ANALYSIS OF SELECTED TOXIC HEAVY METALS AND PESTICIDE RESIDUES IN *Catha edulis* FROM SELECTED REGIONS IN MERU COUNTY, KENYA

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this university or any other for the award of a degree.

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Recommendation

This thesis has been submitted with our approval as University supervisors.

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DEDICATION

It is with sincere regards that I dedicate this work to my loving parents (Mr. Jephiter Keago Mogaka and Mrs. Milcah Gichemba Ogembo) and my sister Lilian Nyaboke Keago, who have been instrumental in helping me pursue the desires of my heart, motivated, and through their prayers, have propelled me to this level. I am deeply indebted to them.

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ABSTRACT

Heavy metal and pesticide pollutions on environmental matrices and their potential human toxicity have attracted considerable attention worldwide in recent years. The desire to increase food production to satisfy the ever rising population has resulted in the use of potentially toxic pesticides and fertilisers. The application of these chemicals in modern farming technologies is a source for potentially toxic heavy metals and pesticide residue levels in human consumables such as foodstuffs, which may ultimately trigger adverse health effects. Accordingly, this study focused on the concentration profiles of cadmium (Cd), lead (Pb), chromium (Cr), iron (Fe), nickel (Ni), and copper (Cu) and pesticide residues in 11 khat (*Catha edulis*) samples randomly sourced from 11 farms within regions of Meru County, Kenya. These analyses are meant to evaluate any associated human health risks through comparison with the World Health Organisation/ Food and Agriculture Organisation (WHO/FAO) safe limits. Human health risk assessment was done using target hazard quotient (THQ) and health index (HI). Inductively coupled plasma-atomic emission spectrometry (ICP-AES) analysis gave results of heavy metal concentrations (mg/kg) in dry khat samples as follows; Cd (7.81 ± 1.56), Cr (15.98 ± 2.22) and Pb (32.35 ± 9.95), indicating all was above the acceptable WHO/FAO limits. The levels of Ni, Cu and Fe heavy metals were below permissible limits and may benefit important human biological functions. The Pb and Cd THQ values and the HI of all investigated heavy metals in the khat samples exceeded the threshold value of 1.0. This suggests that the excessive consumption of Meru khat-based products, poses a potential health risk to the consumers. Besides, the gas chromatograph-mass spectrometry (GC-MS) results showed the detection and identification of pyrethroid and organophosphate pesticides representing 54.5% pesticide contamination prevalence rate of the sample size. The Ultraviolet-Visible (UV-Vis) quantification results revealed that acephate and cypermethrin residue levels in khat samples were below maximum residue limits (MRLs) thus, there is no acephate and cypermethrin pesticide contamination reported from this study. Consequently, Meru khat farmers should regularly be educated on safe post-harvest practices to protect consumers. Therefore, the results of this work are important in sensitizing khat farmers that the use of agro-chemicals should significantly be minimized or avoided on farms and encourage on alternative farming practices that do not potentially increase potentially toxic heavy metals and residues in khat. Regular monitoring and evaluation of pesticide residues and heavy metals in khat products is necessary to ensure public and environmental health.

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LIST OF ABBREVIATIONS AND ACRONYMS

AAS	Atomic absorption spectroscopy
AChE	Acetylcholinesterase
CAS	Chemical abstracts service
FAO	Food and Agriculture Organisation
GC/EI-MS	Gas chromatography/electron impact-mass spectrometer
GC-MS	Gas chromatography-mass spectrometry
HCA	Hierarchical cluster analysis
HI	Hazard index
HQ	Hazard quotient
HPLC	High performance liquid chromatography
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MRLs	Maximum residue limits
NACOSTI	National Commission for Science, Technology and Innovation
NIST	National Institute of Standard Technology
PCA	Principal component analysis
PCC	Pearson correlation coefficient
SPSS	Statistical package for social science
THQ	Target hazard quotient
UV-Vis	Ultraviolet-visible
WHO	World Health Organisation
XRF	X-ray fluorescence

CHAPTER ONE

INTRODUCTION

1.1 Background information

The use of psychoactive substances has been on the rise with the aim of seeking gratification to psychological and euphoric needs. For instance, the increase in khat (*Catha edulis*) consumption has been ascribed to the presence of addictive alkaloid chemicals. However, khat has faced health concerns across the world due to pesticide toxicity and heavy metal contamination (Atnafie *et al.*, 2021; Kassab & Moustafa, 2017). These toxins are believed to originate from natural sources and human activities including application of fertilisers and pesticides in khat farming. Accordingly, little or no research has been carried out to determine potentially toxic heavy metals and pesticide residues in khat plants collected from Meru County. Therefore, this study aims to determine concentration profiles of these metals including Cd, Fe, Ni, Cu, Pb, and Cr, and characterise pesticide residues including, acephate, cypermethrin, cyhalothrin, chlorfenvinphos, cyfluthrin, and chlorpyrifos in young khat shoot samples obtained from Meru County in Kenya and assess their associated non-carcinogenic health risks.

Basically, khat is both a dicotyledonous, and perennial plant found in East African nations including Kenya and Ethiopia and in the Arabian Peninsula, including Yemen and Saudi Arabia (Algabr *et al.*, 2014; Tadesse & Kebede, 2015). It has been shown to produce higher returns than other crops (Asmamaw & Tadesse, 2018), such as coffee and tea. In Kenya, khat is grown in the highlands of Meru County and for many decades its consumption has been on the rise in these regions. Locally, khat is known as miraa, gomba, veve, or muguka. Remarkably, it provides long-term livelihoods through high profits from khat farming and attractive agri-business ventures for most people (Carrier, 2005; Krueger & Mutyambai, 2020; Michuki & Kivuva, 2013). At the same time, it serves as the indigenous people's cultural identity, complete with rich historical traditions such as dowry negotiations, wedding ceremonies, and conflict resolution (Patel, 2015). The young leaves of khat, shoots, and soft stems are typically chewed because their alkaloid compounds offer amphetamine-like stimulating and euphoric qualities, which is the primary motive for their intake (Ademe *et al.*, 2020; Jayed & Al-Huthi, 2016).

According to research and literature, anthropogenic activities have elevated heavy metal levels in the environment to concentrations that pose etiological concerns (Artwell *et al.*, 2017). These negative impacts are serious for living species such as humans, who may consume contaminated edible plants, including khat (Atlabachew *et al.*, 2010). Khat like other plants, can accumulate heavy metals such as Cu, strontium (Sr), manganese (Mn), cobalt (Co), Ni, beryllium (Be), Pb, and cadmium (Cd) from their surroundings. Their composition in khat is mostly determined by their contents in soil and in the contaminated atmosphere (Al Bratty *et al.*, 2019), through frequent fertiliser usage, combustion of a scrap of biomass or waste rich in metals, and pesticide application (Ashenef *et al.*, 2014; Atlabachew *et al.*, 2010). According to research, both Ethiopian and Yemen khat products contain heavy metallic ions (Tadesse & Kebede, 2015; Yimer & Khan, 2015). Their bioaccumulation, particularly in the food chain, may cause a health concern by contaminating food and causing heavy metal poisoning (Kassab & Moustafa, 2017).

Modern farming is characterised by an overreliance on synthetic pesticides to quickly manage farm pests, hence enhancing global food supply and security (Krueger & Mutyambai, 2020; Zhang, 2018). Unfortunately, their widespread use has resulted in increased food and environmental contamination, putting human health at risk due to their chemical stability and refractory abilities (Adamu *et al.*, 2019; Bempah *et al.*, 2012). The khat plant's susceptibility to pest invasion, weeds, and sometimes poor growth necessitates the use of fertilisers to boost khat yield and pesticides to control pests (Atlabachew *et al.*, 2010; Weyesa, 2021). Generally, the consumption of khat parts is preferred when not rinsed, raw, and fresh thus exposing consumers to harmful organics such as pesticide residues and potentially toxic heavy metals (Al-Rajab *et al.*, 2016; Daba *et al.*, 2011). Hygienically, chewable khat leaves and shoots must be rinsed with clean running water before consumption to remove some of the residues (Ademe *et al.*, 2020; Hailemariam *et al.*, 2018). This healthy habit is critical because pesticides are toxic, persistent, and stable in the environment, making them hazardous to animals and humans (Masia *et al.*, 2015; Tiryaki & Temur, 2010).

Previous studies have demonstrated that pesticide use causes human poisoning, which poses a health risk to khat users and the general environment (Masoud *et al.*, 2012; Regassa & Regassa, 2018). As a result, World Health Organisation (WHO) recorded approximately 1-5 million pesticide poisoning cases per year, with over 20,000 fatalities (Karunamoorthi *et al.*, 2012; Weyesa, 2021). This poisoning has also resulted in the deaths of numerous non-target species, which is significant in agricultural settings (Hedlund *et al.*, 2020). It is thus critical to enact, monitor, and implement agricultural policies such as insurance schemes aimed at reducing the negative effects of pesticide use on the environment settings and human (Mohring *et al.*, 2020), as farmer safety must ultimately be prioritized (Damalas *et al.*, 2019).

Because application of pesticides in crop production is largely unregulated (Hassan *et al.*, 2016), farmers utilise a variety of fertilisers and pesticides to increase production to meet local and international khat market demand. Heavy metals including as Cu, Co, Pb, Fe, and Zn have been found in traces in edible khat products (Ashenef *et al.*, 2014; Desta & Ataklti, 2015). As a result, in one of its publications, WHO showed that metals such as Fe, Mn, Zn, and Cu can promote healthy growth and sustainable development. However, their levels should not exceed permissible levels, since this could lead to life-threatening disorders (Al Bratty *et al.*, 2019). To ensure safety, periodic determination of potentially toxic heavy metals, both macro and micro nutrient elements in foods, water bodies, and soils is critical for monitoring their concentrations and keeping them within permissible standards (Al Bratty *et al.*, 2019; Kohzadi *et al.*, 2019).

The analytical techniques including inductively coupled plasma optical emission spectrometry (ICP-OES), atomic absorption spectrometry (AAS) and ICP-AES which possess commendable characteristics such as high sensitivity, fast analysis and user friendly, can be used to detect and quantify the metallic elements in various samples (Gebeyehu & Bayissa, 2020; Llorent-Martínez *et al.*, 2011). The mathematical based statistical techniques including PCA and hierarchical cluster analysis (HCA) are useful in identification and evaluation of similar patterns in data, data reductions to simple interpretable forms, and assess sources of metallic elements (Bhuiyan *et al.*, 2011; Moura *et al.*, 2021).

1.2 Statement of the problem

The khat (*Catha edulis*) plant contains various natural chemicals and can adsorb and accumulate metal ions, pesticide residues, and other substances from the environment. The regular intake of this plant, particularly chewing, has increased due to the amphetamine-like stimulating and euphoric qualities of the alkaloid compounds that motivate consumers. However, over time, toxic heavy metals and persistent synthetic pesticide residues in khat extracts bio-accumulate in the consumer's body. They are believed to attack vital body organs such as kidneys, heart, and liver, among others, becoming contributing factors in cancer development, hypertension, and liver cirrhosis, among other serious health conditions. Studies in khat-growing regions worldwide have linked heavy metal poisoning to adverse health risks, especially when their concentrations exceed the WHO/ Food and Agriculture Organisation (FAO) allowable limits. The levels of these toxins in organisms increase as that organism's position increases in the food chain. The current study investigated heavy metal concentration profiles and pesticide residue levels in khat samples from Meru County, Kenya, and assessed the human health risk associated with their intake using WHO/FAO acceptable limits, THQ, and HI threshold values.

1.3 Objectives

1.3.1 General objective

To contribute to knowledge on potentially toxic heavy metals and synthetic pesticides found in consumed khat (*Catha edulis*) parts in selected regions of Meru County, and assess the human health risks associated with khat usage.

1.3.2 Specific objectives

i) To determine the concentration profiles of pesticide residues in samples of khat obtained from selected regions in Meru County.

ii) To determine the concentration of potentially toxic heavy metals (Pb, Ni, Cr, Fe, Cu and Cd) in khat samples collected from selected regions in Meru County.

iii) To assess health risks of selected heavy metals and pesticide residues in khat samples using hazard quotient, target hazard quotient and hazard index non-carcinogenic parameters.

1.4 Research Questions

i) How will the concentrations of pesticide residues in khat samples obtained from selected regions in Meru County compare with the threshold limits?

ii) What are the concentration profiles of heavy metals in khat samples collected from Meru County?

iii) What are the non-carcinogenic parameter values of hazard quotient, target hazard quotient and hazard index of heavy metals and pesticide residues in khat samples?

1.5 Significance of the study

Statistics show a rapid increase in consumption of locally cultivated khat among members of all segments of the population. Unregulated khat usage means the consumers are exposed to the potential health hazards arising from abuse or long-term use of khat. The potential adverse health effects of bioaccumulation of pesticide residues and toxic heavy metals are more likely to target perennial users. They include cancer cases, liver cirrhosis, and heart complications, among others. The focus on levels of these toxins and their characterisation in samples of khat originating from regions of Meru County has not been adequately addressed in the literature. Accordingly, a periodical determination is essential in monitoring their concentrations and maintaining them within permissible limits that guarantee the safety of consumers. This work has generated scientific information to fill the knowledge gap on the concentrations of potentially toxic heavy metals and pesticides in the khat cultivated in selected regions of Meru County, Kenya. This information aligns with the third sustainable development goal (SDG No. 3) of Good Health and well-being, which aims to promote the extension of life expectancy. Lastly, the study is important to khat farmers, consumers and policymakers since the levels of these toxins provided will inform on best agricultural practices that ensure the safety of these products to meet international standards.

1.6 Limitations of the study

The soil elemental analysis from selected khat farms where the samples were obtained was not investigated in this study because of limited financial resources. Factors such as age of the sampled khat plant and post-harvest period after pesticide applications were not studied since there was time constraints and unwillingness of farmers to disclose this information. The temporal season variations in all the regions of Meru County were not investigated. Challenges encountered during sampling include language barrier with some of the khat farmers and expensive khat given it is the main economic venture and income earner for most people. Some farmers were not willing to engage on dissemination of information regarding khat in terms of whether they apply fertilisers or the variety planted for fear of defaming their profitable khat products limiting customer preference. In some cases, it was necessary to use a local to facilitate sampling and to get relevant information since some farmers were reluctant to share with strangers' despite formal introduction making the process expensive.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Khat (*Catha edulis*) is a green plant whose tender soft leaves and young shoots are consumed to achieve euphoric and psychostimulative properties (Gezon, 2012). In general, the chemical content of this plant and the type of variation to be grown are determined by the growing region, prevailing meteorological conditions, and environmental suitability (Tadesse & Kebede, 2015). In most cases, khat variations vary in terms of size of leaves, their colour, and the height they possess (Atlabachew *et al.*, 2010). These characteristics commonly result in varying pharmacological adaptation levels (Hailemariam *et al.*, 2018). Due to high domestic demand and widened international market for khat, this product's yield is exported mainly for foreign exchange (Kandari *et al.*, 2014; Tadesse & Kebede, 2015). This has caused its prices and production levels to exponentially increase with an aim to meet the soaring khat needs (Gebrehiwot *et al.*, 2016). The Figure 2.1 shows khat shoots and tender leaves samples that are commonly chewed. Khat is not only a recreational tree with medical properties, but also a foreign exchange generator with economic significance. For example, its production provides more than 70% of personal income for farmers in the region of Oromia in Ethiopia (Kandari *et al.*, 2014).

Figure 2.1 Khat samples of tender leaves and young soft shoots

Chewing of khat is regarded both as a tradition and habitual social practice in communities that consume khat (Ayano *et al.*, 2019). In general, khat is utilised for religious, psychological, and recreational purposes, and it is well-known to possess medicinal benefits. Roots of khat and its leaves can be utilised for asthma treatment, influenza nursing, and to treat coughs (Yimer & Khan, 2015). In addition, khat infusion is utilised for the treatment of premature ejaculation in males and cure boils (Mohammed & Engidawork, 2011). Fresh tender leaves and young soft shoots of khat are preferably chewed for its euphoric effects, particularly in the Sub-Saharan African countries such as Djibouti, Kenya, Ethiopia, Eritrea, Somalia, and Uganda and in the middle east countries such as Yemen, Saudi Arabia, and Oman (Al-Motarreb *et al.*, 2002). Nevertheless, khat has spread to other nations, including Madagascar, Democratic Republic of Congo, USA, UK, and Netherlands, courtesy of immigrants. For example, in the early twentieth century, Yemeni dock workers brought khat to Madagascar, and it is now primarily grown in Northern Madagascar (Gezon, 2012).

Nevertheless, regular khat consumption, particularly chewing, has been linked to a higher risk of high blood pressure (Atlabachew *et al.*, 2011; Nigatu & Libsu, 2019), liver problems, and an elevated pulse rate. Chewing fresh khat and using contaminated khat products with either pesticide residues or potentially toxic heavy metals has been linked to an increased potential of cardiovascular problems including cardiac arrest and oxidative stress (Atnafie *et al.*, 2021; Kassab & Moustafa, 2017). Consumption of Cd-contaminated khat over time can result in bioaccumulation of this metal to elevated levels in the blood of a khat chewer leading to higher blood pressure (Garner & Levallois, 2017). Lead's non-biodegradability makes it potent in the human body for an extended length of time, potentially causing neuronal breakdown, hypertension, cancer lesions, cardiovascular disease. Accordingly, blood with Pb concentrations of between 40 ug/dL and 60 ug/dL causes serious health concerns except if immediate treatment is implemented (Flora *et al.*, 2012). As a result, this work is critical in articulating concerns such as potentially toxic heavy metals discovered in khat as a result of potential fertiliser, composite manure, and pesticide use. Heavy metal concentrations in khat products are under legal limits set by the WHO and the Food and Agriculture Organisation (FAO), however, bio-accumulation from khat consumption can pose health risks. Furthermore, this study provides a full account of life-threatening health conditions such as cancer disease, liver cirrhosis, and high blood pressure that have been linked to chewing of khat and its smoking. This has produced a possible health problem related to human poisoning caused by the use of agrochemicals and heavy metals that have bioaccumulated in khat parts.

On the contrary, because there is weak or no proper regulation on the use of pesticides, particularly in nations that are developing (Hassan *et al.*, 2016), some farmers use a variety of agrochemicals which may possess potentially toxic trace metals with an aim to increase khat production that will meet food demands in both domestic and international markets. The determination of potentially toxic heavy metals including chromium (Cr), cadmium (Cd), zinc (Zn), and lead (Pb) in consumed khat is extensively recognised in previous studies. Although their concentrations maybe low (Ashenef *et al.*, 2014; Atlabachew *et al.*, 2010; Nigatu & Libsu, 2019), their ongoing bio-accumulation and bio-magnification in biological systems may be detrimental.

2.2 Methodology

This review focused on English-language literature. Databases including Medley, published reports, Google Scholar, Cochrane, and Web of Science were used to search for pertinent topics, including khat plant, pesticides, chewing of khat, farming chemicals, potentially toxic heavy metals, various toxicities, cancer, high blood pressure, and liver damage. A detailed and reliable literature search was conducted online following the techniques outlined in prior survey of literature standards (Palmatier *et al*., 2018). The authors configured the following online databases to deliver regular notification updates of search results that met the established search criteria: Science Direct, Google Scholar, Cochrane, PubMed, and Medley. The data was kept on personal computers and analysed using bibliometric methods.

2.3 Main text

Apart from the potential adverse health risks related with substance misuse caused by igh intake of khat, there are possibly negative consequences related with methods of cultivation and practices that involve incorrect and/or inappropriate applications of pesticide. Chewing of khat is linked to major health problems like cancer, liver cirrhosis, and high blood pressure. This can be due in part to the excessive use of contaminated khat, which may contain farming pesticides and heavy metals (Geta *et al.*, 2019; Yang & Chen, 2021). The chemical ingredients of khat are undisclosed to most government officials and the khat-consuming population. Here, we look at the adverse health risks that consumers of khat experience as a result of habitual chewing of khat's young shoots and tender leaves contaminated with heavy metals and agricultural pesticides. However, the compounds present in khat cannot be connected to ailments such as high blood pressure, liver damage, and cancer on their own. This research provides adequate evidence that trace metal and pesticide absorption by the khat can have major health effects for the khat-consuming population. Notably, farmers' use of inexpensive pesticides has gained recognition as an effective way to control farm pests and increase global food supply (Bempah *et al.*, 2012; Daba *et al.*, 2011; Karunamoorthi *et al.*, 2012; Krueger & Mutyambai, 2020). This has led to both improved food yield and increased health issues in the same magnitude (Adamu *et al.*, 2019). For example, organochlorines pesticides are inexpensive, versatile against large populations of pests, but stay longer in the environmental surfaces and can cause serious health problems such as endocrine issues and brain damage (Bempah *et al.*, 2012).

It is crucial to highlight that khat tree is mostly vulnerable to insect-borne diseases, weeds, and pest attack, hence fertiliser and pesticide applications are required (Atlabachew *et al.*, 2010; Tadesse & Kebede, 2015). These pesticides are thought to ensure protection of khat trees against pest attack and disease (Hassan *et al.*, 2016), whilst fertilisers applied to improve their produce (Masoud *et al.*, 2012; Weyesa, 2021). Nevertheless, unregulated and indiscriminate pesticide applications might be dangerous due to their toxicity, environmental stability and persistence (Masiá *et al.*, 2015; Tiryaki & Temur, 2010). These substances, when exposed for long periods of time, can lead to major health concerns. They introduce trace metals into the soil and eventually entering khat plant tissues and are later absorbed into the biological organs and systems of consumers of khat, potentially causing injury (Matloob, 2003; Woldamanuel, 2019).
Some studies have demonstrated that pesticides can cause human poisoning, denaturation of physiologically necessary enzymes, and overall environmental contamination (Hassan *et al.*, 2016; Masoud *et al.*, 2012). According to the WHO, there are approximately 1-5 million pesticide poisoning cases each year, with over 20,000 deaths (Karunamoorthi *et al.*, 2012; Weyesa, 2021). These findings are consistent with elevated concentrations of pesticide toxicity in farm products including usable khat parts (Hassan *et al.*, 2016). Various pesticides commonly utilised in farming previously have been reported to contain significant levels of metals. For example, approximately 10% of chemical compounds ratified for use as fungicides and insecticides in the UK contained Pb, Zn, Hg, and Cu (Wuana & Okieimen, 2011).

The soils utilised for khat cultivation can be slightly acidic or alkaline in nature, with pH values approximated to range from 6.0 to around 8.8 (Desta & Ataklti, 2015). Naturally, soils contain metallic elements; thus, hazardous Cd, cobalt (Co), and Pb cations are absorbed and may bioaccumulate in various areas of the khat plant via active transport. Potentially toxic heavy metals accumulate in the soil structure because of modern agricultural methods, despite the fact that some of these elements occur naturally in soils. The trace metal contents in the soil may determine the plant's absorption levels. Particularly, elevated levels of potentially toxic heavy metals and other elements may penetrate the food chain, causing negative health impacts in living organisms including humans (Tadesse & Kebede, 2015).

2.4 Economic benefits of khat plant

Given that personal income benefits of khat plants are significant, farmers are increasingly encroaching on natural forests to produce this new cash crop. Moreover, because attractive economic rewards, khat growers have extended to use of pesticide compounds to increase its yield, which unfortunately pollutes the khat young shoots and tender leaves, which are typically demanded for the possess of amphetamine-like stimulating properties (Derso & Dagnew, 2019). Khat crop and its produce make a significant contribution to personal income from the sales and national GDP through exports (Patel, 2015) and taxation on local consumption. These products are connected with large demand, higher readiness to pay, and high profits for khat farmers, leading to greater flexibility of household economies of scale (Gezon, 2017; Yahya *et al.*, 2016). Farmers of khat and sectoral entities have demonstrated that this plant provides significantly superior returns than other well-known crops such as tea and coffee (Carrier, 2008). The cultivation of khat is essential in Yemen, Ethiopia, and Kenya because it supports both economic growth and providing farmers and dealers with a variety of direct or indirect job opportunities (Megerssa *et al.*, 2014; Njiru *et al.*, 2013; Teklie *et al.*, 2017). The growing khat market globally has forced farmers to utilise alternative agricultural practices to increase production in order to meet demand (Woldamanuel, 2019; Yimer & Khan, 2015). Furthermore, the high khat returns accrued were previously projected to be around 2.7 times greater than those from food crops including beans and maize (Feyisa & Aune, 2003).

