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Evaluation Content of Selenium, Mercury and Chromium in Commonly Consumed Food (Vegetable and Staple Foods) in Addis Ababa

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LIST OF ABBREVIATIONS

AA	Atomic Absorption
ANOVA	Analysis of Variance
BAM	Bacteriological Analytical Manual
CCA	Copper, Chromium and Arsenic
CVDs	Cardio Vascular Diseases
DMDSE	Dimethyl Diselenide
DMSE	Dim Ethyl Selenide
DNA	Deoxyribonucleic Acid
ED	Exudative diathesis
FAO	Food and Agricultural Organization
FI-HG-AAS	Flow injection Hydride Generation Technique combined with Atomic Absorption Spectrometry
GPX	Glutathione Peroxides
HG-AAS	Hydride Generation Atomic Absorption Spectrometry
HG-AFS	Hydride generation-atomic fluorescence spectrometry analysis
HNO ₃	Nitric acid
HR-ICP-MS	High resolution inductively coupled plasma mass spectrometry
ICP	Inductively Coupled Plasma
ICP	MS -Inductively Coupled Plasma Mass Spectrometry.
KBD	Kashin-Beck Disease
KD	Keshan Disease
LSD	Least Significant Difference
MI	Mil Litter
MSS	Municipal Sewage Sludge
PH	Potential of Hydrogen

RDA	Recommended Dietary Allowances
RDI	Recommended Daily Intakes
SPSS	Statistical Package for Service Solution
TPE	Teflon, Polyethylene
UK EGVM	United kingdom European Grapevine Moth
UL	Upper Limit
USDA	United State Development Association

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ABSTRACT

Food contains a wide range of essential metallic and other mineral elements like zinc, selenium (Se), mercury (Hg), chromium (Cr). The presence of such minerals in food matrix has positive and negative impact on the consumer. Therefore, determination of the content of mineral in food matrix. To determine the above minerals, first samples were collected from Nifas silk Lafto, Akaki and yeka sub-cities and then samples got digested. Finally the total chromium, mercury and selenium were determined. The total content of mercury in tomato, red onion, soil are 75.25 ± 0.375 ng/g, 77.68 ± 0.975 μ g/kg, 49.23 ± 0.275 ng/g respectively (This is mean of Nifas Silk Lafto and Akaki Sub-cities). It wasn't detected in waste water, bread and injera. The level of chromium in tomato, red onion wastewater, and soil are 18.50 ± 2.00 mg/kg, 14.50 ± 1.00 μ g/g, 187.25 ± 4.25 mg/L, 122.00 ± 7.50 μ g/g in that order. It wasn't detected in bread and injera. And also the concentration of selenium in red onion, tomato, soil, wastewater, injera and bread are mean \pm SD 8.015 ± 0.035 μ g/kg, $4.80 \pm$ ng/g, 2.40 ± 0.15 μ g/kg, 1.275 ± 0.125 ng/mL, 7.875 ± 1.475 μ g/kg and 1.55 ± 0.05 ng/g respectively. In all minerals, there is no significant difference within column (area), that implies rejection of alternative hypothesis at $p < 0.05$. But there is significant difference within row (sample) indicating the non-acceptance of the null hypothesis at $p < 0.05$ except in the case of chromium where no significant difference was seen between red onion and tomato. Therefore, it is possible to conclude that the content of chromium in wastewater is very high. The level of selenium in red onion and none fermented injera is also high. The same conclusion goes to mercury content in tomato and red onion. The recommended level of mercury found in food matrix is very small or nil (WHO), where as in the case of selenium the recommended content is for most vegetables and fruits 1-20 ng/g, for cereal based food 10-550 ng/g (WHO/FAO 2001) and Maximum limitation of chromium in vegetable and fruit 2.3 mg/kg (WHO/FAO, 2001).

Key words: Selenium, Mercury, Chromium, Red onion, Tomato, Soil, Wastewater, Injera and Bread.

1 INTRODUCTION

1.1 Background

Food contains a wide range of essential metallic elements such as copper, zinc, selenium etc.; Food could cause problems for consumers, if mineral found in food matrix in excess. Other metallic elements like cadmium, mercury, lead, chromium are harmful to health if food-stuffs containing them are regularly consumed. The majorities of heavy metals are natural components of the earth's crust and can enter as a result of human activity like industrial and agricultural processes (Kenness, 1992).

A heavy metal is a general collective term which is applied to the group of metals and metalloids with an atomic density greater than 4g/cm^3 . Heavy metals are often grouped in to the essential micronutrients (Fe, Mn, Zn, Cu, Ni, and Mo) which are of importance to plants. The toxic heavy metals (Cd, Cr, Pb, and Hg) are dangerous for consumer even if they exit in small amount. Metals and other elements can naturally be found in food or can enter food as result of human activities like industrial processes. Metals of particular concern related to harmful effects on human health are mercury, lead, and cadmium, tin and arsenic. The toxicity of these metals is in part due to the fact that they accumulate in biological tissues, a process known as bioaccumulation. This process of bioaccumulation of metals occurs in all living organisms as a result of exposure to metals in food and the environment.

The main concern in relation to the toxicity of mercury in the general population is the potential effect of organic mercury, for example, on the brain and intellect of young children. While long term exposure in both children and adult can cause damage to the kidney, reproductive system, immune system and nervous system (food safety Authority of Ireland, 2009). Mercury is a global pollutant with complex and unusual chemical and physical properties. The major natural source of mercury is the degassing (the removal of gas from a liquid or solid or from a vacuum system) of the Earth's crust, emissions from volcanoes and evaporation from natural bodies of water.

Mercury is highly toxic to human health posing a particular threat to development of children *in utero* and early in life. It occurs naturally, and exists in various forms: *elemental* (or metallic); *inorganic* (e.g. Mercuric chloride); and *organic* (e.g., methyl- and ethyl-mercury). All these forms have different toxicities and implications for health and also for measures to prevent exposure. Elemental mercury is a liquid that vaporizes readily. It can stay for up to a year in the atmosphere where it can be transported and deposited globally. It ultimately settles in the sediment of lakes, rivers or bays where it is transformed into methyl-mercury, absorbed by phytoplankton, ingested by zooplankton and fish; it also get accumulated especially in long-lived predatory species such as shark and swordfish (WHO, 2007).

The term “trace minerals” refers to minerals which may be found in foods amounting well below 50 mg/kg. It would have some toxicological or nutritional significance when going above 50 mg/kg (WHO, 1987). Trace elements are needed throughout life by humans and other living organisms in small quantities. Both insufficient and excessive intakes of trace elements can have negative consequences on human health (Zinaye, 2014).

Selenium is a trace element that naturally exists in many foods, and available as a dietary supplement. It is a well known growth factor for plants and essential for human even though it may a manifest toxic effect at exceedingly high amounts. Selenium is deemed to play antioxidant role like vitamin E. It would protect the cell membrane from the pernicious (harmful) action of peroxide. It also exists in two forms: inorganic and organic. Both forms can be good dietary sources of selenium.

Elemental Se is very stable and highly insoluble in water. Under reduced conditions selenides and other soluble Se compounds occur in certain soils. These can be converted into elemental Se, and therefore become unavailable for absorption by plants. This process can also remove Se from active recycling, and thus reduce the possibility of environmental pollution (WHO, 1987). According to world health organization recent finding about selenium has enormous significance for health of humans. determination).

1.2 Statement of Problems

Pollution of Environment occurs mostly in areas where anthropogenic activities are high. That is why the urban areas are more likely to experience metal poisoning sources of anthropogenic activities. These anthropogenic activities range from mining to smelting of non-ferrous metals, from addition of manures to sewage sludge, from fertilizers to pesticides to soil. A number of studies identify the risks in relation to increased soil metal concentration and consequent crop uptake. These heavy metals do not only pollute the surface water but also the soil and ground water that subsequently enter the plants. human exposure to metals may be through diet or absorption into the skin. Heavy metals are there in foods in minute quantities playing important roles in the metabolism and life processes of man.

Soils contain both macro and micro-elements which are all necessary to support the growth of plants. Plants take up metals by absorbing from the soil, and also from parts of plant exposed to air with heavy metals. It has been reported that more than average of heavy metal consumption by human (lead, cadmium, arsenic, chromium and mercury) happens through food from plant origin. Urban soil contamination is mainly caused by anthropogenic activities such as traffic emission, industrial emission, and domestic emission, weathering of building and asphalt road surface and so on. However, in agricultural soil, the presence of heavy metals is due to smelting, waste disposal, vehicle exhaust, pesticides and use of fertilizers (Larbie *et al.*, 2014). Cd, Hg, Cr, Pb and As, are present in industrial effluents released to the lakes and river (Larissa *et al.*, 2011).

Determination the level of mercury (Hg) leads (Pb) and cadmium (Cd) in Hawassa and Ziway Lake and edible fish. The result showed that, due to bio-accumulation and bio-magnification of these metals in the body of the fish, the concentration of fish samples was higher than that of the water concentration (Kiflom and Tarekegn, 2014). The concentration of mercury and selenium in other food matrix is not determined in Ethiopia. The level of selenium and mercury was also expected higher in vegetable than soil and water because due to bio-accumulation and bio-magnification of these elements.

Tomato (*Solanum lycopersium* L.) is one of the most widely grown vegetable in the world. Asia and Africa account for about 79 percent of the global tomato area with about 65 percent of world output (FAO, 2009). Although tomato requires a relatively cool and dry climate for high yield and better quality, it is adapted to a wide range of climate conditions from temperate to hot and humid to tropical (Naika *et al.*, 2009).

Tomato contains nutrients like vitamin A, vitamin C, potassium, phosphorus magnesium and calcium (USDA, 2009). It also contains lycopene, which is a good antioxidant compound that reduce the risk of cancer (AVRDC, 2010). This study was aimed at determining the levels of heavy metals and other trace mineral like selenium in tomato, red onion, surrounding soil, water, injera and bread.

