AKENTEN APPIAH-MENKAH UNIVERSITY OF SKILLS TRAINING AND ENTREPRENEURIAL DEVELOPMENT

HUMAN HEALTH RISKS ASSOCIATED WITH SELECTED ELEMENTS IN SOME TOBACCO PRODUCTS AND THEIR NICOTINE DELIVERY CAPABILITIES

OSEI – OWUSU CHARLES
(8211440004)

JULY, 2023

AKENTEN APPIAH-MENKAH UNIVERSITY OF SKILLS TRAINING AND ENTREPRENEURIAL DEVELOPMENT

HUMAN HEALTH RISKS ASSOCIATED WITH SELECTED ELEMENTS IN SOME TOBACCO PRODUCTS AND THEIR NICOTINE DELIVERY CAPABILITIES

OSEI – OWUSU CHARLES
(8211440004)

Thesis in the Department of Chemistry Education,

Faculty of Science Education,

Submitted to the School of Graduate Studies in partial fulfilment of the requirements for the award of the Degree of

MASTER OF PHILOSOPHY
(Chemistry Education)
In the Akenten Appiah-Menkah University of Skills Training and Entrepreneurial Development

JULY, 2023

DECLARATION

STUDENT'S DECLARATION

I, Osei-Owusu Charles hereby declare that this thesis is the outcome of my original research with the exception of quotations and references contained in published works where due acknowledgments has been made and have not been submitted either in part or whole for the award of any degree elsewhere.

SIGNATURE: ……………………………………………

DATE: ……………………………………………………

SUPERVISOR'S DECLARATION

We hereby declare that the preparation and presentation of this work were supervised in accordance with the guidelines for supervision of thesis as laid down by the Akenten Appiah-Menkah University of Skills Training and Entrepreneurial Development.

Dr. Opoku Gyamfi (Principal Supervisor)

Signature: ……………………………………………

Date: …………………………………………………

Prof. Kofi Sarpong (Co-Supervisor)

Signature: ……………………………………………

Date: …………………………………………………

ACKNOWLEDGEMENTS

My profound gratitude goes to my supervisor Dr. Opoku Gyamfi for his guidance, support and benignity from the commencement of this research study to its conclusion.

I am also thankful to Pastor Enoch Bessah (PhD) for his encouragement and support. I also appreciate the support and cooperation of my wife; Priscilla Osei-Owusu, my mum Selina Darkwa and my kids; Dayshaun, Katana, and Enoch-Perez.

I would also like to say a very big thank you to Mr. Emmanuel Buasilenu, my Head of science department; St Monica's Senior High School; Mampong for the hand of benevolence he extended me throughout this programme. I say God richly bless you.

DEDICATION

This work is dedicated to the Most High God for His grace and mercies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Introduction: Smokeless tobacco use is on the increase among the population especially the youth in Ghana partly because of its perceived medicinal benefits and relatively less harmful and non-addictive effect.

Objective: This study aims at a systematic classification of 51 smokeless tobacco product samples and 6 cigarette brands with different flavors and from different manufacturers from 11 different locations within the Ejisu Municipality.

Methodology: Moisture, pH, total nicotine, and percentage free base nicotine using previously published analytical methods from CORESTA. Target elements for analysis were Ca, Cu, Fe, K, Mn, Rb, Sr, Mo, V, S, U, Zr, Tl, and Zn using XRF analyzer.

Results: The nicotine delivery capability of the locally produced snuff products; with mean pH 96.98 and percentage free base nicotine (% A) 96.98% was the highest, followed by dried tobacco leaves (mean pH: 25.93, % A: 25.93%) and the least being the cigarette products (mean pH: 5.49, % A: 0.33%). Menthol flavored snuff products (pH of 9.96; % A of 98.8%) had higher nicotine delivery capabilities than moringa flavored snuff products (pH of 9.77; % A of 98%). Users of C6, a smuggled cigarette product are liable to increased non-cancer health effects (HI value of 1108.35). C4 (highest pH of 5.58 with corresponding % A of 0.70) are liable to be dependent on the product than other examined cigarette products. Conclusions: Locally produced snuff products and dried tobacco leaves have potentially very high addictive levels of nicotine that may favor tobacco dependence. They can also lead to non-cancer health effects due to high hazard quotients and hazard index.

Recommendation: It is recommended that further research on smokeless tobacco products focusing more on the carcinogenic nitrosamines and other identified mutagens be undertaken.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Tobacco is projected to kill over a billion people if there is no intervention in place (Cahn et al., 2013). The use of smokeless tobacco products is increasing among the youth (Veda & Aïssata, 2022). It is also projected that annual deaths from tobacco use by the year 2030 is going to be around ten million, most of which will occur in developing countries (Veda & Aïssata, 2022). Tobacco is the general name of a group of plants of the genus Nicotiana and belongs to the Solanaceae family (IARC, 2009). It's also the general name given to any product made from the dried or fermented leaves of these plants (IARC, 2009). It is the leading cause of preventable deaths in the world (Dahlawi et al., 2021). Tobacco use is linked to diseases such as cancer, heart disease, stroke, chronic lung disease as well as other non-communicable diseases" (Dahlawi et al., 2021).

Tobacco products come in two main forms, viz the smoked and smokeless tobacco products (Dahlawi et al., 2021). Smoked tobacco products include cigarettes, cigar, shisha; also known as hookah or water pipe, bidis (a small, thin, hand-rolled cigarettes which comprise tobacco wrapped in tendu or temburni leaf) and kreteks (an unfiltered cigarette with a blend of tobacco, cloves and other flavours) (Dahlawi et al., 2021).

Cigarettes are the most common type of smoked tobacco products in Ghana (Owusu-Dabo et al., 2009). Cigarettes contain tobacco itself, fillers which include stems and other waste products, water, flavourings and additives (Owusu-Dabo et al., 2009). There are about 600 different additives permitted for use in the United Kingdom (Owusu-Dabo et al., 2009). Majority of cigarette brands consumed in Ghana are sourced from the UK marketed by British American Tobacco (BAT) Ghana Limited (Owusu-Dabo et al., 2009). About 10%

of cigarette brands in Ghana are available from neighboring countries Owusu-Dabo et al., (2009). It is safe to conclude that cigarettes consumed in Ghana have similar chemical compositions as that of their counterparts in the UK. The additives include moisturizers; to prolong shelf life, sugars; to enable the smoke to be more easily inhaled, and flavours such as chocolate, vanilla and menthol (Robson & McNeill, 2021). The additives can be toxic when combined with other substances or burned during smoking (Robson & McNeill, 2021). More than 2000 chemical compounds have been identified in processed tobacco (IARC, 2009). These includes original tobacco constituents and chemicals applied during cultivation, harvesting and processing (IARC, 2009). Major classes of compounds identified in tobacco include aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols, phenols, amines, amides, alkaloids, metals and radioelements (IARC, 2009).

However, Smokeless tobacco is a non-combustible tobacco product consumed by either inhalation or by ingestion. Smokeless tobacco comes in two forms in the West African Sub-Region. The chewing tobacco; in the form of loose leaf, plug or twist and the Snuff, in dry or wet form.

In Ghana, predominantly used smokeless tobacco product is the snuff, locally known as “Tawa or Asraha”. It is prepared by mixing dried tobacco leaves with some known additives such as such as saltpetre (potassium nitrate), moringa, menthol, Indian hemp and unknown additives and grinding into fine powder (Verma et al., 2010). Manufacturers of these smokeless tobacco classify these unknown additives as “trade secrets” and are always reluctant to reveal them apart from the generally known additives which gives the various snuffs their identity. These additives have the potential to introduce harmful substances that have carcinogenic and other life-threatening tendencies (Verma et al., 2010). Aside possible introduction of harmful chemicals through the additives used in the preparation of snuff, there are a number of inorganic toxic elements, essential elements and organic carcinogens (Verma et al., 2010).

Tobacco plant can absorb and accumulate heavy metal species from the soil into its leaves or deposited on tobacco leaves from air (Verma et al., 2010).

Dried tobacco leaves, are also a form of smokeless tobacco (SLT), and are chewed by users. After roasting the tobacco leaves, users dip the roasted tobacco into an alkaline agent (fly ash of wood) before inserting it between the lower gum and lip (Addo et al., 2008). This is done to improve delivery of free (Addo et al., 2008).

1.2 Statement of the Problem

Developing countries such as Ghana are at high risk of epidemic due to increased tobacco use, however, the extent of this problem, especially in locally produced smokeless tobacco is not clearly defined because of lack of interest and difficulty in research (Owusu-Dabo et al., 2009). Limited studies have been conducted on the elemental composition, concentration and toxicity of potentially harmful elements in tobacco leaves and locally produced ready-to-use snuff products in Ghana (Owusu-Dabo et al., 2009). The few that have been done were focused in the Greater Accra Region and the northern parts of the country. A study by Owusu-Dabo et al., (2009), has revealed that research done on tobacco has been focused on its prevalence and usage and not on toxicological assessment chemical determination.

Literature has revealed that elemental concentrations in chewable tobacco and snuff may exceed levels found in other consumer products by two orders of magnitude (Pershagen, 1996). Studies done in South Africa and Nigeria have shown that deaths due to tobacco consumption are on the rise (5.4 million in 2005; projected to rise to 6.4 million in 2015 and expected to reach 8.3 million in 2030 (Etu et al., 2017). Over 80% of these deaths will be in developing countries such as Ghana (Etu et al., 2017).

Whiles snuff was presumed to be consumed mostly by older adults in Ghana, the youth are now becoming more interested in it (Addo et al., 2008). The increasing interest among the youth is consistent with the findings from Global Youth Tobacco Survey (GYTS) conducted in the year 2005 on Ghana (Addo et al., 2008). The survey showed that 10.4% of youths are using tobacco products other than cigarettes (Addo et al., 2008). Further inquiries suggest that any time responders referred to "other tobacco products", they were predominantly referring to snuff (Addo et al., 2008).

Exposure to second-hand tobacco smoke is associated with numerous adverse health effects, even among children and unborn babies infection (Cahn et al., 2013). Exposure to second-hand tobacco smoke is linked to substantial mortality and morbidity globally infection (Cahn et al., 2013). Although second-hand tobacco smoke usually comes from cigarettes, other tobacco products such as snuff is common in some homes and can substantially contribute to second-hand exposure infection (Cahn et al., 2013).

In many populations, homes are the main place of exposure to second-hand tobacco smoke for women and children infection (Cahn et al., 2013). For example, globally, second-hand tobacco smoke causes more deaths in women (573,000 in 2016) than in men (311,000 in the same year) (Cahn et al., 2013) and more lung cancer deaths among women (40,000 in 2013) than men in China (12,000 in the same year) (Cahn et al., 2013). These statistics call for an evaluation of toxicity of second-hand exposure to tobacco products in our homes and other enclosed environments

The lack of data on characterization and toxicity of locally produced snuff and dried tobacco leaves is very worrying. Hence, there is an urgent need to investigate them.

Information on elemental profile of dried tobacco leaves and snuff will assist in knowing whether toxicological strength of tobacco reduces or increases when it is converted into snuff. From the above, determination of elemental composition, exposure concentration of selected elements and potential toxicity of tobacco leaves and locally produced ready-to-use snuff products in Ghana and specifically, the Ashanti Region is long overdue. This will help to mitigate the negative effects of tobacco use on the populace.

1.3 Objectives of the research

The main objective of this study is to perform toxicological assessment and evaluate nicotine delivery capabilities of selected tobacco products.

1.3.1 Specific objectives

In order to achieve the main objective, the following objectives are to be done:

1) Determine concentration of selected elements (14 metals: Mo, Zr, Sr, U, Rb, Zn, Cu, Fe, Mn, V, Ti, Sc, Ca, K and 1 nonmetal: S) in cigarettes, dried tobacco leaves and locally manufactured snuff in the Ejisu Municipality of the Ashanti Region of Ghana.
2) Determine the chronic exposure and daily exposure of elements and intake of elements through cigarette smoking, inhalation of locally manufactured snuff, and sucking/chewing of dried tobacco leaves
3) Determine pH, nicotine delivery capabilities using established tobacco nicotine concentrations and Henderson–Hasselbalch equation
4) Determine short and long-term effects (toxicological assessment) of exposure to cigarette smoke and snuff products on active and nominal users.

1.4 Justification

Mustpaha, (2019) listed socioeconomic status, low levels of education, ethnicity and gender as the factors that influence the use of tobacco. The Ejisu municipality has a higher rural population of 101,731 (GSS, 2021. This constitutes 56.3% of the total population in the municipality (GSS, 2021). This confers on Ejisu a low to middle socio-economic status with diverse ethnic representation (GSS, 2021).

About 58.3% of people within 15 to 64 years are prone to smoking and other forms of tobacco use (Composite budget, 2021). The economy of Ejisu municipality mimics the national macro economy (Composite budget, 2021). Thus, research done in the Municipal will be a fair representation of the Region and the country as a whole. There is an increasing number of hotels, pubs and drinking spots in the Municipality. Therefore, these recreational points are primary sale outlets of tobacco products. Site reconnaissance also revealed that the use of smokeless tobacco (Snuff) in the Municipality is increasing at an alarming rate to the extent that snuff can be purchased from provision shops, public places of convenience, lotto kiosks and residential apartments.

These calls for the need to characterize tobacco products, (especially smuggled cigarette brands and locally prepared tobacco products) so as to ascertain their elemental profile, nicotine delivery capability and their toxicological effects on humans.

1.5 Significance

There is a rising interest in smokeless tobacco as a relatively safer option to tobacco consumption (Mustpaha, 2019). This is because, the use of snuff, which was once considered outmoded by many African people (Mustpaha, 2019), is now being marketed heavily to smokers, including women, young people, and men (Mustpaha, 2019). Smokeless tobacco products are advertised to women, as an alternative to smoking in cultures where smoking by women is socially unacceptable; to young people, for whom flavoured and milder-tasting “starter” products have been developed; and to addicted smokers, for use where smoking is prohibited (Mustpaha, 2019). For tobacco dependent individuals, snuff products are the most affordable and accessible way of getting sufficient nicotine (Mustpaha, 2019).

Also, it is assumed that since smokeless tobacco products do not produce harmful chemicals such as carbon monoxide, Tar, benzene, arsenic and formaldehyde just to mention a few, it is safer to use them as compared to the smoked tobacco variant (Mustpaha, 2019). However, this assertion is not wholly accurate because, aside the addictive nature of nicotine in tobacco products and all health issues associated with its use, locally produced snuff is not regulated (Mustpaha, 2019). There are many additives such as moringa, menthol, lime etc in the ground tobacco leaves that retailers and consumers are not aware of (Mustpaha, 2019). Some of these additives account for the more than 30 chemicals found in smokeless tobacco products which are linked with cancer (Mustpaha, 2019).

Heavy metals easily get incorporated into bodies of users through the use of smoked and smokeless tobacco products (Verma et al., 2010). Existing data gives evidence that metals exist in higher concentration in tissues of tobacco consumers (Verma et al., 2010). This contamination in tobacco products varies from country to country (Pourkhabbaz et al., 2012).

Consumers of tobacco products are prone to many diseases (Charlton, 2004). For example, in a 2019 study in the Tamale Municipally by Mustpaha, (2019), where data were collected from 408 adults to investigate the health effects of smokeless tobacco on users, it was established that about 46 (11%) of the respondents had receding gums whilst 56 (13.7%)

of them had decayed cavities. An approximated 80 (26.5%) of the respondents also had decayed tooth, 44 (10.8%) of them showed signs of abrasions in the mouth. In terms of Leukoplakia, 72 (23.8%) showed signs, 4 (1.3%) also had other signs which included coloration of teeth (Mustpaha, 2019). In addition, Smokeless tobacco may cause death in kids because of nicotine poisoning when mistakenly taken for candy (Mustpaha, 2019).

Therefore, it is very worrying that, with all the dangers associated with smokeless tobacco use in Ghana, the local production and selling of tobacco products are not regulated nor monitored (Owusu-Dabo et al., 2009). No data exists on manufacturers, brands, types of products sold, flavours, active ingredients, possible side effects and contraindications associated with their use.

Since it's an established fact that tobacco use is very harmful, toxic and genotoxic to human health (Pappas et al, 2008) and its contamination is site, process and country specific (Boffetta et al., 2008), it is important to conduct researches that look into both qualitative and quantitative chemical characterization and toxicological assessments identify elements present in selected locally produced tobacco and cigarette products in Ejisu and assess their potential toxicological risk. These will be used as a guide for policy formulation by relevant authorities in the Ashanti Region and the country as a whole.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

In Ghana, scanty literature exists on physicochemical characterization of tobacco some products and heavy metals contents in smokeless tobacco products (Addo et al., 2008). However, much literature exists on the physicochemical characterization of cigarettes than smokeless tobacco products (Addo et al., 2008). This chapter seeks to review existing literature on physicochemical characterization; risk assessment, nicotine delivery capabilities of tobacco products, pH and metal concentrations of common tobacco products (snuff, cigarettes and dried tobacco leaves), consumed in the research area within the research location.

2.2 Definition of Tobacco

The tobacco plant belongs to the family Solanaceae (Nightshade family), and is of the same family as potato and tomato (Whitty, 2009). It's from the Genus, Nicotiana tabacum, and Nicotiana rustica. Tobacco is an annual crop, it has long fibrous roots, it is erect, round, hairy, and has viscid stem (Whitty, 2009). Its leaves are large, numerous, alternate, pointed, hairy, pale green in colour, brittle, and has a narcotic odour with a nauseous, bitter acrid taste (Whitty, 2009)

2.3 Factors influencing bioavailability of trace elements in soil, and their uptake by tobacco plant

Soil is a major repository of trace elements over geologic time (Abdushukurov et al., 2022). Some patterns of relationship between bioavailability of nutrients in general and heavy metals and their uptake by crops have been proposed (Abdushukurov et al., 2022).

In Type 1 soils, uptake increases as crop grows and then falls when crop reaches maturity (Kelepertzis et al, 2015). This pattern is seen in uptake of major nutrients such as nitrogen, potassium and phosphorus by the soil ((Donkor., 2011)).

In type 2 soil, a similar but steeper peak (Donkor., 2011). This is seen in uptake of micro nutrients such as copper or zinc (Donkor., 2011). In Type 3, uptake is highest at the early growing stages, and falls during the later stages of growth (Donkor., 2011). This pattern is seen for heavy metals such as arsenic, cadmium, chromium, lead, nickel and mercury uptake by plants (Donkor., 2011). Cadmium is soluble in soil under oxidized conditions (Donkor., 2011). However, it precipitates under reducing conditions as cadmium sulphate (Donkor., 2011). It may be concluded that physicochemical parameters of soil are most important in influencing solubility of metals, solution composition (organic and inorganic soluble), pH, oxidation potential (Eh type), density of charge on soil colloids and reactive surface area of soil (Donkor., 2011). These phenomena are dependent on soil properties such as metal concentration and form, particle size distribution, quantity and reactivity of hydrous oxides, mineralogy, microbial activities and aeration (Dube et al., 2001). These soil properties vary geographically and depends on the combined effects of parent material, topography, climate, time, anthropogenic activities and biological processes (Dube et al., 2001).

Verma et al., (2010) reported that tobacco plant easily takes up metals from soil and concentrate them in the leaves. The plant can preferentially accumulate metals such as Pb, Cd, Zn, Ni and Cu (Rong et al., 2020). According to Pourkhabbaz et al., (2012), more studies showed high levels of toxic elements in cigarettes tobacco. Terrestrial accumulation of heavy metals and their subsequent migration into the human body is a matter of world concern (Lindberg et al., 2010).

The tobacco plant can accumulate metals from different sources including soils through the root, dry and wet atmospheric deposition on exposed part of the plant, and agricultural practices during tobacco growing, and treatments during curing, and processing (Saha et al., 2016). Biogeochemical cycle of heavy metals in agricultural soil are influenced by anthropogenic activities such as industrial effluents, agricultural practices, municipal wastes, vehicular emissions, and atmospheric deposition of dust and aerosol (Saha et al., 2016). However, accumulated heavy metals in tobacco plant can enter the human body through inhalation and consumption (Saha et al., 2016).

A pathway of heavy metal transportation from soil to tobacco plant and subsequently to human body has been depicted (Figure 2.1). After entering in a human body, the metals react with enzymes to slow or inhibit essential physiological reactions (Saha et al., 2016). Eventually, they can cause different types of serious problems, e.g., anemia, kidney dysfunction, and brain damage (Saha et al., 2016). Exposure to high levels of heavy metals may also destroy testicular tissue, cause cancer, tumor, and even paralysis (Saha et al., 2016). Due to their persistent and non-biodegradable nature, heavy metals, even at low levels, heavy metals intake over an extended period can lead to chronic toxicity (Saha et al., 2016). Whilst high exposure levels can pose detrimental effect to human health (Saha et al., 2016).

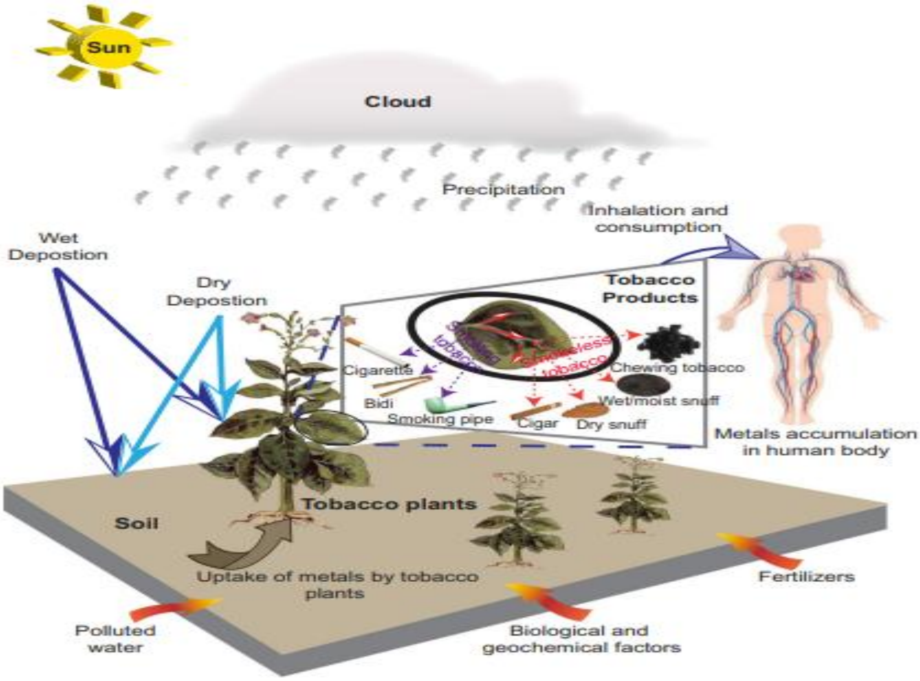

Figure 2.1 Conceptual model of transport of heavy metals by tobacco plants
(Saha et al., 2016)

2.4 Tobacco products in Ghana

Metals and metalloids occur naturally in tobacco (Zhang et al., 2018). The amounts of these substances in tobacco is influenced by soil pH, soil composition, and industrial contamination (Zhang et al., 2018). Due to differences in the absorption capacities minerals by tobacco plant, mineral contents of tobacco vary greatly (Pourkhabbaz et al., 2012; Zhang et al., 2018). Several studies have been conducted to determine trace amounts of elements in tobacco and tobacco products, especially cigarettes (Pourkhabbaz et al., 2012.). However, potential toxicological effects of locally produced SLT (snuff and dried tobacco products) are poorly known by the Ghanaian public, as studies on its elemental contents have been scarce (Addo et al., 2011). However, a review of studies on SLT products from Ghana as reported Addo et al., (2011), including some other countries, found detectable concentrations of arsenic (0.1–3.5 μg/g), beryllium (0.01–0.), nickel

(0.84–13.1 μg/g), lead (0.23–13 μg/g), and cobalt (0.056–1.22 μg/g). They are known to contain nicotine as the active ingredient (Addo et al., 2008).

2.5 Smokeless tobacco (snuff and dried tobacco leaves)

In Ghana, the predominantly used (smokeless tobacco) SLT is Snuff (Gordon & Lamoreux, 2021). Snuff is a tobacco product. Like cigarettes, it contains harmful chemicals such polycyclic aromatic hydrocarbons and heavy metals that can bring about many health problems. To produce snuff, tobacco is dried and finely ground. There are two main types of snuff: dried and moist and two main ways of consumptions; chewing (dipping) and inhalation (sniffing) (Gordon & Lamoreux, 2021). The study area predominantly uses dry Snuff and consumes by inhalation (sniffing) so this study will focus on dry snuff.

2.5.1 Additives and Preparation of snuff

Local snuff is prepared by mixing the dried tobacco leave (*Nicotiana tabacum L.*) indigenous to the forest areas with some chemicals (especially saltpetre) and then grinding it into fine powder (Addo et al., 2008). As a smokeless tobacco product, it is intended for use by being sniffed or snorted into the nose. Snuff is also consumed by placing it between the cheek and gum, thereby enabling it to seep gradually into the mouth and body through mixing with saliva (Addo et al., 2008). In Ghana, snuff and tobacco leaves are used among others for medicinal purposes such as to induce sneezing to 'lighten" the head, as a depressant and stimulant Addo et al., (2008) and also to enhance energy, and for sexual enhancement (Addo et al., 2008). It is consumed mostly by the Ghanaian ageing population. At present careful observation reveals that the attitude of the youth towards snuff consumption is on the increase (Addo et al., 2008).

According to Abdallah, (2003); Baker & Smith, (2003) and as reported by IARC, (2009), during preparation of tobacco products, tobacco leaves, stems and other ingredients are blended to achieve a specific nicotine content, pH, taste, flavour, and aroma. These features are critical for the acceptance of the product by the user. For cigarettes, it has been demonstrated that the type of tobacco blend significantly affects these features as well as the toxicity of the product (Addo et al., 2008). The pH strongly influences concentration of unprotonated nicotine; the bioavailable form of nicotine according to Monika et al., (2020) while the nitrite content influences the levels of nitrosamines in the product as stated by Jin et al., (2021). Tobacco types, plant parts, cultural practices, degree of maturity and fertilizer treatment are among some prominent factors that determine the level of alkaloids in Nicotiana plants (Djordjevic & Doran, 2009). Every step-in tobacco production that affects plant metabolism influences the level of alkaloid content to a certain degree (Djordjevic & Doran, 2009). Dried tobacco leaves can contain between 0.2 and 4.75% nicotine by weight, depending on plant genetics, growing conditions, degree of ripening, fertilizer treatment and leaf position on the stalk (*Production, Composition, Use and Regulations*, 2004). The chemical composition of tobacco undergoes substantial changes during growing, curing, processing, and storing (Zong et al., 2022) The purpose of curing is to produce a dried leaf of suitable physical properties and chemical composition. At the beginning of curing, a tobacco leaf is metabolically active and continues to live until biochemical processes are arrested by thermal effects or desiccation (WHO, 2009).

In curing, the starch content of the leaves declines drastically, while the amount of reducing sugars increases by 100 % (Laldinsangi, 2022). Also, protein and nicotine contents decrease slightly (Laldinsangi, 2022). The bulk of the processed tobacco leaf before fermentation consists of carbohydrates (about 50%) and proteins (Laldinsangi, 2022). Fermentation of dried tobacco causes the contents of carbohydrates and polyphenols in the leaves to diminish (Laldinsangi, 2022). Other major components are alkaloids (0.5–5.0%), which include nicotine as the predominant compound (85–95% of total alkaloids), terpenes (0.1–3.0%), polyphenols (0.5–4.5%), phytosterols (0.1–2.5%), carboxylic acids (0.1–0.7%), alkanes (0.1–0.4%), aromatic hydrocarbons, aldehydes, ketones, amines, nitriles, N- and O-heterocyclic hydrocarbons, pesticides, alkali nitrates (0.01–5%) and at least 30 metallic compounds (Laldinsangi, 2022). According to Sharma et al., (1991) and as cited by IARC, (2009), because of the disappearance of carbohydrates and polyphenols during fermentation, heavy casings (additives applied during processing such as molasses, liquorice and fruit extracts) are done to meet consumer's requirements (e.g., they improve taste, flavour and aroma, and prolong shelf-life).

Many smokeless tobacco formulations use plant extracts or chemicals as flavouring agents (IARC, 2006). Tobacco additives may include methyl or ethyl salicylate, β-citronellol, 1,8-cineole, menthol, benzyl benzoate, eugenol and possibly coumarin, among others (IARC, 2006). Eugenol and menthol are used to numb the throat and facilitate tobacco use (IARC, 2006). Also, they also work to indirectly enhance the addictiveness of tobacco products by numbing the throat, thereby increasing tolerance towards tobacco products, especially in amateur users (IARC, 2006).

Ascorbic acid is added to tobacco as an antimicrobial agent whereas the addition of sodium propionate serves as a fungicide (IARC, 2006). Other additives, such as ammonia, ammonium carbonate, and sodium carbonate are applied to control nicotine delivery by raising pH. These subsequently increases the level of unprotonated nicotine. Unprotonated nicotine is the form of nicotine that is most readily absorbed through the mouth into the bloodstream (IARC, 2006). However, the formulation of most of the additives, including flavours, remains a trade secret.

2.5.2 Toxic metals in smokeless tobacco products

It has been reported that a number of metals that are IARC Group 1 carcinogens (such as arsenic, beryllium, chromium VI, cadmium, nickel compounds, and polonium-210) or Group 2B carcinogens (such as cobalt and lead) are detectable in smokeless tobacco products (NCI, 2014). The pathological risks to which a consumer is exposed by consumption of smokeless tobacco arise from total exposure to all the toxic, irritant and carcinogenic substances that are biologically available from the products (Pappas, 2011). However, as toxicity and carcinogenesis are complex processes, different substances are usually studied individually (Pappas, 2011). Smokeless tobacco usage exposes users to higher levels of toxic metals (Cadmus & Ayo-Yusuf, 2018). Even so, less research has been done on the possible health implications of this exposure (Cadmus & Ayo-Yusuf, 2018). The oral cavity's epithelial tissues have a high proximal transfer potential, allowing for the absorption and spread of harmful metals from smokeless tobacco products (WHO, 2009). Metals that are dissolved in saliva and ingested are exposed to the esophagus and stomach; systemic exposure most likely results from direct oral absorption or absorption of swallowed saliva or cigarette particles in the digestive tract (Pappas et al., 2008). Studies on metal extraction from synthetic saliva show that metals are carcinogens and toxicants that are physiologically accessible in the mouth cavity (Pappas, 2011; Pappas et al., 2008).

2.5.3 Toxicity of moist and dry snuff products

The toxicity of smokeless tobacco products results from continued exposure to toxic compounds contained in or generated upon the consumption of these products, some of which include cancer-causing chemicals (World Health Organization, 2020). The following class of chemicals contribute to the toxicity of tobacco products:

1. Heavy metal ions, such as lead, arsenic, mercury and nickel (World Health Organization, 2020)
2. Aldehydes, which are known carcinogens, produced from the burning of sugars added as sweeteners in tobacco products (World Health Organization, 2020)
3. Tobacco-specific nitrosamines (TSNAs), namely NNN and NNK, which are the most potent carcinogens in SLT (World Health Organization, 2020)
4. Nicotine (World Health Organization, 2020)
5. Polyaromatic hydrocarbons (World Health Organization, 2020)
6. Nitrates and nitrites. (World Health Organization, 2020)

In a 2014, the National Cancer Institute and the Center for Disease Control and Prevention (CDC) found that over 40 types of smokeless tobacco products are used via nose or mouth by more than 300 million people around the world. To use dry snuff, the ground tobacco is ground tobacco and inhaled into the nasal cavity. To use moist snuff, the tobacco product is put between the lower lip or cheek and gum, also known as "dipping" (CDC, 2014). (*CDC*, 2023) and (Stanfill et al., 2022) also warn that using smokeless tobacco products may:

1. increase risk of death from heart disease and stroke
2. increase the chance of premature birth and stillbirth in pregnant women.
3. cause nicotine poisoning in if children accidentally exposed to tobacco via ingestion of the substance (CDC,2020)

Using moist snuff can also cause yellow your teeth, bad breath and also lead to tooth decay and gum infections, and in some cases jaw complications or loss of teeth resulting in bone loss and face disfigurement (CDC,2020). The CDC also warns that smokeless tobacco products can raise risk of several types of cancer, including Oral cancers, esophageal cancer, pancreatic cancer (CDC,2020).

A 2019 research review by Gupta et al., (2019) involving 20 studies over 4 global regions found a significant association between smokeless tobacco use and risk of death from coronary heart disease, especially among European users. The researchers pointed to the need to include smokeless tobacco in public tobacco cessation efforts.

2.6 Toxic metals in cigarette products

Smoke from conventional cigarettes is inhaled by mouth, throat, and lungs, where a significant amount of the particulate particles and volatile compounds are absorbed or deposed internally(Baker & Dixon, 2006). Many of these compounds are promptly absorbed from the lungs, readily transported to the bloodstream, and swiftly dispersed throughout the circulatory system (Taylor et al., 2021). Other components of smoke, such as 60–80 % particulate matter, are retained, build up in the lungs, and eventually divide between the lung tissue, airways, and circulatory or lymphatic system (WHO, 2009). The majority of metals and metalloid ions are comparatively inert at ambient temperature (WHO, 2009). While only a handful of compounds of mercury's ionic forms are flammable at temperatures below 100 °C. Over 900 °C may be reached by the tobacco burning at the tip of a cigarette (Maiyo et al., 2022). Cigarettes smoke inhaled via the mouth (mainstream smoke) can reach about 30 °C, and about 10 cm from the burning tip, the temperature of sidestream smoke drops below 100 °C (Longest & Xi , 2008). The hot enough temperature of a burning cigarette tip can therefore cause numerous metal ions to volatilize or to interact with other substances to generate volatile compounds and complexes (Stavrides, 2006). Due to this, certain metals may be in the gas phase in metallic or complex form while others may condense into the particle phase of the smoke (Stavrides, 2006). A puff of cigarette smoke may contain around 70% air, 17% gas-phase species, 8% particulate matter, and 5% other substances, however the precise composition varies on the smoker's habits (WHO, 2009). When smoke is inhaled or rises in a plume as sidestream smoke from the cigarette, the majority of the metal ions have consolidated with other substances to create aerosol particles, which make up the majority of the particulate matter in the smoke aerosol (Sheu et al., 2020).

The majority of exposure to ultrafine and fine particulate matter comes from cigarette smoking (CDC., 2010). Although the ultrafine particle component of tobacco smoke does not have the highest mass, its tiny size makes it easier for cells to absorb. Also, because of its higher surface area-to-mass ratio, it is considerably more hazardous and causes more oxidative stress per unit mass (Pappas , 2011). The levels of metals and metalloids present in tobacco are inversely correlated with the amounts carried by smoke from combustion products (Ali et al., 2019; Moradnia et al., 2021). The third most common cause of lung cancer, second-hand smoking, also known as environmental tobacco smoke, is thought to be the cause of 3,000 lung cancer deaths annually (Anupozu & Vanamala, 2018; Res et al, 2020);. By exposing them to second-hand smoke, smoking has an impact on non-smokers. The risk of lung cancer from second-hand smoke exposure is 20–30% higher for people who share a household with smokers (Anupozu & Vanamala, 2018). Breathing in other people's tobacco smoke is a strong risk of lung cancer, according to epidemiological and biochemical research on environmental tobacco smoke (ETS) (Hashim et al., 2015). Combining the results of several research, they show that ETS exposure increases the number of lung malignancies seen in non-smokers (Dubin et al., 2020) .

The amounts of hazardous metals in smokeless tobacco are greater than in smoke, and a smoking machine is not necessary, making analysis easier (Pappas, 2011). Much of metals or metalloids' health impact is determined by what happens to it once it enters the lung (Fischer & Pomati, 2013). While certain metals, including cadmium and chromium, may become caught in mucus, be coughed up, and be eaten, they can accumulate and stay mostly in lung tissue for a long biological period (Gaur & Agnihotri, 2019). People who are in an enclosed space with a smoker are exposed to hazardous compounds through second-hand smoke from cigarettes (Zhou et al., 2014). It's also conceivable that those who are near a smoker, including young infants, might be exposed to low levels of harmful metals, including aluminum, cadmium, lead and other metals, that are exhaled in moist aerosols for some time after smoking (Zhou et al., 2014).