Khat plant is dubbed "green gold" in some areas of Northern Madagascar, and its associated economic viability has made most khat households to purchase other essential products with large sums of cash from khat sales (Gezon, 2017). For instance, khat is one of Ethiopia's most lucrative exports, generating an estimated US$80 million in the year 2005 (Gebissa, 2008; Mekuria, 2018). Similarly, Somaliland collected nearly US$5.5 million from khat import duties in the year 2005, accounting for over 30% of the country's total budget. These estimates may have risen over time due to the legal position that khat has in certain nations. Important to note is that farmers in Mbeere sub-county region of Eastern Kenya earn approximately $20,000 annually (Njiru *et al.*, 2013). Khat accounts for almost one-third of Yemen's GDP (Lamina & Lamina, 2013). A bouquet of approximately 200 g of khat's tender leaves and young shoots could readily be acquired in the UK for around £3.00 about ten years ago (Pickering, 2010), however khat may now be purchased online. These statistics indicate that cultivation of khat is a profitable agribusiness, despite adverse health risks involved.

It is assumed that producers of khat have higher living standards particularly in terms of annual food spending against family income and can also meet all family demands (Feyisa & Aune, 2003), although this can be contested. Constant reports claim that other crop producers in Kenya are considering khat farming since it has a significantly higher income per year than other crops especially near known khat growing regions (Njiru *et al.*, 2013). This entails using contemporary farming methods to maximise khat yield. Khat growers are least influenced by seasonal weather variations, hence khat cultivation can help alleviate poverty and hunger (Ruder, 2018). The great prevalence of the use of khat has boosted daily rates of consumption due to substantial revenue gains, motivating farmers to employ any available unconventional tactics such as the use of illegal agricultural chemicals, to improve production. In general, farmers who cultivate khat, obtain greater pricing for its sale as compared to other commerce activities (Gudata *et al.*, 2019).

2.5 Prevalence of the consumption of khat

In some countries, including Djibouti, mostly men are strongly correlated with heavy khat usage, which undermines the society's economic and social position. As a result, regular khat users have reported issues with oral hygiene, such as dental caries (Bennani & Mohamed, 2021). In Ethiopia, the incidence of khat consumption is significant, particularly among students of the university which stands at around 23.22%. This has been a source of concern because it is propagated by factors including peer pressure, alcohol consumption, and family chewing habits (Al-Maweri *et al.*, 2018; Gebrie *et al.*, 2018). Similarly, the region of Jazan in Saudi Arabia has a 33.2% lifetime chewing of khat prevalence rate. Furthermore, Kenya's consumption of khat figures are relatively high because the plant has a favourable legal position, and its exports earns personal income and taxes as a source of revenue to the government which can spur its economic growth (Al-Maweri *et al.*, 2018; Ongeri *et al.*, 2019). Table 2.1 summarises the prevalence of khat among khat users across different countries (Gebrie *et al.*, 2018; Ongeri *et al.*, 2019).

Factors such as location and gender may contribute to the high incidence of khat use (Ageely, 2009). In some regions like Kisenyi in Uganda, it was revealed that factors including khat availability and family history of khat usage greatly influence the prevalence of khat consumption. It is important to note that social norms and variety in the culture of khat-growing regions have a significant impact on its prevalence (Abdinasir, 2013). For instance, the habit of chewing khat which is widespread in Yemen has proven to be integral activity of their social life, with research indicating that the number of women adopting this social routine is increasing (Sadeq-Ali & AlAkhali, 2017). It is emerging that consumed khat is chewed on a regular basis in Kenya because it keeps users busy, relieves stress and stimulates their brains (Njuguna *et al.*, 2013). Furthermore, khat parts have been consumed for so long in Harar, and its negative impacts are more noticeable than in other regions of Ethiopia due to its history of growing and usage (Gudata *et al.*, 2019). Particularly, in Ethiopia and other Eastern African nations, khat usage is a growing health concern with the lifetime rates of khat chewing, specifically among Ethiopian women, is largely associated with sociodemographic concerns (Yitayih & van Os, 2021).

Table 2.1: Prevalence rates of khat usage in various countries

Study country	Type of study	Population segment	Khat usage prevalence (%)
Ethiopia	Systematic review and meta-analysis	University students	23.22
Djibouti	Cross-sectional epidemiological survey	Patients (129)	48.1
Eastern region, Kenya	Cross-sectional household survey	individuals above 10 years	36.8
Jazan region, Saudi Arabia	Cross-sectional survey	students (15-25 years)	21.4

Kisenyi, Uganda	Cross-sectional study	male adults	5.53

2.6 Biological uses and medicinal benefits of khat

Khat may serve as an immunosuppressant substance. Despite being a well-established stimulant, the primary ingredient in khat, particularly cathinone with concentration of 0.625 to 1.25 mg/kg plays an important function in inhibiting unpleasant immune responses against disease-causing pathogens (Ketema *et al.*, 2015). Studies have shown that a khat chewer is believed to consume around 50 to 200 g of fresh khat per day, which is similar to an oral dosage of 5 mg amphetamine, resulting in a significant intake of cathine and cathinone (Attafi *et al.*, 2018), with potential health implications including hypertension, cardiac arrest, kidney failure, and stroke. Furthermore, a high khat intake exceeding 500 g per week has the potential to significantly reduce testosterone levels and affect sperm quality (Mohammed & Engidawork, 2011), as revealed in baboon study (Gorfu, 2006; Nyachieo *et al.*, 2013). It is especially crucial for expectant women since chewing of khat increases intimacy and, as a result, strengthens connections with their spouses while also allowing them to manage with stress associated with pregnancy (Mekuriaw *et al.*, 2020). The structural similarity of alkaloid compounds present in consumed khat, particularly cathinone, to amphetamine has showed immunomodulatory properties in immunosuppressive disorders including tuberculosis disease (Alvi *et al.*, 2014). Accordingly, mild khat usage promotes testosterone synthesis, which boosts sexual activity in men (Mohammed & Engidawork, 2011).

Table 2.2 summarises the known biological characteristics of khat extracts and the benefits they provide to biological tissues. Consequently, treatment with cathinone compounds is effective in the control of obesity, which is a human health concern for most individuals globally. Furthermore, research with animal models have shown that khat extracts can prevent weight gain by activating lipolysis in adipose tissues (Alshagga *et al.*, 2020). Interestingly, a study conducted on male albino mice which used khat extracts from leaves and shoots showed it’s antidepressant characteristics are useful in curing of asthmatic disorders (Alfaifi *et al.*, 2017). Khat's anti-oxidative activity make it a promising substance for medical uses and the food industry applications, with the ability to serve as an antioxidant capacity reference (Dudai *et al.*, 2006).

Table 2.2: Biological benefits of khat

Form of khat used	Study specimen	Property of khat samples	Perceived benefits of the body
Khat ethanolic extract	Animal models (male albino mice)	Antidepressant characteristics	treat diseases including asthma (Alfaifi *et al.*, 2017).
Fresh khat shoots	Pregnant women	Enhances intimacy	Relieves pregnancy related stresses and boosts intimacy (Mekuriaw *et al.*, 2020).
Fresh khat leaves	Adult mice	Reduce gain in body weight	Obesity management and reduction in body weight (Alshagga *et al.*, 2020).
Khat extract and cathinone	Swiss albino mice	Immunomodulatory roles	Helps in combating infectious diseases (Ketema *et al.*, 2015).
Khat extract and/or cathinone	Sprague–Dawley rats	Enhance production of testosterone levels	Enhance sexual activity (Mohammed & Engidawork, 2011).

2.7 Heavy metals in the samples of blood

Whole blood is the most preferred specimen for determining heavy metal exposures like, which ones are strongly bound to the intracellular protein molecules (Karri *et al.*, 2016; Keil *et al.*, 2011). Furthermore, this specimen is ideal for deteemining exposure to Hg metal since the most toxic Hg forms, including diethylmercury, and dimethylmercury, are not typically removed in urine (Anyanwu *et al.*, 2018; Keil *et al.*, 2011). Potentially toxic heavy metals in different settings of the environment do not degrade and can enter the animal and plant biological systems primarily through soil, water exposure, and air, eventually accumulating in human via the food chain, such as chewing of khat (Li *et al.*, 2019). A tangible evidence from literature demonstrates that exposure to a variety of heavy metals are likely to have major neurotoxic effects (Alrobaian & Arida, 2019; Cusick *et al.*, 2018; Karri *et al.*, 2016). For instance, exposure to lead metal is linked to intellectual deficits, neurodevelopmental delays, and higher chances of cognitive impairments, such as Attention Deficit Hyperactivity Disorder (ADHD) problems (Canfield *et al.*, 2005; Cusick *et al.*, 2018). Similarly, excess Mn results in neuronal cell damage (Yin *et al.*, 2018), whereas arsenic (As) toxicity in drinking water is dependent on dosage thereby, inhibiting antioxidant activity and increasing cognitive oxidative stress (Cusick *et al.*, 2018; Jomova *et al.*, 2011).

Children receiving blood transfusions, particularly those with reduced birth weight, are at a higher likelihood of exposure to lead (Bearer *et al.*, 2000). A reported study carried out in Dhaka, Bangladesh, examined the prospect of health concerns posed by potentially toxic heavy metals to the plastic industry workers and discovered that Pb metal was present in significant proportions in their blood samples as shown in Table 2.3 (Ahmed *et al.*, 2020). It is worth noting that the International Agency for Research on Cancer (IARC) classifies Cd element as a group 1 human carcinogen, whereas Pb metal is classified as a group 2A potential human carcinogenic (Ahmed *et al.*, 2020). As a result, changes in blood cells seen under a microscope or the deletion of thick lines in children's bones as seen on an X-ray are indicators of Pb poisoning instances (Wani *et al.*, 2015). In the year 2012, the Centres for Disease Control and Prevention (CDC) established high blood Pb levels for adults at 10.0 μg/dL and for children at 5 μg/dL of blood. It is believed that beyond these standards, the human system fails, and the effects can be catastrophic (Wani *et al.*, 2015). It is worth noting that Pb poisoning causes damages to the human heart, brain, kidneys, neurological system, and liver, with initial symptoms including headaches, dullness, irritability and memory loss (Flora *et al.*, 2012; Kinuthia *et al.*, 2020).

In France (Table 2.3), for example, the 106 subjects' whole samples of blood were collected and analysed for a variety of heavy metals due to their toxicity concerns in human, as well as to gain information for clinical concerns and also for public interest, and for utilisation in monitoring of industrial hygiene practices (Cesbron *et al.*, 2013). China has recently been a major contributor of heavy metal contamination in the world, owing to industrialization. As shown in Table 2.3, blood samples from residents of China's Pearl River delta contained a high amount of Pb (Li *et al.*, 2019). Lead poisoning, for example, is a serious environmental concern with serious consequences for the human body since lead toxicity affects practically every function in the body (Wani *et al.*, 2015). Although each metallic element has its own toxicology, there are common toxicity mechanisms such as oxidative damage, mimicry, and DNA adduct formation; for example, non-essential metallic elements may cause enzymatic damage, disruption of endocrine, and cellular damage (Keil *et al.*, 2011).

Heavy metals are classified as those with a specific density greater than 5 g/cm^3, including Pb, Hg, As, Cd, nickel and aluminium (Alissa & Ferns, 2011). Remarkably, as toxic load become a serious clinical concern, precise assessment is critical for not only determining interventions including recognised metal toxicity tests, which frequently depend on urine or blood samples and are well-known to be primarily important for acute exposure but undependable for human body toxic load (Pizzorno, 2016). Generally, heavy metal toxic load (HMTL) assesses the presence of potentially toxic heavy metals in the biological system and their impact on human health. It informs health authorities about the extent of harm to the body (Saha & Paul, 2018). Heavy metals are likely to be harmful as they are not metabolised and therefore, bioaccumulate in soft tissues of an organism (Alissa & Ferns, 2011; Jose & Ray, 2018). Their poisoning causes common symptoms including abdominal pain, nausea, shortness of breath, diarrhoea, body weakness, and confusion (Jarup, 2003).

Table 2.3: Selected concentrations of heavy metals in the blood samples from different countries

Country	Target group	Heavy metal in blood	Heavy metal level in blood (%)
Uganda	Children	Antimony	99.0
		Lead	97.0
		Manganese	36.4
		Cobalt	19.2
		Cadmium	17.0
		Copper	12.0
Bangladesh	Industrial	Lead	32.78 ± 9.47

	workers		
		Zinc	9.08 ± 1.95
		Nickel	1.42 ± 1.01
		Cadmium	1.08 ± 0.47
India	0 – 30 years	Arsenic	2.45 ± 1.72
		Lead	31.6 ± 12.5
		Mercury	1.31 ± 0.83
	31–45 years	Arsenic	2.05 ± 1.45
		Lead	29.8 ± 16.3
		Mercury	1.87 ± 1.42
	45 – 60 years	Arsenic	1.54 ± 1.00
		Lead	41.0 ± 12.7
		Mercury	1.52 ± 1.55
France	Adults	Arsenic	0.02
		Lead	2.26
		Mercury	0.05
		Cadmium	0.06
		Copper	7.00
China	Inhabitants of Pearl River delta	Zinc	11.50 ± 8.03

Copper	0.67 ± 0.09
Chromium	92.83 ± 32.63
Lead	158.84 ± 160.38
Mercury	1.19 ± 1.40
Antimony	1.92 ± 1.09

2.8 Heavy metals in khat products

Basically, heavy metals are generally non-biodegradable, making them potential candidates to cause long-term toxicity in both humans and animals (Al Bratty *et al.*, 2019). These metallic elements in khat products may develop as a result of regular fertiliser use, metal scrap incineration (Ashenef *et al.*, 2014), and pesticide application faming practise (Atlabachew *et al.*, 2011). These metals can also be taken up by the khat plants through polluted soil (Al Bratty *et al.*, 2019). Finally, long-term adverse health concerns from exposure of heavy metals in both environmental and occupational set ups include multi-organ toxicities such as disruptions of nervous system, immune system dysregulation, cognitive breakdown, carcinogenesis, and homeostatic disruptions (Offor *et al.*, 2021). Previous studies found that consumed Ethiopian khat contained heavy metals including Zn, Fe, Cr, Cu, and Co (Tadesse & Kebede, 2015; Yimer & Khan, 2015). Similarly, a study conducted in Yemen found that khat had substantial amounts of Pb, Zn, Cd, and Cu elements (Matloob, 2003). This is because khat plant can gather both essential elements needed for growth and non-essential trace heavy metals that play no function in growth and development (Atlabachew *et al.*, 2011). These elements can be analysed in different samples of khat using analytical methods including X-ray fluorescence spectroscopy (XRF), inductively coupled plasma-mass spectroscopy (ICP-MS) (Al Bratty *et al.*, 2019), and flame atomic absorption spectrometry (FAAS) (Woldamanuel, 2019; Yimer & Khan, 2015).

Cultivation of both food and cash crops such as khat plant, vegetables and fruits in areas that are polluted is routinely practised by farmers utilising fertilisers and illegal agrochemicals to maximise output. This may result in a gradual heavy metal accumulation in the African environment, perhaps exceeding WHO permitted standards (Anyanwu *et al.*, 2018). Potentially toxic heavy metals including Hg, Pb, and As are well-known systemic contaminants that are harmful to human; these metallic elements are becoming more prevalent in environmental matrices as a result of chemicalized agricultural activities and industrialization, resulting in metal bioaccumulation in the human biological system (Jose & Ray, 2018). Table 2.4 displays the analysis of Hg, Pb, and As levels in samples of blood from the specified individuals to determine how age affects heavy metal accumulation (Chowdhury *et al.*, 2018; Dobson *et al.*, 2004; Sane *et al.*, 2018; Tinkov *et al.*, 2021).

According to Table 2.4, plants such as khat can bioaccumulate trace heavy metals and essential elements from environmental matrices, with the former providing no direct benefit to plants (Atlabachew *et al.*, 2011). Soils enhanced with fertilisers contain more heavy metals than the natural soils. Pesticide application in a proportional manner raises their level in soils, increasing the probability of plant uptake and subsequent entry into the food chain. It is important to note that, heavy metal concentrations in plants, such as khat, is influenced by factors including soil level, geographical variations, pesticide and fertiliser applications, prevailing climatic conditions (Atlabachew *et al.*, 2011), and related atmospheric deposition (Tadesse & Kebede, 2015).

Despite the fact that khat tender leaves and young shoots are regularly consumed to attain mental stimulation, long-term misuse has been linked to a variety of health concerns and diseases caused by the deposition of leftover chemicals and metals in the human blood system. According to research, khat has the capacity to bioaccumulate heavy metals in its tissues when growing (Jose & Ray, 2018). As a result, long-term usage may predispose regular chewers of khat to a variety of serious health issues associated with heavy metals buildup, toxicity concerns, and/or poisoning cases.

Table 2.4: Health effects associated with toxicities of heavy metals

Heavy metals determined in khat	**Health effects**	**Heavy metal physiological symptoms**
Pb, Cd, Cu	Cardiovascular diseases	Blurred vision, increased heart rate, dry mouth (Chowdhury *et al.*, 2018).

Cu, Mn, Fe, Co, Cd	Gastrointestinal disorders	Diarrhea, nausea, vomiting, abdominal pain (Li *et al.*, 2019; Pizarro *et al.*, 1999; Sane *et al.*, 2018; Tinkov *et al.*, 2021).
Pb	Reproductive problems	Impede fetal growth and fertility disorders (Kumar *et al.*, 2020).
Cd	Carcinogenic effects	Tumor growth (Genchi *et al.*, 2020).
Pb, Cu, Mn, Fe	Neurological dysfunction	Affect intelligence quotient in children, mental breakdown, behavioral disorders and suicide attempts (Dobson *et al.*, 2004; Organization, 2019; Sane *et al.*, 2018; Santos *et al.*, 2019).

Table 2.4 summarises relevant surveys, clinical diagnostics, and studies on the unfavourable health consequences linked to heavy metal toxicity (Genchi *et al.*, 2020; Santos *et al.*, 2019). Heavy metal deposition and toxicity have been linked to a variety of health effects, including cardiovascular illness, digestive diseases, reproductive disorders, malignancies, and neurotoxicity. The physiological symptoms associated with the biological impacts are presented in Table 2.4. Regular chewers consuming significant amount of khat may be more susceptible to harmful biological effects after long time (Kumar *et al.*, 2020). Table 2.5 displays heavy metal contents in various samples of khat, as well as the recommended daily allowance (RDA) and provisional tolerable weekly intake (PTWI) in mg for some potentially toxic heavy metals, as given by the WHO and FAO (Al Bratty *et al.*, 2019; Woldamanuel, 2019). When these metal concentrations do not exceed PTWI and RDA levels, no detrimental health effects are documented, yet bio-accumulation in bodily tissues can be harmful (Woldamanuel, 2019).

High quantities of Zn and Fe can be found in khat-growing soils, which can be both acidic and alkaline, with approximate pH values ranging between 5.6 and 7.3 (Atlabachew *et al.*, 2011). Iron's high level is connected with elevated organic matter levels in the soil as well as human activities (Desta & Ataklti, 2015). As a result, increased Fe levels in khat can damage the liver and heart (Ashenef *et al.*, 2014). Copper levels in khat plant are regulated by soil concentrations

(Woldamanuel, 2019). Previous research found Pb emissions from car exhaust, Cd emissions from incineration techniques, and the use of manure and fertilisers as other major sources of Cd and Pb, resulting in very high amounts in khat produce (Ashenef *et al.*, 2014; Woldamanuel, 2019). Long-term exposure to Cd causes liver cirrhosis (Kang *et al.*, 2013). The WHO/FAO PTWI and RDA levels for metallic elements do not pose adverse health risk when consumed in moderation, approximately 100 g per day of khat parts; or else, exceeding this limit may cause negative health effects (Tadesse & Kebede, 2015; Woldamanuel, 2019) on regular adult chewers. Accordingly, khat chewing is a precursor to possible metal poisoning instances (Tadesse & Kebede, 2015). Table 2.5 summarises the levels of several heavy metals discovered in khat and their acceptable limits (Al Bratty *et al.*, 2019; Ashenef *et al.*, 2014; Tadesse & Kebede, 2015; Woldamanuel, 2019).

The detection of Cd and Pb heavy metals, which are typically introduced either anthropogenically or naturally to promote khat development, has the potential to have serious health problems (Ashenef *et al.*, 2014). For example, Cd is toxic and can cause persistent poisoning in the human body. In addition, it attaches to metallothioneins to form a Cd-metallothionein complex, which is carried to all organs and causes substantial cell injuries and later damage. Cadmium is primarily deposited in the liver, lungs, pancreas, and kidneys (Suhartono *et al.*, 2014), and it is thought to enter biological structures by cutaneous contact, ingestion mode and inhalation (Wu *et al.*, 2016).

Table 2.5: The concentrations of heavy metals in samples of khat grown in selected countries and the WHO/FAO limits

Heavy metal	Concentration range of heavy metal in khat samples	Country of study	WHO/FAO recommended values
Pb	0.18 ± 0.87 mg/kg	Jazan, Saudi Arabia	1.75 mg PTWI (Al Bratty *et al.*, 2019).
	0.18 ± 0.87 mg/kg	Ethiopia	1.75 mg PTWI (Woldamanuel, 2019).
	0.97 ± 0.37 µg/g	Ethiopia	214 µg RDA (Ashenef *et al.*, 2014).
Cd	ND	Ethiopia	2 µg/g PL (Tadesse & Kebede, 2015).
	0.00–0.08 mg/kg	Jazan, Saudi Arabia	0.49 mg PTWI (Al Bratty *et al.*, 2019).
	0.15 ± 0.90 µg/g	Ethiopia	0.49 mg PTWI (Woldamanuel, 2019).
	0.87 ± 1.44 µg/g	Ethiopia	60 µg RDA (Ashenef *et al.*, 2014).
Mn	ND	Ethiopia	0.02 µg/g PL (Tadesse & Kebede, 2015).
	339.63–1284.43 mg/kg	Jazan, Saudi Arabia	6.68 mg PTWI (Al Bratty *et al.*, 2019).
	11.64 ± 4.28 µg/g	Ethiopia	2000 µg RDA (Ashenef *et al.*, 2014).
Co	0.51–1.73 mg/kg	Jazan, Saudi Arabia	1.33 mg PTWI (Al Bratty *et al.*, 2019).

Cu	254.60–1757.37 mg/kg	Jazan, Saudi Arabia	245 mg PTWI (Al Bratty *et al.*, 2019).
	0.10–41.80 µg/g	Ethiopia	245 mg PTWI (Woldamanuel, 2019).
Zn	NG	Ethiopia	10 µg/g PL (Tadesse & Kebede, 2015).
	7.17 ± 1.11 µg	Ethiopia	3000 µg RDA (Ashenef *et al.*, 2014).
	351.95–1240.93 mg/kg	Jazan, Saudi Arabia	490 mg PTWI (Al Bratty *et al.*, 2019).
	25.15–73.95 µg/g	Ethiopia	490 mg PTWI (Woldamanuel, 2019).
Fe	6.80–8.96 µg/g	Ethiopia	50 µg/g PL (Tadesse & Kebede, 2015).
	529.13 ± 350.03 µg	Ethiopia	10,000 µg RDA (Ashenef *et al.*, 2014).

RDA: recommended daily allowance by WHO/FAO, PTWI: provisional tolerable weekly intake, PL: plant limit as per WHO, ND: not detectable, NG: not given

Children and young people need to be discouraged from using khat since determination of Pb in it may have negative impact on their mental health, growth and development (Woldamanuel, 2019). The heavy metal concentration in khat crops, particularly the leaves, is determined by agricultural practices such as fertiliser use, soil chemical composition, meteorological conditions, and location (see Table 2.5) (Tadesse & Kebede, 2015). Nevertheless, the majority of the health concerns experienced by the khat-consuming population are caused by alkaloid chemicals in khat and some associated with pesticides sprayed on khat plants, especially chewable tender leaves and young shoots (Nigatu & Libsu, 2019). Figure 2.2 depicts the main alkaloids of khat.

