DETERMINATION OF THE CONCENTRATION OF ZINC AND CHROMIUM IN TEA LEAVES (Camellia sinensis) AND INFUSED TEA SAMPLES

BY

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CERTIFICATION

This is to verify that this research work was carried out by OYEMAH FAVOUR ALFRED (Miss) of the Department of Chemistry at the University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

This project is dedicated to the all sovereign and merciful God for helping me through the course of this work. This work is also dedicated to my family for their consistent support and care.

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ABSTRACT

The study was designed to assess the concentrations of zinc and chromium in some commercially available green and Black sold teas they include Lipton, Top tea, Richmond tea, Cinnamon Tea and Natural green tea within Benin City, Nigeria. Five of the most popular brands among consumers were purchased in the open market. They were digested, infused (cold and hot) and analyzed for their heavy metal content using the atomic absorption spectrophotometer. The heavy metal concentration varied among the different brands of tea in the study. In the tea samples zinc concentration ranged between 35mg/ kg to 70mg/kg while chromium gave the lowest value of 0.65mg/kg and maximum concentration of 22mg/kg. The cold and hot infusion samples revealed very low concentrations of both zinc and chromium (most of them below detectable limits) ranging between 0.03 mg/l to 0.10 mg/l.

In conclusion, the risk of heavy metal exposure via the consumption of these tea is low, with no significant health implications to consumers and thus does not pose a threat to food safety.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Tea is a product of the leaves of the Camellia sinensis plant, it is an aromatic beverage prepared by pouring hot or boiling water into the cured (processed) or fresh leaves of the Camellia sinensis plant. This ever green plant is native East Asia but however, grown in about 45 countries globally. It is cultivated in countries like China, India, Kenya, Zimbabwe, Sri Lanka, Turkey, Mexico, Korea, Phillipines (Li *et al*.,2015) etc. However, it is recorded historically that tea has been cultivated in China for over 2000 years (Mondal, 2009).

Tea is one of the most popular caffeinated nonalcoholic beverages consumed throughout the world (Zhang *et al.*, 2018a). It is said to have numerous beneficial effects including prevention of many diseases, according to pharmaceutical and epidemiological studies (Katherine., 2001). It is also used in folk medicine for headaches, digestion, and enhancement of immune system, as an energizer and to prolong long life. Generally, tea is consumed because of its attractive and sdistinct colors, flavor, aroma, and taste and health benefits.

Heavy metals are those metals that are relatively denser and can be poisonous in tiny amounts. Anthropogenic activities like mining, vehicular emissions, machining, wastes and effluent from building and manufacturing industries, fossil fuels, fertilizer application etc. are known sources of heavy metal pollution in the environment (air, water and soil). The accumulation of heavy metals in tea plants depended primarily on the physiological properties and the metal absorption mechanism of the plants, the chemical speciation of metals in soil and the physiochemical properties of the soil (Fang *et al*., 2007). Both essential element and risks metals are extracted during tea infusion (Dambiec *et al.,* 2013), Serious health damage can result from toxic metal accumulation in different parts of the human body like liver, kidney etc. (Shaheen *et al*., 2016) as a result of the continuous ingestion of food, beverage etc. with even as a little of these toxic metals.

1.2 Background of Study

Several countries have imposed laws to restrict the concentration of heavy metals which are harmful to human health by the intake of food and beverages. Reports have implied the presence of some heavy metals in tea, particularly since tea plants are known to accumulate trace metals (Bosque *et al*., 1990). Subsequently, (Jain *et al.*, 2013) reported that although tea has various health benefits, analysis of the potential toxicities and possible harmful effects of high dose consumption on human health remains largely ignored. A vast range of articles about tea components suggest the presence of heavy metals, catechins, other nutrients, and antioxidant compounds (Cabrera.,2003, Welna *et al*.,2012). It is well known that economic development and human activities are responsible for serious environmental issues caused by heavy metal accumulation in agricultural system around the world (Li et al., 2015).

1.3. Statement of Problem

Toxic heavy metals from different industrial operations that contaminate the environment is a global challenge. Several physical, chemical and biological methods have been adapted consistently to remediate this global menace. Food and beverage consumption are pathways by which humans are exposed to the toxic metals (De Oliveira *et al*., 2018). Several studies have reported the concentration and possible health risks of heavy metals in tea leaves, made tea, and tea infusion (Salahinejad and Aflaki., 2010; Seenivasan *et al*.,2008).

Risk assessment regarding heavy metals in tea is crucial to potential heavy metal exposure of the ever increasing tea consumers, hence the need for this study.

1.4 Relevance of Study

The quality of tea is important for the development of the tea industry, the farmers' income and the health of the consumers. In particular, heavy metals in tea are very important indicators in the process of tea quality evaluation; because it can enter the human body by means of tea consumption and this pose potential risk to human health. A majority of the elements available in food at major, minor, and trace amounts are seen to be essential to the wellbeing of humans.

However, the appropriate concentration required for this purpose depends on the type of metal, the age and sex of consumers(Ahmed *et al.,*1989). Both metallic and non-metallic elements are needed by the human body for growth, development, and proper functioning, thus the determination of these elements in food, beverage, water, plants and soil has become of utmost importance and is currently the subject of study by various researchers (WHO, 1998).

1.5 Scope of Study

This study was designed to determine the concentration of Zinc and chromium in tea leaves *(Camellia Sinensis*) and tea infusions. The various samples were digested and the concentrations of heavy determined using Atomic Absorption Spectrophotometer (AAS).

