ANALYSIS OF FLUORIDE AND SELECTED HEAVY METALS IN KALES *(Brassica oleracea)* AND TOMATOES (*Lycopersicum esculentum*) FROM HORTICULTURAL FARMS IN NAKURU COUNTY, KENYA

BY

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DECLARATION

DECLARATION BY THE STUDENT

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DEDICATION

To my husband, Mr. Kosgey, and to my children, Joylyne, Victor, Jayden and Blessing; who have filled my cup with joy and for their moral support permitting me to pursue my dreams. Equally to my mum Mrs. Veronica Maina, and to my late dad Mr. Tito Maina, for their determined efforts and sacrifices, they gave me their best that I could become the best in life .

ABSTRACT

Recently field-grown vegetables including curly kales and endive have been reported to accumulate high fluoride levels in their leaves. As vegetables normally form an integral portion of the human diet, the occurrence of high fluoride (F) in their edible portions can cause significant human exposure to these fluoride residues. Nonetheless, chemical residues normally do not occur in isolation under natural environments and their availability is strongly controlled by their chemical and biochemical interactions with co-existing residues. In this respect, heavy metals and other contaminants have therefore been studied extensively especially in recent years. However, the interactions between metal contaminant ions with fluoride in polluted environments remains unclear. In this study levels of fluoride and selected heavy metals in kales and tomatoes from Nakuru County have been investigated and compared with the World Health Organization/Food and Agriculture Organization standards. Kales and tomatoes samples were obtained from selected horticultural farms from all the eight regions in Nakuru County. Fluoride in kales and tomatoes was extracted using a 6M sodium hydroxide solution and determined using the F ion-selective electrode. The levels of heavy metals in the two sets of samples were determined using atomic absorption spectrophotometry. The F and heavy metal measurements were based on calibrations prepared from respective standards in the linear ranges of 0.0–20.0 mg/L and 0.0 – 5.0 mg/L, respectively. The overall mean concentrations of F levels in tomatoes was 12.94 ± 0.35 mg/kg while the overall mean ranges for the selected heavy metals were: 0.23±0.00–0.38±0.06 mg/kg for Cu; 0.18±0.00–0.31±0.02 mg/kg for Zn; 0.69±0.20–0.91±0.01 mg/kg for Cr; 0.08±0.00–0.13±0.01 mg/kg for Mn and 0.71±0.05–0.87±0.04 mg/kg for Fe. In kales, the corresponding average F level was 13.33±0.29 mg/kg while mean concentrations ranges for the selected heavy metals were: 0.05±0.00–0.07±0.02 mg/kg for Cu; 0.20±0.01–0.37±0.03 mg/kg for Zn; 0.80±0.08–1.11±0.04 mg/kg for Cr; 0.10±0.01–0.13±0.01 mg/kg for Mn and 0.30±0.02–0.97±0.1 mg/kg for Fe. Fluoride concentrations in all the samples were above the WHO/FAO maximum allowable limits but those for the metals in the samples were within the recommended limits. There was a significant negative correlation between fluoride concentration with Zn and Cu concentration in tomatoes, whereas in kales significant positive correlation was found between fluoride concentration with Cr ion concentration.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYNMS

AAS	Atomic Absorption Spectrometer
AG	Analytical grade
EPA	Environment Protection Agency
F	Fluoride
FAO	Food and Agriculture Organization
NaOH	Sodium hydroxide,
pH	Concentration of Hydrogen Ions
TISAB	Total Ionic Strength Adjustment Buffer
WHO	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Fluoride is known to play a vital role in the development of strong teeth and bones when it is consumed in adequate amounts. It contributes to good dental hygiene by mitigating against dental carries. However, the consumption of high levels of fluoride in water and food leads to disease conditions that adversely affect the skeletal structures (Dhar & Bhatnagar, 2009).Excess fluoride primarily accumulates in bony tissues of animals resulting in dental and skeletal degradation. Prolonged exposure to fluoride in drinking water has therefore been linked to fluorosis both in domestic animals (Choubisa *et al.*, 2011) and in human beings (Kumar & Puri, 2012).

The primary manifestation of fluorosis is osteosclerosis of the skeletal structures and mottling of teeth which are respective usual symptoms for dental and skeletal fluorosis (Mishra & Pradhan, 2007). Nonetheless, fluorosis that does not affect skeleton causes teratogenic effects, reproductive dysfunctions, neurological disorders, and gastrointestinal disturbances as typical non-skeletal manifestations of fluoride toxicity in man and animals (Haenlein & Anke, 2011). Still, some researchers indicate that fluoride exposure could be the cause of low birth-weights in certain animal species (Haenlein & Anke, 2011).

The chronic toxicity effects, prevalence, and severity of fluoride vary in proportion to the consumed amounts, duration of exposure, and the frequency of contact with toxic levels of fluoride (Choubisa, 2012). Naturally, all foodstuffs contain some amounts of fluoride (F) (Fawell *et al*., 2006) but some of the highest fluoride levels in Kenyan foodstuffs have been found in vegetation from parts of Nakuru County, Kenya (Kahama *et al*., 1997). Field-grown vegetables from these areas including curly kales (40 mg/kg fresh weight) and endive (0.3–2.8 mg/kg fresh weight) have been found to accumulate elevated fluoride levels (McLaughlin *et al.*, 2011). Besides vegetables, other foods that have been reported to contain high levels of F include fish (0.1-30 mg/kg) and tea (Jackson & Alloway, 2017) particularly high amounts (100 mg/kg) of F have been reported.

High concentrations of fluoride in farm plants result from plants taking up high natural concentrations F from polluted soils and waters and through the application of F contaminated farm inputs. Soil F may accumulate in growing plants by absorption through roots in the amount that is dependent upon the quantity and type of F speciation found in the particular soils, type of soil, and the type of plant. Fluoride absorbed by plants through a gaseous exchange is another accumulative poisoning process to the plants that occurs through plant foliage (Nagajyoti *et al.,* 2010).

However, it is well known that chemical residues do not normally exist in isolation in natural environments. The bioavailability of essential minerals, as well as potentially harmful chemical residues in the environment, is controlled by many chemical and biochemical interactions among co-existent residues in the environmental systems. For these and their ecological and public health effects, heavy metals, and other contaminants have extensively been studied (Zhao *et al*., 2012). Nonetheless, the interaction of fluoride with typical heavy metals in co-polluted environments remains unclear. Furthermore, heavy metal pollution of foodstuffs remains a major environmental concern that is increasingly becoming severe all over the world and more so, among peri-urban and urban environments in the developing nations (Akinyele & Shokunbi, 2015). This is because plants are inclined to absorb and retain heavy metals over prolonged periods due to their biodegradability and persistent nature in the environment (Ashraf *et al.,* 2011). Thus, the concentrations of heavy metals tend to increase through the trophic levels by bio-magnification processes. Human beings at the tail end of the food chain are, therefore, always at the greatest risk of exposure to heavy metals at unhealthy concentrations.

Vegetables form an integral portion of the human diet and humans require large volumes of vegetables in their diet as a source of vital vitamins, essential minerals, other nutrients, and fiber. People who eat more vegetables and fruits as part of an overall healthy diet are likely to have a reduced risk of chronic diseases including stroke, cancer, heart diseases, and type-2 diabetes (Schwingshackl *et al.,* 2019) . Kales and tomatoes are the two leading vegetable items consumed in large proportions by the population in Kenya and the entire Eastern Africa region. It is estimated that 96% and 82% of urban households in Kenya purchase tomatoes and kales respectively for their everyday consumption (Van der Lans *et al.,* 2012; Gemechis *et al.,* 2012).

The current study was therefore designed to study the occurrence of F and selected heavy metals in kales and tomatoes from Nakuru County , Kenya. The study aimed to provide information on F and heavy metals that is Fe, Zn, Cu, Cr, and Mn levels in kales and tomatoes consumed by farmers and consumers in central Rift Valley in Nakuru County, Kenya. Information on levels of F and heavy metals could be useful to policymakers to influence positive intervention policies on F and heavy metal pollution in Nakuru County, Kenya.

1.2 Statement of the problem

Fluoride is an essential micronutrient in animal diets and adequate amounts of ingested F is important for the healthy development of teeth and bone and it is also desired for the prevention of dental caries. However, ingestion of high F leads to disease conditions, and millions of people globally are affected by the toxic effects of fluoride overexposure (Ayoob & Gupta, 2006). Fluoride toxicity arises from excessive prolonged F intake from a variety of natural or man-made sources and it can lead to skeletal and dental fluorosis, which are the most known hazardous effects of high F levels in humans.

Fluoride has a long-term toxic effect on plant growth through its accumulation and retention in vegetative tissues. The representative data about the role of leafy vegetables in human overexposure to fluoride and its impacts on human health in many parts of the world including Kenya remains inadequate. Similarly, heavy metals such as Fe, Zn, Cu, Cr, and Mn may be present in the environment through natural and anthropogenic causes and their presence in trace amounts is essential to both animal's and plants' life. Nonetheless, over-exposure to metals is detrimental to chronic health effects (Kolnagou *et al.,* 2013).

The present investigation was, therefore, established to determine the levels of F and heavy metals in kales *(Brassica oleracea)* and tomatoes (L*ycopersicum esculentum*) from horticultural farms in Nakuru county, Kenya and to investigate relationships that exist between levels of these environmental residues in co-polluted areas. It was anticipated that the resulting data would be relevant for awareness creation, among the scientific community and in the general public within the affected areas about the potent risk of human over-exposure to F and metal residues.

1.3 Justification

Vegetables, especially tomatoes and kales contain phytochemicals that have anti-inflammatory, enzyme inhibiting and bioactive properties capable of combating the activities of oxidants in the human body. They are also the most affordable vegetables in Kenya as they are fast-growing and they normally have high yields at minimum inputs. This makes them the most convenient horticultural products for low-income rural communities throughout the country. For this reason, the average consumption of tomatoes and kales in Kenya is very high and it has been estimated that at least 96% and 82% households in Kenya purchase and consume tomatoes and kales daily respectively (Van der Lans *et al.,* 2012; Gemechis *et al.,* 2012).

Nakuru County, which is the focus of the current study is among the most populous counties where huge volumes of tomatoes and kales are cultivated and consumed in Kenya. Nonetheless, the area is also a well known high fluoride area in the world (Wambu & Muthakia, 2011), and persistent heavy metal residues have also been reported there (Jirsa *et al*., 2013). Exposure to toxic heavy metals and high levels of F residues present in edible vegetables may pose a risk to the human body by resulting in maladies that affect the brain, heart, liver, kidney, lungs, and central nervous system (Prasad, 2013).

The present work studied the levels of heavy metals and F in tomatoes and kales. It was anticipated to reveal data that would contribute to a deeper understanding of the distribution of toxic residues in food chains through agricultural products. It was hoped that the accrued data would be found relevant in informing ongoing efforts to design strategies to enhance food safety and safeguard public health among the affected communities.

1.4 Significance of the study

The results from this study, it was projected, would help in-depth understanding of the existence of possible relationship between the levels of heavy metals and F in plants resulting in a transfer to human beings. This way, the current findings could disclose further information that could be useful in designing corrective and preventive actions to lessen or eliminate the threat of F and heavy metals contamination in Nakuru County.

Apart from enhanced awareness among the stakeholders, it was anticipated that such information would be useful to policymakers in formulating policies for F and heavy metals remediation in the affected areas.

Environmental authorities could also utilize the data in educating environmental stakeholders and creating awareness among the larger human population on potential overexposure to F and heavy metals through vegetables cultivated from polluted sites. From these results, they would then propose mitigation measures to safeguard public health as well as mitigate the impacts of F and heavy metal pollution in the environments. It was also hoped that the data obtained would form a background upon which significant subsequent studies in related fields would be anchored.

1.5 Objectives

1.5.1 General objectives

The broad objective of this study was to analyze fluoride and heavy metals in kales *(Brassicca oleracea)* and tomatoes (L*ycopersicum esculentum*) from Nakuru county, Kenya.