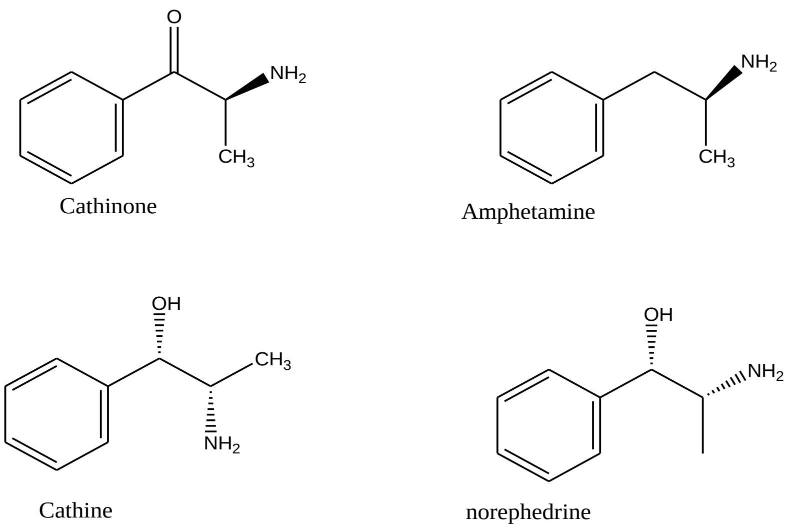


Figure 2.2 Chemical structures of natural psychostimulants in khat

2.9 Common pesticides used in farms of khat and their health effects

Pesticides are chemicals that are routinely used on farms to control pests and reduce disease infestations (Richardson *et al.,* 2019; Weyesa, 2021), as well as growth regulators and boosters (Regassa & Regassa, 2018). These pesticidal chemicals can be designed as liquids, dusts, or pellets (Weyesa, 2021). In addition, they are classed according to the target pests, such as rodenticides (rodents), herbicides (weeds), and fungicides (moulds and fungus), insecticides (insects), (Meftaul *et al.,* 2020). Furthermore, classification can be based on the application techniques and chemical identification. Pesticides serve as the main pillar of agriculture, particularly in highly mechanised farms (Richardson *et al.,* 2019). Consequently, these chemicals can enhance the quantity of production, quality, and yield, which is why they are so widely used. Their use and manufacturing are regulated globally by government authorities and regulatory agencies (Hassan *et al.,* 2016; Meftaul *et al.,* 2020), despite the fact that their contaminating impacts are severe worldwide. Since they are highly toxicity, can also affect non-target pests including birds and honeybees, posing environmental health effects. The susceptibility of khat plants to attack by pests and assault is the primary reason why farmers of khat have changed their focus to regular pesticide use in farms of khat in order to increase returns and prevent farm produce loss (Regassa & Regassa, 2018; Weyesa, 2021).

Previous research has indicated that farmers of khat use pesticides such as dichlorodiphenyltrichloroethane (DDT), malathion, wuhagare, actelic, sevin, and organophosphates to dissuade pests from attacking khat plants (Ademe *et al.,* 2020; Regassa & Regassa, 2018). The inexpensive and high efficacy of a pesticide justifies its widespread usage, regardless of its illegal status and accompanying environmental problems (Regassa & Regassa, 2018; Weyesa, 2021). Previous research carried out in Ethiopia revealed detection of diazinon organophosphate and DDT organochlorine pesticides in samples of khat, as well as their link into causing fatalities. Malathion, DDT, and actelic pesticides, as depicted in Figure 2.3, are frequently sprayed on consumed crops to suppress pest infestation. Malathion has been shown in experimental animal models to cause micronuclei damage and chromosomal aberrations (Daba *et al.,* 2011; Hassan *et al.,* 2016). Voluntary disregard of pesticide application practices can result in pesticide poisoning and related deaths, with khat growers being the most vulnerable (Regassa & Regassa, 2018).

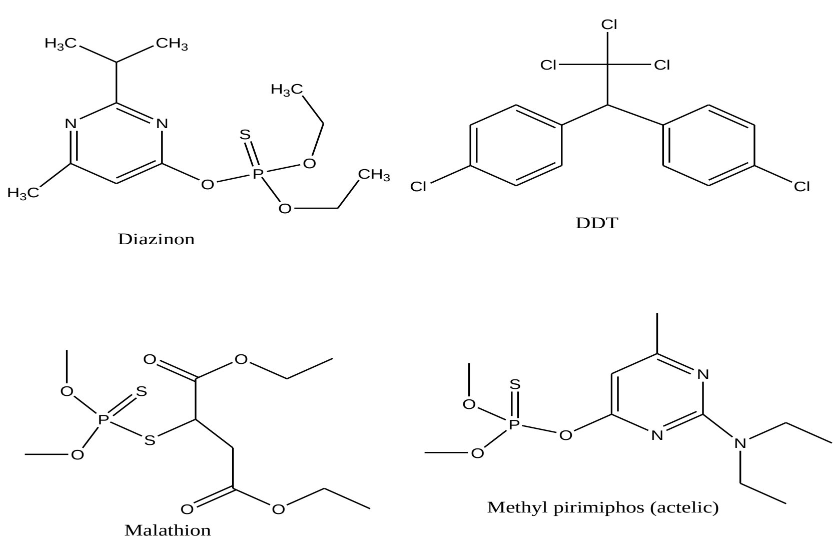


Figure 2.3 Pesticidal chemicals commonly used in farming of khat

Some of the pesticidal chemicals are carcinogenic (Weyesa, 2021), and their chronic exposure can cause endocrine disruptions and genotoxic effects (Hassan *et al.*, 2016; Swaddiwudhipong *et al.*, 2010), and immune system dysfunction (Karunamoorthi *et al.*, 2012). Furthermore, pesticide use is linked to ailments like respiratory problems, digestive system abnormalities, and liver disease (Regassa & Regassa, 2018). In addition, there have been reports of eye irritations, headaches, and elevated heart rate after chemicals spraying when not wearing protective equipment (Weyesa, 2021). Furthermore, skin irritations, sadness, breathing problems and dizziness have been documented in the previous studies (Derso & Dagnew, 2019). Some users experienced stomach irritation after eating pesticide-contaminated khat (Regassa & Regassa, 2018).

Table 2.6 presents an overview of the potential adverse health risks connected with certain agricultural chemical pesticides typically used in production of khat (Colovic *et al.*, 2013; Lemaire *et al.*, 2006; Nicolopoulou-Stamati *et al.*, 2016; Park & Dublin, 2011; Tchounwou *et al.*, 2015). Clinical research has also demonstrated that chemicals naturally produced by khat plant can enter the human blood plasma and digestive tract after chewing the leaves and young shoots (Widler *et al.*, 1994). Organic pesticides may survive through bioaccumulation mechanisms and can be deposited as residuals in the circulatory system after extended khat use.

Previously conducted studies revealed that concentrations of pesticide residues such as DDT, diazinon and aldrin in chewable khat from specific khat-growing farms (Adamu *et al.*, 2019). This signals that consuming an excessive amount of farmed khat may result in the accumulation of these residues in human organs, disrupting normal biochemistry and metabolic functioning (Nicolopoulou-Stamati *et al.*, 2016). This can have negative impact on health if consumed in excess of the permissible levels, as documented in literature (WHO, 2013). Generally, DDT is an artificial chemical created in 1940s primarily for use as an insecticide (pesticide) (Hussein, 2015). Technical-grade DDT can contain DDE (dichlorodiphenyldichloroethylene) and DDD (dichlorodiphenyldichloroethane) (Scheme 2.1), which are DDT breakdown products (Fossi *et al.*, 2014). The DDT is a persistent, deadly toxin that accumulates in live organisms' tissues (Hussein, 2015). The Stockholm Convention prohibits its usage in farming, but it is nonetheless utilised in developing nations, notably in grain production. In nations where DDT is still used, most of it is used as an insecticide (Ali *et al.*, 2014). It can reach the atmosphere through vapourisation and pollute surface water via soil runoff (McKnight *et al.*, 2015).

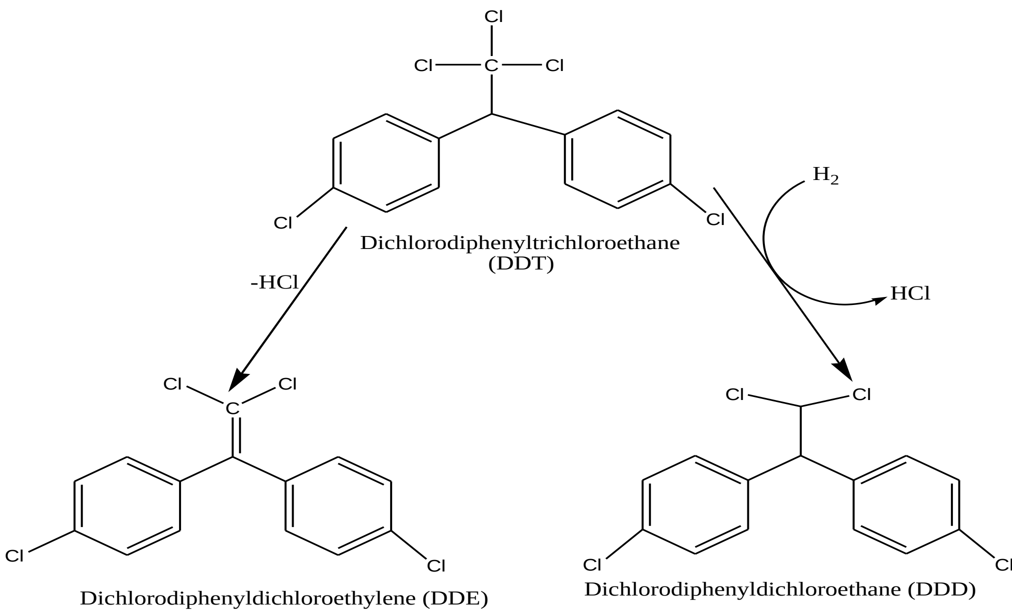
Table 2.6: Potential adverse health effects related with pesticides applied in farming of khat

Classification of pesticide	Trade name	Molecular formula	Name of the organic active ingredient	Potential health effects and hazards related to elevated levels of exposure
Organophosphates	Actellic	$C_{11}H_{20}N_3O_3PS$	Primiphos-methyl or *O*-[2-(Diethylamino)-6 methylpyrimidin-4-yl] *O*,*O*-diethyl phosphorothioate	Cholinesterase inhibitor (Park & Dublin, 2011).
	Malathion	$C_{10}H_{19}O_6PS_2$	O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate	Eye and skin irritant, cholinesterase inhibitor, dermal toxicity, reproductive and some developmental effects in mammals and disruption of endocrine (Colovic *et al.*, 2013; Organization, 2013; Tchounwou *et al.*, 2015).
	Diazinon	$C_{12}H_{21}N_2O_3PS$	O,O-Diethyl O-[4-methyl-6-(propan-2-yl)pyrimidin-2-yl] phosphorothioate	Endocrine disruption (Nicolopoulou-Stamati *et al.*, 2016).
Organochlorines	DDT	$C_{14}H_9Cl_5$	Dichlorodiphenyltrichloroethane	Possible carcinogen, disruption of endocrine and poor embryonic development (Garabrant *et al.*, 1992; Nicolopoulou-Stamati *et al.*, 2016;

				Tiemann, 2008; Wallace, 2015).
	DDE	$C_{14}H_8Cl_4$	Dichlorodiphenyldichloroethylene	A likely carcinogen, neurodevelopment effect in children and disruption of endocrine (Eskenazi *et al.*, 2006; Nicolopoulou-Stamati *et al.*, 2016).
	Aldrin	$C_{12}H_8Cl_6$	Hexachlorohexahydrodimethano-naphthalene	Endocrine disruption (Lemaire *et al.*, 2006).
	BHC	$C_6H_6Cl_6$	Benzene hexachloride	Disruption of endocrine (Nicolopoulou-Stamati *et al.*, 2016).

The DDT and its breakdown products have persisted in the environment, contributing to bioaccumulation process and bio-magnification (Vorkamp & Riget, 2014). Aside from its metabolites, DDT is widely present in food and environmental settings (Hussein, 2015). DDT exposure has been associated to liver cirrhosis, reproductive issues, breast cancer, and neurodevelopmental impairments in early life (Ali *et al.,* 2014). As a result, pollution of khat young shoots and tender leaves with DDT and its byproducts may be harmful to human health. DDT's non-biodegradability characteristics makes it a precursor for the vast health concerns and diseases due to its widespread applications in pest management, despite being outlawed in developed nations (Regassa & Regassa, 2018; Wallace, 2015).

Basically, organochlorine pesticides like DDT induce oesophageal and oral malignancies in chewers of khat. (Daba *et al.*, 2011; Woldetsadik *et al.*, 2021). Consequently, DDT pesticide is totally banned in the majority of developed nations, including the United States and Europe, due to detrimental health impacts including endocrine problems (Karunamoorthi *et al.*, 2012; Meftaul *et al.*, 2020). Ethiopia and Kenya are among the other nations that have outlawed DDT use for agricultural purposes. Pesticide residues on chewable khat parts can cause poisoning and, fatalities (Ademe *et al.*, 2020). As a result, adequate pesticide application management measures must be implemented to prevent possible pesticide poisoning, deaths, and environmental contamination (Derso & Dagnew, 2019). Furthermore, acceptable limitations established by regulatory organisations must be observed and followed with an aim to reduce the serious effects of these pesticides and their residues. The European Union (EU) regulates DDT and diazinon pesticides with a maximum residue level (MRL) of 10 µg/kg, especially for vegetables and fruits (Adamu *et al.*, 2019; Daba *et al.*, 2011).



Scheme 2.1 Suggested mechanistic degradation of DDT pesticide to its metabolites

2.10 The toxicological concerns of use of khat

Regular chewing of khat has been connected with human medical diseases including decreased libido, liver damage, high blood pressure, depression, dental caries, and cancer (Al Bratty *et al.*, 2019; Tarboush *et al.*, 2019). It is believed that psychotropic compounds in khat are precursors for oral and oesophageal cancer in regular chewers of khat (Omare, 2020; Yadeta *et al.*, 2020). A surge in cathinone levels in plasma raises the blood pressure (Geta *et al.*, 2019). Notably, khat contains significant levels of toxic metal ions, which can harm key body organs including kidneys and liver (Al Bratty *et al.*, 2019). Moreover, consuming not-rinsed khat tender leaves likely containing pesticide residues induces genotoxicity and cancer disease (Atnafie *et al.*, 2021). The body's liver organ is critical in performing metabolic and detoxification tasks (Djurasevic *et al.*, 2019). Because of its prominent position in the bodily system, it is extremely susceptible to the serious consequences of khat consumption. Chewing khat is associated with significant liver damage and fibrosis (Ademe *et al.*, 2020; Orlien *et al.*, 2018). Fibrosis develops in some cases as a result of significant Cd deposition in the liver following repeated low dosage exposure (Baba *et al.,* 2013). Regrettably, the human liver may be completely damaged and not recover even after stopping use of khat (Makeen *et al.*, 2021), necessitating a liver transplant in extreme situations. Medical research has demonstrated that severe liver damage is a silent illness, with majority of patients tend to remain asymptomatic until decompensation happens (Ademe *et al.*, 2020; Heidelbauch & Bruderly, 2006).

The harm to the liver can occur as a result of the metabolization of harmful khat components such as heavy metals and insecticides. This may culminate in psychological symptoms including insomnia and sadness (Heidelbauch & Bruderly, 2006). Studies on mice fed on a diet including khat products revealed elevated liver enzymes and histological necro-inflammatory changes, implying that these products caused liver injury (Orlien *et al.*, 2018). Plausibly, the liver is crucial to the maintenance and balance of Cu and Fe in human body, which mostly enhances lipid peroxidation. Nonetheless, excessive Fe and Cu absorption (Djurasevic *et al.*, 2019) can lead to elevated bio-accumulation concentrations, which can be harmful to the liver organ (Ashenef *et al.*, 2014).

Correspondingly, Cd element is very hazardous with biological half-life of around 6–39 years that can cause major health problems such as neurological breakdown, failures of the kidney and liver (Egger *et al.*, 2019). Cadmium metal is a well-known carcinogen that has been shown to reduce bone mineral density at elevated levels of exposure (Egger *et al.*, 2019). This element can enter living organism's system via the respiratory airway and the gastrointestinal tract, with exposure time and dosage being critical factors in liver toxicity (Djurasevic *et al.*, 2019). Epidemiological studies conducted in humans have shown a link between liver dysfunction and Cd exposure (Kang *et al.*, 2013). It decreases the liver Fe content, lowering enzymatic activities and resulting in mechanisms which does not regulate proteins (Djurasevic *et al.*, 2019). Furthermore, Cd element has the ability to harm the adrenals, cause death of cells, and is a well-established precursor to kidney dysfunction (Egger *et al.*, 2019). Unregulated utilisation of banned pesticides on khat plants including DDT, a carcinogenic agent, causes serious liver damage in khat consumers (Weyesa, 2021). Liver cirrhosis disease can cause death by impeding liver function (Tsochatzis *et al.*, 2014). Also, liver injury to the parenchyma caused by an infusion of acute or chronic inflammatory cells is a prelude to hepatitis, a fatal disease (Adamu *et al.*, 2019).

2.11 Khat use as a potential candidate for causing cancer

Oral and oesophageal cancers that are known to cause fatalities mostly in developing nations including Somalia, Ethiopia, Kenya and Djibouti have been discussed (Ram *et al.*, 2011; Yang & Chen, 2021). First, oral cancer may be caused by excessive alcohol consumption and chronic smoking of cigarettes notwithstanding use of khat products (Warnakulasuriya, 2009). As a result, chewing of fresh khat is related to oral cancer, periodontitis and oesophagitis (Yang & Chen, 2021). Oral cancer is one the cancers worldwide which severely affect the cheek , the mouth’s lower side, and tongue (Ram *et al.*, 2011; Warnakulasuriya, 2009). Some of these symptoms are cell carcinomas (Morse *et al.*, 2007). Accordingly, cancer is related with dental factors and dietary deficiencies (Ram *et al.*, 2011). The other types of cancers are related with trematode and bacteria infestations and parasitic diseases (De Flora & Bonanni, 2011). Khat chewing routine practices has been related with oral cancers among regular chewers of khat leaves and young shoots (Lu *et al.*, 2017; Nigatu & Libsu, 2019). The khat chewer's regular chewing side of the mouth may develop radical changes including keratotic white lesions (Schmidt-Westhausen *et al.*, 2014), oral epithelial dysplasia (OED) and irritation of the oesophagus which form a basis for reactive process or cancer development (Leon *et al.*, 2017). The OED is associated to cause cellular changes evocative of malignancy development which can become oral carcinogenesis (Morse *et al.*, 2007).

Table 2.7 illustrates some studies undertaken in khat-growing areas such as the Horn of Africa and Arabia (Leon *et al.*, 2017; Njuguna *et al.*, 2013; Schmidt-Westhausen *et al.*, 2014; Sinba, 2017). These research have found a relationship between khat use and the onset of cancer-linked physiological signs. In a pilot study conducted in Ethiopia, it was established that more than one third of cancer patients were younger users of khat (Leon *et al.*, 2017). The research has also reported that smoking, peer pressure, cultural beliefs, social influences, religion, and traditions contribute to the surge in intake of khat and potential abuse of the recreational stimulative herbal narcotic.

High amounts of khat toxins, as well as the development of precancerous keratotic white lesions in the cheek where boluses of khat are inserted during khat chewing process, are precursors to oral cancer (Kassab & Moustafa, 2017; Yarom *et al.*, 2010). The alkaloid chemicals in khat cause precancerous chromosomal alterations and cell death via apoptosis. Furthermore, pesticide residues on consumed khat parts are the leading cause of cases of cancer among chewers who prefer to chew unclean tender leaves and young shoots (Karunamoorthi *et al.*, 2012; Weyesa, 2021). Prolonged excessive usage of khat contaminated with high residues might result in genotoxicity, increasing the risk of developing diabetes mellitus disease (Atnafie *et al.*, 2021). Correspondingly, the use of organochlorine pesticides like DDT raises the risk of causing cancer disease (Al-Akwa *et al.*, 2009; Regassa *et al.*, 2020). Particularly, DDT pesticide has a slow degradation rate, a high lipophilic nature, and a high affinity for tissues of human and animals, resulting in more harmful effects (Regassa *et al.*, 2020). Oral cancer causes oral cavity and oropharynx malignancies as reports in the year 2012 projected roughly 300, 000 instances of cancer and about 145, 000 deaths worldwide, with rate of survival of about 60% (Kassab & Moustafa, 2017). Studies have shown that other malignancies, such as oesophageal cancer, can develop as a result of prolonged oesophageal irritation caused by extract of khat when swallowing it (Yadeta *et al.*, 2020).

Regular khat chewing can cause white lesions on the oral mucosa (Table 2.7) due to chemical reactions (Gorsky *et al.*, 2004; Schmidt-Westhausen *et al.*, 2014). Despite several research contradicting this correlation, the link between khat chewing and oral cancer is strongly suspected (Gorsky *et al.*, 2004). Nonetheless, newer research suggest that this connection is mostly linked to oesophageal and oral malignancies (Leon *et al.*, 2017). Chewing of khat leaves and young shoots sprayed with diazinon and DDT pesticides is more likely to cause human cancer because these pesticide residues are among the most dangerous organic compounds in the environment (Leon *et al.*, 2017). Tobacco smoking is clearly associated with diseases such as cancer due to the presence of numerous carcinogenic chemicals (Molla *et al.*, 2017).

Undoubtedly, khat users account for a large number of cases (Table 2.7), with studies indicating that khat extract is carcinogenic to khat users (Defar *et al.*, 2017; Nasher *et al.*, 2014). According to the literature, more than half of khat households in Djibouti, over 70.0% of Yemenis, and approximately 30.0% of Ethiopians are users. With these figures, it is clear that the possibility of consuming khat polluted with agrochemicals and potentially toxic heavy metals is a serious that should be examined further. In one of its reports, WHO has revealed that for instance in Yemen, around 30 to 50% of female adults use khat on a regular basis (Naji *et al.*, 2015), which is a concerning finding.

Table 2.7: Demographic characteristics of user of khat, associated physiological symptoms of cancer types and the factors influencing khat use

Type of study	Country of study	Age	Descriptive statistics linked to cancer cases		Physiological abnormalities linked with development of cancer	Factors that influence khat use among the users
			Recorded number of participants	Khat users in terms of percentages (%) of the respective populations		
Administered interviews	Ethiopia	< 18 years	NP*	NP*	Mouth sores	Peer pressure, religious beliefs, and Social influences (Sinba, 2017).
Cross-sectional hospital study	Yemen	20–65 years	82	75.20%	White lesions on oral mucosa	Social influences (Schmidt-Westhausen *et al*., 2014).
Population survey	Israel	< 30 years	39	83%	White lesions on skin sections	Global spread of khat usage and smoking (Gorsky *et al*., 2004).
Pilot case–control study	Ethiopia	< 18 years	73 CC*	35.40%	CC*	Traditions, culture and religious beliefs (Leon *et al*., 2017).

Cross-sectional study	Ethiopia	31.86 years (Mean Age	NP*	NP*	Lesions	Leisure activities, family history of tobacco use and peer pressure (Molla *et al.*, 2017).
Case–control study	Yemen	56.95 years (Mean Age)	NP*	NP*	Lesions, lymph node, mucosal burns	Smoking and experimental Khat chewing (Nasher *et al.*, 2014).
Cross-sectional population based study	Ethiopia	15–69 years	NP*	NP*	NP*	Khat chewing that accompany heavy alcohol consumption (Defar *et al.*, 2017).
Descriptive study	Kenya	Adults	51	75	*NP	Mouth sores, dental carries, alcohol consumption (Njuguna *et al.*, 2013).

*NP: not provided, CC: cancer case

2.12 High blood pressure and use of khat

High blood pressure is a medical disorder that occurs when blood flows at a high pressure in the artery walls—diastolic blood pressure ≥ 90 mmHg and/or systolic blood pressure ≥ 140 mmHg (Abdissa & Kene, 2020; Chuka *et al.*, 2020; Niriayo *et al.*, 2019). This can cause long-term health difficulties, physical disability (Huang *et al.*, 2021), and even early mortality (Woldetsadik *et al.*, 2021). Currently, high blood pressure is a global health problem (Niriayo *et al.*, 2019), mostly impacting the populations of developing nations including Ethiopia (Kebede *et al.*, 2020), with instances anticipated to increase by 2025 (Ibrahim & Damasceno, 2012). Hypertension can potentially cause heart and blood vessel damage (Tessema & Zeleke, 2020). Primarily, it is a leading cause of cardiovascular and kidney diseases, such as myocardial infarctions, renal failures (Kebede *et al.*, 2020; Niriayo *et al.*, 2019), maternal death during pregnancy and dementia. Alkaloid compounds like cathinone and cathine are known to cause cardiovascular problems (Badego *et al.*, 2020; Jayed & Al-Huthi, 2016).

