ASSESSMENT OF HUMAN HEALTH RISK DUE TO ACCUMULATION OF HEAVY METAL IN AFRICAN GREEN LEAFY VEGETABLE IRRIGATED BY WASTE WATER IN ARUSHA MUNICIPALITY

NAIMANI SARIKIEL PALLANGYO

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL STUDIES IN OPEN UNIVERSITY OF TANZANIA

2019

CERTIFICATION

The undersigned certifies that he has read and here by recommends for acceptance by the Open University of Tanzania a dissertation entitled: ***"Assessment of Human Health Risk Due to Accumulation of Heavy Metal in African Green Leafy Vegetable Irrigated by Waste Water in Arusha Municipality*,"** in partial fulfillment of the requirements for the degree of Master of Science in Environmental Studies (MES) of the Open University of Tanzania.

…………………….……………………

Dr Josephat Alexander Saria

(Supervisor)

…………………………………………

Date

COPYRIGHT

DECLARATION

I, **Naimani Sarikiel Pallangyo,** do hereby declare that this thesis is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

....

Signature

....

Date

DEDICATION

Where would I be without my family members? My parents deserve special mention for their inseparable support and prayers. My Parents Mr. and Mrs. Sarikiel Aseri Pallangyo, in the first place the persons who put the fundament my learning character, showing me the joy of intellectual pursuit ever since I was a child.

I certainly must thank my wife Lusila Eugen Mushy, A Chemistry and Biology Teacher at Kisimiri High School, who played a paramount role in shaping my career and my two lovely children (Naaman and Diana). Blessings, patience, moral support, love and sacrifice of my wife have always provided me the strength to counter all problems, without her constant and never ending concern, support and encouragement, this would have not been realized.

ACKNOWLEDGMENT

I would like to express my deepest gratitude to my major advisor, Dr. Josephat Alexander Saria and co- advisors Prof Shabani Mbogo, Pof Emmanuel Kigadye Dr. Joel Mihale, Dr James Kisoza, Dr Emmanuel Muhache, Dr Irene, Dr Yohana Lawi, Dr Dews Ngaruko and Mr Gervas Yeyeye for their careful supervision, excellent guidance and encouragement from the very beginning of the proposal development up to the final thesis write up. I have a special respect and appreciation to them for their fatherly advice in all aspects and achievements of today’s success.

I would like to express my gratitude to the Arusha Technical College (ATC) especially Mr. Meshack Daniely Timoth who is the Director of Research Laboratory, and Mr Joseph Raymond Mruma (Laboratory Technician) of Water quality, Soil and Plant at Arusha Technical College, for offering me the opportunity of using their Laboratories for analysis of Heavy metals.

I have great pleasure in thanking The Arusha Urban Water Supply Authority (Auwsa), specifically The Sewage water department, with respect to Engineer Ansila. Also The Nelson Mandela African Institute of Science and Technology (NM-AIST), especially the Agricultural and Nutritional Research Laboratory Quality Management Representative, Mr. Paulo Martin Sanka, Miss Theresia Justine Kimario and all staff members of this section, finance staffs, transport section and human resource personal for their remarkable cooperation. My thanks also goes to Mr. David Bahati who is the Assistance Lecture at Dodoma University in Department of Physics and to all my best friends and classmates for their moral support, encouragement and sharing me idea in our stay for graduate study.

I would like to express my thanks to the Department of Environmental Studies, Open University of Tanzania, for providing me with the necessary knowledge and assistance to conduct the thesis work.

ABSTRACT

The present study was conducted to assess the heavy metals (Cu, Cd, Pb, and Cr) concentrations in irrigated water, the concentration of the given metal from soil and vegetables (*Amaranths sp* and *Ipomoea sp*), as a result of wastewater irrigation around Arusha Municipal wastewater stabilization pond were determined using Flame Atomic Absorption Spectrophotometer (FAAS). The results obtained from the edible parts of sampled vegetables (*Amaranths sp* and *Ipomoea sp*) in this study, showed concentration of Cr, Cd, Pb, and Cu was (1.40, 4.21), (309.87, 152.60), (0.50, 0.48), (281.69, 248.54) mg /Kg, respectively. Where as, concentration in the wastewater samples (mg /Kg) were Cr (0.32), Cd (607.61), Pb (0.15) and Cu (237.82). Similarly, concentrations (mg/Kg) of the metals in the soil samples were found to be 0.65, 595.90, 48.00 and 234.52 for Cr, Cd, Pb and Cu, respectively. The THQ values showed that Cd was 225.121and 457.132, Pb was 0.7081 and 0.7081, Cr was 0.0014 and 0.0041, and Cu was 9.17 and 10.39 for *Amaranths sp* and *Ipomoea sp* respectively. This study has shown that soil quality is considerably influenced by effluent discharge from the considered industries, household, hospitals. The government intervention is needed to monitor the effluents prior to discharge into receiving surroundings. Also the concerned official bodies to take necessary precaution measures for cleaning the polluted factory effluents prior to discharge.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
FAO	Food and Agricultural Organization
USEPA	United State Environmental Protection Agency
WHO	World Health Organization
FAAS	Flame Atomic Absorption Spectrophotometer
EPA	Environmental Protection Agency
HQ	Hazard Quotients
HHIR	Human Health risk index
TF	Transfer factor
Cd	Cadmium
Cu	Copper
Pb	Lead
Cr	Chromium
HMs	Heavy Metals
AGLV	African green leaves vegetables
ATC	Arusha Technical College
IQC	Internal Quality Controls
CRM	Certified Reference Materials
DIM	Daily Intake Of Metal
DIR	Daily Intake Rate
RFD	Oral Reference Dose

CHAPTER ONE

INTRODUCTION

1.1 Chapter Overview

Heavy metal contamination of farming soil and vegetables is among the most serious ecological problems in the world and Tanzania is not exceptional. The heavy metal contamination of African green leafy vegetables cannot be underscored, as these foodstuffs are important components of African diet and the rest of the world. These vegetables are known to be rich sources of minerals, fibers and vitamins as well as having beneficial ant-oxidative effects. However, intake of heavy metal-contaminated vegetables may pose a risk to the human health.

1.2 Background of the Study

Potentially harmful heavy metal contents in soils may come not only from the bedrock itself, but also from anthropogenic sources like solid or liquid waste deposits, agricultural inputs, and fallout of industrial and urban emissions (Wilson and Pyatt, 2007). Excessive accumulation in agricultural soils may result not only in soil contamination, but has also consequences for food quality and safety. So, it is essential to monitor food quality, given that plant uptake is one of the main pathways through which heavy metals (HMs) enter the food chain (Antonious and Kochhar, 2009). Heavy metals can degrade air, water, and soil quality, and subsequently cause health issues in plants, animals, and people, when they become concentrated as a result of human activities as well as natural events. Vegetables take up heavy metals and accumulate them in their edible and non-edible parts at quantities high enough to cause health problems to both animals and human beings. As an example, the consumption of contaminated food can seriously deplete some essential nutrients in the body causing a decrease of immunological defenses, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer (Oliver, 1997), reported that soil and vegetables contaminated with Pb and Cd in Copsa Mica and Baia Mare, Romania, significantly contributed to decrease human life expectancy (9-10 years) within the affected areas. Cadmium and Lead are the most toxic elements for man (Volpe et al., 2009).

In terms of environmental concentration, lead is the heavy metal closest to the level in which toxic signs manifest than any other substance (Baird, 2002). Others elements such as Cr, Co and Ni, although essential for human being, at concentrations higher than those recommended, may cause metabolic disorders. Moreover, an increasing awareness in terms of the importance of vegetables and fruits to human diet suggests that the monitoring of heavy metals in food crops should be carried out frequently, however information concerning this issue is scarce, especially in African countries. Most major cities have been concerned with heavy metal contents in vegetables (Ferré-Huguet et al., 2008).

In Sub-Saharan Africa, heavy metal contamination of widely consumed African green leafy vegetables has become a major challenge (Saria and Irene 2011), because these heavy metals are regarded as serious pollutants of aquatic ecosystems due to their environmental persistence, toxicity, bioaccumulation and bio-magnifications in the food chain (Saria, 2016). Though these heavy metals may be present at low concentration, the deposits of anthropogenic origin have raised their concentration, creating environmental problems in lakes, rivers, streams as well as households. Some of these pollutants are directly discharged into the environment or water bodies by industrial plants and municipal sewage. Another potential source of pollutants is human activities such as mining and agricultural activities (Saria and Irene 2011).

The food chain pollution is a major pathway of heavy metal contact for humans (Ahmad, 2010). The ingestion of vegetables is one of the most central pathways for heavy metals that interferes human health (Rahman, *et al.,* 2012). Municipal and Industrial wastewater irrigation are normally a mutual reality in many cities of Africa, Asia, and North America (Xu, *et al.,* 2013). Many cities in Asia and Africa, more than 90 varieties of vegetables and about 60 fruits are cultivated (Ahmad, *et al.,* 2010).

Other sources of these heavy metals in African green leafy vegetables include ordinary activities of industrialization, civilization, agriculture and natural sources (Olowoyo, *et al.,* 2011; Kisamo, 2003). The heavy metals from contaminated soil can be absorbed by vegetables that are consumed by humans (Mutune, *et al.,* 2013; Ghosh and Singh, 2005). Heavy metals such as chromium, cadmium, copper, lead and zinc as well as mercury have been obtained in most of African green leafy vegetables planted or consumed within urban areas, sometimes is above allowable limits specify by WHO/FAO or any other bodies (Yebpella, *et al.,* 2011). In most cases, most of these plants have been shown to hyperaccumulate metals in their edible parts (Kumar, *et al.,* 2007). Metal elements like zinc and copper are essential micronutrients and can be found from vegetables in a good amount (Sinha, *et al.,* 2010; Iyaka, 2007). Though considered to be important, but still at high levels they cause oxidative stress through redox reactions (Sinha *et al.,* 2010). Lead also causes oxidative stress and in young children it causes mental retardation. Chronic exposure to cadmium causes glomeruli damage and also alters Zn, Cu and Se metabolism (ATSDR, 2012) while some oxidative forms of chromium (Cr VI) are carcinogenic.

Therefore, the risks linked with the consumption of polluted food grown around areas of wastewater pond, may pose health to consumers (Pandey, *et al.,* 2012). However, very few studies have been conducted on the contamination of African green leafy cultivated around Arusha Municipal oxidation pond.

The present work analyzes the concentration of African green leaves vegetables (AGLV) grown around the Arusha Municipal oxidation ponds. The elements of interest were Cr, Cu, Cd and Pb, in two different vegetable species (*Amaranths sp* and *Ipomoea sp*) around the wastewater pond in Arusha city, Tanzania. The intention was to find out the levels of heavy metals concentration in wastewater, soil and edible parts of vegetables, and finally to evaluate the health risk associated with vegetable consumption of those vegetables.

1.3 Statement of the Problem

In recent years, due to rise in awareness of vegetables nutritional values, demand of green vegetables is increasing day to day among different families in different African countries including Tanzania, but there is compromising possibility on its quality due heavy metal contamination in those vegetables. Prolonged human consumption of vegetables contaminated with heavy metal may lead to disturbance of many biological and biochemical progressions in the human body. It may result into various chronic diseases such as cancer. Heavy metal contamination of African Green Leafy Vegetables (AGLV) cannot be underrated, as these foodstuffs are vital components in the human diet. These vegetables are valuable bases of vitamins A and C, iron, calcium, and dietary fiber. In current years their consumption is growing gradually, chiefly among the urban community. Heavy metals elements, ranks high among the chief contaminants of leafy vegetables.

