MEKELLE UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

MSc Thesis on:

Assessment of Heavy Metal Levels in Soil, Vegetables and Wastewater used for Irrigation in Wukro, Tigray, Ethiopia

Submitted to the Department of Chemistry in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry (Analytical Chemistry)

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September, 2025

Mekelle, Ethiopia

APPROVAL SHEET

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DECLARATION

First, I declare that this thesis entitled "***Assessment of Heavy Metal Levels in Soil, Vegetables and Wastewater used for Irrigation in Wukro, Tigray, Ethiopia***" is my original work and has not been presented for any other award, and that all sources used in this thesis are duly acknowledged. This thesis was carried out under the supervision of Mebrahtu Hagos (Ph.D.) Department of Chemistry, Mekelle University in the academic year of 2025 G.C.

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This thesis has been submitted for examination with my approval as University advisor.

Mebrahtu Hagos (Ph.D.) Signature with date: ________________________

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Date of submission: ________________________________

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to my supervisor Dr. Mebrahtu Hagos, Mekelle University for his guidance and valuable suggestions throughout this study.

More thanks go to Mekelle University for admitting me to pursue my master’s degree and also to Mines and Geology of Mekelle for allowing the use of their laboratories and facilities such as AAS.

I also deeply thank technicians in the Department of Chemistry, Mekelle University particularly Mr. Abadi who was always available to issue the required chemicals and apparatus; more thanks go to my husband for his assistance in sample collection and transportation of samples.

In the same strength, I thank all my family members and friends for their encouragement as well as moral support throughout the study.

Lastly, I thank the almighty Lord for his wisdom and knowledge.

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ABBREVIATIONS AND ACRONYMS

FAO	Food and Agricultural Organization
EFMOH	Ethiopian Federal Ministry of Health
EHDA	Ethiopian Horticulture Development Agency
EFDA	Ethiopian Food and Drug Authority
EPA	Environmental Protection Authority
EPHI	Ethiopian Public Health Institution
ETFRUIT	Ethiopian Fruit and Vegetables Marketing Enterprise
HQ	Hazard Quotient
HRI	Health Risk Index
RfD	Reference Dose
WHO	World Health Organization

ABSTRACT

Fresh vegetables and fruits are the most common foods of human diet all around humankind often used for balanced diet, prevention and treatment of various diseases. However, if these vegetables and fruits grow in a polluted environment they can be a major public concern due to their toxic property which leads to acute and chronic health effects. The aim of this study was to determine the level of manganese, chromium, copper, zinc and lead and to estimate their health risks associated with their daily intake in vegetables grown in Wukro town, Tigray. FAAS was used to determine concentrations of those heavy metals in selected vegetables (cabbage, onion, spinach, lettuce and tomato). 1 gm of each sample was digested by wet digestion method using a mixture of HNO_3 *(69%),* $HClO_4$ *(70%) and* H_2O_2 *(30%) until a clear solution was prepared. After proper dilutions, the solutions were aspirated into the FAAS. The absorbance value of heavy metals was converted in to concentration using linear calibration curve equation. Finally, the concentration of each metal was expressed in mg/Kg of dry weight of each sample and health risk was estimated by using estimated daily intake (EDI), target hazard quotient (THQ), and hazard index (HI) for selected heavy metals. The validity of the method was checked by the analysis of spiked samples whose recovery was found in the range of 90.8−107.0% and %RSD value in the range of 0.03−7.7%. The average concentrations of Cr and Cu in all of the selected vegetables were lower than the maximum limit of normal values. However, concentrations (mg/kg) of Pb in cabbage (2.05), spinach (2.47), and lettuce (1.56) had exceeded the safe limit, 0.3 mg/Kg set by FAO/WHO. Hence, consuming plant products grown from contaminated water sources such as municipal wastes, industrial effluents, engine fuels, garages disposals, constructions and car washes are potentially toxic to plants, animals and humans. Therefore, people and concerned bodies should take care of the sources of water for irrigation.*

Keywords: *Heavy metals, Toxic metals, Vegetables, Wet digestion, Pollution, FAAS*

CHAPTER ONE

1. INTRODUCTION

1.1Background of the study

Water is a common chemical substance that is essential for the survival of all known forms of life. The major proportion of all water quality degradation worldwide is due to anthropogenic activities causes. Water resources are the main areas which are very closely associated with the daily life of masses. The municipal and industrial wastewater discharge constitutes the constant polluting source, on the concentration of pollutants in the river water. It is imperative to prevent and control the rivers pollution and to have reliable information on the quality of water for effective management. In view of the variations in the hydrochemistry of rivers, regular monitoring programs are required for reliable estimates of the water quality. [Jayalakshmi, Lakshmi, 2011].

Rivers worldwide serve as the recipients of great quantities of waste discharged agricultural, industrial and domestic activities. Agriculture seems to be the most considerable sources of pollution due to run off of from fertilized land. Industrial processing and solid waste dumps are considered the main anthropogenic activities sources of metal pollution. Shortage of freshwater throughout the world could be attributed to human abuse, which most commonly is in the form of pollution [Shmi, amsath, 2012]. The contamination by hazardous substance can pose risk to human health in particular via the food chain. However, it becomes more and more difficult to meet such water quality standards because of continuous economic expansion, urban development and growing population pressure. [Kolawole, Ajayi, 2011]. One such resource which is faced the same problem is the Genfel River, the river system of Wukro town in Tigray.

Heavy metal contamination of environment is worldwide phenomenon that has attracted a great deal of attention. Heavy metal contamination of soil resulting from urban river water irrigation cause serious concern due to impacts on human welfare. In urban areas, the careless disposal of industrial effluents and other municipal wastes in rivers may contribute greatly to the poor quality of rivers water. Most of the rivers in the urban areas of the developing countries are the ends of effluents discharged from the industries and municipal wastes [Raymond Wuanaand Felix, 2011].

1.2 Statement of the problem

World Health Organization (WHO) estimated that about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution and is increasing worldwide. The most commonly found heavy metals pollutants include Cd, Cr, Cu and Pb which cause risks for human health and the environment. Their toxicity is a problem of increasing impact for ecological, evolutionary, nutritional and environmental reasons. Therefore, vegetables and fruits quality and safety has become a major public concern worldwide mainly in a developing country like Ethiopia [WHO, 2012].

Heavy metals are among the major contaminants of vegetables and fruits and may be considered the major problem to our environment such problem is getting more serious all over the world especially in developing countries such as South Africa, Turkey, Yemen, Zimbabwe, Nigeria, Tanzania and Egypt. Dietary exposure to heavy metals, namely Cd, Pb, Cr, Cu and others have been identified as a risk to human health through the consumption of vegetable, fruit and other crops [Mahurpawar, 2015; Sharma et al., 2016].

Smith et al estimated that about 25–30% of the total burden of disease in the world is related to environmental factors, including chemical toxicants [Smith et al., 1999]. Lead and Cadmium are the most toxic and the most abundant metals in food. Excessive accumulation of these heavy metals in human bodies creates these problems. Cadmium can lead to kidney damage, high blood pressure, nervous system disorders and carcinogenesis [WHO, 2012]. The prevalence level of lead in the blood of children worldwide is estimated at 40%, where the risk is more concerning in developing countries [Mamtani et al., 2011].

Ethiopia is one of the developing and fastest growing countries in sub-Saharan Africa, which has attracted and being attracting many investors from all over the world. As a result, small and medium scale industries (mainly working on production of brews, fabrics, chemicals, floriculture and tanneries) are growing in a fastest rate and are generally established around urban and sub-urban areas and sideways of rivers. The wastewater being released from these businesses are reportedly encompasses elevated levels of toxic metals including cadmium (Cd), arsenic (As), mercury (Hg), copper (Cu) and lead (Pb). These toxic metals have been long regarded as serious environmental contaminants even at smaller concentration because of their detrimental effect to public health [Gizaw Z., 2019].

1.3 Significance of the Study

Heavy metals contamination is more prevalent in under developed and developing countries due to inefficient food regulatory policies, inadequate environmental monitoring, and enforcement strategies.

In the past, different researchers have investigated the concentrations of some heavy metals, such as Pb, Mn, Cu, Zn and Cr in different vegetables from the market sites in some developed and developing countries. Nevertheless, limited published data are available in Wukro, Tigray, Ethiopia. Hence, there are not any established papers as well as well documented safety precaution and environmental safety. Therefore, this study is to insight the selected heavy metal concentration in cabbage, onion, lettuce, spinach and tomato samples cultivated in Wukro town, Tigray, Ethiopia. In addition, their harmful effects on living organisms related with the consumption of these vegetables have also been appraised through the determination of estimated daily intake (EDI), target hazard quotient (THQ), and hazard index (HI) for selected heavy metals. The study finding also may have significant value for policy makers and development partners who lend a hand in the growth of good quality foods and to apply environmental protection policy and food quality policies and regulations can be implemented to boost and stimulate both factors. The study sets to benefit the population and concerned bodies as it may appreciate the benefit of adopting various food quality control practices that may enable them improve the quality of food production and also used for scholars and researchers who might have an interest in developing the findings as a source of reference.

1.4 Scope of the Study

The study had two scopes, the first one being the geographic scope which only focuses in Wukro town but not address outside of that town. The second scope of the study is the conceptual scope which focuses on determination of the level of manganese, chromium, copper, zinc and lead and to estimate their health risks associated with their daily intake in selected edible vegetables of Wukro town, Tigray, Ethiopia.

The study was selective on heavy metals of Mn, Pb, Cr, Zn and Cu because these heavy metals have high background concentrations plus additional concentrations from use of pesticides, fertilizers, wastewater in farming and herbicides in regions where vegetables and fruits are grown which are cumulative poisons which leads to environmental hazards and reported to be highly toxic. Further, these heavy metals are of great interest in toxicological research as their elevated uptake in the human food chain are thought to be precursors to non-contagious diseases of which are of concern in the modern-day research [Orish et al., 2012].

1.5 Research Questions

The research questions of this study are;

1) Does the level of manganese, chromium, copper, zinc and lead in each selected vegetables of spinach, tomato, onion, lettuce and cabbage above the FAO/WHO tolerable limit?

2) Does the daily intake of these elements within the Recommended Dietary Allowance (RDA) values set by FAO/WHO?

3) Does each selected heavy metal have health risks associated with their daily intake in selected vegetables?

1.6 Research Hypothesis

H01: The levels of manganese, chromium, copper, zinc and lead in selected vegetables sold in Wukro town are within FAO/WHO recommended health standards.

H02: The daily intakes of these elements via selected vegetables are within the recommended dietary allowance values set by FAO/WHO.

H03: The uptake of heavy metals via consumption of selected fresh vegetables sold in Wukro town possesses insignificant health risks to consumers.

1.7 Objectives

1.7.1 General Objective

Aim of the present study was to assess the level of manganese, chromium, copper, zinc and lead and to estimate their health risks associated with their daily intake in selected vegetables of spinach, tomato, onion, lettuce and cabbage.

1.7.2 Specific Objectives

The specific objectives of this study were:

a) To determine the level of manganese, chromium, lead, zinc and copper on selected edible vegetables in Wukro town using Atomic Absorption Spectrophotometer.

b) To compare levels of manganese, zinc, chromium, lead and copper on selected edible vegetables with recommended limits set by FAO/WHO.

c) To compare the daily intake of these elements with the recommended dietary allowance values set by FAO/WHO.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Vegetables

Vegetables and fruits have recently attracted great interest as potential therapeutic agents against a variety of diseases like those involving radical damage due to the presence of lip tropic, antioxidant and also anti-tumor properties which have a wide range of activities such as antimicrobial, anti-inflammatory, anti-mutagenic and anti-oxidative activities. These are very important to boost immunity, bone strength, lower cholesterol levels anemia prevention, alleviation of symptoms associated with gastrointestinal disorders (gastritis, peptic and duodenal ulcers, irritable bowel syndrome), improve digestive health and also support to eye, hair, skin and heart health. They also show beneficial effects in age-associated disease such as cardiovascular diseases, some forms of cancer and Alzheimer’s diseases. They are also widely used industrially as food colorant and source of dietary fiber due to its different color from the pigment [USDA, 2009; FAO/WHO, 2012].

Table 2. 1 Vegetables commonly used in Wukro town, Tigray, Ethiopia

No.	Common name	Scientific name
1	Cabbage	*Brassica oleracea*
2	Onion	*Allium cepa*
3	Lettuce	*Lectica sativa*
4	Tomato	*Lycopersicon esculentum*
5	Spinach	*Amaranthuscaudatus*

2.2 Vegetables in Ethiopia

The Ethiopian Fruit and Vegetables Marketing Enterprise (ETFRUIT) is one of trading organization established in 1980 under the Horticulture Development to deal with domestic and export trade of fresh fruits, vegetables, flowers and processed horticultural products [EHDA, 2011]. Ethiopia has a comparative advantage in the processing of horticultural products due to its favorable climate and edaphic conditions for the production of tropical, sub-tropical and temperate varies vegetables and fruits in the lowlands, midlands and highlands, respectively [EHDA, 2011]. Vegetable and fruit production is practiced both under rain fed and irrigation systems. The irrigated vegetable and fruit production system is increasing because of increasing commercial farms and development of small-scale irrigation schemes. Furthermore, access to regional and international markets due to its nearness to European and Middle Eastern countries makes the country favorable for investors in the sector [Ethiopian Investment Agency, 2012]. In addition, its production and consumption are increasing in Ethiopia because of increasing export to Djibouti, Somalia, South Sudan, Sudan, Middle East and European markets. In these countries there is a sustained demand for products such as onions and cabbages, resulting in export increase from 25,300 tons in 2002/03 to 63,140 tons in 2009/10 [EHDA, 2011]. Nowadays, the nutritional and health value of vegetables and fruits are also well recognized in Ethiopia because they play important roles in human health [EHDA, 2011; Ethiopian Investment Agency, 2012].

