2019-12-04

DETERMINATION OF THE LEVELS OF SELECTED ESSENTIAL AND NONESSENTIAL METALS IN KETAR RIVER WATER AND THE ONION (Allium Cepa L.), IRRIGATED SITE þÿ W I T H I N   K E T A R   Q O T E   B U L A KEBELE (GOLJA) IN THE CASE OF TIYO WOREDA USING ICP-OES

MUZEYEN, ADEM

BAHIR DAR UNIVERSITY

OFFICE OF GRADUATE STUDIES

COLLEGE OF SCIENCE

DEPARTMENT OF CHEMISTRY

DETERMINATION OF THE LEVELS OF SELECTED ESSENTIAL AND NON-ESSENTIAL METALS IN KETAR RIVER WATER AND THE ONION (Allium Cepa L.), IRRIGATED SITE WITHIN ‘’KETAR QOTE BULA’’ KEBELE (GOLJA) IN THE CASE OF TIYO WOREDA USING ICP-OES

BY:-MUZEYEN ADEM

September, 2019

BAHIR DAR, ETHIOPIA

BAHIR DAR UNIVERSITY

OFFICE OF GRADUATE STUDIES

COLLEGE OF SCIENCE

DEPARTMENT OF CHEMISTRY

DETERMINATION OF THE LEVELS OF SELECTED ESSENTIAL AND NON-ESSENTIAL METALS IN KETAR RIVER WATER AND THE ONION (Allium Cepa L.), IRRIGATED SITE WITHIN ''KETAR QOTE BULA'' KEBELE (GOLJA) IN THE CASE OF TIYO WOREDA USING ICP-OES

A THESIS SUBMITTED TO COLLEGE OF SCIENCE,

DEPARTMENT OF CHEMISTRY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

BY

MUZEYEN ADEM

ADVISOR: ***TESFAYE SHIFERAW (PhD)***

September 2019

BAHIR DAR UNIVERSITY

Approval Sheet

The thesis entitled "Determination of the levels of selected essential and non-essential metals in Ketar river water and the onion (Allium cepa L.), irrigated site within ''Ketar Qote bula'' Kebele (GOLJA) in the case of Tiyo Woreda using ICP-OES" by Ms. Muzeyen Adem is approved for the degree of Master of Science in Chemistry.

Board of Examiners	Signature	Date
Name of adviser: Tesfaye Shiferaw (PhD)	______________	______________
External examiner: __________________	________________	________________
Internal examiner: _____________________	______________	________________
Chairman: __________________	________________	________________

Place and date: Bahir Dar University, Bahir Dar

September, 2019

Declaration

I hereby declare that, this thesis entitled "Determination of the levels of selected essential and non-essential metals in Ketar river water and the Onion (Allium Cepa L.), irrigated site within ''Ketar Qote bula'' Kebele (Golja) in the case of Tiyo woreda using ICP-OES" and the work presented in it are my original work and has not been presented for a degree in any other university and that all sources have been appropriately acknowledged.

Student's Name: Muzeyen Adem

Signature: ______________________________

Date: ______________________________

Name of adviser: Tesfaye Shiferaw (PhD)

Signature: ______________________________

Date: ______________________________

ACKNOWLEDGEMENTS

Firstly, I would like to express my gratitude to my thesis advisor, Tesfaye Shiferaw (PhD) for his deep suggestion and guidance throughout this work from the beginning to the final editing and correcting of the work. I am also grateful to Department of Chemistry, Bahir Dar University, Ethiopia, for providing the laboratory facilities and sponsoring this study.

Secondly, I would like to thank my families; my mother w/ro Meko Mamo, my brother Muktar Adem, my sister Fetuma Adem and all others at Arsi, Digelu and Tijo woreda for their moral and financial support, special appreciation and credit goes for their tolerance and support for my study. My deep felt thanks also goes to Ato Dawud Abdurehman for his support during preparation of equipment, the sample collection and his being pivotal for my planning and execution of the work.

Finally I would like to thank, Ato Seyida Kaweti (Munessa woreda administration) and I also would like to thank my friends who were encouraging me with strong moral during my work.

Abstract

The concentration of essential elements K, Ca and Mg and non-essential elements Cr, Cd and Pb were investigated in onion bulb and irrigation water used for cultivating onion at Ketar Kotebula Kebele (Golja) irrigation site in Tiyo Woreda, Arsi Zone, Oromia region, Ethiopia. A composite sample of irrigation water were taken from the two farm sites during 28-29/01/2019 and 23-24, and while a composites of Onion bulb sample were taken from two plots of each two vegetable farm sites during 23-24, May 2019. Dissolution of onion samples was carried out by wet digestion with a total volume of 2mL HNO_3 (65%) and 2 mL $HClO_4$ (70%) mixture on hot plate apparatus. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to determine the levels of essential and non-essential heavy metals. The method validated by performing parameters such as instrumental calibration, spiking sample, method detection limit and precision of analytical procedures. The levels of major metals were K (20.05 ± 2.19 mg/L), Ca (12.84 ± 0.0216 mg/L) and Mg (3.71 ± 0.104 mg/L), where the levels of non-essential metals were Cr (0.01±0.001 mg/L), Pb (0.0036 ± 0.004 mg/L) and Cd (not detected) in irrigation water from Ketar River. The levels of major metals were K (4726 ± 0.025 mg/Kg), Ca (1730.56 ± 0.98 mg/Kg) and Mg (899.09 ± 0.99 mg/Kg), where the level of non-essential metals were Cr (1.367 ± 0.009 mg/Kg), Pb (4.1 ± 0.015 mg/Kg) and Cd (not detected) in onion irrigated with water from Ketar River. The results showed that Cr and Pb concentrations exceeded the safety limits given by Food and Agriculture Organization (FAO) or the World Health Organization (WHO) for human consumption with the exception of cadmium that was lower than the permissible leveling in all of the samples. It is recommended that, appropriate measures should be taken by population around the area and the government to minimize excess uses of agrochemicals and anthropogenic activities.

Keywords: heavy metals, essential metals, irrigation, concentration

Table of contents

CONTENTS **PAGE**

Lists of figures

Lists of table

LISTS OF ACRONYMS AND ABBREVIATIONS

EPA	Environmental Protection Agency
EU	European Union
FAO	Food and Agricultural Organization
ICP-OES	Inductively coupled optical emission spectroscopy
KQK	Ketar Qote bula Kebele
ND	Not Detected
RSD%	Percentage Relative Standard Deviations
SD	Standard Deviations
UAE	United Arab Emirates
USDA	United States Department of Agriculture
WHO	World Health Organization

1. INTRODUCTION

1.1 Background of the study

Onion (Allium cepa L.) is vegetable species consumed worldwide in large quantities. All the plant parts are edible, but the bulbs and the lower steam sections are the most popular as seasonings or as vegetables in stews (MoARD 2009). Common Onion exists as bulb and shallot shapes where it is also available in three varieties: yellow/brown, red and white. In Ethiopia**,** Onion is the top most important vegetable crop produced almost in all parts of the country by smallholder farmers due to its requirements in the daily diet of peoples and as a source of livelihood of most people's (Berhanu *et al.*, 2014). Not only does the vegetable lend an excellent taste to dishes, but also is associated with imparting a number of health benefits to users. As numerous health benefits have been attributed to onion, it has been used traditionally in Ethiopia as well as in different parts of the world as medicine. China, India and USA are world's leading onion producing countries. Fresh onion contains about 86.6% moisture, 11.6% carbohydrate including 6-9 soluble sugar, 1.2% protein, 0.1% fat, 0.2 - 0.5% Ca, 0.05% P, traces of Al, Cu, Fe, Mn, Zn, pantothenic acid and vitamins (A, B, and C) (El-Tantawy EM et al 2009, Malik MN 2000). It is one of the richest sources of flavonoid in the human diet and flavonoid consumption has been associated with a reduced risk of cancer, heart disease and diabetes. Onion is usually used as ingredient of savory dish, chutneys and or eaten raw. Onion is used as food, flavor and medicines. This vegetable food is full of chemicals that can help protect a person from a variety of infections, diseases and illnesses such as cardiovascular disease, respiratory concerns, diabetes, and a variety of cancers (Johnson et al., 2006). Onion has high flavonoid component (mainly quercetin and its conjugates) and sulphur compounds which possesses antioxidant capacity. Flavonoids which are a large group of phenolic plant constituents that have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals, which are involved in DNA damage and tumor promotion (Miean and Mohamed, 2001). Alliums are known to exhibit antibacterial and antifungal properties and contain powerful antioxidants, sulphur and other numerous phenolic compounds.

In addition, it is known for anti-bacterial, antiviral, anti-allergenic and anti-inflammatory potential and used as preservative and medicinal plant (MoARD, 2009, Vohra SB, et al 1994).

Scientific research studies well support the fact that onions are worthy of being eaten every single day in order to provide optimum health benefits. Although pungent and giving off a sulphurous odour, these are not foods that should be easily brushed aside (Johnson et al., 2006). Most of the heavy metals exist naturally at low concentrations in soils, rocks, water, and biota, sufficient to provide living systems with essential nutrients but at levels too low to cause toxicity. The most common environmental pollutants in the world are heavy metals. Irrigation with sewage water (water contaminated by heavy metals sources from fertilizers, pesticides fungicides and so on.) increases the concentrations of heavy metals in plant and soil (Brar, M.S et al., 2000). Thus excessive use of agrochemicals (pesticides, herbicides, fertilizers and fungicides) are the great cause of contamination of soil, water and plants. Mostly this contamination is due to the presence of toxic substances like heavy metals (a group of chemical elements in elemental form as conjugate) like Ag, As, Cd, Cu, Cr, Hg, Ni, Pb, Zn and etc. Once these heavy metals have been released into the environment, they cannot be easily degraded and will be accumulated in the environment persistently, including the food chain. Exposure to heavy metals through uptake of drinking water or foods can lead to their accumulation in plants, animals and humans (Mulligan et al 2001). The chronic problems associated with long term heavy metals exposure include; serious hematological, brain damage, anemia and kidney malfunctioning (Sonayei et al., 2009). Heavy metals such as Pb and Cd are lethal even in very small doses. Lead has a negative influence on the somatic development, decreases the visual acuity and auditive thresholds (Simeonov et al., 2010). Acute exposure to lead causes brain damage, neurogical symptoms, brain damage and could lead to death (Simeonov et al., 2010). Cd exposure on the other hand, causes renal dysfunction, malignant, neoplasia and skin ulcers have been reported due to various occupations with exposure to chromium compounds. Chromium (VI) inhalation is responsible for bronchial asthma (Sakar, 2005).

Some of the heavy metals (Fe, Cu and Zn) are essential for plants and animals (Wintz H. et al., 2002) where their availability in medium varies, and metals such as Cu, Zn, Fe, Mn, Mo, Ni and Co are essential micronutrients (Reeves RD. et al., 2000). But if their uptake is in excess to the plant requirements they may result in toxic effects (Monni S. et al 2000). Some of the heavy metals act as micronutrients for the growth of animals and human beings when present in trace quantities, whereas others such as Cd, As, and Cr act as carcinogens (Feig et al., 1994; Trichopoulos, 1997). The contamination of vegetables with heavy metals due to soil and irrigation of wastewater contamination poses a threat to its quality and safety. Dietary intake of heavy metals also poses risk to animals and human health. High concentrations of heavy metals (Cu, Cd and Pb) in fruits and vegetables were related to high prevalence of upper gastrointestinal cancer (Turkdogan et al., 2002). This research is thus a study on the determination of the level of some essential metals (Ca, K, Mg) and heavy metals (Cr, Cd, Pd) in the onion samples collected from vegetables locally grown in Oromia Region, Tiyo woreda of the Arsi Zone, particularly in Golja irrigation site near Ketar river by inductively coupled plasma optical emission spectroscopy (ICP-OES).