1.6 Aim and Objectives

The aim of this study was to determine the concentration of chromium and zinc in tea leaves and infused tea from locally available tea brands. .

To achieve the aim, the following objectives were set:

- Collection of selected tea brands from local markets in Benin City metropolis.
  Digestion of the tea leaves using acids, and infusion of the tea in both hot and cold deionized water for 15 minutes.
- Determination of the heavy metal concentration in the samples using AAS.

1.2. Literature Review

1.2.1. Tea

Tea is an infusion made from leaves plucked from the tropical evergreen plant *Camellia sinensis*. (Karimi *et al*., 2008). Tea is the second most widely consumed beverage because of its taste, aroma and health benefits. It has been reported to be valuable in the treatment and prevention of many diseases. It's economic and social importance is unprecedented. It is estimated that over 18 billion cup of tea are consumed daily globally (Fernandez *et al*., 2001).

1.2.2. Types of Tea

Some of the most popular tea produced are given below, there are currently six primary types of tea each with unique trademark characteristics and processing techniques (Atalay *et al*., 2017).

1). Black Tea

Black tea is the most commonly consumed type of tea globally. It is known for its bold robust flavor and dark color (Khan and Hassan., 2013). The leaves of black tea are fully fermented and oxidized which gives them their distinctive taste and color. China and India are the leading producers of black tea. Countries like Vietnam, Nepal, Sri Lanka and Kenya are emerging tea producing nations that export black tea. Black tea is relatively caffeinated, so it is necessary to limit it's consumption (Peluso *et al*., 2016)

It is enriched with flavonoids, which enhance immunity and reduce inflammation (Kodagoda *et al*., 2017). It also alleviate pain and lowers swelling from small cut, scrapes, and bruises when it is heated cooled and applied (Khan and Hassan., 2013).

2. Green Tea

The green tea is another variety of tea produced by the Camellia sinensis plants. This tea is delicate and mild, it is minimally oxidized (steamed or pan fried) this helps to preserve its natural green color and retain its subtle (vegetal) flavor. China and Japan are the two countries where green tea is largely produced. Green tea is said to have about half the amount of caffeine as compared to black tea (Reygaert., 2017). It contains high concentration of flavonoids which can improve heart health, by reducing blood clotting and bad cholesterol (Reygaert., 2017). It is shown to have antiinflammatory properties which support healthy and clear skin.

3). Oolong Tea

It is partially fermented, it falls between black tea and green tea in terms of flavor and oxidation level (which ranges between 10-80%) and can brew up anywhere from a pale yellow to a rich amber cup of tea. Many oolongs can be re-infused many times with subtle differences and nuance in each successive cup (Pan-Pan *et al*., 2018). China and Taiwan are the main countries that manufacture oolong tea.

4). Pu-erh Tea

It is an aged particularly fermented tea (i.e. the leaves are aged using longer and more gradual process) that is similar to black tea in character (Haipeng *et al*., 2013) containing around the same amount of caffeine as black tea, this improve the richness and flavor of the tea overtime.

5). Herbal tea

Also known as tisane, it is not technically a type of tea as it is not made from tea leave (Camellia sinensis plants). Instead it is made from a variety of herbs, flowers, fruits, and spices. Herbal tea has a wide range of flavors and health benefits depending on the ingredients used (Poswal *et al*., 2019). Some popular herbal tea include: chamomile, peppermint, turmeric, ginger. Olive leaves lavender etc. among many others. Herbal tea typically has zero caffeine (Chandrasekher *et al*., 2018).

6). White Tea

This tea is minimally processed (oxidized and withered), which results in a light and refreshing taste. White tea is often characterized by its silver white bud and young leaves and it's usually brewed at lower temperatures for a shorter duration compared to other types of tea. They contain very little caffeine, while certain silver tip teas could have a little bit more (Sanlier *et al*., 2018). White tea is majorly produced in China especially in the Fujian province, where it has a long history.

Because of its high antioxidant content it may be the best tea for preventing many types of cancer, it's has high concentration of fluorides, tannins, catechins etc. which can strengthen teeth, combat plaque and increase their resistance to acid and sugar (Dias *et al*., 2012).

Beyond these categories however, there's a vast array of different tea varietal, growing conditions and processing methods. Even when using the same varietal of tea, and similar growing and processing conditions, tea grown in different locations will develop different characteristics.

Ultimately, there are as many different types of tea as there are tea producers.

1.2.3. Processes involved in Tea Production

Specific tea processing methods have been developed over hundreds of years, each of these tea has its unique characteristics through the different harvesting and processing methods. Some of them are listed below:

1). Growing/Harvesting

Camellia sinensis plants must be grown and harvested; this is the first step in making tea. Growing conditions and Harvesting methods can have a huge impact on the flavor of the finished tea. The environment of the tea can be one of the most fundamental sources of tea's flavor. Changes of climate, soil or even surrounding vegetation can subtly affect the tea leaves and the resulting flavor in the cup.

Premium leaves are plucked by hand to preserve natural sweetness but mass producers harvest by machine, this process does speed up production but exposes more surface area of the leaf enhancing the quick release of bold and dark flavors.

Farmers can also manually change the growing conditions of the plant to exert control over the tea's chemical composition.