Vegetables and fruits also constitute a source of cash income for the households and an opportunity to increase smallholder farmers' participation in the market. Both fresh and processed fruits and vegetables have a huge domestic market in Ethiopia which is by far significant than that of the export volume and also used as source of raw material for local processing industry. Products like tomato paste, tomato juice and ground spice of Capsicum are produced for exports making a significant contribution to the national economy [Ethiopian Investment Agency, 2012].

2.3 Heavy Metals in Vegetables

Vegetables and fruits can take up and accumulate heavy metals in quantities high enough. Especially, vegetables and fruits grown on heavy metal contaminated soils accumulate higher amounts of metals than those grown in uncontaminated soils because of the fact that they absorb the heavy metals through their roots which persist in the environment and are subject to bioaccumulation in food-chains through edible parts of vegetables, fruits and other food crops. Therefore, dietary intake of food results in long-term low level body accumulation of heavy metals and the harmful impact becomes real after several years of exposure [Alam et al., 2003].

2.4. Sources of Heavy Metal Pollution in Vegetables

Sources of pollution from heavy metals and heavy metal compounds are natural constituents of all ecosystems, moving between atmosphere, hydrosphere, lithosphere, and biosphere. They exist naturally in the soil from the soil forming processes of disintegration of parent resources at rare levels (less than 1000 $mgKg^{-1}$) and infrequently poisonous. However, their distribution in the environment is a result of natural processes such as volcanoes, erosion, spring water, bacterial activity and anthropogenic activities like agricultural practices, such as pesticide and herbicide application, contaminated irrigation water, municipal waste used for fertilization, even mineral fertilizer containing traces of heavy metals. Additional anthropogenic sources of heavy metals include direct waste disposal on farmland, mining activities, use of lead as antiknock in petrol, traffic emissions, cigarette smoking, metallurgy and smelting, aerosol cans, sewage discharge and building materials, such as painting. Long-term use of excessive chemical fertilizers and organic manures or long-term simultaneous application in the bare vegetable field and the greenhouse vegetable field contributed to higher accumulation of heavy metals in the soils and water [Engwa et al., 2019; Kumar et al., 2019].

2.5 Wastewater

Industrial or municipal wastewater is mostly used for irrigation of crops mainly in perturbing ecosystem. In various African and Asian cities, studies propose that agriculture relied on the water with wastewater contributes to 50 % of the vegetable source to urban areas. Because waste water is easily available coupled with disposal problems and scarcity of fresh water [Arora et al.,2008]. The waste water from the industries of mining, electroplating, and paint or chemical laboratories often contains high concentrations of heavy metals, including cadmium, copper and lead. Waste water is known to contribute significantly to the heavy metal contents of soils; hence disposal of sewage and industrial waste into agricultural lands leads to contamination of crops including vegetables and fruits grown on that land. This is because these effluents are considered a rich source of organic matter and other nutrients also have high levels of heavy metals such as iron, manganese, copper, zinc, lead, cadmium, nickel and cobalt. Most of the heavy metals are extremely toxic because of their solubility in water [Arora et al., 2008; Khanna, 2011].

2.6 Soil

When plants decay, heavy metals that had been taken into the plants are redistributed and the soil is then again enriched with the pollutants. It has been established that heavy metals in soil are associated with various chemical forms that relate to their solubility which directly bear on their mobility and biological availability. Heavy metals in soluble form have high relation to their uptake by plants. Apart from the source of heavy metal, the physical and chemical properties of the soil also affect the concentration of heavy metals in soils. The uptake and bio-accumulation of heavy metals in vegetables and fruits is influenced by many factors such as climate, atmospheric depositions, the concentrations of heavy metals in soils, the nature of soil and the degree of maturity of the plants at the time of harvest [Arora et al., 2008].

2.7 Common Heavy Metal Toxicants and their Health Risks

2.7.1 Copper (Cu)

Copper is a naturally occurring metallic element that occurs in soil at an average concentration of about 50 parts per million (ppm). It is present in all animals and plants and is an essential nutrient for humans and animals in small amounts. The major sources of environmental Cu releases include the mining, smelting and refining of copper, industries producing products from copper such as wire, pipes and sheet metal, and fossil fuel combustion [Mahurpawar, 2015].

Copper is an essential element required for the normal functioning of more than 30 enzymes. The ability of copper to cycle between an oxidized state, Cu (II), and reduced state, Cu (I), is used by cuproenzymes involved in redox reactions. Cu homeostasis involves regulation of absorption, cellular uptake, intracellular transport, cellular efflux, and excretion from the body [ATSDR, 2004].

The circulation and proper utilization of copper in the body requires good functioning of the liver, gall bladder and adrenal glands. If any of those organs are impaired, the body cannot properly excrete and utilize Cu. Initially, the Cu will build up in the liver, further impairing its ability to excrete Cu. As Cu retention increases, it will build up in the brain, the joints and the lungs, adversely affecting the structure and function of the tissues. It is a powerful oxidant causing inflammation and free radical damage to the tissue [Ashish et al., 2013]. Studies have also shown that is required for infant growth, host defense mechanisms, bone strength, red and white cell maturation, iron transport, cholesterol and glucose metabolism [Commission, 2003].

Toxicity of copper can be classified as short term/acute toxicity and long term/chronic toxicity. Short-term (acute effects) poisoning from ingestion of excessive copper can cause temporary gastrointestinal distress with symptoms such as nausea, vomiting, and abdominal pain. Liver toxicity was seen in doses high enough that resulted in death. High levels of exposure to Cu can cause destruction of red blood cells, possibly resulting in anemia. Long term (chronic effects). Mammals have efficient mechanisms to regulate copper stores in the body such that they are generally protected from excess dietary copper levels. However, at high enough levels, chronic overexposure to Cu can damage the liver and kidneys. Wilson’s disease is an inherited (genetic) disorder in which copper builds up in the liver [Commission, 2003; Mahurpawar, 2015]. But, the carcinogenicity of copper has not been adequately studied. An increase in cancer risk has been found among copper smelters; however, the increased risk has been attributed to concomitant exposure to arsenic. Increased lung and stomach cancer risks have also been found in copper miners when a high occurrence of smoking and exposure to radioactivity, silica, iron, and arsenic which has the association of copper exposure with carcinogenesis. The international agency for research on cancer (IARC) has classified the pesticide, copper 8-hydroxyquinoline, in Group three, unclassifiable as to carcinogenicity in humans and EPA has classified Cu in Group D, not classifiable as to human carcinogenicity [US EPA,2015; Mahurpawar, 2015].

2.7.2 Manganese (Mn)

Manganese makes up about 1000 ppm (0.1%) of the Earth's crust, thus making it the 12th most abundant element (Emsley, 2001). Manganese occurs principally as pyrolusite (MnO_2), psilomelane $(BaH_2O)_2Mn_5O_{10}$, and to a lesser extent as rhodochrosite ($MnCO_3$). Manganese compounds are powerful oxidizing agents with various oxidation states (+4and +7) and can directly combine with boron, carbon, sulphur, silicon and phosphorous [Emsley, 2001]. Among the several oxidation states, the +2-oxidation state is the most stable state and the one used in living organisms for essential functions while other states are toxic for the human body. Depending on their oxidation state, Mn ions have various colors and are used industrially as pigments while MnO_2 is used as the cathode material in standard and alkaline disposable dry cells and batteries. As a free element, it is a metal with important industrial metal alloy uses, particularly in stainless steels and Manganese phosphate is used as a treatment for rust and corrosion prevention on steel. [Zhang and Cheng, 2007]. Though it is a required trace mineral for all known living organisms, in larger amounts, and apparently with far greater activity by inhalation, Mn can cause a poisoning syndrome in mammals, with neurological damage which is sometimes irreversible (ATSDR, 2002). Mnrelated complications also include psychiatric and motor disturbances, termed manganese which has occurred in people employed in the production and processing of Mn alloys (Nusseyet al.2000). People exposed to high levels of environmental pollution by Mn suffer from cerebella dysfunctions, neurological damage as was once observed in inhabitants of Groote Eylandt off the North coast of Australia (Reilly, 2002). Studies in fish for Mn have been recorded in fish from different rivers (Oguzie, 2003; Obasohan, 2008). Mn mean levels in fish from Ogba River ranged from 0.0 to 0.75mg/Kg and also Mn concentration of 17.37 mg/Kg in fish gills from river Nile have been reported (Oguzie, 2003; Obasohan, 2008; Alaa and Osman, 2010). The high concentrations of Mn are attributed to the gills being the dominant site for contaminant uptake because of their anatomical and/or physiological properties that maximize absorption efficiency from water (Alaa and Osman, 2010). Studies of Mn in sediments from Nairobi River recorded levels that ranged from 1598.33 to 4322.83 mg/Kg (Kage, 2003). Mn concentration in sediments from River Nile ranged from 139.8 to 351.8 $mgKg^{-1}$ (WHO, 2003; Alaa and Osman, 2010).

Various levels of Mn in river water that have fluctuated from the WHO recommended limit of 0.4 mg/L have been reported (WHO, 2003; Wachira, 2007; Alaa and Osman, 2010). Mn levels from river Nile that was within the recommended limits fluctuated between 0.033 and 0.14 mg/L while higher levels of 2.5 mg/L and 0.423 mg/L were recorded from Nairobi River and river Ganga respectively (WHO, 2003; Wachira, 2007; Kar et al., 2008; Alaa and Osman, 2010). The higher levels than the recommended limit of 0.4 mg/L was attributed to a sudden rainfall followed by high river discharge from upstream environment, industrial effluents and municipal wastes, geology of river bed and catchment area (Wachira, 2007; Kar et al., 2008). Adoption of adequate measures to remove the heavy metal load from the industrial waste water and renovation of sewage treatment plants are suggested to avoid further deterioration of the river water quality (Kar et al., 2008).

2.7.3 Zinc (Zn)

Zinc makes up about 75 ppm of the Earth's crust, making it the 24th most abundant element with a density of $7.14g/cm^3$. Zn is normally found in association with other base metals such as Cu and Pb in ores and has a low affinity for oxygen and prefers to bond with sulphur and occurs as ores such as sphalerite (ZnS), calamite ($ZnCO_3$) and zincite (ZnO). Zn forms alloys such as brass and bronze and has been used in construction of buildings, roofing and cladding (Emsley, 2001). Other uses of Zn include making circuit boards, photocopiers, dry cell batteries and its compounds are used in chemical and pharmaceutical industries such as paints, medicines and nutritional supplements (Reilly, 2002).The toxicity of Zn is as a result of excessive absorption which suppresses copper and iron absorption while free Zn^{2+}ion in solution is highly toxic to plants, invertebrates, and even fish (FAO/WHO, 2011). Zinc salts are intestinal irritants and can cause nausea, and abdominal pain (ATSDR, 2002). Prolonged exposure to high intakes of Zn results in copper deficiency and subsequent anemia (Reilly, 2002). There is also a condition called the zinc shakes or "zinc Chills" that can be induced by the inhalation of freshly formed Zn oxide formed during the welding of galvanized materials. It has been reported that zinc is able to damage nerve receptors in the nose, which can cause anosmia and recommended that consumers should stop using zinc based intranasal cold products and ordered their removal from store shelves (Johnson et al., 2007;Safty et al., 2008) Levels of Zn in rivers flowing through industrial or mining areas can be as high as 20 mg/l while soils contaminated with Zn through the mining of zinc-containing ores, refining, or where zinc containing sludge is used as fertilizer, can contain several grams of zinc per kilogram of dry soil (Emsley, 2001). A higher Zn mean level of 76.25 mg/l than the 3 mg/l recommended limits was recorded from Forecaddies River while lower mean levels of 0.085 mg/l during dry season and 0.716 mg/l during wet season from River Ganga's water and 1.0 mg/l from Nairobi River's water were recorded (WHO, 2003; Kithiia, 2006; Kar et al., 2008; Agatha, 2010). This level was attributed to land use activities such as agriculture system and effluent from residential and industrial area. Downstream decrease in water pollutants was observed and was attributed to the dilution effect and self-purification. Constant monitoring of the levels of contamination to assess the impact of the heavy metal in the aquatic system and use of reverie vegetation was recommended as useful in absorbing heavy metals as a means of purification (Kithiia, 2006; Agatha, 2010).

2.7.4 Lead (Pb)

Lead is an abundant heavy metal in the earth about 14 parts per million by weight or 1 part per million by moles which is usually found in ores mostly withCu, Zn and Ag. The most common Lead mineral is galena, which is Lead sulfide (PbS). Other minerals include Lead carbonate ($PbCO_3$) and Lead sulfate ($PbSO_4$) [Wilson et al., 2015].

Lead is widely distributed and ubiquitous element in the environment since it was discovered and used by humans for a long time. Natural Lead pollution occurs from volcanic explosions and forest fire. Non-natural sources were from human activities, mainly referring to the lead emission from the industry and transportation. The major sources of lead emissions to the environment today are from ore and the processing of metals, as well as leaded aviation gasoline. The highest air lead levels occur close to lead smelters. Other sources are from manufacturing batteries, coal burning, type casting, and in older houses and buildings. Since lead is not degraded by microbial activity, it is persistent in the environment and accumulates in soils, water bodies and sediments through deposition, leaching and erosion [Wilson et al., 2015].

Lead is used mainly in the production of lead-acid batteries, plumbing materials and alloys. Human occupational exposure can take place during the application and removal of protective lead containing paints, during the grinding, welding and cutting of materials painted with lead containing paints such as shipbuilding, construction, demolition industries, fabrication of lead glass and crystal, and in crystal carving. Mining, smelting, and informal processing and recycling of electric and electronic waste can also be significant sources of exposure [Derakhshan et al., 2016].

