HAWASSA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTEMENT OF CHEMISTRY

M.Sc. THESIS

ASSESEMENT OF THE LEVEL OF HEAVY METALS IN DIFFERENT VARIEETIES OF KHAT (CATHA EDULIS) AND SUPPORTING SOIL SAMPLES COLLECTED FROM TULLA AREA, SIDAMA REGION; ETHIOPA

BY

GETAHUN MENGIST GELLA ID

GPCHEMK/008/10

ADVISOR: ALEMAYEHU PAULOS (Ph.D.)

NOVEMBER , 2024

HAWASSA ETHIOPIA

ASSESEMENT OF THE LEVEL OF HEAVY METALS IN DIFFERENT VARIEETIES OF KHAT (CATHA EDULIS) AND SUPPORTING SOIL SAMPLES COLLECTED FROM TULLA AREA, SIDAMA REGION; ETHIOPA

M.Sc. THESIS SUBMITTED TO HAWASSA UNIVERSITY COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE DEPARTEMENT OF CHEMISTRY POST GRADUATE PROGRAM IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

BY

GETAHUN MENGIST GELLA

ADVISOR: ALEMAYEHU PAULOS (Ph.D.)

NOVEMBER , 2024

HAWASSA, ETHIOPIA

DECLARATION

I do hereby declare that the thesis work entitled "**Assessment of the Level of Heavy Metals Pollution in Different Varieties of Khat (Catha Edulis) and Supporting Soil Samples Collected From Tulla Area, Sidama Region; Ethiopia,**'' is my original work and has not been submitted for a degree or diploma in any other universities by any other researchers or any students and that all sources of materials used for this thesis have been duly acknowledged.

Name: Getahun Mengist

Signature: ___________________________________

Date---

This thesis has been submitted for examination with my approval as thesis Advisor

Advisor: Alemayehu Paulos (Ph.D.)

Signature: ____

Date---

SCHOOL OF GRADUATE STUDIES

HAWASSA UNIVERSITY

Approval sheet-1

This is to certify that the thesis entitled "**Assessment of the Level of Heavy Metals Pollution in Different Varieties of Khat (Catha Edulis) and Supporting Soil Samples Collected From Tulla Area, Sidama Region; Ethiopia**" submitted in partial fulfillment of the requirements for the degree Master of Science in Chemistry the graduate program of the Department of Chemistry, Hawassa University, and is a record of original research carried out by Getahun Mengist, under our supervision, and no part of the thesis has been submitted for any other degree or diploma. The assistance and help received during the course of this investigation have been duly acknowledged. Therefore, I recommend that it is accepted as fulfilling the thesis requirements.

Alemayehu Paulos (Ph.D.) ____________________ _______________

.

Name of advisor Signature Date

SCHOOL OF GRADUATE STUDIES

HAWASSA UNIVERSITY

Approval sheet-2

We, the undersigned, members of the board of Examiners of the final defense by Getahun Mengist have read and evaluated this thesis entitled "Assessment of the level of heavy metals in different varieties of khat (catha edulis) and supportive soil in Tulla area Sidama region Ethiopia " examined the candidate. This is therefore to certify that, the thesis has been accepted for partial fulfillment

of the requirements for the degree of Master of Science in Chemistry.

________________________	________________	_________________
Name of Chairperson	Signature	Date
________________________	________________	_________________
Name of Major Advisor	Signature	Date
________________________	________________	_________________
Name of Internal Examiner	Signature	Date
________________________	________________	_______________
Name of External Examiner	Signature	Date
________________________	________________	_______________
SGS Approval	Signature	Date

ACKNOWLEDGEMENTS

Above all I want to say thanks all praises due to God the sustainer of the heavens and the earth's for everything throughout my life and helping me reach this stage of success. Next, I would like to express my deepest gratitude to my major advisor Dr. Alemayehu. P for his dedicated advice, closer help and warmest treatments beside the idea and suggestion he shared.

His support and encouragement from the beginning up to the completion of this study is kindly appreciated. I have a special respect and appreciation to him, for his help, advice and Friendly approach. Doctor, you deserve a word of appreciations, next to God, without your regular Follow up and guidance, it would not have been possible to accomplish this thesis successfully.

I am also thankful to Hawassa with the staff member of the Chemistry department for providing me all resources University necessary to complete my study from the very beginning to an end. I would like to thank Ministry of Education for allowing me to pursue my M.Sc. Study. Finally, I am extremely grateful to all family members for their appreciation, continuous encouragement and for their supply with whatever resource they have. I should not also forget their care to me that brought me up to this success next to God.

Table of Content

Contents **Page**

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
FAAS	Flame Atomic Absorption Spectrometry
FAO	Food and Agricultural Organization
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
IOM	Institute of Medicine
IQ	Intelligence Quotient
LOD	Limit of Detection
LOQ	Limit of Quantitation
MDL	Method Detection Limit
NACADA	National Agency for the Campaign against Drug Abuse,
% R	Percentage Recovery
USDA	United States Department of Agriculture
WHO	World Health Organization

ABSTRACT

Heavy metal pollution of the environment has been a global concern due to its acute and chronic health effects. The consumption of contaminated food and beverages is among the major route of exposure. Khat (Catha edulis) is one of the major cash crops that are widely cultivated and consumed as stimulant in the study area (Tulla Sidama regional state).This work focuses on the physicochemical analysis and determination of selective heavy metals (Cu, Co, Ni, Cr and Pb) concentration in chewable parts of the leaves of the selected Khat varieties (Nole, Dume and Megala) and its support soil samples. Samples were randomly collected from the selective kebeles of the study area and digested using mixture of acids. Analysis of the physicochemical parameters (pH, electrical conductivity and ash content) was carried out by standard method. The determination of the heavy metals was carried out using Flame Atomic Absorption Spectroscopy (FAAS). The physicochemical analysis showed pH (6.21-6.82), electrical conductivity (1.42-1.86), and ash content (1.72-2.17) in soil samples and pH (5.93-6.43), electrical conductivity (1.17-1.54), and ash content (0.63-0.93) in khat samples. The heavy metal analysis showed concentration of Cu, Ni and Co to be 4.33-4.93mg/kg, 2.52-3.26mg/kg and 1.84-2.85mg/kg respectively in khat samples. The levels of these metals in the corresponding soil samples were (8.54-9.96mg/kg), (3.67-4.95mg/kg) and (3.37-4.85mg/kg for Cu, Ni and Co respectively. Pb and Cr were not detected in soil and khat varieties samples. Among the analyzed heavy metals Cu was detected relatively at the highest concentration followed by N and Co in both soil and Khat samples. These findings indicated a positive correlation between the levels of the heavy metals in the soil and the Khat samples. Although Pb, Cr, Cu and Ni were detected below the permissible level, Co was detected above the permissible level stipulated by WHO. Therefore, it can be concluded that continuous consumption of Khat as stimulant might lead to health risks associated with heavy metals.

Keywords: Physicochemical Analysis, Heavy Metals, Flame Atomic Absorption Spectroscopy, Catha edulis, Soil samples

UNIT ONE

1. INTRODUCTION

1.1. Back ground of the study

Khat (Catha edulis) is an evergreen perennial shrub plant that belongs to the Celastraceae family. It is widely cultivated in East Africa and Arabian Peninsula more specifically in Yemen [1]. Its young leaves and stem tips contains higher proportions of cathinone, which is responsible for much of the stimulant effect of khat [2]. Khat chewing is highly prevalent in East African and some Middle Eastern countries. Its use is both a social and a culture-based activity and it is said to enhance social interaction. It is chewed by both the young and old people in Ethiopia. On average, almost 70 % of households in Yemen and 50 % in Djibouti use khat [3], and more than 30 % of Ethiopians have been reported to use khat [4].

It is a major source of income besides being chewed for its stimulating effect. The employment created through khat farming is very high as a large number of people are involved in growing harvesting, parking, transporting and selling. The varieties of khat depend upon the geographical area of cultivation [1]. They differ in color, size and height of the leaves and size and height of the plant as a whole [5]. The soil on which khat grows is a good source of essential elements for human beings. However the presence of non-essential elements such as Pb and Cd in the soil leads to their uptake by khat. These metals end up in the soil from different sources which include agricultural practices such application of phosphatic fertilizers, pesticides and refuse derived composts [6] reported that heavy metals in fertilizer amended soils were higher when compared to those in natural soils due to soils retaining heavy metals sourced from the applied fertilizers.

The input of heavy metals to soil from various sources may prove detrimental to plant through its uptake to toxic limit, thereby facilitating its entry into food chain. These metals have the capacity to affect adversely the activity of a living organism, its growth, health, life span and reproduction performance [7]. They are extremely persistent in the environment, non-biodegradable and thus readily accumulate to toxic levels [8] and therefore pose a significant health risk to human particularly in elevated concentrations. Pb and Cd are among the most abundant trace heavy metals and are particularly toxic [9]. Other metals, such as copper (Cu), chromium (Cr) and zinc (Zn) are essential for important biochemical and physiological functions and necessary for maintaining health throughout life. However at elevated levels these metals can also lead to metal poisoning. For example Zn accumulation causes vomiting and renal damage while Cu toxicity may induce hypertension, coma and sporadic fever whereas hexavalent Cr may induce gastrointestinal ulceration and cancer [10]. The accumulation of high quantities of metals in plants parts that are consumed is an important environmental problem. There is also a paucity of information on the levels of heavy metals in khat. Therefore this study was carried out to determine the levels of selected heavy metals in different varieties of khat and supporting soil in Tula town Sidama, Ethiopia.

1.2. Statement of the Problem

In Ethiopia, khat is sold commercially in many parts of the country and Chewing khat is a common recreation activity among many individuals of all age groups [11]. Despite objection of khat by National Agency for the Campaign against Drug Abuse, (NACADA), and its cultivation is fast expanding in different parts of the country. As a result, wide areas of fertile land are now being used to grow khat. However, agricultural officers do not include khat in the list of scheduled crops and therefore no technical advice on how to grow and apply agrochemicals to khat is provided to the farmers.

In order to meet its ever demand for both domestic consumption and for the export market, farmers are now employing different methods of farming such as application of fertilizers, pesticides, sewage sludge, compost materials, manure and irrigation to improve and protect the khat. However, agricultural practices such application of phosphatic fertilizers; pesticides and refuse derived composts can be important source of heavy metals in the soil [12]. [13] Reported that nonstop application of fertilizers to the soil may increase the heavy metal contents making it exceed the natural abundances in soils, and transfer of these metals into the human food chain despite the fact that these heavy metals may be present in minute quantities in fertilizers.

Some of these metals such as Pb and Cd have no known use in the body and are toxic even at low levels. Extreme content of these metals in food is associated with a number of diseases, especially those of the cardiovascular, renal, nervous and skeletal systems [14, 15] during the last two decades, important progress has been made in understanding the pharmacological and social effects of khat in Ethiopia, but less attention has been paid to concentration of heavy metals in khat. The presence of high levels of heavy metals such as Pb and Cd in khat could have serious health effects especially to the young users.

Tulla, located in the Sidama Region of Ethiopia, is known for its fertile agricultural land, particularly for the cultivation of khat (Catha edulis), a popular stimulant among the youth. The region's diverse climatic conditions support various crops, but concerns have arisen regarding the potential contamination of khat with heavy metals due to extensive use of agricultural chemicals. Therefore this study aimed at assessing the levels of selected heavy metals in khat plants growing in the different parts of selected areas and their supportive soils so as to address the above issues. Furthermore, the findings of this study would provide adequate information on the distribution of selected heavy metals in the edible portion of the khat plant in order to establish whether the users are exposed to high levels as well as to create awareness. Therefore, in this study the researcher analyzed the concentration of five elements (Cu, Co, Ni, Pb and Cr) from edible portion of khat growing in the study area as well as from the supportive soil.

1.3. Objectives

1.3.1. Main objective

The major objective of this study was to assess the level of heavy metal pollution of Khat varieties and support soil in Tula area, Sidama Ethiopia.

1.3.2. Specific objective

The specific objectives of this study were to:

- Characterize the physicochemical parameters of the various types of the Khat and supporting soil samples
- Develop optimum procedure for the digestions of khat and soil samples for the analysis.
- Determine the levels of Ni, Co, Cu, Pb, and Cr in Khat (Catha edulis) and support soils.

1.4. Significance of the study

The analytical results from this study would provide important baseline data on the levels of selected heavy metals in khat besides being an important assessment of environmental pollution in Tulla areas where khat farming is predominant. The study also would provide awareness of the health effects of khat to the public.

1.5. Scope and limitations of the study

This study determined the levels of Pb, Co, Ni, Cu and Cr in soil and khat samples though there are many other heavy metals. The roots and the stems of the khat plant were not studied. This is because the consumption of khat only involves chewing of young leaves and stem tips. Only the khat varieties grown in the studied region was considered since khat variety depends on the geographical location.

