Kottke, I. Subcellular Localization of Cadmium in the Roots of *Allium Cepa* by Electron Energy Loss Spectroscopy and Cytochemistry, *Journal of Bioscience*, **2004**, 29(3), 329-335.

Lokeshwari, H; Chandrappa, G.T. Impact of Heavy Metal Contammination of Bellandur Lake on Soil and Cultivated Vegetation, *Current Science*, **2006,** 91(5), 622-627.

MAFF, Welch Office Agriculture Department Code of good agricultural practice for the protection of soil. Draft Consultation Document, MAFF London (1992), in Abdullahi, M.S.; Uzairu, A.; Okunola, O.J *African Journal of Biotechnology*, **2008**, 7(10), 1526-1529.

Malak, A. E and Al-Ashkar, E.A.The Effect of Different Fertilizers on the Heavy Metals in Soil and Tomato Plant, *Australian Journal of Basic and Applied Sciences*, **2007**, 1(3), 300-306.

Mann, S. Definition. *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry*, Oxford, **2001**.

Margen, S. Onions, *The Wellness Encyclopedia of Food and Nutrition*, New York, REBUS, **1992**.

Martin, D., Vollenweider, P., Buttler, A. and Gunthardt-Goerg, M.S. Bioindication of Heavy Metal Contamination in Vegetable Gardens. *Forest, Snow and Landscape Res*earch **2006**, 80 (169:2), 169–180.

Malesev, D. and Kunti, V. Investigation of Metal–Flavonoid Chelates and the Determination of Flavonoids via Metal–Flavonoid Complexing Reactions, *Serbian Chemical Society*, **2007**, 72(10) 921–939.

Melaku, S., Taddese Wondimu, T., Dams, R. and Moens, L. Simultaneous Determination of Trace Elements in Tinishu Akaki River Water Sample, Ethiopia, by ICP-MS, *Canadian Journal of Analytical Sciences and Spectroscopy*, **2004**,49(6), 374-384.

Mendez, C.V.; Rodri-Guez, E. M.; Romero, C.D.; Gonza Lez2, M.G.; Isasa, M. T. Comparison of the Mineral and Trace Element Concentrations Between 'Gazpacho' and the Vegetables Used in its Elaboration, *International Journal of Food Sciences and Nutrition*,**2007** 1(1).

Miller, J.N. and Miller, J.C. *Statistics and Chemometrics for Analytical Chemistry* (5th ed), Pearson, Harlow, England, **2005**.

Mitra, S. *Sample Preparation Techniques in Analytical Chemistry: Chemical Analysis,* Vol. 162, Wiley and Sons, USA, **2003**

Mocko, A. and Wacławek, W. Three-Step Extraction Procedure for Determination of Heavy Metals Availability to Vegetables, *Analytical and Bioanalytical Chemistry*, **2004**, 380, 813–817.

Mor, F. and Ceylan, S. Cadmium and Lead Contamination in Vegetables Collected From Industrial, Traffic and Rural Areas in Bursa Province, Turkey, *Food Additives and Contaminants*, **2008**, 25(5), 611–615.

Nabrzyski, M. Mineral Components in Foods, Szefer, P.; Nriagu, J. O. (ed). (Chemical and Functional Properties of Food Components Sereies), CRC Press, Boca Raton, **2006**.

Nassef, M., Hannigan,R., Sayed, K.A. and Tahawy, M.S. Determination Of Some Heavy Metals In The Environment Of Sadat Industrial City, *Proceedings of the 2nd Environmental Physics Conference,* Alexandria, Egypt, 18-22 Feb**. 2006**.

Nemeth, K. and Piskula, M.K. Food Content, Processing, Absorption and Metabolism of Onion Flavonoids, *Critical Reviews in Food Science and Nutrition.* **2007**, 47, 397-409.

Nicholas, D.J.D. and Egan, A.R. Trace Elements in Soil-Plant-Animal Systems, Academic Press, USA, **1975**.

Nigussie, K. Correlation among Trace Metals in Tilapia (Oreochromis niloticus), Sediment and Water Samples of Lake Awassa and Ziway, *Master of Science Thesis unpublished*, Addis Ababa University, Ethiopia, **2004**.

Nnorom, I.C., Osibanjo, O. and Ogugua, K. Trace Heavy Metal Levels of Some Bouillon Cubes, and Food Condiments Readily Consumed in Nigeria, *Pakistan Journal of Nutrition* **2007**, 6 (2), 122-127.

Okunola, O. J., Uzairu, A. and Ndukwe, G. Levels of Trace Metals in Soil and Vegetation along Major and Minor Roads in Metropolitan City Of Kaduna, Nigeria, *African Journal of Biotechnology*, **2007**, 6(14), 1703-1709.

Olajire, A.A., and Ayodele, E.T. Study of Atmospheric Pollution Levels by Trace Elements Analysis of Tree Bark and Leaves, *Bulletin of Chemical Society of Ethiopia*, **2003**, 17(1), 11-17.

Oliveira, E. Sample Preparation for Atomic Absorption Spectroscopy: Evolution and Future Trends, *Journal of Brazilian Chemical Society*, 2**003**, 14(2), 174-182.

Perk, M. Soil and Water Contamination: from molecular to catchment scale, Taylor and Francis, London, **2006**.

Plaster, E. J. Soil Science and Management, (4^{th} ed.), Thomson, Australia, **2003**.

