ASSESSMENT OF ZINC, LEAD AND CADMIUM CONTRIBUTIONS TO DIABETES AND HYPERTENSION: A CASE STUDY OF MARAKWET EAST AND MARAKWET WEST SUB COUNTIES

BY

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE IN CHEMISTRY, TO THE SCHOOL OF SCIENCE, UNIVERSITY OF ELDORET, KENYA

OCTOBER, 2022

DECLARATION

Declaration by the candidate

This is my original work and has not been presented elsewhere for the award of a degree programme in any university. This thesis may not be reproduced in its entirety or parts without prior permission from the author and or University of Eldoret**.**

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This thesis has been submitted with our approval as University Supervisors

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I dedicate this thesis to my parents, husband and children.

ABSTRACT

Globally, chronic heavy metal poisoning is of global concern, aggravated by human activities in the world leading to food and drinking water quality issues. This study assessed the relationship between heavy metals and insulin contributing to diabetes and hypertension within Marakwet East and West. A total of thirty six (36) each of water and soil samples from rivers Chepkaitit, Moiben, Embobut, Arror, Mon and Mosongu were purposefully selected. Water samples were collected from each of the three sites of the six rivers during wet and dry seasons. Twelve (12) samples of beef were also collected within the study area. The samples were digested using wet methods to establish levels of Zn, Cd and Pb in water and soil using AAS and in beef samples using ICP-MS. Exploratory research design using a questionnaire was also undertaken for a comparative study of diagnosed diabetic and hypertensive patients. The interaction of the metal divalent cations with insulin was done in neutral and mildly alkaline solutions. Majority (76.5%) of the respondent pointed out that they had heard about heavy metals (χ^2 = 31.76, d.f.=2P-value = 0.0001). For hypertension condition, majority of the respondents (45.0%) pointed out that presence of other diseases was the contributing factor to those having the condition. All respondents pointed out that they had been exposed to fertilizers. Few of the subjects responded regarding the issue of health of their family members. There was no significant difference in response pertaining to the type of condition and those who were affected (χ^2 = 2.5283 d.f =2, P-Value = 0.2825). For those who were suffering from cancer and hypertension, majority were young women (35.7%) and 43.8%) of the respondents respectively. There was no significant difference in responses concerning how respondents were related to the cancer victims (χ^2= 4.57143 d.f =4, P-value = 0.3342) as well as for hypertension (χ^2= 9.0, d.f=4, P-value = 0.0611). For diabetes victims, majority (52.2%) were young men while a few were old respondents with a significant difference (χ^2= 18.087, d.f =4, P-Value = 0.0012). In water samples, Zn levels were higher during wet season than dry season (p>0.05). Significant increase in Zn levels from upstream (0.212 ppm) to midstream (0.225 ppm) were recorded in water samples (P>0.05). There was significant increase in Cd levels from upstream (0.066 ppm) to midstream (0.068 ppm) (P >0.05). Soil samples collected during wet season from R. Chepkaitit recorded Cd levels of 0.071, 0.078 and 0.053 ppm upstream, midstream and downstream, respectively. Similarly, Pb levels increased from 0.470 ppm to 0.720ppm to 0.791 ppm in water samples correspondingly collected upstream, midstream and downstream of R. Mon. The concentrations of Pb and Cd in some water and soil samples exceeded the WHO standards. The binding strength of Zn, Pb and Cd with histidine resulted in a significant difference (p<0.05) when spiked showing the interaction with histidine. The results from this study would inform the policy decision makers in agriculture and healthcare sectors to sensitize farmers on the use of chemicals and regulate fertiliser applications.

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LIST OF ABBREVIATIONS AND SYMBOLS

µg/L	Micro gramme per litre
AAS	Atomic Absorption Spectrophotometer
Ag	Silver
ANOVA	Analysis of variance
ASR	Alkali–silica reaction
Cd	Cadnium
Co	Cobalt
Cr	Chromium
CTAB	Cetyltrimethyl ammonium bromide
Cu	Copper
CVD	Cardiovascular Disease
FAO	Food and Agricultural Organization
GDM	Gestational Diabetes Mellitus
HDL	High Density Lipoprotein
ICP-MS	Induced Coupled Plasma-Mass Spectrometry
mL	Milli liter
Mn	Manganese
MT	Metallothionein
NHES	National Health Examination Survey
Ni	Nickel
NIST	National Institute of Standards and Testing Materials
Pb	Lead
pH	Hydrogen ion concentration
ROS	Reactive Oxygen Species
Sn	Tin
T2DM	Type 2 Diabetes Mellitus
WHO	World Health Organization
Zn	Zinc

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Heavy metals possess higher atomic number, atomic weights and density. Due to their deleterious human health effects and threats to the environment, pollution by heavy metals have become among the world's top environmental problem (Hembrom *et al*., 2020). Sources of the heavy metal pollution can be both anthropogenic and natural. Anthropogenic sources are mainly linked to industrialization and agriculture. As such therefore, levels of environmental contamination vary greatly around the world. Further, level of contamination may vary among the different heavy metals (Mansour, 2014). Generally, highly industrialized economies are associated with higher levels of pollution. A study that assessed the various levels of contamination (Zn, Pb, Cu, Cd, Ni, Cr, Mn and Fe) in some cities in all the continents, revealed that soils in Asia are more heavily polluted (Kapoor, & Singh, 2021). In many instances Africa is the least polluted however, due to the poor economic status of its inhabitants, they are more exposed to the dangers of heavy metals especially those derived from agricultural activities. As a study by Fewtrell *et al*. (2004) showed, African cities inhabitants have some of the highest blood lead levels (Children 110 µg/L; Adults 118 µg/L). However, the worst affected according to this data is North Africa and parts of Asia.

Increased anthropogenic activities in Kenya over the past few years have led to massive pollution of the environment (Plessl *et al.,* 2017). The use of agricultural inputs, waste, industrial fumes and spills of petroleum products has led to increases in heavy metals in soil (Sankhla *et al*., 2016; Subhashini & Swamy, 2013; Maurya *et al*., 2018). Intensified agricultural practices in Kenya in recent years have led to increased and continued use of fertilizers, pesticides, manure and machinery to increase yields for economic development and to achieve Vision 2030 goals. Fertilizers are regularly applied in large amounts to soils in intensive farming systems to ensure adequate levels of potassium, phosphate, and nitrate for plant growth.

These activities involve use of chemicals, metals, machines, fertilizers, pesticides, fuels such as petroleum and many others (Hertwich, 2010). Heavy metals can be discharged into the environment including soils and surface waters as well as ground water where they persist for a long time (Mahurpawar, 2015).

The scientists Buzea and Pacheco (2020), Adal and Tarabar (2013), Morais *et al*., (2012) have elucidated heavy metals based on their high atomic weights and relative toxicity. They are also classified with a density above 5 g/cm^3 (Konuk *et al*., 2010; Issazadeh *et al*., 2013). Heavy metals are composed of elements such as metalloids, transition metals, actinides, and lanthanides (Khan & Parvez, 2015). These include metals such as lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), chromium (Cr), and mercury (Hg), which are significant environmental pollutants (Asati *et al*., 2016; Gil, 2014; Ogunkunle *et al*., 2014). While most heavy metals are found in rocks, developments have increased human-made heavy metal contributions to the biosphere. Heavy metals occur in trace amounts in the atmosphere as particles or vapors (Ali *et al*., 2016).

Herbicides, pesticides and inorganic fertilizers can contain varying amounts of heavy metals; when used in agriculture for the purpose of providing nutrients to plants and controlling weeds, pests and diseases, these products can result in heavy metal deposition in the biosphere (Heshmat *et al*., 2012). Heavy metals can enter human and animal food chains through plant uptake (Kumar *et al*., 2019). Colloids, particles and dissolved heavy metals are detected in surface waters. The oxidation state of minerals and the redox environment of the system controls trace metal solubility in surface waters and hence heavy metal loading of surface and groundwater.

The chronic non-communicable disease diabetes has increased significantly, especially in developing countries (Guariguata *et al*., 2014). Environmental pollutants (such as heavy metals) can be involved in diabetes in addition to poor diet and lack of exercise (Thayer *et al*., 2012). Metals are ingested through food, water, ambient air and skin contact with consumables (Nordberg *et al*., 2018; Rehman *et al*., 2018). These trace elements are involved in numerous metabolic and biological processes (Nordberg *et al*., 2018).

Hypertension (cardiovascular disease) is a major contributor to global disease and mortality. By 2025, 1.56 billion people will be diagnosed with hypertension (Judd & Calhoun, 2014). It is difficult to estimate the occurrence of high blood pressure in the overall population using standard etiological factors (age, smoking/alcohol use, and obesity). Previous research has linked environmental metal exposure to hypertension (Oliver-Williams *et al.*, 2018). Heavy metals can bind to sulfhydryl groups of antioxidants and enzymes, changing their function and activity. Cadmium, arsenic, and lead can bind to glutathione, causing depletion and oxidative stress. Metals like lead and mercury can inhibit antioxidant enzymes like paraoxonase, increasing free radicals and oxidative stress. Only a few studies have looked into the link between cadmium, lead, and hypertension.

Elgeyo Marakwet county being one of the agricultural regions of Kenya, it is highly probale that the water bodies from this region is polluted with heavy metals. This study therefore seeks to establish the extent to which such contamination influence the prevalence of hypertension and diabetes in Marakwet west and East sub-counties.

1.2 Statement of the Problem

Individual variations in blood pressure and diabetes have been linked to environmental exposure to heavy metals such as zinc, calcium and lead (Lin *et al*., 2020). A number of research papers have been published on this topic (Li *et al*., 2017; Feng *et al*., 2015). The molecular processes underlying the heavy metal-induced changes in blood pressure include Cd-induced nephrotoxicity (Mizuno *et al*., 2021; Nair *et al*., 2013) as well as the rapid increase in cardiac output vasoconstriction induced by subjection is induced by Pb in the sympathetic system. In addition, As, Cd and Pb can induce inflammation and/or oxidative stress in endothelial cells (Mizuno *et al*., 2021). The concept that dangerous heavy metals such as cobalt, arsenic, selenium, cadmium, iron and copper are linked to the development of diabetes has been supported by new epidemiological evidence.

Metal poisoning and its associated risk of hypertension and diabetes have been extensively investigated in developed countries; however, very few studies have been conducted in Africa's tropics. Due to low resources, metal poisoning is typically difficult and expensive to detect or screen in these nations, implying that policies, guidelines, legislation, and institutional management are also limited.

Marakwet East and West Sub Counties have the highest prevalence of diabetes in Kenya (12.6 percent), compared to the national rate of 5.6 percent (WHO, 2015). In addition, ther is no reported work in this region. This study's main purpose was therefore, to document diabetic information and to assess the role of heavy metals in confirmed diabetes cases.

The prevalence of heavy metals in soil, water and food in the Marakwet region has been documented by (Maiyo *et al*., 2014; Kemboi *et al*., 2018; Akenga *et al*., 2020), and no study has been conducted to assess the heavy metals contribution to diabetes and hypertension due to high prevalence in the region. It is therefore hypothesized that heavy metals found in soils and water could as well be a factor contributing to diabetes and hypertension in human. This study was to provide an explanation about heavy metals as contributors to diabetes and hypertension in Marakwet East and West Sub counties.

1.3 Objectives

1.3.1 General objective

To assess heavy metals (Zn,Cd and Pb) as contributors to diabetes and hypertension in Marakwet East and West Sub counties

1.3.2 Specific Objectives

i. To estimate the prevalence of diabetes and hypertension in Marakwet East and Marakwet West

ii. To determine concentration of Zn, Pb and Cd in selected meat, soil and water in Marakwet East and Marakwet West.

iii. To analyze the interaction of different levels of Zn, Pb and Cd with insulin contributing to diabetes and hypertension.

1.4 Hypothesis

i. Ho_1: One percent of the total population of diagnosed diabetic and hypertensive patients are in Marakwet East and Marakwet West

ii. Ho_2: Zn, Pb and Cd levels in soil and water samples obtained from the study area were below permissible limits of WHO

iii. Ho_3: Zn, Pb and Cd do not interact with insulin and so do not contribute to diabetics and hypertension.

1.5 Justification of Study

By 2030, Sustainable Development Goal 3 aims to ensure and promote ensuring and promoting healthy lifestyles for people of all ages; while Goal 4 aims to reduce mortality from four major NCDs (cardiovascular diseases, cancer, diabetes, chronic respiratory diseases) has the goal. Kenya Vision 2030 aims to help Kenya become an emerging middle-income country by 2030, with a good quality of life for all its residents in a clean and safe environment. This can only be achieved through continuous monitoring of toxic products in the environment. The presence of pollutants in water can result in an economic burden on the population from the treatment of diseases caused by heavy metals caused by their toxicity. The quantification of heavy metals and their interaction is crucial to enable the development of cures to suppress their introduction or initiate strategies to remove them.

1.6 Significance of the study

It is anticipated that this data will assist to bring into focus some of the effects of excessive heavy metal intake by human beings. The data will also be useful in providing information to the county government of Elgeyo Marakwet about the occurence of heavy metals, hence enabling the county government come up with mitigation strategies of reducing heavy metal toxicity in soil and water. Findings of this study may be used to inform the communities living within the study area about the potential risk they face on consumption of water containing high heavy metal levels. The local population will also be advised on how to reduce heavy metal uptake through monitoring their levels. The findings of this study will also lead to reduced economic burden for treatment of the diseases.

1.7 Scope of the Study

The study was limited to the water sources and soil in Marakwet East and West sub-counties. In particular, the study focused on the assessment of heavy metals in soil and water. The research was conducted between January and December 2018.

1.8 Limitation of the Study

Several constraints hampered the study. The study only covered two sub counties. A study of only two sub counties could suffer failures of not getting information which could be used to generalize across the country and other counties. However, a larger sample from the study area was quite representative. The study was also constrained by very few reported studies that have been done on heavy metals in Elgeyo Marakwet. Therefore, studies that have been done in other countries on heavy metals were used to make inferences.

1.9 Assumptions for the Study

The study made the following assumptions: it was assumed that the study variables were equally distribution. It was expected that the research variable would follow a regularly distributed probability distribution. It was also believed that the respondents would be able to correctly interpret the study instruments and provide truthful responses to the questions.

1.10 Conceptual Framework

Figure 1.1 illustrates the effect of heavy metals and their interaction with insulin for diabetes and hypertension cases.

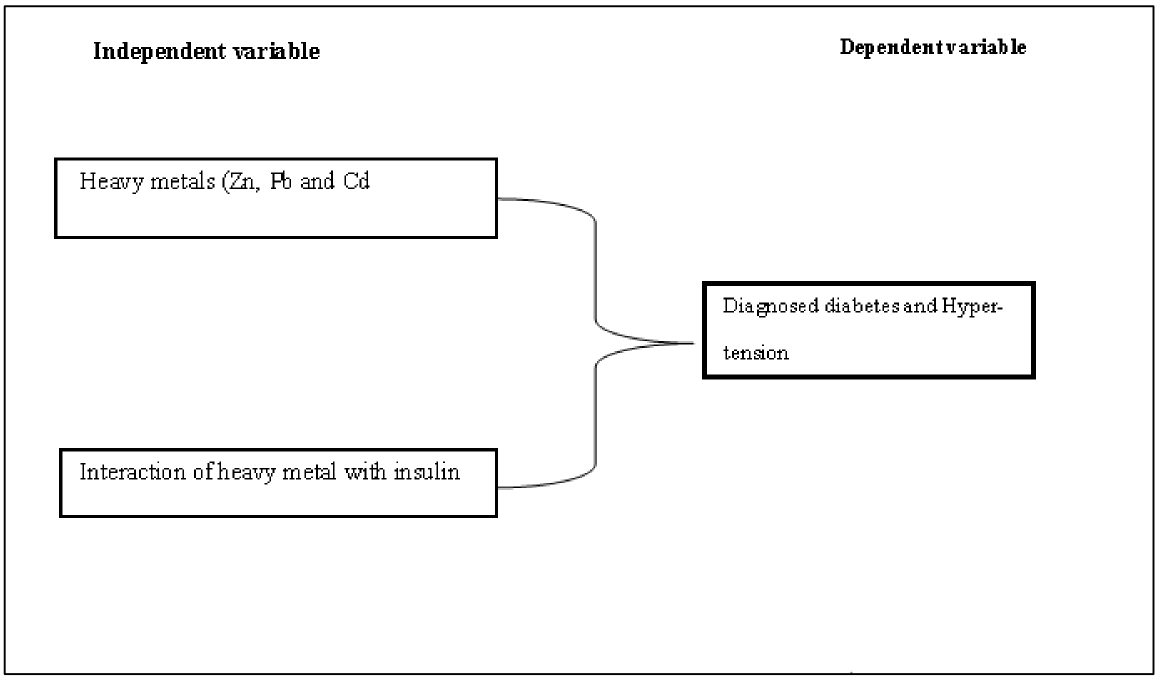

Figure 1.1: Conceptual Framework

CHAPTER TWO

LITERATURE REVIEW

2.1 Chapter Overview

The chapter was reviewed based on work done elsewhere. The apparent relationship between chronic heavy metal exposure and cardiovascular disease has a number of implications. There are few reported reviews on heavy metals as cardiovascular toxins, and the majority of assessments focus on the imbalance in antioxidant defense systems caused by environmental exposure, which results in oxidative stress in the cells. Gene expression changes as a result of environmental influences, most notably diet (Feil, 2006).

2.2 Heavy metals

Heavy metals consist of elements such as metalloids, transition metals, actinides and lanthanides. Bennet and Sinnott (1986) and Harrison and Waites (1998) defined heavy metals based on their high atomic weight and relative toxicity. In addition, they can be classified as having a specific density greater than 5 g/cm^3 (Jarup, 2003). Heavy metals can be defined as elements with an atomic mass greater than 40 and a density of at least four grams per cubic centimeter (Adelekan & Abegunde, 2011). Metals such as lead, cadmium, copper, zinc, chromium and mercury contribute significantly to environmental pollution (Nagajyoti *et al*., 2010).

Ecosystem is greatly affected by Heavy metal contamination. They are found in smelting, mining, sewage sludge, and fertilizers (Filgueiras *et al.*, 2002). Heavy metal contamination is caused by mining, phosphate fertilisation, lime application, and biosolids amendments (Alloway, 1995). Heavy metals enter the soil via fertilizer, sludge, liming materials, compost manures, pesticides, and air deposition. They accumulate in agricultural soils; negatively influence food safety and marketability (Nagajyoti *et al.*, 2010). Heavy metals are essential for biological functions but can become harmful and detrimental to the environment when in excess (Pandey & Madhuri, 2014). While metals such as Manganese, Molybedinum, Cobalt, Zinc, Iron and Copper are required in varying proportions by living creatures, excessive levels can be detrimental to the organisms (Singh, Gautam, Mishra & Gupta, 2011). While heavy metals occur naturally in the ecosystem, anthropogenic activities have contributed a significant amount of these metals to the environment.

2.2.1 Cadmium

Cadmium is the last transition element in the second row with an atomic number of 48, an atomic weight of 112.4, a density of 8.65 g/cm^3, a melting point of 320.9 °C and a boiling point of 765 °C. The specific gravity of Cd is 8.65 and the ionic forms of the metals (Cd^{2+}) combine with oxygen to form (CdO). In addition, it is able to combine with other elements such as chlorine and sulfur (Muzyed, 2011).

Cadmium is a non-essential, naturally occurring element. In most cases, rock and soil samples contain less than 1 microgram/g cadmium, although those from marine black shales and lead-zinc minerals often have much higher levels. These can increase the uptake of cadmium by food crops and vegetables. Anthropogenic causes such as smelter emissions, fertilizer and sewage sludge application can contaminate soils and crops. Cd is also used in rechargeable nickel-Cd batteries, which end up in sewage sludge and increase Cd levels in the environment (Hayat, Nauman, Nazir, Ali & Bangash, 2019). Cadmium is a contaminant in fertilizers, detergents and refined petroleum products (Nziguheba and Smolders, 2008). Zarcinas et al. (2004) found higher Cd levels in soils where cocoa (Theobroma cocoa) was grown due to the use of phosphate fertilizer. Ca, P and chelating chemicals in soil inhibit uptake of cadmium by plants.

Plants collect Cd to amounts that are not hazardous to them but are to humans. Cd absorbed by plants from the soil accumulates first in the rocks, then in the stems and seeds (Wieczorek *et al.,* 2004). Cancers of the various parts of the body; the breast, lung, prostate, nasopharynx, pancreas, and kidney have been linked to occupational and environmental cadmium exposure. Osteoporosis and environmental cadmium have also been connected.

Cadmium in trace amounts biochemically replaces zinc and results in elevated blood pressure and unfavorable alterations in the arteries of the human kidney. The WHO drinking-Water Quality Guidelines prescribe a maximum Cd contamination level of 3 g/l (Monudu & Anyakora, 2010; Adeleken & Abegunde, 2011). Aspartate and glutamate ligands cause iron deficiency when cadmium is present (Castagnetto *et al.,* 2002). Metallothionein is a cell free radical scavenger and cadmium is oxidised like zinc.

2.2.2 Lead

In the periodic table, lead is a Group IV and Period 6 metal with an atomic mass of 207.2, a density of 11.4 g/cm^3, a melting point of 327.4 ^oC degrees Celsius and a boiling point of 1725 ^oC degrees Celsius (Elizondo- lvarez, Uribe-Salas & Nava-Alonso, 2020). The amount of this naturally occurring, bluish-grey metal in the earth's crust is between 10 and 30 mg kg-1. Lead sulfide (PbS), lead sulfate ($PbSO_4$) and lead carbonate ($PbCO_3$) are the three most common forms of this metal (Elizondo-lvarez *et al*., 2020).

Lead is found in the environment for a range of natural and man-made reasons. Lead is an element of a mineral that occurs naturally when put together with other elements such as oxygen ($PbCO_3$) and Sulphur (PbS, $PbSO_4$) (Elizondo-Álvarez *et al.*, 2020). Lead is an insoluble metal that forms complexes regularly with sulfates, phosphates, organic compounds, and clay minerals (Olaniran, Balgobind & Pillay, 2013).

Anthropogenic sources of lead include dust from lead paint in older homes, leaded gasoline, and lead-contaminated tap water from soldered pipes (Brown & Margolis, 2012). Additionally, garbage incineration leads to the increased availability of lead in metropolitan areas. Automobile exhausts, sewage sludge, mining and smelting, shooting, and urban soils have all been identified as anthropogenic sources of lead pollution (Steinnes & Friedland, 2006). Indoor chemicals and smoking contribute to pollution as well (Vardoulakis *et al.*, 2020). A wide variety of products utilize it, including sound and vibration absorbers as well as storage batteries (Barkouch *et al.*, 2007).

Lead inhibits enzymes, interferes with nerve transmission and brain development, and competes with calcium for bone incorporation (Patočka & Černý, 2003). The consequences of lead exposure by inhalation and ingestion are similar (Wuana & Okieimen, 2011). Behavioural abnormalities and cognitive impairments have been linked to lead exposure in both humans and animals (Shannon & Graef, 1992). Lead exposure of 100 g/l causes an increase in Pb blood level in youngsters, causing a 1–5-point loss in intelligence quotient (IQ) (Goyer & Clarkson, 1996). These studies found that children exposed to lead have delayed development, poorer IQs, shorter attention spans, hyperactivity and mental decline. Children under six are at high risk. An adult's reaction time is slowed due to impaired haem synthesis and discomfort caused by lead exposure (Adelekan & Abegunde, 2011). Pb accumulation in the body organs affects the central nervous system (CNS).

Bioaccumulation of lead and hence exposure to high Pb concentrations in the body can result in long-term damage to the CNS, brain, and kidneys, as well as mortality (Pandey & Madhuri, 2014). As a result of this damage, people suffer from behavioural issues (such as hyperactivity), memory and attention issues, hearing issues, migraines, decreased growth, infertility in both sexes, digestive issues, and muscle and joint. Acute exposure to Pb causes hearing loss, anaemia, irritability, headache, exhaustion and kidney damage according to (Charkiewicz & Backstrand, 2020).

