UNIVERSITY OF NAIROBI

FACULTY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF CHEMISTRY

ASSESSMENT OF COPPER, MANGANESE, POTASSIUM AND ZINC LEVELS IN TEA LEAVES AND THEIR INFUSIONS

BY

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DECLARATION

I declare that this Thesis is my original work and has not been submitted elsewhere for examination, award of degree or publication. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

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ABSTRACT

Macrominerals and microminerals serve as electrolytes, metabolic regulators and also have a structural function in the human body. Deficiency of essential elements may lead to a list of healthy complications which includes growth impairments, birth defects, reduced fertility, weak bones and affected metabolism of lipid, proteins and carbohydrates. Copper is an essential cofactor for collagen and extracellular matrix formation. Zinc plays a critical role in cell division, growth and protein synthesis hence it is important for infants and pregnant women. Delayed wound healing, impaired taste sensitivity; severe deficiency: retarded growth and sexual development and dwarfism are traded some of the signs of zinc deficiencies. Potassium is essential for normal muscular and digestive function, proper functioning of tissues and cells in the human body and also plays a crucial role in heart function, skeletal and smooth muscle contraction. Potassium deficiency in the human body can lead to electrolyte imbalance. Manganese which is a Cofactor of large number of enzymes acts as an antioxidant during normal brain function and animal reproduction. Tea infusions may be a rich source of some macrominerals and microminerals which are essential to human health. Though in trace amounts, these elements are known to play critical roles in various body mechanisms though too much of this elements may also be a health hazard. The tea plant is a perennial evergreen plant with three species i.e. *Camellia sinensis*, *Camellia assamica* and *Camellia Cambodiensis* which is believed to have been dispersed either naturally or by human agencies into tropical and subtropical regions. Tea leaf which is the raw material for tea infusions is composed of different chemical compounds which comprises of caffeine, catechins, volatile compounds, amino acids, trace elements, proteins, and minerals. The aim of this study was to determine the levels of manganese, potassium, zinc and copper elements in tea leaves. Black tea, green tea and purple tea which are the centre of this research are products of the leaves of *Camellia Sinensis*. During processing of black tea, tea leaves are fully oxidised before being dried. Green tea is a non- fermented product of the leaves from the tea plant and purple tea is a variety that is rich in some specific nutrients. In this study, 24 samples of tea leaves were randomly purchased from selected supermarkets, hawkers and shops in Nairobi Central Business District. The samples were analysed for total potassium, zinc, manganese and copper using Atomic Absorption Spectroscopy (AAS) and Inductively coupled plasma mass Spectrometry (ICP-MS) techniques. The data obtained was interpreted/analysed using the one-way analysis of variance (ANOVA) tool and Kruskal Wallis test under the Statistical Package (STATA) software. The levels of potassium ions in all the tea leaves and their respective tea infusions was significantly higher compared to the levels of manganese, copper and zinc. Levels of copper, manganese, potassium and zinc metal ions in the tea leaves ranged from 41.20 ± 12.47 µg/g to 48.50 ± 19.40 µg/g, 913.6 ± 471.95 µg/g to 1342.10 ± 310.26 µg/g, 12716.30 ± 2541.56 µg/g to 12837.90 ± 3517.60 µg/g and from 111.10 ± 29.98 µg/g to 116.60 ± 20.25 µg/g respectively. Levels of copper, manganese, potassium and zinc metal ions in the tea infusions ranged from 15.1 ± 4.1 µg/g to 43.3 ± 19.9 µg/g, 231.7 ± 3.8 µg/g to 938.2 ± 96.4 µg/g, 5715.4 ± 64.2 µg/g to 16628.7 ± 246.2 µg/g and from 50.6 ± 8.5 µg/g to 144.2 ± 5.9 µg/g respectively. The levels of metal elements in the tea leaves and tea infusions analyzed arranged in the following order: K>Mn>Zn>Cu hence all the three types of tea leaves are reliable sources of potassium and manganese. There was a significant statistical difference in the manganese levels in the three types of tea.

DEDICATION

I dedicate this work to the employees of Government Chemist Department Nairobi. I recognize their endless support and encouragement during the period of my study.

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LIST OF ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectroscopy
CAN	Calcium Ammonium Nitrate
DAP	Diammonium Phosphate
FAO	Food and Agriculture organisation
ICP-MS	Inductively coupled plasma mass spectrometry
KEBS	Kenya Bureau of Standards
NPK	Nitrogen, Phosphorus Potassium
SSP	Single Super Phosphate
WHO	World Health Organisation

UNITS OF MEASUREMENT

g	Gram
mg/Kg	milligram per Kilogram
mL	Millilitre
µg/g	microgram per gram
ppm	Parts per million

CHAPTER ONE

INTRODUCTION

1.1: Background of the study

United Nations has set an agenda of transforming the world by the year 2030 and in order to achieve this, agenda sustainable development goals have been set (Walker *et al*., 2019). Good health and well-being for all is one of the goals so as to ensure a productive work force. Public health experts have realized that micronutrient malnutrition is the cause of many non-specific physiological impairments, leading to low immunity and metabolism difficulties. Foods products processed from plants contain almost all the mineral nutrients required for numerous body processes in humans (Organization and FAO, 2004). Micronutrient malnutrition has been identified as a leading contributor to the global burden of disease. Ensuring good health and promotion of well-being is required in enabling sustainable development all over the world. Recently, nutritional science has not only focused on the discovery of vitamins but it has also focused on description of mineral deficiency diseases and as a result there has been efforts to fortify foods that are commonly consumed by humans with vitamins and minerals (Zimmermann, 2011). There are two major categories of minerals i.e. macro minerals which are also known as major minerals; examples include magnesium, nitrogen, potassium, sulphur, calcium and sodium and micro minerals also known as trace minerals, examples consisting of boron, copper, iron, iodine, manganese, and zinc (Medeiros and Wildman, 2013). With an aim of ensuring that the food consumed by the world population meets the dietary requirements for specific micronutrients, product specifications have been established with guidance from FAO (Ottaway, 2008) and WHO (Fortin, 2016).

The African Union's Agenda 2063 recognises technical solutions in healthcare as one of key requirements for its realization (Vickers, 2017). Nutritive levels of mostly consumed foods in the African region is an information that the public health experts require in the process of policy development. Tea is consumed by two-thirds of the people in the world as a refreshing healthy drink (Khan & Mukhtar, 2013). Originally tea consumers used to chop the tea leaves as vegetable, chewed the leaves but eventually learned how to prepare a drink by extracting nutrients from the leaves using boiling water (Driem, 1957). Tea drinking is a constituent part of Chinese culture and in some countries tea is drunk in social events. Though there are various legends in relation to where tea originated, tea is believed to have been discovered by Shen Nung in 2737 BC who was an expositor of curative properties of plants. Tea leaves are still used in traditional Chinese medicine in China. According to the legend, a tea leaf which was hanging from a wild tea tree fell into the water which Shen Nung was boiling in a pot in the garden. The leave coloured the water brown but Shen Nung went ahead and drunk the accidentally prepared drink. He was surprised when he discovered that the drink had pleasing flavour and aroma (Weinberg *et al*., 2001). The many different kinds of tea products throughout the world depend on the variety of the tea plant from which the unprocessed tea leaves are harvested from, the curing and processing of the leaves, and the grade of the leaves (Ho *et al*., 2008). In India, tea remains the beverage of choice for most people across all walks of life.

1.2: The origin of the tea plant

Tea is believed to have dispersed either naturally or by human agencies into tropical and subtropical parts, covering tropical rain forest, tropical savannah and summer rain areas (Han *et al*., 2021). Tea is usually grown in acidic soils with pH values ranging between 5.00 and 5.60. The acidic conditions of the soils favours the solubility/availability of most metals ions (Zhen, 2002). Tea leaves contains elements Ca, k, mg, F, Al and Mn in levels of milligram per gram. Tea leaves contain Cr, Fe, Co, Ni, Zn, Cd, Pb, As and Hg in levels of nanograms per gram ((Guardia and Garrigues, 2015). Tea plant is always green and remains productive throughout the year. The tree has three species i.e. *Camellia sinensis*, *Camellia assamica* and *Cambodiensis* (Driem, 2019). The different tea leaves for human consumption are produced from the buds and young leaves of *Camellia sinensis* species. The plant is indigenous throughout the forests of south-east Asia and though the tea plant is maintained as a shrub, it has the potential of developing into a tree between 30 and 40 feet (Willson and Clifford, 2012). As a practice, tea plant is pruned and maintained at a height of about 3-5 feet in order to make tea picking easier and increase the yield of the plant. The soils on which tea grows also vary widely hence tea has a wide range of distribution. Though tea has ability to grow under relatively diverse set of environmental conditions, it’s cultivation requires certain definite criteria for optimal productivity (Ahuja *et al*., 2013).

1.3 Tea infusion

Tea drink is the infusion of the leaves of *Camellia Sinensis* and it is undoubtedly among the best-loved drinks, it is known to have a unique aroma and flavour. It is the oldest non-alcoholic beverage in the world (Kurian and Peter, 2007). Tea infusions are thought to contain a mixture of organic and inorganic compounds (Justino, 2020). It has been established that tea infusions contain volatile products, flavonoids, proteins, carbohydrates, alkaloids, trace elements, minerals and other compounds which are yet to be identified (Guardia and Garrigues, 2015).

Tea leaves manufacturers use young tea leaves which are composed of two leaves and a bud to produce commercial tea. Trace elements distribution in various types of commercial teas from around the world are highly variable. Tea has been recognized as a useful source of some essential elements in the human diet, hence moderate consumption of tea is unlikely to have any harmful effects to healthy humans (Preedy, 2013).

Most of the time tea consumers are after the stimulating effect of tea but are rarely interested with the nutritional value of tea especially the minerals which are equally of importance (Driem, 2019).

1.4: Food fortification and supplementation

Activities of food fortification and supplementation has existed since 1824 when native Indians of South America and Columbia used to treat goitre using a particular type of salt. It was later established that the salt contained high levels of iodine ions (Ottaway, 2008). The objective of fortification programs is to raise the level of micronutrients foods that are mostly consumed by many people. The exercise can involve fortification of several foods or fortification of a single element e.g. the iodization of table salt. Vulnerable population groups mostly in developing countries may not be in a position to access food that meet all the micronutrients requirements (WHO and FAO, 2004). Though cereals are poor dietary sources of micronutrients, they are usually fortified with minerals like iron, copper, manganese and zinc (Szefer and Nriagu, 2006). Food fortification play important role in developing countries in helping them to achieve their health policies and ensure the nutritional health of their citizens (Ottaway, 2008).

1.5: Counties growing tea in Kenya

Tea is currently grown in many countries in the world including China, India, Kenya, Sri lanka, Turkey and Vietnam (Christenhusz *et al.*, 2017). Originally tea in China was consumed as a medicinal drink, hence the Chinese regarded tea as an integral part of their health and well-being (Jain and Priyadarshan, 2008). China produces the largest amounts of tea in comparison to other countries in the world. India comes second after China followed by Kenya. In Kenya small scale holder's tea accounts for two-thirds of Kenya's crop and the other fraction is grown on the large estates (Driem, 2019). The small scale tea growers cultivate tea on smallholdings of about 10-12 hectares. Kenya Tea Growers' Association (KTGA) is an organisation formed by several large plantations tea farmers and they provide a significant amount of tea. Nandi, Kericho and the Great Rift Valley; Mt Kenya, the Nyambene Hills and the Aberdares in the Central Kenya; the Cherangani Hills and the Mau escarpment are areas where the tea plantations are situated. Counties where tea is grown lying in the East of Rift Valley include; Kilifi, Laikipia, Nakuru, Nyeri, Kiambu, Meru, Murang'a, Kirinyaga and Embu. Those in the West of the Rift Valley include; Kericho, Nandi, Trans-Nzoia, Kakamega and Kisii (Ali, 1997).

1.6: Fertilizers used in tea production

Just like other plants, tea plants require nutrients for growth. Required nutrients include primary plant food elements, secondary plant food elements and micronutrients (Han *et al*., 2018). The tea plant requires potassium, phosphorus and nitrogen (NPK) as nutrients for growth. The tea plant also requires zinc, calcium, sulphur, magnesium, and other micronutrients (Ahuja *et al*., 2013). Fertilizers are some of the sources of the elements and hence most tea farmers apply fertilizers so as to raise tea production *(*Han *et al*., 2018). Such fertilizers include, Calcium ammonium Nitrate (CAN), NPK, single super phosphate (SSP) and diammonium phosphate (DAP) (Willson and Clifford, 2012). Use of fertilizers though continually practised by most tea growers raises cost of production but most farmers have no choice than to hope that the extra costs in production will be offset by the profits they make (Han *et al.*, 2018).

1.7: Diseases affecting tea production

There are several diseases which usually affect the roots, stems or even leaves of the tea plant, these include; Blister Blight, Red Rust, *Armillaria* root rot diseases. Management of these diseases by use of chemicals is practiced in order to ensure that high yields are obtained (Rangaswami and Mahadevan, 1998). In controlling Blister Blight disease, the affected plants are sprayed with fungicides such as Cuprous Oxide or Copper Oxychloride (Gour and Purohit, 2004). Red Rust is also a disease known to appear in the form of minute rusty spots on the leaves of the tea plant (Driem, 2019). Through studies, it has been established that healthy leaves of the plant contain more nitrogen and potassium carbonate in relation to infected leaves (Mehrotra, 2013). In this regard, application of potassium carbonate, foliar spray with Copper hydroxide and Carbendazin are the methods mostly used to control the disease (Mehrotra, 2013). The tea plant is also affected by a root and stump disease known as *Armillaria* root rot disease (Ahuja *et al*., 2013). Once diagnosed, it is advisable to remove the stumps of infected trees followed by fumigation of the tea fields with Chloropicrin, carbon disulphide, and methyl bromide (Han *et al.*, 2018).

1.8: Pest affecting tea production

The tea plant can be infested by different types of pests e.g. anthropods, including insects and mites (Hall, 2000). The eriophyid mites, *Calacarus carinatus* and Acaphylla theae and the tenuipalpid, *Brevipalpus phoenicis* usually infest the tea plants at the same time (Willson and Clifford, 2012)**.** Red rust which is an algal disease is another threat to the tea plant attacking the leaves of the plant and mostly appears in the form of minute rusty spots on the leaves. The pathogen which causes this disease in tea is *Cephaleuros Parasiticus Karst* (Mehrotra, 2013). Factors favouring the algae include inadequate shade, waterlogging, drought, poor fertility and lack of aeration of the soil (Mehrotra, 2013).

1.9: Statement of the problem

Tea infusions may be a rich source of some macrominerals and microminerals which are essential to human health. Though in trace amounts, these elements are known to play critical roles in various body mechanisms though too much of this elements may also be a health hazard. Consumption of herbal infusions has greatly increased in the recent years due to their alleged health benefits. Tea infusion is an example of these herbal infusions and is consumed by a large part of the Kenya's population. The infusion contain volatile products, flavonoids, proteins, carbohydrates, alkaloids, trace elements, major minerals and compounds which are yet to be identified as stipulated by Guardia and Garrigues, (2015). Most of these components play critical role in human health hence they are recognized as essential by various researchers (Ho *et al.*, 2008).

Deficiency of essential elements may lead to a list of healthy complications which includes growth impairments, birth defects, reduced fertility, weak bones and affected metabolism of lipid, proteins and carbohydrates (Erkekoglu and Kocer-Gumusel, 2016). In some cases, supplement consumption is usually recommended to people having deficiencies in these major nutrients. The aim of this study was to determine the levels of manganese, potassium, zinc and copper ions in tea leaves. These metal ions are required in small amounts by humans and too much of these ions is also a health hazard (Reilly, 2008). The study was important considering the increasing habit of consuming tea infusions as a non-alcoholic drink among most of the population so as to determine the safety and the nutritional value of the beverage.

1.10: General objective of the study

The general objective of the study was to assess levels of copper, manganese, potassium and zinc elements in different types of tea leaves and tea infusions.

1.10.1: Specific objectives

The specific objectives of the study were to:

i. Determine levels of copper, manganese, potassium and zinc elements levels in black, green and purple tea leaves.

ii. Determine levels of metal nutrients; copper, manganese, potassium and zinc elements in black, green and purple tea infusions.

iii. Compare the levels of copper, manganese, potassium and zinc elements in black, green and purple tea leaves.

1.11: Justification of the study

Though in trace amounts, some trace elements are known to play critical roles in various body mechanisms though too much of this ions may also be a health hazard. Examples of major inorganic nutrients elements that have been detected in tea leaves include iron, copper, zinc, potassium, sodium, manganese, nickel, and magnesium (Justino, 2020). The findings of this research will inform policy makers, Public health experts, the academia, food traders and consumers of tea products.

1.12: Significance of the study

Though some elements found in tea infusions play critical roles in various body mechanisms, Countries, regions and health organisations have specified levels of most elements in food products (Fortin, 2016). This is meant to ensure safety of the consumer hence traders are expected to ensure that the foods they trade in meet the set specifications. This study therefore sought to determine whether the levels of potassium, copper, manganese and zinc in packed tea leaves marketed in Nairobi County meets the regulators specifications in the tested parameters.

CHAPTER TWO
LITERATURE REVIEW

2.1: Sources of potassium, zinc, copper and manganese

Most foods consumed by humans contain the amount of potassium required by the human body and it has been established that fresh fruits and vegetables, bananas, meat and milk are possible sources of potassium (Roza, 2007). Other foods found to contain potassium include; pork loin, sea food, cow milk, processed dairy products and honey (Szefer and Nriagu, 2006). Zinc is required by human body in small amounts hence it is an example of a micromineral. Zinc plays a critical role in the human body systems and is present in most body tissues and fluids. Commonly consumed foods which include; meat, peanuts, beans, cheese and eggs contain zinc in different amounts (Gray, 2006). Foods which contain high concentrations of zinc i.e. about 25-50 mg/Kg include, whole grain cereals, pulses, lean red meat and legumes (WHO, 2004; FAO, 2004).

Most food products derived from animals contain low concentrations of manganese; in the range of 0.003 to 0.140 mg/100g though crab meat is known to have levels of manganese of up to 8.190 mg/100g (Szefer and Nriagu, 2006). It has also been established that though food of plant origin contain low levels of manganese as compared to food derived from animals, tea has levels of manganese of up to 139 mg per 100 g in tea leaves (Szefer and Nriagu, 2006). Other sources of manganese include; avocados, nuts, green vegetables and grain products (Reilly, 2008). Copper is widely distributed in different kinds of animal foods in varying levels. Examples of foods which contain copper in considerable levels include; mammalian liver, offal, kidney and liver of hens. Milk from humans, cows, goats and sheep contain copper though at low levels (Szefer and Nriagu, 2006). Whole grain cereals, legumes, cocoa, shellfish and nuts are other types of food that have also been identified as possible sources of copper though in low concentrations (Wilson, 2009).

Tea has a complex chemical composition of both essential macroelements and microelements in varying levels. Some of the nutrients found in tea leaves include polyphenols, caffeine, amino acids, vitamins, carbohydrates and a group of inorganic elements (Yamamoto *et al*., 1997); (Bidlack *et al*., 2000). Examples of major inorganic nutrients elements that have been detected in tea leaves include iron, copper, zinc, potassium, sodium, manganese, nickel, and aluminium (Guardia and Garrigues, 2015). The ability of the tea plant to take up elements from the soil contributes greatly to the content of these elements in the leaves. According to the contents of iron, manganese, zinc and copper depends on age of the leaf harvested (Ahuja *et al*., 2013).