More than 80% of the daily intake of lead is derived from the ingestion of food, dirt and dust. The amount of lead in food plants depends on soil concentrations and is highest around mines and smelters. The use of lead-soldered food and beverage cans may considerably increase the lead content, especially in the case of acidic foods or drinks. Lead also comes to unintentionally contaminate food as the result of contamination with soil [WHO, 2012; Derakhshan et al., 2016].

The dietary level of lead was originally set in 1982 for infants and children, based on studies conducted with children, infants and fetuses are particularly at high risk for lead neurotoxin and developmental effects. Acute exposures to Pb may cause gastrointestinal disturbances, hepatic and renal damage,hypertension and neurological effects (malaise, drowsiness, encephalopathy) that may lead to convulsions and death, and chronic exposure commonly causes hematological effects such as anemia, or neurological disturbances, including headache, irritability, lethargy, convulsions, muscle weakness, ataxia, tremors and paralysis. There is some evidence that long-term occupational exposure to lead may cause to the development of cancer. The International Agency for Research on Cancer (IARC) has classified inorganic lead compounds as probably carcinogenic to humans (Group 2A), meaning that there is limited evidence for carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals [WHO, 2010].

2.7.5 Chromium (Cr)

Chromium is the seventh most abundant element on earth; and the second largest contributor of ground water, soil and sediment contamination. Contamination of agricultural fields with Cr is very toxic to both human being and plants and has been led a major environmental concern over the last few decades. Release of Cr compounds to the environment, water and soil is mainly due to electroplating, leather tanning, metal finishing, corrosion control and pigment manufacturing industries because it is found in fresh foods, copy machine toner and nickel in coins, kitchen utensils and milk [Tiwari et al., 2013; US EPA, 2015].

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

Adverse health effects associated with Cr (VI) exposure include occupational asthma, eye, skin and respiratory irritation, kidney and liver damage, pulmonary congestion and edema, upper abdominal pain, nose irritation and damage, respiratory cancer and discoloration of the teeth [United States Hexavalent Chromium Occupational Safety & Health Administration, 2010].

All hexavalent chromium Cr chemical compounds are considered carcinogenic to workers. The risk of developing lung, nasal, and sinus cancer increases with the amount of chromium (VI) inhaled and the length of time the worker is exposed. Studies on workers who participate in chromate production, chromate pigment, and chrome electroplating industries employed before the 1980s show increased rates of lung cancer mortality. Certain chromium (VI) compounds produced lung cancer in animals that had the compounds placed directly in their lung. Direct eye contact with chromic acid or chromate dusts, fumes and smoke containing chromium (VI) can cause permanent eye damage. Chromium (VI) can irritate the nose, throat, and lungs. Repeated or prolonged exposure can damage the mucous membranes of nasal passages and result in ulcers. In severe cases, exposure causes perforation of the septum (the wall separating the nasal passages). Some employees become allergic to hexavalent chromium so that inhaling the chromate compounds can cause asthma symptoms such as wheezing and shortness of breath and also prolonged skin contact can result in dermatitis and skin ulcers. Some workers develop an allergic sensitization to chromium. In sensitized workers, contact with even small amounts can cause a serious skin rash's [United States Hexavalent Chromium Occupational Safety & Health Administration, 2010].

2.8 The permissible guideline for heavy metals in Vegetables

Based on the effect of heavy metals on consumer's different organizations have proposed maximum permissible limits of the metals in edible vegetables and fruits. FAO/WHO set the maximum limit and the safe values and safe limits for heavy metal intake in vegetables and fruits based on body weight for an average adult (70 kg body weight) in terms of Provisional Tolerable Daily Intake (PTDI) are mentioned in the table 2.2 [Source: FAO/WHO, 2012]

Table 2. 2 Recommended daily intake and safe limits of heavy metals in vegetables

Heavy metals	**FAO/WHO safe limits (mg/kg)**	**FAO/WHO recommended daily intake**
Manganese	0.2	60 mg
Chromium	2.3	200 mg
Copper	40	3mg
Lead	0.3	214µg

2.9 Heavy metal contents in selected vegetables

Taghipour et al carried-out research on heavy metals in the vegetables collected from production sites in North-West of Iran (Tabriz). Samples of vegetables including lettuce, onion, and tomato were collected from production sites and analyzed for presence of Mn, Cr, Cu, Ni, Pb and Zn by Atomic Absorption Spectroscopy (AAS) after extraction by aqua regia method (drying, grounding and acid digestion). Mean ± SD (mg/kg) concentrations of Mn, Cu, Cr, Ni and Zn were: 0.32 ± 0.58, 1.75 ± 2.05, 6.37 ± 5.61 and 58.01 ± 27.45, respectively. Cr, Cu and Zn were present in all the samples and the highest concentrations were observed in kurrat (leek). Levels of Cd, Cr and Cu were higher than the acceptable limits [Taghipour et al., 2013].

Ametepey et al monitored the levels of heavy metals and their associated health risk in frequently consumed vegetables in the Tamale Metropolis, Ghana. Manganese concentration in cabbage, carrot, green pepper, onion and tomato ranged from 0.04 to 0.07 mg/Kg, 0.01 to 0.06 mg /Kg, 0.04 to 0.06 mg/Kg, 0.03 to 0.06 mg/Kg and 0.03 to 0.07 mg/Kg, respectively. Lead concentration in cabbage, carrot, green pepper and onion ranged from below detectable limit (BDL) to 0.03 mg/Kg, BDL to 0.02 mg/Kg BDL to 0.04 mg/Kg, and BDL to 0.05mg/Kg, respectively but the concentration of heavy metals in the various vegetables were below the World Health Organization standard [WHO,2012; Ametepey et al.

Brhane et al. determined the level of heavy metals in edible vegetables (tomato, onion and green pepper) collected from Mekelle market and garden of Mekelle city and Agricultural Research center (near river) in Ethiopia. Levels of Pb, Cd, Cu and Znwere determined using Flame Atomic Absorption Spectrometry (FAAS) after dry ashing process. The average concentrations of Pb, Cd, Cu and Zn were in the range of 0.244 - 0.987, 0.115 - 0.536, 0.962 3.430 and 2.344 - 4.136 mg/Kg in tomato, 0.241- 0.43, 0.12- 0.441, 0.879-3.428 and 2.197-3.259 mg/Kg in onion and 0.28- 0.392, 0.128- 0.573, 1.229- 2.991 and 3.081-4.242 mg/Kg, respectively in green pepper. The levels of those metals in all vegetables collected from the market site was higher than Agricultural Research center but lower than garden in Mekelletown. The concentration of zinc and copper were within WHO guideline in all analyzed samples, while samples collected from the market and gardens of Mekelle city showed high increment in concentration of lead and cadmium from the FAO/WHO permissible level [FAO/WHO, 2012; Brhane et al., 2014].

Benti assessed the levels of heavy metals in vegetables irrigated with Awash River in selected farms around Adama town, Ethiopia. Three leafy vegetable samples, namely, cabbage, lettuce and spinach from Melka Hida and Wonji Gefersa farms were examined for heavy metal (Mn, Cr and Pb) contamination using atomic absorption spectroscopy. The results indicated that the heavy metals in vegetables of Melka Hida farm were higher than those of the vegetables in Wonji Gefersa farm. In all the samples analyzed, the concentration of Pb and Mn was more than the maximum limit and their levels varied from 0.31 to 0.65 and 0.21 to 0.40 mg/kg, respectively. However, the level of chromium was generally within the normal range in cabbage (0.85 and 0.29 mg/kg) and spinach (1.30 and 1.06 mg/kg) from Melka Hida and Wonji Gefersa farms, respectively, except in lettuce from MelkaHida farm, 2.4 mg/kg. The high levels of these heavy metals place the consumers of these vegetables grown within the study area at health risk with time unless an urgent step is taken by relevant agencies to address this issue [Benti, 2014]

Gebeyehu et al determined the levels of heavy metals (Cr, Cd, Zn, Fe, Pb, As, Mn, Cu, Hg, Ni and Co) in vegetables (tomato and cabbage) and associated health risks in Mojo area, Ethiopia by using Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES). The levels of As, Pb, Mn, Cr and also Hg exceeded the recommended values in vegetable samples with concentrations ranging from 1.93-5.73,3.63-7.56,0.56-1.56,1.49-4.63 and 3.43-4.23mg/kg, respectively. It was observed that leafy vegetable (cabbage) has accumulated heavy metals to greater extent compared with tomato. The estimated daily intakes (EDI) of toxic metals were below the maximum tolerable daily intake (MTDI). However, the total health quotient (THQ), were found>1for as and Hg due to tomato consumption and for As, Hg and Co due to cabbage consumption. The health indexes (HI) of both vegetables were much greater than one, with HI values of 7.205 and 15.078 due to tomato and cabbage consumption, respectively. The total cancer risk (TCR) analysis has also revealed the potential adverse cancer risk induced by As, Mn, Hg, and Ni from the consumption of both tomato and cabbage as their TCR values were above the threshold level. Based on the result, there would be a significant health risk to the consumer associated with the consumption of cabbage and tomato being cultivated in Mojo area [Gebeyehu et al., 2020].

Ejigu et al determined the levels of selected heavy metals (Cr, Mn and Pb) in cabbage, onion Awash River used for irrigation have been investigated. The cabbage, onion and Awash River water samples were collected from the farmlands around Ginchi town of West Shoa Zone in which the Anmol Ethiopia PLC, a paper industry, were there. The samples were digested using properly optimized wet digestion procedures. The metal contents were analyzed using Flame Atomic Emission Spectroscopy (FAES). However, andPb were not detected in all samples. While the highest level in onion samples was, Cr (0.59 mg/L) which exceeded the FAO/WHO limit of maximum recommended level [FAO/WHO,2012; Ejigu et al.,2020]. Berihun et al determined the level of metallic contamination in irrigation vegetables (Ethiopian kale, cabbage, Swiss chard, lettuce, onion, tomato, and potato) and the water in Gondar city, Ethiopia. The concentrations of Cu, Mn, Zn, Cr, Cd, Ni, and Pb were determined using flame atomic absorption spectrometry. In the vegetable’s samples, the mean concentrations of Mn, Ni, and Pb (0.23-6.25, 7.41-51.85, and 0-9.52 mg/kg, respectively) were found to be above the limits set by the joint WHO/FAO. Swiss chard and potato were found to contain the highest levels of Pb, while Ethiopian kale was highly contaminated with Mn and Cr [Berihun et al., 2021].

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 METHODS

3.1.1 Study Area

The studies were carried out in Genfel River which is found in Western side of Wukro town, Tigray, Eastern Ethiopia. The climate of the region is generally sub-tropical with an extended dry period of nine to ten months and a maximum effective rainy season of 50-60 days. Considering the rainfall, atmospheric temperature and evapo-transpiration, more than 90% of the region is categorized as semi-arid. The temperature in Wukro ranges from 11.5 to 30.65 °C and annual rainfall varies from 24.0 to 486.0 (mm/month) [BerihunBT., Amare DE., et al. (2021.)]. This town has a latitude and longitude of 13°46'N 39°21' E with an elevation of 1960 to 2050 meters above sea level. Most of the vegetable leaves are used in the preparation of several delicacies in Eastern Tigray and these vegetables are irrigated with waste water from urban drainages contaminated through processes such as defecations, urination, bath, washing, ago-chemicals and industrial effluents.

The sampling points of the study area are taken according nearby pollution sources and three points are selected using the global positioning systems (GPS) reading to locate the points. These different sites are:

Site 1**:** Belesa approximately the sources where the river inters to the town.

Site 2**:** Waste disposal enters to the river and some metal work takes place around it.

Site 3**:** The final point is where the river leaves the town.

3.2 Materials

3.2.1 Equipment, Instrument and Apparatus

Polyethylene bags were used for handling, transporting and preserving the collected samples. Ceramic mortar with pestle (Haldenwanger, Germany) was used for grounding samples. A digital analytical balance (Model E11140, Switzerland) was employed to weigh the processed samples while measuring cylinders, beaker, conical flask, pipette and volumetric flasks used to measure different volumes of sample solutions, acid reagents and metal standard solutions. Conical flask, hot plate (Stuart scientific, UK) and biological safety cabinet were used to digest the dried and powdered samples, and the blank solution. The digested samples were filtered with WhatmanNo.42 (whatman limited, England) filter paper. Other equipment, instrument and apparatus such as refrigerator for keeping the digested sample till analysis, funnel, porcelain crucibles, hot air oven and plastic knife were used. Atomic absorption spectrophotometer was used for the analysis of metals (Pb, Mn, Zn, Cr and Cu) in vegetable samples.

3.2.2 Chemicals, Reagents and Solvents

Analytical graded chemicals, reagents and solvents were used throughout the experiment. deionizer water was used throughout the experiment for preparing sample and standard stock solutions, dilution and rinsing apparatus prior to analysis; chemicals like: HClO4 (70%, Sisco Research Laboratory Pvt Ltd, India), HNO_3 (69%, Oxford Lab. chem., India) and H_2O_2 (30%, Scharlab S.L, Spain) were used for sample digestion procedures and also standard stock solution that were used for preparation of working standard solutions for calibration and spiking experiments were: $Mn(NO_3)_2$, $Pb(NO_3)_2$, $Zn(NO_3)_2$,$Cu(NO_3)_2$ and $Cr(NO_3)_2$ (99.99%, Merck, Germany).