In the course of chewing of khat, the blood pressure and heart rate are reported to rise proportionally as the chewing progresses, leading to an increase in alkaloid chemical plasma levels (Geta *et al.*, 2019). It has been shown that blood pressure can exponentially rise about 14 times compared to that of non-chewers (non-users) (Birhane & Birhane, 2014). Table 2.8 summarises several characteristics and khat chewing processes. The increase in blood pressure is due to the alkaloid cathinone's multiple vasoconstrictor activities. Khat's psychotropic components also raise heart rate (Geta *et al.*, 2019). Hypertension is one of the leading causes of global disease burden (Haye & Agama, 2020; Kebede *et al.*, 2020). It affects over 1 billion people globally, with an approximated 7.1 million fatalities anually. Individual genetics, dietary factors, behavioural characteristics, and medical conditions all contribute to hypertension (Geta *et al.*, 2019; Ibrahim & Damasceno, 2012). It can also be caused by other risk factors such as obesity, lack of physical activity, stress, and frequent alcohol consumption. Hypertension has been linked to increased risk of heart failure and stroke (Geta *et al.*, 2019). Consequently, it is critical to treat high blood pressure early through identification, prevention, and sensitization (Badego *et al.*, 2020). Heavy metal contamination of khat with farming pesticides like DDT may exacerbate cancer, high blood pressure, liver damage, and kidney illness. Furthermore, the WHO has approved a global action plan for decreasing hypertension risk through awareness programs (Chuka *et al.*, 2020).

Table 2.8: Some studies on the relationship between high blood pressure and chewing of khat

Type of study	Region/ Country of study	Association with hypertension	Mean age of participants	Prevalence rate of hypertension
Comparative study	Butajira, Ethiopia	Yes	NP*	Prevalence of 13.4% for chewers of khat while 10.7% for non-khat chewers (Miller *et al.*, 2010).
Facility-based cross-sectional	Addis Ababa, Ethiopia	Yes	41.17 years	Prevalence was around 14.0% for chewers of khat who were patients in health centers (Niriayo *et al.*, 2019).
Cross-sectional based	Ethiopia	Yes	NP*	No information on prevalence was provided. Prevalent conditions include low salt intake, lack of enough physical exercise and high weight (Niriayo *et al.*, 2019).
Institution-based cross-sectional	Sidama zone, Ethiopia	Yes	NP*	Prevalence was around 24.5%. Chewing of khat, obesity, old age and alcohol consumption are prevalent conditions (Badego *et al.*, 2020).
Hospital-based cross-sectional	Ethiopia	Yes	42.30 years	Prevalence was 13.2%. Family history of high blood pressure, diabetes mellitus, oral contraceptive and overweight are prevalent conditions (Gudina *et al.*, 2013).

Community-based	Gurage zone, Ethiopia	Yes	34.36 years	The prevalence was approximately 17.40% for chewers of khat and around 8.70% for non-chewers. Alcohol intake, gender and age are prevalent conditions (Geta *et al.*, 2019).

*NP: not provided

2.13 Proposed improvement practices in the farming of khat

The widespread pesticides use and manufactured fertilisers endangers khat farmers' health, the environment, and their livelihoods, particularly in the Arabian Peninsula and the Horn of Africa (Krueger & Mutyambai, 2020). Because khat tender leaves and young shoots are mostly exported and chewed in western countries including the UK, the Netherlands, and the United States, the impacts may be magnified for a larger population. It is also critical to use khat farming practices that are devoid of pesticides and artificial fertilisers. Organic farming is thus a management system for improving soil fertility and facilitating quality production of crops, ultimately reducing the bio-accumulation of potentially toxic heavy metals and pesticide residues in crops, where both are well-known to have negative effects on health of humans. This farming strategy for production of crops does not utilise synthetic fertilisers, growth regulators, animal feed additives or pesticide application (Yuvaraj *et al.*, 2020).

Improved agricultural practices and methods are critical in alleviating environmental-based problems caused by traditional farming methods, and this approach can be viewed as a good development in the food production system (Drimie & Pereira, 2016). Basically, organic farming relies on farm-wide practices with the goal of increasing biological diversity, augmenting soil biological activities, maintaining soil quality and fertility for long time, returning soil nutrients, and promoting the healthy use of air, water, and soil while minimising pollutions including bio-accumulation of potentially toxic heavy metals and fertilisers (de Haes & de Snoo, 2010). Since organic farming offers several significant benefits and encouraging properties, such as a favourable impact on local biodiversity and improved productivity levels, it is ideal not only for crop production but also for farming of khat (Seufert *et al.*, 2017).

CHAPTER THREE

GC/EI-MS AND UV-VIS ANALYSIS OF PESTICIDE RESIDUES IN CULTIVATED *Catha edulis* (KHAT) FROM SELECTED FARMS IN MERU COUNTY, KENYA

Abstract

In the present study, pesticide residues were analysed using a gas chromatography/electron impact-mass spectrometer (GC/EI-MS) to determine and characterise these residues in khat *(Catha edulis)* collected from khat farms of selected regions in Meru County, Kenya. A solid-phase microextraction (SPME) procedure followed by the GC/EI-MS qualitative analysis resulted in the determination of six pesticidal chemicals in khat samples from total ion chromatograms. These pesticide compounds include cyhalothrin, cyfluthrin, cypermethrin, chlorfenvinphos, chlorpyrifos, and acephate. This translated to pesticide contamination prevalence rate of about 54.50% of the total sample size. Of the detected residues, 50% were pyrethroid-based compounds and the other half were organophosphate-based. Four of the six detected pesticide residues were chlorinated chemicals. A rapid, simple, and effective UV-Visible (UV-Vis) double beam spectrophotometric technique based on Cu (II) chelation reactions resulting in coloured Cu-pesticide complexes was developed, validated, and used to quantify and compare the concentrations of acephate and cypermethrin residues. UV-Vis wavelength-scan measurements of pesticide chemicals chelated to Cu (II) ions found that Cu-acephate and Cu-cypermethrin had maximal absorptions at 207 nm and 321 nm, respectively. UV-Vis quantitation calibration curves for cypermethrin and acephate standards demonstrated good linearity in the concentration range of between 0.5 µg/L and 10 µg/L (R^2 > 0.9900). The calculated limits of quantification (LOQ) were 0.25 and 0.26 µg/L, respectively. UV-Vis quantitation of acephate demonstrated that its residue levels were in the range 2.897-7.978 µg/L which were clearly higher than cypermethrin level (2.145 µg/L). The levels of pesticide residues quantitatively analysed in the selected khat samples were lower than the maximum residue limits (MRLs). The hazard quotients (HQs) ranged from 0.247 and 0.797. Thus, there is no likelihood of adverse health risks from the consumption of Meru khat.

3.1 Introduction

The khat (*Catha edulis* Forsk), is one of the most extensively ingested psychotropic plants globally. This plant is grown/cultivated in large farms in the Horn of Africa, southwestern regions of the Arabian Peninsula, and Eastern Africa. Commonly, tender leaves and twigs of khat (locally known as miraa) are chewed daily by nearly 20 million people for their psychostimulatory benefits. The routine practice of chewing khat has become firmly established in the history, custom, and culture of the local community where it is planted (Patel, 2015). The regions of Meru County host the cradleland of Kenya's large khat farms. This County has a population of 1,545,714 as per the 2019 census results and a substantial part of them not only consume khat but also draws their livelihoods from khat production as a very lucrative agri-business endeavour (Carrier, 2005; Kobia *et al.*, 2019). Consequently, pesticides use in farming for khat protection purposes is widely spread in these regions.

Although pesticides protect agricultural crops from pest attack and disease infestation towards improving productions and quality, they are chemically stable in the environment, bioaccumulate in tissues of plants, and later expose their adverse effects to the consumers. Some of these effects include loss of consciousness, respiratory system, asthma attacks, nervous system, depression, seizures, and mental disorientation (Aktar *et al.*, 2009; Jayaraj *et al.*, 2016; Sharma *et al.*, 2020). Numerous monitoring and assessment research have in the past determined residues of pesticides in vegetables and fruits. Sometimes, these residue concentrations have been above the MRLs (Jallow *et al.*, 2017; Mebdoua *et al.*, 2017). The consumption of substantial pesticide-contaminated food produce can possibly pose adverse health concerns if the pollution is not timely determined. Thus, there is need for periodic determination of pesticide residues in the farm produce with the aim of alerting the public or specified demographic on the state of pollution and the likelihood of health threats that the users may be exposed to.

The GC/EI-MS and a UV-Vis double beam spectrophotometer are analytical instruments that have increasingly become indispensable in analysis of pesticide residues in plants including consumed crops (Collimore & Bent, 2020; dos Anjos & de Andrade, 2014; Pakade *et al.*, 2013; Zhu *et al.*, 2016). In this investigation, the former technique was utilised for the detection, identification, and characterisation of the residues contained in samples of khat. A developed and validated UV-Vis spectrophotometric approach based on Cu chelation with acephate and cypermethrin pesticides was utilised to quantify the levels of the aforementioned residues in samples of khat. This investigation was designed to document analytical data and develop scientific reports by recording the different kinds of pesticide residues present in the samples of khat collected from 11 farms within the Meru County. The prevalence rates of the determined residues based on the present investigation are also presented herein.

3.2 Experimental

3.2.1 Sample collection

The sampling of khat for the present study was carried out in accordance with the general guidelines and methodology outlined in the European Commission (EC) regulation 2002/63/EC for establishing MRLs in food produce. They were randomly obtained from the chosen farms of khat in Meru County, as shown in Figure 3.1.

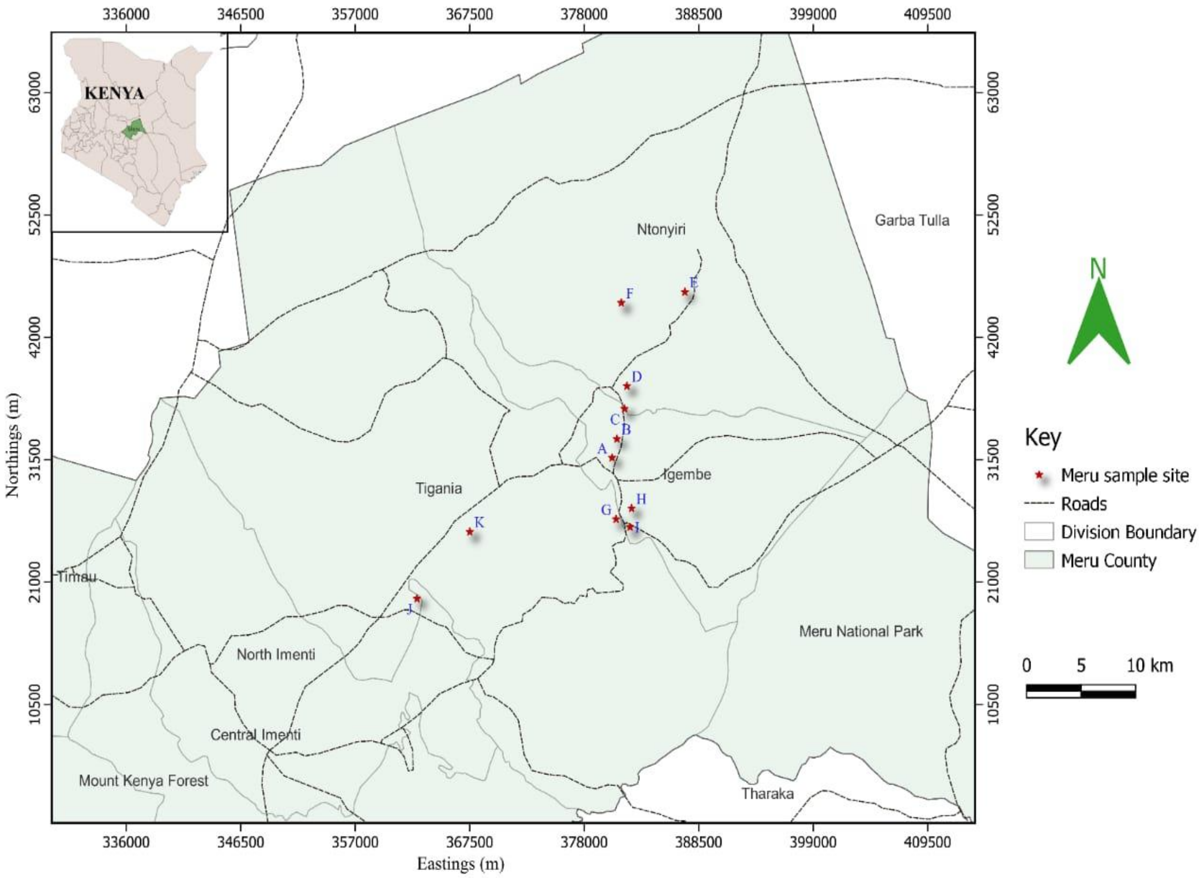


Figure 3.1 A map of the khat sampling farms within Meru County, Kenya.

3.2.2 Reagents and chemicals

The extraction procedures used analytical grade solvents such as n-hexane, acetone, and ethyl acetate and salts such as magnesium metasilicate ($MgSiO_3$) and anhydrous sodium sulfate (Na_2SO_4) were all obtained from Kobian Scientific, Nairobi, Kenya. The pesticide standards including acephate and cypermethrin, used in the quantitative assays were all of high purity (> 98.50%) and obtained from Organix Limited Suppliers in Nairobi, Kenya. The standard stock solutions of the two pesticides were prepared in the mixture solvent system of n-hexane, acetone and ethyl acetate in a 1:1:2 ratio and stored in a refrigerator at -5 °C. Analytical grade C18-bonded silica was bought from Sigma-Aldrich (Germany).

3.2.3 Extraction of pesticides

Approximately 1.0 g ground fresh sample of khat was placed in a glass mortar and 1.0 g of C18-bonded silica was added. The ground khat sample and the C18-bonded silica substance were then thoroughly mixed. The resulting homogenized mixture was introduced into a 100 mm × 20 mm ID polypropylene column, filled with 0.1 g of glass wool fitted at the tap base, followed by, a 1.0 g layer of anhydrous sodium sulfate (Na_2SO_4) and then 1.0 g of magnesium metasilicate ($MgSiO_3$). A 50 mL homogenous solution containing 40 mL of *n*-hexane and 10 mL of acetonitrile (MeCN) was added to the packed column to elute dropwise. The eluate was collected in an Erlenmeyer flask, transferred to a round bottom flask, and concentrated using a rotary vacuum evaporator (water bath temperature 40 °C) to a reduced volume of 1 mL, then a 1 µL part of the concentrate was siphoned for GC-MS qualitative analysis.

3.2.4 Gas chromatography-mass spectrometer (GC-MS) analysis

The SPME extraction technique was used to adsorb the prepared extracts of khat sample. The coating fibre used was polydimethylsiloxane-divinylbenzene (PDMS/DVB). A Shimadzu QP2010 SE series GCMS (Kyoto, Japan) equipped with an electron capture detector was used for the pesticide residue analysis. A volume of 5 mL of the sample was placed in a 20 mL headspace vial. The PDMS/DVB fibre was inserted into the headspace with heating (40 °C) and agitation (250 rpm) for 30 min. After the extraction process, it was loaded into the sample injection port of the GC–MS instrument equipped with an SE 30 capillary column (50 m × 0.25 mm ID at 0.25-µm film thickness) in split mode where the chromatographic separation of the volatile components of the extracts of khat sample was conducted. The carrier gas used was helium at a flow rate of 1 $mL\ min^{-1}$. The oven temperature was programmed as follows: initially held at 40 ℃ for 4 min, increased at a rate of 10 ℃/min raised to 250 ℃, then raised at a rate of 10 ℃/min to 300 ℃ and held for approximately 15 min. The MS fragmentation was operated in electron impact (EI) mode (electron energy, 70 eV; ionization temperature, 230 ℃). The transfer line temperature was 230 ℃. The mass acquisition range was set to 33–450 amu. The National Institute of Standards and Technology (NIST 11) database was used to confirm the identities of the hits in the mass spectra. Then the identification of the pesticide compounds was confirmed by the injection of the authentic compounds into the GC–MS based on comparing mass spectra and GC retention times.

3.2.5 Ultraviolet-Visible (UV-Vis) spectrophotometric quantitative analysis

A double beam K9000 UV-Visible spectrophotometer equipped with 10 mm path length quartz cuvette cells, fitted with silicon diode detector and deuterium and tungsten/halogen lamps were utilised to obtain the spectral data. As a result, analytical grade acetone, ethyl acetate, and n-hexane solvents were used in the development of the solvent system for extraction of the pesticide compounds from the extracts of khat sample. Stock solutions of cypermethrin (100 µg/L) and acephate (100 µg/L) pesticides were prepared from which aliquots were siphoned to prepare a set of working standards in the ranges between 0.5 µg/L and 10 µg/L in a solvent system of acetone: n-hexane: ethyl acetate in a ratio 1:1:2.

The analytical grade chemicals were used to prepare solutions of 0.1 M $Cu(NO_3)_2$, 0.1 M potassium chloride, KCl, and 20% of 0.1 M sodium hydroxide, NaOH, and phosphate buffer of pH 7 including double-distilled water were prepared for the quantitative analysis. Exactly 2 mL of the solution containing 10 mL $Cu(NO_3)_2$, 4 mL KCl, and 4 mL NaOH was added to the standards solutions shaken in a warm bath (50 ℃ for exactly 15 min) to form the coloured Cu(II)-pesticide complexes. The wavelength scans were done on these freshly prepared standards in order to obtain a linear relationship of the absorbance over a range of varying concentrations. This was necessary for the construction of calibration curves.

3.2.6 Extraction of pesticides for UV-Visible analysis

Approximately 1.0 g ground fresh sample of khat was placed in a glass mortar and 1.0 g of C18-bonded silica was added. The khat sample and the C18-bonded silica substance were then thoroughly mixed with using a pestle. The homogenized mixture was introduced into a 100 mm × 20 mm ID polypropylene column, filled with 0.1 g of glass wool at the tap base, followed by, a 1.0 g layer of anhydrous sodium sulfate (Na_2SO_4) and then 1.0 g of magnesium metasilicate ($MgSiO_3$). A 50 mL solution containing 40 mL of *n*-hexane and 10 mL of acetonitrile (MeCN) was added to the packed column to elute dropwise. The eluate was collected in an Erlenmeyer flask, transferred to a round bottom flask, and dried using a rotary vacuum evaporator (water bath temperature 40 °C). The dried khat sample was dissolved in 1 mL of the solvent system of acetone, hexane, ethyl acetate in a 1:1:2 ratio and stored in a refrigerator at -5 °C prior for UV-Vis quantitative analysis.

3.3 Results and Discussion

3.3.1 GC-MS qualitative results of pesticide residues in khat samples

The quantitative release of chemical compounds was monitored using a GC-MS and identified from the NIST database and enhanced data libraries. Table 3.1 shows the pesticide residues identified in khat samples. Their chemical formulae, molecular masses, and chemical abstract service (CAS) numbers are listed. Six of the eleven khat samples analysed contained pesticide residues which were spread across all of the selected regions. They were classified into organophosphates, including chlorpyrifos, chlorfenvinphos, and acephate and also pyrethroids comprising of cypermethrin, cyfluthrin, and cyhalothrin each representing 50% prevalence in this study area. This research demonstrates use of pesticides in khat farms to control insects that destroy khat trees and reduce the quality of young khat shoots. Other khat samples registered no pesticide residue, indicating they either were not applied or had degraded below the detection limit by the time of sampling and analysis. Previous studies show that pesticide levels such as acephate can degrade to below detection limits, indicating that crops are safe for human consumption (Mohapatra, 2014). Accordingly, pesticide contamination rate in this study area was approximately 54.50% of the total sample size. All of these residues are oxygenated compounds, with five of them being chlorinated aromatic except acepahte residues. The high molecular weights of these residues (Table 3.1) and their ability to attach to biological structures can make them toxic and cause serious health effects in humans (Glover & Schumacher, 2016). Additionally, the presence of heteroatoms in these pesticide compounds significantly alters their interactions with biological tissues, contributing to the health risks.

The pesticide residues have unending poisonous effects, for instance, acephate pesticides may cause human poisoning and result in genotoxic and cytotoxic effects on human sperm, ranging from when in trace to elevated levels in foods and water (Brovini *et al.*, 2024). Cypermethrin and cyhalothrin residues are believed to persist in the environmental matrices depending on the prevailing physiochemical conditions and microbial metabolic activities in the soil (Bhatt *et al.*, 2020; Muhamad *et al.*, 2015). In addition, literature studies have shown that cypermethrin can damage human organ systems and in animals where in the latter it damages male and female reproductive systems (Zhou *et al.*, 2018). Notably, chlorfenvinphos pesticides weakly poisonous to mammals; however, their widespread use may hinder the functionality of the liver as reported in rats (Mahdy & El-Maghraby, 2010). Apart from increased adverse effects of cyfluthrin to fish and other invertebrates, its residues especially cyanohydrins metabolites on agricultural foods and in water may cause toxicity to humans (Li *et al.*, 2017).

Table 3.1: Summary of GC/EI-MS analysis of pesticide residues present in samples of khat collected from Meru region, Kenya.

Region in Meru County	Sample	Pesticide residue present	Classification	Molecular formula	Molar mass (g/mol)	CAS
Kangeta	A	ND	-	-	-	-
	B	Cypermethrin	Pyrethroid	$C_{22}H_{19}Cl_2NO_3$	415	52315-07-8
Laare	C	ND	-	-	-	-
	D	Chlorpyrifos	Organophosphate	$C_9H_{11}Cl_3NO_3PS$	349	2921-88-2
		Chlorfenvinphos		$C_{12}H_{14}Cl_3O_4P$	358	470-90-6
		Lambda-cyhalothrin	Pyrethoid	$C_{23}H_{19}ClF_3NO_3$	449	91465-08-6
Mutuati	E	ND	-	-	-	-
	F	Chlorpyrifos	Organophosphate	$C_9H_{11}Cl_3NO_3PS$	349	2921-88-2
		Cyfluthrin	Pyrethroid	$C_{22}H_{18}Cl_2FNO_3$	433	68359-37-5
Maua	G	Acephate	Organophosphate	$C_4H_{10}NO_3PS$	183	30560-19-1
	H	ND	-	-	-	-
	I	Acephate	Organophosphate	$C_4H_{10}NO_3PS$	183	30560-19-1
Kianjai	J	ND	-	-	-	-
	K	Chlorpyrifos	Organophosphate	$C_9H_{11}Cl_3NO_3PS$	349	2921-88-2

Legend: ND: Not Detected

Therefore, regular quantification of these residues needs to be done to guarantee surety of human safety and need to maintain them within MRLs set by European Union (EU) through a European Commission, 2006, which pronounced itself as follows; EU MRLs of some pesticides are as follows; cyfluthrin 0.30 mg/kg, cyhalothrin 0.1 mg/kg, cypermethrin 0.5 mg/kg, chlorfenvinphos 0.05 mg/kg and chlorpyrifos 0.5 mg/kg (Blankson *et al.*, 2016) and acephate 5 mg/kg and methamidophos 1 mg/kg set by WHO/FAO for leafy vegetables (Chai *et al.*, 2009).

3.3.2 GC/EI-MS total ion chromatograms (TIC) of the samples of khat with residues

Figures 3.2 (A-F) show the GC-MS TIC obtained from the six samples of khat from which pesticide residues were detected. The aromatic-based pesticides were most prevalent and they were identified in four khat samples including B, K, F, and D while the non-aromatic residues were identified in samples G and I of Figure 3.2. All the aromatic pesticide residues were chlorinated based on their structural identities. The pesticide compounds containing phosphate esters were identified and are shown in Figure 3.2: C, D, E, and F. A previous study revealed that organophosphate residues such acephate and chlorpyrifos were detected and quantified in khat samples obtained from Igembe region of Meru County, Kenya (Krueger & Mutyambai, 2020). The present work showed that the majority of the studied regions, four out of five, use organophosphate pesticides to control pests in the khat plant possibly because they are readily available in local markets.