UNIT TWO

2. LITERATURE REVIEW

2.1. Organ of khat (Catha edulis)

According to existing tradition the use of khat was first discovered by a herder who noticed the effect of the plant on his goats and who tried it and experienced wakefulness and added strength. The distribution of chat in tropical Africa extends from north Arabia to South Africa. In Africa it is well established in Ethiopia, Eritrea, Somalia, etc. despite efforts 'of the respective governments to discourage its cultivation. In east Africa it grows in the range of 1500-2500 m above sea level, Outside Africa it is planted in the Arabian Peninsula, Yemen, and Afghanistan etc. for consumption and in the USA, UK and France for experimental purposes [16].

2.2. The Plant

Khat is an evergreen perennial shrub plants belong to the celastraceous family. There are several names for the plants depend on its origin chat Ethiopia qat, Yemen. The dried leaves of chat are known as Abyssinia tea or the Arabian tea [17]. Khat usually grows up to 7 meters but occasionally reaches as a high as 15 to 25 m. The khat plant is polymorphic and the branches have either opposite or alternate leaves. The leaves are 2-5 cm wider and 5-10cm long. The shape of the leaves euiptical and have started edges. Old leaves are leathery in texture highly polished on their upper surface and deep green in color [18]. The leaf peduncle is around 3-7mm long [19]. The leaf odor is faintly aromatic and the taste is astringent and slightly sweet .The buds and leaves contain an alkaloid and chewed in a fresh or dried condition as a stimulant. Flowers are small and white. The fruit is smooth and narrow splitting to release narrowly winged reddish seeds when matured. The steam is straight and slender. The bark has different colors depending on the variety and age of the steam and branches. The young branches are smooth and green to pinkish but grey and sometimes rougher and darker on older branches and seams. The root systems can grow as deep and as long as 3-5m [20].

2.3. Distribution in Ethiopia

The total area of land under chat cultivation in Ethiopia in the year 1997/98 was estimated at 78,570 hectare [21]. Oromia, mainly east and west Harerghe zones is the most important center of khat production (east Harerghe zone alone contributes 53.4 percent of the total production area) in Ethiopia. Harerghe is considered to be the most important producer of the quality chat in the world [22] .Despite silent support and objection against the crop by development institutions. Khat is cultivated and expanding in different parts of Ethiopia.

It can be grown rain fed and irrigated through the cater covers less than 20 percent of the total khat area. The crop could be planted both in home garden and in the field [23]. Now a day khat farmers are practicing use of paste chemicals and fertilizers to protect the plant from pastes and to increase the yield of their product in addition to this, they sometimes use traditional pastes control mechanisms. There is an extensive literature about khat, providing information about its history, chemistry and pharmacology and exploring the social, economic psychological and oral aspect of its use. Despite this literature studies that have investigated its mineral nutrients are much less than one may expect. Thus khat is becoming more popular all over the country and other parts of the world.

Having detail documents focusing on minerals is very important to evaluate the total mineral intake of the individual who is using this plant regularly. This is a mild- stimulant plant that has a history of at least seven centuries in Ethiopia. Its chemical, medical and pharmacological aspects are emphasized in the global literature whereas studies on its economic, social, cultural and ecological aspects have received a little attention. The plant is an important aspect of the agricultural, social and religious lives of many Ethiopians especially the Oromo of Harare and its cultivation has expanded as a result opening up with increasing land shortage and drought.

There is already some concern as it has been taking away land previously used for the production of food crops and coffee. Its rejection as a narcotic in some countries may also pose threat to the livelihood of many growers and traders but there has been little concern about this so far. Employing an anthropological perspective, this monograph explores and discusses in detail these aspects of khat in Ethiopia. It is useful to professionals, policy makers and other interested parties in the fields of production, distribution and consumption of khat and affecting programmed social change about the khat culture [24].

2.4. Chemistry of Khat

Khat contains more than 40 alkaloids, glycosides, tannins, amino acids, vitamins and minerals [25, 26]. Cathine and cathinone are major active ingredients of khat which are Phenylalkylamine, meaning they are in the same class of chemicals as amphetamines. In fact cathinone and cathin e have a very similar molecular structure to amphetamine. Especially cathinone has been termed as „natural amphetamine‟. It produces central nervous system stimulation analogous to the effects of amphetamine [27]. The stimulant effect of the plant attributed to cathinone which is more behaviorally active than Cathine. In 1975, the related alkaloid cathinone was isolated, and its absolute configuration was established in 1978. Cathinone is mainly found in the young leaves and stem tips. During maturation and drying, cathinone is metabolized to Cathine and Norephedrine. This is because Cathinone is unstable and undergoes decomposition reactions after harvesting, during maturation, drying or extraction of the plant material [28]. The structures of Cathinone and Cathine are illustrated in Figure 1.

Cathinone ($C_9H_{11}NO$) Cathine. Norephedrine ($C_9H_{13}NO$)

Figure 1.The chemical structures of cathinone and cathine

2.5. Essentiality and Toxicity of Metals in Plants and Animals

In plants nutrition, mineral nutrients (elements) are classified in to essential and beneficial. The term essential mineral element (or mineral nutrient) was proposed by Arnon and Stout [29]. They concluded that three criteria must be met for an element to be considered essential. These criteria are: (1) a plant must be unable to complete its life cycle in the absence of the mineral element; (2) the function of the element must not be replaceable by another mineral element; and (3) the element must be directly involved in plant metabolism. Beneficial elements are those that can compensate for toxic effects of other elements or may replace mineral nutrients in some other less specific function such as the maintenance of osmotic pressure [29, 30].

On the other hand, from the biological viewpoint, metallic elements that are considered to be mineral nutrients of plants are most commonly classified into four [30, 31-32]; (i) essential macronutrients, (Ca, Mg and K), are those without which plants cannot complete their life cycle;

(ii) Essential micronutrients or trace metals, (Fe, Mn, Cu, Zn, Mo, Co, V, Na, and Ga), which are needed by plants in quantities much smaller than the macronutrients and they are usually functioning in plants as components of enzyme systems [30, 33]; (iii) essentiality not demonstrated metals, (Cr, Sr and Ni); and (iv) beneficial metals, (Al, Sr and Rb). Thus, the metal content of plants frequently controls the amount of these elements available in different animal bodies. The functions of elements in the animal's body are so diverse, valid statements of principle concerning their functions are rear. On the other hand, heavy metals in foods and beverages are classified into two; based upon their essential and toxic nature. For example Fe, Zn, Cu, Mn, Cr, Co, and V are essential. While Pb, Cd. Ni, As and Hg are toxic at a certain levels [34-35].

2.5.1. Transportation of Nutrients into the Plants and their importance's

In higher plants, the elements enter the root of the plant from the soil and move across the root, either through or between cells, until they reach specialized conducting cells of the vascular system. The cells of the vascular system are generally tubular, and connect end to end with similar tubular cells in a network reaching throughout the plant. In many instances trace elements move through biological membranes in the form of complexes of chelates with organic ligands [36]. In general, most plants grow by absorbing nutrients from the soil. Their ability to do this depends on the nature of the soil. Depending on its location, a soil contains some combination of sand, silt, clay, and organic matter.

The makeup of a soil (soil texture) and its acidity (pH) determine the extent to which nutrients are available to plants. Soil Texture (the amount of sand, silt, clay, and organic matter in the soil) affects how well nutrients and water are retained in the soil. Clays and organic soils hold nutrients and water much better than sandy soils. As water drains from sandy soils, it often carries nutrients along with it. This condition is called leaching. When nutrients leach into the soil, they are not available for plants to use. An ideal soil contains equivalent portions of sand, silt, clay, and organic matter. Sometimes, the nutrients that plants need occur naturally in the soil. Other times, they must be added to the soil as lime or fertilizer. Soil pH (a measure of the acidity or alkalinity of the soil) is one of the most important soil properties that affect the availability of nutrients [38, 39]. Macronutrients tend to be less available in soils with low pH. Micronutrients tend to be less available in soils with high pH. Lime can be added to the soil to make it less sour (acid) and also supplies calcium and Magnesium for plants to use. Lime also raises the pH to the desired range of 6.0 to 6.5. In this pH range, nutrients are more readily available to plants, and microbial populations in the soil increase. Microbes convert nitrogen and sulfur to forms that plants can use. Lime also enhances the physical properties of the soil that promote water and air movement [37].

The life processes of every living cell are conditioned by its content of trace elements. In some instances, this trace element control over life processes is dramatically reflected in human health, the success of agriculture in a region, or in shifts of the equilibrium between organisms in ecological competition. The major reason of interest of studying the elements in plants is that plants transfer elements from the soil to animals and humans. Thus, the mineral content of human and animal diet is frequently dependent upon the mineral content of plants. The physiological roles of Ni, Cu and Co are briefly described below.

2.5.2. Selected Heavy Metal

2.5.2.1. Nickel (Ni)

Nickel (Ni) is the 24th most abundant element in the earth's crust, comprising about 3% of the composition of the earth. It is the 5th most abundant element by weight after iron, oxygen, magnesium and silicon. It is a member of the transition series and belongs to group VIII B of the periodic table. It has the symbol Ni and atomic number 28. Nickel is a naturally occurring element that can exist in various mineral forms. Natural nickel is a mixture of five stable isotopes; nineteen other unstable isotopes are known. The prevalent oxidation state under environmental conditions is Ni (II), nickel in the +2 valence state. Other valences (-1,+1,+3 and+4) are also encountered, though less frequently (40,41,42) . Nickel is a nutritionally essential trace metal for at least several animal species, micro-organisms and plants, and therefore either deficiency or toxicity symptoms can occur when, respectively, too little or too much Ni is taken.

Nickel and nickel compounds have many industrial and commercial uses. Most nickel is used for the production of stainless steel and other nickel alloys with high corrosion and temperature resistance. Nickel metal and its alloys are used widely in the metallurgical, chemical and food processing industries, especially as catalysts and pigment (42).

The nickel salts of greatest commercial importance are nickel chloride, sulphate, nitrate, and carbonate, hydroxide, acetate and oxide (43, 44). Nickel is one of many trace metals widely distributed in the environment, being released from both natural sources and Anthropologic activity, with input from both stationary and mobile sources. It is present in the air, water, soil and biological material. Natural sources of atmospheric nickel levels include wind -blown dust, derived from the weathering of rocks and soils, volcanic emissions, forest fires and vegetation. Environmental sources of lower levels of nickel include Tobacco, dental or orthopedic implants, stainless steel kitchen utensils and inexpensive jeweler (45).

Nickel is normally present in human tissues and, under conditions of high exposure; these levels may increase significantly (43, 46 and 47). The absorption of nickel is dependent on its physicochemical form, with water -soluble forms (chloride, nitrate, sulphate) being more readily absorbed. Nickel is a ubiquitous metal frequently responsible for most common causes of allergic skin reactions (48, 49, 44, 46 and 50). Its hypersensitivity also causes asthma, conjunctivitis, inflammatory reactions. Nickel metal dusts and some nickel compounds are extremely potent carcinogens after inhalation but also that the carcinogenic risk is limited to conditions of occupational exposure (51, 52, 47) . Differences in the carcinogenic activities of nickel compounds may reflect variations in their capacities to provide nickel ions (e.g. Ni^{2+}) at critical sites within target cells (52,47).

2.5.2.2 Copper (Cu)

The essential role of copper in maintaining normal health in both animals and humans has been recognized for many years. The average daily dietary requirement for copper has been reported by many scholars. Copper is required with iron for synthesis of hemoglobin. It works with many enzymes such as those involved in protein metabolism and hormone synthesis. Deficiency of Copper causes low white blood cell count and poor growth. Excess intake of copper can cause vomiting, nervous system disorder and Wilson’s diseases [53].

2.5.2.3 Cobalt (Co)

Cobalt has the symbol Co and atomic number 27. Occurring Co consists of a single stable isotope: 59Co, whereas 60Co is an artificial isotope that is an important g-ray source. Cobalt has two main oxidation states (2+ and 3+). The common oxidation state for simple compounds is CO^{2+}. It has a high melting point of $1495^{0}C$ and is ferromagnetic. Cobalt is often associated with Ni, Ag, Pb, Cu, and Fe -Mn ores, from which it is most frequently obtained as a by - product [54]. The main known ore deposits are found in Katanga (Democratic Republic of Congo), almost 50% of the world's cobalt supply in 2014 was mined in the Democratic Republic of Congo.

Mining and smelter activities in Katanga have contaminated soil, water, and urban environments [55]. Cobalt has been utilized mainly to impart a rich blue color to glass and ceramics. Cobalt, like Fe, can be magnetized and so is used to make magnets. It is alloyed with Al and Ni to make particularly powerful. Other alloys of Co are used in jet turbines and gas turbine generators, where high temperature strength is important. Cobalt metal is sometimes used in electroplating because of its attractive appearance, hardness and resistance to oxidation, and thus corrosion. Cobalt salts have been used for centuries to produce brilliant blue colors in paint, porcelain, glass, and pottery. Cobalt is a critical metal to clean energy over the next 5-15 years, because of its use in lithium ion batteries: each electric- powered vehicle will demand 9.4kg of Co [56].

