QUALITY ASSESSMENT OF SOME SELECTED TOOTHPASTES USED IN NIGERIA.

BY

ELIZABETH OGBEH

DEPARTMENT OF CHEMISTRY

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA.

JUNE 2014.

QUALITY ASSESSMENT OF SOME SELECTED TOOTHPASTES USED IN NIGERIA.

By

Elizabeth OGBEH, B.Sc (Hon) CHEMISTRY (A.B.U) 2008
(M.Sc/SCIE/2827/2010-2011)

A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A
MASTERS DEGREE IN ANALYTICAL CHEMISTRY

DEPARTMENT OF CHEMISTRY, FACULTY OF SCIENCE

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA.

June, 2014

DECLARATION

I declare that the work in this thesis titled **"Quality assessment of some selected toothpastes used in Nigeria"** has been carried out in the Department of Chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at this or any other institution.

OGBEH ELIZABETH	Signature	Date

CERTIFICATION

This thesis entitled **"Quality assessment of some selected toothpastes used in Nigeria"** by ELIZABETH OGBEH meets the regulations governing the award of the degree of Masters of Science in Analytical Chemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. C.E Gimba	Signature	Date
Chairman, Supervisory committee		
Dr. S.E Abechi	Signature	Date
Member, Supervisory committee		
Prof. V.O Ajibola	Signature	Date
Head of Department		
Prof. A.A Joshua	Signature	Date
Dean, School of Postgraduate Studies		

ACKNOWLEDGEMENTS

My sincere gratitude goes to Almighty God for his love and protection granted to me during this research work.

I wish to express my profound gratitude to my project supervisors Prof. C.E. Gimba and Dr. S.E. Abechi, whose constructive criticisms and encouragement made it possible for the successful completion of this project. Your patience, and invaluable contribution guided me throughout the research period and I highly appreciate this.

I sincerely acknowledge the contributions of Lecturers in the Department of Chemistry, Ahmadu Bello University. I thank, Prof. G.I. Ndukwe, Mr. G.A. Shallangwa, Dr. E.D. Paul for their moral support and encouragement.

My gratitude goes to Head of Chemistry Department, Prof. V.O. Ajibola, the Coordinator of Post Graduate Studies in the Department, Dr. S.O. Idris and other officers in the department who worked hard at facilitating the programme. My sincere appreciation goes to Mr. Oche, Mr. Silas and Mallam Bashir for their contribution and support.

My utmost appreciation goes to my parents, especially my mother, Mrs. Veronica Ogbeh for her prayers and encouragement.

I sincerely appreciate the support and encouragement of my sister and her husband, Mr. and Mrs. R.O. Onoja, for their untiring efforts to see that i achieve the best.

I must not fail at this time to express my hearty thanks to my brothers and sisters, nieces, nephews, in-laws, friends and well wishers; you are great and I thank you all. To God be the glory, Amen.

ABSTRACT

In this research work, Quality assessment of varieties of toothpastes used in Nigeria was investigated in this study. Ten different brands of toothpastes were purchased from the open market in Kaduna, Nigeria and their physicochemical properties, bacterial load, fluoride, heavy metal levels, titanium, saccharin and sodium lauryl sulphate (SLS) concentrations were determined using Standard methods. The pH of these toothpastes was found to range from between 5.20 to 8.42. 60% of the sampled toothpastes fell within the NAFDAC recommended pH range of 6.5 to 7.5. The moisture and volatile constituents of the toothpastes were between 28.03% and 46.19%, which is within the range set by NAFDAC. The fluoride concentration of the sampled toothpastes ranged from 770 ± 13.46 ppm to 1220 ± 5.00 ppm. Although none of the toothpastes was above the maximum limit of 1500 ppm fluoride as set by NAFDAC, three were found to be below the minimum set value of 850 ppm as required by NAFDAC. The results from the microbial load of the toothpastes showed that they were all sterile as no bacterial or fungal isolates were identified in any of the sampled toothpastes. Traces of titanium metals were found in all the sampled toothpastes ranging from 1.44 to 6.25 mg/g. While traces of heavy metals like Cd, Mn, Ni, Co and Zn were detected in the toothpaste samples. Lead was absent in all the sampled toothpastes. The concentration of saccharin in the analyzed toothpastes ranged between 83.3 ± 17.29 to 232.86 ± 11.47 mg/g while SLS ranged between 15.64 ± 10.11 to 21.30 ± 10.22 mg/g. The concentrations of the heavy metals and saccharin in the sampled toothpastes where compared with the level for their acceptable daily intake. It was deduced that users of this products were likely to be exposed to high risk of health issues if toothpaste which was meant for brushing the tooth is swallowed mistakenly.

A comparative study of the concentration of heavy metals in local toothpastes with the foreign toothpastes showed that foreign toothpastes contained higher concentration of these heavy metals and may pose greater health problem to consumers. Therefore there is the need to sensitize consumers on the dangers involved in swallowing toothpastes and based on the results obtained from this research work, it is recommended that the importation of foreign toothpastes should not be encouraged.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

ABBREVIATIONS

ADI: Allowed Daily Intake

ASFC: Actual Sample Fluoride Concentration.

CAFC: Claimed Active Fluoride Content.

CAFI: Claimed Active Fluoride Ingredient.

CEFC: Claimed Equivalent Fluoride Concentration.

CFC: Calculated Fluoride Concentration.

FDA: Food and Drug Administration

M&VM: Moisture and Volatile Matter

MAXWHO: Maximum value set by World Health Organization.

MINWHO: Minimum value set by World Health Organization.

$NaF.PO_4$: Sodium monofluorophosphate.

NaF: Sodium Fluoride.

NAFDAC: National Agency for Food and Drugs Administration

NIL: Not Detected

NLD: Not Detected

NS: Not Stated.

SON: Standard Organization of Nigeria

TVC: Total Viable Count

WHO: World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Preamble

Toothpaste is a substance in molten form used with a toothbrush as an accessory to clean and maintain the aesthetics and health of teeth. Toothpaste is a personal hygiene product used on daily basis by children and adults. The chemical composition of toothpaste varies from one brand to another and even among several presentation of the same brand. They are composed of substances which act as abrasive, pigment, foam inducer, humectants, thickener, stabilizer, solvent, sweetener, therapeutic agent and enamel hardener.

The primary purpose of brushing the teeth with a dentifrice (toothpaste) is to clean the accessible tooth surface of dental plaque, stains and food debris. Tooth cleaning with dentifrices dates back over 2000 years, while cleaning of teeth with toothpicks and brushes is an older practice. Abrasive dentifrice materials came to be used when it was found that brushes while facilating the cleaning of soft deposit from teeth, were inadequate for the removal of harder deposits and stains. Dentifrices have been prepared in several forms such as powder, paste and gels. The most popular forms are the pastes and gels with over 5 billion tubes used worldwide each year (Eric, 1994).

Several studies have shown that toothpaste has a key role in helping to remove dental plaque, the major cause of dental caries and periodontal diseases (Forward, 1991). Apart from aiding cleaning of teeth directly, tooth paste has a role in encouraging people to clean their teeth. Modern developments in toothpaste formulation have led to the addition

of agents to provide therapeutic, as well as cosmetic benefits. Frequently used modern toothpaste can help prevent dental caries and limit the re growth of dental plaque.

A typical chemical composition of toothpaste includes: abrasives (20% - 50%), flavor (20%) which is pleasant and must be associated with a clean feeling e.g. mint flavor, colouring (<1%), sweetening agents (2%) and water (20% - 40%).

Modern toothpaste is a heavy mix of chemical and other synthetic materials to clean, polish and maintain healthy teeth and gums. Most commercial toothpaste has ingredients such as sodium lauryl sulphate, sodium saccharin, titanium oxide, and fluoride and in some cases, heavy metals and microbial contamination, which may cause health challenges with time.

Saccharin (1,2-benzisothiazolin-3-one-1,1-dioxide) is an artificial sweetener. It is believed to be an important discovery, especially for diabetics, as it goes directly through the human digestive system without being digested. Although saccharin has no food energy, it can trigger the release of insulin in human and rats, presumably as a result of its taste (Just *et al.*, 2008; Lonescu *et al,* 1988; Berthoud *et al,* 1980), as can other sweeteners like aspartame. Consumption of saccharin and other artificial sweeteners have been shown to increase the risk of bladder cancer in humans (Howe *et al,* 1977; 1980; Hoover and Hartge, 1982). It has also been tied to a variety of allergic reactions including headaches, breathing issues, skin rashes and diarrhea (Stewart, 2011). Recent studies show that saccharin can actually increase body weight (Polyak *et al.*, 2010). In infants, it can cause irritability and muscle dysfunction (Tulika, 2011). Pregnant women have also been advised to avoid the use of saccharin in pregnancy, as saccharin has the ability to pass through the

placenta and it may remain in the fatal tissue, which is harmful for the unborn child (Janet, 2002).

Sodium lauryl sulphate (SLS) is a surfactant, detergent and emulsifier, used in thousands of cosmetic products, as well as in industrial cleaner. It is present in nearly all shampoos, scalp treatments, hair color and bleaching agents, toothpaste and body washes.

Studies on SLS shows that it can cause irritation of the skin, eyes, respiratory system if inhaled (Lee *et al.,* 2004). SLS in mouth rinses can cause desquamation of oral pithelium and a burning sensation in humans (Babich and Babich, 1997). SLS is associated with increased aphthous ulcers (cancer sores) due to the denaturing effect and irritation of the oral mucosa (Herlofson and Barkvoll, 1994).

Fluoride, a key ingredient in toothpaste has broad benefits for people, both young and old. The fluoride in toothpaste heals and remineralizes microscopic cavities as they form, it hardens the tooth surface, making it more resistant to the acid attack of bacteria and slows down the action of these acid producing bacteria. Though a small amount of fluoride is beneficial, it has been shown to cause mottled teeth and bone damage at about 5mg/L in water (Tokalioglu *et al.,* 2004). Studies have shown that bone cancer in male children and uterine cancer deaths are linked to water fluoridation due to fluorides gradual build up in the bones, thereby causing adverse changes to the bone structure (Varner *et al.,* 1998). Fluoride has also been reported to cause birth defects and perinatal deaths, impaired immune system, acute adverse reaction, severe skeletal fluorosis at high levels, osteo-arthritis, acute poisoning and contributes to the development of repetitive stress injury (Holistic, 2009).

Titanium dioxide is widely used to provide whiteness and opacity to products such as paints, plastics, papers, ink, food and toothpaste. It is also used in cosmetic and skin care products. It has been reported that high concentration of titanium dioxide dust may cause coughing and mild, temporary irritation, long-term inhalation of high concentration of pigmentary (powdered) or ultrafine titanium dioxide may cause lung cancer based on animal evidence (Han *et al.,* 2012).

Titanium dust, has been classified by the international Agency for Research on Cancer (IARC) as an IARC group 2B carcinogen, meaning it is possibly carcinogenic to human (IARC, 2006). It has also been shown to cause respiratory track cancer in rats exposed by inhalation and intra tracheal instillation (Kutal and Serpone, 1993). Recent data indicated that TiO_2-nanoparticles are cyto- and genetoxic against several lineages of cell cultures. They also present high carcinogenic potential in animal models (Huang *et al.*, 2009; Trouiller *et al.*, 2009). Wide spread use and its potential entry through dermal, ingestion and inhalation routes suggest that nano sized TiO_2 poses considerable exposure risks to humans, livestock and eco-relevant species (Long *et al.,* 2006).

Many heavy metals are not acceptable as ingredient in oral products, as they may pose health challenges to users. Yet, they may still be found in oral products as impurities due to the persistent nature of these substances and the fact that they are found naturally in the environment. Heavy metals, being added deliberately as ingredient in toothpaste, has been curtailed, however, the presence of these substances as impurities in toothpaste calls for concern. Popova and Marinova, 2007, in a recent research, reported that metals used in dentistry are known to cause contact dermatitis in the mouth. The metals of primary toxicological concern are lead, cadmium, titanium, nickel, manganese, copper and zinc.

Bacterial contamination can pose a potential health risk for humans. The illness associated with bacterial contamination includes gastro-intestinal infections and other life threatening infections. Microbial contamination can be found in food, water, cosmetics and toothpastes (Jennifer and David, 2012). Although microbial contamination in toothpaste is not common, it has recently been reported in some counterfeit toothpaste sold in the US (Jennifer and David 2012). Hence, it is necessary to test these brands of toothpaste products against microbial contamination.

1.2 Research Problem

Various companies now manufacture toothpastes under different brand names without giving the percentage composition of the ingredient used. Some toothpaste sold in Nigeria today and some imported ones do not carry the registration numbers from regulatory bodies, meaning they have not been screened. Some of the constituent chemicals used include SLS, Sodium Saccharin, Fluoride, Titanium dioxide which have been proved to be hazardous to the human health, if above a certain limit.

Recently, most manufacturers make use of plant extracts to make herbal toothpaste. This can further lead to contamination of the toothpaste by microorganisms as well as heavy metals due to their persistent nature.

Despite the health implication of the presence of these substances in food, they are still used in food products in minimum amounts.

In Nigeria, toothpaste is used by both adults and children. Reports show that children and some adults (pregnant women especially), swallow a substantial amount of

toothpaste in the process of brushing , which could lead to any of the effects associated to the ingestion of these substances. (Hernandez *et al.,* 1985)

1.3 Aim

This work aimed at assessing the quality of some selected locally and foreign manufactured toothpastes, used in Nigeria Markets.

1.4 Objectives

The aim of this research will be achieved through the following objectives;

i. Evaluation of some physical parameters of toothpaste (colour, moisture and volatile content, pH) using recommended methods.
ii. Investigation of the bacteriological and microbial status of the toothpaste, using standard methods.
iii. Investigation of fluoride concentration in toothpaste samples.
iv. Determination of Heavy metals levels (Pb, Cd, Mn, Cu, Ni and Zn) and TiO_2 contamination in toothpastes using AAS and x-ray Spectrometer respectively.
v. Investigation of sodium saccharin, sodium lauryl sulphate concentration in the selected toothpaste using UV-Spectrophotometer.
vi. Carry out correlation analysis to compare the levels of these substances in the foreign and local toothpastes.
vii. Comparing the obtained results with set standards by regulatory bodies.

1.5 Justification of Research

Toothpaste is used on daily basis by children and adults to promote oral hygiene. Available evidence shows that children and pregnant women are exposed to high risk of health problem if they continue to consume toothpaste. There is therefore need for continuous monitoring of levels of potentially hazardous substances in toothpaste and also to check the quality of this product available to consumers. Recommendation(s) can then be made to manufactures of toothpastes on how to adjust their formulations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Saccharin

Saccharin (1, 2-benzisothiazolin-3-one-1, 1-dioxide) is an artificial sweetener. Saccharin and its salts are intense sweeteners, being about 300-500 times sweeter than sucrose in aqueous solution (Martindale, 2002). Saccharin is not only soluble in water but its commercially available sodium salt, used as a non-nutritive sweetener, is freely soluble in water. Saccharin, a petroleum- based sugar substitute is used in soft drinks, diet food and personal hygiene products such as lip balm and tooth paste. Saccharin derives its name from the word saccharine, meaning, relating to or resembling that of sugar. Saccharin is unstable when heated but it does not react chemically with other food ingredients. As such, it stores well. Blends of saccharin with other sweeteners are often used to compensate for each sweeteners weakness or faults. Saccharin is believed to be an important discovery, especially for diabetics, as it goes directly through the human digestive system without being digested. Although saccharin has no food energy, it can trigger the release of insulin in human and rats, presumably as a result of its taste (Just *et al.,* 2008; Lonescu *et al.,* 1988), as can other sweeteners like aspartame.

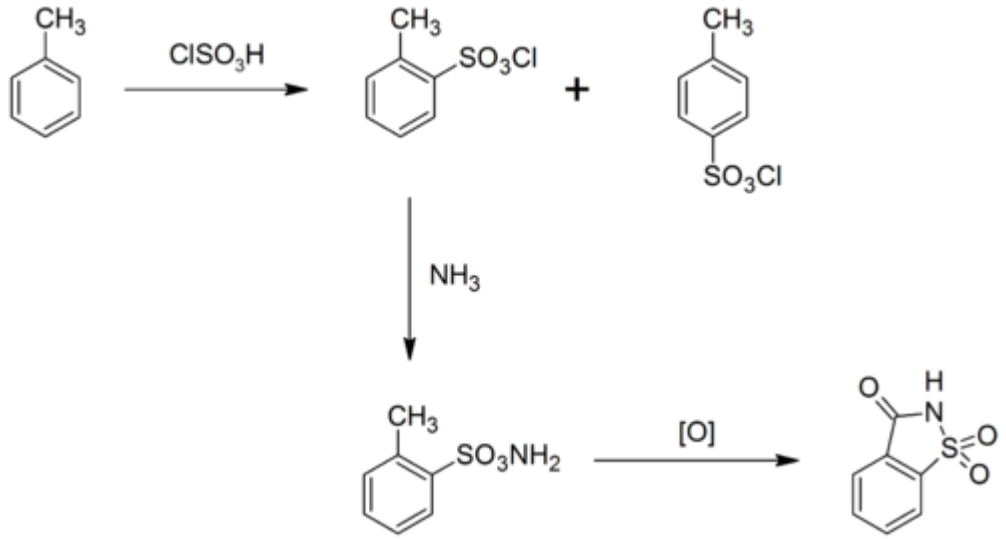
2.1.1 History

Saccharin was first produced in 1878 by Constantin Fahlberg, a Chemist working on coal tar derivatives in Ira Remsen's Laboratory at the Johns Hopkins University. The sweet taste of saccharin was discovered when Fahlberg noticed a sweet taste on his hand

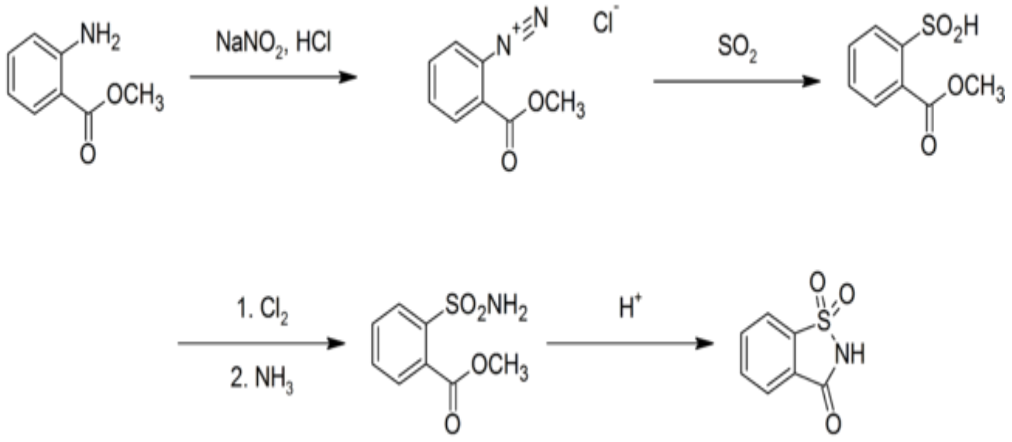
one evening, and connected this with the compound that he had been working on that day (Myers and Richard, 2007). Although saccharin was commercialized not long after its discovery, it was not until the sugar shortage during World War I that its use became widespread. Its popularity further increased during the 1960s and 1970s among dieters, since saccharin is a calorie-free sweetener.

2.1.2 Chemistry

Saccharin can be produced in various ways. The original route by Remsen & Fahlberg (1880) starts with toluene. Sulphonation by chlorosulphonic acid gives the *ortho* and *para* substituted chlorosulphonic acids. The *ortho* isomer is separated and converted to the sulphonamide with ammonia. Oxidation of the methyl substituent gives the carboxylic acid, which cyclicizes to give saccharin free acid (Gert-Wolfhard, 2005).



In 1950, an improved synthesis was developed at the Maumee Chemical Company of Toledo, Ohio. In this synthesis, anthranilic acid successively reacts with nitrous acid (from sodium nitrite and hydrochloric acid, sulphur dioxide, chlorine, and then ammonia to yield saccharin (Gert-Wolfhard, 2005).



Saccharin can be used to prepare exclusively disubstituted amines from alkyl halides via a Gabriel synthesis.