1.5.2. Specific Objective

The specific objectives of this study were:

i. To determine the levels of F in samples of kales and tomatoes obtained from horticultural farms in Nakuru County, Kenya.

ii. To determine concentration levels of selected heavy metals that is Fe, Zn, Cu, Cr, and Mn in samples of kales and tomatoes obtained from horticultural farms in Nakuru County, Kenya.

iii. To establish the relationships that exist between levels of F and those of the selected heavy metals in kales and tomato samples from horticultural farms in Nakuru County, Kenya.

1.6. Hypothesis

Ho: There is low occurrence of fluoride in kales and tomatoes from Nakuru County

Ho: Heavy metals concentration in samples of kales and tomatoes obtained in horticultural farms in Nakuru County is high.

Ho: Their is no relationships that exists between levels of F and those of selected heavy metals in kales and tomato samples from horticultural farms in Nakuru County, Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Extent of vegetable production in Nakuru County, Kenya

Because of its rich agricultural hinterland, Nakuru County is often reffered to as the 'farmers capital' of Kenya and is famous for its agro-based industries. There is a lot of agriculture, which is practiced in the county both for food and for commercial purposes. Agriculture is, therefore, the main land use and the economic mainstay of the county (Kinambuga, 2010). Nakuru County Integrated Development Plan of 2015 showed that the total land area under agriculture in Nakuru County was about 71,400 and 243,700 ha for cash crops and food crops, respectively. A study conducted by Foeken *et al.*, (2002) showed that the three crops that stand out by household frequency (around 60%) are: kales commonly called *sukuma wiki* (*Brassica oleracea*), maize (*Zea mays*) and beans (*Phaseolus vulgaris*). Other common crops of horticultural importance are onions (*Allium cepa*), tomatoes (*Lycopersicum escaletum*), Irish potatoes (*Solanum tuberosum*), bananas (*Musa ssp.*), cowpeas (*Vigna unguiculata*) and spider plant (*Cleonegynandra*). Vegetables contain variable amounts of fluoride ions, depending on location of origin, age and part of the plant (Owuor, 1985).

The primary contributing factors for prevalence of high agricultural activities in these areas include the conducive climatic conditions with adequate rains in most parts of the county, rich volcanic soils that provide great perspective for crops, well-developed road network, enabling structures available to the farming communities, readily available labor force from its high population and the availability of market for farm produce from both its own urban centers and from other major ones such as Nairobi County (Nakuru County Integrated Development Plan, 2015).

2.2 Fluoride occurrence in environmental samples

Fluoride (F) is generally used in environmental terms to refer to all forms and speciation fluorine in the environment (Jha*et al.,* 2011). This is because fluorine is the most electronegative and most reactive of all the elements. It reacts supremely with all known natural materials and it has never been detected in its elemental state in nature, except under highly controlled industrial environments (Halka & Nordstrom, 2010).

Soils, water, plants, and animal tissues contain inorganic F (Soetan *et al.,* 2010). The notable soil fluoride minerals in nature include: fluorspar, CaF_2; Cryolite, Na_3AlF_6; and fluorapatite, $3Ca_3\ (PO_4)_2Ca\ (FCl_2)$ (Ochieng, 2014). Most of the geogenic fluoride results from past and present volcanic action. The contribution of volcanicity to the F content of the earth's atmosphere is 1.7×10^6 tonnes per year (Amini *et al.,* 2008). However, most airborne F comes from industrial sources associated with aluminum smelting in urban areas, ceramic production, and manufacture of phosphatic fertilizers (Jha *et al.,* 2011). The greatest single atmospheric F contaminant is hydrogen fluoride (HF) because it is extensively used in industries creating a major atmospheric pollution hazard (Amini *et al.,* 2008).

Anthropogenic F emissions results from among other sources, the combustion of fluorine-containing materials that release HF as well as particulate F into the air. Coal, for example, contains small but significant amounts of F. This has led to coal-fired power plants constituting the largest source of anthropogenic HF emissions (Haneef & Akıntuğ, 2016). (Brougham *et al., (*2013) also added that other major sources of industrial F emissions are aluminum production plants and phosphate fertilizer plants which both emit HF and particulate F. Other industries releasing HF are: chemical production, magnesium, steel, structural clay products and brick-making (Förstner & Wittmann, 2012). Hydrogen fluoride would also be released by municipal incinerators as a consequence of the presence of F-containing material in their waste stream (Förstner & Wittmann, 2012).

In soils, F is found in minute trace amounts up to about 0.1% but heavy use of phosphatic fertilizers and limestone can avertedly increase the F content of agricultural soils (Otta, 2009).

Brindha & Elango (2011) indicated that areas of extensive deposits of rocks containing high F cause elevated F content in water and food, which leads to human exposure to the contaminant. Some water sources in Kenya, especially in Rift Valley contain elevated F concentrations of up to 33 mg/L (Syume & Chandravanshi, 2015; Nigus & Chandravanshi, 2016), which is higher than the WHO recommended maximum of 1.5 mg/L for household water (Ochieng, 2014). For that reason, a recent analysis of hydrochemical, economic, and demographic factors about spatial dissemination of high-F domestic water sources indicates that fluorosis is increasingly becoming a serious public health concern in the Rift Valley (Kebede *et al*., 2016), Fluoride concentrations above 5.0 mg/L have been found in hot springs (100% of all sources), lakes (78%), shallow wells (54%) and in boreholes (35%) (Kut *et al.*, 2016).

In the same way, all vegetation contains some F which is absorbed from soil and water. The highest levels of F up to 40 mg/kg fresh weight in field grown vegetables are found in curly kales (Sudjalim *et al.*, 2006). However, the F levels of vegetation and in food depend upon the nature of soil and quality of irrigation water and on the specifc plant species (Saxena & Sewak, 2015).

Fluoride is taken up by plants passively through a process which is dominantly diffusion controlled. However, the plants uptake of F through roots is largely dependent on the concentration of F ion in the soil, the type of soil and on the soil pH. At neutral pH, F is bound to soil surfaces and its generally unavailable to plants. In an acidic environment ($pH < 6$) however, F leach into water and form $(AlF)^{2+}$ complexes (Neal, 1995). Thus, fluoride ion (F^-) is more labile in acidic soils due to which its uptake by plants is enhanced.

In F polluted atmospheres, significant amounts of F is taken up by plants through the stomata (Álvarez-Ayuso *et al*., 2011). The absorbed fluoride mainly accumulates in the leaves and leads to visible leaf injury, damage to fruits and changes in the yield of crops because it interferes with efficiency of the leafy plants to photosynthesize (Yadav *et al.*, 2012). Good correlations have been found between F concentrations in air and F content in leaves in the vicinity of Al smelters (Saini *et al.,* 2013). Research by Manahan (2017) indicates that people living near hazardous waste sites have a higher chance of being exposed to high levels of F by similar routes. He added that respiratory F is almost negligible under occupational exposure conditions.Vegetables and fruits grown near such areas may contain higher levels of F principally from F^{-}containing dust settling on the plant (Spittle, 2018).

Additionally, fluoride is a strong ligand and F pollution can lead to increased solubility and uptake of Al and other metals by plants (Spittle, 2018). The objective of the current work is therefore to investigate the existence of synergy if any, between the occurrence of fluoride and common metal pollutants in two of the most common vegetables that are, kales and tomatoes from the high fluoride areas in Nakuru, Kenya.

2.2.1 Concentration levels of fluoride in samples of kales and tomatoes

Fluoride content in different vegetables in Nairobi- Kenya ranged from 1.2 to 5.4 mg/kg (Njenga *et al.,* 2005). Some vegetables contained higher F levels than the limits recommended by the WHO/FAO and particularly high F concentrations of 25.7 mg/kg (Gautam *et al.,* 2010; Bhargava & Bhardwaj , 2009; EFSA 2010). Also, Saxena & Sewak (2015) have reported 29.15 mg/kg of F in kales and also in cabbage ranging between 2-20 mg/kg. The latter levels would easily exceed the recommended daily F intake of 3 mg/day for 60 kg body weight for food (EFSA , 2010).). High levels of F in vegetables are usually due to the F in water used in their irrigation.

2.2.2 Fluoride health risk

An individual's exposure to F varies significantly (Sudjalim *et al.*, 2006) depending on the person's age, weight, diet, drinking-water source, frequency of use of F containing products, and climate (Ayoob & Gupta, 2006). Also, the potential of intakes of F through water, beverages, and foods varies from place to place according to the background levels of fluoride in soil, water, atmosphere, and diet (Syume & Chandravanshi, 2015; Nigus & Chandravanshi, 2016). Prolonged fluoride over-exposure, the pathway, and origin not withstanding have been linked to serious maladies that affect bones and leads to mottled teeth (Shomar *et al*., 2004). Ullah *et al.*, (2017) indicates that F toxicity generally depresses thyroid activity and it has been associated with premature aging of the human body. Nonetheless, the most profound health effect of fluoride appears to be dental and skeletal fluorosis.

2.2.3 Dental fluorosis

Mehrotra *et al.*, (2014) in their review titled “Tell-Tale Shades Of Discolored Teeth” have indicated that enamel fluorosis is a dose-related mottling of enamel that can range from mild discoloration of the tooth surface to severe staining and pitting as portrayed in Figure 2.1a. Dental fluorosis is permanent after it develops in children during tooth formation, from birth to about the age of 8 years (Mascarenhas, 2000). Long-lasting enamel fluorosis is characterized by dark yellow to brown tint, discrete, and confluent pitting which constitutes enamel loss (EPA, 2006). In severe cases, it can result in loss of tooth function. Many of the studies from various parts of the world reported the development of dental fluorosis even in areas where the people consumed drinking water with F less than 1.0 mg/L, which implies that the optimal F dose level in drinking water may vary with various features like local climatic conditions, methods of food processing and cooking, amount of food and water intake (Viswanathan *et al.*, 2009).

2.2.4 Skeletal fluorosis

Skeletal fluorosis (Figure 2.1b) is a bone and joint disorder associated with long exposure to high concentrations of F. Petrone *et al.* (2011) indicated that Fluoride increases bone density and appears to exacerbate the growth of osteophytes present in the bone and joints which results to joint stiffness and pain. There are four stages in which the conditions are categorized: a preclinical stage and three clinical stages that increase in severity. At the preclinical stage, mobility is not significantly affected, but it is characterized by chronic joint pain, arthritic symptoms, slight calcification of ligaments, and osteosclerosis of the cancellous bones. The most severe stage is the three clinical-stage which historically has been referred to as the "crippling" stage. Environmental Protection Agency, Maximum Contaminant level Goal of 4 mg/L protects against these precursors of more serious mobility problems (EPA, 2006).

[redacted]

Figure 2.1: Cases of fluorosis, dental (a) and (b) skeletal fluorosis (Shaw , 2015)

2.3 Heavy metals Concentration in vegetables

2.3.1 Human health and Heavy Metals

Although there is no clear definition of what a heavy metal is, density is in most cases taken to be the main criterion. Heavy metals are thus commonly defined as those having a specific density of greater than 5 g/mL (Jarup, 2003). The key sources of the heavy metals in plants are the plants' growth media, which include: the soil and water (Ashraf & Mian, 2008). Agro-inputs such as herbicides, insecticides, and fertilizers may be absorbed through the leaves, roots, and barks of the plant Moseti *et al.* (2013). Rainfall in atmospheric polluted areas due to high traffic density and industrialization are other sources of heavy metals (Lozak *et al.*, 2002; Sobukola *et al.*, 2010).

Levels of essential elements in plants are conditional, the content is mainly controlled by the characteristics of the soil and the ability of plants to selectively accumulate some metals (Sobukola *et al.*, 2010). Also, heavy metals are not biodegradable, have long biological half-lives, and persist in different body organs, a phenomenon called bioaccumulation, where they eventually lead to unwanted side effects (Jarup, 2003; Sathawara *et al.*, 2004). Such elements include Pb and Zn and are toxic to humans even at very low concentrations and have been associated with the etiology of several diseases especially cardiovascular, kidney, nervous as well as bone diseases (Steenland & Boffetta, 2000; Jarup, 2003). Thus, the biological importance, toxicological information, and possible sources of selected heavy metals are briefly mentioned below.