The artificial radionuclide 60Co is widely used in cancer treatment, as a tracer, and for radiotherapy. Cobalt is also a naturally occurring element in air, soil, planets, and water. CO^{2+} is the principal aquatic species in seawater (57). Cobalt tends to be associated with either Mn or Fe ox hydroxide (58). Although there is a lack of comprehensive data on aqueous Co concentration in soil pore waters, groundwater, and surfaces water, natural Co concentrations vary mostly from 0.006mg/L to 0.43mg/L[59].

In soil, Co is fixed by Mn- oxides in a non -extractable form, and its bioavailability is inversely proportional to the MN content of the soil [60]. High and frequent Co exposures can affect nervous system and cause an axonopathy [61]. Chronic inhalational intake of cobalt dust can lead to diffuse -inflammatory reactions of the bronchial mucosa and chronic respiratory tracdisorders [62]. In large doses some Co forms carcinogenic. Some plant species, also called metallophytes, have adapted to natural and contaminated Co - rich soils [63] .Among these metallophytes, some are able to hyper accurate Co in plant shoots (>300ppm, without toxicity symptoms and growth inhibition). Natural sources of cobalt are fish, nuts, green leafy vegetables such as broccoli and spinach, cereals such as oat. Elevated level of cobalt in water may result from anthropogenic activities such as the mining and processing of cobalt bearing ores, the application of cobalt containing sludge or phosphate fertilizers to soil, the disposal of cobalt containing wastes [64]

2.5.3. Toxic metals

Heavy metals are potential environmental contaminants with the capability of causing human health problems if present to excess in the food we eat. They are given special attention throughout the world due to their toxic effects even at very low concentrations. Several cases of human disease, disorders, malfunction and malformation of organs due to metal toxicity have been reported [65].

2.5.3.1 Lead (Pb)

Lead serves no useful purpose in the human body, and its presence in the body can lead to toxic effects, regardless of exposure pathway. Lead toxicity can affect every organ system. On a molecular level, proposed mechanisms for toxicity involve fundamental biochemical processes. These include lead's ability to inhibit or mimic the actions of calcium (which can affect calcium-dependent or related processes) and to interact with proteins (including those with sulfhydryl, amine, phosphate, and carboxyl groups). Acute high lead exposure can cause serious physiol effect, including death or long-term damage to brain function and organ systems. Effects of lead exposure vary according to exposure timing and levels, and other factors, and some effects may be latent [66].

2.6. Soil pH

Soil pH is a determination of the soil solution’s acidity and alkalinity. By definition, pH is the negative logarithm (base 10) of the hydrogen ion concentration in a solution, that is,

$$PH = -\log [H^+] \qquad 1$$

Soil pH applies to the H^+ ions concentrations in the solution present in soil pores which is in dynamic equilibrium with the predominantly negatively charged surface of the soil particles. Hydrogen is added in the form of ammonia-based fertilizers, urea-based fertilizers and protein (amino-acids) in organic fertilizers. Transformation of these sources of nitrogen to nitrate releases hydrogen ions $[H^+]$ to create soil acidity. Therefore, application of large quantities of both chemical and organic fertilizers may eventually make the soil more acidic. The increased use of nitrogenous fertilizers generally increases soil acidity [67]. Reported that fertilizer amended soils have generally lower pH than natural soils. Other factors that do affect soil acidity include soil organic matter and rainfall. Soil organisms are continuously decomposing organic matter. The net effect of their activity is that hydrogen ions are released and the soil becomes more acidic. Soils under high rainfall conditions are more acidic than soils under dry conditions. Soil pH greatly influences the availability of both nutrients and toxins for uptake by plant roots [68].

2.7. Methods of analysis of heavy metal

Several techniques for determination of metallic elements are currently in use. These include the atomic absorption spectroscopy (AAS) [69], inductively coupled plasma mass spectroscopy (ICP-MS) [70], the inductively coupled plasma atomic emission spectroscopy (ICPAES) [71] and energy dispersive X-ray fluorescence (EDXRF) spectroscopy [72]. For this study, the AAS was used because of its availability, reproducibility and time efficiency. The AAS has high sensitivity and selectivity. It is a single elemental method in which one element is determined in a series of samples and instrumental parameters optimized for the next element and can easily be automated.

UNIT THREE

3. MATERIALS AND METHODS

3.1. Chemicals

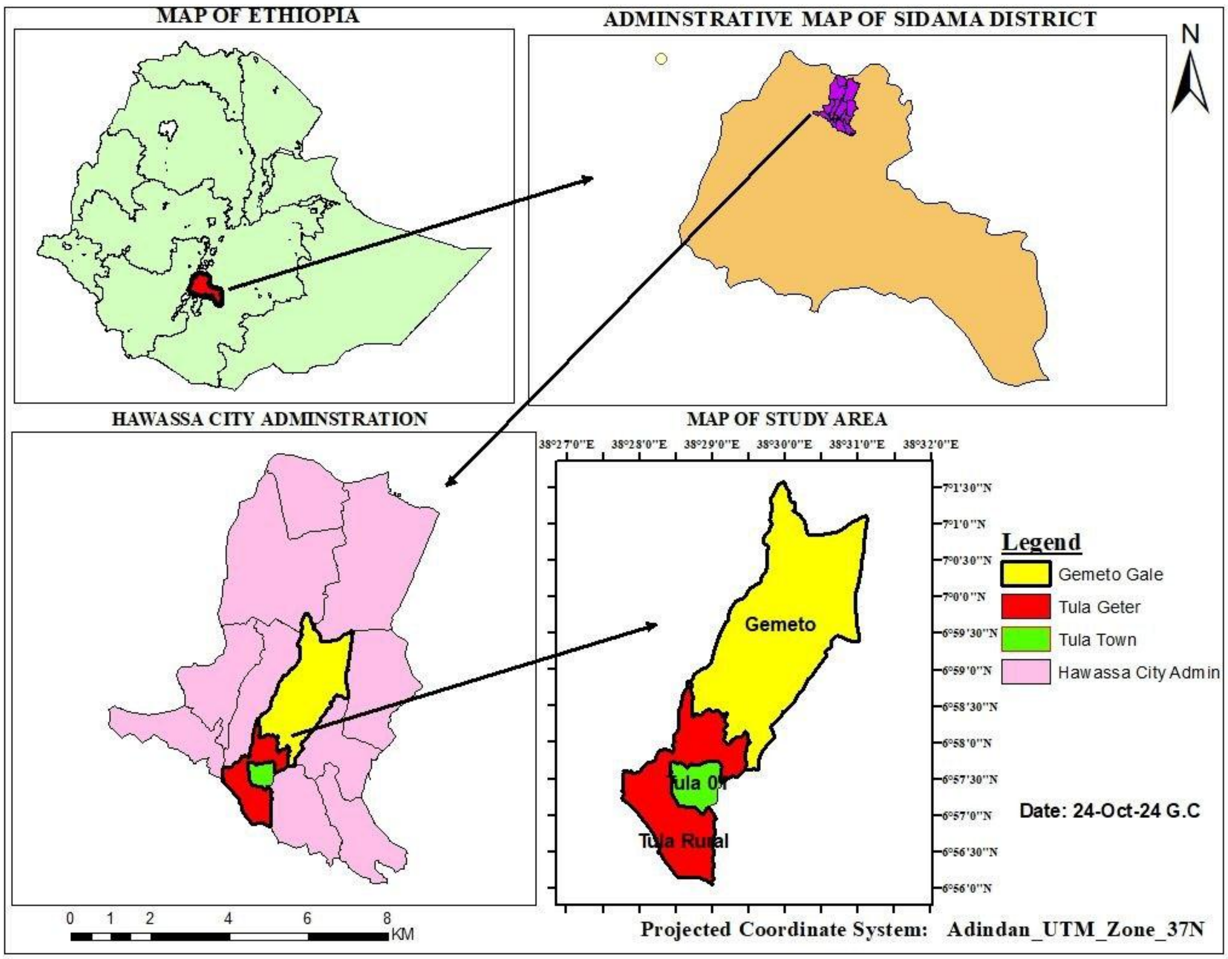
All the reagents used were of analytical grade. Concentrated HNO_3 (69-72 %,) SPECTROSOL, BDH, UK), concentrated $HClO_4$ (70%, India) and H_2O_2 (30%, Scharlaw, Chemie. S.A) were used for the digestion of khat leaf samples. Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals (Ni, Cu, Cr, Co and Pb) were used for the preparation of calibration standards and in the spiking experiments. Deionized water was used throughout the experiment.

3.2. Instruments

A drying oven 24h at 50^0c (Genlab ENGLAND) was used to dry khat samples. Ceramic pestle and mortar were used to ground and homogenize the dried khat sample. All of the khat leaves were weighed on a digital analytical balance .Round bottom flask (100 mL) fitted with reflux condenser were used for digestion of khat samples .Whatman paper (No 42) was used to filter the soil sample. The concentrations of Ni, Cu, Cr, Co, and Pb in khat sample were determined by flame atomic absorption spectrophotometer (Buck Scientific Model 210 VGP, using an air - acetylene flame.

3.3 Description of the study area

The study area namely Tulla, located in the Sidama Region of Ethiopia, is known for its fertile agricultural land, particularly for the cultivation of khat (Catha edulis), a popular stimulant among the youth. The region's diverse climatic conditions support various crops, Hawassa city of the capital city Sidama region is located 275 km from Addis Ababa Capital city of Ethiopia. It is surrounded by the Lake Hawassa in west, Hawassa Zuria woreda in the south east, Melga woreda in the north east & Oromia region in the north. It is located at 7'30" latitude north & 33'30" east longitude with an altitude of 168 meter above sea level (www.southinvest.gov.et/potential Hawassa.Htm). Hawassa city administration comprises 8 sub cities namely:-Hayek dar, Tabor, Menaheria, Misrak, Mehalketema, Bahil aderash, Addis ketema and Hawela Tulla. Tulla sub city is surrounded by Hawassa Zuria woreda in the south east, Tabor sub city in the west Melga woreda in the north, Addis ketema in the North West& Oromia regional state in the north. According to Tulla sub city administration, Tula sub city has a total population of 129,507 of whom 65,018 are men & where 64,499 are women. The study area experiences moderate types of tropical climate. Although it is situated in rift valley it has relatively high altitudes about 1800 above sea level means that the climate is mild round. The land form is plain with reddish volcano so on.



Source: **- CSA, 2024**

Figure 2 Map of the study area

3.4 Sampling

Samples of khat leaves and supporting soils were collected from different appropriate sampling sites. The samples were gathered from Tula area (specific Kebeles namely Gemeto, Tula 01 and

Tula rural) Sidama region Ethiopia. 18gBulk samples of Khat with supporting soils were gather ed from three different farms of each site. Generally, the Khat plants growing in these selected districts are locally named as Nole, Dume and Megala, respectively.

3.4.1. Khat leaves sample collection

Three varieties of khat were used in this study that was distinguished by the local farmers of Tula area Sidama regional state based on their exiting rate and their colors. The upper recently matured leaves which are free of physical damage and injury from insects were selected for this study. The three varieties of khat were collected from three different sites of the kebeles with a total amount of nine sample (1g each sample in fresh weight).

3.4.2. Soil Samples Collection

Soil samples were collected from a given field at depth of 0-30cm. 10-15 increments (holes) were collected so as to get a representative sample of 1g from a particular field. A total amount of nine soil samples were stored in plastic bags and transported to the laboratory for analysis. The soil samples were first air dried, ground in ceramic mortar and pestle, and then were passed through a 2mm sieve. And these soil samples were packed in other plastic bags until the beginning of the laboratory experiment.

3.4.3 Sample preparation

The Khat sample was washed with tap water and edible parts of all the khat was selected. All of the khat samples were oven dried for 24 hour at 50°C and then Vacuum packed until analysis. The vacuum dried material was then ground in to a fine powder by using mortar and pestle. The powdered samples were sieved to obtain fine powder which was later digested. Finally the powdered material was sealed in an aluminum foil bag until used for analyses.

3.5. Methodologies Optimization of Digestion Procedure:

To select an optimum procedure for digestion, parameters like digestion time, reagent volume, volume ratio of reagents, and digestion temperature were optimized by varying one parameter at a time and keeping the others constant. Parameters giving clear solution at lower temperature, requiring minimum reagent volume and digestion time were selected as an optimum procedure for digestion of khat sample. Finally, for a complete digestion of 1 g the dry sample, 6 ml HNO_3

(69– 72%), 2ml $HClO_4$ (70%) and 2 ml H_2O_2 (30%) for a total of 3 h at variable interval was selected as optimized procedure for the digestion [73].