Over the past years, there has been growing issues on the safety of consuming fresh vegetables such as cabbage, tomato, onion, etc. As result there is questionable food safety, due to their possible health risk associated with pesticide and fertilizer applications. Mostly tomato and red onion are cultivated among the urban and pre-urban dwellers. Bread and injera are may be contaminated by different human activities.

The walleyes leather and leather production private share company, kangaroo shoe factory private company, Ethiopian leather and leather production institute and others industries are disposing their wastes to rivers found near to the industries. Along those rivers, there are many farmers cultivating vegetables like cabbage, tomato, potato; onion and chili are more common. It is normally assumed that these vegetables are more exposed to be contaminated by heavy metals and trace minerals from the soil. Therefore if tomato, onion which are cultivated nearest that river are contaminated by mercury and chromium, injera and bread also may contaminated by heavy metals and trace minerals due to different human and natural activities.



Figure 1: Releasing of waste to the river from leather factories

This figure could show that the releasing of wastage from leather factories. This waste matters are contaminated the environment.

2 Objectives

2.1 General objective

The objective of this study is to quantify the amount of selenium, mercury and chromium in injera, bread, tomato, wastewater, soil and red onion in Addis Ababa.

2.2 Specific objectives

The study specifically intends to:

- ❖ To determine the content of selenium in injera, bread, tomato and red onion.
- ❖ To identify the content of mercury (Hg) in injera, bread, tomato, soil wastewater and red onion that is lower, similar or higher to the other research of selenium content in food.
- ❖ To determine the level of chromium in wastewater, soil, red onion, tomato, injera and bread.
- ❖ To see how contaminated tomato, onion, bread and injera are/ are not with chromium, mercury and selenium or not.

3 LITERATURE REVIEW

3.1 Mineral of Food

Food contains some of the minerals that play essential roles in the body. Mineral content of foods may vary considerably depending on many factors. However, additional factors may influence the quantitative and qualitative composition of mineral content. In the case of plant it is well known that deficiency of some element in the soil, in plant and oppositely a higher concentration, if soil rich in a given. The element may cause increased levels of this element in plant tissue. As an example, selenium content of soil may be mentioned. In Finland very low content of selenium was determined in soil causing very low selenium concentration of in cereals (Lasztity, 1999).

Minerals in food comprise large and diverse groups of elements and complex ions. Foods are containing generally more than, 50 different elements that may be confirmed by up to date analytical techniques. Many of these are nutritionally required by humans. One part of elements is found in the body in significant concentration (e.g. Calcium, phosphorus, magnesium, sodium, potassium, and sulfur). Many of the other elements are present in very small concentration known as a microelement or trace elements. According to our present knowledge one part of the elements fulfill physiological function in the body. To this group belongs e.g. Iron, copper, cobalt, manganese, zinc, iodine, selenium, chromium, molybdenum, fluorine, vanadium, silicon and nickel. These elements are called essential trace element. Whether other trace elements are contaminated or their biological role is until now unknown.

Minerals are common constituents of all foods of animals and plant origin. The concentration of minerals in foods and in drinking water varies depending, on the case of plants, on species, soil composition, conditions of growing, etc. (Lasztity, 1999).

In another way, there are also toxic minerals like; - mercury, lead, cadmium, chromium

3.1.1 Chemistry of Minerals

Minerals component may be present in food in different forms. Elements free form don't occur in food (some pharmaceutical products or food supplement may contain reduce ions) in tissue fluids the metals are present as cations. Among anions the chloride, some phosphate, hydrocarbon and sulfate may be mentioned. Many metals are present in the food bounded to an organic compound.

3.1.2 Minerals can be Toxic

Like some fat-soluble vitamins, minerals can be toxic if ingested in high amounts. However, mineral toxicity from an excess dietary intake is rare in healthy individuals because the amounts found in foods are not that high, and most Americans do not generally exceed the UL (upper limit) for minerals. Also, the body can adapt its absorption or excretion of many minerals according to its needs. In other words, the small intestine can reduce absorption of a particular mineral if the body already contains an adequate amount, and the kidneys can filter excess minerals from the blood and excrete them through the urine.

However, ingesting more than the UL of a mineral, such as by taking large amounts of supplements, the food may have excess amount of such minerals, that may lead to illness and even death. Excessive levels of magnesium in the blood can result in heart problems or an inability to breathe, 8 while excessive amounts of calcium may cause nausea, vomiting, loss of appetite, increased urination, kidney toxicity, confusion, and irregular heart rhythm. Although mineral toxicity is more likely to occur in individuals with certain conditions, such as acute or chronic kidney failure, even in healthy people ingesting excessive amounts of minerals will lead to unwanted side effects. Though minerals have much in common with each other and work together to support numerous body functions, they are each important for individual reasons (Nel *et al.*, 2006).

3.2 Contaminations of Food

Food contamination can come from multiple sources, including antibiotics and hormones in meat and dairy products, as well as microbial contamination that can lead to illness. A

wide variety of chemicals from man-made sources may be found in or on foods. Contaminants in foods may come from the application of pesticides to crops, from transport of industrial chemicals in the environment, or from chemicals used in food packaging products. Children's diets are an important pathway of exposure to some environmental contaminants. Children may be at a greater risk for exposures to contaminants because they consume more food relative to their body weight than do adults. Additionally, children's dietary patterns are often less varied than those of adults, suggesting that there are greater opportunities for continuous exposure to a food borne contaminant than in adults (America's Children and the Environment, 2011).

3.2.1 Food Contamination by Heavy metals

Heavy metals are toxic metals having density five times greater than water. They are toxic for all living organisms. In humans, they enter into body through various ways like ingestion, absorption, etc. They become harmful when their accumulation rate is more than their discharge. They accumulate gradually in the body over a long time and are toxic.

Heavy metal contamination is a major problem in the environment and of medium sized cities due to anthropogenic activities.

Human activities may contribute largely in their production such as burning of fossil fuel, mining and use of many chemicals for crop growth, etc. Wastewater also contains heavy metal and when it is applied to crops it can cause threat to soil and plants growing in that soil. Wastewater, health risks can be determined by different indices. Generally, heavy metals cannot be removed from wastewater and when they enter into the soil, interfere with the plant roots - these plants when eaten by animals or humans they enter into the food chain. Plants along with other nutrients also uptake lead, cadmium and others; their accumulation may be affected by the concentration time of exposure and climatic factor.

Heavy metals affect the quality and production of crops and influence atmospheric and water quality. These contaminations are important and of concern because of increasing

demand for food safety. There are different sources of heavy metal such as natural and man-made, as industries and airborne sources. These heavy metals have severe effects on plants, animals, humans and ultimately on the environment (Sardar *et al.*, 20

3.2.2 Sources of Heavy Metals:

Heavy metals exist naturally in the soil from the soil forming processes of disintegration of parent resources at rare levels ($<1000\text{mg kg}^{-1}$) and infrequently poisonous (Kabata, 2001). Heavy metals come from man-made sources trying to be more moving in the soil, hereafter biologically available than soil forming phenomena (Kuo *et al.*, 1983). Metal-containing solids at polluted places can originate from an extensive variation of manmade sources in terms of metal mine stakeouts, leaded gasoline and paints that are lead based, application of fertilizer, discarding high metal wastes in inappropriately protected landfills, animal manures, bio solids (sewage sludge), coal combustion remainders, compost, petrochemicals, pesticides and deposition in atmosphere (Khan *et al.*, 2008). are discussed here.

A)Fertilizers:

Huge amounts of fertilizers are frequently applied to soils in concentrated farming systems to deliver suitable N, K and P for crop growth. The complexes used to offer these elements comprise rare quantity of heavy metals (for example Cadmium and Lead) as contaminations that, after continual fertilizer application may meaningfully reproduced their quantity in the soil. Metals like Cadmium and Lead have no recognized physiological actions. Certain phosphate fertilizer applications unintentionally add Cod and other possibly dangerous elements for the soil, including F, Pb and Hg (Raven *et al.*, 1998).

B) Pesticides:

Numerous known insecticides used widely in agriculture in the past contained considerable amounts of metals. As in the recent past, near 10% of the chemicals have permitted for use as pesticides and in UK fungicides were based on complexes which comprise Cu, Hg, Pb, Mn or Zn. For Example, such pesticides that are fungicidal spray

that contained copper like copper oxychloride and Bordeaux mixture that consists of copper sulphate (Jones and Jarvis, 1981). Lead arsenate was applied in fruit orchards for several years to regulate some parasitic pests. Compounds that consist of arsenic were applied widely to control livestock ticks and for the control of insects in banana in New Zealand and Australia, wood has been well-preserved with preparations of Copper, Chromium and Arsenic (CCA), and there are currently many neglected sites where soil amounts of these elements largely surpass background focuses. Such pollution has the capacity to result in problems, especially if sites are improved for other agricultural or non-agricultural purposes. Linked with fertilizers, the application of these ingredients has been more restricted, being localized to special sites or crops (McLaughlin *et al.*, 2000).

C) Bio solids and Manures:

The supply of various bio solids, for example., composts, cattle manure and municipal sewage sludge (MSS) to land unconsciously points towards the build-up of heavy metals like Arsenic, Cadmium, Chromium, Copper, Lead as well as Hg, Se, Ni, Mo, Zn, Sb, in the soil. Some animal wastes like livestock, poultry and pig manures created in agriculture are usually supplied to crops and meadows either in the form of solids or semi solids (Sumner, 2000).

D) Wastewater:

The supply of wastewater coming from industrial land municipal sources and associated wastes to soil dates back 400 years and is a usual practice now in different parts of the globe. Internationally, it is projected that 20 million (20m) hectares (ha) of arable land are watered with some sort of wastewater. In various African and Asian cities, studies propose that agriculture relied on the water with wastewater contributes to 50 % of the vegetable source to urban areas (Bur, 2007). Farmers usually are not worried about environmental welfares or dangerous and are principally concerned with maximizing their productions and profits. Though the metal amounts in wastewater discharges are comparatively low, long-term watering of land with such wastewater can finally cause heavy metal build-up in the soil.