2.3.2 Zinc

Zinc is a vital trace element to all organisms and is present in many enzymes. It is found in dehydrogenase, functioning as a catalyst in hydride transfer in co-enzymes such as flavin coenzymes (Bugg, 2012). The two Zn enzymes which have received much attention are carboxypeptidase which catalyzes the hydrolysis of the terminal peptide bond in proteins during the digestion process and carbonic anhydrase which catalyzes the equilibrium reaction:

$$CO_2 + H_2O \longrightarrow HCO_3 + H^+ \ldots\ldots\ldots\ldots \qquad (1)$$

In mammalian erythrocytes, the forward reaction (hydration) occurs during the uptake of CO_2 by blood in tissues, while the backward reaction (dehydration) takes place when CO_2 is subsequently released in the lungs. The enzyme increases the rates of these reactions by a factor of about one million (Ranjith-Kumar *et al*., 2003).

The metal is non-cumulative and the amount absorbed is believed to be inversely proportional to the amount ingested. Zinc deficiency causes lesions of the skin, abnormalities of the skeleton, and defects in the reproductive organs especially testicular development in males (Soetan *et al.,* 2010). Zinc is moderately toxic, causing mental fume fever and pulmonary disorders, which have was observed in industrial workers exposed to the fumes (Plum & Haase, 2010). Casual ingestion of $ZnSO_4$ causes drowsiness, lethargy, and increased serum lipase and amylase levels. Patients with renal failure resulting from hemodialysis have been observed to have acute toxicity of zinc. Vomiting, fever, and severe anemia have also been observed (Alala, 1981).

Zinc tends to be found only in trace amounts in uncontaminated surface and ground waters. However, it is often found in domestic supplies at consumers' taps as a result of corrosion of galvanized iron piping or tanks and dezincification of brass fittings. Zinc has a threshold taste at approximately 5 mg/L and can also cause opalescence above this value (Otta, 2009).

2.3.3 Copper

The element is essential to all organisms and is a constituent of redox enzymes and O_2-transport pigments in certain animal species. The element is essential in the maintenance of vascular and skeletal integrity and the central nervous system. It occurs in metalloproteins such as hemocyanin (an oxygen carrier in mollusks and anthropoids), cytochrome oxidases, and plastocyanin (Otta, 2009). The cupric ion is the one that serves as an oxidative catalyst for example, in the oxidation of ascorbic acid by molecular O_2 to form dehydroascorbic acid. Copper deficiency causes anemia, neonatal ataxia, lack of pigmentation, connective tissue defects including bone disorders and cardiovascular failure (Soetan *et al.*, 2010).

Copper is however very toxic to most plants, highly toxic to invertebrates and moderately so to mammals. In trace amounts, copper is beneficial and essential for the nutrition in aquatic environment but increased levels of copper in receiving waters have profound and detrimental impact on the resident biota (Stauber & Davies, 2000).

Copper naturally enters the water from soils and mineral deposits by erosion action of water (Rickson, 2014). The main anthropogenic sources of copper are power plants, municipal wastewater discharge, industrial processes, and agricultural activities. Copper is toxic to humans in large quantities, resulting in Wilson's disease Parmar & Thakur, (2013). This is a hereditary metabolic disorder, which results in the accumulation of copper in some organs of the body, mainly brain and liver being the most sensitive. The disease is transmitted as an automatic recessive trait. Sheep can also be affected by copper toxicosis under practical husbandry (Scheiber *et al.,* 2014).

2.3.4 Chromium

Humans and animals need Chromium in the form of Cr^{3+}. The most prevalent human effect is chromium allergy caused by occupational exposure to Cr (VI) compounds (Hansen *et al.,* 2003). It is necessary for the metabolism of insulin and essential for animals (Soetan *et al*., 2010). For plants, it is not known whether it is an essential nutrient although plants contain the element (Soetan *et al*., 2010). Further, human occupationally exposed to high levels of the element may include respiratory, circulatory, digestive excretory systems effects and increased risk of death from lung cancer (Mishra & Bharagava, 2016). Chromium in high concentration can be toxic to plants (Singh *et al.*, 2013).The main feature of Cr intoxication is chlorosis, which is similar to Fe deficiency (Singh *et al.*, 2013). Chromium (III) is not considered carcinogenic while Chromium (VI) is more toxic to organisms than Cr (III). Chromium (VI) is some how inert in air , but it is reduced to Cr (III) when it interacts with organic matter in biota, soil and water (Singh *et al.*, 2013).

2.3.5 Manganese

Manganese, whose compounds are very common on earth, is a toxic essential trace element. The uptake of manganese by humans mainly takes place through food such as vegetables, tea, grains, rice, olive oil, eggs, and nuts (Miano *et al.*, 2014). Shortages of manganese in the human diet can cause health effects such as fatness, glucose intolerance, blood clotting, skin problems, lowered cholesterol levels, skeleton disorders, birth defects, changes of hair color, and neurological symptoms (Datta, 2004). Manganese is necessary for the production of manganese superoxide dismutase, which is one of the key antioxidants in the body. Enzymes involved in cholesterol synthesis are manganese dependent hence deficiency can decrease sex drive. Manganese is required for adrenal gland activity, normal thyroid, and is essential for the formation of thyroxine that is necessary for vitamin K production. Manganese deficiency can cause dizziness and deafness. Manganese helps treat myasthenia gravis and is important in the treatment of multiple sclerosis and diabetes (Ghasemi, 2016).

Toxic effects of manganese occur mainly in the brain and in the respiratory tract (Normandin *et al.,* 2004).Common symptoms of manganese poisoning include forgetfulness, hallucinations and nerve damage (Wongwit *et al.,* 2004). Manganese can also cause Parkinson disease, bronchitis and lung embolism (Lucchini *et al.,* 2009). When men are exposed to manganese for a longer period of time, they may become impotent. Manganese poisoning also manifests itself by weak muscles, headaches, schizophrenia, insomnia and dullness. Further, the conseqeunces of chronic manganese poisoning may result from prolonged inhalation of fumes and dust. Thus, central nervous system is the chief spot of damage from the disease, which may result in permanent disability. Symptoms include emotional disturbances, spastic gait, languor, sleepiness, weakness, paralysis and recurring leg cramps. Incidences of pneumonia and other upper respiratory infections has been found to be high in workers exposed to dust or fume of manganese compounds (Al-Terehi *et al.,* 2015). Manganese uptake through the skin can cause tremors and coordination failures.

Manganese ions in plants are transported to the leaves after uptake from soils (Singh *et al.,* 2013). Manganese can cause both deficiency and toxicity symptoms in plants (Al-Terehi *et al.,* 2015). Manganese deficiencies are more common when the pH of the soil is low. Highly toxic concentrations of manganese in soils can cause brown spots on leaves, swelling of cell walls and withering of leaves. The concentration of manganese required for optimal plant growth can be detected between toxic concentrations and concentrations that cause deficiencies (Aref, 2011).

2.3.6 Iron

Iron is found in several proteins, it oxidizes compounds in many enzymes whose reactions are widespread in metabolism where absorption occurs principally in the duodenum and proximal jejunum (Jomova & Valko, 2011; Gulec *et al.*, 2014). According to Jomova & Valko (2011), iron is essential for making new cells, hormones, amino acids, and neurotransmitters.

Hemoglobin which is the protein in red blood cells which carries oxygen to tissues has almost two-thirds of Fe in the body (Smith, 2013). Smaller amounts of Fe are found in myoglobin, in enzymes that assist biochemical reactions and a protein that helps supply oxygen to muscle (Smith, 2013). Iron is also found in proteins that transport Fe in blood and that which store Fe for future use. Iron is found in animal foods that originally contained hemoglobin, such as fish, red meat and poultry. Iron in plant foods such as vegetables, lentils and beans are arranged in a chemical structure called nonheme Fe (Smith, 2012). Heme Fe is absorbed better than nonheme Fe about 10% to 15% of dietary Fe is absorbed by healthy adults by single absorption (Abbaspour *et al*., 2014). Storage levels of Fe have the highest influence on Fe absorption, absorption increases when body stores are low while when Fe stores are high, absorption declines to help protect against toxic effects of Fe overload (Hurrell & Egli, 2010). Iron absorption is also influenced by the type of dietary consumed. Absorption of heme Fe from meat proteins is effective. Absorption of heme Fe ranges from 15% to 35%, and is not expressively affected by diet (Sharp, 2010). In contrast, 2% to 20% of nonheme Fe in plant foods such as rice, maize, black beans, soybeans and wheat is absorbed (Teucher *et al.,* 2004). Nonheme Fe absorption is significantly influenced by various food components such as fruit, vegetables, tea, wine, and spices (Abbaspour *et al*., 2014).

According to a research done by Pereira & Vicente, (2013) results indicated that meat proteins and vitamin C improve the absorption of nonheme Fe (Tannins, polyphenols, calcium, and phytates) can decrease absorption of nonheme Fe (Petry *et al.,* 2015). Some proteins found in soybeans also inhibit nonheme Fe absorption (Miret *et al.,* 2003). Nonheme Fe is important to be included in foods to improve absorption when daily Fe intake is less than recommended, when Fe losses are high (which may occur with heavy menstrual period), when Fe requirements are high (as in pregnancy), and when only vegetarian nonheme sources of Fe are consumed (Hurrell & Egli, 2010).

2.4 Concentration of selected heavy metals in vegetables

Vegetables constitute essential diet components by contributing Fe, manganese, copper, chromium and other nutrients, which are usually in minute quantity (Lyimo *et al.,* 2003). Türkdogan *et al., (*2003) added that they function as buffering agents during the digestion process. On the other hand, they contain both essential and toxic elements over a wide range of concentrations (Türkdogan *et al.*, 2003).

Plants take up heavy metals by absorbing them from contaminated soils, water and air (Zurera-Cosano *et al.*,1989). Moreover, some groups of people seem to be more exposed especially vegetarians (Zurera-Cosano *et al.*, 1989).

According to Chronopoulos *et al. (*1997), metal contamination of garden soils may be widespread in urban areas due to past industrial activity and the use of fossil fuels. Soil is also polluted through application of chemical fertilizers (like phosphate and Zn fertilizers), and herbicides (Demirezen & Aksoy, 2004). Heavy metal accumulation in soils is of concern in agricultural production. Heavy metals accumulation in plants depends upon plant species, and the efficiency of different plants in absorbing metals is evaluated by either plant uptake or soil-to-plant transfer factors of the metals (Khan *et al.*, 2008). Fazli (2016) reported that those vegetables, grown in heavy metals contaminated soils have higher concentration of heavy metals than those grown in uncontaminated soil. Voutsa *et al.*, (1996) reported that there have been a number of studies which have investigated atmospheric deposition of heavy metals in soil and/or vegetables growing in vicinity of industrial areas.

The resulting food chain contamination is one of the important pathways for the entry of these toxic pollutants into the human body (Yadav *et al.*, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

3.1.1 Study Area: Location and Size

The current study was based in Nakuru County as shown in Figure 3.1, which is one among the 47 Counties that were formed after the promulgation of a new consititution in Kenya in the year 2010. It is situated at a distance of 167 km from Nairobi the capital city of Kenya within latitude 0°18'11.27"S and longitude 36° 4'18.44"E. Nakuru County has an area of 2,325.8 km^2 (KPHC, 2009).

3.1.2 Administrative Sub-divisions

There are nine sub-counties in Nakuru county. These are Nakuru North, Gilgil, Nakuru, Rongai, Naivasha, Subukia, Molo, Njoro, and Kuresoi (Hussein & Wanyoike, 2015).

3.1.3 Population Size and Composition

The total population of Nakuru County was 2.162 million people in 2019 .Where males were 1,077,272, females were 1,084,835 and unisex were 95 (Kenya National Population and Housing Census, 2019).