From the GC-MS analysis and illustration of Figure 3.2, a single pesticide residue was detected in four khat samples B, G, I, and K whereas sample D and sample F contained many pesticide compounds. Accordingly, three pesticide residues were detected from sample D while sample F registered two different kinds of residues. The pesticide compounds containing cyano-groups (cyfluthrin, cyhalothrin, and cypermethrin) were common after identification from more than half of the khat samples that recorded residues. Figure 3.2 show that cypermethrin was detected in sample B at retention time of 26.50 min, which is relatively similar to the retention time of 26.0 minutes recorded in a study involving soil samples (Muhamad *et al.*, 2015).

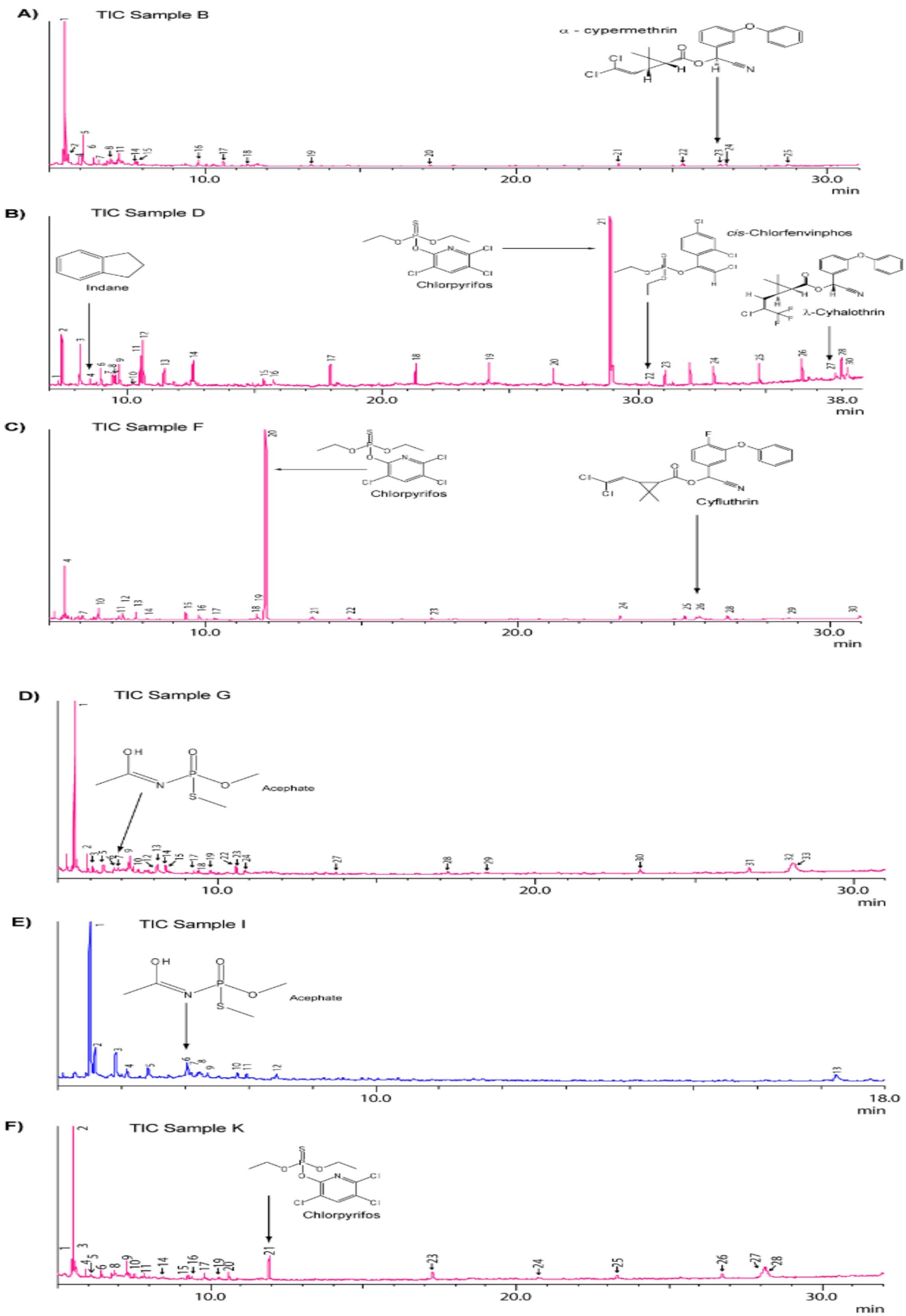


Figure 3.2 GC-MS TIC of the samples of khat with pesticide residues

3.3.3 Characterisation of pesticide residues in khat samples using mass fragmentations

Figure 3.3 illustrates the mass spectra of each of the organophosphate pesticides identified using GC-MS analysis. The mass fragmentations are shown using hatched lines on the molecular structures in the respective spectra. The molecular ion base peaks for each pesticide compound are identified and illustrated by the fragmentation patterns. In all the compounds identified, the molecular fragments arising from the cleavage of phosphorus-oxygen (P-O) bonds (for chlorpyriphos and chlorfenvinphos) and phosphorus-sulfur (P-S), and phosphorus-nitrogen (P-N) bonds (in the case of acephate residue compound) can be deciphered in the respective mass spectra. The rupture of these bonds is as a result of their low energies (Ramu & Seetharaman, 2014). For example, the fragmentation of acephate (Figure 3.3 (A)) along the P-N bond results in an ion peak at 125 *m/z* whereas the P-S bond rupture gives the molecular ion base peak at 136 *m/z*. The base peak of chlorfenvinphos (Figure 3.3 (B)) observed at 267 *m/z* arises due to the breakage of the P-O bonds as shown in the molecular fragmentation figure. Regarding chlorpyrifos (Figure 3.3 (C)), the P-O cleavage leads to the detected 197 *m/z* peaks.

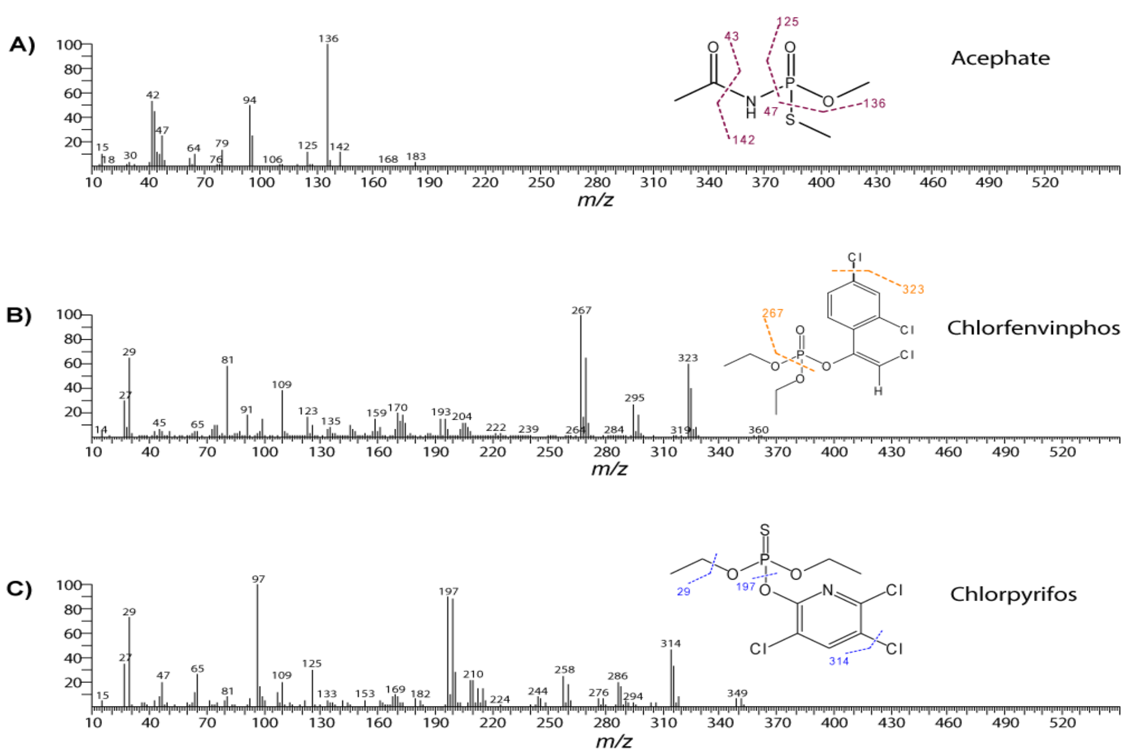


Figure 3.3 Molecular fragmentations of organophosphate pesticide residues in khat samples

Figure 3.4 illustrates the mass spectra of each of the three detected pyrethroid-based pesticidal compounds identified from the khat samples using the GC/EI-MS qualitative analysis. These compounds include cyfluthrin, cyhalothrin, and cypermethrin. In addition, structures of these compounds all have biphenyl ether aromatic rings, a cyano and cyclopropyl groups. The molecular ion base peaks detected at 163 *m/z* in the spectra of both cyfluthrin and cypermethrin arises from the fragments that have cyclopropyl groups attached to the halogen-substituted ethylene groups in the respective compounds as shown in the respective spectra. The molecular ion base peak of cypermethrin recorded in this study (Figure 3.4) is similar to the one reported in a study that used soil samples (Muhamad *et al.*, 2015). Accordingly, the m/z ion base peaks of cypermethrin and cyfluthrin residues gave similar mass ions and m/z ratios which closely compares to a research done on similar compounds (Gopal *et al.*, 2015).

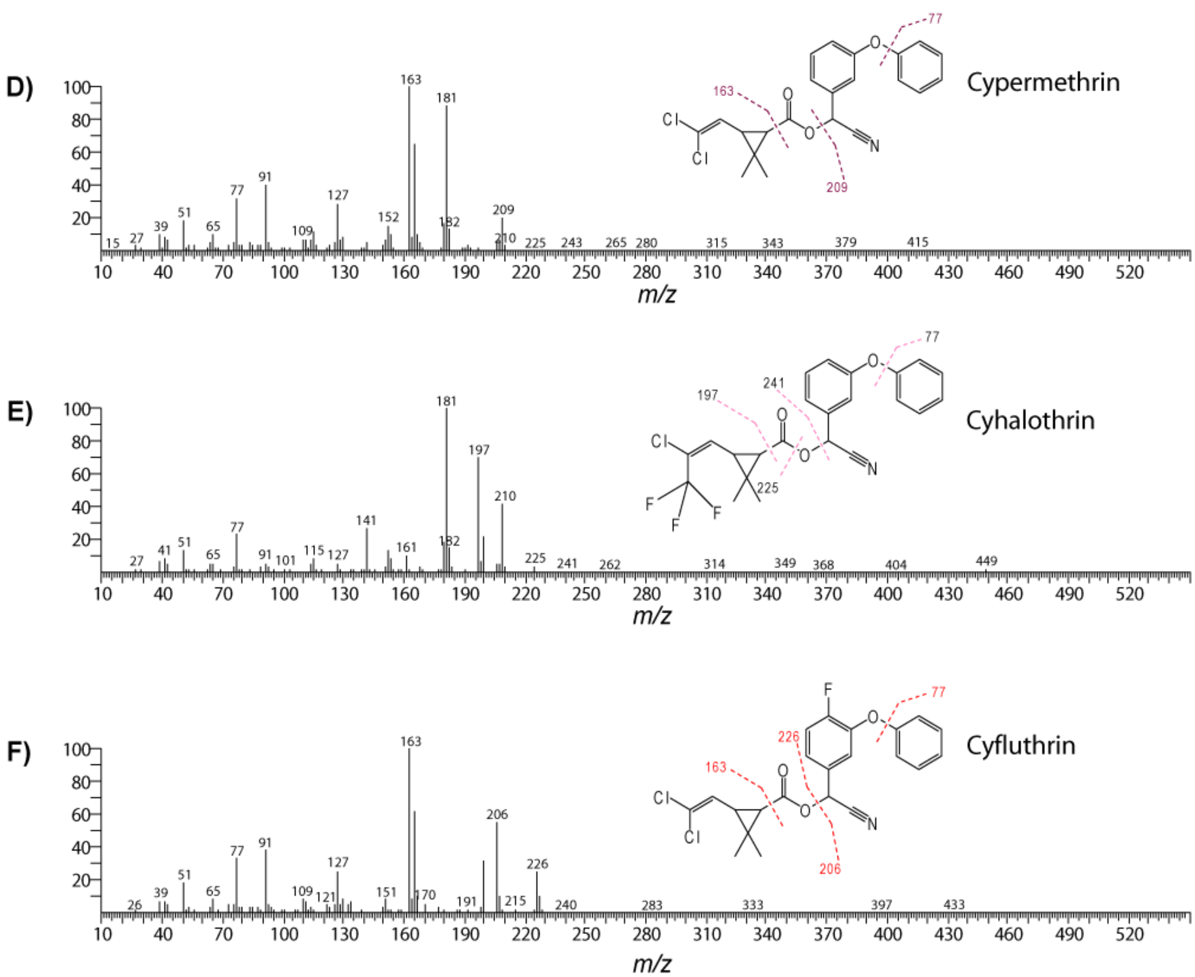


Figure 3.4 GC/EI-MS mass spectra of the pyrethroid-based pesticide compounds in khat samples

A similar cyclopropyl side-chain fragmentation on cyhalothrin pesticide compound yields the prominent ion peak detected at 197 *m/z*. The molecular ion peak detected at 77 *m/z* in all the mass spectra of the pyrethroids is as a result of fragmentation of a phenyl group from the bulkier diphenyl-ether group found present in all three pesticidal compounds.

Table 3.2 summarises all the detected pesticide compounds in samples of khat leaves using the GC-MS separation and subsequent qualitative analysis. The identities are characterised by the presented observed base-peak MS data in the table. Three organophosphate pesticide compounds and three other pyrethroid-based pesticides were identified. The observed molecular ion base peaks in the spectra were used in conjunction with the molecular fragmentations to verify their structural identities using the NIST database.

Table 3.2: The detected pesticide compounds based on GC/EI-MS analytical data

Pesticide compound	m/z ion base peak (observed)	m/z ion peaks (confirmatory)	Prevalence rate (%)*
Acephate	136	142, 94	18.18 %
Cypermethrin	163	209, 77	9.09 %
Cyfluthrin	163	206, 77	9.09 %
Chlorfenvinphos	267	205, 145	9.09 %
Chlorpyrifos	97	244, 153	27.27 %
Cyhalothrin	181	197,77	9.09 %

* = number of particular detected pesticide occurrences/total number of all the pesticide-contaminated khat samples

Out of the eleven khat samples analysed in this study, two of the samples confirmed traces of acephate representing a 18.18% prevalence rate. The pesticide compounds: cyfluthrin, cyhalothrin, chlorfenvinphos, and cypermethrin had similar prevalence rates of about 9.09%. The organophosphate chlorpyrifos pesticide recorded the highest prevalence rate of about 27.27%.

3.4 UV-Visible spectroscopic analysis

In UV-Visible spectroscopy, light is used to determine the absorbance or transmission of any chemical species in either an aqueous or solid state in the visible or adjacent ranges. In this regard, the colour of the chemicals under scrutiny influences the absorption in these ranges since the molecules undergo electronic transitions (Sahu & Saxena, 2013). Previous research has demonstrated that spectrophotometric measurement of pesticide residues in foods is a simple and fast method that uses inexpensive and easily available reagents (Elgailani & Alghamdi, 2018). The sub-sections that follow detail the determination of pesticide residues in prepared khat samples.

3.4.1 Maximum UV absorptions for the cypermethrin and acephate standards

This section presents a UV-Visible spectroscopic study into the quantification of acephate and cypermethrin pesticide residues in khat samples. As such, Figures 3.5 and 3.6 illustrates the UV-Visible wavelength scan absorption spectra obtained from pesticide standards of the Cu (II) complexes of cypermethrin and acephate respectively. These two pesticides are reportedly the most commonly applied in the growing of khat in different farms and regions of Meru County. The choice of these pesticides for quantification studies is also informed by previous studies that recorded their use on khat plants in various parts of Igembe region in Meru County (Krueger & Mutyambai, 2020). Therefore, considering their widespread use, this study will provide vital information about their levels in consumable young parts of khat for policy formulation and safety.

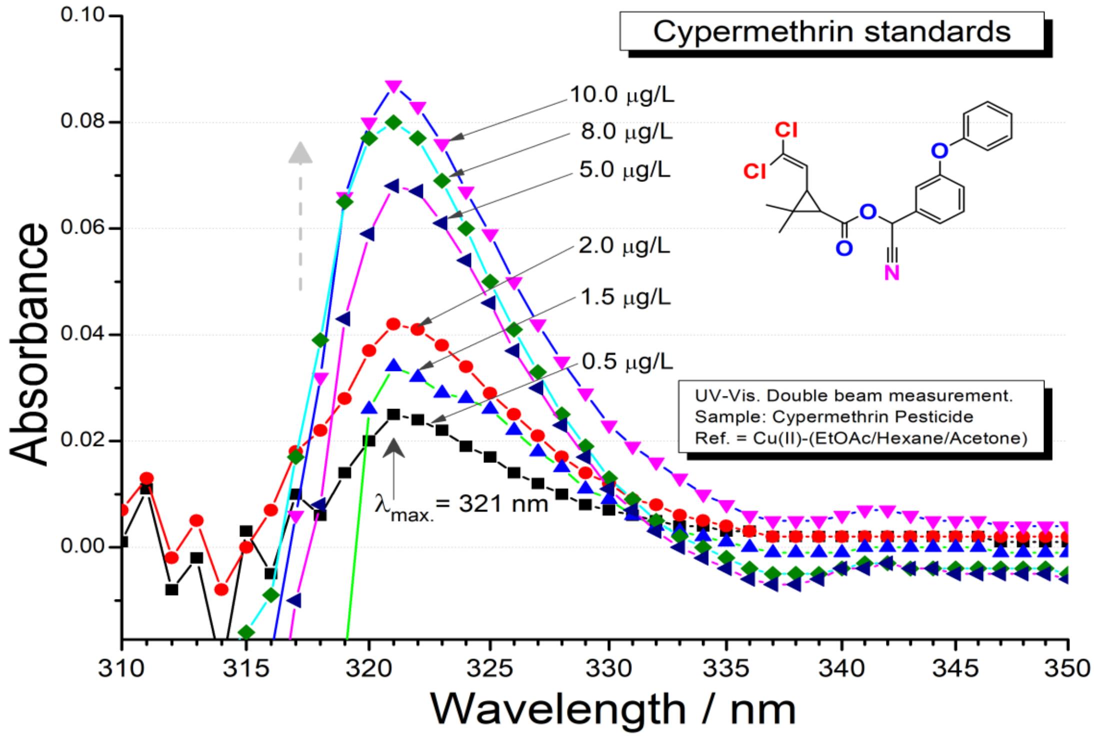


Figure 3.5 UV-Visible absorption spectra of Cu (II) complexes formed with cypermethrin standard in EtOAc/acetone/n-hexane solvent system

In both Figures 3.5 and 3.6, the normalized absorption spectra obtained from each of the respective pesticide standards are plotted as a function of the scanned wavelengths in the UV-Visible region. Notably, both organometallic Cu(II)-cypermethrin and Cu(II)-acephate complexes exhibit absorptions in the UV region. Under the measurement conditions, the Cu-Acephate complex has its maximum absorption at about 207 nm while the Cu-Cypermethrin complex exhibits maximum absorption at about 321 nm. These maximum wavelength values are due to the spectra sharpness and proper baselines. Remarkably, the positioning of these spectra (Figure 3.5 and 3.6) and the intensities of their maxima gives important information about these two pesticides. Quantification methods that exploit the formation of heavy metal chelates have been performed using UV-Visible spectrophotometry. The binding of Cu(II) ions in the current case plausibly occur via the oxygen, nitrogen, and sulfur-ligating donor atoms that are present in the pesticide compounds based on the chelate effect (Jallow *et al.*, 2017).

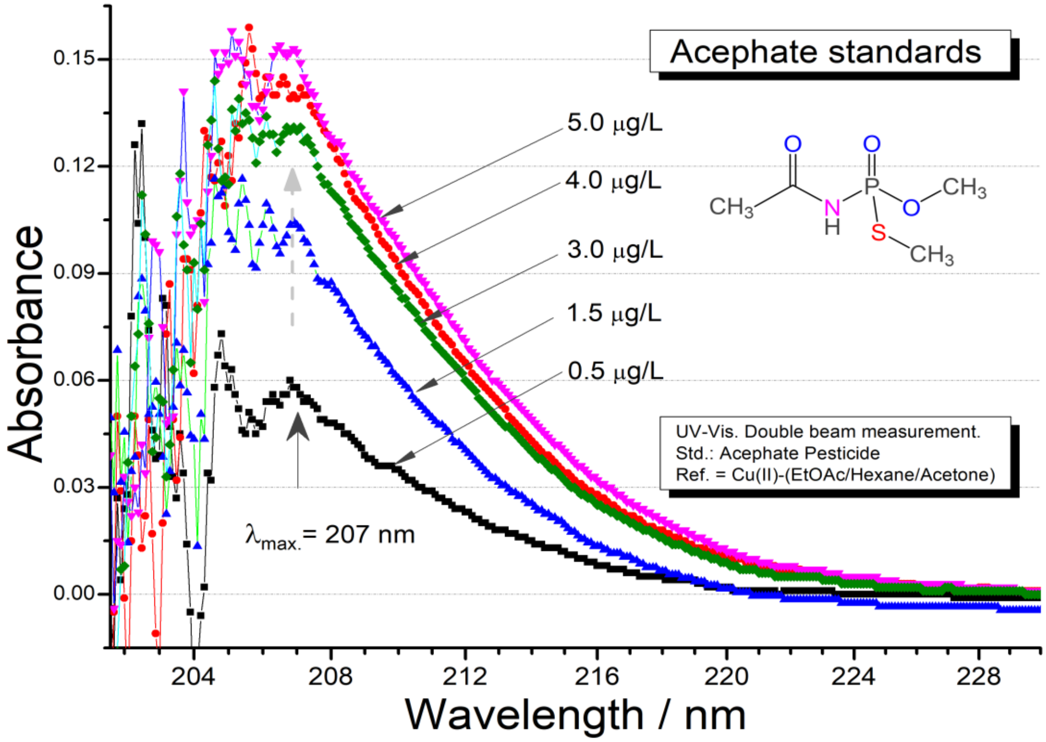


Figure 3.6 UV-Visible absorption spectra of Cu (II) complexes formed with acephate standard in EtOAc/acetone/hexane solvent system

3.4.2 Calibration curves of cypermethrin and acephate standards

Figure 3.7 (a and b) shows the calibration plots derived by recording the absorptions of the standard solutions of Cu-cypermethrin and Cu-acephate at 321 nm and 207 nm respectively. Individual photometric measurements based on each wavelength for the determination of these pesticide residues in various samples of khat. The linearity of the standard curves was determined by plotting the mean absorbance values (including the standard errors) against the concentration (in µg/L) of the respective standards. The plots were fitted by applying linear regressions, both of which showed a good fit with a very strong coefficient of determination $R^2 > 0.99$ and very low standard errors in the intercepts and gradients. The respective calibration plots indicate the linear regression equations used to determine the concentrations (µg/L) of the mentioned pesticides. The good linearity demonstrated herein is similar to that of a previous study used for the validation of assay of formulation of paracetamol tablet (Behera *et al.*, 2012).