3.2.3 Cleaning of Glassware and Apparatus

All glassware, plastic containers and other apparatus used were washed with liquid soap, rinsed with water, soaked in 10 % nitric acid for 24 hours then cleaned thoroughly with deionizer water. The glassware were then dried in hot air oven for 90 minutes to ensure that any contamination does not occur then take out the glassware from the hot air oven and cooled and stored in clean dry places free of contamination till use. All pipette rinsed immediately prior to use three times with deionizer water [Khan et al., 2009; Gebeyehu et al., 2020].

3.3 Sampling and Analysis

3.3.1 Collection, preparation and analysis of water samples

Samples were collected in pre-cleaned plastic containers, which are cleaned by detergent and de-ionized water and then rinsed using HNO_3 appropriately. During sampling, sample bottles were rinsed with sampled water three times and then filled to the brim from each of three sampling points. Ones liter of the waste water used for irrigating each farm were collected and treated with 1.5 ml of concentrated HNO_3. 50 ml of the water sample was transferred to an evaporating dish and evaporated on a steam bath to about 20 ml. 10 ml of 8 M HNO_3 of 98 % purity were added and evaporated on a hot plate to near dryness. The residue was quantitatively transferred using two aliquots of 10 and 15 ml of concentrated HNO_3 into a 250 ml flask. 20 ml of $HClO_4$ were added and boiled until the solution became clear and white fumes of $HClO_4$ appeared. It was cooled and de – ionized distilled water (about 50 ml) was added and the solution filtered. The filtrate was quantitatively transferred to a 100 ml volumetric flask with two portions of 5 ml of de – ionized distilled water. The solution was diluted to mark and mixed thoroughly by shaking [Muhammad, Farooq and Umer, 2008].

3.3.2 Collection, preparation and analysis of soil samples

Soil samples will be collected from three sampling points within the study of vegetable farms from the river using plastic spade vertically from0-5 cm borehole. The collected samples were thoroughly mixed to give representative samples. All the well mixed soil samples were dried in an oven to remove excess moisture and then were ground and sieved with 200 mesh (75 mm) sieve. The dried samples were kept packed until analysis **[**Vigneswaran andSundaravadivel, 2004].

A triplicate sample of 0.5g of air-dried ground soil were transferred to 250 ml conical flask; 5ml of concentration H_2SO_4 was added following the addition of 25ml of concentrated HNO_3 acid and 5ml of concentrated HCl. The mixtures were heated at 200^0C for one hour in a fuming hood and then cooled to room temperature. After cooling, 20 ml of distilled water were added and the mixture will be filtered to complete the digestion. Finally, the mixture will transfer to a 50 ml volumetric flask, filled to the mark and let to settle for at least 15 hours. The filtrate was analyzed for total, Cu, Pb, Zn, Cr and Mn by AAS [Vigneswaran andSundaravadivel, 2004].

3.3.3 Collection, preparation and analysis of vegetable samples

The vegetables were mostly grown around Genfel River like cabbage (*Brassica oleracea*), swisscard (*BetaVulgarisL.var.cicl*tomato, Spinach (*Amaranthuscaudatus*), lettuce (*Lactuca sativa*), onion (*Allium cepa*), were collected for analysis using polyethylene plastic containers, and the collected samples were washed with distilled water to remove dust particles and air dried. The dry vegetable samples were crushed in a mortal and the resulting powders were packed for analysis of heavy metals. A triplicate of approximately5g of the vegetable powder was put to a 250 ml conical flask, 5 ml of concentrated H_2SO_4 was added following the addition of 25 ml of concentrated HNO_3 and 5 ml of concentrated HCl. The mixtures were heated at 200 °C or 1 hour in a fuming hood and then cooled to room temperature. Then, 20 ml of distilled were added and the mixture filtered using filter paper to complete the digestion of organic matter. Lastly, the mixture was transferred to a 50 ml volumetric flask, filled to mark and let to settle for at least 15hrs. The resultant supernatant used for analysis of total Cu, Pb, Zn, Cr and Mn.

3.4 Research Design

This study was a case study that used experimental research design. The study made use of an experimental approach to carry out the laboratory analysis to collect relevant data. The laboratory analysis was done in accordance with scientific guidelines. The research objectives, questions, samples, sampling sites, and analytical procedures were predetermined and numbers and statistics used to analyze and explain its findings.

3.5. Calibration and quality control analysis

Standard series of 0, 10, 20, 30, 50 and100 ppb of the AAS were used to prepare a calibration curve that had a minimum of five points inclusive of the blank. The standards were prepared for each metal from their stock solution to calibrate the instrument. The detection limit of AAS was 0.5 ppb. Analytical Grade chemicals were used in sample preparation. Double de-ionized water was used for solution preparation and glassware washing with 10% HNO_3. Precision and accuracy of analysis was checked through repeated analysis against European standard reference material (CEC278K) for vegetables, water and soil for heavy metals.

3.5.1. Statistical Data Analysis

The data was analyzed using Minitab Statistical Software, version 19. The significant difference ($p \leq 0.05$) of heavy metal concentrations in vegetables, soil, and water among the different sampling sites was tested using One-way Analysis of variance (ANOVA). Where there were significant differences in heavy metal means, post- hoc Turkey test was used to separate the means. Pearson correlation analysis was done to determine the correlation between heavy metals in soil, water and vegetables. A comparison of data collected against

WHO set limits for heavy metals in water for irrigation use and in vegetables for human consumption was done.

Data analysis also involved computation of soil to plant metal ***transfer factor (TF)*** using the equation**;**

$$\boldsymbol{Transfer\ Facor\ (TF) = \frac{C_{plant}}{C_{soil}}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textbf{(3.1)}$$

Where'C_{plant}' is the concentration of heavy metals inplants and 'C_{soil}' is the concentration of heavy metals in soil.

3.5.2 Standard Solution and Working Standard Solution Preparation

Apure analytical grade reference standard solution containing Pb, Mn, Zn, Cr and Cu in 1000 mg/L of their corresponding salts: Pb $(NO_3)_2$, Zn $(NO_3)_2$, Mn $(NO_3)_2$, Cr $(NO_3)_2$ and Cu $(NO_3)_2$, respectively were used for preparing intermediate and working standard solutions by using de-ionized water.

Working standards of metals solutions were prepared by diluting the intermediate standards solutions of the metal with de-ionized water. 50 mL of 10 mg/L intermediate standard solution of each analyzed heavy metal (Cr, Mn, Zn, Pb and Cu) was prepared freshly from each stock standard solution containing 1000 mg/L by diluting appropriate amount of the corresponding salt in deionizer water, while five series of working standard solution was prepared by serial dilution with de-ionized water from the respective intermediate standard solutions.

3.5.3. Calibration curve and Heavy Metal Analysis in Selected Vegetables

Before analysis, the instrument was calibrated using calibration blank and its analytical wavelengths, energy, lamp current and slit width for each element were adjusted using the manual to attain its better sensitivity. After properly calibrating the instrument, a five points of calibration curve was constructed by plotting the standard concentration (mg/L) of each heavy metal against the absorbance of heavy metals after running the five series of the prepared working standard solutions in AAS. The calibration standard concentrations were within the working linear range of the instrument used for analysis. All the calibration procedures were evaluated based on their corresponding correlation coefficients (R^2) of the calibration curves which were found to be ≥0.998 for the linearity of the regression line [Christian, 2003].

After establishing calibration using the standard solutions, the vegetable sample solutions and blank solutions were aspirated into AAS (Buck Scientific Model 210VGP AAS) at University of Mekelle. The absorbance of each sample's Cr, Mn, Zn, Pb and Cu was recorded and three replicates were taken for each determination to confirm precision of the result then converted in to concentration using linear calibration curve equations. Finally, the average concentration value of each metal was expressed in mg/kg of dry weight of 1g sample by using ASEAN manual of food analysis formula presented as equation (3.1) [Puwastien et al., 2011]:

$$Concentration\ in\ dry\ weight\ (mgKg^{-1}) = \frac{(C_s - Conc_b) \times V \times DF \times CF}{W} \text{………………..} \quad (3.2)$$

Where'C_s' and 'C_b' are concentrations of metals in sample and blank in (mgL^{-1}), respectively; 'V' is final Volume (50 ml) of digested sample solution in liter; 'W' is initial weight (1 g) of sample measured in kilogram; 'DF' is Dilution factor if there is and 'CF' is conversion factor (0.085) is to convert fresh vegetable weight to dry weight as described by Puwastien et al. [Puwastien et al., 2011] and Rehman et al. [Rehman et al., 2019].

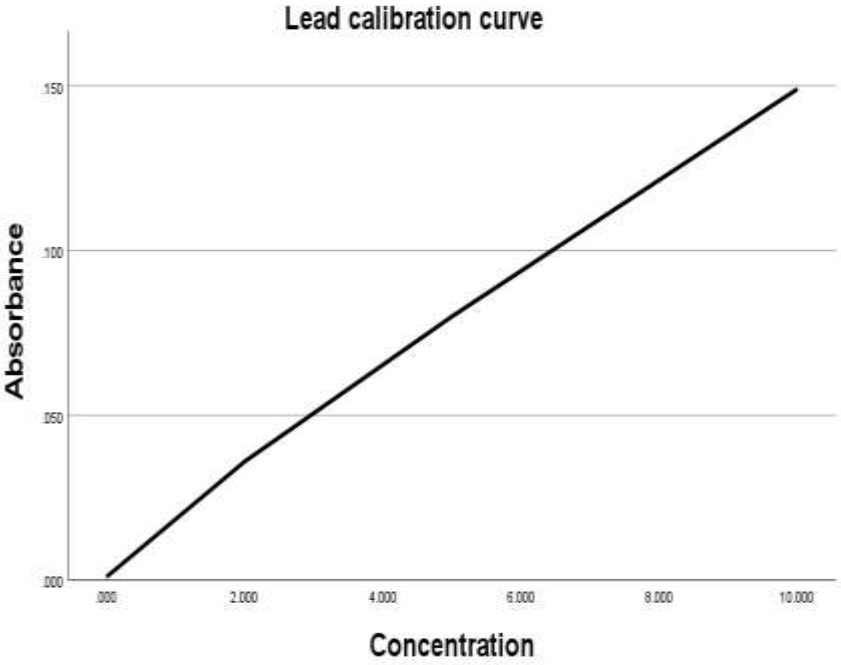
3.5.4. Calibration curves of heavy metals

The calibration curves for the selected metals showed a good linearity with coefficients of determination (R^2) 0.9999 as shown from A − D. The R^2 value is within the acceptable limit, 0.998, for the linearity of the regression line [Christian, 2003] which indicates that there is a good correlation between concentration and absorbance and also good calibration of the instrument. The regression calibration curve equation was expressed in terms of, Y= mx + b, (R^2) where, Y= absorbance and x= concentration of Pb, Cr, Mn, Zn and Cu obtained at their corresponding wavelength 283.3, 357.9, 228.8 ,322.7 and 324.7 nm, respectively and also b is the Y intercept. The concentration of Pb, Mn, Cr and Cu in given vegetable samples were calculated from regression equation of the standard solution whose absorbance of equivalents of each heavy metals.

A. Calibration curve of Lead

$y=0.0072x+0.036$

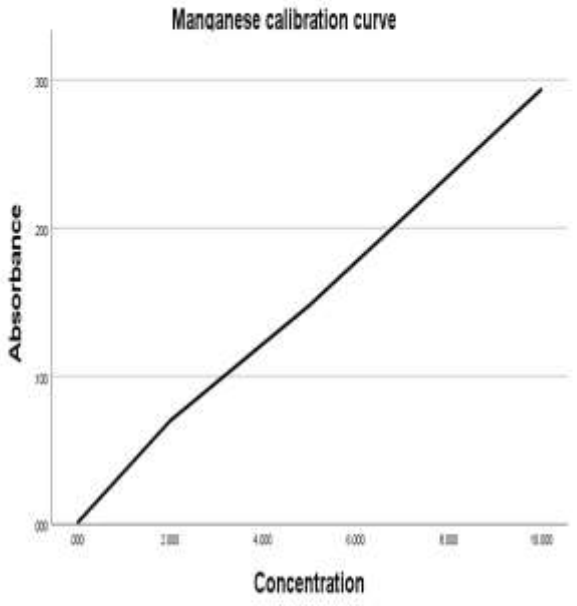
$R^2=0.9999$



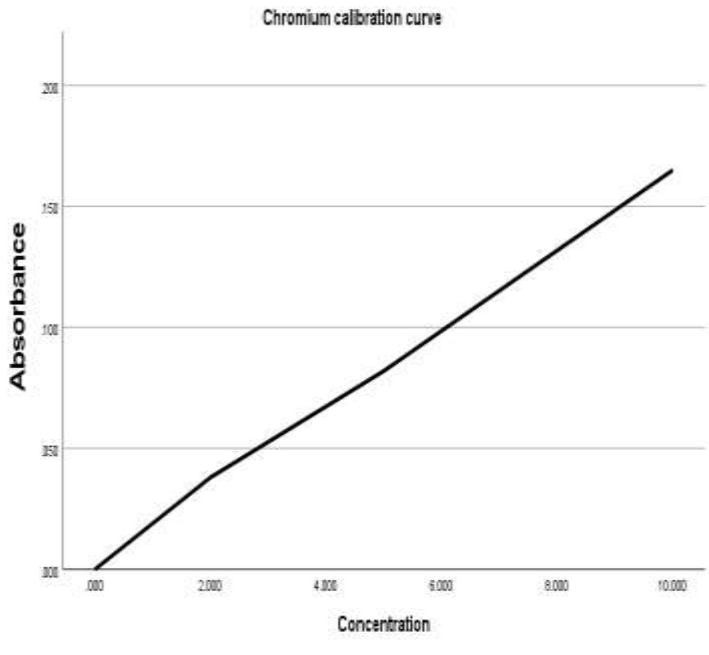
B. Calibration curve of Manganese

$y=0.0304x-0.0272$

$R^2=0.9999$



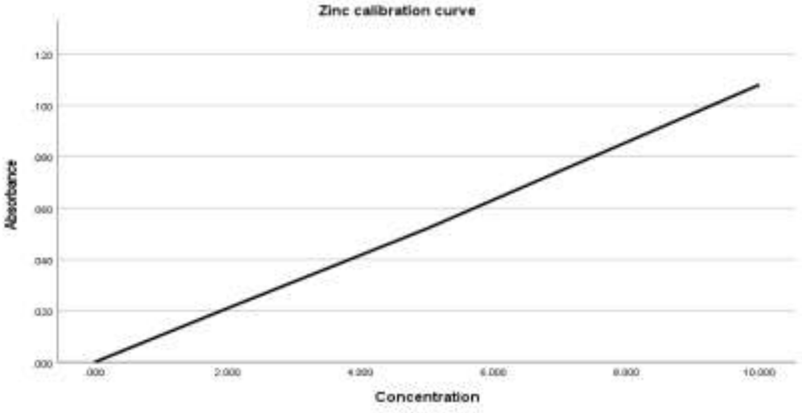
C. Calibration curve of Chromium



$Y=0.0157X+0.004$

$R^2=0.999$

D. Calibration curve of zinc



Y=0.0107X+0.047

R2=0.999

3.5.5. Estimation of daily intake (EDI) of heavy metals

The estimated daily intake of the metals considered in this study was determined based on their mean concentration in each cabbage, onion, Spinach, lettuce and tomato and the estimated daily consumption of the vegetables in Ethiopia within specified bodyweight of a consumer. The health risk posed to the population was determined by dietary intake of heavy metals and compared with the maximum permissible risk level for human population. The value of EDI was calculated by using equation (3.2) [US-EPA, 2015].