1.2 Statement of the problem

Onion is the most cultivated vegetable in many countries and has great economic importance, for food, flavor and medicines. It is also the most consumed vegetable type worldwide and makes a significant contribution to the nutritional value in the human diet. Onion is rich in nutrients such as calcium, iron and vitamin B among others.

It is used for salads (bunching onion or sliced full-grown bulbs), pickling (e.g. silver skin onions), cooking (such as in soups) and frying (for example, with meat). Onion is particularly suited to small holder farming in most countries (USAID-KAVES, 2014). It is also mainly consumed for its unique flavor or for its ability to enhance the flavors of other foods and medicinal value. The implication associated with heavy metal contamination is of great concern, particularly in agricultural production system.

Heavy metals are not biodegradable, have long biological half-lives, toxic in nature and potential for accumulation in the different body organs leading to unwanted side effects (Gebregziabher B. et al, 2014). Dietary exposure to heavy metals, namely chromium (Cr), cadmium (Cd), lead (Pb), and others has been identified as a risk to human health through the consumption of vegetable crops (Fisseha I., 1998)**.**

Many studies have shown that waste water irrigation has elevated the levels of heavy metals in receiving soils (Singh *et al*., 2004; Sharma *et al*., 2006). Since all population around the river uses fertilizers, pesticides, herbicides and fungicides to increases their crop production, and hence these chemicals from the surrounding areas leached or drained into water and it might be the source of heavy metals that pollute water of Ketar River and the soil. It was also assumed that it is the source of heavy metals for the vegetable onion cultivated by the irrigation of this river water in Golja irrigation site. This vegetable is grown in large amount in these areas but there is no research done and reported on the determination of toxic metals in onion grown in the sampling area. Thus determination of the level of toxic metals (Cr, Cd, and Pb) content of these vegetables grown in Tiyo woreda Golja irrigation areas is very important to understand the relationship of dietary intake and human health. The result obtained from this study will be used to indicate the toxicity level of heavy metals in both river water and onion bulb samples and provide the reference for future study. Therefore, a scientific study was needed to determine toxicity level of heavy metals in onion that caused by waste soil and irrigation with waste water, due to the excessive use of agro-chemicals (fertilizer, pesticides, herbicides and fungicides) by local population affects health of consuming people. Agronomy management practice for onion on the field has high significant effect on onion nutritional quality parameters (Kebeda et al., 2017; Variety in Ethiopia). By the excess use of these chemicals, plants can accumulate these metals in their tissues in concentration above the standard levels which poses a risk to human and animal's life. The contamination of vegetables with heavy metals due to soil and irrigation of waste water contamination poses a threat to its quality and safety. Dietary intake of heavy metals also poses risk to animals and human health (Turkdogan et al., 2002).

The concern of this research work was thus the determination of the level of some essential (K, Ca, Mg) and heavy metals (Cr, Cd, Pd) in the river water used for the irrigation and onion bulb samples collected from vegetables locally grown in Oromia Region, Tiyo woreda of the Arsi Zone, particularly in Golja irrigation site near Ketar river by ICP-OES.

The problem that the research address was to see the effect of excessive use of the chemicals used to increase crop productions, on the concentration level of toxic metals in Onion bulb.

This study is targeted to determine:

1. Irrigation water chemical make up
2. Content of essential metals in Onion
3. Content of toxic metals in Onion

1.3 Objectives of the study.

1.3.1 General Objective

The main objective of this study is to determine some essential elements (K, Ca and Mg) and level of toxic metals (Cr, Cd & Pb) in Ketar river water used for irrigation and Onion grown near the river in Ketar Kotebula Kebele (Golja) site of irrigation using Inductively Coupled Optical Emission Spectroscopy (ICP-OES) technique.

1.3.2 Specific Objectives

The specific objectives of this study are:

- To determine essential (K, Ca, Mg) and toxic metals (Cr, Cd and Pb) in irrigation water sample taken from Keter River.
- To determine essential (K, Ca, Mg) toxic metals (Cr, Cd & Pb) in onion bulb sample collected from Golja irrigation site by ICP-OES.
- To compare the results with respective literature values.

2. LITERATURE REVIEW

2.1. Onion

Onion (*Allium cepa* L.) is a vegetable species known as the bulb onion or common onion that is the most widely cultivated species of the genus *Allium*. Its close relatives include the garlic, shallot, leek, chive, and Chinese onion (Block, E. 2010). Most of the diversity within A. *cepa* occurs within this group, the most economically important *Allium* crop, and they were form large single bulbs, and are grown from seed or seed-grown sets. The majority of cultivars grown for dry bulbs, salad onions, and pickling onions belong to this group (Rabinowitch, L. et al., 2002*)*. As the onion matures, food reserves begin to accumulate in the leaf bases and the bulb of the onion swells.

2.1.1 Onion production in the world and Ethiopia

Onion is the most cultivated biennial vegetable grown in temperate zones as an annual crop. In 2017, world production of dried onion was 97.9 million tones, led by China, India and USA producing 25%, 23% 3.77% of the total, respectively. It is the most consumed vegetable in Ethiopia produced by farmers and some commercial growers.
There are five common varieties of onion found in Ethiopia; which are; Adama red, Bombey red, Red creole, Melkam, and Nasik red (Dereselegn) to farmer (CSA, 2011). Oromia is the most important production region for onion (64%), followed by Amhara (30%) (CSA, 2008).

2.1.2 Characteristic of onion and its chemical contents

Onion has characteristic taste, a taste like onion and garlic. Although pungent and giving off a sulphurous odour, these are not foods that should be easily brushed aside (Johnson et al., 2006). Freshly cut onions often cause a stinging sensation in the eyes of people nearby, and often uncontrollable tears. This is caused by the release of a volatile liquid, *syn*-propanethial-S-oxide and its aerosol which stimulates nerves in the eye (Eric Block, 2010).

The contents of onion bulb described by USDA Nutrient Database were, the raw onion bulbs Nutritional value per 100 g (3.5 oz):- Energy 166 kJ (40 kcal), Carbohydrates (9.34 g), Sugars (4.24 g), Dietary fibre (1.7 g), Fat (0.1 g), Protein (1.1 g), Vitamins:-Thiamine (B1) (0.046 mg), Riboflavin (B2) (0.027 mg), Niacin (B3) (0.116 mg), Pantothenic acid (B5) (0.123 mg), Vitamin B6 (0.12 mg), Folate (B9) (19 μg), Vitamin C (7.4 mg), Minerals Quantity:-Calcium (23 mg), Iron 0.21 mg, Magnesium (10 mg), Manganese (0.129 mg), Phosphorus (29 mg), Potassium (146 mg), Zinc (0.17 mg) ,Other constituents (Quantity Water 89.11 g and Fluoride 1.1 μg). Most onion cultivars are about 89% water, 9% carbohydrates (including 4% sugar and 2% dietary fibre), 1% protein, and negligible fat. Onions contain low amounts of essential nutrients and have an energy value of 166 kJ (40 Calories) in a 100 g (3.5 oz) amount. Both constituents of essential and non-essential metals in onion are functional for human being if it exists in allowed level of concentration, where the deficiency and the excess of these non-essential metals affects the health of human being and hence excess of the heavy metals leads to toxicity.

2.1.3. Healthy benefits of onion

Vegetables are essential for human nutrition and health, particularly as source of vitamin C, folic acid, minerals, niacin, thiamine, pyridoxine and dietary fiber, their biochemical role and their anti-oxidative effects (Siegel et al. 2014). Onion is used for food, flavor and for medicines. Scientific research studies well support the fact that onion is worthy of being eaten every single day in order to provide optimum health benefits. Onion is used as a food; it is the source of various nutrients, vitamins and some essential and toxic metals which is used for different purposes. It constitutes important elements of human food chain because of high content of flavonoid, fructans, carbohydrate, sugar, protein, vitamins, macro and micro nutrients. They have high flavonoid component (mainly quercetin and its conjugates) and sulphur compounds which possesses antioxidant capacity. Onion is also used as flavors, since the vegetable lend does an excellent taste to dishes and is mainly used as pot herb. Onion contributes savoury flavour to dishes without contributing significant caloric content (US, NOA 2011).

Onion is consumed in the world as natural food for medicine since it is full of chemicals that can help protect a person from a variety of infections, diseases and illnesses such as cardiovascular disease, respiratory concerns, diabetes, and a variety of cancers. Alliums are known to exhibit antibacterial and antifungal properties and contain powerful antioxidants, sulphur and other numerous phenolic compounds. Both constituents of essential and non-essential metals in an onion are functional for human being if it exists in permissible level of concentration, were the deficiency and the excess of these non-essential metals affects the health of human being and hence excess of the heavy metals leads to toxicity. One of the most essential aspects of food quality assurance is heavy metal contamination of the food items (Wang et al. 2005).

2.2. The Essential Metals under study and their benefits

Essential metals are metals that are important for life of living things. These essential metal elements are Ca, K, Mg, Zn they have their own role in development and growth of plants. Metal accumulation in plants depends on the plant species, types of soil, environment and agricultural practice. In fact some of the trace element deficiencies in plants can cause nutrient deficiencies in the animals that graze those plants (Tuzen M. et al 2005). The following are some of the essential elements under the study and their roles

Potassium (K): Potassium is a very significant body mineral, important to both cellular and electrical functions. It is one of the main blood minerals electrolytes. It has been found that an average human body weighing 70 kg contains 0.25 kg of potassium were its deficiency result in various neurological dysfunction, influences vascular volume and led increase in blood pressure.

Calcium (Ca): In human body calcium has different roles, such as building and strengthening the bones and teeth, cell signaling, muscle contraction, blood clotting, transmitting of the nerve impulses and regulating heart's rhythm. 99% of calcium in a human body is stored in bones and teeth. The remaining one percent is found in the blood and cellular and extracellular fluid. The body gets calcium by pulling it from the bones when blood levels of calcium drop too low, usually when quite a long time passes since having taken calcium with meal.

Calcium can also be found in soybean,dark green leafy vegetables, spinach, dried beans and legumes, calcium fortified juice in addition to milk powder and dairy products are a convenient source of calcium for many people (Uždavinienė D. et al 2007)

Magnesium (Mg): Magnesium ions regulate over 300 biochemical reactions in the body. Magnesium produces energy in our body. It exists in the average of 0.042 kg in 70 Kg human body. Its deficiency results in a permanent state of muscles contraction in our body and we could not adjust the levels of cholesterol produced and released in to the blood stream.

2.3. Heavy metals

The term heavy metal refers to any elements with metallic properties having a density of 5 g/cc and an atomic number >20. These metals occur in soil, minerals, rock, plant tissue and fluid and animal tissue and fluid. These metals may be present in the food obtained from plant and animals as a result of contamination by various sources. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb, and Zn. The total metal content in soil is the result of parent materials, fertilizers, atmospheric deposition, agrochemicals, and organic wastes (Kanakaraju, D. et al 2007). They have positive and negative roles in human life. Some of heavy metals are considered essential including iron, zinc and copper, while some metal ions like cadmium, lead and Cr (IV) are non-essential metals which have toxic roles in biochemical reactions in our body (M.Soylak et al 2004). Of the nonessential metals, mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As) are recognized as health hazardous and all have caused major health problems as a result of environmental pollution (Duffus, 2002). Trace elements have great significance due to their tendency to accumulate in the vital human organs over prolong period of time. It is possible to have toxicities of trace elements, as well as deficiencies. Some plants may accumulate toxic metals at levels which may be harmless to the plant but could be harmful to humans if ingested.

2.3.1. The source of heavy metals in environment

Some human activity like waste discharge from industry, domestic, business and mining, fertilizers, using metal based pesticides, herbicides, fungicides are the source of heavy metal that polluted water, soil, environmental air. Hence the plant or vegetable produced in heavy metal contaminated soil and water also uptake the Heavy metals from the soil and water, which results in polluting the plant or vegetable food consumed.

