UNIVERSITY OF NAIROBI

EVALUATION OF TRACE ELEMENTS' CONCENTRATIONS IN NAILS OF ESOPHAGEAL AND STOMACH CANCER PATIENTS: AS AN EARLY DIAGNOSTIC SCREENING TOOL

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

This thesis is dedicated to my beloved parents who were my first teachers on earth.

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ABBREVIATIONS/ACRONYMS AND SYMBOLS

AAS - Atomic Absorption Spectroscopy

DNA - Deoxyribonucleic Acid

EPA - Environmental Protection Agency

IAEA - International Atomic Energy Agency

IARC - International Agency for Research in Cancer

ICP - Inductive Coupled Plasma

NCCS - National Cancer Control Strategy

NCI - National Cancer Institute

PC - Personal Computer

TXRF - Total Reflection X-ray Fluorescence

WHO - World Health Organization

CE - Cancer of the Esophagus

CS - Cancer of the Stomach

CN - Control Group

OPERATIONAL DEFINITIONS

Cancer	A group of diseases characterized by abnormal cell growth capable of spreading and invading other cells.
Heavy Metals	Metals with high atomic density and atomic numbers
Trace Elements	Chemical elements required in very low/minute quantities by living organisms
Esophageal Cancer	Buildup of abnormal cells arising from the food pipe that runs between the throat and the stomach
Stomach Cancer	Buildup of abnormal cells that develop in any part of the stomach and can spread from the stomach to other organs

ABSTRACT

This study aimed at investigating a possible early screening diagnostic tool for cancer. Cancer is ranked third among the leading causes of deaths in Kenya with an average of 75% of cancer patients being diagnosed at advanced stages of the disease. This late diagnosis is attributed to most patients only presenting themselves for check-ups when the disease has progressed. Information from various literature established that among the several causes of cancer, is prolonged exposure to toxic heavy metals and high concentrations of trace elements to the body. The study therefore aimed at evaluating the concentrations of Cr, Hg, Se, Pb, Cu, Zn, and Fe elements in the human body from esophageal and stomach cancer patients in Kenya. It was expected that this research work would identify a correlation between trace elements' concentrations with esophageal and stomach cancers, which can be used as a prediction of the disease. This will allow for early intervention measures thereby reducing and mitigating the disease burden. It was a case-controlled study comprising 95 esophageal and stomach cancer patients and 31 non-cancer volunteers as a control group. Fingernail clippings were obtained by the researcher from newly diagnosed stomach and esophageal cancer patients at the Kenyatta National Hospital, Nairobi, Kenya who had consented to take part in the study. The study participants were recruited from adults aged between 32 to 65 years and were randomly selected from the health records at the Kenyatta National Hospital, Cancer Treatment Centre. The control group comprised of adult volunteers of matched age and sex and without any known cancer history, from orthopedic surgery, general surgery and ophthalmology wards at the Kenyatta National Hospital. Informed consent was obtained and written questionnaires administered by the principal investigator before participating in the study. The total number of esophageal cancer patients who participated in this study were 72, while 23 were stomach cancer patients and 31 non-cancer control group. An average of 50 mg of nail clipping samples were obtained from each participant using sterilized stainless nail cutters. Each of the nail samples were then cleaned by putting under continuous stirring according to sample washing procedure as suggested by International Atomic Energy Agency. Drying of the samples was done between filter papers for 24 hours and weighed before digestion. Wet acid digestion method using concentrated nitric acid was applied. The accuracy of the procedure was validated by analyzing three replicate samples; with Yttrium as the internal standard. Analysis of the samples was done using Total Reflection X-Ray Fluorescence technique for detection of the seven selected trace elements for the study; while R statistical software was used to analyze the data. Trace elements in finger nail clippings varied in concentrations. The mean concentrations of Cr, Fe, Cu, Zn, Se, Hg and Pb in esophageal cancer patients were 8.32 $\mu g\ g^{-1}$, 212 $\mu g\ g^{-1}$, 21. 8 $\mu g\ g^{-1}$, 211 $\mu g\ g^{-1}$, 2.04 $\mu g\ g^{-1}$, 2.24 $\mu g\ g^{-1}$, 9.01 $\mu g\ g^{-1}$respectively; and 11.6 $\mu g\ g^{-1}$, 209 $\mu g\ g^{-1}$, 18.3 $\mu g\ g^{-1}$, 265 $\mu g\ g^{-1}$, 1.70 $\mu g\ g^{-1}$, 2.56 $\mu g\ g^{-1}$, 10.1 $\mu g\ g^{-1}$ respectively in stomach cancer patients; while in the non-cancer patients, the mean concentrations were 3.17 $\mu g\ g^{-1}$, 213 $\mu g\ g^{-1}$, 28.1 $\mu g\ g^{-1}$, 258 $\mu g\ g^{-1}$, 1.06 $\mu g\ g^{-1}$, 1.39 $\mu g\ g^{-1}$, 10.5 $\mu g\ g^{-1}$for Cr, Fe, Cu, Zn, Se, Hg and Pb respectively. The specific objectives of the study were therefore achieved. These findings revealed that evaluation of Cu, Cr, Pb, Se and Hg in nail clippings using TXRF can be used as an early screening diagnostic tool for cancer. There were no correlations established for Fe and Zn concentrations. Further research on analysis of other heavy metals and trace elements using different analytical tools are recommended to ascertain the conclusions of this study.

CHAPTER ONE

Introduction

1.1 Background

Cancer is currently a global burden as it exerts too much pressure on demographic and health systems across all income levels in a population. Local and international news report that both the rich and the poor, rural and urban populations of all faiths and lifestyles are dying of one form of cancer or the other. According to Kenya Ministry of Health, 2017, cancer is ranked third among the leading causes of deaths, after cardiovascular and infectious diseases (Bray *et al*., 2018).

Moreover, estimated new cancer cases now stand at 47,887 reported annually with a mortality of 32,987. In the year 2012, it is reported that 28,500 people died of cancer and cancer related ailments (KMH, 2017). Clearly, there is as increase of the cancer epidemic.

As reiterated by Muinao and Co-workers, 2018, survival rates for cancer are typically lower due to late stage of diagnosis and lack of accessibility to quick and efficient as well as standard treatment. Therefore, correct and timely disease detection is critical for clinical diagnosis, proper toxicity monitoring, and ultimately ineffective cancer treatment (Muinao *et al.,* 2018).

Moreover, the key obstacle in treatment for cancer is early-stage disease identification, which would significantly improve cancer treatment efficiency and survival. Consequently, minimally invasive tests are ideal for detecting early phase melanoma, and developing these techniques in clinical applications is desirable. Fortunately, recent efforts have been made to develop a variety of chemical tools for detecting cancer-related biomarkers with high sensitivity, such as protein molecules, nucleic acids, enzymes, organic molecules and cancerous cells (Muinao *et al.*, 2018). Current diagnostic screening methods include the use of Cancer Protein Biomarkers, Enzyme-linked Immunosorbent Assay methods, Electrochemical and Electrical Detection methods, Optical methods, Enzyme-induced Conformational Change method, Electrophoresis-based methods and fluorescent methods (Muinao *et al.*, 2018).

However, the future of cancer biomarker detection lies in the development of efficient screening platforms with highly sensitive and selective, smaller size, highly flexible, elevated, and the discovery of new biomarkers that explicitly state the need for earlier detection (Ferlay *et al.*, 2012).

It is observed that among the major causes of cancer, are toxic heavy metals which have been confirmed to be carcinogenic (Karimi *et al.*, 2012 & Mulware, 2013). Due to the physiological and chemical properties of these heavy metals, chronic exposures to them are almost unavoidable in daily life. Consequently, due to the use of these metals in, drug manufacturing, food additives, industrial applications, mining, manufacturing of semiconductors, cement-manufacturing plants and refining of metal ores, leading to the release of heavy metals into the environment. These in addition, raise the population's exposure to these metals, thereby contributing to the environmental contamination and also human body accumulation (Abo El-Atta, 2011).

Trace elements are dietary nutrients required in minute quantities of an organism's mass; normally not up to 0.01%. They have a vital role in health maintenance, proper growth, development and are also components of enzymes in living organisms. They are inorganic micronutrients involved in many cellular functions; hence their deficiency may cause malfunctions, diseases and possibly result in death (Mehri & Marjan, 2015).

According to Chitturi et al. (2015), there are nineteen trace elements known which are categorized as either essential, probably essential or potentially toxic elements.

Requirements for essential elements in humans per day, which include Co, Cu, F, Fe, Zn, Se, Mn, Mo and I ranges from 50 µg to 10 mg. Their imbalance in the body is considered in many diseases as a risk factor. Probably essential elements have very little or no beneficial function in the humans' life process and very little is known about them and they include Sn, Ni, B, and V; while potentially toxic elements' excessive concentrations are considered hazardous to human health and can also inhibit growth in plants. There may however be some possibility with essential functions for these elements, and they include Au, Al, Pb, Cr, Cd, and Hg (Chitturi *et al*., 2015).

Hence, in the bodily concentration of trace elements, a balance needs to be maintained for proper maintenance of life and health of living organisms (Mehri & Marjan, 2015).

Heavy metals on the other hand, are elements that occur naturally with high atomic mass and weight. They are considered toxic in the human body even at low concentrations because they are

understood to cause numerous organ harm, even at minimum levels of exposure. They are widely dispersed throughout the environment as a result of their numerous applications in agriculture, household life, industry, and medicine. This factor gives rise to worries about their possible negative consequences on human health. Their toxicity is dependent on the method of exposure, the dosage, the chemical species, as well as the exposed person's weight, age, gender, and nutritional status. Due to their high toxicity levels, As, Cd, Cr, Pb, and Hg are designated as priority metals of concern for public health (He *et al*, 2005).

According to Liang and his Co-workers, 2017, Hg, Pb and Cd represent significant health concerns and are categorized as heavy metal pollutants. This is mainly due to their ability to induce adverse health effects, more serious one being their role in carcinogenesis. Trace metals on the other hand such as Se, Zn, Fe and Cu are essential micronutrients, but at concentrations higher than the amount required by the body, they become toxic just as heavy metals whose roles are unknown in living organisms; making them toxic no matter how low their concentration, for example Cadmium, Mercury and Lead. They are therefore non-essential micronutrients. This poses serious risks to human health and ecosystem (Liang *et al,* 2017).

However, due to their occurrence in small amounts in the environment, heavy metals are also regarded as trace elements. (Kabata, 2001).
Hence, assessing the concentrations of heavy metals and trace elements is crucial, in order to check their potential health risks.

Consequently, in studies involving these elements' status in the body, nail measurements have demonstrated to be useful. For instance, in assessing Se status, toenail Se level has always provided a time-integrated and a more superior measure than other biomarkers. In a certain case-control study on investigation of trace elements, nail specimens were employed as biomarkers. The study examined Fe, Co, Zn, Ca and Cr in association to cancer of the upper digestive tract. The results showed that persons who got certain upper aerodigestive tract carcinomas and those who did not, consumed different amounts of minerals. Thus, measuring the concentration of heavy metals and trace elements in nails continues to be vital in clinical research (Janbabai *et al*., 2018).

This study only used nail clippings because most of the body tissues are in a flux state due to metabolic activities, except the nails and hair. For example, after the nail formation, what follows is its complete expulsion from the nail-bed before its isolation from the continuing metabolic activities of the body. Therefore, nails represent the body's exposure or intake during the past few months or so; thus, one millimeter of the nail sample could correspond roughly to one month of the body's nutritional status (Abdulrahman, 2012). It then follows that analysis of heavy metals and trace elements in the nails can provide information about the body intake for a given period of time.

The current study therefore, used using nail clippings of cancer patients and a non-cancer control group in evaluating the concentration of selected heavy metals and trace elements in esophageal and stomach cancer patients. These patients were those being attended to at the Kenyatta National Hospital. The effort of this work was to find a non-invasive and method of fostering early cancer detection, diagnosis and treatment.

1.2 Problem Statement

In Kenya, breast, prostate, cervical, esophageal, stomach and colorectal cancers are the leading new cancer type cases in both males and females across all ages; most of which have no family histories of cancer. Approximately, 70-80% of the Kenyan cancer patients are discovered when the disease is quite advanced, and is nearly impossible to cure (Bray et al., 2018).

In Kenya, there is a rising demand for cancer treatments, but the capacity for detection and treatment is extremely constrained. This presents the government with major health-care policy difficulties. The fundamental issue arises from the fact that the number of patients has been steadily increasing and is predicted to do so going forward, particularly with regard to malignancies of the esophagus, prostate, cervix and breast. However, access to equipment and facilities continues to be a major obstacle. (Wambalaba *et al*, 2019).

Between January 1999 and September 2007, all pathology-confirmed cancers identified in Tenwek Hospital, Bomet County, Kenya, were examined retrospectively. The study found that the stomach, esophagus, prostate, cervix, and colorectum were the five most frequent cancer locations since 1999. 914 of the 2643 newly diagnosed cancer cases, with a growing tendency both inside

and outside the catchment region, were esophageal cancer cases. The youngest patient was 14 years old at the time of diagnosis, and 58 (6.3%) patients were under 30 and 9 (1%) were under 20. (Parker *et al*, 2010).

The study in Tenwek further revealed that cancer of esophagus was the most prevalent cancer seen in Western Kenya affecting even younger generations; and hence highlighted the need for additional research on the environmental and genetic predispositions to esophageal cancer.

According to a study carried out by Lodenyo and Co-workers, 2018, information about stomach cancer remains low in developing countries and especially in Africa; yet it a major killer across the globe. According to the study, 990,000 people worldwide are diagnosed with stomach cancer each year, and 938,000 of them pass away as a result of the illness. Aside from that, this condition has one of the highest burdens of cancer in terms of years of life lost with a disability. Multiple studies have found that nutrition has a key role in stomach cancer, especially in Africa, where incidence rates have continued to increase (Lodenyo *et al*, 2018).

In cancer inhibition and development, heavy metals possess a complex character in their roles; thus, creating a lot of concerns due to their importance to human health and potential toxicity. Their carcinogenic capability depends mainly on their chemical structures and oxidative states. Thus, the complexes they form catalyze redox reactions within DNA, thereby oxidizing the DNA. This leads to DNA damage, promoting the onset of carcinogenesis in most cases (Mulware, 2013).

Heavy metals and trace elements have also been shown to have beneficial effects in biological systems notably in some enzymes involved in metabolism, detoxification, and damage repair, as well as in cellular organelles and portions such as the nucleus, cell membrane, lysosomes, and mitochondria. (Wang, 2001).

However, Beyersmann & Hartwig, 2008 observed that the metal ions engage in interactions with nuclear proteins and DNA that make up cells. Due to DNA damage and mutations brought on by this, cell cycle regulation, carcinogenesis, or apoptosis may result. Their research also showed that oxidative stress and the generation of reactive oxygen species are crucial factors in the toxic and carcinogenic nature of metals such As, Cd, Cr, Pb, and Hg (Beyersmann & Hartwig, 2008).

Having experienced very rapid growth in industrialization and economic development in the urbanization process over the past few years, Kenya has with no doubt pollution issues. These environmental changes affect human health. There is absolutely no research in Kenya that has biologically monitored heavy metals or trace elements levels in humans which can be a direct indicator of the cancer disease in the human body. Hence, the current study focused at determining the concentration of these elements in the human body and establishing their association to the growth of esophageal and stomach cancer cells.

1.3 Objectives

1.3.1 General Objective

To evaluate the concentrations of Cr, Fe, Zn, Cu, Pb, Se, Hg in esophageal and stomach cancer patients in search of a non-invasive method of early cancer screening in Kenya.

1.3.2 Specific Objectives

1) To determine concentrations of selected trace elements in nail clippings of esophageal cancer patients at the Kenyatta National Hospital.
2) To determine concentrations of selected trace elements in nail clippings of stomach cancer patients at the Kenyatta National Hospital.
3) To evaluate the monitoring of concentrations of the trace elements as an early cancer screening method.

1.4 Justification of the Study

International Agency for Research in Cancer, 2012 classified, among other heavy metals, As, Cd, Ni and Cr as group one carcinogens. Ironically, some of the trace elements such as Zn and Cu are biological co-factors for enzymes that are necessary for many intracellular processes. They also have DNA-binding domains (Mulware, 2013). Thus, it is important to critically analyze and quantify, despite their essential biological functions, specific concentration levels in the human body and their effects on the growth of cancerous cells in cancer patients (IARC, 2012).

In the year 2017, Kenya Ministry of Health highlighted the estimated cancer cases, in Kenya in 2012 as 37,000 cases and 28,500 deaths. The highlight informed the basis of drawing the National Cancer Control Strategy 2017-2022 in which the first of the documented five priorities is prevention, early detection and screening of cancer and the second includes diagnosis. However, even though it is curable, most of the cancer patients in Kenya are diagnosed while at advanced stages. The findings of this study will therefore help the health professionals in early presentation, fasten referrals, diagnosis and treatment as revealed by the levels of various elements in the body. Through early detections, most cancers may be optimally cured with complete surgical removal. This is because, at late diagnosis when the symptoms occur, the cancer is no longer localized making surgery not an option.

In addition, no study has been carried out in Kenya that used biomarkers to correlate the levels of trace elements in human population with the prevalence of cancer, hence, this study will be a gateway to environmental monitoring and earlier diagnosis of the cancer.

1.5 Scope and Delimitations of the Study

The current study only focused on trace elements. This is due to the fact that in daily life, it is almost unavoidable not to expose any human population to heavy metals. Thus, analysis of their intake and concentration levels in the body is of great importance. In addition, levels of exposure to these elements can be potentially modified. Consequently, the study only compared the levels of these elements among esophageal and stomach cancer patients with a control group.

Since the elements; Cr, Fe, Zn, Cu, Pb, Se and Hg have been identified among other trace elements by IARC (2012) as having an impact on cancer, they were the only ones analyzed in this study.
In sample collection, cancer patients who had been on chemotherapy treatment for more than one month and those who would have undergone any form of esophageal or stomach surgery were excluded from the study. In addition, any participant engaged in any drug and substance abuse was also be excluded. These measures were meant to make the study more objective.

The study was conducted using nail clippings as the samples since they are bio-accumulators of toxic heavy metals and trace elements. As compared to other tissues, they accumulate elements over longer periods of time without any changes thereby serving as surrogate in measuring the

status of critical body organs. Moreover, these samples don't need special storage conditions, allowing analysis to be performed securely without element loss. In addition, the collection of samples is non-invasive; a factor that is expected to increase the participation rate of the target population. The collection is also easy and economical; which made the study to be time and cost effective.

CHAPTER TWO

Literature Review

2.1 Total Reflection Xray Fluorescence (TXRF)

Total Reflection Xray Fluorescence spectroscopy is a method of non-destructively analysing materials to determine their elemental composition. It entails identifying constituents of a sample. This is done by taking a measurement of the fluorescence X-ray that a sample emits after being excited by a main X-ray source. Individual elements in the sample emit distinctive fluorescent X-rays that are peculiar to that element; consequently, this method can be utilized for both qualitative and quantitative material composition analysis (Beltran et al., 2019).

In conventional XRF technique, a regulated X-ray tube emitting high energy X-rays is used to irradiate a sample of a solid or liquid. When an atom in the sample is attacked by an X-ray with enough energy, which is always more than the atom's K or L shell binding energy, one electron from the innermost shell of the atom gets dislodged (Figure 1). An electron from one of the atom's higher energy orbitals fills the hole left in the inner shell of the atom in an attempt to restore stability. This electron moving to a lower energy state, releases a fluorescent X-ray whose energy is the exact difference in energies between two electron quantum states. Therefore, this energy measurement serves as the foundation for the XRF analysis. (Antosz *etal*.,2012).

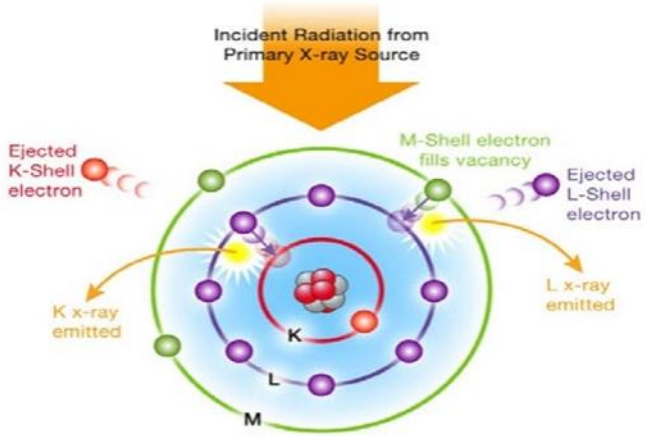


Figure 1 X-ray Fluorescence Process

In TXRF, the radiation coming from the Xray is incident on a given sample at extremely low angle with total reflection; this makes TXRF quite different from the conventional XRF technique (Figure 2). The uniqueness of this technique makes it advantageous because the element being analyzed and its concentration is not affected by matrix effects, the technique is sensitive and can detect concentrations at parts per billion, requiring little amounts of the sample for analysis hence low quantities of reagents. The background noise levels of TXRF spectroscopy are low allowing for, improved limits of detection. (Beltran et al., 2019).

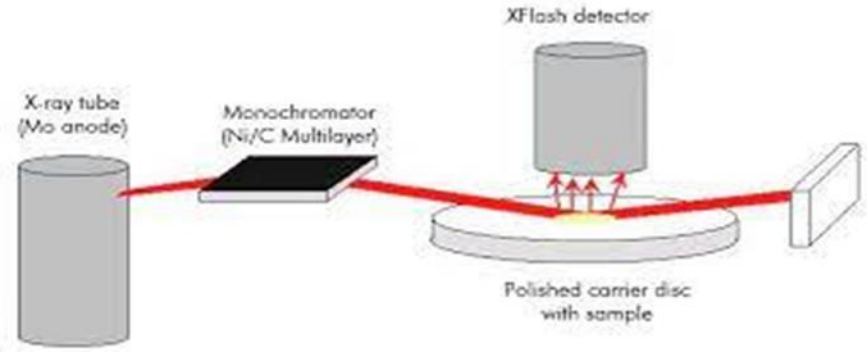


Figure 2 Schematic Working Principle of S2 PICOFOX TXRF Spectrometer

This analytical technique is well established for determination of multi-elements in many sample types, especially micro-powdered and liquid samples. As seen in Figure 3 below, the produced X-ray spectrum is unique to each individual component found in the sample, with intensities inversely proportional to concentration of each element (Antosz *et al*., 2012).

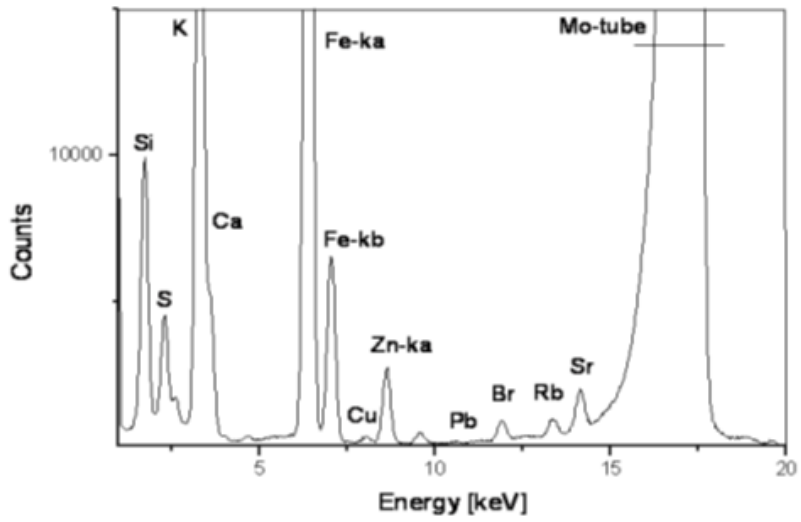


Figure 3 Typical Spectrum Showing Peaks of Several Elements

2.2 Nail Bed Trace Elements Analysis for Early Cancer Detection

Various studies on analysis of soil samples, air and water provide insufficient data to assess environmental pollution's impact on health hazards because the range of contamination by heavy metals may vary in given areas depending on the status of the environment (Liang *et al*., 2017). Analysis of hair and nail clippings is significant in the monitoring of heavy metals in the environment, making them important biomarkers. (Environmental Protection Agency, 2018) considers them as very important biomarkers. As metabolic products, nails and hair are able to accommodate heavy metals in their structure (Liang *et al*., 2017).