E) Industrial wastes and metal mining processes:

3.3 Effect of heavy metals on consumers

Withdrawal, tailings (larger particles settled at the end of the flotation cell while mining) are straight discharged in naturally occurring depressions, as well as onsite wetlands resulting in high amounts (Devolve, 2003). Widely, Lead (Pb) and zinc (Zn) mineral withdrawal and smelting have resulted in pollution of soil that causes danger to human and environmental health.

Arsenic, cadmium, mercury, lead and inorganic tin account for a majority of heavy metal poisoning cases involving food products. Levels of arsenic are naturally high in fish and seafood. Found in soil due to the presence of insecticides, fungicides, sludge and commercial fertilizers, cadmium can contaminate agricultural food products. Mercury is an industrial pollutant as well as a by-product of volcanic emissions.

In recent years, a number of products, ranging from protein shakes to baby formula to fresh produce, have been linked to heavy metal contamination. Spurred by consumer calls for safer products, regulatory agencies around the globe are taking steps to detect and minimize the presence of hazardous heavy metals in food

3.4 Types of Foods

3.4.1 Tomato

Tomato is one of the most important vegetable in Africa and Asia and the contents account for more than 65% of globe production (FAO, 2008). Tomato is rich in nutrients such as vitamin A, vitamin C, potassium, Calcium, magnesium, phosphorus and antioxidant, which are important to well balanced diets. Tomato is also an important dietary component because it contains high levels of lycopene, antioxidants that reduce

the risk associated with several cancers and neurodegenerative diseases (USDA, 2009). Tomato is susceptible to several insect and mite pests as well as plant diseases. Chemical pesticides are being used indiscriminately to manage these pests in South and South Asia and part of Africa. In addition to, chemical fertilizers and insecticides are sometimes overused in tomato production, which may be contaminating ground water. Intensive agrochemical use in tomato husbandry substantially increased the production cost and may pose a serious risk to producer, consumer and the overall health of the environment.

AVRDC-The world vegetable center development and promoted a safer tomato production strategy in Taiwan from 2005 to 2007. This strategy has reduced reliance on chemical pesticides. The strategy is being promoted in South Asia, and is ready for broad-scale adoption in other tomato growing countries in tropic.

3.4.1 Onion

The germination of onions is slow at 6 to 7°C, the optimum germination temperature range is 10 to 35°C, and the maximum temperature is 40°C. The onion crop is adapted to a growing season with air temperatures at 13 to 24°C. Low temperatures early in the season are desirable with higher temperatures after bulb formation. The onion is tolerant of frost but seedlings are generally only tolerant down to -1°C. Some bunching onion cultivars overwinter in the milder areas of Atlantic Canada. Adequate soil moisture is required due to the relatively small root system. Onions are sensitive to photoperiod. Long days are favorable to onion production as this enhances leaf development and formation which, in turn, is directly related to bulb size.

Early varieties require 13 hours for bulb initiation while late varieties require 16 hours for bulb initiation. Onions begin to form bulbs when day length reaches the appropriate duration for the cultivar, providing temperatures are high enough. Early seeding or transplanting is therefore essential. Cool weather during early growth of the plant promotes formation of seed stalks (bolting). The onion bulbs are quicker in warm than at cool temperatures. At 40°C bulbing is retarded. In the onion there is an interaction between day length and temperature in the bulbing process of an individual cultivar

Onions have a shallow and limited root system which explore mainly the upper 30 cm of the soil. This crop should be irrigated frequently throughout the growing season. The soil moisture should not be allowed to fall below 50%. Most soils should receive 2.5 cm of water per week from the combination of rainfall and irrigation. Soil moisture is important in the growth of new roots; the soil moisture must reach the base of the bulb periodically if the newly formed roots from the stem are to grow into the soil. New roots will not grow into dry soil (UN Advisor committee on vegetable and crops, 2009).

3.4.2 Bread

Bread is a food prepared from a dough flour and water, usually by baking. Throughout recorded history it has been popular around the world and is one of the oldest artificial foods, having been of importance since the dawn of agriculture.

There are many combinations and proportions of types of flour and other ingredients, and also of different traditional recipes and modes of preparation of bread. As a result, there are wide varieties of types, shapes, sizes, and textures of breads in various regions. Bread may be raised by many different processes ranging from the use of naturally occurring microbes to high-pressure artificial aeration methods during preparation or baking. However, some products are left unleavened, either for preference, or for traditional or religious reasons. Many non-cereal ingredients may be included, ranging from fruits and nuts to various fats. Commercial bread in particular, commonly contains additives, some of them non-nutritional, to improve flavor, texture, color, shelf life, or ease of manufacturing.

Depending on local custom and convenience, bread may be served in various forms at any meal of the day. It also is eaten as a snack, or used as an ingredient in other culinary preparations, such as fried items coated in crumbs to prevent sticking, or the bland main component of a bread pudding, or stuffing designed to fill cavities or retain juices that otherwise might drip away.

Partly because of its importance as a basic foodstuff, bread has a social and emotional significance beyond its importance in nutrition; it plays essential roles in religious rituals and secular culture. Contains wheat 9-grain wheat Whole wheat flour, enriched flour (wheat flour, niacin, iron, thiamine mono nitrate, riboflavin, folic acid), yeast, sugar, wheat gluten, contains 2% or less of: calcium carbonate, soybean oil, salt, wheat, rye, yellow corn, oats, triticale, brown rice, barley, flax seed, millet, sorghum, refinery syrup, vitamin D2, sunflower lecithin, natural flavor, honey, ascorbic acid, yeast extract, enzymes (U.S.A. product ingredients, 2015).

3.4.3 Injera

Injera is thin, fermented Ethiopian traditional bread made from flour, water and starter (*ersho*), which is a fluid, saved from previously fermented dough. Teff (*Eragrostis tef* (Zucc) Trotter) is the most popular grain for making *injera*, although other grains such as sorghum, maize, barley, wheat and finger millet are sometimes used. Teff [*Eragrostis tef* (Zucc) Trotter] has the largest share of area (23.42%, 2.6 million hectares) under cereal cultivation and third (after maize and wheat) in terms of grain production (18.57%, 29.9 million quintals) in Ethiopia. There is a growing interest in teff grain utilization because of nutritional merits (whole grain); the protein is essentially free of gluten. About two-third of Ethiopian diet consists of *injera* and it accounts for about two-third of the daily protein intake of the Ethiopian population.

„*Wot*“ in the Ethiopian national language (Amharic) means a stew which is made from plant and animal products is served with *injera*. The preparation of teff *injera* consists of two stages of natural fermentation, which last for about 24 to 72 hours, depending on ambient temperatures. The only required ingredients are the teff flour and water. The method of processing of *injera* from its raw materials to the final product involves preparing and mixing the ingredients to dough, which is fermented and subsequently thinned to a batter. The batter is then poured onto a hot griddle in a thin layer to cook, and to develop color, flavor and texture.

The major quality attribute of a good *injera* is its slightly sour taste, which is due to the acidic (low pH) nature of *injera*. Unfortunately, *injera* storage period does not usually exceed three days at ambient temperature (temperature in the highlands of Ethiopia is between 17 and 25⁰C) under the traditional storage conditions essentially due to mould spoilage. It is a common practice to discard moldy *injera*. However, in times of food scarcity, mould *injera* is sun dried and prepared for consumption (Ashgrie and Dawit 2012).

3.5 Types of Heavy Metals and Trace Minerals

Heavy metals are present naturally in relatively low amounts in the earth's crust. Through food, drinking water and the air, humans absorb trace amounts of these elements. Trace amounts of some heavy metals, such as selenium, zinc, and copper, are keys to maintaining the metabolism of the human body (Brandl, 2012). Toxic metals, including "heavy metals," are individual metals and metal compounds that negatively affect people's health. In very small amounts, many of these metals are necessary to support life. However, in larger amounts, they become toxic. They may build up in biological systems and become a significant health hazard. Streambed sediments exhibit the same binding characteristics found in the normal soil environment. As a result, many heavy metals tend to be sequestered at the bottom of water bodies. The aquatic environment is more susceptible to the harmful effects of heavy metal pollution because aquatic organisms are in close and prolonged contact with the soluble metals

3.5.1 Chromium (Cr)

Heavy metal contamination is one of the major ecological problems worldwide, leading to losses in agricultural yields and harmfully affects human health when contaminants enter the food chain. Chromium (Cr) is the seventh most abundant element on earth; and the second largest contributor of ground water, soil and sediment contamination (Shrivastava and Thakur, 2006). Contamination of agricultural fields with Cr is very toxic to both human being and plants and has been led a major environmental concern over the last few decades (Tiwari *et al.*, 2013). Release of Cr compounds to the environment is

mainly due to electroplating, leather tanning, metal finishing, corrosion control and pigment manufacturing industries (Liu *et al.*, 2011).

Cr is released to the atmosphere, water and soil, respectively (Shen, 2002). Cr has two stable forms, i.e. trivalent Cr (III) and hexavalent Cr (VI) form, later one is more toxic. These two forms are inter and convertible in soil due to various microbial activities. High amount of Cr in the soil reduces plant growth. Moreover, at high concentrations, Cr acts as a mutagen, teratogen and carcinogen. Cr also causes deleterious effects on physiological processes of plants such as the photosynthesis and mineral nutrition (Diwan *et al.*, 2010). Thus, there is an urgent and imperative need to develop efficient techniques for Cr removal from the environment. However, some plants are able to withstand a very high level of Cr through their physiological mechanism. Phytoremediation has recently attracted a great deal of attention as an alternative means of soil decontamination. This process is cost-effective, eco-friendly and can be applied to large areas

3.5.1.1 Health Effects of Chromium (Cr)

Adverse health effects associated with Cr (VI) exposure include occupational asthma, eye irritation and damage, perforated eardrums, respiratory irritation, kidney damage, liver damage, pulmonary congestion and edema, upper abdominal pain, nose irritation and damage, respiratory cancer, skin irritation, and erosion and discoloration of the teeth. Some workers can also develop an allergic skin reaction, called allergic contact dermatitis. This occurs from handling liquids or solids containing Cr (VI) such as portal and cement. Allergic contact dermatitis is long-lasting and more severe with repeated skin exposure. Furthermore, contact with non-intact skin can lead to ulceration of the skin sometimes referred to as chrome ulcers. Chrome ulcers are crusted, painless lesions showing a pitted ulcer covered with fluid (U.S. Hexavalent Chromium Occupational Safety & Health Administration, 2010).