2.2: Macrominerals and microminerals

Essential minerals are grouped into two namely macrominerals and microminerals. Macrominerals are elements which the human body require in amounts greater than 100 milligrams per day (100 mg/day) while microminerals are required in amounts of less than 15 mg/day (Zohoori and Duckworth, 2019). These minerals serve as electrolytes, metabolic regulators and also have a structural function. Sodium, potassium, chloride, calcium, phosphorus and magnesium are examples of macrominerals (Berdanier *et al*., 2008). Copper, iron, iodine, zinc, selenium and manganese are examples of trace minerals (Medeiros and Wildman, 2013). With an aim of ensuring that the food consumed by the world population meets the dietary requirements for specific micronutrients, product specifications have been established with guidance from FAO (Ottaway, 2008)and WHO (Fortin, 2016).

2.3: Major minerals and trace minerals

Minerals are inorganic substances and they are divided into two general categories i.e. major minerals and trace minerals (Medeiros and Wildman, 2013).

2.3.1: Trace minerals

Essential microelements are a group of nutrients that are required in small amounts by the body; examples include selenium, iron, zinc, copper, fluorine, cobalt, manganese, nickel, iodide, boron, and chromium (Szefer and Nriagu, 2006).

2.3.2: Major minerals

The estimated average daily dietary needed for major minerals is 100 milligrams (Wildman and Medeiros, 1999). Presence of metals in foods may have toxicological or nutritional significance. Determination of inorganic components in foods is done by spectrophotometric methods including atomic absorption spectrometry (AAS) using a flame or electrothermal device for atomization, and atomic emission spectrometry (AES) using a flame or inductively coupled plasma technique with an appropriate detector.

According to a codex standard i.e. Codex stan193-1995, a contaminant is defined as a substance that is not purposely added to a food product but is present in such food as a result of the production process or as a result of environmental contamination.

Determining the levels of minerals present in food is important for food safety, trade and nutritional considerations. Macroelements or macrominerals are minerals that occur in relatively high amounts and are required in quantities of about 100 mg per day. Potassium is an example of a macromineral. Tea is known to contain a variety of phytochemicals and phytonutrients and tea infusion is a rich source of minerals (Yamamoto *et al*., 1997).

2.4: Inorganic nutrients

2.4.1: Copper (Cu)

Copper (atomic number 29) and is distributed in nature as sulphide ores, chalcocite, covellite, chalcopyrite, cuprite, malachite and azurite (Beatty, 2001). Copper is required for bone formation, connective tissue development and tissue pigmentation. Along with other micronutrients such as iron and zinc, copper is important to the health of the body from foetal growth through to old age (Gropper *et al.,* 2016). Copper is also an essential cofactor for collagen and extracellular matrix formation.

2.4.2: Manganese (Mn)

Manganese is known to activate certain metalloenzymes and is an important nutrient for various metabolic functions involved in human development (Klimis-Zacas, 1993). Manganese which is a Cofactor of large number of enzymes acts as an antioxidant during normal brain function and animal reproduction. Inadequate daily intake of manganese can lead to reduced fertility, birth abnormalities, impaired bone formation, modified metabolism of proteins and generalized growth impairments are some of health repercussions which are caused by inadequate daily intake of manganese (Avila *et al*., 2013).

2.4.3: Potassium (K)

Potassium is ranked seventh most abundant in earth’s crust (McKinney, 2018). Naturally occurring potassium compounds occurs as crystals called field spars and micas (Garrett, 2012). In most plants tissues, potassium is almost exclusively present in the ionic form and only a very small portion of potassium is bound by organic ligands (Pandey & Mahiwal, 2020). Potassium is an important ion in the human body and its deficiency can lead to electrolyte imbalance (Nieder *et al*., 2018). The large amount of potassium (17.70–24.80 mg/g) found in tea leaves is beneficial to hypertensive individuals hence tea infusions can supplement diets with inadequate potassium. Potassium is essential for normal muscular and digestive function, proper functioning of tissues and cells in the human body and also plays a crucial role in heart function, skeletal and smooth muscle contraction.

2.4.4: Zinc (Zn)

Zinc occurs naturally as Zinc sulphide (sphalerite) which is the most common zinc mineral (Lew, 2008). Zinc is known to promote human health in that it plays various roles which includes catalytic , structural and regulatory (Driskell, 2009). Zinc is constituent of many enzyme systems, carbon dioxide transport and vitamin A utilization. Zinc is needed for healthy skin and bones; it also plays an important role in a healthy immune system. Zinc deficiency is accountable for physical and intellectual retardation, preventing children from developing to their full potential (Erkekoglu and Kocer-Gumusel, 2016).

Some of the foods which are mostly consumed e.g. roots and tubers, fish, green leafy vegetables and fruits only contain low concentrations of zinc in the range of less than 10 mg/kg (WHO and FAO, 2004). Zinc plays a critical role in cell division, growth and protein synthesis hence it is important for infants and pregnant women. Delayed wound healing, impaired taste sensitivity; severe deficiency: retarded growth and sexual development and dwarfism are traded some of the signs of zinc deficiencies. Effects of zinc deficiency such as retarded growth and development can be addressed by initiating zinc fortification programmes and zinc supplementation (Ottaway, 2008). Zinc fortification programmes are being studied, especially for populations that consume predominately plant foods. An amount of 45 mg/day is the upper level of zinc intake for an adult man. Taking higher levels of zinc than required can interfere with the activity of copper in the body. After ingestion of 4-8 g of zinc, toxicity signs such as vomiting, fever, nausea, lethargy and diarrhoea have been observed (WHO and FAO, 2004). National and international food regulators set maximum levels of contaminants in foods so as to ensure adequate consumer protection.

In the tea market, tea quality is usually based on composition of tea infusions produced from tea products which have been placed in the market for purchasing. Professional quality assessors determine the quality of tea by use of eyes, nose, and tongue approximations but this method is exposed to biasness, inaccuracy and variability (Mason *et al*., 2013). Though these methods of analysing tea may still be reliable to some extent, employing a more accurate method and reliable method is expected to give findings which are more acceptable. Though the cations in tea infusions may be of great importance at some levels, but at certain levels the elements may be a health hazard hence there was need to determine their levels in tea infusions.

2.5: Tea production and processing

The tea plant is currently cultivated in many countries around the world. Propagation of the tea plant can be either by seed or cuttings. Tea processing involves withering, rolling, fermentation and drying of the plucked tea leaves. The chemical components in the tea leaves go through chemical reactions during processing which leads to change in their original chemical composition (Sarkar and Nout, 2014). Tea producers have been able to manufacture different types of tea products in relation to the demand in the world market by using different processing procedures despite them using the same raw material. Tea products can be grouped into three i.e. non-fermented green tea, the partially fermented tea products and the fully fermented black tea. The three categories are possible sources of a number of elements which are required by the body for various body mechanisms. All the three types are produced from young leaves of the tea plant (*Camellia Sinensis*). The three main products of tea have distinct qualities and appearance (Ho *et al*., 2008). The quality of tea is determined by a large number of factors which includes temperature, rainfall, humidity, type of leaves, altitude and soil conditions.

Most consumers of tea infusion believe that it possesses refreshing, stimulating and medicinal properties. Tea products can be grouped into four main types i.e. green, white, black(red) and oolong. These products have been used for preparation of different tea infusions worldwide (Hall, 2000). Tea infusion may be a rich source of some minerals and trace elements which are essential to human health. Tea leaves infusion have been consumed from the ancient of age for their specific aroma and taste. The tea plant, *Camellia sinensis* is an example of plants that has the ability of taking up metal ions from the soil and accumulating the metals ions in it’s leaves and stems (Mehrotra, 2013).

2.6: Tea types

There are three aspects that determine the tea quality i.e. the variety of the tea plant, the environment in which it grows, and how the leaves are processed. The method of processing is the key element that determines the tea quality (Chen *et al*., 2013). Traditionally, there are six different tea types i.e. black (red), green tea, white tea, dark tea, yellow tea, and oolong tea. Green and black are the most popular types of tea. Green tea is a non-fermented product and hence most of the properties of the raw materials are retained (Muirhead, 2008).

2.6.1: Black tea

Black tea is a product of fermentation of the leaves of *Camellia Sinensis* plant. In order to obtain black tea, oxidation of the tea leaves is fully accomplished before drying. During the fermentation process, enzymic oxidation takes place which leads to colour change and the development of the desired flavour (Sarkar and Nout, 2014). Though the aim of the fermentation process may be to achieve specific characteristics in the final product, the composition of elements in the final product is different from a product that is partially fermented or not fermented at all and this results in different compositional levels of most components in the different tea categories.

2.6.2: Green tea

Green tea is a non-fermented tea product and its production involves harvesting, withering and steaming. The tea leaves are slightly steamed after the harvest to prevent oxidation (Ho *et al*., 2008). Steaming deactivates an enzyme that is responsible for oxidation and the green colour of the leaves is maintained. The leaves are then shaped into different forms by use of shaking or rolling machines after which they are dried. Green tea has more health benefits compared to black tea (Muirhead, 2008). It owes much of its benefits to the method used when steaming the flesh tea leaves (Muirhead, 2008). Fermentation products are expected to be part of the composition of tea infusions but since green tea is non-fermented, most of the components found occurring naturally in tea leaves are expected to be present in green tea making this type of tea to be preferred by a group of tea consumers. Japan, China, Taiwan and Indonesia are known consumers of green tea (Jain and Priyadarshan, 2008).

2.6.3: Purple tea

Purple tea is both drought and frost-resistant and highly yielding (Battle, 2017). Purple tea variety targets new tea products diversification and value addition and its production is expected to lead to economic growth. It is expected that since purple tea is a variety that is rich in some specific nutrients, levels of other elements are affected to some extent making this type of tea to have a composition of elements which is different from other types (Al-Khayri *et al*., 2019). Together with other components, tea has been found to contain minerals which include Boron(B), Aluminium (AL), Copper (Cu), Manganese (Mn), Sodium (Na), Phosphorous (P) and potassium (Hall, 2000).

2.7: Problems affecting tea production

2.7.1: High cost of production

Tea production is currently faced with various challenges which includes but not limited to poor infrastructure, high cost of production, inadequate research, incomplete value addition, competition from similar products, and reduction of global tea prices (Chen *et al.*, 2013).

2.7.2: Diseases affecting tea plants

Reduction of quality and quantity of the tea product due to infestation of insect pests and diseases is one of the problems facing farmers. Diseases affecting the tea plant can be classified as stem and branch diseases, leaf diseases and root diseases. Blister blight is the most serious disease which affects the buds and young leaves and buds of the *Camellia Sinensis* plant (Pettigrew, 2014). It is a fungal disease caused by *Exobasidium vexans Massee* and manifests itself in the form of small, pale or pinkish, round spots on the young buds and leaves (Rangaswami and Mahadevan, 1998). Red rust which is an algal disease is another threat to the tea plant attacking the leaves of the plant and mostly appears in the form of minute rusty spots on the leaves. The pathogen which causes this disease in tea is *Cephaleuros Parasiticus Karst* (Mehrotra, 2013). Factors favouring the algae include inadequate shade, waterlogging, drought, poor fertility and lack of aeration of the soil (Mehrotra, 2013). *Armillaria* root rot disease which is severe in Africa is of great concern to tea growers since it is among the most challenging factors in tea growing areas (Sharma and Sharma, 2020). The disease is caused by *Armillaria mellea* fungus which spreads and grow in old stumps forming rhizomorphs which also grow through the soil and infect the roots of new tea plants (Willson and Clifford, 2012).

2.8: Spectroscopic methods for metal determination

It is assumed that electromagnetic radiation or light is made of particles known as photons. Based on the type of molecular or atomic transition, the electromagnetic radiation is divided into different regions. Atomic absorption spectroscopy, atomic emission spectroscopy and fluorescence spectroscopy are analytical techniques or instrumental methods that are based on absorption, emission and fluorescence phenomena, respectively in the visible and ultraviolet regions.

2.8.1: Atomic absorption spectroscopy (AAS)

AAS technique is based on electronic transitions between electronic energy levels of atoms. The technique involves absorption of electromagnetic radiation by atoms of a sample which are at the ground state. When using the flame as the method of atomisation, the sample is held by the flame as the light passes through the atoms and the flame simultaneously (Harvey, 2000). The information about the sample is obtained by ascertaining the radiation released as the excited atoms revert to the ground state. During the interaction of the atoms and the photon, the energy carried by the photon is absorbed by the atom, promoting the valence electron to an excited state (Lajunen *et al*., 2004). Most of the gaseous metal atoms normally remains in the ground state hence they absorbed electromagnetic radiation of their own specific resonance wavelength when they interacted with light (Lajunen *et al*., 2004).

The absorbing atom do not absorb the whole range of the electromagnetic radiation, hence some of the radiation is transmitted; the level of absorption is proportional to the number of atoms in the ground state present in the flame hence the amount of the energy absorbed is directly proportional to the concentration of the absorbing atoms in the sample. Quantitative measurements in atomic absorption are based on Beer's Law, which states that concentration is proportional to absorbance ($C = kA$). Figure 2.1 shows ContrAA 700 High Resolution Continuum Source (AAS) which was used for the analysis of the metal ions. Atomic Absorption spectroscopy technique is suitable for determining levels of the metallic ions in various products. The technique which is very reliable, sensitive and simple is based on the fact that vaporised atoms can absorb electromagnetic radiation at specific wavelength (Kerio *et al.,* 2012).

When using the graphite furnace as the means of atomization, for the sample injection occurs in the cylindrical graphite tube that is open at both ends and that has a central hole for introduction of sample by means of a micro-pipette. The graphite tube fits closely into a pair of cylindrical graphite electrical contacts located at the two ends of the tube. Two inert gas streams are provided. The external stream prevents outside air from entering and incinerating the tube. The internal stream flows into the two ends of the tube and out the central sample port. This stream not only excludes air but also serves to carry away vapours generated from the sample matrix during the first two heating stages. When the tube temperature is raised rapidly, atomization is delayed since the sample is no longer in contact directly with furnace wall. As a result, atomization occur in the environment in which temperature is not changing rapidly as in other atomizers, hence the resulting signals are more reproducible.

Figure 2.1: ContrAA 700 High Resolution Continuum Source (AAS) machine

2.8.1.1: Detector

The detector technology is based on charged coupled device (CCD) chip. The charged coupled device is capable of turning light photons into an electrical signal. A CCD is sensitive to very low light levels and has a quantum efficiency of about 70%. The photons free electrons and so the more photons land, the more electrons are released. The greater the number of pixels, the better the resolution. A photon of light which falls within the area defined by one of the pixels will be converted into one or more electrons and the number of electrons will be directly proportional to the intensity. A CCD consists of millions of tiny photosites which generate and store charge when photons land on them (Janesick, 2001).

2.8.1.2: Radiation Sources

All forms of spectroscopy require a source of energy and in absorption spectroscopy this energy is supplied by photons. There are two types of sources of radiation which are suitable in spectroscopic studies i.e. continuum and line sources. Line sources emit limited number of lines or bands of radiation each of which spans a limited range of wavelengths while continuum sources emit radiation that changes in intensity smoothly as a function of wavelength.

2.9: Inductively coupled plasma mass spectrometry

Inductively coupled plasma mass spectrometry (ICP-MS) is a type of mass spectrometry technique employed in analysis of samples/elements that can easily form positively charged ions. The main components in ICP-MS instrument includes a sampler, nebulizer, spray chamber, plasma torch, quadrupole and detector. The liquid sample is converted into an aerosol by the nebulizer (Thomas, 2013). Within the plasma torch, the aerosol is dried, decomposed, atomised and finally ionised. ICP-MS instrument requires argon gas as cooling gas as well as to generate the inductively coupled plasma. Mass separation takes place in the quadrupole on the basis of mass-to-charge ratio. The ICP source operates at atmospheric pressure while quadrupole and detector require a high vacuum for optimum performance. Each element has its own characteristic isotopes and masses and will therefore produce its own mass spectrum.

The ICP source operates at atmospheric pressure while mass analyzer and detector require a high vacuum for optimum performance. The iCAP Q ICP-MS instrument requires argon gas as cooling gas as well as to generate the inductively coupled plasma and for controlling internal functions with the aid of pneumatics. The sample aerosol is generated by the nebulizer, in the spray chamber, larger aerosol droplets are filtered out into the drain while smaller droplets are carried into the plasma torch. The generated ions are then accelerated to the Right Angle Positive Ion Deflection lens, where they are deflected (Amarowicz, 2021). The neutral particles from the plasma pass directly out of the lens. The ions are then introduced into the quadrupole mass analyzer, where they are filtered out in relation to their specific mass to charge ratio (m/z).

CHAPTER THREE

MATERIALS AND METHODS

3.1: Study area

The study area is located in Nairobi County in Kenya. Tea samples were sampled using the stratified random method (Greenfield & Southgate, 2003). The subgroups were black, green and purple tea leaves. Representative samples were acquired by purchasing different brands from various shopping sites as shown in figure 3.1. Whole sale and retail markets are efficient marketing infrastructure for various food products including tea. Tea consumers purchase the tea products from the supermarkets, shops and hawkers, hence sampling from various supermarkets, shops and hawkers within the county gave reliable information on the levels of copper, manganese, potassium and zinc ions in the tea infusions consumed by the people in the county. Green, black and purple tea leaves were purchased in various sites in Nairobi County as shown in Table 3.1.

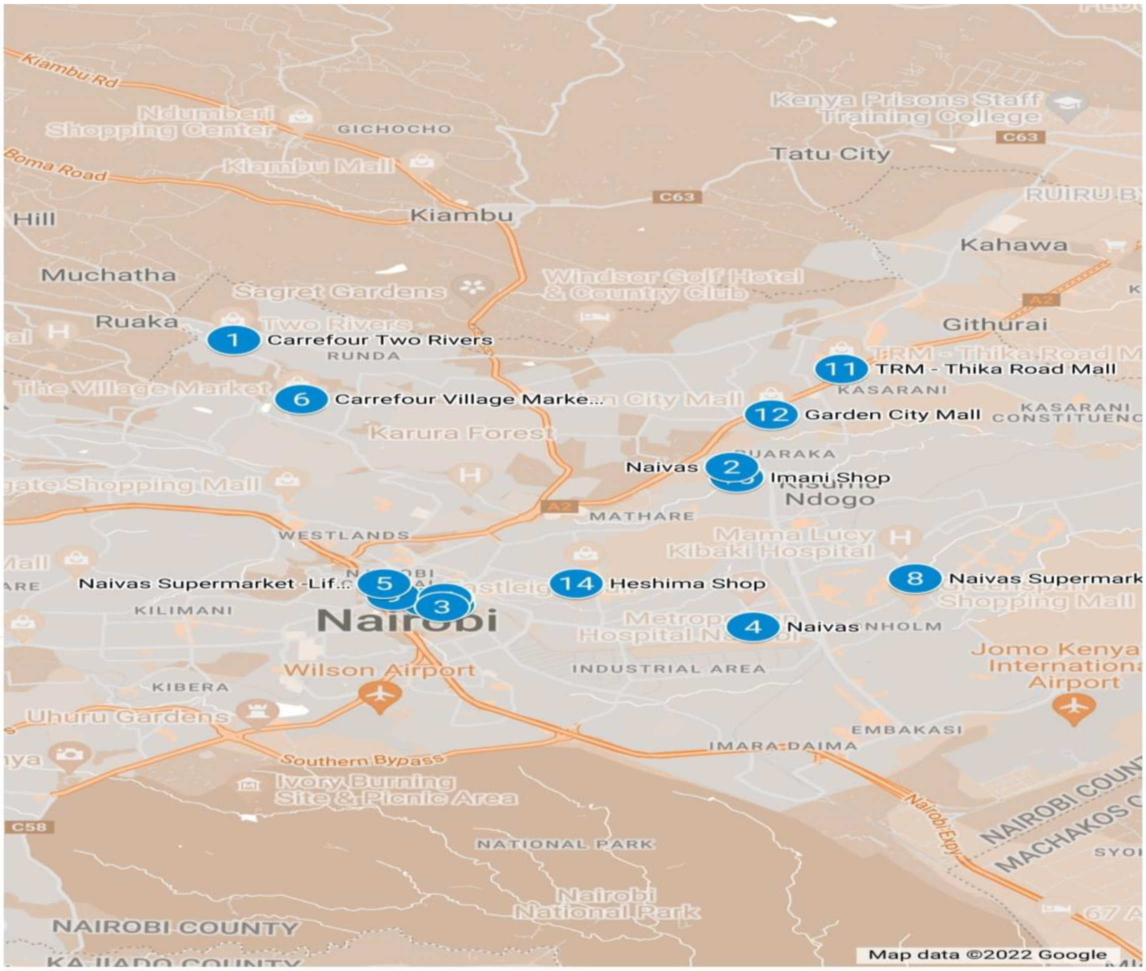

Figure 3.1: Sampling sites

3.2: Chemicals and reagents

Stock solutions of each of 1000 ppm of zinc ($ZnSO_4$), potassium (KCl), manganese ($MnCl_2.4H_2O$) and copper ($CuSO_4$ $5H_2O$) standards solutions were prepared by dissolving their respective salts (99.9%) in distilled water. Analytical quality chemicals and reagents were obtained from the local suppliers of Fluka and BDH laboratory reagents. Cleaning of glassware was done thrice with deionized water and then immersed in dilute nitric acid overnight. The apparatus was then rinsed thrice with deionized water and then dried in a Mermert oven (Mueller-Harvey & Baker, 2002).