A number of studies have shown heavy metals as important chief pollutants of the vegetables (Saria, 2016). Vegetables take up metals by absorbing them from contaminated soils, as well as from deposits on various parts of the vegetables exposed to the air from contaminated environments. Regular monitoring of these metals in vegetables is important for avoiding excessive accumulation of the metals in the food chain worldwide including in Arusha city-Tanzania.

The main objectives of the present work was to determine concentration levels of heavy metals in African green leafy vegetables (*Amaranths sp* and *Ipomoea sp*) grown around Arusha Municipality oxidation ponds, levels of heavy metals in wastewater and soil where those vegetables are being grown around Arusha municipal wastewater oxidation pond. At the end recommendations on human diet foodstuff was made to insure significant improvement in food safety and advise policy makers and consumers appropriately.

1.4 General Objectives

The purpose of this study was to assess levels of heavy metal concentration in wastewater, Soil and two African green leafy vegetables species (*Amaranths sp* and *Ipomoea sp*).

1.5 Specific Objectives

(i) To investigate the levels of Cr, Cu, Cd and Pb concentration in wastewater from Arusha municipal oxidation pond.

(ii) To assess the levels of Cr, Cu, Cd and Pb concentration in soil where vegetables are being grown and irrigated by wastewater from Arusha municipal oxidation pond.

(iii) To analyze the levels of Cr, Cu, Cd and Pb concentration in *Amaranths sp* and *Ipomoea sp* irrigated by the wastewater from Arusha municipal oxidation pond.

1.6 Research Question

(i) The water from Arusha municipal wastewater oxidation pond is not fit for irrigation of vegetables consumed by human.

(ii) The soil around Arusha municipal wastewater oxidation pond is not fit for cultivating vegetables consumed by human.

(iii) iii. Concentrations of heavy metals in African green leafy vegetables grown around the Arusha Municipal wastewater oxidation pond exceeds acceptable level limits.

1.7 Significance of the Study

The study on assessment of heavy metals concentrations from Arusha municipal wastewater oxidation pond, the surrounding soil used for cultivation and African green leafy vegetables grown around the Arusha municipal wastewater oxidation pond will be used to identify appropriate stakeholders for intervention to reduce health risks from heavy metals. This will be through abolishing the use of the wastewater or by advancing the contents of wastewater. Worthwhile people who consume African green leafy vegetables irrigated by wastewater grown around Arusha municipal oxidation pond will have an assurance on their health per heavy metal concentrations standards required.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Metals are elements with high electrical conductivity, softness, and shine, which freely lose their electrons to form cations. Metals are found naturally on the earth's crust and their compositions vary among different areas, resulting in spatial variations of surrounding concentrations. The metal dispersal in the atmosphere is monitored by the properties of the given metal and by various environmental factors (Khlifi & Hamza-Chaffai, 2010).

Populations are increasingly demanding a cleaner environment in general and reductions in the amounts of contaminants reaching people as a result of increasing human activities. The contamination chain of heavy metals almost always follows a cyclic order: industry, atmosphere, soil, water, foods and human (Castro-González and Méndez-Armenta 2008). Soil contamination which results in the underground water pollution has become a very important topic worldwide in environmental protection as it is difficult in remedial compared to air contamination.

Heavy metals, such as arsenic, cadmium and lead can be found in plants that grow in healthy contaminated soil because they are natural constituents of the Earth’s crust and have existed on earth since its formation (Agency for Toxic Substances and Disease Registry 2011). Heavy metal contamination is difficult to be noticed, as it does not explicitly damage the environment in a short period, so is a chemical time bombs (Zhang, *et al*., 2014).

Water percolating through soils picks up naturally occurring minerals, salts, and organic compounds. As the water migrates downward, the concentrations of dissolved heavy metals in the underground water increase (Botwe, *et al.,* 2012). Atmospheric deposition is responsible for 43–85% of the total as, chromium (Cr), mercury (Hg), nickel (Ni), and lead (Pb) inputs into agricultural soils in China (Luo *et al*., 2013). The ashes from volcanic eruptions are transported from volcanic mountain and eventually settle down and contained in the layers of the soil.

A number of studies have been carried out in various volcanic mountains in the world to analyze the levels of heavy metals in soil associated with volcanic eruptions (Kara *et al.* 2003). All of these studies have reported that, high concentration of heavy metals exists in the soils located close to the volcanic mountains. A good example is study of Morocco; distribution of heavy metals in shallow wells was affected by the location of the wells (Kara *et al.* 2003, Baba *et al*. 2008, Lar and Usman 2012).

Green leafy vegetables are popular around the world. Heavy metal contamination of vegetables cannot be under estimated, as these foodstuffs are important components of the human diet. Heavy metal contamination of the food items is one of the most important aspects of food quality assurance (Khan, *et al.,* 2009; Radwan and Salama, 2006; Wang, *et al.,* 2005). These vegetables are valuable sources of vitamins A and C, iron, calcium, folic acid, and dietary fiber. In recent years their consumption is increasing gradually, particularly among the urban community. Heavy metals ranks high among the chief contaminants of leafy vegetables. A number of studies have shown heavy metals as important contaminants of the vegetables (Sinha *et al.,* 2006; Singh and Kumar, 2006; Sharma *et al.,* 2006, 2007, 2008. Mapanda *et al.,* 2005).

Vegetables take up metals by absorbing them from contaminated soils, as well as from deposits on different parts of the vegetables exposed to the air from polluted environments (Sobukola *et al.,* 2010). However, intake of heavy metal contaminated vegetables may pose a risk to the human health. Prolonged human consumption of unsafe concentrations of heavy metals in food stuffs may lead to the disruption of many biological and biochemical processes in the human body, it have been reported to affect cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair (WHO, 1992; Jarup, 2003). Intake of vegetables is an important path of heavy metal toxicity to human being. Dietary intake of heavy metals through contaminated vegetables may lead to various chronic diseases. Legislation on environmental protection should become more and more strict and supports manufacturers to reduce the concentration of certain metal ions released into effluents.

Regular monitoring of these metals in vegetables and in other food materials is essential for preventing excessive buildup of the heavy metals in the food chain. The aim of this study is to find the concentrations of heavy metals in vegetables grown around Arusha city wastewater pond so as to estimate their contribution to the daily intake of the metals.

Health Effects and Benefits Associated with Cr, Cd, Pb, and Cu

It is necessary to have a certain amount of heavy metals in our diet and body in order to maintain a good health. However, large concentrations of heavy metal cause toxicity and it can cause a lot of damages to living organisms. For example, it can cause reduced mental activity or damage it. Further it can damage lungs, kidneys, liver and other important organs. Heavy metals can be accumulated in side living organisms over food chains. So it is important to know the sources of heavy metals and control their release to the natural environment. There are about eight common heavy metals, which are arsenic, cadmium, barium, chromium, mercury, selenium, lead, and silver. Normally these are all naturally occurring substances that are commonly present in the environment at very low concentrations. When in larger amounts, they can be dangerous to consumers. Normally, humans are exposed to these metals by ingestion (eating or drinking) or breathing (inhalation). Working or living near to an industrial site which produces these metals and their compounds increases risk of exposure, as does living near a site where these metals have been improperly disposed. Subsistence lifestyles can also impose higher risks of exposure and health impacts because of hunting and gathering activities.

Trace amounts of some heavy metals required for certain biological processes. Some of these heavy metals like iron and copper can be used in oxygen and the electron transport while metal like cobalt can be used in cell metabolism and zinc is used in hydroxylation process (Moore and Ramamoorthy 1984). Vanadium and manganese is used in enzyme regulation as well as functioning while chromium is used in glucose utilization; nickel (cell growth); arsenic (metabolic growth in some animals and possibly in humans) and selenium (antioxidant functioning and hormone production) (Wiberg 2001).

Chromium (Cr) is an element that can exist in six valence states, 0, II, III, IV, V and VI, which represent the number of bonds an atom is capable of making. Its symbol Cr and has atomic number 24. It is the first element in group 6. Trivalent (Cr-III) and hexavalent (Cr-VI) are the most common chromium species found environmentally. Trivalent is the most stable form and its compounds are often insoluble in water. Trivalent chromium (Cr (III)) ion is an essential nutrient in trace amounts in humans for insulin, sugar and lipids metabolism, although the issue is debated. Hexavalent chromium is the second most stable form, and the most toxic. Many of its compounds are soluble. Chromium-VI has the ability to easily pass into the cells of an organism, where it exerts toxicity through its reduction to Cr-V, IV and III. Most Cr-VI in the environment is created by human activities.

Chromium is one of common element found in animals, rocks, plants, and soil. It can be in the form of gas, solid, or liquid. Soil, sediment, water and air can all become contaminated with chromium through industrial activities. Dust from industry operations such as mining and smelting settles out of the air, polluting soils and surface water. Most soluble chromium eventually settles onto sediment. Contamination of soil, surface and groundwater can also occur through release of industrial wastewater and leaching of soluble Cr-VI compounds from wastes such as mine tailings, waste rock, dust and slag piles. The Chromium compounds bind to soil and are not likely to migrate to ground water, but they are very persistent in sediments in the water. Chromium is used in metal alloys such as stainless steel; protective coatings on metal (electroplating); magnetic tapes; and pigments for paints, cement, paper, rubber, composition floor covering and other materials. Its soluble forms are used in wood preservatives. The chromium (VI) compounds have been declared as a powerful occupational carcinogen among workers in chrome plating, stainless steel, and pigment industries (Shrivastava *et al*. 2006). A study by Saria and Irene (2011) noted that chromium is an element that is essential to the human body in trace amounts as it is found as a co-factor in glucose tolerance factor (GTF). Concentration of Cr for vegetables Maximum contaminant level according to USEPA is 2.2 mg/kg, FAO/WHO-Guideline value is 2.3 mg/kg and BIS-permissible limit is 2.1 mg/kg (Sharma and Tyagi, 2013).

Cadmium is a metal element with atomic number 48 and its symbol is Cd. The average concentration of cadmium in Earth's crust is between 0.1 and 0.5 parts per million (ppm). It was discovered in 1817 simultaneously by Stromeyer and Hermann, both in Germany, as an impurity in zinc carbonate. It is widely distributed in humans, the chief sources of contamination being cigarette smoke, welding, and contaminated food and beverages. Cd enters the environments through natural and various anthropogenic sources. However, the accumulation of Cd in the soil–plant environment mainly through anthropogenic activities such as application of phosphate fertilizers, wastewater, sewage sludge and manures.

The high mobility in soils make Cd accumulation in plants poses a serious threat to animal and human health. The possible pathways of human exposure to Cd via the food chain. Cd is a common contaminant found in most human foodstuffs due to the high transfer factor properties of plants. The bio concentration of Cd from soil to the foodstuffs makes diet a primary source of Cd exposure among non-smoking, non-occupationally exposed populations. Although cadmium has no known biological function in higher organisms, a cadmium-dependent carbonic anhydrase has been found in marine diatoms. Cadmium is predominantly found in fruits and vegetables due to its high rate of soil-to-plant transfer (Satarug *et al.*, 2011). Cadmium is a naturally occurring toxic heavy metal with common exposure in industrial area, plant soils, and from smoking.

Due to its low permissible exposure to humans, overexposure may occur even in situations where trace quantities of cadmium are found, Cadmium is a highly toxic nonessential heavy metal that is well recognized for its adverse influence on the enzymatic systems of cells, oxidative stress and for inducing nutritional deficiency in plants (Irfan *et al.*, 2013). Cadmium is primarily toxic to the human kidney, especially in the proximal tubular cells; it causes bone demineralization and itai-itai disease (a painful degradation of bone and joints). Although cadmium accumulates in bone, the bone disease that results from excessive cadmium exposure is believed to be secondary to changes in calcium metabolism due to cadmium-induced renal damage (ATSDR 1999). Clinically significant bone scratches usually occur late in severe chronic cadmium poisoning and include pseudofractures and other effects of osteomalacia and osteoporosis. Pseudofractures are spontaneous fractures that follow the distribution of stress in normal skeleton or occur at sites where major arteries cross the bone and cause mechanical stress through rhythm. The maximum permissible limit for Cd in vegetables is 0.2 mg/kg WHO (Nazir *et al*. 2015). TBS and BIS (2012) acceptable limits of the Cd is 0.1 mg/kg (Hussain *et al*. 2015).