2). Withering

The Camellia sinensis plants are thick and waxy, therefore they have to be softened or withered to make them pliable for crafting. The leaves are laid out on fabric or bamboo mats and left to wilt. The withering process reduces the water content of the leaves by much as half, without it, subsequent heating steps would produce something likened to cooked vegetables rather than dried tea. Farmers can also control variables like temperature etc. in this process with great precision. **3). Bruising**

Oolong tea, black tea and pu-erh tea usually undergo some sort of bruising process like rolling, twisting, or crushing. This is done so as to break down cell walls in the leaves and facilitate the next step oxidation.

4). Oxidizing

Oolong and black tea are left to turn brown (the cell walls that have been broken, an enzymatic reaction turns the leaves brown just like a cut apple).

Leaves must be carefully monitored during this process, because missing the correct moment can mean ruining the tea or crafting something completely different from what was intended. This Browning process is the primary differentiating factor between different types of tea, Green tea, pu-erh tea crafting skip this process, i.e. it is completely unoxidized.

5) Fixing

Fixing is carried out to stop the oxidation process. The tea leaves is heated, heat application denatures the enzymes and stops the leaf from continuing to turn brown. It is applied to all leaf types except black tea which oxidation is halt by drying instead. This process actually serve to preserve whatever green color is left in the leaf at this stage.

6). Drying

Finally, all tea leaves must be dried to remove any residual moisture and create a shelf stable leaf. Again this method can dramatically change the flavor of the tea.

After it is dried, the tea is ready to be packaged and shipped all over the world.

Fig. 1.1: Schematic diagram showing the various processes in tea production.

1.2.4. Components of Tea Leaves and It's Importance (Health Benefits)

Tea is said to contain polyphenol, catechins, flavonoids like quercetin, myricetin, kaempfenol etc. , amino acids, tannic acids and other antioxidants (Brzezicha, 2016). It contributes 63%of the flavonoids in the diet and 69-85% of flavonoid content is soluble in hot water brewing within the first 3-5 minutes. Theaflavins which are condensation products of flavan-3-ols contribute to tea taste (Hicks *et al*., 1996).

The polyphenols such as epigallocatechin are responsible for the medicinal properties of tea which includes: anticancer activities, hepatoprotective, cholesterol lowering, prevention of obesity, Alzheimer's disease and high blood pressure. Tea is also useful in the elimination of alcohol and toxins from the body (Serafini *et al.,* 2012). They are used In folk medicine for headaches, digestion, diuresis, enhancement of immune defense, as an energizer and to prolong life. Tea leaves also contain minerals such as potassium, manganese, selenium, boron, zinc, strontium and copper which are very important minerals needed by the body (Seenivasan *et al*.,2008). Other health benefits of tea consumption are outlined below:

- Boosts immune system.
- Aid in weight loss by helping to boost metabolism and burning fats.
- Improve heart health by reducing the risks of heart diseases, lower cholesterol levels and improve cardiovascular health.(Chung *et al*.,2003).
- Reduces stress and anxiety by enhancing a sense of calm and relaxation.
- It also helps to improve sleep quality and promote better mental health.
- Boosts brain function by improved alertness and mental performance.

Promote digestion by alleviating digestive issues such as bloating, constipation, and nausea. It also reduces inflammation in the digestive tract (Chandrasekara *et al*., 2018)

Researchers have opined that increase in the consumption of tea may be beneficial in cancer prevention. (Salahinejad and Aflaki., 2010).

The daily dietary requirements of some elements can be met via the daily consumption of tea (Karak and Bhagat., 2010).

1.2.5. Heavy Metals

Members of an ill-defined subset of elements that show metallic properties are referred to as heavy metals (Reena *et al*., 2011). Any metal (or metalloids) species can be seen as contaminants if it occurs where it's unwanted or in forms or concentration that poses to harmful to humans and the environment. Such metals or metalloids include lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr), copper (Cu), selenium (See), nickel (Ni), silver (Ag), zinc (Zn), other less common contaminants include: aluminum (Al), cesium (Ce), cobalt(Co), magnesium (Mg), molybdenum (Mo), strontium (Sr), and uranium (U) (Chronopoulos *et al*., 1997). Some essential heavy metals like iron(Fe) whose deficiency can result in anemia, fatigue, perinatal mortality

(Anand *et al*., 2014, Ahmed *et al*., 2014)etc. cobalt (Co) required for vitamin B12 synthesis

(Varela-Moreiras *et al*.,2012) , manganese (Mg) which helps in bone and cartilage development (Leach.,1988), it also acts as an antioxidant, aids wound healing (Marrotte *et al*.,2010), molybdenum (Mo) known to reduce dental caries (Burguera and Burguera., 2007) and also necessary in small amount for bounding molydo-enzyme in the body, zinc (Zn) etc. are heavy metals needed by the human body but are required in varying amounts (Reena *et a*l.,2011). Others such as mercury, plutonium and lead (non-essential) have been seen to have no beneficial effects on the human body and their accumulation over time can results in several illness like reduction of cognitive development and intellectual performance in children, the liver and kidney can also be damaged (Baars *et al.*,2001). From various study, it has seen that all metals are toxic at higher concentrations (Kumar *et al*., 2005).