Analytical grade reagents were used throughout the analytical process i.e. during sample preparations, sample dilutions, standard preparations, generation of calibration curve and determination of each element concentration in the samples.

3.3: Instrumental and apparatus

Hot plate (Gallonkamp 180 model), analytical balance (Shimadzu AUW 220 D model) capable of weighing to an accuracy of 0.0001 g, fume cupboard, water deionizer (Ionizer Mk 8), Memmert laboratory oven, desiccators, Contr AA 700 High Resolution Continuum Source (AAS) machine, ICP-MS and sampling plastic containers were used. The apparatus used include; 50 ml, 250 ml beakers, measuring cylinder 10 ml and 1000 ml, volumetric flasks and watch glass, muffle furnace, crucibles, filter funnels, filter papers (No. 42), pipette (5 ml, 10 ml), pipette fillers, concentrated nitric acid, concentrated hydrochloric acid, argon gas, compressed Air, acetylene gas and distilled water. Determinations of potassium, copper, zinc and manganese elements in the tea leaves and that of potassium, copper and manganese elements in the tea infusions were achieved by use of Contr AA 700 High Resolution Continuum Source Atomic Absorption Spectrometer (Figure 2.1). Determination of zinc in the tea infusions was achieved by use of ICP-MS technique. The light source for the spectrometer was a xenon short-arc lamp which is a single continuum source of radiation capable of providing very high radiation density and continuous emission throughout the entire spectral range (190-900 nm). The atomic absorption spectrometer was equipped with graphite tube atomizer which housed the graphite tube furnace (Welz *et al*., 2006). Zinc was determined using electrothermal atomization method, while copper, manganese and potassium were determined using flame atomization method. Changing from flame method to graphite furnace method was readily possible.

Inert gas, in this case argon was used to protect the graphite components of the atomizer which are subjected to extreme temperatures. The selectivity of the analysis was realized by a high-resolution double monochromater based on a prism and an echelle grating monochromator. The spectrophotometer was connected to an autosampler (MPE 60) which simplified the loading of the samples.

Inductively coupled plasma mass spectrometer (iCAP Q ICP-MS model) was used to determine the concentration of zinc ions in the tea infusions. The sample aerosol was generated by the nebulizer, in the spray chamber, larger aerosol droplets were filtered out into the drain while smaller droplets were carried into the plasma torch. Zinc ions were generated in the plasma. The ions were then accelerated to the Right Angle Positive Ion Deflection lens, which deflected the analyte ions by 90° and the lens ensured that neutral particles from the plasma passed directly out of the lens (Montaser, 1998). The ions were introduced into the quadrupole mass analyzer, where they were filtered out in relation to their specific mass to charge ratio (m/z). Figure 3.3 shows a basic illustration of an ICP-MS.

Figure 3.2: Basic illustration of Inductively coupled plasma mass spectrometer

3.4: Tea leaves samples

A total of 24 tea leave samples were purchased; 9 samples of black, 10 of green and five of purple tea. All green tea samples, 5 black tea samples and 4 purple tea samples were purchased from different supermarkets. Two black tea samples were purchased from retail shops, two black tea samples and one purple tea sample were purchased from tea hawkers as shown in Table 3.1. After purchase, each sample was wrapped in a brown paper bag, labelled and packed in cooler box containing ice and transported to the Government Chemist department, Nairobi laboratory for sample preparation and analysis. Table 3.1 shows the different brands of tea leaves samples that were analysed in this study.

Table 3.1: Different types of tea samples purchased from different out lets

Sample number	e Green Tea Leaves		Black Tea Leaves		Purple Tea Leaves	
	Brand BBA	Source	Brand	Source	Brand	Source
1	Brand GA	Supermarket	Brand BA	supermarket	Brand PA	Supermarket
2	Brand GB	Supermarket	Brand BB	supermarket	Brand PB	Supermarket
3	Brand GC	supermarket	Brand BC	supermarket	Brand PC	supermarket
4	Brand GD	supermarket	Brand BD	supermarket	Brand PD	supermarket
5	Brand GE	supermarket	Brand BE	Retail Shop	Brand PE	Hawker
6	Brand GF	Supermarket	Brand BF	Hawker		
7	Brand GG	Supermarket	Brand BG	Supermarket		
8	Brand GH	supermarket	Brand BH	Hawker		
9	Brand GJ	supermarket	Brand BJ	Retail shop		
10	Brand GK	supermarket				

3.5: Laboratory sample storage, preparation and analysis

In the laboratory the samples were stored on clean shelves spaces preserved for food products. All the analytical work was carried out at the Government Chemist Department, Nairobi laboratory.

3.5.1: Sample preparation (tea leaves)

Each of the tea leaves sample was labelled before weighing. About 0.5 g of each tea leaves sample were accurately weighed in triplicates in silica crucibles using a calibrated analytical balance (Shimadzu AUW 220 D model). The weighed sample was initially heated in a hot plate in order to volatilise as much organic matter as possible and finally transferred to a temperature controlled muffle furnace where it was ashed at 550 °C till no carbon remained.

After cooling in a desiccator, the ash was dissolved in 5 ml of concentrated nitric acid and 2 ml of concentrated hydrochloric acid under a fume cupboard. The solution was diluted using distilled water before reconstituting it in a 100 ml volumetric flask using deionized water, finally, the sample taken for Atomic Absorption Spectroscopy analysis. The analytical process is summarised in Figure 3.1. The blanks samples were prepared using the same reagents used during the sample preparation as described in subsection 3.5.3.

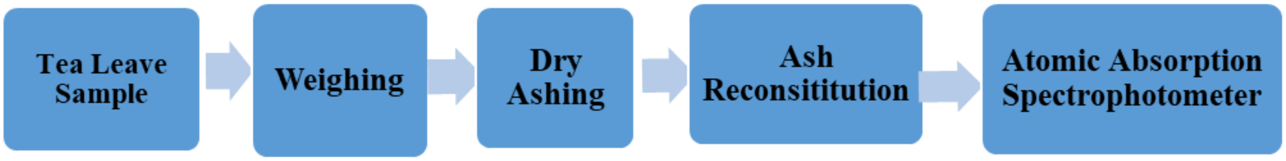

Figure 3.3: Analytical process for samples preparation and analysis

3.5.2: Preparation of tea infusions

About 0.20 g of each tea sample was accurately weighed in triplicates in 50 ml beaker using a calibrated analytical balance. 20 ml of boiled distilled water was added to the weighed tea leaves and mixed, after which the process of infusion was allowed to take place for about ten minutes. The mixture was stirred from time to time within the ten minutes using a glass rod and then covered with a lid with an aim of allowing the metal nutrients to infuse into the water. The average infusion period for tea leaves is 3-5 minutes i.e. portion of 2-4 grams in a cup (M.Sc, 2012). The mixture was filtered through Whatman filter paper No. 42 into a 100 ml volumetric flask after which a mixture of 5 ml concentrated nitric acid and 2 ml hydrochloric acid was added. The mixture was thoroughly mixed and finally filled to the mark with distilled water and taken for AAS or ICP-MS analysis. Determination of potassium, copper and manganese elements in all the tea infusions was achieved by use of the AAS technique while that of zinc element was achieved by use of ICP-MS technique.

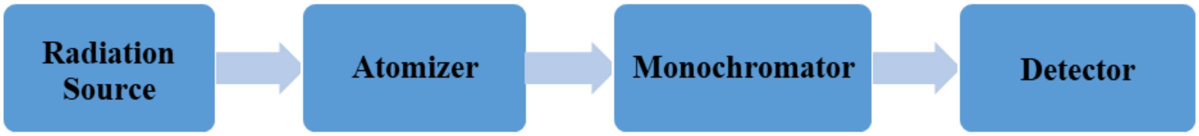

Figure 3.4: Basic illustration of atomic absorption spectrometer

3.5.3: Blank preparation

During the determination of copper and manganese elements, a blank was prepared which involved boiling 20 ml of distilled water in a beaker, cooling the water for one hour, transferring the water into a 100 ml volumetric flask, adding 5ml of concentrated nitric acid, adding 2ml of concentrated hydrochloric acid and finally filling the volumetric flask to the mark with distilled water. This was the blank that was used during the process of determination of copper and manganese elements in the tea infusions for the generation of calibration curves. Blank for the determination of potassium element in the infusions was prepared by diluting the blank that was used during the determination of copper and manganese twenty times while the blank that was used during the determination of zinc elements was obtained by diluting the blank that was used during the preparation of copper and manganese one hundred times.

3.6: Standard solutions preparation

The concentration of each of the stock standard solution for each element in this research was 1000 ppm. Standard solutions were prepared in sub-sections 3.6.1-3.6.4 and further suitable dilutions of the stock standard solutions to obtain the working standard solutions of 1.0, 2.0, 3.0, 4.0 and 5.0 ppm were performed. A calibration curve for each element was generated and the best line suited depending on the element was selected.

3.6.1: Copper standard preparation

To prepare 1000 ppm stock solution of copper, 3.929 g of copper sulphate ($CuSO_4$ $5H_2O$; 98.5 %) was dissolved in 50 ml of dilute nitric acid in a one litre volumetric flask and then made to the mark with distilled water.

3.6.2: Manganese standard preparation

To prepare 1000 ppm stock solution of manganese, 3.602 g of manganese chloride ($MnCl_2.4H_2O$) (98.5%) was dissolved in 50 ml of dilute nitric acid in a one litre volumetric flask and then made to the mark with distilled water.

3.6.3: Potassium standard preparation

To prepare 1000 ppm stock solution of potassium, 1.9069 g of dry potassium chloride (99.0%) was dissolved in 50 ml of dilute nitric acid in a one litre volumetric flask and then made to the mark with distilled water.

3.6.4: Zinc standard preparation

To prepare stock solution of zinc, 4.398 g of zinc sulphate ($ZnSO_4$) (98.5%) was dissolved in 50 ml of dilute nitric acid in a one litre volumetric flask and then made to the mark with distilled water.

These standard solutions were used to generate the calibration curve for each metal ion (appendix I, Figures 1A-1D). The limits of detection i.e. the smallest amount of analyte that can be determined with confidence for each metal element in the samples was established.

3.7: The analytical process

During the determination of elements using the flame AAS, a sample volume of at least one millitre (1ml) was aspirated at different wave length (ƛ) specific for each metal ion (Table 3.2). An aerosol mist was produced from the sample solution by use of a nebulizer. After mixing the mist with the combustion gases and then passing the mixture to the burner, the mist was desolvated by the flame's thermal energy thereby forming a dry aerosol of small, solid particles (Nielsen, 2003). The gases used when using the flame method were acetylene which was the fuel and compressed air was the oxidant. The range of temperature that was achieved was 2100–2400 ˚C. The flame's thermal energy further volatilized the particles and a vapour was produced which contained free atoms.

Table 3.2: Absorption radiation wavelengths and gases used during analysis

Element	Wavelength (nm)	Flame atomization
Potassium	769	Air-acetylene
Manganese	279	Air-acetylene
Copper	324	Air-acetylene

Source: Laboratory data 2021

3.7.1: Atomization process during determination of zinc

A few microliters of sample were deposited into the furnace by an autosampler after which the sample was subjected to a series of heatings that included drying, ashing and finally atomization of the sample. Drying was done at low temperatures; (80-110˚C), the temperature in the electrically heated graphite tube was increased to between 300-1200˚C thereby ensuring that all the organic matter was ashed and converted to carbon dioxide and water vapour. After ashing, the current was rapidly increased to several hundred amperes, this caused the temperature to rise to about 2450°C at which the sample was atomized (Skoog *et al.*, 2017). For graphite furnace measurements, argon was used as inert gas. Figure 3.2 shows a basic illustration of an atomic absorption spectrophotometer and Table 3.3 shows the wavelength at which zinc absorbs the electromagnetic radiation.

Table 3.3: Absorption radiation wavelength and gas used during analysis

Element	Wavelength (nm)	Graphite furnace atomization
Zinc	213	Argon gas

Source: Laboratory data 2021

3.8: Quality assurance

In order to ensure validity of results of the research, blanks solutions were also analysed alongside the samples. Glassware and plastic ware was cleaned thoroughly, rinsed with distilled water and then immersed in dilute nitric acid overnight. Distilled water was used throughout the experiment for the preparation of all samples and standards solutions. Analytical grade reagents were purchased from the local suppliers of Fluka and BDH laboratory reagents. All vessels were soaked in 10% v/v nitric acid (HNO_3) and rinsed three times with purified water before use. Type 1 water generated from Puranity TU 12 was used for rinsing all apparatus, sample preparations and analyses. Puranity TU 12 system is a purification water purification system; which purifies portable water further to ultrapure water which is required to minimize errors during analytical procedures. Blank samples were prepared by following the same procedures but without the tea leaves. Blanks and standard solutions of all monitored analytes were prepared in milligram per litre (mg/L) or microgram per litre (µg/L) concentration ranges for the various analytes (Prichard & Barwick, 2007). Levels of metal elements in the samples solutions was then determined by interpolation on the respective calibration curves.

3.9: Instrument conditions

Air-acetylene (C_2H_2-Air) flame was used to atomize the samples during determination of copper ions. The burner was adjusted to a height of six millimetres (6mm) and the rate of flow of fuel was maintained at 50 litres per hour while that of oxidant was maintained at 470 litres per hour. During

the atomization process, the fuel/oxidant ratio was maintained at 0.106. (Burner height (mm), 6; Fuel flow (L/ h), 50; Oxidant flow (L/h), 470; Fuel/oxidant, 0.106).
The absorbance of standard solutions of concentrations of 0.00, 2.00, 4.00, 6.00, 8.00 and 10.00 ppm was measured at 324 nm and the measurements obtained were used to generate a calibration curve. The limit of detection for copper i.e. the smallest amount of analyte that can be determined with confidence, was 0.3276 mg/L and the product moment correlation coefficient (R^2) was 0.9976. Levels of copper in the samples solutions was then determined by interpolation on the calibration graph.

Air-acetylene (C_2H_2-Air) flame was used to atomize the samples during determination of manganese ions. The burner was adjusted to a height of six millimetres (6mm) and the rate of flow of fuel was maintained at 65 litres per hour while that of oxidant was maintained at 470 litres per hour. During the atomization process, the fuel oxidant ratio was maintained at 0.138. (Flame; air- acetylene, (C_2H_2-Air); Burner height (mm), 6; Fuel flow (L/h), 65; Oxidant flow (L/h), 470; Fuel/oxidant, 0.138). The absorbance of a series of standard solutions of concentrations; 0.00, 2.00, 4.00, 6.00, 8.00 and 10.00 mg/L were measured at a wavelength of 279 nm and a calibration graph was generated. The limit of detection for manganese was 0.3328 mg/L and the product moment correlation coefficient (R^2) was 0.9970. Concentrations of manganese atoms in the sample solutions was determined by interpolation on the calibration curve.

Air-acetylene (C_2H_2-Air) flame was used to atomize the samples during determination of potassium ions. The burner was adjusted to a height of eight millimetres (8mm) and the rate of flow of fuel was maintained at 80 litres per hour while that of oxidant was maintained at 470 litres per hour. During the atomization process, the fuel oxidant ratio was maintained at 0.170. (Flame; air- acetylene (C_2H_2-Air); Burner height (mm), 8; Fuel flow (L/ h), 80; Oxidant flow (L/h), 470; Fuel/oxidant, 0.170). Potassium atoms can absorb electromagnetic radiation at 769nm, hence the absorbance of a series of standard solutions of 0.00, 1.00, 2.00, 3.00, 4.00 and 5.00 mg/L were measured at this wavelength. The limit of detection for potassium was 0.4422 mg/L and the product moment correlation coefficient (R^2) was 0.9840. Concentrations of potassium atoms in the prepared sample solutions was determined by interpolation on the calibration curve after diluting each of the sample solutions twenty times with distilled water.

The absorbance of a series of zinc standard solutions of concentrations 0.00, 2.00, 4.00, 6.00, 8.00 and 10.00 µg/L were measured at 213nm and a calibration curve developed. The limit of detection for zinc was 0.6531 µg/L and the product moment correlation coefficient (R^2) was 0.9941. Concentrations of zinc in the prepared sample solutions was determined by interpolation on the calibration curve after diluting each of the sample solutions one hundred times.

3.10: Data analysis

A total of 24 tea leaf samples of different brands were purchased from selected supermarkets, and shops in the city of Nairobi and hawkers (Table 3.1) with an objective of determining the levels of minerals namely, copper, manganese, potassium and zinc. All measurements were carried out in triplicate. Results of the parameters determined were expressed as a mean and standard deviation of the triplicates samples. The data was analyzed using the one-way analysis of variance (ANOVA) tool and Kruskal Wallis test under the Statistical Package (STATA) software (Miller & Miller, 2005)**.**

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1: Data analysis and results interpretation

24 tea samples were analysed in triplicate and the results compiled using the one-way analysis of variance (ANOVA) tool and Kruskal Wallis test under the Statistical Package (STATA) software**.** The levels of the parameters analysed were expressed as a mean and the standard deviation of the triplicate values.

The limits of detection (LOD) and product moment correlation coefficient (R^2) for copper, manganese, potassium and zinc analytes were determined and are tabulated in Table 4.1. The plot of absorbance against concentration gave the best line of fit that resulted to correction factors (R^2) of more than 0.98 showing that the relationship between the instrument response and analyte concentration was good. Calibration curves for copper, manganese, potassium and zinc are shown in appendix 1; Figures 1A, 1B, 1C and 1D respectively which produced straight lines with correlation factors (R^2) of 0.9976, 0.9970, 0.9841 and 0.9941 respectively (Table 4.1), showing a good association between instrument response and analyte concentration.

Table 4.1: Limits of detection and correlation coefficient for metal analystes

Metal ion	Limit of detection (LOD)	Correlation coefficient (R^2)
Copper	0.3276 µg/g	0.9976
Manganese	0.3328 µg/g	0.9970
Potassium	0.4422 µg/g	0.9841
Zinc	0.6531 µg/Kg	0.9941

The limit of detection for copper was 0.3276 µg/g and the product moment correlation coefficient (R^2) was 0.9976.