3.5.1 Digestion of Khat leaves

Applying the optimized procedure 1 g of dried and ground khat leaves sample was digested using 10 ml of 6:2:2 mixture of HNO_3 (69-72%), $HClO_4$ (70%) and H_2O_2 (30%) under reflux at150^{0}C for 2:30 hour and the optimization process were given in below (table 3.1), The digests were used to determine concentration of Cu, Cr, Ni, Co and Pb by FAAS [73].

3.5.2 Digestion of Soil sample

One grams of soil (dry weight) was weighed using a digital weighing scale and the homogenized samples were poured into a 250 ml refluxing block digester. A mixture of HNO_3 (70%), H_2SO_4 (98%), and H_2O_2 (30%) was added in a volume ratio of 6:2:2 followed by mild heating for 3:00 hours at 100°C. Then the temperature was set to 150°C and heating continued for 3: 00 hours until a clear solution observed and the optimization process were given by below (table 3.2). Then, the samples were cooled, filtered with Whatman No.42 filter paper and diluted to 50 mL by distilled water.

Table 3.1.Optimization of digestion procedure for khat sample.

Trial	Reagent volume mL			Total	Temperature ^{0}C	Time (h)	Observation
	HNO_3	H_2O_2	$HClO_4$				
1	3	2	2	7	50	2:30	Red brown
2	4	3	2	9	130	2:20	Yellow
3	6	1	3	10	120	2:10	Slightly Colorless
4	**6**	**2**	**2**	**10**	**150**	**2:30**	**Colorless**
5	6	2	2	10	150	0:30	Cloudy Suspension
6	6	2	2	10	150	1:30	Yellow
7	**6**	**2**	**2**	**10**	**150**	**2:30**	**Colorless**
8	6	2	2	10	60	2:10	Brown
9	6	2	2	10	120	1:30	Yellow
10	**6**	**2**	**2**	**10**	**150**	**2:30**	**Colorless**

Table 3.2: Optimization of digestion procedure for soil sample

Trial	Reagent volume mL			Total	Temperature °C	Time (h)	Observation
	HNO_3	H_2O_2	H_2SO_4				
1	3	3	2	8	150	2:30	Red brown
2	4	3	2	9	150	2:30	Yellow
3	6	1	3	10	150	2:30	Slightly Colorless
4	**6**	**2**	**2**	**10**	**150**	**2:30**	**Colorless**
5	6	2	2	10	150	0:30	Cloudy Suspension
6	6	2	2	10	150	1:30	Yellow
7	6	2	2	10	150	2:30	Slightly Colorless
8	**6**	**2**	**2**	**10**	**150**	03:00	**Colorless**
9	6	2	2	10	60	03:00	Brown
10	6	2	2	10	120	03:00	Yellow
11	6	2	2	10	150	03:00	Colorless
12	**6**	**2**	**2**	**10**	**150**	**03:00**	**Colorless**

3.5.3 Digestion of blank sample

In addition to the samples, 6 each reagent blanks were prepared maintaining the same digestion Parameters for the analysis of soil and khat samples. All digested blanks were stored in the refrigerator until analysis.

3.6. Operating conditions of FAAS for analysis of metals

In this study a total of five metals for each khat and soil sample were analyzed using FAAS with external calibration curve after the parameters such as burner and lamp alignment, slit width and wavelength adjustment were optimized for maximum signal intensity of the instrument. For each metal, the respective hollow cathode lamp was inserted in to the atomic absorption spectrophotometer, and the solution was successively aspirated into the flame. Hallow cathode lamp for each metal, operated at the manufacturer's recommended conditions, and were used at its corresponding primary source line. The acetylene and air flow rates were managed to ensure suitable flame conditions. Five elements (Cr, Ni, Cu, Co and Pb) were analyzed by absorption mode of the instrument. The operating conditions of the instrument employed for each analyte are shown in Table 3.3.

Table 3.3. Instrumental operation conditions for determination of selected metals using FAAS.

Metals	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Energy (Ev)	Instrumental detection limit (mg/L)
Cr	357.9	0.7	2.0	2.712	0.04
Ni	341.5	0.2	7.0	2.624	0.020
Cu	324.7	0.7	1.5	3.938	0.005
Pb	283.2	0.7	2.0	2.874	0.040
Co	240.7	0.2	4.5	3.106	0.050

3.6.1. Instrument calibration and measurement of selected metals concentration

Determination of trace metals concentration was done by using FAAS (BUCK SCIENTIFIC MODEL 210VGP) in Hawassa University Chemistry Laboratory. Intermediate standard solutions (10 mg/L) of metals of interest were prepared from the 1000 mg/L standard stock solutions. These solutions were diluted to the desired concentrations to calibrate the instrument (A 10 ppm multi-element solution containing Cr, Co, Ni, Pb, and Cu was utilized to prepare elemental calibration solutions). This multi-element solution was diluted with 2% nitric acid to obtain working standards for each metal of interest).These intermediate standards were diluted with deionized water to obtain four working standards of each metal. After shaking and homogenizing the solutions, the selected metals were analyzed after the instrumental operating conditions were optimized for maximum signal intensity of the instrument [74]. Calibration curves for Cr, Co, Ni, Pb and Cu were obtained by using suitable standard solutions prepared from stock solutions. Calibration curves for each selected metal was set to ensure the accuracy of the instrument and to confirm that the results of determination were true and reliable. Calibration standards for the elements analyzed were prepared in concentration range expected for the analyte in the samples analyzed. In addition, the calibration standards were prepared by taking into consideration the optimum working ranges of the elements. The prepared standard concentration and the corresponding correlation coefficients of the calibration curve for each metal were presented in **Table 3.4**.

Table 3.4. Concentrations of the standard solutions used to establish calibration graphs for the determination of metals in khat and soil samples and their corresponding correlation coefficients.

S.no	Analyte (metal)	Intermediate Standard concentration in mg/L	Standard concentration in mg/L	Correlation coefficient of calibration curve in %
1	Cr	10	0.04, 0.5, 1, 2	0.998
2	Cu	10	0.005, 0.1, 0.5, 1	0.999
3	Ni	10	0.02, 0.5, 1, 1.5	0.999
4	Pb	10	0.005, 0.5, 1, 2	0.998
5	Co	10	0.05, 0.5, 1, 2	0.995

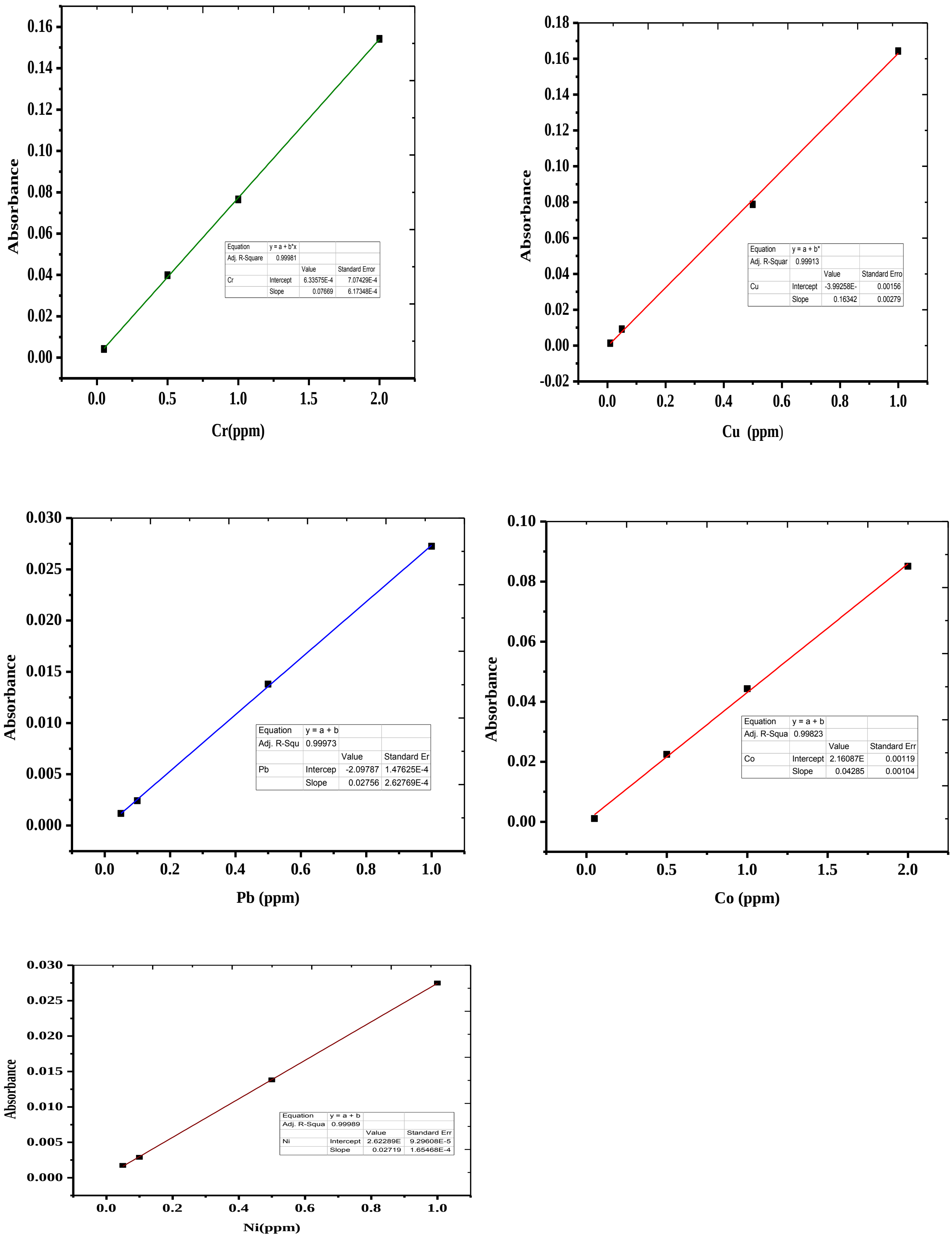


Figure 3 The calibration curve for Cr, Cu, Pb, Co, and Ni respectively

3.6.2 Method Detection Limit

Method detection Limit is the lowest concentration level that can be determined to be statistically different from an analyte blank or that gives a minimum concentration that can be detected by the analytical method with a given confidence limit[74]. In other words, it is the lowest analyte concentration that can be distinguished from statistical functions in a blank which usually correspond to three times the standard deviation of the blank (MDL = 3SDBlank). In this study, after the digestion of six blank solutions containing HNO_3 (69-72%) and $H_2 O_2$ (30%) and $HClO_4$ (70%) six readings were taken from FAAS and the standard deviation was calculated. The method detection limit of each element was calculated as three times the standard deviation of the blank (MDL = 3SDblank, n = 3) and the result was presented by table 3.5. The method detection limit of the metal was obtained by using the equation used in reference [68].

MDL=3xSD (where: MDL is method detection limit and SD is standard deviation of the blank reading.

Table 3.5. Method and instrument detection limits values for each metal analyzed

Analyte	Pb	Cr	Co	Ni	Cu
MDL(mg/L) for soil	0. 070	0.073	0.089	0.095	0.040
Khat MDL(mg/L) for khat	0.074	0.089	0.085	0.091	0.024
IDL (mg/L)	0.040	0.040	0.050	0.050	0.005

3.6.3. Method Validation

The validity of the digestion procedure, precision and accuracy of FAAS were assured by spiking the raw khat samples with standard of known concentration. The spiked and non-spiked khat samples were digested following the same procedure employed in the digestion of the respective samples and analyzed in similar condition. All the spiked samples were digested in triplicate following the optimized digestion procedure of khat samples. The digested spiked samples were analyzed for their respective metals content using FAAS. Then the percentage recoveries of the analyte were calculated by E Equation (2) [68]:

$$\%\text{Recover} = \frac{\text{C(spiked)} - \text{C(non spiked)}}{\text{C(added)}} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(\mathbf{3})$$

Where; C (spiked) is metal content of the spiked sample, C (non-spiked) metal content of non-spiked sample and C (added) is metal content of metal added.

3.6.4 Spiking Khat Sample

Known amount of each metal was added from the 1000 mg/L of stock solution in to flasks containing 1 g of raw khat (catha edulis) sample to check the efficiency of digestion procedure. The procedure was as the following: 0.03 mg/L of 10 mg/L intermediate solution each metal (Ni, Co and 0.1 mg/L of Cu) which are prepared from the intermediate 10 mg/L of the elements prior by taking 1 ml from the stock standard solutions containing 1000 mg/L of the metals were spiked at once in to triplicate round bottomed flasks containing 1 g of khat (catha edulis) and soil. Then, after the spiked samples were digested simultaneously with the unspiked samples based on the digestion method for khat (catha edulis) and soil samples, each sample was then determined for their respective metals (Ni, Co and Cu) by FAAS[75].