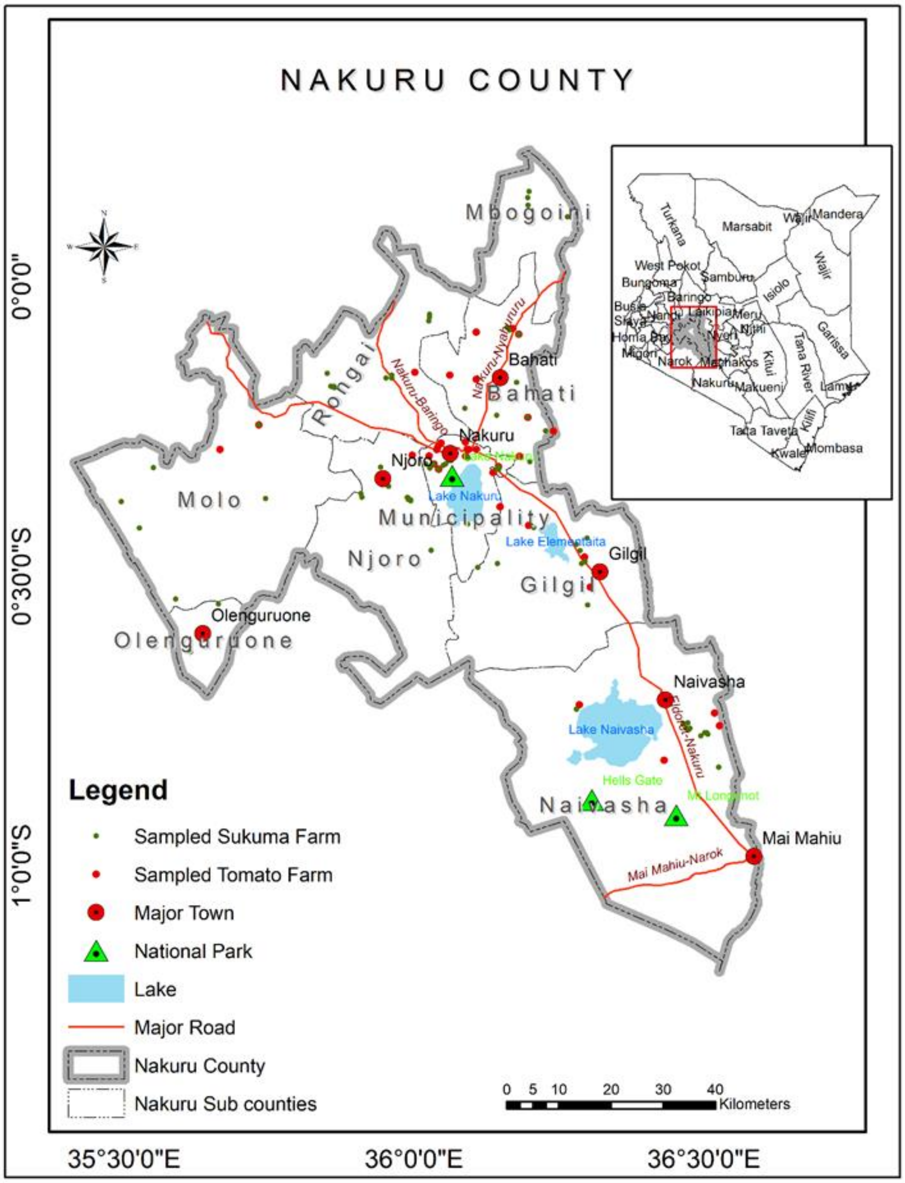

Figure 3. 1: The map of the study area Nakuru County (Source:www.google maps).

3.1.4 Climatic Conditions

The area lies within the Great Rift Valley and receives annual rainfall that averages 800 mm (Ogeto *et al.,* 2012). The climate of Nakuru County is strongly influenced by the altitude and physical features, particularly the Rift Valley itself. There are three broad climatic zones (II, III and IV). Zone II covers areas with an altitude between 1980m and 2700 m and it receives minimum rainfall of 800 mm per annum. It covers most of the county moreso Upper Subukia, Rongai and Mau Escarpment. Zone III covers areas of altitudes of 1850 m and it receives rainfall of between 950–1500 mm per annum Wakachala *et al.,* 2015. This zone covers most parts of the county and is the most significant for agricultural cultivation. Zone IV occupies more or less the same elevation (900–1800m) as Zone III but it has lower rainfall of about 500–1000 mm per annum. This zone dominates Solai and Naivasha (Ogeto *et al.*, 2012). The county has a bimodal rainfall pattern Wakachala *et al.*, 2015. The short rains are experienced between October and December while the long rains between March and May. Temperatures range from 29.3^{o}C between the months of December to March to as low as 12 ^{o}C during the month of June and July. Molo and Kuresoi Sub-Counties are relatively cold while Nakuru East, Nakuru West, Naivasha, Gilgil and parts of Rongai Sub-County experience extreme hot weather. However, with the deforestation experienced in the county's forest blocks and influence from climate change, variant rainfall patterns and higher temperatures may be experienced (Ogeto *et al.,* 2012).

3.1.5 Human economic activities

Nakuru County is well endowed with economic resources that include; small and large scale agriculture, livestock production, fisheries management, forestry and agroforestry, mining, textile industries, food processing, sawmills, engineering works others are manufacturing industries and tourism activities. It is a major producer of food, cash, and horticultural crops. The leading food crops include maize, wheat, beans, potatoes, and various fruits and vegetables whereas the main cash crops are: tea, coffee, pyrethrum, and flowers.

Types of fruits and vegetables which include grown are tomatoes, peas, carrots, onions, French beans, citrus fruits, peaches, apples, asparagus, and leeks. Most of these are grown in Bahati, Rongai, Mbogoini, Olenguruone, Njoro, Molo, Nakuru Municipality, and Gilgil Divisions (Ogeto *et al.*, 2013).

3.2 Research approach

A questionnaire was formulated to know and analyse vegetables grown in the area. This study was carried out within the eight sub-counties of Naivasha, Nakuru West, Nakuru East, Gilgil, Njoro, Molo, Rongai, and Subukia. One hundred structured questionnaires containing close-ended and open-ended questions were administered to the households selected by random sampling.

3.3 Experimental design

The schematic representation of the procedure that was followed in sample collection, drying, grinding, and extraction in this study is as shown in Figure 3.2. Kales (sukumawiki) and tomatoes were obtained from study areas in cross-sectional study design. Samples were picked from different locations within the study area randomly. Fluoride and heavy metal ion contents were determined in the samples.

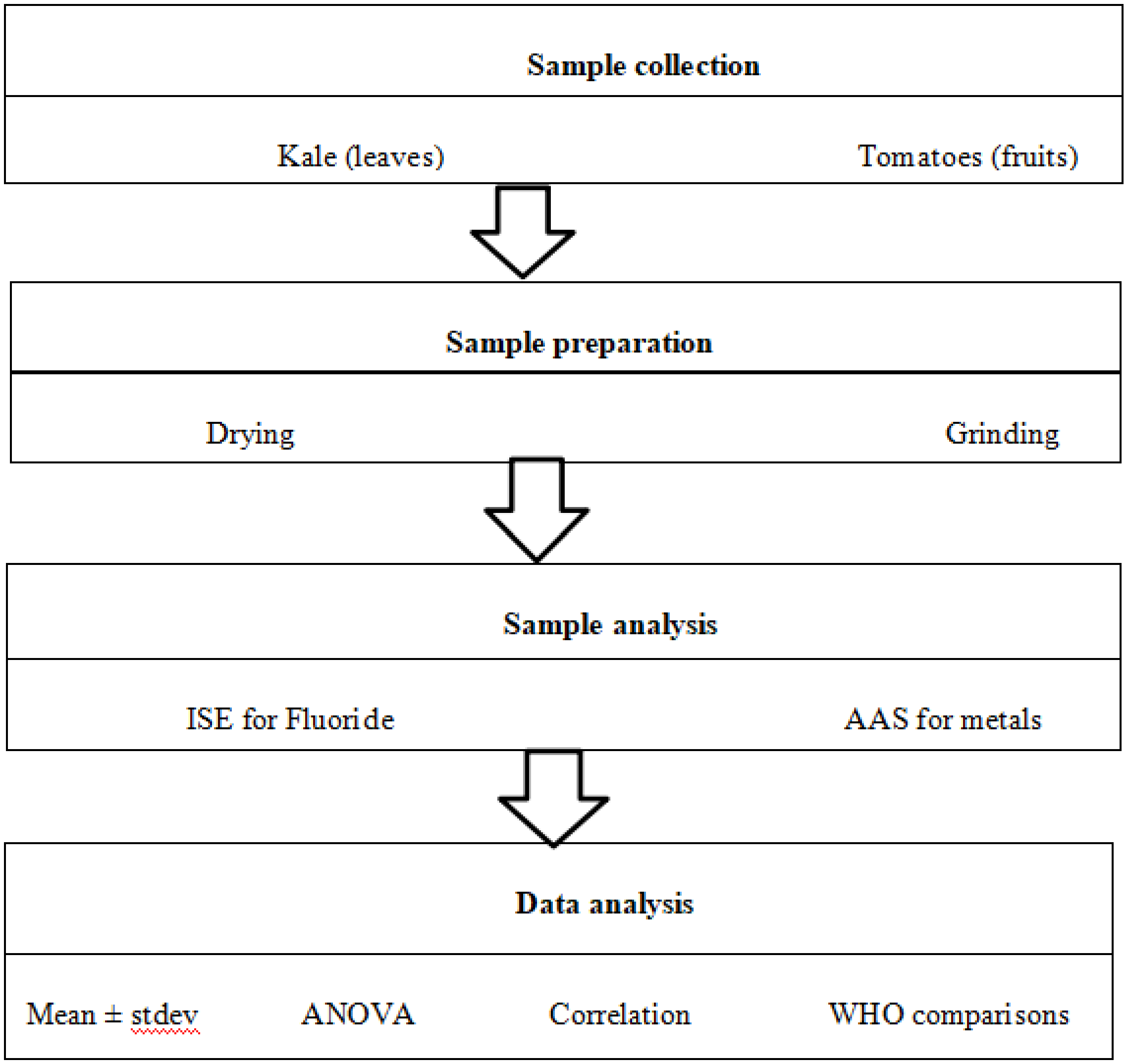

Figure 3.2: Schematic representation of the procedure which was followed to analyse kales and tomatoes.

3.4 Reagents used

All the standard solutions used in the current study were prepared from analytical reagent grade in doubly distilled water. These reagents include; zinc (II) chloride ($ZnCl_2$); iron (III) chloride ($FeCl_3.6H_2O$); chromium (III) nitrate ($Cr(NO_3)_3$. $9H_2O$); manganese chloride ($Mncl_2$); copper sulphate ($CuSO_4$); concentrated nitric acid (HNO_3); hydrochloric acid (HCl); distilled water, TISAB-buffer, standard sodium fluoride (NaF) solution, F–electrode filling solution. All the reagents were obtained from Kobian chemicals Ltd, Nairobi.

3.5 Equipment and Materials

An oven (model ULM-400, Germany), was used in sample drying; while an electric miller (MP-45E, Appleton, USA) was used in pulverizing the dry samples. An atomic absorption spectrometer, AAS, (spectra-AA 200, Australia) was then used in the measurement of metal ion concentrations; digestion block (PB512L, Tamil Nadu, India), was used in the digestion of kales and tomato samples; Hot plate (model HP 205N, New Taipei, Taiwan), was used for heating and F ion-selective electrode meter (model B20P1, USA) was used to determine F concentration in the samples. Analytical balance (model AUW220, Shimadzu Corporation, Japan) was used to determine the weights of the dried kales and tomato samples.

3.6 Sample collection

3.6.1 Plant Samples

Kales (*sukumawiki*) leaves and tomatoes ripe fruits were collected in May 2017. A total of 161 vegetable samples comprising 120 kales samples and 41 tomato samples were randomly collected from Njoro, Molo, Naivasha, Nakuru East, Nakuru West, Subukia, Rongai and Gilgil sub-counties within the study area. This was done randomly from representative farms to cover the entire study area and Table 3.1 summarizes the sample collection criteria. The samples were collected in clean polythene ziplock bags and stored frozen at –10 ^{o}C.

Table 3.1: Kales and tomatoes collected from the study area for fluoride and heavy metal analysis

Sampling regions	Farms where kales where collected	Farms where tomatoes where collected	Total samples
Njoro	34	3	37
Molo	3	-	3
Naivasha	20	4	24
Nakuru East	11	10	21
Nakuru West	19	11	30
Subukia	6	2	8
Rongai	22	6	28
Gilgil	5	5	10
Total	**120**	**41**	**161**

3.7 Sample pretreatment and preparation

3.7.1 Plant samples

Kales (*sukumawiki*) and tomato samples were washed in excess distilled water to remove any contaminants on their surface. The samples were sliced and dried to constant weight in an oven at 70 ^{0}C . They were then ground separately using an electric miller to pass through a 150-µm sieve and preserved in khaki bags for further analysis (Okalebo *et al.*, 2002).