2.6.1 Second-Hand Exposure to tobacco products

In 2016, an estimated one-fifth of males and one-third of females globally were exposed to second-hand smoke. Although second-hand smoke usually comes from cigarettes, smoking other tobacco products (such as waterpipe) is common in some populations and can substantially contribute to second-hand smoke exposure (Cahn et al., 2013). Exposure to second-hand smoke is associated with numerous adverse health effects, even among children and unborn babies, and causes substantial mortality and morbidity globally (Cahn et al., 2013).

In 2016 alone, for example, second-hand exposure caused an estimated 884,000 deaths (Cahn et al., 2013). The years of life lost due to ill-health, disability, or early death because of second-hand smoke was 6.4 million years for lower respiratory infections, 2.5 million for chronic obstructive pulmonary disease, and more than 200,000 for middle ear infection (Cahn et al., 2013). Within countries, some groups demonstrate higher exposure levels and related burden, such as those of lower socioeconomic groups and non-smoking women (Cahn et al., 2013). For example, second-hand smoke causes more deaths in women than in men globally (573,000 vs. 311,000 in 2016) and more lung cancer deaths among women than men in China (40,000 vs. 12,000 in 2013 (Cahn et al., 2013).

2.7 Biological responses to selected metals

2.7.1 Essentiality of selected elements

Certain elements are nutritionally essential to humans and play a key role in physiological or biochemical processes (USEPA, 2007). Elements essential to other organisms may not be essential to humans and vice versa. Adverse nutritional effects can occur if essential metals are not available in sufficient amounts, and nutritional deficits also can be adverse and increase the vulnerability of humans to other stressors, including those associated with other metals (USEPA, 2007). Essentiality should be viewed as part of the overall dose-response relationship, and reference doses designed to protect from toxicity of excess should not be set below doses identified as essential (USEPA, 2007). Metals that are currently deemed nutritionally essential for humans are Co, Cr III, Cu, Fe, Mg, Mn, Mo, Se and Zn (Table 2.1) (USEPA, 2007). Some metals (e.g., B, Ni, Si, V, and perhaps As), while not essential to human health, may have some beneficial effects at low levels of exposure (USEPA, 2007).

Table 2.1 Metals essential to humans

Nutritionally essential metals	Nutritionally nonessential metals	
Cobalt II, III	Aluminum III	Nickel II
Chromium III	Antimony III, V	Niobium* V
Copper 0, I, II	Arsenic III, V	Palladium* 0, II
Iron II, III	Barium II	Platinum* 0, II, IV
Magnesium II	Beryllium II	Rubidium I
Manganese II, IV	Bismuth III, V	Silicon* IV
Molybdenum IV, VI	Boron III	Silver 0, I, II
Selenium II, IV, VI	Cadmium II	Strontium II
Zinc II	Cesium* I	Tellurium* II, IV, VI
	Chromium VI	Thallium I, III
	Gallium* III	Tin II, IV
	Germanium* IV	Titanium IV
	Gold 0, I, III	Tungsten VI
	Indium* III	Uranium IV, VI
	Lead II, IV	Vanadium III, V
	Lithium I	Zirconium* IV
	Mercury 0, I, II	

* Limited human data for these metals.

(USEPA, 2007)

2.7.2 Exposure levels of selected elements

Exposure is classified as "acute" if it lasts for a short time, but it can also be classified as "chronic" if it lasts a long time or is repeated frequently (Fischer et al., 2013). The biological response of exposure to a stressor, depends on both the intensity and length of exposure, but bioaccumulation and sensitization are additional elements that may result in the effects of exposure to hazardous metals (Fischer et al., 2013). Even while a single acute exposure or a brief period of low-level chronic exposure would not have any clinical consequences, bioaccumulation may eventually cause the pathological response to become more pronounced (Pappas, 2011). As a result of tobacco smoking, a number of the metals and metalloids (Table 2.1) bioaccumulate in the lung and other tissues (Pappas, 2011). A biological reaction will frequently be seen later at much lower doses if a tissue is sensitized to a metal (Pappas, 2011). The following sections that follow includes a number of well-known powerful sensitizers, some of which also bioaccumulate (Pappas, 2011).

2.7.3 Copper

Low quantities of copper are necessary for good nutrition (Bhattacharya et al., 2016). Copper is a respiratory irritant that causes macrophage migration into the alveoli, eosinophilia, the development of histiocytic and noncaseating granulomas containing copper inclusions, and pulmonary fibrosis (Pappas, 2011).

Iron and copper are both oxidation-reduction (redox) active metals. Because iron (80-7859 µg/g) is present in tobacco in greater quantities than copper (0.24 µg/g), the importance of redox activity is primarily investigated with reference to iron (James, 2014; Valko et al., 2016). Copper has been observed to be statistically considerably higher in the blood of smokers than of non-smokers according to Sundblad et al., (2016)., despite Chronic obstructive pulmonary disease (COPD) patients' exhaled breath condensate having copper contents that are much lower than healthy controls who don't smoke (WHO, 2009; Sundblad et al., 2016).

2.7.4 Iron

Low amounts of iron are necessary for nourishment (Abbaspour et al., 2014). Iron has been reported at lower concentrations in the exhaled breath condensate of people with COPD than that of nonsmoking healthy controls as reported by the WHO Study Group on Tobacco Production Regulation, (2009) and Sundblad et al., (2016). Padmavathi et al., (2009) found iron at statistically significantly higher concentrations in the serum of long-term smokers than of nonsmokers. As such, tobacco use increases the level of iron that the respiratory system is exposed. However, it alters the biology of the lung cells, causing both an increase in iron absorption and a decrease in iron release (Zhang et al., 2019). Although not directly etiologically linked, both cigarette smoke and iron status affect epithelial and immune cells in the lungs. It also alters the microbial ecology, and is connected to a number of respiratory diseases, including hemochromatosis, an iron-related disease brought on by a systemic iron excess, sideroblastic anemia, an iron-related disease brought on by a mitochondrial iron overload, and iron deficiency anemia (IDA), an iron related disease caused by a shortage of iron storage. Smoking tobacco is closely linked to IDA (Vivek & Kaushik, 2023). Cigarette and beedi smoking both significantly increase the risk of developing IDA. However, light smokers are at a higher at risk of IDA, and the risk is duration-dependent (Vivek & Kaushik, 2023). Additionally, iron inhalation adds to lung damage caused by free radicals (Vivek & Kaushik, 2023). The presence of traces of iron in particulates has also been shown to augment the pulmonary inflammatory response to silica (WHO Study Group on Tobacco Production Regulation, 2009). These studies show that iron oxidation-reduction chemistry enhances oxidative stress and lung damage caused by smoking (Jomova & Valko, 2011).

2.7.5 Manganese

Another essential element for humans is manganese (Erikson & Aschner, 2019). Both mineral deficit and excessive exposure to the mineral might have negative consequences (Montes et al., 2008; Tran et al., 2002). There have been clinical studies that suggest negative neurological consequences after prolonged exposure to very high levels of Mn in drinking water (Montes et al., 2008; Tran et al., 2002). It is known to produce neurological effects following inhalation and exposure, particularly in workplace settings (Montes et al., 2008; Tran et al., 2002).

According to research from the US Environmental Protection Agency cited by Leikauf, (2002), compounds of manganese are thought to cause or exacerbate asthma. Bast-Pettersen & Ellingsen, (2005) discovered that smokers who were occupationally exposed to manganese experienced more tremor than nonsmokers

2.7.6 Nickel and cobalt

Nickel is an IARC group 1 human carcinogen and cobalt is an IARC group 2B (WHO Study Group on Tobacco Production Regulation, 2009). This implies it may be carcinogenic to humans. This is despite the fact that both cobalt and nickel are nutritionally necessary at trace doses. The two metals are discussed together because they both cause immunological sensitization, including epidermal and oral allergic contact sensitization, contact dermatitis, pulmonary inflammation, pneumoconioses, and asthma as reported by Ahlström & Thyssen, (2009); Hostynek, (2006) and Yoshihisa & Shimizu, (2012). Given that they both activate the endothelium inflammatory pathway, if a person is sensitized to one of these metals, immunological cross-sensitization to the other is frequently seen

(Kelleher et al., 2000; ATSDR., 2013). All five lobes of smokers' lungs were found to have considerably greater tissue nickel concentrations than those of nonsmokers (Afridi et al., 2021; Tsuchiyama et al., 1997).

2.7.7 Radioactive elements

2.7.7.1 Uranium

According to Addo et al., (2011), tobacco may be the single biggest source of carcinogenic ionizing radiation in the world when it comes to radioactive elements. One of the hazardous elements found in SLT products is uranium (McAdam et al., 2017). The discovery of both uranium and thorium in tobacco samples highlight the radiological risks associated with using smokeless tobacco products (Addo et al., 2008). One of the most radioactive naturally occurring isotopes, uranium-238, is known to be both radiotoxic and chemotoxic (Escareño-Juárez et al., 2019). Lead, Radium, and radon are crucial components of the Th and U decay series (Moinester & Kronfeld, 2014; Vengosh et al., 2022).

2.7.7.2 Radon and radium

The uranium and thorium decay series include both radon and radon (Addo et al., 2008). 226-Radium is a long-lived, α-emitter nuclide and its daughter is 222-Radon, a short-lived (3.8 days) α-emitter nuclide that has very short-lived daughters (from seconds to microseconds), it’s also a form of ionizing radiation and proven carcinogen (Addo et al., 2008).

In the majority of uranium mines, this radionuclide poses the greatest radiation risk due to the simultaneous emission of α-and β particles (Salas et al., 2014). More than half of the effective dosage from natural sources is thought to be derived from the inhalation of short-lived radon daughters (Inácio et al., 2017; Skubacz et al., 2019). The carcinogenic potential of radon has been proven by several cohort, case-controlled, and experimental investigations as reported by Syed, (2012).

Long-term radon exposure may harm human health, increasing the risk of lung cancer and harming bronchial tissue (Abu-Jarad, 1997; Ghany, 2006). Soil, construction materials, and water supplies are the primary sources of indoor radon and its decay products (Abu-Jarad, 1997; Ghany, 2006). Due to the high electric charges in the decay products, they easily adhere to interior dust particles (Abu-Jarad, 1997; Ghany, 2006).

It was estimated in 1989 that globally, 9000 cancer deaths occurred as a result of radon exposure, which rose to 21,000 in 2012 (Samet, 2006). Also, the National Academy of Sciences BEIR VI committee in 1999, estimated that radon exposure results in 21,800 lung cancer cases each year, making it the second leading cause of lung cancer in the country (Lubin, 1994; Samet, 1989; Sethi & El-Ghamry, 2012).

Another study by Alavanja, (2002) found that approximately 90% of the 157000 lung fatalities globally in 2000 may be traced to smoking cigarettes, while 30% of lung cancer deaths in non-smokers can be attributable to home radon exposure. Although outnumbered by lung cancer caused by smoking, lung cancer in lifelong non-smokers is a major cause of mortality in the US and many other nations, accounting for around 16,000 deaths annually (Alavanja, 2002). This means that cancer and death cases resulting from Radom exposure are increasing exponentially. Radiation interacts with DNA in the cell nucleus directly or indirectly through the action of free radicals, causing damage to lung epithelial cells (Alavanja, 2002; Sethi & El-Ghamry, 2012). USEPA estimates radon to the leading cause of lung cancer among non-smokers (USEPA, 2020). According to the World Health Organization (WHO), radon is responsible for up to 15% of lung cancer cases globally (WHO., 2021). Thus, it becomes even more clear how harmful cigarettes and other tobacco products may be to people's health. Lung cancer, skin cancer, and renal ailments are among the health impacts of radon decay products inhalation. (Addo et al., 2008).

2.8 Toxicity of selected elements based on consumption of tobacco product samples

Second-hand tobacco smoke has been associated with development of asthma in children (WHO, 2009). Particulate samples with high concentrations of transition metals such as iron and vanadium appeared to be most active in promoting sensitization and triggering existing asthma (Willers et al, 2005). Willers et al, (2005) in their study investigated associations between exposure to environmental tobacco smoke, heavy metals and nicotine (as urinary metabolite, cotinine) in households of 23 children with asthma. They concluded that the children with asthma were being exposed to 'heavy metals' in side-stream smoke. An increasing number of non-cancer lung diseases and other diseases such as respiratory bronchiolitis, desquamative interstitial pneumonia, cough, restrictive pulmonary function, bronchiole-centred lesions, interstitial and airspace inflammation and fibrosis extending to the alveoli are associated with smoking (Selman, 2003). Chronic inflammatory response may, in turn, increase cancer risk (WHO, 2009). Suppression of the immune response, especially to pulmonary pathogens, has been observed as a consequence of heavy smoking (WHO, 2009). Toxic metals are available in saliva and are transmitted to oral epithelial tissues when smokeless tobacco is used. Oral contact inflammation is known to be a consequence of smokeless tobacco use. Toxicity resulting from exposure to metals extracted by saliva from tobacco held close to oral tissues have been found to contribute to the oral stomatitis inflammatory lesions observed as a consequence of smokeless tobacco use. Feron et al., (2001) and Mueller (2006) described the risk for carcinogenesis resulting from chronic inflammation of various epithelial and mucosal tissues. Thus, chronic oral inflammation is a consequence of smokeless tobacco consumption due to acute or chronic exposure to metals (WHO, 2009).

From the analysis of metals found in tobacco products in this study, the following elements exceeded safe limits for human exposure or intakes and may lead to non-cancer health effects;

1. Ca exceeded safe limits for short term exposure (snuff and cigarette samples); Fe and Mo (dry tobacco leaves) exceeded their Recommended daily allowance by 145.62 % and 136 % respectively.
2. For active users using dry tobacco leaves, long term or chronic exposure may lead to non-cancer health effects due to exposure to Zr, for snuff products users, non-cancer health effects may arise from long term or chronic exposure to all elements examined except Mo, Cu and Ti whiles for cigarette samples, non-cancer health effects may arise from chronic exposure to Mo, Rb, Zn, Fe, Cu (for C4 and C6), and Ti (for C1 and C2)
3. For chronic second-hand exposure to snuff samples, non-cancer health effects may arise due to long term exposure to Zn, Fe, V (except for "S1" products from commercial exposure), Mn (except for "S2" and "S3" products from both residential and commercial exposure) and Rb (with the exception of "S1" and "S2" products from commercial exposure). For chronic exposure to cigarette samples, non-cancer effects can arise from exposure to Fe, Zn (C4, Pall Mall, C6 from residential exposure), Mn (C4 and C6 from both residential and commercial exposure).

Some elements with acceptable safety values may bioaccumulate in the lung and other tissues, as reported by Pappas., (2011) and may cause some toxic effects.

Studies of workers chronically exposed to Mo have indicated a high incidence of weakness, fatigue, headache, irritability, lack of appetite, epigastric pain, joint and muscle pain, weight loss, red and moist skin, tremor of the hands, sweating, and dizziness (Gad et al, 1998). Elevated levels of Mo in blood plasma and urine and high levels of ceruloplasmin and uric acid in blood serum were reported for workers exposed to Mo (8-hr TWA 9.5 mg Mo/m^3) (National Research Council, 2000). Nineteen workers exposed to levels of 1 to 25 mg/m3 metallic molybdenum and molybdenum trioxide for 4 to 7 years, 3 were reported to suffer from symptoms such as breathing difficulty and frequent coughing. Pneumoconiosis (early stages) was verified by lung x-rays (14). In a subsequent study, 43 workers with inhalation exposure to molybdenum trioxide were compared with 23 unexposed workers. Respiratory symptoms (chest pains, breathlessness, coughing) lasting more than 6 weeks were reported by 33 of the exposed workers (Montelius & Sant, 2011).

Zirconium may be toxic to the upper respiratory tract. Prolonged exposure to Zr can lead to target organs damage (Ghosh et al., 1992a). Zr has been associated with degenerative disorders due to reports of deposition in the human brain, as found with aluminum, which has very similar properties, and Alzheimer's disease (Ghosh et al., 1992b).

Exposure to uranium can result in both chemical and radiological toxicity. The main chemical effect associated with exposure to uranium and its compounds is kidney toxicity. This toxicity is be caused by breathing air containing uranium dusts or by eating food containing uranium, which then enters the bloodstream. Once in the bloodstream, uranium compounds are filtered by the kidneys, where they can cause damage to the kidney cells. Very high uranium intakes (ranging from about 50 to 150 mg depending on the individual) can cause acute kidney failure and death. At lower intake levels (around 25 to 40 mg), damage can be detected by the presence of protein and dead cells in the urine, but there are no other symptoms (Thakur et al., 2012).

Several possible health effects are associated with human exposure to radiation from uranium. Because all uranium isotopes mainly emit alpha particles that have little penetrating ability, the main radiation hazard from uranium occurs when uranium compounds are ingested or inhaled. At the exposure levels typically associated with the handling and processing of uranium, the primary radiation health effect of concern is an increased probability of the exposed individual developing cancer during their lifetime (Boice et al., 2019). Cancer cases induced by radiation are generally indistinguishable from other "naturally occurring" cancers and occur years after the exposure takes place. The probability of developing a radiation-induced cancer increases with increasing uranium intakes (Thakur et al., 2012).

Zinc is believed to cause adverse nutrient interactions with copper and other minerals. It also suppresses immune functions and reduces high density lipoprotein (HDL) levels in the body (Saper & Rash, 2009). Inhalation of Zinc dust may cause irritation of the respiratory system, cough, dyspnoea and may also cause circulatory collapse and fever (*ATSDR*, 2014). Although "metal fume fever" occurs in occupationally exposed workers, it is primarily an acute and reversible effect that is unlikely to occur under chronic exposure conditions when zinc air concentrations are less than 8-12 mg/m^3 (*ATSDR*, 2014). Gastrointestinal distress, as well as enzyme changes indicative of liver dysfunction, have also been reported in workers occupationally exposed to zinc (Kerr et al., 1998a).

Although iron and copper are essential minerals for humans, exposure to iron can cause iron oxide deposition in Parkinson patients while high levels of copper have been shown to cause liver damage (Montes et al., 2014). The lethal dose of ferrous sulfate for a 2-year-old child is approximately 3 g, this value is of particular concern for children at homes were second-hand exposure is prevalent; for adults, it ranges from 200 to 250 mg/kg body weight (National Research Council, 1989) which is approximately 14g to 17.5g for a 70kg adult.

Acute inhalation exposure to copper dust or fumes at concentrations of 0.075-0.12 mg Cu/m^3 may cause metal fume fever with symptoms such as cough, chills and muscle ache (Kerr et al., 1998b)

Some people are genetically at risk from iron overload or hemochromatosis. Idiopathic hemochromatosis; which can result in the failure of multiple organ systems, is the result of an inborn error of metabolism, which leads to enhanced iron absorption. There is evidence for the carcinogenicity from inhalation of Fe dust, as well as for Fe-compounds being vectors for other carcinogens (PAHs, Ni, Cr etc.) (James, 2014). Inhalation of Fe could be more cytotoxic to humans, as Fe^{2+} oxidizes readily in $pH<7$ environments such as the lung, creating ROS. Iron overload plays a role in hepatic carcinogenesis, which is already associated with tobacco consumption (James, 2014).

Continued exposure to Mn can damage the lungs, liver, and kidneys. Exposure to manganese dust or fumes can also lead to a neurological condition called manganism. Manganism's symptoms which is similar to those of Parkinson's disease, may include the following: trembling, stiffness, slow motor movement and potentially severe depression, anxiety and hostility (Kiersma, 2014). Manganese is an essential trace element in humans that can elicit a variety of serious toxic responses upon prolonged exposure to elevated concentrations either orally or by inhalation. The central nervous system is the primary target. Initial symptoms are headache, insomnia, disorientation, anxiety, lethargy, and memory loss. These symptoms progress with continued exposure and eventually results in motor disturbances, tremors, and difficulty in walking. These motor difficulties are often irreversible. Effects on reproduction (decreased fertility, impotence) have been observed in humans with inhalation exposure and in animals with oral exposure at the same or similar doses that initiate the central nervous system effects. An increased incidence of coughs, colds, dyspnea during exercise, bronchitis, and altered lung ventilatory parameters have also been seen in humans and animals with inhalation exposure (RAIS, 2022).

Correlations have been found in the United Kingdom for Vanadium in air pollution and incidence of respiratory disease (bronchitis, pneumonia and lung cancer) (James, 2014). Soluble V absorbs in the lung well; once in the body it exhibits insulin-like effect on animals and humans. Acute toxicity may lead to irritation of the eyes and the upper respiratory tract, with neurobehavioral impairments suspected in chronic exposure. Though all the Vanadium compounds have not been widely described as health hazards, they pose some level of toxicity. Adverse health impacts of Vanadium include loss of appetite, nausea, vomiting, abdominal pain, diarrhea, and loose stool, liver and nervous system damages, kidney failure, and disrupted growth (Wilk et al., 2017). Other symptoms associated with Vanadium toxicity are irritation of mucous membranes and the upper respiratory tract, dizziness, abdominal and intestinal inflammations, headaches, skin rashes, nose and internal bleeding, damage in the cardiovascular system, and behavioral changes. Paradoxically, high levels of Vanadium can contribute to anemia, high cholesterol, reduction in blood sugar (hypoglycemia) and white blood cells, and fertility and birth challenges. There are also indications that the accumulation of Vanadium in breast tissues high-risk breast cancer. Inhaling Vanadium could result in throat, lung, and nose irritation, bronchitis, and pneumonia (Amuah et al., 2021).

TiO_2 has been classified by the International Agency for Research on Cancer (IARC) as an IARC Group 2B carcinogen, “possibly carcinogenic to humans” by inhalation. Although the IARC working group concluded that the epidemiological studies on TiO_2 provide inadequate evidence of carcinogenicity, they considered that the results from animal studies of inhalation and intratracheal instillation provide sufficient evidence to classify TiO_2 in Group 2B. Also, the National Institute for Occupational Safety and Health (NIOSH) has recently classified TiO_2 as a potential occupational carcinogen but considered that there is insufficient evidence at this time to classify submicron-sized TiO_2 as a potential occupational carcinogen (Skocaj et al., 2011).

Although no adverse effects have been observed in many healthy adults consuming up to 2,500 mg of calcium per day, high intakes may induce constipation and place up to half of otherwise healthy hypercalciuric males at increased risk of urinary stone formation. A high calcium intake may inhibit the intestinal absorption of iron, zinc, and other essential minerals (National Research Council, 1989). Ingestion of very large amounts may result in hypercalciuria, hypercalcemia, and deterioration in renal function in both males and females (National Research Council, 1989).
Potassium can affect you when breathed in. Potassium can severely irritate the skin and eyes. Exposure to Potassium can irritate the nose, throat and lungs with sneezing and coughing. Prolonged exposure to Potassium fumes can cause sores of the inner nose. Repeated exposure may cause bronchitis to develop with cough, phlegm, and/or shortness of breath (Sheet, 2003).

People can be exposed to small levels of radioactive Sr through inhalation and oral ingestion or dermal contact with contaminated soils (Amuah et al., 2021). It could pose disruptions in bone development in adults as it may be attached to the surface of bones and in it also creates hard bone mineral which impedes bone growth in children. The bone marrow, where red blood cells are formed is also damaged by the high levels of Sr. This reduces the production of red blood cells. Therefore, proper respiration could be disrupted (Amuah et al., 2021). Radioactive Sr contributes to anaemia and oxygen shortage in people exposed to high concentrations. According to Amuah et al., (2021), studies have also associated genetic disruptions in humans which could results in a wide range of cancers to Sr. Sr has also been linked to other debilitating health and psychological impacts including severe skin reactions, disturbances in thinking, inflammation of the liver, and seizures (Amuah et al., 2021). Strontium has been shown to inhibit sensory irritation after continuous dermal contact (Amuah et al., 2021). Since there has not been any derived reference value for toxicological assessment of strontium, toxicological assessment of strontium was not determined in this study but from literature, it has been observed to have toxicological effects on humans through ingestion and inhalation.

2.9 Nicotine

Nicotine is a biogenic yet non-physiological compound in the human body (The German Federal Institute for Risk Assessment, 2021). At certain levels it can be acutely toxic. An estimated acute toxicity value (5 mg/kg bodyweight) has been defined for oral exposure (The German Federal Institute for Risk Assessment, 2021). Depending on the dose incorporated, nicotine increases the number of stillbirths and may affect the cardiovascular system (The German Federal Institute for Risk Assessment, 2021). Nicotine is a natural component of tobacco leaves (Ayo-Yusuf et al., 2004). Cigarette tobacco contains up to 1.5 % nicotine (The German Federal Institute for Risk Assessment, 2021). The addictive substance in tobacco, nicotine, keeps users from quitting and encourages continued usage over time (Ayo-Yusuf et al., 2004).

In an earlier US study, the cardiovascular effects of cigarette consumption were compared with the use of American “snuff” in ten healthy smokers (The German Federal Institute for Risk Assessment, 2021). In one study part, a cigarette was smoked with one puff every 45 seconds for twelve puffs, in the other study part, a pouch of American “snuff” weighing 2.5 g was placed between the upper lip and the gum for 30 minutes. Blood samples were taken at different time points and heart rate and blood pressure were measured (The German Federal Institute for Risk Assessment, 2021). The absorbed dose was calculated based on nicotine measurements; it was 1.8 mg nicotine for cigarette consumption and 3.6 mg nicotine for snuff consumption. The heart rate increased by 26 beats per minute after cigarette consumption and by 18.2 beats per minute after snuff consumption one puff every 45 seconds for twelve puffs. The blood pressure increased by 18.6 mm (systolic) and 12.2 mm (diastolic) for cigarette consumption and 15.6 mm (systolic) and 11.4 mm (diastolic) for snuff consumption (The German Federal Institute for Risk Assessment, 2021). The study shows that consumption of a cigarette or a pouch of American “snuff” leads to a relevant increase in heart rate and blood pressure.

In a review article, relationship between nicotine and type 2 diabetes was pointed out (Piano et al., 2010). Cigarette smoking is an important risk factor for developing type 2 diabetes. Compared to non-smokers, smokers show an increased insulin resistance, but there is no evidence for an effect on insulin secretion (Piano et al., 2010). However, nicotine can increase insulin resistance by increasing levels of insulin antagonists (catecholamines, cortisol and growth hormones) (IARC, 2006).

2.9.1 pH and unprotonated nicotine

Of particular interest in pH analysis of tobacco products is the free base (unionized) nicotine, which is the form that is most easily absorbed. The pH of SLT has a significant impact on nicotine absorption by the nose and mouth (Ayo-Yusuf et al., 2004). Therefore, unlike cigarettes where nicotine delivery is dependent on the smoking habits of the user, the nicotine dose acquired from a unit (or "dip" or "sniff") of SLT is mostly influenced by the properties of the product itself. Also, to a lesser extent, nicotine delivery is dependent on the amount of snuff used, as well as behavioral and physiological aspects specific to each user (Ayo-Yusuf et al., 2004; Fant et al., 1999; Lunell & Lunell, 2005; S. Tomar & Henningfield, 1997). One study used four US brands of SLT and they found out the nicotine level of the products examined ranged from 7.5 to 11.4 mg/g. pH also ranged from 6 to 9; after using the high pH product, plasma nicotine concentrations increased by 20 ng/ml, but only by 4 ng/ml after using the lower pH product (Fant et al., 1999). It was shown that their pH, not the amount of nicotine, was principally responsible for the variations in nicotine absorption, as well as the subjective and physiological consequences (Stanfill et al., 2021).

The amount of unprotonated nicotine impacts the efficiency and extent of nicotine absorption, according to (Stanfill et al., 2021; Wilhelm et al., 2022). Both investigations demonstrated that nicotine-dosing capability differ significantly between products and that nicotine concentration and pH level play a major role in determining it (Tomar & Henningfield, 1997). Nicotine absorption appears to be primarily determined by pH (Tomar & Henningfield, 1997). As reported by IARC., (2009), several salts (e.g., ammonium, sodium and potassium salts) have the potency to alter the pH of smokeless tobacco. As such, they are included among the 562 additives for smokeless tobacco products. Additionally, smokeless tobacco contains elements that are designed to regulate the body's absorption of nicotine have been reported by the House of Representatives in 1994 and 1996 respectively (Etu et al., 2017). However, the amount of tobacco toxins that users are exposed to depends not only on the specific product they are using, but also on how they are used (Etu et al., 2017). Users of smokeless tobacco who dip or chew eight to ten times per day might receive the same amount of nicotine exposure as people who smoke 30 to 40 cigarettes per day (Etu et al., 2017).

According to research by Hecht et al. (2005) and Lemmonds et al. (2005) which has been quoted by (IARC., 2009), snuff users are exposed to 3.08 times as much nicotine as cigarette smokers. The amount of nicotine absorbed across the oral mucosa is greatly reliant on the pH of the product, which affects the proportion of total nicotine present in the unprotonated form (IARC., 2009). Unprotonated nicotine is significantly easier to pass through epithelium than protonated nicotine, which causes an increase in blood nicotine levels (Tomar and Henningfield, 1997; Fant et al., 1999; CDC., 1999).

It has also been demonstrated that raising the nicotine content while consuming alcohol causes N-Nitrosonornicotine (NNN) to penetrate the oral mucosa at a substantially higher rate (Du et al., 2000). In a report to the WHO South-East Asian Regional Office cited by IARC., (2009), information on the chemical make-up of 14 different types of smokeless tobacco products used in India, including pH and nicotine concentration, showed that some products had up to 10.1 pH and 10.2 mg/g of nicotine (Gupta, 2004; Table 9). The pH and nicotine level of wet snuff products used in South Africa had nicotine levels varying from 0.8 to 1.6 percent wet weight (11.6-29.3) which were reported by Ayo-Yusuf et al. in 2004. pH ranged from 7.1 to 10.1 (Ayo-Yusuf et al., in 2004); the nicotine content from 0.8 to 1.6% wet weight (11.6–29.3 mg/g dry weight, as adjusted for moisture content) (Ayo-Yusuf et al., in 2004) and from 10.1 to 99.1% in the unprotonated form (Ayo-Yusuf et al., in 2004).

Even small changes in pH of a tobacco product have a significant impact on the unprotonated nicotine concentration in SLT products (Tomar et al., 2019).. For instance, recent tests of 79 variants of US wet snuff products revealed that, while overall nicotine levels varied only by around 2-fold, unprotonated nicotine concentration varied by more than 1,000-fold in the same products (Tomar et al., 2019). Such a large range of unprotonated nicotine concentrations would make it easier for customers to switch to utilizing products with more potent nicotine content and develop severe addictions (Tomar et al., 2019).

2.9.2 Relationship between pH, moisture content and nicotine delivery in tobacco products

Dry snuff has the lowest moisture content and middle-to-high nicotine levels, while wet snuff has the highest percentage of pH and nicotine concentration (NCI, 2014). Chewable tobacco had the lowest nicotine (NCI, 2014). Average pH of snuff is 7.43 (moist snuff), 6.36 (dry snuff), and 5.82 (chewable tobacco) (NCI, 2014). Amounts of unprotonated nicotine in wet snuff are roughly five folds than those in dry snuff and 32 folds than those in chewable tobacco due to the high pH (NCI, 2014). Amounts of unprotonated nicotine, however, differ from product to product and from year to year due to the continual interaction between pH, nicotine concentration, and moisture in tobacco products (NCI, 2014).

pH of tobacco product (cigarette filler/SLT) is of important in determining amounts of unprotonated or free nicotine bioavailable to the consumer (WHO, 2020). Since the unionized form of nicotine obtained at basic pH values is more readily absorbed by the human body (WHO, 2020). The pH of a tobacco product plays a crucial role in deciding its efficacy, which provides a good basis for a reason it should be measured (WHO, 2020). Thus, it is a useful and necessary parameter for researchers/regulators, as it can help to inform policy (WHO, 2020). Determination of moisture content is so that the concentration of nicotine and other constituents can be reported both on dry and wet weight basis, as variation in moisture can effect of other importance parameters (WHO, 2020).

2.10 Human health risk assessment of selected elements

Human health risk assessment of chemicals refers to methods and techniques that apply to the evaluation of hazards, exposure and harm posed by chemicals. In some cases, human health risk assessments of chemicals may differ in the approaches used (Robinson, 2014). The risk assessment process begins with problem formulation and includes four additional steps: 1) hazard identification, 2) hazard characterization, 3) exposure assessment and 4) risk characterization (Robinson, 2014).

According to the Integrated Risk Information System (IRIS) and quoted by Benson et al., (2017), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) are classified as non-carcinogenic metals. The purpose for conducting toxicity assessment is to check the likelihood that potentially toxic metals present in the samples could pose serious health effects to users and non-users through direct and indirect inhalation exposure and oral exposure. The long term human health exposure assessment and risk characterization associated with non-carcinogenic were calculated using USEPA's methodology Retrieved from (https://www.epa. gov/expobox/exposure-assessment-tools-routesinhalation) on 2022-12-28.

According to the and quoted by USEPA and quoted by Marano et al., (2018), the averaging time (AT) depends on the type of toxic effects assessed (i.e. cancer or noncancer). That is, for long term exposure to non-carcinogens, exposure concentrations are calculated by averaging intakes over the period of exposure (Marano et al., 2018). For carcinogens, exposure concentrations are calculated by prorating the total intake over a lifetime (USEPA, 1989). Thus, the averaging time for noncancer effects (ATNC) is the same as the exposure duration, (20987.5 days (57.5 years × 365 days/year) for cigarette smokers and 18615 days (51 years × 365 days/year) for smokeless tobacco product users (Marano et al., 2018). In accordance with the most updated USEPA guidance (USEPA, 2014), default life expectancy of 7(0 years) is used as the average time for assessing carcinogens (ATC), (25550 days i.e., 70 years × 365 days/year) for both cigarette smokers and smokeless tobacco product users (Marano et al., 2018).

The purpose of hazard assessment is to evaluate whether an agent poses a carcinogenic hazard to humans and under what circumstances an identified hazard may be expressed (USEPA, 2003). Hazard assessment is performed by comparing calculated contaminant dose from ingestion and dermal exposure routes with the reference dose (RfD) to develop the hazard quotient (HQ). RfC and RfD are derived in a manner to ensure that they are unlikely to underestimate the potential for adverse non-cancer effects to occur (Marano et al., 2018).

USEPA guidance documents (USEPA, 1989; 2003, 2005; 2009b) that address of cancer risk and noncancer hazard estimation generally provide a hierarchy of sources from which representative toxicity factors should be identified.

In particular, toxicity factors generated by USEPA's Integrated Risk Information System (IRIS) are generally specified as the primary source within the hierarchy; other scientifically reasonable sources for toxicity assessment exist. It is recommended by USEPA (2003) that selection of toxicity factors in the Toxicity Assessment of tobacco products follow a similar hierarchy. This ensures that the most conservative (health protective) approach is observed. Based on guidance from USEPA (2003), the hierarchy for selection of toxicity factors includes:

1. Tier 1—EPA's Integrated Risk Information System (IRIS).
2. Tier 2—EPA's Provisional Peer Reviewed Toxicity Values (PPRTVs): The Office of Research and Development/National Center for Environmental Assessment/Superfund Health Risk Technical Support Center develops PPRTVs on a chemical-specific basis when requested by USEPA's Superfund program.
3. Tier 3—Other Toxicity Values: Tier 3 includes additional USEPA and non-USEPA sources of toxicity information, such as the California Environmental Protection Agency (CalEPA, 2018a; 2018b), Agency for Toxic Substance and Disease Registry (ATSDR, 2018), Texas Commission of Environmental Quality (TCEQ, 2017), and the peer reviewed literature. Priority should be given to sources of information that are current, the basis for which is transparent and publicly available, and which have been peer-reviewed (Marano et al., 2018).