Lead compounds can be found naturally in all parts of our environment as a result of human activities, such as fossil fuel burning, mining, and manufacturing, this includes water, soil and air. Lead released from natural sources, such as volcanoes, windblown dust, and erosion, are minor compared with anthropogenic sources. In the air, lead is in the form of particles and is removed by rain or gravitational settling. The sink for lead is the soil and sediment, because it is strongly adsorbed to soil, it is generally retained in the upper layers of soil and does not leach substantially into the subsoil and groundwater. Lead compounds may be transformed in the environment to other lead compounds; however, the solubility of lead compounds in water is a function of pH, hardness, salinity, and the presence of humus material. Lead is an element and cannot be destroyed. Lead compounds can be used in many different ways like production of batteries, ammunition, metal products like solder and pipes (Martin and Griswold, 2009). Lead is a highly toxic metal and, as a result of related health concerns its use in several products like gasoline, paints, and pipe solder (Thürmer *et al.*, 2002).

In the late 19th century, today the most common sources of lead exposure are lead-based paint and possibly water pipes in older homes, contaminated soil, household dust, drinking water, lead crystal, lead in certain cosmetics and toys, and lead-glazed pottery. Lead quantities found in diets are larger in industrial areas, particularly those where the metal and its components are widely used. The preoccupation with lead outcomes on human health and the environment have led many countries to adopt laws limiting or prohibiting its use (Meyer *et al.*, 1999). However, developing nations are not in the frontline of monitoring lead use (Olivero-Verbel *et al.*, 2007).

In view of the global situation of lead exploitation, it is estimated that the production of batteries is accountable for about 70% of the lead consumed worldwide. The large international growth in the automobile manufacturing in the last decades (Paoliello & Chasin, 2001), as well as the expansion of the market for batteries, makes the use and reprocessing of batteries one of the primary methods of lead pollution in soils. Lead can happen naturally in plants as a result of the processes of taking up the lead normally found in the soil. Lead forms and contents in vegetables vary greatly with the species and depend principally on the environmental circumstances, because polluted soils can induce lead build-up by crops (Nan *et al.*, 2002). Lead accumulation by vegetable crops grown in soils with unusually elevated levels of the metal poses a risk to human health. Therefore, the capacity of these plants to accumulate lead and its presence in edible parts should be evaluated.

Lead's toxicity was recognized, and its use has since been phased out of many applications. Lead is a neurotoxin that accumulates in soft tissues and bones, damages the nervous system, and causes blood disorders. It is particularly problematic in children, as permanent brain damage may result, even if blood levels are promptly normalized with treatment.

Exposure to lead is cumulative cause death or permanent damage to the central nervous system, the brain and kidneys. There is no known level of lead exposure that is considered safe (WHO 2015). Maximum contaminant level according to USEPA is 0.2 mg/kg, WHO and BIS permissible limits is 0.3 mg/kg in vegetables (Sharma and Tyagi 2013).

Copper is a chemical element with symbol Cu and atomic number 29. It is a soft, malleable, and ductile metal with very high thermal and electrical conductivity. It is an essential element for living organisms, including humans, and-in small amounts-necessary in our diet to ensure good health. It is a key constituent of the respiratory enzyme complex cytochrome c oxidase. In molluscs and crustaceans, copper is a constituent of the blood pigment hemocyanin, replaced by the iron-complexed hemoglobin in fish and other vertebrates. In humans, copper is found mainly in the liver, muscle, and bone.

Consumption of vegetables contain higher than normal concentration of copper a person can cause adverse health effects, including vomiting, diarrhea, stomach cramps, and nausea. It has also been associated with liver damage and kidney disease (Eastwood 2003). The human body has a natural mechanism for maintaining the proper level of copper in it. Like all essential elements and nutrients, too much or too little nutritional ingestion of copper can result in a corresponding condition of copper excess or deficiency in the body, each of which has its own unique set of adverse health effects. However, children under one year old have not yet developed this mechanism and, as a result, are more vulnerable to the toxic effects of copper. People with Wilson's disease also have a problem with maintaining the proper balance and should also exercise particular care in limiting exposure to copper.

Higher intakes can cause liver and kidney damage, even death and with adolescents results into decline their intelligence (WHO 2015). Daily dietary standards for copper have been set by various health agencies around the world. Standards adopted by some nations recommend different copper intake levels for adults, pregnant women, infants, and children, corresponding to the varying need for copper during different stages of life.

Generally the maximum permissible limit for Cu in vegetables is 0.2 mg/kg WHO (Nazir *et al*. 2015). Zinc has been reported to cause gastrointestinal bleeding to patient after ingestion of Zinc Sulphate 220 mg twice daily as a treatment (Mohammed 2000). TBS Maximum Tolerance limit of Zinc in vegetables is 2 mg/kg and Lower Limit is 5.0 mg/kg Categorized as less toxic. Acceptable limit in vegetables according to the Bureau of Indian Standards (BIS) 2012 of Zn is 2 mg/kg (Hussain *et al*. 2015).

Iron is the second most abundant metal on the earth's crust (EPA, 1993). Iron occupies the 26th elemental position in the periodic table. Iron is an essential element in human nutrition, is most crucial for the growth and survival of almost all living organisms (Valko *et al.*, 2005). It is one of the vital components of organisms like algae and of enzymes such as cytochromes and catalase, as well as of oxygen transporting proteins, such as hemoglobin and myoglobin in most mammals, including human being (Vuori, 1995). Estimates of the minimum daily requirement for iron depend on age, gender and iron bioavailability and range from about 10 to 50 mg/day. When in excess in our body, iron produced hydrogen free radicals attack DNA, resulting in cellular damage, mutation and malicious transformations which in turn cause an array of diseases (Grazuleviciene *et al.*, 2009). TBS Maximum Tolerance limit in vegetables is 5.0 mg/kg and Lower limit is 1.0 mg/kg Bureau of Standards (BIS) 2012 acceptable limits is of Fe is 0.3 mg/kg and WHO (Hussain *et al*. 2015 and Nazir *et al*. 2015).

Therefore, this study aims at assessing the concentration of heavy metal inedible part of vegetables grown/cultivated around the area of Arusha municipal wastewater pond and the assessment of their associated health risks. This is because farmers of the mentioned area are using water from the pond for irrigation of their vegetables. It shows that there is an intensive uncontrolled operation of various industries and sewage systems in releasing their effluents into the respected pond, which is believed to have high concentrations of heavy metals. The heavy metals to be investigated include Lead, Chromium, Cadmium, Iron and Copper.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

For the present study, the agricultural fields was selected around the wastewater oxidation pond area of Arusha municipality, located eastern side of the city. Agricultural fields surrounding the pond was selected based on a farmer's interview where irrigation with contaminated wastewater is a common practice for many years.

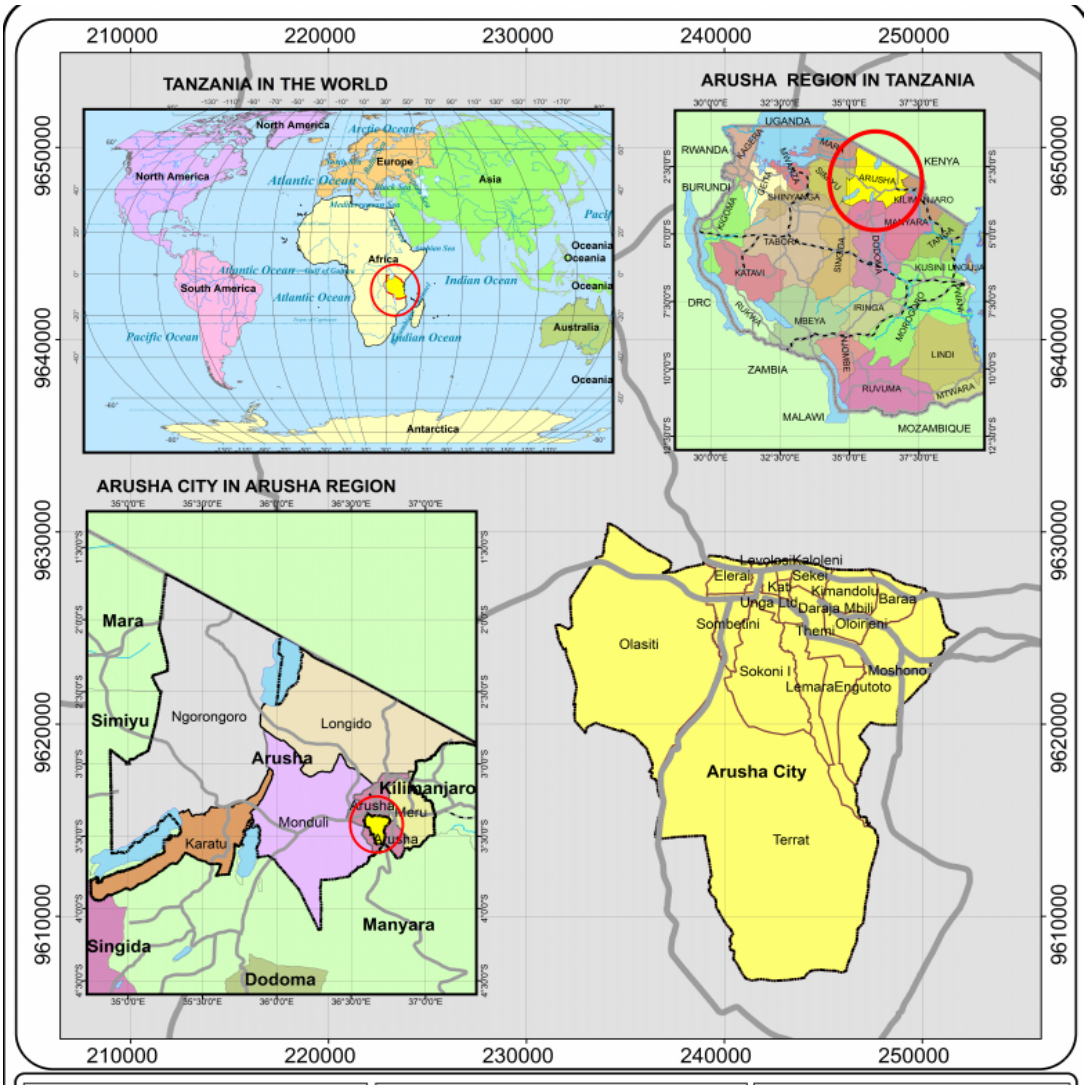

Figure 3.1: Map showing the Study Area

Numerous industries (Breweries, textiles, metals processing, paper mills, electronic goods, fertilizers, pharmaceuticals, dyeing, battery manufacturing, ink manufacturing, etc.) are situated near the selected fields. Most of the treated and untreated industrial effluents are being discharged into the pond. Several acres of agricultural land irrigated by contaminated wastewater and farmers cultivate various types of vegetable crops for their daily use and economic importance.

The Arusha city (Figure 3.1) is one of the most four densely cities in Tanzania, with the population of approximately 1.694 million people, of which most of them are served by the sewage treatment facility (National census-2012). The Arusha Municipal wastewater oxidation pond receives domestic raw sewage as well as industrial effluents from the surroundings and nearby industrial belt.