2.1.3 Biochemical data

Saccharin and saccharin salts (sodium, ammonium, and calcium) have been in use since the late nineteenth century, salt forms being more soluble but of the same sweetening power as the acid form. The absorption of ingested saccharin in animals and man occurs rapidly. At pKa of 2.2, saccharin exists in acidic media predominantly in the unionized form, which is the more readily absorbed form in a number of animal species. Saccharin is more completely absorbed from the guinea-pig (pH 1.4) and rabbit (pH 1.9) stomach, than from the rat's stomach (gastric pH 4.2) (Minegishi *et al.*, 1972). In vitro perfusion of rat stomach and small intestine with a solution of saccharin demonstrated

considerable absorption from the stomach at pH 1.0 and slow absorption from the small intestine, (less than 9% in 2 hours) (Kojima and Ichibagase 1966). The gastrointestinal absorption of saccharin appears to be somewhat greater in monkeys than in rats (Pitkin *et al.*, 1971). In monkeys, and also most probably in man, both gastric acidity and degree of absorption are intermediate between those of the rabbit and guinea-pig on one side, and the rat on the other. This also means that the degree of absorption of saccharin could be dependent on food intake which affects the acidity of the gastric contents.

It has long been assumed that saccharin undergoes very little metabolic conversion under normal dietary usage in animals and man. Studies conducted with human subjects suggested that some degree of saccharin metabolism may occur in man, since oral doses of saccharin could not be recovered quantitatively as saccharin in the urine (McChesney and Golberg, 1973). Subsequently, more definitive studies, using ^{14}C-labelled saccharin demonstrated that the reduced recovery was an artifact thought to be due to the binding of a portion of the urinary saccharin to unidentified urinary constituents (Byard *et al.*, 1974).

Four men received 500 mg of ^{14}C-saccharin (uniformly labeled in the benzene ring) and their excreta were collected at intervals up to 96 hours post-dosing. More than 98% of the ^{14}C was recovered within 48 hours (92.3% in urine, 5.8% in faeces). An additional 0.3% was excreted in the 48-72-hour collection interval. None of the detected saccharin was found to have been metabolized. Consequently, it was concluded that man, like other species, does not metabolize saccharin (Byard *et al.*, 1974).

Consumption of saccharin and other artificial sweeteners have been shown to increase the risk of bladder cancer in humans, most especially smokers. Increased risk

were observed by Howe *et al* (1977, 1980) in men who smoked more than 40 cigarette daily and women who never smoked and were never exposed occupationally to known bladder carcinogens by Hoover and Hartge (1982).

Analysis of nearly 1500 cases found that heavy use of artificial sweeteners was associated with increased risk of bladder cancer (Sturgeon, 1994). A second analysis of more than 600 cases also found an increased risk of cancer in Canadian men who consumed either more artificial sweeteners or consumed them for a long period of time (Howe and Burch, 1981).

A British study found that English women who consumed more than 10 tablets of artificial sweeteners (mostly saccharin) also had high risk of cancer (Morrison, 1982).

2.1.4 Research works on saccharin

The determination of concentration of saccharin in food, drinks and pharmaceuticals using different methods of analysis have been reported widely in literature. Miracakar and Gordana (2006) reported a simple, accurate and selective high performance thin layer chromatographic method for the determination of saccharin in pharmaceuticals. The chromatography was performed on silica-gel 60F254 plates with ethylacetate–carbon tetrachloride–acetic acid (3 + 4 + 0.5 v/v/v) as the mobile phase. The chromatographic zones corresponding to the saccharin spots were scanned in the reflectance/absorbance mode at λ_{max} = 230 nm. Two series of saccharin sodium salt solutions were prepared in methanol (solvent 1) and in ethyl acetate–acetic acid (9:1, v/v) mixture (solvent 2) for the standard curves. A linear calibration relationship was observed within the concentration range from 300.00 – 1200.00 ng saccharin sodium salt per spot, correlation coefficients being 0.998 (solvent 1) and 0.995 (solvent 2). The relationship

between the peak area and the amount of saccharin sodium salt was evaluated by linear regression analysis. The limits of detection and quantification of saccharin sodium salt were 35.00 ng and 110.00 ng per spot (solvent 1), respectively, and 45.00 ng and 150.00 ng per spot (solvent 2), respectively. Mean recovery values of 103.50 % (solvent 1) and 102.30 % (solvent 2), and RSD values of 4.42 % (solvent 1) and 2.53 % (solvent 2) were obtained. The proposed method was applied for saccharin determination in two pharmaceutical preparations, effervescent tablets and a carbomer-based gel.

In another research, Saccharin, a synthetic sweetener constituent of several pharmaceutical and cosmetic preparations, was analyzed qualitatively and quantitatively in the presence of interferences like active ingredients and excipients (Vianna-Soares and Jorge, 2002). A derivative ultraviolet spectrophotometric method employing the second and the fourth orders was used. The calibration data obtained were satisfactory, with a relative precision range of 0.53%-0.72%, and linearity between 0.9998-0.9999. The recovery test results for the tooth gel sample ranged between 97.80% and 104.95% in the second order of derivative and between 98.04% and 101.32% in the fourth derivative, respectively, using zero-peak (ZP) and peak-peak (PP) methods. The suspension sample showed values between 97.35% and 104.72% in the second derivative and between 95.94% to 104.41% in the fourth derivative, respectively employing the zero peak (ZP) and peak-peak (PP) methods. The proposed method showed precision, accuracy and simplicity (Vianna-Soares and Jorge, 2002).

Maja and Zorka (2011), presented two high performance liquid chromatographic (HPLC) methods used for the separation and determination of artificial sweeteners

aspartame, acesulphame K, sodium saccharin, and sodium cyclamate in beverages and special nutritional products (special food intended for specific population groups). All four compounds are soluble in aqueous solutions and can easily be separated and determined by HPLC with a diode array detector (DAD). The first method involved separation of aspartame, acesulphame K, and sodium saccharin on a C_{18} column with an isocratic elution of phosphate buffer and acetonitrile as mobile phase. The second method was used to separate sodium cyclamate on a C_{18} column with methanol and water as mobile phase. Under optimum conditions, both methods showed good analytical performance, such as linearity, precision, and recovery. The methods were successfully applied for the analysis of real samples of soft drinks and special nutritional products.

The determination of concentration of saccharin in low calorie and dietary products using a simple, rapid and inexpensive monoparameter flow-through sensor was developed by Captain-Valley *et al.,* (2004). The method is based on the transient adsorption of the sweetener on Sephadex G-25 solid phase packed to a height of 20 mm in the flow cell. The optimal transient retention of the synthetic sweetener, in terms of sensitivity and sampling frequency, was obtained when pH 2.75 citric acid-sodium citrate buffer 5×10^{-3} M was used as a carrier at a flow-rate of 1.50 cm^3 min^{-1}. Saccharin was determined measuring its intrinsic absorbance at 217 nm at its residence time. Calibration graphs for peak height and peak area were linear over the range 5.00 – 200.00 µg cm^{-3}, RSD 1.18%, and 1.00–200.00 µg cm^{-3}, RSD 0.78%, respectively. Saccharin was determined in several food samples measuring height or area peak, obtaining recoveries ranging between 98 – 104 and 99 – 102% for height and area peak, respectively. The procedure was validated for use in the

determination of saccharin in low calorie and dietary products giving reproducible and accurate results.

Saccharin has also been determined in different materials by solvent extraction methods. The formation and extraction of ion-association compounds of saccharin with basic dyes offered a simple alternative for rapid spectrophotometric determination of saccharin. Nile blue was used as an extractive reagent in the spectrophotometric determination of saccharin in different materials by Hernandez *et al.,* (1985). They reported that in a slightly acidic medium, (sodium acetate-acetic acid buffer, pH 4) saccharin and Nile blue form an ion –association compound which is extractable into methyl isobutyl ketone and allows the spectrophotometric determination of saccharin. At 630nm, Beer's law was obeyed over the saccharin concentration range 0.10-3.50µg/ cm^3 in the aqueous phase and the apparent molar absorptivity was 5.80×10^4 $1.mole^{-1}.cm^{-1}$. The method showed good selectivity and can be applied to the determination of saccharin in artificial sweeteners, soft drinks and toothpastes. In the preliminary experiments, several basic dyes (phenosafranine, Acridine Yellow, Acridine Orange, Rhodamine B, new Methylene Blue, Brilliant Cresyl Blue and Nile Blue) where tested at different pH values as ion-pairing reagents for extraction of saccharin. Chloroform, carbon tetrachloride, benzene, toluene, isobutyl acetate, ethyl acetate, cyclohexanone, isobutyl alcohol and methyl isobutyl ketone were tried as solvents. None of these systems showed better results than the Nile blue and methyl isobutyl ketone system. The absorbance of the reagent blank was not high and this system gave the highest degree of extraction of saccharin.

2.2 Sodium Lauryl Sulphate (SLS)

SLS has the chemical formula $C_{12}H_{25}SO_4Na$, and has many other names such as sodium monolauryl sulphate, sodium dodecyl sulphate, sodium dodecane sulfate, lauryl alcohol, sulphuric acid mono lauryl ester sodium salt. It is used as ingredient in household and industrial items.

SLS is a surfactant, detergent and emulsifier used in thousands of cosmetic products, as well as in industrial cleaners. It is present in nearly all shampoos, scalp treatments, hair colour and bleaching agents, toothpastes, body washes and cleaners, make-up foundations, liquid hand soaps, laundry detergents and bath oils/bath salts. It is formed by combining sulphonic acid and dodecanol in a process known as esterification. This product is then neutralized with sodium carbonate to give sodium lauryl sulphate.

SLS is a surfactant, which means a molecule that has ampiphilic properties. This means the sulphate head group is hydrophilic and water soluble, while the 12-carbon-long chain is hydrophobic and water insoluble. It is an anionic surfactant as defined by the sulphate head group, since it has a negative charge. The head of the group must be sufficiently soluble in water to be classified as a surfactant. Surfactants are wetting agents that lower the surface tension of a liquid, allowing for easier spreading of a droplet on the surface, thus lowering interfacial tension between the two liquids (Barnes and Gentle, 2005).

2.2.1 Side effect of SLS.

According to the Environmental Working Group's Skin Deep: Cosmetic Safety Reviews (2006), research studies on SLS have shown links to:

i. Irritation of the skin, eyes, respiratory system if inhaled;

ii. Organ toxicity;

iii. Developmental/reproductive toxicity;

iv. Neurotoxicity, endocrine disruption, ecotoxicology and biochemical or cellular changes; and

v. Possible mutations and cancer.

High levels of SLS intake, either orally or through the skin, are not ordinarily experienced in normal cosmetic use; it's the gradual cumulative effects of long-term, repeated exposures that are of the real concern. There is a serious lack of long term studies on all of the chemicals in these products, hence it is not really known, what the long-term effects are. It is not just repeated exposure to one chemical, it is the combined effect of thousands of little chemical exposures, day in and day out, and that is of concern.

A number of studies report SLS being damaging to the oral mucosa and skin. This is not at all surprising since SLS is actually used as a skin irritant during studies where medical treatments for skin irritation require an intentionally irritating agent.

A study at the stern college for women at Yestiva University in New York in 1997 examined SLS in mouth wash. They found that SLS in mouth rinses caused desquamation of oral epithelium and a burning sensation in human volunteers (Babich and Babich, 1997).

In another study, Herlofson and Barkvoll (1996) reported that SLS can cause adverse effects on oral soft tissues. This double-blind cross-over study describes the oral mucosal effects of SLS-containing toothpastes and pastes containing a zwitterionic detergent, cocoamidopropyl-betaine (CAPB) in an experimental model in 28 healthy females. Seven toothpastes, differing only in detergent concentration and/or type, were used: SLS (0.50, 1.00, 1.50%), CAPB (0.64, 1.27, 1.90%) and a placebo. Each participant

applied 1.00 cm of assigned test toothpaste via a cap splint to the teeth and the mucosa of the upper jaw. The splints were used twice daily for 2 min during a period of 4 days, after which the participants were examined for oral desquamation. No other oral hygiene was allowed during the test periods. Ten days brushing with detergent-free toothpaste was performed between each test period. Forty-five desquamative reactions were observed in 21 of 27 subjects (one was excluded) during the trial. Forty-two reactions were recorded during the SLS periods and the remaining three during the CAPB periods. The detergent-free toothpaste did not result in oral desquamation. SLS in toothpastes significantly increased the incidence of desquamation of the oral mucosa compared with toothpastes containing the detergent CAPB.

The model used is not directly relevant to normal tooth brushing with toothpaste, but indicates that sensitive patients may contract mucosal irritation through SLS in toothpastes. Less toxic detergents, e.g. CAPB, are desirable in oral hygiene products.

A study was carried out to investigate whether a difference in susceptibility to chemically induced irritation of the oral mucosa in 14 pre- and 14 post-menopausal women exists, following local exposure to SLS in toothpastes. 4 different pastes differing only in detergent concentration present, or not, were used. The participants applied 1.00 cm of the different test toothpastes to a cap splint covering the teeth and the oral mucosa of the upper jaw two times daily for two minutes during a period of 4 days. 42 desquamative reactions of the oral mucosa (in 20 subjects) were observed. 29 and 13 were in the pre- and the post-menopausal group, respectively. Desquamation was only experienced following exposure to SLS-containing toothpastes, not succeeding SLS-free paste. The results demonstrated that oral mucosa of pre-menopausal women was significantly more sensitive to SLS than

oral mucosa of post-menopausal women. The difference in mucosal irritation between the 2 groups increased with increasing concentration of SLS (Herlofso and Barkvoll, 1996).

A study by Lee *et al.*, (2004), appearing in Exogenous Dermatology confirmed SLS to be a very "corrosive irritant" to the skin, irritation which persisted in research subject for 3 weeks. SLS exerts its damage by stripping the skin of protective oils and moisture.

Sodium lauryl sulfite (SLS), asynthetic detergent commonly used in dentifrices, is an effective denaturant. SLS was found to be associated with increased aphthous ulcers (cancer sores) due to the denaturing effect and irritation of the oral mucosa (Herlofson and Barkvoll, 1994).

In their study to investigate the effect of SLS in patients with recurrent aphthous ulcers (RAU), ten patients with multiple minor RAU participated in the study. The mean incidence of RAU was 17.8 during a 3-month period before the study. The patients used a dentifrice containing 1.2% SLS for a test period of 3 months followed by a dentifrice without SLS for the same time period. The results showed a statistically significant decrease in the number of aphthous ulcers from 14.3 after using the SLS-containing dentifrice to 5.1 ulcers after brushing with the SLS-free dentifrice ($p<0.05$). It was then suggested that the denaturing effect of SLS on the oral mucin layer, with exposure of the underlying epithelium, induces an increased incidence of RAU mucosa (Herlofson and Barkvoll, 1994).

Swallowing SLS will likely lead to nausea and diarrhea and is even used as a laxative in enemas, hence, based on the above findings by other researchers, toothpaste containing SLS should not be swallowed.

The evidence linking SLS to cancer is a bit challenging due to the paucity of scientific studies. However, carcinogenic effect are quiet possible when one consider that SLS is often contaminated by two known carcinogens.

i. Ethylene oxide (which is what the 'E' in SLES represents) reveals a high hazard rating which appears as an impurity in thousands of personal care products. It is used to ethoxylate SLS and other chemicals to make them less harsh.

ii. 1,4 dioxane, a by product of ethylene oxide, also receives a high hazard rating, it is also described as "probably carcinogenic to humans," toxic to the brain and central nervous system, kidneys and liver. It is also a leading ground water contaminant.

2.2.2 Analysis of SLS

SLS have been analysed in water and cosmetic products. A simple spectrophotometric method applicable to waste water bodies was developed for the determination of anionic surfactant (AS), in which an ion-association complex was formed between an anionic surfactant Sodium dodecyl sulphate (SDS) and a cationic dye Crystal Violet (CV). The dark blue colored complex can be easily extracted in organic solvent, benzene. The absorbance of the complex in benzene layer is then measured spectrophotometrically at maximum wave length (λ_{max}) of 565 nm. Under the optimal experimental conditions, absorbance of the organic extractant was found to obey Beer's law over the range of 0.75-10.00 μg cm^{-3} of SDS and the LOD was 0.01312 μg L^{-1}. It was reported that this method was much easier, less time consuming and applicable to accelerated urbanization and industrial development found in newly formed Chhattisgarh State in Central India. The validity of the method has been tested in regionalized industrial and domestic waste water runoff (Santosh *et al.*, 2008).

A simple and rapid spectrophotometric method was developed by Asok *et al.*, (2005) for the determination of sodium dodecyl sulphate (SDS). SDS forms a yellow coloured complex with acridine orange (ACO), a cationic dye. The complex was extracted into toluene and the absorbance of the complex in toluene layer was measured at maximum wave length (λ_{max}) of 467 nm. The calibration curve was found to be linear in the range of 0 - 6.00 ppm of SDS concentration. The method was compared with the standard MBAS method in terms of time, ease and accuracy. It was noticed that the method was much easier and less time consuming. The method required chemicals, which are less toxic than those required for the standard method. The method was reported to be applicable to waste water analysis.

2.3 Fluoride

Fluorine is a trace element. Trace elements occur in tiny amounts or traces. They play a major role in health, for even minute portions of them can significantly affect health. Fluoride is usually found naturally in low concentration in drinking water and foods. Fluoride also is frequently added to some consumer products, such as toothpaste, toothpowder, mouth wash and vitamin supplements for various reasons.

2.3.1 Cavity prevention

Fluoride-containing compounds are used in topical and systemic fluoride therapy for preventing tooth decay. They are used for water fluoridation and in many products associated with oral hygiene (McDonagh *et al.,* 2000). Originally, sodium fluoride was used to fluoridate water; hexafluorosilicic acid (H_2SiF_6) and its salt sodium hexafluorosilicate (Na_2SiF_6) are more commonly used additives, especially in the United States. The fluoridation of water is known to prevent tooth decay (Griffin *et al.,* 2007;

Winston and Bhaskar, 1998). In some countries where large, centralized water systems are uncommon, fluoride is delivered to the populace by fluoridating table salt.

2.3.2 Toxicology

Fluoride-containing compounds are so diverse that it is not possible to generalize on their toxicity, which depends on their reactivity and structure, and in the case of salts, their solubility and ability to release fluoride ions.

Soluble fluoride salts, of which sodium fluoride is the most common, are mildly toxic but have resulted in both accidental and suicidal deaths from acute poisoning (Aigueperse *et al.*, 2005). The minimum fatal dose in humans is not known, however, the lethal dose for most adult humans is estimated at 5.00 to 10.00 g (which is equivalent to 32 to 64 mg/kg elemental fluoride/kg body weight) (Baselt, 2008; IPCS, 2002a). A toxic dose that may lead to adverse health effects is estimated at 3.00 to 5.00 mg/kg of elemental fluoride (Nochimson, 2011). For Sodium fluorosilicate (Na_2SiF_6), the median lethal dose (LD_{50}) orally in rats is 0.125 g/kg, corresponding to 12.50 g for a 100 kg adult (Merck Index, 1996). The fatal period ranges from 5 minutes to 12 hours. The mechanism of toxicity involves the combination of the fluoride anion with the calcium ions in the blood to form insoluble calcium fluoride, resulting in hypocalcemia; calcium is indispensable for the function of the nervous system, and this condition can be fatal. Treatment may involve oral administration of dilute calcium hydroxide or calcium chloride to prevent further absorption, and injection of calcium gluconate to increase the calcium levels in the blood. Hydrogen fluoride is more dangerous than salts such as NaF because it is corrosive and

volatile, and can result in fatal exposure through inhalation or upon contact with the skin; calcium gluconate gel is the usual antidote (Muriale *et al.*, 1996).

In the higher doses used to treat osteoporosis, sodium fluoride can cause pain in the legs and incomplete stress fractures when the doses are too high; it also irritates the stomach, sometimes so severely as to cause ulcers. Slow-release and enteric-coated versions of sodium fluoride do not have gastric side effects in any significant way, and have milder and less frequent complications in the bones (Murray and Ste-Marie, 1996). In the lower doses used for water fluoridation, the only clear adverse effect is dental fluorosis, which can alter the appearance of children's teeth during tooth development; this is mostly mild and is unlikely to represent any real effect on aesthetic appearance or on public health (Yeung, 2008).