Recommended inhalation toxicity factors identified per the hierarchy are presented (Appendix Table E) and recommended oral toxicity factors identified per the hierarchy are also presented (Appendix Table F). Consistent with the proposed hierarchy, for harmful and potentially harmful constituents (HPHC), when toxicity factors were not available from USEPA, other sources were identified.

CHAPTER THREE

METHODOLOGY

3.1 Chemicals/Apparatus

1. Analytical balance accurate to 4 decimal places manufactured by Mettler Toledo, MS Series; Switzerland
2. Desiccator
3. Thermostatically controlled oven manufactured by Infitek, DON-HE Series, China
4. Glass dishes
5. Stainless steel containers with approximate dimensions of 50 mm deep by 90 mm wide with lids.
6. Digital pH meter M.K 6, Systronics, Ahmedabad; India, equipped with a temperature probe.
7. Orbital shaker or magnetic stirrer.
8. All reagents were analytical grade and comply with existing national regulations.
9. Distilled water, complying with grade 2 of ISO 3696:1987.
10. Standard pH buffers (4.00, 7.00 and 10.00)

3.2 Study Area

The study was conducted in the Ejisu Municipality in the Ashanti Region of Ghana. The Ejisu Municipal is one of the 261 administrative districts in Ghana (GSS, 2021). It forms part of the 43 districts in the Ashanti Region with Ejisu as its administrative capital (GSS, 2021). The Municipal is known globally for its rich cultural heritage and tourists' attraction site notably the booming kente weaving industry making it a potential center for cultural exchange (GSS, 2021).

The Municipal covers an area of 637.2 km^3 constituting about 10% of the entire Ashanti region (GSS, 2021). The Municipality is located in the central part of the Ashanti Region and provides enormous opportunity for creating an inland port for Ghana to serve the northern section of the country (GSS, 2021). It is located within longitudes 1°5W and 1°39' W and latitudes 7°9' N and 7°36'N. It is the fifth largest district in Ashanti region (GSS, 2021). Ejisu Municipal shares boundaries with six other Districts in the Region (GSS, 2021). To the north east and north west of the Municipal are Sekyere East District and Kwabre East Municipal respectively (GSS, 2021), to the south are Bosomtwe District and Asante Akim South Municipal, to the east is the Asante Akim North Municipal and to the west is the Kumasi Metropolitan (GSS, 2021).

The population of the Municipality according to the 2021 Population and Housing Census stands at 180,723 with 87,836 males 92,887 females (GSS, 2021) with a high population of 58.3% between the ages of 15 to 64 years as stated in the Composite Budget (EMA, 2021; GSS, 2021) within the age bracket that is prone to smoking and other forms of tobacco use.

3.3 Sample Collection

A total of 56 samples were collected from eleven (11) different locations within Ejisu Municipality in the month of April, 2022. Three different types of tobacco products were sampled (two smokeless tobacco products, namely; snuff and dry tobacco leaves and one combustible tobacco product, namely, cigarettes). In all forty-five samples of snuff were purchased from eleven different locations within the Ejisu Municipality. They were transported to the analytical laboratory in labelled zip lock bags prior to analysis. A total of five samples of dried tobacco leaves were also purchased whiles six different brands of cigarettes including one which was being sold illegally (smuggled) were also purchased from different vendors within the Ejisu township. The collected samples were not more than 6 months old from the date of production.

Table 3.1 Sampling locations and their GPS coordinates of tobacco product samples

TOBACCO PRODUCT	FLAVOUR	MANUFACTURER (CODE)	LOCATION	LATITUDE	LONGITUDE
Snuff	No flavour	S1	Addadientem	N6.713522	W1.432263
Snuff	Menthol	NA	Ampabame	N6.706933	W1.419680
Snuff	Moringa	NA	Onwe	N6.682541	W1.450804
Snuff	Unknown flavour	S4	Onwe	N6.683374	W1.450919
Snuff	Moringa	NA	Achinakrom	N6.662196	W1.469884
Snuff	Unknown flavour	NA	Essienimpong	N6.654711	W1.447939
Snuff	Moringa	NA	Essienimpong	N6.655008	W1.451407
Snuff	No flavour	S1	Kwaso	N6.632145	W1.450730
Snuff	Moringa	S2	Ejisu	N6.721790	W1.471675
Snuff	Moringa	NA	Akyawkrom	N6.732017	W1.460422
Snuff	Unknown flavour	S3	Akyawkrom	N6.730010	W1.459027
Snuff	Unknown flavour	S2	Donaso	N6.696253	W1.454019
Snuff	Menthol	S1	Besease	N6.726064	W1.454293
Snuff	Moringa	NA	Besease	N6.726064	W1.454293
Dry tobacco leaves	-	L1	Ejisu	N6.720800	W1.473139
Dry tobacco Leaves	-	L2	Ejisu	N6.721370	W1.472793
Dry tobacco leaves	-	L3	Ejisu	N6.720798	W1.473144
Dry tobacco leaves	-	L4	Ejisu	N6.720709	W1.473251
Dry tobacco leaves	-	L5	Ejisu	N6.721790	W1.471675
Cigarette	-	C1/C2/C3/C5	Ejisu market	N6.722260	W1.471400
Cigarette	-	C4/C6	Ejisu	N6.720709	W1.473251

NA= Unknown / Not Available
S1= Power Snuff
S2= KD Power
S3= Power Must Change Hands
S4= Okatakyie
NF= No flavour
C1= London Menthol
C2= Rothmans King Size
C3= London Filter
C4= Oris
C5= Pallmall Menthol
C6= Gold seal (Illicit)

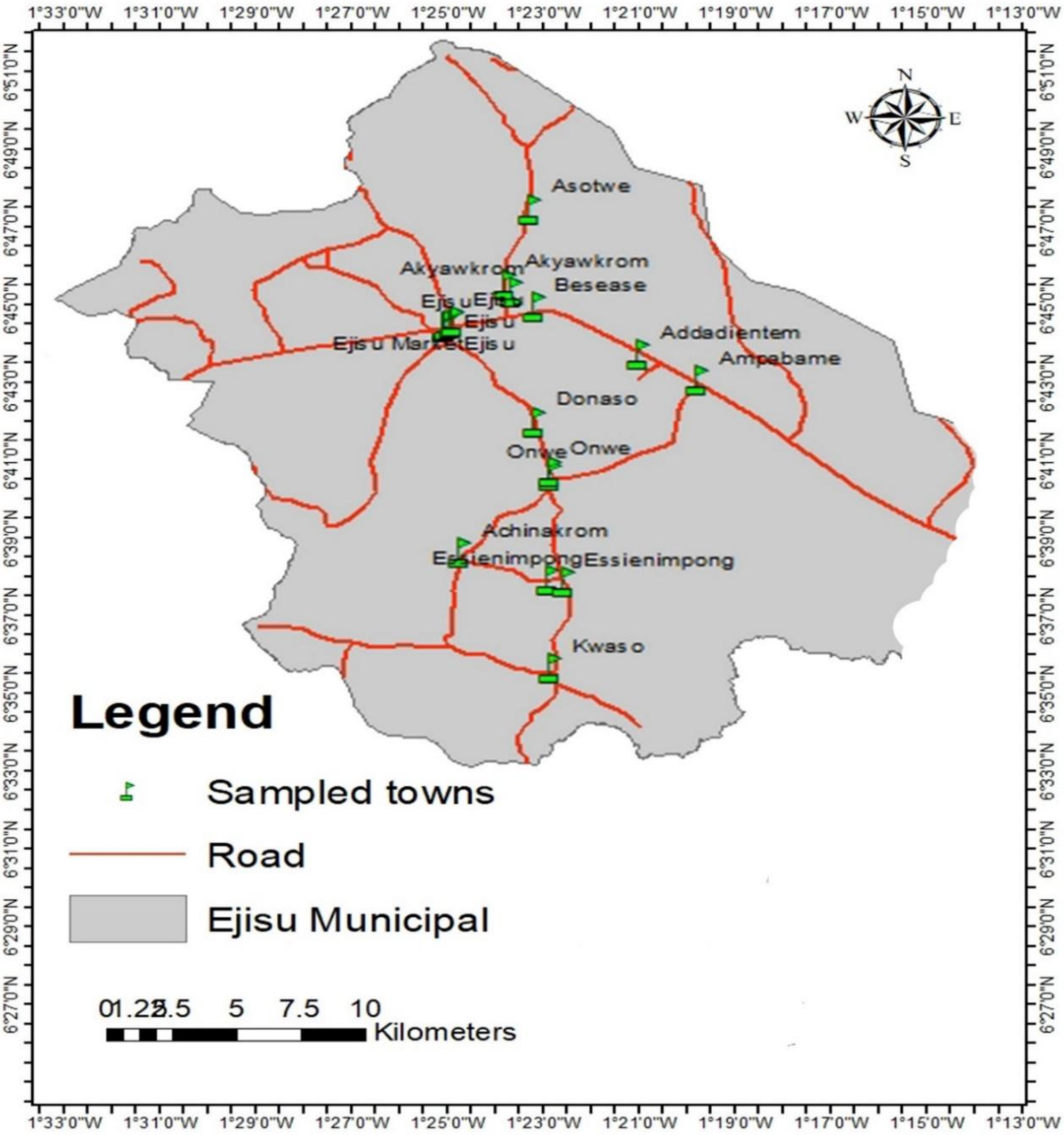

Figure 3.1 Map showing sampling locations within the Ejisu municipality

3.3.1 Sample Preparation

The whole sample preparation, precaution against contamination and handling procedures followed the guidelines described by CORESTA guide number 11 (CORESTA, 2020).

3.3.2 Grinding of samples

The dried tobacco was ground with a mortar and a pestle into fine powdery particles to simplify weighing and facilitate chemical and instrumental analysis. The remaining tobacco particles were wiped off from the mortar and pestle before proceeding to the next sample to avoid cross contamination.

3.4 Physical Properties of Samples

3.4.1 Weight

The average weight of each cigarette brand was measured by weighing 5 sticks of each brand before and after removing the filters and papers, whereas that of the snuff (SLT) was measured by emptying the smokeless tobacco (ST) from its container unto a pre-weighed filter paper and measured on a chemical balance, by Mettler Toledo, MS Series.

3.4.2 Moisture content determination

Section 3.4.2.2 describes the procedure moisture content was determined as described by CORESTA Recommended Method number 76 (CORESTA, 2021).

3.4.2.2 Procedure

The stainless-steel tin was tarred and placed oton a balance (Mettler Toledo, MS Series) with its lid on the balance and its weight recorded as Tare weight of stainless-steel tin and lid (WT). The stainless-steel container was removed from the balance and then filled with 5.0 g of tobacco. After, the tin was covered with the lid and it was then placed onto the balance again. Reading was allowed to stabilize and weight recorded as W1. The container was then placed in an oven at 100 ^{0}C for 3 hours. After which it was removed and put into a desiccator to cool. The cooled sample was weighed and the data collected as W2. Percentage loss of weight was calculated and then expressed as M %.

3.4.2.3 Calculation

The percentage moisture content (oven volatiles) of the tobacco was calculated as equation 2:

$$M\ (\%) = \frac{W1 - W2}{W1 + WT} \times 100 \quad , \qquad 2$$

where: M = Moisture content , W1 = Initial weight of tobacco sample, including stainless steel tin and lid (g), W2 = Weight of dry tobacco sample after test, including stainless steel tin and lid (g), and WT = Tare weight of stainless-steel tin and lid (g).

3.4.3 Determination of pH of tobacco samples

pH of the samples were determined by following the procedure as described by CORESTA Recommended Method number 69 (CORESTA, 2021).

3.4.3.3 Calibration of pH Meter

1. The pH meter was initially calibrated by placing the pH electrode into a buffer solution of pH 4. The asymmetric potential control of the instrument was then adjusted until the meter read the known pH value of the buffer solution.
2. The electrode after rinsing with copious amounts of distilled water was further immersed in a second buffer solution of pH 7 and the instrument adjusted to read the pH value of the buffer solution. A three-point calibration procedure was used to cover the range of pH of the tobacco samples.
3. The procedure was repeated for the standard buffer solution of pH 10.

4. This procedure was used to calibrate the instrument before its use in pH determination of the tobacco samples.

3.4.3.4 Procedure used in determination of pH of tobacco products

Two grams of the ground sample was weighed into a 50 ml container. Twenty milliliters of distilled water were added to the sample. The sample shaken gently for thirty minutes. The pH of the samples was then measured in triplicates by placing the electrode in the sample and allowing a constant value to display on the pH meter and the mean values recorded.

3.5 Elemental Screening

Tobacco samples were screened using a Niton XL3 GOLDD+ X-ray fluorescence (XRF) analyzer from Thermo Scientific; USA. The tobacco analysis followed the United States Environmental Protection Agency USEPA, Method 6200 as used by (Darko et al., 2019).

3.5.1 Procedure for elemental determination in tobacco samples

Two grams of the sieved sample was placed into a small polyethylene container of approximately 30 mm to three quarters of its capacity and then sealed at both ends using a mylar film. It was then placed in the XRF shroud and scanned for 180 seconds for elemental analysis (Darko et al., 2019).

3.6.1 Determination of percentage Free Nicotine

The free nicotine content was calculated using 12 mg/g and pH values for each product. The percentage of total nicotine available as free nicotine (% free nicotine) was calculated by substituting the appropriate pKa value for nicotine and the pH of each product into the Henderson–Hasselbalch equation and then multiplying by 100.

The Henderson-Hasselbalch curve (Figure 3.2) for nicotine models the sigmoidal relationship between alkalinity and free nicotine (%). The α_{fb} value refers to the fraction of nicotine in the freebase form. At pH 8.02, 50% of nicotine is in the protonated form and 50% in the un-protonated form.

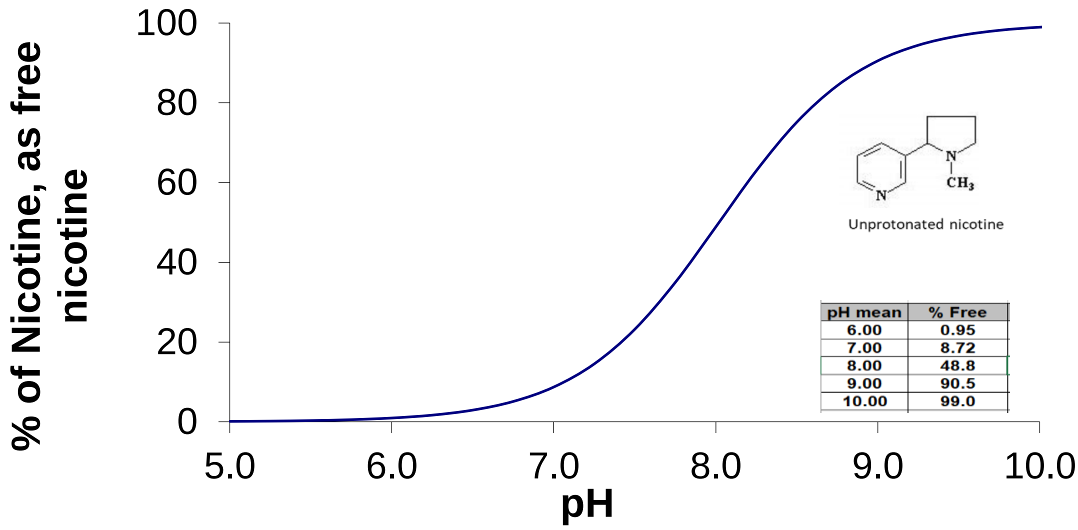

Figure 3.2 Graph of pH against % free nicotine

3.7. Quality Assurance

Determination of physical properties of tobacco samples adhered to quality control and assurance protocols as outlined in CORESTA, (2020) and CORESTA, (2021). To ensure quality assurance in elemental screening with the XRF, tobacco samples were air-dried in the sun to ensure that they were free from moisture. Each of the dried tobacco samples was then sieved to < 250 µm using a USA Standard Testing Sieve ASTME11. The XRF was also calibrated using NIST 2709a certified reference material and system checked each day prior to analysis. This gave recovery of $\geq 75 \pm 5$ always. Each sample analysis was done in triplicate and the average of the readings computed.

3.8 Statistical Analysis

Statistical analysis of the data was performed using Minitab 20.0 (Minitab, LLC, Pennsylvania; USA) and JASP 0.17.2, University of Amsterdam, (Netherlands). Some charts were also generated from of Microsoft Excel, 2019; (Microsoft Corporation, Washington; USA)

3.9 Human Health Risk Assessment

A human health risk assessment identifies hazards, which can be anything that's likely to cause harm or illness (USEPA, 2000). A risk assessment helps determine whether significant risks to peoples' health may exist at or near a contaminated site or from exposure to certain substances and also helps determine a risk-based cleanup level for the site (USEPA, 2000).

3.9.1 Long-Term Non-cancer Risk Assessment

The human health risk assessment was used to characterize the nature and magnitude of possible human health risks from non-carcinogenic elemental contaminants in the tobacco samples. The long-term health risk assessment for active tobacco users due to exposure to elemental contaminants in the tobacco samples will be estimated based on the USEPA risk assessment model (USEPA, 1989; 2003, 2005; 2009). Values employed in the calculation of various parameters were presented (Table 3.2 and 3.3).

3.9.2 Short- Term Non-cancer Risk Assessment

Human health risk assessment for short-term exposure estimates Average Daily Exposure to potential toxicants over a period of 8 hours (ADE_8) as a percentage OSHA Time weighted average (TWA_8) for cigarette and snuff products (Behera et al., 2013). The purpose for estimating ADE_8 was to examine whether any short-term health effects could occur due to exposure to snuff particulates and cigarette smoke as indicated in a study by (Behera et al., 2013) and also to examine whether any short-term ADE_8 health effect could occur on second-hand tobacco users, especially for individuals working in an area that is exposed to much tobacco use ADE_8 (Behera et al., 2013). For example, if ADE_8 as percentage OSHA TWA_8 of any element is below 100%, then the area is safe, (no acute health effect(s) due to second-hand exposure). However, if ADE_8 is above than 100%, then the area is not a safe environment to work (Behera et al., 2013).

ADE_8 as a percentage of OSHA TWA_8 elements in second-hand tobacco exposure (STE) was estimated to represent short-term health effects of selected elements on both active and passive second-hand smokers. Two possible cases (Case 1 and Case 2) for health risk assessment were evaluated by considering two environmental conditions, in which non-smokers are exposed to cigarette smoke and snuff products. Case 1 represents residential settings while case 2 represents a commercial settings.

All OSHA TWA_8 values were sourced from Cal/OSHA, (2021). Except Ti data; which was sourced from US/OSHA, (2017). Case 1 refers to risk estimation with the presence of one smoker with 10 dilution factor for indoor air. Case 2 refers to risk estimation with the presence of five smokers with 100 dilution factor for partial indoor air.

$$ADE_8 \text{ as \% of OSHA } TWA_8 = ADE_8\ (mg/m^3) \times 100 \div TWA_8\ (mg/m^3), \qquad 3$$

The average daily concentration for an 8 h workday (ADC_8) in mg/m^3 was estimated by assuming a breathing volume of 10 m^3 of air during working hours in a day and smoking 10 cigarettes/inhaling 10 grams of snuff during that period of time.

The Estimated Daily Intake (EDI), Chronic daily Intake (CDI), Exposure Concentration, hazard quotient (HQ), Average Daily Exposure) (ADE_8), %OSHA TWA_8 and hazard index (HI) were estimated using Equations. 4-13 in the sections below.

3.9.3 Estimated Daily Intake (EDI), Chronic daily Intake (CDI), and Exposure Concentration of selected elements

For dried tobacco leaves, (a form of SLT), which people use by either chewing or sucking by inserting them between the lower gum and lip, in accordance with USEPA guidance USEPA, (1989), and exposure to constituents in a medium is expressed either as estimated daily intake (EDI), which calculated by using equation (5), the chronic daily intake (CDI), which is also calculated using equation (6). EDI is used to assess short term (acute) health effects whereas CDI is used to assess long term health effects (Behera et al., 2013). Similar to cigarette smokers, smokeless tobacco product users are assumed to be lifetime receptors (Behera et al., 2013).

Hazard quotients (HQ) for snuff and cigarette samples were estimated as:

$$HQ = \frac{EC}{RfC}. , \quad 3$$

Estimation of exposure concentration was made by smoking 10 cig/day and a breathing volume of 20 m^3. Hazard quotient (HQ) for dry tobacco leaf samples was estimated as:

$$HQ = \frac{CDI}{RfD} , \quad 4$$

Estimation of chronic daily intake (CDI) was made by chewing 10 g of dry tobacco leaves /day.

$$EDI = C \times CF \times TC , \quad 5$$

$$CDI = \frac{(C \times CF \times TC \times ABS \times EF \times ED)}{Bwt \times At} , \quad 6$$

where:

EDI – estimated daily intake (mg/day)

CDI – chronic daily intake (mg/kg/day)

C – toxicant concentration (nanogram per gram [μg/g] tobacco)

CF – conversion factor (10^{-3} mg/μg)

TC – tobacco consumption rate (gram per day [g/day])

ABS – toxicant absorption rate (unitless)

EF – exposure frequency (days/year)

ED – exposure duration (years)

BW – body weight (kg)

AT – averaging time (days)

However, in 2009, the USEPA (2009a) provided an update to the Superfund Programme's approach to determining risk of inhaled constituents. This guidance updated the USEPA's original approach, established in 1989, to determine exposure estimates in terms of chronic, daily "air intake" (mg/kg/day). Nasal snuff is prepared from dry fermented tobacco pulverized to fine particles and then inhaled in Ghana (Addo et al., 2008). Estimates of risk via inhalation of cigarettes and snuff products was calculated using concentration of the constituent in air; exposure concentration as the exposure metric (mg/m^3), rather than estimated intake of constituents. Details of the standards employed in the calculation of various parameters are presented (Table 3.2) (Marano et al., 2018).

$$\text{exposure concentration} = \frac{(C \times CpD \times EF \times ED \times CF)}{\mathrm{IR} \times \mathrm{At}} , \qquad 7$$

where:

exposure concentration – exposure concentration (mg/m^3)

C – HPHC measured yield (μg/g)

CF – conversion factor (10^{-3} mg/μg)

CpD – number of cigarettes per day

ED – exposure duration (years)

EF – exposure frequency (days/year)

IR – inhalation rate (m^3/day)

AT – averaging time (days)

3.9.4 Short-term non-cancer Hazard assessment

The exposure concentration for an 8 h workday (ADE_8) in mg/m^3 was estimated by assuming a breathing volume of 10 m^3 during working hours in the day and smoking 10 cigarettes/inhaling 10 g of snuff during that period of time. The equations used are as follows:

$$\% \text{ OSHA } TWA_8 = \frac{\text{ADE8}}{\text{OSHA TWA8}} \times 100 \quad , \qquad 8$$

$$ADE_8 = \frac{(C \times CpD \times CF)}{\text{IR}} \quad , \qquad 9$$

$$\text{Case 1} = \frac{\% \text{ OSHA TWA8}}{10} \quad , \qquad 10$$

$$\text{Case 2} = \frac{\% \text{ OSHA TWA8}}{100} \times 5 \quad , \qquad 11$$

where

TWA_8 – 8 h time weighted average

ADE_8 - Average Daily Exposure over a period of 8 hours

C – HPHC measured yield (µg/g)

CF – conversion factor (10^{-3} mg/µg)

CpD – number of cigarettes per day

IR – inhalation rate (m^3/day)

3.9.5 Long-term non-cancer Hazard assessment

Metrics of non-cancer effects were calculated via hazard quotients (Hazard Quotient, for individual constituents) or a hazard index (HI, for multiple constituents). Hazard Quotient, representing the ratio of estimated exposure to the toxicity value, are calculated for each constituent associated with non-cancer health effects using the Equations12-15:

$$HQ = \frac{\text{EC or CDI}}{\text{RfC or RfD}} \quad , \qquad 12$$

Case 1=$\frac{HQ}{10}$, 10

Case 2= $\frac{HQ}{100}\times 5$, 11

$HI = \sum HQ$, 12;

HQ – hazard quotient (unitless)

EC – exposure concentration (mg/m^3)

CDI – chronic daily intake (mg/kg/day)

RfC – reference concentration (mg/m^3)

RfD – reference dose (mg/kg/day)

For non-cancer risk characterization, HI greater than 1 indicates there is a potential for adverse health effects. On the other hand, if HI < 1, this suggests that it is unlikely for a smoker to have non-carcinogenic health effects. Recommended input assumptions for the exposure assessments for cigarettes and smokeless tobacco products are presented in Table 3.2

3.9.6 Assumptions

Differences in pulmonary bioavailability are known to exist between the exposure situation and the critical study used to develop the inhalation toxicity value (NASEM, 2003). This can be addressed through the use of a relative bioavailability or absorption factor, as with exposure by oral ingestion (NASEM, 2003). However, there are few obvious examples of situations where such an adjustment is required, and consequently absorption factor for inhaled constituents is rare in risk assessments (NASEM, 2003). Instead, the implicit assumption is that the relative bioavailability or absorption factor associated with exposure through inhalation is 100 percent (NASEM, 2003).

Based on the above, the researcher made the following assumptions and estimates:

1. 100% of the elements present in tobacco products will be transferred and deposited into the human respiratory system through inhalation for both cigarettes and SLT.
2. Case 1 refers to risk estimation with for one smoker with 10 dilution factor for indoor air.
3. Case 2 refers to risk estimation for five smokers with 100 dilution factor for partial indoor air.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 pH, Moisture Content, Free Nicotine, % Unprotonated Nicotine

The mean values of moisture content, pH, % free nicotine and free nicotine (mg/g) and physicochemical properties of the analyzed products were summarized (Tables 4.1-4.3). A value of 12 mg\g was assigned as the total nicotine content in tobacco samples (CDC, 2010; Nicotinell, 2023). Some of the snuff manufacturers had multiple products offerings distinguished by flavour (menthol, moringa, no flavour (NF) as informed by vendors. Some of the vendors could not identify the flavours of some of the snuff products (denoted as not available (NA)).

4.1.1 Percentage moisture content

Based on the percentage moisture values, tobacco leaves have the highest moisture content with the highest being 28.46 % and the lowest being 15.46 %. Cigarettes have the second highest percentage moisture content with the highest being 15.62 % and the lowest being 13.37 %. Sample C6, a smuggled cigarette product had the highest moisture content and C5 had the lowest moisture content. Snuff products had the least moisture content, 15.86 % as the highest percentage moisture content and 3.88 % the lowest percentage moisture content. Overall, the mean moisture content of tobacco products analyzed are 21.39, 14.59, and 8.31% for tobacco leaves, cigarettes and snuff respectively.

The mean moisture content for dried tobacco leaves is lower than the value obtained for twist (twist chewing tobacco consists of dried tobacco leaves twisted together into a rope-like mass. Unlike most loose-leaf tobaccos, twist chewing tobacco is usually not sweetened studied by Lawler et al., (2013) with moisture content of 30.64 % whiles that of cigarettes was slightly above the range (7.2 to 13.4 %) obtained in the analysis of 23 cigarette products by Mahmood & Zaman, (2010), moisture content of snuff was higher than the value reported by the Lawler et al., (2013) with a value of 6.52 %. Below is a graph comparing the mean % moisture content of snuff, cigarette and dried tobacco leaves.

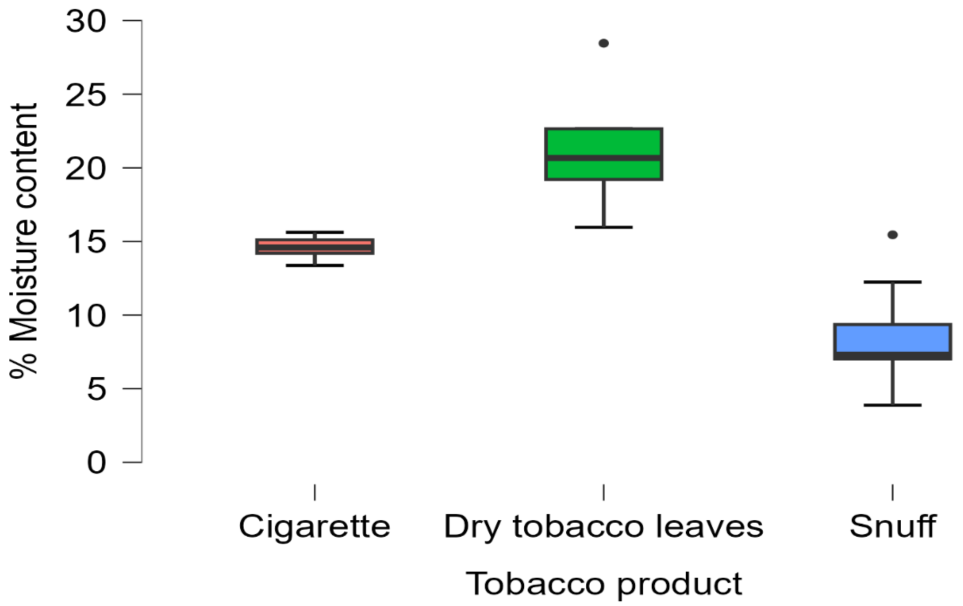

Figure 4.1 Graph comparing moisture content of tobacco products

4.1.2 pH

Among all the tobacco products, pH ranged from5.21 (C3 cigarette) to 10.16 (snuff) for tobacco acquired from Ampabame with unknown manufacturer and menthol flavour). This pH range translated to 0.15%–99.28% of nicotine in the free nicotine form.. Snuff had pH values ranging from 9.07 (sourced from Donaso; Flavour: NA) to 10.16 (snuff acquired from Ampabame with menthol flavour. These corresponded to 91.81% and 99.28% free nicotine, respectively. The pH values for tobacco leaves ranged from 6.63 to 8.0, which corresponds to 3.91% and 48.84% free nicotine, respectively. Cigarette samples had pH 5.21 (C3) and pH 5.87 (C4) which corresponded to 0.15% and 0.70% free nicotine, respectively.

Overall, the mean pH value for tobacco products analyzed were 9.73, 7.39, and 5.49, for snuff, dried tobacco leaves and cigarettes respectively. The mean pH for dried tobacco leaves is higher than the tobacco leaves studied by Hegde & Nanukuttan, (2017) with pH value of 5.24. however, pH of cigarettes is comparable with the 5.46 obtained in the studies conducted by Lawler et al., (2017). The pH of snuff was slightly higher than the value reported by the National Cancer Institute and Centers for Disease Control and Prevention, (2014) with a value of 9.24 and 9.46 for traditional snuff studied in South Africa and Nigeria respectively. Below is a graph comparing the mean pH of snuff, cigarette and dried tobacco leaves.

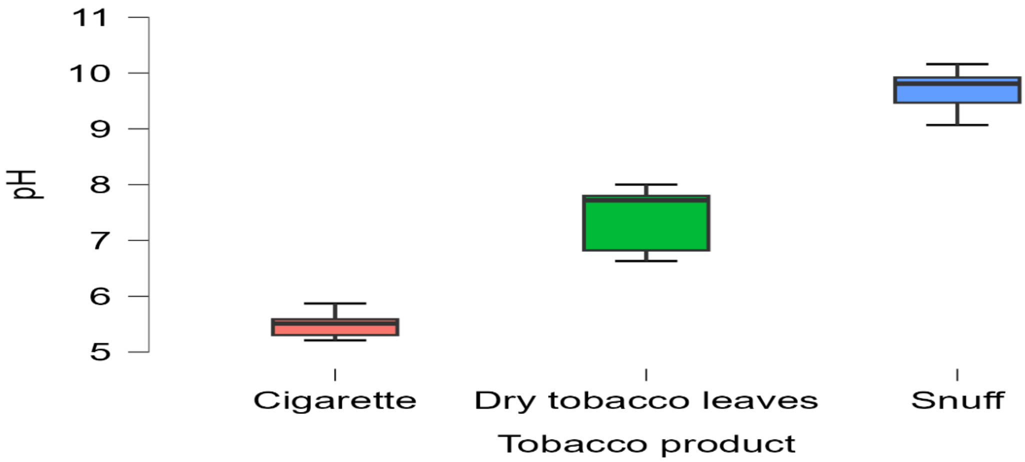

Figure 4.2 Graph comparing pH of tobacco products

4.1.3 Nicotine and % Free nicotine

This study assumed a value of 12 mg/g as the average of nicotine concentration in tobacco products (CDC, 2010; Nicotinell, 2023).

The free nicotine content was calculated using the stated total nicotine (12 mg/g) and measured pH values for each product. The term total nicotine represents the sum of nicotine in all of its ionic forms—both protonated and unprotonated (Stanfill et al., 2021). Protonated nicotine predominates at pH lower than 6. Unprotonated nicotine (free or free base nicotine) is the form that is most readily absorbed across oral membranes, and this form increasingly predominates at pH levels above 6. The percentage of total nicotine available as free nicotine (% free nicotine) is calculated by substituting the appropriate pKa value for nicotine and the pH of each product into the Henderson–Hasselbalch equation and then multiplying by 100 (Stanfill et al., 2021). Because a plot of the Henderson–Hasselbalch equation yields a sigmoidal curve, the percentage of nicotine as free nicotine (%) rises sharply between pH 7 and pH 9 (Stanfill et al., 2021).

The α_{fb} value refers to the fraction of nicotine in the freebase form (Stanfill et al., 2021). Measurement of pH along with total nicotine allows the calculation of both % free nicotine and amount of free nicotine (mg/g). Using the assumed values of 12 mg/g for tobacco products as the total nicotine content in this study, free nicotine ranged from 0.019 (C3) to 10.16 mg\g (snuff) acquired from Ampabame with menthol flavour. Snuff had free nicotine values ranging from 10.93 mg/g with 91.81 % free nicotine (sourced from Donaso; 2022, Flavour: NA) to 10.16 mg\g with 99.28 % free nicotine (snuff acquired from Ampabame with menthol flavour). The free nicotine values for tobacco leaves ranged from 0.46 to 5.86 mg/g, which corresponded to 3.91% and 48.84% free nicotine, respectively. Cigarette samples had free nicotine values of 0.018 (C3) and 0.084 (C4) mg/g which corresponds to 0.15 and 0.70% free nicotine, respectively.

Overall, % mean free nicotine for tobacco products analyzed were 98.09, 18.99 and 0.29 % corresponding to 11.65, 3.11, and 0.041mg/g for snuff, tobacco leaves and cigarettes respectively. Since nicotine absorption through cell membranes is pH dependent (Beljan et al., 2008), these values indicate that the snuff samples analyzed in this study have very high cell membrane absorption ability, followed by dry tobacco leaves with the least being cigarette. This is due to the decreasing order of % free base nicotine from snuff to cigarette samples. The mean % free nicotine for dried tobacco leaves obtained was higher than the tobacco leaves studied by Hegde & Nanukuttan, (2017) with % free nicotine value of 0.17 (pH 5.24) whiles that of cigarettes is comparable with the 0.24 % (pH 5.64) obtained in the studies conducted by Lawler et al., (2017). Snuff has slightly higher % free nicotine than the value reported by the National Cancer Institute and Center for Disease Control and Prevention, (2014) with a value of 94.32 (pH= 9.24) and 96.50 (pH= 9.46) for traditional snuff studied in South Africa and Nigeria respectively.

Below is a graph comparing % free nicotine concentration and free nicotine content of snuff, cigarette and dried tobacco leaves.

Figure 4.3 Graph comparing % free nicotine concentration and free nicotine content of tobacco products

% moisture concentration, pH, % unprotonated nicotine and free nicotine (Table 4.1-4.3) based on adopted average total nicotine concentration (12 mg/g) for tobacco products analyzed in this study.