Levels of copper ions in the samples solutions was then determined by interpolation on the calibration curve appendix 1; Figure 1A. The limit of detection for manganese was 0.3328 µg/g and the product moment correlation coefficient (R^2) was 0.9970. Levels of manganese ions in the sample solutions was determined by interpolation on the calibration curve appendix 1; Figure 1B. The limit of detection for potassium was 0.4422 µg/g and the product moment correlation coefficient (R^2) was

0.9841. Levels of potassium ions in the prepared sample solutions was determined by interpolation on the calibration curve appendix 1; Figure 1C after diluting each of the sample solutions twenty times with deionized water. The limit of detection for zinc was 0.6531 µg/Kg and the product moment correlation coefficient (R^2) was 0.9941. Levels of zinc in the prepared sample solutions was determined by interpolation on the calibration curve appendix 1; Figure 1D after diluting each of the sample solutions one hundred times.

4.2: Conversion of concentrations from calibration curve to the concentration of elements in tea leaves

Levels of metal elements in the prepared sample solutions was determined by interpolation on their respective calibration curves. The instrumental readings were either in mg/L, this meant that a certain amount of elements was present in a litre of the solution. The reconstitution of the sample solutions was done in a 100ml volumetric flask. This meant that the weight of the tea leaves taken (sample) was contained in the 100ml volumetric flask. By dividing each measurement by 10, concentration of the metal element in 100ml solution was obtained. This amount of element was contained in the weight of the tea leaves taken in each case, hence the concentration of the element in microgram per gram was obtained.

4.3: Metal ions in black, green and purple tea leaves.

Mean levels of copper metal ions in purple, black and green tea brands were found to be 41.20 ± 12.47 µg/g, 41.40 ± 11.89 µg/g and 48.50 ± 19.40 µg/g respectively. Among the green, black and purple tea samples manganese levels were 1161.70 ± 338.38 µg/g, 1342.10 ± 310.26 µg/g and 913.60 ± 471.95 µg/g respectively. The levels of manganese in all the three types of teas were higher compared to the levels of copper but there is no considerable difference in relation to the tea types. The mean concentrations of potassium metal ions in green, black and purple tea samples were 12 801.7 ± 4 065.66 µg/g, 12 716.30 ± 2 541.56 µg/g and 12 837.90 ± 3 517.60 µg/g respectively. The mean concentrations of potassium were the highest in relation to all the other elements. The mean concentrations of zinc in green, black and purple tea samples were 111.10 ± 29.98 µg/g, 113.90 ± 48.33 µg/g and 116.60 ± 20.25 µg/g respectively. The levels of zinc ions were much lower compared to potassium and manganese though they were slightly higher than the levels of copper ions. These findings have been tabulated in table 4.2.

Table 4.2: Metal ions levels in black, green and purple tea leaves

Concentration of Metal elements (µg/g)	Black tea	Green tea	Purple tea
Copper	41.40 ± 11.89	48.50 ± 19.40	41.20 ± 12.47
Manganese	1 342.10 ± 310.26	1 161.70 ± 338.38	913.60 ± 471.95
Potassium	12 716.30 ± 2 541.56	12 801.70 ± 4 065.66	12 837.90 ± 3 517.67
Zinc	113.90 ± 49.38	111.10 ± 29.98	116.60 ± 20.26

4.3.1: The metal elements levels in black tea leaves brand

The levels of copper obtained in this research ranged from 19.90 ± 2.69 µg/g to 57.70 ± 18.24 µg/g (Table 4.3). Brand BA had the lowest value while brand BJ had the highest. These values are slightly higher than those obtained by (Ashraf & Mian, 2008) of 9.40 - 31.00 µg/g and (McKenzie *et al*., 2010) at 12.00 - 35 µg/g for black tea leaves brand sampled from United Kingdom outlets. The maximum limit for copper in the Kenya and East Africa standards for black tea products is set at 150 ppm hence the levels in all the black tea samples analyzed were within the two regulations but there is no value specified in the Codex standard as shown in Table 4.4.

Table 4.3: Copper, manganese, potassium and zinc metal ions levels in black tea brands

Sample	Cu (µg/g)	Mn (µg/g)	K (µg/g)	Zn (µg/g)
Brand BA	19.90 ± 2.69	953.80 ± 201.63	11 953.30 ± 2 830.01	93.60 ± 13.76
Brand BB	47.90 ± 7.32	1 525.50 ± 31.52	1 3935.10 ± 44.56	64.10 ± 9.88
Brand BC	38. 60± 7.21	1 390.50 ± 41.69	14 861.80 ± 883.84	117.90 ± 0.70
Brand BD	38.70 ± 8.28	1 369.90 ± 7.13	14 245.2 ± 259.08	213.40 ± 13.90
Brand BE	56.50 ± 1.62	1 389.70 ± 23.93	9 316.50 ± 702.90	104.50 ± 23.60
Brand BF	37.60 ± 2.95	1 258.40 ± 31.68	14 093.60 ± 544.29	93.00 ± 15.57
Brand BG	41.005 ± 2.96	184.40 ± 26.79	12 158.60 ± 241.84	132.10 ± 8.94
Brand BH	45.30± 0.82	787.20 ± 18.20	14 489.50 ± 1 380.98	115.30 ± 12.79
Brand BJ	57.70 ± 18.24	1 561.30 ± 25.24	11 015.20 ± 3 666.85	56.60 ± 3.35

The mean manganese levels in black tea leaves ranged from 184.40 ± 26.79 µg/g to 1 561.30 ±25.24 µg/g. Brand BG tea sample had the lowest value while BJ had the highest level. Manganese levels in all the black tea leaves were higher than copper levels (Table 4.3). There was no maximum level of manganese set in the Kenyan, East Africa or codex standards (Table 4.4).

Levels of potassium ranged from 9 316.50 ± 702.90 µg/g to 14 861.80 ± 883.84 µg/g. Sample BE had the lowest value while BC had the highest level. Potassium levels in all the black tea leaves samples were higher than copper values (Table 4.3). There were no set limits for potassium levels in the Kenyan, East Africa or codex standards (Table 4.4).

The mean zinc levels ranged from 56.60 ± 3.35 µg/g to 213.40 ± 13.90 µg/g. Sample BJ had the lowest level while BD recorded the highest (Table 4.3). The maximum zinc levels set in the Kenyan and East Africa standard are 50 ppm and 150 ppm respectively however no limits have been specified in the codex standard. Kenya has set a lower maximum zinc levels for tea products in relation to the east African standard and this difference is a possible source of trade barrier between the East African countries hence call for harmonization is recommended. Zinc levels for all the black tea leaves samples analyzed were above the recommended value in the Kenya standard (KS 65: 2016) for black tea (Table 4.4). The zinc values were much lower than potassium and manganese but slightly higher than copper levels (Table 4.3).

Table 4.4: Metal element specifications

Recommended Levels				
	Copper (µg/g)	Manganese(µg/g)	Potassium(µg/g)	Zinc(µg/g)
Kenyan Standard	150	Not specified	Not specified	50
East Africa Standard	150	Not specified	Not specified	150
Codex Standard	Not specified	Not specified	Not specified	Not specified

Kenya Standard (KS 65: 2016), East Africa Standard (EAS 28: 2018), Codex (Stan 193-1995)

In the Kenya standard (KS 65: 2016) for black tea, zinc level is set at a maximum limit of 50 ppm, hence all the black tea samples analyzed (56.60 ± 3.35 to 213.40 ± 13.90) (Table 4.3) do not comply with the set specification (Table 4.4). In the Kenya standard for purple tea, the required levels for zinc are not specified. In the tea products specifications referenced, zinc and copper have been categorised as contaminants though the same elements have been categorised as nutritive elements in the literature. In the Kenya standard (KS 2744:2017) for orthodox tea, arsenic, cadmium and lead have been listed as contaminants. No limit is specified for copper, manganese, potassium and zinc in the ISO standard for black tea (ISO 3720: 2011) and in the ISO standard for green tea (ISO 11287: 2011). Arsenic, cadmium, lead, mercury, methyl mercury and tin are listed as heavy metals in the codex general standard for contaminants and toxins in foods (Codex Stan 193-1995). This is an indication that the national institutions which are responsible for the development of Kenya standards and codex standards put a lot of emphasis on metal contaminants but not on nutritive metal ions found in tea products.

4.3.2: Levels of metal ions in green tea leaves

The copper ion levels ranged from 27.60 ± 3.18 µg/g to 87.00 ± 10.86 µg/g (Table 4.5). The highest levels in green tea samples was detected in Brand GK (87.00 ± 10.86µg/g) while brand GB had the lowest (27.60 ± 3.18 µg/g).

In the case of manganese content in the green tea brands analyzed, brand GJ had the highest mean level (1 569.20 ± 38.15 µg/g) while brand GB had the lowest value (598.00 ± 12.46 µg/g). The highest potassium level (18 390.60 ± 109.40 µg/g) was from brand GA and the lowest value (6 759.90 ± 117.97 µg/g) from brand GC. Brand GD had the highest mean zinc level (175.40 ± 27.37 µg/g) while brand GJ the lowest value (75.60 ± 2.72 µg/g). Potassium content in the green tea leaves was the highest followed by manganese, zinc and finally copper (Table 4.5).

Table 4.5: Levels of copper, manganese, potassium and zinc metal ions in green leaves

Sample	Cu (µg/g)	Mn (µg/g)	K (µg/g)	Zn (µg/g)
Brand GA	63.00 ± 0.78	1 295.5 ± 10.95	18 390.60 ± 109.40	115.00 ± 1.98
Brand GB	27.60 ± 4.11	598.00 ± 12.46	13 410.60 ± 193.07	99.10 ± 15.43
Brand GC	38. 40± 2.62	1 197.00 ± 1.78	6 759.90 ± 117.97	101.50 ± 0.39
Brand GD	45.10 ± 4.97	1 420.30 ± 56.90	14 307.80 ± 149.34	175.40 ± 27.37
Brand GE	42.50 ± 3.74	754.90 ± 23.00	11 990.20 ± 188.15	105.20 ± 2.24
Brand GF	45.70 ± 9.22	1 405.50 ± 39.55	14 529.70 ± 348.67	106.00 ± 37.06
Brand GG	42.40 ± 4.01	961.40 ± 25.26	16 436.90 ± 573.30	96.70 ± 9.14
Brand GH	47.50± 1.64	1 568.10 ± 27.08	13 300.80 ± 114.39	105.20 ± 3.11
Brand GJ	50.50 ± 6.96	1 569.20 ± 38.15	12 132.70 ± 697.64	75.60 ± 2.72
Brand GK	87.00 ± 40.86	847.20 ± 57.65	6 961.10 ± 248.28	145.00 ± 21.46

4.3.3: Levels of metal ions in purple tea leaves

As shown in Table 4.6, the level of 52.50 ± 8.67 µg/g of copper ions was the highest from brand PB while brand PA had the lowest value i.e. 26.30 ± 4.78 µg/g. Brands PB and PD had the highest and lowest levels of manganese ions i.e. 1 367.20 ± 136.01µg/g and 135.70 ± 27.29 µg/g respectively. Brand PE had the highest mean potassium levels of 16 498.50 ± 834.91µg/g while PD contained the lowest levels i.e. 7 852.90 ± 1 401.23 µg/g.

Brand PB had the highest mean levels of zinc metal ions at 140.50 ± 14.72 µg/g while PC had the lowest value of 91.60 ± 0.29 µg/g from the purple tea leaves brands that were analyzed (Table 4.6).

Table 4.6: Copper, manganese, potassium and zinc levels in purple tea leaves brands

Sample	Cu (µg/g)	Mn (µg/g)	K (µg/g)	Zn (µg/g)
Brand PA	26.30 ± 4.78	919.80 ± 24.75	13 441.00 ± 1 590.44	109.60 ± 4.18
Brand PB	52.50 ± 8.67	1 367.2 ± 136.01	11 379.10 ± 1 920.91	140.50 ± 14.72
Brand PC	48.10± 9.64	1 357.80 ± 26.81	16 240.90 ± 313.40	91.60 ± 0.29
Brand PD	27.60 ± 3.18	135.70 ± 27.29	7 852.90 ± 1 401.23	99.50 ± 6.51
Brand PE	46.50 ± 3.91	787.30 ± 49.21	16 498.50 ± 834.91	125.60 ± 7.93

In the Kenya standard for purple tea, the required levels for copper are not specified. In an Indian standard for green tea, copper is categorised as a contaminant at a maximum level of 150 ppm (IS 15 344:2003).

4.3.4: Comparison of metal element levels in green, black and purple tea leaves

As shown in Figure 4.1, the levels of copper in purple, black and green tea leaves samples brands varied from 26.30 ± 4.78 µg/g to 52.50 ± 8.67µg/g, 19.90 ± 4.69 µg/g to 57.70 ± 18.24 µg/g and 27.60 ± 3.18 µg/g to 87.00 ± 40.86 µg/g respectively. The levels of copper were considerably low in all the samples and were within Kenya and East African Standards (Table 4.4).

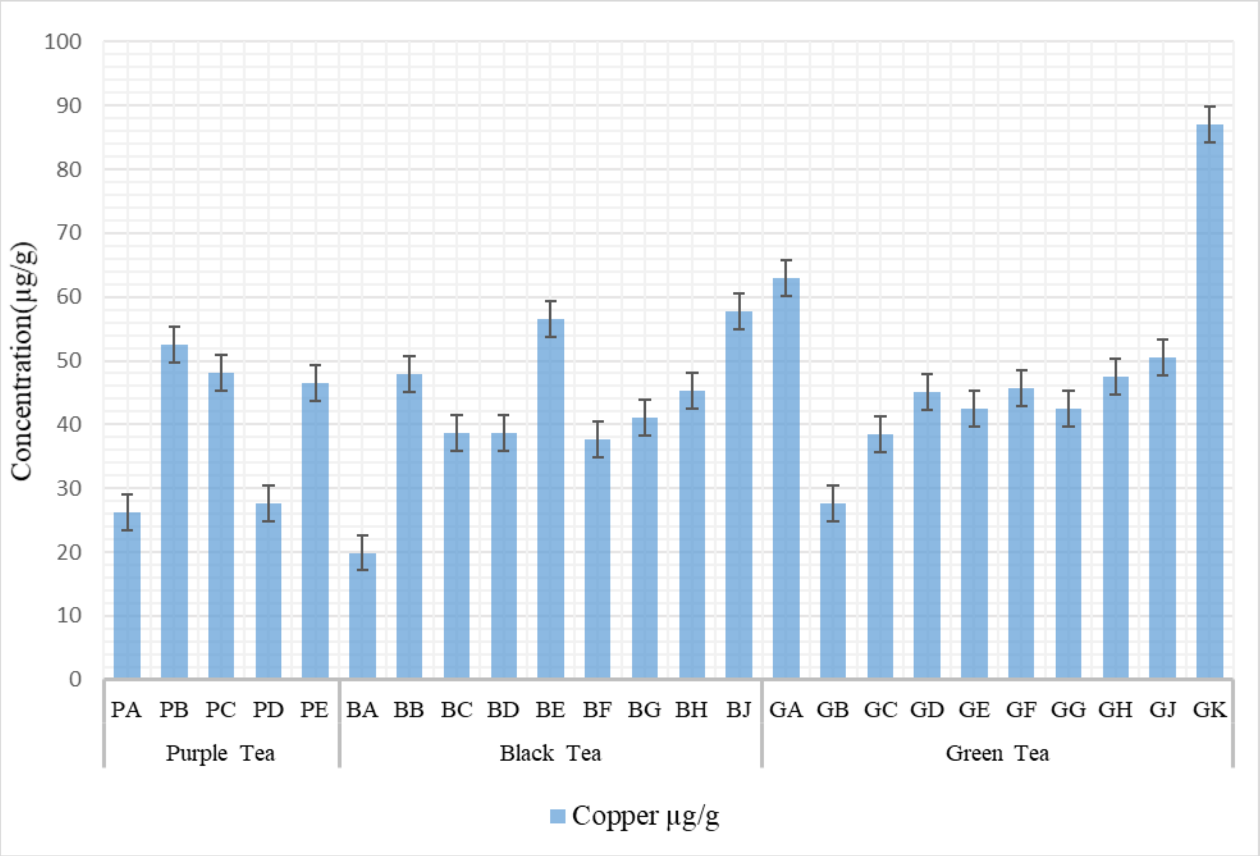

Figure 4.1: Comparison of copper levels in purple, black and green tea leaves samples

Brand GK was the tea product which had the highest value of copper levels at 87.00 ± 10.86 µg/g (Table 4.5) while brand BA had the lowest level of 19.90 ± 4.69 µg/g (Table 4.3). Brand GK was a green tea sample while brand BA was a black tea sample (Figure 4.1).

Other researchers, (Street *et al.*, 2011), (Tsushida & Takeo, 1979) and (Crosby, 1977) have also acknowledged the presence of copper in tea leaves with levels ranging from 9.00 µg/g to 65.10 µg/g.

4.3.5: Comparison of the manganese levels in green, black and purple tea leaves

As shown in Figure 4.2, the levels of manganese in purple, black and green tea leaves samples brands varied from 135.70 ± 27.29 µg/g to 1 367.20 ± 136.01 µg/g, 787.20 ± 18.20 µg/g to 1 842.40 ± 26.79 µg/g and 598.00 ± 12.46 µg/g to 1 569.20 ± 38.15 µg/g, respectively.

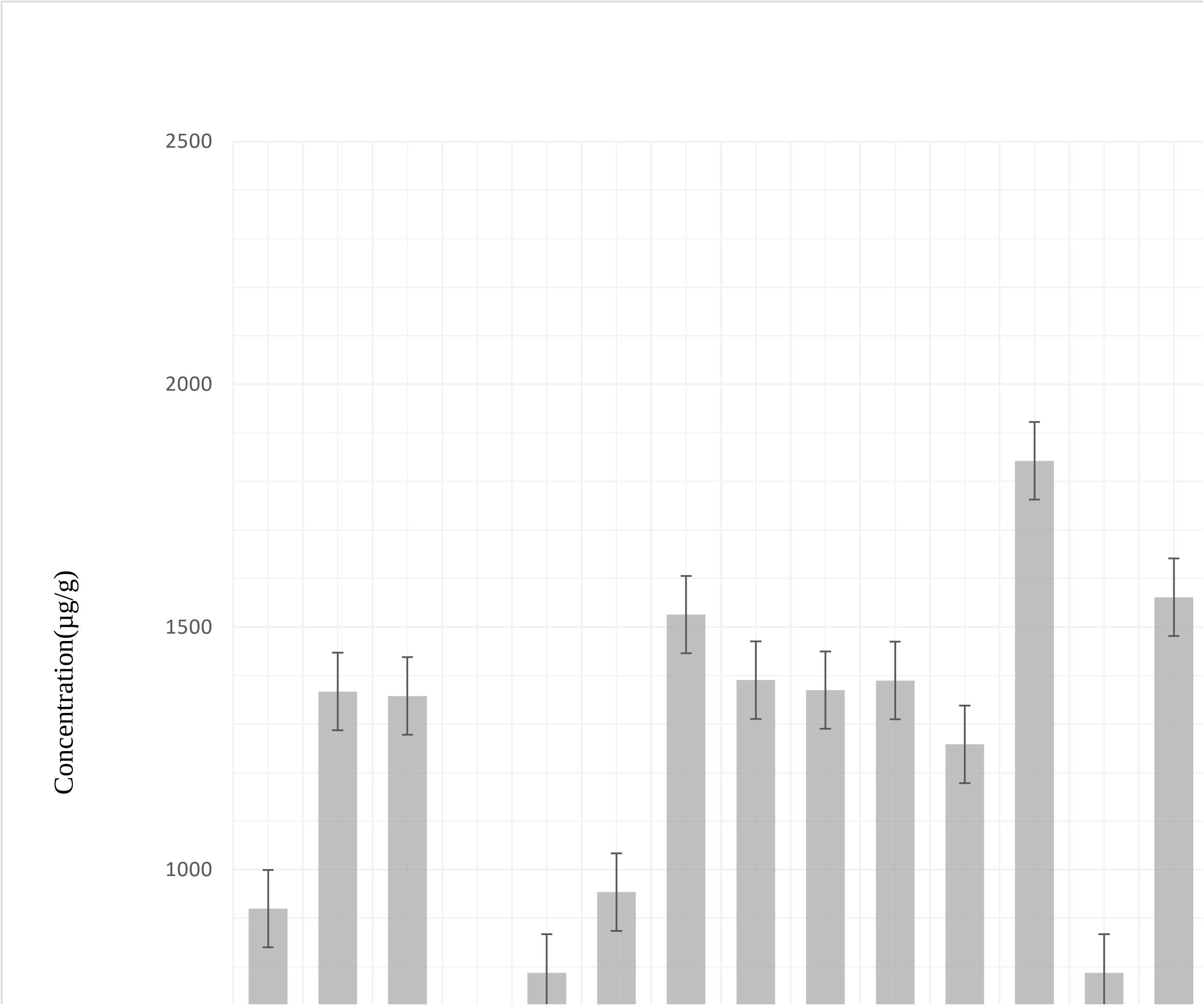

Figure 4.2: Comparison of manganese levels in purple, black and green tea leaves

The highest level of manganese (1 842.40 ± 26.79 µg/g) was measured in brand BG which was a black tea sample (Table 4.3) while lowest value (135.70 ± 27.29 µg/g) was measured in a purple tea sample brand PD (Table 4.6). Manganese levels in purple tea were highest in brand PB (1 367.20 ± 136.01 µg/g) while the lowest value (135.70 ± 27.29 µg/g) was from brand PD. This study has confirmed a report from Szefer & Nriagu, (2006) which stated that tea leaves contains up to 139 mg per 100g manganese levels. The tea plant has the ability to accumulate metals such as manganese, aluminium and selenium to levels far higher than those found in other plants (Reilly, 2008). (McKenzie *et al.*, 2010) determined levels of manganese in 36 samples of black tea leaves samples from Ceylon, China, Georgia, India, and Kenya and detected manganese ions levels ranging from 190 µg/g –1570 µg/g (Preedy, 2013). These findings are generally similar to those reported in this study (184.40 ± 26.79 µg/g to 1 561.30 ±25.24 µg/g.) as shown in Table 4.3.