CHROMIUM

Chromium is a transition metal with a complex chemistry. Chromium In water and soil it exhibits an oxidation number ranging between -2 to +6 (Unceta *et al.,* 2010). The most stable being Cr

(VI) and Cr (III) this states are interconvertible depending on the solution's PH value(Sharma *et al*.,2008). The +6 oxidation state is more stable and of great concern due to its toxic effect on humans, animals and microorganisms (Alemayehu *et al.,* 2011). For humans this is dependent on the dosage, exposure level, and duration of exposure (Shanker *et al*., 2019). Chronic inhalation can result in damaged respiratory tracts, nasal septum, nasal itching and soreness (Saha *et al*., 2011). Ingestion via food, drinks, beverages etc. can have adverse effects on the liver, kidney, gastrointestinal, immune system, and blood (Ray., 2016). A dose of 2-5 grams of soluble Cr^{6+} can be fatal to an adult human (Sharma *et al*., 2008).

ZINC

Zinc is an essential trace element which plays keen role in various important biological processes in the human body (Maret ., 2017). It is the second most abundant macronutrient in the body after iron (Maret .,2016 ; Lim *et al.,* 2013), being a major component of more than 3000 enzymes, and even greater number of proteins, which shows how indispensable it is to human health (Vallee *et al*., 1993). The divalent cation is potentially required for catalytic, structural and regulatory functions in the body (King *et al*., 2011; Andreini *et al*., 2005), zinc is also essential for several cellular processes such as differentiation, apoptosis and proliferation which influences growth and development of an organism (Maret and Sandstead., 2006).

The acute effects of zinc are usually the result of short term-high dose exposure and depends strongly on the point contact, also chromic somatic effects tend to be associated with low-dose exposure over an extended period of time (Jerome.,2007). Sideroblastic anaemia, hypochromic microcytic anaemia, leukemia, tymphadenopathy, neutropenia, hypocupraemia and hypoferriamia are some of the most common effects associated with long term excessive zinc intake ranging 150mg/day to 12mg/day. Oral uptake of small amount of zinc is essential for survival. The recommended dietery allowance (RDA) for zinc is 11mg/day for men, 8 mg /day for women (Scott *et al*., 1983), lower intake is recommended for infant 2-3mg/day. 225-400mg has been seen to be an emetic dose (Brown *et al*., 1964). Patient often recover to normal blood patterns after cessation of zinc intake with or without copper supplementation (Jerome., 2007).

1.2.6. Effects of Heavy Metals on Human Health

Ideally, tea should be free from contaminants such as heavy metals, some of these heavy metals which are toxic and harmful because of their non-biodegradable nature and long biological half life can accumulate in different parts of the body like the liver, kidney, heart, e.t.c after prolonged consumption of food and beverages containing such heavy metals. This can results in bone diseases, cardiovascular disorder, risk of different cancer, coronary artery disease (Hiranta *et al*., 2003), Parkinson disease (Richard., 2001) etc.

1.2.7. Possible Sources of Heavy Metals in Tea Leaves

Tea can be contaminated during growth period and manufacturing process which might increase the metal body burden in humans (Seenivasan *et al.,* 2007). The metallic constituents of tea leaves are normally different varying according to the type of tea leaves and the geological source (Marcos ., 1996). . Other sources of heavy metals in tea leaves include their growth media, nutrients, agro input such as organic and inorganic fertilizer application especially phosphate fertilizer which are known to have variable levels of Cd, Cr, Ni, and Pb, pesticides and fungicide used in agricultural lands (soils). However, the rate of these contaminants depends on the rate of application of the contributors with its elemental concentrations and the soil characteristics to which it is applied to (NAFDAC, 2003).Irrigation waters, as well as industrial activities such as mining, high temperature processing of metals (smelting and casting) emits metal in particulate and vapor forms which can be disperse by wind or precipitated in rainfall causing contaminant in soil. Other industrial activities include: processing plastic, textile, microelectronics, coal burning power plants, petroleum combustion etc.

1.2.8. Determination of Heavy Metals in Tea

The atomic absorption spectroscopy (AAS) is an analytical technique used to determine the concentration of metal atom/ion in a sample. It analyses the concentration of element in a sample base on energy absorbed from certain wavelength of light (electromagnetic radiation). 75% of the Earth's Chemical elements are metal, sometimes the content of metal in a substance is desirable, but at other times can also be contaminants/dangerous. As a result measuring the metal content in various matter compositions is critical in many different applications.

1.2.9. Principle

All atoms can absorb light (electromagnetic radiation), at specific wavelength. When a sample containing a metal ion is exposed to light at the Characteristics wavelength, only the metal atom will absorb this light. The amount of light absorbed at this wavelength is directly proportional to the concentration of the absorbing ion or atom.

The electrons within an atom exist at various energy levels, when the atom is exposed to it's own wavelength of light, it can absorb energy (photons) and electron move from a ground state to excited. The radiant energy absorbed by the electron is directly related to the transition that occurs during this process. The radiation represents a unique property of each individual element and can be measured.

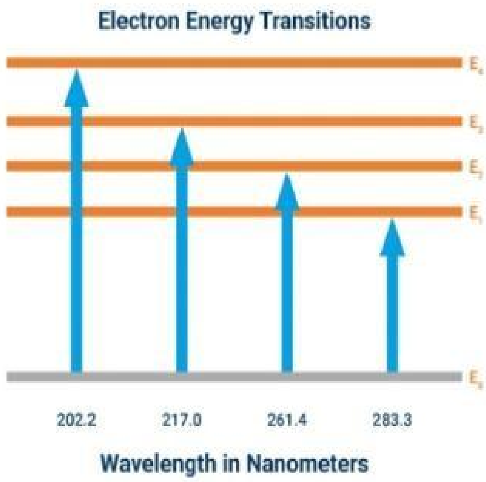


Figure 1.2: Diagram showing electron energy transition at various wavelength. **(***Thermofisher scientific)*

The Beer Lambert's law describes the relationship between light absorption and the concentration of the element. According t No the law, the amount of light absorbed is proportional to the number of atoms excited from the ground state in the flame.