Cancer;-All hexavalent chromium Cr (VI)) chemical compounds are considered carcinogenic to workers. The risk of developing lung, nasal, and sinus cancer increases with the amount of hexavalent chromium inhaled and the length of time the worker is

exposed. Studies of workers in chromate production, chromate pigment, and chrome electroplating industries employed before the 1980s show increased rates of lung cancer mortality. Certain hexavalent chromium compounds produced lung cancer in animals that had the compounds placed directly in their lungs (U.S. Hexavalent Chromium Occupational Safety & Health Administration, 2010).

Eyes;-Direct eye contact with chromic acid or chromate dusts can cause permanent eye damage. Avoid eye contact with dusts, fumes, smoke, liquids, mists, and aerosols containing hexavalent chromium.

Respiratory Tract;-Hexavalent chromium can irritate the nose, throat, and lungs. Repeated or prolonged exposure can damage the mucous membranes of the nasal passages and result in ulcers. In severe cases, exposure causes perforation of the septum (the wall separating the nasal passages). Some employees become allergic to hexavalent chromium so that inhaling the chromate compounds can cause asthma symptoms such as wheezing and shortness of breath.

Skin;-Prolonged skin contact can result in dermatitis and skin ulcers. Some workers develop an allergic sensitization to chromium. In sensitized workers, contact with even small amounts can cause a serious skin rash.

3.5.1.2 The level of Chromium in Vegetable

The vegetable farm at Ejersa is among the vegetable farms of the area, where a substantial amount of vegetables are being produced seasonally. The farm is irrigated with the wastewater from the effluent by the tannery and from underground water that may be contaminated by the effluent of the tannery. Before several decades, the water from the river in the area was clean. However, with the increase in the urban population and industrialization, the water has now become contaminated with various pollutants among which are heavy metals (Aklilu et al., 2013). The result of study showed that the concentration of chromium in matured vegetable is 2.4mg/kg.

3.5.2 Mercury

Mercury is readily absorbed via respiratory tract. Inflammation of mouth and gums, swelling of salivary glands, excessive flow of saliva, loosening of teeth; kidney damage; muscle tremors, jerky gait, spasms of extremities; personality changes, discouragement, depression, irritability, nervousness, dementia, loss of motor coordination are common among people exposed to mercury. Mercury accumulation in fish may harm the fish and other animals that consume them. Birds and mammals that eat the fish are then exposed to. Some effects on wildlife can include reduced fertility, damaged kidneys, slower growth and development, abnormal behavior and even death. Whales and dolphins may also be at high risk from mercury exposure.

Mercury is highly toxic to human health, posing a particular threat to the development of the child *in utero* and early in life. It occurs naturally and exists in various forms: *elemental* (or metallic); inorganic (e.g. mercuric chloride); and organic (e.g., methyl- and ethyl mercury). These forms all have different toxicities and implications for health and for measures to prevent exposure. Elemental mercury is a liquid that vaporizes readily. It can stay for up to a year in the atmosphere, where it can be transported and deposited globally. It ultimately settles in the sediment of lakes, rivers or bays where it is transformed into methyl mercury, absorbed by phytoplankton, ingested by zooplankton and fish, and accumulates especially in long-lived predatory species, such as shark and swordfish (WHO, 2007).

3.5.2.1 Health Effects of Mercury

- ✓ Elemental and methyl mercury are toxic to the central and peripheral nervous system. The inhalation of mercury vapor can produce harmful effects on the nervous, digestive and immune systems, lungs and kidneys, and may be fatal. The inorganic salts of mercury are corrosive to the skin, eyes and gastrointestinal tract, and may induce kidney toxicity if ingested
- ✓ Neurological and behavioral disorders may be observed after inhalation, ingestion or dermal application of different mercury compounds...

- ✓ Children are especially vulnerable and may be exposed directly by eating contaminated fish (WHO, 2007).

3.5.2.2 The way of Plant Types of Food Contaminated with Mercury

Soils contain both macro and elements which are all necessary to support the growth of plants. It stake up metals by absorbing from the soil and also from parts of the plant that is exposed to air contaminated with heavy metals. It has been reported that more than average of heavy metal consumption (lead, cadmium, arsenic and mercury) is through food from plant origin. Urban soil contamination is mainly caused by anthropogenic activities such as traffic emission, industrial emission, and domestic emission, weathering of building and pavement surface and so on. However, in agricultural soil, the presence of heavy metals is due to smelting, waste disposal, vehicle exhaust, pesticides and use of fertilizers. Among these fruit tomato and onion are the most affected (Larbiea et al., 2014).

3.5.3 Selenium

Selenium has an atomic weight of 78.96 and its atomic number is 34. It belongs to Group six along with oxygen, sulfur, tellurium and polonium, between arsenic and bromine in Period 4 of the Periodic Table of the elements. The chemical properties are intermediate between those of sulfur and tellurium, and its compounds resemble the corresponding sulfur and tellurium compounds in behavior. The outer electronic configuration of Se is $3d^{10}4s^24p^2$, with three completely filled inner shells. Its position in the Periodic table and electron configuration place Se is in the important group of half metals or metalloids, which are neither fully metallic nor non-metallic.

3.5.3.1 Selenium isotopes

Selenium has six naturally occurring stable isotopes, ^{74}Se , ^{76}Se , ^{77}Se , ^{78}Se , ^{80}Se , and ^{82}Se . The two most abundant are ^{80}Se (49.8%) and ^{78}Se (23.5%). The availability of these isotopes has enabled researchers to make significant advances in our understanding of the biological roles of Se. They have been particularly useful in the analysis of Se

bioavailability. The ability to label specific chemical forms of Se with stable isotopes has also helped to understand the role that these different species play in the complex biochemistry of the element (Crews, 2001).

3.5.3.2 Physical properties of selenium

Selenium has unique electrical properties. Its conductivity, which is low in the dark, is increased by 100-fold on exposure to light which also generates a small electrical current in the element. Due to the ability to conduct more easily in one direction than in the other, it is known as an asymmetrical semiconductor (Reilly, 2006). Elemental Se is very stable and highly insoluble. Under reducing conditions, selenates and other soluble Se compounds that occur in certain soils can be converted into elemental Se and therefore become unavailable for absorption by plants. This process can also remove Se from active recycling and thus reduce the possibility of environmental pollution (WHO, 1987).

3.5.3.3 Organic and Inorganic selenium compounds

The organic compounds of Se have similar properties, but not identical with organo sulfur compounds both physically and chemically. Some volatile organo-Se compounds, such as dimethyl selenide (DMSE) and dimethyl diselenide (DMDSE) are the result of biomethylation of inorganic Se by microorganisms. More complex seleno amino acids (e.g. seleno methionine and selenocysteine) can be found in biological tissues as a consequence of biological pathways by which Se is incorporated into proteins, like sulfur. Se reacts both with metals and nonmetals, gaining electrons to form ionic compounds containing the selenide ion, Se^{2-} , for example FeSe , Al_3Se_2 , and Na_2Se . Se also forms covalent compounds with the other substances. Naturally occurring oxidation states of Se in elemental and combined forms are -2 (e.g. Na_2Se , sodium selenide), (Se, elemental Se), +4 (e.g. Na_2SeO_3 , sodium selenite), and +6 (e.g. Na_2SeO_4 , sodium selenate (Reilly, 2006)). Se in the +6 states is stable under both acidic and alkaline conditions. This is of significance with regard to the availability for absorption by plant roots in alkaline soils in which selenates naturally occur. Se forms halides by direct combination with fluorine, chlorine, and bromine, but not with iodine. It also forms oxyhalides, namely oxychloride

(SeOCl₂), which is a powerful chlorinating agent and oxidant, capable of reacting with other substances explosively (Elis, 2008).

3.5.3.4 Importance of selenium for human and animals

Selenium is one of the rarest elements. It was once known only for its toxicity, but has come to be recognized for its importance in human and animal health. Se is a key component of the so-called Factor 3, an active principle found in brewer's yeast able to replace vitamin E in preventing liver necrosis in rats and chickens. This therefore indicated that Selenium plays a vital role in the metabolism of animals. Furthermore, researchers were able to demonstrate that a variety of enzootic in cattle and sheep, as well as exudative diathesis in chickens could be controlled effectively by Se, replacing vitamin E.

Selenium was shown to be an essential component of glutathione peroxides (GPX), an enzyme that provides antioxidant protection by reducing levels of hydro peroxides in cells. Subsequent findings showed that Se, acting through the expression of a wide range of seleno-proteins, has diverse roles in mammals. It is involved in thyroid hormone homeostasis, immunity and fertility, and many other activities in addition to its antioxidant activities. It has also been shown to have anticancer properties, to act as a growth factor and to play important roles in the regulation of synthesis of leukotrienes, thromboxanes, and prostaglandins, as well as other metabolic functions.

Se levels in foods can vary widely, not only between countries but also between regions in a country. This variation appears to be a result of differing availability of Se in the soils on which an animal is raised or a plant is grown. Typically the major dietary sources in many diets are cereals, meat products, and sea foods. Only small amounts are usually contributed by dairy products, and still less by vegetables and fruits. It is noted that Se levels in cereal grain products can be quite high. According to the surveys performed by Lyons et al. (2004), Se concentration in cereals were ranging from <5 to 750µg/kg, with most in the 100 to 300µg/kg range. Much of this variation was associated with spatial variation in soil Se (Elis, 2008).

3.5.3.5 Endemic Diseases Related to Selenium Deficiency in Humans

The findings of various Se-responsive conditions in farm animals directed the attention of investigators to the possibility that humans might also be at risk from an inadequate intake of the element. Keshan disease (KD) attracted a great deal of attention in the mid 1930s. Women of childbearing age and children 2 to 10 years of age are the most susceptible to the disease. The main features of KD include acute or chronic cardiac insufficiency, cardiac enlargement, congestive heart failure, and cardiac arrhythmias. KD is classified into four types: acute, sub-acute, chronic and latent. Depending on which type is present, symptoms can vary widely.