The most important water pollution problems related to agriculture are: (i) excess nutrients accumulating in surface and coastal waters that cause eutrophication, hypoxia and algal blooms; (ii) accumulation of nitrates in groundwater; and (iii) Pesticides accumulated in groundwater and surface water bodies. Water pollution caused by nutrients (particularly nitrate) and pesticides has increased as intensive farming methods have proliferated, such as increased use of chemical fertilizers and higher concentrations of animals in smaller areas. The 1980s saw a progressive worsening of water quality owing to the growth of intensive livestock farming (chickens, pigs) in areas that were already saturated, and of intensive crop-growing involving the use of chemical weed killers and over fertilization. Developed countries have had major problems of water pollution from agriculture and trends indicate that intensified farming systems and agrochemical consumption are being extended in emerging economies.

Agriculture is by far the greatest water user in the world and consequently a major cause of water pollution. Agricultural pollution is commonly non-point source; however, agricultural operations sometimes include identifiable point source discharges, particularly for concentrated livestock operations. The main pollutants from agriculture are excess nutrients and pesticides. (Thematic Report - TR08, Javier, M., NRL, FAO)

Heavy metal contamination may occur due to factors including irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, harvesting process, storage and/or sale (Radwan and Salama 2006). Heavy metal contamination of the food items is one of the most important aspects of food quality assurance (Khan *et al*., 2008).

International and national regulations on food quality have lowered the maximum permissible levels of toxic metals in food items due to an increased awareness of the risk, these metals pose to food chain contamination (Radwan and Salama, 2006).

2.3.2 The source of heavy metals in onion

Anthropogenic activities are a major source of heavy metal contamination which includes agricultural crop residue, emission from industries and vehicular emissions. Generally, wastewater contains significant amounts of useful nutrients and heavy metals that create opportunities and problems in terms of agricultural production (Chen et al. 2005). Wastewater used for irrigation has many contaminants mainly heavy metals depending upon the source of discharge (Pedrero et al. 2010). Many studies across the globe have reported high content of heavy metals in vegetables cultivated with wastewater (Boamponsem et al. 2012; Flores-Magdaleno et al. 2011; Mathur et al. 2006). The consumption of vegetables contaminated with heavy metals may pose a risk to the health of humans. Heavy metals are deleterious due to their long biological half-lives, non-biodegradable nature, and their ability to accumulate in different body parts (Monu et al. 2008; Heidarieh et al. 2013).

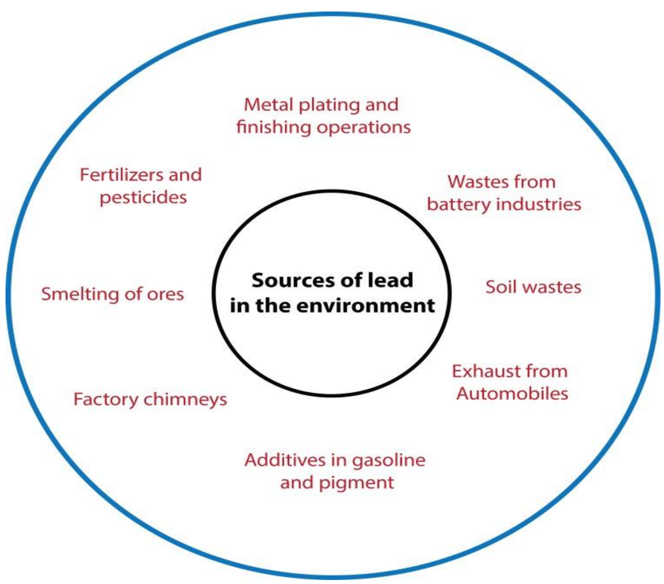


Figure 1: Sources of heavy metals (eg., Pb).

2.3.3. Heavy metals up-taken by onion

Vegetables also act as buffering agents for acidic substances obtained during the digestion process. However, these plants may contain both essential and toxic elements, such as heavy metals, at a wide range of concentrations (Bahemuka TE, at al. 1999). Metals, such as lead, chromium, cadmium and copper are cumulative poisons. These metals cause environmental hazards and are reported to be exceptionally toxic (Ellen G, et al. 1990). 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). Living organisms (plants, animals and microorganisms), store and transport metallic elements, as both to provide appropriate concentrations of them for later use in metallo-proteins or cofactors and to protect themselves against the toxic effects of metal excess (Reta, B.et al., 2009).

Contamination of vegetables with heavy metal may be due to irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, the harvesting process, storage or at the point of sale. It is well known that plants take up metals by absorbing them from contaminated soil as well as from deposits on parts of the plants exposed to the air from polluted environments (Khairiah, J. 2004, Chojnacka K, et al 2005). Heavy metals can be readily adsorbed by vegetable roots, and can be accumulated in the edible parts of vegetables at high levels, regardless of the heavy metal concentration in the soil (Jolly et al. 2013). 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).

2.3.4 The uses of heavy metals

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). 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)

2.3.5 The Effects of Toxic Metals in this Study

The accumulation of the heavy metals can decline the physical health and mental cognitive of the individual (Sandeep et al., 2012). Cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), and lead (Pb) are heavy metals which are harmful for human health. Cd has toxic effects on many organs and tissues, especially on kidneys, bones, and lungs (ATSDR, 2012a).

Cr may cause bad effects on gastrointestinal tract, such as abdominal pain, vomiting, peptic ulcer, hemorrhage and necrosis, and bloody diarrhea (ATSDR, 2012b). Accidental ingestion of large doses of Cu causes gastrointestinal bleeding, haematuria, and acute renal failure amongst other symptoms. The lower doses of Cu have similar effects, which caused headache, nausea, vomiting, and diarrhoea (Agarwal et al., 1993). Ni may cause gastrointestinal and cardiovascular disorder, liver damage, and carcinogenic effect (ATSDR, 2005). Pb nephrotoxicity is characterized by proximal tubular nephropathy, glomerular sclerosis and interstitial fibrosis (Loghman-Adham, 1997). The effects of some selected metals in this study are given below.

Chromium: The most common forms of chromium are chromium VI and chromium III. Cr (III) is an essential element required for normal sugar and fat metabolism. It is effective to the management of diabetes and it is a cofactor with insulin. Cr (III) and its compounds are not considered a health hazard (Hilgenkamp, 2006. Chromium III is an important component of a balanced human and animal diet and its deficiency causes disturbance to the glucose and lipid metabolism in humans while chromium VI is carcinogenic (Chernoff, 2005).

Among the health effects brought about by the exposure to chromium VI include lung cancer, malignant neoplasia, chromium dermatitis and skin ulcers (Sarkar, 2005).)The prevalence of chromium in drinking water above 5 mg/l results in bleeding of the gastrointestinal tract, cancer of the respiratory tract, ulcers of the skin and mucus membrane (Adeleken and Abegunde, 2011).

Cadmium (Cd): Cadmium is highly toxic non-essential heavy metal and it does not have a role in biological process in living organisms. The permissible limit 0.2 mg/kg by FAO/WHO, Cadmium is water soluble and can be transferred efficiently from soil to plants, which may affect human health if there is excessive intake from a contaminated food source. Thus, even in low concentration, cadmium could be harmful to living organisms (Ambedkar G.et al 2012). Cadmium poisoning in man could lead to anemia, renal damage, bone disorder and cancer of the lungs (Edward, JB. et al 2013). Cadmium and lead are among the most abundant heavy metals and are particularly toxic.

The excessive amount of these metals in food is associated with etiology of a number of diseases, especially with cardiovascular, kidney, nervous as well as bone diseases (Sanchez-Castillo et al 1998).

Lead (Pb): Lead is the most widely and evenly distributed trace metals absorbed by plants to varying degrees depending on the form of the metal present, and the pH and the temperature of the soil exists in many forms in the natural sources throughout the world. The lead contamination of soil and plants is caused by various sources such as the gases from various industries, Car and dust. Once the soil is contaminated by $Pb2+$ which is not biodegradable for long period of time was found to be acute toxic to human beings when present in high amounts. Its permissible limit in onion vegetable is 0.3 mg/kg by FAO/WHO. Lead toxicity is known to cause musculo-skeletal, renal, ocular, neurological, immunological, reproductive and developmental effects (Ambedkar G. Muniyan M 2012). Chronic exposure to Pb can affect physical growth and can cause anemia, kidney damage, and headache, hearing problems, speaking problems, fatigue or irritable mood (Simeonov *et al*., 2010). The toxicity of Pb is multiple biochemical effects. It has the ability to inactivate enzymes, compete with calcium for incorporation into bones and interfere with nerve transmission and brain development (Ediin *et al*., 2000). Lead is non-essential element for living organism and it is highly toxic to man and animal (Fifield and Haines, 1997). Lead toxicity influences brain, heart, kidney, liver, nervous system and pancreas. It may cause many signs and symptoms such as abdominal pain, anemia anorexia, anxiety, bone pain, brain damage, confusion, Fatigue, headaches and hypertension (Fifield, F.W. et al., 1997).

Many research studies Shows that the onion bulb and leaf grown by heavy metals contaminated irrigation and the soil contaminated by heavy metals and unsafe for consumption. Some of the results of the researches done on the determination of heavy metals in an onion grown by the contaminated water and soil are listed below. Determination of Heavy Metal Concentration in Cultivated Vegetables - A Case Study of Mysore District(Shobha M S1 and Shiva Kumar D*2 *1 2016)* and they obtained results declared that concentrations of major studied metals were exceeding than the recommended maximum acceptable levels proposed by the Joint FAO/WHO Expert Committee on Food Additives. Bedassa M., Abebaw A. and Desalegn T. study, onion bulb were analyzed for the concentration of heavy metals (Cu, Zn, Cr, Fe, Mn, Pb and Cd). Out of the tested metals Cr, Cd and Pb concentration were found to be above the permissible limit collected from Mojo, Meki and Ziway site in onion bulb; which indicates that consumption of onion from this area could be a health risk set by FAO/WHO. Yebirzaf Yeshiwas, 2017 study on the review on heavy metal contamination in vegetables grown in Ethiopia and its economic welfare implications. The result obtained is vegetable grown in the vicinity of industrial areas and contaminated irrigation water in Ethiopia indicated that vegetables grown in such lands, contaminated with heavy metals and unsafe for consumption. Dietary exposure to heavy metals, namely chromium (Cr), cadmium (Cd), lead (Pb), and other has been identified as a risk to human health through the consumption of vegetable crops (Fisseha I. 1998).

3 MATERIALS AND METHODS

3.1 Apparatus and Instrumentation

Chopping board and Teflon knife were used to cut onion bulb sample. Analytical balance (Nimbus, ADAM), was used to weigh the onion bulb sample. Oven was used to dry the onion bulb. Mortar and pestle were used to fine powder the oven dried onion bulb samples. Sieve 50 mm pores was used to sieve the powdered onion bulb samples. Beakers were used for digesting irrigation water and onion bulb sample on hotplate. Borosilicate volumetric flasks (50 ml and 100 ml) were used during dilution of sample and preparation of metal standard solutions. Measuring cylinders, pipettes, and micropipettes (100 -1000 μL) were used during measuring different quantities of volumes of sample solution, acid reagents and metal standard solutions. A metal concentration determination was done by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Prkin Elmer Optima 8000).

3.2 Chemicals and Reagents

All the reagents and chemicals used in this study are analytical grade. Nitric acid (65 %) (N43725-4K, UNI-CHEM) and 70% $HClO_4$ both purchased from Research-lab Fine Chemical Industries, Addis Ababa, Ethiopia.