$A = \varepsilon * c * l$

A is there absorbance

E is the molar absorption coefficient; it is the absorptivity of the sample at a particular wavelength (L/mole/cm). c is the determined concentration of the element (mol/liter).

l is the path length, it's fixed for all measurements (cm)

1.2.1.1. Components of A.A.S.

The atomic absorption spectrophotometer consist of five major parts

Atomizer: here heat energy is utilized in atomic absorption spectroscopy to covert metallic elements to atomic dissociated vapor. It is carried out using either by a flame it furnace.

Light source: to give continuous source of radiation in the Atomic absorption spectrophotometer. Hollow cathode glow discharge lamps are usually used to give sharp emission lines for a specific element in the AAS instrument.

Monochromator: this is an optical device that transmits a narrow band of wavelength of light and other radiation from a wider range of wavelengths. The Spectra through a monochromator can be shown by a curve.

Detector: it converts light source coming from a monochromator into amplified electrical signal which can be recorded. The amount of light getting to the detector is a function of the concentration of the element of interest in the sample being analyzed.

Recorder: this usually a computer system and software, it receives the analog signals from the detector and converts it to readable responds.

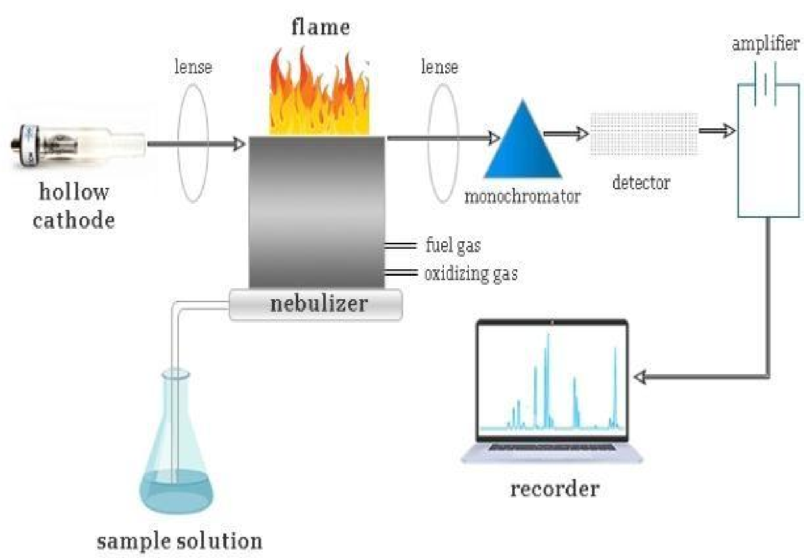


Figure 1.3: Schematic representation, showing the various components of the AAS machine

1.2.1.2. Application of A.A.S.

The Atomic absorption spectroscopy has unlimited number of applications in various field of science, this may include: agriculture (food and beverage), water, clinical research and pharmaceutical analysis, mining operations to determine the percentage of precious metals contained in ores, environmental control etc.

1.2.1.3. Basic Advantage of the Technique

Some of the advantages of the Atomic absorption spectrophotometer include the following;

1) It is easy to operate
2) It has high sensitivity and accuracy
3) It is void of most free element interference
4) It has a wide range of applications with low cost charge for analysis

CHAPTER TWO

MATERIALS AND METHODOLOGY

2.1 Sampling Collection

Four different brands of locally available teas samples were purchased from local markets in Benin City, Edo State, Nigeria.

2.2 Chemicals and Reagents

All reagents used for this study were of analytical grade and was obtained from the Department of Chemistry University of Benin, Benin City, Edo State. The chemicals were used without further purification.

Concentrated nitric acid (HNO_3)

Concentrated hydrochloric acid (HCl)

Deinoized and distilled water (H_2O)

2.3 Equipment/Apparatus

The equipment and apparatus used for this study and their function is given below

Atomic absorption spectrophotometer (210 VGP, BUCK SCIENTIFIC)

Electric balance (AR 2130, Ohaus corporation)

Measuring cylinder (100ml).

Digestion vessels (500ml).

Spatula

Beaker (500ml)

Volumetric flask (100ml)

Electric heater

Funnel.

2.4 Sample Preparation

The glassware used for this analysis was pre-washed to remove any impurities before use. The wares were then washed with a little nitric acid solution and finally rinsed with deionized water before oven dried..

2.5 Digestion of Tea Samples

One gram of the tea samples was digested using concentrated nitric acid and hydrochloric acid. The digested sample was mixed using a measured volume of a mixture of 15 ml nitric acid and 5 ml hydrochloric acid (3:1). The solution was then heated until clear white fumes were observed. After cooling, the sample was transferred into 50ml volumetric flask and the volume adjusted to the mark using deionized water.