The etiology of KD remains uncertain. Xie and coworkers concluded that the disease was the result of a combination of several factors, one of which is Se deficiency. This view has been further supported by Vanderpas *et al.* 1990 who found that low levels of Se intakes in Zaire, Africa do not cause KD. This implies that additional factors, besides Se deficiency, are involved; notably Coxsackie B3 virus, a cardiophilic RNA virus. In China, KD has almost been eliminated largely through the provision of selenised salt. Kashin-Beck disease (KBD), also known as Urov disease, is an osteoarthropathy. It is characterized by chronic disabling degeneration and necrosis of the joints and the epiphysial-plate cartilages of the arms and legs. It becomes evident in childhood and adolescence and leads to varying degrees of disability throughout adult life (Elis, 2008).

3.5.3.6 Recommended intakes

Selenium is an essential element, and therefore various national and international organizations have established recommended daily intakes of selenium (FAO/WHO, 1998). Recommended intakes of 6–21µg of selenium is per day for infants and children, according to age, 26 and 30µg of selenium per day for adolescent females and males, respectively, and 26 and 35µg of selenium per day for adult females and males, respectively. In 2000, the United States National Academy of Sciences Panel on Dietary Oxidants and Related Compounds revised the recommended intake of selenium to 55µg/day for both men and women and 70µg/day for women during pregnancy and

lactation. Recommended selenium intakes for children are between 15µg/day for infants 0–6 months of age and 30µg/day. Na₂Se

3.5.3.7 Adequacy of Selenium Intakes

The dietary intakes of Se in different countries based on published results vary considerably, depending on the methods used to assess intakes, as well as on several other causes. These include the difference in food consumption patterns, especially in the types of staple food consumed in addition to representative population samples which might vary from one investigation to another. A selection of estimated daily Se intakes by adults in a number of countries has been compiled and presented in. These range from as low as 3 µg/day in parts of China where endemic Se deficiency occurs, to as high as nearly 5 mg/day in another part of the same country where selenosis has been reported. In between these extremes, intakes in many countries appear to be both safe and adequate according to the (US Food and Nutrition Board, 1980).

3.5.3.9 Enhancing Selenium Content in Foods

A relatively simple way to achieve an increase in Se levels in food crops is by adding Se to fertilizers. This has been advocated as a practical way of addressing the problem of falling Se intakes in the UK. Also suggested that it is indeed a convenient and economical way of increasing dietary intake and can be implemented without introducing toxic levels of Se in crops, animals fed on them, or in the human population. Animal foodstuffs are also enriched with Se. This is achieved by adding Se supplements, both inorganic and organic, to their rations. According to a recent report, these value added products include Se-enriched milk developed in Korea, the “Megaegg” which has added vitamin E in addition to Se in Ireland, as well as selenium enriched chicken and pork (Foley, 2005).

4 MATERIALS AND METHOD

4.1 Study Area

The study is focused on areas commonly cultivating onion and tomato in the city, and also on bread and injera available for consumption in Addis Ababa, Ethiopia. Addis Ababa is located at $9^{\circ} 1' 44''$ north of the equator and $38^{\circ} 44' 24''$ east longitude with an area of 540 km^2 and 2500 meters above sea level found in 1886. According to the census estimate, the city had a population of about three million (CSA, 2010). Its average temperature is 16°C while its annual rainfall is 121 millimeter. Addis Ababa is the seat of the Africa Union, the United Nation Economic Commission for Africa and more than 92 embassies and consulates. The city is divided into ten administrative sub-cities (Gruma *et al.*, 2006).

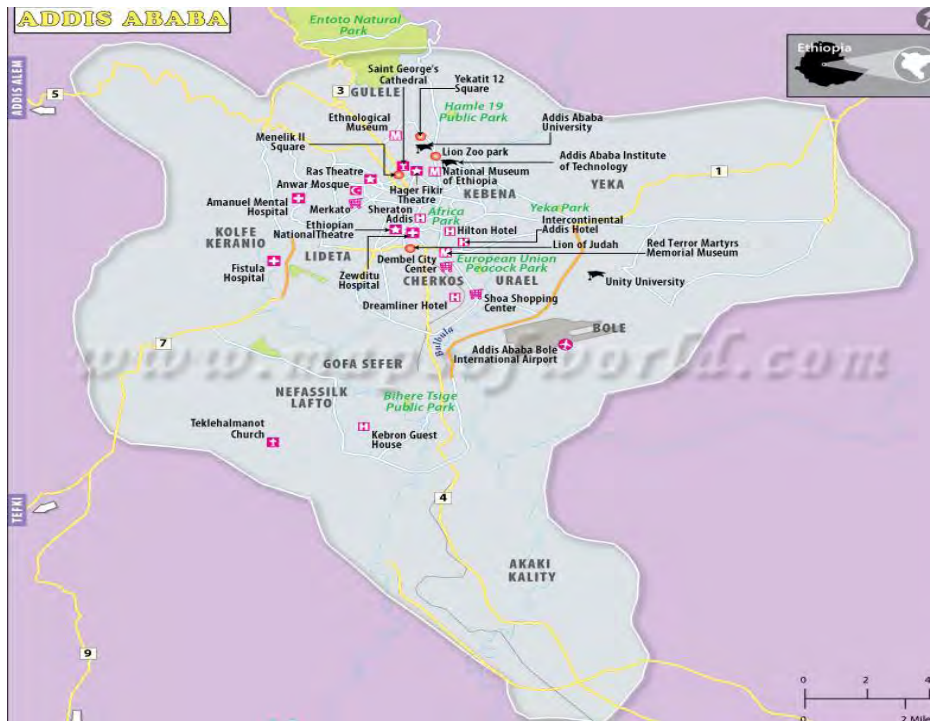


Figure 2: Addis Ababa city carat shows samples collection. Source www.maps of world.com Addis Ababa

4.2 Study Design

Laboratory cross-sectional study design was there to determine the level of mercury, chromium, and selenium in tomato, red onion, relative soil, wastewater, injera and bread. The determination of these minerals was done at three laboratories: Addis Ababa University food science and nutrition laboratory, Bless Agri food Laboratory services PLC and Geological Survey of Ethiopia Laboratory.

4.3 Sampling Design.

Samples of tomatoes, red onion plus their respective soils and wastewater were collected from two different locations in the Akaki and Nifa Silk Lafto sub city. Two kilograms of each sample of tomatoes and red onion were randomly collected at each site to obtain representative samples. They were wrapped in pre-cleaned aluminum foil, packed in polyethylene bags and transported to food and nutrition department of Addis Ababa University for pre-treatment. The collected samples were transported by ice box to Addis Ababa University food science and research laboratory for analysis.

4.4 Sample Preparation

All except wastewater samples were dried by oven until almost all moisture evaporated from the samples. Then the dried samples were grinded by grinder to small fines. After that the wet digestion (with microwave digester) was applied.

4.5 Microwave Digestion.

At the beginning 0.498g of dry sample powder tomato, red onion, injera and bread was accurately weighed into the vessel. Then after 5ml 67.5% of HNO₃ and 2ml of 30% of H₂O₂ in weighed tomato, red onion, injera and bread samples was added. And for soil sample 10ml of 67.5% of HNO₃ and 5ml of 30% of H₂O₂ were added on an inside vessel sample for soil digestion. The vessel placed on rotating turntable of microwave oven, and the six steps digestion procedure the instruction information that was used as shown in table 2.

Table 1: Manufacturing standard microwave digestion instruction information

No.	Steps	Pressure in kg/cm ²	Temperature in °c	Power in watt (w)	Time in minute
1	1	20	120	2000	300
2	2	20	120	2000	180
3	3	35	180	2000	300
4	4	35	180	2000	300
5	5	45	200	2000	300
6	6	45	200	2000	600

All sample result stayed on until the light changes yellow. Then the resulting solution was cooled and filtered through paper in volumetric flask, and made up to 25 ml volume with distilled water. Blank of selenium , chromium and Mercury were run in each procedure for quality assurance.

4.6 Wet Digestion with Kjeldahl apparatus chromium analysis

4.6.1 Food Sample Digestion

Food samples were then dried with oven and grinded. About 2.56g was weighed by using analytical balance and placed in a flask; 15ml of NH_3O was added to the samples, then ashed for 50^oc for 10 mints and for 100^oc for 25 mints. After the mixture got cooled down, 5ml of nitric acid was added and then the mixture heated again at 150^oc for 30 mints. The mixture was placed on a Whatman filter paper No.42 into 50ml volumetric flask. The filter paper was washed with distal water. The solution in the flask was diluted to 50ml with distal water. The solution was used to determine level of chromium in the samples.

4.6.2. Soil Sample Extraction

Large particles of soil were sorted out. The soil was then dried with oven and grid. 2.56g of soil was weighed by using analytical balance and placed in a flask 15ml of NH_3O then added to the sample. The mixture was placed on a mechanical shaker, and shaken for 15 minutes. Filtration was carried out through. The mixture was placed on a Whatman filter paper No.42 in into 50ml volumetric flask. The filter paper was washed with distal water. The solution in the flask was diluted to 50ml with distal water. The solution was used to determine level of chromium in the soil (Drocio and Rodriguez, 1996).

4.7 Moisture Content Determination

To determine the percent of the moisture content, AOAC method (AOAC) was used. Brief duplicates of 5.00g of samples were placed in pre-washed and dried oven; then heated in the oven at 105°c for 3.5 hours. They were, then, cooled in desiccators and weighed in to constant weight.