2.3.3 Fluoridated toothpaste

Most toothpaste today contains between 0.22 % (1000 ppm) and 0.312 % (1450 ppm) fluoride, usually in the form of sodium fluoride or sodium monofluorophosphate (MFP); 100.00 g of toothpaste containing 0.76 g MFP equates to 0.10 g fluoride. Prescription strength fluoride toothpaste generally contains 1.10% (5,000 ppm) sodium fluoride toothpaste. This type of toothpaste is used in the same manner as regular toothpaste. It is well established that 1.10% sodium fluoride is safe and effective as a prevention of cavities. This prescription dental cream is used up to three times daily in place of regular toothpaste. Consumption of large amounts of fluoride can lead to fluoride poisoning and death, the lethal dose for most adult humans is estimated at 5.00 to 10.00 g, which is equivalent to 32.00 to 64.00 mg/kg elemental fluoride/kg body weight (Gosselin

et al., 1984; Baselt, 2008; IPCS, 2002a). Ingestion of fluoride can produce gastrointestinal discomfort at doses at least 15 to 20 times lower (0.20 – 0.30 mg/kg) than lethal doses (Bradford *et al.,* 1994). Chronic intake and topical exposure may cause dental fluorosis and excess systematic exposure can lead to systemic effects such as skeletal fluorosis. Young children are at risk for receiving excess fluoride, and the ADA has recently issued an interim guidance on their fluoride consumption (ADA, 2006).

2.3.4 Analysis of fluorides in toothpaste

Spectrophotometric determination of fluoride in dosage forms and dental preparation have been reported (Sandulescu *et al.,* 1996). The method is based upon the reaction between fluoride ions and the coloured complex of Fe (III) with methyl salicylate to form the stable, colourless hexafluoride complex of iron. The standard curve was obtained for 0.01 - 0.08 mg NaF cm^{-1} at 525 nm. The method was validated and the results showed good precision (100.16 ± 2.33%), comparable with that of other analytical methods. Good results were obtained in the spectrophotometric determination of fluoride in a stomatological gel and in toothpaste (Sandulescu *et al.,* 1996).

The determination of the total fluoride concentration of 13 brands of fluoridated toothpastes marketed in Nigeria was performed using a fluoride ion-selective electrode (ISE). Eleven adult and 2 paediatric toothpastes of different brands were purchased from local markets/supermarkets in Lagos, Nigeria and their fluoride concentrations were determined using an Orion Bench top pH/ISE meter. The fluoride concentrations of the adult toothpastes were found to be between 348.790 ± 1.00 and 925.780 ± 1.00 ppm, whilst the paediatric toothpastes samples were found to be between 349.100 ±0.495 and 726.19 ±

0.50 ppm respectively**.** The comparison of these results with the permissible values given by the National Agency for Food and Drug Administration and Control (NAFDAC), Standard Organization of Nigeria (SON) and the World Health Organization (WHO), however revealed that none of the paediatric toothpastes fell within the approved range of 425.00 – 625.00 ppm. On the other hand, three of the adult toothpastes samples conformed to the recommended fluoride concentration of between 825.00 – 1250.00 ppm. This study established that the fluoride content of only three out of thirteen toothpaste samples conformed to the SON and NAFDAC standards (Adejumo *et al.*, 2009).

2.4 Heavy Metals

Heavy metals are stable elements that cannot be metabolized by the body and therefore bioaccumulate as it passes up the food chain to human. Most of these do not have any basic function in the body but can be highly toxic. Once the metals are released into the environment through the air, drinking water, food, or countless man-made products and chemicals, they get into the body through inhalation, ingestion and skin absorption (Elas and Haans, 2003). If the heavy metals accumulates in the body tissues faster than the body's detoxification pathways can dispose of it, a gradual build up of this toxins will occur. This can lock into the enzymes sulphuric attachments and block out other molecules from correctly attaching and then suffocate the enzymes, making it useless, resulting in weakness of some physiological process in the body (Singh, 2010).

Heavy metal over load can lead to neurological disease, depression, aggravate conditions such as osteoporosis and hyperthyroidism, memory loss and poor coordination, muscle pain, anorexia, constipation, vomiting anemia, kidney and liver damage (Amal, 2012). Heavy metals occur naturally in rock forming and are minerals but human activities

have given rise to anomalous high concentration of the metals relative to the normal background levels (Ashworth and Alloway, 2004).

Heavy metals are defined as those metals whose density is above 5 g/cm^3 (Pattabhi *et al.*, 2008). High concentration of heavy metals in the environment can be detrimental to a variety of living systems. Excessive ingestion of these metals by humans can cause accumulative poisoning, cancer, nervous system damage and ultimately death (Corapcioglu and Huang, 1987; Issabayeva *et al*., 2007). The toxicity of these metals is due to their ability to bind to oxygen, nitrogen, and sulphur groups in proteins, resulting in alterations of enzymatic activity (Issabayeva *et al*., 2007).

Nearly all organ systems are involved in heavy metal toxicity; however, the most commonly involved include the hematopoietic, renal, and cardiovascular. To a lesser extent, lead toxicity involves the musculoskeletal and reproductive systems. The organ systems affected and the severity of the toxicity vary with the particular heavy metal involved, the chronicity and extent of the exposure, and the age of the individual (Schwartz and Hu, 2007).

Main sources of heavy metal contamination include urban industrial aerosols, solid wastes from animals, mining activities, industrial and agricultural chemicals. They are found as impurities in cosmetic and oral products due to their persistent nature. In all cases, however, the prevailing concentration of heavy metals in toothpaste should not be above 20mg/kg (Popova and Marinova, 2007).

2.4.1 Sources and toxicity of selected heavy metals

2.4.1.1 Lead

One of the ingredients in most tooth pastes, silica, contains natural levels of Pb in trace amounts. Lead has long been recognized as one of the harmful environmental pollutants. There are many ways in which humans are exposed to lead: these are through air, drinking water, food, contaminated soil, deteriorating paint, and dust. Airborne lead enters the body when an individual breathes or swallows lead particles (EPA, 1990). Lead poisoning has been a recognized health hazard for more than 2,000 years. Characteristic features of lead toxicity, including anemia, colic, neuropathy, nephropathy, sterility and coma. Exposure to low-levels of lead has also been associated with behavioral abnormalities, learning impairment, decreased hearing, and impaired cognitive functions in humans and in experimental animals (Saxena *et al.*, 2006).

There have been a number of reports in the media and on internet about the presence of lead in some toothpaste products, especially those manufactured from China. Lead poisoning from environmental exposure is thought to have caused mental retardation in many children. Mild lead poisoning causes anaemia. The victims may have headaches and sore muscles and may feel generally fatigued and irritable (Bhatia, 2006).

EPA has set a regulatory limits for lead to be 15 parts per billion (ppb) in drinking water and 0.15 micrograms per cubic meter in air (Sabine and Wendy, 2009).

2.4.1.2 Cadmium

Cadmium ingestion by human beings may occur through drinking cadmium contaminated water and drinks. Sewage sludge has been known to contain high concentrations of cadmium and the use of it in agriculture, as source of nutrient, has

resulted in the deposition of high amount of this metal in vegetable and edible tissues. The sources of cadmium in water include the use of galvanized pipes, cisterns and cadmium containing solders in water hectares (Bhatia, 2006). Cadmium is widely used in batteries. It is also used in alloy and metal plating, as pigments and stabilizers for polyvinyl chloride (Shin *et al*., 2006). Cadmium is not an ingredient in toothpaste, but due to the persistent nature of this element, traces of it can be found in toothpaste and other cosmetic products. Water, which is about 20 - 40% 0f the toothpaste composition, is as major source of cadmium in toothpaste. The effects of acute cadmium poisoning in humans are very serious. Among them are high blood pressure, kidney damage, destruction of testicular tissue, and destruction of red blood cells. It is believed that much of the physiological action of cadmium arises from its chemical similarity to zinc. Specifically, cadmium may replace zinc in some enzymes, thereby altering the stereo- structure of the enzyme and impairing its catalytic activity. Disease symptoms ultimately result (Bhatia, 2006). The regulatory limits set for cadmium by regulatory bodies are as follows;

i. EPA – 5.00 parts per billion (ppb) or 0.005 parts per million (ppm) of cadmium in drinking water.
ii. Food and Drug Administration (FDA) – concentration in bottled drinking water should not exceed 0.005 ppm (5 ppb). (Sabine and Wendy, 2009).

2.4.1.3 Manganese

Manganese is one of the most abundant metals in Earth's crust, usually occurring with iron. It is a component of over 100 minerals but is not found naturally in its pure (elemental) form (ATSDR, 2000). Manganese is an element essential for the proper functioning of both humans and animals, as it is required for the functioning of many

cellular enzymes (e.g. manganese superoxide dismutase, pyruvate carboxylase) and can serve to activate many others (e.g. kinases, decarboxylases, transferases, hydrolases) (IPCS, 2002b). Manganese can exist in 11 oxidative states; the most environmentally and biologically important manganese compounds are those that contain Mn^{2+}, Mn^{4+} or Mn^{7+} (USEPA, 1994). Manganese is used principally in the manufacture of iron and steel alloys and manganese compounds and as an ingredient in various products (IPCS, 1999; ATSDR, 2000). Manganese dioxide and other manganese compounds are used in products such as batteries, glass and fireworks. Potassium permanganate is used as an oxidant for cleaning, bleaching and disinfection purposes (ATSDR, 2000; HSDB, 2001). Manganese greensands are used in some locations for potable water treatment (ATSDR, 2000). An organic manganese compound, methylcyclopentadienyl manganese tricarbonyl (MMT), is used as an octane-enhancing agent in unleaded petrol in Canada, the United States of America (USA), Europe, Asia and South America (Lynam *et al.*, 1999). Other manganese compounds are used in fertilizers, varnish and fungicides and as livestock feeding supplements (HSDB, 2001). Aside water, Manganese occurs naturally in many food sources, such as leafy vegetables, nuts, grains and animal products (IOM, 2002). Food is the most important source of manganese exposure in the general population (ATSDR, 2000; USEPA, 2002). Traces of manganese are found in toothpaste ingredients such as peppermint-herb, sorbitol and water. Manganese is an essential element for many living organisms, including humans. Adverse health effects can be caused by inadequate intake or overexposure.

The primary targets of manganese toxicity are the brain and central nervous system (US-CDC, 2000). Manganese has been shown to be deposited in certain regions of the

brain, and exposure to high concentrations in occupational studies was associated with permanent damage, with symptoms of impaired neurological and neuromuscular control, mental and emotional disturbances, muscle stiffness, lack of coordination, tremors, difficulties with breathing or swallowing, and other neuromuscular problems (US-CDC, 2000). Exposure to very high doses of manganese in experimental animal studies has resulted in impaired male fertility, and birth defects in offspring including cleft palate, impaired bone development, and other effects (Elbetieha, 2001; Golub, 2005). Manganese deficiency in humans appears to be rare, because manganese is present in many common foods.

Animals experimentally maintained on manganese-deficient diets exhibit impaired growth, skeletal abnormalities, reproductive deficits, ataxia of the newborn and defects in lipid and carbohydrate metabolism (Hurley and Keen, 1987).

The neurological effects of inhaled manganese have been well documented in humans chronically exposed to elevated levels in the workplace (Roels *et al.,* 1999; ATSDR, 2000). The syndrome known as "manganism" is caused by exposure to very high levels of manganese dusts or fumes and is characterized by a "Parkinson-like syndrome", including weakness, anorexia, and muscle pain, apathy, and slow speech, monotonous tone of voice, emotionless "masklike" facial expression and slow, clumsy movement of the limbs (Mergler *et al.*, 1994). By the oral route, manganese is often regarded as one of the least toxic elements, although there is some controversy as to whether the neurological effects observed with inhalation exposure also occur with oral exposure. Several case reports of oral exposure to high doses of manganese have described neurological impairment as an effect, but the quantitative and qualitative details of exposure necessary

to establish direct causation are lacking. An individual who took large mineral supplements over several years displayed symptoms of manganism (Banta and Markesbery, 1977).

Another individual who ingested 1.80 mg of potassium permanganate per kilogram of body weight per day for 4 weeks developed symptoms similar to Parkinson disease 9 months later (Bleich *et al.*, 1999).

2.4.1.4 Nickel

Nickel is primarily found combined with oxygen or sulphur as oxides or sulphides that occur naturally in the earth's crust. Nickel combined with other elements is present in all soils, in meteorites, and is emitted from volcanoes. As for most metals, the toxicity of nickel is dependent on the route of exposure and the solubility of the nickel compound (Coogan *et. al.*, 1989).

The major sources of nickel contamination of toothpaste come from industrial processes. It is used in making batteries, welding rods, adding pigments to paints, and producing dental and surgical prostheses (Mayflor, 2009). The occurrence of nickel is clearly connected with alkaline magma rock as well as silty sedimentary rock. It most often accompanies rock formative magnesium - iron silicates. Due to its sulphurphillic tendency, it combines with arsenic and sulphur to form various minerals of its own. In the course of weathering processes, nickel easily undergoes activation, and in the cationic form of Ni^{2+} it can migrate together with its solutions over great distances. Often, however, it is quickly combined with iron and manganese hydroxides. A considerable part of nickel finds its way into the environment as a result of the burning of diesel oil containing nickel. In nature, it generally occurs in an oxidation state of +2, but its valence may change from -1 to +4. The most common adverse effect of nickel in human is that it causes an allergic reaction at the

site of contact. Acute nickel poisoning in human causes headache, nausea and vomiting, dizziness, chest pain, tightness of the chest, dry cough, and shortness of breath and rapid respiration (Csuros and Csuros, 2002).

2.4.1.5 Zinc

Zinc is one of the most common elements in the Earth's crust. Zinc is found in the air, soil, and water and is present in all foods. In its pure elemental (or metallic) form, zinc is a bluish-white, shiny metal. Powdered zinc is explosive and may burst into flames if stored in damp places. Metallic zinc has many uses in industry. A common use for zinc is to coat steel and iron as well as other metals to prevent rust and corrosion; this process is called galvanization. Metallic zinc is also mixed with other metals to form alloys such as brass and bronze. Metallic zinc is also used to make dry cell batteries. Zinc enters the air, water, and soil as a result of both natural processes and human activities. Most zinc enters the environment as the result of mining, purifying of zinc, lead, and cadmium ores, steel production, coal burning, and burning of wastes. These activities can increase zinc levels in the atmosphere. Waste streams from zinc and other metal manufacturing and zinc chemical industries, domestic waste water, and run-off from soil containing zinc can discharge zinc into waterways. The level of zinc in soil increases mainly from disposal of zinc wastes from metal manufacturing industries and coal ash from electric utilities. Zinc is an essential element needed by your body in small amounts. We are exposed to zinc compounds in food. The average daily zinc intake through the diet ranges from 5.20 to 16.20 milligrams (milligram = 0.001 gram). Food may contain levels of zinc ranging from approximately 2 parts of zinc per million (2.00 ppm) parts of foods (e.g., leafy vegetables) to 29.00 ppm (meats, fish, poultry). Zinc is also present in most drinking water. Drinking water or other

beverages may contain high levels of zinc if they are stored in metal containers or flow through pipes that have been coated with zinc to resist rust (ATSDR, 2005). Zinc chloride is added to toothpaste as the anti bacteria agent. Other sources of zinc in toothpaste include peppermint-herb, water, humectants used in toothpastes. If the amount of zinc taken in is more than the recommended daily amount of supplements containing zinc, one may have higher levels of zinc exposure.

The levels of zinc that produce adverse health effects are much higher than the Recommended Dietary Allowances (RDAs) for zinc of 11.00 mg/day for men and 8 mg/day for women. EPA has stated that drinking water should contain no more than 5 mg of zinc per liter of water (5 mg/L or 5.00 ppm) because of taste. If large doses of zinc (10–15 times higher than the RDA) are taken by mouth even for a short time, stomach cramps, nausea, and vomiting may occur (IOM 2002; WHO 1996; EPA 1987). Ingesting high levels of zinc for several months may cause anemia, damage the pancreas, and decrease levels of high-density lipoprotein (HDL) cholesterol. Symptoms of high-concentration zinc chloride exposure include dyspnea, cough, pleuritic chest pain, bilateral diffuse infiltrations, pneumothorax, and acute pneumonitis, resulting from respiratory tract irritation (ATSDR, 2005; Brandao-Neto *et al.,* 1990). Gastrointestinal symptoms reported in cases of excess zinc exposure include vomiting, abdominal cramps, and diarrhea, in several cases with blood. In general, oral exposure levels associated with gastrointestinal effects of zinc have not been reliably reported, but the limited available data suggest that oral concentrations of 910.00 mg zinc/L or single-dose exposures of ~140.00 – 560.00 mg zinc (acute oral doses of 2.00 – 8.00 mg/kg/day) are sufficient to cause these effects (Lewis and Kokan 1998).

2.4.1.6 Copper

Copper is a metal that exists in the environment as a mineral in rocks and soil. It is Commonly found at low levels in natural water bodies. It is also an essential trace element that is required to maintain good health.

The normal adult requires approximately 2.00 to 3.00 milligrams of copper per person per day. More than 90% of a person's dietary need for copper is provided by food. Drinking water usually provides less than 10% of your daily copper intake. Consumption of high levels of copper can cause nausea, vomiting, diarrhea, gastric complaints and headaches. Long term exposure over many months and years can cause liver damage and death.

High levels of lead, copper, zinc and chromium in the body tissues may tend to increase the tooth's susceptibility to decay (Agarwal *et al.*, 1993). A high tissue copper level, by causing a relative zinc deficiency, predisposes one to hypertension, heart attacks and strokes. Elevated copper levels are frequently associated with migraine headaches. The acute lethal dose for adults lies between 4.00 and 400.00 mg of copper (II) ion per kg of body weight, based on data from accidental ingestion and suicide cases (Agarwal *et al.*, 1993). Individuals ingesting large doses of copper present with gastrointestinal bleeding, haematuria, intravascular haemolysis, methaemoglobinaemia, hepatocellular toxicity, acute renal failure and oliguria (Agarwal *et al.*, 1993). At lower doses, copper ions can cause symptoms typical of food poisoning (headache, nausea, vomiting, diarrhoea). Records from case-study reports of gastrointestinal illness induced by copper from contaminated water or beverages plus public health department reports for 68 incidents indicate an acute onset of symptoms. Symptoms generally appear after 15 – 60 min of exposure; nausea and

vomiting are more common than diarrhoea (US EPA, 1987; Knobeloch *et al.*, 1994; Low *et al.*, 1996; Stenhammar, 1999). Among outbreaks with quantitative data, the lowest copper concentrations associated with effects were about 4.00 mg/litre or higher; background information on the amount of beverage consumed, sample collection and analytical methods is limited. Some studies (Knobeloch *et al.*, 1994; Stenhammar, 1999) reported lower effect levels in children.

2.1.4.7 Titanium

Titanium element occurs within a number of mineral deposits, principally rutile and ilmenite, which are widely distributed in the earth crust and it is found in almost all living things, rocks, water bodies and soils (Encyclopedia, 2006). Its common compound, titanium dioxide, is a popular photocatalyst used in the manufacture of white pigments (Kurebs, 2006). It is used in paints, paper, toothpastes and plastics (USGS, 2011). When used as a whitening agent, titanium dioxide does not really improve the quality of the product. Its function is to mask the inferior quality of the product.

Titanium dioxide, when inhaled, can be carcinogenic, research has shown that it can cause respiratory track cancer in rats exposed by inhalation and intra-tracheal instillation (Kutal and Serpone, 1993). Studies have also found that titanium dioxide nanoparticles cause inflammatory response and genetic damage in mice. Also, there is evidence that the rare disease yellow nail syndrome may be caused by titanium, either implanted for medical reasons or through eating various foods containing titanium dioxide (Berglund and Carlmark, 2011).

Although, there is no limit for titanium dioxide in cosmetics products, it has been found to cause pulmonary irritation in chronically exposed workers.

Workers exposed to it in the production of titanium dioxide have shown signs of fibrosis although described as slight. It also causes mild irritation to skin with intermittent contact for three days. Human skin penetration studies have shown that titanium dioxide can penetrate hair follicles up to 50 microns deep (Hostynek and Maibach, 2002).

2.5 Other Research Works on Toothpastes

The appearance of potentially counterfeit ‘‘Colgate’’ toothpaste on the American market prompted a criminal investigation by the United States Food and Drug Administration (FDA), 60,000 tubes of toothpaste from retail outlets and product distributors were collected for analysis.