$$EDI = \frac{(C_{metal} \times IR)}{BW} \text{ ………………………………………….……….. } (3.3)$$

Where:‘EDI’ is the estimated daily intake of heavy metals ($mgKg^{-1}day^{-1}$); ‘C_{metal}’ is the average concentration of heavy metals in the edible portion of studied vegetables and fruits ($mgKg^{-1}$, dry weight); IR (Ingestion rate) is the average daily vegetable and fruit consumption rate for the Ethiopian people (adult) is 115g $person^{-1}day^{-1}$[Worku et al., 2017] and ‘BW’ is reference body weight for an adult which is 70 kg [Woldetsadik et al., 2017; Aschale et al., 2019].

3.5.6. Health risk assessment of heavy metals in the selected vegetables

Health risk assessment was computed based on the average contents of carcinogenic and no carcinogenic metals determined in the vegetable and fruit samples using probabilistic risk assessment model of United States Environment Protection Agency [US EPA, 2015]. In this study the risk of intake of Pb, Mn, Zn, Cr and Cu through mango, orange, cabbage, onion spinach, lettuce and tomato to human health was evaluated based HazardQuotient (HQ) and

Hazard Index (HI) for non-carcinogenic and Target Cancer Risk (TCR) for carcinogenic health risk and compared with FAO/WHO standard limits [FAO/WHO, 2012].

3.5.7. Target Hazard Quotient (THQ)

Risk of intake of metal-contaminated vegetables and fruits to human health was characterized by Target Hazard Quotient (THQ) which used to assess the non-carcinogenic risk to humans from long-term exposure to heavy metals from vegetables and fruits. If THQ is<1, the population were posing no non-carcinogenic risk are expected from exposure, while THQ≥1 signifies that the population would experience potential non-carcinogenic risks due to life time exposure [US EPA, 2015]. The THQ was calculated as a fraction chronic daily dose to the reference oral dose level (RfD) by using equation (3.3) [US EPA, 2015]:

$$\text{THQ} = \frac{EDI}{RfDo} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(3.4)}$$

Where EDI is estimated daily intake of heavy metals via vegetables and fruits in mg/kg/day determined by Eq (3.3) and RfD is the reference oral dose of the metal ($\text{mgKg}^{-1}\text{day}^{-1}$) is an estimated exposure of metal to the human population per day that has no hazardous effect during a life time, generally used in EPA’s non-cancer health assessments. The values of reference dose (RfD) (mg/ kg per day) for Pb, Mn Zn, Cr, and Cu are: 0.0035, 0.001, 0.003 and 0.04 respectively [US EPA, 2015].

3.5.8. Health Risk Index (HRI)

Health Risk Index (HRI) helps to evaluate the overall non-carcinogenic risk to human health through more than one heavy metal in the same vegetable or fruit which means exposure to more than one pollutant results in additive effects. It is determined by using Estimated Daily Intake (EDI) of heavy metals via vegetables and fruits ($\text{mgKg}^{-1}\text{day}^{-1}$) and Reference oral Dose (RfD) or it is the sum of the Target hazardous quotient (THQ) of all heavy metals as described in equation (3.4) [US EPA, 2015]:

$$HRI = \sum THQ = THQ_{Pb} + THQ_{Cu} + THQ_{Cr} + THQ_{Mn} + THQ_{Zn} \ldots\ldots\ldots\ldots \text{(3.5)}$$

Where: ‘Pb, Cu, Cr,Zn and Mn’ are the individual heavy metals found in one vegetable or fruit species. If **$\sum$THQ<1**, the exposed population are considered to be **safe**. However, **HRI value >1.0** indicates that potential health implication while a serious chronic health impact has been suggested for HI greater than 10.0 [US EPA, 2015].

3.6. Method Validation

In order to validate the analytical method and efficiency of the AAS instrument, the following method validation parameters such as instrumental detection limit, method detection limit, limit of quantification, precision (in terms of repeatability) and accuracy (in terms of recovery) studies were carried out [Chauhan et al.,2015].

3.*6.1* Accuracy

The recovery results for spiked metals in vegetable samples were used as estimates of the accuracy of the method. The accuracy of the method was assessed by spiking pre-analyzed samples of 1gm vegetable and fruit samples with known amounts of standard metals (0.5 ppm or 1 ppm) and the percentage recovery was calculated to evaluate the accuracy of the analytical procedure by using equation (3.5) [Singh et al., 2011].

$$Recovery\ (\%) = \frac{CM_{in\ the\ spiked\ sample} - CM_{in\ the\ unspiked\ sample}}{CM_{added\ for\ spiking}} \times 100 \ \ldots\ldots\ldots\ldots\ldots \ (3.6)$$

Where: ‘CM’ is concentration of the metal in mg/L.

3.6.2. Precision

The repeatability of the analytical procedure was checked by carrying out a triplicate analysis from a triplicate digested samples (n=5) and calculating the relative standard deviations of the mean for each metal based on equation (3.6). The obtained results as the average of three replicates of each sample was showed that the validity of the employed methods and good repeatability for the analysis of fruits and vegetables samples.

$$RSD\ (\%) = \frac{SD}{Mean} \times 100 \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ (3.7)$$

3.6.3. Method detection limits and quantification limit

Method detection limit is the minimum concentration of a substance that can be measured. The generally accepted and common definition of method detection limit is the concentration that gives a signal three times the standard deviation of the blank or background signal. In this work, after digestion of three blank solutions a mixture of 10 mL conc. HNO_3, 4.0 mL conc. $HClO_4$ and 4.0 mL of H_2O_2 was prepared following the same digestion procedure used for the samples and diluted to 50 mL with de-ionized water which were used for wet digestion of the selected vegetable and fruit samples and each of the samples were determined the level of Pb, Cr, Mn, Zn, and Cu by AAS model 210VGP. Triplicate readings of absorbance were recorded for each of the sample at their respective wavelength and then converted to concentration. The standard deviation for each element was calculated from the nine blank measurements to determine method detection limit and limit of quantification for each metal by using equation (3.7) and (3.8) [Chauhan et al.,2015].

$$MDL = 3 \times S_{blank} \quad \text{……………………………………………………….. (3.8)}$$

$$LOQ = 10 \times S_{blank} \quad \text{……………………………………………………….. (3.9)}$$

Where: 'S_{bank}' is the standard deviation of nine blank measurements

3.7 Data quality assurance

Appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results. At the end of each laboratory work, generated data on each laboratory work was recorded and checked for its completeness and consistency through preferred data analysis or review the collected data. The reliability of the experimental results also guaranteed by: handling the sample carefully, cleaning of the instruments properly, and all glass wares were immersed in HNO_3 (10%) for 24 hours, washed with distilled water before use to minimize the risk of contamination. All analytical graded reagents, chemicals and solvents were used throughout the study. The other one is performing calibration using analytical grade reagents before doing the experiment and undertaking repeated measurements of standards to ensure the system is working properly or not to take corrections for the instrument readings. A spike-and-recovery analysis test was performed to assess the accuracy of the analytical techniques used and the obtained value in this study should be falls within the normal acceptable range of 80-120 % for a good recovery study [Getachew et al., 2019] and also by triplicate analysis of samples the coefficients of variation was determined for precision of analysis. If the method is precise, the triplicate results did not differ by more than 10% of the mean [Gouthami et al, 2015].

3.8. Method of data processing, analysis and presentation

Quantitative data was collected and those analytical data obtained from the experiment was checked for its completeness and consistency every day and then recorded on sheets of Microsoft Excel 2010 before analysis. Descriptive statistics was used in analyzing the data quantitatively. Results of the study were organized by tables in the form of mean, range and standard deviation in $mgKg^{-1}$ of dry weight at 95 % confident limit (Mean ± SD) of triplicate determination. The statistical analysis of data was carried out by employing statistical package for the social sciences (SPSS) version 23 for analysis of quantitative data and also to summarize descriptive statistics. Microsoft Excel 2010 was used for performing calibration curve and summarizing the data while one-way ANOVA used to examine the existence of significance difference between means as the level of heavy metal contamination might vary with sample site. Besides, correlation coefficients were calculated to investigate the association between the targets and also P-value < 0.05 was considered statistically significant. Finally, these quantitative data were summarized and described using tables and figures.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Heavy metal concentration in Water

Heavy metal concentration (Cu, Zn, Mn, Pb, and Cr) range in water from the different sampling sites were; 1.08−1.28, 0.57−0.56, 0.06−0.43, 2.31−2.08, and 0.004−0.007 mg/L, respectively. All mean values were below WHO permissible limits for irrigation water in all the sampling sites (Table 4.1) hence the water was safe for agricultural use. The mean heavy metal concentration in water varied in the order Pb > Cu > Zn > Mn > Cr.

The concentration of Cr in water ranged from 0.004 to 0.007 mg/L with Cr having the lowest concentration in all the sampling sites. Cr concentration in site 1(Bellesa upstream) varied between BDL – 0.003mg/L with a mean concentration of 0.0056±0.0001 mg/L. Site 2 (Genfell middle stream) had a mean Cr level of 0.006±0.01 mg/L with the levels ranging between BDL – 0.002 mg/L. The mean Cr concentration for site 3 (Dengollo downstream) was 0.007±0.015 mg/L with a range of BDL – 0.001 mg/L. Site 1 (Bellesa) had the least mean concentration while site 3 (Dengollo downstream) had the highest Crmean concentration. At site 3, there was raw sewage released directly into stream at this point. The sewage effluent could be containing chromium compounds that could be responsible for Cr in water at site 3. Lower Cr concentrations in the downstream could be due to dilution of wastewater by river water.

Table 4. 1 Mean ± standard deviations of heavy metal concentration (mg/L) in water and WHO set limits (WHO, 2008)

Heavy metals in river water (mg/L)	**Mean ± standard deviations of heavy metal concentration (mg/L)**				
	Site 1	**Site2**	**Site3**	**WHO Limit**	**p-value**
Cu	1.08±0.04	1.23±0.018	1.28±0.050	2.0	0.870
Zn	0.57±0.05	0.52±0.035	0.56±0.02	3.0	0.210
Mn	0.06±0.01	0.11±0.020	0.43±0.014	0.01	0.417
Pb	2.31±0.01	2.40±0.010	2.08±0.087	0.01	0.417
Cr	0.004±0.001	0.006±0.010	0.007±1.58	0.05	0.494

The levels of Cr in water for all the different sampling sites were within WHO recommended limits for surface water; hence the water was safe for agricultural use. There was no significant difference ($p > 0.05$) in Cr concentration in the different sampling sites. These values were lower than Cr concentrations (0.004 mg/L) detected in genfell river and 0.007 – 0.16 mg/L recorded for wastewater in Embu (Sayo et al., 2020; Njuguna et al., 2017). Contrary to Cr levels obtained in this study, Mohsin et al. (2019) reported higher Cr concentration (0.02 – 0.39 mg/L).

The average Cu concentration in the different sampling sites ranged from 1.08 to 1.28mg/L with the highest value recorded at site 3. Variation of Cu concentration in the sampling site was; BDL – 0.020 mg/L for site 1, BDL – 0.05 mg/L for site 2 andBDL – 0.018 mg/L for site 3. Discharges from industries and sewage treatment plants and natural weathering of rocks are some pathways through which copper is released to water (Nzeve et al., 2015). The high level of copper at site 3, could be attributed to raw sewage effluent at site3, pesticides and fertilizers that leached into the water from the surrounding farms and erosion of minerals from rocks and soil. Generally, the concentration of Cu was within the recommended level of Cu in surface water (WHO, 2008). The Cu levels in the different sampling sites showed no significant variation ($p>0.05$). The levels of Cu were lower than previous levels (1.29-0.727 mg/L) determined in river Chawalla, Nigeria (Bichi et al., 2010). Kacholi (2018) also reported higher Cu values than those obtained in this study for urban stream water in Dar es Salaam, Tanzania.