Percent moisture = $100 - (\text{wt of dry sample} / \text{wt of wet sample}) \times 100$

4.8 Instrumental Analyses

The metals and minerals (Hg, Cr and Se) analyses were conducted using hydride generation with vapor generation accessory (VGA- 76) (made in Australia) and flame Atomic absorption Spectrophotometers (AAS) at Geological Survey of Ethiopia. Concentration working standard of selenium are (Se) 0.02, 0.04 and 0.08 ($\mu\text{g}\ \text{L}$), for Mercury (Hg) 0.001, 0.0025, 0.005 and 0.010($\mu\text{g}\ \text{L}$), and for Chromium (Cr) 2.50, 5.00 and 10.00($\text{mg}\ \text{L}$).

4.9 Nutrition Information Analysis

4.9.1 Protein Content Determination

To determine the protein content AOAC method (AOAC. 2000) was used. 0.5g of sample powder red onion, tomato, bread and injera were placed in washed and dried Kjeldahl flask HYP- 1008 six ml of H₂SO₄ were added to each sample and kept for overnight. About 3.5 ml H₂O₂ and 3g mixture of CuSO₄ were added to each flask and digested at 370^oc for four hours. The digested was diluted with 25ml distilled water and neutralized by 40%NaOH. The ammonium formed by digestion was distilled into boric acid solution. Borate anion (proportional to the amount of nitrogen) was titrated with standardized 0.1 N of HCl; the nitrogen content was calculated

$$\text{Nitrogen \%} = (1.4007 \times (V_s - V_b) \times N) / W$$

Where V = volume of HCl by the sample and blank

N = normality

W= weight of sample percent of protein. This can be calculated by multiplying amount of nitrogen by factors of 6.25% for vegetable and bread; 5.38% for injera. Blank was run in each procedure to assure the quality of analyses.

4.9.2 Fat Content Determination

AOAC method (AOAC. 2005) was used to determine the fat content. Brief duplicate of 2.00 g of powder sample was weighed in pre-dried porous ceramic extraction thimble plugged with cotton. Sample was weighed in vacuum dried oven. Fifty ml of hydrous diethyl ether was added in pre-dried and weighed in extraction beaker. The beaker was pushed up into condenser apparatus of soxhlet and put on heater extraction for four hours at 55^oc (2 hours soaking and extraction time for each). Then, the heater was lowered and then cooled. The extraction beaker was removed and dried at 105^oc for 10 minutes in an oven. Then cooled down in a desiccator and weighed. The crude fat content was calculated and expressed as percentage. The weight of fat is in gram in the sample = (weight of beaker + weight of sample) - weight of beaker.

Crud fat % of = (g of fat in sample /g of dried sample) x 100

4.9.3 Ash Determination

To determine the ash content AOAC (AOAC- 2005) was used. Duplicates of 5.00 g of powder sample were placed in pre-washed, dried, weighed and marked crucibles to be ashed at 550°C in Muffle furnace for eight hours. Then sample were cooled down in desiccators and weighed again.

The ash content was calculated as follows:

% ash (dry basis) = [(wt after ashing –wt of crucible)/ (original sample wt)] x100.

4.9.4 Determine of Carbohydrate

The carbohydrate content was determined by a different method. Subtraction of the sums of the weights of the crude protein, total fat moisture, and ash in the total weight of the sampled food were subtracted from 100%

% total carbohydrate = 100%- (% moisture +%protein +%fat +%ash

4.9.5 Calculate of the Energy Value

The energy yield bread, injera, tomato and red onion were calculated as:

Energy value =carbohydrate = fat = protein; and fat 9 kcal/ gram;

Protein and carbohydrate for each = 4kcal/gram

4.10 Statistical Analyses

Data analysis was computed using SPSS software version 20. Descriptive statistics like range and arithmetic mean \pm SD were applied to express the concentration of each metal and the trace minerals (selenium). One-way ANOVA was employed to test the available minerals among vegetables, foods, wastewater, soil and between the two areas(sample collection area) .

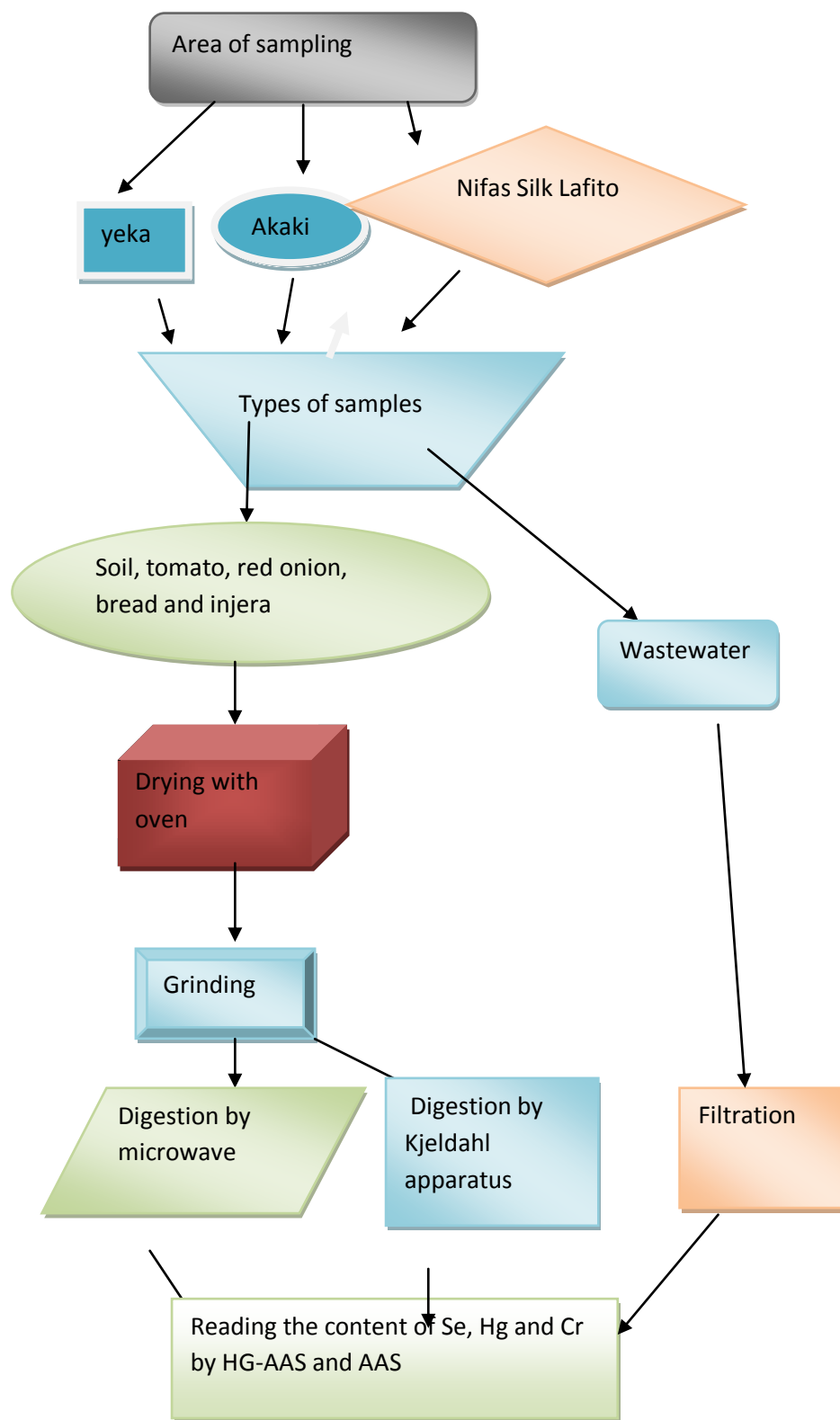


Figure 3: Summary of methodology

5 RESULTS AND DISCUSSIONS

5.1 Nutritional Value of Bread, Injera, Tomato and Red onion

The nutritional composition of injera, bread, tomato and red onion were determined.

Table 2: Comparison of observed nutritional value for tomato, red onion, injera and bread.

Samples	Nutritional status			
	Fat	Protein	Ash	Carbohydrate
Tomato	4.40±0.99 ^a	18.35±0.035 ^a	9.90±0.50 ^a	67.32 ^a
Red onion	3.00±0.50 ^b	11.80±0.77 ^b	12.80±0.02 ^b	72.40 ^b
Injera	2.94±0.44 ^k	9.91±.075 ^k	2.60±0.40 ^k	84.55 ^k
Bread	6.66±0.23 ^{abk}	8.48±0.01 ^{abk}	5.20±1.00 ^{abk}	79.66 ^{abk}

5.2 Heavy Metals and Trace Minerals Values of Tomato, Red Onion Wastewater, Soil, Bread and Injera

Table 3: Comparison level of mercury (Hg) in tomato, red onion, wastewater, soil, bread, and injera (in $\mu\text{g}/\text{kg}$ or /L)

Samples	No. of trail	Area of samples was collected	
		Nifas Silk Lafito- sub city	Akaki –sub city
Red onion	2	77.75 \pm 0.25 ^a	79.65 \pm 0.35 ^a
Tomato	2	75.00 \pm 0.00 ^{bb}	75.75 \pm 1.25 ^{bb}
Soil	2	49.50 \pm 0.50 ^k	48.95 \pm 0.25 ^k
Wastewater	2	ND	
Injera	2	ND	
Bread	2	ND	

ND= not detected

There is no a significant difference within column (area), there is rejection of alternative hypothesis at $p < 0.05$. But there is significant difference within row (sample), which means that not accepted the null hypothesis at $p < 0.05$

The level of mercury (Hg) in red onion, the one from Akaki sub-city, is greater than that of Nifas Silk Lafto sub-city which was 1.81 $\mu\text{g}/\text{kg}$ in mean difference. However the concentration mercury (Hg) in red onion, which was collected from Akaki sub-city, is not significantly different from the one collected in Nifas Silk Lafto sub-city sample area. There is also considerable difference in concentration of mercury (Hg) in tomato, which was 75.00 \pm 0.00 in Nifas Silk Lafto sub-city and 75.75 \pm 1.25 in Akaki sub-city. This shows that the level of mercury (Hg) in tomato from Akaki sub-city is higher than that of Nifas Silk Lafto sub-city. In the case of soil sample, the level of mercury (Hg) in Akaki sub-city is lower than that of Nifas Silk Lafto.