Microbiological testing was performed based on the FDA Bacteriological Analytical Manual, which determined the presence and number of bacteria present in the products. Bacteria were isolated from each ‘‘Colgate’’ variety; up to 2×10^6 cfu / g were isolated from some of the collected samples. Using conventional microscopic and biochemical bacterial identification methods, most of the bacteria isolated from these samples were Gram-negative rods of several genera, including *Pseudomonas, Serratia,* and *Klebsiella*. Most of the organisms isolated represent opportunistic pathogens and therefore, counterfeit ‘‘Colgate’’ toothpaste containing high levels of bacteria pose a human health hazard (Jennifer and David, 2012). Hence the need to analyze toothpastes sold in Nigeria.

Vulnerable persons were advised to exercise caution while using some toothpaste after a research was carried out on some selected toothpaste brands sold in Nigeria. The fluoride, phosphate, calcium, sodium, potassium, magnesium, moisture content and pH of the randomly sampled toothpaste were analyzed and the health implication for tooth care

and of assimilation of the elements during the mouth cleansing process especially when present at unacceptable levels were also discussed (Oyewale, 2005).

2.6 Analytical Techniques

2.6.1 Theory of X-ray spectrometer

XRF is an analytical method for determining the chemical composition of all kinds of materials; solid, liquid, powder, filtered or other forms. In XRF, x-ray is produced by a source to irradiate the sample; the elements present in the sample will emit fluorescent x-ray radiation with discrete energies that are characteristic for these elements. Coupled with a detector and a multi-channel analyzers, it is possible to measure the energies of the radiations emitted by the sample to determine which element is present and by measuring the intensities of the emitted energies it is possible to determine how much of each element is present in the sample. The method is fast, accurate and non-destructive and usually requires only a minimum of sample preparation. The applications of XRF are very broad and include the metal analysis. The range of elements that can be analyzed is wide (Be-U). The concentration range goes from sub-ppm levels to 100%. Elements with high atomic numbers have better detection limits than lighter elements. The precision and reproducibility of XRF analysis is very high, very accurate results are possible when good standard specimens are available, but also in application where no specific standard can be found.

A compact energy dispersive X-ray Spectrometer designed for elemental analysis of a wide range of samples known as the Mini Pal was used for this analysis. The system is controlled by a PC running the dedicated Mini Pal analytical software.

The Mini Pal 4 version in use is PW 4030 X-ray Spectrometer, which is an energy dispersive microprocessor controlled analytical instrument designed for the detection and measurement of elements in a sample (solids, powders and liquids), from sodium to uranium.

The sample for analysis is weighed and ground in an agate morta and a binder (PVC) dissolve in Toluene) is added to the sample, carefully mixed and pressed in a hydraulic press into a pellet.

The pellet is loaded in the sample chamber of the spectrometer and voltage (30kV maximum and a current (1mA maximum) is applied to produce the X-rays to excite the sample for a preset time (10 minutes in this case). The spectrum from the sample is now analyzed to determine the concentration of the element in the sample.

2.6.2 Theory of UV-spectrophotometer

Jenway 6405 UV/Visible spectrophotometer was used for the determination of sodium lauryl sulphate and saccharin. The spectrophotometer is an instrument that will resolve polychromatic radiation into different wave lengths. Like all spectrometer, it require a source of continuous radiation over the wave length of interest, a monochromator, for selecting a narrow band of wavelength from the source spectra, a sample cell, a detector or transducer, for converting radiant energy into electrical energy and a device to read out the response of the detector. The sample may precede or follow the monochromator. Each of these except the read out device will vary depending on the wave length region.

This method of analysis involves measuring the absorbance of the reactants or products as a function of time and relating it directly to its concentration using the Beer-Lambert relation

A = εcl where A is the absorbance, ε is the molar absorptivity, c is the concentration and l is the path length.

One of the most common applications of spectrophotometry is to determine the concentration of an analyte in a solution. The experimental approach exploits Beer's Law, which predicts a linear relationship between the absorbance of the solution and the concentration of the analyte (assuming all other experimental parameters do not vary). In practice, a series of standard solutions are prepared (A standard solution is a solution in which the analyte concentration is accurately known). The absorbance of the standard solutions are measured and used to prepare a calibration curve, which is a graph showing how the experimental observable (the absorbance in this case) varies with the concentration. For this experiment, the points on the calibration curve should yield a straight line (Beer's Law). The slope and intercept of that line provide a relationship between absorbance and concentration ie:

$$A = slope\, c + intercept \tag{2.1}$$

The unknown solution is then analyzed. The absorbance of the unknown solution, A_u, is then used with the slope and intercept from the calibration curve to calculate the concentration of the unknown solution, c_u.

$$c_u = \frac{A_u - intercept}{slope} \tag{2.2}$$

2.6.3 Theory of atomic absorption spectrophotometer (AAS).

The AAS is a form of optical analysis that is used primarily for detecting the presence of trace metals. AAS relies on the absorption, emission or transmittance of light by atoms in an excited state to allow the determination of the concentration of atoms in the sample. The liquid samples are nebulized and the resulting aerosol passes through a very hot flame which vaporizes the solvent, leaving a cloud of atoms. The light is beamed at a particular wavelength by a hollowed cathode lamp specific for the element of interest. The light beam passes through the hotter part of the flam and the amount of light absorbed/emitted /transmitted by the excited atom cloud is detected by the instrument and the concentration is of atoms present can be measured. AAS gives accurate measurement of very small amounts of elements (typically 1mg/l detection limit for flame mode), but it can only measure the atoms specific to the cathode lamp used.

2.6.4 Principle of operation of HI 83200 photometer

HI 83200 is a multiparameter bench photometer dedicated for laboratory analysis. It measures 44 different methods using specific liquid or powder reagents. The amount is precisely dosed to ensure maximum reproducibility. The principle behind its operation is also based on Beer-Lambert law:

$$-\log I/I_o = \varepsilon_\lambda \, c \, d \qquad (2.3)$$

$$\text{or} \quad A = \varepsilon_\lambda \, c \, d \qquad (2.4)$$

Where:

$-\log I/I_o$ = Absorbance (A)

I_o = intensity of incident light beam

I = intensity of light beam after absorption

ε_λ = molar extinction coefficient at wave length λ

c = molar concentration of the substance

d = optical path through the substance

Therefore the concentration of the analyte can be calculated from the absorbance of the substance as other factors are known.

Photochemical analysis is based on the possibility to develop an absorbing compound from a specific chemical reaction between sample and reagents.

The optical system of HI 83200 is based on special subminiature tungsten lamps and narrow-band interference filters to guarantee both high performance and reliable results. Five measuring channels allow a wide range of tests. A microprocessor controlled special tungsten lamp emits radiation which is first optically conditioned and beamed through the sample contained in the cuvette. The optical path is fixed by the diameter of the cuvette. Then the light is spectrally filtered to a narrow spectral bandwidth, to obtain a light beam of intensity Io or I, the photoelectric cell collects the radiation I that is not absorbed by the sample and converts it into an electric current, producing a potential in the mV range. The microprocessor then uses this potential to convert the incoming value into the desired measuring unit and to display it on the Liquid Crystal Display (LCD).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS / EQUIPMENT

3.1.1 List of apparatus and equipment

i. AAS: Varian AA240FS. Fast Sequential Atomic Absorption Spectrometer.

ii. Analytical balance: Sartorius Analytical balance.

iii. Beaker – 50 cm^3, 100 cm^3, 250 cm^3

iv. Conical flask – 12 cm^3 , 250 cm^3

v. Crucibles

vi. Desiccators

vii. Filter paper – whatman no. 42 grades

viii. Fume cupboard

ix. Funnel

x. Furnace

xi. Glass rod

xii. Hot plates

xiii. Measuring cylinder – 100 cm^3 , 10 cm^3, 5 cm^3

xiv. Mortar and pestle

xv. Oven : Schutzart DW 40050-IP20

xvi. Petri-dish

xvii. pH meter: Jenway 3505 pH meter.

xviii. Photometer: HI 83200.

xix. Pipette 1 cm^3, 5 cm^3, 10 cm^3 and 25 cm^3.

xx. Plastic bottles for digest – 50 cm^3

xxi. Spatula

xxii. Steam bath

xxiii. UV Spectrophotometer: Jenway 6405 UV/Vis. Spectrophotometer.

xxiv. Volumetric flask 50 cm^3, 100 cm^3 , 250 cm^3, 1000 cm^3

xxv. Wash bottles

xxvi. X-ray Spectrometer: PW 4030

.

3.1.2 List of reagents and chemicals (Analar grade)

i. 4-nitroaniline

ii. Acetic acid

iii. Acetone

iv. Acridin Orange

v. Alizarin red

vi. Ammonia

vii. Hydrochloric acid

viii. Nitric acid

ix. Sodium hydroxide

x. Sodium Lauryl Sulphate

xi. Sodium nitrate

xii. Sodium Saccharin

xiii. Toluene

xiv. Zirconyl acid

3.2 Reagents and Preparation of Solutions

All chemicals used were BDH products of analytical grade purity and were obtained from chemical stores in Kaduna State, Nigeria. All solutions used for this analysis were prepared by dissolving appropriate amount in distilled water. Deionized water was used for solution preparations.

3.2.1 Sodium nitrite solution (1%): prepared by dissolving 1.000 g of sodium nitrite in 100cm^3 distilled water in a 100cm^3 volumetric flask. This solution was kept in a brown bottle and is stable for at least, one week.

3.2.2 Diazotization of 4-nitroaniline (25 millimole): 4-nitroaniline (0.691 g) was dissolved in 50.00 cm^3 of distilled water, containing glacial acetic acid (2.00 cm^3), then 6.60 cm^3 of concentrated HCl was added and the solution heated. The mixture was then transfer to a 200 cm^3 volumetric flask and cool to about 5^oC. A 34.50 cm^3 of 1% $NaNO_2$ was added and the mixture was stirred occasionally for 5 minutes and the volume was made up to 200.00 cm^3 with additional (about 5^oC) cold distilled water. This solution was stored in darkness over ice and used after 15 minutes. This reagent solution when kept in the refrigerator (about 5^oC) is stable for 3 days at least (Younis, 1998).

3.2.3 Alizarin solution: Alizarin red (0.70 g) was weighed and dissolved in about 700.00 cm^3 distilled water in a 1000 cm^3 volumetric flask. The solution obtained was shaken and made up to mark.

3.2.4 Zirconyl Acid: Zirconyl acid (0.354 g) was weighed into a 1000 cm^3 volumetric flask containing 800.00 cm^3 distilled water, conc. H_2SO_4 (33.30 cm^3) was added slowly with stirring and made up to mark with distilled water.

3.2 Sampling

Toothpaste samples (six local and four foreign) of different brands were randomly sampled and purchased from the open/local markets in Zaria, Kaduna State, Nigeria.

3.3 Analysis of Physical Parameters

3.3.1 Colour

The colour of each of the sampled toothpaste was determined by visual method.

3.3.2 Moisture and volatile content

Exactly 2.00 g of toothpaste was weight in a suitable tarred dish. It was then dried at 105 ± 2^oC in oven to constant weight. The moisture and volatile matter percent by weight was then calculated thus;

$$\frac{(M-M_1)\ x\ 100}{M} \qquad (3.1)$$

Where, M_1 = weight in grams of the material after drying.

M = weight in grams of the paste taken for the test.

3.3.3 pH value

Exactly 10.00 g of the toothpaste was weighed into 100 cm^3 beaker. 45.00 cm^3 of freshly distilled and cooled water was then added and mixed well. The pH of the solution was determined using a pH meter.

3.4 Microbial Purity Test

Nigerian Industrial Standard (NIS) for microbiological examination for toothpaste was used to determine the approximate bacterial load of each sample. The first 5.00 cm^3 of toothpaste was squeezed out and discarded, so as to ensure that the paste used for this analysis is not contaminated, since it is possible that the paste might have been exposed to bacteria from the environment. Exactly 1.00 g of the paste was then weighed into 9.00 $cm^3$of 0.10% peptone water (diluents). It was then shacked until the paste was evenly suspended. Petri dishes containing the sterile plate count agar were heated in boiling water until the agar has completely melted. The container were then allowed to cool to approximately 40 - 45^oC and kept in a water bath at 40 - 45^oC until required. 0.10 cm^3 aliquots of the sample after shaking was transferred to a Petri dish each and incubated for the specified periods to permit the development of visual colonies for counting (NIS, 2006).

3.5 Determination of Fluoride Content

Fluoride concentration of the sampled toothpaste was determined using the HI 83200 multiparameter bench photometer. Exactly 1.00 g of dried toothpaste was dissolved in 100.00 cm^3distilled water in a flask. 1.00 cm^3 of the obtained solution was carefully transferred into a sample cuvette, 4 drops of alizarin and zirconyl acids were then added

and the solution made up to the mark of the cuvette. The blank solution was prepared in the same way but without the sample. 4 drops of alizarin and 4 drops of zirconyl acid were added into another cuvette and made up to the mark with distilled water. The cuvette caps are then replaced and inverted several times to mix. The fluoride content was then determined using the HI 83200 multiparameter bench photometer.

3.6 Determination of Titanium

Five grammes (5.00 g) of the toothpaste was weighed and oven dried. It was then crushed and one gramme (1.00 g) was weighed and pelletized. The concentration of Titanium was determined using the XRF.

3.7 Heavy Metal Analysis

This was carried out according to the method described by Popova and Mariniva, 2007. Exactly 1.00 g of toothpaste, weighed in a crucible was heated cautiously on a sand bath until the evolution of vapour seizes. After which, 1.00 cm^3 of conc. HNO_3 was added until the absence of nitrogen oxide was detected. The obtained residue was introduced into a cold furnace and the temperature was increased at 100^oC / hour up to 800^oC. The obtained ash was treated with 1.00 cm^3 HNO_3 (1:1) and heated carefully on a sand bath to dry. The residue obtained was then dissolved in hot distilled water, acidified by the addition of several drops of HNO_3, and filtered through a filter into a 50.00 cm^3 volumetric flask and made up to the mark. The concentrations of Pb, Cd, Mn, Zn, Ni and Cu in the toothpaste were then determined using AAS (Popova and Marinova, 2007).

3.8 Determination of Sodium Lauryl Sulphate (SLS)

One gramme (1.00 g) of the toothpaste was dried at a temperature of about 105^{o}C. It was then dissolved in 100.00 cm^{3} deionized water and used for the analysis. Briefly, 10.00 cm^{3} of sample solution, to which 100.00 µl of acridine orange (5×10^{-3} M) and 100.00 µl of glacial acetic acid was added, and the resultant was extracted with 5.00 cm^{3} of toluene. It was then shaken for 1 minute and allowed to stand for 5 minutes, followed by isolation of the organic layer and its subsequent measurement at 467 nm using a spectrophotometer. A calibration curve was plotted for SLS concentration, ranging from 1.00 ppm to 9.00 ppm. (Santosh *et al.*, 2008).

3.9 Determination of Saccharin

One gramme (1.00 g) of toothpaste was dried at a temperature of about 105^{o}C. It was then dissolved in 100.00 cm^{3} distilled water and used for the analysis. To 10.00 cm^{3} of the prepared solution, 5.00 cm^{3} of 25 mM diazotized 4-nitroaniline was added and the mixture was shaken. After 25 minutes, 6.00 cm^{3} of 40% sodium hydroxide solution was added and the volume was made up to 100.00 cm^{3} with distilled water. The absorbances were measured at 427 nm against the corresponding reagent blank, using 1.00 cm cell. A calibration curve was plotted for saccharin concentration ranging from 1.00 ppm to 9.00 ppm (Thaira and Suad, 2006).

CHAPTER FOUR

RESULTS AND ANALYSIS

4.1 General Characteristics of Samples

A total of ten (10) toothpaste samples, consisting of six (6) local and four (4) foreign toothpastes were obtained for the study. All the samples were in molten form (gel-like). The toothpastes sampled were labeled T1 to T10. The parameters analyzed were compared with set standards were applicable and the results obtained are as reported. Appendix A gives general characteristics of the sampled toothpaste.

4.2 Bacteriological Examination

The result obtained from the analysis of the microbial load of the toothpastes is as shown in Table 4.1. The result shows that they were all sterile as no bacterial or fungal isolates were identified in any of the sampled toothpaste.

4.3 Physicochemical Parameters of the Samples

The pH of the toothpastes was found to be within 5.20 and 8.42 (Figure 4.1). T8, a herbal toothpaste was found to be alkaline; it had the highest pH value of 8.42. Some of the sampled toothpaste (40%) were acidic (i.e. their pH values are less than 7), 40% were found to be neutral and 20% were found to be basic (i.e. their pH values are greater than 7).

Table 4.1: Results obtained from bacteriological examination of toothpaste.

Sample label	TVC	E.Coli	Salmonelia	*Peudomonas arugenisa*
T1	0.00	ND	ND	ND
T2	0.00	ND	ND	ND
T3	0.00	ND	ND	ND
T4	0.00	ND	ND	ND
T5	0.00	ND	ND	ND
T6	0.00	ND	ND	ND
T7	0.00	ND	ND	ND
T8	0.00	ND	ND	ND
T9	0.00	ND	ND	ND
T10	0.00	ND	ND	ND
WHO/SON	300	ND	ND	ND

ND: Not detected.

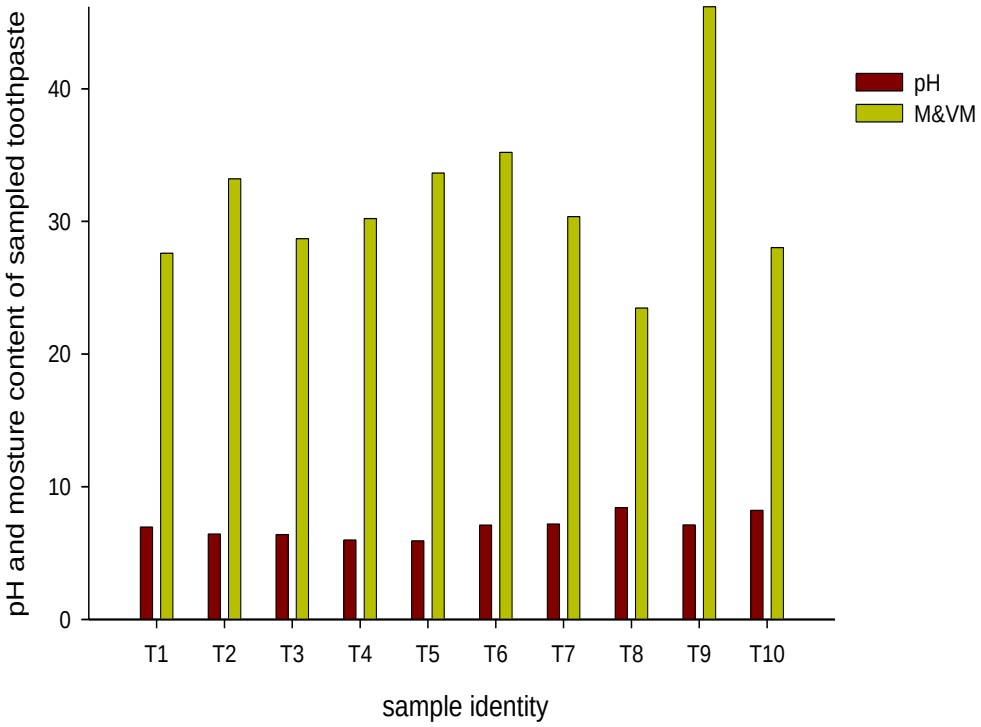


Figure 4.1 pH and moisture content of toothpastes.

4.4 Analysis of Fluoride Concentration in Toothpaste

The average results obtained from three determinations for each toothpaste is as shown in Figure 4.2. The fluoride concentration for each toothpaste was found to be between 770.00 ± 13.46 ppm and 1220.00 ± 5.00 ppm. The claimed active fluoride ingredient and their fluoride content in percentages (%) as well as the claimed equivalent fluoride concentration in ppm, calculated fluoride concentration for each of the ten toothpaste sampled and the allowed permissible levels of fluoride in toothpaste as set by NAFDAC is as given in Appendix C.