Stock standard solution of concentration 1000 mg/L in 2% HNO_3 of the metals Ca, Mg, K, Cr, Cd and Pb were used to prepare intermediate standard solutions. Distilled water was used for dilution of samples, intermediate metal standard solutions, and for rinsing glassware and bottles.

3.3 Description of the Study Area

The study was carried out in Arsi Zone of Oromia region in Tiyo Woreda at Ketar Kotebula Kebele (*Local name, Golja irrigation site or Ketar Genet***)**. This study area is located 210 Km south east of Addis Ababa at 6° 59' to 8° 49' N latitudes and 38° 41' to 40° 44' E longitudes. The altitude of the area ranges from 2500 to 3000 ml.

Tiyo district characterized by mid subtropical weather, with minimum and maximum temperature ranging from 8.4 to 22.6°C and the relative humidity ranging from 43 to 60%. The average rainfall is 2000 mm (Girma Q.et al., 2017).

The area was selected for the study for four main reasons: (i) The areas are under continuous cultivation throughout the year and have been supplying large production of a wide variety of vegetables like onion, cabbage, green pepper, potatoes, tomatoes etc to the markets and for local consumption since long time; (ii) The area uses agrochemicals, fertilizers, pesticides and herbicides to increase the yield which enhance salt and minerals deposit in the area. The area was also closest to Lole government farm and selected seed production which uses excess agrochemicals and fertilizers. (iii) The Ketar River water used for irrigating the Ketar Kotebula Kebele (KQK) irrigation site has many small tributaries that were polluted by domestic and agricultural wastes. (iv)The area uses frequent irrigation for cultivation of various vegetables since long time, while frequent irrigation after evaporation causes salt and mineral deposits.

3.4 Sample collection, preparation and pretreatment

Figure 2: Sample collection at the sampling site

3.4.1. Irrigation water sampling, preparation and pretreatment

The irrigation water chemical makeup affects the onion metal levels. The Ketar river water used for irrigating vegetables at KQK irrigation site was collected to determine the level of the concentration of some selected essential and toxic metals using ICP-OES.

The irrigation water samples were collected from two sites; ”Ketar Qote bula” and ”Hamsa gasha” in two rounds, where selection of the area was based on the irrigation farm sites. The first round was during establishment of the onion plants (December-January) and the second round was during harvesting of onion bulbs where in each round, the irrigation water samples were collected in two successive days. In the first round, the prepared, pre cleaned labeled two polyethylene bottles (500 ml) were used to collect the irrigation water samples while about 500 mL homogenized irrigation water samples were collected from the two farm sites in two successive days (28-29/01/2019). The collected samples were immediately transported to lab and preserved by adding 2 mL HNO_3 and stored in refrigerator at about 4 ^oC without freezing.

In the second round, about 500 mL irrigation water samples were collected from the two farm sites in two successive days during harvesting of the onion bulb (23-24/05/2019).

While irrigation water was collected, the prepared bottled were rinsed three times by water sample before the final sample filled to container. Then the bottles were dipped below the surface and filled with water sample up to 500 ml with the measured pH value of 6.5 and temperature of 19.3^OC. The polyethylene plastic bag that contains two bottles with sufficient headspace placed in plastic bag (transportation bag) and the other plastic bags filled with ice water were also placed on the samples plastic bag inside transportation bag to maintain at about 4 ^0C or less. To avoid potential of melting ice water seeping into the sample bottles in the use of plastic bag to contain the samples.

3.4.2 Onion bulb sampling, preparation and pretreatment

The Onion from KQK (Golja) irrigation site was collected to determine the level of the concentration of some selected essential and toxic metals by ICP-OES. Recently matured eight onion bulb samples were graped from four farmer’s plots of two farm sites, which appeared healthy were freshly collected from location of N; 7^o 52’733’’ E; 39^{o}1’10.8’’ N; 7^{o}51’57.9’’ E; 39^{o}00’57.9’’ by grapping the base of plant by pulling up with hand from each of the four (two were taken from Halko, and two from 50 Gasha) farm sites by putting pair of nitrile gloves on hand.

The four farmers plot were selected in such a way that from each division of the plot prepared for irrigation suitability centers and corners were involved. The soil was removed from the onion bulb by clean decontaminated soft bristle brush, washed with water to remove remaining soil, followed by distilled water, and then dried using air. Leaf and roots removed from the onion bulb by setting the bulb on the clean chopping board using Teflon knife.

The bulb samples were put in clean plastic bags labelled according to the water samples, while the large onion bulb were cut in to smaller pieces as suitability for container and transportation bag. After samples were put in to the sample container the sample were stored in cooler of ice at approximately 4^{0}C, and brought immediately to the laboratory for further pretreatment.

After transportation to BDU laboratory onion bulb was placed in standard refrigerator at 4^{0}C (±2). Peeling the outer dry skin, the bulbs were cut in to nearly equal size to facilitate uniformly drying. About 250 g of the sliced bulbs were put on acid-washed crucibles labelled according to the sample and dried in air oven at 80 ^{0}C for 48 h till it got brittle and crisp. Cooling to ambient temperature, the dried samples were ground in to fine powder with porcelain mortar and pestle and sieved with 0.5 mm sieve. The powdered sample was then placed in pre-cleaned screw capped polyethylene container and stored in desiccators containing calcium chloride to keep to constant dry weight till digestion.

3.5 Digestion of samples

3.5.1 Water sample

The water sample taken from Ketar River was digested by using HNO_3 to dissolve the total metals in the samples. A 50 ml of irrigation water sample were added in the beaker (100 ml) and 5 ml of HNO_3 was added in the sample. The triplicates of sample solutions and the blank solution were placed on hot plate that was adjusted with 100 ^{o}C and continuously heated, until clear solution approximately with 20 ml remain. Then the digested solutions were cooled down at room temperature. Finally the walls of the beaker were washed down by 5 mL distilled water; filter the solution in to 50 ml Erlenmeyer flask and diluted to mark by distilled water.

3.5.2 Wet digestion of onion bulb sample

The powdered onion bulb sample was digested using HNO_3 and $HClO_4$ to soluble the total metal in the sample. The wet digestion procedures of onion bulb were done according to Awofolu1, O. Z. 2005.

0.5 g of powdered onion bulb sample was placed in 50 ml beaker, 5 ml of HNO_3 were added in to the sample. The triplicates of onion bulb sample solutions and blank reagent were placed on hot plate and heated at about 80 ^oC while the sample were heated about 15 minutes, 5 ml HNO_3 and 5 ml $HClO_4$ were added and heated until the colorless solution were obtained. Finally the samples were cooled down at room temperature, the beakers were washed down by 5 ml distilled water and the samples were filtered in to 50 ml volumetric flask and diluted up to the mark by distilled water.

3.6. Analysis

The analysis described in this method involves multi elemental determinations by ICP-OES using sequential or simultaneous instruments. The instruments measure characteristic atomic-line emission spectra by optical spectrometry. Samples are nebulized and the resulting aerosol is transported to the plasma torch. Element specific emission spectra are produced by radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer, and the intensities of the line spectra are monitored at specific wavelengths by a photosensitive device. Photocurrents from the photosensitive device are processed and controlled by a computer system. A background correction technique is required to compensate for variable background contribution to the determination of the analytes. Background was measured adjacent to the analyte wavelength during analysis. ICP-OES is method of choice for analysis of heavy metals in food and pharmaceutical products because of its low detection limits and its high degree of selectivity (Gaur et al., 2011).

3.6.1 Analysis of irrigation water and Onion bulb metal level concentration

The total metal content was analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Prkin Elmer Optima 8000) at Chemistry department of Bahir Dar University. The digested samples were thoroughly mixed and the total metal content in the samples were analyzed using ICP-OES. A reagent blank having the same reagents with the same volume and concentration were digested by following the same procedures as the sample were also analyzed by ICP-OES. All measurements of samples were done in triplicate and the mean of the measured values are reported.

Then the concentration of metals in each digested samples were reported and concentration in irrigation water and onion bulb sample were calculated. Concentration of onion bulb sample:-

$$\textbf{Conc. (mg/Kg) (in dry base weight bases)} = \frac{CxVxD}{W},$$

Where: C = Concentration in extract (mg/L), V = Volume of extract (L, 50 mL = 0.05 L), D = Dilution factor (undiluted = 1), W = Weight of sample aliquot extracted (0.5 g x 0.001 = 0.0005 kg) (Martin, TD. et al 1994).

3.7 Method Validations

Validation of analytical method or sample preparation technique needs to be performed so as to evaluate its merits with respect to the purpose and necessity of the type of analysis. Method validation Analytical method validation of ICP-AES for analysis of trace metals was assessed by determining several analytical parameters according to International Conference on Harmonization (ICH, 2005).

The method of preparation in this study was validated by performing parameters such as instrumental calibration, spiking sample, method detection limit, precision of analytical procedures and statistical method.

3.7.1 Instrumental calibration

Six appropriate working standards 0.1, 15, 30, 45, 60, 75 mg/L of K, Mg and Ca, and 0.05, 1.05, 2.05, 3.05, 4.05, 5.05 mg/L of Cr, Cd and Pb were prepared for each of the metals from an intermediate standard solution of 10 mg/L which was first prepared by diluting the 1000 mg/L stock metal standard solutions within the concentration range of elements in the samples and used to calibrate ICP-OES.

The linearity of analytical response was assessed by plotting the intensity values (y-axis) of diluted series of potassium, magnesium, calcium, chromium, cadmium and lead standard solution versus its final concentration (x-axis). The dynamic concentration ranges used were 0.1-75 mg/L for K, Ca and Mg and 0.05–5.05 mg/L Cr, Cd and Pb. The linear relationships were established for all six regression equations and were checked by coefficient of determination (r^2) values. The analytical response was linear over certain concentration ranges, if the r^2 value obtained is higher than 0.995 (Eurachem, 1998). Linear regression data of potassium, magnesium, calcium, chromium, cadmium and lead calibration curves Table 1.

3. 7.2 spiking of Samples

In order to further validate the method developed, post digestion spike recovery studies were performed by adding 10 ppm standard analyte concentration for all analytes in irrigation water samples to monitor the accuracy of the digestion procedure used. The samples spiked by adding the known concentration of standard solution of analyte in to the sample and run with ICP-OES to calculate the percent of recovery, which tell us the accuracy of the method we used.

3.7.2.1 Spiking of water sample

For spiking the irrigation water sample, a digested 50 ml of one irrigation sample were taken and separated in to two 25 mL flasks. In each of the two flasks, 5 mL amount of sample were removed, and then 5 ml of 10 mg metals concentration were added to one flask (spiked sample solution), while 5 mL of distilled water were added to another flask (un-spiked sample solution) and diluted to the mark by distilled water.

The accuracy of method and the efficiency of the instruments were evaluated by calculated percentages of recovery of each metal (Maxfield, R., EPA method 200.7) which is given in Table 2.

3.7.2.2 Spiking of Onion bulb sample

For spiking the onion bulb sample, a digested 50 ml of one onion bulb sample was taken and separated in to two 25 mL flasks. In each of the two flasks, 5 mL amount of sample were removed and then 5 ml of 10 mg/L metals concentration were added to one flask (spiked solution), while 5 mL of distilled water was added to another flask (un-spiked solution) and diluted to mark by distilled water. The spiked and un-spiked samples were run on ICP-OES to determine the concentrations of metals in the same manner as the onion bulb sample was.

The accuracy of method and the efficiency of the instruments were evaluated by calculated percentages of recovery of each metal in the sample as shown in Table 3.

3.7.3 Method detection limit

One of the method validations that tells us whether a systematic process that, the analytical method under question is acceptable for its intended purpose were, the analytical sensitivity of ICP-OES which was evaluated by determining the values of method detection limit (MDL) and limit of quantification (LOQ). MDL and LOQ are terms used to describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure. Limit of quantification (LOQ) is the lowest concentration of analyte that can be determined with an acceptable level of uncertainty (Prichard and Barwick, 2007).