3.7 Analysis of soil samples

3.7.1. Soil

Before the total elemental analysis in soils, the soil pH was measured in water suspension. 10gm of air dried ground was weighed into 100ml beaker. And 25ml of deionized water was added to this beaker, then after stirring by a magnetic stirrer for 30 minutes the sample was allowed to equilibrate by standing for 1 hr. The pH of the suspension was measured after calibrating the pH meter using a Standard buffer analytical concentrate solution with pH values of 4.00, 7.00 and 9.2 [52].

3.8. Statistical Analysis

Microsoft Excel was used for statistical analysis. Statistical evaluations such as mean, standard deviation and relative standard deviation have done for all parameters of khat (catha edulis) samples collected from the different sources. All analyses were carried out in triplicate. Methodological precision was therefore evaluated with standard deviation (SD). Statistical analysis of the data were carried out using one-way analysis of variance (ANOVA) to assess significant variation in the mean concentrations of selected metals. A probability level of $p < 0.05$ was considered statistically significant. Pearson’s correlation coefficient was used to determ ine the association between the selected metals.

UNIT FOUR

4. RESULTS AND DISCUSSION

4.1. Physicochemical properties of Soil of Experimental sites

4.1.1. pH, Electrical Conductivity and Ash Content of soil

Heavy metals such as cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn) can increase soil acidity. These metals often become more bioavailable in acidic conditions, which can further exacerbate soil acidification. Acidic soils enhance the solubility of these metals, making them more accessible to plants and potentially leading to increased uptake and accumulation in the food chain [76, 77 and 78]. The application of certain fertilizers can also contribute to this process by altering soil pH and promoting heavy metal mobility [79, 80].

The pH range determined for the target soil samples in this study was between 6.21 and 6.82 (Table 4.1), indicating that the soil samples can be classified as slightly acidic to nearly neutral and it is in the range for normal agricultural soils. Based on the measured pH values, it could be reasonable to conclude that the soils of the study areas are appropriate for crop production and safe from the factor that may arise due to the extreme pH. It is a common practice, nowadays, for the farmers to use fertilizers to enhance the agricultural productivities, and frequent use of nitrogen fertilizers application normally escalates soil acidity. Therefore, extensive use of nitrogen, phosphorus, and potassium (NPK) fertilizers might be one of the possible reasons for the slight acidity of the investigated soils.

It is also worth describing that soil pH is among the parameters which significantly determine bioavailability of metals in soil system. Low soil pH favors the metals solubility and bioavailability, and the approximate pH range of normal agricultural soils is 5.0 – 7.Therefore, pH of the soils considered in this study can be described as convenient for agriculture.

Electrical conductivity (EC) in soil is closely related to its mineral content, primarily because minerals dissociate into ions that facilitate electrical current flow. Higher mineral concentrations, particularly soluble salts like sodium, calcium, and potassium, increase EC values, indicating greater nutrients availability and salinity levels [81]. Soil texture also influences EC; clay-rich soils generally exhibit higher conductivity due to their larger surface area and moisture retention capabilities compared to sandy soils [82, 83]. Thus, measuring EC can provide insights into soil fertility and potential plant health [82, 83]. EC measures the ion contents and salinity of the soil solutions that it gives a clear idea for the presence of soluble salts in the solution of soil samples. EC of the studied composite soil samples were found to be 11.42 ± 0.01, 1.86 ± 0.01, and 1.63 ± 0.02 (μScm^{-1}) for Gemeto, Tula 01, and

Tula rural respectively (Table 4.1). Accordingly, the soil samples can be classified as soils with very low EC and not salt affected.

Ash content in soil is directly related to its mineral content, as ash represents the total mineral residue remaining after organic matter combustion [84, 85 and 86]. Higher ash content typically indicates greater mineral presence, including essential nutrients like calcium, potassium, and magnesium. However, variations in ash content can also reflect contamination from soil or other materials, affecting the perceived mineral levels [87, 88, and 89] .Thus, measuring ash content serves as a practical proxy for assessing the overall mineral composition of the soil.

The p^H, electrical conductivity and ash content of the analyzed varied in the soil samples. High value of the P^H (6.82 ± 0.01) was observed for Tulla rural site and the least value of (6.21 ± 0.02) was recorded for the Gemeto site. However, the P^H value in soils of Tulla 01 site was (6.64 ± 0.30) next higher value was recorded. Electrical conductivity was recorded relatively highest in Tulla 01 soil site (1.86± 0.01 μScm^{-1}) whereas the least concentration was recorded at Gemeto soil site (1.42 ±0.01 μScm^{-1}) and (1.63± 0.02μScm^{-1}) was observed in Tulla rural soil site. High ash content (2.17 ± 0.015%) was observed for Tulla 01 site and the least concentration (1.72 ± 0.02%) was recorded for the Tula rural site. However, the ash content in soils of Gemeto site was (1.93 ± 0.025 %) next higher concentration was recorded. The ash content of the analyzed soils ranged from 1.72 % to 2.17 % for khat varieties

Table 4.1: Selected physicochemical property of the soils

Soil site	pH	Conductivity (μScm^{-1})	Ash content (%)
Gemeto	6.21 ± 0.02	1.42 ± 0.01	1.93 ± 0.025
Tula 01	6.64 ± 0.03	1.86 ± 0.01	2.17 ± 0.015
Tula rural	6.82 ± 0.01	1.63 ± 0.02	1.72 ± 0.020

4.2 Physicochemical analyses of khat varieties (Nole, Megala, Dume)

The pH determined for the khat varieties in this investigation was in the range of 5.93 to 6.43 (Table 4.2), indicating that khat samples can be slightly acidic and it is in the normal range . It is also value telling that pH is among the parameters which meaningfully control bioavailability of metals in plant. Electrical conductivity (EC) measures the ion contents and salinity of the solutions that it gives a clear idea for the presence of soluble salts in the solution.

The p^H, electrical conductivity and ash content of the analyzed varied in the soil samples. High value of the P^H (6.43 ± 0.01) was observed in Megala khat varieties and the least value of (5.93 ± 0.02) was recorded in Nole khat varieties. However, the P^H value in Dume khat variety was (6.24 ± 0.01) next higher value was recorded. Electrical conductivity was recorded relatively highest in Dumekhat variety (1.54± 0.01 μScm^{-1}) whereas the least value was recorded in Nole khat variety (1.17 ±0.01 μScm^{-1}) and (1.33 ± 0.02μScm^{-1}) was observed in Megala. High ash content (0.93 ± 0.01%) was observed in Megala khat variety and the least concentration (0.63 ± 0.02%) was recorded for Nole khat variety. However, the ash content in Dume khat variety was (0.85 ± 0.01 %) next higher concentration was recorded.EC of the composite khat varieties were in the range of 1.17 to 1.54 ($mScm^{-1}$) for khat varieties of Nole, Dume and Megala (Table 4.2). The ash content of khat sample was ranged from 0.63 % to 0.93 % for khat varieties.

Table 4.2: Selected physicochemical property of the khat varieties

Khat varieties	pH	Electrical conductivity	Ash content%
Nole	5.93 ± 0.02	1.17 ± 0.01	0.63 ± 0.02
Dume	6.24 ± 0.01	1.54 ± 0.01	0.85 ± 0.01
Megala	6.43 ± 0.01	1.33 ± 0.02	0.93 ± 0.01

4.3 Levels of heavy metals in soil sample

Determination of the levels of heavy metals in soil were analysis using the producer describe methodology section using FAAS. The result is indicated in Figure 4.The concentration levels of metals (Cu, Co, Ni, Cr and Pb) measured in soil sample. The concentrations of the metals analyzed varied in the soil samples. High concentration of Co (4.85 ± 0.02 mg/kg) was observed for Tulla 01 site and the least concentration (3.37 ± 0.02 mg/kg) was recorded for the Tulla rural site. However, Co concentration in soils of Gemeto site was (4.233 ± 0.30 mg/kg) next higher concentration was recorded. The concentration of Cu was recorded relatively highest in Tulla rural soil site (9.96± 0.20 mg/kg) whereas the least concentration was recorded at Tulla 01 soil site (8.54 ± 19 mg/kg) and 9.23 ± 0.15 mg /kg was observed in Gemeto soil site. High concentration of Ni (4.95 ± 0.04 mg/kg) was observed for Tulla rural site and the least concentration (3.67 ± 0.02 mg/kg) was recorded for the Tula 01 site. However, the concentration of Ni in soils of Gemeto site was (4.57 ± 0.02 mg/kg) next higher concentration was recorded. The concentration of, Pb and Cr were below detection limits.

The level of metals in different soil types are shown in Figure 4 indicating concentration order of metals in Gemeto soil from which Nole khat variety was collected, is as follows: Cu > Ni> Co and Cr and Pb were not detected, for Tula 01 Cu > Co >Ni and also Cr and Pb were not detected whereas for Tula rural soil type from which Megala khat variety was collected is Cu > Ni>Co whereas Cr and Pb were not detected. Among the heavy metals Cu found to be the highest although Co was lowest concentration in soils.

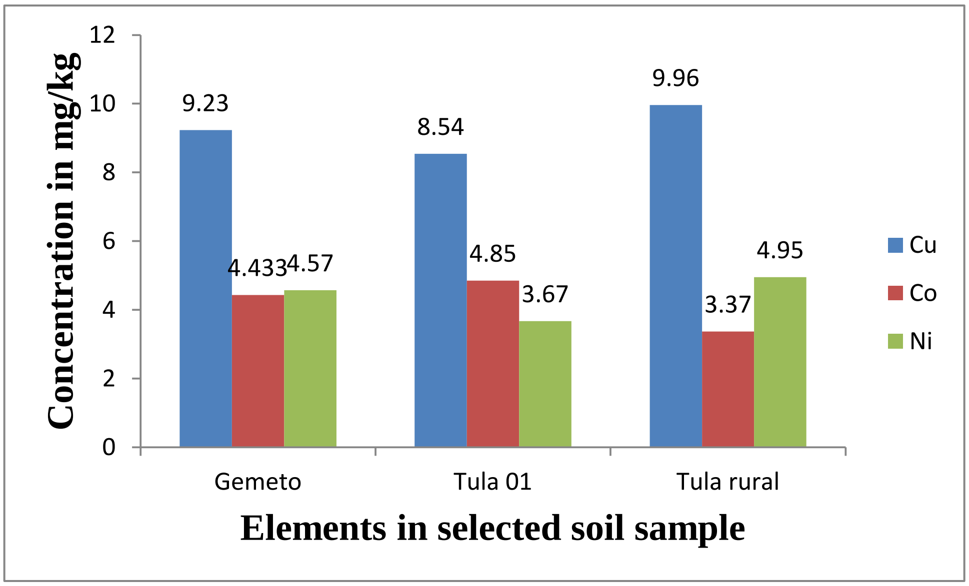


Figure: 4 The level of selected metals in soil sample

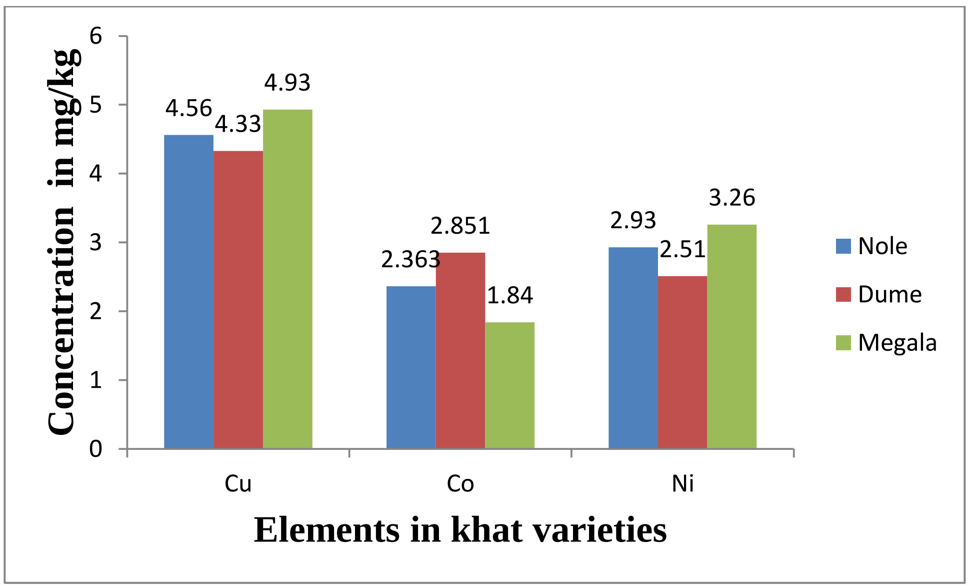
4.4 Levels of heavy metals in khat varieties

Determination of selected heavy metals in khat varieties were carried out to assess the level of pollutant. The level of heavy metals was detected by using FAAS as described in the methodology section. Based on this study result, the concentrations of heavy metals (Cu, Co, Ni, Pb and Cr) for khat varieties are shown on the Figure 5. The concentrations of metals studied also varied in the khat varieties samples. The concentration of Cu recorded was relatively the highest in Megala khat varieties which was (4.93 ± 0.15 mg/kg) and the least concentration was recorded in Dume khat variety (4.33 ± 0.20 mg/kg) whereas the concentration recorded to Nole khat variety was 4.56 ± 0.15 mg/kg.