The concentration of mercury (Hg) among samples is also different. In the red onion, the level of mercury (Hg) is greater than in the tomato by $2.75\mu\text{g}/\text{kg}$ in Nifas Silk Lafto. The difference in Akaki sub-city is $3.90\mu\text{g}/\text{kg}$. The level of mercury (Hg) in soil is less than that of red onion by $28.25\mu\text{g}/\text{kg}$ in Nifas Silk Lafto sub-city; it is $30.70\mu\text{g}/\text{kg}$ in Akaki sub-city. The level of mercury (Hg) in tomato is greater than that of in the soil by $25.50\mu\text{g}/\text{kg}$ in Nifas Silk Lafto and in Akaki sub-city by $26.80\mu\text{g}/\text{kg}$.

Generally the level of mercury (Hg) in red onion and tomato is higher in Nifas Silk Lafto sub-city and lower in both samples, from Akaki sub-city, whereas the level mercury (Hg) in soil is higher in Nifas Silk Lafto that of Akaki sub-city soil. (Kaur *et al.*, 2014).

In a previous study by (Moskářová and Zemberyová, 1996), the level of mercury (Hg) was $86.00\mu\text{g}/\text{kg}$. In case of presence study, the level of mercury in tomato is $75.375\pm 0.375\mu\text{g}/\text{kg}$. This difference may be associated with extreme soil contamination. (kameyama, 1992).



Figure 4: Different types of activities, which are the source of mercury (Hg). These figures show that this waste matter is the main source of contamination of the environment with mercury.

The level of mercury in tomato is higher than the findings in the previous study. But the difference isn't that significant. In another study (Husain *et al.*, 1995), the level of mercury in imported tomato in Kuwait was $4.17\mu\text{g}/\text{kg}$.

The present study finding, the level of mercury in red onion is $78.7 \pm 0.95 \mu\text{g}/\text{kg}$. In case of the previous study (U.S. food and Drug administration, 2014), the level of mercury in red onion is $10 \mu\text{g}/\text{kg}$. In both studies the level of mercury in red onion has great difference. The most possible reason for that would be the difference of contaminated levels samples with mercury.

The concentration of mercury in my study in relative soil is $49.225 \pm 0.275 \mu\text{g}/\text{kg}$ whereas another study (USEPR, 1997) found it to be $29 - 133 \text{ng}/\text{g}$. This shows that the result of this study is in between the result of the above study by the United States Environmental Protection Agency. So it can be concluded that determination of mercury in relative soil is satisfactory. But the conclusion can't change the difference in result between the two studies in consideration here. The level of mercury in vegetable is higher than relative soil. These happened due to bio-accumulation and bio-magnification of metal.

However, wastewater and bread samples level of mercury are $3.00 \text{ng}/\text{g}$ in hospital wastewater (after treatment with activated charcoal method) (Kaur, *et al.*, 2014) and $20.00 \text{ng}/\text{g}$ (U.S. food and Drug administration, 2014) respectively.

Table 4: Comparison level of chromium (Cr) in tomato, red onion, relative soil, wastewater, injera and bread in (mg/kg or L)

Samples	No. of trials	Area of sampling	
		Nifas- Silk Lafito sub-city	Akaki sub city-
Tomato	2	20.50±0.5 ^a	16.50±1.50 ^a
Red onion	2	15.50±1.5 ^a	13.50±1.50 ^a
Wastewater	2	191.50±1.5 ^k	183.00±2.00 ^m
Soil	2	129.50±1.5 ⁿ	114.50±2.50 ^b
		Yeka sub- city	
Injera	2	ND	
Bread	2	ND	

There is no a significant difference within column (area), that impels there is accepting of null hypothesis at $p < 0.05$.

But there is significant difference within row (sample), excluding between red onion and tomato, which means that not accepted the null hypothesis at $p < 0.05$.

Maximum limitation of chromium in vegetable and fruit 2.3mg/kg (WHO/FAO, 2001)

The level of chromium (Cr) in red onion is 15.50±1.5 in Nifas Silk Lafto and 13.50±1.5 in Akaki sub-city. This shows that the level of chromium (Cr) in red onion, which was cultivated in Akaki sub-city, is less than that of Nifas Silk Lafto sub-city by 2.00µg/kg. The concentration of chromium (Cr) in red onion, the one from Akaki is significantly different from Nifas Silk Lafto sub-city. The concentration of chromium (Cr) in tomato, which was collected from Nifas Silk Lafto is greater than Akaki by 4.00µg/kg. The level of chromium in tomato is considerably different between both areas and other samples.

The level of chromium in wastewater was average in both areas ($187.25 \pm 4.25 \mu\text{g}/\text{kg}$) twelve fold greater than both the tomato and red onion $18.50 \pm 2.00 \text{ng}/\text{g}$ and 14.50 ± 1.00 in that order. Whereas the concentration Cr in wastewater is greater at $62.00 \mu\text{g}/\text{kg}$ given the result of the soil concentration is. This implies there is highly significant difference in the levels of the samples in this study. The concentration of chromium in wastewater collected from Nifas Silk Lafto is also significantly different from that of Akaki. This shows that the release of wastewater by leather and leather related factories is the main cause of for the contamination of the nearby river waters with chromium.

The concentration of chromium in the soil is almost six fold greater than both tomato and red onion. These results lead me to conclude that the level of chromium decreases from wastewater to soil to vegetable. As table 3 shows, the concentration of chromium in injera and bread samples not detected.

Chromium exists almost exclusively in the Cr (III) or Cr (VI) oxidation state. Predominate use of chromium in industry unfortunately intrudes environmental concern. In the environment Cr (III) can't cause a significant problem. That is why it is soluble in acidic solution; it typically precipitates as hydroxide in alkaline solutions. And it is somewhat important for mammalian physiological activities. In contrast, Cr (VI) seems to serve no useful biological purpose to living things. It is also harmful (toxic) in biological system (Dionex, 2014).

In a previous study (Shadreck and Tawanda, 2013), the level of total chromium in wastewaters is $40.96 \pm 0.012 \mu\text{g}/\text{g}$ whereas in case of my study, the concentration of total Cr in wastewater is $187.25 \pm 4.25 \text{mg}/\text{kg}$ mean in both area. The result of total chromium in the pervious and this study showed huge difference. The result of this study exceeds the previous result by $(146.29) \mu\text{g}/\text{g}$. This means the result of the present study is almost four fold of the result of the study (Shadreck and Tawanda, 2013). In Nifas Silik Lafto and Akaki sub-cities, there are different leather and leather product factories, for instance, Waliya's leather and leather product factory, kangaroo shoes, factory etc. These factories often release their wastes to the nearby river. As I saw it for myself in person, many

farmers cultivate different kinds of vegetables along the river through irrigation. The above results showed in both areas the river wastewater has been highly contaminated with chromium.

The concentration of total chromium in relative soil is 122.00 ± 7.50 mg/kg whereas in a previous study (MDE, 2008) the result of total chromium in the soil was 23,00 mg/kg. The result of the previous study is less than the present study $119.00 \mu\text{g/g}$. This indicates the sample, which was studied by my thesis, is highly contaminated with total chromium.

Another study (Shadreck and Tawanda, 2013) showed that the level of chromium is 1.16 ± 0.03 mg/kg. The result of the concentration of total chromium in soil in both the other previous studies is much less than the findings in this thesis. However the study by (Fisseha, 2008) showed the concentration of chromium in soil is 115.00mg/kg. The result of Fisseha's study and this study are almost the same. The reason for that could be the fact that both studies got conducted in Addis Ababa but different location.

The concentration of total chromium in tomato in the present study taking the average of both areas is 18.50 ± 2.00 mg/kg whereas in the study of (Aklilu *et al.*, 2013), the level of chromium in tomato is 2.33 mg/kg. The result of the present study exceeds at $16.17 \mu\text{g/g}$. This indicates that the samples incorporated in my study are found much contaminated with chromium. But the result of the study by (Fisseha, 2008) Show the concentration of chromium in lettuce was 9.47mg /kg. The result of this study is almost similar with the findings in my study. Still there is difference. Perhaps the difference happens due to the sample differences, difference in season, and difference in area of samples.

In my study the concentration of chromium in red onion average of both areas is 14.50 ± 1.00 $\mu\text{g/g}$. But in the study of Aklilu *et al.*, 2013), the level of chromium is 1.55mg/kg. The result of both studies has a big difference. This could be because of the present study sample is highly contaminated. The concentration of chromium in injera and bread is not detected. And I found no research conducted in this area despite my search for such a thesis.

Table 5 Comparison level of selenium (Se) in tomato, red onion, relative soil and wastewater in ($\mu\text{g}/\text{kg}$ or L)

Sample type	NO. OF trail	Area of samples was collected	
		Nifas Silk Lafito	Akaki
Red onion	2	8.05 ± 0.05^a	7.98 ± 0.08^a
Tomato	2	4.45 ± 0.15^b	5.15 ± 0.05^b
Soil	2	2.25 ± 0.15^m	2.55 ± 0.05^m
Wastewater	2	1.15 ± 0.05^k	1.40 ± 0.10^k

There is no a significant difference within column (area), that impels the rejection of alternative hypothesis at $p < 0.05$.

But there is significant difference within row (sample), which means that not accepted the null hypothesis at $p < 0.05$.

The recommended content of selenium is for most vegetables and fruits $1-20\text{ng}/\text{g}$, for cereal based food $10-550\text{ng}/\text{g}$ (WHO/FAO 2001).

The level of selenium in red onion is $8.015 \pm 0.035\text{ng}/\text{g}$ (the average of both areas). That means, it is higher by $3.215\mu\text{g}/\text{kg}$ than concentration of selenium in tomato. The concentration of selenium in the soil is almost four fold less than that of the concentration of selenium in red onion. Of course the source selenium, which exists in vegetable, is the concentration and the availability selenium in the soil. However the pervious seed selenium concentration, irrigation water and fertilizer may also be the source of selenium existing in the vegetable. Therefore the level of selenium in vegetable must be greater than that of the soil because of due to bio-accumulation and bio-magnification of the element.