The mean Pb concentrations varied between 2.31 and 2.08 mg/L (Table 4.1). Pb concentration in water samples from site 1 (Bellesa upstream) varied from BDL to 0.05 mg/L with a mean Pb concentration of 2.31±0.01 mg/L recorded for the period of study. Site 2 (Genfell middle stream) recorded a minimum Pb concentration of BDL and a maximum of 0.14 mg/L. The average Pb concentration at site 2 was 2.40±0.010 mg/L, while Pb concentration in water samples from site 1 (Bellesa upstream) ranged from BDL to 0.050mg/L with a mean value of 2.08±0.080 mg/L. Highest Pb levels were noted in site 1 while the lowest levels in site 2. Lead in water samples from site 1 could be coming from; petroleum products and metallic waste from garage located next to middle stream, sewage effluent released directly into the stream and airborne Pb deposition. Concentration of lead in all the sampling sites was within acceptable Pb levels in river water hence there was high Pb pollution presently. The Pb in the different sampling sites did show a significant variation ($p >0.05$).

A similar study carried out in previous studies recorded higher values than those obtained in this study mean Pb value of 8.60 mg/L reported in Riyadh city river waters (Badr et al., 2020) and 0.113 mg/L of Pb was reported from Yucuambi river (Villa et al., 2018). Xiao et al., (2019) reported 0.25 mg/L–0.25 mg/L while Kacholi (2018) recorded 0.46 – 0.55 mg/L from Temeke, Dar es Salaam.

Concentration of zinc in water in the sampling sites was found to be in the range 0.550–0.020 mg/L and was found to be the lowest among the heavy metals investigated. Zn had the highest concentration of 0.570 mg/L at site 1, where raw sewage drains into stream thus explaining the highest level of zinc at that site since the raw sewage contains dissolved zinc compounds. Lowest Zn levels (0.520 mg/L) were recorded at site 2 (Genfell middle stream). On the other hand, average Zn mean of 0.560 mg/L was recorded in water samples from site 3 (Dengollo downstream). Erosion of minerals from rocks and soil could also be another natural pathway of introduction of zinc in river water (Saskatchewan, 2007). Additionally, other sources of Zinc could be steel products, burning of waste materials, leaching of fertilizers and pesticides into the water. Zn in water samples from all the sampling sites was within acceptable WHO limits. This was an indication that the water was not zinc polluted. There was no significant variation in concentration of Zn in the sampling sites ($p > 0.05$). Ahmed et al., (2018) in a similar study in Bangladesh also reported Zn out of the six heavy metals assessed as having the highest concentration. Comparable mean Zn values in surface water were reported in previous studies; 0.092 – 0.132 mg/L were reported in the study carried out in Masinga dam, Kenya (Nzeve et al., 2015); 0.99 – 1.26 mg/L obtained in Temeke municipality in Dar es Salaam (Kacholi, 2018); 0.34 – 1.85 mg/L detected in Sahiwal district, Pakistan (Mohsin et al., 2019). In the contrarily, the values of Zn (2.0 – 13.7 mg/L) reported in Bangladesh (Ahmed et al., 2018) were higher compared to Zn level values obtained in this study.

The concentration of manganese ranged from 0.060 – 0.40mg/L (Table 4.1). Mn levels at site 1 (Bellesa upstream) ranged with an average mean of 0.20 ± 0.10 mg/L. At site 2 (Genfell middle stream) the mean Mn concentration was 0.110 ± 0.020mg/L with Mn levels varying between BDL and 0.090 mg/L during the period of study. Mn range at site 3 (Dengollo downstream) was BDL – 0.230 mg/L while the average mean was 0.430 ± 0.014 mg/L at that site. The highest mean concentration of manganese was at site 3. This could be associated to raw sewage drained in to the stream at site 3 as the sewage could contain dissolved chromium compounds from paints.

Additionally, washing of motor bikes in stream could also be attributed to Mn in water as some motorbikes have plating and the Mn gets deposited in the water (Njuguna et al., 2017). One-way ANOVA showed that there was no significant variation ($p > 0.05$) in Mn concentration in the different sampling sites. Concentration of Mn in the water from all the sampling sites was lower than WHO acceptable standards for irrigation water, hence this river is not polluted with manganese. Similar values (0 – 0.32 mg/L) to those obtained in this study were recorded in Cauvery River (Begum et al., 2009). Mn mean concentrations obtained in this study were lower than those recorded in previous studies.For instance,Njuguna et al., (2017) reported 0.245 mg/L in Nairobi river, Xiao et al., (2019) reported 5.13 mg/L in river water in Chinese Loess plateau and Woldetsadik et al., (2017) detected 2.26 – 6.76 mg/L in Tinishu and TelekuAkaki rivers in Addis Ababa.

4.2 Heavy Metal Concentration in Soil

Heavy metal mean concentration values in water are already reported in Table 4.1 above. The concentrations were all below WHO set limits for heavy metals in agricultural soil for all the sampling sites. However, the levels of Mn, Pb, Zn, Cu and Cr observed in soil were more elevated compared to metal concentration in water. This could be due to continuous irrigation of the agricultural farms using polluted water.

The mean values for Mn ranged from 85.90±1.10 – 26.50±3.38 mg/Kg as indicated in Table 4.2. At site 1 (Bellesa), Mn range was BDL – 59.4 mg/kg, with the mean concentration at that site being 26.5±3.38 mg/kg. Site 2 (Genfell middle stream) had a lower Mn mean concentration (±1.10 mg/kg) with the minimum and maximum Mn concentration during the period of study for this site recorded as 3.38and mg/kg respectively. Site 3 Dengollo downstream) had the lowest Mn concentration (26.50±3.38 mg/kg). Mn levels at site 3 ranged between BDL and -19.8 mg/kg during the period of study. Mn levels in all the sampling sites were lower than WHO acceptable standards for Mn in agricultural soil implying that the soil at that time was not Mn contaminated. There was no significant difference of heavy metals’ concentration in the different sampling sites (p value was greater than 0.05).

Table 4. 2 Mean ± Standard deviations of heavy metal concentration in soil samples (mg/kg) and WHO set guidelines

Heavy metals in soil	**Mean ± Standard deviations of heavy metal concentration in soil (mg/Kg)**				
	Site 1	**Site 2**	**Site 3**	**WHO Limit**	**p-value**
Mn	85.9 ± 1.10	26.5 ± 3.38	26.5 ± 3.38	2000	0.051
Cu	10.06 ± 0.231	134.5 ± 3.95	134.5 ± 3.95	100	0.624
Pb	27.4 ± 0.46	27.30 ± 1.62	27.30 ± 1.62	84	0.581
Zn	11.1 ± 0.346	16.4 ± 2.466	16.4 ± 2.466	300	0.894
Cr	0.033 ± 0.217	0.041 ± 0.001	0.041 ± 0.001	30	0.319

Mn in soil could be originating from sewage and domestic effluents, organic matter, phosphate fertilizers, mineralization and atmospheric deposition (Abraham, 2020; Birke et al., 2017). Compared with the current study, similar Mn levels were reported in previous studies i.e., 0.45 mg/Kg in New Zealand, 0.185 mg/Kg in Europe and 0.11 mg/Kg in Switzerland agricultural soils [Abraham, 2020; Birke et al., 2017; Bigalke et al., 2017].

The level of Cu in soil in this study ranged between 10.06 and 134.5 mg/Kg. Range of Cu concentration in the sampling sites in the period of study were as follows; 3.907 – 16.64 mg/Kg in site 1 (Belesa up stream), 0.00 – 103.857 mg/Kg in site 2 (Genfel upstream) and 0.00 – 55.47 mg/Kg in site 3 (Dengollo downstream). All the values were found to be lower than the WHO accepted standards of Cu in soil thus the soil was not copper polluted. Hence, there was no significant difference ($p > 0.05$) in the concentration of Cu in the sampling sites.

Application of sewage on agricultural soil, fungicides and pesticides used during farming and atmospheric deposition could be associated to the presence of Cu in soil (Panagos et al., 2018). Similar Cu levels to those detected in this study were reported in previous studies; 16.7 mg/Kg in European agricultural soils (Panagos et al., 2018) and 28.74 mg/Kg for waste water irrigated soils in Lahore, Pakistan (Mahmood & Malik, 2014). Values for Cu in soil (40.961 mg/kg) noted in Yangtze River delta were higher than the values of Cu in soil obtained in this study (Mao et al., 2019). In the contrarily, lower Cu levels were detected in previous studies on concentration of Cu in cropland soils; 1.33 – 3.33 mg/Kg in Kericho West sub county and 1.661 – 3.781 mg/Kg in Embu, Kenya (Sayo et al., 2020; Bett et al., 2019).

Concentration mean values of Pb were between 4.23±3.71 and7.56±5.59 mg/Kg as shown in Table 4.2. Pb levels ranged from 0.00 – 15.47 mg/Kg, 0.00 – 15.6 and 0.00 – 6.9398 mg/Kg at site 1 (Bellesa), site 2 (Bellesa upstream) and site 3 (Dengollo downstream), respectively. Highest mean Pb levels were at site 2 and the lowest at site 3. All Pb values were within WHO permissible limits for agricultural soils in the different sampling sites.

There was no significant variation in the Pb levels in the different sampling points ($p > 0.05$). Similar results were reported by Bett et al., (2019) who recorded 5.00 ± 0.58 – 5.67 ± 0.88 mg/Kg of Pb in soil in Kericho West sub-county, Kenya. Lower mean concentrations of Pb were reported in soil irrigated with sewage contaminated water in western region of Saudi Arabia (0.3 mg/Kg), 0.034 – 0.985 mg/Kg in Embu and 0.25 – 0.7 mg/Kg along Liheriver, Tahiu (Sayo et al., 2020; Chen et al., 2018; Balkhair& Ashraf, 2016). On the other hand, Ikenaka et al., (2014) reported higher values (5 – 7.76 mg/Kg) than those obtained in this study.

Zinc generally had the highest mean values in the soil samples (20.1 – 28.8 mg/kg) in the different sampling sites. Zn levels at Site 1 (Bellesa upstream) ranged between 0.00 – 64.17 mg/Kg. Variation of Zn at site 2 (Genfel middle stream) was 0.00 to 63.01 mg/Kg while at site 3 (Dengollo downstream) the variation was 0.00 – 38.7572 mg/Kg. Highest Zn concentration was at site 2 and the lowest at site 3. The mean concentration of Zn from all the sampling sites was below permissible standard set by WHO for agricultural soils. A one-way analysis of variance (ANOVA) showed that there was no significant variation in Zn levels in the different sampling points ($p > 0.05$). The source of Zn in soil could be associated to fertilizers and pesticides used in the farm fields, naturally occurring in rocks in the soil and heavy metals in the wastewater used to irrigate the farms.

The results in this study can be compared to those reported in previous studies which were below FAO/WHO permissible limits; Sayo et al., (2020) reported Zn levels lower than those detected in this study (3.011 – 4.679 mg/kg) in Embu, Kenya for wastewater irrigated soils while Woldetsadik et al., (2017) reported higher values (119 – 203mg/Kg) than those obtained in this study from vegetable farming sites in Addis Ababa.

Concentration of Cr in the study area ranged from 8.17±5.92 to 10.03±6.02 mg/Kg as indicated in Table4.2. The concentration of Cr during the period of this study ranged as follows: 0.00 – 15.47, 0.00 – 34.753 and 4.465– 14.9982 mg/Kg in site 1 (Genfell), site 2 (Bellesa upstream) and site 3 (Dengollo downstream), respectively. The highest Cr mean concentration (10.03 mg/kg) was recorded at site 1 while site 3 records the lowest Cr mean concentration (8.17 mg/Kg). All Cr values were within WHO recommended levels hence the soil in the sampling sites was safe for agricultural use. Chromium in the different sampling sites had no significant difference ($p>0.05$). Cr in soil could be originating from the sewage contamination of irrigation water, atmospheric emissions, and industrial effluents (Toxicology & Medicine, 2011) These values are lower compared to Cr levels detected in previous studies; 30.67 –172.75 mg/Kg for wastewater irrigated soils in suburban areas of Varanasi India (Kumar et al., 2007), 47.0 mg/kg in Mitidja plain, Algeria and 17–39 mg/Kg at Kafue River, Zambia (Ikenaka et al., 2014). Khan et al., (2019) however reported higher Cr concentration in soils (0.9 – 1.8 mg/Kg) than those obtained in this study.