Lead is absorbed more readily through the gastrointestinal tract in children (30–50%) than in adults (5–10%). Lead is absorbed through the skin, blood, and soft tissue. The red blood cells bind almost all of the lead in the blood (98-99 percent), leaving only 1-2 percent in the plasma (Carocci *et al.,* 2016). Absorption and retention of lead via the gastrointestinal tract, the primary route of lead intake, vary significantly with age, gastrointestinal lumen chemistry, and iron storage (nutritional status of the subject). Protein, fat, and lactose may increase lead solubility and thus absorption (Alissa & Ferns, 2011).

Lead's total body content has no absorption feedback. Lead absorbed is excreted in urine

(Martens *et al.*, 2018). Lead, a calcium-like element, mimics calcium's mobility in the body and is generally affected by calcium metabolism regulators. Increased bone turnover during pregnancy or lactation or osteoporosis releases lead from bone. If calcium competes for transport and binding sites, lead is remobilized from bone. This allows bone led to re-equilibrate, little is known about cadmium toxicity (Patrick, 2003). It triples in metallothionein, a cystein-rich protein.

Long-term exposure to Pb caused decreased reaction time, headache, lethargy, dizziness, poorer cognitive and vasomotor performance, and slower nerve conduction (Lille *et al.* (1988). Adults who have been exposed for a long time may develop nervous system dysfunction, weakness in their fingers, wrists, or ankles, elevated blood pressure, and anemia (Wuana & Okieimen, 2011). The EPA reports lead is a potential human carcinogen. This metal can harm every organ and system.

2.2.3 Zinc

This is a chemical element in Periodic Table Group II B with atomic number 30, atomic mass 65.4, density 7.15 g/cm^3, melting point 520 ^oC and a boiling point of 970 ^oC (Wieser & Berglund, 2009). The most common ores of zinc are ZnS, ZnO and $ZnCO_3$. Zinc is the fourth most used metal after Fe, Al and Cu (Anees *et al.*, 2011).

A naturally occurring metal, zinc is present in foods, soil, water, and the atmosphere (Nazir *et al.*, 2015). Zinc is a trace element that is biologically necessary in small amounts for nutritional value (Hilmy *et al.*, 1987). Zinc is a common component of industrial effluents and can be found in a variety of alloys, such as brass and bronze, batteries, and pigments (Hellawell, 2012). In agriculture, zinc is used to create fertilizers, insecticides, composted materials, and liquid manure (Bhagure & Mirgane, 2010). By limiting the activity of bacteria and earthworms, zinc in water can make it more acidic, preventing the degradation of organic compounds (Wuana & Okieimen, 2011).

Excess Zn in plants can induce system malfunctions and development retardation (Duruibe *et al.*, 2007). Studies have indicated that low soil pH increases zinc uptake by terrestrial plants, while excessive organic matter reduces zinc uptake. Zinc poisoning in plants involves metabolic problems distinct from zinc shortage. Most species' essential leaf tissue zinc concentration for growth is 200–300 mg/kg (WHO, 2008). Zinc poisoning is rare but can occur at amounts up to 40 mg/l, causing agitation, muscle rigidity, and pain (Al-Weher, 2008). Fever, nausea, vomiting, stomach cramps, and diarrhea occurred 3–12 hours after consumption of Zn (Elinder *et at.*, 1987).

Elevated zinc has also been shown to reduce leukocyte quantity and function. Some researchers have revealed declines in HDL levels in persons exposed to high doses of zinc, however not all investigations have verified this (Hughes & Samman, 2006). Although the mechanism is uncertain, prolonged zinc consumption may decrease iron reserves. Zinc or zinc compounds used topically often have no negative effects. Since zinc is a necessary component of the human diet, its absence may have negative effects on people's health (Hughes & Samman, 2006).

Zinc deficiency causes neurosensory alterations, oligospermia, reduced cognitive skills, growth retardation, delayed wound healing, immunological problems, and dermatitis in humans. Zinc supplementation generally reverses these problems. Humans with a zinc-to-copper imbalance develop copper insufficiency, with increased copper needs, excretion, and status. Zinc pharmacological consumption has been linked to leukopenia, hypochromic microcytic anemia, and low blood (HDL) concentrations. Cessation of zinc and copper therapy reversed these problems. The World Health Organization (WHO) recommended a zinc concentration in drinking water recommendation of 3mg/L (Ali *et al*., 2019).

2.3 Case studies of heavy metals pollution in soil, food and water studies in Kenya

Moywaywa (2018) conducted a study on the presence of heavy metals in sediments, water and plants of the Thika River. Several metals have been discovered, including (gl-1) Mn (53.5–605), Cu (10–303), Zn (22–325), Ni (15–77), and Pb (10–84). Cu (65-129), Zn (153-434), Ni (35-235), Mn (3719-21200) and Pb (35-235). The concentrations of heavy metals varied greatly between the three media. In kale, soil, and water samples from irrigated farms along the Moiben River in Uasin-Gishu County, Kenya, Akenga *et al*. (2020) examined the amounts of heavy metals. In soil and water, Fe was highest at 85.37–250.22 mg/kg, while Zn was highest in kale at 0.007–0.0154 mg/kg. Metal ion concentrations were higher in soil and irrigation water than in kale, and the order of metal concentrations was Zn > Fe > Mn > Cu > Cr > Pb > Cd. Metal ion concentrations in soil and water were in the order Fe > Mn > Zn > Cu > Cr > Pb > Cd. Soil samples had higher metal ion concentrations (mg/kg) than water and kale. However, Cd, Fe, Zn and Pb were found to be statistically significant in water samples. Kale has been shown to have metal ion levels below WHO recommendations. The ions Fe, Pb and Mn in water samples exceeded WHO recommendations.

Heavy metal concentrations have been determined in five Rift Valley lakes in Kenya: Nakuru, Elementaita, Naivasha, Bogoria, and Baringo (Ochieng *et al.*, 2007). Anthropogenic contamination is indicated by greater quantities of heavy metals at specific places, including Lake Nakuru. Co, Ni, and Cu concentrations in remote Lake Baringo silt, as well as Pb and Mn concentrations in remote Lake Bogoria sediment may have been altered by geochemical processes. Temperature, pH, salinity, and electrical conductivity are among the information.

In Bungoma Central Sub County, Kenya, Wekesa (2015) analyzed heavy metals in water from the River Kuywa and neighboring wells. Pb (0.57, 0.09, 3.36, 1.15), Mn (0.15, 0.14, 0.25, 0.03), Cu (1.01, 0.12, 1.92, 0.14), and Cd are the results for two seasons (0.32, 0.02, 0.99, 0.67) Manganese levels were found to be below KEBS and WHO recommendations, however Pb, Cu, and Cd levels were found to be above those recommendations. The culprits were deemed to be local industrial, agricultural, and domestic sources of pollution. The findings reveal excessive amounts of heavy metals in sub-county water, putting human health at risk. Heavy metal levels in water varied between seasons ($p = 0.05$) (dry and wet).

Heavy metals were found in unprocessed honey, soil, and floral samples from Kenya's Baringo and Keiyo counties, according to Maiyo *et al.* (2014). Raw honey samples had Pb, Zn, Fe, Cu, Cd, and Cr. In comparison to raw honey and floral samples, earth samples included higher levels of heavy metals. The WHO, FAO, and KEBS tolerance levels for heavy metal concentrations in raw honey were all met. Raw honey contains excessive amounts of Cd, Pb, and Cr (Keiyo).

Kemboi *et al.* (2018) investigated heavy metal levels in grains grown in Kenya's Fluorspar Mining Belt, Elgeyo Marakwet County. The levels of arsenic, cadmium, and lead in millet varied greatly between the upper and lower parts of Kimwarer, as well as in between and lower Kimwarer ($P = 0.05$). The CODEX Alimentarius International Food Standards (CODEX Alimentarius) were surpassed in all zones. Consumption of selected heavy metals at levels over the prescribed limits poses health concerns to residents of the Fluorspar mining area.

2.4 Interaction of Heavy Metals with Insulin Contributing to Diabetes

Diabetes is a chronic disease that is more common (particularly type 2) in developed countries. Chronic hyperglycemia with abnormal carbohydrate, lipid, and protein metabolism due to insulin secretion. Type 1 (T1DM) affects 10% of the population, while type 2 (T2DM) affects 85% (Ergun-Longmire *et al.*, 2021). In 2008, there were 1.3 million deaths, with over 2 million anticipated by 2030. Diabetes affects low- and middle-income countries disproportionately. Diabetes is increasing morbidity, mortality, and health care expenses (Mayosi *et al.,* 2009). The WHO estimate Kenya's diabetes prevalence at 3.3%, with a 4.5 percent growth predicted by 2025 (Okube & Gichuki, 2018). Prevalence has increased dramatically over the last decade as a result of variables such as average age, hereditary influence, bad food, and sedentary lifestyle.

Risk factors for type 2 diabetes mellitus (T2DM) include obesity, a high caloric intake, a lack of exercise, and high blood pressure. Oxidative stress caused by heavy metals eventually damages islet cell succession (Hudec *et al*., 2020). As pancreatic cells exhibit a high number of metal transporters and a weak antioxidant system, numerous studies suggest that reactive oxygen species play a significant role in the death of pancreatic cells (Valko *et al*., 2006). Due to the weaker immune system, cells are more vulnerable to the impacts of heavy metals, which can lead to pancreatic cell dysfunction, damage, or even death. An autoimmune effect of humoral, cellular, and damaging immune regulation may appear as a result of the death of cell islets (John *et al*., 2010).

Reduced insulin synthesis or insulin resistance can lead to type 2 diabetes. The pathophysiology and development of diabetes mellitus, especially gestational diabetes mellitus, can be influenced by environmental pollution (GDM). Pregnancy can lead to the disorder known as GDM. Certain dangerous metals have been linked to T2DM patients' impaired glucose regulation and absorption (Franzago *et al*., 2019). Numerous studies have been done to find out how much more heavy metals are found in diabetic patients than in non-diabetic controls. The results show that different concentrations of specific heavy metals contribute to the development of diabetes mellitus. As a result of unregulated pollution and industrialization, exposure to hazardous metals such as Pb, Ni, Cd, and Hg contributes to glucose absorption disturbance and other issues (Peng *et al.*, 2015). Human exposure occurs via a variety of mechanisms however, there is currently no evidence linking heavy metals with type 2 diabetes, with the exception of arsenic (Reichard & Puga, 2010).

2.5 Heavy Metal with Hypertension

Every year, 7.5 million people die from high blood pressure, a known risk factor for cardiovascular disease. In Bangladesh, hypertension is common in adults, but it is unknown how common it is in children. 3.5% of children in the US have hypertension, with the prevalence being higher among children who are overweight or obese (Arjunan, 2013).

Obesity in childhood, sleep apnea, chronic kidney disease, and early birth have all been associated to hypertension in adulthood (Abuyassin *et al*., 2015). Environmental pollutants may change the blood pressure of youngsters, according to new research (Trasande *et al*., 2013). Although the effects of metal exposure on childhood blood pressure are unknown, previous research has connected dangerous metals and essential elements (such as zinc and iron) to increased blood pressure or hypertension in adulthood. Given the strong link between hypertension in childhood and hypertension later in life, it's critical to look into this in youngsters (Shih *et al*., 2021).

New methods for evaluating the health impacts of environmental mixes have recently rekindled attention (Geiger & Cooper, 2010). Due to synergistic effects, alterations in toxicodynamics and toxicokinetics, many metals on a characteristic may increase toxicity. All previous studies focused on single metal exposures. No studies on metal combinations and blood pressure in adults or children aged 4–6 can be found. Cystein-metallothionein complex causes hepatoxicity, then nephrotoxicity.

2.6 Coordination of Amino Acids through Functional Groups and Heavy Metals

Because transition-metal ions have open valence-shell orbitals that can accept pairs of electrons from a ligand, they can form coordination complexes (Shih *et al*., 2021). At least one pair of non-bounding electrons that can be transferred to a metal ion must be present in the ligands. A chemical complex where a predetermined number of atoms, molecules, or ions are attached to the central ion or atom (or the coordination center).

The structure of insulin is depicted in Figure 2.1, which has 51 amino acids, including histidine. Among all the amino acids, histidine is one of the most active. Heavy metals can impair the activity of insulin-producing -cells, which is important in the development of T2DM.

Insulin structure

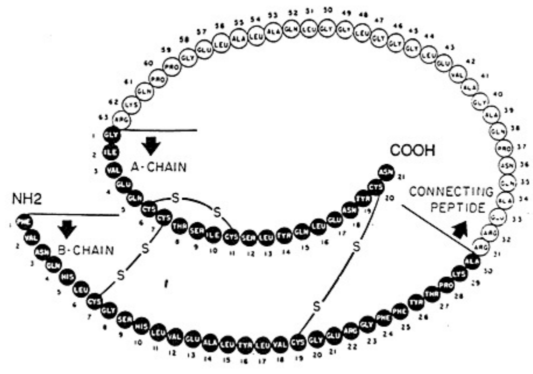

Figure 2.1: Insulin structure showing 51 amino acids (Weiss et al., 2014).

As shown in figure 2.2, Histidine is an essential amino acid required in the production of proteins. It qualifies as a positively charged amino acid at physiological pH since it has a -amino group, a carboxylic acid group, and an imidazole side chain (Fig 2.2 a). Through one or both of the depronated N atoms, histidine can coordinate a wide range of divalent transition metal ions, including Zn, Cd, and Pb. Only when a proton is removed from at least one nitrogen atom does metal (M^{2+}) binding take place (Fig 2.2 b).

Heavy metals interfere with natural protein biological activity in a variety of ways, including binding to functional groups in proteins, displacing metal ions in proteins, and catalyzing amino acid side chain oxidation. The number of coordination’s and geometries of metal binding sites vary. Many metal sites in proteins are surrounded by a shell of hydrophilic ligands and a shell of carbon-containing groups (Ihara *et al*., 2014).

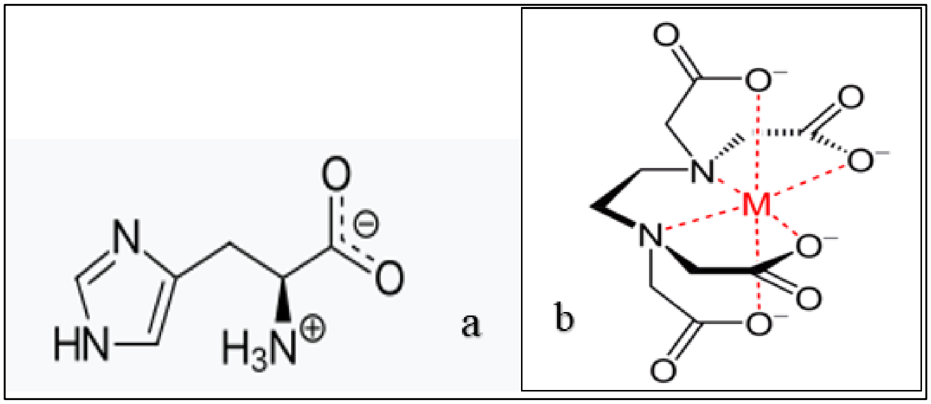

Figure 2.2: Histidine structure in its natural state is depicted in 'a' while fig b, shows histidine bound to a metal ion ***(Ihara et al., 2014).***

The O-H stretch is more intense than N-H stretch because it is more polar. The polarity of N-H bond is weaker than O-H bond. The stretching frequency increases with increase in the number of *pi*-bond which present in C-O bond. Introduction of metal ion (M^{2+}), lone pair of electrons are transferred from functional groups through N-H, O-H, O-C, hence, affecting stretching and vibration frequency (Boukaoud, Chiba, & Sebbar, 2021). If M^{2+} ….N-H, for example is strengthened, the N-H is weakened, stretching and absorption frequency drop (or wavelength increase).

The UV-vis spectrometry works by extracting the metals d-electron from the ground state to an excited state using light (Passos & Saraiva, 2019). It measures the abundance of transition metal atoms with excited electrons at specific wavelength of light.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

3.1.1 Location and Size

The study area covered the Marakwet East and Marakwet West Sub Counties approximately latitude 1° 6'27.25"N and longitude 35°34'1.81"E which are found in the Rift valley region. Marakwet East covers an area of approximately 784 km^2 while Marakwet west is 804.6 km^2.

3.1.2 Climate

The rainfall is approximately 30 % reliable, ranging from 400 to 1000 mm annually with a significant degree of unpredictability, with an average of 600 mm per year (Wasonga *et al.*, 2011). In typical conditions, rainfall is bimodal, with long rains beginning in May and ending in August, and short rains beginning in late September and early November, with intermittent dry seasons during the months (Chebet, Odenyo & Kipkorir, 2017). The annual average temperature ranged between 14 and 24 ^oC. Between December and March is the hottest time of the year, with lengthy droughts.

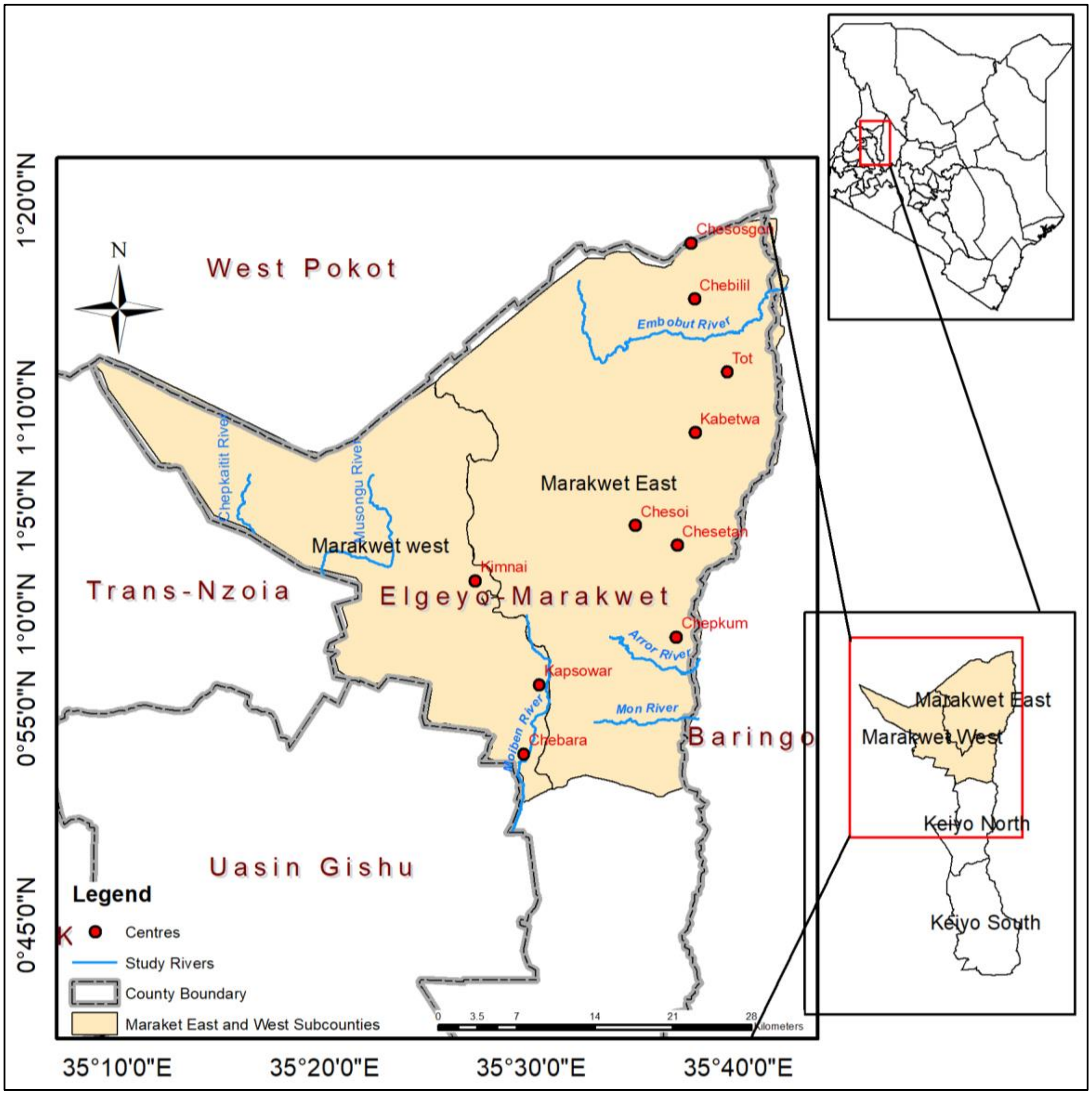

Figure 3.1: Map of Elgeyo Marakwet County showing the sampling points

3.1.3 Geology and soils

The soils in the county's upper section are very productive. However, some of the hills and valley floor soils are unsuitable for agriculture because the soils lack humus and most plant nutrients.

Highland soil erosion in Marakwet East occurs due to steep slopes and low ground cover, especially during the rainy season. The Kerio River runs through the county and the topography rises from the alluvial plain towards the west. The Elgeyo escarpment is notable for its 1500 m relief differential. The county's geography is mountainous in the north and south, giving way to more gentle relief changes in the west (Morongâ *et al*., 2018).

3.1.4 Flora and fauna

Acacia nubica, Acacia reficiens, and *Vachelliatortilis* were dominant on the valley floor while *Boscia angustifolia, Boscia coriacea,* and *Boscia mossambicensis* dominated the escarpment (*Diospyros abyssinica* and *Euclea divinorum*). Trees ferns, scrubby, grassland, *Acacia abyssinica*, *Cyathea manniana*, rocky and water bodies (Moore *et al*., 2020). Elephants are the only large mammals in Rimoi and Kamnarok, and they come and go as they choose, depending on water and food availability. Monkeys, civets, genets, and pangolins are among the smaller mammals. Crocodiles, lizards, tortoises, and snakes are the reptiles present.

3.1.5 Socio economic activities

The county’s total population is 454,480 in 2019 according to National Population and Housing Census (2019). Grazing, pastoralism, agricultural and dairy farming are the principal human activities in the study area. Its principal crops are maize, beans, cabbage, kales, and mangoes (*Mangifera indica*).

3.2 Data collection

A questionnaire was formulated containing the following details; the name and location history of disease and the main source of water of the diagnosed hypertensive and diabetic patients. This aided in ascertaining the interaction of different concentrations of heavy metals with insulin contributing to diabetes and hypertension.

3.3 Research Design

The researcher utilized an experimental design in which laboratory tests were carried out to establish occurrence of Zn, Cd and Pb in water and soil. Further, experiments were done to find out whether there was interaction of Zinc, Cadmium and Lead with insulin contributing to diabetes and hypertension. This design was appropriate because it involved performing lab tests to ascertain the quantity of heavy metals.

3.4 Materials

The study's instruments and equipment were calibrated before and during the tests. Heavy metals were removed from all glassware using 10 % concentrated nitric acid (HNO_3). Detergent-washed and deionized-water rinsed apparatus including volumetric flasks, measuring cylinders and digestion flasks. A solution of one percent (w/v) potassium dichromate in 98 % (v/v) H_2SO_4 was used to soak the digestion tubes for 24 hours, followed by rinsing with deionized water and drying in an oven until the analysis began. Each device was immersed and cleaned in deionized water before use.

3.4.1 Equipment and Apparatus

Round-bottomed flasks, borosilicate volumetric flasks, measuring cylinders (Duran, Germany), pipettes (Pyrex, USA), and micropipettes were among the tools and apparatus utilized (Dragon med 1-10 ml, 100-1000 ml, Shanghai, China). Atomic Absorption Spectrophotometer (AAS) (Shimadzu AAS model 6800, USA), Mann No 1 filter paper and other glassware used. Meat analysis was carried out using Induced Coupled Plasma-Mass Spectrometry (ICP-MS).

Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Perkin Elmer, NexION 300, and American) was used to analyze target elements. The ICP-MS system was calibrated using external standards and an internal standard of Rh, Re. One percent HNO_3 was present in the reagent blank solution. In reagent blank solutions, mixed standard solutions for Zn, Pb, and Cd were created. Air entrainment, solvent, and background plasma gas interferences were all removed. It is possible to avoid, correct, or reduce isobaric spectrum interferences caused by polyatomic ion species involving the sample matrix elements by choosing the right isotope.

External standards were used to calibrate AAS 254. The reagent blank solution had 1% concentrated HNO_3 and 0.01% $K_2Cr_2O_7$. The Mercury Analyzer is designed to measure mercury directly in solid samples without pre-treatment.

UV/VIS was used for vibrational and rotational absorption because their values fall within range. Their spectra were recorded on a UV-1700 Pharma spectrophotometer (Shimadzu, Japan). Standard solutions were diluted with a buffer pH 10 to c = 4×10^{-3} mol dm^{-3} and recorded from 200 nm to 1000 nm using standard 1.00 cm quartz cells. During sample preparation, the UV-Vis spectrophotometer was warmed up and set up, with the wavelength range set to 200 nm to 600 nm and the auto-tracking component activated. Before placing the sample in the sample chamber, the equipment was zeroed to the imputed parameters. A cuvette of methanol was placed in the inner sample chamber to serve as a blank, while a cuvette of the sample solution was placed in the outer sample chamber. In seconds, the graph was plotted and the peak of the result auto-tracked showing points of histidine absorption at specific wavelengths of absorption, and then the displayed result on the screen was printed.

3.4.2 Reagents and Chemicals

To prepare sample and intermediate metal standard solutions for analysis, analytic grade HNO_3, $HClO_4$, Pb $(NO_3)_2$, and Cd $(NO_3)_2$ were used.

To prepare working solutions for UV-Vis, 0.47 g of Cd $(NO_3)_2$ weighed accurately and dissolved in 100 ml of distilled water. Zinc nitrate (0.34g) was as well dissolved 100 ml of distilled water. The same was done for 0.62 g of lead nitrate. Methanol was used as the blank solution. Each of the metal ions (solutions prepared) were divided into separate portions measuring 10 ml and transferred into the cuvets in the machine. Then the histidine wavelength was also measured the the metal ions were reacted with histidine and the wavelengths recorded.

3.5 Collection and Preparation of Water Samples

The sampling sites consisting of rivers Moiben, Arror, Mosongu, Mon, Chepkaitit, and Embobut were purposefully selected based on the research objectives. The six rivers were opted for because they are permanent and therefore capable of serving the residents all year round. A geospatial location of the six rivers was carried out to enable collection of water samples from the source or points of entry, middle and end or exit for the rivers within the two sub counties.

Water samples were taken from the three sites of the three rivers (source, middle and mouth) during rainy (March and April) and dry seasons of the year (December). A total of 36 samples of water was therefore collected during the period of study (that is 6 rivers x 3 samples x 2 seasons = 36). The 6 river sites were selected to represent sites that were more impacted by Zn, Pb and Cd metals. The samples were taken to the University of Eldoret and filtered by whattman No.40 filter papers. Alkali–silica reaction (ASR) was measured in water samples. Water samples were reacted with sodium hexametaphosphate to check for metal precipitation after basic analysis. An Atomic Absorption Spectrophotometer (AAS) was used to establish the presence of heavy metals (Thermoelectric S-Series).

3.6 Collection and Preparation of Soil Samples

Six composites consisting of three subsets of soils (triplicates) were collected from the Marakwet East and Marakwet West during the rainy and dry seasons after every 4 km from 0-15 cm and 15 – 30 cm making a total of 36 samples. The soils were sampled from the same areas as the water. Soil samples were collected from three grid locations and mixed properly in a plastic bucket. A 2 mm stainless steel sieve was used to determine the purity of the air dried samples. Soil samples were extracted and analyzed with an atomic absorption spectrophotometer (Model Thermoelectric S-Series) for heavy metals.

Lastly, 70 diagnosed diabetic and 70 hypertensive patients were sought in this study period. The sampling intended to capture the interaction of insulin and heavy metals in the sampling sites and thus enabled capturing the spatial contributor of heavy metals to diabetes and hypertension (Appendix I).

Meat (beef), samples were collected from the butcheries in the study area. Assurance was sought to ensure that the meat samples collected were from the cattle in the area.

3.7 Preparation of the Sample

3.7.1 Digestion Mixture Preparation Procedure

The following ingredients were added to 175 mL of 30 percent Hydrogen Peroxide: 0.21 g of selenium powder, 70 g of lithium sulphate, and thoroughly mixed. While the solution was chilling in an ice bath; 210 mL of concentrated sulphuric acid was also added. Thereafter, it was placed in a freezer at 4 °C to ensure long-term stability.

3.7.2 Procedure for digestion

A total of 0.3 g of dried finely ground sample (soil and meat) were weighed and placed in a clean, dry digestion tube that had been labeled. Both samples were obtained from study area. Each tube had 4.4 mL digestion mixture and reagent blanks for each batch of samples. The tubes were then heated for 2 hours at 350 °C in a block digester. This was the point at which the digest become colorless. The tubes were then removed from the digester and allowed to cool. A 25 mL distilled water was added, mixed and volume increased to 50 mL and lastly the solution was transferred to a 50 mL volumetric flask to settle.

3.7.3 Preparation of Working Standard

Each heavy metal's working standard solution was created, and an MS Excel calibration graph of absorbance against concentration was plotted. Graphs equation was used to calculate the concentration of the sample. For each element in the linear range, a set of standard solutions were run, followed by triplicate analyte sample solutions, and the mean absorbance value was recorded. The working standard was made in the same manner using sodium fluoride standard solutions in the linear range of 0.0 to 20.0 mg/l (Adriano & Doner, 1983).

3.8 Procedures for Absorbance

Four procedures of determining absorbance of zinc, lead, cadmium ions and histidine are described in the following sub sections.

3.8.1 Hydrochloride Histidine Monohydrate (HHM) stock solution (Histidine assay)

A 55 mL/g of HHM was dissolved in 60 ml of 1 M NaOH in a 250 ml volumetric flask and adjusted to the mark with 1 M NaOH.

3.8.1.1 Working Solution

A 4.4 mg of HHM was rinsed twice with 5 mL of 1 M NaOH and the volume made to 100 mL with 1 M NaOH. A 25 mL of the solution was then diluted to 100 mL with 1 M NaOH solution.

3.8.1.2 Colouring Agent for histidine

This was prepared by diazotization reaction with sulphuric acid. A diazonium salt of sulphuric acid was formed by sodium nitrite reacting with an amino group in HHM. In 6–7 minutes, the colouring agent reacted with the carboxylic group on HHM to form a yellow complex. A 200 mg sodium nitrite in 60 mL distilled water after 1 hour, 500 mg HCl was added and final volume topped up to 500 mL with purified water, and the resultant reagent was then ready for use.

3.8.2 Procedure for Zinc Ions

A 500 mL of zinc Sulphate containing 3.3 µg /L of zinc ions was taken and added to 50 mL (0.1% w/v of dithizone) prepared for dissolving 10 mL of dithizone in 100 mL chloroform. Same volume of a buffer solution of alkaline ammonium citrate (prepared by dissolving 5 g of dibasic citrate in 100 ml of 5 % ammonium solution and the mixture shaken. The organic layer was transferred to a 250 mL volumetric flask keeping over anhydrous $CaSO_4$ followed by the filtering of the chloroform layer to obtain dark cherry red colour.

3.8.3 Procedure for Lead Ions

Neutral aqueous solutions containing 0.6-600 µg of lead ions in 10 mL was weighed and mixed with 75-130-fold molar excess of dithizone solution followed by 3-6 ml of 0.3M Cetyltrimethyl ammonium bromide (CTAB).

3.8.4 Procedure for Cadmium Ions

Cadmium ions was reacted with Alizarin red S in acidic media (0.005-0.05 M H_2SO_4) to give a deep greenish yellow chelate. The ratio of cadmium to alizarin was 1:2.

3.9 Sample Analysis

Samples of water were collected using trace metal clean methods (APHA, 1998). To get rid of trace metals, pre-clean with GFS Chemicals Inc.'s high-purity nitric acid before washing with Milli-Q water. The rinsed bottles were double-bagged in polyethylene. The cleaning and storing methods ensured that the sample equipment was free of metal contamination (Shelton, 1994). A pH of 2 was achieved by adding ultra-pure HNO_3 to the samples collected in polypropylene bottles and storing them at 4 °C prior to heavy metal analysis. According to APHA (1998), dissolved oxygen, pH, and electrical conductivity were determined in the field.

Atomic absorption spectrophotometer AAS measured metal concentrations. An internal standard comprising 40 g/l ^{209}Bi, ^{75}Ge, ^{115}In, and ^{6}Li was applied to 15 ml of sample. The sample solutions were stabilized with 40 g/l ^{196}Au. A linear range of 1 ppb to 100 ppb was used to generate standards for all analytes. All heavy metals were tested using NIST 1640 reference material and procedure blanks. Dialyze insulin (4 g/l) in 0.0 1 M HCl for 36 hours with 1.0 M HCl to remove Zn^{2+}, then added 0.01 M NaOH to reach alkaline pH. Each measurement used a stock solution diluted with 0.1 M NaCl and pH adjusted. In a two-cell set up, one cell contained insulin solution with a perturbant (metal chloride), the other insulin solution alone. To ensure the identical insulin concentration in both cuvettes, the metal chloride solution was added first, then the insulin solution.

Spiking was done by adding a known concentration of the analysis parameter to the sample before digestion was carried out and then evaluating the spiked solution using the appropriate cuvette test (E1). At the same time a non-spiked sample was measured (E2). The spiking amount was then calculated: (A = E1 – E2/2). If the spiking amount was within the given confidence interval, the sample contained no interference substances and was analyzed without diluting it any further.

Extractable Zn, Cd, and Pb (0.005 mol of DTPA pH: 7.2 1:10 w/v ratio) and total (digested by 4 M HNO_3 for 12 hours). Sequential extraction method is most extensively used for meat Tessier *et al. (*1979).

3.10 Statistical Analysis

Differences between mean concentrations of different samples were calculated using ANOVA. Correlation analysis and the data were represented using graphs, figures and distribution tables. The data were checked for normality and homogeneity of variance using MINITAB®Statistical Software for Windows ver.14 and the Kolmogorov-Smirnov Normality Test (P = 0.05). Analysis of variance (ANOVA) was used to examine the relationship between heavy metal concentrations in water and samples from selected study locations that had been diagnosed with diabetes and hypertension. The interaction of heavy metals with insulin in beef and pharmaceutical histidine was studied using ultraviolet difference spectroscopy.

CHAPTER FOUR

RESULTS

4.1 Statistical and comparative study of diagnosed diabetic and hypertensive patients in Marakwet East and Marakwet West

4.1.1 Introduction

This chapter contains survey and interview results. The t-test and descriptive statistics were used to analyse the data and explore relationships between variables.

4.1.2 Social- demographic Information of the Respondents

Out of 140 questionnaires administered, 139 (99.29 %) were completed and returned as shown in (Table 4.1). There were sixteen villages whose participants were victims of either diabetes or hypertension. Majority of the respondents were from Kapcherop village (9.4 %) followed by those from Chesingei (8.6 %) and Chebai (7.9 %). Few of the respondents were from Kilang'ata (2.9 %) with no significant difference (χ^2 = 6.07003, d.f.=15, P-Value = 0.9785). Males (61.5 %) made up the majority of responses. The majority (60.9 %) were over 36, with only 39.1 % being under 36 and over 18 years. In regards to the level of education, most of the respondents (79.8 %) had formal education, with 42.4 % having a secondary education. Table 4.1 also shows that farming (69.1 %) was the most common occupation.

Table 4.1: Respondent's socio-demographic profile

Variable	**Respondents**	**Frequency**	**Percentages**
Village of residence	Tot	9	6.5
	Embobut	8	5.8
	Kapcherop	13	9.4
	Mosongu	7	5.0
	Chukor	9	6.5
	Chebai	11	7.9
	Kipkundul	7	5.0
	Kipsero	8	5.8
	Kiplenge	10	7.2
	Chesingei	12	8.6
	Kaptalamwa	9	6.5
	Kapkanyar	10	7.2
	Cheptongei	8	5.8
	Chorwai	7	5.0
	Chebiemit	7	5.0
	Kilang'ata	4	2.9
	Total	**139**	**100.0**
Gender	Male	80	61.5
	Female	50	38.5
	Total	**130**	**100**
Age	below yrs.	13	9.4
	26-30 yrs.	19	13.8
	31-35 yrs.	22	15.9
	36-40 yrs.	28	20.3
	41-45 yrs.	43	31.2
	Above 45 yrs.	13	9.4
	Total	**138**	**100.0**
Education	None	28	20.1
	Primary	33	23.7
	Secondary	59	42.4
	Tertiary	10	7.2
	University	9	6.5
	Total	**139**	**100**
Occupation	Employed (private/government)	20	14.4
	Farmer	96	69.1
	Others	23	16.5
	Total	**139**	**100.0**

4.1.3 Diagnosed Diabetes and Hypertension Background

4.1.3.1 Common Sources of Heavy Metals in the Area

Majority (76.5%) of the respondents pointed out that they had heard about heavy metals such as lead, zinc and cadmium. As shown in Figure 4.1, majority of the study participants indicated that water (56.4%) followed by soils (33.6%) were the common sources of heavy metals in areas where they lived. A somewhat lower but roughly same proportion of respondents pointed out that plants were the most prevalent sources of heavy metals in the locations where respondents lived. There was a significant difference in responses pertaining to common local sources of heavy metals (χ^2 = 31.76, d.f =2 P-Value = 0.0001).

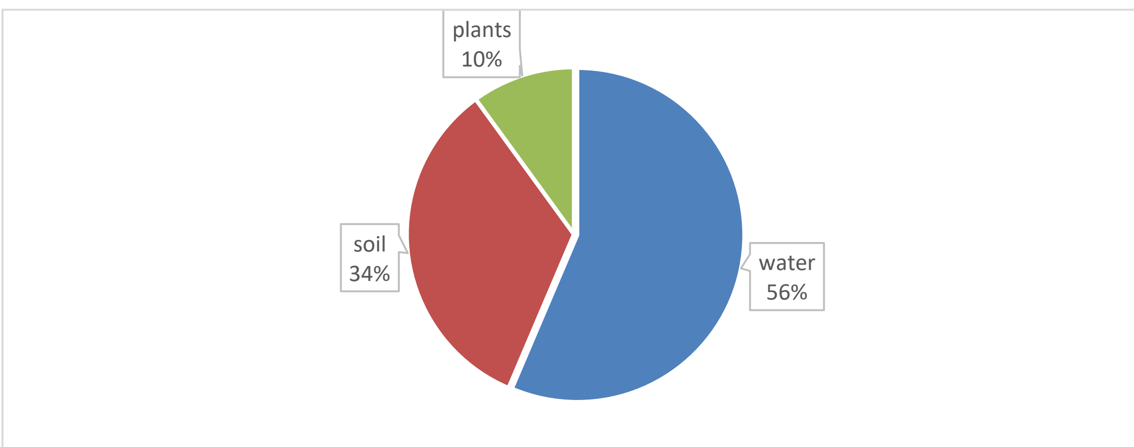

Figure 4.1: Common sources of heavy metals in the area

In cross tabulation with education levels (Figure 4.2) of the respondents, majority (21.0%) of those who indicated that water was the source of heavy metals had tertiary level of education with few (6.1%) with primary level of education thus indicating a significant difference (χ^2 = 24.1786, df = 4   P-Value = 0.0001). The study results found no significant difference in responses pertaining to soil as well as plants as the sources of heavy metals when they were cross tabulated with education level, respectively (Soil: χ^2= 9.8787, df = 4   P-Value = 0.0425; Plants: χ^2 = 2.0, df = 4, P-Value = 0.7358).

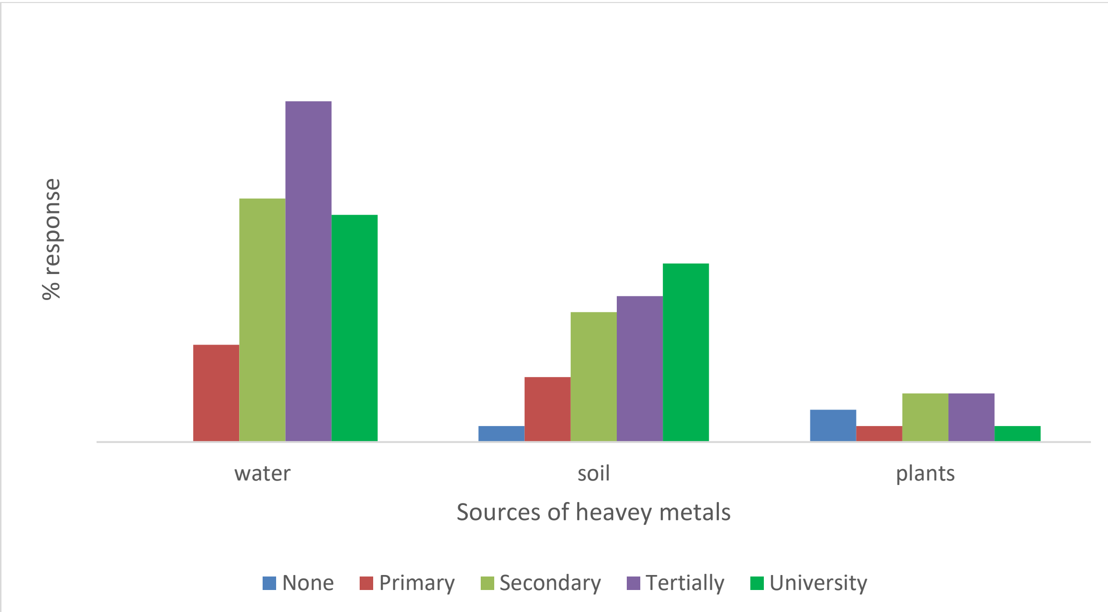

Figure 4.2: Cross tabulation of causes of heavy metals with education level

Respondents were asked to mention the nearest reliable water source that they relied on for domestic purposes. Majority (35.5%) of those interviewed were near river Embobut followed by those who were near the Moiben and Mosongu. A few (11.8%) significant number of respondents were from near Mon River as shown in figure 4.3 below. There was a significant difference in responses pertaining to proximity to reliable river water source for domestic purposes (χ^2 = 15.95, d.f.=4, P-Value = 0.0031).

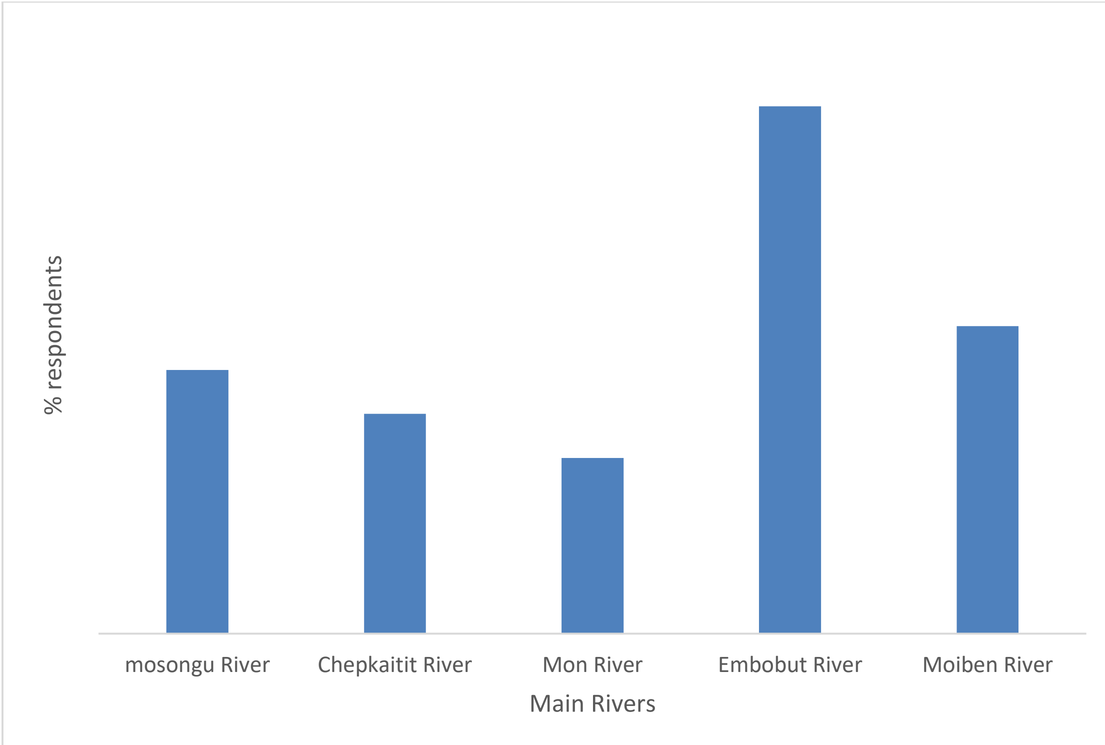

Figure 4.3: Nearest water source for domestic purposes

4.1.3.2 Disease Diagnosed

The study involved those participants who had acquired diabetes as well as hypertension from Kapsowar Mission hospital records. From figure 4.4 majority (71.4%) respondents were suffering from diabetes while the rest were suffering from hypertension with a significant difference (χ^2 = 17.64, d.f. =1, P-Value = 0.0001). Majority of the respondents pointed out that they were diagnosed between the year 2015 and 2018.

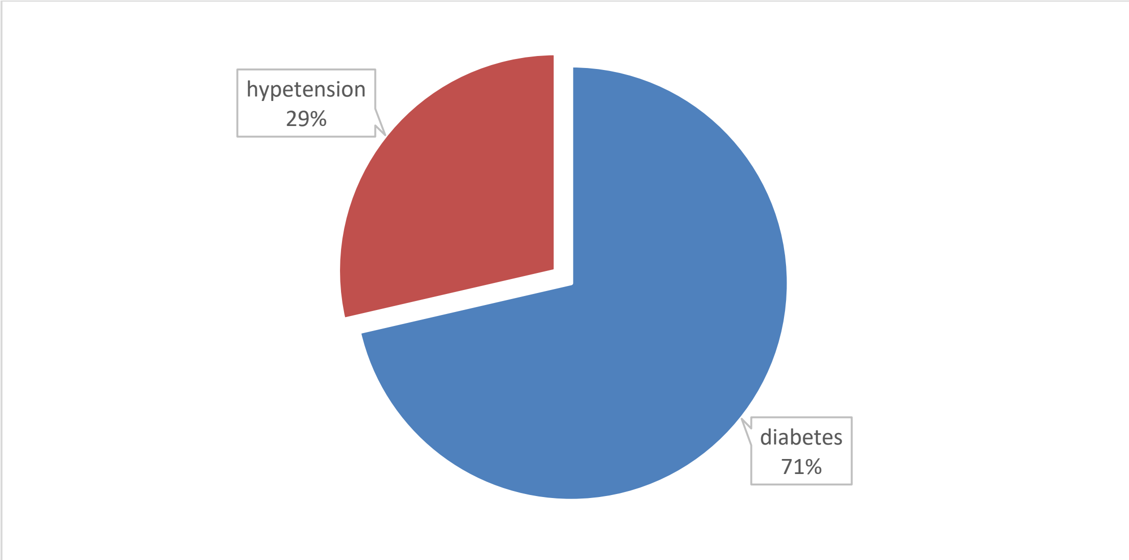

Figure 4.4: Disease diagnosed

Respondents were asked to point out what they thought could have contributed to their contracting the disease condition. For those who were suffering from diabetes, majority of them indicated that consumption of sugary foods (31.3%) followed by failure to do physical exercises (24.2%) contributed to their contracting the disease. Few of those suffering from diabetes indicated that they contracted the disease due to having shock from death of a family member (6.1%), as well as family disagreements as reported in Figure 4.5. The respondents views differed significantly pertaining to what might have contributed to the victims to contract diabetes (χ^2= 32.4545, d.f.=5 P-Value = 0.0001).