The main constituents of human nail clippings are keratin-rich proteins. Trace metals are accommodated in these proteins in proportion to how many different processes are consuming them; for example, synthesis of proteins. As a result, the nails are increasingly being used in clinical trials as markers for trace elements analyses. It is feasible to collect and preserve them for several months before analyzing them in epidemiological research for trace element concentration measurements (Janbabai *et al.,* 2018).

Consequently, a relationship may be established between trace element detection in nail beds and early cancer detection. A study to investigate trace elements in hair and nails of patients with

stomach cancer, for example, revealed that trace elements' average concentration increased significantly as the disease progressed. The study's findings revealed a relationship between an increase in potassium, copper, phosphorus, lithium, selenium, and iron with the spreading of carcinoma of the stomach. As a result, high concentration of trace elements in nails could serve as a diagnostic indicator of the development and cause of cancer. (Janbabai *et al*., 2018).

In another research, Blaurock-Busch *et al.* (2015), levels of metals in healthy people's nails from a certain region in India with high cancer prevalence were randomly compared with a European group. The study revealed that 13% of the healthy Indian population had nails with Cd concentrations above the accepted reference range as compared to a similar healthy European population where only 6% exceeded that range. The study revealed that for the concentration of As in 4.8% of the healthy population exceeded the reference range as compared to 0% in the European group. The study therefore concluded that the healthy Indian group showed a considerably higher metal burden for several toxic heavy metals than the healthy European group. As a result, people who live in places where harmful metals are present are likely to experience more chronic metal exposure which can cause rising cancer rates in such regions; this is because such metals could possibly lead to DNA damage and oxidative stress (Blaurock-Busch *et al.*, 2015).

Metal concentrations in healthy and cancer patient nails can therefore be compared and use to assist medical organizations in establishing early intervention and appropriate treatment plans with the intentions of reducing cancer statistics (Blaurock-Busch *et al.*, 2015).

2.2 Trace Elements and Heavy Metals and Human Biological Systems

World Health Organization's report of 1996 indicated that essential trace elements have a significant influence in the biochemistry and physiology of plants, animals, and other living things. They participate significantly in oxidation-reduction reactions and are components of the majority of significant enzymes. The report also highlighted how some heavy metals and trace elements are involved in these redox reactions. Copper for example, has the ability to cycle between its oxidized and reduced states; thus, Copper II and Copper I respectively. Due to the creation of superoxide and hydroxyl radicals during the transitions between the oxidation states, which change an organism's DNA, copper is potentially hazardous due to this feature. (WHO, 1996).

In another study conducted by Shelnutt and Co-workers in 2007, the findings revealed that the lung is the principal organ affected by chromium exposure, which primarily occurs through inhalation and contact with the skin (Shelnutt *et al*, 2007).

Due to its role in the metabolism of proteins, fats, and glucose through enhancing the action of insulin, chromium III is a vital nutrient for humans. The increased risk of chromium-induced diseases among industrial employees, who are typically exposed to Chromium VI, has caused a significant focus on occupational exposure, though. Most regulatory and non-regulatory organizations classify this species of chromium as a dangerous industrial contaminant and human carcinogen. (Guertin, 2005).

On the other hand, in human body, Pb occurs primarily as a result of breathing in dust or aerosols that contain lead. As reiterated by Flora and Co-workers, 2006, in contrast to children, who may consume more than 50% of the lead in drinking water, adults absorb 35 to 50% of it. Therefore, factors including physiological state and age have an impact on how well it is metabolized. Liver, kidney, and other soft tissues including the heart and brain absorb majority of the substance after it has been absorbed by the body (Flora *et al*, 2006).

Experimental studies by Goyer, 1996 however indicated that Pb is carcinogenic, considering that it triggered kidney cancers in rats and mice. Therefore, IARC considers this metal as a human carcinogen. Additionally, exposure to Pb causes sister chromatid swaps and gene alterations, and most Studies conducted *in vitro* and *in vivo* revealed that Pb compounds harm DNA through a variety of implicit processes, which include interference with DNA synthesis and repair, oxidative damage, and interactions with proteins that bind to DNA and tumor suppressors (Goyer, 1993).

In Kenya, there are reported cases of high iron content in water being consumed. In a study conducted in Athi River which serves about four million people with drinking water, a mean of 2.5 mg/L of iron was recorded, against United States Environmental Protection Agency approved concentration of 0.3 mg L^{-1}. This analysis reveals overexposure of the surrounding population to iron consumption. This is alarming because majority of residents use the water from Athi River

without any form of treatment (Njuguna *et al*, 2021). Being a trace element, the concentration of Fe in human body becomes toxic at elevated levels.

A study conducted by Sankar, 2005 revealed that Hg is a frequent toxin and environmental contaminant that changes bodily tissues and has detrimental effects on health. Distinct chemical varieties of mercury are present in the environment, exposing people to them. The many forms of mercury include organic mercury compounds, inorganic mercuric, mercuric oxide, and elemental mercury vapor. Because mercury is so widespread in the environment, it is impossible for plants, animals, and people to avoid coming into contact with it (Sankar, 2005).

In identifying mercury as a genotoxic agent, studies have shown that its toxicity has been linked to oxidative stress as the molecular mechanism. The creation of Reactive Oxygen Species, which is known to damage DNA in cells, has therefore been demonstrated to be induced by mercury. This action triggers the beginning of cancer-causing pathways. (Valco *et al,* 2006).

2.3 Concentration Trends of Trace Elements in Cancer Patients

According to Abo El-Atta (2011), a certain study conducted in Cairo, Egypt, assessing the estimates of human health risks in residents from different regions of the city gave the following conclusion at $p < 0.05$: The levels of cadmium, copper, chromium, lead, arsenic, vanadium, manganese, antimony, nickel and titanium were found to be elevated beyond what is considered to be generally safe for the general human population. The study further revealed that Cd concentrations were significantly increased in cancer patients' tissue and urine samples than the corresponding cancer-free control group. Increased concentration of Cu was also observed in the cancer patients' urine sample in relation to the control group. Fe concentration was so much reduced in urine samples of the victims of cancer in comparison to their corresponding control group. However, Pb and Zn levels were high across the board in the samples taken but there was no obvious difference between the cancerous and non-cancerous groups.

In another study which estimated the concentrations Zn, Cu, Cd, Pb and Fe in urine and tissue samples of breast cancer patients, concurring results were found. The basis of the study was mainly on previous studies which had shown that the development of breast cancer and heavy metals are

closely related. It was discovered in this study that the mean urinary Cd concentration levels in those with cancer of the breast was significantly higher as compared to patients with benign breast diseases (Abo El-Atta, 2011).

A study done by Antila et al. (1996), however gave a contradictory conclusion that the mean concentration of Cd in forty-three cancer patients didn't differ so much from thirty-two healthy control; even though the content of Cd in both groups reached high concentrations. The high Cd concentration in both groups was attributed to Cd's characteristic behavior of always being bound tightly to adipose tissue and its poor excretion in milk.

Further research works were conducted to confirm the findings of Antila et al. (1996) as per the literature above. For example, Ionescu et al. (2006) carried out an analysis for Cd in twenty frozen biopsies of breast cancer. The results revealed higher concentration of Cd in cancer biopsies as compared to the samples from a healthy comparison group. In addition, assessment of the concentration of Cd in samples from breast tissues of twenty-one breast cancer patients in comparison to nineteen who had benign breast diseases, the results showed that as compared to the benign group, Cd concentration had significantly increased in the malignant breast tissues. These authors therefore concluded that the findings of Antila et al. (1996) on the non-significant Cd concentration in breast cancer could be attributed to the location of sampling. Moreover, in the human body, there is no homeostatic control for Cd. It is therefore considered to be very toxic even in small amounts. Introducing Cd ions in the body replaces Zn ions causing even more metabolic disorders (Mulware, 2013).

The research findings given by Karimi *et al.* (2012), in determining the association between prostate cancer risk among men living in Klang Valley of Malaysia and heavy metals also gives consistent results. In the study which involved one hundred men aged fifty to eighty-six years, composed of fifty prostate cancer patients and fifty controls. The study revealed that the levels of Se and Zn in the nail and hair samples in both cases was low as compared to controls. Prostate cancer patients on the contrary, had significantly high concentrations of Copper, Iron and Manganese in their nail and hair samples as compared to the non-cancer control groups. The results were as shown in Table 1 with the values resulting from Mann-Whitney U-test and Independent-sample t-test.

Table 1 Concentrations of Trace Elements in Study Participants' Hair and Nails

Parameters	Case Mean±SDev	Median (25%-75%)	Control Mean±SDev.	Median (25%-75%)	P[a]
Concentrations in hair					
Selenium	7.15±3.5	6.0 (4.48-9.74)	10.4±4.52	11.35 (6.88-14.00)	0.001
Zinc	3.29±2.22	3.10 (1.48-3.99)	4.29±2.53	3.75 (2.92-4.85)	0.018
Cupper	0.09±0.03	0.08 (0.06-0.1)	0.07 ±0.02	0.07 (0.05-0.08)	0.029
Ferrus	1.23±0.968	0.97 (0.65-1.5)	1.21±1.472	0.72 (0.41-1.09)	0.25
Manganese	0.07±0.04	0.07 (0.05-0.09)	0.055±0.05	0.02 (0.01-0.10)	0.001
Concentrations in nails					
Selenium	7.23±3.11	6.92 (5.26-8.98)	9.03±3.69	10.17 (5.90-11.69)	0.001
Zinc	2.70±1.49	2.83 (1.31-4.07)	3.97±4.06	3.32 (2.73-4.36)	0.01
Cupper	0.07±0.03	0.06 (0.05-0.09)	0.06±0.02	0.05 (0.04-0.08)	0.08[b]
Ferrus	1.58±0.88	1.50 (0.89-2.04)	0.92±0.74	0.65 (0.41-1.44)	0.001
Manganese	0.10±0.06	0.1 (0.06-0.13)	0.05±0.04	0.05 (0.01-0.08)	0.001

The reviewed literatures so far also relate to a study done in Bomet County in Kenya where the prevalence of esophageal cancer in the region was assessed in association with Se concentration in serum. The study's findings reported a positive association between the prevalence of esophageal cancer and higher Se concentration in serum (Pritchett et al., 2017). These results are also supported by the reports of other authors, who determined the concentration of Se and Zn of esophageal cancer patients and healthy subjects' serum samples, using different Atomic Absorption Spectroscopy technique. The mean concentrations were Se = 117.3 μgL^{-1} and Zn = 1020.2 μgL^{-1} in the healthy subjects and Se = 98.4 μgL^{-1} and Zn = 620.6 μgL^{-1} in the esophageal cancer patients (Goyal *et al.* (2006).

Similarly, in another study to establish trace elements and cancer risks, the findings revealed a negative correlation between Se exposure and prostate cancer risk. There was also an observed reduction in risk as far as lung cancer was concerned in relation to Se exposure. In addition, an inverse association was established between breast cancer and Zn. Stomach, colorectal and breast cancers were however reported not to have any association with Se; while prostate cancer also had no association with Zn. The findings also revealed a strong association between As in bladder and lung cancers (Silvera & Rohan, 2007).

Therefore, based on reviews of various literature, it is evident that there is a disparity in the levels of heavy metals between cancer patients and healthy subjects. These distinct patterns could be of significance in early detection and diagnosis of cancer.

2.4 Specific Trace Elements Associated with Different Cancers

In another case-controlled study carried out among thirty patients with cancer of the bladder and a control group composed of thirty volunteers, trace elements in urine were determined. The analyzed trace elements were: Pb, As, Se, Cu, and Zn where Atomic Absorption Spectroscopy (AAS) technique was used. A comparison of these elements in the urine of bladder cancer patients and the healthy control group gave the levels of Zn and Se as Zn = 0.15 mgL^{-1} and Se = 0.06 mgL^{-1} in the patients ($p < 0.05$) and Zn = 2.54 mgL^{-1} and Se = 0.03 mgL^{-1} in the control group. There was however no significance difference noted for urinary Cu, As, and Pb. This study therefore suggested that proliferation of cancer bladder cells could be associated with the concentration of Zn and Se. This is due to excretion of these two elements in the urine of the patients of cancer of the bladder (Lin *et al.,* 2009).

According to a study by Lin and Co-workers (2009), low Se levels (0.04 mg/L) in serum in esophageal cancer cases was also observed. This was attributed to the involvement of Se in numerous biological pathways during its metabolism. After its methylation, it is eliminated from the body through the urine as trimethyl-selenomnium ions. The levels of Se in urine are also observed to be lower than in serum because its volatile forms are exhaled, while the one in the urine varies with intake (Lin et al., 2009). Concurring results were also obtained by Goyal and Co-workers (2006) in a study conducted in India which indicated that there was a close link between Se, Zn and Cu and esophageal cancer. There was however lower Se and Zn levels in serum but higher levels of Cu as compared to the controls.

As reported by Goyal and Co-workers (2006), Zn and Cu as microelements, regulate the physiological functions of many organs of the body and produce pathological changes in them. Thus, in many diseases, the values of serum Cu are significantly high e.g., in Chronic Obstructive Pulmonary Disease (COPD), psychosis and also malignancy. As an element, Zn is specifically required for cell division, DNA and protein synthesis. Se on the other hand, serves as an active site for the Selenium-dependent Glutathione peroxidase (GSH-Px) enzyme. This is an enzyme with

four subunits in which one of them contains a single Se atom. Se inhibits free oxygen radical production, thus protecting the cell. In addition, seleno-proteins also transport Vitamin E which is an important antioxidant. Thus, there is an obvious correlation between GSH-Px activity and carcinogenesis (Goyal et al., 2006).

Low amounts of Se in serum also induces breast, prostate, stomach, esophagus, lungs and colon cancer risks in quite a number of human epidemiologic studies. Hence from Goyal and Co-workers (2006) study, reduced levels of Cu and Se together with high Zn levels in patients of esophageal cancer in comparison to the corresponding controls supported the existing correlation with cancer of esophagus in the Indian population.

According to another study among Egyptian females, Cd concentrations significantly increased in tissue and urine samples of cancer patients in comparison to the non-cancer control group. It was carried out on one hundred females composed of seventy-five breast cancer patients and twenty-five non-cancerous control group with benign breast diseases. The study aimed at investigating the role played by a few heavy metals Cu, Cd, Fe, Pb and Zn, in inducing breast cancer. Analysis was done using Inductive Coupled Plasma (ICP) – mass spectrometer technique. In comparison to the corresponding non-cancerous control group, the outcome revealed a great significant increase in urine Cu concentration in breast cancer patients. Iron concentration in urine samples was however significantly reduced among the cancerous group as compared to the non-cancerous group ($p < 0.05$). Among the studied population, Pb and Zn showed no change that was statistically significant at p 0.05. In conclusion, a link between breast cancer and a rise in Cd and Cu with a decrease in Fe was discovered (Abo El-Atta, 2011).

Abo El – Atta (2011) further investigated the possible mechanism for breast cancer induced by Cd. This involved an *in-vitro* study by use of cells from breast cancer and normal cultured mammary cells. It was reported that the compound, Cadmium Chloride not only triggered damage of DNA in both breast cancer cells and mammary cultured ones, but it was also cytotoxic. This was as a result of mutations in the sequence of their nucleotide since it originates from metastatic breast cancer. Hence, Cd can trigger or promote the growth of mammary cells therefore it is also considered as a chemical carcinogen. The significant reduction in the concentration of iron in urine may be associated with its reduced storage in female patients. This is as a result of the gradual

increase in the absorption of Fe during periods of pregnancy. In addition, increased Cd concentration and decreased Fe could be due to vaginal bleeding disorders or menstruation. This suggests a general mechanism of Cd and Fe uptake through a duodenal metal which is a transporter protein whose role involves uptake of Fe into the mucosa cells and transportation of Cd; it is however upregulated by Fe deficiency. Hence, depletion of Fe stores and their deficiency initiates increased Cd uptake and accumulation. Since there was also increased Cu in tissue and urine samples of breast cancer patients, it was concluded that Cu has a close association with breast cancer and its action mechanism is similar to Cd breast cancer induction (Abo El – Atta, 2011).

In an assessment correlating different stages of cancer of the thyroid with the concentration of heavy metals in human tissue and blood, Cd, Se, and Zn levels in the tissues were elevated in stages III and IV patients as compared to those in stage I. It was observed that the level of Cd was greater in patients with higher stages of the tumor as compared to lower stages. This gave a conclusion that thyroid Cd accumulation could be attributed to the progression and aggravation of thyroid cancer (Pellegriti *et al.*, 2013).

Therefore, literature reveal that each cancer is unique to excess or deficiency of particular trace elements in the human body; creating a correlation between various cancers with specific elements.

2.5 Lifestyles and Regional Prevalence of Some Cancers in Kenya

Various studies have investigated the relationship between the presence of heavy metals and people's lifestyles such as cigarette smoking so as to establish whether or not such habits could be a source of any of these elements. Ma and Co-workers in 2017 carried out a study to analyze the levels of heavy metals in smokers' and non-smokers' nails to determine if there is a link between these elements' presence in the body and smoking in a Saudi Arabian population. Inductively Coupled Plasma Mass-Spectroscopy was used to measure the concentration of toxic metals. The analysis showed an association between smoking and concentrations of heavy metals as compared to non-smokers. In addition, there was also a direct relationship between the duration of smoking and the concentration of the elements in the body (Ma *et al*., 2017).

It has been established that the use of tobacco contributes to a number of chronic diseases which include cardiovascular disorders, lung diseases and cancer. Due to this factor, many regulations have been set up worldwide to restrict the use of tobacco (Mulware, 2013).

Many researchers have observed that carcinogenic materials and other toxic substances present in tobacco and its products, spread to the bloodstream and accumulate in many body organs, thus causing many diseases. For example, Ni leads to kidney and cardiovascular diseases; and it also produces carcinogenic complexes when it reacts with carbon monoxide produced during tobacco smoking. Heart, nerves and blood vessels' problems are associated with Pb while Cd is associated with kidney failure. In addition, the Agency for Toxic Substances and Disease Registry (ATSDR) highlights Pb, Hg and Cd as among the top ten toxic metals in comparison to other hazardous substances (Mulware, 2013).

An appreciable proportion of Cd is contained in all forms of tobacco. This makes the smoking of tobacco the main source of Cd exposure in humans and its impact on cancer. These effects are reported to be so pronounced because absorption of Cd into the body cells from the lungs is much greater as compared to absorption into the gastrointestinal tract (Mulware, 2013). These findings support what the previous researchers have revealed; that, heavy metals have negative effects on biochemical processes as they compete for sites with essential elements and take their place thus causing damage to the cell membrane (Jaishankar *et al*., 2014).

Subsequently, other studies have also described the mechanism through which these heavy metals react by forming complexes with proteins in places where amine, carboxylic and thiol groups are present. These biological molecules end up losing their ability for proper functioning as a result of the modifications made by the heavy metals; thus, leading to cell malfunctions or cell death. These cell modifications may also produce free radicals which can cause the biological molecules to be oxidized thus triggering the growth of cancer cells (Jaishankar *et al.*, 2014).

Another study was conducted with the aim of determining levels of the heavy metals' levels in human nail and hair samples in Borno State, Nigeria (Abdulrahman *et al*., 2012). This was to correlate the workplace and environment with heavy metals' concentration in the body. The samples for the study were obtained from welders in iron workshop and liquor users. A comparison of the analyzed results between the two groups was done. Highest concentrations were observed

in Zn and lowest in Cu levels in both groups. There was, however, no relationship between liquors and the concentration of heavy metals in the samples. Consequently, the concentration of these heavy metals among the iron welders was significantly high as compared to the liquor subjects. It was therefore concluded that work place influences the concentration of heavy metals in the body. Thus, there is need for creation of public awareness as far as hazards associated with various occupations are concerned so that precautions can be taken (Abdulrahman *et al*., 2012).

Odera and Co-workers (2017) further highlights that Kenya as a region is now termed as esophageal cancer corridor in Africa. This is attributed to her being among the highest in esophageal cancer incidence rates worldwide; even though very limited research has been done on this matter. Esophageal cancer in Kenya is also identified to be so unique due to high percentage of younger generation suffering from it even without any family history of such. Hence, their study concluded that factors such as intake of hot drinks, malnutrition, genetic factors and drinking of alcohol should be critically looked into as potential risk factors (Odera *et al*., 2017).

Awichi (2019) reported in the Kenya's National Cancer Institute detailed prevalence rates of cancer in eleven counties namely; Uasin Gishu, Meru, Nairobi, Kisumu, Mombasa, Kakamega, Kiambu, Nyeri, Nakuru, Bomet and Embu in Kenya. The exercise was a move towards shaping the government's intervention to combat the scourge.

The reports as shown in Figure 4 revealed that esophagus cancer, was most prevalent in Kisumu (8.6%), Kakamega (9.9%), Nyeri (8.2%), Nakuru (7.1%), Bomet (21.8%) and Uasin Gishu counties. It is still the leading killer cancer, affecting both women and men. Moreover, the report showed that men living in Mombasa (16.3%), Nairobi (32.1%), Embu (24.1%) and Meru (24.5%) counties were prone to prostate cancer than any other type of cancer (Awichi, 2019).

Breast cancer was still ranked as the most prevalent cancer among the Kenyan women from the selected eleven counties, even though according to the report, it was more prevalent in Mombasa, Nakuru, Nairobi, Meru, Kiambu and Embu; while in Uasin Gishu (21.1%), Nyeri (8.2%), Bomet (21.8%), and Kakamega (8.2%) counties, most women patients suffered from esophagus cancer. This report supported the fact that different cancers are unique to different environments and the regional cancer prevalence is evident in Kenya with esophageal cancer being noted to be so much on the increase in specific regions (Awichi, 2019).