4.5 Analysis of Titanium and Other Heavy Metals in Toothpaste

Titanium metal and other selected heavy metals were detected in all the sampled toothpaste, with the exception of lead, which was below the detection limit of the instrument used for analysis. X-Ray spectrophotometer was used to analyze titanium, while Atomic Absorption Spectrophotometer was used to determine Pd, Cd, Ni, Cu, Zn and Mn in the samples.

The result obtained from these analysis showed that all the toothpastes sampled contained some levels of titanium in the range of 1.44 mg/g to 6.25 mg/g, with toothpaste labeled T4 having the highest titanium concentration of 6.25 mg/g (Figure 4.8). Traces of Cd, Ni, Zn, Cu and Mn were also detected (figure 4.3 – 4.7) while Pb was absent in all the sampled toothpaste.

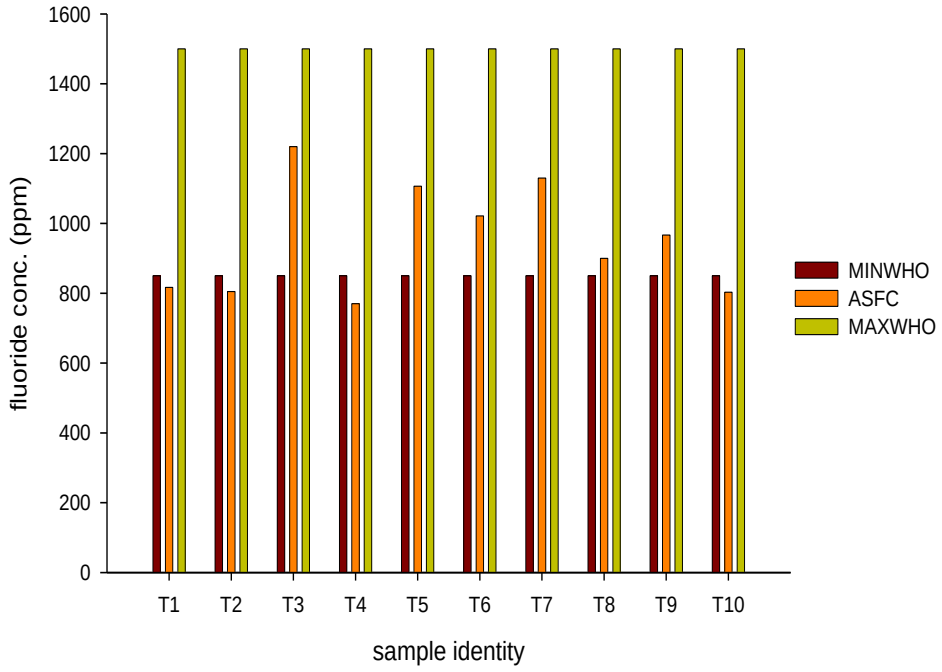


Figure 4.2: Comparision between the fluoride concentration of the sampled toothpaste with the minimum and maximum limit set by WHO/SON.

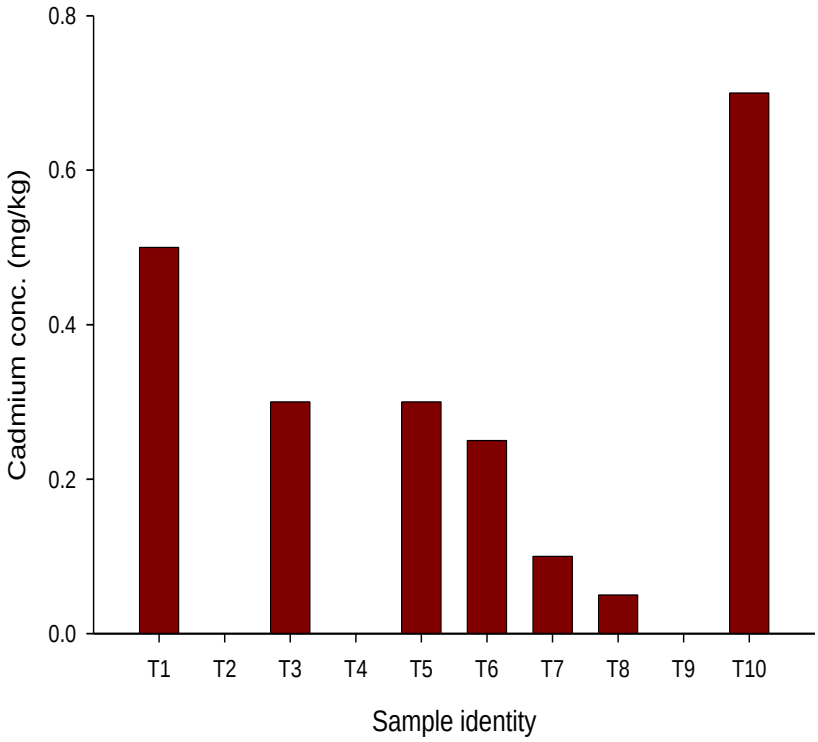


Figure 4.3: Concentration of Cadmium in the sampled toothpastes.

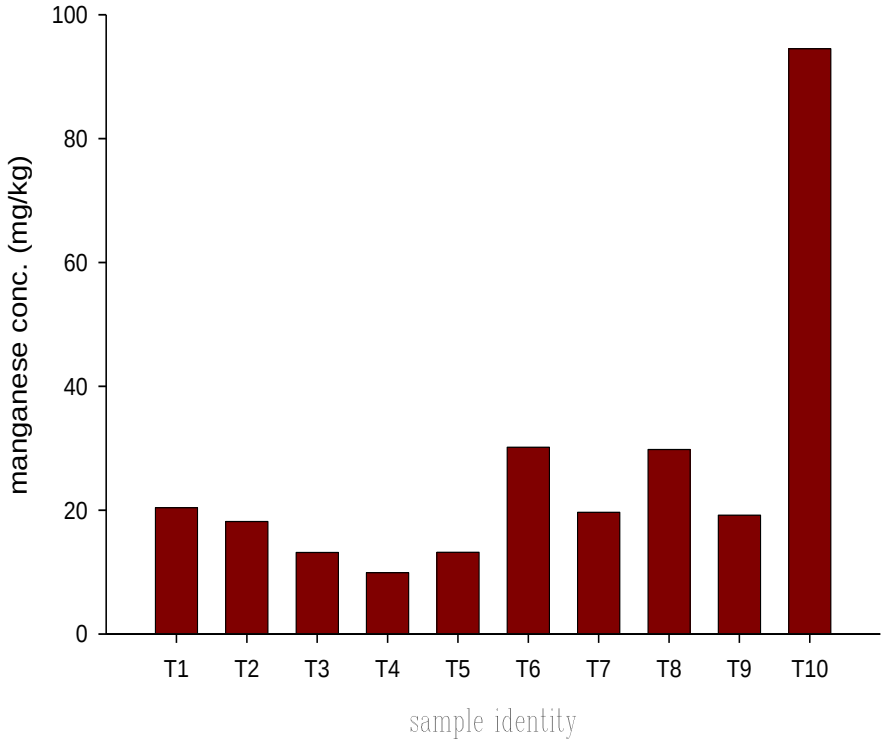


Figure 4.4: Concentration of manganese in the sampled toothpastes.

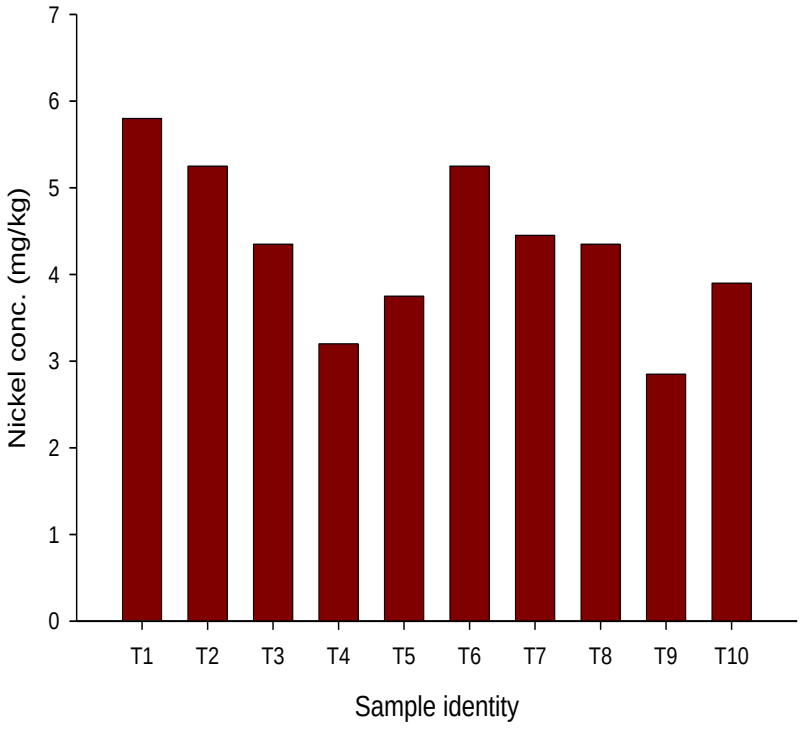


Figure 4.5: Concentration of nickel in the toothpastes samples.

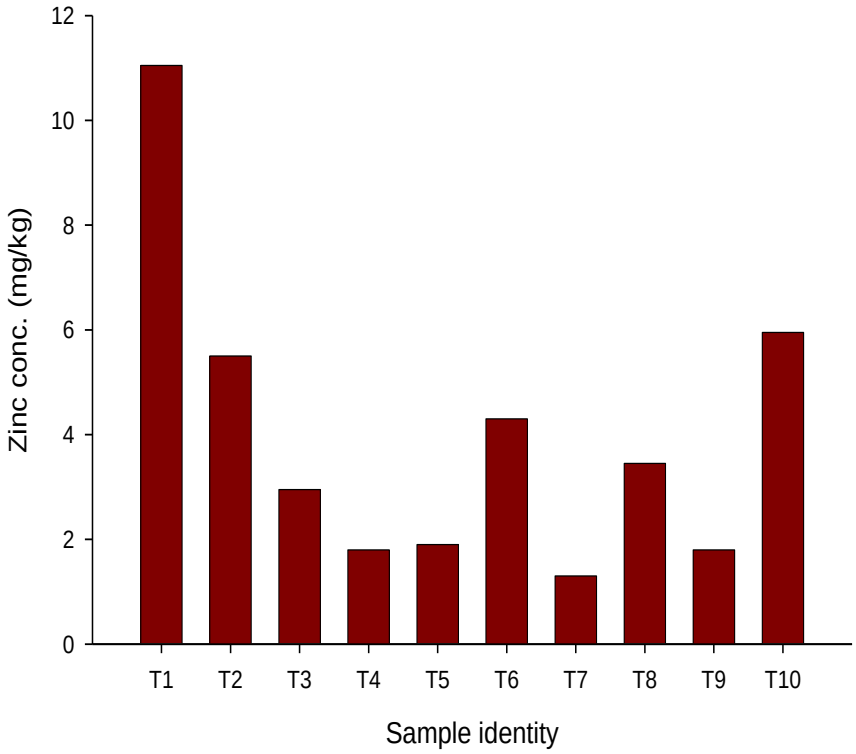


Figure 4.6: Concentration of zinc in the toothpastes.

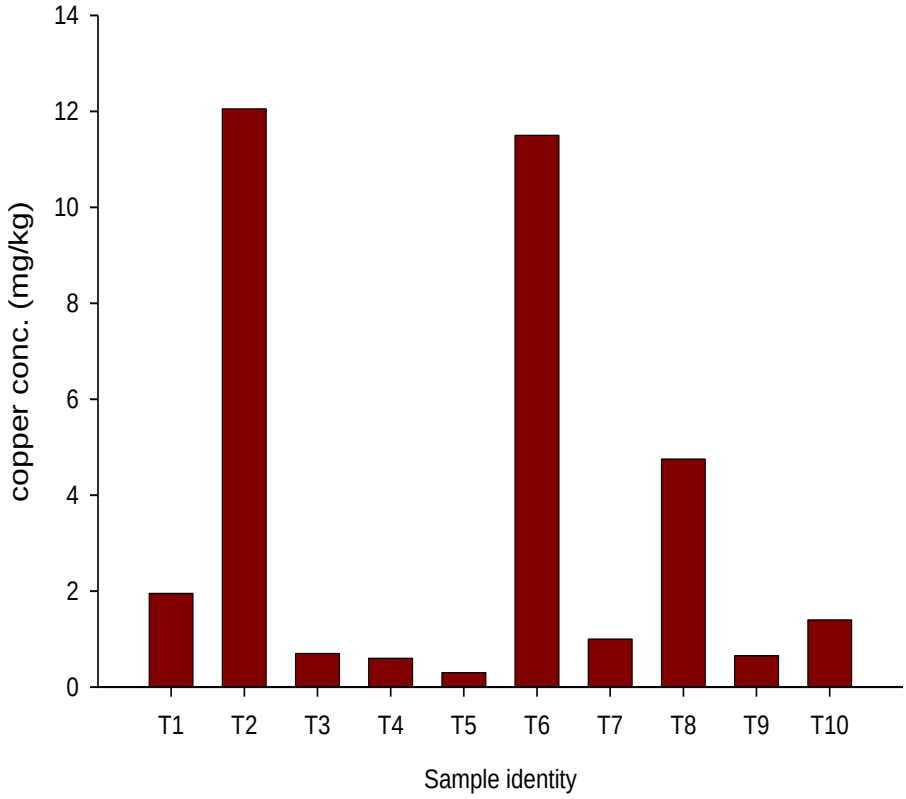


Figure 4.7: Concentration of copper in the toothpastes.

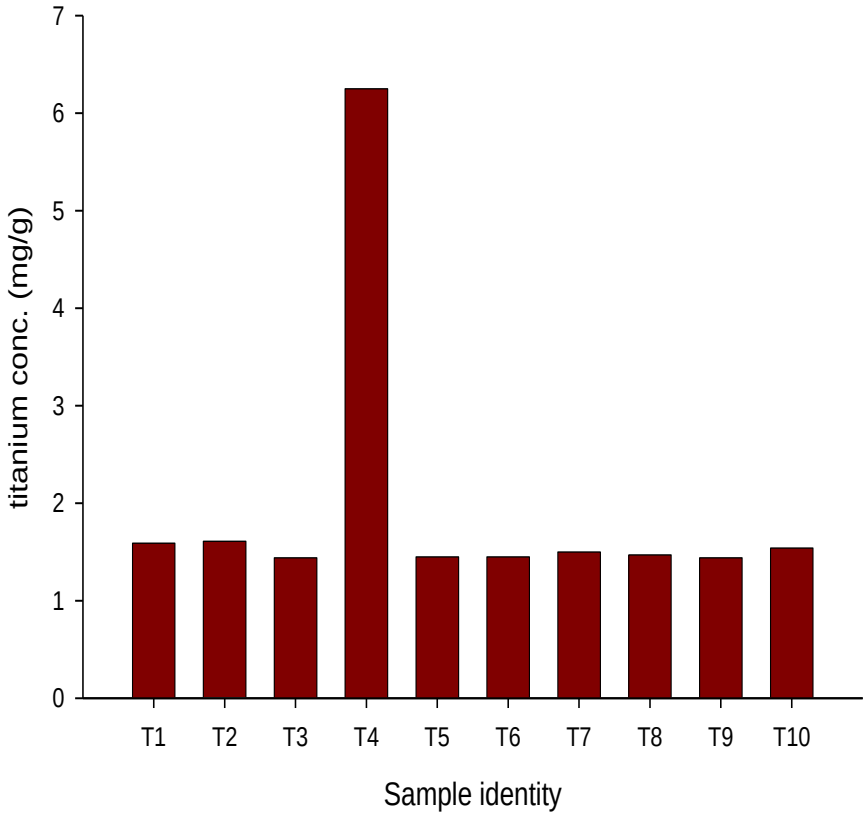


Figure 4.8: Concentration of titanium in the toothpastes.

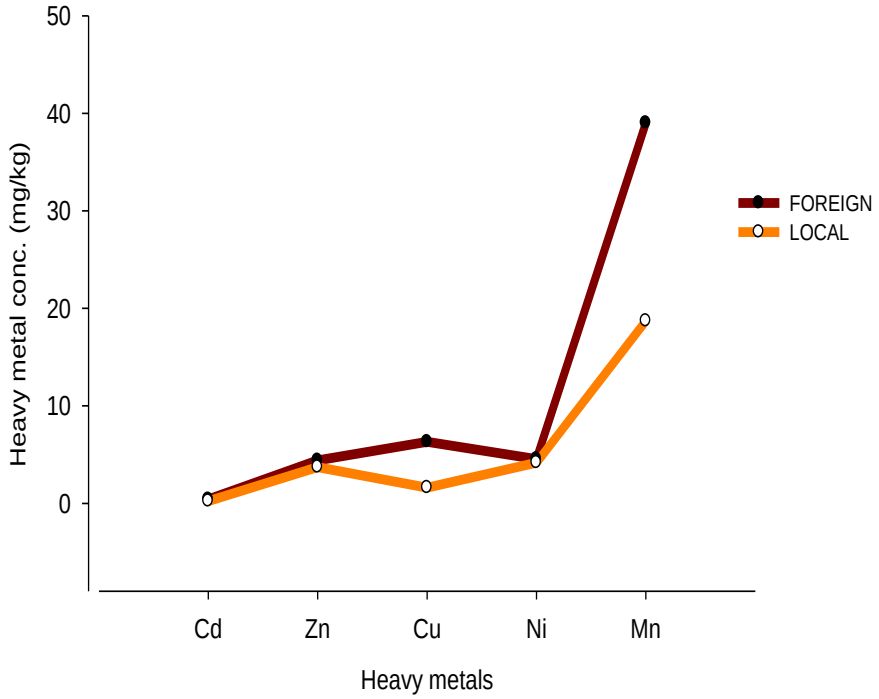


Figure 4.9: Variation study of Cd, Zn, Cu, Ni and Mn in both foreign and local toothpastes.

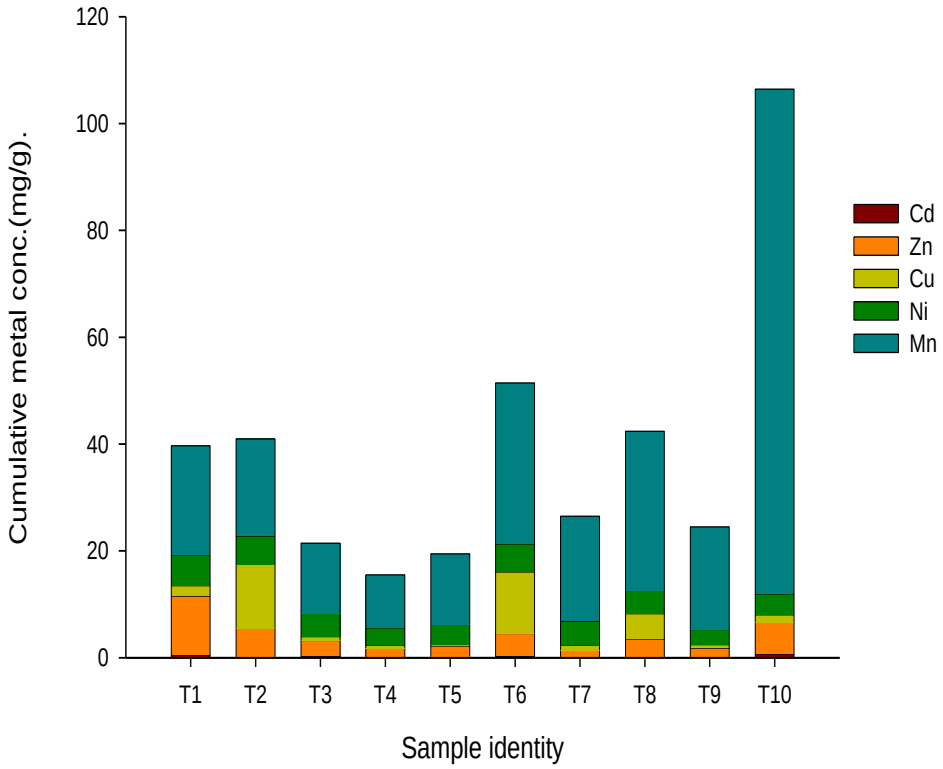


Figure 4.10: Cumulative metal concentration in the toothpastes.