2.6 Tea infusion

A total of 3 g of each tea sample was weighed (balance name and model) and then was added to 500 ml of boiling deionized water and allowed to infuse for 15 minutes. The sample was then filtered using a Whatman No.42 filter paper. Another set of tea samples solution was prepared in a similar way using room temperature deionized water as described by Karami *et al*.,(2008).

2.7 Determination of Heavy Metals in Tea

The concentration of chromium and zinc was determined for all the samples after the digestion of the tea leaves and preparing the tea infusion samples using the atomic absorption spectrophotometer (AAS).

2.8 Data Analysis

The concentrations of the Chromium and Iron in the various tea samples were analyzed employing mean, standard deviation and analysis of variance (ANOVA) using Microsoft Excel.

Figure 2.1: Tea samples used for the experiment

Figure 2.2: Digestion and infusion samples.

Figure 2.3: Showing some of the apparatus used in the study

CHAPTER THREE

RESULTS AND DISCUSSION

The concentrations of selected heavy metals (zinc and chromium) in tea leaves as well as infused tea samples were studied in order to assess potential human exposure from these metals. Results obtained from the experiments are presented below. Table 3.1 (Figure 3.1 and Figure 3.2) shows the concentrations of zinc and chromium in the selected brands of locally available tea. The concentration of zinc was found to range between 35.00 mg/kg to 70 mg/kg representing Sample A and Sample E respectively. However, the values obtained were found to fall within the permissible limits of 99.4 mg/kg as set by WHO/FAO in 1996

Zinc was not detected in Sample B, Chromium reported the highest concentration of 22 mg/kg for Sample C while the lowest concentration of 0.65 mg/kg was obtained in Sample D. chromium was not detected in Samples A and C. Comparison with set regulatory standards revealed that Sample C exceeded the permissible limit of 1.5mg/kg as prescribed by WHO and FAO and could therefore be a potential threat to human health.

Table 3.1: Heavy metal concentrations (mg/kg) in the selected tea samples:

Sample	**Zinc (mg/kg)**	**Chromium (mg/kg)**
Sample A (black tea)	35	Not detected
Sample B (black tea)	Not detected	2
Sample C (green tea)	40	22
Sample D (black tea)	55	0.65
Sample E (Cinnamon tea)	70	Not detected

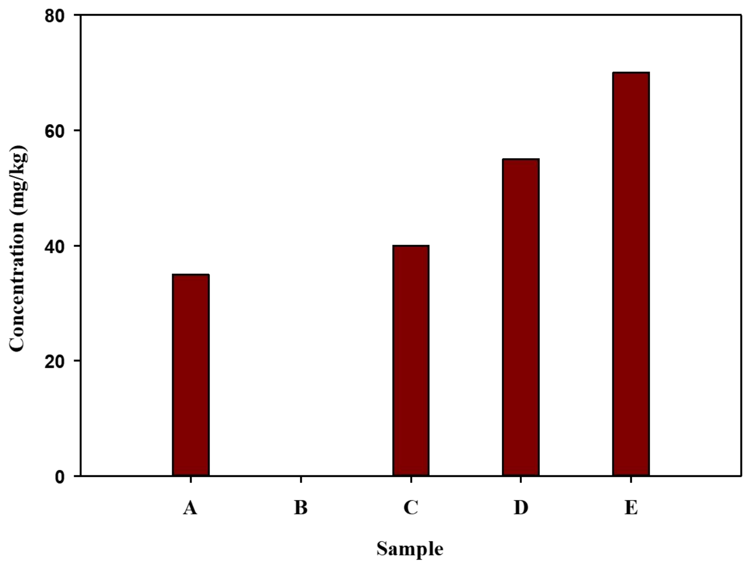


Figure 3.1: Concentration of zinc (mg/kg) obtained in the selected tea samples

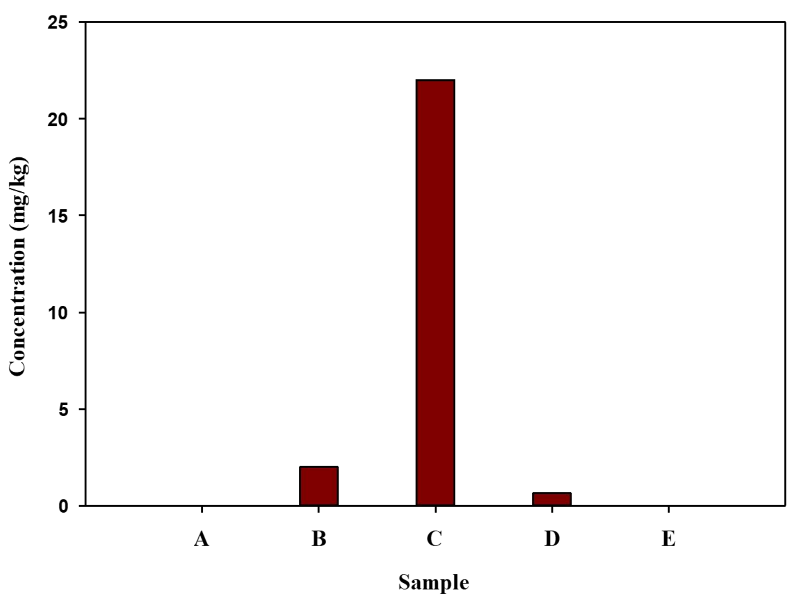


Figure 3.2; Concentration of chromium (mg/kg) obtained in the selected tea samples.