4.3 Heavy metal concentration in vegetables

The results of this study showed that heavy metals (Pb, Mn, Zn, Cr, and Cu) were present in varying concentrations in the ten types of vegetables and fruits commonly used in Wukro town, Tigray Ethiopia. The average concentrations of Pb, Mn, Zn, Cr and Cu in the examined vegetables and fruits in dry weight along with the relevant standard deviation values were determined by using Eqn. 3.1 and presented in Table 4.3. The observed concentrations of Pb, Mn, Zn, Cr and Cu in the vegetables and fruit were compared with the recommended limit established by FAO/WHO (2012) to assess the levels of its contamination and to assess non-carcinogenic and carcinogenic risks. The average concentrations of Cr and Cu in all of the selected vegetables and fruits were lower than the maximum limit of normal values (Table 4.3). However, the concentrations of Pb and Mn were higher by 50% and 20% for vegetable samples than the limit standards, respectively. Therefore, there is a need to estimate the health risks of heavy metals via consumption of vegetables and fruits by the community in Wukro town, Tigray, Ethiopia

Table 4. 3 Mean concentration of heavy metals in selected vegetables in Wukro town, Tigray, Ethiopia (mean ± SD, n = 4) and %SRD

Vegetables	**Mean concentration of Heavy metals in mg/Kg dry weight (X ±SD) and %SRD**				
	Cu	**Zn**	**Mn**	**Pb**	**Cr**
Onion	8.06 ± 0.01	7.96 ± 0.40	2.5 ± 0.30	23.9 ± 1.25	0.012 ± 0.002
	0.1	5.0	12	5.2	16.7
Tomato	7.2 ± 0.10	6.2 ± 0.07	2.3 ± 0.10	22.4 ± 0.69	0.052 ± 0.002
	1.4	1.1	4.3	3.1	3.8
Lettuce	53.4 ± 0.43	11.7 ± 1.55	42.7 ± 2.67	24.5 ± 0.8	0.017 ± 0.003
	0.8	13.2	6.2	3.3	17.6
Cabbage	11.4 ± 1.63	15.6 ±1.06	62.1 ± 1.93	24.9 ± 2.05	0.026 ± 0,002
	14.3	6.8	0.03	8.2	7.7
Spinach	6.2 ± 0.45	12.4 ± 0.6	23.9 ± 1.57	26.5 ± 2.47	0.012 ± 0.002
	7.2	4.9	6.5	9.3	16.6
FAO/WHO Limit (mg/Kg)	73	100	500	0.3	2.3

N.B.: ND = Not detected; SD = Standard deviation; Source: a = FAO/WHO, 2012

It is a dangerous component that can be unsafe to vegetables. In present study, Pb was detected in 80% of vegetable samples. The level of Pb concentration in the edible portions of vegetable samples ranged between 23.9 and 26.5 $mgKg^{-1}$. The highest concentration of Pb was observed in spinach (26.5 $mgKg^{-1}$) and also the concentration ($mgKg^{-1}$) of Pb in tomatoes (22.4), lettuce (24.5), onion (23.9) and cabbage (24.9) were exceeded the safe limits which indicates that 100% of the samples exceeded the permissible limit, hence the products might be unsafe for human being consumption. It may cause problem such as: brain damage, seizure, central nervous system disorders, kidney disease both acute and chronic, gastro intestinal disturbances, slight liver impairment and damage a child’s central nervous system, kidneys, and reproductive system.

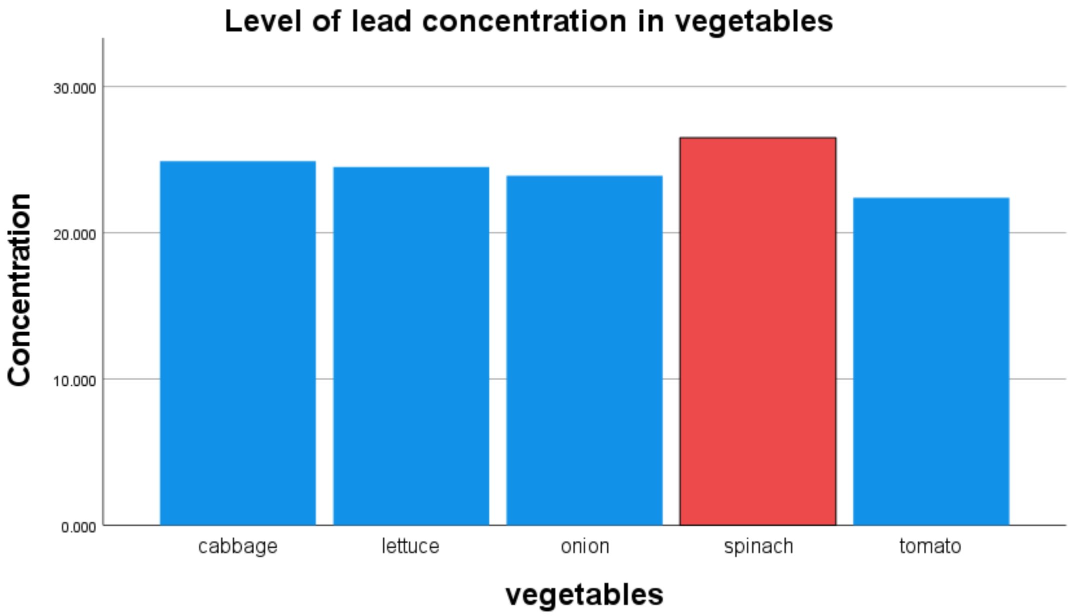


Figure 4. 1 Comparison of lead concentrations in different vegetable samples

It is non-essential and has no advantageous part in plants, animals and people and has no nutritious capacity as they are toxic. In this study Mn was detected in 100% of vegetable samples. The level of Mn concentration in the edible portions of vegetable samples ranged between 2.50 and 23.9 mgKg^{-1}. The maximum concentration was observed in cabbage followed by lettuce which is higher but not with in WHO recommended permissible limit.

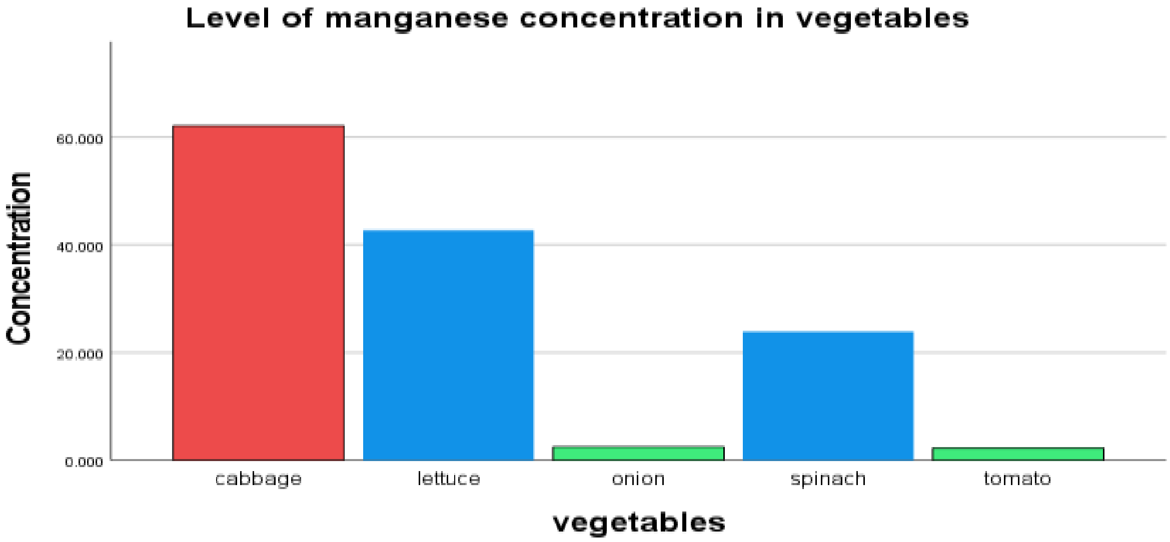


Figure 4. 2 Comparison of Manganese concentrations in different vegetable samples

In this study Cr was detected in 100% of vegetable samples. The level of Cr concentration in the edible portions of vegetable samples ranged 0.0120 − 0.0520 mg/Kg. The maximum and minimum concentration (mg/Kg) of Cr was found in tomatoes (0.0520) and cabbage (0.0260) respectively but the concentration in all types of vegetable samples was below safe limit, 2.3 $mgKg^{-1}$, set by FAO/ WHO [FAO/WHO, 2012]. The Cr concentration values ($mgKg^{-1}$) in the edible portion of vegetable samples were in the following order: tomatoes (0.0520) > cabbage (0.0260) > lettuce (0.017) > Onion = spinach (0.0120) and Figure 4.3.

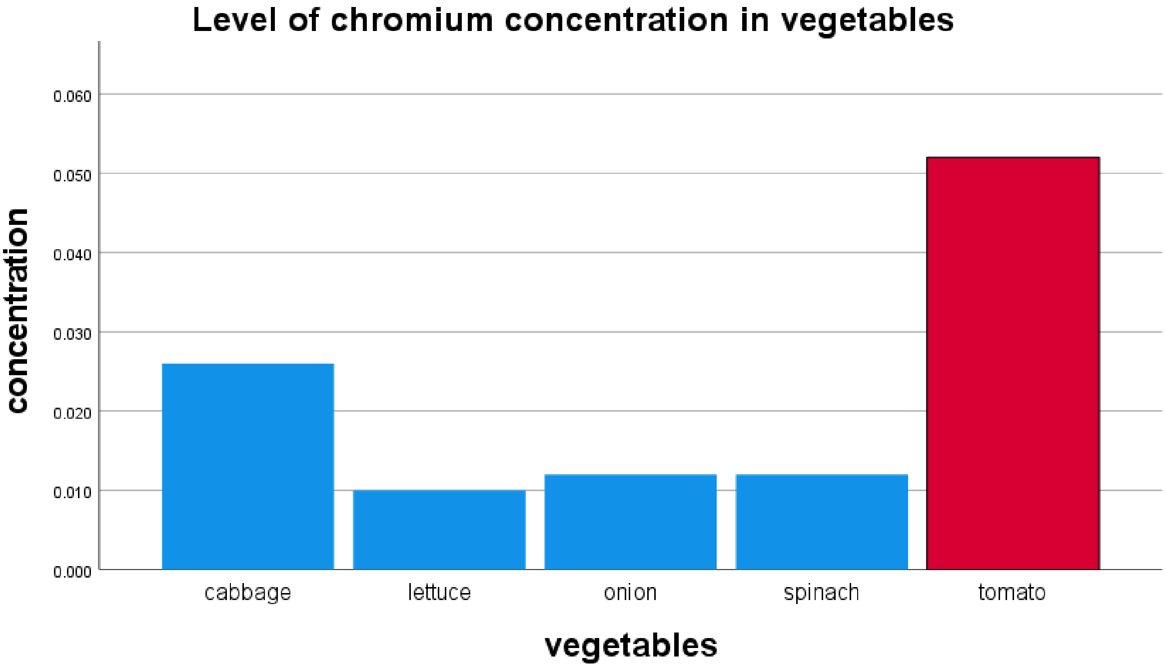


Figure 4. 3 Comparison of chromium concentrations in different vegetable samples

In present study Cu was detected in 100% of vegetable samples in the range between 6.20 mg/Kg and 53.40mg/Kg. The minimum concentration value was recorded in spinach 6.20 mg/Kg) and the maximum was in lettuce (53.40mg/Kg) but not exceeded the safe limit, 73 mg/Kg, set by [FAO/WHO, 2012]. The Cu concentration values (mg/Kg) in the edible portions of vegetable samples were in the following order: lettuce (53.40) > cabbage (11.40)>onion (8.06) >tomatoes (7.20) > Spinach (6.20), (Table 4:4) and Figure 4.4.

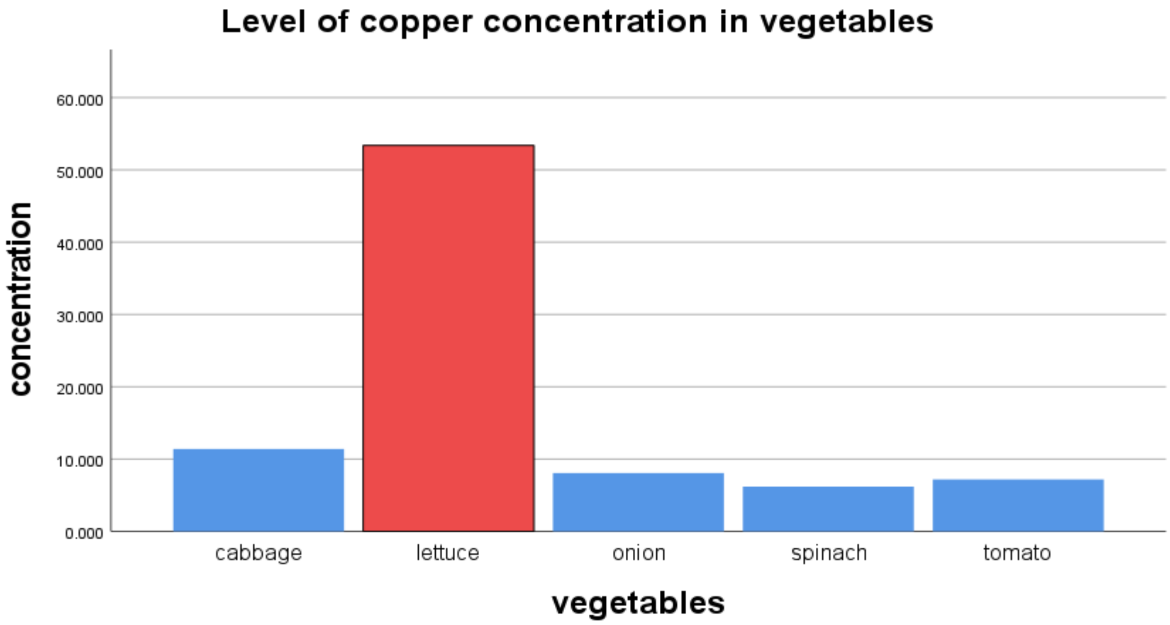


Figure 4. 4 Comparison of copper concentrations in different vegetable samples

In this study Zn was detected in 100% of vegetable samples. The level of Zn concentration in the edible portions of vegetable samples ranged between 6.20 mg/Kg and 15.60 mg/Kg. The maximum and minimum concentration of Zn in cabbages and spinach was found to be15.60 and 12.40 mg/Kg, respectively but the concentration in all types of vegetable samples was below safe limit, 100 mg/Kg, set by FAO/ WHO [FAO/WHO, 2012]. The Zn concentration values (mg/Kg) in the edible portion of vegetable samples were in the following order: cabbage (15.60) > spinach (12.40) > lettuce (11.70) > onion (8.06) > tomatoes (7.20), Figure 4.5.