Table 4.1 Moisture content, pH, % Unprotonated nicotine and free nicotine values of dry tobacco leaves

Dried Tobacco Leaves Code		Moisture	pH	Unprotonated Nicotine (%)	Free nicotine (mg\g)
L1		20.67	8	48.84	5..86
L2		22.65	6.82	5.93	0.72
L3		15.96	7.8	37.59	4.52
L4		19.21	7.72	33.38	4.00
L5		28.46	6.63	3.91	0.46
	Mean	21.39	7.39	18.99	2.28
	SD	4.65	0.62	20.01	1.20
	Min	15.96	6.63	3.91	0.46
	Max	28.46	8	48.84	5..86

Table 4.2 Moisture content, pH, % Unprotonated nicotine and free nicotine values of snuff products

Location	Flavour	Moisture Content (%)	pH	Unprotonated nicotine (%)	Free Nicotine (mg/g)
Besease[1]	Menthol	8.83	9.81	98.4	11.68
Besease[2]	Moringa	10.79	9.41	96.09	11.50
Donaso	NA	7.73	9.07	91.81	10.93
Akyawkrom[1]	NA	4.90	9.96	98.86	11.77
Akyawkrom[2]	Moringa	9.36	9.86	98.58	11.56
Achinakrom	Moringa	7.02	9.87	98.61	11.81
Kwaso	NF	9.08	9.51	96.87	11.43
Essienimpong[1]	Moringa	7.20	10	98.96	11.88
Essienimpong[2]	NA	7.27	9.93	98.78	11.84
Onwe	NA	6.64	9.58	97.32	11.67
Onwe	Moringa	7.30	9.71	98.00	11.70
Ampabame	Menthol	3.88	10.16	99.28	11.84
Ejisu	Menthol	15.45	9.92	98.76	11.83
Adadientem	NF	12.24	9.25	94.44	11.67
Asotwe	NA	7.06	9.47	96.57	11.57
	Mean	8.32	9.73	98.09	11.77
	SD	2.87	0.28	20	0.06
	Min	3.88	9.07	91.07	11.43
	Max	15.45	10.16	99	11.88

Physicochemical parameters of snuff products with different flavours

Flavour	Moisture Content (%)	pH	Unprotonated nicotine (%)	Free Nicotine (mg/g)
Menthol	9.39	9.96	98.81	11.78
Moringa	8.33	9.77	98.05	11.69
NA	6.72	9.60	96.67	11.56

Physicochemical parameters of snuff products with different manufacturers				
Manufacturer	Moisture Content (%)	pH	Unprotonated nicotine (%)	Free Nicotine (mg/g)
S2	11.59	9.50	95.29	11.38
S1	10.05	9.52	96.57	11.59
S4	6.85	9.53	96.95	11.62
NA	7.55	9.85	98.33	11.73
S3	4.90	9.96	98.86	11.77
NF	10.66	9.38	95.66	11.55

Table 4.3 Moisture content, pH, %Unprotonated nicotine and free nicotine values of cigarette products

Cigarette Brands Code	Moisture content (%)	pH	Unprotonated Nicotine (%)	Free nicotine (mg/g)
C1	14.1	5.59	0.37	0.044
C2	14.7	5.26	0.17	0.021
C3	14.5	5.21	0.15	0.019
C4	15.25	5.87	0.70	0.084
C5	13.37	5.58	0.36	0.043
C6	15.62	5.44	0.26	0.031
Mean	14.59	5.49	0.29	0.039
SD	0.81	0.24	0.20	0.010
Min	14.1	5.21	0.15	0.019
Max	15.62	5.87	0.70	0.084

4.2.0 Snuff products

According to Ayo-Yusuf et al., (2004); Benowitz et al., (2009); ACS, (2020), the concentration of total nicotine in SLT products are comparable to that in commercial cigarettes. As such a value of 12 mg/g which is the mean of the range of total nicotine concentration (6 to 18 milligrams per gram (mg/g) or 0.6 to 1.8 percent by weight) in snuff and cigarette products (CDC, 2010; Nicotinell, 2023). This estimated total nicotine concentration value was used to calculate % free base nicotine and concentration of free nicotine in tobacco product samples.

53% of the 45 snuff products examined had labels (name of product) but none of them listed the ingredients used or included any warning signs. Flavour used in the preparation of the SLT were not indicated and the researcher had to enquire from the vendor what flavour was used. Of the SLT used, 13.5% contained menthol as the flavour, 33% had moringa, whereas 13.5% had no flavour. The flavour in the remaining 40% could not be identified since the vendor did not know what flavour was used.

The widespread use of Moringa snuff is as a result of the perceived health benefit of Moringa, as stated by Essa et al., (2014) and Hamza, (2010). They reported that Moringa has been utilized to treat conditions such as: skin diseases, frailty, nervousness, asthma, clogged pores, blood contaminations, bronchitis, catarrh, chest blockage, cholera and numerous different sicknesses.

This result suggests that, there are varied types of snuff used depending on the geographical location, perception and preference of the user. For instance, Nemeth et al., (2012) indicated that 'chemma or shammah' is the most common type of snuff use in Algeria. Bhawna, (2013) also suggested that khaini is the most common snuff in India. Of all types, 'chemma' is the most known type of tobacco used worldwide (Bhawna, 2013).

These types however are not available in Ejisu or Ghana, as none was mentioned by vendors interviewed in the study. Snuff samples (menthol flavor) acquired from Ampabame and Essienimpong[1] (moringa flavor) had the highest and second highest pH (10.16 and 10.00 respectively) (Figure 4.4) and snuff samples acquired from Addadientem (which was personally observed by the researcher during its packaging and observed that no additives/flavour was added) had the second lowest pH of 9.25 (Figure 4.4). This finding suggests that snuff that has flavour/additives recorded high pH values.

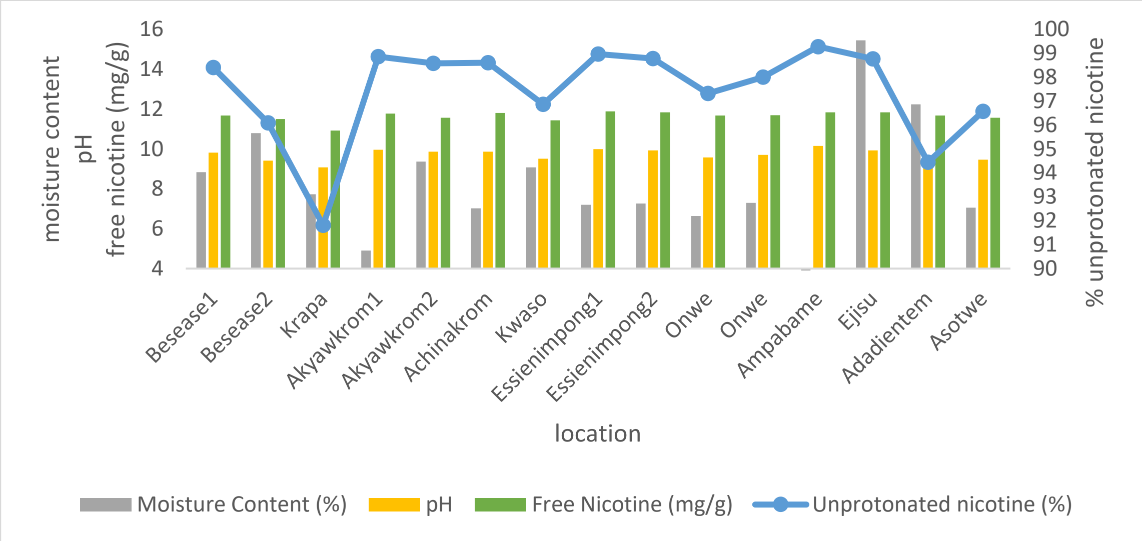

Figure 4.4 Graph showing locations where snuff products were acquired against moisture content, pH, % unprotonated nicotine and free nicotine

4.2.1 Dried tobacco leaves

From the five tobacco leaves analyzed, the highest pH recorded was 8.00. This corresponded to 48.84 % free nicotine and 5.86 mg/g free nicotine concentration whiles the least pH 6.63 also corresponding to 3.91 % free nicotine: and free nicotine 0.46 mg/g. The mean pH (7.39) value calculated corresponded to 25.93 % free nicotine and 3.11 free nicotine (Figure 4.5).

Use of the tobacco leaves also poses more health challenges aside those identified in this study. This is because the ash that is added to the leaves also contain a considerable amount of toxic combustion products (Beljan et al., 2008). Also, the ash that has been added has the potential to increase pH of the product and hence increase the unprotonated nicotine concentrations. This would then increase the nicotine delivery capability of the products and eventually leads to the addiction and dependence by users.

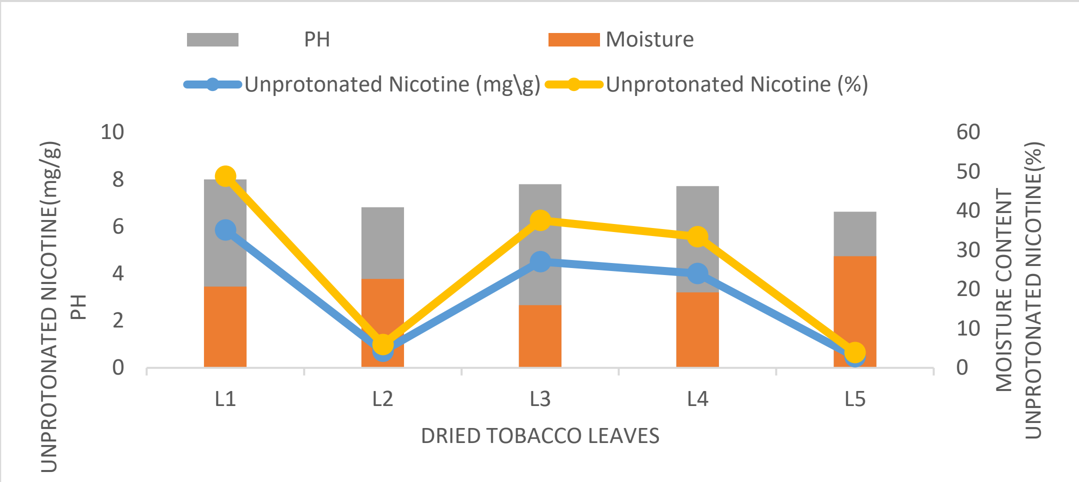

Figure 4.5 Graph showing dried tobacco leaves against moisture content, pH, %Unprotonated nicotine and free nicotine

4.2.2 Cigarette products

Figure 4.3 indicates that, C4 had the highest pH 5.58 and a corresponding % unprotonated nicotine of 0.70 % and unprotonated nicotine of 0.084 mg/g, followed by C1 and C5 with the least being C3 with pH 5.59, 5.58 and 5.21 respectively. These corresponded to % unprotonated nicotine and unprotonated nicotine concentration of 0.37 %, 0.044 mg/g; 0.36 %, 0.043 mg/g; and 0.15 %, 0.019 mg/g respectively. With the assumption of average total nicotine concentration of 12 mg/g in snuff and cigarette samples. C6, a smuggled cigarette product has a pH of 5.44 with percent unprotonated nicotine and unprotonated nicotine of 0.26 % and 0.031 mg/g.

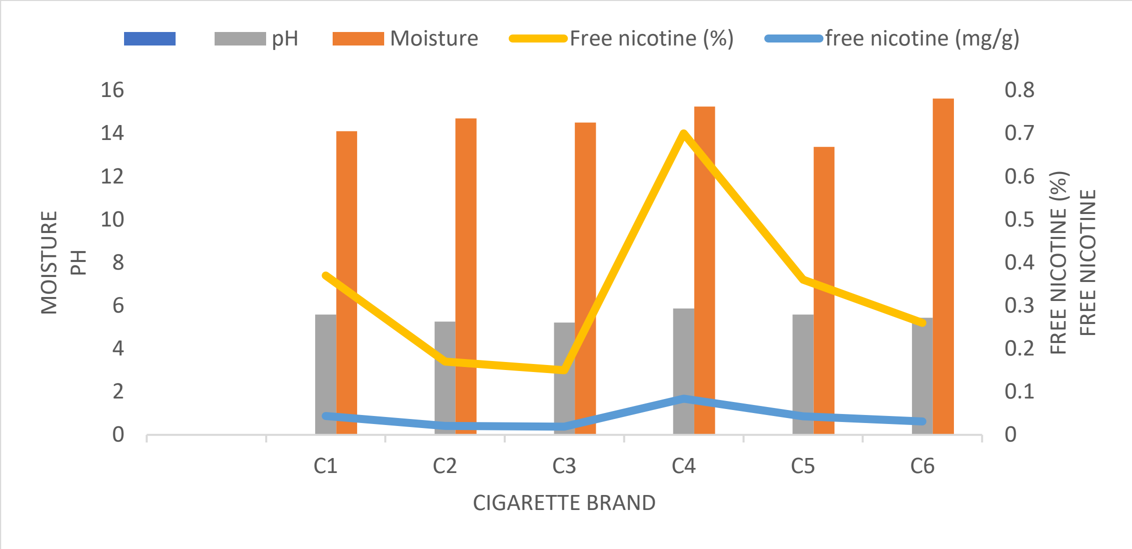

Figure 4.6 Graph of cigarette products against moisture content, pH, % unprotonated nicotine and free nicotine

Table 4.1; 4.2 and 4.3 indicate that a graduated increase of levels (5.5–9.7) exist across the three tobacco products used, with cigarettes having the least mean pH (5.5) and snuff having the highest mean pH (9.7). This indicates snuff has the highest nicotine delivery capability, followed by tobacco leaves and the least being cigarettes. It must however be noted that since ash is added to the leaves before use, its pH has the potential to increase beyond the values stated in this study (Beljan et al., 2008).

4.2.3 Effect of additive/flavours on pH, free nicotine (mg/g) and % unprotonated nicotine

It's observed in this present study that there is considerable variation in pH and nicotine delivery capabilities of tobacco products across nations and among products (even when coming from the same source/manufacture. Table 4.1 indicates that "S2" acquired from Donaso had 9.07 and the product from the same manufacturer acquired from Ejisu market had pH of 9.92 representing % unprotonated nicotine of 91.07% and 98.58% respectively. This may be as a result of additives or flavours added in the preparation of the product as suggested. However, on the average, samples from “S3” manufacturer recorded the highest pH 9.96 corresponding to 98.86 % unprotonated nicotine whiles “S2” recorded the lowest pH 9.49 corresponding to 95.28 % unprotonated nicotine (Figure 4.4).

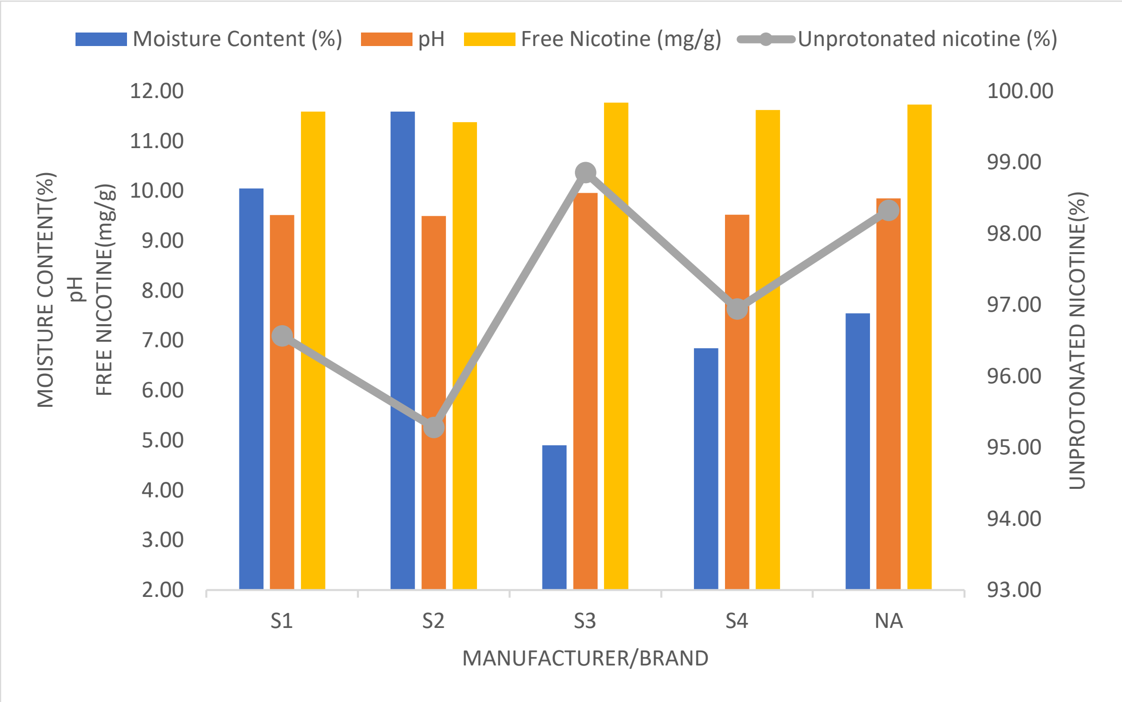

Figure 4.7 Graph showing snuff manufacturers/brand against moisture content, pH, % unprotonated nicotine and free nicotine

The present study (Figure 4.5) confirms findings which suggested that use of menthol flavour in tobacco products may result in even higher free nicotine exposure, as similar observations have been made with mentholated cigarettes (Ayo-Yusuf et al., 2004). Average pH of Menthol flavoured snuff products is 9.96, that of Moringa are 9.77 whiles the ones with no flavour had 9.38. C1 and C5 cigarettes had the second and third highest pH levels of 5.59 and 5.58 respectively with C4 Cigarette having the highest pH of 5.87. Also, dry tobacco leaves, which contains no additives recorded an average pH of 7.39 which is lower than the lowest pH value of 9.07 recorded for snuff products.

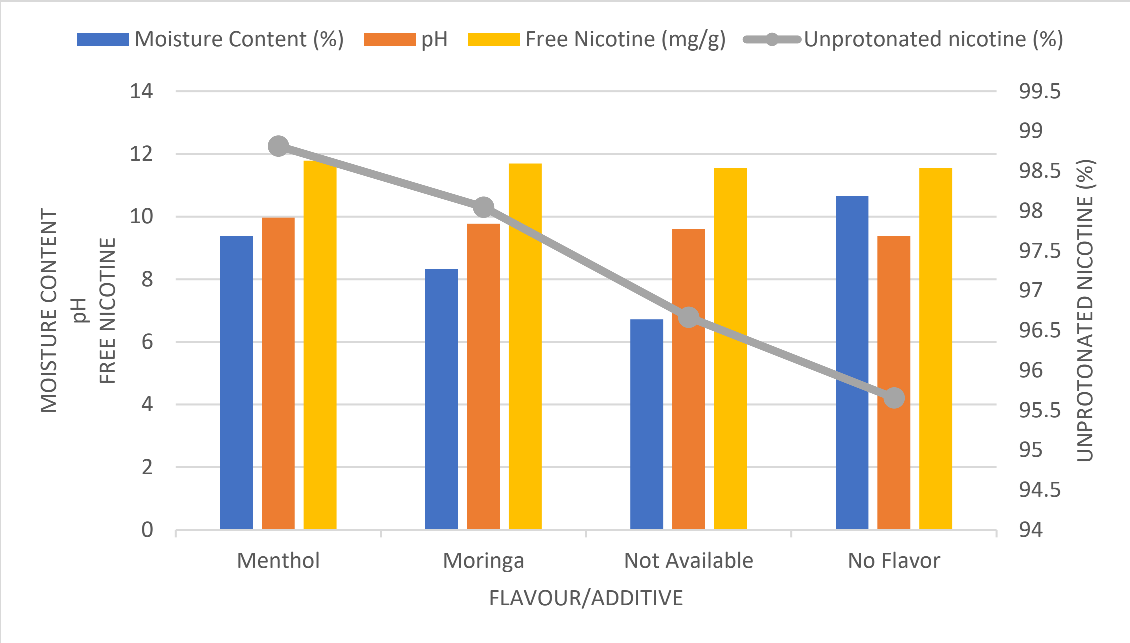

Figure 4.8. Graph showing effect of snuff additives/flavour on moisture content, pH, % unprotonated nicotine and free nicotine

4.2.5 Prevalence of tobacco products consumption, its nicotine delivery and addiction potential on the individual in Ejisu Municipality

Sample site reconnaissance demonstrated that an average smokeless tobacco product user will ‘‘dip’’ (dry tobacco leaves) or ‘‘sniff’’ (nasal snuff) about five times a day and for 30 minutes each time. With an estimated 2 g smokeless tobacco commonly dipped or inhaled per time, daily total nicotine exposure averages 120 mg or 0.12 g per 10 g. Table 4.4 categorizes tobacco products based on their nicotine delivery capability and addiction potential.

Table 4.4 Categorization of tobacco products based on nicotine delivery capability and addiction potential of cigarette product

TOBACCO PRODUCT	Mean pH	%A	Amount consumed in a day (g/day)	total nicotine conc.(mg/day)	free nicotine conc.(mg/day)	free nicotine conc.(mg/week)	Nicotine delivery capability	Addiction potential
Snuff	9.73	98.09	10	120	117.71	823.96	Very high	very high
Leaves	7.39	18.99	10	120	22.79	159.53	High	High
Cigarette	5.49	0.29	10	120	0.35	2.44	very low	High

The table above suggest that those who desire to quit by switching to SLTs such as snuff and dried tobacco leaves may be unintentionally sustaining dependence by exposing themselves to higher nicotine addiction through sustained use of unprotonated/free nicotine tobacco products.

Though amount of free nicotine of cigarette is low, it takes seconds to reach the brain. The time it takes to reach the brain is also an indicator of addiction potential. In all, the variation in time taken for free nicotine to reach brain, which is dependent on the differences in administration duration, as well as absorption rate and time that differs with each route of delivery, determines the addictive potential of a tobacco product.

4.3 Concentration of Elements in Tobacco Products

Tobacco products, both processed and unprocessed have varying levels of elemental compositions. The presence of 15 elements (14 metals: Mo, Zr, Sr, U, Rb, Zn, Cu, Fe, Mn, V, Ti, Sc, Ca, K and 1 nonmetal: S) were identified in some of the tobacco products in this study. The mean concentrations of total elements detected in the 56 tobacco products (snuff, tobacco leaves and cigarettes) with their respective ranges are stated 4.5.

The variations in trace element concentration of tobacco products especially for the snuff products have been observed by others as well (Mohammad, 2014). However, these variations could possibly be influenced by agriculture soil on which tobacco leaves were cultivated, closeness of farming lands to roads and residential areas, the composition of tobacco leaves and processing and handling (Mohammad, 2014).

Tables 4.5-4.7 gives the mean concentrations of determined elements in the tobacco products.

Table 4.5 Mean elemental concentration (μg/g) of snuff products

	Elemental concentration (μg/g) of snuff products with different flavours														
	Mo	Zr	Sr	U	Rb	Zn	Cu	Fe	Mn	V	Ti	Sc	Ca	K	S	TOTAL
Menthol	2.0	71.2	299.3	4.89	53.48	109.5	22.5	3427.3	9.32	11	590.8	173.8	38748.4	46415.68	4039.7	9397.87
Moringa	1.6	68.0	180.7	4.34	148.8	97.89	10.5	4006.4	39.5	9.42	524.2	91.75	23442.6	125523.0	4199.3	15834.79
NA	2.1	68.5	254.5	6.07	84.95	108.3	11.5	2661.8	53.2	5.39	465.6	125.5	28135.2	72646.25	4116.1	10874.48
NF	1.7	79.6	212.4	6.35	145.5	81.11	27.8	3305.6	25	10.5	459.5	100.5	23272.7	114596	4675.3	14699.94

Table 4.6 Mean elemental concentration (μg/g) in dry tobacco leaves

	Elements												
Code	Mo	Zr	Sr	Rb	Zn	Fe	Mn	Ti	Sc	Ca	K	S	TOTAL
mean	3.49	53.03	215.72	27.60	59.844	1456.16	29.36	451.39	143.44	30283.4	45172.4	4442.42	82320.8
SD	1.57	15.84	24.20	9.57	28.28	415.95	26.26	317.33	23.39	4827.37	6440.66	886.29	12113.76
max	5.87	79.69	240.42	43.28	97.64	1941.15	58.72	927.03	166.29	35683.6	54442.4	5157.86	98844.1
min	2.09	42.54	181.71	19.14	26.43	953.74	0	198.52	111.84	22956.2	38604.4	3028.96	66125.5

Table 4.7 Mean elemental concentration (μg/g) in cigarette products

							Elements						
Code	Mo	Zr	Sr	Rb	Zn	Fe	Mn	Ti	Sc	Ca	K	S	TOTAL
mean	6.59	4.99	101.35	27.08	42.326	347.48	82.915	48.415	194.17	37800.57	75319.68	4142.26	118046.4
SD	1.05	0.94	24.21	6.16	9.34	64.72	44.99	30.60	64.84	12957.5	24361.5	1364.70	31639.31
Min	5.14	3.6	67.75	18.2	32.49	267.6	61.1	21.17	87.47	22006.8	52124.7	3018.51	88139.34
Max	7.68	5.85	128.22	35.6	58.74	418	104.7	71.08	280.1	54829.5	116041	6053.28	177734.9

4.3.1 Comparison of concentrations (µg/g) of potentially toxic elements in tobacco products

Snuff products had the highest concentration in 10 out of the 15 elements identified in the tobacco products (i.e., Zr; 88.21 µg/g, Sr; 331.68 µg/g, U; 12.59 µg/g, Rb; 292.58 µg/g, Zn; 153.74 µg/g, Cu; 31.15 µg/g, Fe; 8487.86 µg/g, V; 31.49 µg/g, Ti; 853.63 µg/g and K; 24295.60 µg/g). Cigarettes recorded highest concentration for Mo; 7.68 µg/g, Mn; 104.7 µg/g, Sc; 280.10 µg/g and Ca; 54829.50 µg/g whereas dried tobacco leaves recorded highest concentration for S; 3028.96 µg/g. Uranium, Zinc, and Vanadium were detected in only snuff products.

It is observed that with the exception of Zn; 153.74 µg/g, Cu; 84.70 µg/g, Mn; 87.30 µg/g, and Ca; 44364.60 µg/g (Table 4.8), maximum concentrations of all other metals detected in snuff were above the maximum concentrations of these metals in tobacco products reported by James, (2014). With regard to dry tobacco leaves, only Mo; 5.87 µg/g, Zr; 79.69 µg/g, Ti; 927.03 µg/g and Sc; 166.29 µg/g exceeded concentration of metals in tobacco products published in literature (James, 2014) whereas with metals detected in cigarettes, only Mo; 7.69 µg/g and Sc, 280.10 µg/g recorded maximum concentration than reported concentrations published in literature (James, 2014).

The findings in this study suggest that the mean elemental composition in smokeless tobacco 135.07 mg/g (Table 4.5) is greater than in cigarette products; 118.05 mg/g (Table 4.7) which is in agreement with the findings by Pappas., (2011). Difference in elemental concentration in tobacco products is because contamination of tobacco products is site, process and country specific (Boffetta et al., 2008). Some elemental concentration of snuff products exceeded those published by other researchers because local preparation of snuff is not regulated and does not follow any approved hygienic protocols, as such toxicants from diverse sources might be introduced into the final product. Same reason goes for cigarette products but due to enhanced hygienic protocols and minimal human handling of products due to the incorporation of state-of-the-art technology in the manufacturing process, less toxicants are introduced into the final product.

Below are graphs comparing the concentrations (μg/g) of various elements detected in the tobacco products.

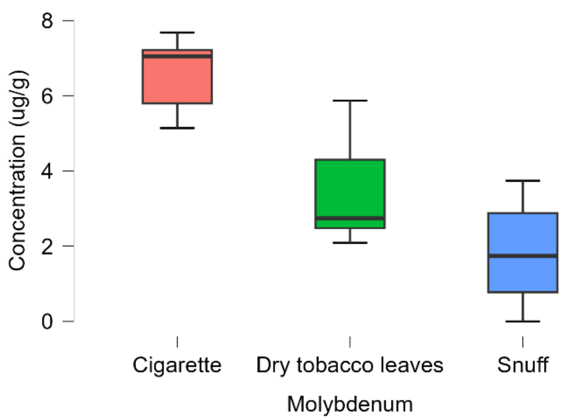

Figure 4.9 Concentration of Mo in tobacco products

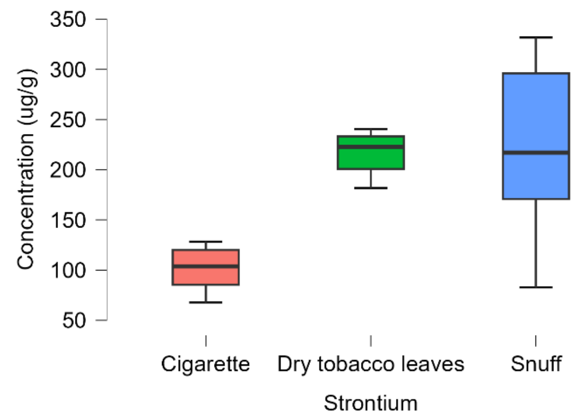

Figure 4.11 Concentration of Sr in tobacco products

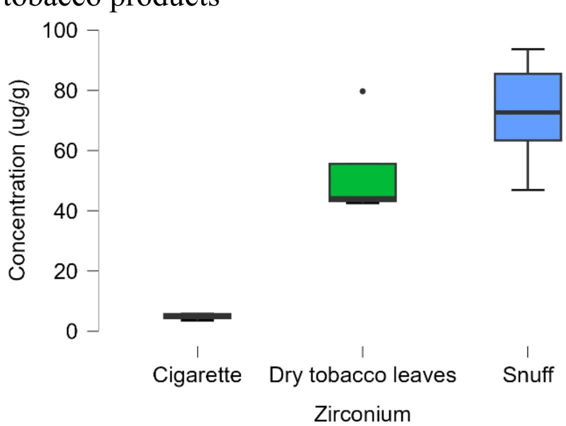

Figure 4.10 Concentration of Zr in tobacco products

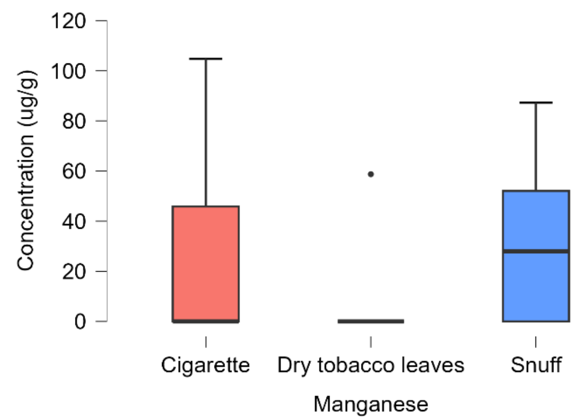

Figure 4.12 Concentration of Rb in tobacco products

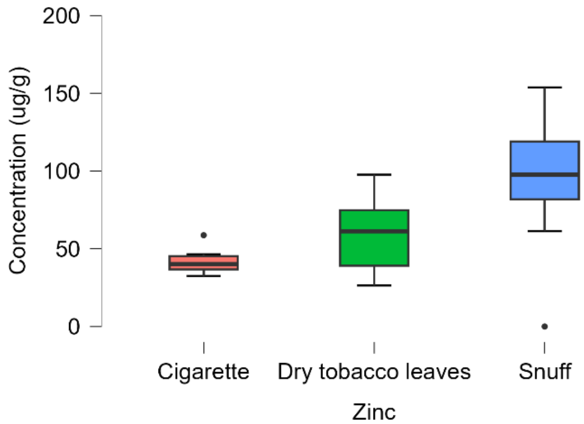

Figure 4.13 Concentration of Zn in tobacco products

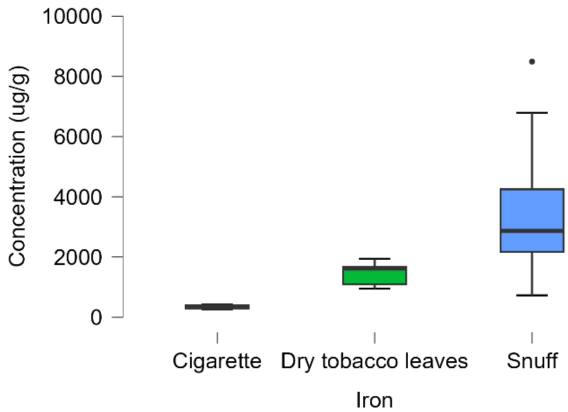

Figure 4.14 Concentration of Fe in tobacco products

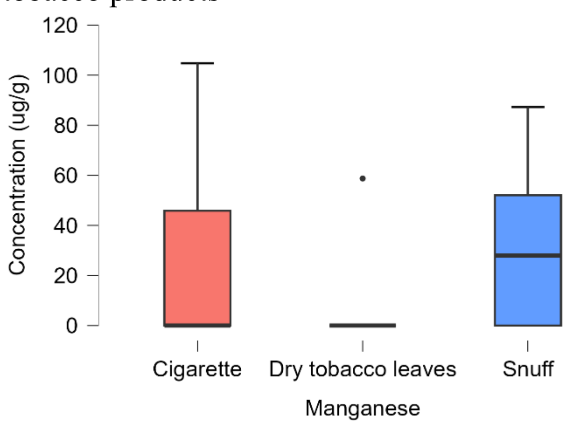

Figure 4.15 Concentration of Mn in tobacco products

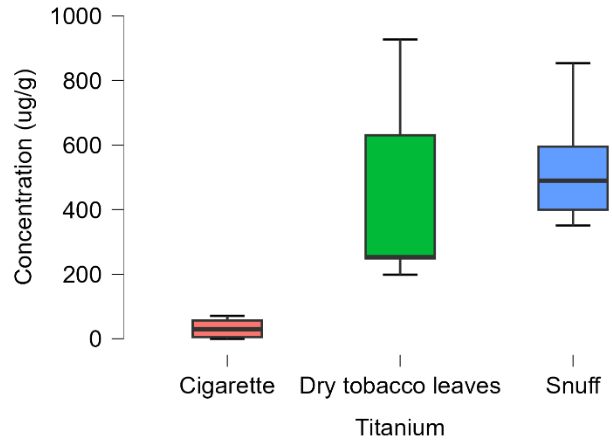

Figure 4.16 Concentration of Ti in tobacco products

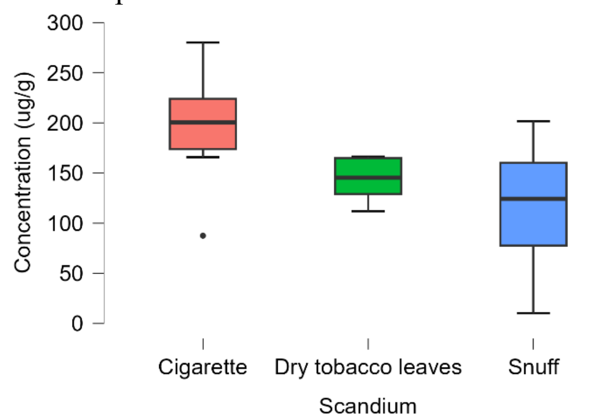

Figure 4.17 Concentration of Sc in tobacco products

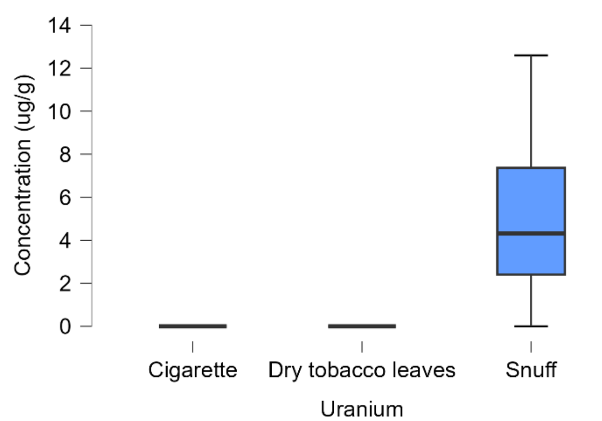

Figure 4.18 Concentration of U in tobacco products

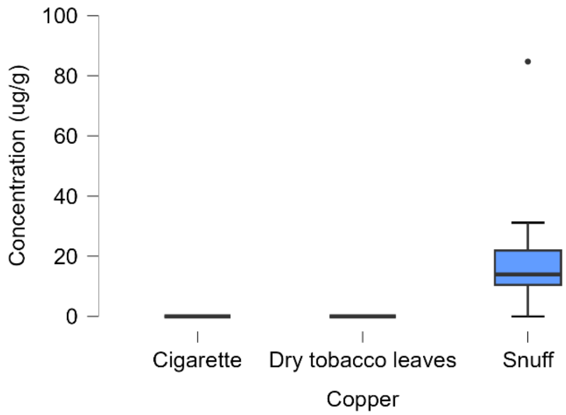

Figure 4.19 Concentration of Cu in tobacco products

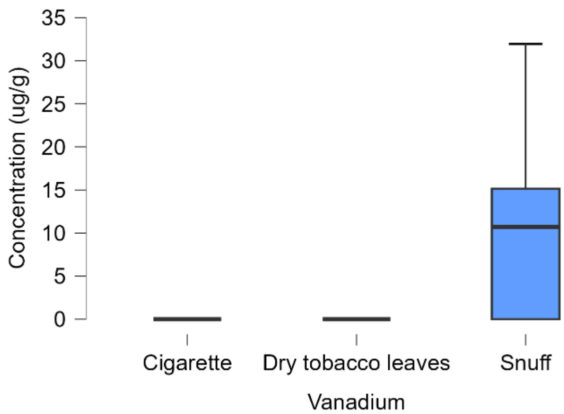

Figure 4.20 Concentration of V in tobacco products

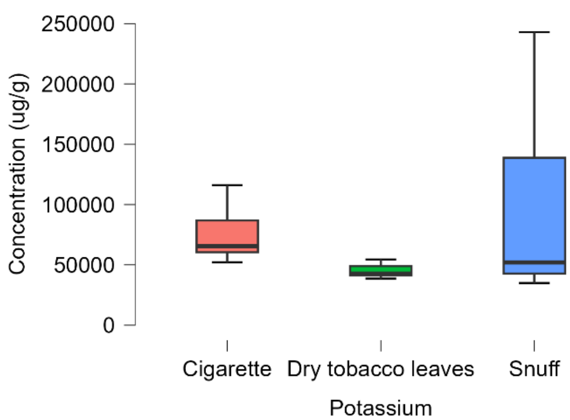

Figure 4.21 Concentration of K in tobacco products

Table 4.8 is a representation of mean (SD), minimum and maximum concentrations of 15 elements examined in snuff products, dried tobacco leaves and 6 cigarette brands; compared with reported concentration of elements in tobacco products from literature (James, 2014).