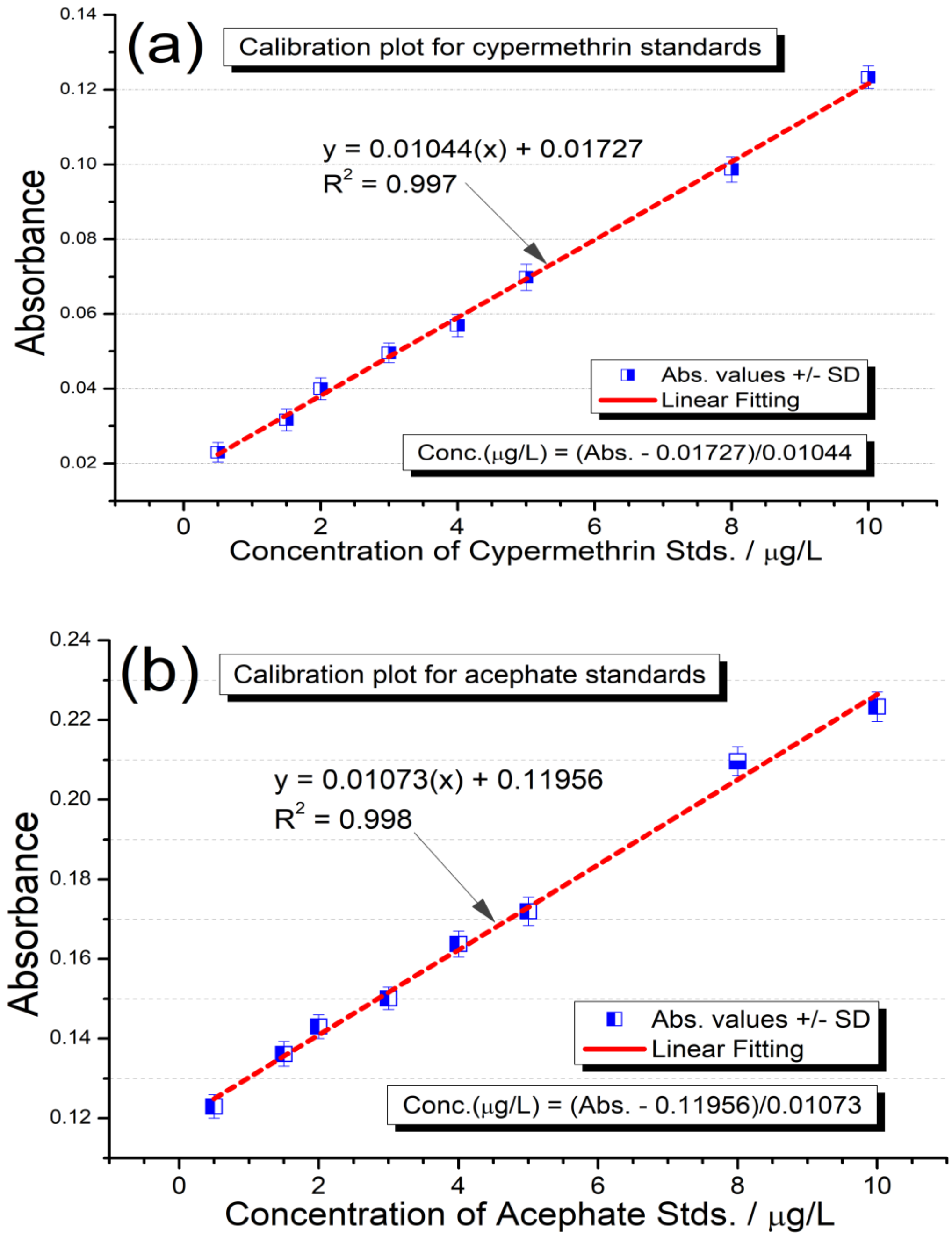


Figure 3.7 Standard curves for the Cu(II) complexes formed with (a) cypermethrin and (b) acephate both in EtOAc/hexane/acetone solvent system

The linearity data is shown in Table 3.3. There was a strong linear relationship between the mean absorbance values and the concentration of analyte in the working/calibration range of between 0.50 µg/L and 10.0 µg/L for both acephate and cypermethrin standards. The estimated linear dynamic range (LDR) ranged from 1.5 to 6.5 µg/L for cypermethrin and between 1.8 µg/L and 7.0 µg/L for acephate. The limits of quantitation (LOQ) based on non-linear data extrapolation were estimated to be 0.252 μg/L, and 0.261 μg/L for cypermethrin and acephate, respectively. The estimated LDR and LOQ values were determined by applying validation procedures that consider the standard deviation of the signal response (in this case, the measured absorbance values) and the calibration curve slopes (Rao, 2018; Sengul, 2016).

Table 3.3: Linearity data of the calibration curves for acephate and cypermethrin standards

Pesticides	Working/Curve range (μg/L)	Slope	R^2	LDR (μg/L)	LOQ (μg/L)
Cypermethrin	0.50 – 10.0	0.01044	0.9987	1.5 – 6.5	0.252
Acephate	0.50 – 10.0	0.01073	0.9997	1.8 – 7.0	0.261

The results of this study indicate that the developed spectrophotometric techniques may act as a useful alternative analytical quality control laboratory method for the trace level determination and comparison of pesticide residue levels (PRLs) within the concentration ranges of between 1.0 μg/L and 10.0 μg/L.

Table 3.4: Estimated concentrations of acephate and cypermethrin in the samples of khat

Khat sample	Cypermethrin (μg/L)	Acephate (μg/L)	MRL**	HQ***
Sample B	2.145 ± 0.047	ND*	<	0.247
Sample D	ND*	2.897 ± 0.034	<	0.289
Sample F	ND*	7.978 ± 0.056	<	0.797

ND* means Not detected by the GC-MS qualitative analysis; **According to EU-EPA standards; *** Exposure Concentration/Reference MRL value

Table 3.4 shows this study's results from the UV-Vis spectrophotometric quantitation measurements of the concentrations of acephate and cypermethrin pesticide residues as determined from the khat samples from which they were detected using GC-MS qualitative analysis. From the khat samples analysed, acephate was found to exist at a higher concentration than residues of cypermethrin. There was a significant variation in the acephate residue content between khat sample D and sample F. The concentrations were all below the MRL values set by Codex (Janta *et al.*, 2022). According to the quantitative analysis, the HQ are computed relied on MRL values (Li *et al.*, 2021). They were 0.247 for cypermethrin and between 0.287 and 0.797 for acephate. These results showed that the consumption of Meru khat is safe and will not pose any significant health threat to humans. Accordingly, to limit any possible pesticide intake, thorough washing of the young chewable khat leaves and shoots with clean water to remove any pesticide residues adhered to them is strongly recommended.

Conclusion

In the present study, a modified GC/EI-MS analytical technique was successfully employed to carry out chromatographic separation, detection, and profiling of pesticide residues present in the samples of khat. Three organophosphate pesticides (chlorfenvinphos, chlorpyrifos and acephate) and three pyrethroid-based compounds (cyfluthrin, cyhalothrin, and cypermethrin) were identified and characterised. A UV-Visible spectrophotometric method was developed, optimised, and applied for quantitative analysis of detected pesticide residues, demonstrating a rapid and cost-effective procedure for estimating and comparing pesticide concentrations in the samples of khat. The Cu-pesticide chelate complexes had the highest absorptions in the UV range, according to double-beam wavelength scan spectrophotometric tests. The intensities of maximum absorptions for the prepared Cu-cypermethrin and Cu-acephate pesticide standards were found to be linearly related to their concentrations. The relationship followed the Beer-Lambert equation with high linearity ($R^2 > 0.99$) in the ppb range of between 0.5 µg/L and 10.0 µg/L. The UV-Vis quantitative analysis of the samples revealed acephate concentrations ranging from 2.897 to 7.978 µg/L, whereas cypermethrin concentrations were 2.145 µg/L. Their concentrations were below the MRLs, and the hazard quotients were also moderately low. The developed UV-Visible analytical method may find application in food safety control laboratories as a methodology for quantifying residues in the samples of various vegetables.

CHAPTER FOUR

ANALYSIS OF THE CONCENTRATION OF HEAVY METALS IN KHAT GROWN IN MERU COUNTY AND THE ASSESSMENT OF THEIR ASSOCIATED HEALTH RISKS

Abstract

The contamination of agricultural products with potentially hazardous heavy metals has emerged as a major global problem. Plants can accumulate these metals, posing serious health risks including cancer and brain damage. Khat (*Catha edulis*) plant has the ability to accumulate these metals and micronutrient elements in its tender leaves and young shoots, which have been shown to cause psychedelic characteristics when consumed. Consequently, this study aimed to investigate the concentrations profiles of the selected metallic elements, namely cadmium (Cd), iron (Fe), copper (Cu), chromium (Cr), lead (Pb) and nickel (Ni) in samples of Meru khat, compare these levels to WHO limits in order to predict related health risks, and estimate their non-carcinogenic risks on khat consumers using total health quotient (THQ) and health index (HI). Approximately 1.0 g of dry ground khat was acid digested and stored for 5 hours before being analysed for potentially toxic heavy metals by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The mean concentrations (mg/kg) of the selected elements in dry khat samples were as recorded as follows: Pb (32.36 ± 9.95), Cd (7.81 ± 1.56), Cu (15.81 ± 2.84), Ni (0.37 ± 0.02), Cr (15.98 ± 2.22), and Fe (97.35 ± 32.67). According to these results, the mean levels of Pb, Cr, and Cd surpassed the WHO acceptable limits. Furthermore, the Pb and Cd THQ values, as well as the HI of the studied metals in the khat samples, toppled the 1.0 standard. These THQ and HI values revealed that Pb and Cd elements were the major contributors to non-carcinogenic risks for habitual khat users. The excessive intake of Meru khat is cause for concern, since it may result in a toxicological response over time. Based on the study's findings, the application of agrochemicals in khat growing should be drastically reduced. As such, farmers should be sensitised on alternate farming practices that significantly limit heavy metal contamination in khat.

4.1 Introduction

The farming of khat and its related trading activities are multi-million dollar businesses in regions which grow khat, and they contribute significantly to the gross domestic product (GDP) of the growing nations (Atlabachew *et al.*, 2010; Tolcha, 2020). Meru is not only Kenya's leading county which produces khat for domestic and foreign markets, but it is also the country's largest consumer (Krueger & Mutyambai, 2020; Njuguna *et al.*, 2013). Nevertheless, the consumption of young shoots and tender leaves of khat for stimulative and psychotropic effects is to a great extent uncontrolled, and pollutants including potentially toxic heavy metals and pesticides residues in khat are rarely evaluated and monitored prior to consumption. This exposes consumers of khat to the health risks that can arise from the contaminated khat. This work is thus motivated by the potentially toxic heavy metals in khat, which are regarded a consequential health danger caused by their bioaccumulation and may pose severe etiological risks. As a result, a lack of awareness regarding the levels of heavy metals in foods and consumed plants, particularly khat, has raised numerous health issues which can be detrimental to the well-being of the public (Khaleeq *et al.*, 2022).

Periodic determination of potentially trace heavy metals and other essential elements in crops, soils samples, and water is critical for monitoring their concentrations and keeping them below acceptable limits (Al Bratty *et al.*, 2019; Kohzadi *et al.*, 2019) to guarantee health safety. It is worth noting that studies into the elemental characteristics of plant-based and food materials are expanding in response to the need for the development of sustainable agriculture, pollution of heavy metals, and natural phenomena such as lightning and volcanic eruptions (Melkamu *et al.*, 2022). As a result, improved food security should focus on avoiding heavy metal contamination (Islam *et al.*, 2015), poisonous pesticides, and other agricultural-based chemical compounds (Bett *et al.*, 2019).

Undoubtedly, trace heavy metals are key food chain contaminants that can cause negative health effects in humans even at low levels (Khaleeq *et al.*, 2022). Despite widespread concern about heavy metal pollution, human activities have significantly contributed to increase in the utilisation of soil as a reservoir for toxic metals. These metals are non-biodegradable and accumulate in the soil, potentially increasing their toxicity when combined with inorganic and organic matter (Ahmad *et al.*, 2021). The khat plant may absorb micronutrients and heavy metals from polluted soils, including landfills and industrial effluent (Khaleeq *et al.*, 2022). Continuous uptake of these metallic elements bio-magnify in the plant's leafy sections, resulting in varied amounts at different plant parts (Ashenef *et al.*, 2014; Bett *et al.*, 2019). It is indispensable to highlight that khat is likely to be destroyed by pests; therefore, the use of pesticidal chemicals controls them, and fertiliser applications increases its yield (Atlabachew *et al.*, 2010). These techniques introduces potentially heavy metals into soils and leaf surfaces, which are ultimately taken by plants into their tissues (Tadesse & Kebede, 2015). Their bioaccumulation, particularly in plant tissues, is determined by the plant's age and maturity at the time of harvest (Bett *et al.*, 2019). This aggregation of heavy metals easily contaminates consumable khat parts, posing substantial health hazards when consumed (Chabukdhara *et al.*, 2016).

Toxicity of heavy metal has recently become a public health concern, requiring continuous monitoring and assessment (Bett *et al.*, 2019). Thus, human health risk assessment is censorious because it predicts the risk of environmental pollution to health via several means, including inhalation exposure, ingestion administration, and the skin contact (Sajjadi *et al.*, 2022). Correspondingly, research reports from throughout the globe have revealed significant health effects caused by poisonous heavy metals found in plant samples, as well as a potential threat to animal and human lifespans (Kohzadi *et al.*, 2019). This is because these metals are chemically stable and non-biodegradable, persist in the environment for a long time, and can cause major public health issues such as cancer disease, and nerve failure (Tadesse & Kebede, 2015). However, WHO regularly reports that some trace heavy metals, particularly Cu, Fe, and Zn, are not only nutritive elements but also important for growth if their concentrations do not surpass permissible limits, beyond which they can result into the onset of diseases (Kohzadi *et al.*, 2019; Organisation, 2007), intoxication, and chronic toxicity in humans (Islam *et al.*, 2015).

Heavy metals can be detected and quantified in a variety of samples using analytical techniques such as ICP-AES, atomic absorption spectrometry (AAS) (Llorent-Martínez *et al.*, 2011), and inductively coupled plasma optical emission spectrometry (ICP-OES) (Gebeyehu & Bayissa, 2020). To the best of our knowledge, minimal and unreliable study has been undertaken on heavy metals in Meru-khat. This work, therefore, is important as it will evaluate the concentration profiles of six heavy metals in Meru khat leaf shoots - Cd, Cr, Cu, Pb, Fe, and Ni - and compare them to WHO permissible levels in order to conjecture related health hazards from khat use. To evaluate the magnitude of these metals' risks, non-carcinogenic THQ and HI parameters were determined. Consequently, this study forms a basis for alternative yet sustainable agricultural practices in the cultivation of khat plants that are safe. Furthermore, these findings can help researchers better understand the human risks of some heavy metals in farmed plants like khat in the regions of Meru County. This will provide information on contamination prevention in terms of human health concerns and sustainable, human-benign economic growth that will spur development (Taghavi *et al.*, 2023).

4.2 Study area

In this study, 11 khat samples were obtained at random from farmers of khat in each of the regions of Kangeta, Laare, Mutuati, Kianjai, and Maua in Meru County. *Catha edulis* (miraa), also called as *Miwee* by the Ameru people, is a species that is grown for both local consumption and export. Figure 4.1 shows the sample collecting sites. The gathered samples were then brought directly to the chemistry laboratory for treatment and elemental analysis. Meru County, with locational coordinates 0° 21′ 21″ N/37° 48′ 32″ E in Kenya covers a total area of land of 7,006 km^2. According to Kenya Population and Housing Census of 2019, the population of this County was 1,545,714. Moreover, the yearly rainfall ranges from 300 mm to 2500 mm, with long rains falling from March to May and the short rains from October to the month of December. The Meru County is located near the equator, thus, making summers arduous to define. The documented annual temperature range is 12.92 to 23.45 degrees Celsius characterised with the coldest and warmest months. Khat cultivation is the primary source of income for the county's residents, who benefit greatly from this lucrative and profit-making agribusiness venture. This County is one that not only consumes but also exports a lot of khat to neighbouring countries like Somalia, and Uganda (Krueger & Mutyambai, 2020). Khat plant in this county can withstand climate extremes, such as drought and cold conditions. In recent years, the domestic khat demand and the international markets have put a strain on khat supplies. Agricultural practices and animal husbandry are the most common activities in the majority of the studied areas, and fertilisers (both animal dung and artificial fertilisers) and insecticides are commonly applied. The majority of khat farmers in this county use an intercropping method with other food crops (Krueger & Mutyambai, 2020).

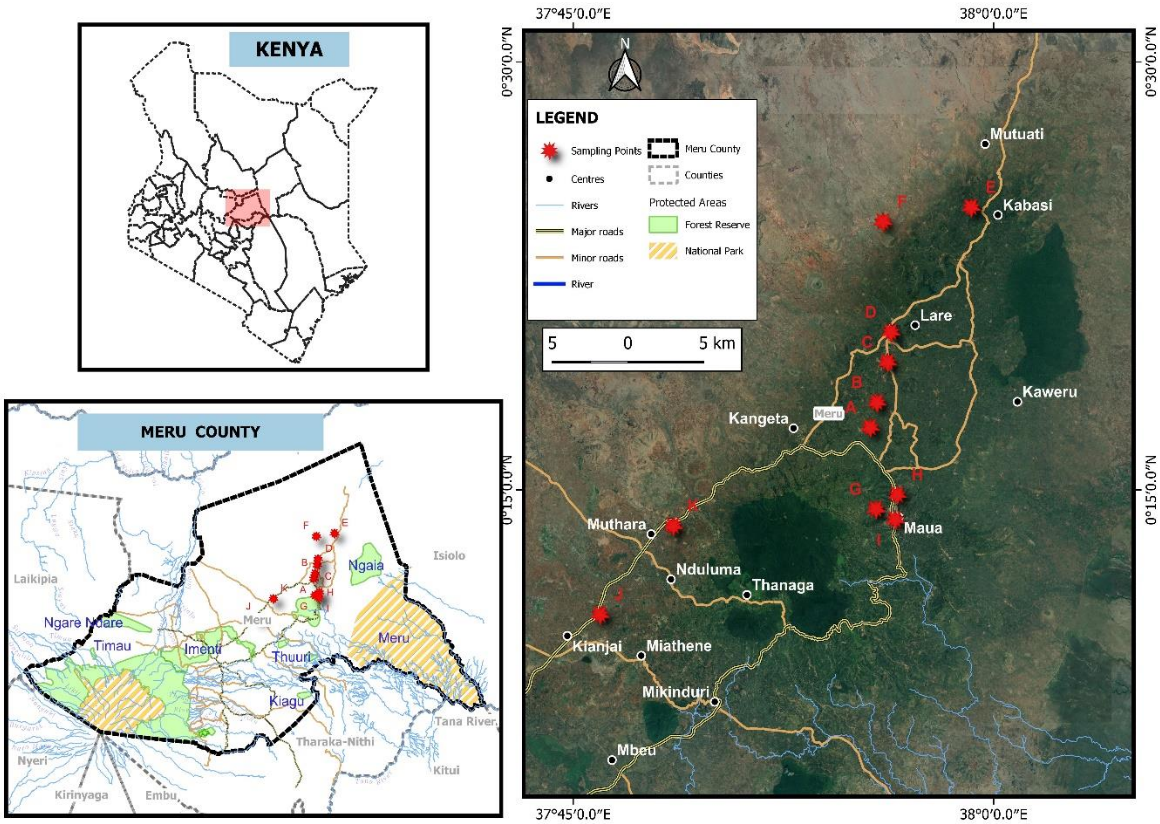


Figure 4.1 A Meru County map illustrating khat sample collection farms.

4.3 Experimental

4.3.1 Reagents and preparation of sample

This study used analytical grade reagents with percentage purity levels of greater than 99.90%. Materials and chemical reagents, such as commercial standard 1000 ppm mixture containing six selected heavy metals, were bought from Kobian Chemical Company, which is a subsidiary of Sigma Aldrich Ltd, South Africa. In addition, 10% nitric (v) acid and 20 volume hydrogen peroxide (H_2O_2) were also used. Throughout the study, deionized water obtained from Sigma Aldrich (Germany) was utilised for rinsing of apparatus, preparation of samples, and in the dilution prior to elemental analysis.

The samples of khat were air-dried in the chemistry laboratory for 7 days, then oven-dried at 70 ℃ for 3 days to obtain a constant mass. The samples of dry khat were homogenised and reduced to 0.25 mm in size with a grinder. They were then placed in sterilised plastic containers awaiting further analysis. The elements content of digested samples were determined using an inductively coupled plasma atomic emission spectrometer (ICPE-9000).

To minimise cross contamination challenges, cleaning of stainless steel grinding systems with acetone was done thoroughly after each grinding cycle. The ground samples of khat were kept in sterilised plastic containers. The deionized water was used to thoroughly clean all the glassware, followed by a powerful 10% nitric (V) acid wash (Manousi & Zachariadis, 2020). During the sampling of khat and analysis stages, the plastic gloves which as disposable were worn. Finally, the digested solutions of khat were refrigerated until the elemental analysis was completed.

The digestion procedure for the samples of khat was optimised for various parameters, including reagent volume, reaction temperature, digestion time, and reagent volume ratio. The trial procedure optimisation was performed through variation of one parameter at a time while maintaining the other parameters constant. All the parameters gave clear khat solutions at lower reaction temperatures, shorter times, and needed a minimal amount of reagent volume and ratio were chosen as the optimal procedure for the digestion of the samples of khat. Exactly, 1.0 g of ground dried khat sample was weighed and placed in sample digestion tubes. The khat sample ashed in a muffle furnace for 5 hours at 450–500 ℃ prior to cooling into ambient temperature in a desiccator apparatus. This kaht sample was taken into a 100 mL conical flask, which was followed by approximately 1 mL of a mixture of H_2O/HCl (1:1 v/v), H_2O/HNO_3 (1:1 v/v), and the 10 cm^3 20 volumes H_2O_2, and the mixture was evaporated on a hot plate to dryness. The mixture was later reconstituted with 25 mL of HCl whose concentration is 0.05 M and let to stand still for a period of 5 hours before heavy metal analysis. The digests of khat were then used to determine the concentrations of Ni, Cu, Cr, Cd, Pb, and Fe using ICP-AES.

4.3.2 Analysis of potentially heavy metals

All standards, blanks, and khat samples were prepared, and analysed in triplicate. A solution for tuning the analytical instrument containing 0.1 mg/kg of HCl was used for quality checking and tuning. A calibration curve was developed that used nine points of a multi-element standard in absorbance mode to estimate the content of heavy metallic elements in samples of khat. The ICP-AES analytical method developed was then validated for linearity, accuracy, durability, and precision. Table 4.1 shows the parameters of the instrument and working conditions for ICP-AES analytical method. Ultra-pure (>99.99%) argon was used as auxiliary gas, plasma, and the nebulizer. Prior to the studies, the calibration of the instrument for different parameters was done.

Table 4.1: The instrument's parameters and working conditions of ICP-AES

ICP-AES parameter	Value
Radio Frequency Generator	1.2 kW
Vacuum pressure	> 10 Pa
Plasma power	1300 W
Pump rate	25 rpm
Nebulizer flow	0.90 L/min
Coolant flow rate	12.00 L/min
Auxiliary gas flow	0.60 L/min
Exposure time	30.0 seconds
Plasma gas flow rate	10.0 L/min
Carrier gas flow rate	0.70 L/min
Pressure of argon gas	450 ± 10 kPa

4.3.3 Evaluation of accuracy of the method and precision

The method's precision and accuracy parameters were evaluated by spiking 10 mL aliquot of 5 μ*g* of each metal analyte into 1.0 g sample of khat followed by the optimized digestion procedure that involved both the spiked and non-spiked samples. The concentrations of the six heavy metals in both khat samples were determined by ICP-AES method, and the recovery percentages were computed using Equation 4.1.

$$\text{Percentage recovery} = \frac{A_{sks} - A_{ks}}{A_a} \times 100 \qquad (4.1)$$

Here, A_{sks} is concentration in spiked sample of khat, A_{ks} denotes the concentration in non-spiked sample of khat, while A_a= Amount of each metal added

4.3.4 Target hazard quotient (THQ)

The THQ was used to evaluate the non-carcinogenic risks of heavy metals to humans from consuming contaminated khat on a daily basis (Peirovi-Minaee *et al.*, 2023). The aggregated risks of collective exposure to the six heavy metals in young khat leaves and shoots were calculated using the HI value. The THQ of the examined heavy metallic elements was calculated using Equation 4.3.

$$THQ = \frac{C^i \times IR \times EF \times ED}{Bw \times RfD \times AT} \times 10^{-3} \quad (4.2)$$

Since $\frac{EF \times ED}{AT}$ = 1, then Equation 4.2 becomes;

$$THQ = \frac{C^i \times IR}{Bw \times RfD} \times 10^{-3} \quad (4.3)$$

Where, Bw represents body weight of the adult considered as 70 kg; C^i stands for the mean concentration of potentially heavy metallic element in the ground dry khat (mg/kg); EF denotes the exposure frequency taken as 365 days/year; ED stands for exposure duration which was taken as seventh years; AT is the average time for possible health risk given as ED × 365 days; IR denotes the rate of ingestion of khat given as 100 g/person on daily basis as determined in previous research (Mawari *et al.*, 2022).