For hypertension condition, majority of the respondents pointed out the that presence of other diseases (45.0%) followed by having family disagreements (22.5%) were the main contributing factors to them having the condition. Few of the victims pointed out that they inherited the condition (5.0%) as shown in Figure 4.5. There was a significant difference in percentage frequency on what could have contributed the hypertension condition among the victims (χ^2= 28.7, d.f.=5, P-Value = 0.0000).

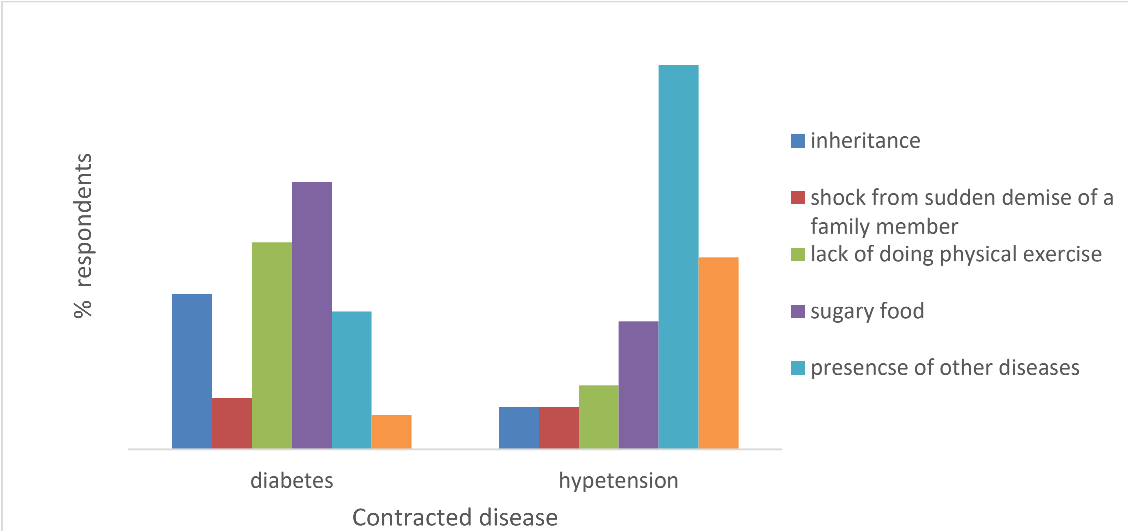

Figure 4.5: Contributing factors to contracting diabetes and hypertension

Cross tabulation was also performed to determine whether the nearest water source had any influence on the type of disease contracted. Contraction of diabetes as well as hypertension disease was dependent on nearest water source. Figure 4.6 shows that majority (36.4%) of respondents suffering from diabetes were near and dependent on Embobut River with a significant difference (χ^2= 40.4545, d.f.=5 P-Value = 0.0001). Few (4.0%) of those who were suffering from diabetes were near and depended on Mon River as a source of water for household consumption. For those who were suffering from hypertension disease, majority (37.5%) were dependent on water from Embobut River with a significant difference (χ^2= 22.1, df = 5 P-Value = 0.0005). Majority of those with diabetes as well as hypertension were near Embobut River with insignificant difference with type of contracted disease (χ^2= 0.0542, df = 1 P-Value = 0.8158).

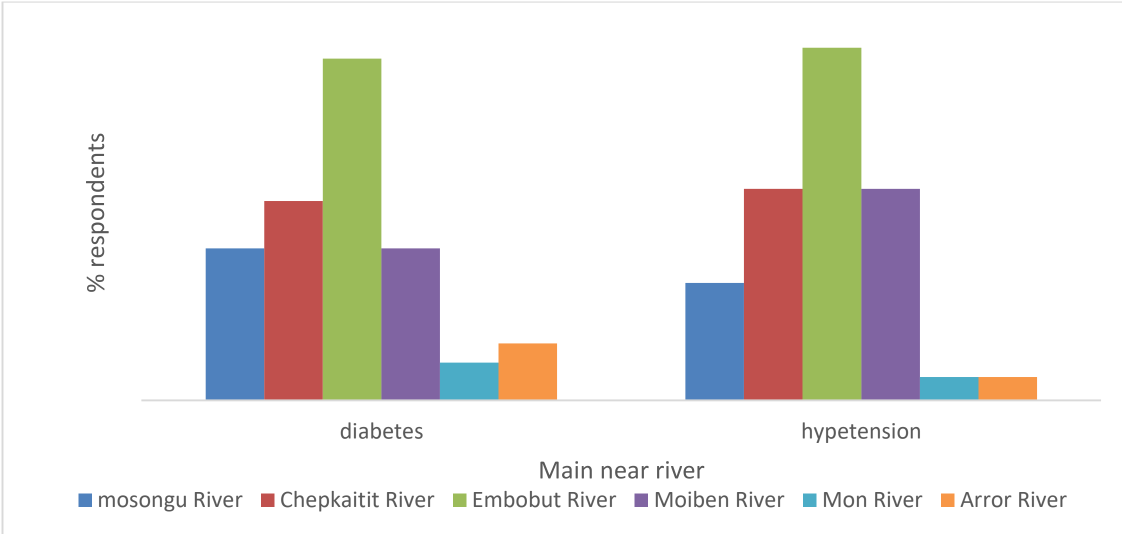

Figure 4.6: Cross tabulation between disease/condition and source of water for domestic use

4.1.4 Heavy metal toxicity screening among diagnosed diabetic and hypertensive patients

Patients with diabetes and hypertension were asked to list symptoms in the questionnaire that were frequently present in those who had ingested high levels of heavy metals such lead, zinc, and cadmium and the responses tabulated below.

Table 4.2: Heavy metal toxicity screening among diagnosed diabetic and hypertensive patients

Symptoms often found in patients who have absorbed excessive amounts of heavy metals	Frequency	% Frequency
Unexplained rashes or skin irritations	105	20.1
Frequent diarrhoea	8	1.5
Get out of breath easily	33	6.3
Get headache just after eating	51	9.8
Burning sensation on the tongue	82	15.8
Frequent urination during the night	37	7.1
Unexplained chronic fatigue	98	18.9
Difficult remembering or use of memory	106	20.4
Total	**520**	**100**

Respondents pointed out on the numerous symptoms frequently observed in people who have ingested high levels of heavy metals as shown in Table 4.2. All respondents (100.0%) pointed out that unexplained rashes or skin irritations and difficult remembering or loss of memory were the most symptoms often found. This was followed by those who pointed out that there was also unexplained chronic fatigue (18.9 %), burning sensation on the tongue (15.8%) as well as having headache just after eating (9.8%).

Few of the respondents pointed out that frequent urination at night (7.1 %), getting out of breath easily (6.3%) and having frequent diarrhoea (1.5) were common symptoms in individuals exposed to high levels of heavy metals such as lead, zinc, and cadmium. This study demonstrated a substantial difference in reactions to symptoms commonly reported in patients exposed to heavy metals as lead, zinc, and cadmium (χ^2= 461.38, d.f.=7, P-

Value = 0.0001).

4.1.5 Scores on Toxicity Levels

All interviewed respondents also added the total score provided to determine the likelihood level of heavy metal toxicity. From Figure 4.7, majority of total scores were between 0-39 (61.3%) with few total score in the range of 86-126 (10.1%). A range of 0-39 total scores indicated low likelihood of heavy metal toxicity. A range of 40-85 total scores indicated a moderate likelihood of heavy metal toxicity while a range of 85-126 total scores indicated strong likelihood of heavy metal toxicity. Chi square goodness of fit test showed that there was a significant difference in total scores (χ^2= 48.1176, d.f.=2, P-Value = 0.0001).

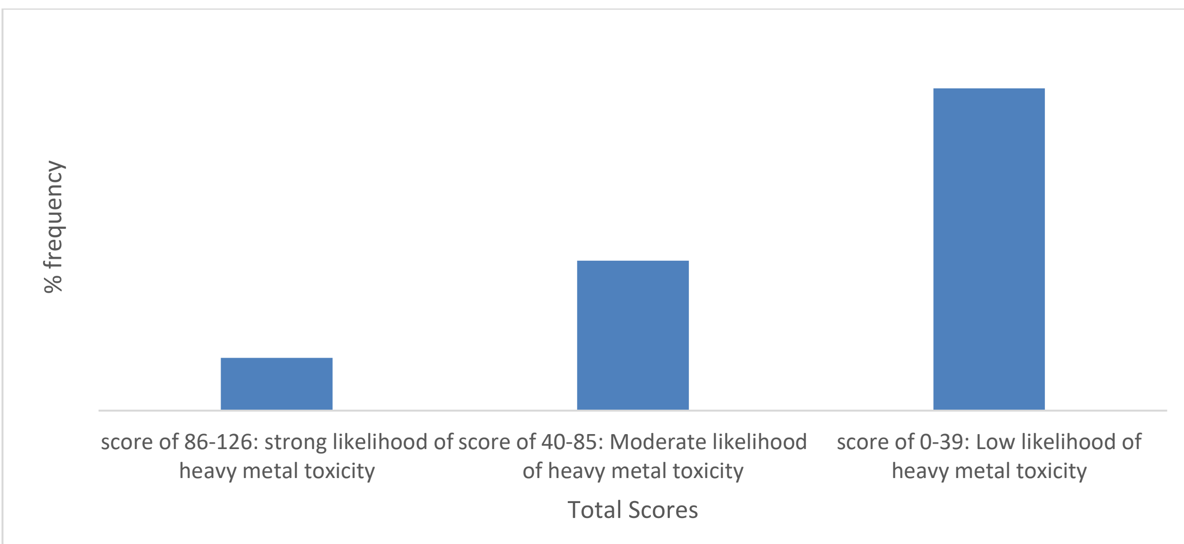

Figure 4.7: Scores on Toxicity Levels

Respondents were also asked what they were significantly exposed to. All the respondents pointed out that they had ever been exposed to fertilizers which explain why most of the study participants were farmers. A significant majority of the respondents also indicated that they were frequently exposed to pesticides (36.4%), herbicides (26.2%), fungicides (16.7%) and batteries (18.7%). Few of the respondents indicated that they were significantly exposed to dyes (1.0%), alloys (4.2%), and wood preservatives (5.0%) as well as paints and thinners (5.0%) as shown in Figure 4.8. responses differed significantly pertaining to what the respondents were significantly exposed to (χ^2= 357.541, d.f = 9, P-Value = 0.0001).

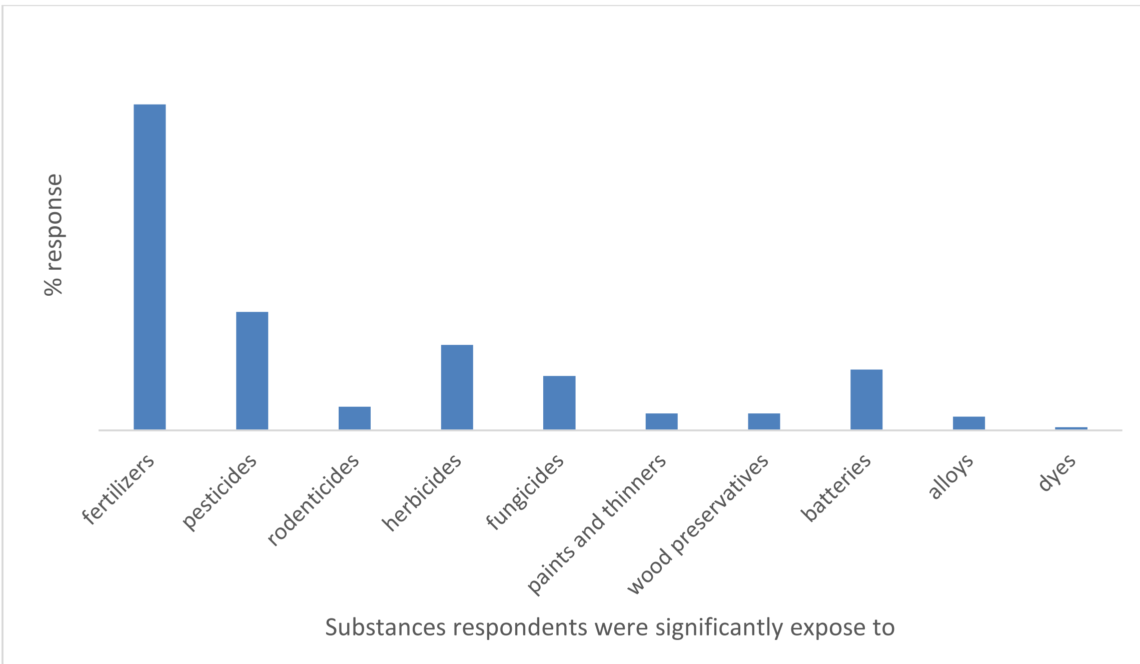

Figure 4.8: Substance's respondents were significantly exposed to

In a question whether the respondents were smokers, only one respondent pointed that he did and that he smoked one packet per week. The other entire respondents, however indicated that they were exposed to second hand smoke.

4.1.6 Relative's health

Few of the respondents responded on the issue pertaining to health of their family members. There was no significant difference in response pertaining to type of condition and who was affected (χ^2= 2.5283, df = 2, P-Value = 0.2825). From Figure 4.9, those who were suffering from cancer and hypertension, majority were mothers and (43.8%) while few (35.7%) were grandmothers and grandfathers to the respondents. There was no significant difference in responses pertaining to how respondents were related to the cancer victims (χ^2= 4.57143 df = 4, P-Value = 0.3342) as well as for hypertension (χ^2= 9.0, df = 4, P-Value = 0.0611). For diabetes victims, majority (52.2%) were fathers of the respondents while a few were grandmother and grandfathers of the respondents with a significant difference (χ^2= 18.087 d.f.=4, P-Value = 0.0012).

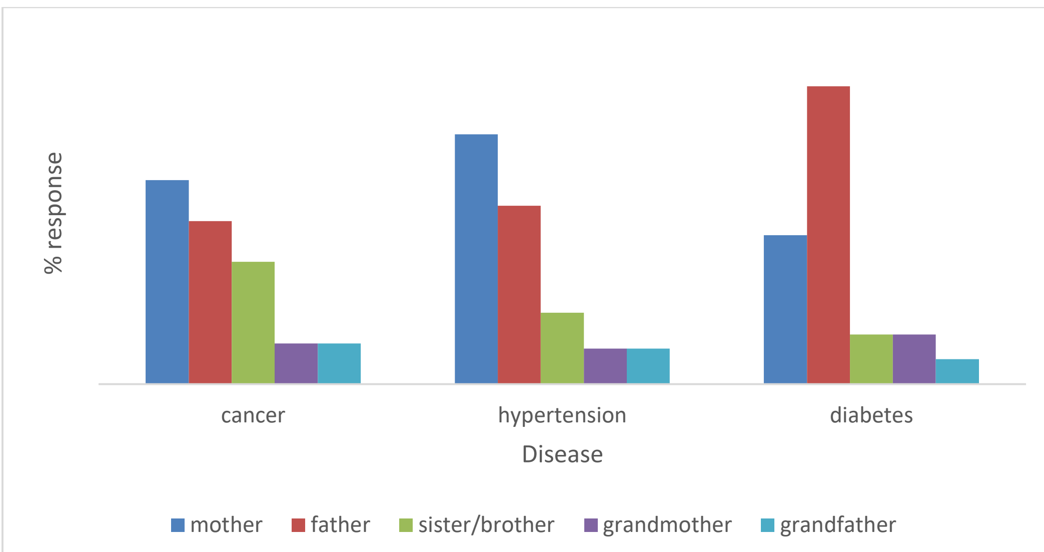

Figure 4.9: Relative's health

4.2 Quantitative analysis of Zn, Pb and Cd in soil and water in Marakwet East and Marakwet West

4.2.1 Concentration of Zn in water and soil samples from selected rivers in Marakwet East during dry and wet seasons

As presented in table 4.3, in water samples, Zn levels generally were higher during wet season than dry season. In addition, Zn levels increased significantly from upstream to downstream in all the three rivers. Furthermore, significant higher Zn levels were recorded in soil samples than in water. During wet season, significant high amount of 0.432 ppm was reported downstream of River Mon, followed by 0.267 and 0.257 ppm mid and upstream respectively. Water samples collected from River Arror showed Zn levels of 0.207 ppm upstream, 0.226 ppm midstream and 0.205 ppm downstream.

Table 4.3: Zinc concentration in water and soil samples selected rivers in Marakwet East during dry and wet seasons

		Water		**Soil**	
	Section	Wet	Dry	Wet	Dry
R. Embobut	Upstream	0.207±0.004a*	0.072±0.001b	0.358±0.005a*	0.583±0.015c
	Midstream	0.225±0.007b*	0.076±0.001c	0.428±0.011b*	0.618±0.008e
	downstream	0.245±0.007c*	0.083±0.002c	0.537±0.008d*	0.659±0.007f
R. Arror	Upstream	0.207±0.003a*	0.080±0.002c	0.566±0.007e*	0.608±0.002d
	Midstream	0.226±0.007b*	0.068±0.001a	0.475±0.006c*	0.710±0.021g
	downstream	0.205±0.019a*	0.082±0.001c	0.705±0.004h*	0.457±0.001b
R. Mon	Upstream	0.257±0.007d*	0.070±0.01b	0.763±0.003i*	0.840±0.025h
	Midstream	0.267±0.002e*	0.078±0.009bc	0.672±0.001g*	0.828±0.019h
	downstream	0.432±0.001f*	0.067±0.001a	0.654±0.003f*	0.436±0.002a

Means followed by varied letters within a column are significantly different at $p<0.05$
** Denote significance between dry and wet season*

Significant increase in Zn levels from 0.207 ppm upstream to 0.225 ppm midstream to 0.245 ppm downstream was reported in samples of water collected from River Embobut. During dry season, water samples from River Embobut recorded Zn levels of 0.072, 0.076 and 0.083 ppm at up, mid and down streams respectively. In River Arror, Zn levels of 0.08, 0.068 and 0.082 ppm were, respectively recorded up, mid and downstream. In river Mon the Zinc levels drastically reduced downstream to 0.436 ppm from an initial 0.84 ppm upstream and 0.828 midstream.

During wet season, soil samples collected from River Embobut correspondingly recorded for Zn levels were 0.358, 0.428 and 0.537 ppm for up, mid and down streams while from River Arror were 0.566, 0.475 and 0.705 ppm up, mid and downstream. On the other hand, samples collected from River Mon showed higher Zn levels of 0.763 ppm upstream than midstream (0.672 ppm) and downstream (0.654 ppm). During dry season, corresponding Zn levels in soil samples from River Embobut were 0.583, 0.618 and 0.659 ppm for up, mid and down streams, while for River Arror, reported respective values of 0.608, 0.710 and 0.457. However, Zn levels in sampled soil from River Mon decreased insignificantly from 0.840 ppm at upstream to 0.828 ppm midstream followed by a further significant decrease to 0.436 ppm downstream.

4.2.2 Concentration of Zn in water and soil samples from selected rivers in Marakwet West during dry and wet seasons

Concentrations of Zn in river water (Chepkaitit, Moiben and Mosongu) and soil within Marakwet west during wet and dry seasons are presented in Table 4.4. Wet season reported significant higher Zn levels than dry season. Similarly, soil samples reported higher Zn levels than water samples. During wet season, water samples collected from River Chepkaitit recorded correspondingly Zn levels of 0.204, 0.227 and 0.299 ppm for up, mid and down streams.

Water samples collected up, mid and down streams of R. Moiben reported respective Zn levels of 0.218, 0.204 and 0.208 ppm.

Table 4.4: Concentration of Zinc in water and soil samples from selected rivers in Marakwet West during dry and wet seasons

		Water		Soil	
	Section	**Wet**	**Dry**	**Wet**	**Dry**
R. Chepkaitit	Upstream	0.204±0.005a*	0.062±0.011cd	0.757±0.003d*	0.474±0.016a
	Midstream	0.227±0.014ab*	0.055±0.002c	0.662±0.003c*	0.682±0.004e
	Downstream	0.299±0.006c*	0.145±0.025	0.844±0.002g*	0.503±0.002a
R. Moiben	Upstream	0.218±0.024a*	0.064±0.004d	0.543±0.242a*	0.504±0.031a
	Midstream	0.204±0.013a*	0.091±0.003f	0.778±0.001f*	0.607±0.017b
	Downstream	0.208±0.011a*	0.098±0.001g	0.882±0.006h*	0.628±0.006c
R. Mosongu	Upstream	0.212±0.001a*	0.043±0.001a	0.440±0.014a*	0.634±0.029c
	Midstream	0.225±0.006b*	0.049±0.001b	0.456±0.011a*	0.651±0.002d
	Downstream	0.345±0.007d*	0.079±0.011e	0.776±0.001e*	0.822±0.000f

*Means followed by different letters within a column are significantly different at $p<0.05$, * denote significance between dry and wet season*

Significant increase in Zn levels from 0.212 ppm upstream to 0.225 ppm midstream and finally to 0.345 ppm downstream was recorded in water samples collected from R. Mosongu. During dry season of the study, Zn levels in water samples from R. chepkaitit correspondingly were 0.062, 0.055 and 0.145 ppm for up, mid and down streams. While those from R. Moiben were 0.064, 0.091 and 0.098 ppm correspondingly for up, mid and down streams. Similarly, Zn levels increased from 0.043 ppm upstream to 0.049 ppm midstream and finally to 0.079 ppm downstream in R. Mosongu.

Respective soil samples collected from R. Chepkaitit during wet season were; 0.757, 0.662 and 0.844 ppm for up, mid and down streams. In R. Moiben, Zn levels generally increased correspondingly from 0.543 ppm to 0.882 ppm up to downstream of the river. Similarly, significant increase in Zn levels from 0.440 ppm to 0.456 ppm to 0.776 ppm was recorded up, mid and down streams of R. Mosongu, respectively. During dry season, R. Chepkaitit recorded corresponding Zn levels of 0.474, 0.682 and 0.503 ppm for up, mid and down streams. Significant increase in Zn levels (0.504, 0.607 and 0.628 ppm) were recorded up, mid and down streams of R. Moiben, respectively. However, respective Zn levels in soil samples collected from up, mid and down streams of R. Mosongu were 0.634, 0.651 and 0.822 ppm.

4.2.3 Concentration of Cd (ppm) In Water and Soil Samples from Selected Rivers in Marakwet East during Dry and Rainy Seasons

Levels of Cd in water and soil obtained from three rivers (Embobut, Arror and Mon) within Marakwet East during wet and dry seasons are presented in Table 4.5. Water samples obtained from up, mid and down streams of R. Embobut during wet season recorded Cd levels of 0.1, 0.105 and 0.105 ppm, respectively. Up, mid and down streams of R. Arror Cd recorded corresponding Cd levels of 0.132, 0.118 and 0.116 ppm. However, water samples from R. Mon recorded Cd levels of 0.099, 0.060 and 0.082 ppm up, mid and down streams, respectively. During dry season, samples of water collected from R. Embobut upstream, midstream and downstream had respective Cd levels of 0.146, 0.031 and 0.033 ppm. Analysis of variance revealed that these levels were significantly different ($P < 0.05$). Average Cd levels of up, mid and downstream of R. Arror were 0.025; 0.028, 0.019, respectively while for R. Mon they were 0.034 and 0.035; 0.044 ppm. Cd levels in soil samples collected from up, midstream and downstream of R. Embobut were 0.439, 0.475 and 0.158 ppm, respectively during wet season. Varied corresponding concentrations of 0.046, 0.055 and 0.071 ppm were recorded up, mid and down streams of R. Arror. Soil samples from R. Mon showed Cd levels of 0.076, 0.047 and 0.088 ppm up, mid and down streams respectively. The results were found to be significantly different statistically ($P < 0.05$).