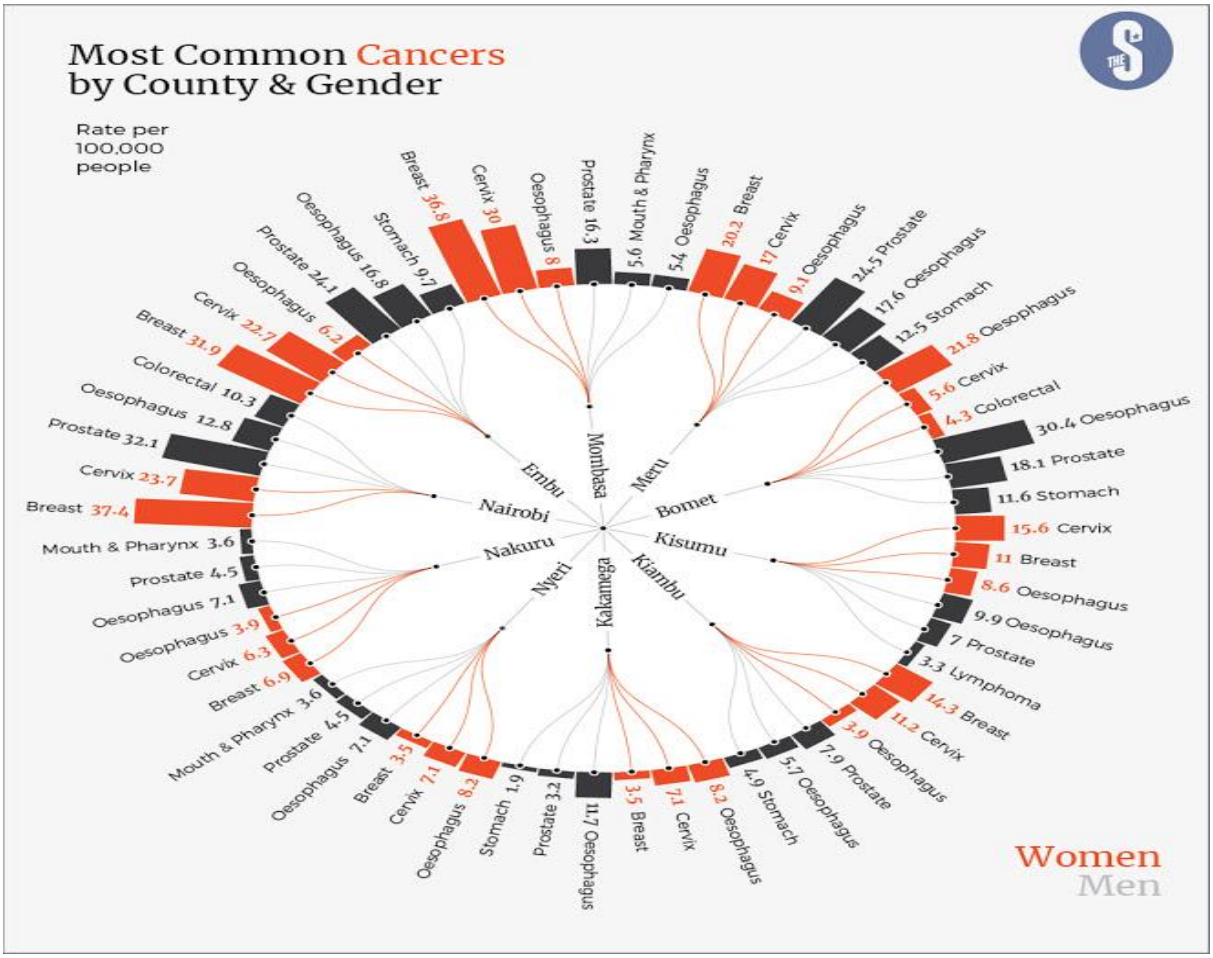


Figure 4 **2017 National Cancer Institute Report on Cancer Prevalence per County**

Further, evaluation of heavy metals in Lake Naivasha, Kenya sediments, was carried out and the results revealed higher values near the surface which corresponded to a water depth of 6 to 8 meters. The analysis also showed that heavy metals from natural sources included Pb, Ni, Cu, Cr and Al; while those from both anthropogenic and natural sources were As, Fe, Mn and Zn. This study therefore concluded that other than leaching from agricultural fertilizers and runoffs, other activities by the population which are anthropogenic such as improper disposal, also lead to increased concentration of trace elements in lakes (Maina *el at*., 2019).

CHAPTER THREE

Research Methodology

3.1 Study Design

This was designed as a case-control study which involved administration of written questionnaires accompanied by a consent form to obtain nail clippings from esophageal and stomach cancer patients being attended to, at the Kenyatta National Hospital, Nairobi, Kenya. Simple random sampling technique was applied in selecting the cases through the assistance of KNH Health Information Department, while the control group were randomly selected from KNH patients at the Orthopaedic surgery, General surgery and Ophthalmology wards, with no known cancer history and had not been admitted for any digestive disease or cancer.

3.2 Ethical Considerations

The purpose and nature of the study was explained to the participants and their consent obtained. They were assured of confidentiality of the information they provided, and was maintained throughout the study. In addition, the participants were made to understand that participation in the study was voluntary and no procedure could be carried out without their consent. There were also no cost implications to the participants at any point throughout the study.

An official approval for this study was obtained from Kenyatta National Hospital-University of Nairobi (KNH-UoN) Ethics and Research Committee with approval number P124/02/2020. It was then endorsed by the Deputy Director of Medical Research, while the Director of the Department of Health Records and Information gave a written permission to access the daily records of patients' files in order to identify the appropriate cases for the study.

3.3 Study Site

The study was carried out at the Kenyatta National Hospital Cancer Treatment Centre. This was a suitable location due to the fact that in Kenya, KNH is the largest public referral hospital with comprehensive cancer treatment facilities. Most cancer patients have to travel from all corners of the country to access treatment.

3.4 Study Population

The case participants were recruited from adult esophageal and stomach cancer patients aged 20 to 65 years. The control group comprised of adult volunteers with matched age and sex with no known cancer history from Orthopaedic surgery, General surgery and Ophthalmology wards. Neither the cases nor control participants had any history of drug and substance abuse. An informed consent was obtained and written questionnaires were administered by the principal investigator prior to participating in the study.

The following criteria was used in recruiting participants for the study:

Inclusion Criteria

- Patients at the KNH aged between 20 - 65 years who had been diagnosed with esophageal or stomach cancer; with no history of drug and substance abuse and had given their consent to be included in the study.
- Volunteers from Orthopaedic surgery, General surgery and Ophthalmology wards aged between 20 - 65 years with no chronic disease or drug abuse history who had consented to be included in the study.

Exclusion Criteria

- Patients aged between 20-65 years diagnosed with esophageal or stomach cancer but had declined being included in the study.
- Patients who had undergone either gastric or esophageal surgery.
- Patients who were younger than 20 or older than 65 years old.
- Patients who were considered too ill to consent (mainly in final cancer stages).
- Both cases and control individuals with history of drug and substance use.

3.4 Sample Size Determination

The desired sample size for the cases in this study was established using Cochran's formula as follows:

$$n = Z^2 + pq \,/\, e^2$$

where:

- "n" was the size of the sample
- "e" stands for the required precision level. (i.e., the error margin)
- "p" as the proportion of the population estimated to have the characteristics being measured
- q represented 1-p
- The Z value was obtained from a standard normal table

It was assumed that an eighth (1/8) of the cancer patients in KNH suffer from either cancer of the esophagus or stomach, and are aged between 20-65 years. So, p = 0.125

For 95% Confidence Level, the Z value is 1.96.

Therefore; $n = (1.96)^2 + (0.125)(1 - 0.125) \,/\, (0.05)^2$

n = 168. A random sample of 168 cases in the target population was therefore to give 95% confidence level. For the control group, the sample size was assumed to be thus, 84, that is half of the of the cases. Therefore, the estimated sample size of 168 was the minimum necessary to achieve the required representative population. This was however not achieved during the sample collection because of the time – frame of the research period and COVID-19 restriction measures by the Ministry of Health in the month of April, 2021. There were to be very minimal visits to any healthcare institution due to the increased cases in the country. Sampling was abruptly called off in March, 2021 with a total sample collection from 126 participants; 95 being cancer patients and 31 non-cancer control group.

Therefore, with the same assumptions used in sample size determination, the new confidence value was therefore:

$$n = Z^2 + pq \,/\, e^2$$

$$126 = Z2 + \frac{(0.125)(1 - 0.125)}{(0.05)2}$$

Z= 9.069.

The Z value obtained gave a P-value < 0.0001 from Z-score calculator. Thus, the result was significant at p < 0.05. The assumption that an eighth of the cancer patients in KNH suffer from

either cancer of the esophagus or stomach, and are aged between 20-65 years was therefore rejected; implying that less than an eighth of the total number of patients suffered from either of these cancers.

3.5 Data Collection Procedures

After obtaining an informed consent from each of the participants to the study, written questionnaires were administered before sample collection to capture the respondents' demographic information, socio-economic status, dietary habits, lifestyles and cancer history. No enumerators were hired in data collection because the questionnaires captured very minimal information which were only intended to be used in inclusion and exclusion criteria. There was no questionnaire pretesting carried out.

Data quality was ensured by conducting continuous checks of the completeness of questionnaires. The data was entered and managed in Microsoft Access database where it was saved in a password protected database.

Fingernail samples of approximately 50 mg were collected from each of the participants at the Kenyatta National Hospital, Cancer Treatment Centre, Orthopedic and General surgery wards using stainless sterilized nail cutters. The samples of each individual were preserved in a small plastic bag and properly labelled pending cleaning, drying, weighing, digestion and analysis at the Institute of Nuclear Science and Technology, University of Nairobi.

3.5.1 Sample Carrier Preparation

Sample preparation was preceded by cleaning of the sample carriers according to Bruker S2 PICOFOX ^{TM} Manual. This was done in 800 ml laboratory glass beakers. The procedure involved mechanical pre-cleaning of the sample carriers with a fluffy-free tissue and acetone, mounting of the washing cassette with the pre-cleaned sample carriers, transferring of the washing cassette into the glass beaker filled with cleaning solution and heating on a heating plate for five minutes before rinsing thoroughly with distilled water. This was followed by transferring into another glass beaker half-filled with 10% nitric acid and heating for two hours. The sample carriers were then transferred into a beaker filled with distilled water and heated for five minutes before rinsing

thoroughly with distilled water, drying on a heating plate and careful short wiping of the sample holders using a fluffy-free tissue soaked in acetone. Eventually, 10 μl silicon solution was applied into the centre of the sample carriers and the droplets allowed to spread into a circle of about 20 mm after which they were dried at about 80^0 C for 30 minutes on a heating plate. For purity control measurement, analysis of the cleaned blank sample carriers for any trace element content was then done using Total Xray Reflection Fluorescence (Bruker AXS Microanalysis GmbH) technique.

3.5.2 Sample Preparation

Each of the nail samples was cleaned under continuous stirring according to sample washing procedure proposed by International Atomic Energy Agency, 2003. This was done repeatedly using Triton X-100 as a nonionic detergent, deionized water, and acetone. The samples were then dried between filter papers for 24 hours and weighed before digestion.

Wet acid digestion method using nitric acid was used. This involved weighing an average of 25 mg of the already cleaned and dried nail samples and placing them into polypropylene tube and adding 1 ml of concentrated HNO_3. The mixture was then left overnight at room temperature for complete digestion. Finally, the digest was diluted to 10 ml using deionized water ready for TXRF analysis. The accuracy of the procedure was validated by three replicate measurements.

3.5.3 Quality Assurance Protocols

Quantification of the elements in the sample and to improve the precision of qualitative analysis, 0.3 ppm of Yttrium (Yttrium ICP Standard, Merck KGaA, Germany) liquid was used as a mono-element standard for internal standardization. This was after determination of the qualitative element distribution in the sample using TXRF to avoid spectral interferences between the added internal standard element and the elements already present in the sample. Thus, it was ensured that the element used as an internal standard was not present in the sample itself.

The volume of the internal standard to be pipetted into the sample solution was obtained as follows: $M_1V_1=M_2V_2$, where, M_1 was the concentration of the stock internal standard solution in ppm, V_1 was the volume to pipette, M_2 was the concentration of the sample solution in ppm while V_2 represented the volume of the sample solution in mililitres. The internal standard solution was added to each of the sample solutions and thoroughly homogenized.

A measured volume of the homogenized mixtures was then transferred to sample carriers by means of an automatic micropipette, dried on a heating plate and finally analyzed for elemental content using TXRF (Bruker AXS Microanalysis GmbH) technique to detect the selected trace elements.

3.6 Experimental Procedures and Instrumentation

The TXRF instrument used has molybdenum X-ray tube anode and a silicon-drift detector. It was operating at a tube current of 1000 µA, working at 50 kV and set at 200 seconds for each sample analysis. Only one sample was loaded and analyzed at a time by the automatic sample carrier. The spectral lines used in this study were L - α and K – α. Arsenic was used as the mono-element standard sample for gain correction and confirmation of any detector artifact like background and escape peaks. This mono-element standard sample compensated for any spectroscopic amplification drift. Once a known fluorescence peak has been measured twice, a correction value is provided to the spectroscopic amplifier (Bruker, 2007). The software application Bruker AXS Microanalysis GmbH was used to interpret the TXRF spectra and evaluate the results.

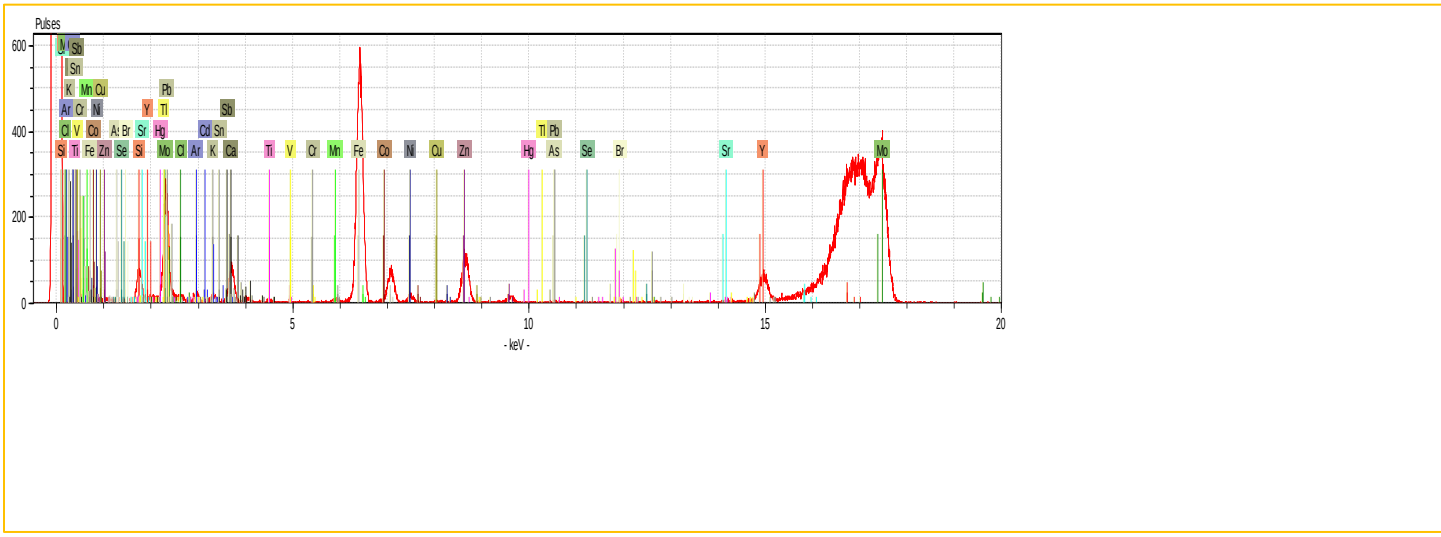


Figure 5 Spectrum of a Finger Nail Clippings Sample

3.7 Data Analysis

Ross Ihaka and Robert Gentleman (R) statistical software was used in analyzing the data obtained from the samples. This involved employing basic statistical analysis using the R statistical package to perform two-sample differences tests. These were presented in interquartile range, mean, median and means' difference significance tested using t test.

The process involved downloading and installing R-studio in Microsoft windows 10 PC, then R Markdown file format for making dynamic documents was used to read and display the data on mean concentrations which had previously been saved in Microsoft excel spreadsheets. These data files were then converted to Comma- Separated Values, CSV files. The data were grouped as data frames and displayed according to participants' cases and the trace elements being analyzed as outlined in Appendix 4. Data sets were outlined for each class of participants, then box plots obtained to identify outliers which were eliminated as seen in Appendix 5.

3.8 Study Limitations

There was language barrier with some of the participants. This was minimized by use of translators. The translators were mainly family members who had accompanied the patient to the hospital.

Some of the participants were not able to read and write; therefore, the investigator had to read out and explain the questionnaires and the consent forms in such situations.

The nails, as elemental biomarkers, could have been contaminated by medications or nail polishes. The nail cutters used to obtain the clippings could also have imparted elemental contamination to the nail samples. These constraints were managed through ultrasonic cleaning procedures using polar and nonpolar solvents, as proposed by the International Atomic Energy Agency in 2003.

3.9 Study Population

The participants for this study comprised of adult patients scheduled for and receiving treatments at the Kenyatta National Hospital. The collection of the nail clipping samples took place from October 2020 to March 2021 and a total of 126 samples were obtained from stomach, esophageal

cancer patients and a non-cancer control group. The distributions of the study participants were as shown in the pie chart below in Figure 6.

CHAPTER FOUR

Results and Discussion

4.1 Results

The total number of newly diagnosed cancer of esophagus patients were 72 (57%) out of 126 participants. Compared to the percentage of stomach cancer which was only (23)18%, there was a clear indication that esophageal cancer is on the rise and affects a large part of the population. This observation agreed with a study by Awichi, 2019 carried out in Bomet County, Kenya which revealed that esophageal cancer ranks 9th and 5th world's most prevalent cancer and developing nations respectively, with an average of 300,000 new cases diagnosed each year.

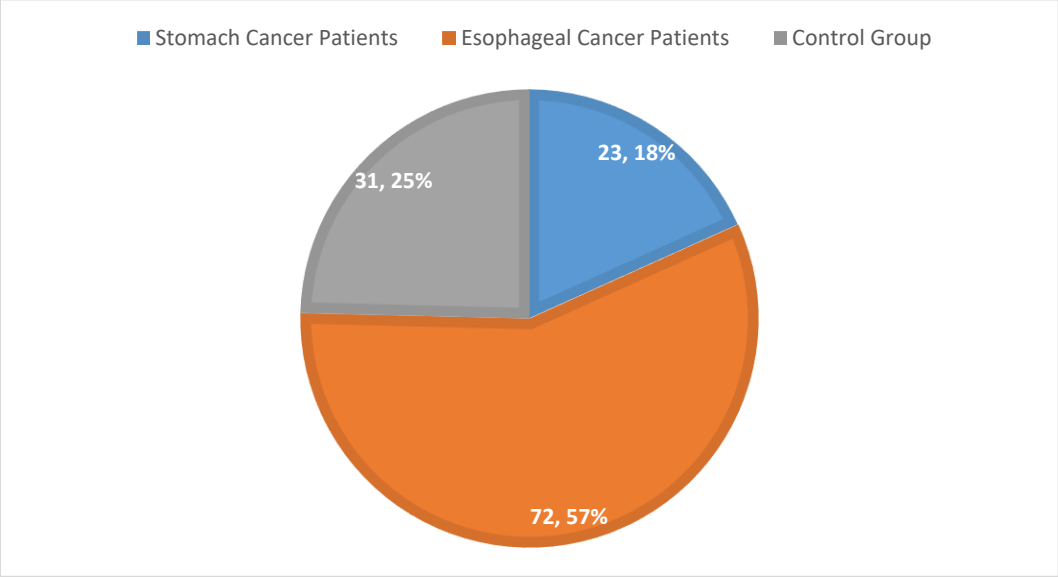


Figure 6 Pie Chart Showing the Study Population

In terms of age distribution, 17 out of the 95 cancer patients who participated in this study were aged between 20 to 45 years, those between 46 to 55 years were 41 while 37 were aged above 55 years. Range of age distributions were as shown in Figure 7.

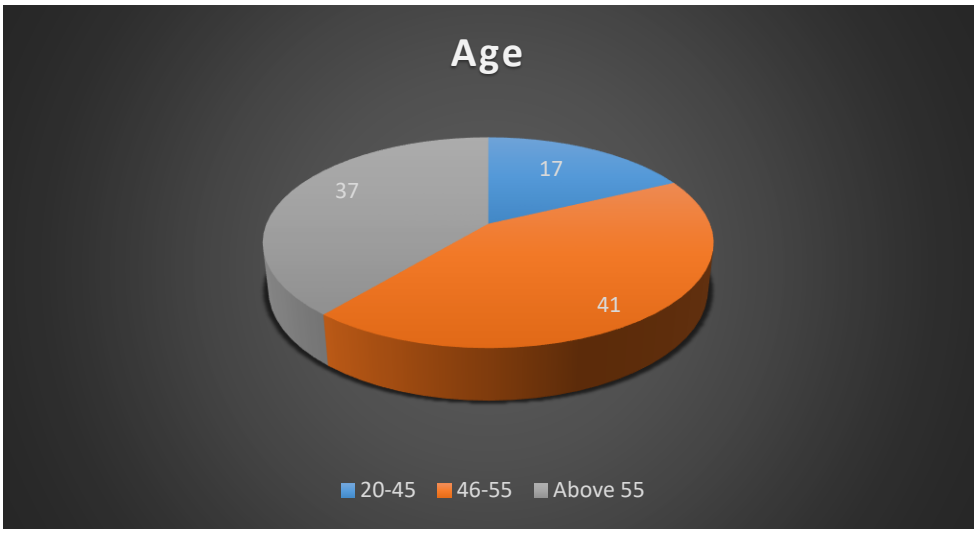


Figure 7 **Age Distribution of Cancer Patients**

In terms of gender, fifty seven out of the ninety-five cancer patients who took part in this study were males, while thirty-eight were females.

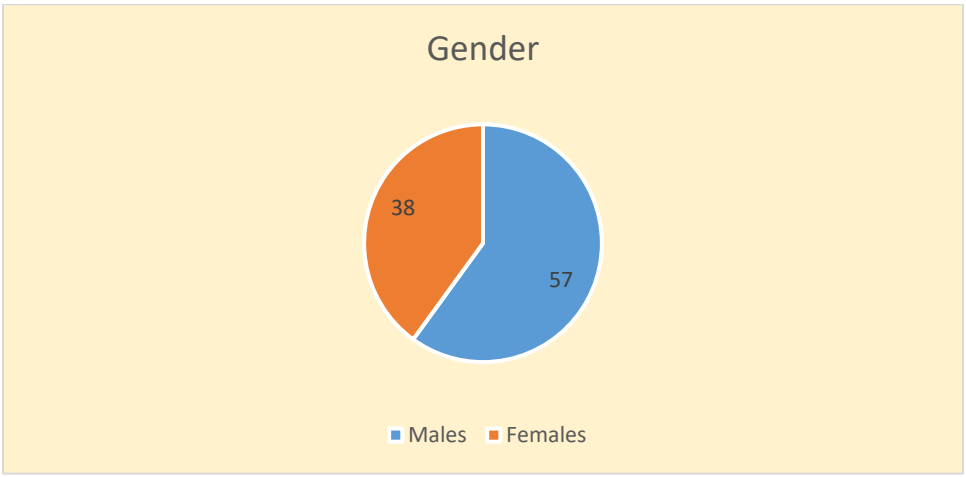


Figure 8 **Distribution by Gender of Cancer Patients**

Distribution of the cancer patients' residence in terms of counties revealed that out of forty-seven counties in Kenya, twenty-seven counties were represented in the study as shown in Figure 9. Kiambu, Nairobi and Murang'a counties had the highest representation of 12, 11 and 11 respectively. This could be attributed to the fact that these are the major counties close to Nairobi region and it could be assumed that patients in western part of Kenya are most likely to be going

to Moi Teaching and Referral Hospital, Eldoret which is also a level six referral hospital and is nearer to them than the Kenyatta National Hospital, Nairobi.