For estimated method detection limits and limits of quantification for the onion bulb samples, the blank reagent equal volume to that added in the onion bulb sample were digested following the same procedures. For irrigation water samples reagent blanks were prepared and digested by following the same procedures as water sample were digested.

The reagent blanks of both onion bulb and irrigation waters were run on ICP-OES for all metals for which was used in similar manners the samples for metals concentrations determination so as to calculate standard deviations and estimate method detection limits. The values of MDL and LOQ for each metals (Table-4) were calculated as 3.3 SD/b and 10 SD/b respectively, where SD is the standard deviation of analytical responses and b is the slope of calibration curve (Shrivastava, A. et al 2011).

3.7.4 Precision of analytical procedures

Precision of analytical procedures expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions (ICH, 2005). Analytical Precision indicates that the analytical method used is precise and reliable which can be determined by triplicate analysis of samples. It is typically evaluated by measuring the values of relative standard deviation (RSD) under the conditions of a set of data (repeatability) and intermediate precision (different day of measurement).

4. RESULTS AND DISCUSSION

4.1 Analysis of irrigation water and Onion bulb concentration metal level

The total metals content in irrigation water samples and onion bulb samples were analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES) (8000 model) at Bahir Dar University Chemistry laboratory.

4.2 Method validation

The method used for preparation of samples and the efficiency of analytical method used in this study were assessed, whether it meets the purposes of the study or not, were performed using different parameters in this study.

The validity of the method of preparation of samples and the efficiency of analytical method used, for it meets to the purpose were assessed by performing different parameters in this study.

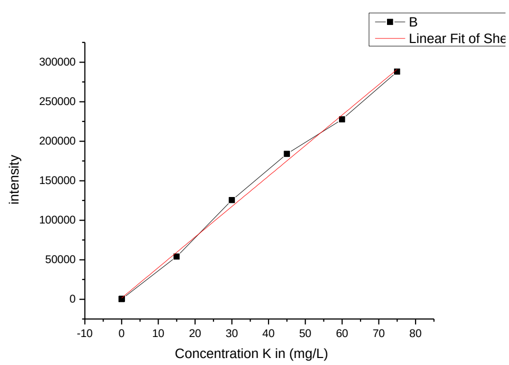
4.2.1. Linearity of the calibration curves

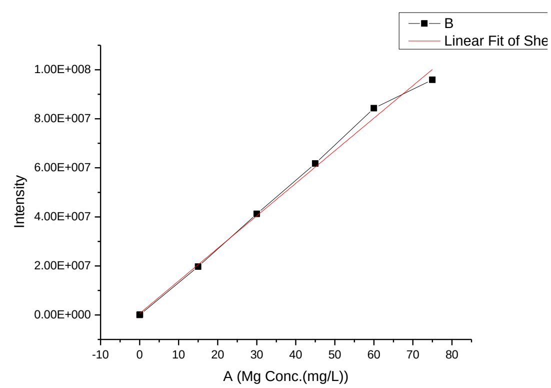
The linearity of the analytical response was assessed by plotting the intensity values (y-axis) of diluted series of potassium (K), calcium (Ca), magnesium (Mg), chromium (Cr), cadmium (Cd) and lead (Pb) standard solution versus its final concentration (x-axis). The analysis of the calibration curve metals of interest, the linear relationship was established for all six regression equations with acceptable coefficient of determination (r^2) values given in Table 1.

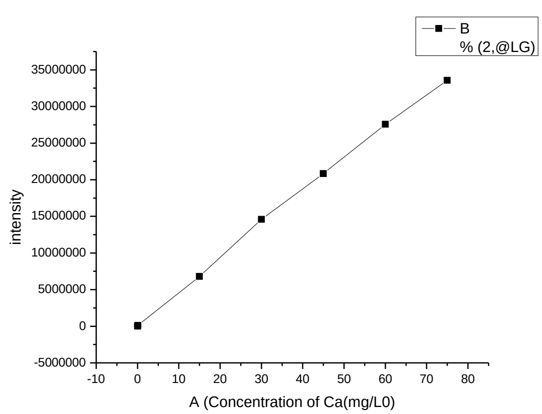
Table 1: Linear regression data of potassium, magnesium, calcium, chromium, cadmium and lead calibration curves

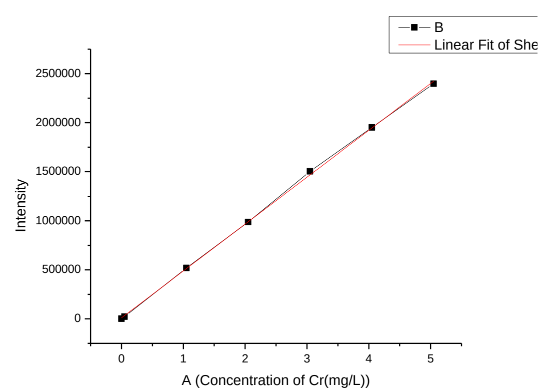
Metals	Wavelength (nm)	Regression equation, (y = ax + b)	Correlation coefficient (r^2)
K	766.490	Y = 1507.247 + 3859.682X	0.99887
Mg	285.213	Y = 605229.68 + 1.32765E6X	0.99786
Ca	317.933	Y = 244491.359+ 452000.592X	0.99945
Cr	267.716	Y = 9617.398 + 478115.439X	0.99978
Cd	228.802	Y = 3603.0165 + 294749.999X	0.99979
Pb	220.353	Y = 1166.1289 + 19738.935X	0.99983

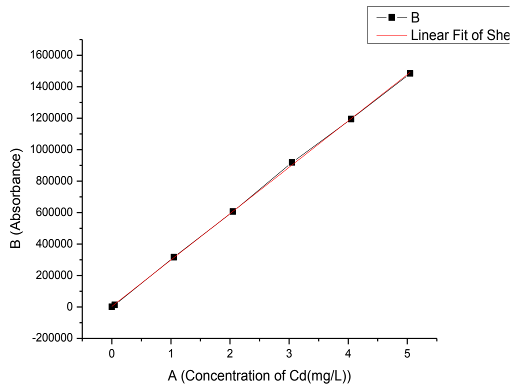
The correlation coefficients of all the calibration curves were higher than 0.998, which shows that there was very good relationship between concentration and intensity.











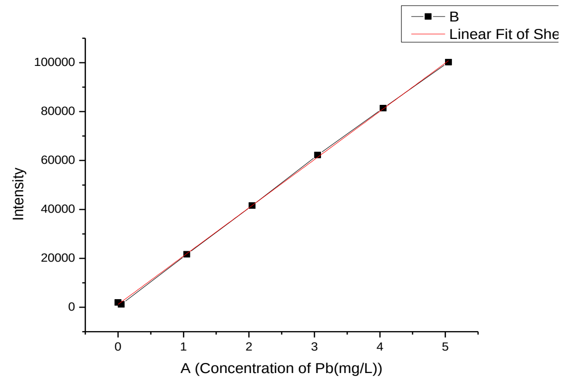


Figure 3: The calibration curves of K, Mg. Ca, Cr, Cd and Pb.

4.2.2. Metals Recovery value

Accuracy of the developed method was assessed using standard addition method and is expressed as recovery. Accuracy can determine the lack of analyte levels due to the losses or contamination during sample preparation, and matrix interferences during the measurement step (Ertas and Tezel, 2004). The recovery determinations were carried out by spiking technique. The recovery values for accuracy of method for irrigation water and onion bulb sample are given in Table 2 and Table 3 respectively.

Table 2: Recovery of potassium, magnesium, calcium, chromium, cadmium and lead content in irrigation water sample with (mean ± SD) in (mg/L)

Metals	Concentration of standard added	Concentration in spiked	Concentration in un spiked	% recovery
K	1.05	2.391	1.281	105.7
Mg	2	3.918	1.936	99.1
Ca	1.25	5.758	4.523	98.8
Cr	0.047	0.349	ND	-
Cd	0.012	0.129	ND	-
Pb	0.05	ND	ND	-

Table 3: The recovery of potassium, magnesium, calcium, chromium, cadmium and lead content in onion bulb sample with (mean ± SD) (mg/Kg)

Metals	Concentration of standard added	Concentration in spiked Onion	Concentration in un spiked Onion	% Recovery
K	11.547	45.25	35.27	107
Mg	0.55	7.886	7.338	99.6
Ca	1.5	10.14	8.591	103.3
Cr	0.062	0.671	ND	-
Cd	0.026	0.264	ND	-
Pb	0.2	ND	ND	-

The obtained percentage recovery varied from 90% to 105% in both the onion bulb and irrigation water samples which were in the acceptable range (Table 2 and 3).

According to previously published study (Huber, 1998), the acceptable recovery percentage range is 80–110% for the analyte level of 1 μg/mL. Therefore, the developed method was accurate for quantification of K, Ca, Mg, Cr, Cd, and Pb in onion bulb.

4.2.3 Sensitivity of analytical method

The analytical sensitivity of ICP-OES was evaluated by determining the values of method detection limit (MDL) and limit of quantification (LOQ). The blanks of both onion and water which were prepared following the same procedures utilized for digestion of irrigation water and onion bulb sample, were analyzed for its metal contents (K, Mg, Ca, Cr, Cd and Pb) by ICP-OES and calculated standard deviation were used to obtain method detection limit and limit of quantification in both samples as shown in Table 4 and Table5.

Table 4: Method detection limit and limit of quantification for metals in irrigation water sample

Metals	Concentration in water (mg/L)	MDL	LOQ
K	20.05	0.081	0.26964
Mg	3.707	0.00334	0.0113
Ca	12.844	0.00437	0.0146
Cr	0.0101	0.00047	0.0016
Cd	ND	0.00012	0.0004
Pb	0.00356	0.00382	0.01272

Table 5: Method detection limit and limit of quantification for metals in onion bulb sample

Metals	Concentration in onion bulb (mg/Kg)	MDL	LOQ
K	4726	0.21547	0.7182
Mg	899.09	0.00281	0.0094
Ca	1730.56	0.11871	0.3957
Cr	1.367	0.00062	0.0021
Cd	ND	0.00026	0.0009
Pb	4.1	0.00016	0.0005

The mean concentration of K, Mg, Ca, Cr and Pb were observed above the MDL in both irrigation water and onion bulb samples, while Cd was not detected (ND) in both samples. In this study MDL were lower than LOQ, this result, indicates that the method was verified and acceptable.

The MDL values found in onion bulb sample were of 0.2154 mg/Kg (K), 0.0028 mg/Kg (Mg), 0.1187 mg/Kg (Ca), 0.00062 mg/Kg (Cr), 0.00026 mg/Kg (Cd) and 0.00016 mg/Kg (Pb).

Mean while, the LOQ values found in onion bulb sample were; 0.7182 mg/K (K), 0.0094 mg/Kg (Mg), 0.3957 mg/Kg (Ca), 0.0021 mg/Kg (Cr), 0.0009 mg/Kg (Cd) and 0.0005 mg/Kg (Pb).

The MDL values found in irrigation water sample were of 0.081 mg/L (K), 0.00334 mg/L (Mg), 0.00437 mg/L (Ca), 0.00047 mg/L (Cr), 0.00012 mg/L (Cd) and 0.00382 mg/L (Pb). Meanwhile, the LOQ values in onion bulb sample found were of 0.26964 mg/L (K), 0.0113mg/L (Mg), 0.0146mg/L (Ca), 0.0016mg/L (Cr), 0.0004mg/L (Cd) and 0.0127 mg/L (Pb).

The MDL for all metals in onion bulb were higher than in irrigation water samples. Based on MDL values, ICP-OES was sensitive enough for analysis of these heavy metals because, the MDLs were high enough to detect the presence of metal of interest at trace levels in both the onion bulb and irrigation water samples and this because MDL values were lower than maximum values of heavy metals allowed to be present in vegetable products, i.e. 0.5 mg/Kg (Roba et al., 2016).