Table 4.5: Concentration of Cd (ppm) in water and soil samples from selected rivers in Marakwet East during dry and wet seasons

		Water		Soil	
	Section	Wet	Dry	Wet	Dry
R. Embobut	Upstream	0.100±0.014b*	0.146±0.176e	0.439±0.006a*	0.088±0.001a
	Midstream	0.105±0.021b*	0.031±0.001c	0.475±0.001b*	0.092±0.001b
	Downstream	0.105±0.007b*	0.033±0.001c	0.518±0.006c*	0.106±0.009d
R. Arror	Upstream	0.132±0.019d*	0.025±0.002b	0.046±0.002b*	0.093±0.001b
	Midstream	0.118±0.004c*	0.019±0.003a	0.055±0.001c*	0.095±0.001c
	Downstream	0.116±0.011c*	0.035±0.001c	0.071±0.001d*	0.109±0.001e
R.Mon	Upstream	0.099±0.007b*	0.028±0.001b	0.076±0.001e*	0.091±0.001b
	Midstream	0.060±0.005a*	0.034±0.001c	0.047±0.015ab*	0.112±0.001f
	Downstream	0.082±0.017b*	0.044±0.001d	0.088±0.007f*	0.096±0.001c

*Means followed by different letters within a column are significantly different at $p<0.05$, * denote significance between dry and wet season*

During dry season, soil samples from R. Embobut showed respective general increase of Cd levels of 0.088, 0.092, 0.106 ppm at up, mid and down streams. There was also a general increase in Cd levels from upstream (0.093 ppm), midstream (0.095 ppm) to downstream (0.109 ppm) observed in soil samples from R. Arror. However, Cd levels increased from upstream to midstream and then dropped down stream of R. Mon with respective values of 0.091, 0.112 and 0.096 ppm.

4.2.4 Concentration of Cd (ppm) in water and soil samples from selected rivers in Marakwet West during dry and wet seasons

The levels of Cd in water and soil from three rivers (Chepkaitit, Moiben and Mosongu) within Marakwet West during both wet and dry seasons is shown in table 4.6. Corresponding Cd levels upstream, midstream and downstream of R. Chepkaitit were 0.102, 0.093 and 0.108 ppm during wet season. Different levels of 0.098, 0.082 and 0.095 ppm of Cd were recorded upstream, midstream and downstream of R. Moiben, respectively. However, there was significant increase in Cd levels observed from 0.066 ppm upstream to 0.068 ppm midstream and finally to 0.081 ppm downstream.

During dry season, Cd levels in water samples collected from R. Chepkaititat upstream, midstream and downstream were; 0.033, 0.032 and 0.024 ppm, respectively. Significant increase in Cd levels from 0.022 upstream to 0.029 ppm midstream and finally to 0.032 ppm was recorded in water samples obtained from R. Moiben. In respect to the study results, it is worth noting that constant concentration of 0.034 ppm was recorded in water samples from R. Mosongu for both up and mid streams, with significant increase to 0.039 ppm downstream.

Table 4.6: Concentration of Cd (ppm) in water and soil samples from selected rivers in Marakwet West during dry and wet seasons

		water		Soil	
R. Chepkaitit	Section	wet	Dry	wet	Dry
	upstream	0.102±0.001e*	0.033±0.001c	0.071±0.008c*	0.099±0.002a
	midstream	0.093±0.004d	0.032±0.001c	0.078±0.011c*	0.102±0.000b
	downstream	0.108±0.001f*	0.024±0.001a	0.053±0.004a*	0.098±0.002a
R. Moiben	upstream	0.098±0.006d*	0.022±0.001a	0.088±0.006d*	0.103±0.001b
	midstream	0.082±0.001c*	0.029±0.001b	0.091±0.001d*	0.099±0.001a
	downstream	0.095±0.004d*	0.032±0.001	0.100±0.002*	0.095±0.007a
R. Mosongu	upstream	0.066±0.001a*	0.034±0.001c	0.066±0.001b	0.069±0.041a
	midstream	0.068±0.002b*	0.034±0.001c	0.057±0.006a*	0.104±0.001b
	downstream	0.081±0.006c*	0.039±0.001d	0.054±0.001a*	0.108±0.001c

*Means followed by different letters within a column are significantly different at p<0.05, * denote significance between dry and wet season*

Soil samples collected during wet season from R. Chepkaitit recorded Cd levels of 0.071, 0.078 and 0.053 ppm upstream, midstream and downstream, respectively. Significant corresponding increase in Cd from 0.088 ppm to 0.091 ppm and finally to 0.1 ppm was recorded upstream, midstream and downstream of R. Mosongu. However, corresponding

Cd levels of 0.066, 0.057 and 0.054 ppm were recorded upstream, midstream and downstream of R. Mosongu. During dry season, respective Cd levels in soil samples collected from R. Chepkaitit upstream, midstream and downstream were 0.099, 0.102 and 0.098 ppm.

Soil samples collected from River Moiben recorded respective Cd levels of 0.103, 0.099 and 0.095 ppm upstream, midstream and downstream. Further, there was a significant increase in Cd levels was recorded from 0.069 to 0.104 and to 0.108 ppm upstream, midstream and downstream of R. Mosongu, respectively.

4.2.5 Concentration of Pb (ppm) in water and soil samples from selected rivers in Marakwet East during dry and wet seasons

The levels of Pb in water and soil samples collected from rivers Embobut, Arror and Mon in Marakwet East during wet and dry seasons are shown in table 4.7.

Table 4.7: Concentration of Pb (ppm) in water and soil samples from selected rivers in Marakwet East during dry and wet seasons

		Water		Soil	
	Section	Wet	Dry	Wet	Dry
River Embobut	Upstream	0.210±0.028a*	0.465±0.021b	0.648±0.004a*	1.031±0.443a
	Midstream	0.421±0.014d*	0.521±0.014c	0.716±0.007b*	1.501±0.453f
	Downstream	0.461±0.014*	0.565±0.007c	0.739±0.008c*	1.560±0.014f
R.Arror	Upstream	0.191±0.042a*	0.581±0.056d	0.715±0.021b*	1.455±0.021e
	Midstream	0.205±0.063b*	0.395±0.021a	0.805±0.063d*	1.420±0.014d
	Downstream	0.355±0.021c*	0.685±0.007e	0.900±0.014e*	1.685±0.078g
R. Mon	Upstream	0.470±0.028d*	0.580±0.042d	1.041±0.084f*	1.235±0.007b
	Midstream	0.720±0.084e*	0.042±0.014a	1.141±0.014g*	1.721±0.042g
	Downstream	0.791±0.057f*	0.555±0.063c	1.190±0.014h*	1.400±0.028c

*Means followed by different letters within a column are significantly different at $p<0.05$, * denote significance between dry and wet season*

According to the findings, Pb levels in water samples taken upstream, midstream, and downstream of R. Embobut were 0.210, 0.421, and 0.461 ppm, respectively. Upstream, midstream and downstream of R. Arror recorded Pb respective increasing trend in levels of 0.191, 0.205 and 0.355 ppm. Similarly, Pb levels increased from 0.470 ppm to 0.720 ppm to 0.791 ppm in water samples collected upstream, midstream and downstream of R. Mon, respectively. During dry season, water samples collected upstream, midstream and downstream of R. Embobut showed respective Pb levels of 0.465, 0.521 and 0.565 ppm. Pb levels were (0.581 ppm) upstream, midstream (0.395 ppm) and downstream (0.685 ppm) of R. Arror, while a general decrease of 0.580, 0.042 and 0.555 ppm levels of Pb were recorded upstream, midstream and downstream of R. Mon, respectively.

Respective Pb levels in soil samples collected during wet season upstream, midstream and downstream of R. Embobut were 0.648, 0.716 and 0.739 ppm. In R. Arror, Pb levels upstream, midstream and downstream were 0.715, 0.805 and 0.900 ppm, respectively. Significant highest levels of Pb were recorded in R. Mon with values of 1.041 ppm (upstream), 1.141 ppm (midstream) and 1.190 ppm (downstream). Generally, high levels of lead were recorded in soil during the dry season. Upstream, midstream and downstream of R. Embobut, lead levels in dry season were 1.031, 1.501 and 1.560 ppm, respectively. Sampled soil collected from rivers Arror and Mon were correspondingly recorded to be 1.455;1.235 ppm (upstream), 1.420;1.721 ppm (midstream) and 1.685;1.400 ppm downstream.

4.2.6 Concentration of Pb (ppm) in water and soil samples from selected rivers in Marakwet West during dry and wet seasons

The result of Pb levels in water and soil sampled from three rivers (Chepkaitit, Moiben and Mosongu) within Marakwet West during both wet and dry seasons are recorded in Table 4.8. During wet season, water samples from R. Chepkaitit recorded levels of Pb upstream (0.510 ppm), midstream (0.580 ppm) and downstream (0.170 ppm). Pb mean concentrations of upstream, midstream and downstream of R. Moiben were 0.470; 0.761, 0.175 respectively while that of R. Mosongu were 0.715, 0.945 and 0.651 ppm respectively.

Table 4.8: Concentration of Pb (ppm) in water and soil samples from selected rivers in Marakwet West during dry and wet seasons

		Water		**Soil**	
	Section	Wet	Dry	Wet	Dry
R.Chepkaitit	upstream	0.510±0.113c	0.531±0.014c	1.015±0.035b	1.461±0.042
	midstream	0.580±0.028c	0.481±0.028b	1.065±0.007b	1.471±0.000
	downstream	0.170±0.001a	0.565±0.035d	0.910±0.056a	1.385±0.063
R. Moiben	upstream	0.470±0.028b	0.415±0.021a	1.015±0.063b	1.460±0.014
	midstream	0.175±0.035a	0.635±0.035e	0.875±0.007a	1.451±0.014
	downstream	0.945±0.007f	0.605±0.021e	1.595±0.021d	1.435±0.007
R. Mosongu	upstream	0.761±0.014e	0.605±0.007e	1.225±0.007c	1.441±0.014
	midstream	0.715±0.049e	0.470±0.014b	1.215±0.007c	1.450±0.028
	downstream	0.651±0.014d	0.540±0.042c	1.075±0.021b	1.515±0.049

*Means followed by different letters within a column are significantly different at $p<0.05$, * denote significance between dry and wet season*

During dry season, water sampled from R. Chepkaitit recorded respective Pb levels of 0.531, 0.481 and 0.565 ppm at recorded upstream, midstream and downstream. However, varied concentrations of Pb were reported upstream (0.415, 0.605 ppm), midstream (0.635, 0.470 ppm) and downstream (0.605, 0.540 ppm) of rivers Moiben and Mosongu, respectively. During wet season, soil sampled upstream, midstream and downstream of R. Chepkaitit recorded lead levels of 1.015, 1.065 and 0.910 ppm respectively. At different points of R. Moiben, varied levels of Pb were recorded upstream (1.015 ppm), midstream (0.875 ppm) and downstream (1.595 ppm). Similarly, different levels of Pb were recorded upstream (1.225 ppm), midstream (1.215 ppm) and downstream (1.075 ppm) in water sampled from R. Mosongu. During dry season, water sampled upstream, midstream and downstream of Chepkaitit recorded Pb levels of 1.461, 1.471 and 1.385 ppm, respectively. Lead levels were 1.460, 1.441 ppm (upstream), 1.451, 1.450 ppm (midstream) and 1.435, 1.515 ppm downstream of rivers Moiben and Mosongui, respectively.

4.3 Interaction of metals Zn, Pb and Cd with histidine through coordination

4.3.1 Zn Absorption

Absorbency verses wavelength (nm) was determined for free histidine, maximum peak occurred at 250 nm while His and Zn^{2+} occurred at 302 nm. Zn^{2+} + His followed by Pb^{2+} occurred at 305 nm and Pb^{2+} + His followed by Pb^{2+} and Cd^{2+} occurred at 301 nm as summarized in the Figure 4.10 below.

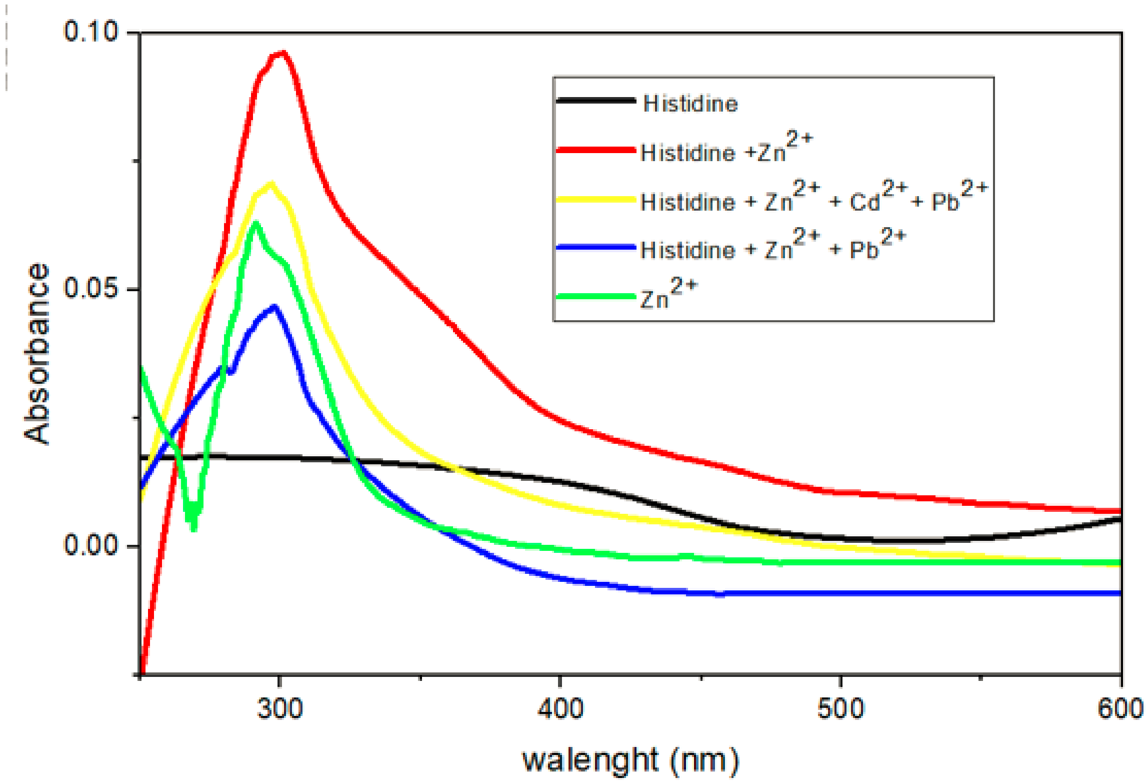

Figure 4.10: Zn Absorbency UV/VIS absorption spectra of L-histidine and their complex in a 1:1 ratio c=10^{-3}mol dm^{-3}

Absorption frequency was lowered when zinc interacted with histidine

4.3.2 Pb Absorption

His maximum peak occurred at 250 nm while for His+Pb^{2+} occurred at 302 nm, Pb^{2+} + His followed by Pb^2+ was recorded at 299 nm and Hs+Pb^{2+} followed by Zn^{2+} + Cd^{2+} absorption peak was at 298 nm as shown in figure 4.11 below, Pb^{2+}+His+Zn^{2+} was recorded at 295 nm and Pb^{2+}+His+Zn^{2+} + Cd^{2+} absorption peak was at 287 nm as summarized in figure 4.11 below.

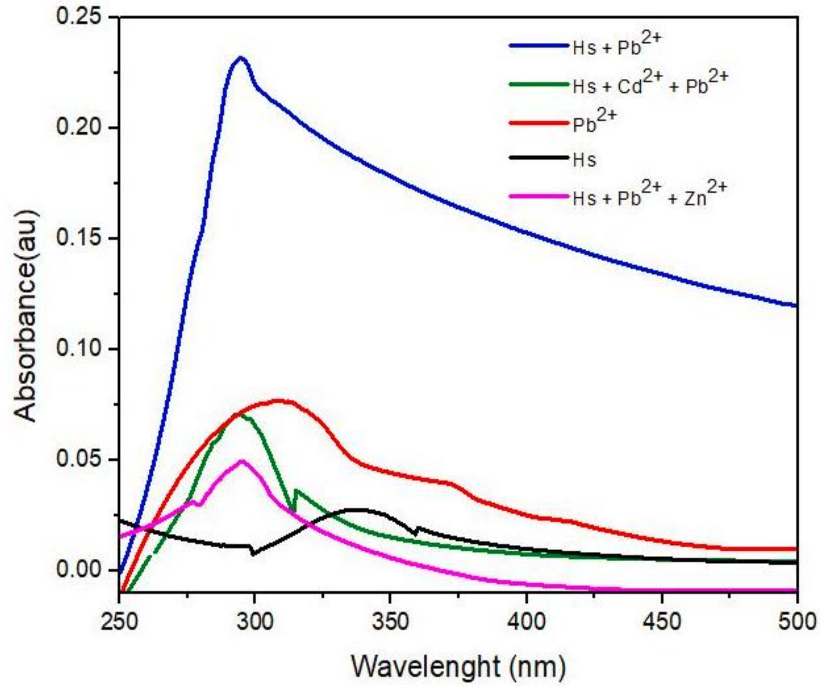

Figure 4.11: Pb absorption UV/VIS absorption spectra of L-histidine and their complex in a 1:1 ratio c=10^{-3}mol dm^{-3}

4.3.3 Cd Absorption

Absorption frequency for His occurred at 250 nm, while for His+ Cd^{2+} maximum peak occurred at 310 nm, Cd^{2+} +His followed by Zn^{2+} occurred at 295 and Cd^{2+} +His+ Zn^{2+} followed by Pb^{2+} occurred at 299 nm as shown in Figure 4.12 below.

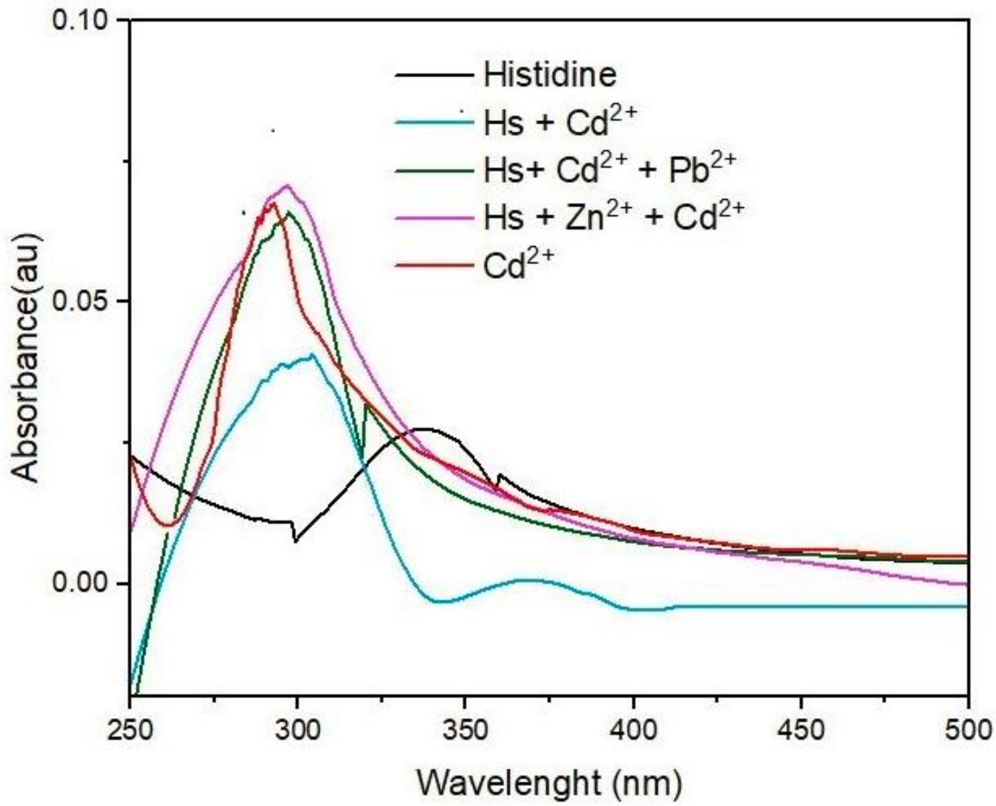

Figure 4.12: Cd Absorbency UV/VIS absorption spectra of L-histidine and their complex in a 1:1 ratio c=10^{-3}mol dm^{-3}

4.3.4 Interaction of Zn, Pb and Cd with histidine

Heavy metal concentration in meat samples was determined. Zn, Cd, and Pb total concentrations were found to be considerably higher. For Zn, the mean concentration was 15.89 mg/ kg; Cd was 0.01 mg/kg while Pb was 0.32 mg/kg before spiking. To determine the binding strength with histidine, spiking with the Zn, Pb and Cd with different concentrations (0.5, 2, 3 and 4 times) was done. Experimental data indicated a significant difference between spiked and un-spiked concentrations for all treatments as shown in

Figure 4.13. For the Zn spiked with Cd resulted in a significant difference ($p<0.05$) but when spiked with Pb resulted in no significant difference ($p>0.05$) for samples from both Marakwet East and West. When Pb was spiked with Cd and Zn, there was no significant difference in change ($p<0.05$) for all levels from both samples for the two regions reported in Figure 4.13. However, when Cd was spiked with Pb and Cd, there was a significant reduction ($p<0.05$) in meat samples.

ICP-MS results for interaction of Zn, Pb and Cd with histidine

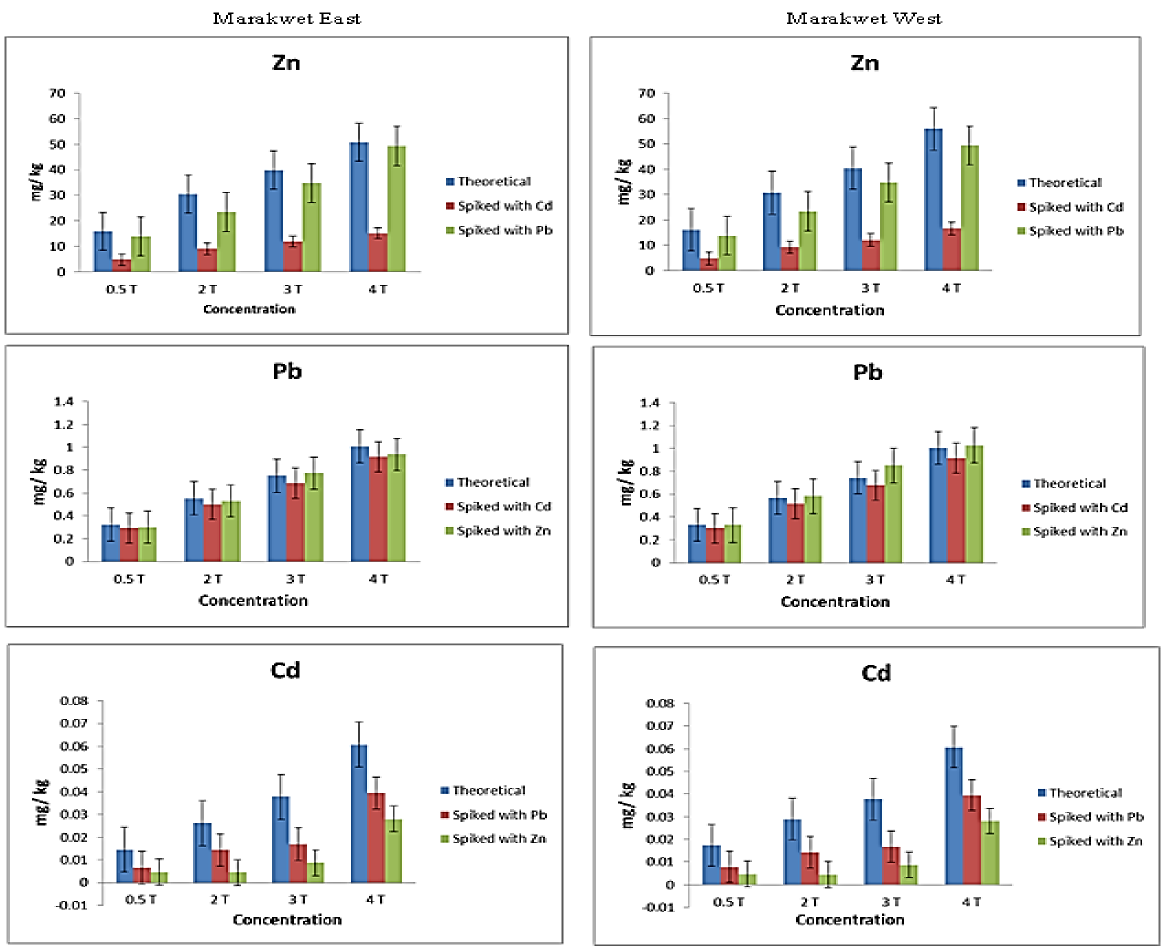

Figure 4.13: Interaction of Zn, Pb and Cd with Histidine

Generally, when a metal was spiked with another metal its concentration was reduced.