Table 4.8 Mean elemental concentration (µg/g) of Snuff, cigarette and dried tobacco products compared to reported values

Element	Reported Conc	Snuff			Dried Tobacco Leaves			Cigarette		
		Mean (SD)	Min	Max	Mean (SD)	Min	Max	Mean (SD)	Min	Max
Mo	0.84-1.08	1.75±1.27	ND	3.74	3.496±1.57	2.09	5.87	6.59±1.05	5.14	7.68
Zr	8-13.9	72.55±14.88	46.89	93.65	53.032±15.83	42.54	79.69	4.99±0.93	3.6	5.85
Sr	27-300	224.83±77.70	82.76	331.68	215.722±24.20	181.71	240.42	101.35±24.21	67.75	128.22
U	0.005-0.01	5.35±4.4	ND	12.59	ND	ND	ND	ND	ND	ND
Rb	4.5-100	120.12±86.89	39.85	292.58	27.602±9.57	19.14	43.28	27.08±6.16	18.18	35.55
Zn	8.1-169	96.02±36.66	ND	153.74	59.844(28.28)	26.43	97.64	42.32667±9.34	32.49	58.74
Cu	0.24-510	18.77±20.18	ND	84.7	ND	ND	ND	ND	ND	ND
Fe	80-7859	3477.68±2046.80	727.53	8487.86	1456.166±415.95	953.74	1941.15	347.48±64.72	267.62	417.97
Mn	55-540	30.44±30.3	ND	87.25	29.36±26.26	ND	58.72	82.915±45.00	61.1	104.73
V	0.49-5.33	9.54±9.4	ND	31.94	ND	ND	ND	ND	ND	ND
Ti	0.76-378	508.17±145.98	350.95	853.59	451.396±317.33	198.52	927.03	48.415±30.60	21.17	71.08
Sc	0.085-9.1	115.56±58.20	10.22	201.58	143.442±23.39	111.84	166.29	194.17±64.84	87.47	280.09
Ca	5000-78000	27072.78 ±10880.09	10017.61	44364.62	30283.44 ±4827.39	22956.22	35683.68	37800.57 ±12957.55	22006.8	54829.5
K	1300-118000	99008.96±70337.18	34891.07	242952.57	45172.48±6440.66	38604.4	54442.45	75319.68±24361.5	52124.71	116041
S	-	4314.96±1375.24	2772.32	7328.19	4442.422±886.29	3028.96	5157.86	4142.263±1364.70	3018.51	6053.28

Reported metal centrantrations by James, 2014. ND= Below detection limit

4.4 Exposure and intake of Potentially Toxic Elements

Exposure concentrations are calculated by averaging intakes over the period of exposure (Marano et al., 2018). For carcinogens, exposure concentrations are calculated by prorating the total intake over a lifetime (USEPA, 1989). The chronic daily intake (CDI), which is the estimated daily chemical dose for an individual averaged over the exposure duration. CDI was used in assessing long term health effects.

4.4.1 Chronic Exposure, Chronic Daily Intake and Daily Intake of selected potentially toxic elements

It was observed through researcher-smoker interaction that 10 sticks of cigarettes are consumed by an average smoker whiles average snuff and tobacco leaves consumed/inhaled by an average smoker were approximately 10 grams in a day.

The mean chronic exposure concentration of Molybdenum for snuff products was 0.00105 mg/m^3 whereas that of cigarette brands were 0.0033 mg/m^3 (Table 4.10). The mean chronic exposure concentration for snuff was lower than the Reference Concentration (RfC) of Molybdenum (0.002 mg/m^3) whereas that of cigarette (0.003 mg/g^3) was higher than its RfD. Tobacco leaves which are ingested orally, had mean chronic daily intake of 0.00045 mg/kg/day (Table 4.10). This value was also observed to be lower than the Reference Dose (RfD) of Molybdenum which is 0.005 mg/kg/day (USEPA, 2022).

The estimated daily intake obtained was 0.035 mg/day (Table 4.13). This value is higher than 0.025 mg/day, the Recommended Daily Intake of Mo as given by EFSA, (2004) which is 136% higher than the stated (0.025 mg/day) Recommended Daily Intake value (Table 4.13). Moringa flavoured Snuff products had lower exposure concentration value than Menthol flavoured Snuff products with values 0.0007 mg/m^3 and 0.001 mg/m^3 respectively whereas Snuff products with unknown flavours had exposure concentration of 0.0008 mg/m^3 (Table 4.9). The different Cigarette brands had similar exposure concentration values with the least being 0.0025 mg/m^3 for C1 and the highest being 0.0038 mg/m^3 for C5 (Table 4.9).

The mean exposure concentration of Zirconium for snuff products was 0.04 mg/m^3 whereas that of all cigarette brands were also found to be 0.003 mg/m^3 (Table 4.10). The mean chronic exposure concentration for snuff and cigarette were much higher than the Reference Concentration (RfC) of Zirconium which is 0.005 mg/m^3. Tobacco leaves which are ingested orally, had mean chronic daily intake of 0.00152 mg/kg/day (Table 4.10). This value was observed to be far higher than the Reference Dose (RfD) of Zirconium which is 0.00008 mg/kg/day (USEPA, 2012). Moringa flavoured Snuff products (0.0340 $mg\backslash m^3$) had lower exposure concentration value than Menthol flavoured (0.0356 $mg/m^{3)}$. Snuff products with values 0.034 mg/m^3 and 0.036 mg/m^3 respectively whereas Snuff products with unknown flavours had highest exposure concentration of 0.04mg/m^3 (Table 4.9).

Among the snuff manufacturers, “S2” had the least exposure concentration value of 0.0351mg/m^3 whiles “S3” had the highest exposure concentration of 0.044mg/m^3 (Table 4.9). The different Cigarette brands had similar exposure concentration values with the least being 0.0018 mg/m^3 for C2 and the highest being 0.0028 mg/m^3 for C6 (Table 4.9).

The mean exposure concentration of Uranium for snuff products was 0.002 mg/m^3 (Table 4.10) whereas there was no detection of uranium in any of the cigarette brands. The mean chronic exposure concentration for snuff was much higher than the Reference

Concentration (RfC) of Uranium which is 0.00004 mg/m^3 (ATSDR, 2018). There was no uranium detected for Tobacco leaves. Moringa flavoured Snuff products (0.0022 mg/m^3) had lower exposure concentration value than Menthol flavoured Snuff products (0.0024 mg/m^3). Snuff products with unknown flavours had highest exposure concentration of 0.00318 mg/m^3 (Table 4.9). Among the snuff manufacturers, 'S3' had the least exposure concentration value of 0.00 mg/m^3 whiles "S4" had the highest exposure concentration of 0.004 mg/m^3 (Table 4.9).

The mean exposure concentration of Zinc for all snuff products was 0.05 mg/m^3 whereas that of all cigarette brands were also found to be 0.02 mg/m^3 (Table 4.10). These values were much higher than the Reference Concentration (RfC) of Zinc which is 0.002 mg/m^3. Tobacco leaves which are ingested orally, had mean chronic daily intake of 0.00256 mg/kg/day (Table 4.10). This value was observed to be far lower than the Reference Dose (RfD) of Zinc which is 0.3 mg/kg/day (USEPA, 2022). The estimated daily intake obtained was 0.6 mg/day (Table 4.13). This value is also lower than 9 mg/day, the Recommended Daily Intake of Zn as given by institute of Medicine, Food and Nutrition Board, (2001). The recorded estimated daily intake was computed to be 6.6% of the stated Recommended Daily Intake (Table 4.13).

Moringa flavoured Snuff products had lower exposure concentration value than Menthol flavoured Snuff products with values 0.06 mg/m^3 and 0.05 mg/m^3 respectively. Snuff products with no flavours had least exposure concentration of 0.04mg/m^3 (Table 4.9). Among the snuff manufacturers, "S4" had the least exposure concentration value of 0.028 mg/m^3 whiles "S3" had the highest exposure concentration of 0.058 mg/m^3 (Table 4.9). The different Cigarette brands had similar exposure concentration values with the least being 0.0016 mg/m^3 for C2 and the highest being 0.029 mg/m^3 for C6 (Table 4.9).

The mean exposure concentration of copper for snuff products was 0.11 mg/m^3 whereas there was no detection of copper in any of the cigarette brands (Table 4.9). This value was observed to be slightly higher than the Reference Concentration (RfC) of Cu which is 0.1 mg/m^3.

Copper was below the detection limit for tobacco leaves for the method used in analysis (Table 4.10). Moringa flavoured Snuff products had lower exposure concentration value than menthol flavoured Snuff products with values 0.005 mg/m^3 and 0.11 mg/m^3 respectively whereas Snuff products with unknown flavours had highest exposure concentration of 0.014 mg/m^3 (Table 4.9). Among the snuff manufacturers, "S1" had the least exposure concentration value of 0.006 mg/m^3 whiles "S4" had the highest exposure concentration of 0.025 mg/m^3 (Table 4.9).

The mean exposure concentration of iron for all snuff products was 1.76 mg/m^3 whereas that of all cigarette brands were also found to be 0.17 mg/m^3 (Table 4.10). The mean chronic exposure concentration for snuff and cigarette were much higher (1.76 and 0.17 mg/m^3 respectively) than the Reference Concentration (RfC) of Fe which is 0.004 mg/m^3. Tobacco leaves which are ingested orally, had mean chronic daily intake of 0.031 mg/kg/day (Table 4.10) which is lower than the Reference Dose (RfD) of Iron (0.7 mg/kg/day).

The estimated daily intake of iron obtained was 14.6 mg/day (Table 4.13). This value is also higher than 10 mg/day, the Recommended Daily Intake of Fe as given by institute of Medicine, Food and Nutrition Board, (2001) which is 145.6% of the stated Recommended

Daily Intake (Table 4.13). Moringa flavoured Snuff products had higher exposure concentration value than menthol flavoured snuff products with values 2.0 mg/m^3 and 1.714mg/m^3 respectively whereas snuff products with no flavours had least exposure concentration of 1.33mg/m^3 (Table 4.9). Among the snuff manufacturers, "S1" had the least exposure concentration value of 1.36 mg/m^3 whiles "S4" had the highest exposure concentration of 2.24 mg/m^3 (Table 4.9). The different Cigarette brands had similar exposure concentration values with the least being 0.134 mg/m^3 for C4 and the highest being 0.209 mg/m^3 for C1 (Table 4.9). Two brands ("S1" and "S4") and brands without label (NA) detected Manganese in their snuff products (0.018, 0.019 and 0.02 respectively). The mean exposure concentration of these three brands was 0.019 mg/m^3 (Table 4.10). Also, two cigarette brands (C4 and C6) recorded manganese in their products. The mean exposure concentration for these two brands was 0.0415 mg/m^3 (Table 4.10). The mean chronic exposure concentration for snuff and cigarette were much higher than the Reference Concentration (RfC) of Mn which is 0.00005 mg/m^3. Out of the five Tobacco leaves examined, only sample L2 was found to contain manganese. Its chronic exposure concentration was calculated to be 0.00007 mg/kg/day (Table 4.10) which is far lower than the Reference Dose (RfD) of Mn (0.14 mg/kg/day).

The estimated daily intake is 0.59 mg/day. This is lower than the Recommended Daily Intake for manganese; 2.3 mg/day by Institute of Medicine, Food and Nutrition Board, (2001). this represents 25.65% of the Recommended Daily Intake (Table 4.13). Moringa flavoured Snuff products had higher exposure concentration value than Menthol flavoured Snuff products with values 0.020mg/m^3 and 0.005mg/m^3 respectively. Snuff products with no flavours had highest exposure concentration of 0.027mg/m^3 (Table 4.9). Among the snuff manufacturers, “S1” had the least exposure concentration value of 0.018mg/m^3 whereas products without any label (NA) had the highest exposure concentration value of 0.020mg/m^3 (Table 4.9). Of the two Cigarette brands that recorded manganese metal, C6 had the highest exposure concentration value of 0.52 mg/m^3 whereas C4 recorded 0.031 mg/m^3 (Table 4.9).

The mean exposure concentration of vanadium for snuff products was 0.006 mg/m^3 whereas there was no detection of vanadium in any of the cigarette brands nor for dry tobacco leaves (Table 4.10). The mean chronic exposure concentration for snuff (0.006 mg/m^3) was much higher than the Reference Concentration (RfC) of vanadium which is 0.0001 mg/m^3 (USEPA, 2022). Menthol flavoured snuff products had higher exposure concentration value (0.0055 mg/m^3) than moringa flavoured Snuff products (0.0047 mg/m^3) whereas snuff products with no flavours had least exposure concentration of 0.0027 mg/m^3 (Table 4.9). Among the snuff manufacturers, “S1” had the least exposure concentration value of 0.002 mg/m^3 whiles “S3” had the highest exposure concentration of 0.009 mg/m^3 (Table 4.9)

The mean exposure concentration of titanium for all snuff products was 0.24 mg/m^3 whereas that of cigarette brands were also found to be 0.016 mg/m^3 (Table 4.10). The mean chronic exposure concentration for snuff (0.238 mg/m^3) was much higher than the Reference Concentration (RfC) which is 0.03 mg/m^3 whereas that of cigarette was much lower than the RfC. Tobacco leaves which are ingested orally, had chronic daily intake of 0.00129 mg/kg/day (Table 4.10). This value was below the Reference Dose (RfD) of Titanium which is 40 mg/kg/day. Menthol flavoured Snuff products had higher exposure concentration value (0.295 mg/m^3) than Moringa flavoured Snuff products (0.262 mg/m^3). Snuff products with no flavours had least exposure concentration of 0.233 mg/m^3 (Table 4.9). Among the snuff manufacturers, "S3" had the least exposure concentration value of 0.193mg/m^3 whiles 'NA' had the highest exposure concentration of 0.274mg/m^3 (Table 4.9). Titanium was not detected in C4 and C5. However, sample C6 recorded the least exposure concentration with 0.011 mg/m^3 whereas C2 recorded the highest exposure concentration with 0.036 mg/m^3 (Table 4.9).

The mean chronic exposure concentration of calcium for snuff products was 12.62 mg/m^3 whereas that of cigarette brands were also found to be 18.90 mg/m^3 (Table 4.10). Tobacco leaves which are ingested orally, had chronic daily intake of 1.195 mg/kg/day (Table 4.10) whereas the estimated daily intake obtained was 302.83 mg/day. which is below the (1000 mg/day) Recommended Daily Intake for calcium; by Institute of Medicine, Food and Nutrition Board, (2001) representing 30.28% (Table 4.13). Calcium as an essential element necessary for proper bone and teeth formation. It has no Reference Concentration (RfC) nor Reference Dose (RfD). However, short-term exposure risk assessment determined that the daily exposure of Calcium from snuff (25.24 mg/m^3) and cigarette (37.38 mg/m^3) is 1262 % and 1869 % of the stated permissible exposure limit set by the Occupational Safety and Health Administration (OSHA) which is 2 mg/m^3.

Menthol flavoured Snuff products had higher exposure concentration value (19.37 mg/m^3) than Moringa flavoured Snuff products (11.72mg/m^3). Snuff products with no flavours had least exposure concentration of 14.07 mg/m^3 (Table 4.9). Among the snuff manufacturers, "S3" had the least exposure concentration value of 5.66 mg/m^3 whiles "S2" had the highest exposure concentration of 16.03 mg/m^3 (Table 4.9).

Comparing the cigarette brands, C1 recorded the least exposure concentration with 11.00 mg/m^3 whereas C2 recorded the highest exposure concentration with 27.42 mg/m^3 (Table 4.9).

Comparing the exposure concentrations of the three tobacco products indicate significant differences between dry tobacco leaves, snuff, and cigarette, ($p = 0.00$, $F = 5.27$). As such, the null hypothesis (Exposure concentration means are equal) can be rejected. Exposure concentration variance noticed between the tobacco products studied may be due site-specific characteristics of soils on which they were cultivated, their bioaccumulation capacity, and additives included during processing (Ndokiari et al., 2021)

.

Table 4.9 Exposure concentration of 15 elements found in Snuff (flavour & manufacturer) and cigarette samples

Elemental exposure concentration (mg/m^3) of snuff samples (additives/flavour)															
SAMPLE	Mo	Zr	Sr	U	Rb	Zn	Cu	Fe	Mn	V	Ti	Sc	Ca	K	**S**
Menthol	0.0010	0.0356	0.150	0.0024	0.027	0.055	0.011	1.714	0.005	0.0055	0.295	0.087	19.374	23.20	2.02
Moringa	0.0008	0.0340	0.090	0.0022	0.074	0.049	0.005	2.003	0.020	0.0047	0.262	0.046	11.721	62.76	2.10
No Flavour	0.0010	0.0342	0.127	0.0030	0.042	0.054	0.006	1.331	0.027	0.0027	0.233	0.063	14.068	36.32	2.05
Unknown	0.0008	0.0398	0.106	0.0032	0.073	0.041	0.014	1.653	0.012	0.0052	0.230	0.050	11.636	57.29	2.33
Elemental exposure concentration (mg/m^3) of snuff samples (manufacturers)															
SAMPLE	Mo	Zr	Sr	U	Rb	Zn	Cu	Fe	Mn	V	Ti	Sc	Ca	K	S
S1	0.0011	0.0363	0.140	0.003	0.037	0.053	0.006	1.363	0.018	0.002	0.260	0.071	15.978	31.990	1.997
S2	0.0015	0.0351	0.144	0.003	0.026	0.055	0.008	1.877	0.000	0.008	0.236	0.073	16.030	18.870	1.956
S3	0.0014	0.0437	0.053	0.000	0.113	0.058	0.007	2.239	0.000	0.009	0.193	0.015	5.664	70.175	2.068
S4	0.0006	0.0369	0.119	0.004	0.065	0.028	0.025	1.468	0.019	0.004	0.226	0.052	12.116	65.151	2.188
No label	0.0006	0.0354	0.098	0.003	0.071	0.048	0.007	1.867	0.020	0.005	0.274	0.055	13.308	58.340	2.288
Elemental exposure concentration (mg/m^3) of cigarette samples (brands)															
SAMPLE	Mo	Zr	Sr	U	Rb	Zn	Cu	Fe	Mn	V	Ti	Sc	Ca	K	**S**
C1	0.0026	0.0021	0.064	0.000	0.015	0.018	0.000	0.209	0.000	0.000	0.031	0.044	11.003	46.600	1.845
C2	0.0027	0.0018	0.052	0.000	0.013	0.016	0.000	0.144	0.000	0.000	0.036	0.140	27.415	58.021	3.027
C3	0.0035	0.0029	0.052	0.000	0.015	0.019	0.000	0.195	0.000	0.000	0.019	0.083	12.468	29.703	1.509
C4	0.0036	0.0029	0.034	0.000	0.009	0.023	0.000	0.134	0.031	0.000	0.000	0.099	20.890	26.062	1.520
C5	0.0038	0.0025	0.063	0.000	0.018	0.021	0.000	0.203	0.000	0.000	0.000	0.101	17.468	33.803	1.684
C6	0.0036	0.0028	0.040	0.000	0.011	0.029	0.000	0.158	0.052	0.000	0.011	0.116	24.157	31.771	2.842

Table 4.10 Exposure concentration (snuff and cigarette in mg/m^3) and CDI (dry tobacco leaves in mg/kg/day) of tobacco products

Element	Mo	Zr	Sr	U	Rb	Zn	Cu	Fe	Mn	V	Ti	Sc	Ca	K	S
Snuff (mg/m^3)	0.001	0.037	0.111	0.002	0.062	0.048	0.011	1.76	0.011	0.006	0.238	0.053	12.62	48.91	2.1
Cigarette (mg/m^3)	0.003	0.002	0.051	0.00	0.014	0.021	0.00	0.17	0.014	0.00	0.016	0.09	18.90	37.66	2.07
Dry tobacco leaves (mg/kg/day)	0.0005	0.0015	0.0117	0.00	0.0039	0.0026	0.00	0.031	0.00007	0.00	0.0013	0.004	1.95	5.808	0.13

In all, snuff products had the highest mean exposure concentration of 65.97 mg/m^3, followed by cigarette products with 59.02 mg/m^3 whereas dry tobacco leaves had mean EDI of 54.88 g/day.

Figure 4.23 compares mean exposure concentrations of the 15 elements examined in both snuff and cigarette samples and their ratios in percentage. Snuff products had the highest mean exposure concentration (65.97 mg/m^3) because of the manner in which the product is prepared and the additives/flavours that are added. This is because dry snuff, which is inhaled, is prepared from dry fermented tobacco pulverized to fine particle. In Ghana, local snuff is prepared by grinding the dry tobacco leaves into fine powder (Addo et al., 2008). Local preparation of snuff is not regulated and does not follow any approved hygienic protocols, as such toxicants from diverse sources might be introduced into the final product. Same reason goes for cigarette products but due to enhanced hygienic protocols and minimal human handling of products, less toxicants are introduced into the final product.

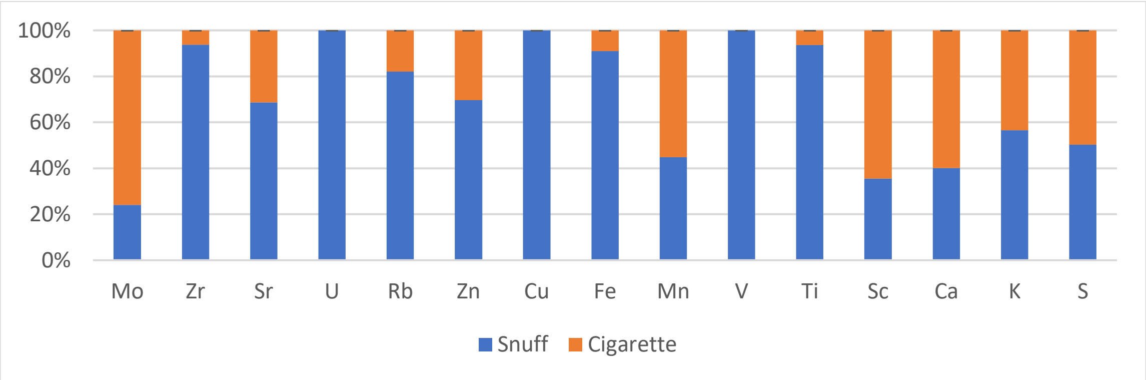

Figure 4.22 Comparison of long-term exposure concentration of elements between snuff and cigarette in percentage

4.4.2 Daily chronic daily intake and Exposure Concentration of metals from tobacco consumption.

Snuff products from Essienimpong[1] had the highest chronic exposure concentration of 130.63 mg/m^3 whiles that of Adadientem had the lowest with 35.09 mg/m^3 (Figure 4.25). Chronic Daily Intake from dry tobacco leaves ranged from and 6.97- 9.60 mg/kg/day with a mean value of 8.46 mg/kg/day (Figure 4.24).

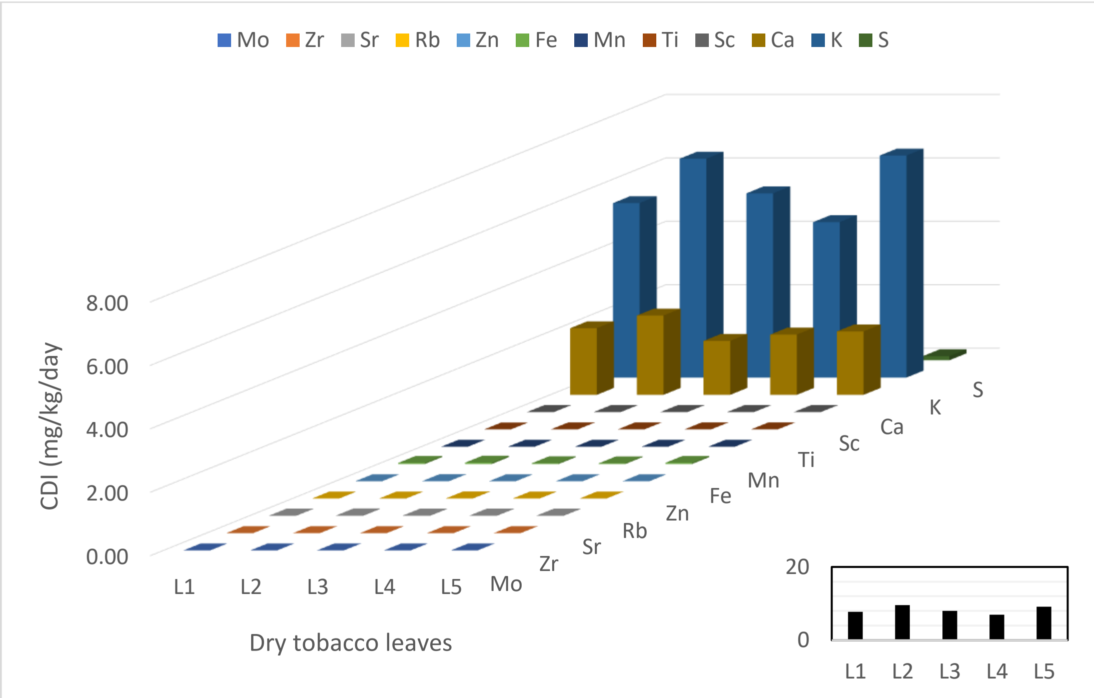

Figure 4.23 A Graph of total chronic daily intake CDI (mg/kg/day) of dry tobacco leaf samples

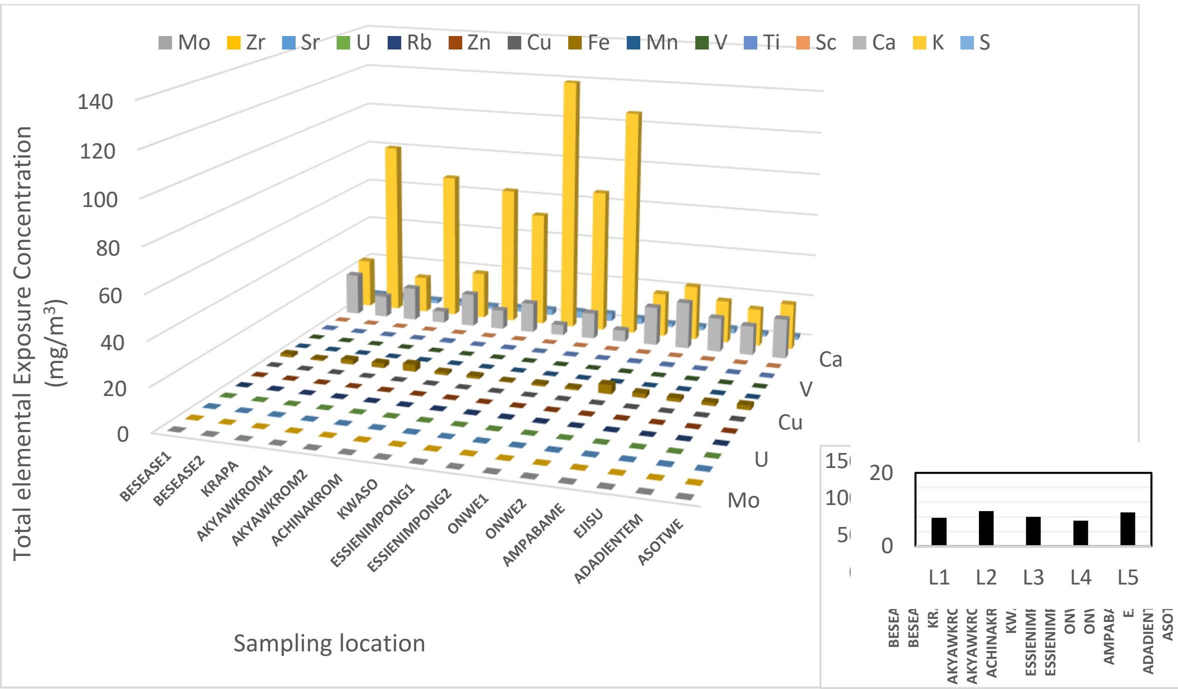

Figure 4.24 Graph of Total elemental exposure concentration (mg/m^3) against snuff sampling location

It is seen from Figure 4.26 that Moringa flavoured Snuff products recorded the highest total exposure concentration 79.17 mg/m^3 whiles Menthol flavoured Snuff products had the lowest exposure concentration of 46.99 mg/m^3

Moringa flavoured snuff products had highest exposure concentration due to high mineral contents of moringa leaves as indicated by Gopalakrishnan et al., (2016), moringa provides 7 folds vitamin C than oranges, 10 folds vitamin A than carrots, 17 folds calcium than milk, 9 folds protein than yoghurt, 15 folds potassium than bananas and 25 folds iron than spinach. From the values obtained, it can be deduced that high potassium contents of moringa leaves is partly responsible for the high exposure concentration of moringa flavoured snuff products (79.17 mg/m^3).

Snuff products with no flavour had total exposure concentration of 54.37 mg/m^3. The higher exposure concentration of non-flavoured snuff products over mentholated snuff products may be attributed to the fact that the snuff products contained other additives which vendors were genuinely not aware. It could also be as a result of the method of processing, farming or handling that added more contaminants to the final product.

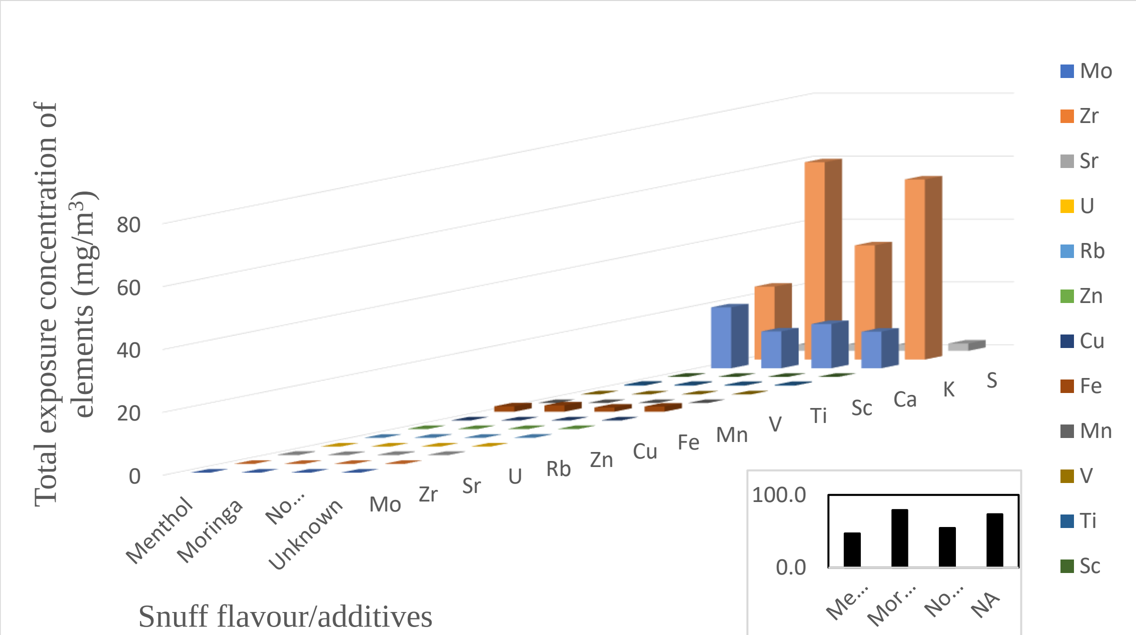

Figure 4.25 Graph of Total exposure concentration (mg/m^3) against snuff flavour/additives

Also, analysis of snuff samples from the various manufacturers as indicated (Figure 4.27) shows that ‘S4” had the highest total exposure concentration (81.50 mg/m^3) and “S2” with the least total exposure concentration (39.32 mg/m^3).

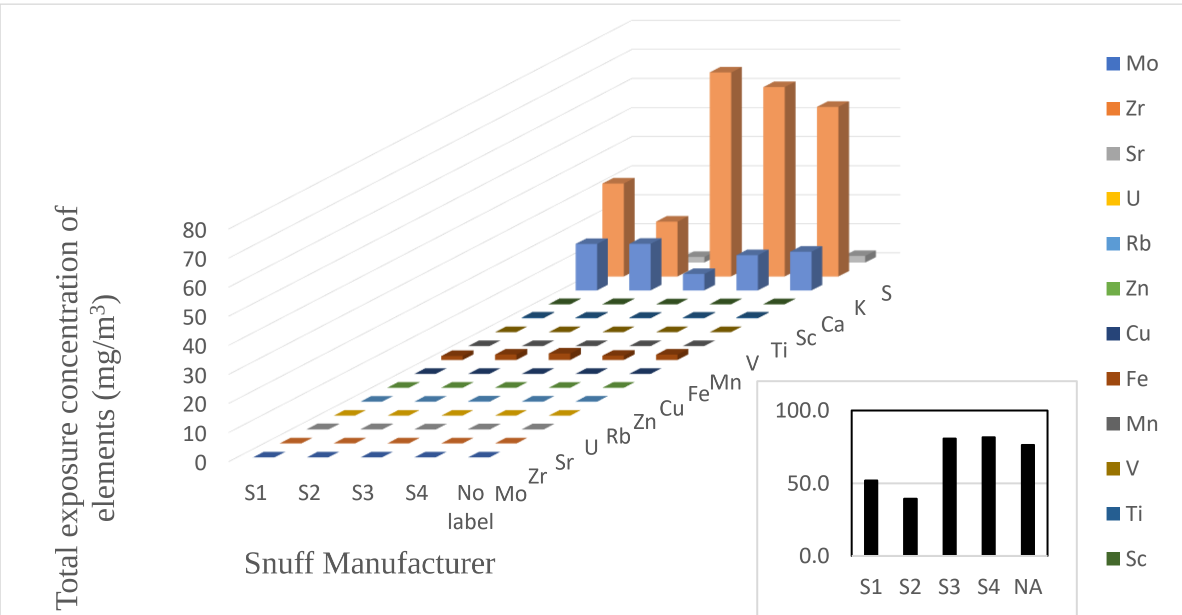

Figure 4.26 Graph of Total elemental exposure concentration (mg/m^3) against snuff manufacturer

Figure 4.28 indicates that C2 cigarettes had the highest total exposure concentration (88.87 mg/m^3) followed by C1; (59.83 mg/m^3). C6, a smuggled cigarette brand had total exposure concentration of (59.02 mg/m^3) whilst the brand with the least total exposure concentration (44.07 mg/m^3) was C3.