3.7.2 Sample analysis

3.7.3 Analysis of fluoride in kales and tomatoes samples

A 1.25 g ground kales and tomatoes samples were each measured and placed into different digestion tubes. They were then treated with 10 mL of 6 M sodium hydroxide solution and the mixture heated over a water bath for 30 min until each of the kales and tomatoes sample was completely dissolved in the molten base. The solutions were allowed to cool to room temperature and then neutralized by careful adding dropwise 8 M sulphuric acid. The solutions were filtered then transferred into a 50 mL volumetric flask and the solution made up to 50 mL with double distilled water. The solutions were then mixed with total ionic strength adjustment buffer (TISAB) solution at a ratio of 1:1 to dissociate fluoride complexes, stabilize the pH at 5.5, and maintain a constant ionic strength (Adriano & Doner, 1982). Fluoride content was measured at room temperature using a fluoride ion-selective electrode.The sample concentrations of fluoride were found using the standard/calibration curve.

3.8 Block digestion samples for heavy metal determination

3.8.1 Digestion mixture preparation procedure

A 0.21 g selenium powder and 7.0 g of lithium sulfate were weighed in a clean vial then added to 175 mL of 30% hydrogen peroxide and mixed well. A 210 mL of concentrated sulfuric acid was then added with care while cooling in an ice-bath. This was then stored at 4 ^{0}C for stability.

3.8.2 Procedures for digestion

A 0.3 g portions of finely ground dried vegetable samples were weighed into separate dry, clean digestion tubes. Then 4.4 mL of the digestion mixture was added to each of the tubes and also to reagent blanks for each batch of samples. The tubes were heated in block digestion at 350 ^{0}C for 2 hours. The digestion was completed when the digest became colorless. The tubes were removed from the digester and allowed to cool to room temperature. About 25 mL of distilled water was added, mixed well, and made up to 50 mL with distilled water. The contents were then transferred into a 50 mL volumetric flask and allowed to settle so that the clean solution was used for the analysis of Zn, Cu, Cr, Mn, and Fe by AAS.

3.8.3 Preparation of working standards

The working standard solutions of individual heavy metals were prepared within the linear range of 0.0 to 5.0 mg/L and a calibration graph of absorbance against concentration plotted using the *MS Excel* program. The equations of the graphs were used to determine the concentration of the sample solutions. The concentration of the heavy metals in the samples was determined by running a series of standard solutions for each element within the linear range, followed by the analyte sample solutions in triplicates and the mean absorbance value recorded.

Similarly, F working standards were prepared within the linear range of 0.0 to 20.0 mg/L using NaF standard solution as stated in the F electrode manufacturer's manual (Adriano & Doner, 1982).

3.8.4 Sample analysis by AAS

Optimum setting for Shimadzu (AAS model SpectrAA-200) for analysis of heavy metal concentration in digested kales and tomato samples is as shown in Table 3.2

Table 3.2: Optimum setting for Shimadzu AAS model SpectrAA-200 for analysis of heavy metal concentration in digested kales and tomato samples.

Parameter	Cu	Mn	Zn	Fe	Cr
Wavelength (nm)	342.7	213.9	279.5	324.7	217.0
Lamp current (mA)	6.0	3.0	5.0	5.0	5.0
Band pass (nm)	0.3	1.0	0.5	1.0	1.0
Burner Height (mm)	20.0	22.0	22.0	24.0	20.0
Fuel and oxidizer flow rate (cm^3/min)	5.0	5.0	5.0	5.0	5.0

3.8.5 Atomic Absorption Spectrometer (AAS)

Atomic Absorption Spectrometer (AAS) is very suitable for the analysis of trace elements. AAS can be used for the determination of major, minor and trace elements using the same basic equipment with the alteration of only instrumental or procedural settings such as analytical line, absorption path length and dilution. Because of its versatility and simplicity of operation, AAS is the most extensively used method for the determination of heavy metals (West & Nurnberg, 1988).

AAS is based on the absorption of radiation at a particular wavelength by neutral excited atoms of the analyte. The narrow bandwidth at which this occurs ensures a high degree of selectivity of determination. The "ground state" atom absorbs light energy of a specific wavelength as it enters the "excited state." As the number of atoms in the light path increases, the amount of light absorbed also increases. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte can be made. The use of special light sources and careful selection of wavelengths allow the specific determination of individual elements (Skoog, 1985).Advantages of AAS include ; low spectral interferences, direct analysis of some types of liquid sample and very small sample size used,while disadvantages include; It is expensive, it has low precision and low sample throughput.

3.8.6 Potentiometric determination

Potentiometric analysis of fluoride content (as F^-) in solutions by using fluoride ion-selective electrode is simple, reliable and cheap. Very small concentrations of fluoride-ions (to 10^{-6} M) can be determined by fluoride selective electrode, with regulation of ion strength of a solution and control of concentration of hydroxide ions and interfering ions of metals (Rajković & Novaković, 2007). Fluoride Ion Selective Electrode (FISE) should be a method of choice for those with little experience in analytical chemistry. It is easy to operate, relatively rapid and require little technical skill (Ward *et al.,* 1987). In direct potentiometry method, concentration can be determined with a single measurement (Bratovčić *et al.*, 2009). Advantages of FISE include; The potential is measured across the analyte, It does not use an indicator etc. Its disadvantages include, highly pH sensitive.The potential is measured across the analyte.

3.9 Statistical analysis

The generated results were analyzed using the *Statistical Package for Social Science* (SPSS) Bryman & Cramer, (2005). Analysis of variance (ANOVA) and *t-test*, using general linear models (GLM) procedure was performed to determine whether there were any significant differences between the concentrations of the selected heavy metals and F in kales and tomatoes from Nakuru county. The triplicate values obtained for each sample were analyzed using the SPSS statistical package and the quantitative values of the mean, standard error, and correlations were used. The output for the least significant difference (LSD) values was obtained at $p \leq 0.05$ confidence levels.

CHAPTER FOUR

RESULTS

4.1 Distribution of F levels in tomatoes and kales by regions

The levels of F in kales and tomatoes collected from different sub-counties in Nakuru County were analyzed and the results are presented in Table 4.1.

Table 4.1: Mean concentration of F levels in tomatoes and kales from different regions in Nakuru County.

	Kales		Tomatoes	
Sub-county	***N***	**Mean F ±S.E (mg/kg)**	***N***	**Mean F ±S.E (mg/kg)**
Njoro	34	13.06 ± 0.33	3	13.39 ±0 .83
Molo	6	5.63 ± 0.38	-	-
Naivasha	20	17.33 ± 0.55	4	15.65±0.45
Nakuru East	11	12.04 ± 0.52	10	11.66± 0.50
Nakuru West	19	12.65 ± 0.49	11	11.72 ±0.53
Subukia	6	12.65 ± 1.24	2	12.97 ± 0.33
Rongai	22	13.33 ± 0.53	6	12.05 ± 0.39
Gilgil	5	16.94 ± 0.53	5	16.70± 0.46
Total	**120**	**13.33 ± 0.29**	**41**	**12.94 ± 0.35**

N= number of samples

The F concentration in tomatoes from 7 regions was found to vary from 11.66 to 16.70 mg/kg, while in kales it ranged from 5.63 to 17.33 mg/kg from 8 sub-counties. Nakuru East recorded the lowest while Gilgil recorded the highest this could be attributed to low rains experienced in Gilgil area which reduces fluoride solubility.Fluoride levels for tomatoes from the 7 different sub-regions in Nakuru County were as follows: Njoro,13.39 ± 0.83 mg/kg; Naivasha, 15.65 ± 0.45 mg/kg; Nakuru East, 11.66 ± 0.50 mg/kg; Nakuru West, 11.72 ± 0.53 mg/kg; Subukia, 12.97 ± 0.33 mg/kg; Rongai, 12.05 ± 0.39 mg/kg; and Gilgil, 16.70 ± 0.46 mg/kg.

For kales, the overall mean concentration for F levels was 13.33 ± 0.29 mg/kg. However, Naivasha had the highest levels of F with a mean of 17.33 ± 0.55 mg/kg, followed by Gilgil 16.94 ± 0.53 mg/kg, Rongai 13.33 ± 0.53 mg/kg , Njoro 13.06 ± 0.33mg/kg, Subukia 12.65 ± 1.24 mg/kg, Nakuru West 12.65 ± 0.49 mg/kg, Nakuru East 12.04 ± 0.52 mg/kg while Molo had the lowest of 5.63± 0.38 mg/kg. In all cases, the mean F levels in tomatoes and kales exceeded dietary recommended daily allowance (RDA) of 4 mg/kg (Dhar & Bhatnagar, 2009).

4.2 Distribution of the metals levels in tomatoes by regions

Tomatoes collected from 7 different regions of Nakuru County were analyzed for the presence of heavy metals. The overall mean levels for each element are shown in Table 4.2.

Table 4.2: Mean concentration of heavy metals in tomatoes from different sampling regions in Nakuru County

Sub-county *n=7*	**Mean metal concentrations ± S.E (mg/kg)**				
	Cu	**Zn**	**Cr**	**Mn**	**Fe**
Njoro	0.23±0.00	0.18±0.00	0.69±0.20	0.11±0.00	0.71±0.05
Naivasha	0.26±0.01	0.28±0.02	0.90±0.04	0.13±0.01	0.72±0.04
Nakuru east	0.26±0.11	0.31±0.00	0.86±0.03	0.08±0.00	0.81±0.04
Nakuru west	0.38±0.06	0.26±0.01	0.91±0.04	0.09±0.01	0.75±0.04
Subukia	0.23±0.01	0.26±0.01	0.88±0.20	0.11±0.01	0.77±0.07
Rongai	0.26±0.02	0.31±0.02	0.89±0.04	0.05±0.00	0.87±0.04
Gilgil	0.23±0.00	0.22±0.02	0.91±0.04	0.07±0.00	0.86±0.01
Overall	**0.28 ± 0.018**	**0.27 ±0.01**	**0.87±0.03**	**0.08±0.00**	**0.79±0.02**

Nakuru West recorded the highest concentration of Cu with 0.38 ± 0.06 mg/kg, while Gilgil, Njoro, and Subukia had the lowest concentration of Cu with the same mean of 0.23 ± 0.00 mg/kg. Zinc levels were highest in Nakuru East and Rongai with a mean of 0.31±0.01 mg/kg, while Njoro recorded the lowest level of 0.18 ± 0.00 mg/kg. The highest chromium concentration was recorded in Gilgil and Nakuru West with the same mean of 0.91±0.04 mg/kg, while the lowest was recorded in Njoro with a mean of 0.69±0.20 mg/kg. Naivasha on the other hand, recorded the highest concentration of Mn 0.13±0.04 mg/kg, while Rongai had the lowest Mn concentration with a mean of

0.05±0.00 mg/kg. The highest Fe concentration was in Rongai with a mean of 0.87±0.04 mg/kg, while Njoro recorded the lowest levels of 0.71 ± 0.05 mg/kg.

4.3 Distribution of the metal levels in kales by regions

The results for the variations in the levels of the heavy metals in kales by region are presented in Table 4.3

Table 4.3: Levels of heavy metals in kales from 8 regions in Nakuru County

Region	Cu (mg/kg)	Zn (mg/kg)	Cr (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
Njoro	0.05±0.00	0.25±0.01	0.86±0.02	0.11±0.00	0.58±0.03
Molo	0.05±0.01	0.20±0.01	0.73±0.04	0.13±0.01	0.30±0.02
Naivasha	0.05±0.01	0.36±0.10	1.11±0.04	0.13±0.01	0.59±0.04
Nakuru East	0.05±0.02	0.25±0.05	0.80±0.08	0.11±0.00	0.89±0.06
Nakuru West	0.07±0.02	0.37±0.30	0.95±0.04	0.11±0.01	0.62±0.05
Subukia	0.07±0.02	0.24±0.55	0.90±0.10	0.11±0.01	0.53±0.12
Rongai	0.05±0.01	0.36±0.14	1.06±0.04	0.12±0.00	0.97±0.10
Gilgil	0.07±0.01	0.32±0.04	1.01±0.06	0.10±0.01	0.74±0.02
Overall	**0.05±0.00**	**0.3±0.3**	**0.94±0.02**	**0.12±0.00**	**0.68±0.03**

The overall mean levels of heavy metals in kales decreased in the order: Cr > Fe > Zn > Mn > Cu.