Concentration of the different heavy metals is in g/l.

The table 4.9 below displays the levels of Cd, Pb and Zn in beef obtained from the study area.

Table 4.9: Heavy metals (Cd, Pb and Zn) levels in cattle meat and comparison with CODEX STAN 193-1995

Parameter analyzed	Levels in mg/kg	Maximum Permitted levels (MPL) in mg/kg	Reference	Remarks
Zn	174.89	0.3-1.0 for meat of cattle		Above threshold thus not fit
Cd	0.01	Less than 0.5 for meat of cattle	FAO/WHO (2001)	Below threshold thus fit
Pb	0.16	0.1 for meat of cattle, pigs and sheep and 0.5 for offals		Above threshold thus not fit

In comparison to the values advised by the FAO, excessive levels of Zn and Pb were discovered in beef.

CHAPTER FIVE

DISCUSSIONS

5.1 Statistical and comparative study of diagnosed diabetic and hypertensive patients in Marakwet East and Marakwet West

Majority of the respondents pointed out that they had ever heard about heavy metals such as lead, zinc and cadmium (Fig 4.1). Respondents were asked to mention the nearest water source that they relied on for domestic purposes. Majority of the interviewed respondents were near Embobut River followed by those who were near rivers Moiben and Mosongu (Fig 4.3). Polluted source of drinking water can contribute to heavy metal poisoning leading to diseases such as diabetes and hypertension (Fernandez-Luqueno *et al.*, 2013).

Hellen (2020) indicated that lead is scarce in rivers and lakes. In water distribution networks and residential or commercial plumbing, it penetrates through corrosion. Brass and chrome-plated faucets, and lead pipes connecting homes and buildings to water mains are soldered with lead solder. It is still used in metal smelting and battery manufacturing. Especially if these sources are nearby, lead in the air mixes with soil. Buildings' lead-based paint can flake and mix with soil. During home remodeling, lead-based paint might accidentally combine with soil. Lead dust from the soil can blow into homes and yards.

The present study involved those participants who had acquired diabetes as well as hypertension. For those who were suffering from diabetes, majority indicated that consumption of sugary food followed by failure to do body exercises contributed to their contracting the disease (Fig 4.5). These findings agree with those reported by other scholars that consuming a lot of sugar raises one's risk of diabetes, but sugar is only one factor (Malik *et al.,* 2010). In this research, consumption of food and water with high amounts of cadmium, zinc and lead prove to be the contributing factor to diabetes in addition to high intake of sugar by the respondents. Other factors that may influence the risk include diet, lifestyle, and genetics.

Patients with diabetes and hypertension were asked to identify symptoms of heavy metal poisoning, such as lead, zinc, and cadmium. Multiple symptoms were pointed out which included tremors and shaking of hands, among others.

Toxic metals accumulated in diabetes patients' biological samples, disrupting glucose uptake and affecting linked molecular mechanisms, producing disorders such as hypertension, according to (Rehman *et al.,* 2018). Long-term exposure to heavy metals like lead and cadmium can cause life-long brain damage. Lead poisoning can be lethal at high amounts. But lead poisoning typically has no evident symptoms.

According to Hayes and Skubala (2009), toxic effects of lead pipes include interference with heme biosynthesis, calcium and vitamin D metabolism interference, digestive irritation, dullness, irritability, poor attention span, headaches, muscle spasms, abdominal cramps and kidney damage, hallucinations memory loss encephalopathy and hearing loss.

Respondents were also asked whether they were significantly exposed to pesticides, herbicides, fungicides and batteries as well as fertilizers. This is in line with findings of O'Connor *et al.(*2018) that there are various sources of lead poisoning such as lead-based paint, fertilizers, soils among others as pointed out by respondents.

5.2 Quantitative analysis of Zn, Pb and Cd in soil and water in Marakwet East and Marakwet West

Results indicated that Zn concentrations in water were higher during wet season than dry season (Tables 4.3 & 4.4). In addition, Zn levels increased significantly from upstream to downstream in the two rivers. This could be attributed to geographical difference, the parent rock and different agricultural activities in the region and high run off, soil erosion and leaching. River Mon had Zn levels decreasing downstream which could be because of high flow rate due to the steepness of the river downstream. Furthermore, significantly higher Zn levels were recorded in soil samples than in water samples (Tables 4.3 & 4.4). Rivers Embobut, Arror, Moiben and Mosongu recorded the highest levels of zinc during the dry and wet seasons. The soil samples in rivers Mon, Mosongu, and Chepkaitit recorded the highest concentrations of Zn during wet and dry seasons. However, the mean concentrations of Zn from the six rivers that is Mon, Embobut, Arror Chepkaitit, Moiben and Mosongu were all below the WHO (2001) maximum permissible limits for water and soil. The results of the study were similar to those of other studies done by Jepkoech (2013) who recorded Zn concentration of 0.70 ± 0.22 ppm in water samples from Sosiani River, Uasin Gishu County, Kenya. Akenga *et al*. (2020) recorded 0.071-0.054 ppm of Zn from water collected along River Moiben, Uasin-Gishu County, Kenya.

Rivers Embobut, Mosongu, Chepkaitit, and Arror recorded the highest levels of Cd concentration in water during the seasons of wet and dry periods. The soil samples in rivers Mon, Embobut, Mosongu, and Moiben recorded the highest concentration of Cd during the dry and wet seasons. The average concentration of Cd from the six rivers that is Mon, Embobut, Arror, Chepkaitit, Moiben and Mosongu were all above the WHO maximum permissible limits for water and soil. Differences in Cd levels in parent rocks and agricultural activities in the region may be to blame for elevated Cd levels in water. The high levels in the area may be due to heavy phosphate fertilizer use in potato and tomatoe fields. Natural contaminants in phosphate fertilizers include Cd (Sodhi, 2019). It has been reported that Ethiopian farms had Cd levels ranging from 0.73 to 1.23 g/g (Atlabachew *et al.*, 2011), showing farmers use similar soil management strategies. The detected soil cadmium levels were well below the 3 g/g acceptable limit for agricultural land.

The results from rivers Arror, Mon, and Moiben recorded the highest levels of Pb in water samples during the dry and wet seasons. The soil samples from rivers Mon, Mosongu, Moiben, and Mon recorded the highest concentrations of Pb during the dry and wet seasons. The mean concentration of Pb from the six rivers that is Mon, Embobut, Arror, Chepkaitit, Moiben and Mosongu were above the WHO maximum permissible limits for water and soil except for some few samples. The increase in Pb levels in agricultural soil could be due to the use of artificial fertilizers and pesticides (Onder *et al.*, 2007). The low levels of lead in the top samples may be attributed to the type of soil and its physical characteristics because lead mobility is greater in sandy soils with less organic matter than in organic soils (Kirmani et al., 2011). But they were substantially below the Pb levels found in uncontaminated soil (10 to 70 mg/g) and far below the acceptable limits of 50 g/g for agricultural land (FAO/WHO, 2001).

5.3 Interaction of Zn, Pb and Cd with histidine contributing to diabetes and hypertension

Heavy metal concentration in the meat samples was determined using ICP-MS. Experimental data indicated a significant difference between spiked and un-spiked concentrations for all treatments (Fig 4.13). For the Zn spiked with Cd resulted in a significant difference, but when spiked with Pb resulted in no significant difference. Pb spiked with Cd and Zn showed no appreciable change. The reduction in Cd with Pb and Zn was significant. Jalali and Khanlari (2006) reported that the second layer's Cd content is nearly eleven times that of the first layer's zinc content.

Zinc has the ability to form protective layers comprising basic carbonates, oxides or hydrated sulfates depending upon the nature of the environment (Wu *et al.*, 2021). When the protective layers have formed and completely covered the surface of the coating, corrosion proceeds at a greatly reduced rate. Cadmium form an oxide layer single crystals on exposure to air at room temperature. Cadmium can also form a layer of cadmium sulphide.

Insulin is a protein composed of two chains, an A chain (with 21 amino acids) and a B chain (with 30 amino acids), which are linked together by sulfur atoms (Lehrman, 2017). Insulin is derived from a 74-amino-acid prohormone molecule called proinsulin. Proinsulin is relatively inactive, and under normal conditions only a small amount of it is secreted (Krikorian & Calimag, 2022). Histidine is one of the amino acids and was chosen because of the reaction with heavy metal such as zinc, lead, and cadmium.

Histidine is one of the 20 naturally occurring amino acids that has the most inter- and intramolecular interactions. L-Histidine (His) is an amino acid that is a strong metal coordinating ligand and is involved in protein metal ion binding. Most biomolecule active sites contain histidine as an amino acid residue (Vandenbossche *et al.,* 2015; Liao *et al.,* 2013; Kowalik-Jankowska *et al.,* 2007). It has three potential metal-binding sites: carboxylate oxygen, imidazole imidonitrogen and aminonitrogen (Mengistu *et al.*, 2019; Kumaravel *et al.,* 2018). Histidine is a reactive imidazole group found in the active site of enzymes and directly involved in catalysis. It regulates metal transmission in biological bases and acts as a neurotransmitter or neuromodulator in the mammalian CNS (Bharath *et al.,* 2012). Blood cell production and histamine biosynthesis are just a few of the many biochemical processes involving histamine. It has vasodilating and hypotensive properties and may increase the brain's alpha wave activity.

Figure 4.10 shows free histidine C—O bond rotational and vibrational absorption frequencies took place at around 250 nm. Introduction of Zn^{2+} resulted in a shift to lower absorption frequency that wavelength 302 nm. This means that Zn^{2+}—his bond was strengthened consequently lowering the C—O vibrational and rotational absorption frequencies. With introduction of Pb^{2+} to Zn^{2+}—his system there was a shift to higher wavelength with a maximum peak at 305 nm implying stronger bonds involving Pb^{2+}. This is in agreement with the results earlier done by (Chang *et al*., 2016).

In Figure 4.11, the standard rotational and vibrational absorption frequency in free histidine had a maximum peak at around 250 nm. On introduction of Pb^{2+} there was a shift to a higher wavelength of 302 nm, imply increased strength of Pb^{2+}--Histidine bond with a consequential weakening of C--O vibrational and rotational absorption frequencies. However, when Zn^{2+} were introduced to the Pb^{2+}--histidine system, the rotational and vibrational absorption frequency increased (drop in wavelength to 299 nm) implying weaker bond formation with Zn^{2+} and strengthening C—O vibrational and rotational absorption frequencies. Therefore Zn^{2+} could not displace Pb^{2+} from the complex.

In Figure 4.12, free histidine had a C—O vibrational and rotational absorption frequency at around 250 nm. Spiking Cd^{2+} into histidine affected rotational and vibrational absorption frequency to a higher value of 310 nm. This shows that Cd^{2+}--histidine bond formed was strengthened causing a decrease of C—O vibrational and rotational strength. Introduction of Zn^{2+} increased absorption frequency that is at lower wavelength of 295 nm. However, further addition of Pb^{2+} resulted in slight drop in absorption frequency at 299 nm. From these results Zn^{2+} could not displace Pb^{2+} from Cd^{2+}—histidine—Pb^{2+} complex because of stronger bond formation between Pb^{2+} and histidine. Addition of Pb^{2+} to Cd^{2+}--His—Zn^{2+} resulted in longer wavelengths or lower absorption frequency implying that Pb^{2+} cannot displace Zn^{2+} in this complex.

Following the rotational and vibrational frequencies with histidine and metals, histidine + Zn^{2+} occurred at 302 nm, histidine + Pb^{2+} at 302 nm, and histidine + Cd^{2+} had a maximum peak at 310 nm, implying cadmium from the weakest bond with histidine and could be easily displaced by Zn^{2+} and Pb^{2+} from the histidine complexes. Zn^{2+} and Pb^{2+} from complexes with histidine having equal frequencies. Pb^{2+} has smallest hydrated radius while Zn^{2+} has the largest hydrated radius (Wang *et al*., 2017).

Histidine is a good metal ion chelator, thanks to the imidazole nitrogen atoms, which can operate as either an electron donor or acceptor depending on the situation. The aromaticity and amphoteric characteristics of histidine are due to its imidazole side chain, which is unique among amino acids.

Zinc (Ar) $3d^{10}$ $4s^2$ with an atomic radius of 0.138 nm loses the two outer electros to form Zn^{2+} which is a Lewis acid with a filled d orbital (d^{10} (Kamel, 2016).

Zinc binding sites are classified as structural, catalytic, or cocatalytic. Histidine is the most common amino acid ligand for Zn^{2+}. Many proteins contain zinc, particularly DNA-binding proteins with the zinc finger domain. Aside from the preferred protein ligand Cys, there are no bound water molecules (Hulugalle, 2013). In all, around 300 enzymes require zinc as a cofactor. Zinc complexes with three N, O, and S donors. Water is always a ligand here. Each metal is linked to the other by an amino acid side chain moiety (Asp, Glu, or His) and sometimes a water molecule and no Cys ligands (Ma *et al.*, 2009). The ligands for the fourth zinc binding site are amino acid residues on two proteins' surfaces. It is usually a catalytic or structural zinc site. All zinc sites have a hydrophilic shell within a larger hydrophobic shell. Moreover, zinc ligand amino acid side chains frequently form hydrogen bonds with other residues (Enke *et al.*, 2015).

Heavy metals (Zn, Cd, and Pb) pollutants damages the organ functions and disrupt physiological homeostasis (Chen *et al*., 2009). The deficiency and efficiency of these metals plays a role in islet function and the development of diabetes mellitus. Toxic metals have been known to be elevated in biological samples of patients with diabetes mellitus. Interactions between serum cadmium, lead levels in pre and postmenopausal women have been found to impact the prevalence of hypertension. Cadmium prevents the release of insulin destroying its receptors. It also disrupts glucose metabolism processes decreasing insulin production (Buha *et al*., 2020).

Zinc is stored in metallothionein reserves in microbes and animals' intestines and livers. Intestinal metallothionein can alter zinc absorption by 15%–40%. Because metallothionein absorbs both copper and zinc, inadequate or excessive zinc intake can be harmful (Osredkar & Sustar, 2011). Dopamine re-uptake is inhibited and amphetamine-induced dopamine efflux is amplified in vitro by the human dopamine transporter's high affinity extracellular zinc binding site. The human serotonin and norepinephrine transporters do not bind zinc. Some EF-hand calcium binding proteins, like S100 and NCS-1, can also bind zinc (Pifl *et al.*, 2009).

Cadmium is (Kr) ($4d^{10}$) ($5s^2$) and radius is 0.154 nm (Mekuria, 2020). Cadmium is a hexagonal close packed crystal with a = 0.297 nm and c = 0.561 nm. Aspartate, cysteine, glutamate, and histidine ligands are all affected by cadmium. Cadmium inhibits bilirubine-conjugating enzymes. It raises urinary Ca^{2+} excretion, causing severe bone disease. Low-level cadmium exposure over time is dangerous because it easily spreads to the liver and renal tissues, which are the principal organs affected by acute and chronic cadmium poisoning (Nordberg et al., 2018).

Calcium and zinc ions, for example, are known to be displaced by lead ions in proteins (Khalid *et al.*, 2013). According to Kirberger and Yang (2008), one-third of lead binding sites were caused by zinc or calcium ionic displacement, while two-thirds were opportunistic. Lead's main ligand is oxygen from amino acids or water, followed by sulphur and nitrogen. When lead replaces zinc at the zinc-binding sites in delta-aminolevulinic acid dehydratase, sulphur acts as the ligand (ALAD). Calmodulin, a calcium binding protein that can be activated by lead, was found to be activated first, and then inhibited by increasing lead concentrations. Further opportunistic binding was thought to cause more pronounced conformational changes.

The findings established that Zn and Cd in the water and soils were within the permissible levels by FAO. Bhowmik, Chiranjib, and Kumar (2010) state that zinc regulates over 100 enzymes involved in gene expression, protein folding, and the creation and neutralization of ROS. It is involved in cell signaling, cell division, apoptosis, and glucose homeostasis in mammals. Zinc is an antioxidant that reduces ROS generation, which is advantageous in ageing and diabetes mellitus and zinc deficiency is found in T2D patients (Chabosseauand & Rutter, 2016). By competing with iron and copper in the cell membrane and inhibiting NAD^+ nicotinamide Adenine Dinucleotide Triphosphate Oxidase, Cruz *et al.*(2015) point out that zinc is an enzyme cofactor for superoxide dismutase. This reduces oxidative stress caused by diabetes mellitus by boosting reactive oxygen species production and/or decreasing antioxidant defense system activity. Hence, if Zn is displaced by any other heavy metal, the functions stated will cease, hence impacting negatively on the affected individual.

Conversely, zinc may contribute to diabetes development. This occurs when zinc homeostasis is disrupted, causing diabetes and insulin resistance. Zinc deficiency can affect immune function by reducing cytokine production. The discovery of antibodies against this zinc transporter in type 1 diabetics opens up new diagnostic possibilities (Bourdineaud, 2010). Type 2 diabetes is linked to genetic variations in zinc transporter 8 and metallothionein (MT)-encoding genes.

Zinc is an enzyme cofactor for superoxide dismutase, according to Cruz *et al.* (2015). Inhibition of reactive oxygen species production and/or antioxidant defence system activity reduces oxidative stress in diabetes. The assessed cadmium levels in meat samples were very low when compared with FAO codex on heavy metals in food. Cd could not be a factor contributor to diabetes and hypertension in the area of study irrespective of increasing interest in how exposure to environmental substances can contribute to the onset of type II diabetes mellitus (T2DM). Human activities discharge a rare element, cadmium, into the air, land, and water (Misra & Misra, 2020). The two major causes of pollution are cadmium production and use, and cadmium waste disposal. The path of human exposure from agricultural crops is vulnerable to increases in soil cadmium concentration (Gill, Khan & Tuteja, 2012). Horses and certain feral terrestrial animals have higher levels of cadmium than humans. Regular intake might increase exposure (Chiari *et al.*, 2015). Insulin release is impaired in type I diabetes and contributes to T2DM development. Edwards and Ackerman (2016) link cadmium exposure to hyperglycemia, T2DM, and decreased serum insulin. Edwards and Prozialeck (2009) found that Cd causes time dependent and statistically significant changes in fasting leptin, GIP, and pancreatic polypeptide hormone levels.

While Cd's separate cellular effects are examined, it is likely that numerous pathways exist and work synergistically to cause islet dysfunction and ultimately dysglycemia. The Pb levels in the beef samples were over the threshold and this may cause diabetes and hypertension. Since the 1950’s, type 2 diabetes was attributed to lifestyle changes, but exposure to pollution and industrial toxins was dismissed (Leff *et al.*, 2018; Tyrrell *et al.*, 2017). Acute Pb exposure raises hepatic triglyceride levels and expression of the gluconeogenic genes PEPCK and glucose-6-phosphatase. Pb treatment of hepatoma cells enhanced PEPCK and glucose-6-phosphatase gene expression (Tyrrell *et al*., 2017).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Majority of the victims suffering from the diabetes and hypertension resides near rivers Mon and Embobut. Patients who had ingested large levels of heavy metals like lead, zinc, and cadmium frequently displayed tremors and trembling of the hands.

Water and soil samples from all the six rivers in the two Sub Counties of Marakwet East and West had the highest levels of Zn, Cd and Pb in both dry and wet seasons. However, Cd and Pb levels were above acceptable WHO maximum permissible limits for water and soil. Zn values were within the acceptable standards. There was a general increasing trend of metal levels downstream along the rivers.

Histidine—Cd^{2+} formed the weakest coordination bonds while Zn^{2+} and Pb^{2+} formed complexes of equal bond strength thus being partial contributors of diabetes and hypertention.

6.2 Recommendations

The study makes the following recommendations, based on the findings:

1. The levels of lead and Cadmium in the water should be constantly monitored because they are slightly above the WHO permissible limits. Lead levels in both surface and ground water are already beyond WHO guidelines.
2. Health personnel from the ministry of Agriculture and health from Elgeyo Marakwet County should train the local residents on the sources and health effects of heavy metals poisoning.
3. The Government through the Ministry of Health should educate the public on the dangers of heavy metals in water and soil and periodically conduct health screening exercises on the residents to check for some symptoms of heavy metals poisoning.
4. Further studies should be done on the interactions of other types of heavy metals with diabetes and hypertensions.

REFERENCES

Abuyassin, B., Sharma, K., Ayas, N. T., and Laher, I. (2015). Obstructive sleep apnea and kidney disease: a potential bidirectional relationship? *Journal of Clinical Sleep Medicine*, *11*(8), 915-924.

Adal, A., and Tarabar, A. (2013). Heavy metal toxicity. *Medscape. Lenntech water treatment and air purification. (2004) Water treatment. Published by Lenntech, Rotterdamseweg, Netherlands (http://www. excelwater. com/thp/filters/Water-Purification. htm*.

Adelekan, B. A., and Abegunde, K. D. (2011). Heavy metals contamination of soil and groundwater at automobile mechanic villages in Ibadan, Nigeria. *International journal of physical sciences*, *6*(5), 1045-1058.

Adriano, D. C., & Doner, H. E. (1983). Bromine, chlorine, and fluorine. *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*, *9*, 449-483.

Akenga, T., Ayabei, K., Kerich, E., Sudoi, V., and Kuya, C. (2020). Evaluation of Levels of Selected Heavy Metals in Kales, Soils and Water Collected from Irrigated Farms along River Moiben, Uasin-Gishu County, Kenya. *Journal of Geoscience and Environment Protection*, *8*(02), 144.

Ali, M. H., Mustafa, A. R. A., and El-Sheikh, A. A. (2016). Geochemistry and spatial distribution of selected heavy metals in surface soil of Sohag, Egypt: a multivariate statistical and GIS approach. *Environmental Earth Sciences*, *75*(18), 1-17.

Ali, U., Batool, A., Ghufran, M. A., Asad-Ghufran, M., Sabahat-Kazmi, S., and Hina-Fatimah, S. (2019). Assessment of heavy metal contamination in the drinking water of muzaffarabad, Azad Jammu and Kashmir, Pakistan. *Int J Hydro*, *3*(5), 331-337.

Alissa, E. M., and Ferns, G. A. (2011). Heavy metal poisoning and cardiovascular disease. *Journal of toxicology*

Alloway B. J., (1995): Heavy Metals in Soils. Chapman & Hall, London.

Al-Weher, S. M. (2008). Levels of heavy metal Cd, Cu and Zn in three fish species collected from the Northern Jordan Valley, Jordan. *Jordan journal of biological sciences*, *1*(1), 41-46.

American Public Health Association (APHA) (1998). Standards methods for examination of water and wastewater. Washington DC: American Public Health Association Inc...