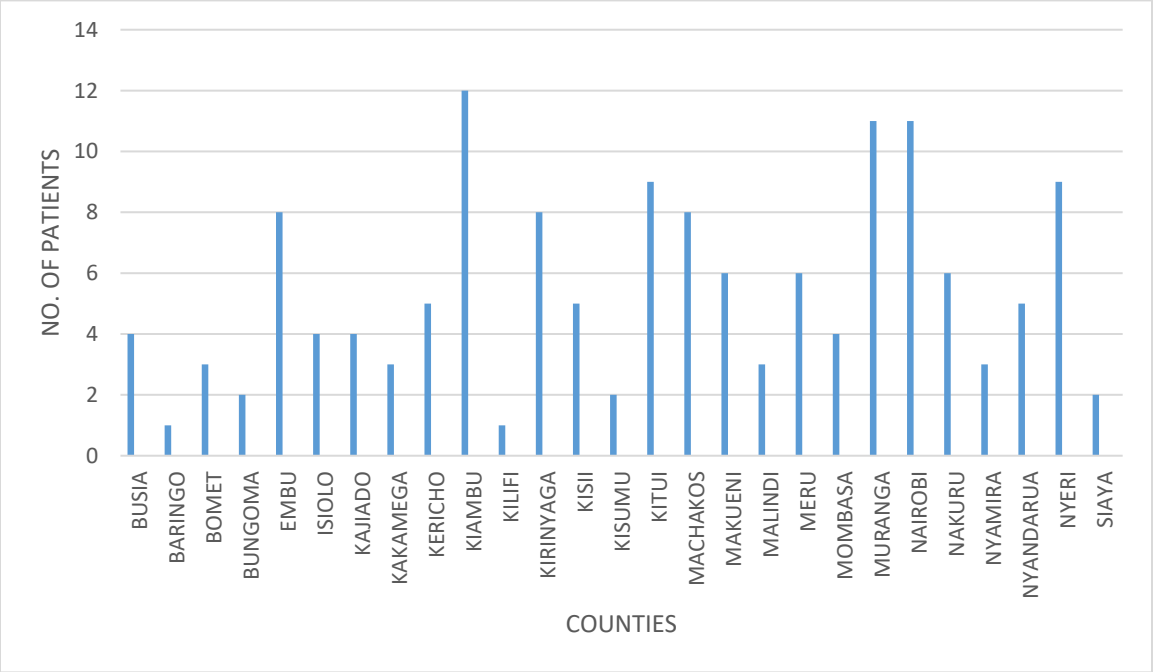


Figure 9 **Distribution by County of Cancer Patients**

4.2 Trace Elements Analysis

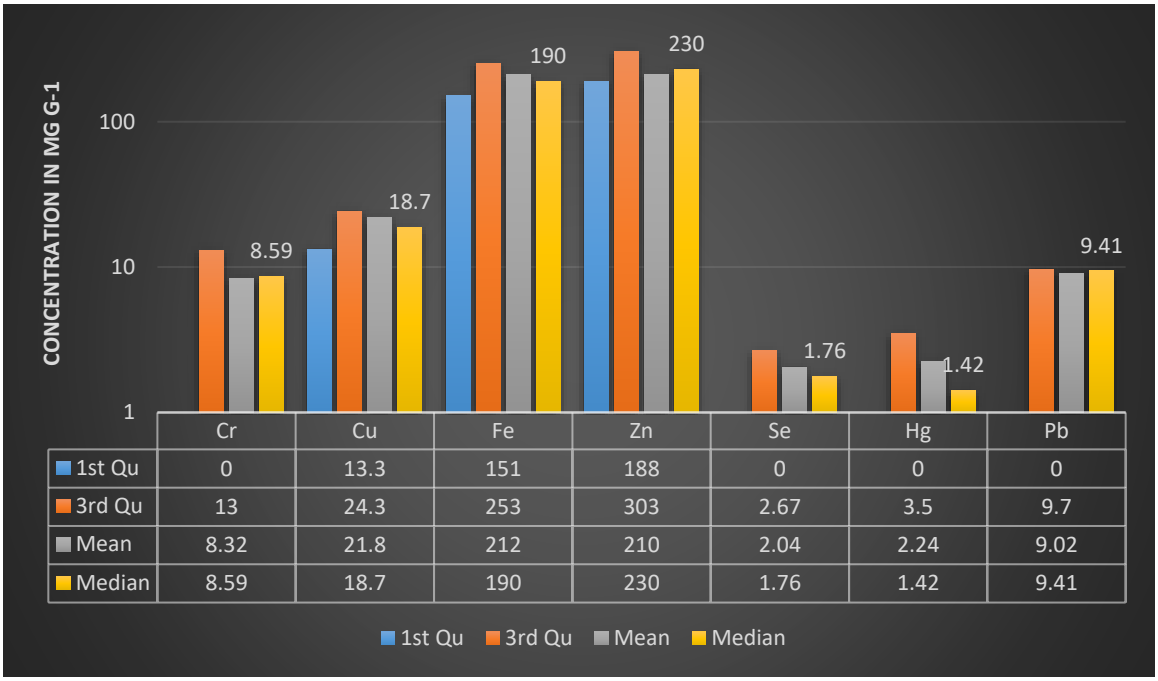
Initially, Cadmium was to be among the heavy metals to be analyzed in this study. However, during analysis, it observed Cd concentrations in the samples were below the detection limits. Therefore, the data was grouped to remove Cd column from all the classes of participants, as shown in Appendix 6. Thus, new subsets were obtained with each class of participants and grouped with all the trace elements being analyzed as illustrated in Appendix 7. This was followed by matching the columns and rows for all the classes of participants as shown in Appendix 8. Rows and columns for each class of participants were filtered so as to have equal variables. A data summery was then generated with the mean concentrations of each of the selected trace elements for each class of participants as seen in Appendix 9 and presented in interquartile range, mean and median and tested using t-test as indicated in Table 1 and 2.

4.2.1 Concentrations of Selected Trace Elements in Nail Clippings of the Study Population

Elemental analysis was done using TXRF (Bruker AXS Microanalysis GmbH) technique on samples from 95 cancer patients and 31 non-cancer control group, all aged between 20 to 65 years. Statistical software R, using unpaired two-sample t-test was used to determine whether there was any significant difference between the mean concentrations, and then presented in interquartile range, mean and median gave results as shown in table 2.

4.2.1.1 Elemental Analysis in Esophageal Cancer Patients

The results of the analysis of trace elements and heavy metals in esophageal cancer patients were as shown in Figure 10.



	Cr	Cu	Fe	Zn	Se	Hg	Pb
1st Qu	0	13.3	151	188	0	0	0
3rd Qu	13	24.3	253	303	2.67	3.5	9.7
Mean	8.32	21.8	212	210	2.04	2.24	9.02
Median	8.59	18.7	190	230	1.76	1.42	9.41

Figure 10 Concentration of Trace Elements and Heavy Metals in Esophageal Cancer Patients

4.2.1.2 Elemental Analysis in Stomach Cancer Patients

The results of the analysis of trace elements and heavy metals in stomach cancer patients were as shown in Figure 11.

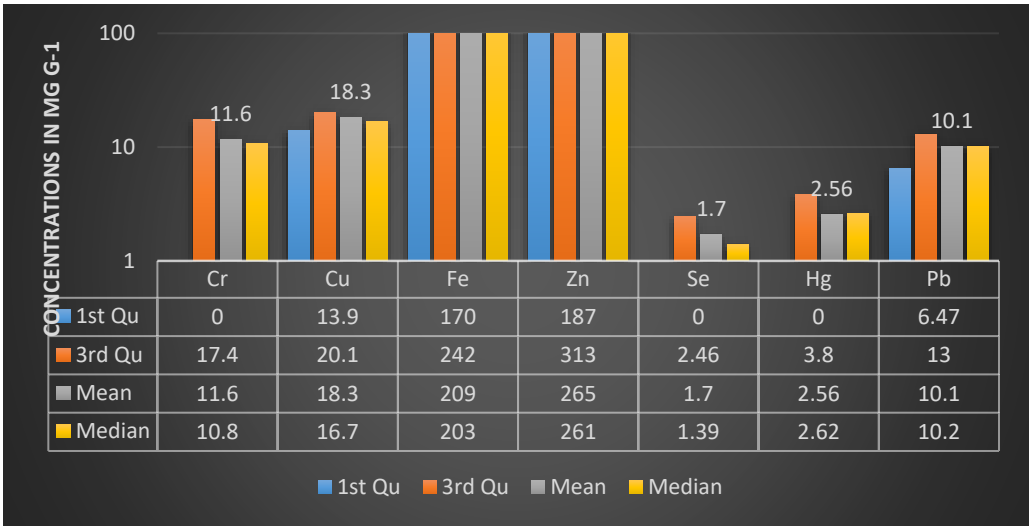


Figure 11 **Concentration of Trace Elements and Heavy Metals in Stomach Cancer Patients**

4.2.1.3 Elemental Analysis in Non-Cancer Control Group

The results of the analysis of trace elements and heavy metals in non-cancer patients were as shown in Figure 12.

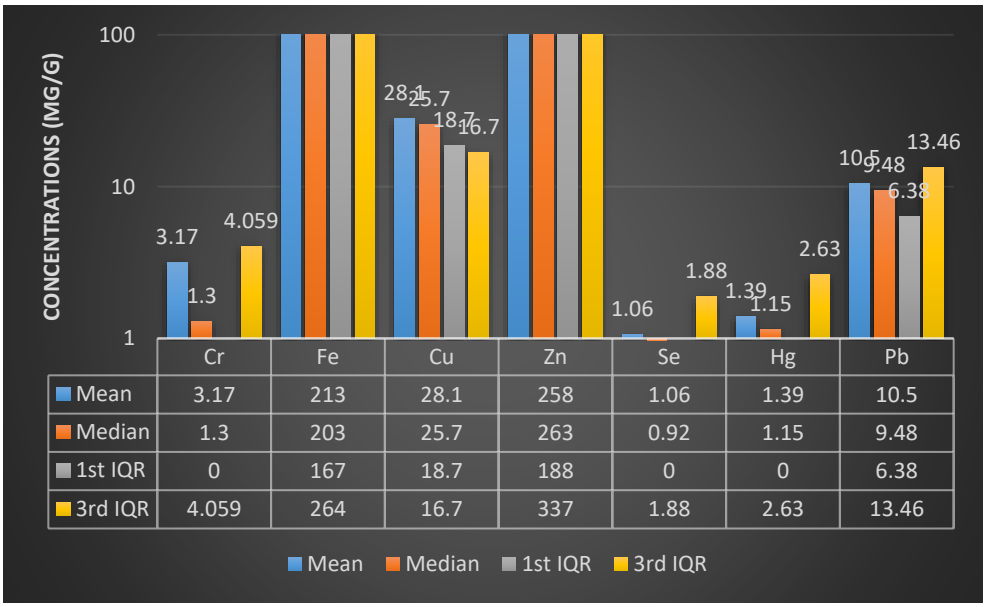


Figure 12 **Concentration of Trace Elements and Heavy Metals in Non-Cancer Patients**

4.3 Discussions

The mean concentrations were compared by unpaired two-sample t-test in two-way variables and $P < 0.05$ was considered significant statistically. This was to test the null hypothesis that there was no significant difference in the concentrations of trace elements between the patients of stomach and esophageal cancer and the non-cancer control group; while the alternative hypothesis supported a difference in the mean concentrations. The results as tabulated in Table 1 concurred with the findings reported by Pritchett and Co-workers, 2017 that indicated a positive association between the prevalence of esophageal cancer and higher Se concentration in serum. Similar reports were also given by other authors, Goyal et al. (2006) who determined the concentration of Se and Zn of esophageal cancer patients and healthy subjects' serum samples, using different analytical techniques.

The mean concentration of Cr in esophageal cancer patients was 8.32 µg g^{-1} as compared to 3.17 µg g^{-1} in the non-cancer control group, with a test statistic of t = -3.04, 39.8 degrees of freedom and p-value = 0.004. The mean concentration of Cu was 21.8 µg g^{-1} in esophageal cancer patients and 28.1 µg g^{-1} in the non-cancer control group; with a test statistic of t = 2.45, 51.6 degrees of freedom, and p-value = 0.02. The mean concentration of Zn was 210 µg g^{-1} while non-cancer control group was 258 µg g^{-1} and the mean concentration of Fe was 212 µg g^{-1} and 213 µg g^{-1} in esophageal and non-cancer patients respectively. The mean concentration of Se was 2.04 µg g^{-1} and 1.06 µg g^{-1} in esophageal and non-cancer patients respectively; with a test statistic t = -2.89, df = 52.3 and p-value = 0.01. Analysis of the mean concentration of Hg in esophageal cancer patients revealed 2.24 µg g^{-1} as compared to 1.39 µg g^{-1} in the non-cancer group with a test statistic t = -1.58, df = 46.6 and p-value = 0.12. The concentration of Pb was 9.02 µg g^{-1} and 10.5 µg g^{-1} in esophageal cancer patients and non-cancer control group respectively, with a test statistic t = 0.97, df = 43.5 and p-value = 0.34.

The mean concentration of Cr in stomach cancer patients was 11.6 µg g^{-1} and 3.59 µg g^{-1} in non-cancer control group with a test statistic of t = -3.65, df = 26.5 and p-value = 0.001. Analysis of the levels of Fe in both stomach cancer and non-cancer patients gave mean concentrations of 209 µg g^{-1} and 215 µg g^{-1}; while the mean concentrations of Zn were 265 µg g^{-1} and 252 µg g^{-1} respectively. With a test statistic t = 3.24, df = 29.6 and p-value = 0.003, the mean concentrations of Cu in both stomach cancer patients and the non-cancer control group were 18.3 µg g^{-1} and 27.1

µg g^{-1} respectively. The mean concentration of Se was 1.7 µg g^{-1} in stomach cancer patients as compared to 1.20 µg g^{-1} in the non-cancer control group at t = -0.97, df = 31.1 and p-value = 0.34. At t = -1.72, df = 32.3 and p-value = 0.09, the concentration of Hg was 2.56 µg g^{-1} and 1.37 µg g^{-1} in stomach cancer and non-cancer patients respectively. The mean concentration of Pb was 10.1 µg g^{-1} as compared to 8.52 µg g^{-1} in non-cancer control group.

From the demographic information, a larger percentage of the cancer patients, 58 out of 95 were below 55 years of age. This implies that cancer of esophagus and stomach does not discriminate on age and affects even younger generation. This observation supports the findings of Bray *et al*., 2018 and Parker *et al*, 2010 who reported incidences of cancer of esophagus and stomach cancer in young people aged below 30 years.

Table 2 Unpaired t-test Results at 95% Confidence Interval

Control Vs Oesophagus

Trace Element	test statistic	df	p-value	CN Mean	CE Mean
Cr	-3.03	39.8	0.004	3.17	8.32
Cu	2.45	51.6	0.017	28.1	21.8
Fe	0.05	46.9	0.96	213	212
Zn	2.36	47.4	0.02	258	210
Se	-2.89	52.3	0.01	1.06	2.04
Hg	-1.58	46.6	0.12	1.39	2.24
Pb	0.97	43.5	0.34	10.5	9.02

Control Vs Stomach Cancer

Trace Element	test statistic	df	p-value	CN Mean	CE Mean
Cr	-3.65	26.5	0.001	3.59	11.6
Cu	3.24	29.6	0.003	27.1	18.3
Fe	0.26	31.5	0.79	215	209
Zn	-0.42	36.9	0.67	252	265
Se	-0.97	31.1	0.33	1.21	1.71
Hg	-1.72	32.3	0.09	1.37	2.56
Pb	-1.01	31.6	0.32	8.52	10.1

Oesophageal Vs Stomach Cancer

Trace Element	test statistic	df	p-value	CN Mean	CE Mean
Cr	-1.44	37.1	0.16	7.89	11.6
Cu	1.34	37.6	0.19	21.8	18.3
Fe	-0.35	34.1	0.73	201	209
Zn	-1.92	33.1	0.06	210	265
Se	0.42	36.1	0.68	1.94	1.71
Hg	-0.61	35.916	0.554	2.12	2.56
Pb	-0.71	41.7	0.48	9.06	10.1

4.3.1 Copper

In comparing the means in control group and esophageal cancer patients, the test statistic was t = 2.45, with 51.6 degrees of freedom, and p-value = 0.02. On average, the concentration in the control group was 6.33 µg g^{-1} higher than the concentration in the cancer patients.

The test statistic in comparing the concentrations between the control group and the stomach cancer patients was t = 3.24, with 29.6 degrees of freedom, and p-value = 0.003. With the p-value being less than α=0.05, the alternative hypothesis which stated that a true difference in means is not equal to 0 was supported. Thus, increased concentrations of Cu were observed in the control group as compared to the stomach cancer patients as observed in Figure 13.

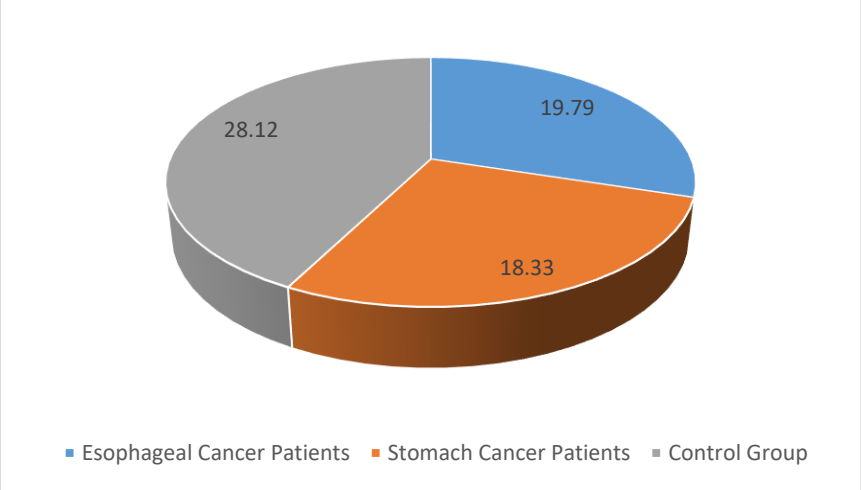


Figure 13 Comparison in the Mean Concentrations of Copper in Test Groups

4.3.2 Chromium

The test statistic comparing the control group and esophageal cancer patients was t = -3.04, with 39.8 degrees of freedom and p-value = 0.004. The mean concentrations were CN = 3.17 µg g^{-1} and CE = 8.32 µg g^{-1}.

A comparison between esophageal and stomach cancer patients' concentrations gave t = -1.44, with 37.1 df and p-value = 0.16. Thus, there was no significant difference between the two means, though CS with mean = 11.6 µg g^{-1} was slightly higher than CE mean = 7.89 µg g^{-1}.

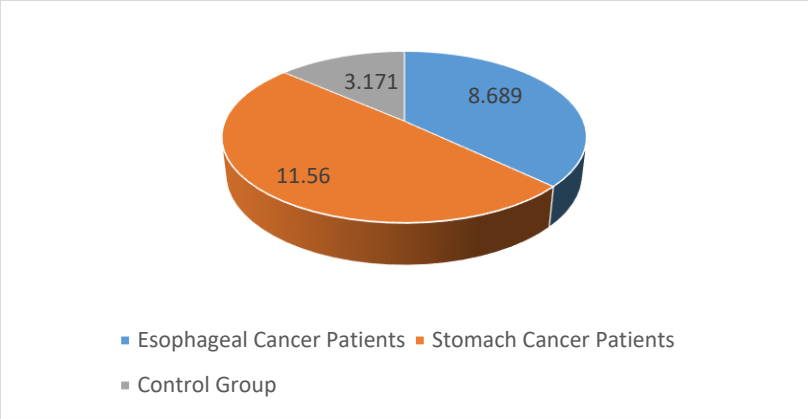


Figure 14 Comparison in the Mean Concentrations of Chromium in Test Groups

4.3.3 Iron

Analysis of the difference in means of Fe between the non-cancer control group and esophageal cancer patients gave a test statistic, t = 0.05, 46.9 degrees of freedom and p-value = 0.96; between the non-cancer control group and stomach cancer patients, t = 0.26, 31.5 degrees of freedom and p-value = 0.797; while between esophageal and stomach cancer patients t = -0.35, 34.02 degrees of freedom, and p-value = 0.73. Since all the p-values were greater than α=0.05, the null hypothesis was accepted and thus stated that there was no difference, on average, in the means between the three samples.

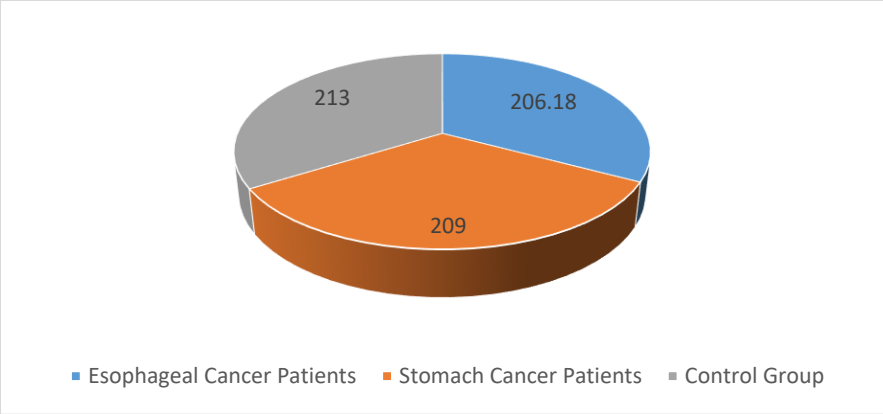


Figure 15 Comparison in the Mean Concentrations of Iron in Test Groups

4.3.4 Zinc

Generally, in all the test groups, the concentrations of Zn and Fe were found to be nearly the same. This concurs with a study done in Iran on trace elements in human nutrition (Mehri & Marjan, 2015). Similar results were also obtained by Lin et al. (2009).

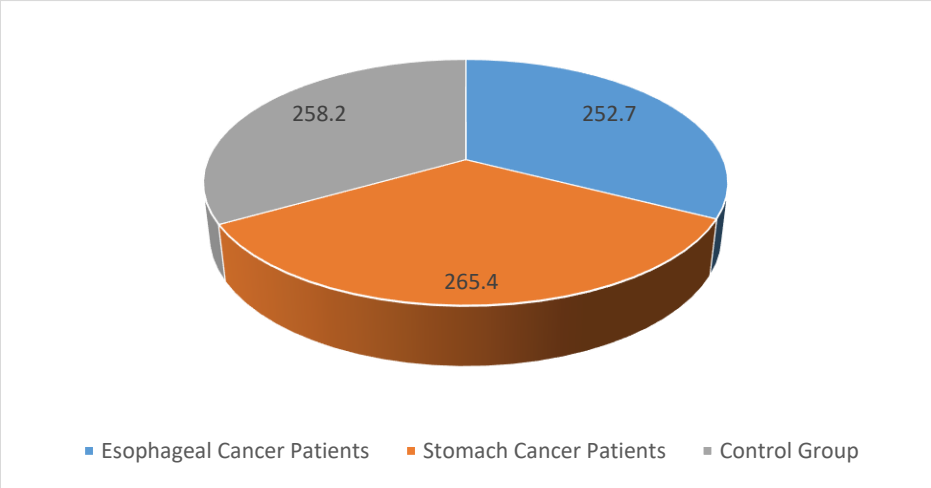


Figure 16 Comparison in the Mean Concentrations of Zinc in Test Groups

4.3.5 Selenium

There was disparity in the levels of Se as detected in the nail samples of esophageal and stomach cancer patients together with the non-cancer control group as shown in Table 1. The concentrations were however reduced in the non-cancer control group as observed in Figure 14.