3.2 Vegetable Sampling and Sample Processing

The samples were collected from four agricultural fields besides the pond. At each sampling site, the same species of vegetables was collected as sub-samples. Twelve samples of two AGLV vegetables, which includes, *Amaranths sp* and *Ipomoea sp*. All vegetables were collected from the selected agricultural fields, and samples was placed in polyethylene zip-bags and transported to the laboratory on the day of sampling.

Each vegetable sample was carefully washed with distilled water and the edible parts cut into small pieces and then oven dried at 70–80°C for four hours to attain constant weight (Tiwari *et al.,* 2011). The moisture contents were calculated by recording the fresh and dry weights. The dried vegetable samples were grinded with mortar and pestle. The processed samples were taken to Arusha Technical college (ATC-Arusha) for chemical analysis.

De-ionized water was used for solution preparation. The polypropylene containers was cleaned, soaked in 5% HNO_3 for more than 24 hours, then rinsed with de-ionized water and dried. For vegetable, 0.2 g of dried sample was digested with 6ml of 69% HNO_3 and 2ml of H_2O_2 in a Microwave Digestion System. The digested samples were then transferred into a beaker and total volume made up to 50ml with de-ionized water. The digested solution was then filtered by using syringe filter and stored in 50ml polypropylene tubes. Thereafter, the microwave vessels were cleaned with de-ionized water and dried with air. Finally, blank digestion using 5ml of HNO_3 was carried out to clean up the digestion vessels.

3.3 Water Sampling and sample Processing

Before sample collection six Empty Kilimanjaro drinking water bottles of size 500 ml were collected. The bottles were washed thoroughly with detergent (soap), dilute HNO_3 and finally being rinsed three times with distilled water (Mohod *et al*. 2013, Wei and Kong 2014) in laboratory. Water samples was drawn from the outlet oxidation pond at about 100-200 meters from the outlet (the discharged out water from the pond to the civilian), three times in a day (morning, afternoon and evening) and transferred into the prewashed bottles, within the period of two weeks in July and August, (dry season) the samples were acidified with 1 ml of 1% HNO_3 (for metal ion preservation) to the PH of below 2 ready for heavy metals examination (Mohod *et al*. (2013), Sharma and Tyagi (2013).

3.4 Soil Sampling and Sample Processing

Immediately after collection of soil from selected fields, stones and other dirtiness were removed and the soil samples were placed in the polythene bag, which were washed with deionized water repeatedly three times and taken to the laboratory as soon as possible.

In the laboratory, the collected soil samples were dried in an electrical oven at a temperature around 90°C for 24 hours to remove the moisture and then grinded in mortar-pestle and reduced size to a fine powder. 2 g of soil sample was placed in a beaker and 10 ml of concentrated nitric acid was added, then the content was placed in hot water bath to allow evaporation for about 5 ml and left to cool for one hour. Then the solution was diluted with 10 ml of deionized water.

3.5 Instrumental Analysis and Quality Check

For heavy metals, which include Cr, Cu, Cd and Pb, the samples of vegetables, soil and water was analyzed by using Flame Atomic Absorption Spectroscopy (FAAS) at The Arusha Technical College (ATC). All test batches were evaluated using an internal quality approach and validated if they satisfied the defined Internal Quality Controls (IQCs). For each experiment, a run included blank, certified reference materials (CRM) and samples were analyzed in triplicate to eliminate any batch-specific error. The samples of irrigated wastewater, soil and vegetables were collected from the field for about ten weeks. These were being done from June to mid of August. At the day of sampling, samples of water were taken three times a day (morning, afternoon and evening).

3.6 Data analysis

3.6.1 Soil-Vegetable Transfer Coefficients

The transfer coefficient quantifies the relative differences in bioavailability of metals to plants and is a function of both soil and plant properties. The coefficient is calculated by dividing the concentration of a metal in a vegetable crop (DW) by the total metal concentration in the soil. Higher transfer coefficient represents relatively poor retention in soils or greater efficiency of plants to absorb metals. Low coefficient demonstrates the strong absorption of metals to the soil colloids. Soil-to-plant transfer is one of the key components of human exposure to metals through the food chain. Transfer Factor (TF) or Plant Concentration Factor (PCF) is a parameter used to describe the transfer of heavy metal elements from soil to plant body and it is also a function of both soil and vegetables properties. The transfer coefficient was calculated by dividing the concentration of heavy metals in vegetables by the total heavy metal concentration in the soil.

$TF = \frac{C\ plant}{C\ soil}$ ……………………………………….Equation (1)

Where, C_{plant}: metal concentration in vegetable tissue, mg kg^{-1} and C_{soil}: metal concentration in soil, mg kg^{-1}. In the present study, the TF of different heavy metal from the soil to vegetables were presented in tabular form. The mobility of metals from soil to plants is a function of the physical and chemical properties of the soil and of vegetable species, and is altered by uncountable environmental and human factors.

3.6.2 Human Health risk from Consuming Vegetable

The Human Health Risk was computed as the ratio of estimated exposure of tested vegetables and oral reference dose by using the following equation:

$$HHRI = \frac{DIR}{RFD}$$ ………. Equation (2)

Where, DIR and RFD represent daily intake Rate of metal and the reference oral dose, respectively. The DIM was evaluated by using the following equation:

$$DIR = \frac{C\ metal\ X\ C\ factor\ X\ D\ food\ intake}{B\ average\ weight}$$ …………..Equation (3)

Where, C_{metal}, C_{factor}, $D_{food\ intake}$ and $B_{average\ weight;}$ denote metal concentration in plant (mg/kg), the conversion factor, daily intake of vegetable and average body mass of the consumers, respectively. The conversion factor of 0.085 was used to convert fresh vegetable weight to dry weight. The average body of the consumer's chosen will be 65 kg. The daily intake of vegetables i.e. Green amaranth and Sweet potato leaves was estimated to be 400 g as recommended by FAO and WHO.

3.6.2.1 Hazard Quotient Calculation

In this study, the health risks associated with the consumption of vegetables by the local inhabitants were assessed based on the Target Hazard Quotients (THQs). This method of estimating risk using THQ was provided in the U.S. EPA Region III risk-based concentration table (USEPA, 2000) and is based on the following equation:

$$THQ = \frac{EF\ X\ ED\ X\ FI\ X\ MC}{RFD\ X\ WAB\ X\ AT}\ X\ 10^{-3}$$ ………. Equation (4)

Where, EF is the exposure frequency (350 days/ year), ED is the exposure duration (65 years), FI is the food ingestion (g/person/day) in this study is estimated to be 100gm/kg per day, MC is the metal concentration in vegetables (mg/kg, on fresh weight basis), RFD is the oral reference dose (mg/kg/day), BW is the average body weight (adult, 65 kg), AT is the averaging time for non-carcinogens (365 days/ year ×

number of exposure years, assuming 65 years in this study). The oral reference doses base on 1.500, 0.04, 0.001, and 0.0035mg/ kg/day for Cr, Cu, Cd and Pb, respectively (USEPA IRIS, 2006). If the THQ is less than 1, the exposed population is unlikely to experience obvious adverse effects. If the THQ is equal to or higher than1, there is a potential health risk, and related interventions and protective measures should be taken.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

Numerous sources of environmental pollution have been concerned as the way heavy metals can enter into diet. Irrigation using wastewater, air deposition, and contaminated liquid discharge are key path to heavy metals accumulation in vegetables and plants (Singh *et al*., 2010; Oluwole *et al*., 2013; Adesuyi *et al*., 2015). Vegetable is a main part of Tanzanian diet and is very vulnerable to environmental contamination due to the actions and progressions going on or practiced in the part where it is grown or found after.

The permissible maximum limit of cadmium, chromium, lead and copper by FAO/WHO (2001) in vegetables is 0.2, 2.3, 0.3 and 40 (mg/kg), in soil maximum limit is100, 50, 3.0 and 50(mg/kg) while in irrigated water the maximum limit is 0.20, 0.10, 0.01, and5.0 (mg/kg) respectively (Maleki and Zarasvand, 2008). The suggested limits for several heavy metals differ depending on the food being considered and the state sometimes or the institute. Although we find safe limits of heavy metals acknowledged by health sectors in some countries, other nations do not have an available record to lead the heavy metal ingesting of its people.

This chapter analyses the results of the heavy metals obtained from the waste water used by the farmers for irrigation discharged from oxidation pond, soil sample from the areas where vegetables are grown, and vegetable samples (*Amaranths sp* and *Ipomoea sp*). These vegetables were collected from the small-scale farmers around the

Arusha wastewater stabilization pond, and the heavy metals of interest were Cd, Pb, Cr and Cu.

4.2 Levels of Heavy Metals Wastewater used for Irrigation

Table 4.1: Heavy Metal Concentration in Irrigated Water Sample (mg/kg)

Parameter	**Results**	**WHO/FAO Standards (2002)**	**TBS**
Copper (Cu)	237.82	0.20	3.0
Chromium (Cr)	0.32	0.10	0.05
Cadmium (Cd)	607.61	0.01	0.005
Lead (Pb)	0.15	5.0	0.05

The chromium concentration is about three times higher than the acceptable limit by WHO/FAO (2002, 2006) for vegetable irrigation. However the value is four times lower than the maximum concentrations of total Cr, detected earlier (Asfaw, *et al.,* 2017). According to literature (Asfaw, *et al.,* 2017) the reason for high level of chromium in wastewater was due to the use of high amount of chrome salt. However, the Cr levels was much lower than the level detected earlier in Tanzania where it was detected to be 502.3 and 174.7 mg/kg at Jangwani and Vingunguti areas respectively in (Mwegoha and Kihampa, 2010). From these studies, higher concentrations of chromium at wastewater oxidation pond is due to anthropogenic activities like plumbing works and manufacture of electroplating garages and car washing sites pour contaminated water containing chromium.

Table 4.1 shows that concentration of cadmium in the wastewater sample was 607.61mg/kg, which is extremely higher than WHO and Tanzanian Standard. The level is 300 times higher the research determined earlier (Amfo-Out, 2012) of 0.2 ppm in the water samples being used for irrigation at the various sites. Cadmium is considered to be hazardous metal because of its toxicity and accumulation capacity in the living system (Singh *et al.,* 2005). Cadmium used in industry finds its way into many water supplies. Old galvanized pipes and new plastic (PVC) pipes are sources of cadmium in water pump (Tyagi, 2014).

Lead has been used widely for application in metal products, cables and pipelines, as well as paints and pesticides. Lead is one of the metals that have the most damaging effects on human health (Bainies, 1999). This value value was below the recommended limit of Pb for irrigation water set by WHO (2006) (Table 4.1). The value is lower than the value detected earlier Saria (2016) of 0.341 mg/kg. The value is also lower than the value detected earlier by Mwegoha and Kihampa (2010) at Msimbazi river ranging from (9.623±1.086) mg/kg to (22.85±1.502) mg/kg. Researchers (ibid) concluded that the highest level of lead is due to pollution from the garage, which utilizes water for car washing and discharging effluents containing oil into oxidation ponds. The value was much higher than recommended limit of Cu for irrigation water set by WHO/FAO (2001) and about 80 times higher than Tanzanian maximum acceptable limit of 3.0 ppm. Also the value is 15 times higher than the values detected earlier (Taghipour, *et al.,* 2012). This could be attributed to the reason of anthropogenic activities and industrial effluent released without treatment.

4.3 Levels of Heavy Metals in Soil

The concentration rank of heavy metal was detected as Cr (0.65/mg) > Pb (48.00 mg/kg) > Cu (234.52 mg/kg) and Hg highest concentration was Cd (595.90 mg/kg) (Table 4.2).