4.6 Analysis of Saccharin and Sodium Lauryl Sulphate (LSs) Concentration in Toothpaste.

The calibration curve obtained for the saccharin analysis using UV-Spectrophotometer in as shown in figure F.11. The concentration of saccharin as determined in each of the sampled toothpaste is as shown in Figure 4.12. The results obtained showed that, saccharin concentration in the sampled toothpastes, ranged from 83.31 ± 17.29 mg/g to 232.20 ± 11.47 mg/g.

The calibration curve obtained for SLS is as shown in Figure 4.13. The concentration of SLS in the sampled toothpaste ranged from 15.64 ± 10.11 mg/g to 21.30 ± 10.22 mg/g. Figures 4.14 shows the variation of SLS in the sampled toothpaste.

4.7 Statistical Analysis of Data

The results obtained from the statistical analysis carried out on the various parameters determined in the sampled toothpaste are as shown in Table 4.2 – 4.5.

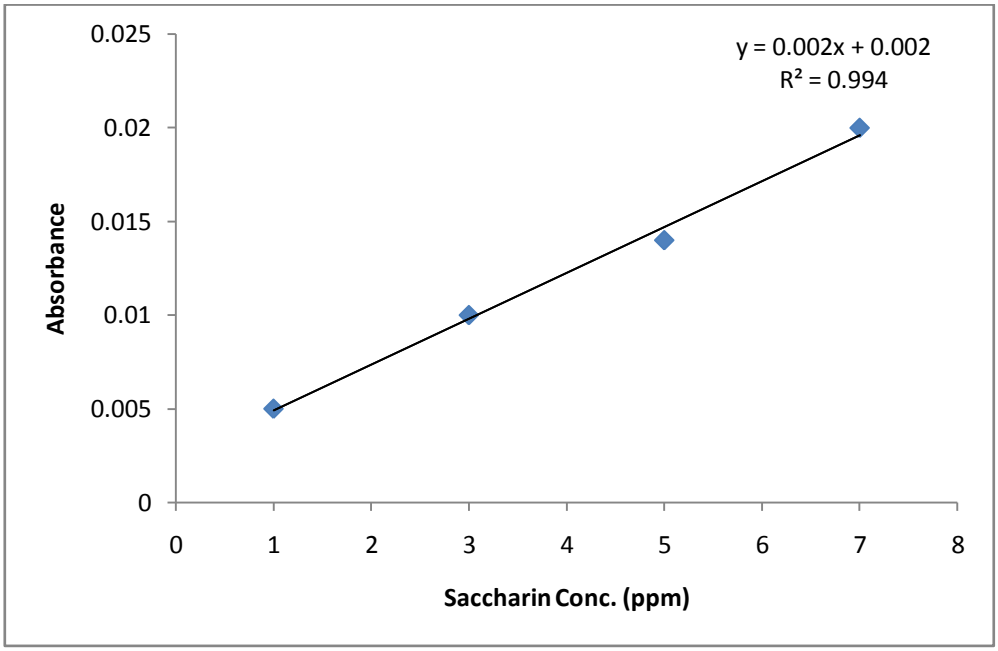


Figure 4.11: Calibration curve obtained for Saccharin.

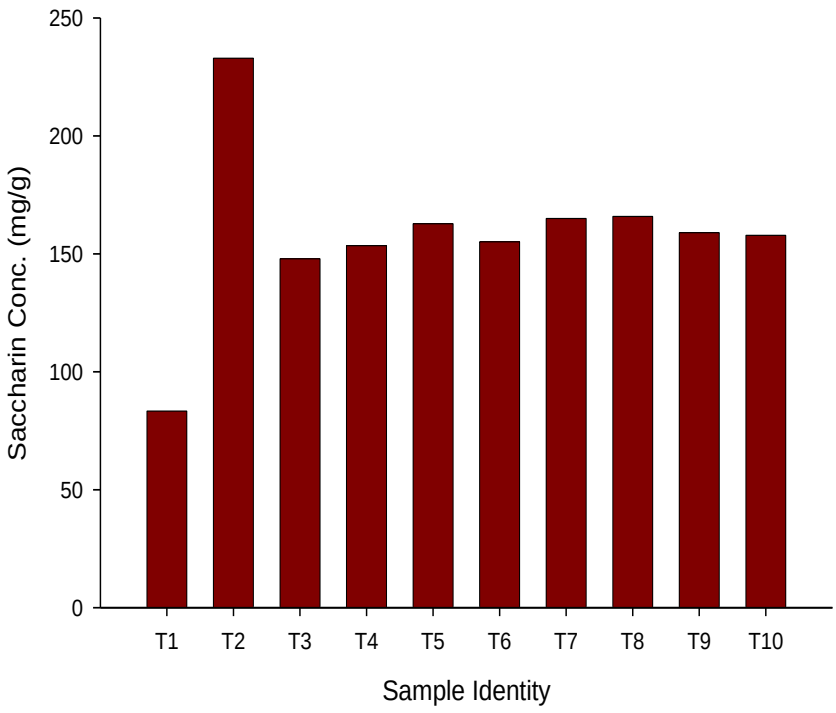


Figure 4.12: Concentration of Saccharin in the toothpastes.

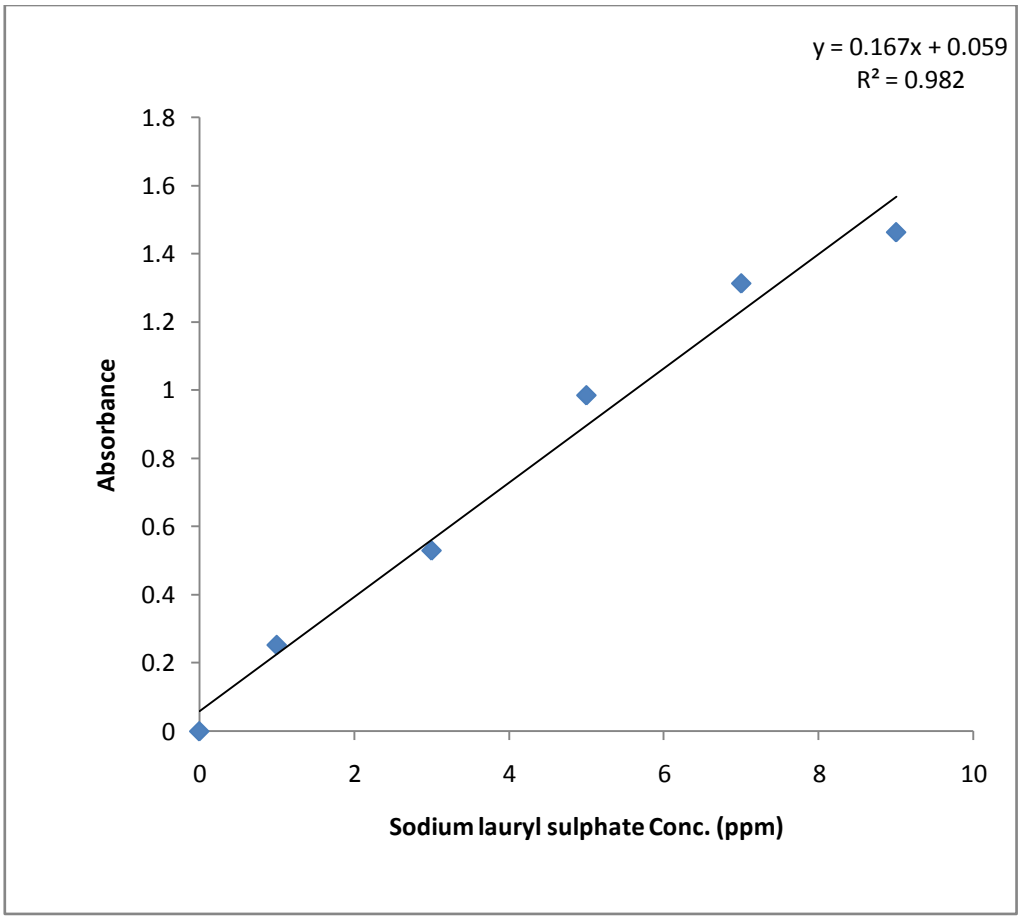


Figure 4.13: Calibration curve obtained for SLS.

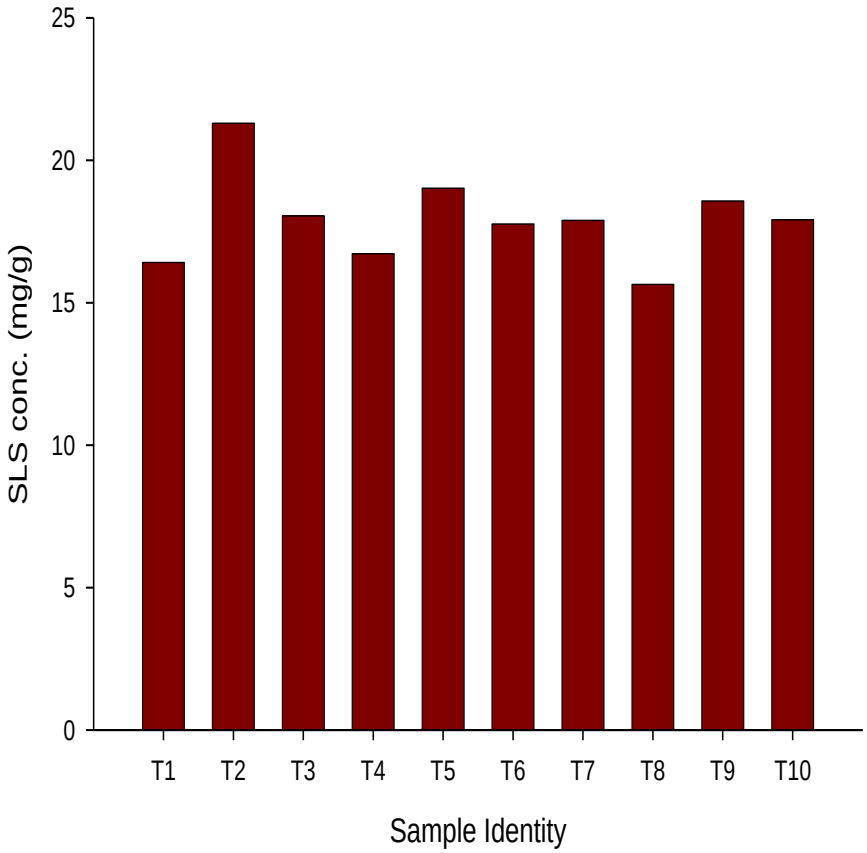


Figure 4.14: Concentration of SLS in the toothpaste

Table 4.2: Correlation coefficient of the physicochemical parameters of the toothpastes.

	pH	M &VM	F
pH	1		
M &VM	-0.294	1	
F	0.221	0.146	1

Table 4.3: The Correlation coefficient (r) of metal concentrations in the different toothpaste.

	Cd	Zn	Cu	Ni	Mn
Cd	1				
Zn	0.587	1			
Cu	-0.227	0.236	1		
Ni	0.271	0.716*	0.598	1	
Mn	0.685*	0.288	-0.005	0.030	1

*correlation is significant at the 0.05 level (2-tailed)

Table 4.4: Analysis of variance table showing saccharin concentration in local and foreign toothpastes.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2387.957	1	2387.957	2.111	0.184
Within Groups	9047.564	8	1130.945		
Total	11435.520	9			

Table 4.5: Analysis of variance table showing SLS concentration in local and foreign toothpastes.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.640	1	7.640	4.251	0.073
Within Groups	14.379	8	7.797		
Total	22.018	9			

CHAPTER FIVE

DISCUSSSION

The result obtained from the bacteriological examination (Table 4.1) shows that all the sampled toothpaste were all sterile as no bacterial or fungal isolates were identified in any of the sampled toothpaste. This result is as expected; toothpaste medium must not support bacterial growth. According to the SON standard for toothpaste, the tolerable limit for the total viable count (TVC) of microorganisms per gram of toothpaste is 300 cfμ/g. *E. coli*, *Salmonella* and *Pseudomonas arugenisa* must be absent. Hence, the result obtained from the present study shows that the microbial load of toothpaste sold in Nigeria meets the criteria set by SON.

The pH value gives an indication of the inorganic constituents in toothpaste. Hight acidic pH encourages the growth of mouth bacterial that causes dental carries (Oyewale, 2005). Recommended pH for toothpaste is between 6.50 and 7.50 (NIS, 2006). Only 60% of the sampled toothpaste fell within this range. The moisture and volatile matter constituents of the toothpastes analyzed fell within 28.03% and 46.19%. The tolerable limit for the moisture and volatile matter in toothpaste as specified by SON is 50% (NIS, 2006). All the sampled toothpastes were found to conform to this specification. The summary of these results are as shown in Figure 4.1.

Figure 4.1 shows that the pH obtained for the foreign toothpastes sampled, conform to the pH specification for toothpastes, except for T5 (5.92 ± 0.001) and T10 (8.22 ± 0.002) which are slightly below and slightly above the minimum and maximum specified values respectively. The average pH of the sampled foreign toothpaste is 6.92 which

conform to toothpaste specification, while that of local toothpaste is 7.01 (neutral), which also conforms to the pH specification for toothpaste.

According to the National Agency for Food and Drugs Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON), the recommended minimal and maximal permissible levels of fluoride in toothpaste are 850.00 ppm and 1500.00 ppm, respectively. As shown in Figure 4.2, the highest fluoride concentration was observed in the sample labeled T3 (1220.00 ± 5.00 ppm) while T4 had the least fluoride concentration (770.00 ± 13.46 ppm). Although, toothpaste samples labeled T1, T5 and T6 were claimed to contain fluoride as high as 1100.00 ppm, 1450.00 ppm and 1450.00 ppm respectively. The result of the analysis showed that the fluoride concentration in these toothpastes were not actually as claimed. The fluoride levels in the toothpastes were 816.67 ± 20.82 ppm, 1106.00 ± 33.00 ppm and 1021.00 ± 33.00 ppm respectively. The concentration of fluoride in T1, T2, T4 and T10 were found to be below the minimum permissible limit set by NAFDAC.

The concentration of fluoride in toothpastes depends on such factors as presence of ingredients which reacts with the fluoride to form insoluble compounds and therefore makes fluoride unavailable to fulfill its therapeutic function, this may as well contribute to the non – conformance of T1, T2, T4 and T10 to the acceptable standard. (Adejumo *et al.*, 2009).

Although fluoride levels in these toothpaste is within the specified limits, it is still risky to swallow even as low as a pea size of the toothpaste since mild exposure of fluoride (6.00 – 8.00 ppm in water) is known to have adverse effect on human, especially children,

pregnant women and old people (Oyewale, 2005). Care must be taken in the formulation and mode of usage of toothpastes, which contain high levels of fluoride.

Many children routinely ingest fluoride from toothpaste. It is now estimated that blood fluoride level of just 95.00 ppb produce an increase in glucose level and a decrease in insulin (Menoyo, 2005). Strikingly, this level is routinely exceeded by about 5 to 10% of children using fluoride toothpaste; particularly those living in fluoridated communities in Nigeria like: Bama community in Borno State, Plateau State and Benue State (Daniel and Agho, 2010; Akpata *et al.*, 2009).

Several researchers have already begun suggesting that diabetic children should use toothpaste with low fluoride content. As noted in a recent study, chronic fluoride intake is capable of decreasing insulin signal and causing insulin resistance. The use of denfrices with lower fluoride content is therefore recommended, especially for diabetic children for whom excessive fluoride consumption may lead to health implications (Chiba, 2012).

Finally, acute fluoride poisoning which occurs at doses as low as 0.10 to 0.30 mg per kg of body weight generally presents in the form of gastric pain, nausea, vomiting, headache, dizziness and flu-like symptoms (Akiniwa, 1997; Gessner, 1994). A child weighing 10.00 kg would only need to ingest 1.00 to 3.00 grams of paste (less than 3% of tube of colgate-for kids) to experience one or more of this symptoms.

The presence of heavy metals in the toothpaste as analyzed can be due to the persistent nature of these elements (Cd, Ni and Cu) as contaminants, while some of them were actually present in some materials used in the manufacture of the toothpaste, for instance, pepper mint herb, used in toothpaste is an excellent source for manganese and

copper, some compounds of these elements are sometimes deliberately added as ingredient in the toothpaste.

Titanium dioxide is added as whitening and thickening agent in toothpaste, control amount of zinc and copper compounds are added to prevent dental plaque formation. Figures 4.3 to 4.8, shows the average results obtained from three determinations of these metals in the sampled toothpastes. A comparative analysis of the levels of these metals in both foreign and local toothpaste is as shown in Figure 4.9. It can be deduced from Figure 4.9 that virtually all the heavy metals analyzed were found in higher concentrations in the foreign toothpaste than in the local toothpastes. This can be due to the advanced level of industrialization in some of these countries. Heavy metal concentrations in some of these countries are high when compared to Nigeria. Hence, most of the ingredient used in the manufacture of the paste can easily be contaminated if care is not taken.

Lead as analyzed was found to be within the safe limit set by standard bodies in all the sampled toothpaste. The presence of cadmium in the samples were in trace amount (0.05 - 0.70 mg/g) in the sampled toothpastes and was absent in others (T2, T4 and T9). Even though cadmium level in the toothpaste is very low, it is good to note that, the slow release of cadmium may also cause harmful effects to the human body. It does not have to be present in abundance in products to produce hypertension. Experimental results showed that minor exposure caused high blood pressure. When cadmium was injected directly into the body system, it caused blood pressure to drop. It targets blood vessels and heart tissue, as well as the kidneys, lungs and brain, and results in heart disease, hypertension, liver damage, suppressed immune system and other nasty symptoms (Godt *et al.*, 2006).

Manganese is required in small amounts in the human body to manufacture enzymes necessary for metabolism of proteins and fats. It has been recommended that an estimated 2.00 to 5.00 mg of Mn per day is safe and adequate intake for adults.

From the results of the analysis, the concentration of manganese in the sampled toothpastes ranged from 9.90 ± 0.0005 to 94.50 ± 0.0005 mg/kg. Meaning that, the consumption of as low as 1.00 g of toothpaste is enough to pose danger to one's health.

Nickel is required by the body in very small amount. It has a very high toxicity, and too much of it can have severe side effects. Hence supplementation with extra nickel is rarely required and one is likely to get enough of it in a normal diet. Small amounts of nickel help the body form enzymes that accelerate chemical reactions in the body especially the formation of new nucleic acids and DNA. Ingesting too much nickel can cause gastrointestinal distress, increased red blood cells, kidney stress, chronic bronchitis, reduced lung function and in some cases lung cancer. There is currently no recommended daily intake for nickel, however, according to the national agricultural library; the tolerable upper in take level of nickel is 1.00 mg in adults. The level of nickel in the sampled toothpaste fall within the range of 2.85 ± 0.0004 mg/kg and 5.8 ± 0.0004 mg/kg with the selected foreign toothpastes having a higher average concentration of 4.538 ±0.827 mg/kg. These concentrations are not very alarming, but it is necessary to avoid swallowing toothpastes while brushing, as this metal have the ability to accumulate in the body and this can be harmful to the human body system.

The level of zinc in the toothpaste analyzed ranged from 1.30 ± 0.0002 mg/kg to 11.05 ± 0.0004 mg/kg. Comparism of the average concentration of zinc in both the foreign

and local toothpastes showed that the foreign toothpaste has higher concentration of zinc (4.413 ±1.814 mg/kg) than the local toothpastes (3.725 ± 3.357 mg/kg). The observed increase in zinc concentration in the sampled toothpaste, compared with the other elements analyzed is not surprising as zinc compound (Zinc citrate) is often used in toothpaste and mouth wash to prevent dental plaque formation and *gingivitis*. Zinc is an essential trace element; hence very small amount of zinc is required by the human body.

The concentration of copper in the sampled toothpastes ranged from 0.30 ± 0.0002 mg/kg to 12.05 ± 0.0001. From Figure 4.7, samples T3, T4, T5 and T9 had very low copper concentration, while T2 and T6 had very high concentration of copper. Although copper is also an essential element, only a little is required by the body as too much of it can be hazardous to the human health. The average daily requirement for copper in the adult human has been established at 2.00 mg and for infants and children at 0.05 mg/kg bw.