4.3.6: Comparison of the potassium levels in green, black and purple tea leaves samples

As shown in Figure 4.3, the potassium levels in purple, black and green tea leaves brands varied from 7 852.90 ± 141.23 µg/g to 16 498.50 ± 834.91 µg/g, 9 316.50 ± 702.90 µg/g to 14 861.80 ± 883.84 µg/g and 6 759.90 ± 117.97 µg/g to 18 390.60 ± 109.40 µg/g respectively. Brands GA had the highest levels (18 390.60 ±109.40 µg/g) and lowest levels (6 759 ± 1 117.97 µg/g) was measured in brand GC (Table 4.5) as shown in Figure 4.3.

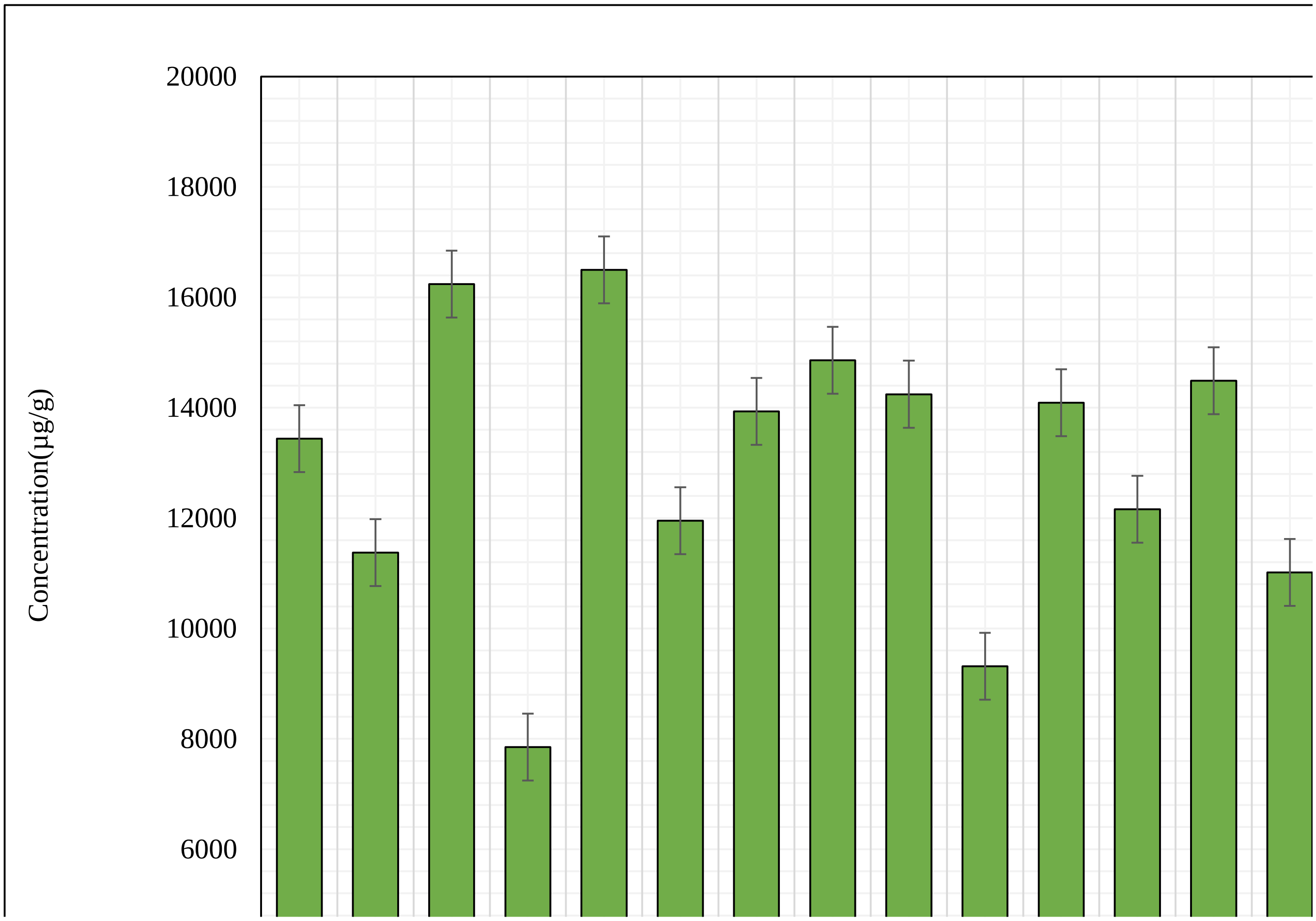

Figure 4.3: Potassium levels in purple, black and green tea leaves

Leafy greens and root vegetables have been found to be good sources of potassium (Medicine, 2006). WHO recommends an increase in potassium intake from food to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart disease in adults. WHO suggests a potassium intake of at least (3 510 mg/day) for adults. Potassium is an example of a macromineral and occurs in plenty of food as shown by America Central Intelligence Agency (CIA, 2013) and this explains the high levels that have been detected in all the tea leaves and the tea infusions in the this study. Potassium is one of the most abundant cation in both plant and animal cells (Pandey and Mahiwal, 2020).

Potassium is found in a wide variety of food and beverages, including fruits, vegetables, nuts, beans, dairy products, meats, poultry and fish (Szefer and Nriagu, 2006). In the U.S, sources such as coffee, tea, dairy products and potatoes are top contributors to potassium intake. Potassium is readily available in many plant foods and healthy adults need about 4,700 milligrams daily (Bennett, 2017).

4.3.7: Comparison of the zinc levels in green, black and purple tea leaves

As shown in Figure 4.4, the levels of zinc in purple, black and green tea leaves samples brands varied from 91.60 ± 0.29 µg/g to 140.50 ± 14.72 µg/g, 56.60 ± 3.35 µg/g to 213.40 ± 13.90 µg/g and 56.00 ± 2.72 µg/g to 175.40 ± 27.37 µg/g respectively.

Zinc levels in black tea leaves were highest in brand BD (213.40 ± 13.90 µg/g) and lowest in BJ (56.60 ± 3.35 µg/g) (Table 4.3). Kabata-Pendias, (2010) reported zinc levels ranging from 26 µg/g to 40 µg/g; these levels were slightly lower than those obtained in this study.

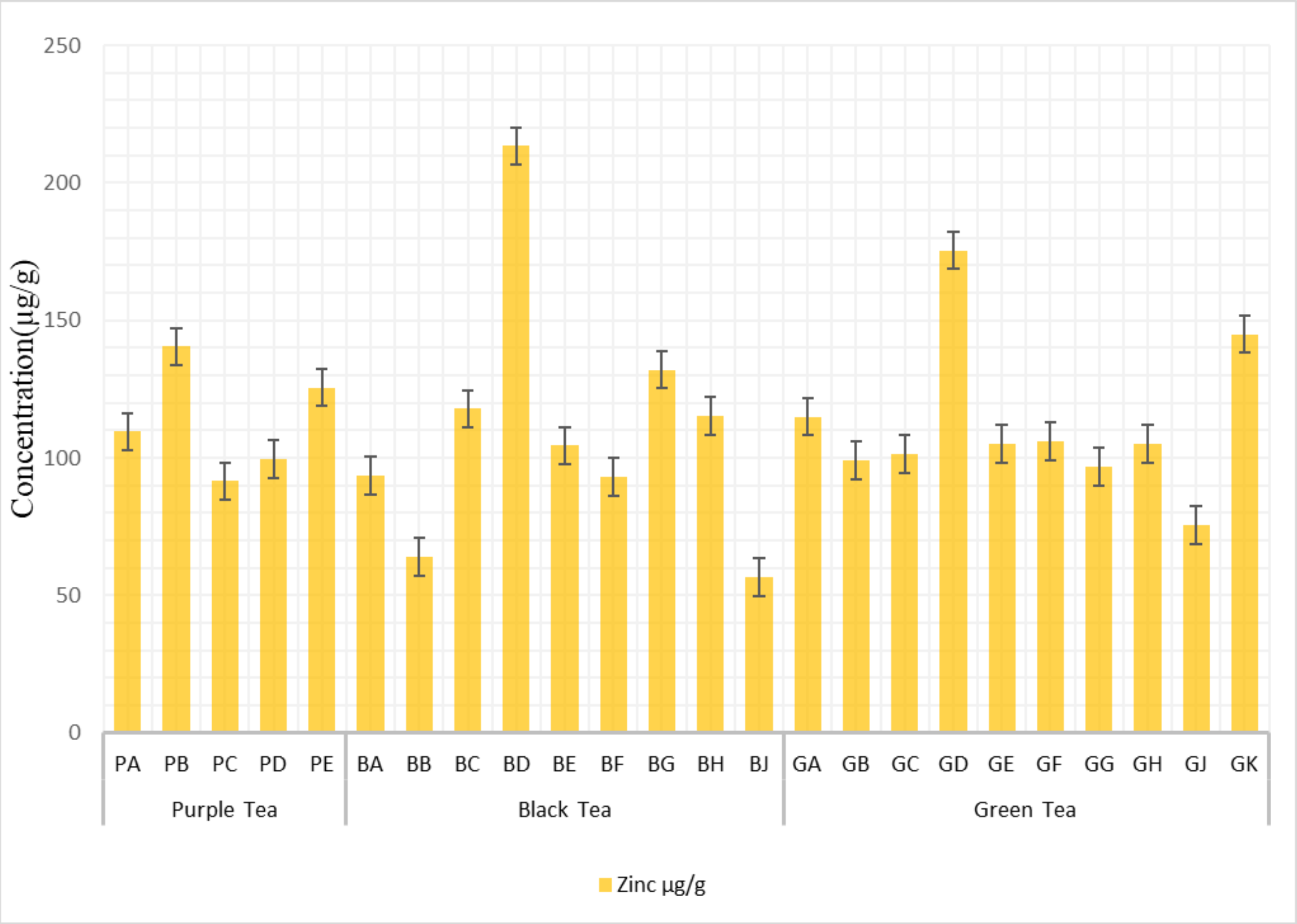

Figure 4.4: Comparison of zinc metal nutrient levels in purple, black and green tea leaves

4.3.8: Comparison of the mean metal ions levels in green, black and purple tea leaves

Table 4.7 shows the average levels of the metal ions analyzed in the three types of tea samples brands. Mean levels of copper metal ions in purple, black and green tea brands were found to be 41.20 ± 12.47 µg/g, 41.40 ± 11.89 µg/g and 48.50 ± 19.40 µg/g respectively. Mean levels of manganese metal ions in green, black and purple tea brands were found to be 1 161.70 ± 338.38 µg/g, 1 342.10 ± 310.26 µg/g and 913.60 ± 471.95 µg/g respectively. Potassium metal ions had mean levels in green, black and purple tea brands of 12 801.70 ± 405.66 µg/g, 12 716.30 ± 241.56 µg/g and 12 837.90 ± 357.60 µg/g respectively. Mean zinc metal ions levels in green, black and purple tea brands were 111.10 ± 29.98 µg/g, 113.90 ± 48.33 µg/g and 116.60 ± 20.25 µg/g respectively. The mean levels of potassium metal ions in all the samples was the highest in relation to manganese, zinc and copper metal ions in all tea samples analyzed (Figure 4.5).

Table 4.7: Mean metal nutrients levels (µg/g) in purple, black and green tea leaves

	Type of tea	n	Mean µg/g	Standard Deviations	Median µg/g	Min	maxi
Copper	Purple tea	14	41.20	12.47	42.50	22.87	59.00
	Black tea	25	41.40	11.89	42.50	15.00	70.60
	Green tea	29	48.50	19.40	45.20	24.20	134.10
Manganese	Purple tea	15	913.60	471.95	920.10	105.60	1 449.30
	Black tea	27	1 342.10	310.26	1 377.90	742.10	1 872.60
	Green tea	30	1 161.70	338.38	1 241.90	587.30	1 612.70
Potassium	Purple tea	14	12 837.90	357.67	12 728.80	6 996.60	17 088.90
	Black tea	22	12 716.30	241.56	13 614.40	8 422.40	17 942.30
	Green tea	29	12 801.70	405.66	12 567.00	4 940.30	20 782.80
Zinc	Purple tea	12	116.60	20.25	116.70	91.40	149.40
	Black tea	24	113.90	48.33	108.70	45.20	226.00
	Green tea	24	111.10	29.98	104.00	72.70	194.70

‘n’ is the total number of laboratory samples analyzed

In general, copper levels in the tea samples were found to be the lowest while potassium had the highest value (Figures 4.5).

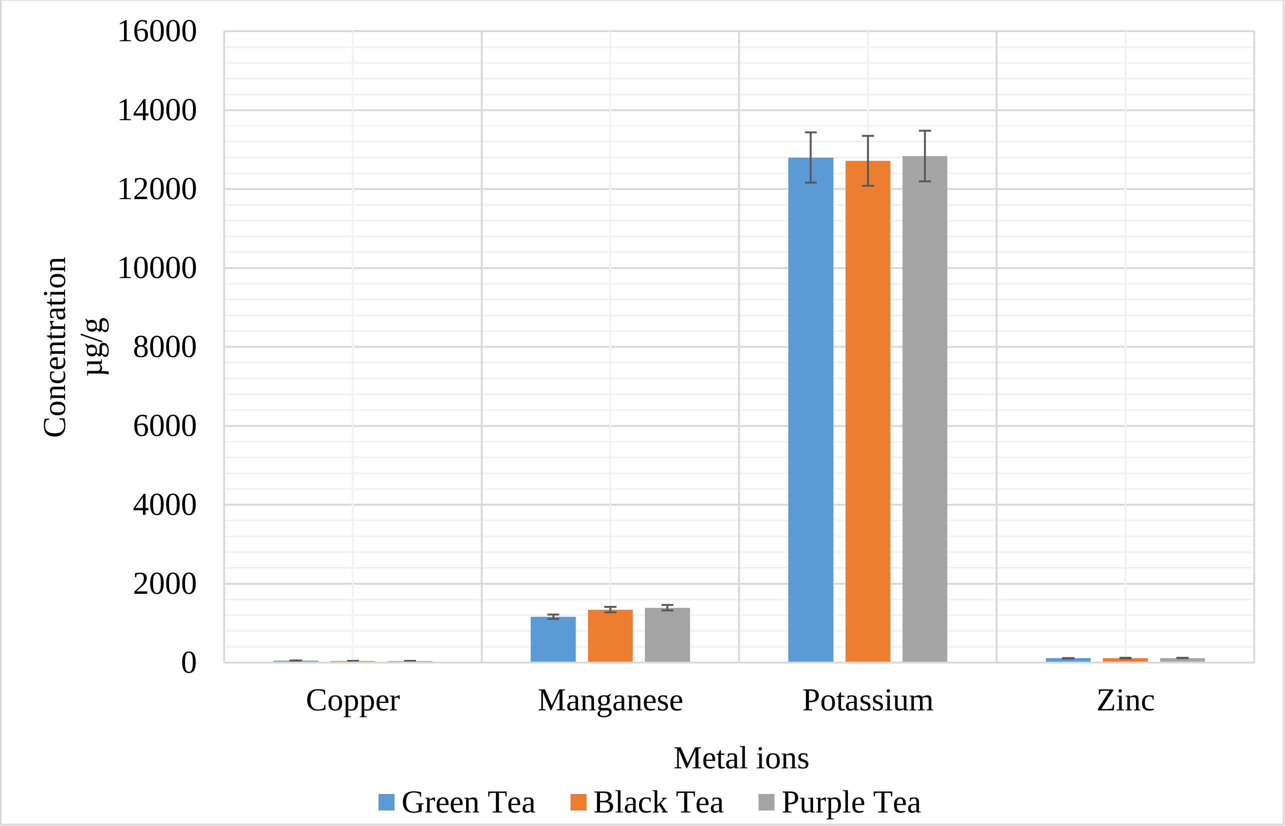

Figure 4.5: Mean metal nutrients levels in purple, black and green tea leaves

Since the levels of copper in all the tea samples analyzed were quite low compared to the levels of the other metal ions, a column chart (Figure 4.6) where potassium levels have been omitted has been provided for easy comparison.