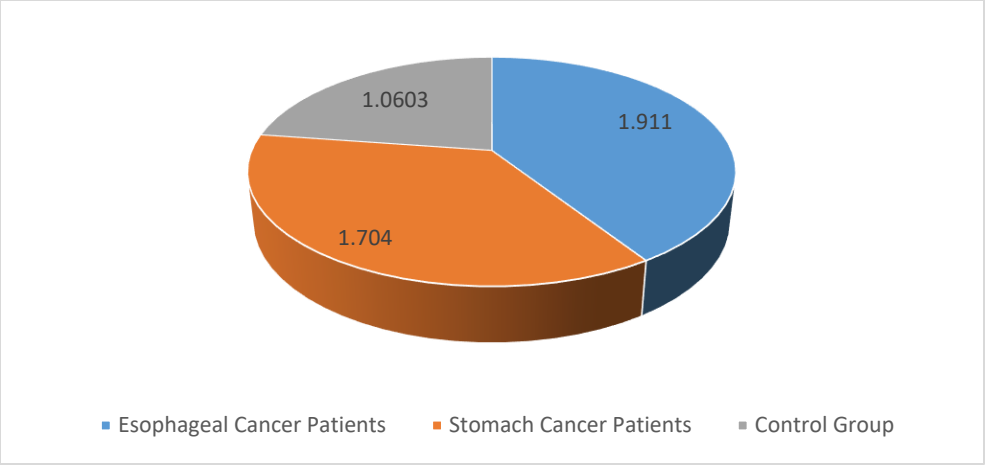


Figure 17 Comparison in the Mean Concentrations of Selenium in Test Groups

4.3.6 Mercury

Figure 15 showed higher mean concentration of Hg in both esophageal and stomach cancer patients as compared to the non-cancer patients. These results concur with those of Valco *et al*, 2006 which indicated that mercury induces the onset of Reactive Oxygen Species which damages the cells DNA; a process that promotes carcinogenic processes.

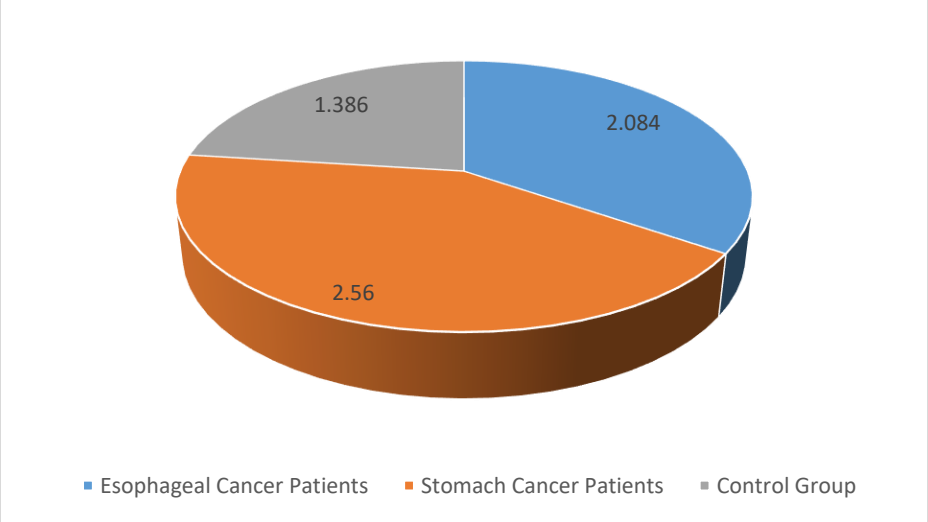


Figure 18 Comparison in the Mean Concentrations of Mercury in Test Groups

4.3.7 Lead

The stomach cancer patients showed an average concentration of 10.1 µg g^{-1}, while the mean concentration of Pb in the non-cancer control group was 8.52 µg g^{-1}. The results therefore revealed a significant decrease of mean Pb concentration in the non-cancer group as compared to the stomach cancer patients. On contrary, a comparison on non-cancer control group with the esophageal cancer patients indicated a significant decrease of mean concentrations among the esophageal cancer patients as shown in Figure 19. These findings are in line with what Lin et al. (2009) revealed in their study where lower levels of Pb and higher levels of Zn were discovered among the esophageal cancer patients as compared to a given healthy control group.

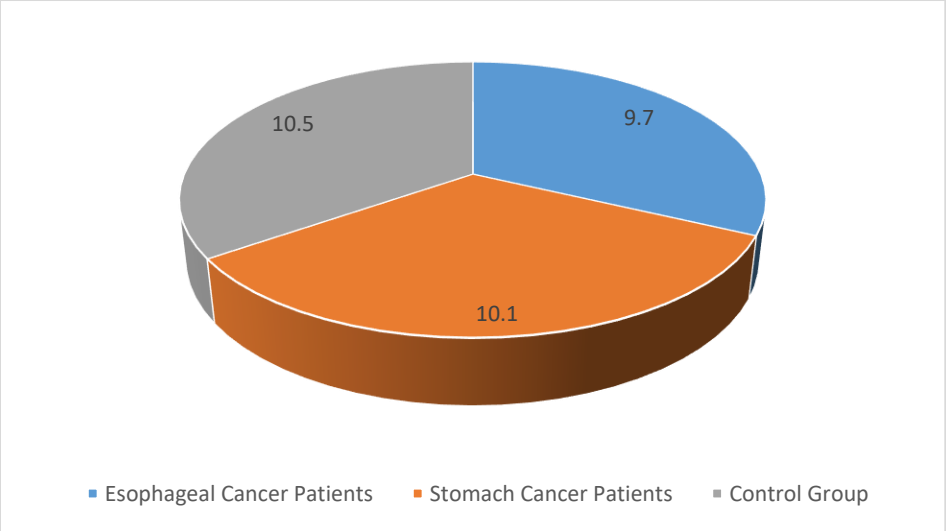


Figure 19 Comparison in the Mean Concentrations of Lead in Test Groups

Table 3 Relationships and Differences on Elemental Concentrations

Participants / Trace Elements	Non-Cancer Control Group (N = 31)	Esophageal Cancer Patients (N = 72)	Stomach Cancer Patients (N = 31)
Chromium			
Mean	3.17	8.69	11.56
Median	1.30	8.58	10.8
IQR	0-4.059	0-13.0	5.1-17.41
Iron			
Mean	213	206	209
Median	203	190	203
IQR	167-264	151-190	170 -242
Copper			
Mean	28.1	19.8	18.3
Median	25.7	18.7	16.7
IQR	22.6-36.2	13.3-24.3	13.9-20.1
Zinc			
Mean	258	253	265
Median	263	240	261
IQR	188-337	188-303	187-313
Selenium			
Mean	1.06	1.91	1.70
Median	0.92	1.76	1.39
IQR	0-1.88	0-2.08	0-2.46
Mercury			
Mean	1.39	2.08	2.56
Median	1.15	1.42	2.62
IQR	0-2.63	0-3.5	0-3.8
Lead			
Mean	10.5	9.7	10.1
Median	9.48	9.41	10.2
IQR	6.38-13.46	6-14	6.47-13

Generally, the results of this study corroborate what Lin et al. (2009) demonstrated in terms of concentrations of trace elements among cancer patients and various healthy populations as reflected in the already reviewed literature.

CHAPTER FIVE

Conclusions

The total number of esophageal cancer patients who participated in this study were seventy-two, representing 57% of the whole study population. Analysis of trace elements in finger nail clippings of esophageal cancer patients in comparison to non-cancer control group gave varied concentrations based on the results of unpaired t-test at 95% confidence interval. Average concentration of Cr was 8.32 µg g^{-1} and 3.17 µg g^{-1} in the non-cancer control group. The mean concentration of Cu was 21.8 µg g^{-1} in comparison to 28.1 µg g^{-1} in the non-cancer control group. The concentration of Zn was 210.3 µg g^{-1} while in the non-cancer control group it was 258 µg g^{-1} and the mean concentration of Fe was 212 µg g^{-1} and 213 µg g^{-1} in esophageal and non-cancer patients respectively. Average concentration of Se was 2.04 µg g^{-1} and 1.06 µg g^{-1} respectively. The mean concentration of Hg was 2.24 µg g^{-1} as compared to 1.39 µg g^{-1} in the non-cancer group, while the concentration of Pb was 9.02 µg g^{-1} and 10.5 µg g^{-1} in esophageal cancer patients and non-cancer control group respectively.

Analysis of heavy metals and trace elements in finger nail clippings of in terms of mean concentrations at 95 confidence intervals in comparison to non-cancer patients revealed that the mean concentration of Cr in stomach cancer patients was 11.6 µg g^{-1} and 3.59 µg g^{-1} in non-cancer control group. Analysis of the levels of Fe gave mean concentrations of 209 µg g^{-1} and 215 µg g^{-1} respectively; while the mean concentrations of Zn were 265 µg g^{-1} and 252 µg g^{-1} respectively. The mean concentrations of Cu in both stomach cancer patients and the non-cancer control group were 18.3 µg g^{-1} and 27.1 µg g^{-1} respectively. The mean concentration of Se was 1.7 µg g^{-1} as compared to 1.20 µg g^{-1} in the non-cancer control group. The concentration of Hg was 2.56 µg g^{-1} and 1.37 µg g^{-1} in stomach cancer and non-cancer patients respectively. The mean concentration of Pb was 10.1 µg g^{-1} as compared to 8.52 µg g^{-1} in non-cancer control group.

Based on the significant differences observed in the concentrations of Cu, Cr, Pb, Se and Hg among the participants of this study, there is a difference in the levels of these elements in cancer patients and non-cancer population. This shows that accumulation of these elements in the human body is a function of the physical state of the body. There was however no much significant variation

observed for the concentration of Fe and Zn among the three test groups in this study since the comparisons of all the tests gave a p-value greater that 0.05; this could possibly imply that these two elements are not associated factors to the prevalence of cancer and their concentrations may not influence early diagnosis of the disease.

Data analysis indicated that in 96% of the tests carried out, the p-value was less than $\alpha=0.05$. The null hypothesis which stated that there was no significant difference, on average, in the mean concentrations between the samples, was therefore rejected.

Hence, this study revealed that using the concentrations of essential trace elements such as copper and selenium; and heavy metals such as chromium and mercury, it is possible to distinguish between cancer of the esophagus and cancer of the stomach since these four trace elements showed greater correlations with these cancers. This study can therefore be a basis to develop an early diagnostic screening tool with Total Reflection Xray Fluorescence as an analytical tool.

Recommendations

This study recommends that further analysis be carried out on other trace elements, using different analytical instruments so as to ascertain the conclusions made, and to establish more relationships between the growth of cancer cells and the concentration of heavy metals and trace elements in the human body.

Further, investigations on the levels of trace elements in cancer patients at different clinical stages could also be done in order to establish the effects of heavy metals and trace elements' concentrations on the growth and development of cancer cells.

Since the current study did not confirm the oxidation states of trace elements and heavy metals analyzed, future research should determine the different chemical species in order to fill in this gap. This is due to the fact that health hazards associated with exposure to most heavy metals and trace elements depend on their oxidation states.

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Appendix 1 Informed Consent Form

Evaluation of Trace Elements' Concentrations in Nails of Esophageal and Stomach Cancer Patients: Investigation of an Early Diagnostic Screening Method in Kenya

SECTION 1: Consent Explanation

I am Nancy Owili, a student pursuing Masters in Nuclear Science at the University of Nairobi.

I am carrying out a study on evaluation of the concentrations of trace elements in nails of esophageal and stomach cancer patients at the Kenyatta National Hospital in partial fulfilment of my master of science program requirements. This study intends to use nail clippings of esophageal and stomach cancer patients in comparison to those of a healthy control group in order to evaluate the concentration of some selected trace elements, especially those reported to have an impact on cancer disease. It aims at fostering early cancer screening, diagnosis and treatment.

The broad objective of this study is to evaluate the concentrations of Cr, Fe, Zn, Cu, Cd, Pb, Se, Hg in esophageal and stomach cancer patients in search of a non-invasive and a more economical method for early diagnostic and screening in Kenya.

You are hereby invited to participate in the study which involves your filling in of the written questionnaire provided and allowing me to get your finger nails using the sterilized stainless nail cutters which I will provide. Participation is entirely voluntary and you are free to withdraw from the study. There are no foreseeable risks to participate or not to participate in this study and refusal to participate will in no way influence your care at the hospital. You will also be accorded the opportunity to request for the results of the study once it is complete.

All questionnaires will be anonymous and any information provided will be highly confidential.

There is no cost or payment expected for your participation in this study.

For any queries arising before and during the course of the study, kindly contact:

Nancy Owili
Tel.: 0723757400
Institute of Nuclear Science & Technology
University of Nairobi
Email: *nyathiowili@students.uonbi.ac.ke*

Prof. Michael Gatari
Tel.: 0723797640
Director, Institute of Nuclear Science & Technology
University of Nairobi
Email: *mgatari@uonbi.ac.ke*

Secretary
Kenyatta National Hospital-University of Nairobi Ethics & Research Committee
Tel.: 2726300 Ext 44102
Email: uonknh_erc@uonbi.ac.ke

SECTION 2: Certificate of Consent

I have read the foregoing information. I understand and agree to the following:

1. My participation in the study is entirely voluntary.
2. I am free to withdraw from the study at any point.
3. Refusal to participate in the study will in no way influence my care at the hospital.
4. I have been accorded an opportunity to ask questions and they have been duly answered.
5. I hereby consent to participate in this research.

Name of Participant: ……

Signature: …… Date: ……

Statement by the Researcher

I confirm that the participant in this study was given an opportunity to ask for clarifications, and all the questions asked by the participant have been correctly answered to the best of my ability. I confirm that the individual has given the consent freely and voluntarily.

Name of Researcher: ……

Signature: …… Date: ……..

Fomu ya Idhini

Tathmini ya Kuzingatia kwa Makini ya Vipimo katika Makucha ya Wagonjwa wa Saratani ya Umio na Tumbo: Kuchunguza Njia ya Upimaji wa Uchunguzi wa Mapema nchini Kenya

SEHEMU YA 1: Ufafanuaji wa Idhini:

Mimi ni Nancy Owili, mwanafunzi wa Masters katika Sayansi ya Nyuklia katika Chuo Kikuu cha Nairobi. Ninafanya utafiti juu ya tathmini ya viwango vya vipengee vya ufuatiliaji kwenye makucha ya wagonjwa wa saratani ya umio na tumbo katika Hospitali ya Kitaifa ya Kenyatta ili kutimiza mojawapo ya mahitaji yangu ya mpango wa sayansi. Utafiti huu unakusudia kutumia mihimili ya makucha ya wagonjwa wa saratani ya umio na tumbo kwa kulinganisha na wenye afya ili kutathmini mkusanyiko wa vipengee vya ufuatiliaji. Itasudia kukuza uchunguzi wa saratani mapema, utambuzi na matibabu.

Kusudi kubwa la utafiti huu ni kutathmini viwango vya vipengee vya chromi, chuma, zinki, shaba, kadnium, risasi, seleniamu na zebaki katika wagonjwa wa saratani ya umio na tumbo kama zana ya utambuzi na uchunguzi wa saratani nchini Kenya.

Unaalikwa kushiriki katika utafiti ambao unajumuisha kujaza dodoso lililotolewa na kuniruhusu kupata makucha zako za vidole kwa kutumia vipandikizi vilivyosafishwa ambavyo nitatoa. Ushiriki ni hiari kabisa na uko huru kujiondoa bila shaka. Hakuna hatari zinazoonekana za kushiriki au kutoshiriki katika utafiti huu na kukataa kushiriki hautashawishi utunzaji wako hospitalini. Pia utapewa fursa ya kuomba matokeo ya utafiti yatakapokamilika.

Habari yote itakayotolewa kwenye dodoso yote itakuwa ya siri sana. Hakuna gharama au malipo yanayotarajiwa kwa ushiriki wako katika utafiti huu.

Kwa maswali yoyote yanayotokea kabla ya na wakati wa utafiti huu, wasiliana kwa anwani:

Nancy Owili

Simu: 0723757400
Chuo Kikuu Cha Nairobi
Barua pepe: *nyathiowili@students.uonbi.ac.ke*

Prof. Michael Gatari

Simu: 0723797640
Mkurugenzi, Taasisi ya Sayansi na Teknolojia ya Nyuklia, Chuo Kikuu cha Nairobi
Barua pepe: *mgatari@uonbi.ac.ke*
Katibu wa Kenyatta National Hospital-University of Nairobi Ethics & Research Committee

Simu : 2726300 Ext 44102

Barua pepe: *uonknh_erc@uonbi.ac.ke*

SEHEMU YA PILI: Hati ya idhini

Nimesoma habari iliyotangulia. Ninaelewa na ninakubali yafuatayo:

1. Ushiriki wangu katika utafiti huu ni wa hiari kabisa.
2. Niko huru kujiondoa kutoka kwa utafiti wakati wowote.
3. Kukataa kushiriki katika masomo hakutashawishi utunzaji wangu hospitalini.
4. Nimepewa nafasi ya kuuliza maswali na yamejibiwa kwa haki.
5. Kwa hivyo ninakubali kushiriki katika utafiti huu.

Jina la Jina la Mshiriki: ……

Sahihi: …… Tarehe: ……

Taarifa ya Mtafiti

Ninadhibitisha kwamba mshiriki katika utafiti huu alipewa nafasi ya kuomba ufafanuzi, na maswali yote aliuliza yalijibiwa kwa kadri ya uwezo wangu. Ninathibitisha kwamba mtu mwenyewe ametoa idhini hiyo kwa hiari.

Jina la Mtafiti: ……..…….....

Sahihi: …… Tarehe: …….

Appendix 2 Questionnaire for the Cases

UNIVERSITY OF NAIROBI

Dear Participant,

I am a Master of Science Student at the University of Nairobi in the Institute of Nuclear Science & Technology. I am currently undertaking a research project on:

"Evaluation of Trace Elements' Concentrations in Nails of Esophageal and Stomach Cancer Patients: As an Early Diagnostic Screening Method in Kenya".

The purpose of this questionnaire is to kindly request for your participation in this study. This will involve filling in this questionnaire and providing fingernail clippings which will be analyzed for this study. The information gathered will be treated with highest confidentiality and will be used only for the intended purpose.

Kindly note that filling of this questionnaire will not take more than five minutes.

Thank you in advance for your cooperation.

Confidential

Instructions:

Place a tick (√) in the bracket after the correct response, and where explanations are required, use the space provided. Do not indicate your name anywhere in the questionnaire.

1) Participant's Age: 20-30 years { } 31-50 years { } Above 50 years { }

2) Gender: Male { } Female { }

3) Area of Residence: _____

a) How long have you lived there?

0-1 year { }

1 to 5 years { }

More than 5 years { }

4) Occupation:

a) Agricultural Sector { }

b) Manufacturing { }

c) Hospitality { }

d) Others _____

5) Kindly indicate what you use mainly in your work:

a) Chemicals { }

b) Paints { }

c) Gases { }

d) Alloys { }

e) Others _____

6) Work Location:

a) Town _____

b) County _____

c) Duration: 0 - 10 years { } 10-20 years { } More than 20 years { }

7) Type of cancer diagnosed with:

a) Stomach Cancer { }

b) Esophageal Cancer { }

8) When were you diagnosed?

a) 0-6 months ago { }

b) 6 months – 1 year ago { }

c) 1 – 5 years ago { }

d) More than 5 years ago { }

9) What type of treatment do you use?

a) Conventional { }

b) Herbal { }

c) Both { }

10) How long have you been on cancer treatment?

a) Conventional: Never { } 1-6 months { } More than 6 months { }

b) Herbal: Never { } 1-6 months { } More than 6 months { }

c) Both: Never { } 1-6 months { } More than 6 months { }

11) Stage of Cancer:

Stage I { } Stage II { } Stage III { } Stage IV { }

12) Do you smoke cigarettes? Yes { } No { }

a) If Yes, please how long have you been smoking?

0-1 year{ } 1-5 years { } 5-10 years { } More than 10 years { }

14) Do you take alcohol? Yes { } No { }

a) If Yes, please how long have you been taking alcohol?

0-1 year{ } 1-5 years { } 5-10 years { } More than 10 years { }

16) Are you on any special diet? Yes { } No { }

17) Do you take mineral or vitamin supplements? Yes { } No { }

If Yes, when last did you take them?

0-1 month ago { } b) 1 to 3 months ago { } c) More than 3 months ago { }

18) Have you lost any member of your family due to cancer? Yes { } No { }

If yes, kindly indicate:

a) Gender of the deceased: Male { } Female { }

b) Age of the deceased:

20-30 years { } 30-50 years { } More than 50 years { }

c) your relationship with the deceased: _____

d) The type of cancer: _____

19) Have you undergone stomach or esophageal surgery? Yes { } No { }
If yes, kindly indicate:

when it was done: 0-6 months { } 6 months – 2years { } Over to years { }

Thank you for your time.

Dodoso

CHUO KIKUU CHA NAIROBI

Mpenzi Mshiriki,

Mimi ni Nancy Owili, mwanafunzi wa masters katika Chuo Kikuu cha Nairobi katika Taasisi ya Sayansi na Teknolojia ya Nyuklia. Kwa sasa ninafanya mradi wa utafiti juu ya:

" Tathmini ya Kuzingatia kwa Makini ya Vipimo katika Makucha ya Wagonjwa wa Saratani ya Umio na Tumbo: Kuchunguza Njia ya Upimaji wa Uchunguzi wa Mapema nchini Kenya".

Madhumuni ya dodoso hili ni kuomba kwa fadhili kwa ushiriki wako katika utafiti huu. Hii itajumuisha kujaza dodoso hili na kutoa makucha ya kidole ambayo itachambuliwa kwa utafiti huu. Habari iliyokusanywa itawekwa kwa usiri mkubwa na itatumika tu kwa madhumuni yaliyokusudiwa.

Tafadhali kumbuka kuwa kujaza dodoso hili hautachukua zaidi ya dakika tano.

Asante kwa ushirikiano wako.

Siri

Maagizo:

Weka alama (√) kwenye braketi baada ya majibu sahihi, na ambapo maelezo inahitajika, tumia nafasi iliyotolewa. Usionyeshe jina lako mahali popote kwenye dodoso.

1) Umri wa Mshiriki: Miaka 20-30 { } Miaka 31-50 { } Zaidi ya miaka 50 { }

2) Jinsia: Mwanaume { } Mwanamke { }

3) Eneo la makazi: _____

a) Umekaa huko kwa muda gani? 0-1 mwaka { } Miaka 1 hadi 5 { }Zaidi ya miaka 5 { }

4) Kazi:
a) Sekta ya Kilimo { }
b) Viwanda { }
c) Ukarimu{ }
d) Mengine _____

5) Tafadhali onyesha kile unachotumia katika kazi yako:
a) Kemikali { }
b) Mchoraji { }
c) gesi { }
d) Vyuma { }
e) Mengine _____

6) Mahali pa kazi:
a) Jiji _____
b) Kata _____
c) Muda: miaka 0 - 10 { } miaka 10-20 { } Zaidi ya miaka 20 { }

7) Aina ya saratani inayotambuliwa na:
a) Saratani ya Tumbo { }
b) Saratani ya Umio { }

8) Uligunduliwa lini?
a) miezi 0-6 iliyopita { }
b) miezi 6 - 1 mwaka mmoja uliopita { }
c) 1 - 5 miaka iliyopita { }
d) Zaidi ya miaka 5 iliyopita { }

9) Je! Unatumia matibabu gani?
a) Kawaida { }

b) Mitishamba { }
c) Zote mbili { }

10) Je! Umekaa kwa matibabu ya saratani kwa muda gani?
a) Kawaida: Sijawahi { } Miezi 1-6 { } Zaidi ya miezi 6 { }
b) Mitishamba: Sijajawahi { } miezi 1-6 { } Zaidi ya miezi 6 { }
c) Zote mbili: Sijajawahi { } miezi 1-6 { } Zaidi ya miezi 6 {}

11) Hatua ya Saratani:
Hatua ya I { } Hatua ya II { } Hatua ya III { } Hatua ya IV { }

12) Je! Unavuta sigara? Ndio { } La { }
a) Ikiwa Ndio, tafadhali umekuwa ukivuta sigara kwa muda gani?
Miaka 0-1 { } miaka 1-5 { } miaka 5-10 { } Zaidi ya miaka 10 { }

13) Je! Unachukua pombe? Ndio { } La { }
a) Ikiwa Ndio, tafadhali umechukua pombe muda gani?
Miaka 0-1 { } miaka 1-5 { } miaka 5-10 { } Zaidi ya miaka 10 { }

17) Je! Unachukua madini au virutubishi vya vitamini? Ndio { } La { }
Ikiwa Ndio, ulichukua lini?
Mwezi 0-1 iliyopita { } b) miezi 1 hadi 3 iliyopita { } c) Zaidi ya miezi 3 iliyopita { }

18) Je! Umepoteza mtu yeyote wa familia yako kutokana na saratani? Ndio { } La { }
Ikiwa ndio, onyesha kwa fadhili:
a) Jinsia ya marehemu: Mwanaume { } Kike { }
b) Umri wa marehemu:
Miaka 20-30 { } Miaka 30-50 { } Zaidi ya miaka 50 { }
c) uhusiano wako na mtu aliyekufa: _____
d) Aina ya saratani: _____

19) Je! Umepata upasuaji wa aina yoyote ya tumbo au umio? Ndio { } La { }
Ikiwa ndio, onyesha kwa fadhili:
wakati ilifanyika:
miezi 0-6 {} miezi 6 - 2years {} Zaidi ya miaka {}

Asante kwa muda wako.