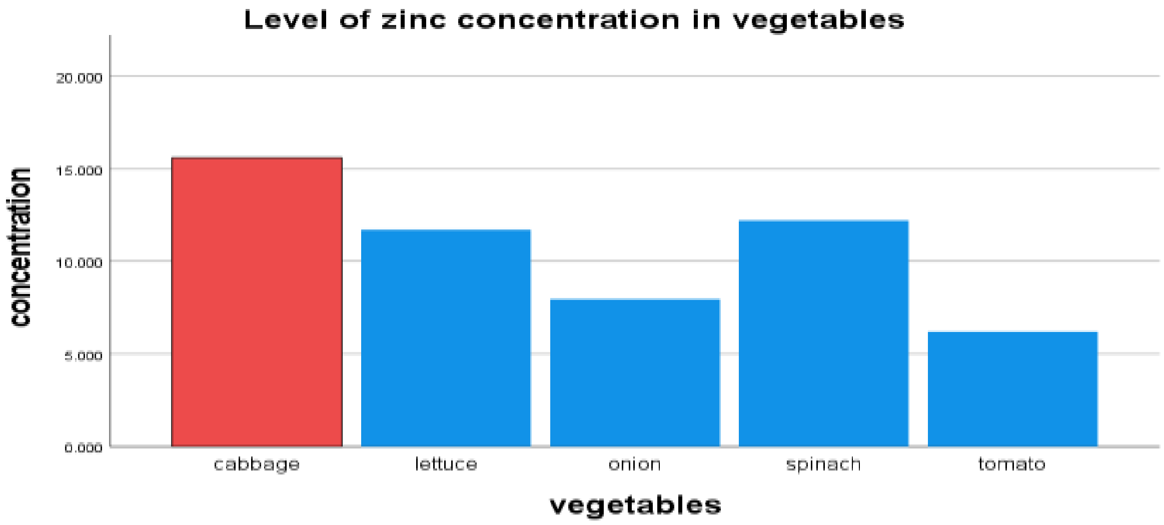


Figure 4. 5 Comparison of Zn concentrations in different vegetables

In order to figure out the heavy metal's concentration, comparisons were made with other literatures done in previous studies. In the present study, Cu and Cr were found in highest concentrations among all heavy metals, however the Cu values were below the results obtained in other parts of Ethiopia (Brhane et al., 2014; Tegegne, 2015), Iran (Taghipour et al., 2013) and Ghana (Ametepey et al.,2018). In this study the maximum and minimum concentration was recorded in cabbage and lettuce, respectively but in other studies onion and tomato have the highest concentration (Taghipour et al., 2013 and Brhane et al., 2014). However, those studies including this study, the Cu concentration did not exceed the standard limit 40-73 mg/Kg set by FAO/WHO [FAO/WHO, 2012]. The maximum Cr concentration was observed in tomatoes (0.052 mgKg^{-1}) which is similar to other studies in Iran (Taghipour et al., 2013) and in Ethiopia (Benti, 2014) but the concentration in all types of vegetable samples as well as in all studies were below standard limit 2.3 mgKg^{-1} set by FAO/ WHO [FAO/WHO, 2012].

The concentrations of Pb identified from the vegetables in study were more than those reported in Ghana (Ametepey et al., 2018) and in Ethiopia (Brhane et al., 2014; Benti, 2014 and Tegegne, 2015) but lower than other studies in Addis Ababa, Ethiopia (Gebeyehu et al., 2020) and Gondar, Ethiopia (Berihun et al., 2021). The level of Mn concentration ranged from 2.30 − 62.10 in this finding which is greater than other findings in Ethiopia (Brhane et al., 2014; Benti, 2014 and Gebeyehu et al., 2020), in Iran (Taghipour et al., 2013) and Ghana (Ametepey et al., 2018) but lower than one study in Gondar, Ethiopia (Berihun et al., 2021). The maximum concentration was observed in cabbage and lettuce in this study which is above the safe limit.

4.4. Estimated daily intake of heavy metals in the selected vegetables

The degree of toxicity of heavy metals to human being depends upon their daily intake. Therefore, the estimated daily intake (EDI) of the metals were estimated based on the mean concentration of each metal in each vegetable and fruit with the respective consumption rate of the vegetables and fruits in Ethiopia. The EDI of heavy metals for the population of the study area is presented in Table 4:4.

Table 4. 4 The estimated daily intake (EDI) and total intake values of heavy metals through consumption of vegetables

Vegetable sample	**Cu**	**Zn**	**Mn**	**Pb**	**Cr**	**Total intake**
onion	13.24	12.95	4.1	39.19	0.019	2.28E-03
Tomato	11.83	10.16	3.77	36.76	0.085	6.22E-03
Lettuce	87.7	19.18	70.02	40.18	0.027	2.02E-03
cabbage	18.7	25.58	101.8	40.83	0.042	6.34E-03
spinach	10.18	20.33	39.19	43.46	0.019	2.07E-03
RfD (mg/kg	4.00E-0	23.00E-01	14.00E-02	3.50E-03	3.0E-03	
FAO/WHO limits mg/Kgday^{-1})	3mg	0.214mg	0.3mg	0.21mg	0.2mg	

The EDI values for Cu, Zn, Mn, Pb and Cr were found to be in the range of 10.18 to 87.7, 10.16 to 25.58, 3.77 to 101.8, 36.73 to 43.46 and 0.019 to 0.085 mgday^{-1}, respectively due to the average daily consumption of, 115gday^{-1}, vegetables in Ethiopia [Worku et al., 2017]. The computed estimated daily intake (EDI) for each element in the studied vegetables was compared with the toxicologically accepted level and oral reference dose value established by US-EPA [US EPA, 2015]. In this study, EDI for Pb, Cr and Cu was below the RfD value in all vegetable samples which is nearly free of risks. The total EDI of Pb, Cu and Cr obtained due to the consumption of all types of vegetables were observed to be more than RfD but less than maximum tolerable daily intake of each metal while the total EDI of Cr is less than both the RfD and also the maximum tolerable daily intake set by FAO/WHO [FAO/WHO, 2012].

A wide variation of heavy metal concentrations was observed in the studied vegetables which affects the intake of each heavy metal where the highest and the lowest metal content (mg/Kg) were Cr with average concentration of 0.0384 and 0.176 with average concentration of Zn respectively. The trend of the heavy metal contents according to average concentration found in the studied vegetables were in the following order: Pb>Mn>Cu >Zn> Cr. More specifically, the consumption of cabbages, spinach and lettuce accounted for high percentage of Pb intake 72.69%, 66.67% and 61.38%, respectively than other metals but tomatoes

(24.9%), onion (21.68%) leads to high percentage of Cu intake and also the consumption of from the total intake of Zn, Mn, Pb Cr and Cu in each vegetable as shown in the figure 4:5. Therefore, it can be inferred that vegetables are the two major sources of heavy metals that enter human body via consumption. Consequently, the heavy metal levels in vegetables should paid special attention and the government supervision should be enhanced to decrease the potential health risks of heavy metals to humans via consumption.

4.5 Health risk assessment of heavy metals in the selected vegetables

To evaluate the health risk of heavy metals via consumption of vegetables by the community, the estimated daily intakes of the toxic metals were analyzed and compared with the provisional tolerable daily intakes (PTDIs) recommended by the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO). As shown in Table 4:5, the calculated estimated daily intakes were used to determine both non-cancer and cancer health risks.

4.5.1. Non-cancer risks (THQ)

The non-cancer risks (THQ) of the intake of a single metal through the consumption of all the studied vegetables as well as the intake of all studied heavy metals via consumption of single type of vegetables for adult inhabitants of the study area were determined.

Table 4. 5 THQ values of individual heavy metals through the consumption of different vegetables in this study area

Types of samples	**Cu**	**Zn**	**Mn**	**Pb**	**Cr**
Onion	331E-3	43.16E-3	29.29E-3	3782..8E-3	6.33E-3
Tomato	295E-3	33.87E-3	26.93E-3	33.80E-3	28.33E-3
Lettuce	2192E-3	63.93E-3	500E-3	25.08E-3	9.00E-3
Cabbage	467E-3	85.27E-3	727E-3	5342E-3	14.0E-3
Spinach	253E-3	67.77E-3	279.9E-3	2908E-3	6.00E-3

As shown in the THQ values varied from 0.295 − 0.467 for Cu, 0.033 − 0.0852 for Zn, 2.69 $\times 10^{-1} - 7.27 \times 10^{-1}$ for Mn, 0.0250 − 3.782 for Pb and $6 \times 10^{-3} - 2.83 \times 10^{-1}$ for Cr and also the total THQ (∑THQ) for Cu, Zn, Mn, Pb and Cr due to consumption was determined by using Eq. (4.4) which was 3.28, 0.293, 1.56, 35.21 and 0.063, respectively. The HI values of all the studied metals were lower than one in each vegetable samples except cabbage and spinach. This suggests that those populations do not face a significant potential non-cancer health risks caused by the intake of all studied heavy metals via consumption of single type of vegetables a except cabbage and spinach in their life time but the populations faced a significant potential non-cancer health risks caused by the intake of either all studied heavy metals via consumption of single type of vegetables or a single metal through the consumption of all the studied vegetables except Pb in their lifetime. The relative contributions of Cu, Zn, Mn, Pb and Cr to the HI from all vegetable's consumption were also calculated. Pb is a major risk contributor for the residents in Wukro TOWN which accounting for 54.0 % of the total HI followed by Cr (31.2%) while the risk contribution from and Cu were accounting 10.7 % and 4.1 %, respectively.

4.6 Analysis of variance

Analysis of variance (ANOVA) is widely used statistical methods to compare the mean of more than two groups of samples or to test the level of significance at $\alpha = 0.05$. ANOVA uses the F statistic to compare whether the differences between sample means are significant or not. In this study, samples were collected from open farmland in Wukro town and the metal levels of each sample were analyzed by AAS. During the processes of sample preparation and analysis a number of random errors may be introduced in each aliquot and in each replicate measurement. The variation in sample mean of the analyzed was tested by using ANOVA, whether the source for variation was experimental procedure or heterogeneity among the samples. The ANOVA results showed that there exist statistically significant differences at 95 % confidence level in mean concentrations of all the metals. The source for this significant difference between sample means may be the difference in mineral contents of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides or other variations in cultivation procedures rather than experimental procedure.

Table 4. 6 Analysis of variance (ANOVA) between and within vegetable samples at 95 % confidence level

Source of variance		**Sum of squares**	**Mean square**	**df**	**F statistic**
Cu	Between Groups	2.13	0.533	4	0.006
	Within Groups	1658.57	82.93	20	
Zn	Between Groups	26.42	6.61	4	0.715
	Within Groups	184.97	9.25	20	
Mn	Between Groups	117.94	29.49	4	0.179
	Within Groups	3287.6	164.38	20	
Pb	Between Groups	73.27	18.32	4	0.969
	Within Groups	378.7	18.9	20	
Cr	Between Groups	248.3	62.08	4	0.995
	Within Groups	1247.6	62.38	20	

4.7 Pearson correlation analysis

A correlation test was carried out between the investigated metals for the vegetable and fruit samples and among metals detected in the vegetables and fruits to associate availability of metals and its accumulation in the vegetables and fruits at $\alpha = 0.05$. In this study, the Pearson correlation matrices using correlation coefficient (r) for the samples were used which examined the relationship between one metal concentration and other metal concentrations in the same sample. If heavy metals concentration via vegetable and fruit samples has high correlation coefficient (near +1 or −1) means a good relation between two metals, and its concentration around zero means no relationship between them at a significant level of 0.05% level, it can be strongly correlated, if $r > 0.7$, whereas r values between 0.5 and 0.7 shows moderate correlation between two different metals. [Sharma et al., 2013].

The Pearson correlation coefficient results revealed a moderately significantly positive relationship between Mn and Cr and there is also moderately positive relationship between Cu and Pb this moderate positive correlation shows that the concentration of each element is moderately associated to each other in the same sample which means that if the concentration of Pb is high in vegetable and samples, the concentration of Cr also high. There was also weak positive correlation between Cu and Mn but the other heavy metals have weak negative correlation between, Mn with Cr and Cu with Cr. Those weak positive or negative correlation of each other indicates that the presence or absence of one element affect in lesser extent to the other.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

In this study, the levels of toxic metals (Pb, Mn, Cr, Zn and Cu) in edible portion of vegetables spinach, tomato, onion, cabbage and lettuce in Wukro town were analyzed and their potential health risks were estimated. The results showed that the average concentrations of Cr and Cu in all of the selected vegetables were lower than the maximum limit of normal values recommended by the WHO. However, the concentrations of Pb in cabbage, onion, lettuce and spinach and also the Mn concentrations in cabbage and spinach were relatively higher than the limit standards. THQ results indicated that there is not additional non-cancerous health risk through the consumption of vegetables except cabbage and spinach in this study area. Therefore, from the health risk assessment of toxic metals, we can conclude that the daily intake of cabbage, lettuce and spinach is unsafe for local consumption.

5.2. RECOMMENDATIONS

Large population residing in rural areas has no information on vegetables and fruits contamination based on outdated agricultural practices, agrochemicals use and under pressure of intense land utilization. This situation has resulted severe deterioration of vegetables and fruits, changing agricultural lands into wastelands therefore, Ethiopian Ministry of Agriculture (EMoA), Ethiopian Horticultural Development Agency (EHDA) and Ethiopian Investment Agency (EIA) should apply concerted efforts in monitoring and regulating the use of agrochemicals in agricultural practice and adopt modern agricultural practices and also the environmental problems and potential health hazard to the population is more pronounced as the environmental safety regulations are not practically in place, so proper environmental monitoring should be done by Ethiopian Environmental Protection Authority (EPA), Ethiopian Food and Drug Authority (EFDA) and Ethiopian Federal Ministry of Health (EFMOH) to avert adverse effects on the environment and the population. Moreover, raising awareness of the public on environmental health is critically important to overcome the problem.

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