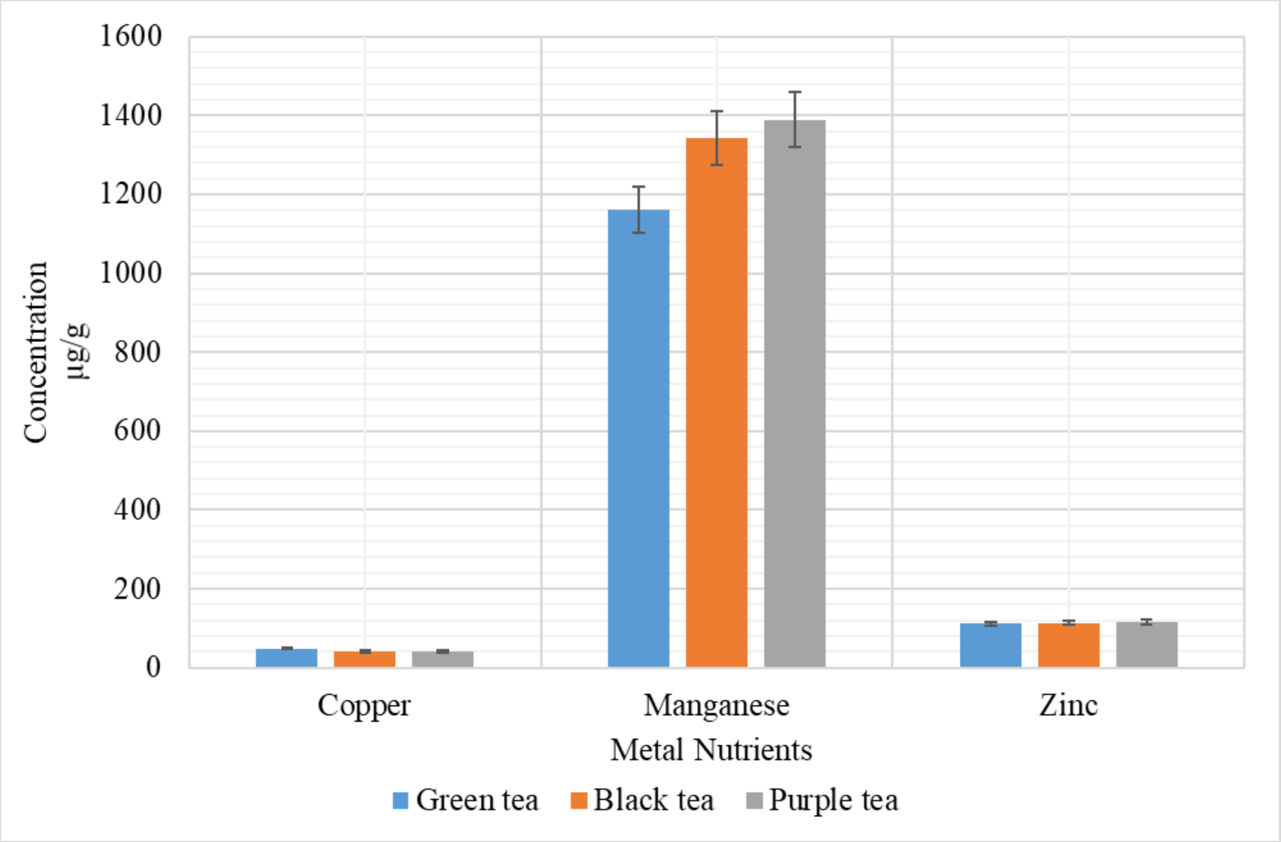

Figure 4.6: Levels of metal nutrients in purple, black and green tea leaves

4.3.9: Kruskal Wallis H test

Kruskal Wallis test was conducted to find out if the difference in the metal ion levels in the three types of tea leaves analyzed were of any statistical significance at 95 % confidence level. Table 4.8 shows the outcome of the Kruskal Wallis of population rank test. There is a significant statistical difference in the manganese levels in the three types of tea (p=0.013). There was no significant statistical difference in the zinc, copper and potassium levels in purple, black and green tea.

Table 4.8: Results of the Kruskal Wallis H rank test

Type of metal	Type of tea	n	Rank sum	Chi Squared Value (at 2.d.f)	P - Value
Copper	Purple	14	447	2.29	0.32
	Black	25	777		
	Green	29	1 122		
Zinc	Purple	12	417	0.91	0.63
	Black	24	716		
	Green	24	697		
Manganese	Purple	15	372	8.74	0.01
	Black	27	1 201		
	Green	30	1 055		
Potassium	Purple	14	489	0.20	0.91
	Black	22	707		
	Green	29	949		

4.3.10: Analysis of variance (One-way ANOVA test)

Similar to Kruskal Wallis H rank test, the analysis of ANOVA showed that there was a statistical significant difference in the means of manganese ions in all the three tea brand samples. There was no significant difference in zinc, copper and potassium mean levels (Table 4.9).

Table 4.9: One-way ANOVA test results

Metal	All samples		
	df	Fishers value	P- Value
Copper	67	1.74	0.18
Zinc	59	0.09	0.91
Manganese	71	6.88	0.00
Potassium	64	0.01	0.99

4.4: Copper, manganese, potassium and zinc element levels in tea infusions

4.4.1: Limits of detection and product moment correlation coefficient for the analytes

The mean potassium metal ions obtained in the tea infusions were considerably high in relation to all the other metal ions determined. The levels of manganese in all the infusions of the three types of tea brands were higher compared to the levels of copper and zinc but there is no considerable difference in metal ion concentrations in the different tea types.

The limits of detection for copper, manganese and potassium was 0.4444, 0.4738 and 0.2866 µg/g respectively while that of zinc was 0.0527 µg /Kg. The product moment correlation coefficient (R^2) for copper, manganese, potassium and zinc was 0.9899, 0.9886, 0.9946 and 0.9013 respectively. Concentrations of the metal elements in the sample solutions were determined by interpolation on the specific calibration curves. (Table 4.10).

Table 4.10: Limits of detection and correlation coefficient for metal analytes

Metal ion	Limit of detection	Correlation coefficient (R^2)
Copper	0.4444 (µg/g)	0.9899
Manganese	0.4738 (µg/g)	0.9886
Potassium	0.2866 (µg/g)	0.9946
Zinc	0.0527 (µg/ Kg)	0.9013

4.4.2: Copper, manganese, potassium and zinc ions levels in black tea infusions

As shown in Table 4.11, the highest value of copper levels of 25.80 ±11.60 µg/g was analysed in brand BG and the lowest level i.e. 15.10 ± 4.10 µg/g was measured in brand BH. After analysis of the black tea for manganese ions, 680.70 ± 45.90 µg/g was the highest level which was measured in brand BE while the lowest manganese ions value of 319.60 ± 26.60 µg/g was from brand BH (Table 4.11).

Table 4.11: Levels of metal ions in black tea infusions

Sample	Cu (µg/g)	Mn (µg/g)	K (µg/g)	Zn (µg/g)
Brand BA	17.0 ± 1.10	500.9 ± 51.20	12 291.5 ± 1 149.20	70.0 ± 1.10
Brand BB	17.7 ± 3.00	676.4 ± 206.30	12 727.3 ± 3 447.70	80.1 ± 10.60
Brand BC	25.6 ± 12.70	544.3 ± 24.70	13 003.5 ± 643.50	107.22 ± 0.50
Brand BD	17.4 ± 2.50	498.1 ± 15.60	10 317.9 ± 2.50	58.4 ± 23.90
Brand BE	109.7 ± 6.40	680.7 ± 45.90	9 557.7 ± 1 786.60	58.0 ± 0.90
Brand BF	18.2 ± 0.80	430.0 ± 2.30	8 404.9 ± 408.00	55.5 ± 10.00
Brand BG	25.8 ± 11.60	596.7 ± 5.20	8 607.7 ± 820.10	69.8 ± 7.20
Brand BH	15.1 ± 4.10	319.6 ± 26.60	11 397.7 ± 1 013.00	98.8 ± 0.70
Brand BJ	15.5 ± 0.10	480.4 ± 42.70	8 873.1 ± 552.80	70.2 ± 14.20

Figure 4.8 shows that brand BC tea infusion contained the highest concentration of potassium ions i.e. 13 003.50 ± 643.60 µg/g while the lowest concentration i.e. 8 404.90 ± 408.00 µg/g was

measured in brand BF. The highest zinc ions concentration i.e. 98.8 ± 0.70 µg/g was measured in brand BE while the lowest concentration of zinc ions i.e. 50.6 ± 8.50 µg/g was measured in brand BJ as shown in figure 4.9.

4.4.3: Copper, manganese, potassium and zinc ions levels in green tea infusions

As shown in Table 4.12, the analysed highest copper metal ions levels in the green tea infusions was from in Brand GF at 43.30 ± 19.90 µg/g while brand GK had the lowest value of 18.70 ± 3.80 µg/g. Brand GH tea infusion had the highest level of manganese of 938.20 ± 96.40 µg/g while the lowest value, 231.70 ± 3.80 µg/g was from GE. Brand GD had the highest potassium ions levels of 16 628.7 ± 246.20 µg/g with brand GC having a value of 10 587.30 ± 447.60 µg/g. After analyzing the infusions from green tea for zinc metal ions, had the highest levels of 99.40 ± 6.30 µg/g from brand GJ while brand GC had the lowest value, 50.60 ± 8.50 µg/g.

Table 4.12: Metal ions levels in green tea infusions

Sample	Cu (µg/g)	Mn (µg/g)	K (µg/g)	Zn (µg/g)
Brand GA	30.80 ± 0.60	474.80 ± 101.40	15 09 ± 2472.90	87.90 ± 3.90
Brand GB	19.00± 3.60	293.00 ± 17.10	14 808.80 ± 319.30	64.10 ± 3.50
Brand GC	24.80 ± 0.30	281.40 ± 22.70	10 587.30 ± 447.60	50.60 ± 8.50
Brand GD	26.80 ± 13.00	642.70 ± 23.20	16 628.70 ± 246.20	54.70± 3.70
Brand GE	27.00 ± 7.40	231.70 ± 3.80	12 378.50 ± 2 298.00	72.70 ± 24.20
Brand GF	43.30 ± 19.90	639.90 ± 25.20	12 691.10 ± 2 253.00	94.10 ± 1.70
Brand GG	28.10 ± 7.80	281.30 ± 42.40	11 410.50 ± 595.80	63.20 ± 0.60
Brand GH	26.70 ± 1.80	938.20 ± 96.40	13 739.30 ± 2 387.60	68.20± 20.90
Brand GJ	26.90 ± 7.20	669.90 ± 3.70	14 200.70 ± 352.30	99.40 ± 6.30
Brand GK	18.70 ± 3.80	428.60 ± 76.30	12 731.80 ± 728.90	51.90 ± 10.20

4.4.4: Copper, manganese, potassium and zinc ions levels in purple tea infusions

The purple tea infusions analyzed, copper, manganese, potassium and zinc levels are shown in Table 4.13. The highest copper level was 27.10 ± 4.10 µg/g from brand PC while brand PB had lowest value of 18.70 ± 3.30 µg/g. Manganese levels of 863.80 ± 3.00 µg/g was obtained from brand PB and the lowest value, 249.50 ± 2.50 µg/g from brand PD. Potassium ions levels of 10 205.20 ± 2 690.40 µg/g was high from brand PA and lower while the lowest at 5 715.40 ± 64.20 µg/g in brand PD. Brand PA was found to have the high level zinc levels of 144.20 ± 5.90 µg/g while brand PC had value of 63.20 ± 1.80 µg/g (Table 4.13).

Table 4.13: Metal ions level in purple tea infusions

Sample	Cu (µg/g)	Mn (µg/g)	K (µg/g)	Zn (µg/g)
Brand PA	22.70 ± 1.30	547.90 ± 4.40	10 205.20 ± 2 690.40	131.40 ± 32.40
Brand PB	18.70 ± 3.30	863.80 ± 3.00	8 988.90 ± 937.80	133.60 ± 61.60
Brand PC	27.10 ± 4.10	771.00 ± 46.20	9 886.10 ± 1 045.80	63.30 ± 1.80
Brand PD	20.40 ± 1.60	249.50 ± 2.50	5 715.40 ± 64.20	144.20 ± 5.90
Brand PE	24.30 ± 1.20	382.10 ± 23.20	7 342.30 ± 3 705.90	92.00 ± 6.40

4.4.5: Comparison of copper, manganese, potassium and zinc levels in tea infusions

As shown in Figure 4.7, copper ions levels in green tea infusions, black tea infusions and purple tea infusions varied from 43.30 ± 19.90 µg/g to 18.70 ± 3.80 µg/g, 25.80 ±11.60 µg/g to 15.10 ± 4.10 µg/g and to 27.10 ± 4.10µg/g to 18.70 ± 3.30 µg/g respectively. Green tea infusion had highest copper levels. Similar results were obtained by Fernandez-Caceres *et al.* (2001). The research involved determination of copper levels in a total of 46 tea infusions. The tea infusions included for 21 green teas, 23 black teas, and 2 instant teas. The tea samples were from China, Japan, India, Kenya, and Sri Lanka and a range of 7.60 µg/g to 37.50 µg/g were also obtained by Preedy (2013).

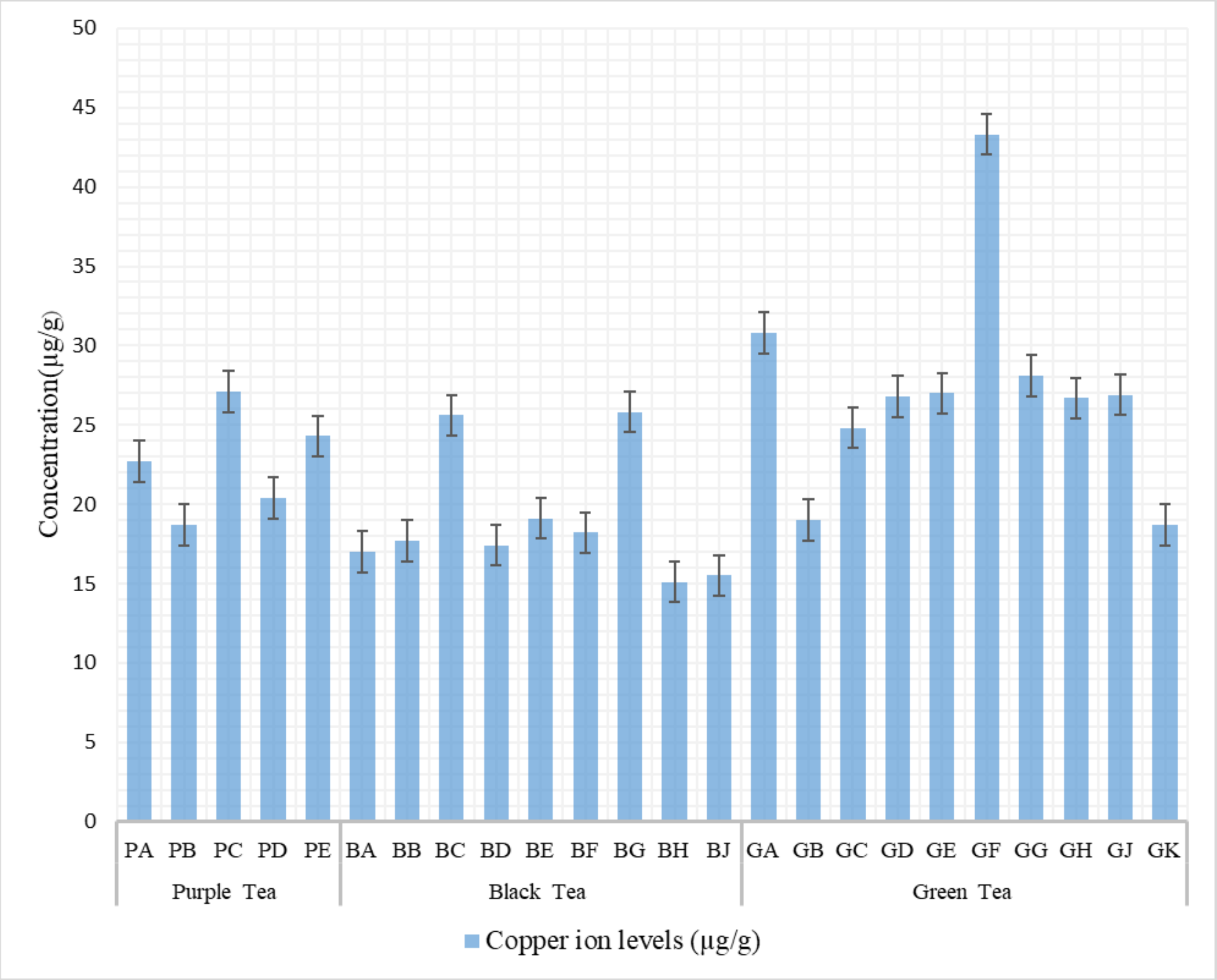

Figure 4.7: Comparison of copper levels in purple, black and green tea infusions

As shown in Figure 4.8, the manganese ions levels in green tea infusions, black tea infusions and purple tea infusions varied from 938.20 ± 96.40 µg/g to 231.70 ± 3.80 µg/g, 680.70 ± 45.90 µg/g to 319.60 ± 26.60 µg/g and 863.80 ± 3.00 µg/g to 249.50 ± 2.50 µg/g respectively. Tea leaves contain considerable levels of manganese element ranging from 200 µg/g to 1 200 µg/g. Manganese ions are easily extracted by water during tea infusion preparation (Zhen, 2002). The findings of this study confirms the report made by Zhen (2002).

According to the study by (Tsushida & Takeo, 1979), black tea infusions contains higher levels of copper than green tea infusions. The range of copper content found in the current study is very close to the findings of the researchers cited.

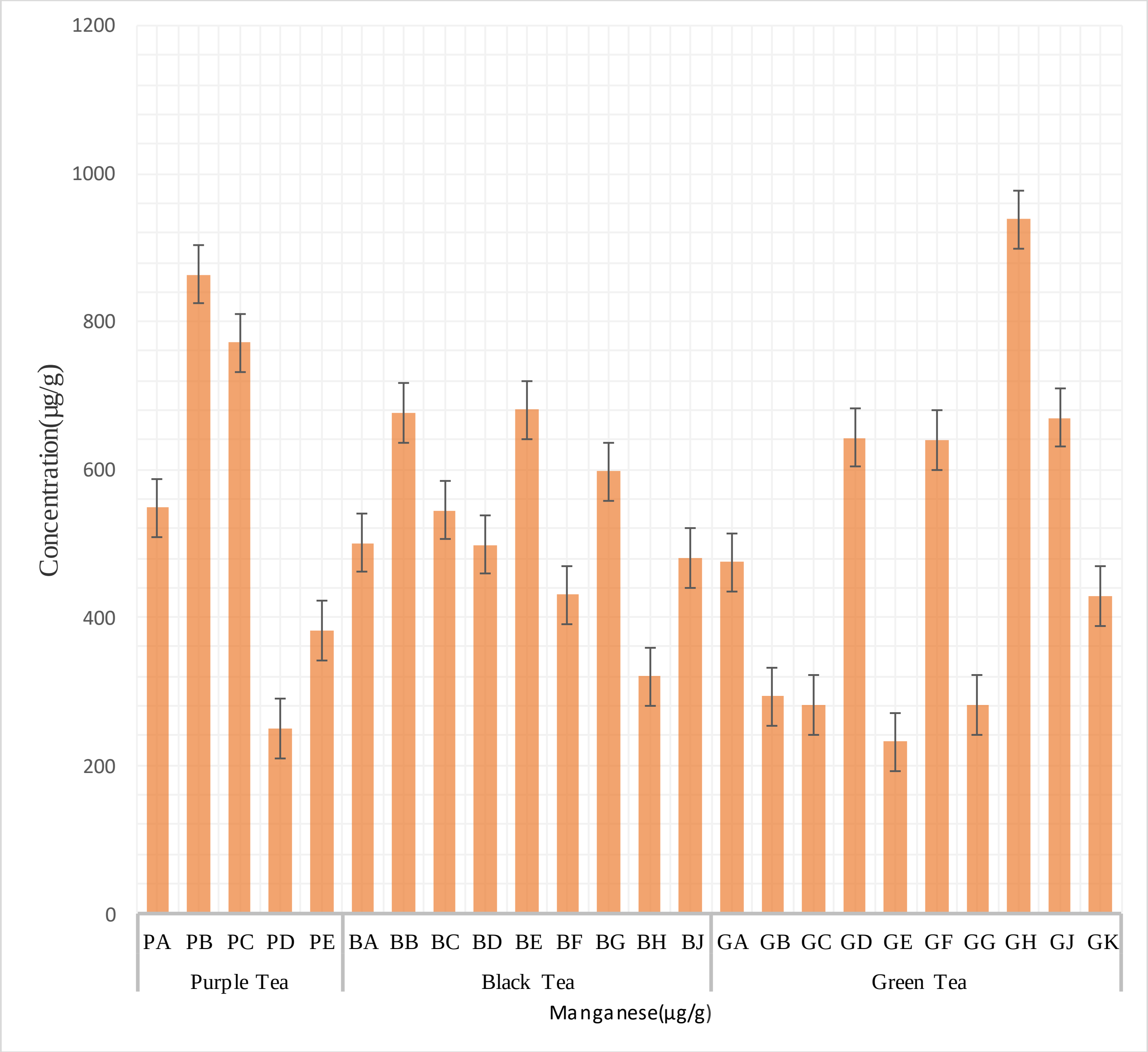

Figure 4.8: Comparison of manganese levels in purple, black and green tea infusions

As shown in Figure 4.9, the potassium ions levels in green tea infusions, black tea infusions and purple tea infusions varied from 16 628.70 ± 246.20 µg/g to 10 587.30 ± 447.60 µg/g, 13 003.50 ± 643.60 µg/g to 8 404.90 ± 408.00 µg/g and 10 205.20 ± 2 690.40 µg/g to 5 715.40 ± 64.20 µg/g respectively. According to numerous data, potassium forms the highest content of total ash of young tea leaves (Han *et al.*, 2018).