Amos, A. F., McCarty, D. J., &Zimmet, P. (1997). The rising global burden of diabetes and its complications: estimates and projections to the year 2010. Diabetic medicine, 14(S5), S7-S85.

Anees, M., Mumtaz, A., Frooqi, S., Ibrahim, M., and Hameed, F. (2011). Serum trace elements (aluminium, copper, zinc) in hemodialysis patients. *Biomedica*, *27*(2), 106-10.

Arjunan, S. P. (2013). *Effects of exercise on cardiovascular disease risk markers in South Asian versus White European men* (Doctoral dissertation, Loughborough University).

Asati, A., Pichhode, M., and Nikhil, K. (2016). Effect of heavy metals on plants: an overview. *International Journal of Application or Innovation in Engineering & Management*, *5*(3), 56-66.

Atlabachew, M., Chandravanshi, B. S., Zewge, F., and Redi, M. (2011). Fluoride content of Ethiopian khat (Catha edulis Forsk) chewing leaves. *Toxicological & Environmental Chemistry*, *93*(1), 32-43.

Azizullah, A., Khattak, M. N. K., Richter, P., &Häder, D. P. (2011). Water pollution in Pakistan and its impact on public health—a review. *Environment International*, *37*(2), 479-497.

Baastrup, R., Sorensen, M., Balstrom, T., Frederiksen, K., Larsen, C. L., Tjonneland, A., Overvad, K. &Raaschou-Nielsen, O. (2008). Arsenic in drinking-water and risk for cancer in Denmark. *Environ. Health Perspective*. 116:231-237.

Barkouch, Y., Sedki, A., and Pineau, A. (2007). A new approach for understanding lead transfer in agricultural soil. *Water, air, and soil pollution*, *186*(1), 3-13.

Bennet, A. J., and Sinnott, M. L. (1986). Complete kinetic isotope effect description of transition states for acid-catalyzed hydrolyses of methyl. Alpha.-and. beta.-glucopyranosides. *Journal of the American Chemical Society*, *108*(23), 7287-7294.

Bexfield, L., & Plummer, L. (2003). Occurrence of arsenic in ground water of the Middle Rio Grande Basin, central New Mexico. *Arsenic in Ground Water*, 295-327.

Bhagure, G. R., and Mirgane, S. R. (2011). Heavy metal concentrations in groundwaters and soils of Thane Region of Maharashtra, India. *Environmental monitoring and assessment*, *173*(1), 643-652.

Bharath, K., Ananda K, B., Raju, S., and Nageswara Rao, G. (2012). Speciation studies of l-histidine complexes of Pb (II), Cd (II), and Hg (II) in dmso-water mixtures. *International Journal of Inorganic Chemistry*, *2012*.

Bhowmik, D., Chiranjib, K., and Kumar, S. (2010). A potential medicinal importance of zinc in human health and chronic. *Int J Pharm*, *1*(1), 05-11.

Boukaoud, A., Chiba, Y., & Sebbar, D. (2021). A periodic DFT study of IR spectra of amino acids: An approach toward a better understanding of the NH and OH stretching regions. *Vibrational Spectroscopy*, *116*, 103280.

Bourdineaud, J. P. (2010). Heavy metals toxicity at very low, yet environmentally relevant doses: the cases of human mercurial contaminations through fish consumption, uranium in drinking water, and gold nanoparticles in aquatic systems. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *157*, S35.

Brown, M. J., and Margolis, S. (2012). Lead in drinking water and human blood lead levels in the United States.

Buamah, R., Petrusevski, B., & Schippers, J. C. (2008). Presence of arsenic, iron and manganese in groundwater within the gold-belt zone of Ghana. *Journal of Water Supply: Research and Technology-Aqua*, *57*(7), 519-529.

Buha, A., Đukić-Ćosić, D., Ćurčić, M., Bulat, Z., Antonijević, B., Moulis, J. M., ... & Wallace, D. (2020). Emerging links between cadmium exposure and insulin resistance: human, animal, and cell study data. *Toxics*, *8*(3), 63.

Buzea, C., & Pacheco, I. (2020). Heavy Metals: Definition, Toxicity, and Uptake in Plants. In *Cellular and Molecular Phytotoxicity of Heavy Metals* (pp. 1-17). Springer, Cham.

Carocci, A., Catalano, A., Lauria, G., Sinicropi, M. S., and Genchi, G. (2016). Lead toxicity, antioxidant defense and environment. *Reviews of environmental contamination and toxicology*, 45-67.

Castagnetto, J. M., Hennessy, S. W., Roberts, V. A., Getzoff, E. D., Tainer, J. A., & Pique, M. E. (2002). MDB: the metalloprotein database and browser at the Scripps Research Institute. *Nucleic acids research*, *30*(1), 379-382.

Chabosseau, P., and Rutter, G. A. (2016). Zinc and diabetes. *Archives of Biochemistry and Biophysics*, *611*, 79-85.

Chang, X., Zhang, Y., Weng, X., Su, P., Wu, W., and Mo, Y. (2016). Red-shifting versus blue-shifting hydrogen bonds: Perspective from ab inito valence bond theory. *The Journal of Physical Chemistry A, 120*(17), 2749-2756.

Charkiewicz, A. E., and Backstrand, J. R. (2020). Lead toxicity and pollution in Poland. *International Journal of Environmental Research and Public Health*, *17*(12), 4385.

Chebet, C., Odenyo, V. A., & Kipkorir, E. C. (2017). Modeling the impact of land use changes on river flows in Arror watershed, Elgeyo Marakwet County, Kenya. *Water Practice and Technology*, *12*(2), 344-353.

Chen, Y. W., Yang, C. Y., Huang, C. F., Hung, D. Z., Leung, Y. M., & Liu, S. H. (2009). Heavy metals, islet function and diabetes development. *Islets*, *1*(3), 169-176.

Chiari, M., Cortinovis, C., Bertoletti, M., Alborali, L., Zanoni, M., Ferretti, E., and Caloni, F. (2015). Lead, cadmium and organochlorine pesticide residues in hunted red deer and wild boar from northern Italy. *Food Additives & Contaminants: Part A*, *32*(11), 1867-1874.

Cruz, K. J. C., de Oliveira, A. R. S., and Nascimento D. (2015). Antioxidant role of zinc in diabetes mellitus. *World journal of diabetes*, *6*(2), 333.

Duruibe, J. O., Ogwuegbu, M. O. C., and Egwurugwu, J. N. (2007). Heavy metal pollution and human biotoxic effects. *International Journal of physical sciences*, *2*(5), 112-118.

Edwards, J. R., and Prozialeck, W. C. (2009). Cadmium, diabetes and chronic kidney disease. *Toxicology and applied pharmacology*, *238*(3), 289-293.

Edwards, J., and Ackerman, C. (2016). A review of diabetes mellitus and exposure to the environmental toxicant cadmium with an emphasis on likely mechanisms of action. *Current Diabetes Reviews*, *12*(3), 252-258.

Elinder, C. G., Nordberg, M., Palm, B., Björk, L., and Jönsson, L. (1987). Cadmium, zinc, and copper in rabbit kidney metallothionein—relation to kidney toxicity. *Environmental research*, *42*(2), 553-562.

Elizondo-Álvarez, M. A., Uribe-Salas, A., and Nava-Alonso, F. (2020). Flotation studies of galena (PbS), cerussite ($PbCO_3$) and anglesite ($PbSO_4$) with hydroxamic acids as collectors. *Minerals Engineering*, *155*, 106456.

Enke, M., Bode, S., Vitz, J., Schacher, F. H., Harrington, M. J., Hager, M. D., and Schubert, U. S. (2015). Self-healing response in supramolecular polymers based on reversible zinc–histidine interactions. *Polymer*, *69*, 274-282.

Ergun-Longmire, B., Clemente, E., Vining-Maravolo, P., Roberts, C., Buth, K., and Greydanus, D. E. (2021). Diabetes education in pediatrics: how to survive diabetes. *Disease-a-Month*, 101153.

FAO/WHO Expert Committee on Food Additives. Meeting. (2001). *Safety evaluation of certain mycotoxins in food* (No. 74). Food & Agriculture Org...

Feil, R. (2006). Environmental and nutritional effects on the epigenetic regulation of genes. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 600(1), 46-57.

Feng, W., Cui, X., Liu, B., Liu, C., Xiao, Y., Lu, W., and Wu, T. (2015). Association of urinary metal profiles with altered glucose levels and diabetes risk: a population-based study in China. *PloS one*, *10*(4), e0123742.

Fernandez-Luqueno, F., López-Valdez, F., Gamero-Melo, P., Luna-Suárez, S., Aguilera-González, E. N., Martínez, A. I., and Pérez-Velázquez, I. R. (2013). Heavy metal pollution in drinking water-a global risk for human health: A review. *African Journal of Environmental Science and Technology*, *7*(7), 567-584.

Fewtrell, L.J., Prüss-Üstün , A., Landrigan, P. and Ayuso-Mateos, J.L. (2004) Estimating the global burden of disease of mild mental retardation and cardiovascular diseases from environmental lead exposure. *Environmental Research* 94(2), 120–133.

Flegal, K. M., Ezzati, T. M., Harris, M. I., Haynes, M. G., Juarez, R. Z., Knowler, W. C., and Stern, M. P. (1991). Prevalence of diabetes in Mexican Americans, Cubans, and Puerto Ricans from the Hispanic health and nutrition examination survey, 1982–1984. *Diabetes care*, *14*(7), 628-638.

Franzago, M., Fraticelli, F., Stuppia, L., and Vitacolonna, E. (2019). Nutrigenetics, epigenetics and gestational diabetes: consequences in mother and child. *Epigenetics*, *14*(3), 215-235.

Geiger, A., and Cooper, J. (2010). Overview of airborne metals regulations, exposure limits, health effects, and contemporary research. *Portland, OR: Cooper Environmental Services*, 1-61.

Gill, M. (2014). Heavy metal stress in plants: a review. *Int J Adv Res*, *2*(6), 1043-1055.

Gill, S. S., Khan, N. A., and Tuteja, N. (2012). Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress (Lepidium sativum L.). *Plant Science*, *182*, 112-120.

Goyer, R. A., and Clarkson, T. W. (1996). Toxic effects of metals. *Casarett and Doull's toxicology: the basic science of poisons*, *5*, 691-736.

Guariguata, L., Whiting, D. R., Hambleton, I., Beagley, J., Linnenkamp, U., and Shaw, J. E. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes research and clinical practice*, *103*(2), 137-149.

Harris, M. I., Flegal, K. M., Cowie, C. C., Eberhardt, M. S., Goldstein, D. E., Little, R. R., and Byrd-Holt, D. D. (1998). Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in US adults: the Third National Health and Nutrition Examination Survey, 1988–1994. Diabetes care, 21(4), 518-524.

Harrison, P., and Waites, G. (1998). *The Cassell Dictionary of Chemistry*. Cassell.

Hayat, M. T., Nauman, M., Nazir, N., Ali, S., and Bangash, N. (2019). Environmental hazards of cadmium: past, present, and future. In *Cadmium toxicity and tolerance in plants* (pp. 163-183). Academic Press.

Hayes, C. R., and Skubala, N. D. (2009). Is there still a problem with lead in drinking water in the European Union? *Journal of water and health*, *7*(4), 569-580.

Hellawell, J. M. (Ed.). (2012). *Biological indicators of freshwater pollution and environmental management*. Springer Science & Business Media.

Hellen, R. (2020). Preliminary study into the reduction and removal of naturally aged varnishes from painted surfaces using an Er: YAG laser in a two-step cleaning process with solvents. *Journal of the Institute of Conservation*, *43*(1), 79-93.

Hembrom, S., Singh, B., Gupta, S. K., & Nema, A. K. (2020). A comprehensive evaluation of heavy metal contamination in foodstuff and associated human health risk: a global perspective. In *Contemporary environmental issues and challenges in era of climate change* (pp. 33-63). Springer, Singapore.

Hertwich, E. (2010). *Assessing the environmental impacts of consumption and production: priority products and materials*. UNEP/Earthprint.

Heshmat, B., Pahlevaninezhad, H., Pang, Y., Masnadi-Shirazi, M., Burton Lewis, R., Tiedje, T., and Darcie, T. E. (2012). Nanoplasmonic terahertz photoconductive switch on GaAs. *Nano letters*, *12*(12), 6255-6259.

Hilmy, A. M., El Domiaty, N. A., Daabees, A. Y., and HA, A. L. (1987). Some physiological and biochemical indices of zinc toxicity in two freshwater fishes, Clarias lazera and Tilapia zilli. *Comparative Biochemistry and physiology. C, Comparative Pharmacology and Toxicology*, *87*(2), 297-301.

Hudec, M., Dankova, P., Solc, R., Bettazova, N., and Cerna, M. (2020). Epigenetic regulation of circadian rhythm and its possible role in diabetes mellitus. *International journal of molecular sciences*, *21*(8), 3005.

Hughes, S., and Samman, S. (2006). The effect of zinc supplementation in humans on plasma lipids, antioxidant status and thrombogenesis. *Journal of the American College of Nutrition*, *25*(4), 285-291.

Hulugalle, D. (2013). *Computational investigation of thiol-based redox modifications in proteins: redox-active disulfides, Zn2+ sites and their associations* (Doctoral dissertation, Victor Chang Cardiac Research Institute & Faculty of Medicine, UNSW Australia).

Ihara, M., Okajima, T., Yamashita, A., Oda, T., Asano, T., Matsui, M., Sattelle, D.

B., and Matsuda, K. (2014). Studies on an acetylcholine binding protein identify a basic residues in loop G on the β1 strand as new structural determinant of neonicotinoid actions. *Molecular Pharmacology*, *86*(6), 736-746.

Issazadeh, K., Jahanpour, N., Pourghorbanali, F., Raeisi, G., and Faekhondeh, J. (2013). Heavy metals resistance by bacterial strains. *Annals of Biological Research*, *4*(2), 60-63.

Jalali, M., and Khanlari, Z. V. (2006). Mobility and distribution of zinc, cadmium and lead in calcareous soils receiving spiked sewage sludge. *Soil & Sediment Contamination*, *15*(6), 603-620.

Järup, L. (2003). Hazards of heavy metal contamination. *British medical bulletin*, *68*(1), 167-182.

Jepkoech, J. (2013). *Selected heavy metals in water and sediments and their bioconcentrations in plant (Polygonum pulchrum) in Sosiani River, Uasin Gishu County, Kenya* (Doctoral dissertation, University of Eldoret).

Jerez, S., Motas, M., Palacios, M. J., Valera, F., Cuervo, J. J., & Barbosa, A. (2011). Concentration of trace elements in feathers of three Antarctic penguins: geographical and interspecific differences. *Environmental Pollution*, *159*(10), 2412-2419.

John, E., Laskow, T.C., Buchser, W.J., Pitt, B.R., Basse, P.H., Butterfield, L.H., Kalinski, P. and Lotze, M.T., (2010). Zinc in innate and adaptive tumor immunity. *Journal of translational medicine*, *8*(1), pp.1-16.

Jovanovic, D., Jakovljević, B., Rašić-Milutinović, Z., Paunović, K., Peković, G., and

Knezević, T. (2011). Arsenic occurrence in drinking water supply systems in ten municipalities in Vojvodina Region, Serbia. *Environmental research*, *111*(2), 315-318.

Judd, E., and Calhoun, D. A. (2014). Apparent and true resistant hypertension: definition, prevalence and outcomes. *Journal of human hypertension*, *28*(8), 463-468.

Kabata-Pendias, A., and Mukherjee, A. B. (2007). *Trace elements from soil to human*. Springer Science & Business Media.

Kahlown, M. A., and Majeed, A. (2003). Water-resources situation in Pakistan: challenges and future strategies. Water Resources in the South: Present Scenario and Future Prospects, 20.

Kamel, S. K. (2016). *New mixed ligand Zn (II) complexes based on the biologically active substituted acetic acid and nitrogen donor ligands: synthesis, crystal structure and biological applications* (Doctoral dissertation).

Kapoor, D., & Singh, M. P. (2021). Heavy metal contamination in water and its possible sources. In *Heavy metals in the environment* (pp. 179-189). Elsevier.

Katsou, E., Malamis, S., and Haralambous, K. J. (2011). Industrial wastewater pre-treatment for heavy metal reduction by employing a sorbent-assisted ultrafiltration system. *Chemosphere*, *82*(4), 557-564.

Kelepertsis, A., Alexakis, D., and Kita, I. (2001). Environmental geochemistry of soils and waters of Susaki area, Korinthos, Greece. *Environmental Geochemistry and Health*, *23*(2), 117-135.

Kelepertsis, A., Argyraki, A., and Alexakis, D. (2006). Multivariate statistics and spatial interpretation of geochemical data for assessing soil contamination by potentially toxic elements in the mining area of Stratoni, north Greece. *Geochemistry: Exploration, Environment, Analysis*, *6*(4), 349-355.

Kemboi, J. O., Simiyu, G. M., Kipkorir, E. C., and Wakhisi, J. (2018). Selected Heavy Metal Levels in Cereals in Fluorspar Mining Belt, Elgeyo Marakwet County in Kenya. *Africa Environmental Review Journal*, *3*(1), 65-73.

Khalid, A., Bukhari, I.H., Riaz, M., Rehman, G., Ain, Q.U., Bokhari, T.H., Rasool, N., Zubair, M. and Munir, S., (2013). Determination of lead, cadmium, chromium, and nickel in different brands of lipsticks. *International Journal of Biology, Pharmacy and Allied Sciences*, *1*(2), pp.263-271.

Khan, M. H. A., and Parvez, S. (2015). Hesperidin ameliorates heavy metal induced toxicity mediated by oxidative stress in brain of Wistar rats. *Journal of Trace Elements in Medicine and Biology*, *31*, 53-60.

King. H., Rewers, M. (1993). Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. *WHO Ad Hoc Diabetes Reporting Group. Diabetes Care* 1993; 16: 157 – 77.

Kirberger, M., and Yang, J. J. (2008). Structural differences between Pb2+-and Ca2+-binding sites in proteins: implications with respect to toxicity. *Journal of Inorganic Biochemistry*, *102*(10), 1901-1909.

Kirberger, M., and Yang, J. J. (2008). Structural differences between Pb2+-and Ca2+-binding sites in proteins: implications with respect to toxicity. *Journal of Inorganic Biochemistry*, *102*(10), 1901-1909.

Kirmani, M. Z. (2011). Determination of some toxic and essential trace metals in some medicinal and edible plants of Karachi city. *Journal of Basic & Applied Sciences*, *7*(2).

Konuk, M., Ciğerci, İ. H., and Korcan, S. E. (2010). ALAD (δ-aminolevulinic acid dehydratase) as biosensor for Pb contamination. *Intelligent and biosensors. Intech, Rijeka*, 363-376.

Kowalik-Jankowska, T., Kozlowski, H., Farkas, E., and Sovago, I. (2007). *Nickel ion complexes of amino acids and peptides* (Vol. 2, pp. 63-108). Wiley & Sons: Chichester, UK.

Krikorian, A. A., and Calimag, A. P. P. (2022). Glycemic Control. In *Diabetes and Kidney Disease* (pp. 443-467). Springer, Cham.

Kumar, S., Prasad, S., Yadav, K. K., Shrivastava, M., Gupta, N., Nagar, S., and Malav, L. C. (2019). Hazardous heavy metals contamination of vegetables and food chain: Role of sustainable remediation approaches-A review. *Environmental research*, *179*, 108792.

Kumar, A, Adak. P., Gurian, P. L., and Lockwood, J. R. (2010). Arsenic exposure in US public and domestic drinking water supplies: A comparative risk assessment. *J. Expo. Sci. Env. Epid*. 20:245-254.

Kumaravel, G., Utthra, P. P., and Raman, N. (2018). Exploiting the biological efficacy of benzimidazole based Schiff base complexes with l-Histidine as a co-ligand: Combined molecular docking, DNA interaction, antimicrobial and cytotoxic studies. *Bioorganic chemistry*, *77*, 269-279.

Leff, T., Stemmer, P., Tyrrell, J., and Jog, R. (2018). Diabetes and exposure to environmental lead (Pb). *Toxics*, *6*(3), 54.

Lehrman, S. R. (2017). Protein structure. Fundamentals of protein biotechnology, 9-38.

Li, X. T., Yu, P. F., Yan, G. A. O., Guo, W. H., Jun, W. A. N. G., Xin, L. I. U., ... and Hang, X. I. A. O. (2017). Association between plasma metal levels and diabetes risk: a case-control study in China. *Biomedical and environmental sciences*, *30*(7), 482-491.

Liao, S. M., Du, Q. S., Meng, J. Z., Pang, Z. W., and Huang, R. B. (2013). The multiple roles of histidine in protein interactions. *Chemistry Central Journal*, *7*(1), 1-12.

Lille, F., Hazemann, P., Garnier, R., and Dally, S. (1988). Effects of lead and mercury intoxications on evoked potentials. *Journal of Toxicology: Clinical Toxicology*, *26*(1-2), 103-116..

Lin, C. Y., Huang, P. C., Wu, C., Sung, F. C., and Su, T. C. (2020). Association between urine lead levels and cardiovascular disease risk factors, carotid intima-media thickness and metabolic syndrome in adolescents and young adults. *International journal of hygiene and environmental health*, *223*(1), 248-255.

Lin, C. Y., Lee, H. L., Hwang, Y. T., Huang, P. C., Wang, C., Sung, F. C., ... and Su, T. C. (2020). Urinary heavy metals, DNA methylation, and subclinical atherosclerosis. *Ecotoxicology and Environmental Safety*, *204*, 111039.

Lisabeth, L. D., Ahn, H. J., Chen. J. J., Sealy-Jefferson, S., Burke, J. F., and Meliker, J. R. (2010). Arsenic in drinking water and stroke hospitalizations in Michigan. *Stroke* 41:2499-2504.

Lugoli, F., Leopizzi, M. I., Bagordo, F., Grassi, T., Guido, M., and De Donno, A. (2011). Widespread microbiological groundwater contamination in the South-eastern Salento (Puglia-Italy). *Journal of Environmental Monitoring*, *13*(1), 192-200.

Ma, Z., Jacobsen, F. E., and Giedroc, D. P. (2009). Coordination chemistry of bacterial metal transport and sensing. *Chemical reviews*, *109*(10), 4644-4681.

Mahurpawar, M. (2015). Effects of heavy metals on human health. *International Journal of Research-Granthaalayah*, *3*(9SE), 1-7.

Maiyo, W. K., Kituyi, J. L., Mitei, Y. J., and Kagwanja, S. M. (2014). Heavy metal contamination in raw honey, soil and flower samples obtained from Baringo and Keiyo Counties, Kenya. *Int J Emerg Sci Eng*, *2*, 5-9.

Malik, V. S., Popkin, B. M., Bray, G. A., Després, J. P., and Hu, F. B. (2010). Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. *Circulation*, *121*(11), 1356-1364.

Manassaram, D. M., Backer, L. C., Messing, R., Fleming, L. E., Luke, B., and Monteilh, C. P. (2010). Nitrates in drinking water and methemoglobin levels in pregnancy: a longitudinal study. Environ. *Health-Glob*. 9:60.

Martens, H., Leonhard-Marek, S., Röntgen, M., and Stumpff, F. (2018). Magnesium homeostasis in cattle: absorption and excretion. *Nutrition research reviews*, *31*(1), 114-130.

Mansour, S. A. (2014). Heavy metal contamination as a global problem and the need for prevention/reduction measurements. *Practical food safety: contemporary issues and future directions*, 257-280.

Mathenge, S. G. (2013). Assessment of selected antibiotics and heavy metals in untreated wastewater, vegetables and soils in Eastern Nairobi, Kenya. *Unpublished PhD thesis, Kenyatta University*.

Maurya, A., Kesharwani, L., and Mishra, M. K. (2018). Analysis of heavy metal in soil through atomic absorption spectroscopy for forensic consideration. *Int. J. Res. Appl. Sci. Eng. Technol*, *6*(6), 1188-1192.