Diet is the main source of selenium for humans. The primary distributor of selenium is plants. The amount of selenium in plants highly depends both on the amount and the availability of selenium in the soil. And this may vary geographically, which in turn can affect the selenium states of entire community (Reilly, 1996). Selenium enters the food

web when plants accumulate it from soil and incorporate it during synthesis of new molecule. Many plant species accumulate large amounts of it from soil and water (North American Metals Council, 2003).

In the case of previous study (Jimenez, and Mateos, 1996), the level of selenium in plants and in soil is 5.80ng/g and 3.30ng/g respectively. Whereas the result of my study showed that, the concentration of selenium in red onion, tomato and soil are $8.015 \pm 0.035 \mu\text{g}/\text{kg}$, $4.80 \pm 0.35 \text{ng}/\text{g}$ and $2.40 \pm 0.15 \mu\text{g}/\text{g}$ (the average of both areas) respectively. Determination of selenium in previous and present study somewhat have similar result. However there is a lit bit difference. The geographical effect may have causes for difference. The selenium content of plant Foods, therefore, varies from countries to countries, being generally low in UK and Europe and higher in china (The British Nutrition Foundation, 2001).

In another research (*M a j a s*, 2003) Selenium content in soil highly varies. It varies from 0.10 to 2.00ng/g in most soils. This also shows that the result of this study has similar selenium content in the soil. In case of wastewater in this study, the selenium content in wastewater is $1.275 \pm 0.125 \text{ng}/\text{g}$. In the case of a previous study (Sarojam, 2010), the level of selenium in wastewater is $3.00 \mu\text{g}/\text{L}$.

Table 6: Comparison level of selenium (Se) in injera and bread (ng/g)

Samples	No. of trials	status samples	
		Fermented yeka	Non fermented yeka
Injetra	2	6.40±0.10 ^a	9.35±0.25 ^a
Bread	2	ND	1.55±.05 ^a

The content of selenium (Se) in fermented injera is 6.40±0.10ng/g whereas in fermented bread can't be detected. Therefore the level of selenium in the two fermented samples is having a significant difference in value. In the case of non fermented injera, the concentration of Se is greater by 2.95ng/g from fermented injera. During fermentation all physicochemical parameters including minerals decreased, except moisture and total titratable acidity contents, which increased after fermentation (Assohoun *et al.*, 2013). The result of my research shows the concentration of selenium increased after fermentation in both injera and bread samples. The level of selenium between bread and injera samples has a significant difference from at $p < 0.05$.

In another pervious study (Elis, 2008), the content of selenium in UK bread is 4.30–9.20µg/kg. In my thesis study, the level of selenium in none fermented bread is 1.55±.05ng/g. The results of the two studies show difference. This difference may have happened due to the selenium concentration of soil, which the wheat consumed during its growth and the sensitivity of read martial may also cause the difference.

6 Conclusions and Recommendation

6.1 Conclusion

Two samples of tomato, red onion, soil and wastewater were taken from two different areas. While other conditions remain constant, the content of chromium (Cr), mercury (Hg) and selenium (Se) were analyzed; so were proximate for tomato and red onion. In addition to these, bread and injera samples were taken from household and got analyzed for their the level of chromium (Cr), mercury (Hg) and selenium (Se) and their proximate. The level of mercury in red onion is higher than that of tomato and soil. However it was not detected in wastewater, bread and injera samples. This may have happened due to the mercury contamination level of samples.

The content of chromium (Cr) in wastewater is higher i.e 65mg/kg than that the one in the soil. It was almost 12 fold greater in tomato and red onion. But it was not detected in bread and injera samples. This indicates the wastewater was highly contaminated by chromium-infested-wastes from the leather industries. Often chromium is used in leather industries for tanning purposes. But the level of chromium decreases as it moves from wastewater to soil, from soil to vegetable. It was not detected at all in bread and injera samples.

The concentration of selenium (Se) in red onion is two times more than in tomato and four folds in soil. Selenium content in wastewater is the least one. In the case of bread and injera, the process of fermentation has an effect on the level of selenium. However in non-fermented-injera, it was almost six times greater than it was in non-fermented bread. But it was not detected in fermented bread. The recommended level of mercury found in food matrix is very small or nil (WHO), where as in the case of selenium the recommended content is for most vegetables and fruits 1-20ng/g, for cereal based food 10-550ng/g (WHO/FAO 2001) and Maximum limitation of chromium in vegetable and fruit 2.3mg/kg (WHO/FAO, 2001)

6.2 Recommendation

- The present study may need further research to exactly confirm the findings, the source and ways of contamination particularly in the case of selenium and mercury content of the samples.
- The effect of grain varieties and soil type may have effect on the level of mineral contents on the samples.
- Food processing may be one the reasons; I assume these limitations could be reasons to go for a further research.
- Industry-wastages are the main causes for contamination of samples, especially in the content of chromium.
- Therefore, the government should establish mechanisms to keep rivers free from industrial-wastages.

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ANNEX

ANNEX 1: The moisture content of red onion, tomato, injera and bread.

Wt Crucible(g)	Wt Samples(g)	Wt of Sa and cr	Measure 1	Measure 2	% Of T Solid	%Moisture 100-%sol
22.15	5.00	27.15	22.68	22.68	10.4	89.6
21.69	5.00	26.69	22.20	23.19		
21.94	5.03	26.97	22.37	22.36	8.7	91.3
22.23	5.00	27.26	22.78	22.78		
21.72	5.03	26.73	23.38	23.38	33.6	66.4
21.81	5.02	26.83	23.37	23.37		
21.48	4.99	26.47	25.05	25.04	34.6	65.4
22.04	5.01	27.03	25.39	25.30		
21.98	5.00	26.98	26.45	26.41	88.2	11.7
22.98	5.00	27.98	27.36	27.36		

ANNEX 2: Summery table for analysis of variance (ANOVA) for mean infestation of.

2.1: Level of selenium in a given samples are significantly different from each other or not

	(I) samples	(J) samples	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	red onion	Tomato	3.21500*	.26396	.000	2.4821	3.9479
		Soil	5.76500*	.26396	.000	5.0321	6.4979
		wastewater	6.74000*	.26396	.000	6.0071	7.4729
	tomato	red onion	-3.21500*	.26396	.000	-3.9479	-2.4821
		Soil	2.55000*	.26396	.001	1.8171	3.2829
		wastewater	3.52500*	.26396	.000	2.7921	4.2579
	soil	red onion	-5.76500*	.26396	.000	-6.4979	-5.0321
		Tomato	-2.55000*	.26396	.001	-3.2829	-1.8171
		wastewater	.97500*	.26396	.021	.2421	1.7079
wastewater	red onion	-6.74000*	.26396	.000	-7.4729	-6.0071	
	Tomato	-3.52500*	.26396	.000	-4.2579	-2.7921	
	Soil	-.97500*	.26396	.021	-1.7079	-.2421	

*. The mean difference is significant at the 0.05 level.

2.2: level of nutrition status in the given samples are significantly different one from each other or not

	(I) samples	(J) samples	Mean Difference (I- J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	tomato	red onion	-7.88500	3.60563	.051	-15.8209	.0509
		injera	-3.37500	3.60563	.369	-11.3109	4.5609
		bread	-70.50667*	3.89452	.000	-79.0785	-61.9349
	red onion	tomato	7.88500	3.60563	.051	-.0509	15.8209
		injera	4.51000	3.60563	.237	-3.4259	12.4459
		bread	-62.62167*	3.89452	.000	-71.1935	-54.0499
	injera	tomato	3.37500	3.60563	.369	-4.5609	11.3109
		red onion	-4.51000	3.60563	.237	-12.4459	3.4259
		bread	-67.13167*	3.89452	.000	-75.7035	-58.5599
bread	tomato	70.50667*	3.89452	.000	61.9349	79.0785	
	red onion	62.62167*	3.89452	.000	54.0499	71.1935	
	injera	67.13167*	3.89452	.000	58.5599	75.7035	

*. The mean difference is significant at the 0.05 level.

2.3: Level of mercury (Hg) in a given samples is significant difference or not

(I) sampl type	(J) sampl type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
red onion	tomato	2.82500*	.95438	.016	.6661	4.9839
	soil	28.97500*	.95438	.000	26.8161	31.1339
Tomato	red onion	-2.82500*	.95438	.016	-4.9839	-.6661
	soil	26.15000*	.95438	.000	23.9911	28.3089
Soil	red onion	-28.97500*	.95438	.000	-31.1339	-26.8161
	tomato	-26.15000*	.95438	.000	-28.3089	-23.9911

*. The mean difference is significant at the 0.05 level.

2.4: Level of chromium in a given samples are significantly different from each other or not.

(I) samples	(J) samples	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Tomato	red onion	4.00000	6.29766	.560	-13.4851	21.4851
	wastewater	-168.75000*	6.29766	.000	-186.2351	-151.2649
	soil	-103.50057*	6.29766	.000	-120.9857	-86.0155
red onion	tomato	-4.00000	6.29766	.560	-21.4851	13.4851
	wastewater	-172.75000*	6.29766	.000	-190.2351	-155.2649
	soil	-107.50057*	6.29766	.000	-124.9857	-90.0155
wastewater	tomato	168.75000*	6.29766	.000	151.2649	186.2351
	red onion	172.75000*	6.29766	.000	155.2649	190.2351
	soil	65.24943*	6.29766	.000	47.7643	82.7345
Soil	tomato	103.50057*	6.29766	.000	86.0155	120.9857
	red onion	107.50057*	6.29766	.000	90.0155	124.9857
	wastewater	-65.24943*	6.29766	.000	-82.7345	-47.7643

*. The mean difference is significant at the 0.05 level.

Annex 3: laboratory working photo





I, the undersigned, hereby declare that this thesis is my original work, has not been presented for a degree in any other university and all sources material used for the study have been correctly acknowledge.

Name Solomon Bereded

Signature -----

Place Addis Ababa University

Date of submission -----

The thesis has been submitted with my approval and advisor

Name Ashagrie Zewdu

Signature -----

Date-----