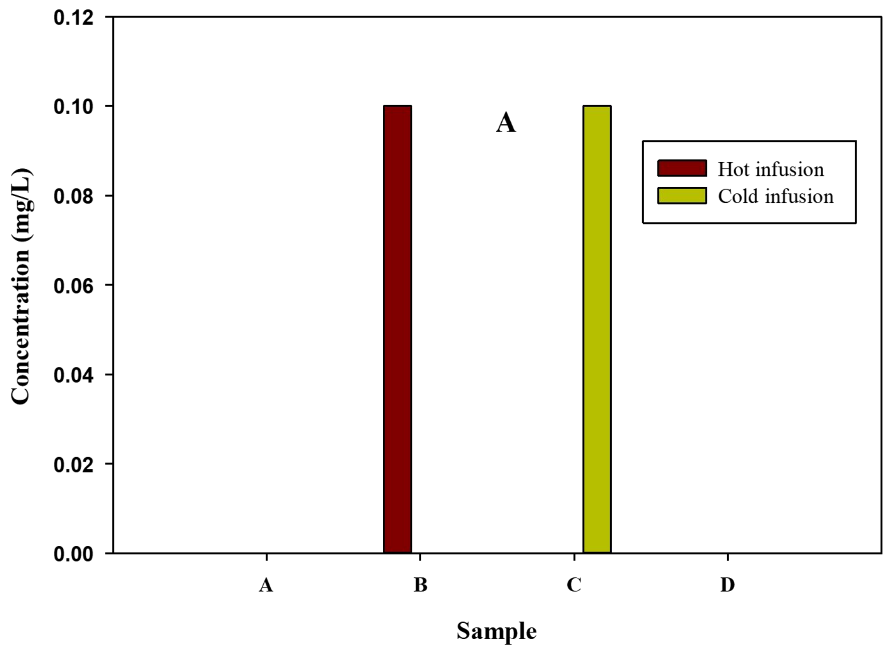
Table 3.2 depicts the concentrations of the heavy metals in tea samples infused in hot water. Assessment of the samples using AAS revealed zinc detected only in Sample B tea with a concentration of 0.1 mg/l detected and was not detected in the other tea samples while chromium was detected in all the samples except Sample B. Chromium concentration range of 0.01 to 0.11 mg/L was obtained for the tea samples for the duration of the experiment.

Table 3.2: Heavy metal content in the hot infusion tea samples:

Sample	**Zinc(mg/l)**	**Chromium (mg/l)**
Sample A	Not detected	0.11
Sample B	0.1	Not detected
Sample C	Not detected	0.02
Sample D	Not detected	0.01

Table 3.3 Heavy metal content in cold Tea infusion:

Samples	**Zinc(mg/kg)**	**Chromium (mg/kg)**
Sample A	Not detected	Not detected
Sample B	Not detected	0.01
Sample C	0.1	Not detected
Sample D	Not detected	0.03



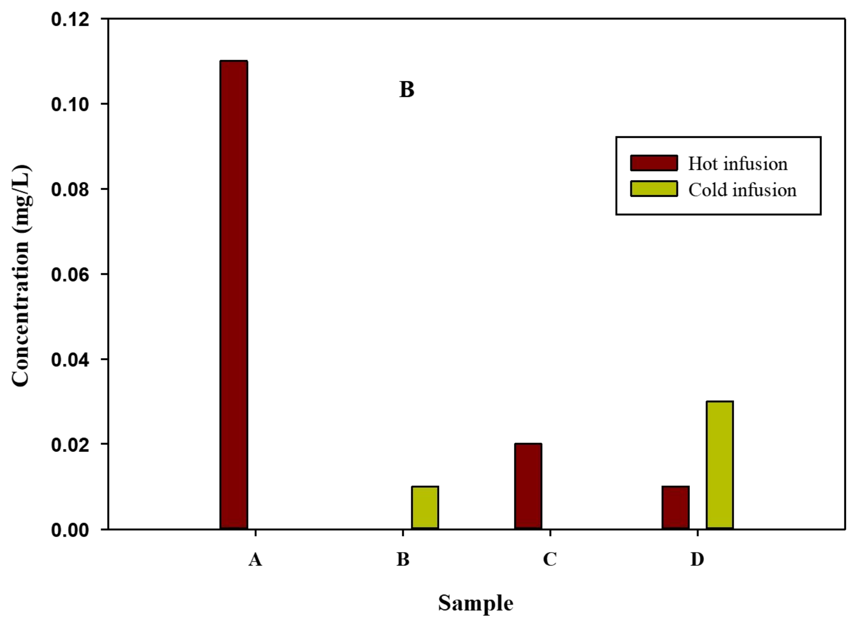


Figure 3.3: Comparison of the concentrations of heavy metals in both hot and cold infusion for (A) Zinc and (B) Chromium

Significantly low concentration, below detectable limits was observed for zinc in sample B and chromium in sample A and E. The metal content of zinc in black and green tea sample is similar but a wide range of difference was observed for chromium in the digested samples.

Assessment made from table 2 and 3 shows the zinc content in hot and cold tea Infusion samples, showed that very low concentrations were obtained for both the cold and hot infusion samples for the various of tea analyzed, this is below the permissible limit of 3mg/l prescribed by WHO (1998). Zinc is an important trace metal which plays crucial role in several cell processes like growth, cell development, muscle and bone formation, part of tooth etc., it's deficiency can as well result in reduced effectiveness of Vitamin A supplementation and cause loss of sense of smell and touch (Hunt 1994) .