Appendix 3 Questionnaire for the Control Group

UNIVERSITY OF NAIROBI

Dear Participant,

I am a Master of Science Student at the University of Nairobi in the Institute of Nuclear Science & Technology. I am currently undertaking a research project on:

"Evaluation of Trace Elements' Concentrations in Nails of Esophageal and Stomach Cancer Patients: Investigation of an Early Diagnostic Screening Method in Kenya".

The purpose of this questionnaire is to kindly request for your participation in this study. This will involve filling in this questionnaire and providing fingernail clippings which will be analyzed for this study. The information gathered will be treated with highest confidentiality and will be used only for the intended purpose.

Kindly note that filling of this questionnaire will not take more than five minutes.

Thank you in advance for your cooperation.

Confidential

Instructions:

Place a tick (√) in the bracket after the correct response, and where explanations are required, use the space provided. Do not indicate your name anywhere in the questionnaire.

1) Participant's Age: 20-30 years { } 31-50 years { } Above 50 years { }

2) Gender: Male { } Female { }

3) Area of Residence: _____

b) How long have you lived there?

0-1 year { }

1 to 5 years { }

More than 5 years { }

4) Occupation:

a) Agricultural Sector { }

b) Manufacturing { }

c) Hospitality { }

d) Others _____

5) Kindly indicate what you use mainly in your work:

f) Chemicals { }

g) Paints { }

h) Gases { }

i) Alloys { }

j) Others _____

6) Work Location:

d) Town _____

e) County _____

f) Duration: 0 - 10 years { } 10-20 years { } More than 20 years { }

7) Do you smoke cigarettes? Yes { } No { }

a) If Yes, please how long have you been smoking?

0-1 year{ } 1-5 years { } 5-10 years { } More than 10 years { }

8) Do you take alcohol? Yes { } No { }

a) If Yes, please how long have you been taking alcohol?

0-1 year{ } 1-5 years { } 5-10 years { } More than 10 years { }

9)Do you have any chronic disease or medical condition?

If Yes, kindly specify:

_____

10) Have you lost any member of your family due to cancer? Yes { } No { }

If yes, kindly indicate:

a) Gender of the deceased: Male { } Female { }

b) Age of the deceased:

0-2 years { } 30-50 years { } More than 50 years { }

c) your relationship with the deceased: _____

d) The type of cancer: _____

Thank you for your time.

Dodoso

CHUO KIKUU CHA NAIROBI

Mpenzi Mshiriki,

Mimi ni Nancy Owili, mwanafunzi wa masters katika Chuo Kikuu cha Nairobi katika Taasisi ya Sayansi na Teknolojia ya Nyuklia. Kwa sasa ninafanya mradi wa utafiti juu ya:

"**Tathmini ya Kuzingatia kwa Makini ya Vipimo katika Makucha ya Wagonjwa wa Saratani ya Umio na Tumbo: Kuchunguza Njia ya Upimaji wa Uchunguzi wa Mapema nchini Kenya".**

Madhumuni ya dodoso hili ni kuomba kwa fadhili kwa ushiriki wako katika utafiti huu. Hii itajumuisha kujaza dodoso hili na kutoa makucha ya kidole ambayo itachambuliwa kwa utafiti huu. Habari iliyokusanywa itawekwa kwa usiri mkubwa na itatumika tu kwa madhumuni yaliyokusudiwa.

Tafadhali kumbuka kuwa kujaza dodoso hili hautachukua zaidi ya dakika tano.

Asante kwa ushirikiano wako.

Siri

Maagizo:

Weka alama (√) kwenye braketi baada ya majibu sahihi, na ambapo maelezo inahitajika, tumia nafasi iliyotolewa. Usionyeshe jina lako mahali popote kwenye dodoso.

1) Umri wa Mshiriki: Miaka 20-30 { } Miaka 31-50 { } Zaidi ya miaka 50 { }

2) Jinsia: Mwanaume { } Mwanamke { }

3) Eneo la makazi: _____

a) Umekaa huko kwa muda gani? 0-1 mwaka { } Miaka 1 hadi 5 { }Zaidi ya miaka 5 { }

4) Kazi:
a) Sekta ya Kilimo { }
b) Viwanda { }
c) Ukarimu{ }
d) Mengine _____

5) Tafadhali onyesha kile unachotumia katika kazi yako:
a) Kemikali { }
b) Mchoraji { }
c) gesi { }
d) Vyuma { }
e) Mengine _____

6) Mahali pa kazi:
a) Jiji _____
b) Kata _____
c) Muda: miaka 0 - 10 { } miaka 10-20 { } Zaidi ya miaka 20 { }

7) Je! Unavuta sigara? Ndio { } La { }
a) Ikiwa Ndio, tafadhali umekuwa ukivuta sigara kwa muda gani?
Miaka 0-1 { } miaka 1-5 { } miaka 5-10 { } Zaidi ya miaka 10 { }

8) Je! Unachukua pombe? Ndio { } La { }
a) Ikiwa Ndio, tafadhali umechukua pombe muda gani?
Miaka 0-1 { } miaka 1-5 { } miaka 5-10 { } Zaidi ya miaka 10 { }

9) Je! Una ugonjwa sugu au hali ya matibabu?

Ikiwa Ndio, taja kwa fadhili:

_____

10) Je! Umepoteza mtu yeyote wa familia yako kutokana na saratani? Ndio { } La { }

Ikiwa ndio, onyesha kwa fadhili:

a) Jinsia ya marehemu: Mwanaume { } Kike { }

b) Umri wa marehemu:
Miaka 20-30 { } Miaka 30-50 { } Zaidi ya miaka 50 { }

c) uhusiano wako na mtu aliyekufa: _____

d) Aina ya saratani: _____

Asante kwa muda wako.

Appendix 4 Data Frame of Participants' Cases and Trace Elements

PARTICIPANTS	CLASS	Cr	Fe	Cu	Zn	Se	Cd	Hg	Pb
C1	CN	12.1	212	22.9	357	2.69	-	2.69	18.6
C2	CN	-	196	10.9	112	-	-	2.61	6.5
C9	CN	-				-	-	-	
C10	CN	-				-	-	-	
C11	CN	11.2	147	22.7	285	1.94	-	5.60	13.2
C12	CN	9.9	264	35.2	370	-	-		-
C13	CN	2.6	105	16.6	167	0.43	-	-	8.5
C14	CN		185	20.2	181	-	-	-	9.9
C15	CN	3.5	167	39.2	364	2.19	-	1.97	9.8
C16	CN	-	296	24.8	276	1.41	-	-	9.2
C17	CN	-	179	21.6	183	1.88	-	2.82	8.7
C19	CN	3.7	236	32.3	149	0.92			5.3
C20	CN	9.2	350		294		-	-	12.3
C21	CN	-	141	28.3	263	4.12	-	1.74	5.6
C22	CN	3.9	124	16.6	137	1.97			2.2
C23	CN	3.9		39.5	370	-	-	-	
C24	CN	-	310	39.5	211	-	-	-	14.4
C25	CN	8.8			248	1.75	-	2.19	
C26	CN	-				-	-	-	
C27	CN	-	359	39.9	303	1.69	-	4.22	
C28	CN	6.8	203	22.7	144	2.72	-	3.63	2.7
C29	CN	-			403	1.36	-	-	
C30	CN	3.2	183	28.7	228	1.38	-	-	9.4
C31	CN	8.2	308	24.7	365	0.91	-	2.74	14.6
C32	CN	-	209	33.5	193	2.02	-	2.79	10.4
C33	CN	3.9	281	42.7		-	-	1.68	20.3
C34	CN	-	167	24.1	215	1.37	-	1.83	6.0
C36	CN	-	109	26.5	282	-	-	0.92	9.5
C37	CN	-	140		198		-	-	9.2
C38	CN	-	238	22.4	353	-	-	-	20.6
C39	CN	4.1	217	39.3	321	-	-	1.37	25.2
CE1	CE	10.1	167	24.1		3.08	-	-	15.4
CE2	CE	12.3	268	23.7		2.64		2.20	12.3
CE3	CE	18.5		11.2	300	-	-	-	14.0
CE4	CE	20.9	350	14.2	225	2.18	-	1.74	8.9
CE5	CE	12.0	124	10.3	197	2.53	-	3.59	5.6
CE6	CE	9.8	140	10.1	205	2.60	-	3.47	3.9
CE7	CE	14.2	177	8.8	280	-	-	-	4.0
CE8	CE	20.0		10.3	209	3.08	-	6.81	10.1
CE9	CE		287	24.3	197	5.91	-		

CE10	CE	9.0	140	13.2	214	-	-	-	10.7
CE11	CE	6.4	88	19.3	96	1.83	-	0.92	3.7
CE12	CE	6.5	177	20.2	167	2.60	-	6.07	-
CE13	CE	-	228	41.0	193	2.42	-	1.45	6.3
CE14	CE	-	240	17.1	239	-	-	4.94	7.6
CE15	CE	-	268	23.8		1.37	-	-	20.3
CE16	CE	19.4					-	2.04	11.2
CE17	CE	7.1		19.7	156	3.97	-	3.31	9.7
CE18	CE	-	121	41.4	142	2.69	-	1.79	9.2
CE19	CE	-	243	29.8	188	-	-	-	5.9
CE20	CE	7.5	291	27.5	157	0.93	-	-	14.0
CE21	CE	-		36.2	404	2.07	-	1.38	15.5
CE22	CE	-	167	24.1	136	1.74	-	4.80	1.7
CE23	CE	-	146	29.8	283	0.94	-		9.0
CE24	CE	-	323	26.5	240	1.30	-	2.61	4.6
CE25	CE		184	42.7	227				3.5
CE26	CE	-		33.5	195	1.40	-	1.86	15.8
CE27	CE	5.4		21.5	174	1.34	-	-	16.3
CE28	CE	7.9	337	15.7	269	2.95	-	5.40	9.8
CE29	CE	4.1	183	10.4	191	1.81	-	-	4.8
CE30	CE	29.0	206	14.2	242	5.23	-	-	8.3
CE31	CE	21.4	234	9.0	150	2.62	-	8.31	8.2
CE32	CE	10.4	166	10.2	144	2.04	-	3.39	3.6
CE33	CE	15.2	152	16.8	231	1.79	-	4.70	9.6
CE34	CE	12.6	203	11.1	161	4.35	-	-	17.4
CE35	CE	5.7	252	8.9	191	4.78	-	-	6.1
CE36	CE		188	14.0	159	-	-	2.19	6.3
CE37	CE	9.3	270	10.4	444	-	-	2.67	6.2
CE38	CE	15.5	333	10.9	345	-	-	4.27	12.5
CE39	CE	12.9	112	6.6	354		-	-	10.2
CE40	CE	14.0	154	7.7	289	-	-	-	20.0
CE41	CE	-	330	25.7		1.76	-	-	6.6
CE42	CE	-	247	24.9		3.11	-	-	10.9
CE43	CE	-	91	23.8		2.46	-	2.46	3.7
CE44	CE	8.9	197	14.4		-	-	3.11	10.0
CE45	CE	-	152	16.0	383	5.82	-	-	5.3
CE46	CE	14.8	175	13.0	494		-		7.0
CE47	CE	11.5	256	8.8	396	3.08	-	3.08	8.1
CE48	CE	-	265	16.6	396	-	-	7.48	17.8
CE49	CE	8.2	238	18.9		-	-	1.82	5.5
CE50	CE	9.2	192	27.0		1.74	-	-	16.6
CE51	CE	8.3	103	26.5		1.74	-	-	8.5

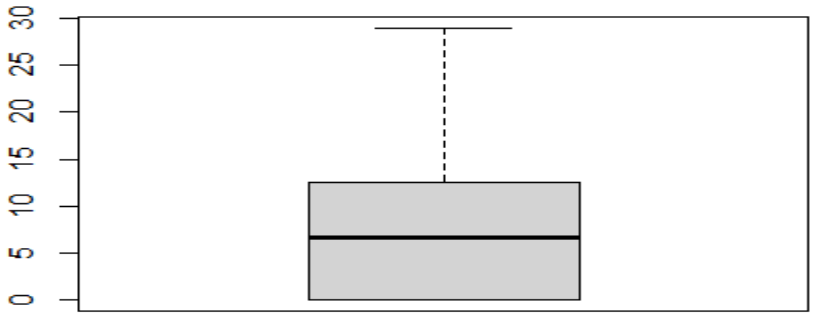
CE52	CE	4.6	138	22.6		-		-	11.1
CE53	CE	-	238	19.0		1.31	-	-	14.0
CE54	CE	9.5	238	21.6		1.29	-	6.47	15.1
CE55	CE			13.6	259	5.82	-	4.92	16.3
CE56	CE	-				-		-	16.9
CE57	CE	-	148	14.4	498	1.31	-	-	8.3
CE58	CE	-	249	21.2	394	1.30		-	3.9
CE59	CE	5.9	135	16.9	352	2.26	-	5.87	4.5
CE60	CE	9.1				3.97		4.53	
CE61	CE	10.7	143			-	-	-	6.6
CE62	CE	18.6		33.4	298	1.73			5.6
CE63	CE	-		18.0	395	2.17		-	8.7
CE64	CE	13.5	99	22.6	261	5.33	-	7.53	14.1
CE65	CE	2.0	21		22	0.30	-	0.56	1.1
CE66	CE	11.9	204	14.1	303	4.39	-	5.65	10.0
CE67	CE	26.4	181	24.7		-	-		16.3
CE68	CE	21.5	388			3.15		-	15.2
CE69	CE	22.1	323	41.3	324		-		14.4
CE70	CE	-	323	23.1	246	1.79	-	-	10.5
CE71	CE	12.7	189	18.6	186	-	-	-	12.0
CE72	CE	24.7	188	15.1	193	-	-		11.7
CS1	CS	12.5	117	11.0	134	-		3.57	5.3
CS2	CS	8.3	246		167	-	-	2.62	18.3
CS3	CS	13.7	174	13.3	314	-	-	3.66	10.5
CS4	CS	27.9	223		237	4.95			19.6
CS5	CS	-	165	28.5	105	2.00	-	7.56	3.6
CS6	CS	6.5	342	31.1	296	1.39	-	-	7.4
CS7	CS	16.8	114	14.6	187	-	-	5.75	13.1
CS8	CS	7.5	200	10.6	108	-	-	1.77	3.1
CS9	CS	9.1	238	10.8	187	2.46	-	3.94	7.1
CS10	CS	23.3		10.8	253	5.01	-	3.01	7.3
CS11	CS	23.3	130	17.5	199	-		6.87	11.5
CS12	CS	24.9		15.1	312	-	-	-	10.9
CS13	CS	17.7	223	20.4	446	1.57		-	
CS14	CS	5.4	258	28.8	496	1.80	-	2.70	10.1
CS15	CS	17.3	203	31.7		5.85	-		15.1
CS16	CS	12.8	168	19.9	392	4.42	-	4.86	5.7
CS17	CS	-	188	16.7	377	-	-	-	13.6
CS18	CS	-	248				-	-	6.3
CS19	CS		353	15.4	244	-	-		8.7
CS20	CS	-	211	16.3	290	1.43	-	-	13.0
CS21	CS				268	4.92	-		15.7

CS22	CS		172	16.8			-	2.33	5.6
CS23	CS	4.2		18.9	295	-	-	-	10.3

Appendix 5 Boxplots

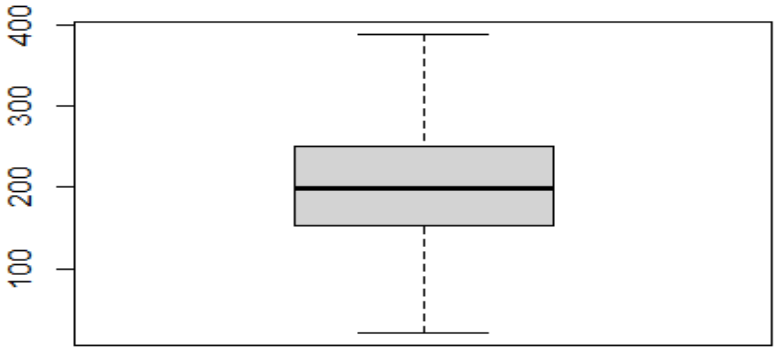
Cr Data

```
dfcr<-data.frame(confi$CLASS, confi$Cr)
dfcr

boxplot(dfcr$confi.Cr)
```



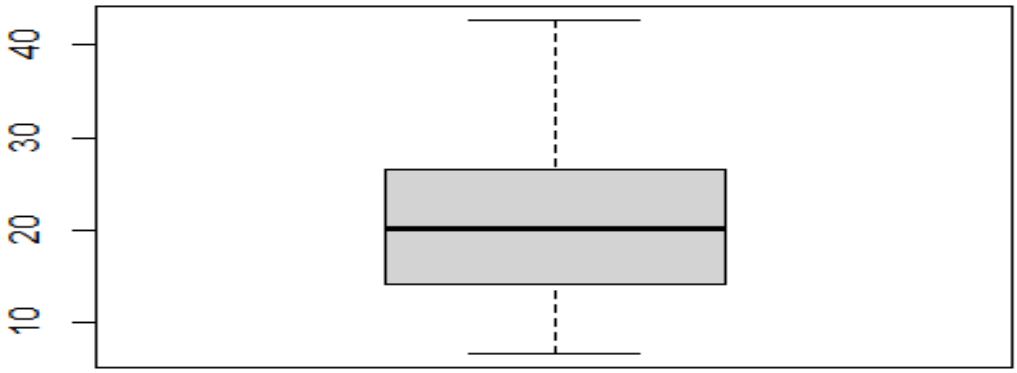
Fe Data

```
dffe<-data.frame(confi$CLASS, confi$Fe)
dffe

boxplot(dffe$confi.Fe)
```



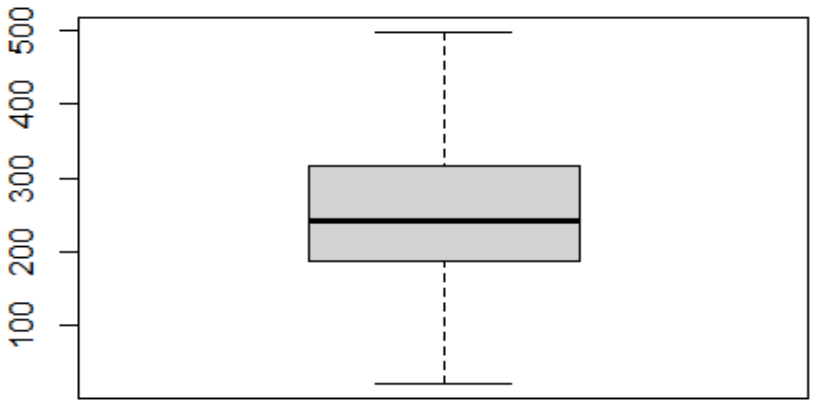
Cu Data

```
dfcu<-data.frame(confi$CLASS, confi$Cu)
dfcu

boxplot(dfcu$confi.Cu)
```



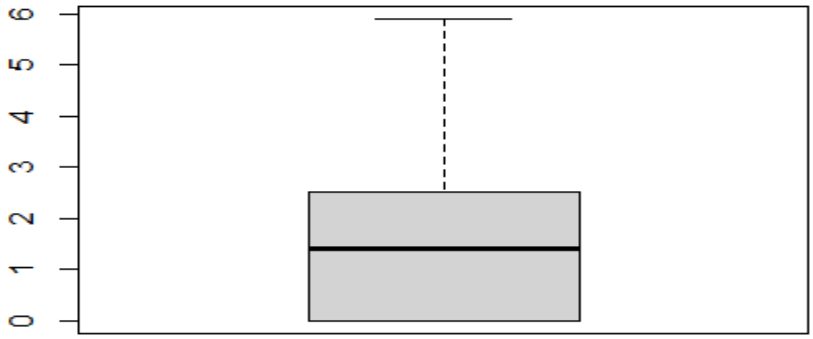
Zn data

```
dfzn<-data.frame(confi$CLASS, confi$Zn)
dfzn

boxplot(dfzn$confi.Zn)
```



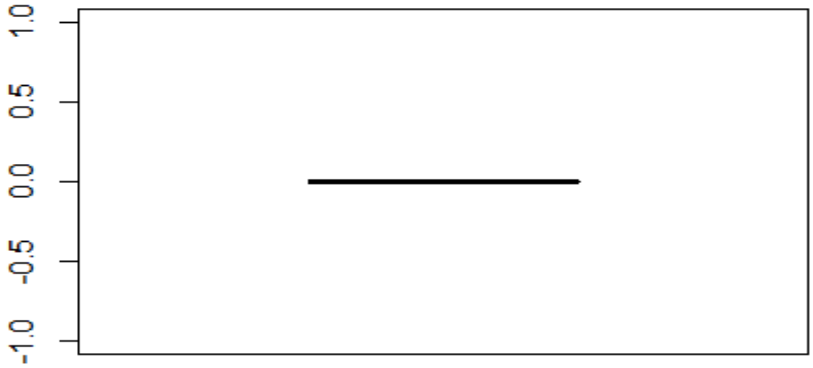
Se Data

```
dfse<-data.frame(confi$CLASS, confi$Se)
dfse

boxplot(dfse$confi.Se)
```



Cd Data

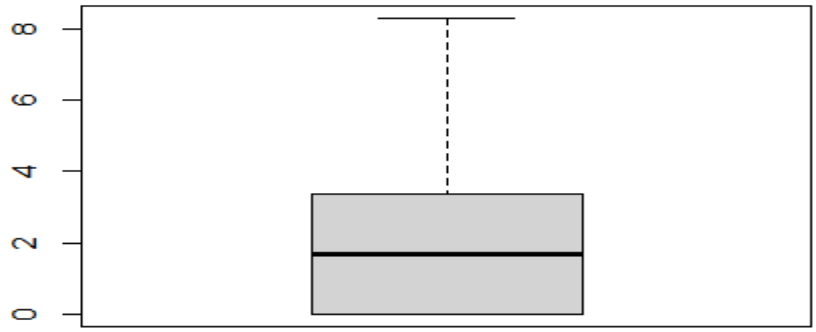
```
dfcd<-data.frame(confi$CLASS, confi$Cd)
dfcd

boxplot(dfcd$confi.Cd)
```