4.2.4. Reproducibility of analytical procedures

The reproducibility of the analytical procedure measure the reliability and precision of obtained data and it was checked by carrying out a triplicate analysis and calculating the relative standard deviations for each metal. In this study, the precision of the results was evaluated by % RSD of the three samples (n=3) and triplicate readings for each sample giving a total of nine measurements for a given bulk sample. In the precision test, the % RSD; 10.9 %(K), 2.8 %(Mg), 1.67 %(Ca) and 10.4%(Cr) for irrigation water sample as shown in Table 6 and where as in onion bulb sample % RSD were 14.86% (K), 11.1% (Ca), 5.69% (Mg), 63.47% (Cr) and 37.47% (Pb) as shown in Table 7. According to Horwitz, the maximum RSD value acceptable for the analyte level of 0.001 mg/L is 16% (Gonzales and Herrador, 2007). AOAC Peer Verified Methods set the maximum acceptable RSD value at 11% for the same analyte level. Therefore, it can be stated that the ICP-OES method showed good precision based on RSD values obtained, while Cr and Pb has less precision.

4.3. Determination of level of metals in irrigation water and onion bulb samples

4.3.1. Determination of level of metals in irrigation water samples

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).

In this study, the irrigation water diverted from Ketar River was collected from the irrigation channel before it enter in to the farmers plot, and then the irrigation water sample were analyzed for major (K, Mg and Ca) and trace non-essential metals (Cr, Cd and Pb) with ICP-OES. The mean concentration of metal along with standard deviation of triplicate analysis is given in Table 6.

Table 6: Metals Mean concentration ± SD mg/L in irrigation water sample

Metal	Concentratin in water sample(Mean ± SD)	% RSD
K	20.05±2.19	10.9
Mg	3.71±0.104	2.8
Ca	12.84±.0216	1.67
Cr	0.01±0.001	10.4
Cd	ND	-
Pb	0.00356±0.0043	121.88

The major metals K, Mg and Ca and non essential metal, Cr and Pb were observed while Cd was not detected (ND) in irrigation water sample. The levels of K, Ca, Mg, Cr and Pb were; 20.05±2.19 mg/L, 12.84±.0216, 3.71±0.104 mg/L, 0.0101±0.001 mg/L and 0.00356±0.004 mg/L respectively. The trends of metal concentration observed in irrigation water sample were, K>Ca>Mg>Cr>Pb. The concentration of major metals in the study area were found to be higher.

4.3.2 Determination of level of metals in onion bulb samples

Table 7: Metals Mean concentration ±SD in Onion bulb sample

Metals	Concentration in dry sample (mg/Kg)	%RSD
K	4726 ± 0.025	14.86
Mg	899.09 ± 0.99	5.69
Ca	1730.56 ± 0.98	11.1
Cr	1.367 ± 0.009	63.47
Cd	ND	-
Pb	4.1±0.015	37.47

Determinations of K, Ca, Mg, Cr and Pb in onion bulb sample are shown in Table 7.
The levels of K, Ca, Mg, Cr and Pb are; 4726±0.025 mg/kg, 1730.556±0.98 mg/kg, 899.089±0.99 mg/kg, and 1.3667±0.009 mg/kg and 4.1±0.015 mg/kg, respectively. K was the most accumulated major metal in the onion bulbs, while Mg was the list accumulated from the analyzed three major metals (K, Ca, and Mg). From the analyzed non-essential metals in an onion bulb samples (Cr, Pb and Cd), Pb were the highest accumulated in onion bulb, while Cr was the list accumulated and Cd was below the method detection limit.

4.4. Comparison of metal concentration in irrigation water sample and onion bulb sample

Table 8: Comparison between metal concentration in irrigation water and onion bulb samples

Metals	Concentration in water (mg/L)	Concentration in Onion (mg/Kg)
K	20.05±2.19	4726±0.025
Mg	3.707±0.104	899.09±0.99
Ca	12.84±.0216	1730.56±0.98
Cr	0.0101±0.001	1.367±0.009
Cd	ND	ND
Pb	0.00356±0.004	4.1±0.015

The metals concentration level in irrigation water from Ketar River and the onion bulb cultivated by this irrigation water have the same trends as shown in Table 8.

Trends in irrigation water were, K (20.05±2.19) > Ca (12.84±.0216) > Mg (3.71±0.104) > Cr (0.0101±0.001) > Pb (0.00356±0.004).

Trends in onion bulb sample were, K (4726+0.025)>Ca (1730.556±0.98) > Mg (899.089±0.99) > Pb (2.533±0.015) > Cr (1.3667±0.009). Cd was not detected in both irrigation water and onion bulb sample.

4.5. Comparison of metal concentration in irrigation water sample

4.5.1 Concentration of some selected essential (major) and non essential metals in literature review

Table 9: Essential and non essential metals in irrigation water in the literature review in mg/L

Country	K	Ca	Mg	Cr	Cd	Pb	Reference
Iran in farm-6	0.025	14.45	9.71	0.005	0.0001	0.001	*Maleki A. 2014*
Ethiopia, Lake Zeway	13±1	10±0.2	8.6± 0.1	0.42± 0.02	0.0062± 0.0004	< 0.1	*Reta, et al.,2011*
Ethiopia, Ketar River	20.05 ±2.2	12.84± 0.022	3.71± 0.104	0.01± 0.001	ND	0.0036 ±0.004	Present
Ethiopia , Meki river	-	-	-	0.015± 0.005	ND	ND	Miheratu, B. et al., 2017
Recommended maximum concentration for irrigation	0-0.05	0-20	0-5	0.1	0.01	5	*WHO/FAO, 2003.*

The concentration K, 20.05±2.2 mg/L, in this study was highest compared with the concentration level of K, 0.025 mg/L in irrigation water that was reported by Maleki A. et al 2014. The level of K in this study was also higher than the level of K, 13±1mg/L, as reported by Reta, B. et al, 2011. The K concentration level in this irrigation water is also higher than the recommended range, WHO/FAO, 2003.

The concentration level of Ca, 12.84±.022 mg/L was lower than the concentration level of Ca reported by Maleki A. et al, 2014, but higher than concentration Ca, 10±0.2 mg/L reported by Reta, B. et al 2011. This level of Ca was within the recommended range 0-20 mg/L, of Ca in irrigation water given by WHO/FAO, 2003.

The level of Mg in this study was lower than the level concentration in irrigation water that was reported by Reta and the concentration level Mg in irrigation water that was reported by Maleki A. et al 2014. The level of concentration of Mg in this was within the recommended range set by WHO/FAO, 2003. The concentration of essential metals in water used for irrigation was highest for K, followed by Ca and Mg, (Table 9). The metal concentrations of the K in Ketar irrigation water and lake Zeway was higher where Ca and Mg found to be lower than the acceptable range recommended for irrigation waters, WHO/FAO, 2003.

The concentration level of Cr in the irrigation water from Ketar was higher than Cr level that was reported by Maleki, A., 2014. The Cr obtained from Zeway lake irrigation water in Ethiopia was higher than the Cr concentration level in this study area. The concentration level of Cr at the present study was nearly similar value with the concentration level of Cr in irrigation water from Meki River as reported by Mihratu, 2017. The Pb level in ketar river irrigation water were higher, Compared with the level of Pb that was reported by Maleki, A, 2014. This level concentration in Pb in the study area was below permissible limit sat by WHO/FAO, 2003. According to table 9, Comparison of the levels of non essential trace metals in irrigation water samples from Ketar river water, Cr, Cd, and Pb were below recommended maximum concentration in irrigation water set by WHO/FAO, 2003. Therefore the irrigation water from Ketar River was safe for irrigation purpose.

4.6. Comparison of metal concentration in onion bulb sample with other literature review

Table 10: Comparison of concentration of major metals mg/Kg, in onion bulb with other literature review values

Country	K	Mg	Ca	Ref.
Spain	1900±17	92.8± 0.64	148±0.8	Khan, S.A. et al., 2006
UAE	86422(2.19)	241(2.15)	84(4.59)	Mendez, C.V. et al., 2007
Saudi Arabia	269	228	849	Nabrzyski, M. et al 2006, Reilly, C. et al., 2006
Ethiopia, Onion irrigated with lake water	3298±5	407±3.0	550±2.0	Reta, B. et al., 2011
Ethiopia, onion irrigated with Ketar River	4726±0.025	899.±0.99	1730.6± 0.98	Present
	0-0.05	0-5	0-20	WHO. 2014 in irrigation water

The concentration level of major metals in onion bulb irrigated with Ketar River is K, 4726±0.03 mg/Kg, Ca, 1730.6±0.98 mg/Kg and Mg, 899.1±0.99 mg/Kg.

The highest concentration of K is obtained in the onion bulb in this study area, where this level was below the concentration level 86422(2.19) reported by Mendez, C.V. et al 2007. The K level in the present study were higher than the concentration level of K, 3298±5 mg/Kg in onion bulb irrigated with lake Zeway water as reported by Reta, B. et al 2011 and the level of K, 1900±17 mg/Kg in Spain that was reported by Khan, S.A. et al 2006. It is essential nutrient of both plants and animals and it is among the primary macronutrients that measure from 1.5−4% in the dry plant matter.

The level of K in onion bulbs in this study is within the proportion proposed in plant materials. It might have originated from the mixed fertilizers applied to soil, mineral deposits in soil, and salts in the irrigation water, which was also containing significant amount.

The concentration level of Ca, 1730.56±0.98 mg/Kg is the 2nd most accumulated metal next to K in onion bulb irrigated with Ketar River water in the studied area.

The level of Ca concentration in this study area is higher than the concentration level of Ca (148±0.8) in an onion bulb reported by Khan, S.A. et al 2006 and the level of Ca (550±2.0 mg/L) in onion irrigated by Lake Zeway water reported by Reta, B. et al 2011. The level of concentration of Ca in the study is also higher than the concentration level of Ca 84 (4.59) in onion bulb in UAE, reported by Mendez, C.V. et al 2007 and the level of Ca, 849 mg/L in onion bulb reported by Nabrzyski, M. et al 2006, Reilly, C. et al 2006 in Saudi Arabia. The amounts of Mg in onion bulb of present study are higher than that obtained by Mendez et al. and Khan et al. The level of Mg in present study are also higher than the level of Mg in onion bulb reported by Reta, B. et al 2011 and Nabrzyski, M. et al 2006, Reilly, C. et al 2006. Higher level of Mg and Ca is observed may be from the salt deposited in the area and it may be due to the common hardness of water. Comparison of levels of trace metals in onion vegetable reported in various parts of the world are summarized in Table 11.

Table 11: Comparison of concentration of trace metals in mg/Kg, in onion bulb with literature values

Country		Cr	Cd	Pb	Ref.
Ethiopia,	Irrigated With lake Zeway	6.6±0.1	0.5±0.06	<0.5	Reta, B. et al., 2011
Ethiopia,	irrigated with Meki river	4.13±0.12	0.03±0.01	<0.1	Miheratu, B, 2017
Ethiopia,	Irrigated by Ketar river.	1.367±0.01	ND	4.1±0.015	Present
Nigeria		3.00-7.1	0.22-0.89	2.00-9.5	Iyaka, 2007, Abdullahi, 2008
Koria			1.88±0.35	4.23±1.1	Jung, 2008
Croatia		-	0.28±0.08	0.17±0.01	Stančić Z, et al., 2016
Accra-Ghana		-	0.02±0.01	0.08±0.02	Crentsil Kofi Bempah et al,2011
For fruit bulb vegetables		0.10	0.05	0.1	FAO/WHO, 2014

From the non-essential heavy metal concentrations were determined based on vegetable dry weight, the non-essential metals Cr, Cd and Pb, while the Cd was not detected in the onion bulb sample irrigated with Ketar river water in Ketar Kote bula kebele of Tiyo woreda of Arsi Zone.