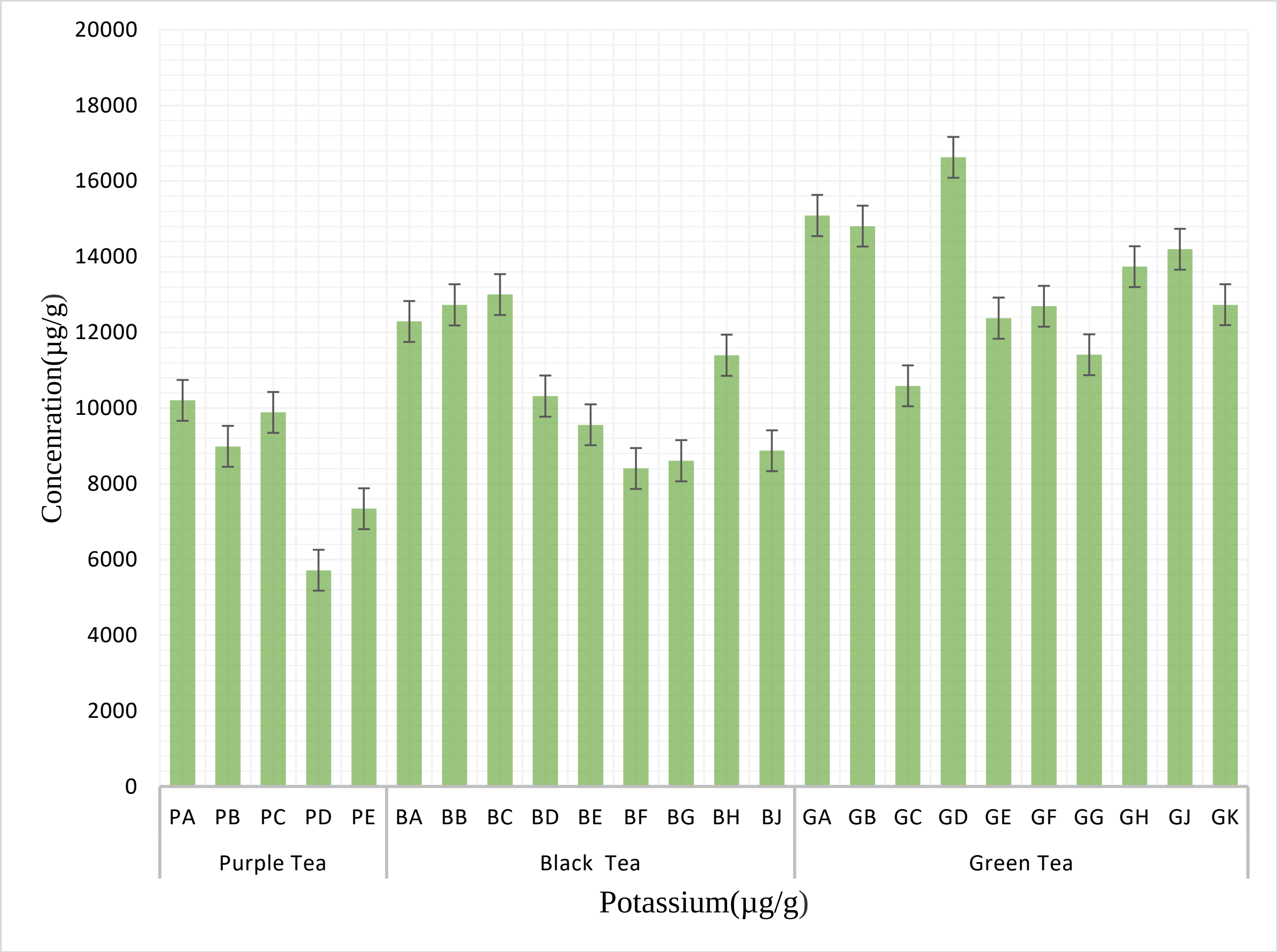

Figure 4.9: Comparison of potassium levels in purple, black and green tea infusions

As shown in Figure 4.10, the zinc ions levels in green tea infusions, black tea infusions and purple tea infusions varied from 99.40 ± 6.30 µg/g to 51.90 ±10.20 µg/g, 98.80 ± 0.70 µg/g to 50.60 ± 8.50 µg/g and 144.20 ± 5.90 µg/g to 70.70 ± 19 µg/g respectively.

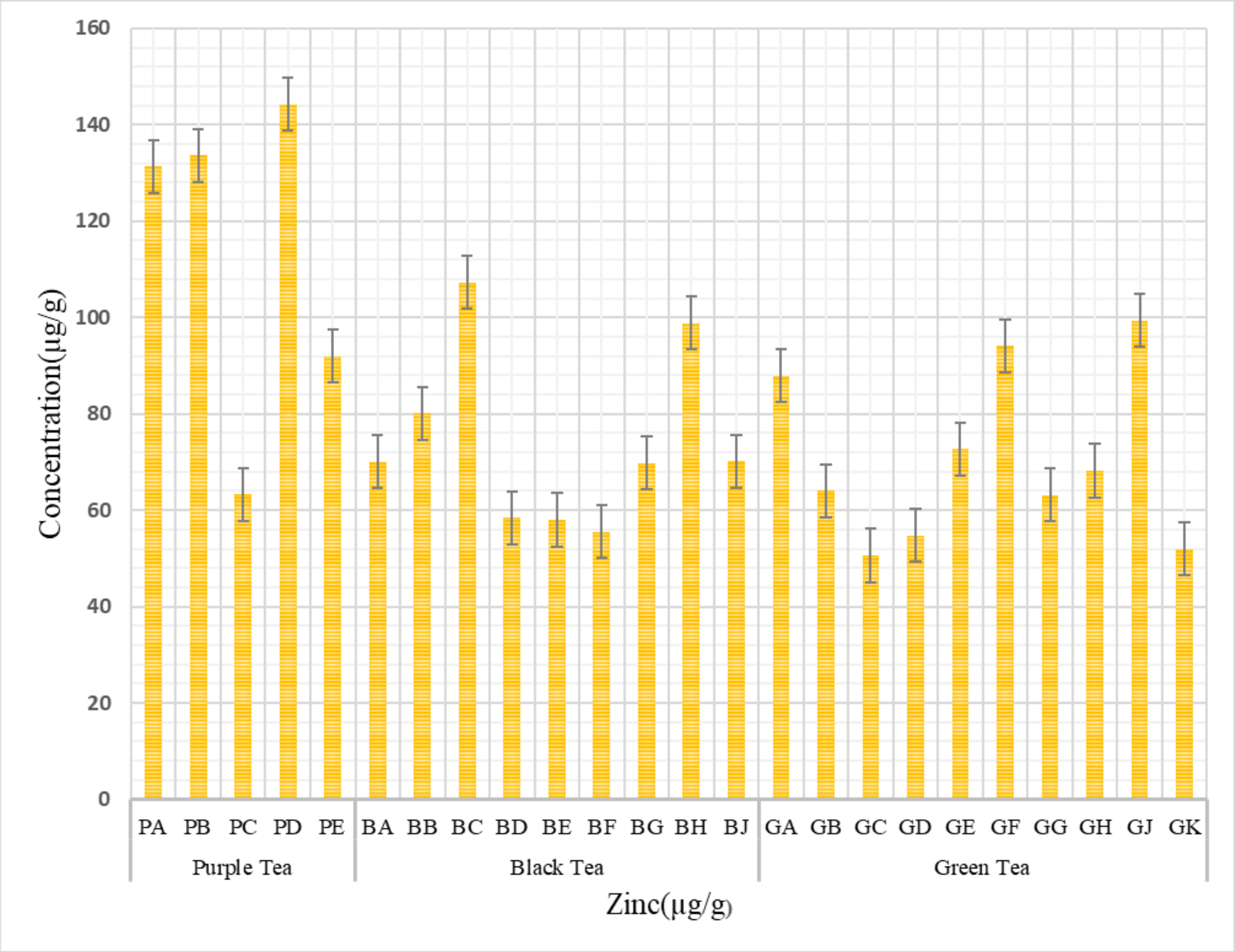

Figure 4.10: Comparison of zinc metal nutrient levels in purple, black and green tea infusions

4.4.6: Comparison of the mean metal ions levels in green, black and purple tea infusions

Table 4.14 shows the average levels of the metal ions analyzed in the three types of tea infusions. Mean levels of copper metal ions in purple, black and green tea infusions were found to be 22.60 ± .36 µg/g, 19.00 ± .59 µg/g and 27.20 ± .92 µg/g respectively. Mean levels of manganese metal ions in green, black and purple tea infusions were found to be 488.10 ± 22.70 µg/g, 525.30 ± 12.40 µg/g and 562.90 ± 24.30 µg/g respectively. Potassium metal ions had mean levels in green, black and purple tea infusions of 13 426.70 ± 209.30 µg/g, 10 575.70 ± 210.20 µg/g and 8 427.60 ± 238.50 µg/g respectively. Mean zinc metal ions levels in green, black and purple tea infusions were 70.70 ± 1.90 µg/g, 74 ± 2.00 µg/g and 112.90 ± 3.90 µg/g respectively. The mean levels of potassium metal ions in all the tea infusions was the highest in relation to manganese, zinc and copper metal ions (Figure 4.11).

Table 4.14: Mean metal nutrient levels in black, green purple tea infusion

Mineral	Type of tea	Mean levels in tea infusions	n	Standard deviations	median	Min	max
Copper	Black	19.00	18	0.59	17.70	12.20	34.60
	Green	27.20	20	0.92	25.20	16.00	57.40
	Purple	22.60	10	0.36	22.60	16.40	30.00
Manganese	Black	525.30	18	12.40	518.70	300.80	822.20
	Green	488.10	20	22.70	442.80	229.00	1 006.40
	Purple	562.90	10	24.30	547.90	247.70	865.92
Potassium	Black	10 575.70	18	210.20	10 485.40	8 027.80	15 165.20
	Green	13 426.70	20	209.30	13 646.50	10 270.80	16 838.60
	Purple	8 427.60	10	238.50	8 736.20	4 721.80	12 107.60
Zinc	Black	74.00	18	2.00	73.70	41.50	107.60
	Green	70.70	20	1.90	63.20	44.60	103.90
	Purple	112.90	10	3.90	102.60	61.90	177.10

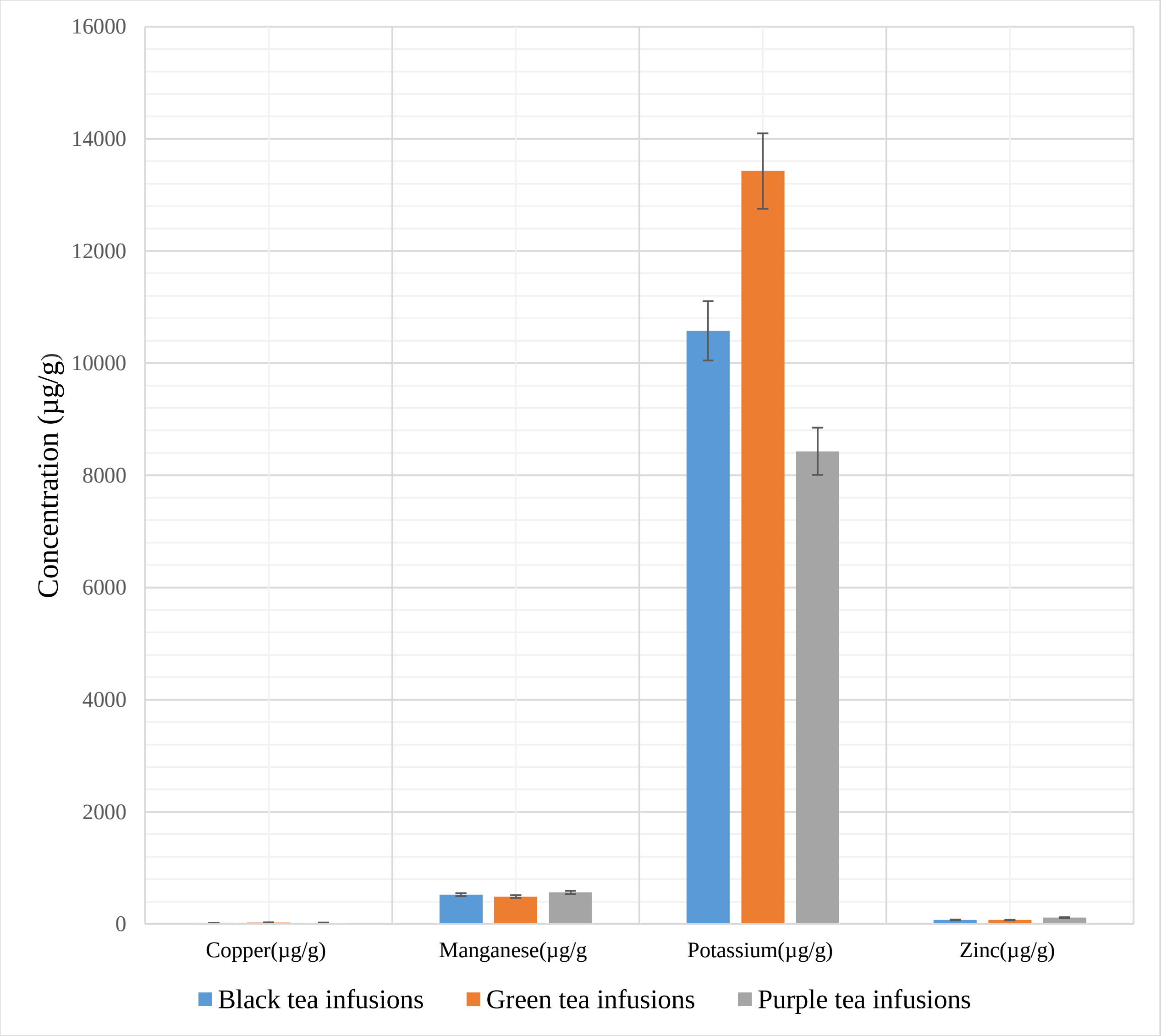

Figure 4.11: Mean Levels of metal nutrient in purple, black and green tea infusions

4.4.7: Kruskal Wallis test

Kruskal Wallis test was conducted to find out if the difference in the metal concentrate in the three types of tea were of any statistical significance at 95 % confidence level. Table 4.15 shows the results of the Kruskal Wallis of population rank test. There was no significant statistical difference in the copper, potassium and zinc levels in the samples drawn from the three types of tea ($p \leq 0.05$). There was a significant statistical difference in the means of tea infusion levels in manganese.

Table 4.15: Results of the Kruskal Wallis of population rank test

Type of metal	Type of tea	n	Rank sum	Chi Squared Value (at 2.d.f)	P - Value
Copper	Black	18	319.50	7.66	0.02
	Green	20	606.50		
	Purple	10	250.00		
Manganese	Black	18	459.00	0.84	0.66
	Green	20	448.00		
	Purple	10	269.00		
Potassium	Black	18	365.00	21,805	0.00
	Green	20	699.00		
	Purple	10	112.00		
Zinc	Black	18	407.00	9.89	0.01
	Green	20	402.00		
	Purple	10	367.00		

4.4.8: Analysis of variance (one-way ANOVA test)

The analysis of variance (ANOVA) showed that there was a statistical significant difference in the means of manganese and copper in the three type of tea in all samples (Table 4.16). In both tests, there was no significant difference in zinc and potassium concentrations.

Table 4.16: Results of the One-way ANOVA test

Metal	All samples		
	df	Fishers value	P- Value
Copper	47	0.36	0.70
Zinc	47	10.60	0.00
Manganese	47	0.49	0.61
Potassium	47	19.60	0.00

4.5: Levels of compliance of metals levels in tea leaves to the established specifications

Copper and zinc are examples of elements which were initially identified as contaminants in most foods including tea products but they have since been established as essential micronutrients (Mahan *et al.*, 2012). As a result, in most Kenya Standard documents these elements have been assigned a maximum value. In the black tea Specification (KS 65: 2016), copper and zinc have been assigned maximum levels of 150 µg/g and 50 µg/g respectively (Table 4.4) hence any food containing higher levels than the ones specified are not recommended for human consumption. Since potassium and manganese are not recognized as potential contaminants in food products, they cause less concern to regulators or policy makers hence their levels have not been specified in food products in Kenya, East Africa and Codex Standards (Table 4.4).

From the findings in this study, the potassium metal ion levels in all the tea leaves brands analyzed were significantly higher compared to those of manganese, copper and zinc as shown in Figure 4.5. Manganese ions levels were also considerably high in all the tea leaves samples though not as high as potassium as shown in Figures 4.6 and 4.11. Relatively similar results were published by Reilly and Wenlock *et al* (Deshpande, 2002). These researchers established that dry tea leaves and their respective tea infusions may contain 900 mg/Kg and up to 38.0 mg/Kg manganese (Szefer and Nriagu, 2006). The levels of zinc were much lower compared to potassium and manganese though they were slightly higher than those of copper.

From the Kenya standard specifications (KS 65: 2016) for black tea the maximum levels for copper and zinc are 150 ppm and 50 ppm respectively (Table 4.4). Levels of copper and zinc have been included in the list of contaminants hence it is the maximum levels that are specified.

None of the metal ions in this study has been specified in the purple tea specification KS 2745: 2017. In the purple tea specification (KS 2745: 2017), only the levels of metals known to be toxic even at low levels such as lead, arsenic and cadmium have been specified. Levels of iron fillings have also been specified in this standard since there is a high possibility of tea products getting contaminated with iron fillings during the production process.

4.6: Availability of minerals in tea leaves and tea infusion

The content of nitrogen, potassium and phosphorus in tea leaves is greater than 2000 µg/g while the level of manganese, aluminium, calcium, magnesium, sodium and fluorine ranges between 500 µg/g and 2000 µg/g and the content of zinc, iron, copper and boron ranges between 5 µg/g to 500 µg/g (Zhen, 2002).

Composition of minerals in tea leaves is related to the concentration of minerals in the respective tea infusions. K, Na, Ni and F are highly extractable, Al, B, Co, Cu, mg, Mn, P, Si and Zn are moderately extractable while Ba, Ca, Fe, Mo, Sr and V are less extractable (Justino, 2020). These levels of efficiency in extraction and the amount of elements in tea leaves partly explains why the levels of metal ions in this study exhibited the following order; K>Mn>Zn>Cu.

This study has established that tea infusions can be important sources of manganese and potassium. This finding is similar to those of Caroli, (2007) who also recognized tea leaves as a source of manganese and went ahead to suggest that the large amount of potassium in tea infusions could be beneficial to hypertensive individuals.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1: Conclusion

This research established that the three types of tea leaves contain low levels of copper and zinc elements in relation to the levels of potassium and manganese. During the analytical process of determining the levels of copper, potassium, manganese and zinc using the AAS technique, it was established that the graphite furnace method of atomization was more reliable when determining the levels of zinc element in the tea leaves.

In all the tea brands analysed, the levels of copper, manganese, potassium and zinc ranged from 41.20 ± 12.47 to 48.50 ± 19.40 µg/g, 913.60 ± 471.95 to 1 342.10 ± 310.26 µg/g, 12 716.30 ± 241.56 to 12 837.90 ± 357.60 µg/g and 111.10 ± 29.98 to 116.60 ± 20.25 µg/g. The levels of metal elements in the tea leaves analyzed arranged in the following order: K>Mn>Zn>Cu. From these research findings, it can be concluded that all the three types of tea leaves are reliable sources of potassium and manganese.