Hg data

```
dfhg<-data.frame(confi$CLASS, confi$Hg)
dfhg
```

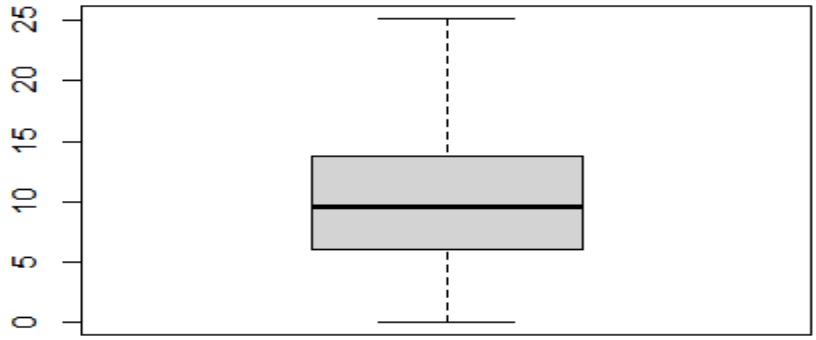
```
boxplot(dfhg$confi.Hg)
```



Pb Data

```
dfpb<-data.frame(confi$CLASS, confi$Pb)
dfpb
```

```
boxplot(dfpb$confi.Pb)
```



Appendix 6 Grouping the data to remove 'PARTICIPANTS' and 'Cd' Columns

```
confi2<-subset(confi,select = -c(PARTICIPANTS, Cd), )
confi2
```

```
##     CLASS        Cr        Fe        Cu        Zn        Se        Hg
Pb
## 1      CN 12.113014 211.84315 22.880137 356.75068 2.6917808 2.6917808
18.573288
## 2      CN  0.000000 195.95175 10.874126 112.22098 0.0000000 2.6097902
6.524476
## 3      CN  0.000000        NA        NA        NA 0.0000000 0.0000000
NA
## 4      CN  0.000000        NA        NA        NA 0.0000000 0.0000000
NA
## 5      CN 11.209969 147.02305 22.707373 285.42305 1.9401869 5.6049844
13.150156
## 6      CN  9.946753 264.02143 35.246104 370.19221 0.0000000        NA
0.000000
## 7      CN  2.590476 104.77037 16.550265 166.79788 0.4317460 0.0000000
8.491005
## 8      CN        NA 185.40690 20.162452 181.02375 0.0000000 0.0000000
9.862069
## 9      CN  3.500752 166.94211 39.237594 363.64060 2.1879699 1.9691729
9.845865
## 10     CN  0.000000 296.32192 24.830137 276.09863 1.4054795 0.0000000
9.213699
## 11     CN  0.000000 178.85833 21.594444 182.92685 1.8777778 2.8166667
8.684722
## 12     CN  3.662428 235.76879 32.275145 149.01503 0.9156069        NA
5.264740
## 13     CN  9.200000 350.04878        NA 293.50244        NA 0.0000000
12.341463
## 14     CN  0.000000 141.45085 28.348023 263.15932 4.1220339 1.7355932
5.640678
## 15     CN  3.940909 124.35758 16.639394 137.05606 1.9704545        NA
2.189394
## 16     CN  3.930882        NA 39.454412 370.37647 0.0000000 0.0000000
NA
## 17     CN  0.000000 310.37899 39.478986 210.55459 0.0000000 0.0000000
14.395652
## 18     CN  8.766284        NA        NA 247.64751 1.7532567 2.1915709
NA
```

```
## 19     CN  0.000000        NA        NA        NA 0.0000000 0.0000000
NA
## 20     CN  0.000000 358.67778 39.900000 303.36667 1.6888889 4.2222222
NA
## 21     CN  6.803571 202.97321 22.678571 143.55536 2.7214286 3.6285714
2.721429
## 22     CN  0.000000        NA        NA 402.55217 1.3630435 0.0000000
NA
## 23     CN  3.211765 183.07059 28.676471 228.26471 1.3764706 0.0000000
9.405882
## 24     CN  8.234043 307.86170 24.702128 364.58511 0.9148936 2.7446809
14.638298
## 25     CN  0.000000 208.57246 33.495652 193.22029 2.0159420 2.7913043
10.389855
## 26     CN  3.917021 280.71986 42.714184        NA 0.0000000 1.6787234
20.331206
## 27     CN  0.000000 167.05769 24.105128 214.65769 1.3730769 1.8307692
5.950000
## 28     CN  0.000000 108.56612 26.487052 281.96391 0.0000000 0.9239669
9.547658
## 29     CN  0.000000 139.80156        NA 198.31958        NA 0.0000000
9.167315
## 30     CN  0.000000 238.27986 22.423675 353.11625 0.0000000 0.0000000
20.611661
## 31     CN  4.098155 216.97454 39.311931 320.87036 0.0000000 1.3660517
25.196064
## 32     CE 10.131174 167.16437 24.079892        NA 3.0834008 0.0000000
15.417004
## 33     CE 12.313253 267.59337 23.746988        NA 2.6385542 2.1987952
12.313253
## 34     CE 18.488889        NA 11.219753 299.77284 0.0000000 0.0000000
13.985185
## 35     CE 20.902128 349.89291 14.152482 224.91560 2.1773050 1.7418440
8.926950
## 36     CE 12.031142 123.89965 10.342561 196.71972 2.5328720 3.5882353
5.628604
## 37     CE  9.767918 140.00683 10.129693 205.34334 2.6047782 3.4730375
3.907167
## 38     CE 14.205882 177.10000  8.760294 280.17157 0.0000000 0.0000000
4.025000
## 39     CE 19.991339        NA 10.251969 208.84724 3.0755906 6.8102362
10.105512
## 40     CE        NA 287.33552 24.323497 196.63388 5.9103825        NA
NA
## 41     CE  8.964706 140.44706 13.198039 213.65882 0.0000000 0.0000000
```

```
10.707843
## 42     CE  6.417341  87.78006 19.252023  96.48931 1.8335260 0.9167630
3.667052
## 43     CE  6.508475 176.59661 20.248588 167.34011 2.6033898 6.0745763
0.000000
## 44     CE  0.000000 228.07288 41.041808 193.41356 2.4237288 1.4542373
6.301695
## 45     CE  0.000000 240.05517 17.071921 239.00690 0.0000000 4.9418719
7.637438
## 46     CE  0.000000 267.56296 23.840741        NA 1.3666667 0.0000000
20.348148
## 47     CE 19.422222        NA        NA        NA        NA 2.0444444
11.244444
## 48     CE  7.055738        NA 19.697268 155.81421 3.9688525 3.3073770
9.701639
## 49     CE  0.000000 121.49179 41.393881 142.11401 2.6898551 1.7932367
9.190338
## 50     CE  0.000000 242.60690 29.805364 187.89068 0.0000000 0.0000000
5.917241
## 51     CE  7.466667 290.96667 27.533333 156.64444 0.9333333 0.0000000
14.000000
## 52     CE  0.000000        NA 36.234343 404.25859 2.0727273 1.3818182
15.507071
## 53     CE  0.000000 166.54101 24.123741 136.31367 1.7438849 4.7956835
1.743885
## 54     CE  0.000000 145.99130 29.765217 283.32077 0.9449275        NA
8.976812
## 55     CE  0.000000 322.82069 26.503448 240.05172 1.3034483 2.6068966
4.562069
## 56     CE        NA 184.07308 42.669231 227.48654        NA        NA
3.473077
## 57     CE  0.000000        NA 33.536842 194.85526 1.3973684 1.8631579
15.836842
## 58     CE  5.366038        NA 21.464151 174.17264 1.3415094 0.0000000
16.321698
## 59     CE  7.854545 337.25455 15.709091 268.77273 2.9454545 5.4000000
9.818182
## 60     CE  4.073684 182.63684 10.410526 191.16140 1.8105263 0.0000000
4.752632
## 61     CE 28.992086 206.21439 14.169065 241.81870 5.2316547 0.0000000
8.283453
## 62     CE 21.421561 233.66989  8.962082 150.09665 2.6230483 8.3063197
8.160595
## 63     CE 10.410526 165.88947 10.184211 143.63509 2.0368421 3.3947368
3.621053
```

```
## 64     CE 15.226794 151.82010 16.794258 230.79043 1.7913876 4.7023923
9.628708
## 65     CE 12.617544 203.18596 11.094737 160.83743 4.3508772 0.0000000
17.403509
## 66     CE  5.652962 251.77422  8.914286 191.33101 4.7832753 0.0000000
6.087805
## 67     CE        NA 188.42230 13.989591 159.13160 0.0000000 2.1858736
6.339033
## 68     CE  9.337500 270.34286 10.449107 443.53125 0.0000000 2.6678571
6.225000
## 69     CE 15.466667 332.53333 10.933333 345.42222 0.0000000 4.2666667
12.533333
## 70     CE 12.855411 112.15238  6.649351 354.04098        NA 0.0000000
10.195671
## 71     CE 13.986755 154.08742  7.692715 289.29272 0.0000000 0.0000000
20.047682
## 72     CE  0.000000 329.86403 25.712253        NA 1.7581028 0.0000000
6.592885
## 73     CE  0.000000 247.33333 24.888889        NA 3.1111111 0.0000000
10.888889
## 74     CE  0.000000  91.21835 23.767584        NA 2.4587156 2.4587156
3.688073
## 75     CE  8.888889 196.88889 14.444444        NA 0.0000000 3.1111111
10.000000
## 76     CE  0.000000 152.45254 15.996610 382.94915 5.8169492 0.0000000
5.332203
## 77     CE 14.750000 175.25000 13.000000 494.25000        NA        NA
7.000000
## 78     CE 11.456911 256.31165  8.813008 395.85095 3.0845528 3.0845528
8.078591
## 79     CE  0.000000 264.87703 16.598649 395.71802 0.0000000 7.4810811
17.767568
## 80     CE  8.194475 237.86740 18.892818        NA 0.0000000 1.8209945
5.462983
## 81     CE  9.155396 192.04532 27.030216        NA 1.7438849 0.0000000
16.566906
## 82     CE  8.266667 102.68070 26.540351        NA 1.7403509 0.0000000
8.484211
## 83     CE  4.645161 138.19355 22.606452        NA 0.0000000 0.0000000
11.148387
## 84     CE  0.000000 237.71277 18.973404        NA 1.3085106 0.0000000
13.957447
## 85     CE  9.494006 238.42902 21.577287        NA 1.2946372 6.4731861
15.104101
## 86     CE        NA        NA 13.645498 259.48815 5.8161137 4.9213270
```

```
16.329858
## 87     CE  0.000000        NA        NA        NA 0.0000000 0.0000000
16.882759
## 88     CE  0.000000 148.44820 14.387050 498.02446 1.3079137 0.0000000
8.283453
## 89     CE  0.000000 248.51667 21.233333 394.18889 1.3000000 0.0000000
3.900000
## 90     CE  5.870103 134.56082 16.932990 351.90515 2.2577320 5.8701031
4.515464
## 91     CE  9.066667        NA        NA        NA 3.9666667 4.5333333
NA
## 92     CE 10.733333 143.11111        NA        NA 0.0000000 0.0000000
6.644444
## 93     CE 18.633333        NA 33.366667 297.55556 1.7333333        NA
5.633333
## 94     CE  0.000000        NA 17.958621 394.72759 2.1724138 0.0000000
8.689655
## 95     CE 13.486364  99.10909 22.581818 260.63182 5.3318182 7.5272727
14.113636
## 96     CE  1.950000  21.49333        NA  21.69556 0.3033333 0.5633333
1.083333
## 97     CE 11.918182 204.17727 14.113636 303.39091 4.3909091 5.6454545
10.036364
## 98     CE 26.429150 181.03968 24.667206        NA 0.0000000        NA
16.297976
## 99     CE 21.525000 387.80000        NA        NA 3.1500000 0.0000000
15.225000
## 100    CE 22.083333 322.85833 41.295833 324.33056        NA        NA
14.354167
## 101    CE  0.000000 323.47937 23.052381 245.51905 1.7904762 0.0000000
10.519048
## 102    CE 12.701408 188.63944 18.581690 185.97371 0.0000000 0.0000000
11.995775
## 103    CE 24.656678 188.16938 15.140065 193.07188 0.0000000        NA
11.679479
## 104    CS 12.478539 116.54064 10.992998 134.14429 0.0000000 3.5652968
5.347945
## 105    CS  8.293431 245.74745        NA 166.95985 0.0000000 2.6189781
18.332847
## 106    CS 13.724138 173.83908 13.266667 313.82529 0.0000000 3.6597701
10.521839
## 107    CS 27.884577 222.62687        NA 236.56915 4.9472637        NA
19.564179
## 108    CS  0.000000 164.74018 28.457143 104.63929 2.0008929 7.5589286
3.557143
```

```
## 109    CS  6.503226 341.57419 31.122581 296.05161 1.3935484 0.0000000
7.432258
## 110    CS 16.817021 114.17872 14.604255 187.34752 0.0000000 5.7531915
13.055319
## 111    CS  7.514286 199.57059 10.608403 108.29412 0.0000000 1.7680672
3.094118
## 112    CS  9.112963 238.41481 10.837037 187.18519 2.4629630 3.9407407
7.142593
## 113    CS 23.296970        NA 10.771717 253.17710 5.0101010 3.0060606
7.264646
## 114    CS 23.272727 130.10563 17.509957 198.59394 0.0000000 6.8709957
11.525541
## 115    CS 24.911111        NA 15.077778 312.04444 0.0000000 0.0000000
10.925926
## 116    CS 17.653846 222.83077 20.400000 446.44615 1.5692308 0.0000000
NA
## 117    CS  5.397015 257.93234 28.784080 495.62587 1.7990050 2.6985075
10.119403
## 118    CS 17.325000 202.95000 31.725000        NA 5.8500000        NA
15.075000
## 119    CS 12.808333 168.49583 19.875000 392.49444 4.4166667 4.8583333
5.741667
## 120    CS  0.000000 187.58649 16.689189 376.65646 0.0000000 0.0000000
13.573874
## 121    CS  0.000000 248.00000        NA        NA        NA 0.0000000
6.250000
## 122    CS        NA 352.75664 15.441259 243.72541 0.0000000        NA
8.699301
## 123    CS  0.000000 211.23636 16.334343 290.21212 1.4272727 0.0000000
13.004040
## 124    CS        NA        NA        NA 268.49174 4.9174312        NA
15.735780
## 125    CS        NA 171.73333 16.800000        NA        NA 2.3333333
5.600000
## 126    CS  4.208108        NA 18.936486 295.03514 0.0000000 0.0000000
10.286486
```

Appendix 7 Filtering the Rows that have "CN" in "CLASS" Column to Obtain New Subsets

```
library(dplyr)

confiCN<-confi2%>%filter(confi2$CLASS=="CN")
confiCN
```

```
##    CLASS        Cr       Fe       Cu       Zn        Se        Hg
Pb
## 1     CN 12.113014 211.8432 22.88014 356.7507 2.6917808 2.6917808
18.573288
## 2     CN  0.000000 195.9517 10.87413 112.2210 0.0000000 2.6097902
6.524476
## 3     CN  0.000000       NA       NA       NA 0.0000000 0.0000000
NA
## 4     CN  0.000000       NA       NA       NA 0.0000000 0.0000000
NA
## 5     CN 11.209969 147.0231 22.70737 285.4231 1.9401869 5.6049844
13.150156
## 6     CN  9.946753 264.0214 35.24610 370.1922 0.0000000        NA
0.000000
## 7     CN  2.590476 104.7704 16.55026 166.7979 0.4317460 0.0000000
8.491005
## 8     CN        NA 185.4069 20.16245 181.0238 0.0000000 0.0000000
9.862069
## 9     CN  3.500752 166.9421 39.23759 363.6406 2.1879699 1.9691729
9.845865
## 10    CN  0.000000 296.3219 24.83014 276.0986 1.4054795 0.0000000
9.213699
## 11    CN  0.000000 178.8583 21.59444 182.9269 1.8777778 2.8166667
8.684722
## 12    CN  3.662428 235.7688 32.27514 149.0150 0.9156069        NA
5.264740
## 13    CN  9.200000 350.0488       NA 293.5024        NA 0.0000000
12.341463
## 14    CN  0.000000 141.4508 28.34802 263.1593 4.1220339 1.7355932
5.640678
## 15    CN  3.940909 124.3576 16.63939 137.0561 1.9704545        NA
2.189394
## 16    CN  3.930882       NA 39.45441 370.3765 0.0000000 0.0000000
NA
## 17    CN  0.000000 310.3790 39.47899 210.5546 0.0000000 0.0000000
14.395652
```

```
## 18    CN  8.766284       NA       NA 247.6475 1.7532567 2.1915709
NA
## 19    CN  0.000000       NA       NA       NA 0.0000000 0.0000000
NA
## 20    CN  0.000000 358.6778 39.90000 303.3667 1.6888889 4.2222222
NA
## 21    CN  6.803571 202.9732 22.67857 143.5554 2.7214286 3.6285714
2.721429
## 22    CN  0.000000       NA       NA 402.5522 1.3630435 0.0000000
NA
## 23    CN  3.211765 183.0706 28.67647 228.2647 1.3764706 0.0000000
9.405882
## 24    CN  8.234043 307.8617 24.70213 364.5851 0.9148936 2.7446809
14.638298
## 25    CN  0.000000 208.5725 33.49565 193.2203 2.0159420 2.7913043
10.389855
## 26    CN  3.917021 280.7199 42.71418       NA 0.0000000 1.6787234
20.331206
## 27    CN  0.000000 167.0577 24.10513 214.6577 1.3730769 1.8307692
5.950000
## 28    CN  0.000000 108.5661 26.48705 281.9639 0.0000000 0.9239669
9.547658
## 29    CN  0.000000 139.8016       NA 198.3196        NA 0.0000000
9.167315
## 30    CN  0.000000 238.2799 22.42367 353.1163 0.0000000 0.0000000
20.611661
## 31    CN  4.098155 216.9745 39.31193 320.8704 0.0000000 1.3660517
25.196064
```

```
confiCN2<-confiCN[1:23,]
confiCN2
```

```
##    CLASS        Cr       Fe       Cu       Zn        Se       Hg        Pb
## 1     CN 12.113014 211.8432 22.88014 356.7507 2.6917808 2.691781 18.573288
## 2     CN  0.000000 195.9517 10.87413 112.2210 0.0000000 2.609790  6.524476
## 3     CN  0.000000       NA       NA       NA 0.0000000 0.000000        NA
## 4     CN  0.000000       NA       NA       NA 0.0000000 0.000000        NA
## 5     CN 11.209969 147.0231 22.70737 285.4231 1.9401869 5.604984 13.150156
## 6     CN  9.946753 264.0214 35.24610 370.1922 0.0000000       NA  0.000000
## 7     CN  2.590476 104.7704 16.55026 166.7979 0.4317460 0.000000  8.491005
## 8     CN        NA 185.4069 20.16245 181.0238 0.0000000 0.000000  9.862069
## 9     CN  3.500752 166.9421 39.23759 363.6406 2.1879699 1.969173  9.845865
## 10    CN  0.000000 296.3219 24.83014 276.0986 1.4054795 0.000000  9.213699
## 11    CN  0.000000 178.8583 21.59444 182.9269 1.8777778 2.816667  8.684722
## 12    CN  3.662428 235.7688 32.27514 149.0150 0.9156069       NA  5.264740
```

```
## 13    CN  9.200000 350.0488       NA 293.5024        NA 0.000000 12.341463
## 14    CN  0.000000 141.4508 28.34802 263.1593 4.1220339 1.735593  5.640678
## 15    CN  3.940909 124.3576 16.63939 137.0561 1.9704545       NA  2.189394
## 16    CN  3.930882       NA 39.45441 370.3765 0.0000000 0.000000        NA
## 17    CN  0.000000 310.3790 39.47899 210.5546 0.0000000 0.000000 14.395652
## 18    CN  8.766284       NA       NA 247.6475 1.7532567 2.191571        NA
## 19    CN  0.000000       NA       NA       NA 0.0000000 0.000000        NA
## 20    CN  0.000000 358.6778 39.90000 303.3667 1.6888889 4.222222        NA
## 21    CN  6.803571 202.9732 22.67857 143.5554 2.7214286 3.628571  2.721429
## 22    CN  0.000000       NA       NA 402.5522 1.3630435 0.000000        NA
## 23    CN  3.211765 183.0706 28.67647 228.2647 1.3764706 0.000000  9.405882
```

```
library(dplyr)
confiCE<-confi2%>%filter(confi$CLASS=="CE")
confiCE
```

```
##    CLASS        Cr        Fe        Cu        Zn        Se        Hg
Pb
## 1     CE 10.131174 167.16437 24.079892        NA 3.0834008 0.0000000
15.417004
## 2     CE 12.313253 267.59337 23.746988        NA 2.6385542 2.1987952
12.313253
## 3     CE 18.488889        NA 11.219753 299.77284 0.0000000 0.0000000
13.985185
## 4     CE 20.902128 349.89291 14.152482 224.91560 2.1773050 1.7418440
8.926950
## 5     CE 12.031142 123.89965 10.342561 196.71972 2.5328720 3.5882353
5.628604
## 6     CE  9.767918 140.00683 10.129693 205.34334 2.6047782 3.4730375
3.907167
## 7     CE 14.205882 177.10000  8.760294 280.17157 0.0000000 0.0000000
4.025000
## 8     CE 19.991339        NA 10.251969 208.84724 3.0755906 6.8102362
10.105512
## 9     CE        NA 287.33552 24.323497 196.63388 5.9103825        NA
NA
## 10    CE  8.964706 140.44706 13.198039 213.65882 0.0000000 0.0000000
10.707843
## 11    CE  6.417341  87.78006 19.252023  96.48931 1.8335260 0.9167630
3.667052
## 12    CE  6.508475 176.59661 20.248588 167.34011 2.6033898 6.0745763
0.000000
## 13    CE  0.000000 228.07288 41.041808 193.41356 2.4237288 1.4542373
6.301695
## 14    CE  0.000000 240.05517 17.071921 239.00690 0.0000000 4.9418719
```

```
7.637438
## 15    CE  0.000000 267.56296 23.840741        NA 1.3666667 0.0000000
20.348148
## 16    CE 19.422222        NA        NA        NA        NA 2.0444444
11.244444
## 17    CE  7.055738        NA 19.697268 155.81421 3.9688525 3.3073770
9.701639
## 18    CE  0.000000 121.49179 41.393881 142.11401 2.6898551 1.7932367
9.190338
## 19    CE  0.000000 242.60690 29.805364 187.89068 0.0000000 0.0000000
5.917241
## 20    CE  7.466667 290.96667 27.533333 156.64444 0.9333333 0.0000000
14.000000
## 21    CE  0.000000        NA 36.234343 404.25859 2.0727273 1.3818182
15.507071
## 22    CE  0.000000 166.54101 24.123741 136.31367 1.7438849 4.7956835
1.743885
## 23    CE  0.000000 145.99130 29.765217 283.32077 0.9449275        NA
8.976812
## 24    CE  0.000000 322.82069 26.503448 240.05172 1.3034483 2.6068966
4.562069
## 25    CE        NA 184.07308 42.669231 227.48654        NA        NA
3.473077
## 26    CE  0.000000        NA 33.536842 194.85526 1.3973684 1.8631579
15.836842
## 27    CE  5.366038        NA 21.464151 174.17264 1.3415094 0.0000000
16.321698
## 28    CE  7.854545 337.25455 15.709091 268.77273 2.9454545 5.4000000
9.818182
## 29    CE  4.073684 182.63684 10.410526 191.16140 1.8105263 0.0000000
4.752632
## 30    CE 28.992086 206.21439 14.169065 241.81870 5.2316547 0.0000000
8.283453
## 31    CE 21.421561 233.66989  8.962082 150.09665 2.6230483 8.3063197
8.160595
## 32    CE 10.410526 165.88947 10.184211 143.63509 2.0368421 3.3947368
3.621053
## 33    CE 15.226794 151.82010 16.794258 230.79043 1.7913876 4.7023923
9.628708
## 34    CE 12.617544 203.18596 11.094737 160.83743 4.3508772 0.0000000
17.403509
## 35    CE  5.652962 251.77422  8.914286 191.33101 4.7832753 0.0000000
6.087805
## 36    CE        NA 188.42230 13.989591 159.13160 0.0000000 2.1858736
6.339033
```

```
## 37    CE  9.337500 270.34286 10.449107 443.53125 0.0000000 2.6678571
6.225000
## 38    CE 15.466667 332.53333 10.933333 345.42222 0.0000000 4.2666667
12.533333
## 39    CE 12.855411 112.15238  6.649351 354.04098        NA 0.0000000
10.195671
## 40    CE 13.986755 154.08742  7.692715 289.29272 0.0000000 0.0000000
20.047682
## 41    CE  0.000000 329.86403 25.712253        NA 1.7581028 0.0000000
6.592885
## 42    CE  0.000000 247.33333 24.888889        NA 3.1111111 0.0000000
10.888889
## 43    CE  0.000000  91.21835 23.767584        NA 2.4587156 2.4587156
3.688073
## 44    CE  8.888889 196.88889 14.444444        NA 0.0000000 3.1111111
10.000000
## 45    CE  0.000000 152.45254 15.996610 382.94915 5.8169492 0.0000000
5.332203
## 46    CE 14.750000 175.25000 13.000000 494.25000        NA        NA
7.000000
## 47    CE 11.456911 256.31165  8.813008 395.85095 3.0845528 3.0845528
8.078591
## 48    CE  0.000000 264.87703 16.598649 395.71802 0.0000000 7.4810811
17.767568
## 49    CE  8.194475 237.86740 18.892818        NA 0.0000000 1.8209945
5.462983
## 50    CE  9.155396 192.04532 27.030216        NA 1.7438849 0.0000000
16.566906
## 51    CE  8.266667 102.68070 26.540351        NA 1.7403509 0.0000000
8.484211
## 52    CE  4.645161 138.19355 22.606452        NA 0.0000000 0.0000000
11.148387
## 53    CE  0.000000 237.71277 18.973404        NA 1.3085106 0.0000000
13.957447
## 54    CE  9.494006 238.42902 21.577287        NA 1.2946372 6.4731861
15.104101
## 55    CE        NA        NA 13.645498 259.48815 5.8161137 4.9213270
16.329858
## 56    CE  0.000000        NA        NA        NA 0.0000000 0.0000000
16.882759
## 57    CE  0.000000 148.44820 14.387050 498.02446 1.3079137 0.0000000
8.283453
## 58    CE  0.000000 248.51667 21.233333 394.18889 1.3000000 0.0000000
3.900000
## 59    CE  5.870103 134.56082 16.932990 351.90515 2.2577320 5.8701031
```

```
4.515464
## 60    CE  9.066667        NA        NA        NA 3.9666667 4.5333333
NA
## 61    CE 10.733333 143.11111        NA        NA 0.0000000 0.0000000
6.644444
## 62    CE 18.633333        NA 33.366667 297.55556 1.7333333        NA
5.633333
## 63    CE  0.000000        NA 17.958621 394.72759 2.1724138 0.0000000
8.689655
## 64    CE 13.486364  99.10909 22.581818 260.63182 5.3318182 7.5272727
14.113636
## 65    CE  1.950000  21.49333        NA  21.69556 0.3033333 0.5633333
1.083333
## 66    CE 11.918182 204.17727 14.113636 303.39091 4.3909091 5.6454545
10.036364
## 67    CE 26.429150 181.03968 24.667206        NA 0.0000000        NA
16.297976
## 68    CE 21.525000 387.80000        NA        NA 3.1500000 0.0000000
15.225000
## 69    CE 22.083333 322.85833 41.295833 324.33056        NA        NA
14.354167
## 70    CE  0.000000 323.47937 23.052381 245.51905 1.7904762 0.0000000
10.519048
## 71    CE 12.701408 188.63944 18.581690 185.97371 0.0000000 0.0000000
11.995775
## 72    CE 24.656678 188.16938 15.140065 193.07188 0.0000000        NA
11.679479
```