Table 4.2: Heavy Metal Concentrations in Soil Sample

Metal	Data Obtained	WHO/FAO Standards (2002)
Copper (Cu)	234.52	100
Chromium (Cr)	0.65	50
Cadmium (Cd)	595.90	3.0
Lead (Pb)	48.00	50

The concentration of cadmium present in soil samples was 595.9 mg/kg. This is 6 times higher than the recommended concentration of 100 mg/kg in soils (TZS, 2003). Cadmium is very soluble and easily leached by rain- water or swept by rainwater especially when the soil pH is in the acidic range. It is believed that the greater level of cadmium is bounded in the organic matter of the soil sample, therefore probably indicates that the greater tendency for cadmium to become unavailable once it is in soils without organic matter. The association of cadmium with organic soil may be due to high formation of organic- complexes chiefly from agrochemicals (Olajire, *et al.,* 2003).

Association of Cd to the residual soil does not generally constitute an environmental risk. This is due to the stable nature of Cd and the fact that the metals are bonded firmly within a mineral lattice that restricts the bioavailability of this metal (Milkessa, 2013).

Concentration of Pb in soil samples from lands irrigated with wastewater around the Arusha Municipal wastewater stabilization pond was found to be 48 mg/kg as indicated in the Table 4.2. This concentrations is about 7 times higher than value detected earlier by Muchuweti, *et al.,* (2006) reported that the level of Pb (6.77 mg/kg). Mwegoha and Kihampa (2010) indicated earlier that the level of lead may be attributed to the inflowing channel from various wastes from domestic effluents; industrial and automobile garages where activities of car wash and discharge mixtures of oil and into the stream leading contaminate the system. Such activities may contribute much to lead contamination-sampling site. Soil organic matter has a large surface negative charge/ cation exchange capacity and elements such as Pb are observed to accumulate in the organic- rich, surface horizons (Zimdahl and Skogerboe, 1977). Therefore, the high concentration of Pb in the soil sample could be attributed to varied nature of industrial activities, sewage from the city.

The concentration of copper in the soil samples detected was 234.52 mg/kg (Table 4.2). This value is three times lower than the one detected earlier (Badilla-Ohlbaum, *et al.,* 2001) where copper levels in agricultural soils of in Chile revealed was 751 mg/kg. The high level of copper in the soil irrigated with wastewater (water with high concentration of organic matter) probably indicates the greater tendency for copper to become unavailable once it is in soils without organic matter. A copper metal in soil sample is the most mobile and is readily available for biological uptake by the plant. Many researchers have reported varying concentrations of Cu in different soil samples. Adekola *et al.,* (2012) reported high percentage concentration of Cu in organic matter in soil with high concentration of organic matter. The dominance of Cu in the organic phase has also been reported by (Chuckwujingu, 2007).

The concentration of (Cr) in soil samples from lands irrigated with wastewater around the Arusha municipal wastewater stabilization pond is 0.65 mg/Kg. The value is three times higher than the value detected earlier (Saria 2016) however much lower compared to the permissible limit recommended by WHO/FAO (2001), which is 50 mg/kg. However, the value is extremely lower compared to the results obtained by Mwegoha and Kihampa (2010), which ranges (502.33±150.991) mg/kg to (174.707±168.278) mg/kg. The source of chromium at this area is due to anthropogenic activities like manufacture of electroplating garages and plumbing works as well as car washing sites, which are very common in Arusha Municipality.

4.4 Levels of Heavy Metals in Vegetables

The concentration of cadmium in this study is very high (309.87 mg/kg) and (152.61 mg/kg) in both *Amaranths sp* and *Ipomoea sp* leaves respectively, compared to the permissible levels by FAO/WHO in vegetable (Table 4.3). These values are extremely high compared to those detected earlier Oluwatosin, *et al.,* (2010) which ranges between 0.5 -1.2 mg/kg in (*Amaranthus hybridus L.)* and about 25 times higher than value detected earlier by Abba and Ibrahim (2017), which recorded 12.5 mg/kg.

Cadmium is a heavy metal with high toxicity and it is not essential element in foods and natural waters, it accumulates principally in the kidneys and liver (Divrikli *et al*., 2006; Adesuyi *et al*., 2015). Higher values have been previously reported for leafy vegetables cultivated along road sides (0.27 mg/kg) by Oluwole *et al.,* (2013). According to FAO/WHO (2001), the safe limit for Cd consumption in vegetables is 0.2 mg/kg**.**

Table 4.3: Heavy Metal Concentration in Green Leafy Vegetables

Parameter	***Amaranths sp***	***Ipomoea sp***	**WHO/FAO (2001)**
Copper (Cu)	281.69	248.54	0.2
Chromium (Cr)	1.40	4.21	2.3
Cadmium (Cd)	309.87	152.60	0.2
Lead (Pb)	0.50	0.48	0.3

The most common sources of cadmium in these two species of green leafy vegetables are sewage effluents released by the Arusha Municipal council to the pond. Also the release of wastewater from industrial zone, release of sewage water from the entire Arusha city, deposition of gasses due to burning of fossil fuel by heavy machines and vehicles as the pond is located along road side of Njiro-Arusha town road and industrial zone. Cadmium accumulates especially in the kidneys leading to dysfunction of the kidney with amplified secretion of e.g. proteins in urine (proteinuria) and other consequences (Waalkes, 2000).

Lead is one of a non-essential heavy metal, which is more persistent metals and is estimated to have a soil retention time for years (Sobolev and Begonia, 2008). Lead causes oxidative stress and contributes to the pathogenesis of lead poisoning by disrupting the delicate antioxidant balance of the mammalian cells. High-level accumulation of Pb in body causes anemia, colic, headache, brain damage, and central nervous system disorder (Rehman *et al.,* 2013).

The highest level of Pb recorded in both *Amaranths sp* was 0.5 mg/kg and *Ipomoea sp* leaf was 0.48 mg/kg. These values were 4 times lower than the value detected before which detect 2.0 mg/kg (Oluwatosin, *et al.,* (2010), but a bit higher than WHO recommended level. There was high variation in Pb level in all the different vegetables across all sampled from cultivated fields. The variation may be due to root ability uptakes. Lead contamination has been exposed to be equal with population/vehicular density (Afolami *et al.,* 2010). Generally, lead pollutions occur in vegetables grown on contaminated soils, through air deposition or through sewage sludge/waste water use (Oluwole *et al*., 2013).

Lead poisoning is a global reality and fortunately is not a very common clinical diagnosis yet in Tanzania except for few occupational exposures (Anetor *et al.*, 1999). In this study, the concentrations of Pb are generally higher than the permissible levels by FAO/WHO in vegetables of 0.3 mg/kg. The high levels of Pb in these vegetables may probably be attributed to pollutants in irrigated water, farm soil (farm site) or due to pollution from the highways traffic (Qui *et al*., 2000; Oluwole *et al*., 2013). Lead is causing concern in particular due to the possible impacts on children. Lead influences the nervous system, slowing down nervous response. This has effects on the learning abilities and behavior of children's (Adesuyi *et al*., 2015).

Chromium plays a vital role in the metabolism of cholesterol, fat, and glucose. Its deficiency causes hyperglycemia, elevated body fat, and decreased sperm count, while at high concentration it is toxic and carcinogenic (Chishti *et al.,* 2011). However, daily uptake of it within a certain range of concentrations (up to 200 μg/day) by human beings and animals is considered to be essential for carbohydrate and lipid metabolism (Girmaye, 2012).

The Cr content in the vegetable samples was found to be 1.40 mg/kg in *Amaranths sp* and 4.21 mg/kg in *Ipomoea sp* leaves. Chromium levels in the *Amaranths sp* vegetables sampled are within safe limits of consumption (WHO/FAO 2001), while the concentration in *Ipomoea sp* leaf is 2 times higher than WHO/FAO (2001) acceptable limit. Chromium depending on the trivalent state can be beneficial or harmful; the hexavalent state of chromium is harmful. The most widespread human effect is chromium allergy caused by exposure to chromium (especially Cr (VI) compounds) and they are assumed to cause cancer (RTI, 2000).

Copper is an essential trace element; it is necessary for many enzymes. It is needed for the normal growth and development. High concentration of Cu causes metal fumes fever, hair and skin de-colorization, dermatitis, respiratory tract diseases, and some other fatal diseases in human beings (Khan *et al.,* 2008).

The concentration of copper in green leafy vegetables ranged between 281.69 mg/kg in *Amaranths sp* and 248.54 mg/kg in *Ipomoea sp* leaves*.* The concentration is twice as much as the levels detected earlier (Shahrzad and Keivan, 2015), which detected 103 mg/kg in *Amaranths sp* (*A. retroflexus*).

Copper (Cu) is essential to human life as metalloproteinase and function as enzymes, however, critical doses leads to health risks such as anemia, diabetes, inflammation, kidney and liver dysfunction and vitamin C deficiency (Lokeshappa *et al*., 2012). JECFA (2005) suggested safe limits of 40 mg/kg in adults, which was significantly higher than maximum copper levels of vegetables in this study.

4.5 Assessment of Health Risks in Consumption of Contaminated Green Leafy Vegetables

Careless use of resources and random urbanization has over-use the natural resources like water and caused harmful effects on the environment. Spontaneous economic development has led to pressure on arable land and make it not fit for cultivation. In order to meet the food demands of the entire exponentially rising population, cultivation of food crops and green leafy vegetables is carried out in areas not suitable for agriculture like along the roadway, along wastewater drains or in other polluted sites like damp sites. To address water crisis in Arusha Municipality, irrigation using wastewater discharged from oxidation ponds, which originated from the industries and many other pollution sites is being used. Huibers and Van Lier (2005), indicated that wastewater used for irrigation has so many contaminants especially heavy metals as well as pesticides depending upon the origin of discharge (Long term apply of wastewater for irrigation especially the green leafy vegetables can cause accumulation of these heavy metals in soil and then translocate to green leafy vegetables and thus enter food chain (Singh and Agrawal 2010, Gupta et al. 2010; Arora *et al.,* 2008;).

Though, this agricultural strategies help in dealing with unemployment as well as increase the crop yield still global food security is not attained. The World Health Organization (WHO), define food security as state of everyone and always have access to sufficient and safe food. To assess the health risk of the inhabitants of Arusha municipal and the neighborhoods due to heavy metal intake from vegetables consumption, soil-plan transfer coefficient, the daily intake of metals (DIM), human health risk index (HHRI), and target hazard quotient (THQ) were calculated.

4.5.1 Soil – Plant Transfer Coeffient of Heavy Metals

Heavy metals in soil are found in several forms, which are involved in their movement from soil to plant. To determine the mobile or bioavailability forms of these heavy metals, several techniques are used such as reagent single extraction and DTPA extraction. The bioavailability of heavy metals in soil is assumed to be available to all plants. As a result, heavy metals can transfer to plant when they are in a mobile form. To estimate the heavy metals transferred to plants, the transfer coefficient, a function of both soil and plant properties, is used due to its representative bioavailability of heavy metals to plants. The transfer coefficient was calculated by dividing the concentration of heavy metals in vegetables by the total heavy metal concentration in the soil. The equation (1) was applied to compute and result being tabulated.

According to Figure 4.1, higher transfer coefficient show the order of Cr > Cu > Cd >Pb in both *Amaranths sp* and *Ipomoea sp* leaf, which represents relatively poor retention of Chromium in soils or greater efficiency of plants to absorb, while the least heavy metals to be absorbed by plants is Pb, TF = 0.01 in both plant species. Low coefficient demonstrates the strong sorption of metals to the soil colloids.