In the present study, the chromium level in onion bulb irrigated with Ketar river water was 1.367±0.01 mg/Kg dry weight, were it was below the range of the literature value of chromium, 3.00-7.1 mg/Kg in dry weight reported by (Akan et al., 2009; Abdullahi et al., 2008; Iyaka, 2007). The result of chromium in this study was also below the level of metal in onion bulb irrigated with Lake Zeway, 6.6±0.1 mg/Kg and the concentration level Cr, 4.9 mg/kg in onion bulb irrigated with well water as reported by Reta, B. et al 2011. This level was also lower than the concentration of chromium metal, 4.13±0.12 mg/Kg in Onion bulb irrigated with Meki River with no effuluent, which reported by Miheratu Bedasa, 2017. The concentration of Cr in onion bulb at Ketar Kote bula irrigation site was above permissible level of (0.10 mg/Kg) FAO/WHO 2014.

The concentration level of Pb, 4.1±0.015 mg/Kg metal in onion bulb irrigated with Ketar river water in the study area was less than the range of Pb, 6-13.00 mg/kg, in dry weight in onion vegetables that was reported Abdullahi et al., 2008. The level lead metal in this study area was as high as reported in the literatures values 4.23 Jung (2008), and it was within the range of Pb, 2.00-9.5 in Nigeria, as reported by Iyaka 2007, Abdullahi, 2008. This Level of lead obtained in this study area was higher than the concentration level of pb, 0.17±0.01mg/Kg that reported in Croutia, by Stančić Z, et al 2016, as well as also higher than the level Pb in Ghana 0.08±0.02 mg/Kg as roported by Crentsil Kofi Bempah et al, 2011. The permissible concentration of lead in fruit, tuberous and bulb vegetables was 0.1 mg/kg, while that in leafy vegetables is 0.3 mg/kg (FAO/WHO 2014). It is observed that, the mean content of lead (4.1±0.015 mg/Kg) in onion bulb in this study area is higher than permissible level of Pb, set by FAO/WHO, 2014.

Therefore the results in the present study showed a high level of Pb and Cr in all the vegetables from the cultivated area according to food and agriculture organization (FAO) or the world health organization (WHO) guidelines WHO/FAO, 2014.

While the results of this study also showed cadmium (Cd) content of the onion bulb not detected that assures the low cadmium exposure of the farm fields as well as waters in the study area.

The Ketar Kote bula farmers (Kebele) vegetable irrigation farm is near the known agricultural area mostly uses excess agrochemicals and fertilizers to increase the yields. This area +is also close to Lole government farm and selective seed production, which uses may affect agriculture areas in the areas for agricultural activities specially vegetables production.

Agricultural activities in this area cause significant alterations in the water resources and increases the toxic metals resulting from fertilizers and metal-based pesticides, transportation and harvesting process. The areas around this vegetable farm are also known for using tractor vehicle to plough land and different machines to harvest crop around the area. So, the levels of Cr and Pb obtained in the present study indicate a toxicity effect to consumers. The most probable reason may be excess use of agrochemicals and it may also be from the emission of gasses and car battery in the cars that is frequently used to plough land and to harvest crop. Anthropogenic activities are a major source of heavy metal contamination which includes agricultural crop residue, emission from industries and vehicular emissions.

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 Ketar River with domestic wastes, livestook (overgrazing, pig and chicken livestock) and Agricultural wastes and others, disposed in to tributary rivers (Ashebeka, Worga, etc) as well as to soils.

5. CONCLUSIONS AND RECOMMENDATION

Moderate onion consumption contributes for the daily nutritional requirements of many essential metals, including major (K, Ca and Mg) and non-essential trace metals (Cr, Cd and Pb). The levels of major metals are K (20.05±2.19 mg/L), Ca (12.84±.0216 mg/L) and Mg (3.71±0.104 mg/L), and non-essential metals are Cr (0.01±0.001mg/L) and Pb (0.00356±0.004 mg/L) in irrigation water from Ketar River. The levels of major metals obtained in onion irrigated with water from Ketar River are K (4726±0.025 mg/Kg), Ca (1730.56±0.98 mg/Kg) and Mg (899.09±0.99 mg/Kg), where the level of non-essential metals obtained are Cr (1.367±0.009 mg/Kg), and Pb (4.1±0.015 mg/Kg). The level of toxic metal Cd is not detected in irrigation waters from Ketar River and the onion bulbs in KQK, vegetable farm site and revealing that the Ethiopian onions obtained from Tiyo woreda, ‘’Ketar Kotebula’’ Kebele of Arsi Zone contain either very low concentration of Cd or may be free from this metal. The results showed that Cr and Pb concentrations exceeded the safety limits given by food and agriculture organization (FAO) or the world health organization (WHO) for human consumption.

A comparison of the levels of heavy metals in the studied vegetables was done with the permissible levels required for safe food. The results clearly showed high Cr and Pb contents which are above the permissible levels by FAO and WHO, are found in onion bulb, these amounts could be toxic and hazardous if taken in large quantities and we would like to recommend that people should not take large quantities of these vegetables so as to avoid large accumulation of the heavy metals in the body.

Appropriate measures should be taken by population around the area and the government to minimize excess uses of heavy metal based fertilizers and pesticides, and as well as to prevent excess discharge of chemicals and anthropogenic activities (agricultural crop residue and vehicular emissions) to soil and water resource.

It is therefore suggested that regular monitoring of heavy metals in plant tissues is essential in order to prevent excessive build-up of these metals in the human food chain.

6. REFERENCES

1. Abdullahi, M.S., Uzairu, A. and Okunola, O.J., 2008. Determination of some trace metal levels in onion leaves from irrigated farmlands on the bank of River Challawa, Nigeria. African Journal of Biotechnology, 7(10).
2. Adelekan, B.A. and Abegunde, K.D., 2011. Heavy metals contamination of soil and groundwater at automobile mechanic villages in Ibadan, Nigeria. International journal of physical sciences, 6(5), pp.1045-1058.
3. Adeyeye, E.I., 2005. Trace metals in soils and plants from Fadama farms in Ekiti State, Nigeria. Bulletin of the Chemical Society of Ethiopia, 19(1), pp.23-34.
4. Agarwal, S.K., Tiwari, S.C. and Dash, S.C., 1993. Spectrum of poisoning requiring haemodialysis in a tertiary care hospital in India. The International journal of artificial organs, 16(1), pp.20-22.
5. Akinola, M.O., Njoku, K.L. and Ekeifo, B.E., 2008. 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, pp.25-31.
6. Ambedkar, G. and Muniyan, M., 2012. Analysis of heavy metals in water, sediments and selected freshwater fish collected from Gadilam river, Tamilnadu, India. International Journal of Toxicology and Applied Pharmacology, 2(2), pp.25-30.
7. ATSDR. Toxological Profile for Cadmium. Atlanta, USA: Agency for Toxic Substances and Disease Registry, U.S. Departement of Health and Human Service, Public Health Service, 2012a.
8. ATSDR. Toxological Profile for Chromium. Atlanta, USA: Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Service, Public Health Service, 2012b.
9. Awofolu, O.R., Mbolekwa, Z., Mtshemla, V. and Fatoki, O.S., 2005. Levels of trace metals in water and sediment from Tyume River and its effects on an irrigated farmland. Water Sa, 31(1), pp.87-94.

10. Bahemuka, T.E. and Mubofu, E.B., 1999. Heavy metals in edible green vegetables grown along the sites of the Sinza and Msimbazi rivers in Dar es Salaam, Tanzania. Food Chemistry, 66(1), pp.63-66.
11. BEDASSA WAKESSA, M.I.H.E.R.E.T.U., 2017. ASSESSMENT OF SELECTED HEAVY METALS IN ONION (ALLIUM CEPA L.), SOIL AND WATER IN SELECTED AREAS OF CENTRAL RIFT VALLEY OF EASTERN SHOA ZONE, OROMIA REGION, ETHIOPIA (Doctoral dissertation, AMBO UNIVERSITY).
12. Bendich, A., Machlin, L., Packer, L. and Fuchs, K., 1997. Vitamin E in Health and Disease. New York, pp.367-379.
13. Allallergy.net. Archived from the original on 15 June 2010. Retrieved 14 April 2010. CABI Publishing. pp. 20–21. ISBN 978-0-85199-510-6
14. Block, Eric, A. John Dane, Siji Thomas, and Robert B. Cody. "Applications of direct analysis in real time mass spectrometry (DART-MS) in Allium chemistry. 2-Propenesulfenic and 2-propenesulfinic acids, diallyl trisulfane S-oxide, and other reactive sulfur compounds from crushed garlic and other Alliums." Journal of agricultural and food chemistry 58, no. 8 (2010): 4617-4625.
15. Bo, S.O.N.G., Mei, L.E.I., Tongbin, C.H.E.N., Zheng, Y., Yunfeng, X.I.E., Xiaoyan, L.I. and Ding, G.A.O., 2009. Assessing the health risk of heavy metals in vegetables to the general population in Beijing, China. Journal of Environmental Sciences, 21(12), pp.1702-1709.
16. Boamponsem, G.A., Kumi, M. and Debrah, I., 2012. Heavy metals accumulation in cabbage, lettuce and carrot irrigated with wastewater from Nagodi mining site in Ghana. International Journal Of Scientific & Technology Research, 1(11), pp.124-129.
17. Brar, M.S., Malhi, S.S., Singh, A.P., Arora, C.L. and Gill, K.S., 2000. Sewage water irrigation effects on some potentially toxic trace elements in soil and potato plants in northwestern India. Canadian Journal of Soil Science, 80(3), pp.465-471.
18. Brhane, G. and Shiferaw, T., 2014. Assessment of levels of lead, cadmium, copper and zinc contamination in selected edible vegetables. International Journal of Innovation and Applied Studies, 7(1), p.78.

19. Chen, Y., Wang, C. and Wang, Z., 2005. Residues and source identification of persistent organic pollutants in farmland soils irrigated by effluents from biological treatment plants. Environment international, 31(6), pp.778-783.
20. Chernoff, R. Getriatic nutrition, glucose lipid: the health professional's handbook. Ontario, Canada: Jones and Bartlett Publishers. 2002, Pp102.
21. Chojnacka, K., Chojnacki, A., Gorecka, H. and Górecki, H., 2005. Bioavailability of heavy metals from polluted soils to plants. Science of the Total Environment, 337(1-3), pp.175-182.
22. Crentsil Kofi Bempah*, Archibold Buah-Kwofie, Anita Osei-Tutu, Dzifa Denutsui and Nash Bentil, Elixir Pollution., 2011.Assessing potential dietary intake of heavy metals in some selected fruits and vegetables from Ghanaian markets. 39, 4921-4926
23. CSA, Area and Production of Major Crops. 2008. Agricultural Sample Enumeration Survey. Addis Ababa, Ethiopia.
24. Duffus, J.H., 2002. " Heavy metals" a meaningless term?(IUPAC Technical Report). Pure and applied chemistry, 74(5), pp.793-807.
25. Ediin, G.; Golantu, E. and Brown, M. Essentials for health and wellness. Toronto, Canada: Bartlett Publishers. 2000, Pp 368.
26. Edward, J.B., Idowu, E.O., Oso, J.A. and Ibidapo, O.R., 2013. Determination of heavy metal concentration in fish samples, sediment and water from Odo-Ayo River in Ado-Ekiti, Ekiti-State, Nigeria. International Journal of Environmental Monitoring and Analysis, 1(1), pp.27-33.
27. Ellen, G., van Loon, J.W. and Tolsma, K., 1990. Heavy metals in vegetables grown in the Netherlands and in domestic and imported fruits. Zeitschrift für Lebensmittel-Untersuchung und Forschung, 190(1), pp.34-39.
28. El-Tantawy, E.M. and El-Beik, A.K., 2009. Relationship between growth, yield and storability of onion (Allium cepa L.) with fertilization of nitrogen, sulphur and copper under calcareous soil conditions. Research Journal of Agriculture and Biological Sciences, 5(4), pp.361-371.