Appendix 8 Matching the Rows and Columns

```
confiCE1<-confiCE[1:31,]
confiCE1
```

```
##    CLASS        Cr        Fe        Cu        Zn        Se       Hg
Pb
## 1     CE 10.131174 167.16437 24.079892        NA 3.0834008 0.000000
15.417004
## 2     CE 12.313253 267.59337 23.746988        NA 2.6385542 2.198795
12.313253
## 3     CE 18.488889        NA 11.219753 299.77284 0.0000000 0.000000
13.985185
## 4     CE 20.902128 349.89291 14.152482 224.91560 2.1773050 1.741844
8.926950
## 5     CE 12.031142 123.89965 10.342561 196.71972 2.5328720 3.588235
5.628604
## 6     CE  9.767918 140.00683 10.129693 205.34334 2.6047782 3.473038
3.907167
## 7     CE 14.205882 177.10000  8.760294 280.17157 0.0000000 0.000000
4.025000
## 8     CE 19.991339        NA 10.251969 208.84724 3.0755906 6.810236
10.105512
## 9     CE        NA 287.33552 24.323497 196.63388 5.9103825       NA
NA
## 10    CE  8.964706 140.44706 13.198039 213.65882 0.0000000 0.000000
10.707843
## 11    CE  6.417341  87.78006 19.252023  96.48931 1.8335260 0.916763
3.667052
## 12    CE  6.508475 176.59661 20.248588 167.34011 2.6033898 6.074576
0.000000
## 13    CE  0.000000 228.07288 41.041808 193.41356 2.4237288 1.454237
6.301695
## 14    CE  0.000000 240.05517 17.071921 239.00690 0.0000000 4.941872
7.637438
## 15    CE  0.000000 267.56296 23.840741        NA 1.3666667 0.000000
20.348148
## 16    CE 19.422222        NA        NA        NA        NA 2.044444
11.244444
## 17    CE  7.055738        NA 19.697268 155.81421 3.9688525 3.307377
9.701639
## 18    CE  0.000000 121.49179 41.393881 142.11401 2.6898551 1.793237
9.190338
## 19    CE  0.000000 242.60690 29.805364 187.89068 0.0000000 0.000000
```

```
5.917241
## 20    CE  7.466667 290.96667 27.533333 156.64444 0.9333333 0.000000
14.000000
## 21    CE  0.000000        NA 36.234343 404.25859 2.0727273 1.381818
15.507071
## 22    CE  0.000000 166.54101 24.123741 136.31367 1.7438849 4.795683
1.743885
## 23    CE  0.000000 145.99130 29.765217 283.32077 0.9449275       NA
8.976812
## 24    CE  0.000000 322.82069 26.503448 240.05172 1.3034483 2.606897
4.562069
## 25    CE        NA 184.07308 42.669231 227.48654        NA       NA
3.473077
## 26    CE  0.000000        NA 33.536842 194.85526 1.3973684 1.863158
15.836842
## 27    CE  5.366038        NA 21.464151 174.17264 1.3415094 0.000000
16.321698
## 28    CE  7.854545 337.25455 15.709091 268.77273 2.9454545 5.400000
9.818182
## 29    CE  4.073684 182.63684 10.410526 191.16140 1.8105263 0.000000
4.752632
## 30    CE 28.992086 206.21439 14.169065 241.81870 5.2316547 0.000000
8.283453
## 31    CE 21.421561 233.66989  8.962082 150.09665 2.6230483 8.306320
8.160595
```

```
confiCE2<-confiCE[1:23,]
confiCE2
```

```
##    CLASS        Cr        Fe        Cu        Zn        Se       Hg
Pb
## 1     CE 10.131174 167.16437 24.079892        NA 3.0834008 0.000000
15.417004
## 2     CE 12.313253 267.59337 23.746988        NA 2.6385542 2.198795
12.313253
## 3     CE 18.488889        NA 11.219753 299.77284 0.0000000 0.000000
13.985185
## 4     CE 20.902128 349.89291 14.152482 224.91560 2.1773050 1.741844
8.926950
## 5     CE 12.031142 123.89965 10.342561 196.71972 2.5328720 3.588235
5.628604
## 6     CE  9.767918 140.00683 10.129693 205.34334 2.6047782 3.473038
3.907167
## 7     CE 14.205882 177.10000  8.760294 280.17157 0.0000000 0.000000
4.025000
```

```
## 8     CE 19.991339        NA 10.251969 208.84724 3.0755906 6.810236
10.105512
## 9     CE        NA 287.33552 24.323497 196.63388 5.9103825       NA
NA
## 10    CE  8.964706 140.44706 13.198039 213.65882 0.0000000 0.000000
10.707843
## 11    CE  6.417341  87.78006 19.252023  96.48931 1.8335260 0.916763
3.667052
## 12    CE  6.508475 176.59661 20.248588 167.34011 2.6033898 6.074576
0.000000
## 13    CE  0.000000 228.07288 41.041808 193.41356 2.4237288 1.454237
6.301695
## 14    CE  0.000000 240.05517 17.071921 239.00690 0.0000000 4.941872
7.637438
## 15    CE  0.000000 267.56296 23.840741        NA 1.3666667 0.000000
20.348148
## 16    CE 19.422222        NA        NA        NA        NA 2.044444
11.244444
## 17    CE  7.055738        NA 19.697268 155.81421 3.9688525 3.307377
9.701639
## 18    CE  0.000000 121.49179 41.393881 142.11401 2.6898551 1.793237
9.190338
## 19    CE  0.000000 242.60690 29.805364 187.89068 0.0000000 0.000000
5.917241
## 20    CE  7.466667 290.96667 27.533333 156.64444 0.9333333 0.000000
14.000000
## 21    CE  0.000000        NA 36.234343 404.25859 2.0727273 1.381818
15.507071
## 22    CE  0.000000 166.54101 24.123741 136.31367 1.7438849 4.795683
1.743885
## 23    CE  0.000000 145.99130 29.765217 283.32077 0.9449275       NA
8.976812
```

```
library(dplyr)
confiCS<-confi2%>%filter(confi2$CLASS=="CS")
confiCS
```

```
##    CLASS        Cr       Fe       Cu       Zn       Se       Hg        Pb
## 1     CS 12.478539 116.5406 10.99300 134.1443 0.000000 3.565297  5.347945
## 2     CS  8.293431 245.7474       NA 166.9599 0.000000 2.618978 18.332847
## 3     CS 13.724138 173.8391 13.26667 313.8253 0.000000 3.659770 10.521839
## 4     CS 27.884577 222.6269       NA 236.5692 4.947264       NA 19.564179
## 5     CS  0.000000 164.7402 28.45714 104.6393 2.000893 7.558929  3.557143
## 6     CS  6.503226 341.5742 31.12258 296.0516 1.393548 0.000000  7.432258
## 7     CS 16.817021 114.1787 14.60426 187.3475 0.000000 5.753191 13.055319
```

```
## 8     CS  7.514286 199.5706 10.60840 108.2941 0.000000 1.768067  3.094118
## 9     CS  9.112963 238.4148 10.83704 187.1852 2.462963 3.940741  7.142593
## 10    CS 23.296970       NA 10.77172 253.1771 5.010101 3.006061  7.264646
## 11    CS 23.272727 130.1056 17.50996 198.5939 0.000000 6.870996 11.525541
## 12    CS 24.911111       NA 15.07778 312.0444 0.000000 0.000000 10.925926
## 13    CS 17.653846 222.8308 20.40000 446.4462 1.569231 0.000000        NA
## 14    CS  5.397015 257.9323 28.78408 495.6259 1.799005 2.698507 10.119403
## 15    CS 17.325000 202.9500 31.72500       NA 5.850000       NA 15.075000
## 16    CS 12.808333 168.4958 19.87500 392.4944 4.416667 4.858333  5.741667
## 17    CS  0.000000 187.5865 16.68919 376.6565 0.000000 0.000000 13.573874
## 18    CS  0.000000 248.0000       NA       NA       NA 0.000000  6.250000
## 19    CS        NA 352.7566 15.44126 243.7254 0.000000       NA  8.699301
## 20    CS  0.000000 211.2364 16.33434 290.2121 1.427273 0.000000 13.004040
## 21    CS        NA       NA       NA 268.4917 4.917431       NA 15.735780
## 22    CS        NA 171.7333 16.80000       NA       NA 2.333333  5.600000
## 23    CS  4.208108       NA 18.93649 295.0351 0.000000 0.000000 10.286486
```

Appendix 9 Data Summary

Participants / **Trace Elements**	**Non-Cancer Control Group (N = 31)**	**Esophageal Cancer Patients (N = 72)**	**Stomach Cancer Patients (N = 31)**
Chromium			
Mean	3.17	8.69	11.56
Median	1.30	8.58	10.8
IQR	0-4.059	0-13.0	5.1-17.41
Iron			
Mean	213	206	209
Median	203	190	203
IQR	167-264	151-190	170 -242
Copper			
Mean	28.1	19.8	18.3
Median	25.7	18.7	16.7
IQR	22.6-36.2	13.3-24.3	13.9-20.1
Zinc			
Mean	258	253	265
Median	263	240	261
IQR	188-337	188-303	187-313
Selenium			
Mean	1.06	1.91	1.70
Median	0.92	1.76	1.39
IQR	0-1.88	0-2.08	0-2.46
Mercury			
Mean	1.39	2.08	2.56
Median	1.15	1.42	2.62
IQR	0-2.63	0-3.5	0-3.8
Lead			
Mean	10.5	9.7	10.1
Median	9.48	9.41	10.2
IQR	6.38-13.46	6-14	6.47-13

Appendix 10 Unpaired t-test Results at 95% Confidence Interval

1. Control Vs Oesophagus

Trace Element	test statistic	df	p-value	CN Mean	CE Mean
Cr	-3.03	39.8	0.004	3.17	8.32
Cu	2.45	51.6	0.017	28.1	21.8
Fe	0.05	46.9	0.96	213	212
Zn	2.36	47.4	0.02	258	210
Se	-2.89	52.3	0.01	1.06	2.04
Hg	-1.58	46.6	0.12	1.39	2.24
Pb	0.97	43.5	0.34	10.5	9.02

2. Control Vs Stomach Cancer

Trace Element	test statistic	df	p-value	CN Mean	CE Mean
Cr	-3.65	26.5	0.001	3.59	11.6
Cu	3.24	29.6	0.003	27.1	18.3
Fe	0.26	31.5	0.79	215	209
Zn	-0.42	36.9	0.67	252	265
Se	-0.97	31.1	0.33	1.21	1.71
Hg	-1.72	32.3	0.09	1.37	2.56
Pb	-1.01	31.6	0.32	8.52	10.1

3. Oesophageal Vs Stomach Cancer

Trace Element	test statistic	df	p-value	CN Mean	CE Mean
Cr	-1.44	37.1	0.16	7.89	11.6
Cu	1.34	37.6	0.19	21.8	18.3
Fe	-0.35	34.1	0.73	201	209
Zn	-1.92	33.1	0.06	210	265
Se	0.42	36.1	0.68	1.94	1.71
Hg	-0.61	35.916	0.554	2.12	2.56
Pb	-0.71	41.7	0.48	9.06	10.1

Appendix 11 Ethics Approval

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Ref: KNH-ERC/A/296

8th September 2020

Owili Nancy Oluoch
Reg. No.S56/15216/2018
Institute of Nuclear Science and Technology
University of Nairobi

Dear Nancy

RESEARCH PROPOSAL – EVALUATION OF TRACE ELEMENTS' CONCENTRATIONS IN NAILS OF ESOPHAGEAL AND STOMACH CANCER PATIENTS: INVESTIGATION OF AN EARLY DIAGNOSTIC SCREENING METHOD IN KENYA (P124/ 02/2020)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 8th September 2020 – 7th September 2021.

This approval is subject to compliance with the following requirements:

a. Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b. All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c. Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e. Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g. Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

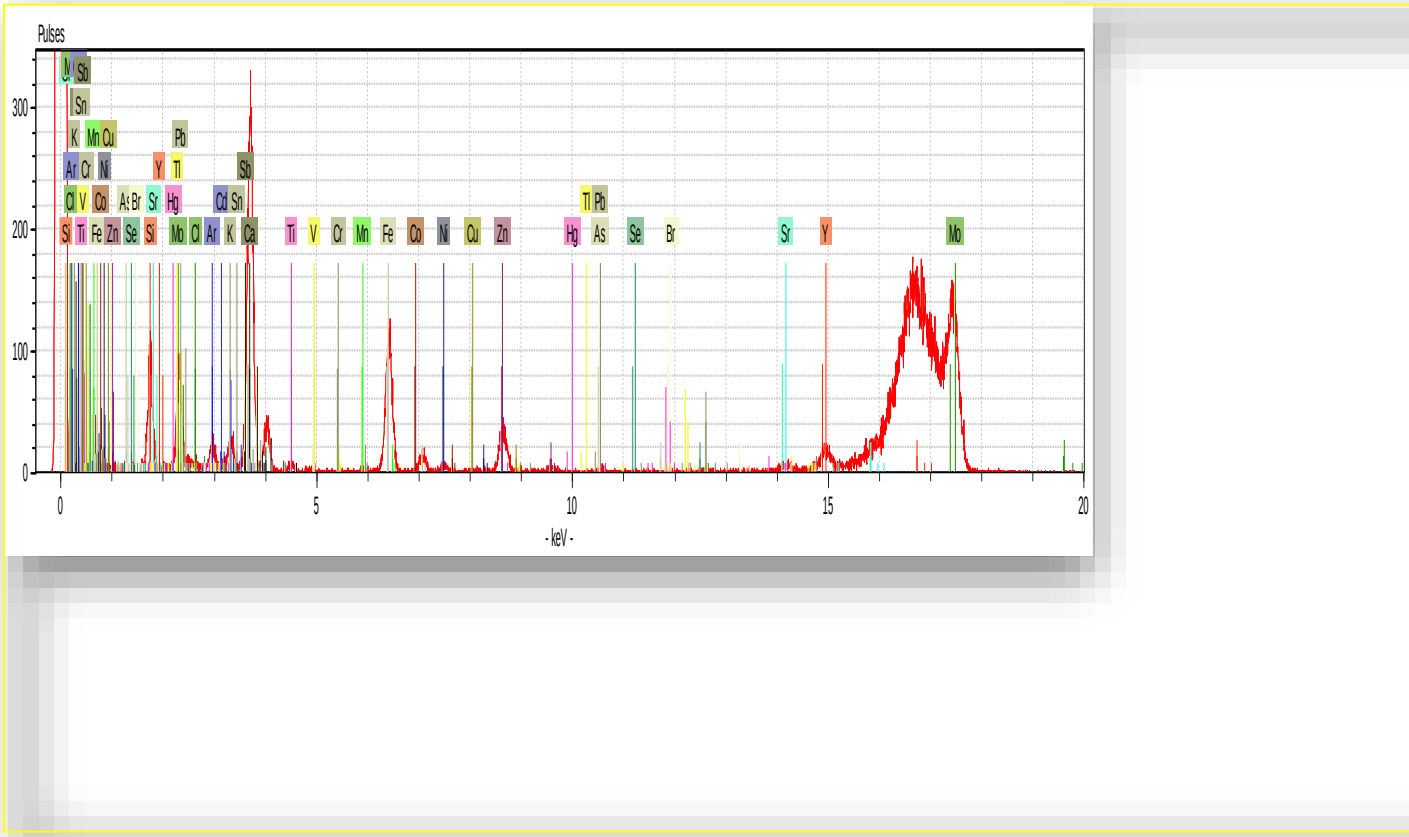
For more details consult the KNH- UoN ERC website**http://www.erc.uonbi.ac.ke**

Yours sincerely,

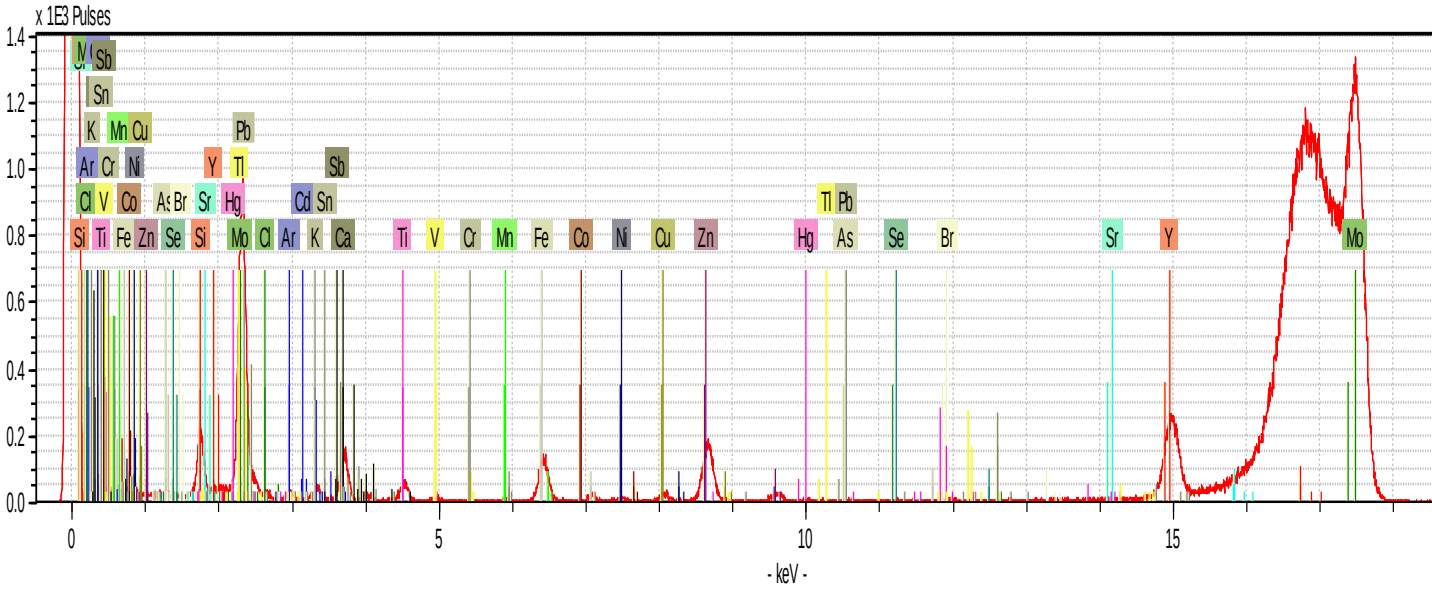
PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Senior Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information, KNH
The Director, Institute of Nuclear Science and Technology, UoN
Supervisors: Prof. Michael Gatari, Institute of Nuclear Science and Technology, UoN
Prof. David K. Kariuki, Dept. of Chemistry, UoN
Dr. Primus Ochieng, Kenyatta N. Hospital

Appendix 12 Sample Spectra of Esophagus Cancer Patient



Appendix 13 Sample Spectra of Stomach Cancer Patient



Appendix 14 Sample Spectra of Non-Cancer Patient