The concentration of titanium in the sampled toothpastes were found to be at very close range, except for T4, a local toothpaste, which had a very high titanium concentration of 6.25 mg/g (Figure 4.8). T3 and T9 had the lowest concentration of 1.44 mg/g. Although research has shown that the exposure to titanium can be hazardous to humans, the ADI for titanium has not yet been established.

A comparison of the level of heavy metal concentration in both local and foreign toothpastes (Figure 4.9) showed that the concentration of these metals are higher in the foreign sampled toothpaste than in the local toothpastes.

The analysis of saccharin concentration in the sampled toothpaste shows that T1 had the lowest saccharin concentration while T2 had the highest saccharin concentration (Figure 4.12). T1 had the lowest saccharin concentration probably because it has been exposed to high temperature since saccharin in unstable when exposed to high temperature. Also, T1 is likely to contain more than one sweetener, as saccharin can also be used with other sweeteners.

European Commission regulations and standards stipulate that: (1) saccharin, sodium saccharin or calcium saccharin is allowed for use in non-alcoholic drinks (maximum useable dose, 80.00 – 100.00 mg/L), in desserts and similar products (100 mg/kg), in confectionery (80.00 – 1200.00 mg/kg) and in vitamins and dietary preparations (1200.00 mg/kg) (European Commission, 1994a); (2) saccharin, sodium saccharin or calcium saccharin may be added as an aromatic and appetizing substance to animal feedstuffs (European Commission, 1970, 1994b). The Scientific Committee for Food of the European Commission increased the ADI for saccharin from 2.50 mg/kg bw to 5.00 mg/kg bw in June 1995 (International Sweeteners Association, 1998); however, it has been recommended that intake of saccharin by children should be minimized, and use of saccharin in infant foods should be prohibited (European Commission, 1978; United Nations Environment Programme, 1998).

The Joint FAO/WHO Expert Committee on Food Additives in 1993 re-allocated a group ADI of 5.00 mg/kg bw for saccharin, sodium saccharin, potassium saccharin and calcium saccharin, singly or in combination (WHO, 1993a). In 1977, it had changed the unconditional ADI for humans of 5.00 mg/kg bw established for saccharin and its potassium and calcium salts to a temporary ADI of 2.50 mg/kg bw (WHO, 1978).

Regulations and standards in the United Kingdom stipulate that saccharin, sodium saccharin or calcium saccharin is a permitted sweetener for food intended for human consumption. The sale, importation, supply and advertisement of any sweetener, of any food containing a sweetener or of the use of any sweetener as an ingredient in the preparation of foods other than as a permitted sweetener is prohibited. The sale of food for babies and young children which contains an added sweetener is restricted. The use of all permitted sweeteners in jam and similar products intended for diabetic patients and in soft drinks is permitted (UNEP, 1998).

In India, sodium saccharin is permitted in carbonated water up to 100.00 ppm [mg/L] (as saccharin) and may be sold as a table-top sweetener (UNEP, 1998).

Regulations and standards in Kenya stipulate that saccharin or sodium saccharin is a food additive permitted as a non-nutritive sweetening agent. The food products in or upon which it is permitted and maximum levels of use are listed (UNEP, 1998).

In the United States, saccharin is currently approved for use under an interim food additive regulation permitting use for special dietary purposes and in special dietary foods. The food additives saccharin, ammonium saccharin, calcium saccharin and sodium saccharin may be safely used as sweetening agents in food in accordance with the following conditions: if the substitution for nutritive sweeteners is for a valid, special dietary purpose and is in accord with current special dietary food regulations and policies or if the use or intended use is for an authorized technological purpose other than calorie reduction. The additives are authorized for use as sweetening agents only in special dietary foods, as follows: (1) in beverages, fruit juices and bases or mixes when prepared for consumption in accordance with directions, in amounts not to exceed 12.00 mg of the

additive, calculated as saccharin, per fluid ounce; (2) as a sugar substitute for cooking or table use, in amounts not to exceed 20.00 mg of the additive, calculated as saccharin, for each expressed teaspoonful of sugar sweetening equivalence; and (3) in processed foods, in amounts not to exceed 30.00 mg of the additive, calculated as saccharin, per serving of designated size. The additives are authorized for use only for the following technological purposes: to reduce bulk and enhance flavours in chewable vitamin tablets, chewable mineral tablets or combinations thereof; to retain the flavour and physical properties of chewing-gum; and to enhance the flavour of flavour chips used in non-standardized bakery products (FDA, 1998).

The amount of saccharin found in the different brands of the toothpastes have similar values except for T1 and T2 whose concentrations where too low and too high respectively when compared to the others. According to the Food and Drug Administration (FDA), the acceptable daily intake (ADI) for saccharin is 5.00 mg/kg of body weight, meaning that a child weighing about 10.00 kg will have a calculated ADI of about 50.00 mg, which is far lower than the concentrations obtained per gram of the toothpaste. When the present results are interpreted in the light of permissible dose, all the subjected samples showed higher amount of saccharin, hence posses high risk to human health.

Although, there is currently no ADI for SLS, it is good to note that swallowing SLS will likely lead to nausea and diarrhea (Herlofson and Barkvoll, 1996), hence one must be careful not to swallow much of toothpaste if it contains SLS.

From the result obtained for the analysis of SLS in the toothpaste samples, T2 had the highest concentration of 21.30 ± 10.22 mg/g while T8 had the lowest concentration of 15.64 ± 10.11. This is shown in Figure: 4.14.

High levels of SLS intake (orally) are not ordinarily experienced in normal toothpaste use, but because of water solubility of this substance, and the fact that the tissue of the mouth are soft and readily absorbs, it gets accumulated in the body, and the effects of long term repeated exposures becomes a real concern.

The correlation coefficient (r) of the physicochemical parameters as shown in Table 4.2 indicates significant difference in all the sampled toothpastes, meaning that they are all independent of one another

The correlation results obtained for the metals as shown in Table 4.3 indicated a significant correlation at the 0.005 level (2-tailed) in some metals, meaning that they could be from the same source

Statistical analysis carried out on the result obtained from saccharin and SLS analysis as shown in Table 4.4 and 4.5 respectively, indicated a significant difference in the concentration of saccharin and SLS in each of the sampled toothpaste.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

In the present study, the pH of various brands of toothpastes analyzed ranged from 5.20 to 8.42, only six (6), out of the ten (10) sampled toothpastes complied with the NAFDAC set standard of 6 to 7. The moisture and volatile matter content of the toothpaste were also less than 50% as specified by WHO. The microbial load of the toothpastes showed that they were all sterile as no bacterial or fungal isolates were identified in any sampled toothpastes and the concentration of the fluoride in the sampled toothpastes were within the specified limits, hence complying to set standards.

Toothpastes of nowadays are a heavy mix of chemicals, some are prepared to suit a particular need of the consumer e.g. T10, is a smokers toothpaste, which is designed to remove stubborn stains from the tooth, hence making it stain free. T8, which is a herbal based toothpaste, contain a lot of plant extracts. It was designed to meet the needs of consumers who prefer to stick to natural products.

In any case, the daily use of these toothpastes can easily result in exposure to thousands of chemicals and many will make their way into the body and remain there, since the body lack means to break them down. This load can become a significant contributing factor to health problem and serious diseases.

The current investigation showed that toothpaste could serve as a source through which humans are exposed to heavy metals, most especially when ingested. The metals

determined were Pb, Cd, Ni, Mn, Zn and Cu. Although lead was absent in all the sampled toothpastes (probably because manufactures were careful during the manufacturing process so as to avoid lead contamination), traces of cadmium were found in some. Cadmium is not an acceptable ingredient in toothpaste, it only exist in some, due to its persistent nature. Hence, only samples T1, T3, T5, T6, T7, T8 and T9 showed traces of cadmium. It was absent in all the others.

Among the different samples analyzed, manganese was found to be at higher concentration than all the other test metals. This is not at all surprising as manganese is found in common toothpaste ingredients like peppermint-herb, sorbitol and water. The concentration of manganese ranged from 9.90 ± 0.0005 to 94.50 ± 0.0005 mg/kg with T10, which is a smoker's toothpaste having the highest manganese concentration. Traces of nickel was also found, and ranged from 2.85 ± 0.0004 to 5.80 ± 0.0004 mg/kg. The level of zinc in the toothpastes analyzed ranged from 1.30 ± 0.002 mg/kg to 12.05 ± 0.0001 mg/kg, while copper, ranged from 0.30 ± 0.0002 mg/kg to 12.05 ± 0.001 mg/kg with samples T3,T4,T5 and T9 having very low copper concentration.

A comparison of the level of these metals in both local and foreign toothpastes showed that the concentrations of these metals are higher in the foreign toothpastes than in the local toothpastes. Statistics carried out to compare the presence of these heavy metals in the different toothpaste brands showed significant relationship in some of the metals, meaning that, they could be from the same source.

The positive test result obtained for titanium in all the samples is not at all surprising, as it is used as an ingredient in toothpastes. It serves as whitening and ticking

agent in toothpaste. All the sampled toothpastes showed some level of titanium in it, with T4 having the highest concentration of 6.25 mg/g.

Hence, based on these results obtained, it can be concluded that toothpastes serve as a source of human exposure to heavy metals.

Aside these heavy metals, all the sampled toothpastes tested positive for the presence of sodium saccharin and sodium lauryl sulphate. They are ingredients added to serve as sweetening and foaming agents respectively. It is important to note that these two substances are hazardous to the human health especially when orally administered. The concentration of saccharin in the sampled toothpastes ranged from 83.31 ± 17.29 mg/g to 232.20 ± 11.47 with T2 having the highest saccharin concentration, while the concentration of SLS ranged from 15.64 ± 10.11 mg/g to 21.30 ± 40.22 mg/g.

In all cases, except for microbial and lead analysis, which conform to set standards, the substances determined in the sampled toothpastes showed higher amount of these hazardous substances, furthermore, the analytical data recorded in the present survey is very alarming, in the sense that the amount of each of these substances determined in the different brands of toothpastes is not uniform, except for lead and microbial contamination which were absent in all the sampled toothpastes. This means that, it is possible that manufactures of these toothpastes, do not use the same guidelines/specifications to manufacture these toothpastes.

Hence, caution must be exercised when using toothpastes, particularly foreign toothpastes. Vulnerable people like children, pregnant women, the elderly and sick, must exercise caution when using toothpastes.

6.2 Recommendations

It is recommended that a guideline be made for the permissible level of saccharin; SLS and TiO_2 used in toothpastes and these should be monitored from time to time to ensure that toothpaste do not pose health challenge to consumers.

The need to stick to the directions given for the use of toothpastes most especially where children and pregnant women are concerned should be emphased.

Further studies should be conducted to include other toxic substances like triclosan, diethylene glycol, xylitol and PEG which are other harmful substances used in some toothpastes.

REFERENCES

Adejumo O.E, George-Tailor O.M, Kolapo A.L, Olubamiwa A.O, Fayokun R and Alawode O.A (2009): Determination of fluoride concentration in various brands of toothpaste marketed in Nigeria using ion selective electrode method *Advances in Medical and Dental Sciences.* 3(2):46-50.

Agarwal S.K, Tiwari S.C and Dash S.C (1993): Spectrum of poisoning requiring hemodialysis in a tertiary care hospital in India *International Journal of Artificial Organs*, 16(1):20–22.

Agency for Toxic Substances and Disease Registry (2000): Toxicological profile for manganes. Atlanta, GA, United States Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Agency for Toxic Substances and Disease Registry (2005): Toxicological profile for zinc. Atlanta, GA, United States Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Aigueperse J, Mollard P, Devilliers D, Chemla M, Faron R, Romano R and Cuer J.P (2005): Fluorine compounds, inorganic *Ullmann's Encyclopedia of industrial Chemistry*. Weinheim, Wiley-VCH, P.307.

Akiniwa K (1997): Re-examination of acute toxicity of fluoride. *Fluoride* 30:89-104

Akpata E.S, Danfillo I.S, Otoh E.C and Mafeni J.O (2006): Geographical mapping of fluoride levels in drinking water sources in Nigeria. *African Health Sciences.* 9(4):227-233.

Amal E.S (2012): Heavy metal overload. http://www.amalelsafty.com content& view=article&id=70:heavy-metal-overload&catid=34:english-articles&itemid=55. Retrieved 11/09/2013.

American Dental Association (2006): Interim guidance on fluoride intake for infants and young children. http://www.wikipedia.com. Retrieved 20/2/2013

American Pregnancy Association (2007): Using Artificial Sweetener during Pregnancy. http://www.americanpregnancy.org/pregnancyhealth/artificialsweetner.htm. Retrieved 6/6/2011.

Ashworth D.T and Alloway B.Y (2004): Soil mobility of sewage sludge-derived dissolved organic matter, copper, nickel and zinc. *Environmental Pollution*. 127(1):137-144.

Asok A, Anjali P and Mana B (2005): Spectrophotometric determination of anionic surfactants in waste water using acridine orange. *Indian Journal of Chemical Technology*. 12:145-148.

Babich H and Babich I.P (1997): Sodium lauryl sulphate and triclosan , invitro cytotoxicity studies with gingival cells. *Toxicology Letter*. 91(3):189-196.

Banta R.G and Markesbery W.R (1977): Elevated manganese levels associated with dementia and extrapyramidal signs. *Neurology,* 27:213–216.

Barnes G.T and Gentle I.R (2005): Interfacial Science. Oxford University Press. Oxford.

Baselt R.C (2008): Disposition of toxic drugs and chemicals in man. *Biomedical Publications* 636-640.

Berglund F and Carlmark B (2011): Titanium Sinusites and the yellow nail syndrome. *Biological and Trace Element Research*. 143 (1):1-7.

Berthoud, H.R. Trimble, E.R. Siegel, E.G, Bereiter D.A and Jeanrenaud B (1980): Cephalic-phase insulin secretion in normal and pancreatic islet-transplanted rat. *American journal of physiology Endocrinology and Metabolism*. 238(4):336 - 340.

Bhatia, S.C. (2006): Environmental pollution and control in chemical process industries. Khanna publishers, Darya gay, New Delli.Pp 1184-1191.

Bleich S (1999): Chronic manganism: Fourteen years of follow-up. *Journal of Neuropsychiatry and Clinical Neuroscience*, 11:117.

Bradfort D.G, Micheal B, John P.M and Gray M.W (1994): Acute fluoride poisoning from a public water system. *New England Journal of Medicine.* 330(2):95-99.

Brandao-Neto J, deMendon C.A, Shuhama T (1990): Zinc acutely and temporarily inhibits adrenal cortisol secretion in humans: A preliminary report *Biological and Trace Element Research.* 24:83-89.

Byard, J. L, McChesney E.W, Golberg L and Coulston F (1974): Excretion and metabolism of saccharin in man. II. Studies with 14C-labelled and unlabelled saccharin. *Food and Cosmetics Toxicology.* 12(2):175-184.

Captain-Valley L.F, Valencia M.E and Arana N. (2004): Flow – through spectrophotometric sensor for the determination of saccharin in low calorie products *Food Aditives and Contamminants.* 21 (1): 32-41.

Chiba F.Y (2012): NaF treatment TNF-a and resisting concentrations and reduces insulin signal in rats. *Journal of Fluorin Chemistry.* 136:3-7

Coogan T.P, Latta D.M, Snow E.T and Costa M (1989): Toxicity and carcinogenicity of nickel compounds. *Critical reviews in toxicology*.19:341-84.

Corapcioglu M.O. and Huang C. P. (1987): The adsorption of heavy metals onto hydrous activated carbon. *Journal of Water Research*. 21 (9): 1031-1044.

Csuros, M., and Csuros, C. (2002): Environmental sampling and analysis for heavy metals Lewis Publishers, Pp. 13 – 40, 221 – 231.

Daniel I.B and Agho M.O (2010): Alarming fluoride concentrations of water samples from boreholes in Bama Community and Remediation with *moringa olifera. International Journal of water and soil resources research*. 1: 1-3.

Daniel M.D (2012): More causes of contact Dermatitis. www. About.com. retrived 01/10/12.

Elas M and Haans M.D (2003): Toxic minerals and heavy metals. Califonia. 44th Ed.

Elbetieha, A (2001): Effects of long-term exposure to manganese chloride on fertility of male and female mice. *Toxicological Letters*. 119(3):193-201.

Encyclopedia Britannica (2006): Titanium. http://en.m.wikipedia.org/wiki/titanium. Retrived 1/1/2013.

Environmental Protection Agency (1987): Ambient water quality criteria for zinc. Washington, DC: U.S. Environmental Protection Agency, Office of Water Regulations and Standards. EPA440587003. PB87153581.

Environmental Protection Agency (1990): Environmental pollution control alternatives Cincinnati, US. Environmental Protection Agency. EPA/625/5-90/025, EPA/625/4-89/023,

Eric C. R (1994): Contents of toothpaste-Safety implication. *Australian Prescriber*. 17: 490-510.

European Commission (1970): Council Directive of 23 November 1970 concerning additives in animal feeding stuffs (70/524/EEC). *Official Journal of the European Communities*, **L270**, 1.

European Commission (1994a): European Parliament and Council Directive of 30 June 1994 on sweeteners for use in food stuffs (94/35/EC). *Official Journal of European Communities*, **L237**,3.

European Commission (1994b): Amendment to the Council Directive of 23 November 1970 concerning additives in animal feedingstuffs (70/524/EEC). *Official journal of European Communities*, **L297**, 27.

Fishberg, A. M., Hitzig, W. M. and King, F. H. (1933): Measurement of the circulation time with saccharin. *Proceedings of the Society for Experimental Biology and Medicine*, 30, 651.

Food and Drug Administration (1998): Saccharin, ammonium saccharin, calcium saccharin, and sodium saccharin. *Code of Federal Regulation*, Title 21(180.37): 422–423.

Forward G.L (1991): Role of toothpaste in the cleaning of teeth. *International Jounal of Dentistry*, 41:164-170.

Gert-Wolfhard von Rymon Lipinski (2005): Sweeteners. *Ullmann's Encyclopedia of Industrial Chemistry*, Weinheim: Wiley-VCH.

Gessner B.D (1994): Acute fluoride poisoning from public water system. *New England Journal of Medicine.* 330:95-99.

Godt J, Scheidig F, Grosse-Siestrup C, Esche V, Brandenburg P, Reich A and Groneberg D.A (2006): The toxicity of cadmium and resulting hazards for human health. *Journal of occupational Medicine and Toxicology.* 1: 22-26.

Golub, M.S (2005): Neurobehavioral evaluation of rhesus monkey infants fed cow's milk formula, soy formula, or soy formula with added manganese. *Neurotoxicology and Teratology*. 27(4):615-627.

Gosselin R.E, Smith R.P and Hodge H.C (1984): Clinical toxicology of commercial products. Baltimore (MD): Williams &Wilkins 111-193.

Griffin S.O, Regnier E, Griffin P.M and Huntley V (2007): Effectiveness of fluoride in preventing caries in adults. *Journal of Dentistry Research*. 86(5): 410-415.

Han H, Wu Z, Young S.H, Chen T.H, Cumpston J.T, Chen F, Kashon M.L and Castranova V (2012): Pulmonary exposure of rats to ultrafine titanium dioxide enhances cadiac protein phosphorylation and substance P synthesis in nodose ganglia. *Nanotoxicology*. 6 (7): 736-745.

Herlofson B.B and Barkvoll P (1994): Sodium lauryl sulphate and recurrent aphthous ulcers: A prelimnary study. *Acta Odontol Scand* 257-259.

Herlofson B.B and Barkvoll P (1996a): Oral mucosa desquamationcaused by two toothpaste detergents in an experimental model. *European Journal of Oral Science*. 104(1):21-26.

Herlofson B.B and Barkvoll P (1996b): Oral mucosal desquamation of pre and post menoposal women. *Journal of Clinical Periodontology*. 23; 567-571.

Hernandez M.C, Lopez I.G and Sanchez-Pedrena C (1985): Spectrophotometric determination of saccharin in different materials by a solvent extraction method using Nile blue as reagent. *Talanta* 32(4):325-327.

Holistic M (2009): Fluoridation/fluoride toxic chemicals in your water. http://www.holisticmed.com/fluorid/. Retrived 04/03/2011.

Hostynek J.J and Maibach H.I (2002): Silver, titanium and zirconium: metals in cosmetic and personal care products. *Cosmetics & Toiletries Magazine.*117 (1): 26-30.