Copper concentration ranged between 0.05±0.00 mg/kg and 0.07±0.01 mg/kg. Nakuru West, Gilgil, and Subukia had the same mean of 0.07±0.02 mg/kg which was the highest concentration, whereas Njoro, Molo, Naivasha and Rongai had the lowest of 0.05±0.00 mg/kg. Zinc concentration ranged between 0.20±0.01 mg/kg and 0.37±0.30 mg/kg. The zinc level was highest in Nakuru West with a mean of 0.37±0.30 mg/kg, while Molo recorded the lowest levels of 0.20±0.01 mg/kg. Chromium concentration ranged between 1.11±0.04 mg/kg and 0.73±0.04 mg/kg . Naivasha recorded the highest level of 1.11±0.04 mg/kg, while Molo recorded the lowest 0.73±0.04 mg/kg compared with other regions. Manganese mean concentration ranged between 0.10±0.01mg/kg and 0.13±0.01 mg/kg. Naivasha and Molo regions had the highest mean concentrations of 0.13±0.01mg/kg, whereas Gilgil had the lowest concentration of 0.10±0.01 mg/kg compared with other regions. Iron concentration ranged between 0.30±0.02 mg/kg and 0.97±0.10 mg/kg. Rongai region had the highest concentration of 0.97±0.10 mg/kg, while the Molo region had the lowest concentration of 0.30±0.02 mg/kg compared to other regions.

4.4 Results of the analysis of variance (ANOVA)

4.4.1 Comparison of fluoride levels in tomatoes and kales between regions

ANOVA was then used to determine if there was a significant difference in F levels in tomatoes and kales across the regions. Results indicated that there was a significant difference in F levels as shown in Table 4.4

Table 4.4: Paired t-test to compare F levels in tomatoes and kales

	Tomatoes		Kales	
Regions	*T*	*P*	*T*	*P*
Njoro-Naivasha	–3.151	0.050	–-6.745	0.000
Molo-Gilgil	–-23.329	0.000	–-14.232	0.000
Molo-Naivasha	–-4.768	0.018	–-10.643	0.000

4.4.2 Comparisons of the metals levels in tomatoes between regions

To determine whether there existed significant differences in the spatial distribution of the heavy metals, a one-way analysis of variance was conducted. According to the results of the analysis of variance presented in Table 4.5, the *p* values of Zn and Mn were both 0.000. They were below 0.05 significance level indicating a significant difference at 95%

in the heavy metals content. The *p* values of Cu, Cr, and Fe were 0.101, 0.428, and 0.104 respectively indicating an insignificant difference as shown in Table 4.5 .

Table 4.5: One - way ANOVA for the concentrations of heavy metals in tomatoes from 7 regions

	Sum of Squares	Mean Square	F	Sig.
Cu	0.130	0.022	1.950	0.101
Zn	0.067	0.011	12.529	0.000
Cr	0.166	0.028	1.021	0.428
Mn	0.019	0.003	18.653	0.000
Fe	0.135	0.022	1.931	0.104

4.4.3 Comparisons of metals levels in kales between regions

One-way analysis of variance was then conducted to determine the existence of any significant differences in the spatial distribution of the heavy metals in kales and the results presented in Table 4.6. According to the analysis of variance of the results in Table 4.6, the *p* values of Cu, Cr, and Fe were all 0.000, while Zn was 0.007. They were below 0.05 significance level indicating a significant difference at 95% in the heavy metals content. The *p* values of Mn were 0.247. It was above the 0.05 significance level indicating insignificance in the content of heavy metals in kales in the different regions from Nakuru County.

Table 4.6: One-way ANOVA for the concentrations of heavy metals in kales from 8 regions

	Sum of Squares	Mean Square	F	Sig.
Cu	.010	.001	8.471	.000
Zn	.427	.061	2.988	.007
Cr	1.548	.221	6.671	.000
Fe	3.854	.551	7.859	.000
Mn	.008	.001	1.320	.247

4.5 Correlation of fluoride and the metals contamination in tomatoes and kales from Nakuru County

4.5.1 Correlation of fluoride and metals contamination levels in tomatoes

The correlation between element pairs in tomatoes found in Nakuru County is shown in Table 4.7

Table 4.7*:* Pearson correlation between heavy metals in tomatoes found in 7 regions in Nakuru County

	F^- (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Cr (mg/kg)	Mn (mg/kg)
Cu (mg/kg)	–-0.398	–-	–-	–-	–-
P (value)	0.009	–-	–-	–-	–-
Zn (mg/kg)	–-0.368	0.183	–-	–-	–-
P (value)	0.016	0.247	–-	–-	–-
Cr (mg/kg)	0.021	–-0.024	0.219	–-	–-
P (value)	0.893	0.881	0.164	–-	–-
Mn (mg/kg)	0.109	0.233	–-0.214	–-0.164	–-
P (value)	0.493	0.138	0.173	0.299	–-
Fe (mg/kg)	–-0.030	0.294	0.302	0.013	–-0.130
P (value)	0.849	0.059	0.052	0.937	0.412

The Pearson correlation showed an insignificant correlation between the elements, except that between, F and Cu, F and Zn which were below 0.05 indicating significant difference at $p \le 0.05$

4.5.2 Correlation of fluoride and the metals contamination levels in kales

The correlation between element pairs in kales found in Nakuru County is shown in Table 4.8

Table 4.8: Pearson correlation between F^- and heavy metals in kales found in 8 regions in Nakuru County

	F^- (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Cr (mg/kg)	Fe (mg/kg)
Cu (mg/kg)	0.044	–-	–-	–-	–-
p (value)	0.636	–-	–-	–-	–-
Zn (mg/kg)	0.112	0.177	–-	–-	–-
p (value)	0.225	0.053	–-	–-	–-
Cr (mg/kg)	0.353	0.258	0.360	–-	–-
p (value)	0.000	0.004	0.000	–-	–-
Fe (mg/kg)	0.096	–-0.077	0.353	0.326	–-
p (value)	0.295	0.004	0.000	0.000	–-
Mn (mg/kg)	0.027	–-0.048	0.040	0.125	–-0.101
p (value)	0.767	0.605	0.665	0.175	0.274

The Pearson correlation shows an insignificant correlation between the elements, except between Cu and Fe, Fe and Zn, Fe and Cr, F and Cr, Cr and Cu finally Cr and Zn were below 0.05 indicating significant difference at $p \leq 0.05$.

4.6 Comparison of F and the heavy metal levels in tomatoes and kales with the WHO/FAO allowable levels

The obtained levels of F and selected heavy metals in tomatoes and kales were compared with standards recommended by both the WHO and FAO and the results of this comparison are presented in Table 4.9.

Table 4.9: Comparison of F and the selected heavy metals in tomatoes and kales with the WHO/FAO/RDA standards

Sample	**Element**	**Elements levels (mg/kg)**	
		Current work	**WHO/FAO/RDA** (WHO/FAO, 2001)
Kales	**Cr**	0.94	1
	Cu	0.05	20
	F	13.33	4
	Fe	0.68	48
	Mn	0.12	0.2
	Zn	0.3	60
Tomatoes	**Cr**	0.87	1
	Cu	0.28	20
	F	12.94	4
	Fe	0.79	48
	Mn	0.08	0.2
	Zn	0.27	60

The results reported in the present study showed that concentration of fluoride in tomatoes and kales from Naivasha, Gilgil, Rongai, Subukia, Nakuru West, Nakuru East, and Molo are exposed to high levels of F compared to the accepted levels of WHO/FAO (2001) with RDA of 4 mg/kg. The concentration of heavy metals, that is, Cr, Fe, Cu, Mn, and Zn in tomatoes and kales from Naivasha, Gilgil, Rongai, Subukia, Nakuru West, Nakuru East, and Molo were recorded as within the permissible limits of WHO/FAO for these metals.

CHAPTER FIVE

DISCUSSION

5.1 Fluoride in raw kales and tomatoes from horticultural farms in Nakuru

Fluoride levels in tomatoes and kales from selected horticultural farms in Nakuru County, were all found to be above the RDA level of 4 mg/kg which poses a health hazard to consumers (Al-Hwaiti & Al-Khashman, 2015). Several studies done on F levels in vegetables and fruits have shown that F has a predisposition to accumulate in plants (Gautam *et al.*, 2010; Bhargava & Bhardwaj, 2009; Chaoke *et al.*, 1997; Jones *et al.*, 1971; Yadav *et al.*, 2012). Nonetheless, in a study done by Moeinian *et al.* (2016) to determine F levels in soil, tomato, and onion from some farms in Zanjan Province of Iranian Azerbaijan, low F levels of just 2.10±0.80 mg/kg were reported in the tomatoes. However, in another study carried out in some villages of India, Bhargava & Bhardwaj (2009) reported mean F concentration in tomatoes (13.48 ± 0.08 mg/kg) that were comparable to those observed in the present study.

High F levels were recorded in tomatoes from all the 7 sub-counties of Nakuru County studied in the present investigation. Gilgil had the highest F levels of 16.70 ± 0.46 mg/kg. This could be attributed to the low rains experienced in these areas, which reduce F solubility and leaching relative to that in the wetter regions. Then, as expected, the F levels in tomatoes from the wetter regions of Rongai (12.05 ± 0.39 mg/kg) and Subukia (12.97 ± 0.33 mg/kg) were lower in comparison to those from Gilgil areas. This was ascribed to greater leaching of fluoride levels in the wetter regions that reduce resident time of labile fluoride in the root zone soils and hence lower its uptake by the plants. This suggests that rainfall amounts strongly control fluoride availability to vegetation.

Surprisingly, some of the driest regions Nakuru East (11.66± 0.50 mg/kg) and Nakuru West (11.72 ±0.53 mg/kg), which also lie close to high-fluoride saline lakes showed lower fluoride levels in their tomatoes than the rest of the sub-counties. However, it was not immediately clear why the studied crops accumulated less F in an area of highest F contamination reported in water sources. It was obvious, however, that F content of agricultural water controlled the crops exposure to excessive F in the environment.

Leafy vegetables such as Raddish leaves (*Raphanus sativus*), Spinach leaves (*Spinaceaoleoracea*) and mustard leaves (*Brassica competes*),which were irrigated with water having 3.54 mg/L to 11.82 mg/L, were found to accumulate 14.96 mg/kg, 29.15 mg/kg and 14.59 mg/kg F respectively (Gautam *et al*., 2010). In the current study, high fluoride was recorded in kales from all the sub-counties of Nakuru County, Kenya. Naivasha had the highest levels of F with a mean of 17.33 ± 0.55 mg/kg. This could be attributed to domination of this regions by alluvial soils, which have been linked to high fluoride content in nature and low rainfall that reduce solubility of F ions in the environment (Wambu & Muthakia , 2011). Molo, on the other hand, had the lowest with a mean F of 5.63± 0.38 mg/kg, due to nitisol soil type which is deep red and has low F accumulation.

5.2 Occurrence of heavy metals in kales and tomatoes from farms in Nakuru County, Kenya

The highest levels of Cu were recorded in tomato samples from Nakuru West (0.38±0.06 mg/kg) and the lowest (0.23±0.00 mg/kg) was recorded jointly in Gilgil, Njoro, and Subukia as shown in Table 4.2. Osma *et al.*, (2012) while determining heavy metal levels in tomato grown in different station types in Instanbul reported 0.08–0.014 mg/kg concentrations for copper which were lower compared to the results of the present study.