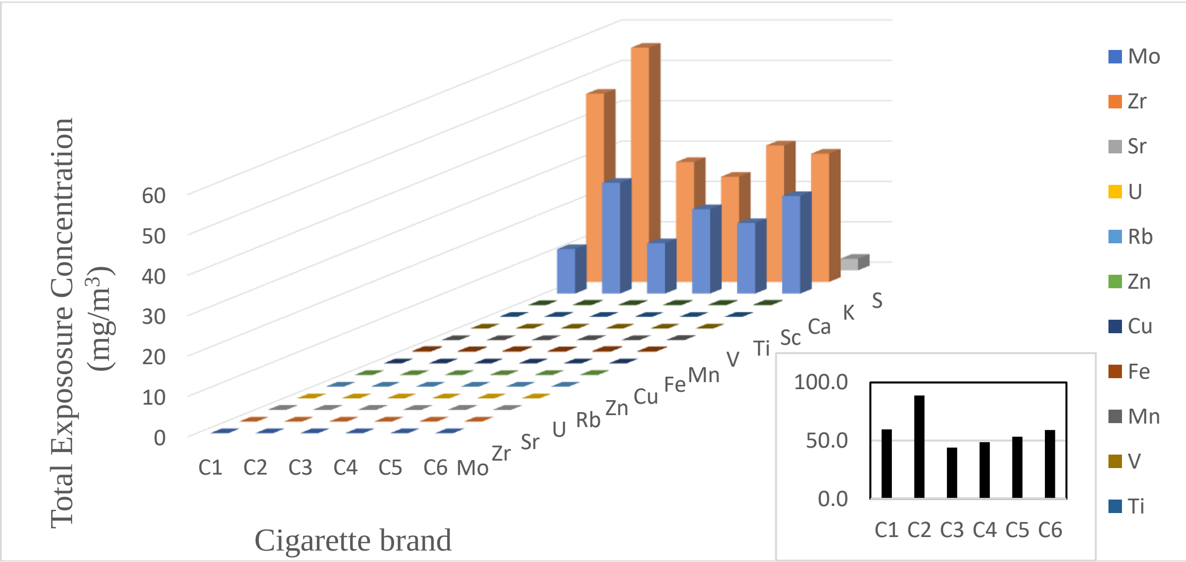

Figure 4.27 Graph of Total elemental exposure concentration (mg/m^3) against cigarette brands

4.5 Human Risk Assessment of selected elements

Indeed, most of the inorganic elements of the periodic table are present in foods and drinking water, usually in trace amounts (National Research Council, 1989). Some elements may occur in amounts that may be toxic under certain conditions (National Research Council, 1989). Examples of inorganic elements in foods that have no biological function in humans are aluminum, antimony, barium, rubidium, vanadium, zirconium, uranium, mercury, silver, strontium, thallium, and titanium (National Research Council, 1989).

4.5.1 Short-term exposure risk assessment of selected elements

In this study, human health risk was estimated for ten elements for active and second-hand tobacco consumers. The purpose for estimating Average Daily Exposure over a period of 8 hours as % OSHA Time weighted average (ADE_8 as % OSHA TWA_8) was to examined whether short-term health effects could occur due to exposure to snuff particulates and cigarette smoke. Secondly, to determine whether for those working in an area that is exposed to cigarette smoke and snuff. For example, if ADE_8 (% OSHA TWA_8) of any element is less than 100%, then the area is safe, as there will be no acute health effects resulting from second-hand exposure present in the vicinity (Behera et al., 2013). On the other hand, if ADE_8 ADE_8 (% OSHA TWA_8)of any element is greater than 100%, then the area is not safe to work. Estimated ADE_8 (% OSHA TWA_8) of elements in STE were to represent short-term health effects of selected elements (Mo, Zr, Zn, Cu, Fe, Mn, V, Ca, Ti, and U) on both active and second-hand tobacco product consumers as indicated in a study by Behera et al., (2013). It is observed (Table 4.11-4.13), there is no possible short term non-cancer health effects from exposure to the elements in the snuff products, cigarettes and dried tobacco leaves. This is because, % exposure to contaminants is below 100. However, Ca with a permissible daily allowance of 2 mg/m^3 (Cal/OSHA, 2021) exceeded the exposure limits for snuff (25.24 mg/m^3) and cigarette products (37.8 mg/m^3) whereas Mo (permissible daily allowance of 0.5 mg/m^3) and Fe (permissible daily allowance of 5 mg/m^3) exceeds the exposure limit for dry tobacco leaves since they all have % exposure more than 100.

Table 4.12 Average Daily Exposure over 8 h period (ADE_8) represented as percentage of OSHA Time Weighted Average for snuff products

		Snuff Flavour				Snuff Manufacturers				
SAMPLE	[a] OSHA TWA_8 (mg/m^3)	Menthol	Moringa	NF	NA	S1	S2	S3	S4	NA
Mo	0.5	0.41	0.31	0.41	0.33	0.44	0.59	0.57	0.26	0.24
Zr	5	1.42	1.36	1.37	1.59	1.45	1.41	1.75	1.47	1.42
U	0.05	9.78	8.68	12.14	12.70	10.96	10.83	0.00	16.14	10.52
Zn	5	2.19	1.96	2.17	1.62	2.13	2.21	2.31	1.14	1.92
Cu	1	2.25	1.05	1.15	2.78	1.20	1.65	1.39	5.04	1.40
Fe	5	68.55	80.13	53.24	66.11	54.50	75.08	89.57	58.72	74.66
Mn	0.2	4.66	19.74	26.62	12.49	17.74	0.00	0.00	18.95	19.60
V	0.05	21.92	18.84	10.78	20.96	7.18	32.16	36.14	15.54	19.02
Ti	15	3.94	3.49	3.10	3.06	3.47	3.14	2.58	3.01	3.65
Ca	2	1937	1172	1407	1164	1598	1603	566	1212	1331

Table 4.11 Average Daily Exposure over 8 h period (ADE_8) represented as percentage of OSHA Time Weighted Average for cigarette samples

SAMPLE	[a] OSHA TWA_8 (mg/m^3)	C1	C2	C3	C4	C5	C6
Mo	0.5	1.03	1.08	1.39	1.45	1.54	1.43
Zr	5	0.08	0.07	0.12	0.12	0.10	0.11
U	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Zn	5	0.72	0.65	0.77	0.93	0.83	1.17
Cu	1	0.00	0.00	0.00	0.00	0.00	0.00
Fe	5	8.36	5.76	7.79	5.35	8.10	6.33
Mn	0.2	0.00	0.00	0.00	30.55	0.00	52.37
V	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Ti	15	0.42	0.47	0.26	0.00	0.00	0.14
Ca	2	1100	2741	1247	2089	1747	2416

TABLE 4.13 Estimated Daily Intake (EDI) of selected elements and their Recommended Daily Allowance (RDA)

SAMPLE	RDI (mg/day)	EDI	%
Mo	0.025[a]	0.035	136
Zn	9[b]	0.60	6.64
Fe	10[b]	14.56	145.62
Mn	2.3[b]	0.117	25.65
Ca	1000[b]	302.83	30.28
K	4700[b]	451.73	0.096

[b]Institute of Medicine, Food and Nutrition Board, 2001; **[a]**EFSA, 2004

4.5.2 Estimated non-cancer risks for non-carcinogenic elements due to short term Second-Hand exposure for (snuff and cigarette products)

Most second-hand exposure studies have been conducted on combustible tobacco products (Addo et al., 2008), particularly cigarette. However, the researcher through reconnaissance observed that non-active users can be exposed to nasal snuff as well, especially in a confined environment. This is because dry snuff, which is inhaled, is prepared from dry fermented tobacco pulverized to fine particle. In Ghana, local snuff is prepared by grinding the dry tobacco leaves into fine powder (Addo et al., 2008). This fine powder and particles when not properly stored can saturate a room and inhaled through second-hand exposure. Therefore, this study deemed it necessary to determine toxicity of snuff as well as cigarette through second-hand exposure. Two possible cases for health risk assessment were evaluated by considering two environmental conditions, in which one can be exposed to cigarette smoke and snuff particulates through second-hand exposure. Case one represents residential settings while case two represents a commercial setting, such as point of sale centers like clubs, ghettos, drinking sports, smoking joints among others).

From the estimated of ADC_8 (% OSHA TWA)$_8$ presented (Tables 4.14 and 4.17), it could be confirmed that:

1. For snuff products, all elements had ADC_8 (% OSHA TWA_8) below 100 % for

each of the possible cases of exposure. Aside Ca which had %OSHA TWA_8 above100 % for all Case 1 scenarios.

2. Elements in cigarette products had estimated values of ADC_8 (% OSHA TWA_8) below 100% for case 1 and 2 scenarios with the exception of Ca which had ADC_8 as %OSHA TWA_8 above 100 % for all Case 1 scenarios and three of the cigarette brands in Case 2 scenario; C2 (137 %), C4 (104 %) and C6 (121 %).

Therefore, it could be inferred that Mo, Zr, Zn, Cu, Fe, Mn, V, Ti and U present in cigarette smoke and snuff had no short-term health effects due to second-hand exposure under the two case scenarios.

Table 4.14 Case 1. Estimated ADC_8 as percentage of OSHA times weighted average from one active snuff user with 10 dilution factor

		Snuff Flavour				Snuff Manufacturers				
Elements	OSHA TWA_8 (mg/m^3)	Menthol	Moringa	NF	NA	**S1**	**S2**	**S3**	**S4**	**NA**
Mo	0.5	0.04	0.03	0.04	0.03	0.04	0.06	0.06	0.03	0.02
Zr	5	0.14	0.14	0.14	0.16	0.15	0.14	0.17	0.15	0.14
U	0.05	0.98	0.87	1.21	1.27	1.10	1.08	0.00	1.61	1.05
Zn	5	0.22	0.20	0.22	0.16	0.21	0.22	0.23	0.11	0.19
Cu	1	0.22	0.10	0.11	0.28	0.12	0.17	0.14	0.50	0.14
Fe	5	6.85	8.01	5.32	6.61	5.45	7.51	8.96	5.87	7.47
Mn	0.2	0.47	1.97	2.66	1.25	1.77	0.00	0.00	1.90	1.96
V	0.05	2.19	1.88	1.08	2.10	0.72	3.22	3.61	1.55	1.90
Ti	15	0.39	0.35	0.31	0.31	0.35	0.31	0.26	0.30	0.36
Ca	2	193.74	117.21	140.68	116.36	159.78	160.30	56.64	121.16	133.08

Table 4.15 Case 1. Estimated ADC_8 as percentage of OSHA times weighted average from one active cigarette smoker with 10 dilution factor

Elements	OSHA TWA_8 (mg/m^3)	C1	C2	C3	C4	C5	C6
Mo	0.5	0.1028	0.1084	0.1386	0.1446	0.1536	0.1434
Zr	5	0.00836	0.0072	0.01152	0.0117	0.00982	0.01134
U	0.05	0	0	0	0	0	0
Zn	5	0.07226	0.06498	0.07694	0.09282	0.08344	0.11748
Cu	1	0	0	0	0	0	0
Fe	5	0.83594	0.57572	0.77922	0.53524	0.8102	0.63344
Mn	0.2	0	0	0	3.055	0	5.2365
V	0.05	0	0	0	0	0	0
Ti	15	0.04184	0.047387	0.025767	0	0	0.014113
Ca	2	110.034	274.1475	124.6811	208.8981	174.6848	241.5716

Table 4.16 Case 2. Estimated ADC_8 as percentage of OSHA times weighted average from five active snuff users with 100 dilution factor

		Snuff Flavour				Snuff Manufacturers				
Elements	OSHA TWA_8 (mg/m^3)	Menthol	Moringa	NF	NA	S1	S2	S3	S4	NA
Mo	0.5	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.01	0.01
Zr	5	0.07	0.07	0.07	0.08	0.07	0.07	0.09	0.07	0.07
U	0.05	0.49	0.43	0.61	0.64	0.55	0.54	0.00	0.81	0.53
Zn	5	0.11	0.10	0.11	0.08	0.11	0.11	0.12	0.06	0.10
Cu	1	0.11	0.05	0.06	0.14	0.06	0.08	0.07	0.25	0.07
Fe	5	3.43	4.01	2.66	3.31	2.73	3.75	4.48	2.94	3.73
Mn	0.2	0.23	0.99	1.33	0.62	0.89	0.00	0.00	0.95	0.98
V	0.05	1.10	0.94	0.54	1.05	0.36	1.61	1.81	0.78	0.95
Ti	2.4	0.20	0.17	0.16	0.15	0.17	0.16	0.13	0.15	0.18
Ca	2	96.87	58.61	70.34	58.18	79.89	80.15	28.32	60.58	66.54

Table 4.17 Case 2. Estimated ADC_8 as percentage of OSHA times weighted average from five active cigarette smokers with 100 dilution factor

Elements	OSHA TWA_8 (mg/m^3)	C1	C2	C3	C4	C5	C6
Mo	0.5	0.05	0.05	0.07	0.07	0.08	0.07
Zr	5	0.00	0.00	0.01	0.01	0.00	0.01
U	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Zn	5	0.04	0.03	0.04	0.05	0.04	0.06
Cu	1	0.00	0.00	0.00	0.00	0.00	0.00
Fe	5	0.42	0.29	0.39	0.27	0.41	0.32
Mn	0.2	0.00	0.00	0.00	1.53	0.00	2.62
V	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Ti	2.4	0.02	0.02	0.01	0.00	0.00	0.01
Ca	2	55	137	62	104	87	121

4.5.3 Long-term exposure risk assessment to selected elements

To assess the long-term health effects due to consumption of tobacco products, risk assessment was performed for ten elements (Mo, Zr, Zn, Cu, Fe, Mn, Ti, V, U and Rb) in active smokers and snuff inhalation to represent the health risk scenario of an active tobacco user (cigarette and snuff. Tables 4.18 - 4.20 present the estimated non-carcinogenic health risk of inhalation of elements through direct tobacco use for the active user. In other words, the values represent the risks that are expected for 10 sticks of cigarettes and a box of snuff product (both estimated to be 10 g in mass) through direct inhalation.

The non-cancer risk, hazard quotient (HQ) for all elements detected in snuff products exceeded 1.0 for menthol and moringa flavoured snuff products including snuff products with no flavour (NF) and those whose flavour are unknown. However, Mo, Cu and Ti exceeded 1.0 for HQ and HI. Also, menthol flavoured snuff products had the least Hazard Index (684.32) whereas moringa flavoured snuff products had the highest HI (1059.16). The higher HI value of moringa flavoured snuff samples could be as a result of higher HQ values of Mn and Fe as compared to menthol flavoured snuff samples (Table 4.18).

The same trend was observed for snuff products assessed according to their manufacturer. Elements obtained had HQ above 1.0 with the exception of Mo, Cu and Ti. Mn was absent in samples "S2" and "S3. However, "S2" had the least HI (664.01) whereas those with unknown manufacturers had the highest HI (1032.53 followed by "S4" with HI value 934.47.

For cigarette brands, HQ for all 6 cigarette brands above 1.0 for all 10 elements. However, Zr which had HQ values below 1.0. Also, for C3 (0.64) and C6 (0.35), HQ values for Ti below 1.0.

Dry tobacco leaves had HQ below 1.0 for all 8 elements examined with the exception of Zr (HQ= 18.94) which had HQ values above 1.0. However, HI for dry tobacco leaves was 20.10.

In all, C6 had the highest HI (1108.35), and cigarette brand which recorded least HI (52.36) was C2 with a value. It must be noted that C6 is an illegal cigarette product in the Ghanaian market.

The extremely high HQ and HI values in this study can be attributed to the high concentration of Zn, Mn, Fe and Rb in the tobacco products. Also the very low reference concentration of these elements led to high HQs and subsequently HI.

Table 4.18 Estimated non-cancer risks of elements due to inhalation of a box (10 g) of snuff a day

		Snuff Flavour				Snuff Manufacturers				
Element	RfC (mg/m^3)	Menthol	Moringa	NF	NA	S1	S2	S3	S4	NA
Mo	0.002	0.51	0.39	0.51	0.42	0.54	0.73	0.71	0.32	0.3
Zr	0.005	7.11	6.8	6.84	7.95	7.25	7.03	8.73	7.37	7
U	4×10^{-5}	61.13	54.25	75.88	79.38	68.50	67.69	0.00	100.8	65.77
Rb	0.0025	10.7	29.76	16.99	29.1	14.63	10.48	45.08	25.84	28.39
Zn	0.002	27.38	24.47	27.08	20.28	26.57	27.58	28.83	14.19	23.99
Cu	0.1	0.11	0.05	0.06	0.14	0.06	0.08	0.07	0.25	0.07
Fe	4×10^{-3}	428.4	500.78	332.7	413.2	340.64	469.24	559.79	366.98	466.62
Mn	5×10^{-5}	93.2	394.69	532.3	249.7	354.86	0	0	379.04	391.92
V	1×10^{-4}	54.8	47.1	26.95	52.4	17.95	80.4	90.35	38.85	47.56
Ti	0.03	0.98	0.87	0.77	0.76	0.86	0.78	0.64	0.75	0.91
	HI	684.32	1059.16	1020.08	853.33	831.86	664.01	734.2	934.47	1032.53

Table 4.19 Estimated non-cancer risks of elements due to inhalation of 10 sticks of cigarette a day

Elements	RfC (mg/m^3)	C1	C2	C3	C4	C5	C6
Mo	0.002	1.28	1.35	1.73	1.81	1.92	1.79
Zr	0.005	0.42	0.36	0.57	0.59	0.49	0.57
U	4×10^{-5}	0	0	0	0	0	0
Rb	0.0025	5.95	5.36	5.98	3.64	7.11	4.45
Zn	0.002	9.03	8.12	9.61	11.6	10.43	14.68
Cu	0.1	0	0	0	0	0	0
Fe	4×10^{-3}	52.25	35.98	48.7	33.45	50.63	39.58
Mn	5×10^{-5}	0	0	0	610.99	0	1047.27
V	1×10^{-4}	0	0	0	0	0	0
Ti	0.03	1.04	1.17	0.64	0	0	0.35
	HI	69.97	52.36	67.25	662.02	99.54	1108.71

Table 4.20 Estimated non-cancer risks of elements due to consumption of 10 grams of dried tobacco leaves

Element	RfD (mg/g)	HQ
Mo	0.005	0.09
Zr	8×10^{-5}	18.94
Sr	0.3	0.0390
Rb	0.004	0.9857
Zn	0.3	0.0085
Fe	0.7	0.0445
Mn	0.14	0.0005
Ti	40	3.22E-05
	HI	20.1

4.5.4 Estimated non-cancer risks of non-carcinogenic elements due to chronic Second-Hand exposure to selected elements

The risks due to exposure assessment of snuff and cigarette products by second-hand exposure were estimated on the basis of the concentration of elements present in STE. This is on the assumption that 100% of the elements present in tobacco products will be transferred and deposited into the human respiratory system through inhalation. Two possible cases were considered to estimate non-cancer risks for snuff and cigarette products. Case 1 was meant to assess exposure risks in residential settings whereas case 2 was also meant to assess the exposure risks in commercial setting such as clubhouse, liquor and smoking joint etc. Table 4.21 and 4.24 presents non-cancer risks of 10 elements (Mo, Zr, Zn, Cu, Fe, Mn, Ti, V, U and Rb)) due to exposure under two scenarios.

4.5.4.1 Case 1 for long term exposure

For flavour/additives effect, HQ was below 1.0 for Mo (0.45), Zr (0.72), Cu (0.01) and Ti (0.088) whereas HQ for Rb (2.04), Fe (39.05), Zn (2.18), Mn (31.75) and V (4.33) were above 1.0. HQ and HI (83.94) of snuff products from the same manufacturers followed same order (i.e., HQ below than 1.0 for Mo (0.5), Zr (0.78), Cu (0.1) and Ti

(0.79) whereas HQ for Rb (2.90), Fe (39.29), Zn (2.70), Mn (22.52) and V (5.51) were more than 1.0. With cigarette products, all elements in the six different brands had HQ lower than 1.0 with the exception of Zn for C4 (1.16), C5 (1.04) and C6 (1.47); Fe also had HQ more than 1.0 for all tobacco products. Mn was detected in only C4 (61.09) and C6 (104.7) respectively.

HI values for all the elements examined were higher than 1.0 with C6 having the highest (110.87) and C2 having the least (5.24).

Since total risk index (HI) were all greater than 1.0, it infers that second-hand exposure to tobacco products (snuff and cigarette products) at homes are likely to cause non-carcinogenic adverse health effects such as irritation of mucous membranes in humans, reduction in respiratory capacity in humans, blood, cardiovascular, and kidney effects (Selman, 2003; Willers et al, 2005; Behera et al., (2013).

.

Table 4.21 Case 1: Estimated non-cancer risks of elements due to indoor exposure from one active snuff user with 10 dilution factor

		Snuff Flavour				Snuff Manufacturers				
Elements	RfC (mg/m^3)	Menthol	Moringa	NF	NA	S1	S2	S3	S4	NA
Mo	0.002	0.05	0.04	0.05	0.04	0.05	0.07	0.07	0.03	0.03
Zr	0.005	0.71	0.68	0.68	0.80	0.73	0.70	0.87	0.74	0.70
U	4×10^{-5}	6.11	5.43	7.59	7.94	6.85	6.77	0.00	10.09	6.58
Rb	0.0025	1.07	2.98	1.70	2.91	1.46	1.05	4.51	2.58	2.84
Zn	0.002	2.74	2.45	2.71	2.03	2.66	2.76	2.88	1.42	2.40
Cu	0.1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.01
Fe	4×10^{-3}	42.84	50.08	33.27	41.32	34.06	46.92	55.98	36.70	46.66
Mn	5×10^{-5}	9.32	39.47	53.23	24.97	35.49	0.00	0.00	37.90	39.19
V	1×10^{-4}	5.48	4.71	2.70	5.24	1.80	8.04	9.04	3.89	4.76
Ti	0.03	0.10	0.09	0.08	0.08	0.09	0.08	0.06	0.08	0.09
	HI	68.43	105.92	102.01	85.33	83.19	66.40	73.42	93.45	103.25

Table 4.22 Case 1: Estimated non-cancer risks of elements due to indoor exposure from one active cigarette smoker with 10 dilution factor

Elements	RfC (mg/m^3)	C1	C2	C3	C4	C5	C6
Mo	0.002	0.128	0.135	0.173	0.181	0.192	0.179
Zr	0.005	0.042	0.036	0.057	0.059	0.049	0.057
U	4×10^{-5}	0	0	0	0	0	0
Rb	0.0025	0.595	0.536	0.598	0.364	0.711	0.445
Zn	0.002	0.903	0.812	0.961	1.16	1.043	1.468
Cu	0.1	0	0	0	0	0	0
Fe	4×10^{-3}	5.225	3.598	4.87	3.345	5.063	3.958
Mn	5×10^{-5}	0	0	0	61.09	0	104.7
V	1×10^{-4}	0	0	0	0	0	0
Ti	0.03	0.104	0.117	0.064	0	0	0.035
	HI	6.997	5.236	6.725	66.2	9.954	110.87

4.5.4.2 Case 2 for long term exposure

For flavour/additives effect, HQ was below 1.0 for Mo (0.22), Zr (0.36), Cu (0.003), Rb (menthol and NF flavoured samples with HQ values 0.53 and 0.85 respectively). HQ was above 1.0 for Rb (moringa and NA flavoured snuff samples with HQ values of 1.48 and 1.45 respectively) (Table 4.23).

Moringa flavoured snuff had the highest HI of 52.96 whiles menthol had the lowest HI of 34.22. Snuff product with no flavour had HI of 51.00 (Table 4.23).

HQ of snuff products from the same manufacturers is as follows; HQ was less than 1.0 for Mo (0.25), Zr (0.39), Cu (0.002), Fe (19.44), Rb (1.44) and Ti (0.38).

Snuff products without any label (NA) had the highest HI (51.63), followed by “S4” (46.72). “S2” had the least HI of 33.20 (Table 4.23).

With cigarette products, all elements (i.e., Mo (0.78), Zr (0.23), Zn (0.46), Rb (0.28) and Ti (0.031) in the six different brands had HQ lower than 1.0. However, HQ of Mn (13.82) and Fe (2.32) were more than 1.0. In all, C6 (55.43) had the highest HI followed by C4 (33.10). the cigarette brand with the lowest HI in this study is C2 (2.61) (Table 4.24).

However, C6, an illegal cigarette product, having the highest HI value for active smokers (1108.07) and all two cases of second-hand exposure; 110.81 and 55.44 for case 1 and 2 respectively should be of special concern to policy makers.

Table 4.23 Case 2: Estimated non-cancer risks of elements due to commercial exposure from five active snuff users with 100 dilution factor

		Snuff Flavour				Snuff Manufacturers				
Elements	RfC (mg/m^3)	Menthol	Moringa	NF	NA	S1	S2	S3	S4	NA
Mo	0.002	0.03	0.02	0.03	0.02	0.03	0.04	0.04	0.02	0.02
Zr	0.005	0.36	0.34	0.34	0.40	0.36	0.35	0.44	0.37	0.35
U	4×10^{-5}	3.06	2.71	3.79	3.97	3.43	3.38	0.00	5.04	3.29
Rb	0.0025	0.54	1.49	0.85	1.46	0.73	0.52	2.25	1.29	1.42
Zn	0.002	1.37	1.22	1.35	1.01	1.33	1.38	1.44	0.71	1.20
Cu	0.1	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00
Fe	4×10^{-3}	21.42	25.04	16.64	20.66	17.03	23.46	27.99	18.35	23.33
Mn	5×10^{-5}	4.66	19.73	26.62	12.49	17.74	0.00	0.00	18.95	19.60
V	1×10^{-4}	2.74	2.36	1.35	2.62	0.90	4.02	4.52	1.94	2.38
Ti	0.03	0.05	0.04	0.04	0.04	0.04	0.04	0.03	0.04	0.05
	HI	34.22	52.96	51.00	42.67	41.59	33.20	36.71	46.72	51.63

Table 4.24 Case 2: Estimated non-cancer risks of elements due to commercial exposure from five active cigarette smokers with 100 dilution factor

Elements	RfC (mg/m^3)	C1	C2	C3	C4	C5	C6
Mo	0.002	0.064	0.067	0.0865	0.0905	0.096	0.0895
Zr	0.005	0.021	0.018	0.0285	0.0295	0.0245	0.0285
U	4×10^{-5}	0	0	0	0	0	0
Rb	0.0025	0.297	0.268	0.299	0.182	0.3555	0.2225
Zn	0.002	0.451	0.406	0.4805	0.58	0.5215	0.734
Cu	0.1	0	0	0	0	0	0
Fe	4×10^{-3}	2.612	1.799	2.435	1.6725	2.5315	1.979
Mn	5×10^{-5}	0	0	0	30.549	0	52.363
V	1×10^{-4}	0	0	0	0	0	0
Ti	0.03	0.052	0.059	0.032	0	0	0.0175
	HI	3.498	2.617	3.361	33.1	3.529	55.45

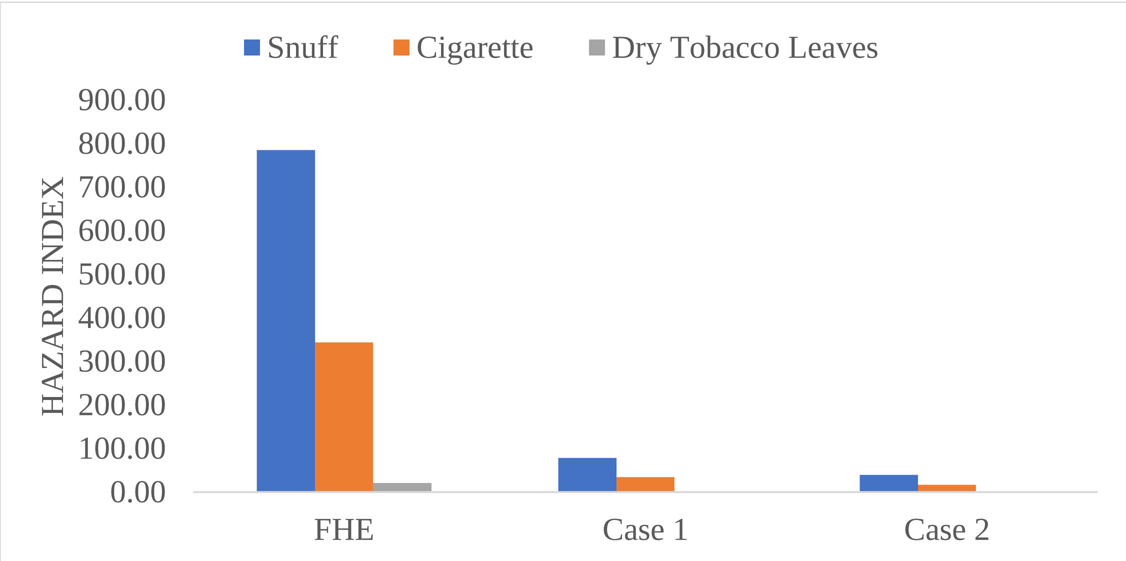

Figure 4.29 Graph showing comparison of HI of snuff and cigarette samples in First Hand Tobacco Exposure (FHE), Case 1 (Domestic tobacco exposure) and Case 2 (Point of sale/Commercial tobacco exposure)

Figure 4.29 represents H1 values for snuff, cigarette and dry tobacco leaves. It indicates that snuff products (784.90) have almost 2 folds HI values as compared to cigarette products (343.32). However, dry tobacco leaves have the least HI (20.1). Comparing the HI of the three tobacco products indicate significant differences between dry tobacco leaves, snuff, and cigarette, ($p = 0.003$, $F = 9.07$).

Figure 4.29 validates the findings of Anupozu & Vanamala, (2018), which states that the risk of lung cancer from second-hand smoke exposure is 20–30% higher for people who share a household with smokers. This is as a result of greater exposure to contaminants to household neighbors than commercial or outdoor exposure.

CHAPTER FIVE

5.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

This study aimed at a systematic classification of 51 smokeless tobacco product samples and 6 cigarette brands within the Ejisu Municipality. The classification was based on their potential toxicity and nicotine delivery capability through chronic and acute exposure to tobacco products. In this study, moisture content, pH, total nicotine, and percentage free base nicotine were determined. Target elements for analysis were Ca, Cu, Fe, K, Mn, Rb, Sr, Mo, V, S, U, Zr, Tl, and Zn using Niton XL3 GOLDD+ X-ray fluorescence (XRF) analyzer.

Toxicological analysis due to elemental exposure concentrations and potential for dependence was inferred from nicotine delivery capabilities determined by the percentage free base nicotine using Henderson-Hasselbalch equation.

The nicotine delivery capability of the locally produced snuff products; with mean pH 96.98 and percentage free base nicotine (% A) 96.98% was the highest, followed by dried tobacco leaves (mean pH: 25.93, % A: 25.93%) and the least being the cigarette products (mean pH: 5.49, % A: 0.33%). Menthol flavored snuff products (pH of 9.96; % A of 98.8%) had higher nicotine delivery capabilities than moringa flavored snuff products (pH of 9.77; % A of 98%). Users of C6, a smuggled cigarette product are liable to increased non-cancer health effects (HI value of 1108.35). C4 (highest pH of 5.58 with corresponding % A of 0.70) are liable to be dependent on the product than other examined cigarette products.

In the case of non-smokers, the risks were estimated under two possible cases of indoor exposure. The human health risk assessment results showed that domestic or residential second-hand exposure to tobacco products (Case 1) resulted in more human health risks compared with point-of-sale centers or commercial settings (Case 2) for short-term exposure.

However, there was increased human health risks for chronic exposure (long-term) for active smokers as compared to acute exposure (short-term). This was due to the influence of dilution of tobacco particulates/emissions prior to inhalation exposure experienced by non-smokers. The cumulative non-cancer health risks for active smokers under the two different cases were greater than the permissible limits (HI greater than 1.0).

5.2 Conclusion

There are several manufacturers of local snuff products in Ejisu Municipality but a high number of these products have no labels to ascertain the manufacturer. Dried tobacco leaves are sold in zip-locks without labels which will indicate the source of packaging though there are no additives/flavours in them.

Snuff products sold in Ejisu have two main flavours/additives. i.e.; menthol and moringa. There is the possibility of other illicit or secret additives such as marijuana added to snuff products. This may account for the high cumulative non-cancer risk (HI) of snuff samples with no flavour. However, there are other snuff products that have no additives/flavours. The most used additives/flavour available in snuff products in the Ejisu Municipality is Moringa.

Snuff and dried tobacco leaves are not healthy alternatives for overcoming smoking addiction. They contain highly unacceptable levels of potentially toxic elements. They also have higher free base nicotine delivery capabilities leading to greater tobacco dependance and consequent health risks. Addition of flavours to mask product harshness also increases pH and elemental burden of the final product. This study shows that menthol flavour increases pH of tobacco products which in turn also enhances addiction potential. Moringa flavour also increases concentration of elements which also increases hazard quotient (HQ) and HI of the product. Among the cigarette samples, C4 cigarette had the highest nicotine delivery capability (0.70 %) whereas C3 had the least (0.15 %). As such, users of C4 cigarette are at a higher risk of tobacco dependence or addiction than the other cigarette products studied.

The cumulative non-cancer risk (HI) of an active snuff user over two folds that of cigarettes. This indicates higher risk of occurrence of non-cancer adverse health effects in snuff products than in cigarette product. Though cumulative non-cancer risk of dried tobacco leaves is less than that of cigarette as found in this study, addition of ash prior to use has potential to increase HI, HQ, and also the pH. This could lead to an increase in % free base nicotine, nicotine delivery capability and subsequently addiction potential. As such, the non-cancer health risks associated with dried tobacco leaves should not be taken for granted. Also from the risk assessment, it became clear that long term use of tobacco products could lead to higher level risk of getting non-cancer diseases as compared to short term (acute) use of tobacco product.

Smuggled cigarette brand in the Ghanaian market (C6), had the highest cumulative non-cancer risk (HI). From the exposure assessment, it was observed that a second-hand smoker who stays very close to the active smoker on a long-term basis will be at a high-level risk of getting non-carcinogenic adverse effects than on a short-term basis. Finally, second-hand exposure to snuff and cigarette products at homes can lead to higher non-cancer health risks as compared to exposure at point-of-sale centers.

This study suggests that those who desire to quit smoking for perceived medicinal or health benefits by switching to smokeless tobacco products such as snuff and dried tobacco leaves may be unintentionally sustaining nicotine dependence. This is as a result of higher exposure to unprotonated/free nicotine tobacco products. This can then lead to nicotine addiction and increased risks to non-carcinogenic adverse health effects.

5.3 Recommendations

Based on the findings of this work and in line with the recently adopted World Health Organization international health treaty (the Framework Convention for Tobacco Control), the following recommendations are made:

1. It is recommended that manufacturers of smokeless tobacco, specifically locally produced snuff be mandated to report to the authorities, and visibly display on package labelling, name of manufacturer, pH and free base nicotine levels so that consumers are better able to make informed health choices.
2. Also, foreign and smuggled cigarette products in the country must be stamped out by the Food and Drugs Authority (FDA), and the Ghana Standards Authority (GSA). The Ministry of Health, the Ghana Medical Association, and the Pharmacy Council of Ghana should raise awareness of the negative health impact of the use of both smokeless and combustible tobacco and nicotine related products to safeguard the health of the populace.
3. It is recommended that the academic scientific community should embark on further research on smokeless tobacco products focusing more on the carcinogenic nitrosamines and other identified mutagens.