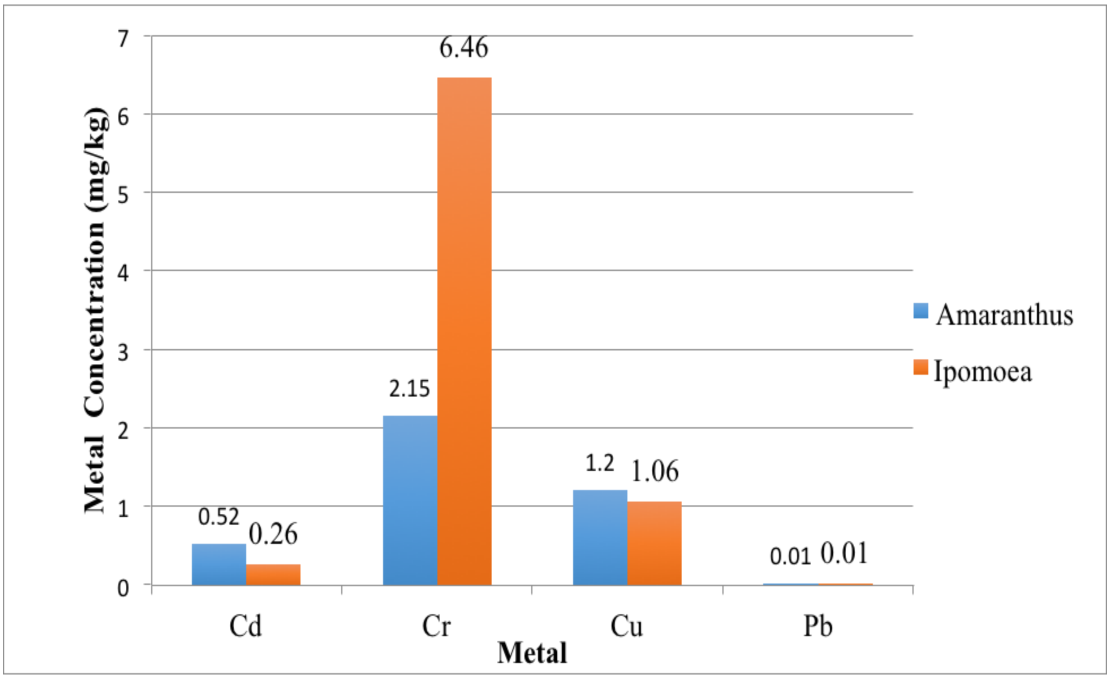

Figure 4.1: Soil- Plant Transfer Coefficient of Heavy Metals

Soil electrolyte plays an important role in the process of metal transfer. Higher transfer coefficient represents relatively poor retention in soils or greater efficiency of plants to absorb metals. Low coefficient demonstrates the strong sorption of metals to the soil colloids (Coutate 1992). The electrochemical properties of soil reflected through the temperature, pH, and electrolyte concentration etc. thus influence the migration transformation ability of toxic metal indirectly (Iyaka *et al.,* 2014).

Soil to plant transfer is one of the key components of human exposure to metals through food chain. Transfer Factor (TF) or Plant Concentration Factor (PCF) is a parameter used to describe not only the transfer of trace elements from soil to plant body but also function of both soil and vegetables properties. The transfer coefficient was calculated by dividing the concentration of heavy metals in vegetables by the total heavy metal concentration in the soil (Kachenko and Singh 2006).

4.5.2 Daily Intake Rate of Heavy Metals

The Daily Intake Rate (DIR) is the average metal content in each vegetable was calculated and multiplied by the respective consumption rate. The DIR was determined by the following equation (Sajjad *et al.,* 2009; Arora *et al.,* 2008):

$DIR = C_{(\text{metal conc in Vegetable})} \times C_{(\text{Factor})} \times D_{(\text{vegetable intake})}$ ....(2)

Where, C (Metal conc.) = Heavy metal concentration in vegetables (mg/kg); C (Factor) = Conversion factor (0.085); D (Vegetable intake) = Daily Intake of Vegetable (kg $person^{-1}day^{-1}$. The conversion factor of 0.085 is set to convert fresh vegetable weight to dry weight based on calculation in literatures (Rattan *et al.,* 2005; USDA 2007).

For daily vegetable consumption was obtained through a formal survey conducted in the Dar es Salaam City. An interview of 60 persons of 35-65 years age group and of 50-77 kg body weight was conducted about their daily consumption rate of vegetables tested. An average consumption rate of each vegetable per person per day was calculated. The average daily intake for Tanzanian adults was set to be 0.246 Kg person^{-1}day^{-1} (expressed as fresh weight). According to WHO (1989) guidelines, the required amount of vegetables in our daily diet must be 0.300 to 0.350 Kg per person. The daily intake of metals (DIM) results in Table 4.4 was compared with the recommended daily intake of metals and the upper tolerable daily intake level (UL) established by the Institute of Medicine for people between the ages of 19 to 70 years (FDA, 2001; Garcia-Rico, 2007).

Table 4.4: Daily Intake Rate (mg per person per day) of Heavy Metals

Vegetables	**Cu**	**Cr**	**Cd**	**Pb**
Amaranths sp	36.84	0.18	40.52	0.065
Ipomoea sp	32.50	0.55	19.96	0.063
DIM (mg per day per person)	0.90	-	0.00	0.00
UL (mg per day per person)	10.00	2.47	0.064	0.240

Recommended daily intake (DI) and upper tolerable daily intake (UL) levels of heavy metals in foodstuffs (FDA, 2001; Garcia-Rico, 2007)

Results show that daily intake of metals in vegetables species for Cr (0.18 – 0.55 mg/kg/person) and Pb (0.065– 0.063 mg/kg/person) are significantly higher than the recommended daily intake of metals and lower than the upper tolerable daily intake level (UL). However, DIM of Cd (40.52 – 19.96 mg/kg) and Cu (36.84 – 32.50 mg/kg) exceed the recommended DIM and the upper tolerable daily level. The DIM of Cr (0.048 – 0.082 mg/kg/person) is lower than the recommended oral reference dose (RfD) of 1.5 mg/kg (USEPA, 2010).

4.5.3 Human Health Risk Index (HHIR) and Hazardous Quotient (HQ)

Human Health Risk Index (HHIR) for the locals (consumers) through the consumption of contaminated vegetables was assessed by the ratio of Daily Intake Rate (DIR) to the oral reference dose (RfDo) for each metal (Oral reference dose (USEP A IRIS, 2006)).

The Human Health Risk Index (HHIR) was calculated using equation (3):

$$HHRI = {}^{DIM}\!/_{RFD} \quad \ldots\ldots\ldots (3)$$

DIM = The Daily Intake of Metals

RFD = Oral Reference Dose

The target hazard quotient (THQ) was calculated using equation (4):

$$THQ = \frac{EF \text{ x } ED \text{ x } FIR \text{ x } C}{RFD \text{ x } WAB \text{ x } TA} \text{ x } 10^{-3} \quad \ldots\ldots\ldots (4)$$

Where EF is the exposure frequency (350 days/year); ED is the exposure duration (According to the latest WHO data published in 2015 life expectancy in Tanzania is 62 years; lifetime); FIR is the food ingestion rate. According to Weinberger and Swai (2006) vegetable consumption values for Northern adult Tanzanian is 63 g/person/day); C is the metal concentration in the edible parts of vegetables (mg/kg). The WAB is the average body weight (65 kg for adults vegetable consumer in Tanzania) and TA is the average exposure time for non-carcinogens (ED x 365days/year). If the THQ value is greater than 1, the exposure is likely to cause obvious adverse effects.

Table 4.5: The Results of Calculated HHRI and HQ for all the Vegetables Species

Vegetables	Cu		Cr		Cd		Pb	
	HHRI	THQ	HHRI	THQ	HHRI	THQ	HHRI	THQ
Amaranths sp	921	405.79	0.12	0.053	40520	17855.56	18.57	8.23
Ipomoea sp	812.5	358.04	0.37	0.16	19960	8793.23	18	7.91
Oral reference dose (RFD)	0.040		1.500		0.001		0.0035	

The HHRI for Cu, Cd and Pb from this study were far greater than 1 (HHRI > 1) except chromium. Generally, HHRI < 1 means that the exposed population is safe of metals health risk while HHRI > 1 means there is potential health risks (Khan *et al.*, 2008). The population is therefore at greater risk of Cu, Cd, and Pb.

The THQ is a ratio between the measured concentration and the oral reference dose, weighted by the length and frequency of exposure; amount ingested and body weight (Tsafe *et al*., 2012). The parameter defines the exposure duration and the risk with that period. The THQ values of Cu, Cr, Cd, and Pb due to vegetable consumption for the population (adults) of the study area are listed in Table 4.5. This result reflected the risk associated with Cd, Pb, Cr, and Cu exposure for the period of life expectancy considered, and the inhabitants are highly exposed to health risks associated to these metals in the order Cd > Cu > Pb > Cr. Generally, Cu which is important nutrients for humans, is considered a much lower health risk to humans than Pb, and Cd (Alexander *et al*., 2006).

In this study, the THQ in Pb and Cr metals is far less than 1 in all the vegetables species; therefore, it does not pose health risk concern. The Cd and Cu have THQ higher than 1 in all vegetables species, therefore pose health risk concern. Higher

THQ for Cd, Pb, and Ni were also reported earlier by Singh *et al*., (2010) in vegetables from wastewater irrigated area. The potential health risks of heavy metal accumulation through vegetable consumption were likely to be higher than for the normal population. However, vegetable consumption was just one part of food consumption. There are other foods stuff such as fishes, meat, rice, and cassava (Osu *et al*, 2015; Iwegbue, 2015; Jolaoso *et al*., 2016). For Arusha population, food consumption, air pollution, drinking water are the important pathways for human exposure to toxic metals. Consequently, the potential health risks for the residents were actually higher.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

The direct use of wastewater from oxidation pond in irrigating vegetables is constrained. The toxicity and level profile of several heavy metals in wastewater and soil is of particular concern. Although many metals are known to be essential micronutrients for humans, still they are toxic in excess due to bioaccumulation in human body. Other metals such as Cd and Pb are not essential, and are known to be harmful if consumed in exceeds certain limits. Unfavorable health effect from chronic ingestion of these metals may only become obvious after several years.

In this chapter we will conclude what we have detected in the study of assessment of human health risk due to accumulation of heavy metal in African green leafy vegetable irrigated by wastewater in arusha municipality. The study succeeds in providing valuable information about potential health risks associated with using wastewater for vegetable irrigation. Furthermore, the recommendations for further study will be provided.

5.2 Conclusion

This study showed that the intensive uncontrolled operation of various industries and wastewater disposal has resulted in the release of trace metals in the local environment surrounding Arusha municipal oxidation pond. Numerous studies have linked excessive bioaccumulation of heavy metals to numerous health abnormalities. They pose both short and long term environmental health risks. Leafy vegetables produced in open-fields or with contaminated irrigation water are known to possess high concentration of heavy metals. Given the importance of vegetables in the food pyramid, their safety is very important from viewpoint of public health. Awareness should be raised about the advantages of organic farming and the dangers of heavy metal pollution in order for farmers to adopt best practices for cultivation of vegetables. Wastewater irrigation for vegetables and food crops should be discouraged as it serves as the major route for heavy metal accumulation in vegetables.

THQ calculations showed that the THQ in Cd and Cu metals is greater than 1 in all the vegetables species, while Pb and Cr has less than 1 THQ, however if all other routes of entry of heavy metal is considered the potential health risks for residents might actually be higher in all heavy metals regardless of having less than 1 THQ of Cr and Pb in this study.

Vegetable contamination by heavy metals can lead bioaccumulation of these toxic and disease-causing elements in the body of consumers. Therefore, regular monitoring of effluents, soils, and vegetables are essential to prevent excessive build-up of the toxic heavy metals in food. This study shows that the implications of using wastewater as an irrigation source and indeed practicable to understanding the risks associated to human health.

5.2 Recommendations

(i) The effluent from Arusha municipal stabilization pond has heavy metal contaminants that are beyond the permissible concentration limit. Therefore the Arusha Municipal town council wastewater effluent should not be used for irrigation purpose.