The oral reference dose (RfD) given in mg/kg/day was taken as Cd (0.001), Pb (0.0035), Ni (0.02), Cu (0.04), Cr (1.5), and Fe (0.07) (Latif *et al.*, 2018; Ogundele *et al.*, 2015; Taghavi *et al.*, 2023; Woldamanuel, 2019). The cumulative HI assumes that the extent of adverse health effects on target human organs and systems will correspond to the summation of THQ of all metal exposures in samples of khat. This was approximated using Equation 4.4.

$$HI = \sum THQ = THQ_{(Pb)} + THQ_{(Cu)} + THQ_{(Cd)} + THQ_{(Fe)} + THQ_{(Cr)} + THQ_{(Ni)} \quad (4.4)$$

If HI is < 1.0, then the non-carcinogenic risks due to the heavy metals exposure are considered to be negligible. However, if the HI value > 1.0, then these risks are detrimental (Peirovi-Minaee *et al.*, 2023).

4.3.5 Statistical analysis

All statistical tests in this study were performed using Microsoft Excel (MS Excel) spreadsheets and IBM's Statistical Package for the Social Sciences (SPSS) version 25.0. The descriptive statistics such as means, standard deviation (SD), and relative standard deviation (RSD) were calculated using MS Excel. Multivariate statistical analysis was used to investigate the variation in heavy metal concentrations in khat plants among various farms. The Pearson correlation coefficient (PCC) was used to examine the relationship between correlated variables (concentration of each metal) in order to determine data divergence and coherence. The principal component analysis (PCA) was used to simplify the data obtained after conducted experiments and provide predictions about heavy metal sources in khat from Meru County. The PCA supports large data sets for consistent dimension reduction while retaining the majority of the information.

4.3.6 Method validation and recovery test

The calibration curve obtained using standards of Cd, Cr, Pb, Fe, Ni, and Cu showed good linearity with a correlation coefficients of $r^2 \geq 0.998$. To assess the ICP-AES method's accuracy, spiked samples of khat were used. The recovery test experiments at various concentrations were examined, with the findings shown in Table 4.2. The percentage recoveries ranged from 91.70% to 109.10% demonstrate that the analytical method adopted show meritorious accuracy.

Table 4.2: Percentage recoveries of heavy metals in samples of khat

Heavy metal	*Concentration in non-spiked khat sample (mg/kg)	Amount added (mg/kg)	**Concentration in spiked khat sample (mg/kg)	Percentage recovery (%)[a]
Cu	16.36	3.50	19.90 ± 0.08	101.1 ± 0.3
Cd	7.64	1.50	9.09 ± 0.11	96.7 ± 0.9
Pb	24.45	2.00	26.43 ± 0.07	99.0 ± 0.2
Fe	91.78	5.00	96.47 ± 0.80	93.8 ± 0.6
Ni	0.36	1.00	1.34 ± 0.01	98.0 ± 0.2
Cr	16.14	2.50	18.59 ± 0.20	98.0 ± 0.7

Legend; *: Average value of three measurements; **: Values are mean ± SD of triplicate analyses; [a] Values are mean ± SD of triplicate

The sensitivity of ICP-AES analytical method was evaluated by computing the limits of detection (LOD) and limits of quantification (LOQ). Consequently, ten different blank solutions were separately prepared and analysed. The calculations of the LOD and LOQ values were done in accordance with the International Union of Pure and Applied Chemistry (IUPAC) recommendations (Manousi & Zachariadis, 2020) using the Equations (4.5) and (4.6);

$$\text{LOD} = 3\left(\frac{\delta}{s}\right) \quad (4.5)$$

$$\text{LOQ} = 10\left(\frac{\delta}{s}\right) \quad (4.6)$$

Where; s = calibration curve slope for each metal, δ = SD of the blank solution measurements.

To evaluate the linearity of the method, calibration curves for each metal were created by plotting the area of the peak of the optimal emission line against the concentration of the standard. The slope, intercept, and coefficient of determination were then calculated using least squares linear regression.

4.3.7 Calibration

The ideal emission lines were chosen based on their intensity, sensitivity, and absence of spectral interferences. Accordingly, the emission lines used for each heavy metal in this investigation were 357.869 nm for Cr, 324.752 nm for Cu, 217.000 nm for Pb, 238.204 nm for Fe, 232.003 nm for Ni, and 226.502 nm for Cd. The performance of the ICP-AES analytical method was assessed under the working conditions and parameters outlined in Table 4.1. Table 4.3 illustrates the calibration curve, linear equations, LOD and the LOQ for all metals investigated in samples of khat. Table 4.3 shows that all heavy metals had good coefficients of determination (r^2 > 0.9800) based on experimental values. The ICP-AES method's LOD and LOQ ranged from 0.072 to 1.193 mg/kg and between 0.220 and 3.616 mg/kg, respectively. The analytical method used demonstrated high sensitivity at lower levels of LOD.

Table 4.3: Calibration curves, LODs and LOQs of the developed ICP-AES method.

Element	**Emission line (nm)**	**Equation**	**slope**	r^2	**LOD (mg/kg)**	**LOQ (mg/kg)**
Cd	226.502	Y=1.7402x - 371	1.7402	0.9855	0.979	2.966
Ni	232.003	Y=1.1564x + 24	1.1564	0.9994	0.195	0.296
Pb	217.000	Y=1.6231x - 199	1.6231	0.9967	0.072	0.220
Cr	357.869	Y=1.3688x - 67	1.3688	0.9953	0.552	1.673
Fe	238.204	Y=1.3819x - 74	1.3819	0.9930	1.193	3.616
Cu	324.752	Y=1.4488x - 32	1.4488	0.9958	0.924	2.801

4.4 Results and discussion

4.4.1 The concentration of metals in khat samples

Table 4.4 shows the levels of six heavy metals (Pb, Cu, Ni, Cd, Cr, and Fe) in samples of khat taken from the regions of Meru County. The results demonstrate a wide variations of heavy metal concentrations in khat sampled from several growing farms in Kangeta, Maua, Laare, Mutuati, and Kianjai regions of this county.

Table 4.4 shows the average concentration and RSD values for all studied metals. The concentrations of these elements vary among samples of khat. The RSD values ranged 4.2 - 33.6. The results of this study revealed that Fe was the most prevalent metallic elements in samples of khat, followed by Cd, Cr, Ni, Cu, and Pb. This results further shows that iron is the most abundant element in samples of khat, with concentrations (mg/kg) ranging between 72.10 ± 2.13 to 180.50 ± 3.02 and a mean level of 97.35 ± 32.67 mg/kg. The highest concentration value was obtained from the Laare region (sample D), while the lowest concentration value came from sample G of Maua region. This could be attributable to khat grown in various geographical regions with varying soil types and profiles, pH, and agrochemical applications. In comparison, investigations on Ethiopian khat found lower levels of Fe than the current study (Tadesse & Kebede, 2015). On the other hand, this work's result was lower than Fe concentration of khat collected from several parts of Ethiopia, including the Amhara, Oromia, and Southern Peoples Nations and Nationalities (Ashenef *et al.*, 2014). Nonetheless, the Fe levels in our study compare consistently with those recorded from several locations of Ethiopia and in Yemen (Desta & Ataklti, 2015).

Soil with high organic matter content contribute to high Fe levels, particularly in khat plant (Desta & Ataklti, 2015; Hagos *et al.*, 2010). Mulching using maize stalks under khat plants not only avoids soil water evaporation, but also undergoes decomposition and therefore reincarnates into soil structure improving fertility thus, increasing Fe levels in the soil. It is believed that a khat consumer chews 50-200g of young fresh khat shoots and tender leaves per day (Ashenef *et al.*, 2014). The computations in Table 4.5 are based on this estimation, which is then compared against permissible standards to determine any attributed health risks.

Table 4.4: The mean concentration of heavy metals in samples of khat collected from farms of Meru County

Region in Meru county	Farm code	Mean concentration (mg/kg) of metals					
		Cr	**Cd**	**Cu**	**Pb**	**Fe**	**Ni**
Kangeta	A	16.40 ± 0.23	5.51 ± 0.11	17.00 ± 0.42	24.14 ± 1.26	92.90 ± 3.12	0.378 ± 0.03
	B	19.10 ± 0.12	5.58 ± 0.10	21.18 ± 0.61	31.11 ± 1.52	139.0 ± 3.64	0.386 ± 0.02
Laare	C	13.90 ± 0.14	9.72 ± 0.08	16.79 ± 0.32	36.29 ± 1.02	83.30 ± 2.11	0.361 ± 0.03
	D	17.60 ± 0.25	8.88 ± 0.13	14.67 ± 0.35	42.55 ± 2.45	180.5 ± 3.02	0.392 ± 0.02
Mutuati	E	14.60 ± 0.18	5.48 ± 0.05	13.03 ± 0.26	15.53 ± 0.33	86.80 ± 2.50	0.373 ± 0.01
	F	19.00 ± 0.31	8.53 ± 0.06	13.70 ± 0.18	25.92 ± 1.92	83.40 ± 2.10	0.367 ± 0.02
Maua	G	12.30 ± 0.15	9.23 ± 0.05	17.23 ± 0.43	48.10 ± 2.82	72.10 ± 2.13	0.352 ± 0.02
	H	18.00 ± 0.27	8.74 ± 0.12	14.06 ± 0.32	<0.001 ± 0.0	78.50 ± 2.13	0.399 ± 0.04
	I	15.60 ± 0.16	8.32 ± 0.23	14.43 ± 0.36	<0.001 ± 0.0	88.50 ± 2.09	0.371 ± 0.03
Kianjai	J	15.00 ± 0.13	7.67 ± 0.04	19.70 ± 0.52	30.10 ± 2.21	80.40 ± 1.89	0.349 ± 0.02
	K	14.30 ± 0.11	8.28 ± 0.05	12.08 ± 0.64	37.47 ± 2.02	85.50 ± 2.05	0.365 ± 0.03
Mean ± SD		15.98 ± 2.22	7.81 ± 1.56	15.81 ± 2.84	32.35 ± 9.95	97.35 ± 32.67	0.3721 ± 0.016
RSD (%)		13.9	20.0	18.0	30.8	33.6	4.2

Legend: SD: Standard Deviation; RSD: Relative Standard Deviation

The results shown in Table 4.5 illustrate that the computed THQ < 1.0 for Fe, indicating that there is no risk of health concerns. As a result, the Fe concentration in all khat samples analysed was less than the WHO/FAO acceptable limit (425.0 mg/kg) (Latif *et al.*, 2018). This demonstrates that khat sourced from the Meru region could be an effective iron supplement. Thus, its determination in khat samples is not only important for users of khat, but keeping its contents below acceptable limits aids in a variety of metabolic activities such as transport of oxygen, as well as DNA synthesis (Abbaspour *et al.*, 2014). Furthermore, in human biological processes, Fe promotes the regular functioning of body cells and other vital organs, as well as the formation of free body radicals that can be beneficial (Milto *et al.*, 2016).

Copper is also a vital trace metal that promotes plant and animal health. This study found Cu concentrations in samples of khat ranging between 12.08 ± 0.64 and 21.18 ± 0.61 mg/kg, where the mean concentration is 15.81 ± 2.84 mg/kg as shown in Table 4.4. Cu contents were highest and lowest in samples collected from a farm in Kangeta region (labeled sample B) and the region of Kianjai (sample K), respectively (Table 4.4). High concentrations of Cu element could be ascribed to the use of pesticides rich in Cu used to control pests, while the khat trees may also draw up it from soil reservoirs (Woldamanuel, 2019). Cu levels in this study are found to be higher than those previously reported in Ethiopia's khat growing regions which ranged between 5.11 to 9.55 mg/kg (Ashenef *et al.*, 2014), but were also lower than those reported in khat obtained from Aweday growing region and other locations of Ethiopia, which had concentrations 0.10-41.80 mg/kg (Woldamanuel, 2019). Interestingly, the Cu levels in this study are lower than the WHO/FAO acceptable limits (73.0 mg/kg) for vegetables (Latif *et al.*, 2018). The THQ calculated in Table 4.5 show that khat used in this study is not polluted with Cu metal and may not pose any health risks when consumed in the Meru County. It is worth noting that, below permissible concentrations, this element is an essential component of various enzymes, including catalase, as well as metabolic reactions. Further, it is also required for the neurological and haematological systems (Al-Fartusie & Mohssan, 2017), and biological processes in living organisms (El-Zomrawy, 2018).

Lead is a well-known toxic heavy metal that can contaminate the environment and provide major health concerns (Beardsley *et al.*, 2021). The concentration of Pb metal in samples of khat in this study ranged between 15.53 ± 0.33 mg/kg and 48.10 ± 2.82 mg/kg, with a mean concentration of 32.35 ± 9.95 mg/kg as shown in Table 4.4. Lead levels in khat samples obtained from farms H and I in the region of Maua were found to be less than 0.001 mg/kg. This could be ascribed to the soil's low content of Pb and the likely absence of lead-based agrochemicals (Abdul, 2010). Contaminated water sources such as rivers, presumably with detergents and other effluents used to irrigate khat trees, may also contribute to reported amounts of heavy metals. Sample G from the Maua region contains a significant amount of lead. The use of synthetic pesticides and fertilisers, as well as compost manure, all contribute to elevated levels of heavy metals. Furthermore, several studies have found that as pH drops, heavy metal solubility increases (Xiong & Wang, 2005), increasing their absorption by plants. In addition, the disposed batteries and waste poisonous paints into khat farms has the potential to increase Pb concentrations in soils (Mahurpawar, 2015). Nevertheless, Pb concentration in this study is lower than that found in the regions of Aweday, Wendo, Haramaya, Indibir, and Bole in Ethiopia that grow khat which ranged 5.0-119.0 mg/kg (Woldamanuel, 2019). The computed THQ values as shown in Table 4.5 which are greater than 1.0, indicate that khat use may have health effects. All of the khat samples contained a high concentration of Pb compared to the WHO/FAO permitted limit (0.3 mg/kg) for vegetables (Woldamanuel, 2019). This demonstrates that the samples of khat investigated in this study were contaminated with Pb metal. As a result, safe khat and farming practices must be recommended in order to minimise lead concentrations to levels below WHO/FAO threshold standards.

Cadmium is a highly toxic metal that is well-known to cause cancer in humans (Nkansah *et al.*, 2016). Previous research has found that excessive Cd exposure can cause severe bone damage, hypertension, and kidney failure (Garner & Levallois, 2017). The study found that Cd levels in samples of khat ranged 5.48 ± 0.05 to 9.72 ± 0.08 mg/kg, with an average of 7.81 ± 1.56 mg/kg. The Cd concentrations were highest in samples of khat collected from region of Laare (farm C) and Maua region (farm G), while lowest in khat samples collected from Kangeta region (farms A and B) and Mutuati region (farm E), with concentrations of 5.51 ± 0.11, 5.58 ± 0.10, and 5.48 ± 0.05 mg/kg, respectively. The determination of Cd in khat could be attributed to the regular application of phosphate fertilisers and manures, particularly animal waste from sheep, cows, chickens, and pigs (Woldamanuel, 2019). This toxic metal might also result due to the disposal of spent dyes and paints, as well as discarded electronics, which are discharged onto farms via surface run-off and then absorbed by khat plant tissues (Mahurpawar, 2015). Groundwater obtained through wells and boreholes primarily used irrigation of crops may include Cd from soil sediments (Engwa *et al.*, 2019). This work highlights the prevalence of mixed farming in Meru County, particularly the intercropping practices involving khat with other food crops such as maize that require utilisation of phosphate fertilisers throughout the time of planting. This kind of growing allows khat to absorb Cd-contaminated fertilisers. Table 4.4 shows that the Cd levels in this research were greater than the concentration which ranged 1.30 to 2.90 mg/kg reported in samples of khat analysed in Addis Ababa (Tilahun, 2009). This could be attributed to the growing of various species of khat in sample collection locations and soil types, as well as the use of agrochemicals during planting in the Meru County. This could also be attributed to the use of domestic manure with traces of Cd element as a byproduct of mixed farming. In this study the concentrations of Cd were lower than those found in local vegetables obtained from farms in Kericho West Sub-County, which ranged between 10.33 to 29.00 mg/kg (Bett *et al.*, 2019). This could be related to differing farming practices, agrochemicals input, and soil types used during vegetable growing, which varies significantly from khat husbandry. According to regular reports given by WHO/FAO, the guideline limit for Cd in vegetables is 0.2 mg/kg (Latif *et al.*, 2018). Consequently, the mean concentration of Cd and THQ exceeded the threshold values. This indicated that the khat analysed in this work may be moderately hazardous to regular users.

Nickel is a trace metallic element that is released into environmental matrices via natural sources as well as human activities including steel and cement manufacturing (Bhalerao *et al.*, 2015), and it may be found in all soil types and profiles (Das *et al.*, 2019). In agricultural soils, it is required in traces for normal plant growth; otherwise, higher concentrations can be severely hazardous to humans, animals, and plants (Bhalerao *et al.*, 2015; Sreekanth *et al.*, 2013). Nickel metal has been linked to carcinogenesis in humans and other mammals, as well as toxicity to the neurological and reproductive systems, liver, and lungs (Sajad *et al.*, 2020). Nickel levels in samples of khat in the current study ranged between 0.349 ± 0.02 to 0.399 ± 0.04 mg/kg, with a mean concentration of 0.37 ± 0.02 mg/kg. The highest Ni content was found in samples D from the region of Laare and sample H from Maua, while the lowest value was found in sample J obtained from the region of Kianjai. This could be attributed to its concentrations in various soil types and the agricultural chemicals used during farming of khat. The results obtained from this investigation show low Ni levels when compared to documented vegetables results from Pakistan's Dera Ghazi Khan District, which ranged from 1.800 to 5.050 mg/kg (Latif *et al.*, 2018). Clearly, this is due to crops' various capacity to acquire Ni from the environment. Table 4.4 further reveals that Ni concentrations in all samples of khat were below WHO's acceptable limits (10 mg/kg) (Ogundele *et al.*, 2015). Associated THQ confirms that Ni intake by khat consumption in all selected regions poses no health concern. When its levels are below WHO permitted limits, it is a vital component in the proper plant growth and development, such as seed germination, as well as in animal species and microbes (Genchi *et al.*, 2020). Furthermore, it is a vital metal for humans since it improves hormonal activity and is used in lipid metabolism (Poonkothai & Vijayavathi, 2012).

Chromium is an essential metallic element in khat plant, which enhances biological activities such as regulation of blood sugar, homeostasis, cholesterol regulation, and fat synthesis in the liver (Woldamanuel, 2019). The Cr levels in samples of khat from this study ranged from 12.30 ± 0.15 to 19.10 ± 0.12 mg/kg, with a mean concentration of 15.98 ± 2.22 mg/kg as shown in Table 4.4. As a result, high Cr levels in khat samples may be due to relatively high concentrations of Cr in the soil, as well as its varied chemical forms in soil, and the agrochemicals used during farming of khat (Woldamanuel, 2019). Furthermore, soil redox potential and pH are significant criteria that influence the properties of Cr in soil, with low soil pH increasing Cr levels in khat (Mondol *et al.*, 2011). The Cr concentrations reported in this study are greater than those from samples of khat in Addis Ababa, which ranged between 3.10 mg/kg and 6.76 mg/kg (Tilahun, 2009), and are comparable to khat grown in eastern Ethiopia, where concentrations ranged between 9.04 mg/kg and 14.54 mg/kg (Deribachew *et al.*, 2015). This is was attributed to variable growing conditions and agricultural chemicals used in farming (Tilahun, 2009). The WHO's recommended limit of 2.30 mg/kg for Cr in vegetables (Woldamanuel, 2019). The mean levels in this study surpassed the allowed limits, and also the THQ values indicating that ingestion of this khat may cause health concerns after bioaccumulation and biomagnification. However, when kept at tolerable levels, this element is essential for various human biological functions.

Most farmers of khat in Igembe region prefer mixed farming, which includes planting bananas, maize, carrot, potatoes, cassava, sugarcanes, pumpkin, millet, and beans in khat farms (Krueger & Mutyambai, 2020). This study attributes that applying heavy metal containing fertilisers to these food crops indirectly allows khat plants to absorb them into their consumed tender leaves and young shoots, raising their levels.

4.4.2 Non-carcinogenic health risk assessment of khat consumption

Target hazard quotient (THQ) is a measure of potential that indicates the potential of developing non-carcinogenic health risks as a result of heavy metal ingestion. The standard THQ value for not developing adverse health concerns is less than 1.0. Exceeding this value may pose non-carcinogenic health threats to humans (Ambedkar & Muniyan, 2011; Bayissa & Gebeyehu, 2021; EPA, 2011). The application of this approach in the current study takes into account the potential of heavy metal exposure through khat consumption, however, it does not always yield quantitative estimates on the exposed population. Table 4.5 shows the calculated THQ values for Ni, Cd, Fe, Cu, Cr, and Pb resulting from habitual khat usage practices in the research area. Clearly, THQ for Pb and Cd are substantially above the 1.0 threshold, indicating high health risk. The THQ values (Table 4.5) of all other studied heavy metals in the analysed khat were found to be within safe limits because they are less than 1.0. The THQ of all selected heavy metals arising from studied young khat shoots and tender leaves intake were observed to be decreasing in order of Pb > Cd > Fe > Cu > Ni > Cr. This implies that consuming khat could cause severe health concerns due to Pb and Cd.

Table 4.5: The THQ and HI values of investigated heavy metals with respect to khat consumption

Non-carcinogenic parameter	**THQ**						**HI**
Heavy metal	Cr	Cu	Ni	Fe	Cd	Pb	
Values	0.015	0.056	0.027	0.20	11.6	13.2	24.90

The cumulative health risks due to numerous metal exposure were calculated by adding the THQ of all the six heavy metals in khat to the computation of HI, which was basic in the estimation of the likelihood of negative consequences. In the present study, the HI obtained for all analysed heavy metals in samples of khat was 24.90, as shown in Table 4.5. According to the current data, Pb and Cd heavy metals were the most significant contributors to total HI. Further, Cd and Pb heavy metals contributed approximately 46.59% and 53.01%, respectively, whereas Cu, Ni, Cr, and Fe together contributed about 0.40% to the overall HI.

4.4.3 Pearson correlation analysis

This is also known as Pearson correlation coefficient (PCC) which was conducted to establish combinations between the levels of the six metallic elements (Cr, Cu, Pb, Fe, Ni and Cd) investigated in this study as its results summarised in Table 4.6. The positive coefficient values suggested a positive link between heavy metals studied, whereas negative coefficient values indicated a negative correlation. It is noteworthy, correlation coefficients near zero (0) suggested a lack of correlation significance. Association coefficients close to 1 suggested a high and significant association between the heavy metals. Iron had the most positive coefficients, which could indicate that it has various contamination sources and origins. Further, it is the most abundant metal in all samples of khat. These results showed that it has positive correlation coefficients with Cu, Cr, and Pb, but were not strong with Ni indicating that its source could be distinct from that of other metals. The metallic elements Pb, Cu, and Cr have the same number of significant positive correlation coefficients, implying that they may have common origins. Additionally, Ni and Cr had strongest positive correlation indicating the same origin in the khat farms and subsequently in samples. Cadmium element had negative correlations with all elements except with Pb (0.216).

Table 4.6: Pearson correlation matrix obtained for six heavy metals

	Ni	Cu	Fe	Cd	Pb	Cr
Ni	1					
Cu	-0.181	1				
Fe	0.565	0.168	1			
Cd	-0.221	-0.264	-0.131	1		
Pb	-0.411	0.293	0.294	0.216	1	
Cr	0.707	0.080	0.495	-0.258	-0.320	1

4.4.4 Principal component analysis

The PCA by Varimax rotation method was used on a data set of six heavy metals for speculation on their sources in Meru khat. Table 4.7 illustrates the results extracted using PCA, which includes the rotated component matrix, component matrix, rotation sums of square loadings and initial eigenvalues. The first three principal components (PCs) selected on the basis of Kaiser-Meyer-Olkin (KMO) criterion measure with eigenvalues > 1 accounted for approximately 83.81% of the total variance. The value of KMO in this study was 0.511 which was above the threshold limit (0.50), indicating that the PCA analysis was effective for this work (Varol, 2011).