Mayosi, B. M., Flisher, A. J., Lalloo, U. G., Sitas, F., Tollman, S. M., and Bradshaw, D. (2009). The burden of non-communicable diseases in South Africa. *The lancet*, *374*(9693), 934-947.

McGuigan, C. F., Hamula, C. L., Huang, S., Gabos, S., and Le, X. C. (2010). A review on arsenic concentrations in Canadian drinking water. *Environmental Reviews*, *18*(NA), 291-307.

Mekuria, T. M. (2020). *Carbon nanotube magnetic nanoparticle composite as nano sensor for monitoring heavy metals in water* (Doctoral dissertation, Morgan State University).

Meliker, J. R., Wahl, R. L., Cameron, L. L., and Nriagu, J. O. (2007). Arsenic in drinking water and cerebrovascular disease, diabetes mellitus, and kidney disease in Michigan: a standardized mortality ratio analysis. *Environmental Health*, *6*(1), 4.

Mengistu, M., Kufa, R. E. M., and Belay, H. H. (2019). Shift In PkaOfl-Histidine And L-Glutamic Acid In Organic Media: A Potentiometric Study. *Journal of Advanced Scientific Research*, *10*(3 Suppl 1).

Milton, A. H., Hasan, Z., Raheavymetalan, A., and Raheavymetalan, M. (2001). Chronic arsenic poisoning and respiratory effects in Bangladesh. *Journal of occupational health*, *43*(3), 136-140.

Misra, B. B., and Misra, A. (2020). The chemical exposome of type 2 diabetes mellitus: Opportunities and challenges in the omics era. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *14*(1), 23-38.

Mizuno, Y., Shimizu-Furusawa, H., Konishi, S., Inaoka, T., Ahmad, S. A., Sekiyama, M., &Umezaki, M. (2021). Associations between urinary heavy metal concentrations and blood pressure in residents of Asian countries. *Environmental health and preventive medicine*, *26*(1), 1-11.

Mokdad, A. H., Bowman, B. A., Ford, E. S., Vinicor, F., Marks, J. S., &Koplan, J. P. (2001). The continuing epidemics of obesity and diabetes in the United States. *Jama*, *286*(10), 1195-1200.

Monudu, M., and Anyakora, C. (2010). Heavy metal contamination of ground water. *The serulere case study Journal*, *2*, 39-40.

Moore, H., Davies, M., Wanjohi, B. K., Njenga, E. W., Sudoi, V., & Kipkore, W. K. (2020). Ecological Knowledge of indigenous plants among the Marakwet Community (Embobut Basin), Elgeyo Marakwet County (Kenya). *Ethnobotany Research and Applications: a journal of plants, people and applied research*, *20*.

Morais, S., Costa, F. G., and Pereira, M. D. L. (2012). Heavy metals and human health. *Environmental health–emerging issues and practice*, *10*(1), 227-245.

Morongâ, D. R., Kariuki, A. C., & Bitok, J. (2018). Strategies Adopted in Adventure Tourism Product Diversification in Sustainable Tourism Development in Elgeyo Marakwet County, Kenya. *Journal of Hospitality and Tourism Management*, *1*(1), 94-112.

Moywaywa, B. A. (2018). *Heavy metal pollution in sediments, water and flora along Thika River* (Doctoral dissertation, University of Nairobi).

Moywaywa, B. A. (2018). *Heavy metal pollution in sediments, water and flora along Thika River* (Doctoral dissertation).

Muzyed, S. K. I. (2011). Heavy metal concentrations in commercially available fishes in Gaza strip markets.

Nagajyoti, P. C., Lee, K. D., and Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental chemistry letters*, *8*(3), 199-216.

Nair, A. R., DeGheselle, O., Smeets, K., Van Kerkhove, E., and Cuypers, A. (2013). Cadmium-induced pathologies: where is the oxidative balance lost (or not)?. *International journal of molecular sciences*, *14*(3), 6116-6143.

Nazir, R., Khan, M., Masab, M., Rehman, H. U., Rauf, N. U., Shahab, S., and Shaheen, Z. (2015). Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water collected from Tanda Dam Kohat. *Journal of pharmaceutical sciences and research*, *7*(3), 89.

Nordberg, G.F., Bernard, A., Diamond, G.L., Duffus, J.H., Illing, P., Nordberg, M., Bergdahl, I.A., Jin, T. and Skerfving, S., (2018). Risk assessment of effects of cadmium on human health (IUPAC Technical Report). *Pure and Applied Chemistry*, *90*(4), pp.755-808.

Nziguheba, G., and Smolders, E. (2008). Inputs of trace elements in agricultural soils via phosphate fertilizers in European countries. *Science of the total environment*, *390*(1), 53-57.

Ochieng, E. Z., Lalah, J. O., and Wandiga, S. O. (2007). Analysis of heavy metals in water and surface sediment in five rift valley lakes in Kenya for assessment of recent increase in anthropogenic activities. Bulletin of environmental contamination and toxicology, 79(5), 570-576.

O'Connor, D., Hou, D., Ye, J., Zhang, Y., Ok, Y. S., Song, Y., and Tian, L. (2018). Lead-based paint remains a major public health concern: A critical review of global production, trade, use, exposure, health risk, and implications. *Environment international*, *121*, 85-101.

Ogunkunle, A. T. J., Bello, O. S., and Ojofeitimi, O. S. (2014). Determination of heavy metal contamination of street-vended fruits and vegetables in Lagos state, Nigeria. *International Food Research Journal*, *21*(5).

Okube, O. T., and Gichuki, N. W. (2018). Life Experience of Patients with Type-2 Diabetes Aged above Forty Years at North Kinangop Catholic Hospital, North Kinangop, Kenya. *EC Diabetes and Metabolic Research*, *1*, 81-88.

Olaniran, A. O., Balgobind, A., and Pillay, B. (2013). Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. *International journal of molecular sciences*, *14*(5), 10197-10228.

Oliver-Williams, C., Howard, A. G., Navas-Acien, A., Howard, B. V., Tellez-Plaza, M., and Franceschini, N. (2018). Cadmium body burden, hypertension, and changes in blood pressure over time: results from a prospective cohort study in American Indians. *Journal of the American Society of Hypertension*, *12*(6), 426-437.

Omar, M. A. K., Seedat, M. A., Motala, A. A., Dyer, R. B., and Becker, P. (1993). The prevalence of diabetes mellitus and impaired glucose tolerance in a group of urban South African blacks. *South African Medical Journal* 1993; 83: 641 – 3.

Onder, S., Dursun, S., Gezgin, S., & Demirbas, A. (2007). Determination of heavy metal pollution in grass and soil of city centre green areas (Konya, Turkey). *Polish Journal of Environmental Studies*, *16*(1), 145.

Osredkar, J., and Sustar, N. (2011). Copper and zinc, biological role and significance of copper/zinc imbalance. *J Clinic Toxicol S*, *3*(2161), 0495.

Ötleş, S., and Çağındı, Ö. (2010). Health importance of arsenic in drinking water and food. *Environmental geochemistry and health*, *32*(4), 367-371.

Pandey, G., and Madhuri, S. (2014). Heavy metals causing toxicity in animals and fishes. *Research Journal of Animal, Veterinary and Fishery Sciences*, *2*(2), 17-23.

Passos, M. L., & Saraiva, M. L. M. (2019). Detection in UV-visible spectrophotometry: Detectors, detection systems, and detection strategies. *Measurement*, *135*, 896-904.

Patočka, J., and Černý, K. (2003). Inorganic lead toxicology. *Acta Medica (Hradec Kralove)*, *46*(2), 65-72

Patrick, L. (2003). Toxic metals and antioxidants: Part II. The role of antioxidants in arsenic and cadmium toxicity. *Alternative medicine review*, *8*(2).

PCRWR (2005). Pakistan Council of Research in Water Resources. Islamabad, Pakistan. Workshop to finalize National Drinking Water Quality Standards, 01-04 March, 2005. Health Services Academy, Ministry of Health, Pakistan. Availableonlineat;(http://www.pcrwr.gov.pk/wq_phase2_report/chapter_no3.pdf)

Peng, S., Liu, L., Zhang, X., Heinrich, J., Zhang, J., Schramm, K.W., Huang, Q., Tian, M., Eqani, S.A.M.A.S. and Shen, H., (2015). A nested case-control study indicating heavy metal residues in meconium associate with maternal gestational diabetes mellitus risk. *Environmental Health*, *14*(1), pp.1-10.

Pifl, C., Wolf, A., Rebernik, P., Reither, H., and Berger, M. L. (2009). Zinc regulates the dopamine transporter in a membrane potential and chloride dependent manner. *Neuropharmacology*, *56*(2), 531-540.

Plessl, C., Otachi, E. O., Körner, W., Avenant-Oldewage, A., and Jirsa, F. (2017). Fish as bioindicators for trace element pollution from two contrasting lakes in the Eastern Rift Valley, Kenya: spatial and temporal aspects. *Environmental science and pollution research*, *24*(24), 19767-19776.

Rahman, A., Vahter, M., Smith, A. H., Nermell, B., Yunus, M., El Arifeen, S., and Ekström, E. C. (2008). Arsenic exposure during pregnancy and size at birth: a prospective cohort study in Bangladesh. American journal of epidemiology, 169(3), 304-312.

Rehman, K., Fatima, F., Waheed, I., and Akash, M. S. H. (2018). Prevalence of exposure of heavy metals and their impact on health consequences. *Journal of cellular biochemistry*, *119*(1), 157-184.

Reichard, J. F., and Puga, A. (2010). Effects of arsenic exposure on DNA methylation and epigenetic gene regulation. *Epigenomics*, *2*(1), 87-104.

Roberts, T. L. (2014). Cadmium and phosphorous fertilizers: the issues and the science. *Procedia Engineering*, *83*, 52-59.

Runcie, J. W., and Riddle, M. J. (2004). Metal concentrations in macroalgae from East Antarctica. *Marine pollution bulletin*, *49*(11), 1114-1119.

Sankhla, M. S., Kumari, M., Nandan, M., Kumar, R., and Agrawal, P. (2016). Heavy metals contamination in water and their hazardous effect on human health-a review. *Int. J. Curr. Microbiol. App. Sci (2016)*, *5*(10), 759-766.

Shannon, M. W., and Graef, J. W. (1992). Lead intoxication in infancy. *Pediatrics*, *89*(1), 87-90.

Shelton, L. R. (1994). *Field guide for collecting and processing stream-water samples for the National Water-Quality Assessment Program* (Vol. 94, No. 455). US Geological Survey.

Shih, Y. H., Howe, C. G., Bryan, M. S., Shahriar, M., Kibriya, M. G., Jasmine, F., and Argos, M. (2021). Exposure to metal mixtures in relation to blood pressure among children 5–7 years old: An observational study in Bangladesh. *Environmental Epidemiology*, *5*(2).

Shirai, N., Birtles, D., Wattam-Bell, J., Yamaguchi, M. K., Kanazawa, S., Atkinson, J., and Braddick, O. (2009). Asymmetrical cortical processing of radial expansion/contraction in infants and adults. Developmental Science, 12(6), 946-955.

Singh, R., Gautam, N., Mishra, A., and Gupta, R. (2011). Heavy metals and living systems: An overview. *Indian journal of pharmacology*, *43*(3), 246.

Smedley, P. L., and Kinniburgh, D. G. (2002). A review of the source, behaviour and distribution of arsenic in natural waters. *Applied geochemistry*, *17*(5), 517-568.

Sobsey, M. D., Stauber, C. E., Casanova, L. M., Brown, J. M., and Elliott, M. A. (2008). Point of use household drinking water filtration: a practical, effective solution for providing sustained access to safe drinking water in the developing world. *Environmental science & technology*, *42*(12), 4261-4267.

Sodhi, K. K., Kumar, M., Agrawal, P. K., and Singh, D. K. (2019). Perspectives on arsenic toxicity, carcinogenicity and its systemic remediation strategies. *Environmental Technology & Innovation*, *16*, 100462.

Steinnes, E., and Friedland, A. J. (2006). Metal contamination of natural surface soils from long-range atmospheric transport: existing and missing knowledge. *Environmental Reviews*, *14*(3), 169-186.

Subhashini, V., and Swamy, A. V. V. S. (2013). Phytoremediation of Pb and Ni Contaminated Soils Using Catharanthus roseus (L.). *Universal Journal of Environmental Research & Technology*, *3*(4).

Tessier, A. P. G. C., Campbell, P. G., and Bisson, M. J. A. C. (1979). Sequential extraction procedure for the speciation of particulate trace metals. *Analytical chemistry*, *51*(7), 844-851.

Thayer, K. A., Heindel, J. J., Bucher, J. R., and Gallo, M. A. (2012). Role of environmental chemicals in diabetes and obesity: a National Toxicology Program workshop review. *Environmental health perspectives*, *120*(6), 779-789.

Tilahun, T. (2009). *Optimization of Anaerobic Co-Digestion of Sewege Sludge and Brewery Yeast Waste* (Doctoral dissertation, Addis Ababa University).

Trasande, L., Sathyanarayana, S., Spanier, A. J., Trachtman, H., Attina, T. M., and Urbina, E. M. (2013). Urinary phthalates are associated with higher blood pressure in childhood. *The Journal of pediatrics*, *163*(3), 747-753.

Tsaih, S. W., Korrick, S., Schwartz, J., Amarasiriwardena, C., Aro, A., Sparrow, D., and Hu, H. (2004). Lead, diabetes, hypertension, and renal function: the normative aging study. *Environmental health perspectives*, *112*(11), 1178-1182.

Tyrrell, J. B., Hafida, S., Stemmer, P., Adhami, A., and Leff, T. (2017). Lead (Pb) exposure promotes diabetes in obese rodents. *Journal of Trace Elements in Medicine and Biology*, *39*, 221-226.

Ullah, S., Skidmore, A. K., Naeem, M., and Schlerf, M. (2012). An accurate retrieval of leaf water content from mid to thermal infrared spectra using continuous wavelet analysis. *Science of the total environment*, *437*, 145-152.

UNFPA, United Nations Populations Founds (2011). Retrieved from http://www.unfpa.org/public/cache/offonce/home/news/pid/7204;jsessionid=6C355D2E24817C8362750097E42535EB. (Verified March 3, 2011)

United Stated Environmental Protection Agency (2011). National primary drinking waterregulations.Retrievedfromhttp://water.epa.gov/drink/contaminants/index.cfm#List (Verified November 15, 2011).

Valko, M., Rhodes, C. J. B., Moncol, J., Izakovic, M. M., and Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-biological interactions*, *160*(1), 1-40.

Vandenbossche, M., Jimenez, M., Casetta, M., and Traisnel, M. (2015). Remediation of heavy metals by biomolecules: a review. *Critical Reviews in Environmental Science and Technology*, *45*(15), 1644-1704.

Vardoulakis, S., Giagloglou, E., Steinle, S., Davis, A., Sleeuwenhoek, A., Galea, K. S., and Crawford, J. O. (2020). Indoor exposure to selected air pollutants in the home environment: A systematic review. *International journal of environmental research and public health*, *17*(23), 8972.

Wang, C., Danovich, D., Shaik, S., and Mo, Y. (2017). A unified theory for the blue-and-red shifting phenomena in hydrogen and halogen bonds. *Journal of Chemical Theory and Computation, 13*(4), 1626-1637.

Wasonga, V. O., Nyariki, D. M., and Ngugi, R. K. (2011). Assessing socioecological change dynamics using local knowledge in the semi-arid lowlands of Baringo district, Kenya.

Weiss, J. G., Yang, Y., Chan, S. J., Phillips, N. B., Smith, B. J., Whittaker, J., ... & Lawrence, M. C. (2014). Protective hinge in insulin opens to enable its receptor engagement. *Proceedings of the National Academy of Sciences*, *111*(33), E3395-E3404.

Wekesa, W. P. (2015). *Analysis of Selected Heavy Metals in Water from River Kuywa and Adjacent Wells in Bungoma Central Sub County-Kenya* (Doctoral Dissertation, School of Pure and Applied Sciences, Kenyatta University).

Wieczorek, J., Guo, Q., and Hijmans, R. (2004). The point-radius method for georeferencing locality descriptions and calculating associated uncertainty. *International journal of geographical information science*, *18*(8), 745-767.

Wieser, M. E., and Berglund, M. (2009). Atomic weights of the elements 2007 (IUPAC Technical Report). *Pure and Applied Chemistry*, *81*(11), 2131-2156.

World Health Organization, WHO/UNICEF Joint Water Supply, and Sanitation Monitoring Programme. (2015). *Progress on sanitation and drinking water: 2015 update and MDG assessment*. World Health Organization.

World Health Organization. (2004). Guidelines for drinking-water quality: recommendations (Vol. 1). World Health Organization

World Health Organization. (2008). Guidelines for drinking-water quality [electronic resource]: incorporating 1st and 2nd addenda, vol. 1, Recommendations.

Wu, B., Wu, Y., Lu, Z., Zhang, J., Han, N., Wang, Y., ... & Zeng, L. (2021). A cation selective separator induced cathode protective layer and regulated zinc deposition for zinc ion batteries. *Journal of Materials Chemistry A*, *9*(8), 4734-4743.

Wu, B., Zhang, Y., Zhang, X., and Cheng, S. (2010). Health risk from exposure of organic pollutants through drinking water consumption in Nanjing, China. *Bulletin of environmental contamination and toxicology*, *84*(1), 46.

Wuana, R. A., and Okieimen, F. E. (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *International Scholarly Research Notices, 2011*.

Yap, L. L., Lin, M. T., Ouyang, H., Samoilova, R. I., Dikanov, S. A., and Gennis, R. B. (2010). The quinone-binding sites of the cytochrome bo3 ubiquinol oxidase from Escherichia coli. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1797*(12), 1924-1932.

Yu, L., Zou, R., Zhang, Z., Song, G., Chen, Z., Yang, J., and Hu, J. (2011). A Zn 2 GeO 4–ethylenediamine hybrid nanoribbon membrane as a recyclable adsorbent for the highly efficient removal of heavy metals from contaminated water. Chemical Communications, 47(38), 10719-10721.

ZalduaEtxabe, I., CambraCotín, K., OnaindiaOlalde, C., and Varela Alonso, J. (2010). Release of lead and other metals from piping into drinking water in the Basque Country (Spain). *Gaceta Sanitaria*, *24*(6), 460-465.

Zarcinas, B. A., Ishak, C. F., McLaughlin, M. J., and Cozens, G. (2004). Heavy metals in soils and crops in Southeast Asia. *Environmental geochemistry and health*, *26*(3), 343-357.

Zierold, K. M., Knobeloch, L., and Anderson, H. (2004). Prevalence of chronic diseases in adults exposed to arsenic-contaminated drinking water. *American Journal of Public Health*, *94*(11), 1936-1937.

APPENDICES

Appendix I: Respondent's Questionnaire

My name is **Loice Chepkorir** a postgraduate student at University of Eldoret. The purpose of this questionnaire was to collect data on the assessment of heavy metal contribution to diabetes and hypertension: a case study of Marakwet East and Marakwet West sub counties. You have been chosen to take part in this study. Further, you are most assured that your identity will be treated with utmost confidentiality and the information will only be used for the purpose of this study.

Part A: Demographic profile of respondents.

1. Place of residence ______________________________Gender_____

   Job Designation____

2. How old are you?

   Below 25 yrs { }

   26-30 yrs { }

   31-35 yrs { }

   36-40 yrs { }

   41-45yrs { }

   Above 46 yrs { }

3. What is your highest academic level?

   KCPE { }

   Certificate { }

   Diploma { }

   Degree { }

Part B: Diagnosed Diabetes and Hypertension Background

4. Have you ever heard about heavy metals such as lead, zinc, and cadmium?

   Yes ( ) no ( ) I don't know ( )

5. If yes, what do you think, are the common sources of heavy metals where you live. Tick accordingly

   Plants ( )

   Water ( )

   Soil ( )

   Industries ( )

   Mining site ( )

   None of the above ( )

6. Mention the nearest water source (River, lake) that you rely on for domestic purposes.

___

___

7. Tick the disease that you were diagnosed to have

   Diabetes ( )

   Hypertension ( )

8. When (year) were you diagnosed

___

___

9. What may have contributed to you contracting the disease/condition?

___

___

Part C; Heavy Metal Toxicity Screening Questionnaire among Diagnosed Diabetic and hypertensive patients

Listed below are symptoms often found in patients who have absorbed excessive amounts of Heavy Metals: These poisonous metals include Lead, Arsenic and Cadmium. All are found in modern environments. Even if you have many of the symptoms below, you may *not* be Heavy Metal Toxic — there may be other causes.

Please put an X by all the following symptoms that apply to you. When you are finished, add the scores next to those you have marked.

Unexplained rashes or skin irritations	_____4
1. Frequent diarrhea	_____1
2. Get out of breath easily	_____4
3. Get headaches just after eating	_____2
4. Burning sensation on the tongue	_____2
5. Frequent urination during the night	_____6
6. Unexplained chronic fatigue	_____6
7. Difficulty remembering or use of memory	_____5

TOTAL _____

Score of 86-126: Strong likelihood of heavy metal toxicity.

Score of 40-85: Moderate likelihood of heavy metal toxicity.

Score or 0-39: Low likelihood of heavy metal toxicity.

(Adapted)

Other sources of Heavy Metal Toxicity apart from soil and water among diabetic and hypertensive patients

Have you worked in manufacturing or fabricating?

Metals ( )

Plastics ( )

Petroleum ( )

Rubber ( )

Textiles ( )

Glass ( )

Ceramics ( )

Paper ( )

Electronics ( )

Hot-type printing ( )

Batteries ( )

Fiberglass ( )

Have you been significantly expose to

Fertilizers ( )

Pesticides ( )

Rodenticides ( )

Herbicides ( )

Fungicides ( )

Paints and thinners ( )

Wood preservatives ( )

Batteries ( )

Alloys ( )

Dyes ( )

Have you done

Health service maintenance

Chemical processing ( )

Electroplating ( )

Soldering ( )

Welding ( )

Metal cutting ( )

Leather tanning ( )

Fireworks ( )

Metal smelting (copper, lead, zinc, etc.) ( )

Photographic darkroom work ( )

Have you ever had 10 or more silver-colored fillings in your teeth? No ( ) Yes ( )

Have you ever worked in a dental office? No ( ) Yes ( )

Do you smoke cigarettes ( )

Never smoked ( )

Smoke now, about packs ( ) a day ( ) week ( ) Quit ( ) *(when)*

If you smoked, how long? About ( ) years. How much? About ( ) packs a day ( ) week ( ).

Other tobacco use: Pipe ( ) Cigars ( ) Snuff ( ) Chew ( )

Are you exposed to second-hand smoke? No ( ) Yes ( )

Tell us about your relatives' health

Has a blood relative had any of the following? If so, check and show exact relationship: Your mother, father, brother or sister; your mother's mother, father, brother or sister; your father's mother, father, brother or sister. If person adopted, note that.

Disease or illness	Relationship
Autism Spectrum Disorder	
Cancer	
Cardiovascular disease	
Mental problems	
Parkinson's disease	
Hypertension	
Diabetes	

Appendix II: Optimum Setting Shimadzu AAS Model 6800 for Analysis of Heavy Metal Concentration in Digested Soil and Water Sample (Meat)

Parameter	Zn	Cr	Pb
Wavelength (nm)	279.5	217.0	248.3
Lamp current (mA)	5.0	5.0	8.0
Band pass (nm)	0.5	1.0	0.3
Burner height in mm	22.0	20.0	20.0
Fuel and oxidizer flow rate cm^3 /min	5.0	5.0	5.0

Appendix III: Similarity Report

Turnitin Originality Report

ASSESSMENT OF ZINC,LEAD AND CADMIUM CONTRIBUTIONS TO DIABETES AND HYPERTENSION: A CASE STUDY OF MARAKWET EAST AND MARAKWET WEST SUB COUNTIES by Loice Chepkorir

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