29. FAO/WHO (2014) General standards for contaminants and toxins in food and feed (CODEX STAN 193-1995)
30. FAO/WHO, Codex Alimentarius Commission (2001). Food additives and contaminants. Joint FAO/WHO food standards programme, ALINORM 01/12A: p. 1-289.
31. Feig, D.I., Reid, T.M. and Loeb, L.A., 1994. Reactive oxygen species in tumorigenesis. Cancer Research, 54(7 Supplement), pp.1890s-1894s.
32. Fifield, F.W. and P.J., 1997. Haines, Environmental Analytical chemistry, Blackie Academic Professional: London, PP: 1997, 323-349.
33. Flores-Magdaleno, H., Mancilla-Villa, O.R., Mejía-Saenz, E., Olmedo-Bolantilde, M.D.C. and Bautista-Olivas, A.L., 2011. Heavy metals in agricultural soils and irrigation wastewater of Mixquiahuala, Hidalgo, Mexico. African journal of agricultural research, 6(24), pp.5505-5511.
34. Gebhardt, S., Lemar, L., Haytowitz, D., Pehrsson, P., Nickle, M., Showell, B., Thomas, R., Exler, J. and Holden, J., 2008. USDA national nutrient database for standard reference, release 21. United States Department of Agriculture Agricultural Research Service.(USDA Nutrient Database)
35. Heidarieh, M., Maragheh, M.G., Shamami, M.A., Behgar, M., Ziaei, F. and Akbari, Z., 2013. Evaluate of heavy metal concentration in shrimp (Penaeus semisulcatus) and crab (Portunus pelagicus) with INAA method. SpringerPlus, 2(1), p.72.
36. Hilgenkamp, K., 2006. Environmental health: Ecological perspectives. Jones & Bartlett Learning.
37. Johnson, L., Strich, H., Taylor, A., Timmermann, B., Malone, D., Teufel- Shone, N., Drummond, R., Woosley, R., Pereira, E. and Martinez, A., 2006. Use of herbal remedies by diabetic Hispanic women in the southwestern United States. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 20(4), pp.250-255.
38. Jolly, Y.N., Islam, A. and Akbar, S., 2013. Transfer of metals from soil to vegetables and possible health risk assessment. SpringerPlus, 2(1), p.385.

39. Jung, M., 2008. Heavy metal concentrations in soils and factors affecting metal uptake by plants in the vicinity of a Korean Cu-W mine. Sensors, 8(4), pp.2413-2423.
40. Kebede D., Jibicho G., Masresha M., 2017. Determination of Nutritional Profile and Physicochemical Properties of Improved Onion (Allium cepaL.) Varieties in Ethiopia. Biochemistry and Molecular Biology.Vol. 2, No. 6, pp. 86-93.
41. Khairiah, J., 2004. The uptake of heavy metals by fruit type vegetables grown in selected agricultural areas. Pakistan Journal of Biological Sciences (Pakistan)
42. Khan, M.A. and Ghouri, A.M., 2011. Environmental pollution: its effects on life and its remedies. Researcher World: Journal of Arts, Science & Commerce, 2(2), pp.276-285.
43. Khan, S.A., Ahmad, I.Q.B.A.L. and Mohajir, M.S., 2006. Evaluation of mineral contents of some edible medicinal plants. Pakistan journal of pharmaceutical sciences, 19(2), pp.148-152.
44. Kitata, R.B. and Chandravanshi, B.S., 2012. Concentration levels of major and trace metals in onion (Allium cepa L.) and irrigation water around Meki Town and Lake Ziway, Ethiopia. Bulletin of the Chemical Society of Ethiopia, 26(1).
45. Loghman-Adham, M., 1997. Renal effects of environmental and occupational lead exposure. Environmental health perspectives, 105(9), pp.928-939.
46. Maleki, A., Gharibi, F., Alimohammadi, M., Daraei, H. and Zandsalimi, Y., 2013. Concentration levels of heavy metals in irrigation water and vegetables grown in peri-urban areas of Sanandaj, Iran. Journal of Advances in Environmental Health Research, 1(2), pp.81-88.
47. Malik, M.N., 2000. Horticulture Biotech Books. Delhi. 586p.
48. Martin, T.D., Brockhoff, C.A. and Creed, J.T., 1994. EMMC Method Work Group-Method 200.7. Revision, 4, pp.2015-08.)
49. Mateo-Sagasta, J. and Burke, J., SOLAW TR08.
50. Mendez, C.V.; Rodri-Guez, E.M.; Romero, C.D.; Gonza-Lez, M.G.; Isasa, M.T. Int. J. Food Sci. Nutr. 2007

51. Miean, K.H. and Mohamed, S., 2001. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. Journal of agricultural and food chemistry, 49(6), pp.3106-3112.
52. MoARD (Ministry of Agriculture and rural Development) (2009).Rural Capacity Building project. Course for Training of trainers on improved horticultural crop technologies. pp. 5-19.
53. Moćko, A. and Wacławek, W., 2004. Three-step extraction procedure for determination of heavy metals availability to vegetables. Analytical and bioanalytical chemistry, 380(5-6), pp.813-817.
54. Arora, M., Kiran, B., Rani, S., Rani, A., Kaur, B. and Mittal, N., 2008. Heavy metal accumulation in vegetables irrigated with water from different sources. Food chemistry, 111(4), pp.811-815.)
55. Mulligan, C.N., Yong, R.N. and Gibbs, B.F., 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Engineering geology, 60(1-4), pp.193-207.
56. Nabulo, G., Young, S.D. and Black, C.R., 2010. Assessing risk to human health from tropical leafy vegetables grown on contaminated urban soils. Science of the Total Environment, 408(22), pp.5338-5351.
57. Nnorom, I.C., Osibanjo, O. and Ogugua, K., 2007. Trace heavy metal levels of some bouillon cubes, and food condiments readily consumed in Nigeria. Pakistan Journal of Nutrition, 6(2), pp.122-127.
58. Pedrero, F., Kalavrouziotis, I., Alarcón, J.J., Koukoulakis, P. and Asano, T., 2010. Use of treated municipal wastewater in irrigated agriculture—Review of some practices in Spain and Greece. Agricultural Water Management, 97(9), pp.1233-1241.
59. Prichard, E. and Barwick, V., 2007. Quality assurance in analytical chemistry (Vol. 25). John Wiley & Sons.)
60. Radwan, M.A. and Salama, A.K., 2006. Market basket survey for some heavy metals in Egyptian fruits and vegetables. Food and Chemical Toxicology, 44(8), pp.1273-1278.

61. Roba, C., Roşu, C., Piştea, I., Ozunu, A. and Baciu, C., 2016. Heavy metal content in vegetables and fruits cultivated in Baia Mare mining area (Romania) and health risk assessment. Environmental Science and Pollution Research, 23(7), pp.6062-6073.
62. Salih, S.A.R., Elsheik, M.A.M. and Haman, B., Pollution of water by heavy metals in Gombe State Nigeria.
63. Sanchez-Castillo, C.P., Dewey, P.J., Aguirre, A., Lara, J.J., Vaca, R., de la Barra, P.L., Ortiz, M., Escamilla, I. and James, W.P.T., 1998. The mineral content of Mexican fruits and vegetables. Journal of Food Composition and Analysis, 11(4), pp.340-356.
64. Sandeep, G., Sangita, A., Kumar, S.S., Rakhi, G. and Dinesh, K., 2012. Biological effect of heavy metal in drinking water samples of Western Uttar Pradesh region in India. Journal of Applied Pharmaceutical Science, 2(7), p.177.
65. Sarkar, B. Heavy metals in the environment. New York, USA: Taylor and Francis. 2005, Pp 33-41.
66. Sharma, R.K., Agrawal, M. and Marshall, F., 2006. Heavy metal contamination in vegetables grown in wastewater irrigated areas of Varanasi, India. Bulletin of environmental contamination and toxicology, 77(2), pp.312-318.
67. Shobha M S1 and Shiva Kumar D*2 1 Department of Microbiology, Maharani's Science College for Women, Mysuru-570 005, Karnataka 2 Department of Studies in Environmental Science, University of Mysore, Manasagangothri, Mysuru-570 006, Karnataka
68. Shrivastava A, Gupta VB., 2011. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chron Young Sci 2011;2:21-5
69. Siegel, K.R., Ali, M.K., Srinivasiah, A., Nugent, R.A. and Narayan, K.V., 2014. Do we produce enough fruits and vegetables to meet global health need?. PloS one, 9(8), p.e104059.
70. Simeonov, L.I., Kochubovski, M.V. and Simeonova, B.G. eds., 2010. Environmental heavy metal pollution and effects on child mental development: Risk assessment and prevention strategies. Springer.

71. Singh, K.P., Mohan, D., Sinha, S. and Dalwani, R., 2004. Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural, and environmental quality in the wastewater disposal area. Chemosphere, 55(2), pp.227-255.
72. Soylak, M., Tuzen, M., Narin, I. and Sari, H., 2004. Comparison of microwave, dry and wet digestion procedures for the determination of trace metal. Journal of Food and Drug Analysis, 12(3), pp.254-258.
73. Srinivas N, Rao SR, Kumar KS. Trace metal accumulation in vegetables grown in industrial and semi-urban areas-a case study. Applied Ecology and Environmental Research, 2009; 7(2): 131-9.
74. Stančić1,Z Vujević1,D., Gomaz1,A. Bogdan1,S. and Vincek2,D. Detection of heavy metals in common vegetable s at Varaždin City Market, Croatia Arh Hig Rada Toksikol 2016;67:340-350.
75. Szefer, P. and Nriagu, J.O., 2006. Mineral components in foods. CRC Press.
76. Tan M, Sudjadi, Astuti, Rohman. Validation and quantitative analysis of cadmium, chromium, copper, nickel, and lead in snake fruit by Inductively Coupled Plasma-Atomic Emission Spectroscopy. J App Pharm Sci, 2018; 8(02): 044-048.
77. Türkdoğan, M.K., Kilicel, F., Kara, K., Tuncer, I. and Uygan, I., 2003. Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey. Environmental toxicology and pharmacology, 13(3), pp.175-179.
78. Tuzen M., Soylak M., Trace Heavy Metal Levels in Microwave Digested Honey Samples from Middle Anatolia, Turkey, Journal of Food and Drug Analysis,2005, 13 (4), 343-347.
79. USAID-KAVES Project 2014 Kenya Agricultural Value Chain Enterprises
80. Uždavinienė, D. and Tautkus, S., 2007. Determination of calcium in mineral waters by flame atomic absorption spectrometry. Chemija, 18(4), 34-37.
81. Vohora, S.B., Rizwan, M. and Khan, J.A., 1973. Medicinal uses of common Indian vegetables. Planta medica, 23(04), pp.381-393.

82. Wang, X., Sato, T., Xing, B. and Tao, S., 2005. Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. Science of the total environment, 350(1-3), pp.28-37.
83. WHO, FAO. Report of the 24th session of the codex committee on nutrition and foods for special dietary uses. Joint FAO/WHO food standards programme codex alimentarius commission. Geneva, Switzerland: World Health Organization; 2003.
84. Yebirzaf Yeshiwas*, Esubalew Tadele. Review on Heavy Metal Contamination in Vegetables Grown in Ethiopia and Its Economic Welfare Implications. JBio,Ag and Healhcare 2017, Vol.7, No.17,2017.