Rafique, E., Mahmood-ul-Hassen, M., Khokhar, K.M., Nabi, G. and Tabassam, T. Zinc Nutrition of Onion: Proposed Diagnostic Criteria, *Journal of Plant Nutrition,* **2008**, 31, 307-316.

Rashad, M. and Shalaby, E. A. Dispersion and Deposition of Heavy Metals Around Two Municipal Solid Waste (MSW) Dumpsites, Alexandria, Egypt, American-Eurasian, *Journal of Agricultural and Environmental Science*, **2007**, 2(3), 204-212.

Reilly, C. Mineral Components in Foods, Szefer, P.; Nriagu, J. O. (ed). (Chemical and Functional Properties of Food Components Sereies), CRC Press, Boca Raton, **2006**.Rouessac, F. and Rouessac, A. (Eds.), *Chemical Analysis: Modern Instrumental Methods and Techniques*, Wiley, Chichester, **2000**.

Sarica, D. Y., Akim, D. and Ozden, T. Determination of Zinc in Aerosol Samples by Discrete Nebulization Flame Atomic Absorption Spectrometry, *Turkish Journal of Chemistry*, **2002**, 26 , 263 -270.

Saxena, R., Venkaiah, K., Anitha, P., Venu, L. and Raghunath, M. Antioxidant Activity of Commonly Consumed Plant Foods of India: Contribution of their Phenolic Content, I*nternational Journal of Food Sciences and Nutrition*, **2007**, 58(4), 250-260.

Sendek, F., Tefera, H. and W/Tsadik, K. Genetic Variability studies in Ethiopian shallot (*Allium cepa* L. var. ascalonicum Backer) Genotype, *East African Journal of Sciences*, **2008**, 2(2), 130-134.

Singh, A.K. Approaches for Removal of Arsenic from Groundwater of Northeastern India, *Current Science*, **2007**, 92(11), 1506-1525.

Soylak,M., Divrikli, U., Saracoglu, S. and Elci, L. Monitoring Trace Metal Levels in Yozgat-Turkey: Copper, Iron, Nickel, Cobalt, Lead, Cadmium, Manganese and Chromium Levels in Stream Sediments, *Polish Journal of Environmental Studies,* **2002,** 11(1), 47-51.

Srinivasan, K. Role of Spices beyond Food Flavoring: Nutraceuticals with Multiple Health Effects, *Food Reviews International*, **2005**, 21, 167–188.

Taiga, A., Suleiman, M.N., Aina, D. O., Sule, W. F. and Alege, G. O. Proximate analysis of some dry season vegetables in Anyigba, Kogi State, Nigeria, *African Journal of Biotechnology*, **2008**, 7 (10),1588-1590.

Tawfik, K.M. A Monitory Field Study at El Saaf - Helwan Faba bean Farms Irrigated by Industrial Waste Water and Polluted Water with Sewage, *Journal of Applied Sciences Research*, **2008**, 4(5), 492-499.

Tokalioglu, S., Kartal, S. and Gultekin, A. Investigation of Heavy metal Uptake by Vegetables Growing in Contaminated Soils Using the Modified BCR Sequential Extraction Method, *International Journal of Environmental Analytical Chem*istry, **2006,** 86(6), 417-4130.

Tomar, M. Qualty Assessment of Water and Wastewater, Lewis publisher, London, **1999**.

Unyayar, S., Keleş, Y. and Cekic, F.O. The Antioxidative Response of Two Tomato Species with Different Drought Tolerances as A Result of Drought and Cadmium Stress Combinations, *Plant Soil and Environment*, **2005**, *51*(2), 57–64.

U.S. Environmental Protection Agency (U.S. EPA), Determination of Metals in Ambient Particulate Matter Using Atomic Absorption Spectroscopy, Compendium Method Io-3.2, *Center for Environmental Research information*, Cincinnati, June **1999**.

Velasco-Reynold, C., Navarro-Alarco N, M., Serrana, H.L., Perez-Valero, V. and Lopez-Martinez, M. Determination of Daily Dietary Intake of Chromium by Duplicate Diet Sampling: in Vitro Availability Study, *Food Additives and Contaminants*, **2008**, 25(5), 604–610.

WHO (World Health Organization) Monographs on Selected Medicinal Plants, vol. 1, *World Health Organization*, Geneva **1999**.

Wikepidia, the free encyclopedia, Allium . http://en.wikipedia.org/wiki/allium.

(Accessed on Aug. 12, **2008**).

Wikepidia, the free encyclopedia, Onion. http://en.wikipedia.org/wiki/onion.

(Accessed on Aug. 12, **2008**).

Worthington, V. Nutritional Quality of Organic Versus Conventional Fruits, Vegetables, and Grains, *The Journal of Alternative and Complementary Medicine*, **2001**, 7(2), 161–173.

Zhou, Z., Fan, Y. and Wang. M. Heavy metal Contamination in Vegetables and their Control in China, *Food Review International*, **2000**, 16(2), 239-255.

DECLARATION

I the Undersigned, confirm that the results reported in this work were obtained by the research carried out by me under the supervision of my Advisor in the Faculty of Science, Department of Chemistry, Addis Ababa University in the academic year 2008-2009 G.C. All the sources of materials used for this study have been duly acknowledged.

Name _____ Signature _____ Date_____

This project has been submitted to the examination with my approval as the university advisor.

Advisor: Prof. B.S. Chandravanshi

Signature: _____

Date: _____

Place and date of submission: School of Graduate Studies

Addis Ababa University

July 2009.