According to WHO reports in Geneva, the permissible limits of heavy metals almost in all edible plants the permissible limit of copper in edible plant is 10 mg /kg [90]. Therefore in all khat varieties the concentration of copper was less than the permissible limits. Lead recorded below detection limits in all khat varieties. Cr also recorded below detection limit in all khat varieties. The concentration of

Ni recorded was relatively the highest in Megala khat varieties which was (3.26 ± 0.31 mg/kg) whereas the least concentration was recorded in Dume khat variety (2.51 ± 0.03 mg/kg) and 2.93 ± 0.02 mg/kg was observed in Nole khat variety, however in all khat varieties, the Ni concentration was below permissible limit .The concentration of Co recorded was relatively the highest in Dume khat varieties which was (2.851 ± 0.04 mg/kg) whereas the least concentration was recorded in Megala khat variety (1.842 ± 0.03 mg/kg) and (2.363 ± 0.023 mg/kg was observed in Nole khat variety, however in all khat varieties, the Co concentration was above permissible limit [90].

The level of metals in different khat varieties are shown in figure 5 indicating the decreasing concentration order of metals in Nole khat variety concentration of Cu > Ni > Co, in Dume khat variety Cu > Co > Ni, Cr and Pb were not detected whereas for in Megala khat varieties Cu > Ni > Co and also Cr and Pb were not detected.



Figure; 5 The level of selected metals in khat samples

4.5. Levels of selected metals in khat leaf and soil samples

The concentration of selected heavy metals (Cu, Co, Pb, Cr and Ni) in soil and khat of the three varieties of khat and their growing soil were determined with FAAS have been identified. the concentrations of five metal ions (mg/kg) were determined in all six samples (three soil and three khat) in triplicate, and the data are presented as Mean ± SD (Table 4.3). The abundance of these elements in different Khat varieties is further depicted in Figure 4 and 5. As it can be seen from Table

4.3 Cu, Co and Ni detected in soil and khat sample whereas Cr and Pb were bellow detection limit of (ND) Plants accumulate minerals essential for their growth from the environment [91]. Various factors affect the concentration of metals in the plant. These are: nature of chemical and physical property of the soil, climatic condition of the region, and application of fertilizers and pesticides.

Increase in population and industrialization results pollution to water, air, and soil which in turn causes in unexpected concentration of trace metals in the plant. Particularly agricultural activities such as use of fertilizers, pesticides, and irrigation with contaminated sewage are the major source of contaminations. As can be seen from Tables 4.3, there is a wide variation in concentration of heavy metals within and among the khat varieties. The pattern of concentration of elements in most of khat varieties analyzed were decreased as Cu > Ni > Co. Cu was found in a higher concentration. It is an essential macronutrient to the plant, can accumulate on top soil and is easily bio-available to the plant with decrease in pH of the soil [92]. Next to Cu, Ni was also found in a relatively higher concentration in analyzed khat plant. As reported by [93].

Table 4.3: Results of the detected mean level of each studied metal (mg/kg dry weight) in the soil and khat samples

Heavy metal	Soil sample	(mg/Kg) n=3	WHO limit in soils(mg/kg) 50 50	Khat varieties	(mg/Kg) n=3	WHO limit in khat (mg/kg)
Cu	Gemeto	9.23 ± 0.15	50	Nole	4.56 ± 0.15	10
	Tulla 01	8.54 ± 0.19	50	Dume	4.33 ± 0.15	10
	Tulla rural	9.96 ± 0.20	50	Megala	4.93 ± 0.20	10
Co	Gemeto	4.433 ± 0.30	5	Nole	2.363 ± 0.023	5
	Tulla 01	4.85 ± 0.02	5	Dume	2.851 ± 0.04	5
	Tulla rural	3.37 ± 0.02	5	Megala	1.842 ± 0.03	5
Pb	Gemeto	ND	10	Nole	ND	0.1
	Tulla 01	ND	10	Dume	ND	0.1
	Tulla rural	ND	10	Megala	ND	0.1
Cr	Gemeto	ND	10	Nole	ND	0.5
	Tulla 01	ND	10	Dume	ND	0.5
	Tulla rural	ND	10	Megala	ND	0.5
Ni	Gemeto	4.57 ± 0.02	20	Nole	2.51 ± 0.31	10
	Tulla 01	3.67 ± 0.02	20	Dume	2.93 ± 0.02	10
	Tulla rural	4.95 ± 0.04	20	Megala	3.26 ± 0.03	10

ND = not detected

4.6. Method validation

The correlation coefficients (R^2) of the calibration curve for the analysis of Co, Cu Cr, Pb, and , Ni in soil and khat leaves by FAAS were in the range of 0.995 to 0.999, indicating a significant positive correlation between metal levels and absorbance. The FAAS was calibrated with 4 standards. The concentrations used for the calibration, instrument working parameters and the correlation coefficients of the calibration curve for each of the metals are shown in Table 4.4.

Table 4.4. Concentrations of the standard solutions used to establish calibration graphs for the determination of metals in khat and soil samples and their corresponding correlation coefficients

S.no	Analyte (metal)	Intermediate Standard concentration in mg/L	Standard concentration in mg/L	Correlation coefficient of calibration curve in %
1	Cr	10	0.05, 0.5, 1, 2	0.998
2	Cu	10	0.01, 0.05, 0.5, 1	0.999
3	Ni	10	0.05, 0.1, 0.5, 1	0.999
4	Pb	10	0.05, 0.1, 0.5, 1	0.998
5	Co	10	0.05, 0.5, 1, 2	0.995

4.7. Recovery test

The concentrations of the five metals (Co, Cu, Pb, Cr and Ni) in khat and its support soil samples collected from Sidama regional state Tula area were determined in triplicate using FAAS. The validity of the method was evaluated by spiking the samples with standards of known levels and calculating the percent recovery expressed as mean ± standard deviation (SD) (n = 3). The obtained percentage recovery for soil and khat sample ranges from (91.16%-116.66% and (98.66%-103.44%), respectively, as shown in Tables 4.5 and 4.6 which were in acceptable range. Thus the procedures used in study for metal analysis in soil and khat variety were in valid range.

Table 4.5. Recovery test results for the determination of the metal in soil samples (mean ± SD)

Metals	Conc.in sample (mg/L)	Amount added (mg/L)	Conc.in spiked sample (mg/L)	% Recovery	RSD
Co	0.081 ±0.0017	0.03	0.112 ± 0.001	103.22± 5.09	4.981
Ni	0.088 ± 0.002	0.03	0.123 ± 0.002	116.66 ± 5.67	6.249
Cu	0.19 ± 0.015	0.1	0.281 ± 0.005	91.16 ± 5.77	5.972

Table 4.6. Recovery test results for the determination of the metal in khat samples (mean ± SD)

Metals	Conc. in sample(mg/L	Amount added (mg/L	Conc. In spiked sample(mg/L	%Recovery	RSD
Co	0.046 ± 0.001	0.03	0.0755 ± 0.001	98.66 ± 3.33	3.448
Ni	0.055 ± 0.003	0.03	0.086 ± 0.0005	103.44± 1.92	2.037
Cu	0.091 ± 0.02	0.1	0.190 ± 0.0057	99.12 ± 5.77	5.412

4.8 Correlation matrix between Transfer factors of metals and Soil pH

Correlation test was carried out among soil parameters and heavy- metal concentrations, in all khat and soil sampling sites (Table 4.7). The correlation study helps to evaluate metal distributions in the soil and their availability and accumulation in the plants and also to answer some of the questions how the metals interact among themselves in plant and soil. Thus Pearson product moment correlation coefficient was calculated. Based on the result of R-values, most the studied soils were found to correlate positively with the levels found in khat leaves.

As can be seen in Table 4.7, in this study Co in the soil correlate positively with copper whereas Ni, were negatively correlated with copper in soil. Copper ions form strong coordination complexes with organic matter [94]. Hence, Cu is often predominantly found bound to the organic matter fraction in the soil and soil organic matter can be the most important soil factor in determining Cu bioavailability [95].

Table 4.7 Correlation matrix between transfer factors of metals in soil with khat

Parametrs	**Cu in soil**	**Cu in khat**	**Co in soil**	**Co in khat**	**Ni in soil**	**Ni in khat**
Cu in soil	1					
Cu in khat	0.975251	1				
Co in soil	0.6583208	0.8084587	1			
Co in khat	0.4711473	0.6545097	0.9741216	1		
Ni in soil	-0.235996	-0.4450107	-0.8868368	-0.9683289	1	
Ni in khat	0.9929235	0.9946066	0.7430541	0.5725624	-0.3497275	1

4.9. Analysis of ANOVA

Statistical analysis of data was made to verify whether there was a significant difference in metal contents between the three khat varieties analyzed and their growing soil. For the present study, the significance of variation within sample and between samples has been studied using one way ANOVA test and calculations were made using SPSS- 20 software. There were no significance difference (($p>0.05$) at 95% confidence interval for Cu, Co and Ni concentrations in all khat varieties and their growing soil (Table 4.8).

Table 4.8. ANOVA table of selected metals in khat and soil sample (at 0.05 levels)

Element	Source of Variation	SS	df	MS	F calculated	P value	F critical
Cu	Between sample	0.541	2	0.2705	0.0028	0.997	3.68
	Within sample	1425	15	95.057			
	Total	1426.4	17				
Co	Between sample	0.0372	2	0.0186	0.0133	0.986	3.68
	Within sample	20.83	15	1.3869			
	Total	20.87	17				
Ni	Between sample	0.03181	2	0.015906	0.01748	0.982	3.68
	Within sample	13.646	15	0.9097			
	Total	13.678	17				

UNIT FIVE

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

This study analyzed levels of some heavy metals (Cu, Zn, Ni, Pb and Cd) in edible part of khat cultivators grown in Tula area, Gemeto,Tulla rural and Tula 01 specific kebeles and their respective supported soil samples using FAAS. The optimized wet- digestion method for khat and soil sample analysis was found to be efficient, and it was evaluated through the recovery experiment, and a good percentage recovery was obtained for the heavy metals determined. From the analyzed metals Pb and Cr were bellow detection limit. The results showed the ability of these plants to accumulate relatively higher amount of Cu and Ni among the determined metals, respectively. Cu was found to be comparatively at higher levels in all of the analyzed khat and soil samples.

The studied metals content of all khat samples followed, generally, similar trend across the varieties that could be arranged in descending order: Cu > Ni > Co. The soils of the study farms were found to contain high levels of, Cu. In general, the levels of most of the metals in the studied soils were found to correlate positively with the levels found in the khat leaves. From anova analysis of data, there is no significant variation in metal contents between intra-site samples of both khat and soil samples. There is no a statistically significant variation in the levels of each metal among the three sampling sites of khat and soil samples. According to this study result, except cobalt concentration in Nole, Dume and Megala khat variety, concentrations of all analyzed metals have below maximum permissible values which stated by WHO [90].

5.2 Recommendations

- Based on this study finding, the concentration of Co was above the permissible limits so that the users should be take care of about Nole, Dume and Megala khat variety as well as special attention should be given to trace metals particularly to those trace toxic metals as they accumulate over time and cause serious health impacts. However; there is a growing interest in the use of khat species recently all over the country. Therefore, further study of relationship between metal levels of khat and soil samples is recommended by taking large number of sites and different methods to come up with a compressive conclusion. Additional investigation on soil pH, nature of agricultural imputes like fertilizers, pesticides, herbicides are also recommended.
- More sensitive analytical instruments must be used to detect at lower level and typical sample based on the above investigation the following recommendation forwarded different varieties from different topographical circumstances (soil type, and climate) has not up still now studied. Further research is recommended to examine the safety and toxicity of these metals, which have concentration more than the FAO/WHO safe limit.

REFERENCES

[1] Tilahun, E. (2009). Determination of trace metals in commercially available Khat (Catha edulis Forsk) in Addis Ababa. M.Sc. thesis. Addis Ababa University. 33-42

[2] Graziani, M., Milella, M. and Nencini, P. (2008). Khat chewing from a Pharmacological point of view: An update. Substance Use and Misuse, 43:762–783.

[3] Milanovic, B. (2008). Qat expenditures in Yemen and Djibouti: An empirical analysis. Journal of African Economies, 17:661.