REFERENCES

Abbaspour, N., Hurrell, R., & Kelishadi, R. (2014). Review on iron and its importance for human health. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*, *19*(2), 164.

Abu-Jarad, F. (1997). Indoor cigarette smoking: Uranium contents and carrier of indoor radon products. *Radiation Measurements*, *28*(1-6), 579-584.

Addo, M. A., Duodu, O. G., Affum, H. A., Gbadago, J. K., Darko, E. O., & Coleman, A. (2011). Determination of minerals profile in Ghanaian local snuffs and an imported snuff using instrumental neutron activation analysis. *British Journal of Pharmacology and Toxicology*, *2*(6), 293-301.

Addo, M., Gbadago, J., Affum, H., Adom, T., Ahmed, K., & Okley, G. (2008). Mineral profile of Ghanaian dried tobacco leaves and local snuff: A comparative study. *Journal of Radioanalytical and Nuclear Chemistry*, *277*(3), 517-524.

Afridi, H. I., Kazi, T. G., Kazi, N., Kandhro, G. A., Baig, J. A., Shah, A. Q., ... & Arain, M. B. (2011). Evaluation of cadmium, chromium, nickel, and zinc in biological samples of psoriasis patients living in Pakistani cement factory area. *Biological trace element research*, *142*, 284-301.

Afridi, H. I., Kazi, T. G., Talpur, F. N., Baig, J. A., Chanihoon, G. Q., Lashari, A., & Channa, G. M. (2021). Elemental concentrations in biological samples of coronavirus disease (COVID-19) and other pulmonary disease patients. *American Journal of Analytical Chemistry*, *12*(5), 162-187.

Ahlström, M. G., Thyssen, J. P., Wennervaldt, M., Menné, T., & Johansen, J. D. (2019). Nickel allergy and allergic contact dermatitis: A clinical review of immunology, epidemiology, exposure, and treatment. *Contact dermatitis*, *81*(4), 227-241.

Alavanja, M. (2002). Biologic damage resulting from exposure to tobacco smoke and from radon: implication for preventive interventions. *Oncogene (2002) 21, 7365 – 7375*

Abdushukurov, D. A., Abdusamadzoda, D., Duliu, O. G., Zinicovscaia, I., & Nekhoroshkov, P. S. (2022). On the Geochemistry of Major and Trace Elements Distribution in Sediments and Soils of Zarafshon River Valley, Western Tajikistan. *Applied Sciences (Switzerland), 12*(6).

Agency for Toxic Substances and Disease Registry; Atlanta (GA): Agency for Toxic Substances and Disease Registry (US); 2020 May. Retrieved July 1, 2022, from https://pubmed.ncbi.nlm.nih.gov/37040459/

Hamza, A. A. (2010). Ameliorative effects of Moringa oleifera Lam seed extract on liver

fibrosis in rats. *Food and Chemical Toxicology*, *48*(1), 345-355.
American Cancer Society. (2020). *Health Risks of Smokeless Tobacco*. Retrieved June 10, 2022, from https://www.cancer.org/healthy/stay-away-from-tobacco/health-risks-of-tobacco/smokeless-tobacco.html

Amuah, E. E. Y., Fei-Baffoe, B., & Kazapoe, R. W. (2021). Emerging potentially toxic elements (strontium and vanadium) in Ghana's pedological studies: Understanding the levels, distributions and potential health implications. A preliminary review. *Environmental Challenges*, *5*, 100235.

Ali, H., & Khan, E., (2021). Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Hindawi.Com*. Retrieved July 4, 2022, from https://www.hindawi.com/journals/jchem/2019/6730305/

Anupozu, R., & Vanamala, M. (2020). Non-small cell lung cancer (NSCLC)-a comprehensive review. *International Journal of Advances in Scientific Research,* 11, 215-223

Atlee, J. L., & Kiviluoma, K. T. (2007). Disorders of potassium balance. *Complications in Anesthesia*, 53–58.

ATSDR, (2018). Minimum Risk Levels. Agency for Toxic Substances and Disease Registry. Retrieved February 8, 2023, from https://www.atsdr.cdc.gov/mrls/index.asp.

Ayo-Yusuf, O. A., Swart, T. J. P., & Pickworth, W. B. (2004). Nicotine delivery capabilities of smokeless tobacco products and implications for control of tobacco dependence in South Africa. *Tobacco Control*, 186–189.

Baker, R. R., & Dixon, M. (2006). The retention of tobacco smoke constituents in the human respiratory tract. *Inhalation Toxicology*, *18*(4), 255–294.

Bast-Pettersen, R., & Ellingsen, D. G. (2005). The Kløve–Matthews static steadiness test compared with the DPD TREMOR: Comparison of a fine motor control task with measures of tremor in smokers and manganese-exposed workers. *Neurotoxicology*, *26*(3), 331-342.

Beljan, J. R., Bertozzi, S., & Bohigian, G. M. (2008). Health effects of smokeless tobacco. *Journal of the American Medical Association*, *255*(8), 1038–1044.
Benowitz, N. L., Hukkanen, J., & Peyton, J. I. (2009). Nicotine psychopharmacology. *Journal of Psychopharmacology*, *6*(4), 545–546.

Benson, N. U., Anake, W. U., Adedapo, A. E., Fred-Ahmadu, O. H., & Ayejuyo, O. O. (2017). Toxic metals in cigarettes and human health risk assessment associated with inhalation exposure. *Environmental Monitoring and Assessment*, *189*(12).

Bhawna, G. (2013). Burden of Smoked and Smokeless Tobacco Consumption in India -

Results from the Global adult Tobacco Survey India (GATS-India)- 2009-2010. *Asian Pacific Journal of Cancer Prevention*, *14*(5), 3323–3329.

Bhattacharya, P., Misra, S., & Hussain, M. (2016). Nutritional aspects of essential trace elements in oral health and disease: an extensive review. *Scientifica, Hindawi.Com*. https://www.hindawi.com/journals/Scientifica/2016/5464373/

Boffetta, P., Hecht, S., Gray, N., Gupta, P., & Straif, K. (2008). Smokeless tobacco and cancer. *The Lancet Oncology*, *9*(7), 667–675.

Boice, J. D., Held, K. D., & Shore, R. E. (2019). Radiation epidemiology and health effects following low-level radiation exposure. *Journal of Radiological Protection*, *39*(4), 14

Borrelli, N., Alvarez, M. F., Osterrieth, M. L., & Marcovecchio, J. E. (2010). Silica content in soil solution and its relation with phytolith weathering and silica biogeochemical cycle in Typical Argiudolls of the Pampean Plain, Argentina—a preliminary study. *Journal of Soils and Sediments*, *10*, 983-994.

Browar, A. W., Koufos, E. B., Wei, Y., Leavitt, L. L., Prozialeck, W. C., & Edwards, J. R. (2018). Cadmium exposure disrupts periodontal bone in experimental animals: implications for periodontal disease in humans. *Toxics*, *6*(2), 32.

Cadmus, E. O., & Ayo-yusuf, O. A. (2018). The effect of smokeless tobacco use and exposure to cigarette promotions on smoking intention among youths in Ghana The effect of smokeless tobacco use and exposure to cigarette promotions on smoking intention among youths in Ghana. *Cogent Medicine*, *5*(1), 1–13.

Cahn, W. Z., Drope, J., Hamill, S., Islami, F., Liber, A., Nargis, N., & Stoklosa, M. (2013). *The tobacco atlas*, 50(5), 50-2422

Campbell, R. (2014). *Speciation of metals and metalloids in tobacco and tobacco smoke: Implications for health and regulation*. Retrieved November 12, 2022, from https://research-repository.st andrews.ac.uk/handle/10023/5728

CalEPA. (2018a). *OEHHA Acute, 8-hour and Chronic Reference Exposure Level (REL) Summary. California Environmental Protection Agency*. Retrieved November 12, 2022, from: http://oehha. ca.gov/air/general-info/oehha-acute-8-hour-and-chronic-reference-exposure-levelrel-summary.

CalEPA. (2018b). *Toxicity Criteria on Chemicals Evaluated by OEHHA. California Environmental Protection Agency*. Retrieved November 10, 2022, from: http://oehha.ca.gov/chemicals.

Caraway, J. W., & Chen, P. X. (2013). Assessment of mouth-level exposure to tobacco constituents in US snus consumers. *nicotine & tobacco research*, *15*(3), 670-677.

California Division of Occupational Safety and Health (Cal/OSHA), (2019) Table AC-1, Permissible Exposure Limits (PELs), in California Code of Regulations (CCR)

Title 8 Section 5155, last viewed October 2, 2019. Retrieved November 7, 2022, from http://www.dir.ca.gov/title8/5155table_ac1.html.

Carvalho, F., Fernandes, S., Fesenko, S., & Holm, E. (2017). *The environmental behaviour of polonium*. Retrieved October 17, 2022, from https://www-pub.iaea.org/MTCD/publications/PDF/D484_web.pdf

Center for Disease Control and Prevention (CDC). (2019). Outbreak of Lung Injury Associated with the Use of E-Cigarette Products. *Centers for Disease Control and Prevention*. Retrieved December 17, 2022, from: https://www.cdc.gov/tobacco/basic_information/e-cigarettes/severe-lung-disease.html

Centers for Disease Control. (1999). Determination of nicotine, pH, and moisture content of six US commercial moist snuff products--Florida, January-February 1999. October 20, 2022, from: https://pubmed.ncbi.nlm.nih.gov/10366135/

Center Control Disease, (2010). How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General. Atlanta (GA): *Centers for Disease Control and Prevention;* 1–101.

Chen, C., Li, Q., Nie, X., Han, B., Chen, Y., Xia, F., Zhai, H., Wang, N., & Lu, Y. (2017). Association of lead exposure with cardiovascular risk factors and diseases in Chinese adults. *Environmental Science and Pollution Research*, *24*(28), 22275–22283.

Chiba, M., & Masironi, R. (1992). Toxic and trace elements in tobacco and tobacco smoke. *Bulletin of the World Health Organization*, *70*(2), 269.

Christobher, S., Periyasamy, M., Athif, P., Syed Mohamed, H. E., Sadiq Bukhari, A., & Shahul Hameed, P. (2020). Activity concentration of polonium-210 and lead-210 in tobacco products and annual committed effective dose to tobacco users in Tiruchirappalli District (Tamil Nadu, India). *Journal of Radioanalytical and Nuclear Chemistry*, *323*(3), 1425–1429.

Chukwuma Sr, C. (1997). Environmental lead exposure in Africa. *Ambio*, 26(6), 399-403.

CORESTA. (2020). *Guide N ° 11 Technical Guide for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products Tobacco and Tobacco Product Analytes* (Issue May). Retrieved October 10, 2022, from https://www.coresta.org/

CORESTA. (2021). *Relative to Tobacco Tobacco and Tobacco Products Analytes CORESTA Recommended Method No . 72 Determination of tobacco- specific nitrosamines in tobacco and tobacco products by LC-MS / MS. 72* Retrieved October 10, 2022, from https://www.coresta.org/

Dayan, A., & Paine, A. (2001). Mechanisms of chromium toxicity, carcinogenicity and allergenicity: Review of the literature from 1985 to 2000. *Human and Experimental Toxicology*, *20*(9), 439–451.

Djordjevic, M. V., & Doran, K. A. (2009). Nicotine content and delivery across tobacco products. *Handbook of Experimental Pharmacology*, *192*, 61–82.

Du, X., Squier, C. A., Kremer, M. J., & Wertz, P. W. (2000). Penetration of N-nitrosonornicotine (NNN) across oral mucosa in the presence of ethanol and nicotine. *Journal of Oral Pathology and Medicine*, *29*(2), 80–85.

Dubin, S. (2020). Lung cancer in non-smokers. *Ncbi.Nlm.Nih.Gov*, Retrieved August 10, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7431055/

Dahlawi, S., Abdulrahman Al Mulla, A., Saifullah, Salama, K., Ahmed Labib, O., Tawfiq Aljassim, M., Akhtar, A., Asghar, W., Kh. Faraj, T., & Khalid, N. (2021). Assessment of different heavy metals in cigarette filler and ash from multiple brands retailed in Saudi Arabia. *Journal of King Saud University - Science*, *33*(6), 101-521.

Darko, G., Boakye, O., Asantewaa, M., Gyamfi, O., Ansah, E., & Acheampong, A. (2019). Human Health Risk and Bioaccessibility of Toxic Metals in Topsoils from Gbani Mining Community in Ghana. *Journal of Health & Pollution*, 9(22), 190602

Dube, A., Zbytniewski, R., Kowalkowski, T., Cukrowska, E., & Buszewski, B. (2001). Adsorption and Migration of Heavy Metals in Soil. *Polish Journal of Environmental Studies*, *10*(1), 1–10.

Ejisu Municipal Assembly. (2021). The population of the Municipality. Min of Local Government, Decentralisation & Rural Dev. and Maks Publications & Media Services. Retrieved July 5, 2022, from ejisujuaben.ghanadistricts.gov.gh/

EPA, (2014), Draft Health Effects Support Document for Strontium (Retrieved January 28, 2023, from: http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OW-2012-0155-0008

Esai, U. S. D., & Arbison, R. A. D. H. (2015). Hamilton & Hardy's Industrial Toxicology. International Encyclopedia of Public Health. Issue 2nd edition. Retrieved December 1, 2022, from https://www.researchgate.net/publication/303783481_Hamilton_Hardy's_Industrial_Toxicology

Essa, M. M., Subash, S., Parvathy, S., Meera, A., Guillemin, G. J., Memon, M. A., & Manivasagam, T. (2014). Brain health benefits of Moringa oleifera. *Food and Brain Health*, *2005*, 113–118.

European Food Safety Authority, (2004). Tolerable upper intake level on vitamins and minerals. In *Wei sheng yan jiu. Journal of hygiene research* Vol. 33, Issue 6.

USEPA. (2022). *Exposure Assessment Tools by Routes - Inhalation* | Retrieved December 28, 2022, from https://www.epa.gov/expobox/exposure-assessment-tools-routes-inhalation

Erikson, K. M., & Aschner, M. (2019). Manganese: its role in disease and health. *Essential Metals in Medicine: Therapeutic Use and Toxicity of Metal Ions in the Clinic*, *19*, 253-266.

Escareño-Juárez, E., Pardo, R., Gascó-Leonarte, C., Vega, M., Sánchez-Báscones, M. I., & Barrado-Olmedo, A. I. (2019). Determination of natural uranium by various analytical techniques in soils of Zacatecas State (Mexico). *Journal of Radioanalytical and Nuclear Chemistry*, *319*(3), 1135–1144.

Etu, E. S., Gemeda, D. H., & Hussen, M. A. (2017). Prevalence and factors that influence smokeless tobacco use among adults in pastoralist communities of Borena Zone, Ethiopia: Mixed method study. *Tobacco Induced Diseases*, *15*(1).

Fant, R. V., Henningfield, J. E., Nelson, R. A., & Pickworth, W. B. (1999). Pharmacokinetics and pharmacodynamics of moist snuff in humans. *Tobacco Control*, *8*(4), 387-392.

Feron VJ et al., (2001). Health risks associated with nasal toxicants. *Critical Reviews in Toxicology*, 3(1), 313–347.

Fischer, B. B., Pomati, F., & Eggen, R. I. (2013). The toxicity of chemical pollutants in dynamic natural systems: the challenge of integrating environmental factors and biological complexity. *Science of the Total Environment*, *449*, 253-259.

Galstyan, S., & Hovhannisyan, L. (2022). Yerevan State Medical University after Mchitar Heratsi. *Ysmubooks.Am*. Retrieved July 5, 2022, from https://ysmubooks.am/uploads/Clinical_Toxicology.pdf

Gardella, C. (2001). Lead exposure in pregnancy:: a review of the literature and argument for routine prenatal screening. *Obstetrical & gynecological survey*, *56*(4), 231-238.

Gaur, S., & Agnihotri, R. (2019). Health Effects of Trace Metals in Electronic Cigarette Aerosols—a Systematic Review. *Biological Trace Element Research*, *188*(2), 295–315.

Gazwi, H. S., Yassien, E. E., & Hassan, H. M. (2020). Mitigation of lead neurotoxicity by the ethanolic extract of Laurus leaf in rats. *Ecotoxicology and environmental safety*, *192*, 110297.

Geiger, A., & Cooper, J. (2010). Overview of airborne metals regulations, exposure limits, health effects, and contemporary research. *Environmental Protection Agency, Portland*, Retrieved July 5, 2022, from https://www.cooperenvironmental.com/wpcontent/uploads/2016/11/otm31appC.pdf

Ghany, H. (2006). The association between indoor radon and tobacco smoke. *Indoor and Built Environment*, *15*(3), 289–293.

Ghosh, S., Sharma, A., & Talukder, G. (1992). Zirconium - An abnormal trace element in biology. *Biological Trace Element Research*, *35*(3), 247–271.

Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness*, *5*(2), 49–56.

Gordon, J. P., & Lamoreux, K. (2021). *Is Snuff Harmful ? Know the Facts*. Healthline Media. https://www.healthline.com/health/what-is-snuff-is-it-harmful#effects-of-quitting. Accessed: 2023-05-21

Gupta, R., Gupta, S., Sharma, S., Sinha, D. N., & Mehrotra, R. (2019). Risk of coronary heart disease among smokeless tobacco users: Results of systematic review and meta-analysis of global data. *Nicotine and Tobacco Research*, *21*(1), 25–31.

Hagedoorn, I.& Gant, C. (2020.). Lifestyle-related exposure to cadmium and lead is associated with diabetic kidney disease. *Mdpi.Com*. Retrieved July 6, 2022, from https://www.mdpi.com/783742

Harvard Medical School. (2020). *Listing of vitamins - Harvard Health*. Harvard Health Publishing. Retrieved July 8, 2022, from https://www.health.harvard.edu/staying-healthy/listing_of_vitamins

Hashim, A. K., Najam, L. A., & Tettey-Larbi, L. (2015). A study of radon concentration in different brands tobacco cigarette in Iraqi market, influencing factors and lung cancer risk. *International Journal of Science and Technology*, *5*(10), 223-245

He, Y., von Lampe, K., Wood, L., & Kurti, M. (2015). Investigation of lead and cadmium in counterfeit cigarettes seized in the United States. *Food and Chemical Toxicology*, *81*, 40-45.

Hegde, V., & Nanukuttan, A. (2017). Comparison of nicotine concentration and pH of commercially available smokeless tobacco products. *Journal of Oral Research and Review*, *9*(1), 21- 39

HERAG fact sheet. (2007). *HERAG Assessment of Occupational Inhalation Exposure and Systemic Inhalation Absorption* (Issue August). Retrieved July 10, 2022, from http://www.icmm.com

Hodson, M. J., & Evans, D. E. (1995). Aluminium/silicon interactions in higher plants. *Journal of Experimental Botany*, *46*(2), 161-171.

Hong, Y., Song, K. (2014). Health effects of chronic arsenic exposure. *Ncbi.Nlm.Nih.Gov*. Retrieved July 5, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4186552/

Hostynek, J. J. (2006). Sensitization to nickel: etiology, epidemiology, immune reactions, prevention, and therapy. *Reviews on environmental health*, *21*(4), 253-280.

Jones, K., Morton, J., Smith, I., Jurkschat, K., Harding, A. H., & Evans, G. (2015). Human in vivo and in vitro studies on gastrointestinal absorption of titanium dioxide nanoparticles. *Toxicology Letters*, *233*(2), 95–101.

Inácio, M., Soares, S., & Almeida, P. (2017). Radon concentration assessment in water sources of public drinking of Covilhã's county, Portugal. *Journal of Radiation Research and Applied Sciences*, *10*(2), 135–139.

International Agency for Research on Cancer- IARC MonograpHs on the Evaluation of Carcinogenic Risks to Humans. (1988). *Man-made mineral fibres and radon*. *43*, 44–46.

Jaishankar, M., Tseten, (2014). Toxicity, mechanism and health effects of some heavy metals. *Ncbi.Nlm.Nih.Gov*. Retrieved July 5, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4427717/

Jia, L., Yu, Y., Li, Z., Qin, S., Guo, J., & Zhang., (2021). Study on the Hg0 removal characteristics and synergistic mechanism of iron-based modified biochar doped with multiple metals. Retrieved July 4, 2022, from https://www.sciencedirect.com/science/article/pii/S0960852421004259

Jin, X. C., Wagner, K. A., Melvin, M. S., Smith, D. C., Pithawalla, Y. B., Gardner, W. P., ... & Karles, G. D. (2022). Influence of nitrite on formation of tobacco-specific nitrosamines in electronic cigarette liquids and aerosols. *Chemical Research in Toxicology*, *35*(5), 782-791.

Jomova, K., & Valko, M. (2011). Importance of iron chelation in free radical-induced oxidative stress and human disease. *Current pharmaceutical design*, *17*(31), 3460-3473.

Jusko, T. A., Canfield, R. L., Henderson Jr, C. R., & Lanphear, B. P. (2005). Comments on "recent developments in low-level lead exposure and intellectual impairment in children". *Environmental health perspectives*, *113*(1), A16-A16.

Kerr, S. B., Bonczek, R. R., McGinn, C. W., Land, M. L., Bloom, L. D., Sample, B. E., & Dolislager, F. G. (1998a). *The risk assessment information system*. Retrieved July 5, 2022, from http://www.osti.gov/servlets/purl/663527-FKtAop/webviewable/

Kerr, S. B., Bonczek, R. R., McGinn, C. W., Land, M. L., Bloom, L. D., Sample, B. E., & Dolislager, F. G. (1998b). Retrieved July 4, 2022, from *The risk assessment information system*. http://www.osti.gov/servlets/purl/663527-FKtAop/webviewable/

Kiersma, M. E. (2014). National Institute for Occupational Safety and Health. *Encyclopedia of Toxicology: Third Edition*, 454–455. Retrieved July 10, 2022, from https://doi.org/10.1016/B978-0-12-386454-3.00340-7

Kazi, T. G., Jalbani, N., Arain, M. B., Jamali, M. K., Afridi, H. I., Sarfraz, R. A., & Shah, A. Q. (2009). Toxic metals distribution in different components of Pakistani and imported cigarettes by electrothermal atomic absorption spectrometer. *Journal of hazardous materials*, *163*(1), 302-307.

Kelleher, P., Pacheco, K., & Newman, L. S. (2000). Inorganic dust pneumonias: The metal-related parenchymal disorders. *Environmental Health Perspectives*, *108*(4), 685–696.

Khlifi, R. & Olmedo, P. (2015). Association between blood arsenic levels and nasal polyposis disease risk in the Tunisian population, *22*(18), 14136–14143.

Kazi, T. G., Jalbani, N., Arain, M. B., Jamali, M. K., Afridi, H. I., Sarfraz, R. A., & Shah, A. Q. (2009). Toxic metals distribution in different components of Pakistani and imported cigarettes by electrothermal atomic absorption spectrometer. *Journal of hazardous materials*, *163*(1), 302-307.

Kowalski, R., & Wierciński, J. (2009). Mercury content in smoke and tobacco from selected cigarette brands. *Ecological Chemistry and Engineering S*, *16*(2), 155–162 .

Landrigan, P. (2011). Children's vulnerability to toxic chemicals: a challenge and opportunity to strengthen health and environmental policy. *Healthaffairs.Org*, *30*(5), 842–850.

Lawler, T. S., Duncan, B. W., & Watson, C. H. (2017). Surveillance of Nicotine and pH in Cigarette and Cigar Filler HHS Public Access Author manuscript. *Tob Regul Sci*, *3*(1), 101–116.

Lawler, T. S., Stanfill, S. B., Zhang, L., Ashley, D. L., Clifford, H., & Branch, V. (2013). Chemical characterization of domestic oral tobacco products: Total nicotine, pH, unprotonated nicotine and tobacco-specific N-nitrosamines. *Food Chem Toxicol.*, *1*(770), 380–386.

Leikauf, G. D. (2002). Hazardous air pollutants and asthma. *Environmental Health Perspectives*, 110(4), 505–526.

Lindberg, A. L., Sohel, N., Rahman, M., Persson, L. Å., & Vahter, M. (2010). Impact of smoking and chewing tobacco on Arsenic-Induced skin lesions. *Environmental Health Perspectives*, *118*(4), 533–538.

Longest, P.W., & Xi, J. (2008). Condensational growth may contribute to the enhanced deposition of cigarette smoke particles in the upper respiratory tract. *Aerosol Science and Technology*, *42*(8), 579-602.

Luckett, B. (2012). Cadmium exposure and pancreatic cancer in south Louisiana. *Hindawi.Com*. Retrieved July 6, 2022, from https://www.hindawi.com/journals/jepH/2012/180186/

Lunell, E., & Lunell, M. (2005). Steady-state nicotine plasma levels following use of four different types of Swedish snus compared with 2-mg Nicorette chewing gum: a crossover study. *Nicotine & Tobacco Research*, *7*(3), 397-403.

Mahmood, T., & Zaman, M. (2010). Comparative Assessment of Total Nicotine Content of the Cigarette Brands Available in Peshawar, Pakistan. *Pakistan Journal of Chest Medicine*, *16*(3), 142

Maiyo, A. K., Kibet, J. K., & Kengara, F. O. (2023). A review of the characteristic properties of selected tobacco chemicals and their associated etiological risks. *Reviews on Environmental Health*, *38*(3), 479-491.

Maiyo, A. K., Kibet, J. K., & Kengara, F. O. (2023). A review of the characteristic properties of selected tobacco chemicals and their associated etiological risks. *Reviews on Environmental Health*, *38*(3), 479-491.

May, D., & Schultz, M. K. (2021). Sources and Health Impacts of Chronic Exposure to Naturally Occurring Radioactive Material of Geologic Origins. *Practical Applications of Medical Geology*,4(13), 403–428.

McAdam, K., Kimpton, H., Porter, A., Liu, C., Faizi, A., Mola, M., ... & Rodu, B. (2017). Comprehensive survey of radionuclides in contemporary smokeless tobacco products. *Chemistry Central Journal*, *11*, 1-20.

McParland, H., & Warnakulasuriya, S. (2012). Oral lichenoid contact lesions to mercury and dental amalgam—a review. *Journal of Biomedicine and Biotechnology*. Retrieved July 10, 2022, from https://www.hindawi.com/journals/bmri/2012/589569/

Marano, K. M., Liu, C., Fuller, W., & Gentry, P. R. (2018). Quantitative risk assessment of tobacco products: A potentially useful component of substantial equivalence evaluations. *Regulatory Toxicology and Pharmacology*, *95*, 371-384.

Stanfill, S., Tran, H., Tyx, R., Fernandez, C., Zhu, W., Marynak, K., ... & Watson, C. (2021). Characterization of total and unprotonated (free) nicotine content of nicotine pouch products. *Nicotine and Tobacco Research*, *23*(9), 1590-1596.

Moinester, M., & Kronfeld, J. (2014). *Health Impact of Uranium in Phosphate Fertilizers-Assessing the radiological impact of using Israeli phosphate fertilizers:*

A critical review. July 15, 2022, from https://www.osti.gov/etdeweb/biblio/22306689

Monika, S., Dineshkumar, T., Priyadharini, S., Niveditha, T., Priyadharshini, S. K., & Rajkumar, K. (2020). Smokeless Tobacco Products (STPs) Harbour Bacterial Populations with Potential for Oral Carcinogenicity. *Asian Pacific Journal of Cancer Prevention*, *21*(3), 815.

Montes, S., Riojas-Rodrı´guezrodrı´guez, H., Sabido-Pedraza, E., & Rıós, C. (2008). Biomarkers of manganese exposure in a population living close to a mine and mineral processing plant in Mexico. *Environmental Toxicology*, *106*, 89–95.

Moradnia, M., Attar, H. M., Heidari, Z., Mohammadi, F., & Kelishadi, R. (2021). Prenatal exposure to chromium (Cr) and nickel (Ni) in a sample of Iranian pregnant women: urinary levels and associated socio-demograpHic and lifestyle factors. *Environmental Science and Pollution Research*, *28*(44), 63412–63421.

Mosad, S. & Ghanem, A. (2010). Lens cadmium, lead, and serum vitamins C, E, and beta carotene in cataractous smoking patients. *Taylor & Francis*, *35*(1), 23–30.

Mudgal, V., Madaan, N., Mudgal, A., Singh, R. B., & Mishra, S. (2010). Effect of toxic metals on human health. *The open Nutraceuticals journal*, *3*(1), 94–99.

Mueller MM., (2006). Inflammation in epithelial skin tumours: old stories and new ideas. *European Journal of Cancer*, 42(5), 735–744.

Muller, C., Mazer, B., Salehi, F., Audusseau, S., Chevalier, G., Truchon, G., ... & Zayed, J. (2010). Urinary levels, tissue concentrations and lung inflammation after nose-only exposure to three different chemical forms of beryllium. *Journal of Applied Toxicology*, *30*(5), 411-415.

Mustpaha Al-Kabir, T. (2019). The use of smokeless tobacco and its health implication among residents in the tamale metropolis. Retrieved July 15, 2022, from www.udsspace.uds.edu.gh

Ndokiari, B., Holly, B., Tamuno-boma, O., & Julie, C. (2021). Inhalation Bioaccessibility of Potentially Toxic Metals in Tobacco Snuff and Related Exposure Risks. *Journal of Environmental Protection*, 237–248.

Nnorom, I. C., Osibanjo, O., & Oji-Nnorom, C. G. (2005). Cadmium determination in cigarettes available in Nigeria. *African Journal of Biotechnology*, *4*(10), 1128–1132.

National Academies of Sciences, Engineering, and Medicine. 2003. Bioavailability of Contaminants in Soils and Sediments: Processes, Tools, and Applications. Washington, DC: Retrieved November 15, 2023, from https://nap.nationalacademies.org/read/10523/chapter/1

National Cancer Institute and Centers for Disease Control and Prevention. (2014). *Smokeless Tobacco and Public Health: A Global Perspective | Division of Cancer Control and Population Sciences (DCCPS)*. Retrieved October 15, 2022, from https://cancercontrol.cancer.gov/brp/tcrb/smokeless-tobacco

National Research Council. (1989). Recommended Dietary Allowances: 10th Edition. Washington, DC: The National Academies Press. Retrieved March 1, 2023, from *Chemicals*. https://www.ncbi.nlm.nih.gov/books/NBK225659/

National Research Council (US), (2000). Subcommittee on Flame-Retardant Chemicals. *Calcium and Zinc Molybdates - Toxicological Risks of Selected Flame-Retardant* Retrieved March 1, 2023, from *Chemicals*. https://www.ncbi.nlm.nih.gov/books/NBK225659/

Nemeth, J. M., Liu, S. T., Klein, E. G., Ferketich, A. K., Kwan, M. P., & Wewers, M. E. (2012). Factors influencing smokeless tobacco use in rural Ohio Appalachia. *Journal of Community Health, 37*(6), 1208–1217.

Nicotinell. (2023). *How much nicotine do I receive from a cigarette?* Accessed on March, 2023 from https://www.nicotinell.co.uk/faqs/how-much-nicotine-is-in-a-cigarette.html

Occupational Safety and Health Administration (OSHA) (2017) Air Contaminants. 29 CFR 1910.1000 [82 FR 2735, January 9, 2017]. Web site accessed on April 4, 2023. Available at https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1000

Office of Health Improvement and Disparities. (2021). *Updated discussion paper on the bioavailability of nicotine and other ingredients from the use of oral nicotine pouches and assessment of risk to users*. Accessed on March, 2023 from https://cot.food.gov.uk/Updated%20discussion%20paper%20on%20the%20bioavailability%20of%20nicotine%20and%20other%20ingredients%20from%20the%20use%20of%20oral%20nicotine%20pouches%20and%20assessment%20of%20risk%20to%20users

OSHA. (2023). *Permissible Exposure Limits – OSHA Annotated Table Z-1 | Occupational Safety and Health Administration*. Accessed on October 7, 2022 from https://www.osha.gov/annotated-pels/table-z-1

Kelepertzis, E., Paraskevopoulou, V., Argyraki, A., Fligos, G., & Chalkiadaki, O. (2015). Evaluation of single extraction procedures for the assessment of heavy metal extractability in citrus agricultural soil of a typical Mediterranean environment (Argolida, Greece). *Journal of Soils and Sediments*, *15*, 2265-2275.

Owusu-Dabo, E., Lewis, S., McNeill, A., Gilmore, A., & Britton, J. (2009). Smoking uptake and prevalence in Ghana. *Tobacco Control*, *18*(5), 365–370.

Omari, M., Kibet, J., Cherutoi, J., Bosire, J., & Rono, N. (2015). Heavy metal content in mainstream cigarette smoke of common cigarettes sold in Kenya, and their toxicological consequences. *Int Res J Environ Sci*, *4*(6), 75-79.

Padmavathi, P., Damodara Reddy, V., & Varadacharyulu, N. (2009). Influence of chronic cigarette smoking on serum biochemical profile in male human volunteers. *Journal of Health Sciences*, *55*(2), 265–270.

Pappas, R. S., Stanfill, S. B., Watson, C. H., & Ashley, D. L. (2008). Analysis of toxic metals in commercial moist snuff and Alaskan iqmik. *Journal of analytical toxicology*, *32*(4), 281-291.

Pappas, R. S. (2011). Toxic elements in tobacco and in cigarette smoke: Inflammation and sensitization. *Metallomics*, *3*(11), 1181–1198.

Park, J. and Lee, B. (2020). Potential protective roles of curcumin against cadmium-induced toxicity and oxidative stress. *Taylor & Francis*, *24*(3), 95–118.

Pourkhabbaz, A. (2012). Investigation of toxic metals in the tobacco of different Iranian cigarette brands and related health issues. *Ncbi.Nlm.Nih.Gov*. Retrieved June 27, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3586865/

Production, Composition, Use and Regulations - Tobacco Smoke and Involuntary Smoking - NCBI Bookshelf (2004). Retrieved June 27, 2022, from https://www.ncbi.nlm.nih.gov/books/NBK316400/

Piano, M. R., Benowitz, N. L., Fitzgerald, G. A., Corbridge, S., Heath, J., Hahn, E., Pechacek, T. F., & Howard, G. (2010). Impact of smokeless tobacco products on cardiovascular disease: Implications for policy, prevention, and treatment: A policy statement from the American Heart Association. *Circulation*, *122*(15), 1520–1544.

Prabhakar, V., Jayakrishnan, G., Nair, S. V., & Ranganathan, B. (2013). Determination of trace metals, moisture, pH and assessment of potential toxicity of selected smokeless tobacco products. *Indian Journal of Pharmaceutical Sciences*, *75*(3), 262–269.

RAIS. (2022). *The Risk Assessment Information System*. University of Tennessee. Retrieved June 27, 2022, from https://rais.ornl.gov/

Robert Charles James, C. (2014). *Speciation of metals and metalloids in tobacco and tobacco smoke : implications for health and regulation*. Retrieved June 28, 2022, from https://research-repository.st-andrews.ac.uk/handle/10023/5728

Robinson, M. (2014). *Risk Assessment Toolkit*. California Department of Technology Retrieved June 27, 2022, from Website. http://www.cio.ca.gov/OIS/Government/risk/toolkit.asp

Robson, D., & McNeill, A. (2021). Tobacco Use Disorders. *Seminars in Addiction Psychiatry; Cambridge University Press*. Retrieved October 20, 2022, from https://books.google.com/books?hl=en&lr

Rahimzadeh, M. and Rahimzadeh, M. (2022). Aluminum poisoning with emphasis on its mechanism and treatment of intoxication. *Hindawi.Com*. Retrieved July 5, 2022, from https://www.hindawi.com/journals/emi/2022/1480553/

Regassa, G., & Chandravanshi, B. S. (2016). Levels of heavy metals in the raw and processed Ethiopian tobacco leaves. *SpringerPlus*, *5*(1), 1-9.

Rao, N. M. (2020). Air pollution effects on health. *Int J Sci Dev Res*, *5*(10), 110-115.