(ii) The current smallholder farmers' irrigation practice using the discharged out wastewater from the municipal stabilization pond effluent as irrigation source showed high concentration of toxic heavy metals in the irrigated water, vegetables as well as the irrigated top and sub-soils surrounding this zone. This concentration level of the heavy metals in all two types of vegetable was harmful that can cause health problem for human/consumers.

(iii) The concerned Administrative bodies, health departments, Arusha Municipal and regional level agricultural offices and of course, farmers in the area should be aware of the problem associated with the effluent coming out of the Arusha Municipal stabilization pond.

(iv) There should be an alternate study on how the farmers can use the effluent from the wastewater stabilization pond safely for irrigation purpose.

(v) Management of Arusha Urban Water Supply Authority must be aware of the situation and they should install and/or establish an urgent mitigation/cleaning measures on the effluents coming out of their wastewater.

(vi) Health science researchers are advised to launch additional assessment and supplementary information on consumers who obtain or buy vegetables harvested from contaminated area.

5.3 Recommendation for Further Studies

(i) We are hereby advocating strongly for continued research along the lines laid down in this study using wider range of heavy metals in food crops, fruits and vegetables to develop even more secure recommendations for practice.

(ii) This study might be repeated using different methods such as GFAAS, ICP-OES, ICP-MS or any other Metal analyzer, which allow speciation to be examined, so as to compare the heavy metal contents of the selected sample types.

REFFERENCES

Abba, A., and Ibrahim, S. (2017). Bioaccumulation of Heavy Metals in Amaranths Sp. L. Sold at Vegetable Farms in Katsina Metropolis. *Science World Journal 12(1),* 1 - 4

Adekola, F. A., Inyinbor, A. A., and Raheem, A. M. O. (2012). Heavy metals distributions and speciation in soil around a Mega Cement Factory in North-Central Nigeria. *Ethiopian Journal of Environmental Studies and Management, 5(1),* 11-12.

Adesuyi, A. A., Njoku, K. L., and Akinola, M. O. (2015). Assessment of Heavy Metals Pollution in Soils and V egetation around Selected Industries in Lagos State, Nigeria. *Journal of Geoscience and Environment Protection*, *3(1),* 11-19.

Afolami, I., Anyakora, C., Ebuehi, O., and Bolawa, O. (2010). Lead levels in some edible vegetables in Lagos, Nigeria. *Scientific Research and Essays*, *5(8),* 813-818.

Agency for Agency for Toxic Substances and Disease Registry (ATSDR), (2012). *Toxicological profile for Cadmium*. Atlanta, GA: U.S. Department of Health and Human Services.

Ahmad. J. U., and Goni M. A. (2010). Heavy Metal Contamination in Water, Soil, and Vegetables of the Industrial Areas in Dhaka, Bangladesh. *Environmental Monitoring and Assessment, 166(14),* 347–357.

Ahmad. M. K., Islam. S., Rahman. S., Haque. M. R., and Islam, M. M. (2010). Heavy Metals in Wlater, Sediment and Some Fishes of Buriganga River, Bangladesh. *International Journal of Environmental Research,* **4(2),** 321–32.

Alexander, P. D., Alloway, B. J., Dourado, A. M. (2006). Genotypic variations in the accumulation of Cd, Cu, Pb and Zn exhibited by six commonly grown vegetables. *Environmental Pollution*, *144(5),* 736– 745.

Amfo-Out, R. (2012). Behaviour of Cadmium and Lead Concentration from Irrigation Water in Soils at Five Urban Irrigation Sites in Ghana. *International Journal of Plan, Animals and Environmental Sciences, 2(2),* 65 – 72.

Anetor J. I., Anetor, G. O., Udah, D. C., and Adeniyi, F. A. (2008). Chemical carcinogenesis and chemoprevention: Scientific priority area in rapidly industrializing developing countries. *African Journal of Environmental Science and Technology, 2(7),* 150-156.

Arora. M., Bala, K., Rani, S., Rani, A., Kaur, B., and Mittal, N. (2008). Heavy Metal Accumulation in Vegetables Irrigated with Water from Different Sources. *Food Chemistry*, *111(2),* 811-815.

Asfaw, T. B. (2017). Determination of Total Chromium and Chromium Species in Kombolcha Tannery Wastewater, Surrounding Soil, and Lettuce Plant Samples, South Wollo. *Ethiopia Advances in Chemistry, 4(2),* 21-27.

Baba, A., Deniz, O., Ozcan. H., Erees. F. S., and Cetiner. Z. S. (2008). Geochemical and Radionuclide Profile of Tuzla Geothermal Field, Turkey. *Environmental monitoring assessment 145(7),* 361-374.

Badilla-Ohlbaum, R., Ginocchio, R., Rodríguez, P. H., Céspedes, A., González, S., Allen, H. E., and Lagos, G. E. (2001). Relationship between soil copper content and copper content of selected crop plants in central Chile, *Environmental Toxicology Chemistry, 20(12),* 2749-2757.

Bainies, J. (1999). Dietary exposure assessments lead. Australia and New Zealand national assessments Annual Report. Canberra, Australia.

Baird, C. (2002). *Environmental Chemistry. Bookman, Porto Alegre, RS, Brazil (in Portuguese.* New Delhi: Ashoka Law House.

Botwe B. O., Addo, M. A., Darko, E. O., Gordon, C., Nyarko, B. J. B., Gbadago, K. J., Nyarko, E., and Affum, H. A. (2012). Evaluation of Heavy Metals Contamination of Soil and Vegetation in the Vicinity of a Cement Factory in the Volta Region Ghana.

Bureau of Indian Standards BIS, (2012). Specification for Drinking Water IS: 10500 New Delhi, Retrieved on 11th May 2018 from: http://cgwb.gov.in/Documents/WQ-standards.pdf.

Castro-Gonzalez, M. I., and Mendez-Armenta, M. (2008). Heavy Metals Implications Associated to Fish Consumption. *Environmental Toxicology and Pharmacology 26(3),* 263-271.

Coutate, T. P. (1992). *Food, the chemistry of its component 2nd edition.* Cambridge: Elsevier.

Divrikli, U., Horzum, N., Soylak, M., and Elci, L. (2006). Trace heavy metal contents of some spices and herbal plants from western Anatolia, Turkey. *International Journal of Food Science and Technology*, 41(3), 712-716.

FAO/WHO, (2011). Food Standards programmer Codex committee on contaminants in foods. 5th Session The Hague, Netherlands, 21-25 March 2011. Working document for information and use in Discussions Related to Contaminants and Toxins in the GSCTFF, prepared by Japan and the Netherlands.

Ghosh. M., and Singh, S. P. (2005). A Review of Phytoremediation of Heavy Metals and Utilization of its by Products. *Applied Ecology and Environmental Research, 3(1),* 1 – 18.

Hussain. J., Mohammad. A., Hussai. I., and Farid, N. (2015). Distribution of Heavy Metals in River Chambal and its Tributaries. *Toxicology and Environmental Health Sciences,* 1(1), 1-14.

Iwegbue, C. M. A. (2015). Metal concentrations in selected brands of canned fish in Nigeria: Estimation of dietary, intakes and target hazard quotients. *Environmental Monitoring Assessment*, 187(8), 1–15.

Iyaka, Y. A., Idris, S., Alawode, R. A., and Bagudo B. U. (2014). Nutrient Content of Selected Edible Leafy Vegetables, *Amer Jour of Appl. Chemistry* **2**(3), 42-45.

Iyaka. Y. A, (2007). Concentration of Cu and Zn in some fruits and Vegetables Commonly Available in North Central Zone of Nigeria. *Electronic Journal of Environmental Agriculture Food Chemestry, 6(6),* 2150-2154.

Jarup, L. (2003). Hazards of heavy metal contamination, *British Medical Bulletin, 68(1),* 167-182.

Jolaoso, A. O., Njoku, K. L., Akinola, M. O., Adesuyi, A. A., and Adedokun, A. H. (2016). Heavy Metal Analyses and Nutritional Composition of Raw and Smoked Fishes from Ologe and Lagos Lagoon, Lagos, Nigeria, *Journal of Applied Science and Environmental Management*, *20(2),* 1355-1371.

Kachenko A. G., and Singh, B. (2006). Heavy Metals Contamination in Vegetables Grown in Urban and Metal Smelter Contaminated Sites in Australia. *Water, Air, and Soil Pollution, 169(4),* 101-123.

Kara. K., Türkdoğan. K. M., Kilice. I. F., 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),* 175-179.

Khan, S., Lin, A., Zhang, S., Huc, Q., and Zhu, Y. (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution, 152(2),* 686– 692.

Kisamo, D. S. (2003). Environmental Hazards Associated with Heavy Metals in Lake Victoria Basin (East Africa) Tanzania. In: *Africa Newsletter on Occupational Health and Safety*, *13,* 67-69.

Kumar. N. J. I. (2007). Characterization of Heavy Meals in Vegetables Using Inductive Coupled Plasma Analyzer (ICPA). *Journal of Applied Science and Environmental Management, 11(3),* 75-79.

Lar. U. A., and Usman. A. M. (2012). Environmental Distribution of Trace Metals in the Biu Volcanic Province, Nigeria: Exposure and Associated Human Health Problems. *International Journal of Scientific and Engineering Research,* **3**(10), 1-16.

Lokeshappa, B., Shivpuri, K., Tripathi, V., and Dikshit, A. K. (2012). Assessment of T oxic Metals in Agricultural Produce, *Food and Public Health*, 2(1), 24-29.

Luo, J. (2013). The Chromium in Timberline Forests in Eastern Tibetan. *Plateau Environmental Science Processes Impacts 15(3),* 1930-1937.

Maleki, A., and Zarasvand, M. A. (2008), Heavy Metals in Selected Edible V egetables and Estimation of their Daily Intake in Sanandaj, Iran, *The Southeast Asian Journal of Tropical Medicine and Public Health*, 39(2), 335 – 340.

Mapanda, F., Mangwayana, E. N., Nyamangara, J., and Giller, K. E. (2005). The effects of long- term irrigation using water on heavy metal contents of soils under vegetables Agriculture. *Journal of Ecosystem and Environment, 107(3),* 151-156.

Mohamed, M. (2015). Assessment of Heavy Metal Concentrations in Water and Sediments from Shallow Wells in Kilwa Masoko Tanzania. (Unpublished) MSc (Physics) Dissertation, University of Dar es Salaam, Tanzania.

Mohammed. N. K, (2000). The Optimization of Energy Dispersive X-ray Fluorescent (EDXRF) Spectrometer for the study of Heavy Metal Pollution. (Unpublished) Master thesis, Department of Physics University of Dar es Salaam, Tanzania.

Mohod. C. V., and Baba. S. G. (2013). Review of Heavy Metals in Drinking Water and their Effect on Human Health. *International Journal of Innovative Research in Science, Engineering and Technology, 2(7),* 2992-2996.

Moore .J. W., and Ramamoorthy, S. (1984). *Heavy Metals in Natural Waters: Applied Monitoring and Impact Assessment.* New York: Springer Verlag.

Muchuweti, M., Birkett, J. W., Chinyanga, E., Zvauya, R., Scrimshaw, M. D., and Lester, J. N. (2006). Heavy Metal Content of Vegetables Irrigated with Mixtures of Wastewater and Sewage Sludge in Zimbabwe: Implications for Human Health. Agriculture, *Ecosystems Environment,* 112(3), 41–48.

Mutune. A. N. (2013). Impact analysis of lead, copper and zinc content in selected African Indigenous and exotic vegetables from Nairobi Markets. *Afr. J. Environ. Sci. Technol*. **7**(6), 540-545.