The PCs indicated that the heavy metals in khat samples from Meru potentially emanated from three distinct sources.

The PC1 explained 39.55% of the overall variation, with high positive loadings of Ni (0.90), Cr (0.83), and Fe (0.82). Regular fertilizer and pesticide applications may have contributed to Pb and Cd in the research area, indicating that these heavy metals occur as a result of anthropogenic activities. Ni and Cr elements had high loadings in PC1 showing they are associated with natural soils where khat plants were grown. This observation agrees with PCC results where Ni expressed strong positive correlation of 0.707 with Cr indicating that these elements have a common origin.

The second PC (PC2) contributed 25.44% of the overall variation, with high positive loadings of Pb (0.93) where Ni (-0.30) and Cr (-0.20) contributed negative loadings. PC3 accounted for 18.83% of the overall variation and had a significant positive Cu loading of 0.77 but a highly significant negative Cd loading. In addition, the results, particularly as shown in Figure 4.2, revealed that Cu had a different origin in the study area. Fe contamination could be the product of numerous contaminations, as considerable loadings are observed in PC1, PC2, and PC3. This observation is in agreement with PCC results. Thus, significant connections between metals within the same PCs demonstrating the importance of both PCA and PCC analysis.

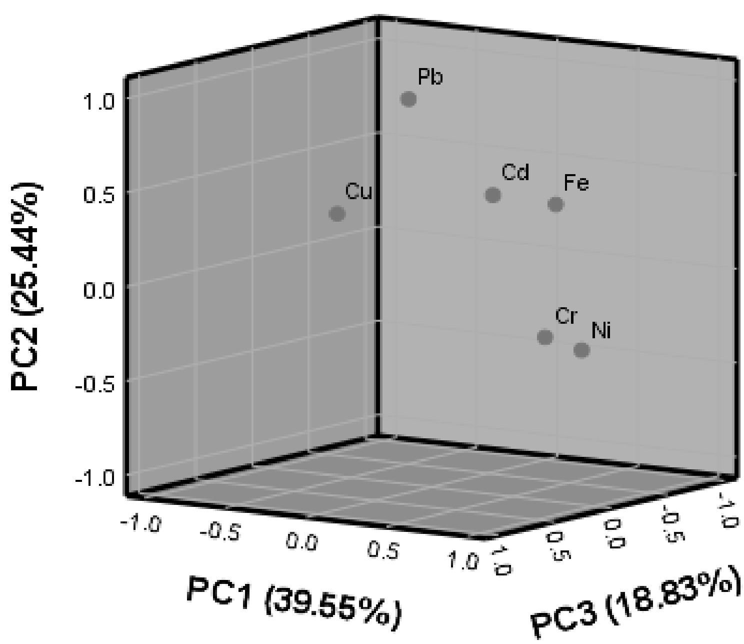


Figure 4.2 Component plot in the rotated space for studied heavy metals in khat samples.

Table 4.7: The PCA results of heavy metals in samples of khat from Meru County

	Principal components matrix				**Rotated components matrix**		
Metals	PC1	PC2	PC3	Metals	PC1	PC2	PC3
Ni	0.91	-0.16	0.21	Ni	0.90	-0.30	-0.46
Cr	0.88	0.03	0.03	Cr	0.83	-0.20	0.19
Fe	0.65	0.57	0.41	Fe	0.82	0.48	0.07
Pb	-0.41	0.78	0.35	Pb	-0.16	0.93	-0.02
Cu	0.01	0.75	-0.49	Cd	-0.19	0.30	-0.81
Cd	-0.44	-0.14	0.75	Cu	-0.47	0.45	0.77

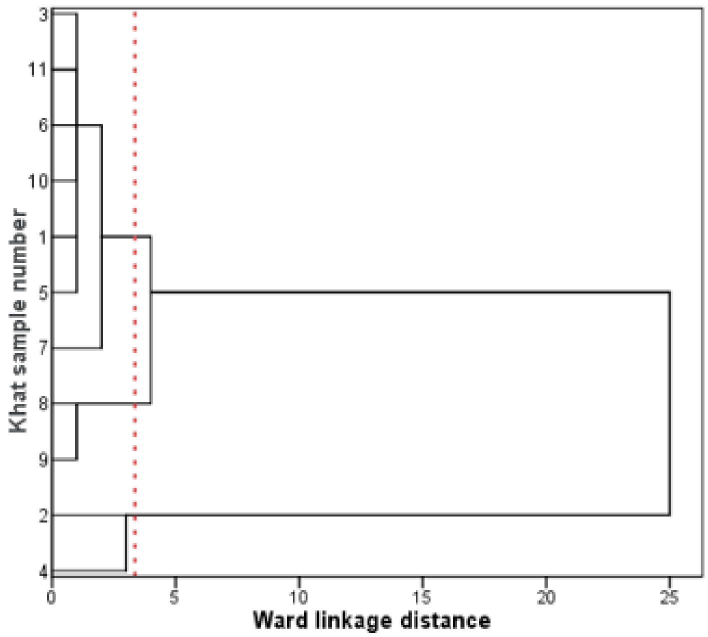
	Initial eigenvalues		
Component	Total	Variance (%)	Cumulative (%)
1	2.37	39.55	39.55
2	1.53	25.44	64.98
3	1.13	18.83	83.81
4	0.56	9.40	93.21
5	0.27	4.51	97.72
6	0.14	2.28	100.00

Extraction sums of squared loadings				**Rotation sums of squared loadings**			
Component	Total	Variance (%)	Cumulative (%)	Component	Total	Variance (%)	Cumulative (%)
1	2.37	39.55	39.55	1	2.23	37.08	37.08
2	1.53	25.44	64.98	2	1.52	25.36	62.44
3	1.13	18.83	83.81	3	1.28	21.37	83.81

4.4.5 Hierarchical Cluster Analysis

In this study, HCA was conducted to further support PCA results as it classifies khat samples in terms of heavy metal content into correlated groups. It was performed by applying the Ward's method where the Squared Euclidean distance taken as similarity measure was used to identify clusters (groups) of khat samples that could be containing similar concentration profiles of heavy metal elements, discover their speciation and correlations in our data. The HCA results are presented as dendrogram in Figure 4.3 which illustrates three clusters at linkage distance shown by red dotted line (x-axis) is perhaps, a measure of closeness of the clusters. The y-axis represents the khat samples in terms of metal speciation as shown in Table 4.4.

Significant correlations were found between khat sample numbers within the same clusters (groups) and the PCs, indicating that PCC, PCA, and HCA results were all important in this study. Consequently, Figure 4.3 shows that khat sample numbers 2 (sample B) and 4 (sample D) are correlated and have high but close Fe levels as shown in Table 4.4 indicating they could be having same source. Khat sample numbers 8 (sample H) and 9 (sample I) obtained from Maua region formed a distinct correlated group as shown in Figure 4.3. This group show similarity (Table 4.4) as the levels of Cu, Cd, Pb, and Ni among these two samples is related. Important to note is that the PCC results (Table 4.6) show iron had the highest number of positive correlation coefficients signaling the two khat farms (where samples B and D were obtained) had more contamination sources responsible for high Fe levels. Both the PCA and HCA demonstrates that there are a total of three clusters (principal components) indicating the analysed metallic elements could have the same sources presumably related soil heavy metal composition and similar application of fertilisers and pesticides practices during khat growing.



Key:

Khat sample	
Number	Label
1	A
2	B
3	C
4	D
5	E
6	F
7	G
8	H
9	I
10	J
11	K

Figure 4.3 Dendrogram from the HCA of khat samples

4.5 Pathophysiological issues of potentially toxic heavy metals

Exposure of human to potentially heavy metals can occur by skin contact, through inhalation, direct contact, or ingestion admistration (Ara & Usmani, 2015). It is becoming increasingly clear that the non-biodegradability and refractory nature of these metals in the environment causes long-term biotoxic effects in biological systems, necessitating a better understanding of the mechanistic pathways that cause them to be harmful to human health (Mahurpawar, 2015). Furthermore, these metallic elements acutely disrupt the normal functioning of organs and systems, ultimately leading to serious health effects and premature death (Das *et al.*, 2019). Some heavy metals disfunction the central nervous system, cause mental impairment, renal damage, and the activation of disease manifestation pathways in the lung (Engwa *et al.*, 2019). Chromium poisoning in humans and animals can lead to respiratory malignancies (Mahurpawar, 2015).

Notably, acute Ni poisoning in humans causes symptoms such as body nausea, weakness, persistent cough, and visual problems, but in mammal animals including rats, it causes kidney failure, body weight loss, and renal damage (Das *et al.*, 2019). In addition, chronic Ni poisoning affects the human skin and lungs, in addition to causing cancer disease and dramatis (Mahurpawar, 2015), causing disruption of human endocrine, and serving as a precursor to cardiovascular disease and genotoxicity (Das *et al.*, 2019). A study reported in North California on children aged between 4 and 5 years found that they are at a significant risk of paediatric obesity owing to elevated concentrations of cadmium in samples of prenatal blood (Tinkov *et al.*, 2021). Epidemiological studies have shown that Cd accumulations in human tissues leads to the development of musculoskeletal illnesses such as osteoarthritis and osteoporosis, as well as renal impairment (Reyes-Hinojosa *et al.*, 2019). Long-term exposure to Pb exposure reduces cognitive abilities, causes anaemia, high blood pressure, and miscarriages in expectant women, and affects the kidneys (Ara & Usmani, 2015). To reduce heavy metal poisoning caused by khat use and other dietary products, metal chelation therapy, which is used to treat chronic iron toxicity, is recommended (Isidori *et al.*, 2018).

4.6 Limitations of the study

This study analysed only 11 khat samples to assess the health risks associated with khat usage among khat consuming population. Given the size of sampling area, the collected data may not be an accurate representation of potentially heavy metal toxicity in khat over the entire Meru County. Furthermore, the non-carcinogenic health risks posed by Cd and Pb cannot be accurately established based on the small sampled area of Meru County. Essentially, the assessment of THQ values and HI of all metals relied on daily khat use; consequently, there are chances that the results could be overestimated. Furthermore, considering the fact that the analytical data were obtained during rainy season, this limits the ability to provide a full overview of heavy metal pollution in the research area, particularly during the dry season. In addition, heavy metal speciation in Meru's khat-growing locations was not studied seasonally or temporally.

Conclusions

This study demonstrated that tender leaves and young shoots of khat obtained from regions of Meru County contaminated with Pb and Cd metals, which is regarded as a health risk among khat users. The THQ values for Pb and Cd substantially surpassed the 1.0 threshold value, suggesting their considerable hazard potential. At the same time, THQ for all other investigated heavy metals in analysed khat was less than 1.0, which is considered safe. In general, the THQ for heavy metals derived from khat shoots and leaves was observed to follow a decreasing order of Pb > Cd > Fe > Cu > Ni > Cr. Nonetheless, the HI of all examined heavy metals in khat above the 1.0 threshold, indicating that khat consumers may face non-carcinogenic health concerns.

The statistical analysis of the data presented in this paper revealed significant variation in the Ni levels in samples of khat from various parts of Meru County, but no significant variations in metals Cd, Pb, Cu, and Fe. This observation could be attributable to a variety of reasons, including farming methods, soil chemical composition, and the use of distinct agricultural tactics followed by different khat producers. Furthermore, the PCs with eigenvalues greater than one accounted for approximately 83.81% of the total variability, indicating that the metals in investigated khat come from three distinct sources. The PCC analysis showed that iron had the most positive correlations, implying that there were various contamination sources and origin. Iron had positive connections with Cu, Cr, and Pb, but only weak associations with Cd and Ni. The observed high levels of Cd and Pb in the research area may be due to the constant application of agrochemicals such as fertilisers and pesticides. This study recommends the adoption of alternative farming practices in which the use of agricultural chemicals should be minimised or entirely avoided. Most importantly, farmers of khat from Meru County should be educated on alternative farming techniques for safer products of khat.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussion

5.1.1 Rationale of the study

This study primarily explored the concentration profiles of selected heavy metals (Ni, Cu, Pb, Cr, Cd and Fe) and pesticide residues in consumable parts of khat (*Catha edulis*) collected from farms in Meru County, Kenya. Khat products from these regions are largely consumed locally and some exported to neighbouring countries such as Somalia, Uganda, Madagascar and Djibouti. The demand for khat has increased significantly, and this has motivated khat farmers into employing modern farming practices to increase yield, thus, there is need for safe products that will guarantee consumer health standards. The motivation behind this study was to probe the levels of selected heavy metals and quantify acephate and cypermethrin pesticide residues in khat samples, and compare them with WHO/FAO and EU limits in order to assess their potential human health effects. Human health assessment was done using THQ, HI and HQ indices to determine associated risks with consumption of khat grown in Meru County.

A total of 11 Meru khat samples used in this study were air-dried for seven days, then oven-dried at 70 ℃ for three days and finally ground to 0.25 mm sizes. They were digested in a mixture of H_2O/HCl (1:1 v/v), H_2O/HNO_3 (1:1 v/v), and 10 cm^3 20 volumes hydrogen peroxide (H_2O_2) to dryness and later reconstituted using 0.05 M HCl. The heavy metal contents in the digests were determined using ICP-AES technique. Comparison of these results was done with similar data from khat-growing regions in the Africa to ascertain metal speciation under the influence of natural and anthropogenic factors. In order to speculate on the possible sources of the investigated heavy metals in khat plants, the PCA and HCA analyses were carried out using the IBM SPSS version 25.0 software.

Another set of 11 fresh khat samples were finely ground. It was later mixed with C18 silica material, and then packed in a polypropylene column. A mixture of acetonitrile and n-hexane (1:4) binary solution was added to elute in a dropwise manner into Elernmeyer flask and was later concentrated using a vacuum rotary evaporator for GC-MS analysis to determine pesticide residues in the khat extracts. The UV-Vis spectrophotometric quantitation measurements of acephate and cypermethrin residues were done in khat extracts using EtOAc/hexane/acetone solvent system. Quantitation studies were performed using a double beam K9000 UV-Visible spectrophotometer.

5.1.2 Findings of the study

5.1.2.1 Analysis of the concentration of potentially toxic heavy metals in khat grown in Meru county and the assessment of their associated health risks

The results obtained from ICP-AES method indicated that Ni, Pb, Cr, Cd, Fe and Cu heavy metals were present in all the khat samples at various concentrations. Associated comparative studies with WHO/FAO threshold limits show that Pb, Cd and Cr concentration profiles exceeded permissible limits whereas the concentrations of Fe, Ni, and Cu were below the acceptable limits. The non-carcinogenic risks associated with exposure of khat consumers to these metals showed that THQ values of Pb and Cd exceeded the threshold value 1.0 indicating their possible hazardous intake capable of causing health effects. Consequently, the collective THQ values of other investigated heavy metals were below the threshold limits. This clearly shows that Pb and Cd are the main contributors to the HI. These findings show that minimising practices such as application of fertilisers and pesticides on khat farms and limiting other sources that potentially increase their concentrations on khat farms will significantly reduce their levels in consumable khat parts hence mitigating etiological risks to the khat consuming community.

5.1.2.2 GC/EI-MS and UV-Vis analysis of pesticide residues in cultivated *Catha edulis* (khat) from selected farms in Meru County, Kenya

The pesticide residues presented in this study from analysis of khat samples were determined using GC/MS and they were found to comprise organophosphate compounds (chlorpyrifos, chlorfenvinphos, and acephate) and pyrethroid chemicals (cypermethrin, cyfluthrin, and cyhalothrin). This is significant evidence of pesticides use in khat farms primarily to protect khat from pest attack which is intended to enhance the quality and yield of khat. UV-Visible spectroscopic quantification studies on acephate and cypermethrin residues show that their levels were below MRLs limits. The analysis was carried out at maximum absorption at about 321 nm for cypermethrin and 207 nm for acephate standards as a result of spectra sharpness and proper baselines. The associated HQ calculated based on MRLs revealed that 0.247 value for cypermethrin and 0.287-0.797 for acephate indicating they were below the threshold limits, therefore based on this observation, Meru khat is safe and may not pose considerable health risk to khat consumers.

5.2 Conclusions

In this study, the concentration profiles of potentially toxic heavy metals and characterization of pesticide residues in consumable parts of the khat obtained from farms in Meru County were analysed. The results of this study showed that:

i) The UV-Vis spectrophotometric quantitative studies conducted showed that cypermethrin and acephate pesticides were below MRLs of EU for the human safe foods, thus, there is no associated acephate and cypermethrin pesticide contamination attributed with consumption of Meru khat.

ii) The concentration of heavy metals such as Cu, Fe and Ni in khat samples were within the WHO/FAO limits whereas Cr, Cd, and Pb levels exceeded the standard threshold limits. Accordingly, THQ and HI values of Cd and Pb were greater than the threshold value 1, and therefore potential for toxicological responses.

iii) The six pesticide residues namely - acephate, chlorpyrifos, and chlorfenvinphos were classified as organophosphates; whereas, cyfluthrin, cyhalothrin, and cypermethrin were pyrethroids. These pesticide residues were detected in the six khat samples out of eleven total sample size representing 54.55% pesticide contamination rate.

5.3 Recommendations

This work has thoroughly assessed the concentration of potentially toxic heavy metals in khat samples obtained from selected farms in Meru County. Human health risks based on THQ and HI threshold limits was also conducted. Spectrophotometric measurements and determination of hazard quotients were exclusively done on acephate and cypermethrin pesticides. Nonetheless, a lot of work needs to be done on Meru khat in order to draw effective conclusions on pesticide residues and elemental composition during the dry and rainy seasons to adequately equip khat-consuming population on the public health concerns of khat consumption. The following are recommendations that can be drawn from this study.

i) There is need to extend this quantitative study to other pesticides such as cyfluthrin, cyhalothrin, chlorpyrifos, and chlorfenvinphos in khat samples.

ii) Further research needs to be done to determine the relationship between levels of toxic heavy metals in the soil and other contamination sources in relation to their concentration profiles in consumable khat leaves and shoots.

iii) Khat farmers and locals in Meru County and other nearby regions should be informed and sensitised on the health risks involved in the use of pesticides through awareness campaigns, mass media and agricultural shows.

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APPENDICES

Appendix I: Copyrights

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Appendix II: Abstracts of published papers

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Research Article

Analysis of the Concentration of Heavy Metals in Khat Grown in Meru County and the Assessment of Their Associated Health Risks

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Contamination of farm produce by toxic heavy metals has become a serious global health concern. These metals can bioaccumulate in plant tissues and are precursors for major public health problems such as cancer and neural impairment. Khat (*Catha edulis*) also referred to as miraa has the potential to sequester and accumulate both micronutrients and potentially toxic heavy metals in its consumable parts—tender leaves and soft barks of young shoots which are known to possess psychoactive properties when consumed. Therefore, the motivation behind this contribution is to determine the levels of six heavy metals, namely, cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), iron (Fe), and nickel (Ni) in consumable Meru khat samples, compare these levels with the permissible limits of World Health Organization (WHO) in order to predict associated health risks, and to estimate the noncarcinogenic risks of these metals by total health quotient (THQ) and health index (HI) on khat consumers. 1.0 g of dry ground khat samples was digested in 0.05 M HCl and allowed to stand for 5 hours before being analyzed for heavy metals using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The mean heavy metal concentrations (mg/kg) in dry khat samples of six toxic heavy metals were Cd (7.81 ± 1.56), Cr (15.98 ± 2.22), Cu (15.81 ± 2.84), Fe (97.35 ± 32.67), Ni (0.37 ± 0.02), and Pb (32.36 ± 9.95). Based on the results, the mean levels of Pb, Cd, and Cr exceeded WHO permissible limits. In addition, the Pb and Cd THQ values and the HI of the six heavy metals investigated in the khat samples exceeded the threshold value of 1.0. Furthermore, the THQ and HI values showed that Pb and Cd were potentially the major contributors to noncarcinogenic risks on regular khat consumers. This is a matter of concern on the excessive consumption of Meru khat-based products, which over time may cause a toxicological response. Based on the findings of this study, the use of agrochemicals should significantly be minimized in khat farming. Accordingly, the Meru khat farmers should be sensitized on alternative farming practices that do not potentially cause heavy metal contamination in khat.

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GC/EI-MS and UV-Vis analysis of pesticide residues in cultivated *Catha edulis* Forsk (Khat) from selected farms in Meru County, Kenya

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ABSTRACT

In this study, an analysis of pesticide residues was performed using a gas chromatography/electron impact mass spectrometer (GC/EI-MS) to qualitatively assess and characterize pesticide residues in *khat* leaves sampled from selected agricultural farms in Meru County, Kenya. A solid-phase microextraction (SPME) procedure followed by GC/EI-MS analysis led to the detection and identification of six pesticide compounds from the sample-ion chromatograms. They include cypermethrin, acephate, cyhalothrin, cyfluthrin, chlorpyrifos, and chlorfenvinphos. The prevalence rate of pesticide contamination was determined to be 54.5% of the sample size. Of the identified pesticide residues, 50% were compounds based on pyrethroids and the other 50% were based on organophosphate. Four of the six identified pesticides were chlorinated compounds. A quick, easy, cheap, effective, rugged, and safe UV-vis double beam spectrophotometric technique based on copper (II) chelation reactions leading to colored copper pesticide complexes was developed, validated, and applied to quantify and compare the levels of selected pesticide compounds found in the *khat* samples. UV-vis wavelength-scan measurements performed on pesticide compounds chelated with copper (II) ions revealed maximum absorption of Cu-cypermethrin and Cu-acephate at 321 and 207 nm, respectively. The standards calibration curves developed from the UV-Vis quantitation technique showed excellent linearity in the concentration range of 0.5-10.0 µg/L (R^2 = 0.99) for both cypermethrin and acephate standards. The estimated limits of quantification (LOQ) were 0.25-0.26 µg/L, respectively. The UV-Vis quantitation results from the selected samples (in which residues were confirmed to be present) revealed that acephate (an organophosphate residue) occurred at higher concentration levels (range 2.897-7.978 µg/L) than cypermethrin (2.145 µg/L). For the pesticides quantitatively analysed in the selected samples, the levels were below the maximum residue limit (MRL). The hazard quotients (HQ) were in the range of between 0.247-0.797.

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REVIEW Open Access

A review of the health implications of heavy metals and pesticide residues on khat users

Albert M. Oyugi, Joshua K. Kibet* and John O. Adongo

Abstract

Background: There is an exponential rise in the use of farming chemicals in agricultural practices ostensibly to increase food production. The chewing of fresh khat leaves and shoots has spread across the world from ancient khat producing regions in East Africa and the Arabian Peninsula. Khat is a well-established socialization substance with stimulating characteristics. In this work, we have reviewed the deleterious impacts of several heavy metals such as lead, cadmium, iron in the khat plant and their health impacts. Survey on the health complications of farming chemicals used in khat production is also presented.

Main body of the abstract: The toxic effects of heavy metals and farming chemicals in plant matter such as khat leaves are a serious health concern. Heavy metals including cadmium (Cd) and lead (Pb), for instance, bio-accumulate in the body and the food chain as precursors for disease. It has been established that blood that has lead levels of 40–60 ug/dL is a precursor for serious health illnesses such as cardiac arrest and cancer. On the other hand, cadmium is reported to bind itself onto metallothioneins hence forming cadmium–metallothionein complex that is transported to all body organs causing deleterious cell damage. The entry of farming chemical into the food chain especially via the chewing of contaminated khat has been known to contribute to health problems such as cancer, hypertension and liver cirrhosis. khat is branded a 'substance of abuse' by the World Health Organization (WHO) because of the adverse health risks it causes to humans. Relevant articles published between 2010 and 2021, and archived in PubMed, Google Scholar, Medley, Cochrane, and Web of Science were used in this review.

Short conclusion: The health implications of heavy metals and farming chemicals arising from the consumption of contaminated khat shoots are a serious concern to the khat chewing community. Consequently, there is need to develop better farming practices that may minimize the absorption of heavy metals and farming chemicals by the khat plant. Information presented in this review is also important in sensitizing policy makers to advance control measures towards safer khat farming practices.

Keywords: Cancer, Contaminated khat, Liver cirrhosis, Pesticides, Hypertension

Appendix III: NACOSTI Permit

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