The infusion samples can be seen to be zinc deficient as they do not provide the necessary RDA needed. Deficiency diseases may surface if the essential trace metal is not obtained from other food sources.

Comparison with literature revealed values for zinc ranging between 0.181 to 163 mg/l, 0.098 to 18.03 mg/l 2.61 to 11. 39 mg/l, 0.59 to 6.20 mg/l, 0.15 to 43.50 mg/l, ad 0.04 to 18.14 mg/l for various infusion samples in China, India, Iran, Pakistan, Sri Lanka, ad Turkey, respectively as shown by (Karak *et al*., 2015). There's a relationship between the concentration of zinc in tea and its percentage transfer to tea infusion in relation to extraction time. Research by Moghadadam *et al*., (2008) of thirty-one tea brands in Iran in relation to extraction rate showed the concentration of zinc in the tea infusion in two minutes, five minutes and ten minutes. The results obtained ranges between; 0.12 to 0.60 mg/l, /, 0.05 to 0.29 mg/l, and 0.02 to 0.14 mg/ l respectively for the various infusion time. From the results obtained the solubility of zinc in the first infusion (2minutes), was significantly higher than that of the second infusion. Concurrently, the second infusion (5minutes), is greater than that of the third infusion (10minutes). (Wong et al., 1998) also had similar findings; he reported that the solubility of zinc in his first infusion was significantly higher than the second infusion in relation to time. This explains the very low concentration of zinc obtained in this analysis for the cold and hot infusion tea samples infused for 15minutes respectively as compared to the content of zinc in the tea samples. Although other factors may apply.

The infusion samples also showed chromium content levels that ranged from 0.03 to 0.44 mg/l in the hot infusion and between 0.01 to 0.11 mg/l for the cold infusion. All the tea infusion samples showed chromium levels below the maximum contaminant level (MCL) of 0.1mg/ l (US EPA., 2002). It has been shown that chromium is essential for normal glucose metabolism and too little chromium in diets may lead to insulin resistance, however, there is still no standard against which chromium deficiency can be established (Garba,2013). Generally, chromium is considered as a local contaminant, it comes in contact with tea mainly through the CTC rollers during the manufacturing of tea. The crush, Tear and Curl (CTC) rollers are made of stainless steel, chromium present (about 17%) in the steel combines with atmospheric oxygen to form a thin invisible layer of chrome - containing oxide called passive film (Seenivasan., 2008). If the metal is cut or scratched the passive film is disrupted and more oxide will be formed which will cover the exposed surface preventing it from oxidative corrosion .

Tea plants are known to have strong ability to uptake and accumulate several micronutrients from soil, tea infusion may serve as a dietary source of different micronutrients for humans. However, debates are being carried out as regarding the safety of drinking tea, considering it can enhance the accumulation of these essential micronutrients like zinc in the human body. Nookabkaw *et al*., (2006), reported that micro nutrient content in tea must be traced and intake controlled on daily basis. Jin *et al*., (2008) concluded that people who consume large amounts of tea, for example 10 gram of tea leaves per day may not necessarily be at any risk since the daily intake of such nutrient is low Polechonska *et al*., (2015) suggested that the amount of trace metal extracted into the tea infusion depends primarily on whether the compound is strongly bound to the matrix or more soluble in the solution used. Gallaher et al., (2006) also reported that copper, manganese, and zinc content in Chinese green tea were relatively low in the infusion when deionized water was used for their study to ensure that water was not contributing to the mineral content of the infusion. However, as consumers are not likely to use deionized water to make any hot beverage, it is recommended the mineral content of the local tap water be known.

CONCLUSION AND RECOMMENDATION

The concentrations of zinc and chromium obtained in both tea and infusion samples were found to be below the permissible limit set by most international bodies for example WHO and FAO. The heavy metal levels in made tea or tea leaves is rather inappropriate to predict the supposedly dangers of the constant consumption of these of this beverage. Therefore the determination of heavy metal levels in tea infusion is paramount as it reflects the possible nutritional effects of the habitual drinking of this beverage and reflect the uptake of element in the gastrointestinal tract (this regulate the actual uptake of element by the human body). More research is required to comprehend the science of the accumulation of heavy metals in tea plants, speciation of their uptake and effects on human health in all tea growing countries. It is also recommended that, beverages consumers constantly check tea labels indicating heavy metal contents in beverages.

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APPENDIX

Fig 1.1: *Camellia sinensis* plant "Wilson bros garden".

Figure 1.2: Grown Camellia sinensis plant ready for Harvest**. INCLUDE SOURCE OF PICTURE!!!!!!!!!!!**

Figure 1.3: Withering of *Camellia sinensis* plant using bamboo mats **INCLUDE SOURCE OF PICTURE!!!!!!!!!!!!!!1**

Figure 1.4: Crushing of Camellia Sinensis plant**. INCLUDE SOURCE OF PICTURE!!!!!!!!!!!!!!!!!**

Figure 1. 5: *Camellia sinensis* plant undergoing oxidation **INCLUDE PICTURE SOURCE!!!!!**

Figure 1.6: Heating of *Camellia sinensis* plant to stop oxidation **INCLUDE PICTURE SOURCE!!!!!!!!**

Figure 1.7: Air Drying of the *Camellia sinensis* plant**. INCLUDE PICTURE SOURCE!!!!**