Richter, P., Faroon, O., & Pappas, R. S. (2017). Cadmium and cadmium/zinc ratios and tobacco-related morbidities. *International journal of environmental research and public health*, *14*(10), 1154.

Rickert, WS., & Kaiserman, MJ. (1994). Levels of Lead, Cadmium, and Mercury in Canadian Cigarette Tobacco as Indicators of Environmental Change: Results from a 21-Year Study (1968–1988). *Environmental Science and Technology*, *28*(5), 924–927.

Rodríguez, J., & Mandalunis, P. (2018). A review of metal exposure and its effects on bone health. *Journal of Toxicology*. Retrieved July 11, 2022, from https://www.hindawi.com/journals/jt/2018/4854152/

Rong, Q., Zhong, K., Huang, H., Li, C., Zhang, C., & Nong, X. (2020). Humic acid reduces the available cadmium, copper, lead, and zinc in soil and their uptake by Tobacco. *Applied Sciences*, *10*(3), 1077.

Rowbotham, A., Levy, L., & Shuker, L. (2000). Chromium in the environment: an evaluation of exposure of the UK general population and possible adverse health effects. *Journal of Toxicology*, *3*(3), 145–178.

Salas, A., Baeza, A., Guillén, J., Muñoz, A., & Muñoz, A. (2014). Removal of high levels of naturally occurring radioactivity from drinking water. *Drinking Water and Water Management*, *133*.

Samejo, S., Kazi, A. G., Afridi, H. I., & Kazi, T. G. (2019). Evaluate the effect of cadmium on levels of zinc in scalp hair and blood samples of smoker and nonsmoker psoriatic patients at different stage. *Environmental Science and Pollution Research*, *26*(31), 31763–31769.

Samet, J. M. (1989). Radon and lung cancer. *JNCI: Journal of the National Cancer Institute*, *81*(10), 745-758.

Samet, J. M. (2006). Residential radon and lung cancer: End of the story? *Journal of Toxicology and Environmental Health - Part A*, *69*(7–8), 527–531.

Schwaiblmair, M. & Behr, W. (2012). Drug induced interstitial lung disease. *Ncbi.Nlm.Nih.Gov*. Retrieved July 5, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3415629/

Sebiawu, G. E., Mensah, N. J., & Ayiah-Mensah, F. (2014). Analysis of heavy metals content of tobacco and cigarettes sold in Wa Municipality of Upper West Region, Ghana. *Chemical and Process Engineering Research*, *25*, 24-33.

Selman, M., (2003) The spectrum of smoking-related interstitial lung disorders: the never-ending story of smoke and disease. *Chest*, 124, 1185–1187

Health Services (2013). Toxicological Profile for. *Oxidative Medicine and Cellular Longevity*, *2013*(205), 24.

Sethi, T. K., El-Ghamry, M. N., & Kloecker, G. H. (2012). Radon and lung cancer. *Clin Adv Hematol Oncol*, *10*(3), 157-164.

Shekhawat, K., Chatterjee, S., & Joshi, B. (2015). Chromium toxicity and its health hazards. *International Journal*, *3*(7), 167–172.

Shelnutt, S. R., Goad, P., & Belsito, D. (2007). Dermatological toxicity of hexavalent chromium. *Critical Reviews in Toxicology*, *37*(5), 375–387.

Sheu, R., Stönner, C., Ditto, J. C., Klüpfel, T., Williams, J., & Gentner, D. R. (2020). Human transport of thirdhand tobacco smoke: A prominent source of hazardous air pollutants into indoor nonsmoking environments. *Science Advances*, *6*(10).

Silvestre, D.& Leme, F. (2016). Direct analysis of barium, calcium, potassium, and manganese concentrations in tobacco by laser-induced breakdown spectroscopy. *Elsevier*. Retrieved July 5, 2022, from https://www.sciencedirect.com/science/article/pii/S0026265X16000163

Skubacz, K., Wysocka, M., & Michalik, B. (2019). Modelling of radon hazards in underground mine workings. *Science of the Total Environment*. https://www.sciencedirect.com/science/article/pii/S004896971933801X

Statistical Service of Ghana. (2021). *Ghana 2021 population and housing census. Genera lreport*. Retrieved August 2, 2022, from https://statsghana.gov.gh/gssmain/fileUpload/pressrelease/2021%20PHC%20General%20Report%20Vol%203A_Population%20of%20Regions%20and%20Districts_181121.pdf

Stanfill, S., Tran, H., Tyx, R., & Fernandez, C. (2021). Characterization of total and unprotonated (free) nicotine content of nicotine pouch products. *Nicotine and Tobacco Research*. Retrieved July 4, 2022, from https://academic.oup.com/ntr/article-abstract/23/9/1590/6285126

Stephen, R. P. (2011). Toxic elements in tobacco and in cigarette smoke: inflammation and sensitization. *Academic.Oup.Com*. Retrieved July 4, 2022, from https://academic.oup.com/metallomics/article-abstract/3/11/1181/6016245

Sundblad, B., Ji, J. & Levänen, B. (2016). Extracellular cadmium in the bronchoalveolar space of long-term tobacco smokers with and without COPD and its association with inflammation. *Ncbi.Nlm.Nih.Gov*. Retrieved July 7, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4869628/

Syed M., F. (2012). study on the radon concentrations in tobacco in Jedda [Saudi Arabia] and the associated health effects. *Med. J. Islamic World Acad. Sci.*, *20*(3), 84–93.

Saha, N., Rahman, M. S., Jolly, Y. N., Rahman, A., Sattar, M. A., & Hai, M. A. (2016). Spatial distribution and contamination assessment of six heavy metals in soils and their transfer into mature tobacco plants in Kushtia District, Bangladesh. *Environmental Science and Pollution Research*, *23*(4), 3414–3426.

Saper, R. B., & Rash, R. (2009). Zinc: an essential micronutrient. *American family physician*, *79*(9), 768.

Skocaj, M., Filipic, M., Petkovic, J., Novak, S., Novak, S., & Stefan, J. (2011). Titanium dioxide in our everyday life; is it safe? *Radiol Oncol*, *45*(4), 227–247.

CDC., (2023). Smokeless Tobacco: Health Effects. Retrieved July 10, 2022, from https://www.cdc.gov/tobacco/data_statistics/fact_sheets/smokeless/health_effects/index.htm

Smokeless Tobacco and Some Tobacco-specific N-Nitrosamines. (2006). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. World Health Organizattion*, *89*, 641.

Stanfill, S. B., Connolly, G. N., Zhang, L., Jia, L. T., Henningfield, J. E., Richter, P., Lawler, T. S., Ayo-Yusuf, O. A., Ashley, D. L., & Watson, C. H. (2022). Global surveillance of oral tobacco products: Total nicotine, unionised nicotine and tobacco-specific N-nitrosamines. *Tobacco Control*, *20*(3), 1–10.

Szukalska, M., Merritt, T.& Lorenc, W. (2021). Toxic metals in human milk in relation to tobacco smoke exposure. Retrieved July 7, 2022, from https://www.sciencedirect.com/science/article/pii/S0013935121003844

Taylor, A. A., Tsuji, J. S., McArdle, M. E., Adams, W. J., & Goodfellow Jr, W. L. (2023). Recommended reference values for risk assessment of oral exposure to copper. *Risk Analysis*, *43*(2), 211-218.

Taylor, A., Dunn, K., & Turfus, S. (2021). A review of nicotine-containing electronic cigarettes—Trends in use, effects, contents, labelling accuracy and detection methods. *Drug Testing and Analysis*, *13*(2), 242-260.

TCEQ. (2017). TRRP Protective Concentration Levels. Texas Commission on Environmental Quality. Retrieved July 10, 2022, from: https://www.tceq.texas.gov/remediation/trrp/ trrppcls.html.

TCEQ. (2023). *TCEQ Toxicity Factors and Protection of Human Health - Texas Commission on Environmental Quality* - Retrieved July 10, 2022, from https://www.tceq.texas.gov/toxicology/faqs/tox

Thakur, S. S., Yadav, J., & Rao, D. (2012). Estimation of uranium isotope in urine samples using extraction chromatography resin. *Radiation Protection and Environment*, *35*(2), 77.

The German Federal Institute for Risk Assessment. (2021). *Health Risk Assessment of Nicotine Pouches* (Issue 042). Retrieved July 10, 2022, from https://mobil.bfr.bund.de/cm/349/health-risk-assessment-of-nicotine-pouches.pdf

Thewe, L. C., Nabuzoka, D., & Paul, R. (2020). Factors Associated with Snuff Usage and its Neurocognitive Effects among Women in Ndola Urban, Zambia. *Medical Journal of Zambia*, *47*(1), 25–32.

Tomar, S. L., & Henningfield, J. E. (1997). Review of the evidence that pH is a determinant of nicotine dosage from oral use of smokeless tobacco. *Tobacco Control*, *6*(3), 219-225.

Tomar, S. L., Hecht, S. S., Jaspers, I., Gregory, R. L., & Stepanov, I. (2019). Oral health effects of combusted and smokeless tobacco products. *Advances in dental research*, *30*(1), 4-10.

Tran, T. T., Chowanadisai, W., Crinella, F. M., Chicz-DeMet, A., & Lönnerdal, B. (2002). Effect of high dietary manganese intake of neonatal rats on tissue mineral accumulation, striatal dopamine levels, and neurodevelopmental status. *Neurotoxicology*, *23*(4-5), 635-643.

Tsuchiyama, F., Hisanaga, N., Shibata, E., Aoki, T., Takagi, H., Ando, T., & Takeuchi, Y. (1997). Pulmonary metal distribution in urban dwellers. *International Archives of Occupational and Environmental Health*, *70*(2), 77–84.

Tubaña, B. S., & Heckman, J. R. (2015). Silicon in soils and plants. *Silicon and plant diseases*, 7-51.

USEPA, (1989). *Risk Assessment Guidance for Superfund Volume 1 Human Health Evaluation Manual (Part a). Interim Final. United States Environmental Protection Agency, Office of Emergency and Remedial Response, Washington, DC EPA/540/ 1–89/002.* Retrieved July 20, 2022, from *https://www.epa.gov/sites/default/files/2015-09/documents/rags_a.pdf*

USEPA, (1991c). *Risk Assessment Guidance for Superfund Volume I – Human Health Evaluation Manual (Part B, Development of Risk-based Preliminary Remediation Goals) Interim. United States Environmental Protection Agency EPA/540/R-92/003.* Retrieved July 20, 2022, from *https://semspub.epa.gov/work/HQ/175137.pdf.*

U.S. Environmental Protection Agency, (2000). Science Policy Council Handbook: Peer Review, 2nd Edition, EPA 100-B00-001, Washington, DC: U.S. Environmental Protection Agency. Retrieved July 20, 2022, from https://www.epa.gov/sites/default/files/201510/documents/osp_risk_characterization_handbook_2000.pdf

USEPA, (2003). Memorandum, Human Health Toxicity Values in Superfund Risk Assessments. Michael B. Cook, Director of Superfund Remediation and Technology Innovation. United States Environmental Protection Agency OSWER Directive 9285.7–53. Retrieved July 20, 2022, from *https://www.ncbi.nlm.nih.gov/books/NBK215034/*

USEPA,(2005). Guidelines for Carcinogen Risk Assessment. United States Environmental Protection Agency. Retrieved July 20, 2022, from*: http://www2.epa.gov/osa/guidelines-carcinogenrisk-assessment.*

USEPA. (2006). Provisional Peer Reviewed Toxicity Values for Iron and Compounds. July 20, 2022, from https://cfpub.epa.gov/ncea/pprtv/documents/IronandCompounds.pdf

USEPA. (2007). *Framework for Metals Risk Assessment* (Issue March). Retrieved *July 20, 2022, from* https://www.epa.gov/sites/default/files/2013-09/documents/metals-risk-assessment-final.pdf

USEPA, (2009a). *Risk Assessment Guidance for Superfund Volume 1 Human Health Evaluation Manual (Part F, Supplemental Guidance for Inhalation Risk Assessment). United States Environmental Protection Agency, Office of Superfund Remediation and Technology Innovation, Washington, DC EPA-540-R-070–002.* Retrieved *July 20, 2022, from* https://rais.ornl.gov/documents/RAGS_F_EPA540R070002.pdf

USEPA, (2009b). Provisional Peer-reviewed Toxicity Values for Complex Mixtures of Aliphatic and Aromatic Hydrocarbons. United States Environmental Protection Agency. Retrieved *July 20, 2022, from: https://hhpprtv.ornl.gov/issue_papers/TotalPetroleumHydrocarbonsAliphaticHigh.pdf.*

USEPA. (2011*). Exposure Factors Handbook: 2011 Edition. United States Environmental Protection Agency. Available at:* Retrieved *July 20, 2022, from https://cfpub.epa.gov/ncea/risk/recordisplay.cfm? deid=236252.*

USEPA. (2014). *Office of Solid Waste and Emergency Response Directive 9200.1-120. Memorandum, Human Health Evaluation Manual, Supplemental Guidance, Update of Standard Default Exposure Factors. United States Environmental Protection Agency.* Retrieved *July 20, 2022, from: https://rais.ornl.gov/documents/OSWER_Directive_9200.1-120_ExposureFactors_corrected2.pdf*

USEPA. (2022). *Integrated Risk Information System (Online Database of Toxicity*

Values). United States Environmental Protection Agency. Retrieved *July 20, 2022, from* https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system

USFDA. (2013). *Toxicology Review of 905(j)(1)(A)(i) Report, Second Cycle Review of Additional Information, SE0003730, SE0003731. United States Food and Drug Administration* Retrieved *July 20, 2022, from* *https://www.fda.gov/media/79702/download*

USFDA. (2017). *Tobacco Product Standard for N-nitrosonornicotine Level in Finished Smokeless Tobacco. Proposed Rule. United States Food and Drug Administration Docket No. FDA-2016-N-2527. Retrieved July 20, 2022, from* *https://www.fda.gov/about-fda/economic-impact-analyses-fda-regulations/tobacco-product-standard-n-nitrosonornicotine-level-finished-smokeless-tobacco-products-proposed*

Veda, F. N., & Aïssata, D. (2022). Comparison of nicotine contents in local and imported cigarettes sold in Abidjan markets in Cte dIvoire: Lessons for regulation. *Journal of Toxicology and Environmental Health Sciences*, *14*(1), 1–9.

Valko, M., Jomova, K., Rhodes, C., & Kuča, K. (2016). Redox-and non-redox-metal-induced formation of free radicals and their role in human disease. *Archives of Toxicology, Springer*, *90*(1), 1–37.

Vengosh, A., Coyte, R. M., Podgorski, J., & Johnson, T. M. (2022). A critical review on the occurrence and distribution of the uranium-and thorium-decay nuclides and their effect on the quality of groundwater. *Science of The Total Environment*, *808*, 151914.

Verma, S., Yadav, S., & Singh, I. (2010). Trace metal concentration in different Indian tobacco products and related health implications. *Food and Chemical Toxicology*, *48*(8-9), 2291-2297.

Vivek, A., Kaushik, R. M., & Kaushik, R. (2023). Tobacco smoking-related risk for iron deficiency anemia: A case-control study. *Journal of Addictive Diseases*, *41*(2), 128-136.

Vlachou, C., Vejdovszky, K., Wolf, J., Steinwider, J., Fuchs, K., & Hofstädter, D. (2021). Toxicological approaches for the quantitative inhalation risk assessment of toxic metals from tobacco smoke: application on the deterministic and probabilistic inhalation risk assessment of cadmium for Austrian smokers. *Inhalation Toxicology*, *33*(4), 128–142.

WHO, (2009). *WHO Study Group on Tobacco Product Regulation. Report on the scientific basis of tobacco product regulation: third report of a WHO Study Group. World Health Organization Technical Report Series*. *Retrieved November 17, 2022, from* https://pubmed.ncbi.nlm.nih.gov/20942227/

Willers, S., Gerhardsson, L., & Lundh, T. (2005). Environmental tobacco smoke (ETS) exposure in children with asthma—relation between lead and cadmium, and cotinine concentrations in urine. *Respiratory medicine*, *99*(12), 1521-1527.

Wilk, A., Szypulska-Koziarska, D., & Wiszniewska, B. (2017). The toxicity of vanadium on gastrointestinal, urinary and reproductive system, and its influence on fertility and fetuses malformations. *Postepy Higieny*, *71*(10), 850–859.

World Health Organization, (2019). *Preventing disease through healthy environments: exposure to arsenic: a major public health concern*. *Retrieved November 17, 2022, from* https://apps.who.int/iris/bitstream/handle/10665/329482/who-ced-phe-epe-19.4.1-eng.pdf

Wilhelm, J., Mishina, E., Viray, L., Paredes, A., & Pickworth, W. B. (2022). The pH of Smokeless Tobacco Determines Nicotine Buccal Absorption: Results of a Randomized Crossover Trial. *Clinical Pharmacology and Therapeutics*, *111*(5), 1066–1074.

World Health Organization. (2021, February 2). *Radon and health fact sheets*. *Retrieved November 17, 2022, from* https://www.who.int/news-room/fact-sheets/detail/radon-and-health

World Health Organization. (2020). *Testing of tobacco and related products*. *November 17, 2022, from* https://www.who.int/publications/i/item/9789290227830

Wu, H., Liu, Q., Ma, J., Liu, L., Qu, Y., Gong, Y., ... & Luo, T. (2020). Heavy Metal (loids) in typical Chinese tobacco-growing soils: Concentrations, influence factors and potential health risks. *Chemosphere*,2020(*245)*, 125591.

Yoshihisa, Y., & Shimizu, T. (2012). *Metal Allergy and Systemic Contact Dermatitis: An Overview. Dermatology Research and Practice, 2012, 1–5.*

Younes, A., Alliot, C., & Mokili, M. (2018). Is Polonium-210 a Good Indicator for Anthropogenic Radioactivity? *Cancer Biotherapy & Radiopharmaceuticals*. Retrieved *November 17, 2022, from* https://www.liebertpub.com/doi/abs/10.1089/cbr.2018.2496

Zaga, V., Lygidakis, C., & Chaouachi, K. (2011). Polonium and lung cancer. *Journal of Oncology*. Retrieved *January 10, 2023, from* https://www.hindawi.com/journals/JO/2011/860103/

Zhang, S., Song, J., Cheng, Y., Liu, G., & Wallace, A. R. (2018). Trace metal(loid)s exposure through soil–tobacco–human pathway: Availability in metal-contaminated agricultural soils, transfer models and health risk assessment. *Ecotoxicology and Environmental Safety, 148, 1034–1041.*

Zhang, W. Z., Butler, J. J., & Cloonan, S. M. (2019). Smoking-induced Iron Dysregulation in the Lung. *Radic Biol Med*, *133*, 238–247.

Zhou, S., Rosenthal, D., Sherman, S., … J. Z.-… (2014). Physical, behavioral, and cognitive effects of prenatal tobacco and postnatal second-hand smoke exposure. Retrieved July 5, 2022, from https://www.sciencedirect.com/science/article/pii/"S1"538544214000492

Zinc; Public Health Statement: ATSDR. (2014). Agency for Toxic Substances and Disease Registry. Retrieved July 5, 2022, from: https://wwwn.cdc.gov/TSP/PHS/PHS.aspx?phsid=300&toxid=54

Zong, J., He, X., Lin, Z., Hu, M., Xu, A., Chen, Y., Zhao, G., Hu, B., Jin, Y., & Zou, C. (2022). Effect of two drying methods on chemical transformations in flue-dried tobacco. *Drying Technology*, *40*(1), 188–196.

APPENDIX

Table A Selected Tobacco products sold in Ejisu Municipality

Label	Type of Tobacco Product	Location	Manufacturer (Code)	Flavour
T1	ST (Snuff)	Besease	S1	Menthol
T2	ST (Snuff)	Besease	S1	Menthol
T3	ST (Snuff)	Besease	S1	Menthol
T4	ST (Snuff)	Besease	NA	Moringa
T5	ST (Snuff)	Besease	NA	Moringa
T6	ST (Snuff)	Besease	NA	Moringa
T7	ST (Snuff)	Donaso	S2	(NA)
T8	ST (Snuff)	Donaso	S2	NA
T9	ST (Snuff)	Donaso	S2	NA
T10	ST (Snuff)	Akyawkrom	S3	NA
T11	ST (Snuff)	Akyawkrom	S3	NA
T12	ST (Snuff)	Akyawkrom	S3	NA
T13	ST (Snuff)	Akyawkrom	NA	Moringa
T14	ST (Snuff)	Akyawkrom	NA	Moringa
T15	ST (Snuff)	Akyawkrom	NA	Moringa
T16	ST (Snuff)	Achinakrom	NA	Moringa
T17	ST (Snuff)	Achinakrom	NA	Moringa
T18	ST (Snuff)	Achinakrom	NA	Moringa
T19	ST (Snuff)	Kwaso	S1	NF
T20	ST (Snuff)	Kwaso	S1	NF
T21	ST (Snuff)	Kwaso	S1	NF
T22	ST (Snuff)	Essienimpong	NA	Moringa
T23	ST (Snuff)	Essienimpong	NA	Moringa
T24	ST (Snuff)	Essienimpong	NA	Moringa
T25	ST (Snuff)	Essienimpong	NA	NA
T26	ST (Snuff)	Essienimpong	NA	NA
T27	ST (Snuff)	Essienimpong	NA	NA
T28	ST (Snuff)	Onwe	S4	NA
T29	ST (Snuff)	Onwe	S4	NA
T30	ST (Snuff)	Onwe	S4	NA
T31	ST (Snuff)	Onwe	NA	Moringa
T32	ST (Snuff)	Onwe	NA	Moringa
T33	ST (Snuff)	Onwe	NA	Moringa
T34	ST (Snuff)	Ampabame	NA	Menthol
T35	ST (Snuff)	Ampabame	NA	Menthol
T36	ST (Snuff)	Ampabame	NA	Menthol
T37	ST (Snuff)	Ejisu Market	S2	Menthol
T38	ST (Snuff)	Ejisu Market	S2	Menthol
T39	ST (Snuff)	Ejisu Market	S2	Menthol

Label	Type of Tobacco Product	Location	Manufacturer	Flavour
T40	ST (Snuff)	Adadientem	S1	NF
T41	ST (Snuff)	Adadientem	S1	NF
T42	ST (Snuff)	Adadientem	S1	NF
T43	ST (Snuff)	Asotwe	S4	NA
T44	ST (Snuff)	Asotwe	S4	NA
T45	ST (Snuff)	Asotwe	S4	NA
C1	Cigarette	Ejisu Market	London Menthol	-
C2	Cigarette	Ejisu Market	Rothmans King Size	-
C3	Cigarette	Ejisu Market	London Filter	-
C4	Cigarette	Ejisu Market	Oris	-
C5	Cigarette	Ejisu Market	Pallmall Menthol	-
C6	Cigarette	Ejisu Market	Gold seal (Illicit)	-
L1	Dried Tobacco leaves	Ejisu Market	NA	-
L2	Dried Tobacco leaves	Ejisu Market	NA	-
L3	Dried Tobacco leaves	Ejisu Market	NA	-
L4	Dried Tobacco leaves	Ejisu Market	NA	-
L5	Dried Tobacco leaves	Ejisu Market	NA	-

NA= Unknown / Not Available NF= No flavour
S1= Power Snuff S2= KD Power
S3= Power Must Change Hands S4= Okatakyie

Table B Elemental concentrations of snuff products

LOCATION	MANUFACURER	FLAVOUR	ELEMENTS (µg/g)														
			Mo	Zr	Sr	U	Rb	Zn	Cu	Fe	Mn	V	Ti	Sc	Ca	K	S	TOTAL
BESEASE[1]	S1	MENTHOL	2.44	80.61	331.68	4.31	49.55	102.27	13.06	2852.02	0	0	628.84	177.4	39598.54	46647.25	3751.96	94239.93
BESEASE[2]	NA	MORINGA	0	48.68	161.34	0	156.58	61.37	6.11	1428.05	87.25	0	350.95	80.24	20509.13	166143.5	4050.33	193083.5
DONASO	S2	NA	2.92	93.65	314.53	3.53	39.85	91.9	9.86	4288.32	0	14.43	501.29	151.18	31837.68	34891.07	3161.07	75401.28
AKYAWKROM[1]	S3	NA	2.84	87.35	106.45	0	225.39	115.31	13.91	4478.39	0	18.07	386.27	29.25	11327.44	140349.6	4135.39	161275.7
AKYAWKROM[2]	NA	MORINGA	3.74	81.25	194.91	0	40.57	153.74	20.65	6786.84	24.06	15.14	853.59	124.2	31494.5	44858.54	2772.32	87424.05
ACHINAKROM	NA	MORINGA	0.98	65.41	201.08	7.43	197.25	78.82	0	2601.59	0	0	412.66	74.98	18479.62	131868.2	4301.32	158289.3
KWASO	S1	NO FLAVOUR	1.46	64.25	216.94	7.08	129.22	122.6	11.8	2455.7	58.31	0	561.42	119.16	28435.88	109737.2	5395.69	147316.7
ESSIENIMPONG[1]	NA	MORINGA	0	56.66	82.76	12.5	292.58	65.25	0	727.53	55.08	0	356.92	10.22	10017.61	242952.6	6621.87	261251.6
ESSIENIMPONG[2]	NA	NA	0	69.28	165.55	12.09	203.83	84.77	14.43	1889.62	49.02	4.36	507.03	113.6	24735.26	137136.3	7328.19	172313.3
ONWE[1]	S4	NA	0.84	62.45	175.95	12.59	212.07	113.55	16.01	1733.98	75.81	4.84	413.4	36.02	10828.25	216940.5	5592.8	236219.1
ONWE[2]	NA	MORINGA	3.04	88.21	263.28	1.75	56.98	130.29	25.54	8487.86	30.98	31.94	646.99	169.12	36712.24	41792.18	3250.72	91691.12
AMPABAME	NA	MENTHOL	0.71	85.94	305.65	3.06	45.96	97.63	31.15	4210.03	27.96	15.14	702.51	201.58	44364.62	52012.18	3705.69	105809.8
EJISU	S2	MENTHOL	2.94	46.89	260.58	7.3	64.94	128.73	23.16	3219.77	0	17.73	441.12	142.38	32281.88	40587.61	4661.33	81886.36
ADADIENTEM	S1	NO FLAVOUR	2.63	72.65	292.08	5.05	40.67	94.01	11.09	2867.79	48.15	10.77	369.83	131.88	27834.43	35555.29	2836.49	70172.81
ASOTWE	S4	NA	1.74	85.01	299.74	3.55	46.33	0	84.7	4137.78	0	10.7	489.7	172.22	37634.6	43662.42	3159.27	89787.76
		MEAN	1.8	72.6	224.8	5.35	120.1	96.02	18.8	3477.7	30.4	9.54	508.2	115.6	27072.8	99008.96	4315	135077.5
		SD	1.3	14.8	77.71	4.41	86.89	36.66	20.2	2046.8	30.4	9.39	145.9	58.20	10880.0	70337.18	1375.2	60714.51
		Min	0	46.9	82.76	0	39.85	0	0	727.53	0	0	351	10.22	10017.6	34891.07	2772.3	70172.81
		Max	3.7	93.6	331.6	12.5	292.5	153.7	84.7	8487.9	87.3	31.9	853.6	201.6	44364.6	242952.6	7328.1	261251.6

Elemental concentration (µg/g) of snuff products with different flavours

	Mo	Zr	Sr	U	Rb	Zn	Cu	Fe	Mn	V	Ti	Sc	Ca	K	S	TOTAL
Menthol	2.0	71.2	299.3	4.89	53.48	109.5	22.5	3427.3	9.32	11	590.8	173.8	38748.4	46415.68	4039.7	9397.87
Moringa	1.6	68.0	180.7	4.34	148.8	97.89	10.5	4006.4	39.5	9.42	524.2	91.75	23442.6	125523.0	4199.3	15834.79
NA	2.1	68.5	254.5	6.07	84.95	108.3	11.5	2661.8	53.2	5.39	465.6	125.5	28135.2	72646.25	4116.1	10874.48
NF	1.7	79.6	212.4	6.35	145.5	81.11	27.8	3305.6	25	10.5	459.5	100.5	23272.7	114596	4675.3	14699.94

Elemental concentration (µg/g) of snuff products with different manufacturers

	Mo	Zr	Sr	U	Rb	Zn	Cu	Fe	Mn	V	Ti	Sc	Ca	K	S	TOTAL
S1	2.93	70.27	287.56	5.42	52.4	110.32	16.51	3754.05	0	16.08	471.21	146.78	32059.78	37739.34	3911.2	7864.38
S2	2.18	72.5	280.23	5.48	73.15	106.29	11.98	2725.17	35.49	3.59	520.03	142.81	31956.28	63979.91	3994.71	10390.98
S3	1.21	70.78	196.37	5.26	141.96	95.98	13.98	3733.07	39.19	9.51	547.24	110.56	26616.14	116680.5	4575.78	15283.75
S4	2.84	87.35	106.45	0	225.39	115.31	13.91	4478.39	0	18.07	386.27	29.25	11327.44	140349.6	4135.39	16127.57
NA	1.29	73.73	237.85	8.07	129.2	56.78	50.36	2935.88	37.91	7.77	451.55	104.12	24231.43	130301.46	4376.04	16300.34

Table C Elemental concentration (μg/g) in dry tobacco leaves

	Elements												
Code	Mo	Zr	Sr	Rb	Zn	Fe	Mn	Ti	Sc	Ca	K	S	TOTAL
L1	4.3	44.17	181.71	27.16	39.12	1621.34	0	198.52	166.29	31644.4	48978.6	5157.86	88063.5
L2	2.48	79.69	240.42	20.64	97.64	1941.15	58.72	927.03	164.78	35683.6	54442.4	4895.77	98554.4
L3	2.74	43.17	222.65	43.28	26.43	1094.01	0	248.74	145.32	32609.8	42690.3	5010.2	82136.8
L4	5.87	42.54	233.12	27.79	61.24	953.74	0	252.46	111.84	22956.2	38604.4	3028.96	66278.1
L5	2.09	55.59	200.71	19.14	74.79	1670.59	0	630.23	128.98	28522.9	41146.4	4119.32	76570.8
mean	3.49	53.03	215.72	27.60	59.844	1456.16	29.36	451.39	143.44	30283.4	45172.4	4442.42	82320.8
SD	1.57	15.84	24.20	9.57	28.28	415.95	26.26	317.33	23.39	4827.37	6440.66	886.29	12113.76
max	5.87	79.69	240.42	43.28	97.64	1941.15	58.72	927.03	166.29	35683.6	54442.4	5157.86	98844.1
min	2.09	42.54	181.71	19.14	26.43	953.74	0	198.52	111.84	22956.2	38604.4	3028.96	66125.5

Table D Elemental concentration (μg/g) in cigarette products

	Elements												
Code	Mo	Zr	Sr	Rb	Zn	Fe	Mn	Ti	Sc	Ca	K	S	TOTAL
C1	5.14	4.18	128.22	29.73	36.13	417.97	0	62.76	87.47	22006.8	93199.58	3689.03	119667.01
C2	5.42	3.6	103.7	26.83	32.49	287.86	0	71.08	280.09	54829.5	116041	6053.28	177734.85
C3	6.93	5.76	103.74	29.94	38.47	389.61	0	38.65	165.73	24936.22	59405.78	3018.51	88139.34
C4	7.23	5.85	67.75	18.18	46.41	267.62	61.1	0	198.04	41779.62	52124.71	3039.93	97616.44
C5	7.68	4.91	125.44	35.55	41.72	405.1	0	0	202.63	34936.95	67605.98	3368.51	106734.47
C6	7.17	5.67	79.26	22.25	58.74	316.72	104.73	21.17	231.07	48314.31	63541.03	5684.32	118386.44
mean	6.59	4.99	101.3517	27.08	42.3266	347.48	82.915	48.415	194.17	37800.57	75319.68	4142.263	118046.4
SD	1.05	0.94	24.21	6.16	9.34	64.72	44.99	30.60	64.84	12957.5	24361.5	1364.70	31639.31
Min	5.14	3.6	67.75	18.2	32.49	267.6	61.1	21.17	87.47	22006.8	52124.7	3018.51	88139.34
Max	7.68	5.85	128.22	35.6	58.74	418	104.7	71.08	280.1	54829.5	116041	6053.28	177734.9

Table E Recommended parameters for exposure assessments of cigarettes and smokeless tobacco products

Parameter	Symbol	Value	Unit	Source
Cigarette consumption	CpD	10	cigarettes/day	This study
Smokeless tobacco consumption	STC	10	grams/day	This study
Oral Absorption factor	OAR	0.1%-100%	Unitless	Chemical-specific, literature
Inhalation Absorption factor	IAR	1	Unitless	(HERAG fact sheet, 2007)
Inhalation Rate	IR	20	m^3/day	USEPA (1991c, 2011)
Exposure frequency	EF	365	days/year	Maximum value
[1]Exposure duration, cigarettes	ED	57.5	Years	USFDA (2013)
[2]Exposure duration, smokeless tobacco	ED	51	Years	USEPA (1989, 2014); USFDA (2017)
Body weight	BW	70	Kg	(USEPA, 2014),
Averaging time, non-cancer, cigarettes	ATNC	20,988	Days	USEPA (1989, 2009a, 2014)
Averaging time, non-cancer, smokeless tobacco	ATNC	18,615	Days	USEPA (1989, 2009a, 2014)

[1]Assumes average lifespan of 70 years, as recommended by USEPA (1989, 2014) and per USFDA (2013), and user initiation at 12.5 years, per USFDA (2013).

[2]Assumes average lifespan of 70 years, as recommended by USEPA (1989, 2014), and tobacco use initiation at or near 19 years of age, as recommended by USFDA (2017) as quoted by (Marano et al., 2018)

Table F Toxicity factors for toxicants following the 3-Tier hierarchy as proposed by USEPA guidance documents (USEPA, 1989; 2003, 2005; 2009)

TOXICANT	RfD (mg/kg/day)	SOURCE	$OSHA_8$ values	RfC (mg/m^3)	SOURCE	OAF	SOURCE
Mo	5×10^{-3}	(USEPA, 2022)	0.5	0.002	(ATSDR *for Molybdenum*, 2020)	90%	(ATSDR *for Molybdenum*, 2020)
Sr	3×10^{-1}	(EPA, 2014)	-	0.002	(TCEQ, 2023)	38%	(USEPA, 2022)
U	3×10^{-3}	(USEPA, 2022)	0.05	4×10^{-5}	(ATSDR, 2018)	6%	(ATSDR, 2018)
Rb	4×10^{-3}	(Dodmane & Wesselkamper, 2016)	-	0.0025	(TCEQ, 2023)	100%	Dodmane & Wesselkamper, 2016)
Zn	3×10^{-1}	(USEPA, 2022)	5	0.002	(TCEQ, 2023)	30 %	(Lewis, 2019)
Cu	0.037	(Taylor et al., 2022)	1	0.1	(ATSDR, 2018)	50 %	(WHO, 2009)
Fe	7×10^{-1}	(USEPA, 2006) PPRTV	5	4×10^{-3}	MOEE,2008	15 %	(Lewis, 2019)
Mn	1.4×10^{-1}	(USEPA, 2022)	0.2	5×10^{-5}	(USEPA, 2022)	5%	(USEPA, 2022)
V	7×10^{-5}	(USEPA, 2022)	0.05	1×10^{-4}	(ATSDR, 2018)	20 %	Textbook of natural medicine (5^{th} ed), 2020
Ti	$4\times10^{+00}$	(Esai & Arbison, 2015)	2.4	3.0E-02	(Esai & Arbison, 2015)	2 %	(Jones et al., 2015)
Zr	8×10^{-5}	(USEPA, 2012)	5	0.005	(TCEQ, 2023)	20 %	(*RAIS*, 2022)

RfD=Reference dose for oral route of exposure   RfC=Reference concentration for exposure through inhalation
$OSHA_8$= Occupational Safety and Health Administration time weighted average for 8 hour exposure (OSHA, 2023)
OAF=Oral Absorption Factor