One-way ANOVA indicated that there was no significant difference in concentration of Cu in tomatoes for the 7 regions ($p>0.05$). This is attributed to the same levels of Cu in the parent rocks related to the normal background concentrations of the metal in natural soil samples. Moreover, the overall mean concentration for Cu in tomato samples from 7 regions of the current study area was 0.28±.018 mg/kg and in all cases, the mean regional levels of Cu in the tomato fruits were lower than the accepted level of 20.00 mg/kg, which showed that tomatoes from the current study area were not contaminated with undue levels of Cu.

In kales, the overall mean concentration of Cu was 0.05± 0.00 mg/kg with the highest levels of 0.07±0.01 mg/kg Cu being reported in Nakuru West, Nakuru East, and Gilgil regions. This could be attributed to the high use of agrochemicals in these areas. Elsewhere, Uwah *et al*., (2011) also reported high Cu concentrations of 0.81 mg/kg in spinach grown in certain sites in Nigeria. The results of both studies showed higher Cu contamination than those reported in the present study. The levels of Cu in kales were within the accepted level of 20.00 mg/kg (WHO/FAO, 2001)

Nonetheless, one-way ANOVA indicated that the difference in the concentration of Cu in kales was statistically significant (*p*<0.05). This difference in the levels of Cu could be attributed to different agricultural practices employed by farmers in each of the regions. The high Cu levels in Nakuru West, Nakuru East, and Gilgil regions were attributed to the application of fertilizers and other horticultural chemicals used in farming. Also, high levels could be attributed to industrial waste discharge from manufacturing industries like the now-defunct Batteries Ltd production industry located in the central business district of Nakuru town.

The overall mean concentration for zinc in tomatoes from the 7 regions was 0.27±0.01 mg/kg. Zinc level was highest in Nakuru East and Rongai with a mean of 0.31±0.02 mg/kg. This was due to anthropogenic activities such as industrial waste and municipal waste dumping. Nonethelss, the concentrations of zinc in tomatoes were within the accepted levels of 60 mg/kg as per (WHO/FAO, 2001); (Kibet *et al*., 2019) requirements. Similarly, one-way ANOVA indicated that the difference in concentration of zinc in tomatoes was not statistically significant (*p*>0.05), this could be attributable to similar geological distribution of parent rock containing zinc.

On the other hand, the overall mean zinc levels in kales from the 8 regions was 0.3±0.3 mg/kg. Zinc levels were highest in Nakuru West with a mean of 0.37±0.30 mg/kg. The results of this study were similar to those reported by Njagi (2013) and by Muhammad *et al.*, (2008) who reported Zn levels of 0.38–2.43 mg/kg and 0.461 mg/kg in certain leafy vegetable including: spinach, cabbage and cauliflower. Nonetheless, somehow higher values of Zn (1.06±0.02–2.82±0.01 mg/kg) were previously reported by Akubugwo *et al.*, (2012) in *Amaranthus hybrid* vegetables. High Zn content in vegetable, especially from Nakuru West could be attributed to anthropogenic activities such as industrial waste and municipal waste disposal and hydrogeological translocation into the farmlands in low-lands surrounding the saline Lake Nakuru.

One-way ANOVA indicated significant difference (F= 2.988, df=7, p=0.007) in concentration of Zn in kales. This is attributed to the same levels of Zn in the parent rocks (Alloway, 2008).

The overall mean concentration for Fe in tomatoes from the 7 regions was 0.79±0.02 mg/kg. Iron level was highest in Rongai with a mean of 0.87±0.04 (mg/kg) this is attributed to different soil type influencing the pH and the pH affects on the absorption of Fe. Rongai area is known to have planosolic soil while Njoro with latosolic soil (Gitagia *et al.,* 2019) recorded the lowest level of 0.71±0.05 (mg/kg) as shown in Table 4.2. Mean concentrations of Fe in kales and tomatoes were within accepted levels of 48 mg/kg (WHO/FAO, 2001). These values of Fe in the present study were slightly higher than those reported by Adegbola, (2013) with mean content of Fe ranging from 0.03 – 0.07 (mg/kg) in fresh tomatoes sold in different markets and farms. One-way ANOVA indicated that there were insignificant difference (*F*=1.931, *df*=6, *p*=0.104 in concentration of Fe in tomatoes for 7 regions. This is attributed to the same levels of Fe in the parent rocks.

As for Fe, the overall mean concentration in kales from the 8 regions was 0.68±0.03 mg/kg and Fe levels were highest in Rongai (0.97±0.10 mg/kg) whereas the lowest levels (0.30±0.02 mg/kg) as shown in Table 4.4 occurred in Njoro and in Molo. Results recorded by Aweng *et al.,* (2011) reported Fe content of 0.65–2.76 mg/kg in vegetables which were similar to those of the current study.

One-way ANOVA indicated significant difference (*F*=7.859, *df*=7, *p*=0.000) in concentration of Fe in kales. Differences in Fe distribution between the regions of the current study area is attributed to different soil type influencing the pH and the pH affects absorption of iron. Similarly, different agricultural practices employed by farmers in each region and different distribution of Fe in parent rock could cause variations in Fe availability and uptake by plants in those regions.

The overall mean concentration for chromium in tomatoes from the 7 regions was 0.87±0.03 mg/kg . Levels of chromium in Gilgil and Nakuru West were (0.91±0.04 mg/kg). This could be due to anthropogenic activities such as use of paints in vehicles industry and engineering works whereas, as shown in Table 4.3, the lowest was recorded in Njoro with a mean of (0.69±0.20 mg/kg). The concentrations of chromium in all the tomatoes were lower than the accepted level of 1 mg/Kg as per WHO/FAO (2001) requirements. Adetogun, (2013) while investigating chromium uptake in tomato plants from Glen Valley farm Gaborone, Botswana, recorded the highest uptakes of chromium in tomato plant to be 0.819 mg/kg. Hellen & Othman (2014), on the other hand, recorded 0.20 mg/kg concentrations of Cr in tomatoes. Banerjee *et al.*, 2010 recorded high concentrations of Cr in cauliflower (2.45 mg/kg) and cabbage (1.35 mg/kg) compared to their respective soils. Results from these studies are abit higher compared to those of the present study.

The overall mean concentration for chromium in kales was 0.94±0.02 mg/kg. The concentrations of chromium in kales were within acceptable limits of 1 mg/kg as per WHO/FAO (2001) requirements. Chromium concentration ranged between 1.11±0.04 (mg/kg) in Naivasha and 0.73±0.04 (mg/kg) in Molo. Studies by Alamin *et al.*, 2007 indicated higher Cr concentrations in cabbages (1.39 mg/kg) than in carrots (0.35 mg/kg) in Libya. Hellen & Othman, (2014) recorded 20.00 mg/kg concentrations of Cr in tomatoes.

One-way ANOVA indicated the difference in concentration of chromium in kales for the 8 regions which were statistically significant ($p<0.05$). Again, variation in Cr levels between regions could be attributed partly to different geological distribution of parent rock and partly to differences in leaching due to varying climatic conditions and annual rainfall.

Manganese in tomatoes, from the 7 regions had overall mean concentration of 0.08±0.00 mg/kg. Naivasha recorded the highest Mn concentration 0.13±0.01 mg/kg as compared with other regions. Rongai, on the other hand, had the lowest Mn concentration with a mean of 0.05±0.00 mg/kg. Ndinwa *et al.*, (2014) while determining heavy metals in tomato (*Solanum lycopersicum*) leaves, fruits and soil samples collected from Asaba Metropolis, Southern Nigeria recorded high concentration of Mn of 3.83 mg/kg in tomato fruit which was higher than those in the present study.The concentrations of Mn in tomatoes obtained in this study were within the accepted level of 0.2 mg/kg as per WHO/FAO (2001) requirements.

One-way ANOVA indicated no difference in concentration of manganese in tomatoes which was not statistically significant (p>0.05). This could be attributed to different geological distribution of parent rock and also low leaching of Mn in Naivasha due to low rainfall .

The overall mean concentration for Mn in kales on the other hand, was 0.12±0.00 mg/kg and Naivasha and Molo recorded the highest concentration of Mn 0.13±0.01 (mg/kg) as compared with other regions. In this case, Gilgil had the lowest Mn concentration with a mean of 0.10±0.01 (mg/kg).

One-way ANOVA indicated no difference in concentration of manganese in kales which was not statistically significant (p>0.05). This is attributed to the same levels of Mn in the parent rocks.

5.3 The relationships between F and heavy metals in kales and tomato from farms in Nakuru County, Kenya.

The correlation between F and Cu, F and Zinc levels in tomatoes were below 0.05 indicating significant difference at $p<0.05$, while the others were above 0.05 and the correlation between Cr and F, Cr and Cu, Cr and Zn, Fe and Zn, Fe and Cu in kales were below 0.05 indicating significant difference at $p<0.05$, while the others were above 0.05

The geochemical behaviors of Zn, Fe, Cr and Cu are known to be similar in most natural processes (Muohi *et al.*, 2003). This could explain the high correlation and suggests minimal or no anthropogenic inputs (Asante, (2005).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the findings of this study it can be concluded that the tomatoes and kales from Naivasha, Gilgil, Rongai, Subukia, Nakuru West, Nakuru East and Molo were contaminated wth high levels of F which were greater than the maximum levels set by WHO/FAO (2001) with RDA of 4 mg/kg. The results reported in the present study showed that concentration of heavy metals Cr, Fe, Cu, Mn and Zn in tomatoes and kales from Naivasha, Gilgil, Rongai, Subukia, Nakuru West, Nakuru East and Molo were below the WHO/FAO permissible limits for these metals. The present study found out that there was a significant negative correlation between fluoride ion concentration with Zn and Cu ions concentration in tomatoes, while kales had a significant positive correlation between fluoride ion concentration with Cr ion concentration.

6.2 Recommendations

From the study, the following recommendations are drawn;

- The Ministry of Agriculture in collaboration with the Ministry of Health should educate the public and farmers on the dangers of consuming kales and tomatoes contaminated with fluoride especially from Nakuru County .

- Sample collection and analysis be done during different seasons of the year in order to assess the effects of seasonal variation in the fluoride and heavy metal content in the studied samples.

- Further research on correlation between fluoride and heavy metals in soil and vegetables to be done.

- Further research to be done to determine the levels of F and heavy metals in human tissues, human milk and animal milk to ascertain the effect of these elements.

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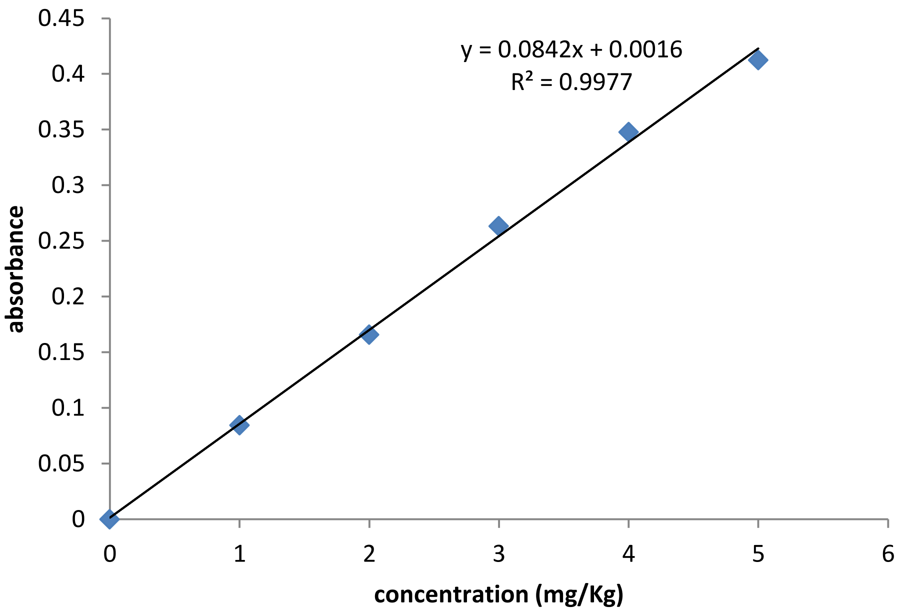
APPENDICES