Tea infusions prepared from the tea leaves analysed in this study had levels of copper, manganese, potassium and zinc ranging from 19.00 ± .59 to 27.20 ± .92 µg/g, 488.10 ± 22.70, to 562.90 ± 24.30 µg/g, 8 427.60 ± 238.50 to 13 426.70 ± 209.30 µg/g respectively. The levels of metal elements in the tea infusions analyzed also had a similar order to that of the tea leaves i.e.: K>Mn>Zn>Cu. This study has informed that the difference in the levels of copper, zinc, potassium and manganese in the tea leaves and tea infusions of black, green and purple tea is insignicant.

5.2: Recommendation

This study has established that tea contains essential minerals which includes copper, manganese, potassium and zinc in varying concentrations.

i) It is recommended that assessment of metal nutrient levels using a larger sample size to in order to explain the significant statistical difference in the manganese levels in the three types of tea.

ii) It is also recommended that similar research be carried out using a less weight of samples during the analytical process so as to minimise the number of dilutions required when determining potassium and zinc. This may result in reducing the experimental error.

iii) Determination of the levels of copper, manganese, potassium and zinc using other elemental analytical techniques other than Atomic Absorption Spectrometry and ICP-MS techniques, is recommended in order to have a more comprehensive assessment.

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APPENDICES

Appendix I: Calibration curves

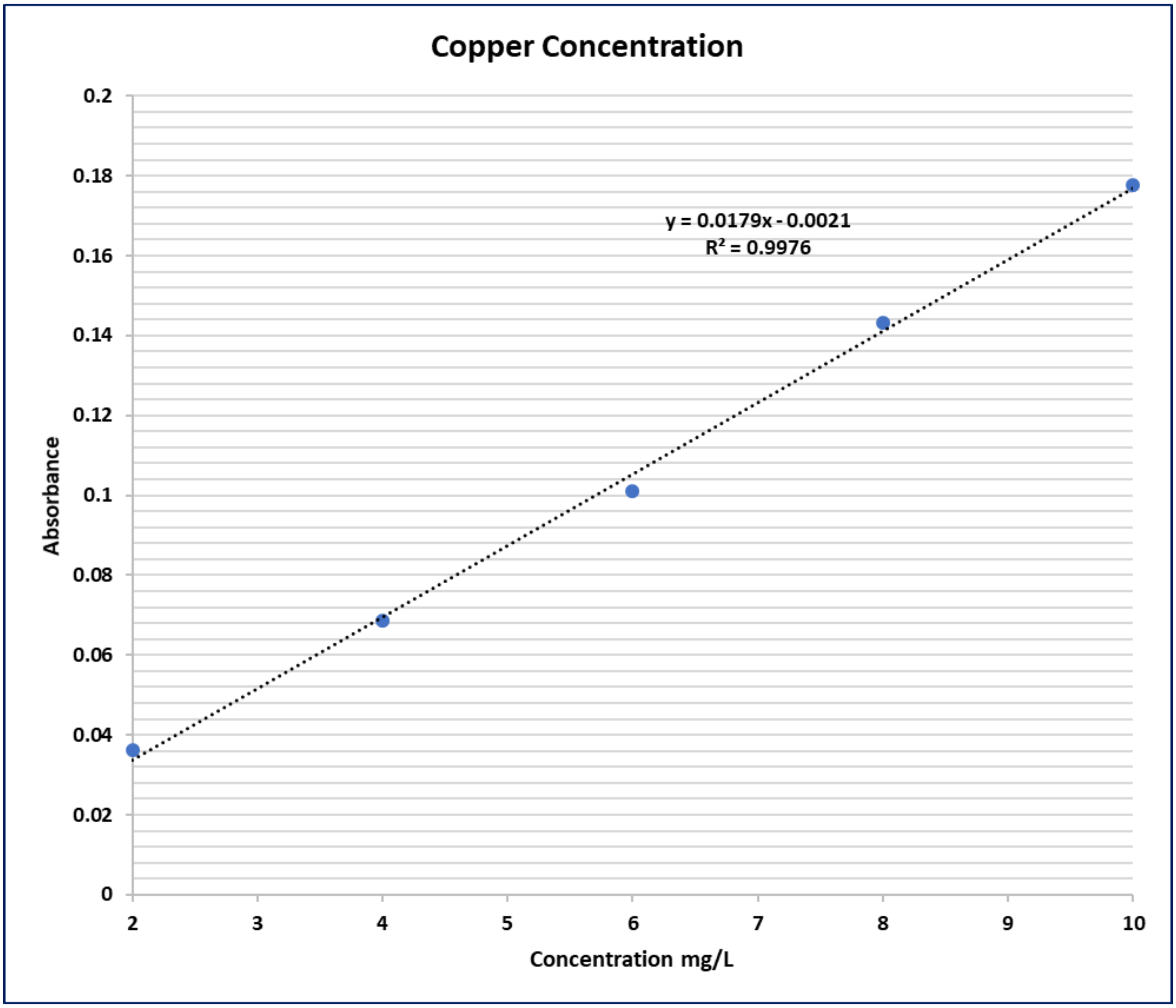

Figure 1A: Copper standard calibration curve

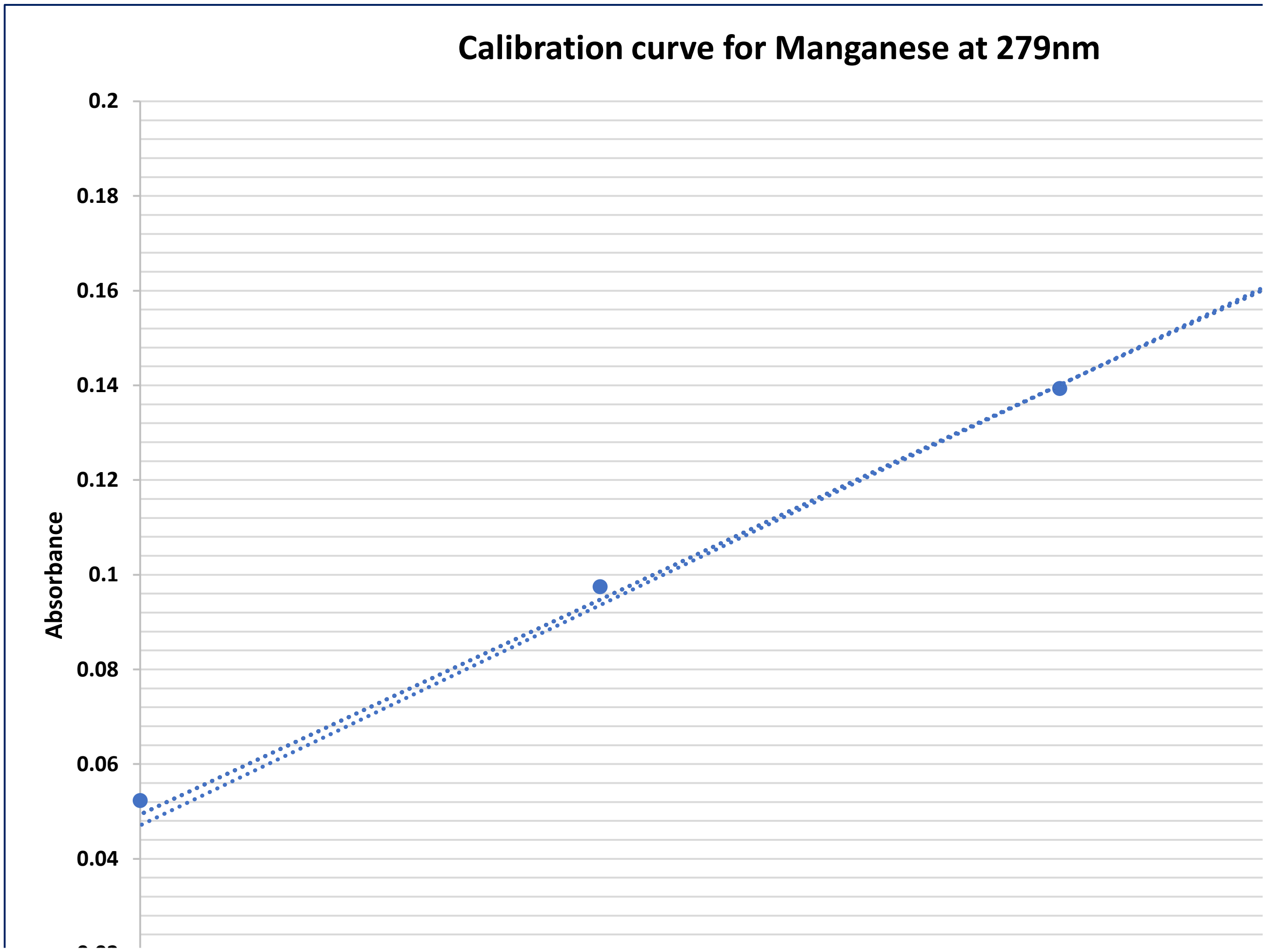

Figure 1B: Manganese standard calibration curve

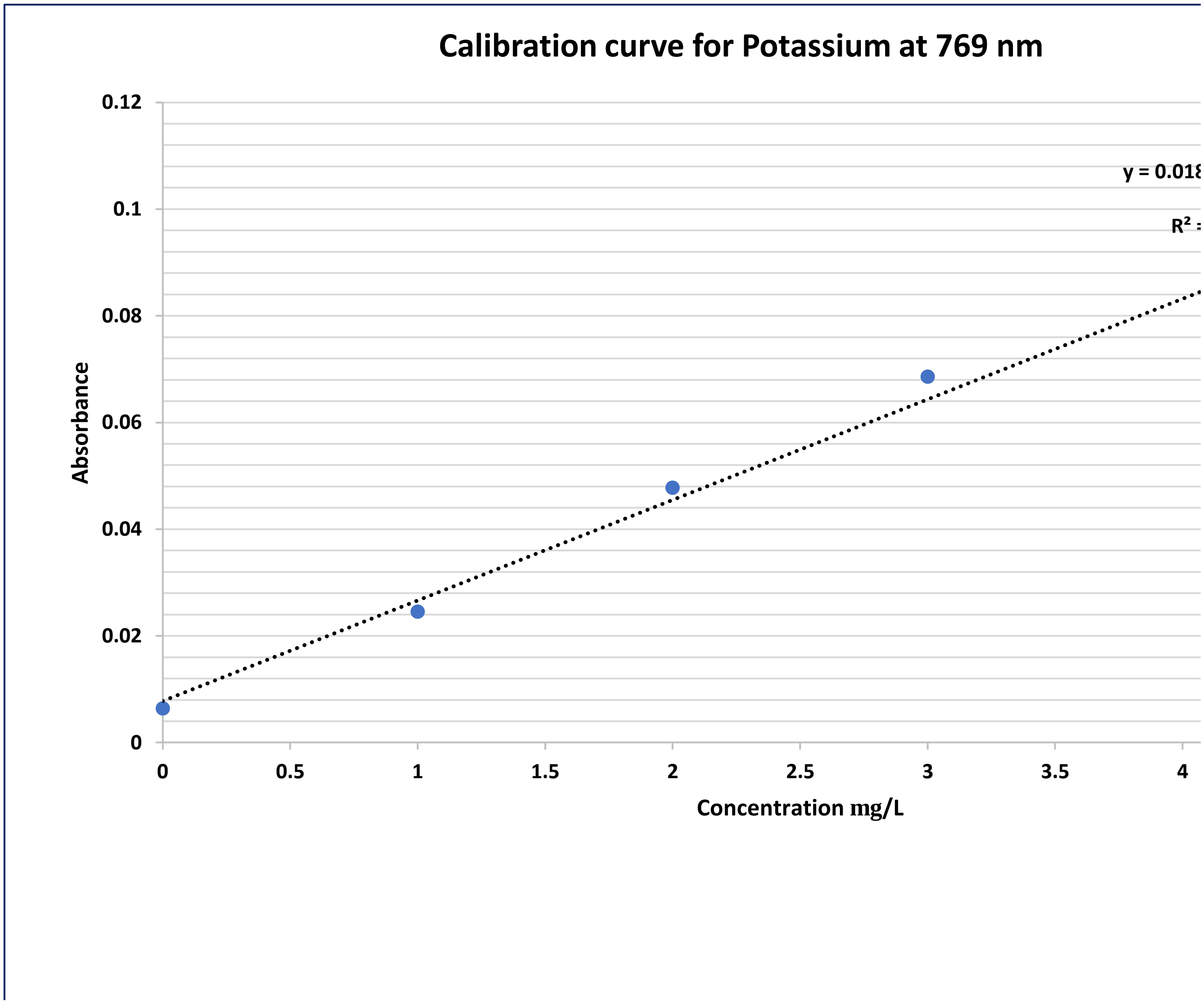

Figure 1C: Potassium standard calibration curve

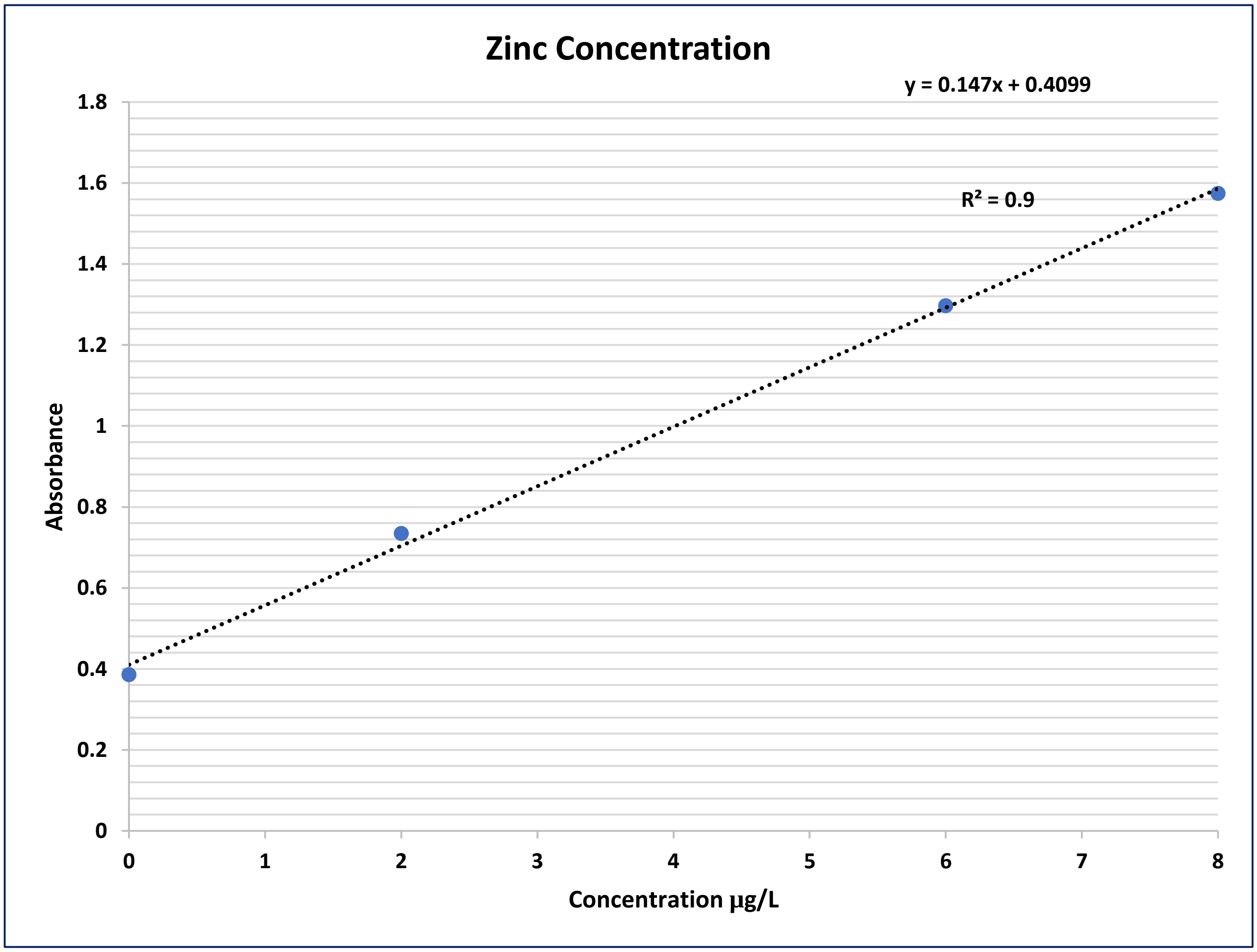

Figure1D: Zinc standard calibration curve

Appendix II: Levels of metal ions in different types of tea

Sample	Cu (µg/g)	Mn (µg/g)	K (µg/g)	Zn (µg/g)
Brand GA	63.00 ± 0.78	1295.5 ± 10.95	18390.60 ± 109.40	115.00 ± 1.98
Brand GB	27.60 ± 4.11	598.00 ± 12.46	13410.60 ± 1193.07	99.10 ± 15.43
Brand GC	38. 40± 2.62	1197.00 ± 1.78	6759.90 ± 1117.97	101.50 ± 0.39
Brand GD	45.10 ± 4.97	1420.30 ± 56.90	14307.80 ± 1491.34	175.40 ± 27.37
Brand GE	42.50 ± 3.74	754.90 ± 23.00	11990.20 ± 188.15	105.20 ± 2.24
Brand GF	45.70 ± 9.22	1405.50 ± 39.55	14529.70 ± 3048.67	106.00 ± 37.06
Brand GG	42.40 ± 4.01	961.40 ± 25.26	16436.90 ± 5373.30	96.70 ± 9.14
Brand GH	47.50± 1.64	1568.10 ± 27.08	13300.80 ± 1014.39	105.20 ± 3.11
Brand GJ	50.50 ± 6.96	1569.20 ± 38.15	12132.70 ± 697.64	75.60 ± 2.72
Brand GK	87.00 ± 40.86	847.20 ± 57.65	6961.10 ± 2148.28	145.00 ± 21.46
Brand BA	19.90 ± 2.69	953.80 ± 201.63	11953.30 ± 2830.01	93.60 ± 13.76
Brand BB	47.90 ± 7.32	1525.50 ± 31.52	13935.10 ± 44.56	64.10 ± 9.88
Brand BC	38. 60± 7.21	1390.50 ± 41.69	14861.80 ± 883.84	117.90 ± 0.70
Brand BD	38.70 ± 8.28	1369.90 ± 7.13	14245.2 ± 259.08	213.40 ± 13.90
Brand BE	56.50 ± 1.62	1389.70 ± 23.93	9316.50 ± 702.90	104.50 ± 23.60
Brand BF	37.60 ± 2.95	1258.40 ± 31.68	14093.60 ± 544.290	93.00 ± 15.57
Brand BG	41.005 ± 2.96	184.40 ± 26.79	12158.60 ± 241.84	132.10 ± 8.94
Brand BH	45.30± 0.82	787.20 ± 18.20	14489.50 ± 1380.98	115.30 ± 12.79
Brand BJ	57.70 ± 18.24	1561.30 ± 25.24	11015.20 ± 3666.85	56.60 ± 3.35
Brand PA	26.30 ± 4.78	919.80 ± 24.75	13441.00 ± 1590.44	109.60 ± 4.18
Brand PB	52.50 ± 8.67	1367.2 ± 136.01	11379.10 ± 1920.91	140.50 ± 14.72
Brand PC	48.10± 9.64	1357.80 ± 26.81	16240.90 ± 313.40	91.60 ± 0.29
Brand PD	27.60 ± 3.18	135.70 ± 27.29	7852.90 ± 1401.23	99.50 ± 6.51
Brand PE	46.50 ± 3.91	787.30 ± 49.21	16498.50 ± 834.91	125.60 ± 7.93