Mwegoha, W. J. S., and Kihampa, C. (2010). Heavy Metal Contamination in Agricultural Soils and Water in Dar es Salaam City, Tanzania, *African Journal of Environmental Science and Technology, 4(11),* 763-769.

Nazir, R., Khan, M., Masab, M., Rehman, U. H., Rauf. U. N., Shahab, S. Ameer, N. Sajed, M., Ullah, M., Rafeeq., M., and Shaheen, Z. (2015). Accumulation of Heavy Metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the Soil, Water and Plants and

Analysis of Physico-chemical Parameters of Soil and Water Collected from Tanda Dam Kohat. *Journal of Pharmaceutical Science and Research,* **7**(3), 89-97.

Olowoyo. J. O. (2011). Uptake and Translocation of Heavy Metals by Medicinal Plants Around a Waste Dump Site in Pretoria, South Africa. *South Afr. J. Bot*. 78(4), 116 - 121.

Oluwatosin, G. A., Adeoyolanu, O. D., Ojo, A. O., Are, K. S., Dauda, T. O., and Aduramigba-Modupe V. O. (2010). Heavy Metal Uptake and Accumulation by Edible Leafy Vegetable (Amaranthus Hybridus L.) Grown on Urban Valley Bottom Soils in Southwestern Nigeria, *Soil and Sediment Contamination,* 19(3), 1–20.

Oluwole, S. O., Makinde, O. S. C., Yusuf, K. A., Fajana, O. O., and Odumosu, A. O. (2013), Determination of Heavy Metal Contaminants in Leafy Vegetables Cultivated By the Road Side, *International Journal of Engineering Research and Development,* 7(3), 1-5.

Osu, S. R., Solomon, M. M., Abai, E. J., and Etim, I. G. (2015). Human health risk assessment of heavy metals Intake via cassava consumption from crude oil impacted soils with and without palm bunch ash additive, *International Journal of Technical Research and Applications*, 3(4), 140-148.

Pandey, R., and Shubhashish, K. (2012). Dietary Intake of Pollutant Aerosols via Vegetables Influenced by Atmospheric Deposition and Wastewater Irrigation. *Ecotoxicology and Environmental Safety, 76(1),* 200–208.

Radwan, M.A., and Salama, A.K. (2006): Market basket survey for some heavy metals in Egyptian fruits and vegetables, *Food Chem. Toxicol*. 44, 1273-1278.

Rahman. S. H., Khanam. D., Adyel. T. M., Islam. M. S., Ahsan. M. A., and Akbor. M. A. (2012). Assessment of heavy metal contamination of agricultural soil around Dhaka Export Processing Zone (DEPZ), Bangladesh, implication of seasonal variation and indices. *Applied Sciences, 2(3),* 584–601.

Rattan, R. K., Datta, S. P., Chhonkar, P. K., Suribabu, K., and Singh, A. K. (2005). Long-term Impact of Irrigation with Sewage Effluents on Heavy Metal Content in Soils, Crops and Goundwater-a Case Study. *Agriculture. Ecosystem and Environment*, *109(5),* 310 - 322.

Rehman, A., Ullah, H., Khan, R. U., and Ahmad, I. (2013). Population based study of heavy metals in medicinal plant Capparis decidua. *International Journal of Pharmacy and Pharmaceutical Sciences, 5(1)*, 108–113.

Sajjad, K., Farooq, R., Shahbaz, S., Aziz, M., and Sadique, M. (2009). Health Risk Assessment of Heavy Metals for Population via Consumption of Vegetables, *World Applied Science Journal*, *6(2),* 1602-1606.

Saria, J. A. (2016). Assessment of Heavy Metals Pollution in the Soil around the Industries in Dar es Salaam City. *African Resource Development Journal,* ***2****(2),* 214 – 228.

Saria, J. A., and Irene, T. (2011). Heavy Metal Levels in Drinking Water: Mining Area vis-à-vis Poorly Planned City in Tanzania, *Huria Journal* ***9****(4),* 70 – 79.

Satter, S. (2012). Transfer of trace elements from Soil to Plants in Industrial Area of Dhaka and its health risk. Savar, Bangladesh: M.Sc Thesis, Department of Environmental Science, Jahangirnagar University, South Africa.

Shahrzad, K., and Keivan, S. (2015). Accumulation and Translocation of Heavy Metals by *Amaranthus Retroflexus J Earth Environ Health Sci* 1(2), 58 – 60.

Sharma. B., and Tyagi, (2013). Simplification of Metal Ion Analysis in Fresh Water Samples by Atomic Absorption Spectroscopy for Laboratory Students. *Journal of Laboratory Chemical Education, 1(3*), 54-58.

Shemdoe, R. S. (2010). Heavy Metal Concentrations in Soils and Leachates of Mtoni Dumpsite Bordering the Indian Ocean in Dar es Salaam. *Tanzania Journals of Science, 5(16),* 2143–2147.

Shrivastava, R. (2006). Effects of Chromium on the Immune System FEMS. *Immunology and Medical Microbiology,* 34(1), 1-7.

Singh, S., Zacharias, M., Kalpana, S., and Mishra, S. (2012). Heavy Metals Accumulation and Distribution Pattern in Different Vegetable Crops. *Journal of Environmental Chemistry and Ecotoxicology, 4(2),* 170-177.

Sinha, S., Gupta, A. K., Bhatt, K., Pandey, K., Rai, U. N., and Singh, K. P. (2006). Distribution of metals in the edible plants grown at Jajmau, Kanpur (India) receiving treated tannery wastewater: relation with physiochemical properties of the soil, *Environ. Monit. Assess*, 115, 1-22.

Sobolev, D., and Begonia, M. F. T. (2008). Effects of Heavy metal contamination upon soil microbes: lead-induced changes in general and denitrifying microbial communities as evidenced by molecular markers. *International Journal of Environmental Research in Public Health, 5(5),* 451-459.

Sobukola, O. P., Adeniran, O. M., Odedairo, A. A., and Kajihausa, O. E. (2010), Heavy metal levels of some fruits and leafy vegetables from selected markets in Lagos, Nigeria. *African Journal of Food Science*, *4(2),* 389 – 393.

Taghipour, H., and Mosaferi, M., (2012). Heavy Metals Concentrations in Groundwater used for Irrigation, *Health Promot Perspect, 2(2),* 205–210.

Tanzania Bureau of Standard, (1997). Finalized Tanzanian Standard Specifications for Natural Mineral Water. Retrieved on 21st May 2017 from: http://www.tbs.go. tz/standards/.

Tanzania Standards for Receiving Water, Effluents and soils (TZS 789:2003). Tanzania Bureau of Standards. Retrieved on 21st May 2017 from: http://extw prlegs 1.fao.org/docs/pdf/tan182368.pdf.

Thürmer. K. (2002). Autocatalytic oxidation of lead crystalline surfaces. *Journal of Science, 297(5589),* 2033–2035.

Tiwari, K. K., Singh, N. K., Patel M, P., Tiwari, M. R., and Rai, U, N. (2011). Metal contamination of soil and translocation in vegetables growing under industrial wastewater irrigated agricultural field of Vadodara, Gujarat, India. *Ecotoxicology and Environmental Safety, 74(2),* 1670–1677.

Tsafe A. I., Hassan, L. G., Sahabi, D. M., Alhassan, Y., and Bala, B. M. (2012). Evaluation of Heavy Metals Uptake and Risk Assessment of Vegetables Grown in Yargalma of Northern Nigeria, *J. Basic. Appl. Sci. Res*. 2, 6708-6714.

U.S. Department of Agriculture (USDA), (2007). USDA Nutrient Database for Standard Reference, Release 20. Riverdale, MD. Retrieved on 21st May 2017 from: http://www.ars.usda.gov/main/site_main.htm?modecode=12-35-45-00.

USEPA, (2010). *Risk-based Concentration Table. United State Environmental Protection Agency.* Washington, DC: An Open Access and Academic Publishers.

Valko, M., and Croninm, M. T. D. (2005) Metals, toxicity and oxidative stress. *Current medical chemistry, 12(10),* 1161–1208.

Vuori, K. M. (1995). Direct and Indirect effects of iron on river eco systems. *Annales*

Zoologica Fennici, 32(2), 317–329.

Waalkes, M. P. (2000). Cadmium carcinogenesis in review, *Journal of Inorganic Biochemistry*, *79(2),* 240-244.

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 and Total Environment*, *350(4),* 28–37.

WHO, (1996). *Trace Elements in Human Nutrition and Health.* Geneva: World Health Organization.

Wiberg, N. (2001). *Inorganic Chemistry.* San Diego: Academic Press.

Wilson, B., and Pyatt, F. B. (2007). Heavy metal dispersion, persistence, and bioaccumulation around an ancient copper mine situated in Anglesey, *UK. Ecotoxicol Environ Saf, 66*: 224-231.

Xu. D., Zhou, P., Zhan, J., Gao. Y., Dou, C., and Sun, Q. (2013). Assessment of Trace Metal Bioavailability in Garden Soils and Health Risks via Consumption of Vegetables in the Vicinity of Tongling Mining Area, China. *Ecotoxicology and Environmental Safety, 90(2),* 103–111.

Yebpella. G. G*,* (2011). Assessment of Cd, Cu, Mn and Zn Levels in Soil, Water and vegetable Grown on Irrigated Farm along River Kubani, Zaria, Nigeria. *J. Appl. Environ. Biol. Sci*. **1**(5), 84- 89.

Zhang. U. W. (2014). A Review on Heavy Metal Contamination in the Soil Worldwide: Situation, Impact and Remediation Technique. *Environmental Skeptics and Critics,* **3**(2), 24-38.

APPENDICES

Appendix I: Research Clearance Letter

THE OPEN UNIVERSITY OF TANZANIA
DIRECTORATE OF, POSTGRADUATE STUDIES

Kawawa Road, KinondoniMunicipality,
P.O. Box 23409
Dar es Salaam, Tanzania
http://www.out.ac.tz

Tel: 255-22-2666752/2668445
Ext.2101
Fax: 255-22-2668759,
E-mail:dpgs@out.ac.tz

Date: 22nd April 2018

Our Ref: PG201703017

Arusha Urban Water and Sanitation Authority,
P .o Box 13600,
Arusha.

RE: RESEARCH CLEARANCE

The Open University of Tanzania was established by an act of Parliament No. 17 of 1992, which became operational on the 1st March 1993 by public notice No. 55 in the official Gazette. The act was however replaced by the Open University of Tanzania charter of 2005, which became operational on 1st January 2007. In line with the later, the Open University mission is to generate and apply knowledge through research.

To facilitate and to simplify research process therefore, the act empowers the Vice Chancellor of the Open University of Tanzania to issue research clearance,on behalf of the Government of Tanzania and Tanzania Commission for Science and Technology,to both its staff and students who are doing research in Tanzania. With this brief background, the purpose of this letter is to introduce to you Mr **Naimani Sarikieli Pallangyo Reg No: PG201703017, (0767391797)** Pursuing **Masters of Science in Envirornmental Studies.(M.Sc.ES)** We here by grant this clearance to conduct a research titled **"Assesment of Heavy Metal Concentration in African Green Leafy Vegetables Irrigated from Arusha Municipal Oxidation Ponds."**. *He* will collect his data at Arusha Region From 23rd April 2018 to 21st September 2018.
Incase you need any further information, kindly do not hesitate to contact the Deputy Vice Chancellor (Academic) of the Open University of Tanzania, P.O. Box 23409, Dar es Salaam. Tel: 022-2-2668820.We lastly thank you in advance for your assumed cooperation and facilitation of this research academic activity.

Yours sincerely,

Prof HosseaRwegoshora
For: VICE CHANCELLOR
THE OPEN UNIVERSITY OF TANZANIA