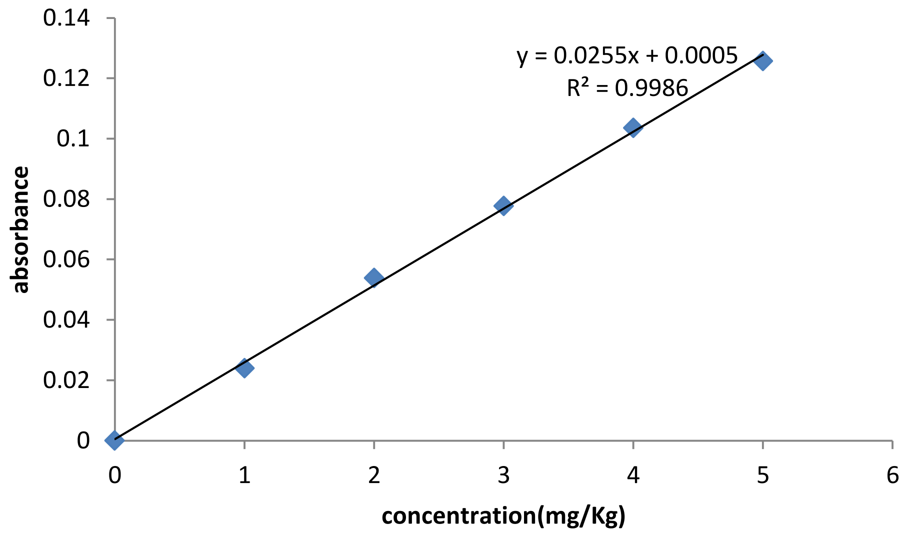
Appendix I. Household consumption of food

ITEM CODE	ASK THIS QUESTION BEFORE MOVING ON TO THE NEXT QUESTIONS FOR ITEMS WITH YES	Yes 1 No 2	How much in total did your household consume ? KILOGRAMS.....1 GRAMS.........2		How much came from own-production? IF NONE WRITE 0 FOR QUANTITY AND LEAVE UNIT BLANK						
			UNIT	QUANTITY	QUANTITY	UNIT					
Vegetables											
001	Kales tomatoes,										

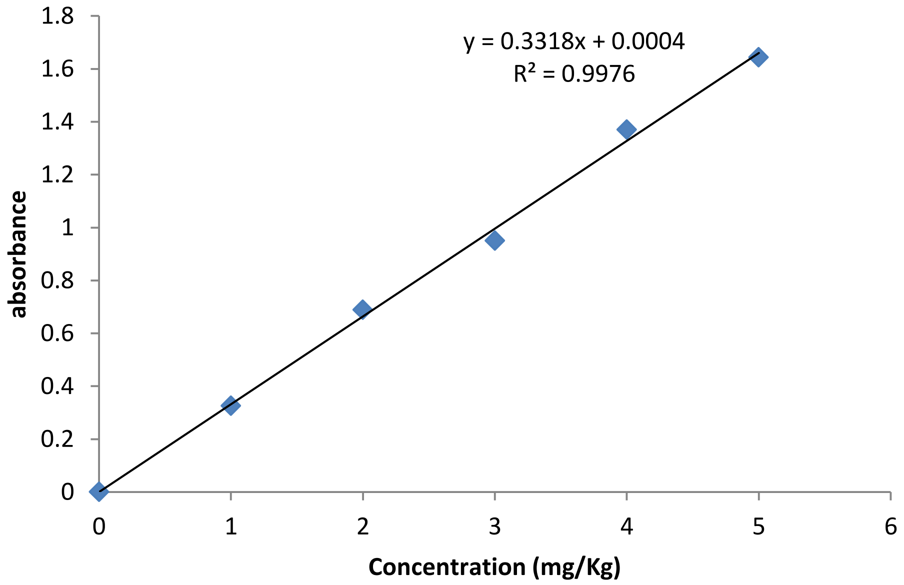
Appendix II: Calibration curve for zinc



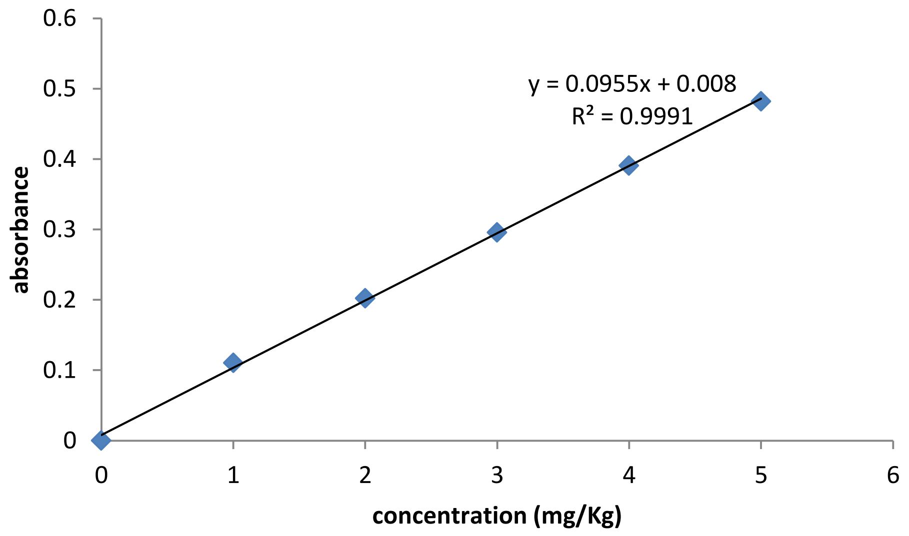
Appendix III: Calibration curve for iron



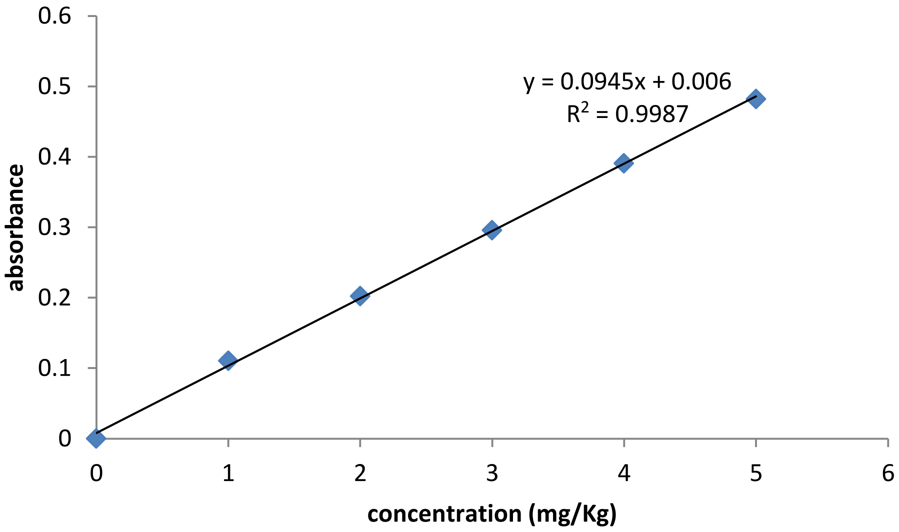
Appendix IV: Calibration curve for chromium



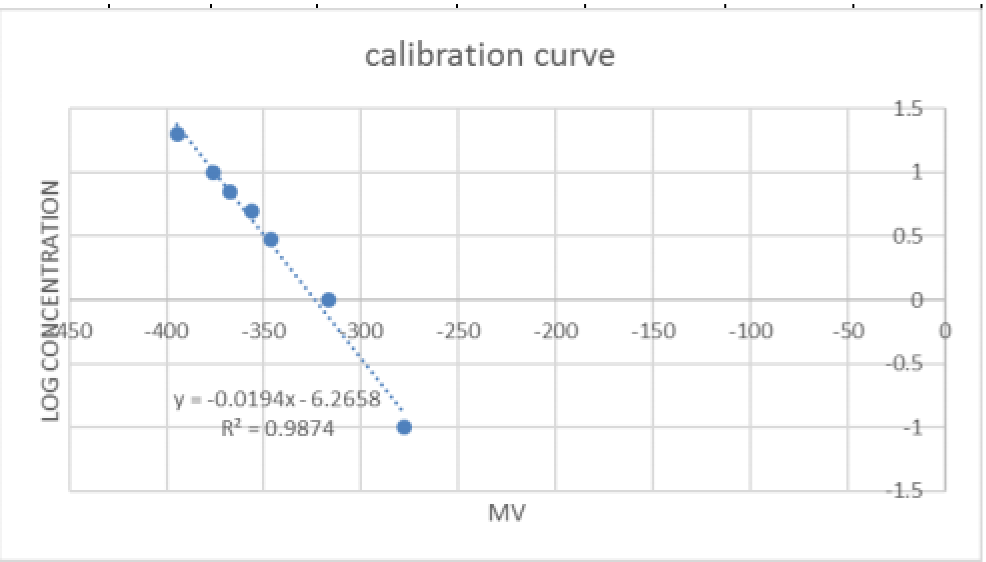
Appendix V: Calibration curve for copper



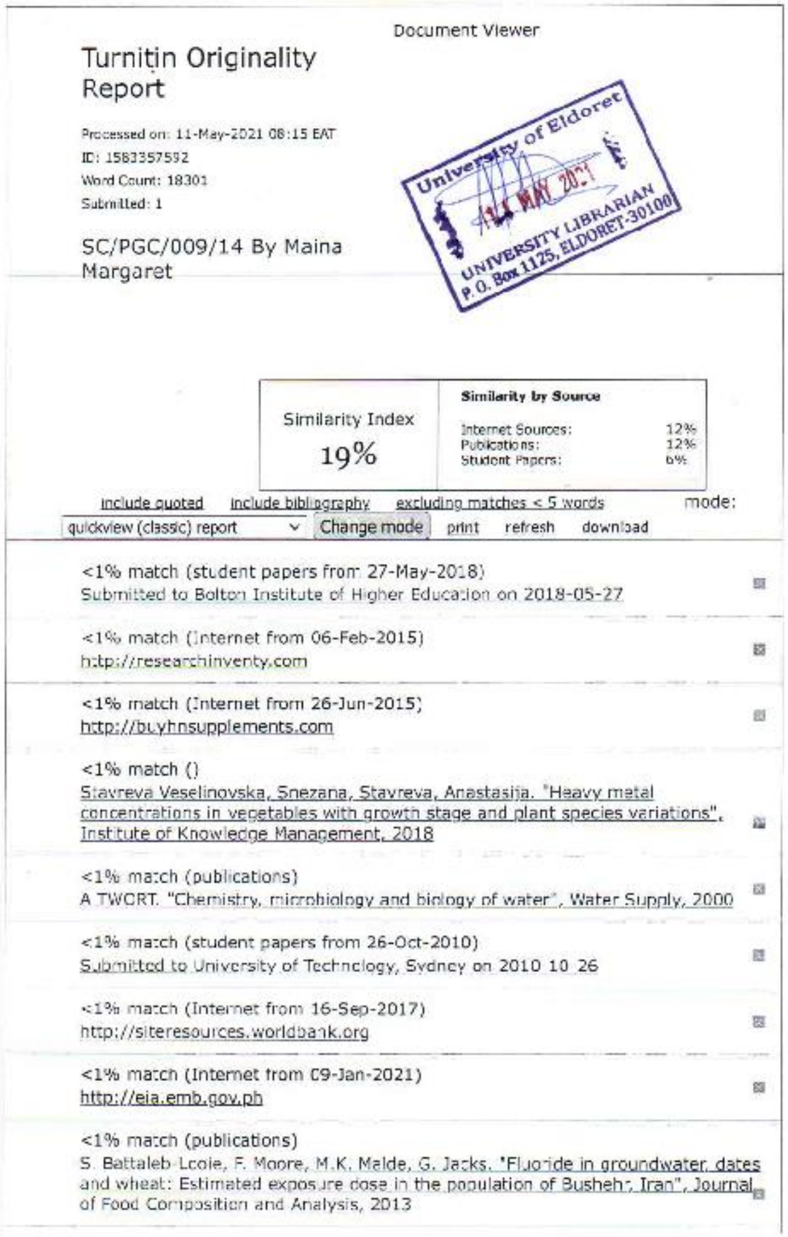
Appendix VI: Calibration curve for manganese



Appendix VII: Calibration curve for Fluoride



Appendix VIII: Similarity Report



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