Howe, G.R and Burch, J.D (1981): Artificial sweeteners in relation to the epidemiology of bladder cancer. *Nutrition and Cancer*. 2 (4): 213-216.

Howe G.R, Burch J.D, Miller A.B, Cook G.M, Esteve J and Morrison B (1980): Tobacco use, occupation, coffee, various nutrients and bladder cancer. *Journal of the National Cancer Institute.* 64(4):701-713

Howe G.R, Burch J.D, Miller A.B, Morrison B, Gordon P and Weldon L (1977): Artificial sweeteners and human bladder cancer. *Lancet.* 2(8038):578-581.

Hoover R and Hartge P (1982): Non-nutritive sweeteners and bladder cancer. *American Journal of Public Health*. 72(4):382-383.

Hazardous Substances Data Bank (2001): Manganese compounds. Bethesda, MD, National Library of Medicine, Hazardous Substances Data Bank. Available at http://toxnet.nlm.nih.gov/cgi- bin/sis/htmlgen?HSDB. Retrived 13/2/13.

Huang, S. Chueh, P.J. Lin, Y. Shih, T. and Chuang. S (2009): Disturbed mitotic progression and genome segregation are involved in cell transformation mediated

by nano-TiO2 long term exposure. *Toxicology and applied pharmacology* 241(2):182-194.

Hurley L.S and Keen C.L (1987): Manganese. *Trace elements in human and animal nutrition,* 5th ed. Vol. 1. New York, NY, Academic Press, pp. 185–223.

International Agency for Research on Cancer (2006): Carbon black, Titanium dioxide and Non-Albestifalc; IARC: Lyon.

Institute of Medicine (2002): Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Institute of Medicine. Food and Nutrition Board, NRC. Washington, DC: National Academy Press, 442-501.

International Programme on Chemical Safety (1999): Manganese and its compounds. Geneva, World Health Organization, International Programme on Chemical Safety (Concise International Chemical Assessment Document 12).

International Programme on Chemical Safety (2002a): Environmental health criteria 227(Fluoride). Geneva: International Programme on Chemical Safety, World Health Organization: 10.

International Programme on Chemical Safety (2002b): Principles and methods for the assessment of risk from essential trace elements. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 228).

Issabayeva, G., Aroua, M. K. and Sulaiman, N. M. (2007): Continuous adsorption of lead ions in a column packed with palm shell activated carbon. *Journal of Hazardous Materials*, 155 (1-2): 109-113.

Janet S.H (2002): Effect of saccharin in pregnancy. www.sweetpoison.com/aspartame-sweeteeners.html. Retrieved 12/03/2011.

Jennifer L.B and David L.C (2012): Characterization of microorganisms isolated from counterfeit toothpaste. *Journal of Forensic Science.* 57(5):1365-1367.

Just, T. Paul, H.W. Engel, U. and Hummel, T (2008): Cephalic phase insulin release in healthy humans after taste stimulation. *Appetite* 238 (4):622-627.

Knobeloch L, Ziaruk K.M, Howard J, Theis B, Farmer D, Anderson H and Protor M (1994): Gastrointestinal upsets associated with ingestion of copper-contaminated water. *Environmental Health Perspectives*, 102(11):958–961.

Kojima, S and Ichibagase. H (1966): Synthetic sweetening agents. X. Thin-layer chromatography of sodium cyclamate, saccharin sodium and dulcin, *Yakuzaigaku*, 26, 115.

Kurebs R.E (2006): The history and use of our earth's chemical elements. A Reference Guide 2nd ed. Westport, CT: Green Wood Press.

Kutal. C and Serpone. N (1993): photosensitive metal organic system; mechanistic principles and applications. *American Chemical Society*, Washington DC.

Lee C.H, Kim H.W, Han H.J and Park C.W (2004): A comparison study of nonanoic acid and sodium lauryl sulphate in skin irritation. *Exogenous Dermatology* 3:19-25.

Lewis M.R and Kokan L. (1998): Zinc gluconate: Acute ingestion. *Journal of Clinical Toxicology*.36:99-101.

lonescu,E. Rohner-Jeanrenaud, F. Proietto,J. R.W and Jeanrenaud, B. (1988): Taste induced changes in plasma insulin and glucose turnover in lean and genetically obese rats. *Diabetes*. 37(6):773-779.

Long T.C, Saleh N, Tilton R.C, Lowry G.V and Veronesi B (2006): Titaniumdioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2); implications for nanoparticle neurotoxicity. *Environmental Science and Technology*. 40:4346-4352.

Low B.A, Donohue J.M and Bartley C.B (1996): Backflow prevention failures and copper poisonings associated with post-mix soft drink dispense. Ann Arbor, MI, NSF International.

Lynam D.R (1999): Environmental effects and exposures to manganese from use of Methylcyclopentadienyl manganesetricarbonyl (MMT) in gasoline. *NeuroToxicology*. 20:145–150.

Maja S. and Zorka K. (2011): Determination of Artificial sweeteners in breverages and special nutritional products using high performance liquid chromatography. *Archives of industrial Hygiene and Toxicology.* 62: 169-173.

Martindale (2002): The Extra Pharmacopoeia, 33rd Ed. *The pharmaceutical press*, London, pp. 1375.

Mayflor, M. (2009): Effects of potentially toxic metals. Niki Fears Publishers, Florida.

McChesney, E. W. & Golberg, L. (1973): The excretion and metabolism of saccharin in Man; Methods of investigation and preliminary results. *Food and Cosmetics Toxicology*, 11,403.

McDonagh M.S, Whiting P.E, Wilson P.M, Sutton A.J, Chestnutt C.J, Misso K, Bradley M, Treasure E. and Kleijnen J (2000): Systemic review of water fluoridation. *British Medical Journal.* 321(7265):855-859.

Menoyo I , Rigalli A and Puche R.C (2005): Effect of fluoride on the secretion of insulin in the Rat. Drug Research. 55(5):455-460.

Merck index. 12th edition. Rahway: Merck, 1996. Pp. 1430.

Mergler D, Huel G, Bowler R, Iregren A, Belanger S, Baldwin M, Tardif R, Smargiassi A and Martin L (1994): Nervous system dysfunction among workers with long-term exposure to Manganese. *Environmental Research*, 64:151–180.

Meyers A.I, McQueen M.J Harbrow D and Seymour G.J (2000): The surface effect of dentifrices. *Australian Dental Journal*. 45 :(2):118-124.

Minegishi, K. I., Asahina, M. & Yamaha, T. (1972): The metabolism of saccharin and the related compounds in rats and guinea pigs. *Chemical and Pharmaceutical Bulletin*, 20, 1351.

Miracakar and Gordana P. (2006): Determination of saccharin in pharmaceuticals by high performance thin layer chromatography. *Journal of the serbian Chemical society.* 71(6):669-676.

Morrison, A.S (1982): Artificial Sweeteners and bladder cancer in manchester, U.K. and Nagoya. *Japan. British journal of cancer*.45 (3) 332-336.

Murray T.M, and Ste-Marie L.G (1996): Prevention and management of osteoporosis; society of Canada. *Fluoride therapy for osteoporosis. CMEJ.* 155(7):949-954.

Muriale L, Lee E, Genovese J and Trend S (1996): Fatality due to acute fluoride poisoning following dermal contact with hydrofluoric acid in a polynology laboratory. *The Annals of Occupational Hygiene*. 40(6):705-710.

Myers, R.L and Richard L.M (2007): The 100 most important chemical compounds: a reference guide. Westport, Conn: Greenwood Press. pp. 241.

Nochimson G (2011): Fluoride toxicity. www.wikipedia.com Retrived 22/02/2013.

Oyewale A.O (2005): Estimation of the essential inorganic constituents of commercial toothpaste. *Journal of Scientific and Industrial Research* 64:101-107.

Pattabhi, S., Madhavakrishan, S., Manickavasagam, K., Rasppan K., Venekatesh R. and Shabudeen, P. S. S. (2008): *Ricinus communis pericarp* activated carbon used as an adsorbent for the removal of Ni (II) from aqueous solution. *Environmental Journal of Chemistry*, 5(4): 761 – 769.

Pitkin, R. M, Andersen D.W, Reynolds W.A and Filer L.J (1971): Saccharin metabolism in Macaca mulatta, *Proceedings of the Society for Experimental Biology and Medicine*, 137, 803.

Polyak E, Gombos K, Hajnal B, Bonyar-Muller K, Szabo S, Kisbenedek A and Marton K (2010): Effects of artificial sweeteners on body weight, food and drink intake. *Acta Physiological Hungarica.* 97(4):401-407.

Popova S and Marinova A (2007): problems in application of Atomic Absorption Spectrometry for control of heavy metals in toothpaste. *Journal of the University of Chemical Technology and Metallurgy.*42 (4):413-418.

Remsen I and Fahlberg C (1880): On the oxidation of orthotoluene sulphamide. *American Chemical Journal.* 1:426-439.

Robichaud C.O, Uyar A.E, Darby M.R, Zucker L.G and Wiener M.R (2009): Estimates of upper bounds and trends in nano-TiO2 production, as a basis for exposure assessment. *Environmental Science and Technology.* 43:4227-4233.

Roels H.A, Ortega E.M, Ceulemans E, Robert A and Lison D (1999): Prospective study on the reversibility of neurobehavioral effects in workers exposed to manganese dioxide. *NeuroToxicology*. 20 (2-3):255–271.

Sabine M and Wendy G (2009): Human health effects of heavy metals. *Environmental Science and Technology Briefs for Citizens.* 15:2-6.

Sandulescu R, Florean E, Roman L, Mirel S, Oprean R and Suciu P (1996): Spectrophotometric determination of fluoride in dosage forms and dental preparations. *Journal of Pharmaceutical and biomedical Analysis*. 14:951-958.

Santosh K.S, Chanda V, Piyush K.P and Ashish B (2008): Reliable technique for the determination of sodium dodecyl sulphate by crystal violet in relation to the effects of drug-Bhilai Region. *Journal of the Chinese Chemical Society.* 56:1250- 1256.

Saxena G, Kannan G.M, Saksenad N, Tirpude R.J and Flora S.J.S (2006): Lead induced oxidative stress and DNA damageusing comet assay in rat blood. *Journal of Cell and Tissue Research*. 6(2):763-768.

Schwartz, B.S. and Hu, H. (2007): Adult lead exposure: time for change. *Journal of Environmental Health Perspective*. 115(3):451-453.

Shin, E.W, Karthikeyan, K.G. and Tshabalala, M.A. (2006): Adsorption mechanism of cadmium on juniper bark and wood. *Journal of Bioresource Technology*. 98: 588-594.

Singh N (2010): Synchronous tumors of the female genital tract. *Hisphathology*. 56(3):277-285.

Stenhammar L (1999): Diarrhoea following contamination of drinking water with copper. *European Journal of Medical Research*, 4:217–218.

Stewart, D. (2011): Risks of saccharin. www.eHowcom. Retrieved 02/15/2011.

Sturgeon, S.R (1994): Assosiation between bladder cancer risk factors and tumor stage and grade at diagnosis. *Epidemology*. 5 (2):218-215.

Thaira, Y.I and suad (2006): Spectrophotometric determination of saccharin by coupling with diazotized 4-nitroaniline. http://www.iasj.net/iasj?func=fulltext&ald=40130. Retrived 18/09/12.

Thompson, M.M and Mayer, J. (1959): Hypoglycemic effects of saccharin in experimental animals, *American Journal of Clinical Nutrition*, 7:80.

Tokalioglu, S; Kartal, S and Sahin, U (2004): Determination of fluoride in various samples and some infusions using a fluoride selective electrode. *Turkish Journal of Chemistry*, 28: 203-211.

Trouiller B, Reliene, R, Westbrook, A, Solaimani P and Schiest R.H (2009): Titanium dioxide Nanoparticles induce DNA damage and genetic instability in vivo in mice. *The Journal of Cancer Research* 68: 8784- 8789.

Tulika N. (2011): Saccharin side effects. www.buzzle.com. Retrived 9/5/2011.

United States Centers for Disease Control (ATSDR) (2000): Toxicological Profile for Manganese. http://www.atsdr.cdc.gov/toxprofiles/tp151.html.

United States Environmental Protection Agency (1987): Drinking water criteria document for copper. United States Environmental Protection Agency, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office.

United States Environmental Protection Agency (1994): Drinking water criteria document for manganese. Washington, DC, United States Environmental Protection Agency, Office of Water.

United States Environmental Protection Agency (2002): Health effects support document for manganese. Washington, DC, United States Environmental Protection Agency, Office of Water.

United States Geological Survey. (2011): Titanium mineral information- United States Geological Survey.

Varner T.A, Jensen K.F, Horvath W and Isaacson R.L (1998): Chronic administration of aluminium-fluoride or sodium –fluoride to rats in drinking water: alternations in neuronal and cerebrovascular intergrity. *Brain Research,* 784 (1&2): 284-298.

Vianna-Soares C.D and Jorge L.S.M (2002): Saccharin analysis in pharmaceuticals and cosmetic preparation by derivative ultraviolet spectrophotometry. *Brazilian Journal of Parmaceutical sciences.* 34 (4).

Winston A.E and Bhaskar S.N(1998): Caries prevention in the 21st century. *Journal of American Dental Association.* 129(11):1579-1587.

World Health Organization (1993a): Evaluation of Certain Food Additives and Contaminants. WHO Technical Report Series 837, Geneva, pp. 17–19 & 46.

World Health Organization (1978): Evaluation of Certain Food Additive. WHO Technical Report Series 617, Geneva, pp. 24–26.

World Health Organization (1993b): Guidelines for Drinking Water Quality, 2nd Edition, (1).

World Health Organization (1996): Zinc, Trace elements in human nutrition and health. *World Health Organization*, 72-104.

Yeung C.A (2008): A systemic review of the efficacy and safety of fluoridation. *Evidence Based Dentistry*. 9(2):39-43.

APPENDICES

Appendix A: General characteristics of sampled toothpastes.

Sample label	Country of manufacture	Claimed ingredients.
T1	Nigeria	Sorbitol, aqua, hydrated silica, SLS, aroma, cellulose gum, trisodium phosphate, sodium phosphate, sodium saccharin, sodium fluoride, limonene.
T2	Indian	Sodium monofluoro phosphate, triclosan, hydrated silica, whitening silica, sorbitol, sodium CMC, SLS, sodium saccharin.
T3	Nigeria	Abrasive silica, tickening silica, sorbitol, sodium carboxy methyl cellulose, sodium saccharin, SLS, PEG, mono sodium phosphate, sodium fluoride, sodium benzoate, color, flavor, DM water.
T4	Nigeria	Sodium fluoride, hydrateted silica, sorbitol, glycerin, PEG-6, SLS, flavor, titanium dioxide, xanthan gum, sodium saccharin.
T5	Ghana	Sodium fluoride, sorbitol, water, hydrated silica, SLS, PEG-32, flavor, cellulose gum, sodium saccharin,

		eugenol.
T6	Ghana	Sodium monofluorophosphate, calcium glycerophosphate, calcium carbonate, aqua, sorbitol, hydrated silica, SLS, aroma, cellulose gum, potassium citrate, trisodium phosphate, sodium saccharin, calcium glycerophosphate, phenyl carbinol.
T7	Nigeria	Sodiummonofluorophosphate, sorbitol, precipitated silica, distilled water, SLS, sodium saccharin, biden, PEG, food grade color.
T8	Nigeria	Basil oil, herbal extract, calcium carbonate, SLS, blend of peppermint, sodium silicate, glycerin, sodium saccharin.
T9	Nigeria	Sodium monofluoro phosphate, sorbitol, silica, sls, sodium CMC, PEG, sodium saccharin.
T10	China	Ingredients were not stated.

Appendix B : pH and Moisture content of the Sampled Toothpaste.

Samples	Sample Types	Colour	pH (Ave.±SD)	Moisture and Volatile Matter Content (%)
T1	Local	Green	6.96±0.001	27.60
T2	Foreign	Red	6.43±0.003	33.21
T3	Local	Red	6.39±0.001	28.70
T4	Local	White	5.98±0.002	30.22
T5	Foreign	Red	5.92±0.001	33.64
T6	Foreign	White	7.11±0.005	35.20
T7	Local	Red	7.18±0.003	30.36
T8	Local	Green	8.42±0.002	23.47
T9	Local	Red	7.12±0.001	46.19
T10	Foreign	Green	8.22±0.002	28.03
WHO/SON	-	-	6.5-7.5	50

Appendix C: Claimed Fluoride Content and Concentrations Determined for each Sampled Toothpastes.

Sample ID	Sample Type	CAFI	CAFC (%)	CEFC (ppm)	CFC(ppm)±SD
T1	Local	NaF	NS	1100	816.67±20.82
T2	Foreign	$NaF.PO_4$	0.76	NS	805.00±8.66
T3	Local	NaF	0.32	NS	1220.00±10.00
T4	Local	NaF	0.306	NS	770.00±26.46
T5	Foreign	NaF	NS	1450	1106.67±11.54
T6	Foreign	NS	NS	1450	1021.67±17.55
T7	Local	$NaF.PO_4$	NS	NS	1130.00±26.45
T8	Local	NS	NS	NS	900.00±20.00
T9	Local	$NaF.PO_4$	0.85	NS	966.67±30.55
T10	Foreign	NS	NS	NS	805.00±33.00
NAFDAC	NS	NS	NS	850-1500	-

Appendix D: Heavy Metal Concentration in the Sampled Toothpastes.

Sam. ID	Typ	Ti (mg/g)	Cd ±SD (mg/kg)	Pb (mg/kg)	Zn ±SD (mg/kg)	Cu ±SD (mg/kg)	Ni ±SD (mg/kg)	Mn ±SD (mg/kg)
T1	L	1.590	0.5000 ±0.0005	ND	11.0500 ±0.0004	1.9500 ±0.0001	5.8000 ±0.0004	20.4000 ±0.0003
T2	F	1.610	ND	ND	5.5000 ±0.0003	12.0500 ±0.0001	5.2500 ±0.0004	18.1500 ±0.0000
T3	L	1.440	0.3000 ±0.0002	ND	2.9500 ±0.0002	0.7000 ±0.0003	4.3500 ±0.0008	13.1500 ±0.0011
T4	L	6.250	ND	ND	1.8000 ±0.0002	0.6000 ±0.0002	3.2000 ±0750	9.9000 ±0.0005
T5	F	1.450	0.3000 ±0.0001	ND	1.9000 ±0.0002	0.3000 ±0.0002	3.7500 ±0.0001	13.2000 ±0.0007
T6	F	1.450	0.2500 ±0.0005	ND	4.3000 ±0.0002	11.5000 ±0.0003	5.2500 ±0.0002	30.1500 ±0.0012
T7	L	1.500	0.1000 ±0.0006	ND	1.3000 ±0.0002	1.0000 ±0.0003	4.4500 ±0.0003	19.6500 ±0.0005
T8	L	1.470	0.0500 ±0.0003	ND	3.4500 ±0.0004	4.7500 ±0.0002	4.3500 ±0.0003	29.8500 ±0.0008

T9	L	1.440	ND	ND	1.8000 ±0.0001	0.6500 ±0.0002	2.8500 ±0.0004	19.2500 ±0.0003
T10	F	1.540	0.7000 ±0.0005	ND	5.9500 ±0.0003	1.4000 ±0.0002	3.9000 ±0.0005	94.5000 ±0.0005

Appendix E: Saccharin and Sodium Lauryl Sulphate Concentration in Toothpastes.

Sample ID	Type	Saccharin (mg/g)	SLS (mg/g)
T1	Local	83.31 ±17.29	16.41 ± 12.05
T2	Foreign	232.86 ±11.47	21.30 ± 10.22
T3	Local	147.86 ±13.74	18.05 ± 12,30
T4	Local	153.44 ±12.01	16.72 ±11.11
T5	Foreign	162.75 ±12.57	19.02 ± 11.51
T6	Foreign	155.14 ±12.35	17.76 ± 10.31
T7	Local	165.00 ±12.39	17.89 ± 15.67
T8	Local	165.80 ±12.39	15.64 ±10.11
T9	Local	159.00 ±12.47	18.57 ± 12.00
T10	Foreign	157.83 ±12.19	17.91 ± 12.51
FDA/WHO	-	5mg/kg/b.wt	-