[4] Belew, M., Kebede, D., Kassaye, M. and Enguoselassie, F. (2000). The Magnitude of khat use and its association with health, nutrition and socioeconomic status. Ethiopian Medical Journal, 38:11–26.

[5] Atlabachew, M., Chandravanshi, B. S. and Redi, M. (2011). Profile of major, minor and toxic metals in soil and khat (Catha edulis Forsk) caltivars in Ethiopia. Treads in Applied Science Research, 6:640-655.

[6] Nartey, V. K., Haizel, H., Doamekpor, L. K. and Dankyi, E. (2012). Studies on the contribution of fertilizers to heavy metal levels in soils and cocoa from some cocoa farms in the western region of Ghana. Journal of Natural Sciences Research, 2:111-120.

[7] Ferner, D. J. (2001). Toxicity. Heavy metals. eMedicine Journal, 2:1

[8] Sharma, P. and Dubey, R. S. (2005). Lead toxicity in plants. Brazilian Journal of Plant Physiology, 17:35-52.

[9] Radwan, M. A. and Salama, A. K. (2006). Market basket survey for some heavy metals in Egyptian fruits and vegetables. Food and Chemical Toxicology, 44:1273-1278.

[10] Prabu, P. C. (2009). Impact of heavy metal contamination of akaki river of Ethiopia on soil and metal toxicity on cultivated vegetable crops. Electronic Journal of Environmental, Agricultural and Food Chemistry, 8:818-827.

[11] Maitai, C. K. (2013). Country report-Kenya. The health and socio economic aspects of khat use.http://www.drugtext.org/The-health-and-socioeconomic-aspect-of-khat-use/country-report-kenya.html (accessed 15/8/2013)

[12] Alloway, B .J. (1995). Heavy metals in soils. Blackie academics professional publishers, London. 7-39.

[13] Lidia, G. L. C., Silvia, R. M., and Liliana, M. (1997). Heavy metals input with phosphate fertilizers used in Argentina. Science of the Total Environment, 204:245-250.

[14] Wild, A. (1993). Soils and the environment: an introduction. Cambridge university press, U.K. 189-210.

[15] Steenland, K. and Boffetta, P. (2000). Lead and cancer in humans: where are we now? American Journal of Industrial Medicine, 38:295-299.

[16] Hönow R, Bongartz D, Hesse A. An improved HPLC-enzyme- reactor method for the determination of oxalic acid in complex matrices. Clin. Chim. Acta 261: 131-139 (1997)

[17] Kassie, F., Darroudi, F., Kundi, M., SchulteHermann, R., Knasmuller, S., Khat (Catha edulis) consumption causes genotoxic effects in humans. International Journal of Cancer,2001, 92, 329– 332.

[18] Alem A., Mental health in rural Ethiopia: study on mental distress, suicidal behavior and use of khat and alcohol. Umeå University, Swede, 2004.

[19] Belew M., The Magnitude of Khat use and its association with health, nutrition and socio-economic status. Community health department, Faculty of Medicine, Addis Ababa Univerity, 1997.

[20] Raman R. Catha edulis Forks, Geographical Dispersal Botanical, Ecological and Agronomical Aspects with Special References to Yemen Arab Republic. PhD Thesis, University of Gottingen: Germany, 1983.

[21] Central Statistics Authority. 1993, Addis Ababa, Ethiopia, 1998.

[22] Murphy, H.F. A report on the fertility status of some of the soils of Ethiopia- College of Agriculture 2000, Experiment station Bulletion No-1- Alemaya, Ethiopia, 1959.

[23] Kabata, P. 1992. Trace elements in soils and plants. Boca Raton, Florida, CRC Press. 365

[24] James LF. Oxalate poisoning in livestock. In: Keeler RF, van Kam- pen, James LF, eds. Effects of poisonous plants on livestock. New York: Academic Press, 1978: 139–145

[25] Eisshusler, S. and Brenneisen, R. The content of psychoactive phenylpropyl and phenylpentenyl khat amines in Catha edulis Forsk of Different origin. J Ethnopharmacol, 1987, 26, 269-277.

[26] MB, Qirbi and AB. Biochemical effects of Catha edulis, cathine and cathinone on adrenocortical Functions. J. Ethnopharmacol, 1993, 39,213–216.

[27] Wikipedia, the free encyclopedia, khat. Accessed on May 20, 1-10.

[28] Kalix, P. and Braenden, O. Pharmacological aspects of the chewing of khat leaves. Pharmacol Rev, 1985, 37,149-164.

[29] Banerji, S. K. Environmental Chemistry, 2nd ed. Prentice – Hall of India: New Delhi, 2005.

[30] http://retirees.uwaterloo.ca/~jerry/orchids/nutri.html, The Orchid House, What Is Plant Nutrition? Accessed on November 10, 2010.

[31] Anderson, K. A., Smith, B. W. Chemical profiling to differentiate geographic growing origins of coffee. Journal of Agriculture, Food Chemistry, 2002, 50, 2068-2070.

[32] Galston, A. W. The Green Plant. Prentice-Hall, In.: New York. 1968, 40 – 53.

[33] Report of a WHO advisory group. Review of the pharmacology of khat. Bull Narc, 1980, 32, 83-93.

[34] Dickinson, A. The name and surname is not clear? Recommended Intakes of Vitamins and Essential Minerals. Council for Responsible Nutrition (CRN), from Nutritional Supplement

[35] Onianwa, P. C., Adetola, I. G., wegbue, C. M., Ojo, M. F. and Tella, O. O. Trace Heavy Metal Composition of some Nigerian Beverages and Food Drinks, Food Chem**,** 1999, 66, 275-279.

[36] Morrison, G. H. Trace Analysis Physical Methods. John Wiley and Sons, New York, 1965, 67- 94.

[37] Taiz, Zeiger. Plant Physiology, 3rd Edition, 2002.

[38] Kimura, M. and Itokawa, Y**.** Cooking Losses of Minerals in Foods and Its Nutritional Significance. J. Nutr. Sci. Vitaminol (Tokyo), 1990**,** 36, 25-33.

[39] Rebouche, C. J., Carnitine, I., Shils, M.E., Olson, J.A., Shike, M. and Ross, A. C. Modern Nutrition in Health and Disease. 9th ed. Philadelphia: Lippincott, Williams and Wilkins, 1999, 505-51233.

[40] YOU NGR.A. Toxicity Profiles.Toxicity summery for nickel and nickel compounds.1995. Website:http://risk.lsd . ornl.gov/tox/profiles/nickel (accessed 03.03.2005).

[41] CLAYTON G. D., CLAYTON F .E.Patty,'s Industrial Hygiene Toxicology, 4th ed.;A Wiley Inter science Publication:New York, pp2157-2173, 1994.

[42] COO GAN T.p.,LATTA D .M .,SNOW E.T., COS TA M.Toxicity and carcinogenicity of nickel compounds.Crit.Rev. Toxicol.19(4),341,1989.

[43] GRANDJEANP.Human exposure to nickel.IARC Sci.Publ.53,469,1984.

[44] CLARKSO N T.W. Biological Monitoring of Toxic Metals;Plenum press:New York,pp265-282,1988.

[45] COLLO IDAL MINERALS. Nickel.1992. Website:http://www.eagle-min.com/faq/faq 101.htm (accessed 06.07.2005).

[46] ENVIRONMENTAL HEALTH CRITERIAL 108. Nickel.WHO, Genewa,1991.

[47] CHANG L.W. Toxicology of Metals; Lewis publishers: New York,pp245-246,1996.

[48] KTAURA H.,NAKAO N., YOS HIDA N., YAMADA T.Induce sensitization to nickel in guinea pigs immunized with mycobacteria by injection of purified protein derivative with nickel.New Microbiol.26(1),101,2003.

[49] CAVANI A . Breaking tolerance to nickel.Toxicology 209(2),119,2005.

[50] IRIS. Nickel, soluble salts.1996.Websites:http//www.epa.gov/iris/subst/0271.htm(accessed 06.07.2005).

[51] HABER L.T.,ERDREICHT L.,DIAMONDG.L.,MAIER A.M.,RATNEY R., ZHAO Q.,DOU RSO N M.L. Hazard identification and dose response of inhaled nickel -soluble salts.Regul. Toxicol. pharmacol.31,210,2000.

[52] SP ECTRUM. Chemical Fact Sheet. Nickel.1998. Website:http//www .speclab .com elements/ nickel.htm(accessed 03.03.2005).

[53] Santos, E.E., Lauria, D.C. and porto da silveria, C. L. Assessment of Daily Intake of Trace Elements Due to Consumption of Food Stuffs by Adult Inhabitations of Rio De Janeiro City, Sci. Total Environ, 2004**,** 329, 69-79.

[54] Gunn,G., 2014.Critical Metal Handbook.Chichester: Wiley.Haynes, W. M.,2015.CRC Handbook of Chemistry and physics, 96th edn .Boca Raton: CRCPress.

[55.] Pourret, O., Lange, B., Bonhoure, J., Colinet, G., Decree, S ., Mahy, G., Selleck, M., Shutcha,M., and Faucon, M.-P.,2016. Assessment of soil metal distribution and environmental impact of mining in Katanga (Democratic Republic of Congo). Applied Geochemistry,64,43-55.

[56] Crundwell , F. K., Moats, M.S., Ramachandran,V ., Robinson,T.G., and Davenport,W. G.,2011.Extractive Metallurgy of Nickel, Cobalt and Platinum Group Metals.Amsterdam: Elsevier.

[57] Bruland, K. W., Middage,R., and Lohan, M. C., 2014. 8.2- Controls oftrace metals in seawater. In Holland, H. D., and Turekian , K. K., (eds.) , Treatise on Geochemistry, 2nd edn. Oxford : Eisevier,pp. 1951.

[58] Brown ,G. E., and Calas , G., 2012.Section 18. Mineral-water interfaces as driving forces for metal concentration : the example of cobalttrapping by Mn-Oxides. Geochemical Perspectives, 1, 667-669.

[59] Gaillardet, J., Viers, J., and Dupre , B., 2014. 7.7-Trace elements in riverwaters. In Holland , H. D., and Turekian , K.K., (eds.), Treatise on Geochemistry , 2nd end. Oxford:Elsevier,pp. 195-235.

[60] Collins.R.N., and Kinsela,A.S.,2010. The aqueous phase speciation and chemistry of cobalt in terrestrial environments. Chemosphere,79,763-771.

[61] Flora, S.J.S., 2014. Chapter 22- Metals.In Aronson, J.K., (ed) Side Effects of Drugs Annual. Amsterdam: Elsevier, pp297-322.

[62] Banza, C.L .N., Nawrot, T.S., Haufroid, V., Decree, S., De Putter, T., Smolders, E., Kabyla, B.I.Luboya , I.S.,Hunga, A.N., Mutombo, A.M., and Nemery,B., 2009.High human exposure to cobalt and other metals in Katanga, a mining area of the Democratic Republic of Congo. Environmental Research, 109,745-752.

[63] Faucon, M.P.,Shutcha, M.N., and Meets, P., 2007. Revisiting copper and cobalt concentrations in supposed hyper accumulations from SC Africa: influence of washing and metal concentrations in soil.plant and soil,301, 29-36.

[64] Pourret, O., Lange, B., Bonhoure,J., Colinet,G., Decree, S., Mathy, G., Séleck, M., Shutcha, M., and Faucon, M. P., 2016. Assessment of soil metal distribution and environmental impact of mining in Katanga (Democratic Republic of Congo) Applied Geochemistry,64,43_55.

[65] Fifield, F.W. and Haines, P.J. (Eds.). Environmental Analytical Chemistry, Blackie Academic and Professional: London, 1997, 323-349.

[66] phyotoavailability of trace elements to vegetables under the field condition. Chemosphere, **55**:811- 822.

[67] Ishibashi, Y., Matsuo, H., Baba, Y., Nagafuchi, Y., Imato, T and Hirata, T. (2004).

Associotion of manganese effluent with the application of fertilizer and manure on tea field. Water Research, **28**:2821-2826.

[68] Brady, N. C. and Weil, R. R. (2002). The nature properties of soil. 13th edition, Pearson education inc. USA. 823.

[69] Taylor, A., Branch, S., Day, M., Patriciarca, M. and White, M. (2006). Atomic spectrometry update. Journal of Analytical Atomic Spectrometry, **21**:114-121.

[70] Conor, R. (2004). Nutritional trace metals. Blackwell publishing ltd. Oxford brookes university, Oxford, UK. 224.

[71] Pavel, Y., Richard, J. S., Morrison, X., Douglas, G. and Chadwick, M. (2005). Spectrochemical acta part B: Journal of Atomic Spectroscopy, 60:986-992.

[72] Beckhoff, B., Langhoff, N., Lanngiefer, B., Wedell, R and Wolff, H. (2006). Hand book of practical X-ray fluorescence analysis. Springer verlag Berlin, Heldelberg. 301.

[73] Griepink, B. and Tolg, G. Sample Digestion for the Determination of Elemental Traces in Environmental Concern. Pure & Applied. Chemistry, 1989, 61, 1139-1146. 45.

[74] Butcher, D. J. and Sneddon J. A Practical Guide to Graphite Furnace Atomic Absorption Spectrometry, John Wiley & Sons, New York, 1998, 34 -149. 46.

[75] Theodore D. M. Method 200.5: Determinations of trace elements in drinking water by axially viewed inductively coupled plasma-atomic emission spectrometry. National Exposure Research Laboratory Office of Research and Development U. S. Environmental Protection Agency Cincinnati, OHIO 45268, 2003,1-36.

[76] Zhang et al., "Heavy metal contamination in soil and on human health: A review," Journal of Environmental Science and Health, Part B, vol. 55, no. 3, pp. 159-169, 2020.

[77] Wang et al., "Soil acidification and its impact on heavy metal solubility and plant uptake," Chemosphere, vol. 278, pp. 225-234, 2021.

[78] Liu et al., "The role of soil pH in the mobility and bioavailability of heavy metals: A review," Journal of Hazardous Materials, vol. 396, pp. 122-133, 2021.

[79] Chen et al., "The impact of fertilizer application on soil pH and heavy metal mobility," Agricultural Research and Technology, vol. 8, no. 2, pp. 45-53, 2020.

[80] Yang et al., "Soil pH and heavy metal mobility in relation to fertilizer application," Journal of Environmental Science and Health, Part B, vol. 56, no. 1, pp. 1-10, 2021.

[81] "Electrical Conductivity and Its Importance in Soil Science," Journal of Soil Science, vol. 45, no. 83, pp. 123-135, 2019.

[82] "The Relationship Between Soil Texture and Electrical Conductivity," Soil and Water Research, vol. 12, no. 2, pp. 45-53, 2020.

[83] "Electrical Conductivity as an Indicator of Soil Salinity and Nutrient Availability," Agricultural Research Journal, vol. 37, no. 4, pp. 215-225, 2021.

[84] "Soil Electrical Conductivity and Its Applications in Precision Agriculture," Computers and Electronics in Agriculture, vol. 172, pp. 113-121, 2020.

[85] "Soil Analysis for Nutrient Management," University of Minnesota Extension, 2018.

[86] "Soil Fertility and Fertilizers," Texas A&M AgriLife Extension, 2020.

[87]. "Soil Testing for Fertilizer Application," University of Wisconsin-Extension, 2019.

[88]. "Understanding Soil Test Results," Iowa State University Extension, 2021.

[89]"Soil Analysis and Interpretation," University of California Agriculture and Natural Resources, 2017.

[90] WHO. Permissible limits of heavy metals in soil and plants. Geneva: world Health organizational, Switzedland ,199644.

[91] Houba, V. J.G., Van der Lee, I.Novozamsky and I. Waling. Soil and plant analysis a series of syllabi. Part5-Soil Analysis procedure. Wageeningen Agricultural University. The Netherlands, 1989.

[92] Raskin, I., Kumar, P.B.A., Dushenkov, S., Salt, D.E. (1994). Bioconcentration of heavy metals by plants. Curr Opin Biotechnol, 5:285–290.

[93] Itanna, F., Brever, J., Olsson, M. (2008). The fate and bioavailability of trace elements applied to two vegetables farms in Addis Ababa. Afr J Agric Res, 3:797–807.

[94] Stevenson, F.J. Organic matter-micronutrient reactions in soil. In "Micronutrients in Agriculture" 2nd. edn. (ed. Mortvedt, J.J., Cox, F.R., Shuman, L.M. and Welch, R.M.). Soil Sciene Society of America, Madison, 1991, 145-186.

[95] Castilho, P., Chardon, W.J. and Salmons, W. Influence of cattle-manure slurry application on the solubility of cadmium, copper, and zinc in a manured acidic, loamy-sand soil. Journal of Environmental Quality, 1993, 22, 689-697.

Appendix

Table 3.1: Optimization of digestion procedure for khat sample.

Trial	Reagent volume mL			Total	Temperature ^oC	Time (h)	Observation
	HNO_3	H_2O_2	$HClO_4$				
1	3	2	2	7	150	2:30	Red brown
2	4	3	2	9	150	2:30	Yellow
3	6	1	3	10	150	2:30	Slightly Colorless
4	**6**	**2**	**2**	**10**	**150**	**2:30**	**Colorless**
5	6	2	2	10	150	0:30	Cloudy Suspension
6	6	2	2	10	150	1:30	Yellow
7	**6**	**2**	**2**	**10**	**150**	**2:30**	**Colorless**
8	6	2	2	10	60	2:30	Brown
9	6	2	2	10	120	2:30	Yellow
10	**6**	**2**	**2**	**10**	**150**	**2:30**	**Colorless**

Table 3.2: Optimization of digestion procedure for soil sample

Trial	Reagent volume mL			Total	Temperature ^oC	Time (h)	Observation
	HNO_3	H_2O_2	H_2SO_4				
1	3	3	2	8	150	2:30	Red brown
2	4	3	2	9	150	2:30	Yellow
3	6	1	3	10	150	2:30	Slightly Colorless
4	**6**	**2**	**2**	**10**	**150**	**2:30**	**Colorless**
5	6	2	2	10	150	0:30	Cloudy Suspension
6	6	2	2	10	150	1:30	Yellow
7	6	2	2	10	150	2:30	Slightly Colorless
8	**6**	**2**	**2**	**10**	**150**	03:00	**Colorless**
9	6	2	2	10	60	03:00	Brown
10	6	2	2	10	120	03:00	Yellow
11	6	2	2	10	150	03:00	Colorless
12	**6**	**2**	**2**	**10**	**150**	**03:00**	**Colorless**

Table 3.3. Instrumental operation conditions for determination of selected metals using FAAS.

Metals	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Energy (Ev)	Instrumental detection limit (mg/L)
Cr	357.9	0.7	2.0	2.712	0.04
Ni	341.5	0.2	7.0	2.624	0.020
Cu	324.7	0.7	1.5	3.938	0.005
Pb	283.2	0.7	2.0	2.874	0.040
Co	240.7	0.2	4.5	3.106	0.050

Table 3.4. Concentrations of the standard solutions used to establish calibration graphs for the determination of metals in khat and soil samples and their corresponding correlation coefficients.

S.no	Analyte (metal)	Intermediate Standard concentration in mg/L	Standard concentration in mg/L	Correlation coefficient of calibration curve in %
1	Cr	10	0.04, 0.5, 1, 2	0.998
2	Cu	10	0.005, 0.1, 0.5, 1	0.999
3	Ni	10	0.02, 0.5, 1, 1.5	0.999
4	Pb	10	0.005, 0.5, 1, 2	0.998
5	Co	10	0.05, 0.5, 1, 2	0.995

Table 3.5. Method and instrument detection limits values for each metal analyzed

Analyte	Pb	Cr	Co	Ni	Cu
MDL(mg/L) for soil	0. 070	0.073	0.089	0.095	0.040
Khat MDL(mg/L) for khat	0.104	0.089	0.115	0.121	0.024
IDL (mg/L)	0.040	0.040	0.050	0.050	0.005

Table 4.1: Selected physicochemical property of the soils

Soil site	pH	Conductivity (μScm^{-1})	Ash content (%)
Gemeto	6.21 ± 0.02	1.42 ± 0.01	1.93 ± 0.025
Tula 01	6.64 ± 0.03	1.86 ± 0.01	2.17 ± 0.015
Tula rural	6.82 ± 0.01	1.63 ± 0.02	1.72 ± 0.020

Table 4.2: Selected physicochemical property of the khat varieties

Khat varieties	pH	Electrical conductivity	Ash content%
Nole	5.93 ± 0.02	1.17 ± 0.01	0.63 ± 0.02
Dume	6.24 ± 0.01	1.54 ± 0.01	0.85 ± 0.01
Megala	6.43 ± 0.01	1.33 ± 0.02	0.93 ± 0.01

Table 4.3: Results of the detected mean level of each studied metal (mg/kg dry weight) in the soil and khat samples

Heavy metal	Soil sample	(mg/Kg) n=3	Khat varieties	(mg/Kg) n=3
Cu	Gemeto	9.23 ± 0.15	Nole	4.56 ± 0.15
	Tulla 01	8.54 ± 0.19	Dume	4.33 ± 0.15
	Tulla rural	9.96 ± 0.20	Megala	4.93 ± 0.20
Co	Gemeto	4.433 ± 0.30	Nole	2.363 ± 0.023
	Tulla 01	4.85 ± 0.02	Dume	2.851 ± 0.04
	Tulla rural	3.37 ± 0.02	Megala	1.842 ± 0.03
Pb	Gemeto	ND	Nole	ND
	Tulla 01	ND	Dume	ND
	Tulla rural	ND	Megala	ND
Cr	Gemeto	ND	Nole	ND
	Tulla 01	ND	Dume	ND
	Tulla rural	ND	Megala	ND
Ni	Gemeto	4.57 ± 0.02	Nole	2.51 ± 0.31
	Tulla 01	3.67 ± 0.02	Dume	2.93 ± 0.02
	Tulla rural	4.95 ± 0.04	Megala	3.26 ± 0.03

ND = not detected

Table 4.4. Concentrations of the standard solutions used to establish calibration graphs for the determination of metals in khat and soil samples and their corresponding correlation coefficients

S.no	Analyte (metal)	Intermediate Standard concentration in mg/L	Standard concentration in mg/L	Correlation coefficient of calibration curve in %
1	Cr	10	0.05, 0.5, 1, 2	0.998
2	Cu	10	0.01, 0.05, 0.5, 1	0.999
3	Ni	10	0.05, 0.1, 0.5, 1	0.999
4	Pb	10	0.05, 0.1, 0.5, 1	0.998
5	Co	10	0.05, 0.5, 1, 2	0.995

Table 4.5. Recovery test results for the determination of the metal in soil samples (mean ± SD)

Metals	Conc.in sample (mg/L)	Amount added (mg/L)	Conc.in spiked sample (mg/L)	% Recovery	RSD
Co	0.081 ±0.0017	0.03	0.112 ± 0.001	103.22± 5.09	4.981
Ni	0.088 ± 0.002	0.03	0.123 ± 0.002	116.66 ± 5.67	6.249
Cu	0.19 ± 0.015	0.1	0.281 ± 0.005	91.16 ± 5.77	5.972

Table 4.6. Recovery test results for the determination of the metal in khat samples (mean ± SD)

Metals	Conc. in sample(mg/L	Amount added (mg/L	Conc. In spiked sample(mg/L	%Recovery	RSD
Co	**0.046**	0.03	0.0755 ± 0.001	98.66 ± 3.33	3.448

Table 4.7 Correlation matrix between transfer factors of metals in soil with khat

Parametrs	Cu in soil
Cu in soil	
Cu in khat	0.975251
Co in soil	0.6583208
Co in khat	0.4711473
Ni in soil	-0.235996
Ni in khat	0.9929235

	± 0.001				
Ni	0.055 ± 0.003	0.03	0.086 ± 0.0005	103.44± 1.92	2.037
Cu	0.091 ± 0.02	0.1	0.190 ± 0.0057	99.12 ± 5.77	5.412

Table 4.8. ANOVA table of selected metals in khat and soil sample (at 0.05 levels)

Element	Source of Variation	SS	df	MS	F calculated	P value	F critical
Cu	Between sample	0.541	2	0.2705	0.0028	0.997	3.68
	Within sample	1425	15	95.057			
	Total	1426.4	17				
Co	Between sample	0.0372	2	0.0186	0.0133	0.986	3.68
	Within sample	20.83	15	1.3869			
	Total	20.87	17				
Ni	Between sample	0.03181	2	0.015906	0.01748	0.982	3.68
	Within sample	13.646	15	0.9097			
	Total	13.678	17				

Calibration data of Cu

Standard	Conc. (ppm)	Abs.
1	0.01	0.00125
2	0.05	0.00912
3	0.5	0.07872
4	1	0.16425

Calibration data of Co

Standard	Conc. (ppm)	Abs.
1	0.05	0.00107
2	0.5	0.02246
3	1	0.04432
4	2	0.08511

Calibration data of Ni

Calibration data of Cr

Standard	Conc. (ppm)	Abs.
1	0.05	0.00175
2	0.1	0.00287
3	0.5	0.01381
4	1	0.02748

Standard	Conc. (ppm)	Abs.
1	0.05	0.00125
2	0.5	0.00912
3	1	0.07872
4	2	0.1642

Calibration data of Pb

Standard	Conc. (ppm)	Abs.
1	0.05	0.00117
2	0.1	0.00242
3	0.5	0.01381
4	1	0.02725

Steps of from sample collection up to laboratory digestion





CEX